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IN OVO VACCINATION AGAINST INFECTIOUS BURSAL DISEASE IN BROIILER CHICKENS

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SUMMARY

A vaccination trial was conducted to determine the safety and efficacy of an attenuated local isolate of infectious bursal disease (IBD) virus seed identified as UPM 93273. *In ovo* vaccination method was used in 18-day-old embryonated commercial broiler eggs. One hundred and eighty, 18-day-old embryonated eggs were divided into 3 groups; A, B and control. The eggs in group A were vaccinated with the IBD virus (IBDV) seed alone while the eggs in group B were vaccinated with the IBDV plus bursal disease antibody (BDA) complex through the allantoic cavity. The control group remained unvaccinated. Serum samples were collected from 18-day-old embryos for IBD antibody titre detection using the enzyme linked immunosorbent assay (ELISA). The hatchability of the eggs was recorded and the day-old chicks were kept separated according to the group. Feed and water were provided *ad libitum*. The chickens were sacrificed at days 0, 7, 14, 21, 28 and 35 of age before the body weight, gross lesions and bursa weight were recorded. At 28 days old, the chickens were further divided within each group into the IBDV-challenged and non-challenged groups. All chickens in the IBDV-challenged groups were inoculated with a highly pathogenic strain of local isolate of IBDV identified as UPM 94283 before all were sacrificed on day 7 post-challenge. The hatchability rate of the control, groups A and B were 93%, 90% and 30% respectively. The body weight of chickens in all groups showed no significant different throughout the trial. The bursar weight continued to increase from days 0 to 21, but reduced significantly ($p<0.05$) at day 28 in group A. However, the weight continued to increase back at day 35 in group A. Histologically, a similar pattern of lesion score was observed. The organ showed mild to moderate lesion in groups A and B with score of 2 to 3 at day 35 in both IBDV-challenged and non-challenged chickens. Severe bursal lesion (score of 4 to 5) was observed in the IBDV-challenged control group. The IBD antibody titre was 2216 ± 534 in the 18-day-old embryo, increased to 4531 ± 388 in day-old chicks, but gradually decreased to 187 ± 45 at day 28 in control group. However, the titre started to increase again at day 21 and reached the highest level at day 35 in the groups A (2818 ± 224) and B (2540 ± 326). The antibody titre remained high in all IBDV-challenged groups at day 35. It was concluded that the *in ovo* vaccination using UPM 93273 IBDV seed either in the form of IBDV plus BDA complex or IBDV alone can give full protection against IBDV challenged at 28 days.

Keywords: *In ovo* vaccination, infectious bursal disease, broiler chickens

INTRODUCTION

Infectious bursal disease (IBD) or Gomboro disease, an acute highly contagious viral disease of chickens, was first recognised as a clinical entity in 1957 in USA (Cosgrove, 1962). Since then, the disease has been reported world wide, although it was relatively under control due to proper vaccination programmes in both hen and their progeny. However, in late 1980's, outbreaks of clinical IBD with high mortality due to highly virulent strains of serotype 1 IBD virus (IBDV) were reported throughout Europe (Chettle *et al,* 1989; Van den Berg *et al.,* 1991). The disease has spread world wide and was first described in Asia in early 1990's (Nunoya *et al.,* 1992), including Malaysia in 1991 (Hair-Bejo, 1992; Loganathan *et al.*, 1992). These highly pathogenic strains of IBDV cause great economic losses through high mortality, impaired growth, excessive carcass condemnation and profound immunosuppression leading to increase susceptibility to other pathogens and interfere with vaccination programmes against other highly virulent diseases (Hair-Bejo, 1994). The virus replicates in the bursa of Fabricius leading to depression of bursal derived lymphocytes and intense immunosuppression. Therapy for IBDV infected chickens is of less value and the incidence of the disease can only be prevented by proper biosecurity and immunisation programmes. To date, more than 46 types of IBD vaccines were imported for used in West Malaysia (Chin, 1993). The virulence of the vaccines range from mild to intermediate to intermediate plus. Mild IBD vaccine is believed to be neutralised by maternal derived antibody (MDA) in some chicks and only a small number of chicks with low MDA levels to IBDV will gain protection from the used of the mild vaccine. In contrast, intermediate-plus vaccine can cause severe bursal lymphocyte destruction within 24 hours post-vaccination in chicks with low MDA level. The live IBD vaccines are usually given in broiler chickens through the eye drops or in drinking water, once to three times throughout their grow out period.

A new concept of vaccination method for IBD, known as *in ovo* vaccination, was introduced to the

poultry industry recently. The vaccination was proposed as a means of priming or stimulating an early immune response that can confer full protection to the chickens at the susceptible age (Sharma and Burmester, 1984). *In ovo* technology has been successfully employed with the development of IBDV plus antiserum complex vaccine. The vaccine was prepared by mixing IBDV with IBD antibody (bursal disease antibody or BDA). This preparation was assumed to allow for the delayed release of IBDV when the ratio of antibody to virus was determined and measured correctly using the viral neutralisation bioassay (Haddad *et al.*, 1994). The BDA can also lower the pathogenicity of the live vaccine.

The IBDV plus BDA complex vaccine is inoculated through the eggshell into the allantoic cavity of 18-day-old embryo, at time of incubation when the eggs are routinely transferred to hatching tray. *In ovo* vaccination eliminates the need for post-hatching vaccination, reduces vaccination stress and decreases the need of manpower (Gildersleeve, 1993). A number of imrnune responses, including antibody responses to antigen, can be induced in the embryo at 12 to 14 days of incubation soon after the emergence of T and B cell progenitors (Jankovic *et al.*, 1975; Houssaint *et al.*, 1991). The efficacy of *in ovo* vaccines against Marek's disease, infectious bursal disease, infectious bronchitis and Newcastle disease have been described previously (Sharma, 1985; Wakenell and Sharma, 1986; Ahmad and Sharma, 1992; Avakian *et al.*, 1994). This study determines the safety and efficacy of *in ovo* vaccination of 18-day-old embryo using vaccine containing local isolate of IBD viral seed (UPM 93273) either given alone or as IBDV vaccine plus BDA complex.

MATERIALS AND METHODS

IBD vaccine

A local live attenuated IBD vaccine, identified as UPM 93273, with a titre of $10^{4.5}$ EID_{50}/0.1 mL was used in this study (Hair-Bejo *et al.*, 1998). The vaccine was found to be safe for broiler chickens.

IBD vaccine and bursal disease antibody (BDA) complex

The IBD antibody was produced in specific-pathogen-free (SPF) chickens using a local isolate of IBDV (UPM 93273). The antisera was filter-sterilised in 0.45 μm filter and heated at 56°C for 30 min to destroy the heat labile non-specific virus inhibitory substance (Beard, 1989). The IBD vaccine and BDA complex (IBD plus BDA complex) was prepared by mixing the live attenuated IBD vaccine ($10^{4.5}$ EID_{50}/0.1 mL) with 500 ELISA unit/mL of BDA. The mixture was incubated at room temperature for an hour to stabilise.

IBD challenge virus

A local isolate of IBDV, identified as UPM 94283, with a titre of $10^{8.5}$ EID_{50}/0.1 mL was used as the IBD challenge virus (Hair-Bejo *et al.*, 1997).

In ovo *vaccination*

One hundred and eighty, 18-day-old embryonated broiler eggs were divided equally into 3 groups; A, B and control. The eggs of groups A and B were inoculated with 0.1 mL of the IBD vaccine (UPM 93273) at a dose of $10^{4.5}$ $E1D_{50}$/0.1 mL and IBDV ($10^{4.5}$ EID_{50}) plus BDA (500 ELISA unit/mL) complex, respectively into the allantoic cavity. The control group remained unvaccinated. The eggs were incubated in separate incubators at temperature between 37.3°C to 38.0°C. Serum samples were collected from ten, 18-day-old embryos via intracardiac route for the detection of IBD maternally derived antibody (MDA).

Experimental broiler chickens

The hatchability of eggs in each group was recorded and the day-old chicks were reared separately. Feed and water were available *ad libitum* and the chicks were monitored for clinical abnormality three times daily. At days 0, 7, 14, 21 and 28 days of age, 5 chickens from each of groups A and control were sacrificed.

When the chicks reached 28 days old, they were further divided into two subgroups; the IBDV-challenged and non-challenged groups. All chickens in the IBDV-challenged group were inoculated intraocularly (0.1 mL) with a highly pathogenic strain of local isolate of IBDV (UPM 94283) at a dose of $10^{8.5}$ EID_{50}/0.1 mL. All chickens were sacrificed at day 7 post-inoculation (pi). The body weights were recorded and serum samples were collected for detection of IBD antibody.

During necropsy, the gross lesions were recorded. The bursa of Fabricius was weighted and fixed in 10% buffered formalin for histopathological examination. The bursa to body weight ratio was determined using a technique previously described (Van den Berg *et al.*, 1991).

Histopathology

The bursa of Fabricius of control and IBD vaccinated groups were fixed in 10% buffered formalin for at least 48 h. Tissues were trimmed to the thickness of 0.5 cm and the blocks were subsequently dehydrated in a series of alcohol, clear with xylene and embedded in paraffin wax using an automatic tissues processor (Reichert-Jung). Tissues were sectioned at about 4 μm and stained with haematoxylin and

eosin (HE) (Lillie., 1965). Tissues were carefully examined using 4x and 10x objectives for histological changes and were subjectively graded by a modified scoring method previously established; 0 (normal), 1 (mild), 2 (mild to moderate), 3 (moderate), 4 (moderate to severe) and 5 (severe) (Muskette *et al.*, 1979; Henry *et al.*, 1980; Sharma *et al.*, 1989; Thu-Zar, 1996).

Enzyme linked immunosorbent assay

The ELISA test was carried out according to the method described by IDEXX'S, Westbrook, Maine, USA. Antigen-coated plates were adjusted to room temperature prior to the assay. One hundred µL of dilution buffer was dispensed into each well. This was followed by addition of 100 µL of the test, positive and negative sera into the well, accordingly. The test sera were diluted 1:500 (v/v) in dilution buffer. The plate was then incubated for 30 min at room temperature. It was then washed three times in distilled water before 100 µL of the goat anti-chicken horseradish peroxidase conjugated IgG was added into the well. The plate was then incubated for 30 min at room temperature and washed three times in distilled water. A hundred µL of substrate solution (TMB) was dispensed into each well and incubated for 15 min at room temperature. Finally, 100 µL of diluted stop solution was added into each test well before the optical density was taken at 650 nm. The IBD antibody titre was calculated automatically using softwear by Blankfard and Silk (1989).

Statistical analysis

Analyses of variance were conducted using the SPSS program (Norusis, 1990).

RESULTS

Hatchability

The hatchability was 90%, 30% and 93% for groups A, B and control, respectively.

Clinical signs

The chickens showed no clinical signs of IBDV infection throughout the trial. One chick from group A died of splay legs and poor body condition at day-old. All IBDV-challenged chickens did not show clinical signs of IBDV infection. However, ruffled feathers, depression, anorexia and diarrhoea were observed in some IBDV-challenged chickens from the control group.

Body weight

The body weight of chickens of all groups increased significantly ($p<0.05$) during the first 35 days (Fig. 1). Similarly, the IBDV-challenged chickens showed no significant ($p>0.05$) differences in the body weight compared to the non-challenge chickens in all groups.

Bursa of Fabilcius weight

The bursal weight increased significantly ($p<0.05$) from 0.06 ± 0.00g at day 0 to 3.90 ± 0.28g at day 28 in the control group (Fig. 2). Similar increase in the organ weight was observed in group A during the first 21 days of age (0.03 ± 0.00g and 2.02 ± 0.24g at days 0 and 21, respectively), but was significantly ($p<0.05$) decreased (0.81 ± 0.05g) at day 28. The organ weight, however, increased slightly on day 35 (0.94 ± 0.12g). Similarly, the bursal weight in group B increased significantly ($p<0.05$) at day 35 (1.30-+0.49g) when compared to day 28 (0.62 ± 0.13g) (Fig. 2).

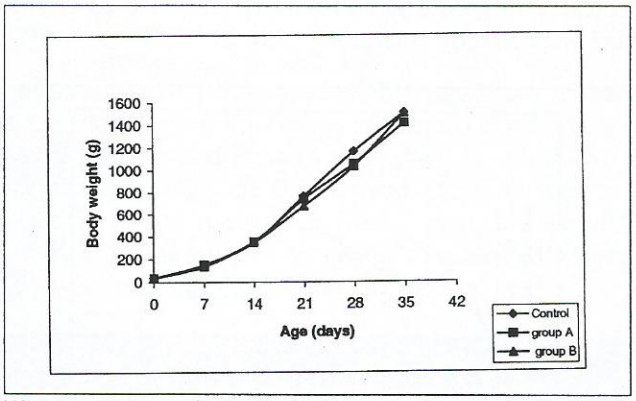

Fig. 1. Body weight of chickens in the control, IBDV (group A) and IBDV plus BDA complex (group B)

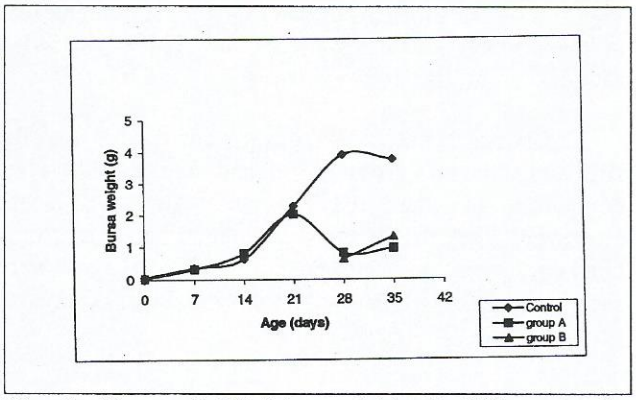

Fig. 2. Bursa of Fibricius weight of chickens in the control, IBDV (group A) and IBDV plus BDA complex (group B)

The bursal weight of the IBDV-challenged chickens (1.52 ± 0.13g) in the control group was significantly low ($p<0.05$) compared to the non-challenged chickens (3.75 ± 0.31g). There were no significant differences ($p>0.05$) in the bursal weight of IBDV-challenged and non-challenged chickens of groups A and B.

Bursal to body weight ratio

The bursal to body weights ratio in the control and group A followed a similar pattern during the first 21

days of age (Fig. 3). However, the ratio in both groups A and B were significantly lower ($p<0.05$) when compared to the control group at days 28 and 35. The ratio in the IBDV-challenged chickens from the control group was significantly lower ($p<0.05$) than the non-challenged chickens but was not significantly ($p>0.05$) different in groups A and B.

Gross lesions

There was no gross lesion of IBDV infection observed in the sacrificed chickens in the control and group A during the first 14 days of age. However, the bursa of Fabricius of two chickens from group A were oedematous on day 21. The serosal surface of the organ was covered with yellowish transudate. The organs of chickens in groups A and B were atrophied on day 28 and were slightly enlarged by day 35.

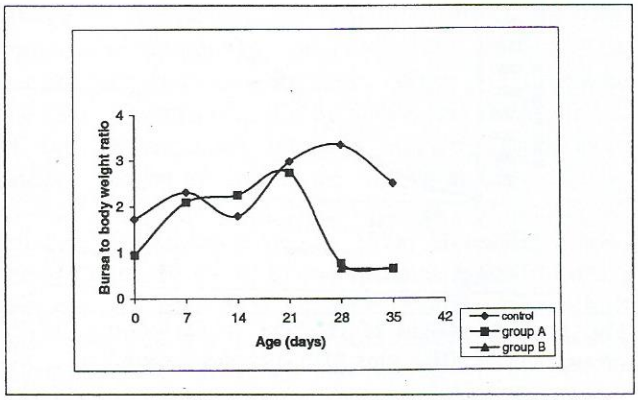

Fig. 3. Bursa of Fibricius to body weight ratio of chickens in the control, IBDV (group A) and IBDV plus BDA complex (group B).

The bursa of chickens from groups A and B of the IBDV-challenged group remained unchanged when compared to the IBDV non-challenged group. However, the organ was atrophied and in some chickens, areas of necrosis and haemorrhages were recorded in the IBDV-challenged chickens of control group.

Histological lesions

The histological lesions in the control group remained mild with the lesion score of 1 throughout the trial (Figs. 4, 6a). A similar lesion scoring was recorded in the group A throughout the first 14 days of age. However, moderate bursal lesion (score of 3) was observed on day 21 and the lesion became moderate to severe (score of 4) on day 28. Sign of recovery was observed on day 35 as the bursal lesions returned to mild to moderate (score of 2 to 3). Sign of bursa recovery was also observed in the group B at day 35 (Fig. 4). Severe bursal lesions (score of 5) was observed in the challenged group on day 7 post-challenged (Fig. 6b). However, groups A and B showed mild to moderate (score of 2 to 3) bursal lesions both in the IBDV-challenged and non-challenged chickens (Figs. 7a, b).

IBD antibody titre

The IBD antibody titre in the 18-day-old embryos was 2216 ± 534 and it increased significantly ($p<0.05$) to 4531 ± 388 at day-old (Fig. 5). However, it started to decrease from day 7 (1947 ± 110) to day 14 (737 ± 198), reached the lowest level on day 28 (187 ± 45) and remained low thereafter. The IBD titre in groups A and B increased significantly ($p<0.05$) from day 28 (2369 ± 326) and day 21 (1336 ± 200), respectively to the highest level on day 35 (2819 ± 224 and 2540 ± 326 in groups A and B, respectively). The antibody titre of the IBDV-challenged chickens in the control group (3092 ± 396) was markedly high compared to the non-challenged chickens (282 ± 68) at day 7 post-challenge. The titre, however, remained unchanged in groups A and B.

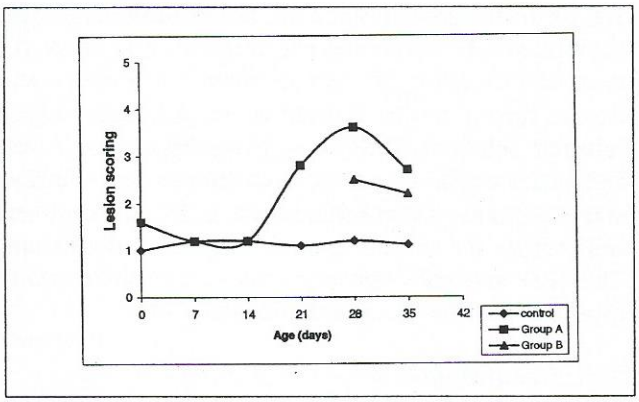

Fig. 4. Histological lesion scoring of the bursa of Fibricius in the control, IBDV (group A) and IBDV plus BDA complex (group B)

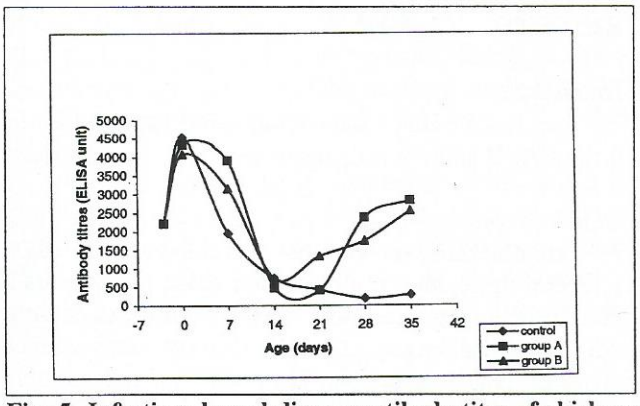

Fig. 5. Infectious bursal disease antibody titre of chickens in control, IBDV (group A) and IBDV plus BDA complex (group B).

DISCUSSION

This study showed that the UPM 93273 IBD vaccine is safe and effective following *in ovo*

vaccination of 18-day-old embryonated commercial broiler eggs. A similar result was reported when the vaccine was inoculated in 14- and 28-day-old commercial broiler chicken (Hair-Bejo *et. al.,* 1998). The IBD vaccine did not affect the hatchability of the vaccinated eggs nor caused gross and histological lesions in the bursa of Fabricius during the first 14 days of age. However, moderate bursal lesions were observed at day 21, suggesting multiplication of the virus in the organ. The bursal damage was not severe enough to cause neither clinical manifestations of IBD nor the changes in body weight. It is interesting to note that a high level of IBD antibody was induced at days 28 and 35 followed by recovery of the bursa of Fabricius at day 35. Similar findings were reported following the use of IBDV plus BDA complex vaccine (Johnston *et al.,* 1997). The chickens in the present study were fully protected against IBDV challenge at the age of 28 days.

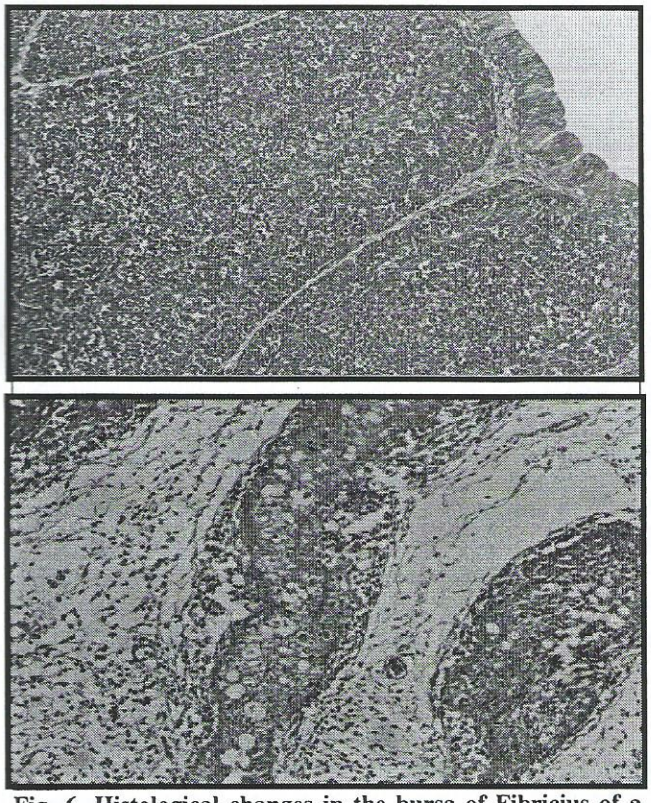

Fig. 6. Histological changes in the bursa of Fibricius of a 35 days old control chicken. A. IBDV non-challenged group (score 1). B. IBDV-challenged group (score of 5). HE x100

These findings demonstrated that the IBD vaccine UPM 93273 can be used for *in ovo* vaccination in 18-day-old embryo, as an alternative vaccination route for the control and prevention of IBDV infection in broiler chickens. This technique could reduce vaccination stress on the chickens due to excessive handling during post-hatching vaccination.

This study also showed that the IBD plus BDA complex vaccine, when used *in ovo* on 18-day-old embryo, is safe and effective in conferring protection against IBDV challenge. The induction of IBD antibody on day 21 was much earlier when compared to the IBD vaccine alone or the IBD plus BDA complex vaccine previously reported (Johnston *et al.,* 1997). The IBD titre was further increased on days 28 and 35. The lesions in the bursa of Fabricius were mild to moderate, which recovered on day 35. The body weight remained unchanged throughout the trial. However, the hatchability of the eggs in this group was significantly low, in contrast to the previous report (Johnston *et al.,* 1997). The IBDV and BDA complex probably caused shock and embryonic death during the incubation period. Several surviving but weak day-old chicks in the group died later.

It is interesting to note that the IBD maternal derived antibody (MDA) is not fully transferred to the 18-day-old embryo. The antibody reached a maximum level at day-old and reduced thereafter. In the *in ovo* vaccinated chickens, the IBD antibody decay seemed to be much slower during the first 7 days of age. It appeared that the vaccination did not affect the MDA of the 18-day-old embryos and day-old chicks.

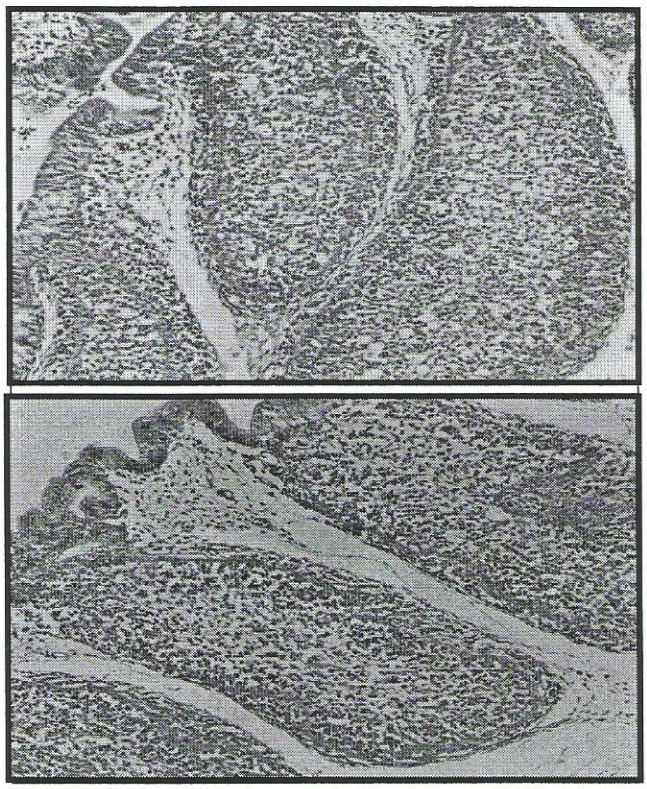

Fig. 7. Histological changes in the bursa of Fibricius of IBD vaccinated broiler chicken at day 7 post-challenge. A. Group A (score of 3). B. Group B (score of 3). HE x100

It was concluded that the *in ovo* vaccination using IBDV alone or IBDV plus BDA vaccine complex can provide full protection against IBDV challenge.

However, the IBDV alone (UPM 93273) is more suitable to be used for *in ovo* vaccination, leading to good hatchability when compared to the IBDV plus BDA complex vaccine.

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RINGKASAN

PEMVAKSINAN IN OVO *TERHADAP PENYAKIT BURSA BERJANGKIT DALAM AYAM PEDAGING*

Satu percubaan pemvaksinan telah dijalankan untuk menentukan keselamatan dan kemujaraban pencilan benih virus tempatan teratenuat penyakit bursa berjangkit (IBD) yang dikenalpasti sebagai UPM 93273. Kaedah pemvaksinan in ovo telah diguna dalam telur ayam pedaging terembrio komersil. Satu ratus lapan puluh biji telur terembrio berumur 18 hari dibahagikan kepada 3 kumpulan; A, B, dan kawalan. Telur dalam kumpulan A divaksinkan dengan benih virus IBD (IBDV) sahaja sambil telur dalam kumpulan B divaksinkan dengan IBDV tercampur kompleks antibodi penyakit bursa (BDA) melalui rongga alantoin. Kumpulan kawalan tidak divaksin. Sampel serum dikumpul daripada embrio umur 18 hari ini untuk pengesanan titer antibodi IBD mengguna asai imunoerap terangkai enzim (ELISA). Kebolehtetasan telur telah direkodkan dan anak ayam sehari diasingkan mengikut kumpulan. Makanan dan air diberikan secara ad libitum. Ayam ini disembelih pada umur 0, 7, 14, 21, 28 dan 35 hari dan berat badan, lesi kasar, berat bursa direkodkan. Pada umur 28 hari, ayam dalam setiap kumpulan ini dibahagikan lagi kepada kumpulan IBDV-tercabar dan kumpulan bukan tercabar. Kesemua ayam dalam kumpulan IBDV-tercabar diinokulatkan dengan strain patogen tinggi pencilan tempatan IBDV yang dikenalpasti sebagai UPM 94283 sebelum disembelih pada hari 7 pasca-cabaran. Kadar kebolehtetasan kumpulan kawalan, A dan B masing-masing 93%, 90% dan 30%. Berat badan ayam dalam kesemua kumpulan tidak menunjukkan perbezaan tererti pada sepanjang percubaan. Berat bursa terus meningkat daripada hari 0 hingga ke hari 21, sambil menunjukkan keertian terkurang ($p < 0.05$) pada hari 28 dalam kumpulan A. Bagaimanapun, berat bursa ini meningkat semula pada hari 35 dalam kumpulan A tersebut. Secara histologi, pola skor lesi yang serupa telah dicerapkan. Organ menunjukkan lesi ringan hingga sederhana dalam kumpulan A dan B dengan skor 2 hingga 3 pada hari 35 dalam kedua-dua kumpulan IBDV-tercabar dan bukan tercabar. Lesi bursa teruk (skor 4 hingga 5) dicerapkan dalam kumpulan kawalan IBDV-tercabar. Titer antibodi IBD ialah 2216 $\pm$ 534 dalam embrio umur 18 hari, meningkat kepada 4531 $\pm$ 388 dalam anak ayam sehari, tetapi menurun secara beransur-ansur kepada 187 $\pm$ 45 pada hari 28 dalam kumpulan kawalan. Bagaimanapun, titer mula meningkat pada hari 21 dan mencapai aras tertinggi pada hari 35 dalam kumpulan A (2818 $\pm$ 224) dan B (2540 $\pm$ 326). Titer antibodi kekal tinggi dalam kesemua kumpulan IBDV-tercabar pada hari 35. Kesimpulannya ialah dalam pemvaksinan in ovo mengguna benih UPM 93273 IBDV sama ada dalam bentuk IBDV bercampur kompleks BDA atau sebagai IBDV sahaja, boleh memberikan perlindungan sepenuh terhadap IBDV pada hari 28.