EFFECT OF DILUENT COMPOSITION ON IBD IMMUNOCOMPLEX VACCINE EFFICIENCY USING COMMERCIAL BIRDS

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Abstract
IBDV remains a major concern for the poultry industry worldwide, causing significant economic losses due to mortality and reduced production. Vaccination has been the most effective strategy for controlling IBDV. However, the effectiveness of the vaccine can be influenced by several factors. This study aims to investigate the efficiency of IBDV immunocomplex vaccine using different diluents.

Introduction
Infectious bursal disease (IBD), also known as Gumboro disease, is an acute highly contagious disease in young chickens caused by infectious bursal disease virus (IBDV). It is characterized by a hemorrhagic syndrome, severe damage in cloacal bursa, in addition to immunosuppression which leads to vaccination failure, E. coli infection, gangrenous dermatitis, and inclusion body hepatitis, in addition to high mortality rates generally at 3 to 6 weeks of age. The disease is easily spread from infected chickens to healthy chickens through food, water, and physical contact.
Objective: to investigate the effect of diluent composition on IBDV Immunocomplex vaccine efficiency.

Experimental Design and Methods
400 commercial birds were randomly divided into two groups:

- **Group 1:** normal saline (0.9% sodium chloride) + IBD Immunocomplex vaccine.
- **Group 2:** the IBD Immunocomplex diluent+ IBD Immunocomplex vaccine, that contains triptose, glucose, sodium chloride, potassium, and sodium phosphate.
- **Serology:** 15 serum samples were taken on day 1, 5, 10, 15 ,20, 25, 30, 35 and 42 days (slaughter day) for each group and analyzed using IDvet- IBD indirect ELISA kit.
- **PCR samples:** 5 bursal tissue samples were taken at 15, 20, 25, 30, 35 and 42 days (slaughter day) for each group and analyzed using real time Anicon IBDV pathotyping kit.
- **Genotyping:** positive PCR samples were sent for genotyping analysis.
- **Histopathology samples:** 5 bursa samples were taken at 15, 20, 25, 30, 35, 42 days (day on the slaughter) for each group.

Results

Serology results showed both groups from day 1-20 days old that maternally derived antibodies (MDA) declined gradually. At 25 days old, IBD Immunocomplex diluent+ IBD Immunocomplexvaccine group showed higher, stable response of antibodies that continued until 42 days old, compared to normal saline + IBD Immunocomplex vaccine group, with an exception at 35 days old for group 1, **Figure 1.**

PCR results: IBD Immunocomplex diluent+ IBD Immunocomplex vaccine group showed the presence of the virus at 20 days old, then disappeared at 35 days old. While normal saline+ IBD Immunocomplex vaccine group showed no detection at 20 days old, but at 25 days old, indicating a delay in immuno-response as shown in **Table 1.**

Genotyping: Specific DNA amplification of the VP2 gene region by RT-PCR - Reverse Transcription Polymerase Chain Reaction, succeeded by the Restriction Fragment Polymorphism (RFLP) study. Results showed the presence of Winterfield strain 2512 **(GM3 - W2512).**

Histopathology: both groups started showing microscopic alterations at 20 days old. In normal saline + IBD vaccine group a slight to discrete change was observed (score 1) **Figure 2.** On the contrary IBD Immunocomplex diluent+ IBD Immunocomplex vaccine group showed an intense to severe inflammatory reaction in bursal tissue, the microscopic alterations observed are the same as those found in cases of the action of IBDV immunocomplex vaccine **Figure 3.**

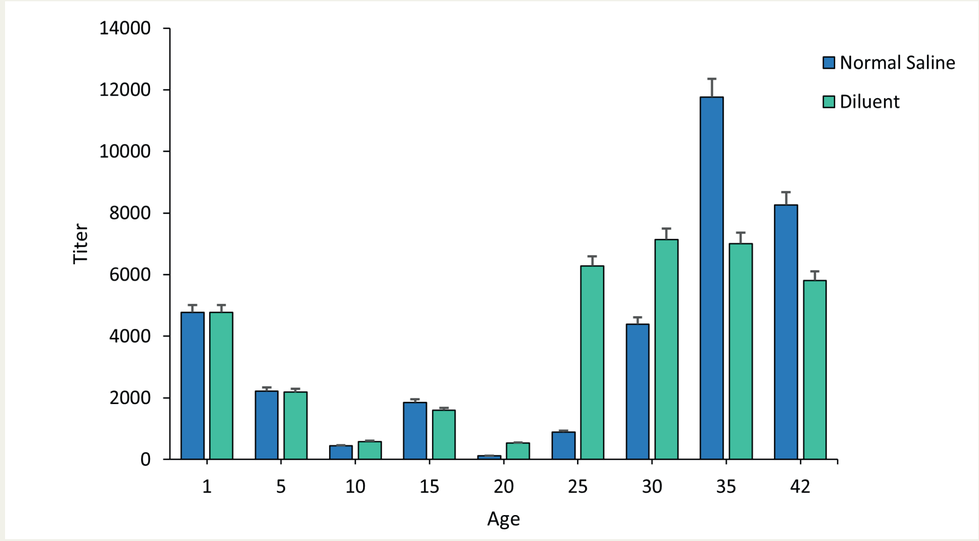

Figure 1. Titers from 25 days old, (diluent) group 2 showed higher, stable response of antibodies that continued until 42 days old, compared to (normal saline) group 1, with an exception at 35 days old for group 1.

Age	Diluent	Normal Saline
15 days	-	-
20 days	+	-
25 days	+	+
30 days	-	-
35 days	-	-
42 days	-	+

Table 1. IBDV PCR Results in Group 1 (Normal saline) and Group 2 (Diluent).

Group 1 (Normal saline + IBD Immunocomplex vaccine)	Group 2 (IBD Immunocomplex Diluent+ IBD Immunocomplex vaccine)
Lymphoid Depletion (3/5), <25% (score 1); (2/5) from 51-75% (score 3)	**Lymphoid Depletion** from 51-75% (score 3) 5/5
Necrosis 1 (0); 2 (0); 3 (0); 4 (0); 5 (+)	**Necrosis** 1 (+); 2 (+); 3 (0); 4 (+); 5 (+)
Interfollicular Inflammatory Infiltration 1 (0); 2 (0); 3 (0); 4 (++); 5 (+)	**Interfollicular Inflammatory Infiltration** 1 (+); 2 (++); 3 (+); 4 (+); 5 (+)
Epithelial Hyperplasia 1 (0); 2 (0); 3 (0); 4 (0); 5 (+)	**Epithelial Hyperplasia** 1 (+); 2 (+); 3 (+); 4 (+); 5 (+)
Hyperemia 1 (0); 2 (+); 3 (0); 4 (+); 5 (++)	**Hyperemia** 1 (+); 2 (++); 3 (++); 4 (++); 5 (+)
Hemorrhage 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)	**Hemorrhage** 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)
Edema 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)	**Edema** 1 (0); 2 (0); 3 (+); 4 (+); 5 (0)
Follicular Regeneration 1 - 3 not applicable; 4 (+++); 5 (++)	**Follicular Regeneration** 1 (++); 2 (++); 3 (++); 4 (++); 5 (+)
Cystic Follicles 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)	**Cystic Follicles** 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)
Intralumial Exudate 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)	**Intralumial Exudate** 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)
Cryptosporidium sp. 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)	**Cryptosporidium sp.** 1 (0); 2 (0); 3 (0); 4 (0); 5 (0)
Labels: 0- absent; + light or discreet; ++ moderate; +++ intense or severe	Labels: 0- absent; + light or discreet; ++ moderate; +++ intense or severe

Figure 2. The histopathology scoring system in both groups shows the differences in inflammatory appearances at 20 days of age.

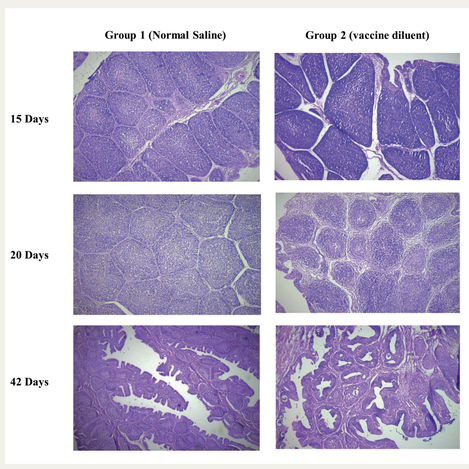

Figure 3. The histopathology results revealed microscopic alterations in both groups at age 20 days. However, the scoring system in **IBD Immunocomplex diluent+ IBD Immunocomplex vaccine group** indicated drastic changes confirming the presence of the virus, that originated from the vaccine.

Discussion:
Serological results showed that antibodies response in diluent group was earlier than those of normal saline group, starting from 25 days old. These results were compatible with PCR test, that showed the presence of the virus in all samples of diluent group at 20 days old, compared to those of normal saline group that showed positive 5 days later at 25 days old, hence indicating a delay of immune response in experimental subjects in which normal saline was used. These above results were confirmed with genotyping results that detected the presence of Winterfield strain 2512 (from vaccination). The histopathological results confirmed the presence of the virus at age 20 days in IBD Immunocomplex diluent+ IBD Immunocomplex vaccine group with an intense to severe inflammatory microscopic alterations that originated from the vaccine, compared to normal saline + IBD Immunocomplex vaccine group that showed slight to discrete microscopic changes at the same age.

Conclusion
This study investigated the effect of other diluents on the efficiency of IBDV immunocomplex vaccine throughout serology and PCR testing. These results indicated that diluent composition has an important effect on the IBD immunocomplex vaccine efficiency.