Comparison study between the efficacy of immune complex and conventionally live vaccine against Gumboro disease in broilers

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Abstract

This study aimed to evaluate the immune response and histological changes of two Gumboro disease vaccines. Two hundred, days old broilers were divided into 4 groups: group A was vaccinated with live attenuated infectious bursal disease (IBD) vaccine at 7 and 21 days of age, group B was vaccinated with Immune-Complex vaccine (Icx) at first day old, then all broilers of the groups A, B and C were vaccinated with ND vaccine at 10 and 24 days, while the group D was negative control. The blood was collected at 1, 7, 14, 21, 28 and 35 days of age to obtain serum for ELISA. Samples of bursa from broilers of all groups at 14 and 28 days of age were submitted for histological examination. As a result of vaccination in group A the antibody titers are elevated after the 1st and 2nd dose of vaccination at 7 and 21 days. In group B this titer is increased from 21 day of age and reaches to peak at 35 day 7810±858 with significant difference, while in unvaccinated groups C and D the titer decreased gradually. The histological examination of bursal sections in group A and B varied at 14 and 28 days post vaccination and showed degeneration and necrosis of follicular lymphocytes compared with group and D. Thus we conclude that (Icx) vaccine improves the immune response after IBD and ND vaccination in comparison with live IBD vaccine.

Introduction

Infectious bursal disease (IBD), also called Gumboro disease is a severe and contagious viral disease of young chicks associated with high morbidity and mortality. It is a major poultry infection worldwide (1). The virus is a cause by birnavirus, double stranded RNA virus, bi-segmented, highly resistant to the environmental circumstances (2). Vaccination is the standard approach that used to control IBD in chickens (3). Parent stock immunization is practical to provoke humoral immune response that will be transmitted to the offspring (passive immune response) that will guard the young chicks for the early stage of life (4). An international trend of poultry industry is to shift to words to hatchery vaccination. Immune complex IBD vaccine is attending the needs for hatchery vaccination (5). Numerous vaccines are available commercially for the control of IBD infection in poultry. Immunization against IBD via drinking water has been practiced in the farm with live attenuated vaccines of much residual pathogenicity. However, the live vaccines, particularly more hostile strains, produce troubles, such as bursal tissue destruction, immunosuppressive effect, weakly protected chickens, and the hazard of mutating to virulence. Furthermore, some of classical vaccines have been described with low efficiency, because of the occurrence of highly virulent variant strains of IBD in latest decade (6). Hatchery vaccination is growing to become appropriate trend because of the accumulative capability of poultry producing concerns, and the commitment to better master of the vaccination processes using automated tools, either via subcutaneous injection at day-old, or in ovo vaccination (7). Novelties in equipment's have allowed the expansion of innovate vaccines generations that are capable to evade the...
neutralizing influence of maternally derived antibodies (MDAbs) and consequently are suitable for use as a hatchery vaccine (8). This study is an effort to evaluate the immune response and the histological changes in broilers using two different genera of vaccines against IBD on the bursal tissue response.

Materials and methods

Broiler chicks

Two hundred, one-day old broiler chicks (Ross 308) were divided randomly in to 4 groups each group 50 chicks. Group A was vaccinated with live attenuated IBD vaccine (Bursine 28ο (Lukert-intermediate strain) (Zoetis-USA) at 7 and 21 days of age via drinking water, group B was vaccinated with Immune-Complex vaccine (lcx) which contains live intermediate plus strain with specific antibodies (Anigen-Antibody complex vaccine) (Zoetis-USA) by subcutaneous injection of 0.2 ml of vaccine/chick in back of neck at 1 day old chicks (DOC), then all chicks of the group A, B and C were vaccinated with Newcastle Disease vaccine (La Sota strain) (Zoetis-USA) at 10 and 24 days of age via drinking water, while the group D considered as negative control (no vaccination).

Blood samples

The blood samples 1-2 ml were collected randomly by slaughtering or from the wing vein of chicks at 1, 7, 14, 21, 28 and 35 days of age to obtain serum. The serum from each sample was separated by centrifugation 1500 rpm/15 min and stored in properly labeled vials at -20ºC for further processing. The serum from blood samples of DOC were assessed firstly for detection of maternally derived IBD antibodies (MDAbs) then to determine the optimal timing of live IBD vaccination in these chicks (9).

Enzyme Linked Immuno-Sorbent Assay (ELISA)

Sera were tested to determine the antibody titer against IBD and ND in these groups to evaluate the humeral immune response against IBD which produced by the 2 different IBD vaccines. The procedure for ELISA Kits of IBD and ND were performed according to the manufacturer instructions (SYNBIOTICS/ ProFLOK /Zoetis/USA).

Chick’s body weight

The body weight of chicks was calculated weekly to determine any differences of this parameter between groups.

Pathology

Selected samples of bursa from the chicks of all groups at 14 and 28 days of age were submitted for histological examination. Tissue section of these organs were removed and fixed in 10% of neutral buffered formalin. All tissue samples were embedded in paraffin wax, sectioned at 5 μm, then passing on clean glass slides and stained routinely with Hematoxylin and Eosin (H&E) stains for detection of histological changes by light microscope (10,11).

Statistical analysis

Statistical analysis was carried out using SPSS version 19, the titers and body weights were analyzed and compared using Duncan’s test (12).

Results

Despite of multiple vaccinations of IBD, the antibody titer against IBD is still different depending on several factors which affect the vaccination process. Currently, vaccines against IBD are selected exclusively on the ability to produce specific Abs. The MDAbs titer in DOC was homogenous between groups, then this titer decreased at 7 days of age in all groups of this study. As a result of vaccination in group A the Abs titer was elevated after the 1st and 2nd booster dose of vaccination at 7 and 21 days of age. In group B this titer was increased from 21 days of age and reached to peak at 35 days 7810±858 with significant difference in comparison with other groups particularly group A. While in both non vaccinated groups C and D the titer decreased gradually till the end of study (Table 1).

Table 1: Symbiotic ELISA Mean of antibodies titer against IBD±SE, with two different IBD vaccines

| Groups | 1    | 7    | 14   | 21   | 28   | 35   |
|--------|------|------|------|------|------|------|
| Group A | 5881±774a | 2917.7±364a | 3864±297a | 2991±650ab | 3827±456b | 4670±412b |
| Group B | 5963±480a | 2895±427a | 3482±258a | 4653±642a | 7200±747a | 7810±858a |
| Group C | 6011±140a | 2951±294a | 1994±431ab | 1244±294bc | 378±226c | 358±206c |
| Group D | 5898±323a | 2936±347a | 1459±348b | 845±169c | 272±163c | 296±172c |

a, b, c : The different superscript in each column means statistically different significantly at P<0.05.

In table 2 the Abs titers against NDV in group A showed significant increase at 14 and 21 days of age 4269±543, 2631±459 in comparison with other groups, no significant difference between groups at 28 days of age except group D, while at 35 days a significant increase in Abs titers 5940±1685 showed in group C when compared with other vaccinated groups A and B with Newcastle disease vaccine.

The significant differences in body weight was appeared at 14 days of age in group C and D. The results at 28 days
of age showed significant increase of body weight in group B, C and D compared with A, while the final results at 35 days of age showed significant increase in body weight in group B and C in comparison with A and D (Table 3).

Table 2: Symbiotic ELISA Mean of antibodies titer against ND±SE, with two different IBD vaccines

| Groups     | Mean of antibodies titer against ND (age/days) |
|------------|-----------------------------------------------|
|            | 1     | 7     | 14    | 21    | 28    | 35    |
| Group A    | 8008±779a | 4572±559a | 4269±543a | 2631±459a | 3024±319a | 1930±553bc |
| Group B    | 8022±1068a | 6501±1970a | 3459±580ab | 1942±172ab | 4220±773a | 3941±571b |
| Group C    | 7960±782a | 4596±1161a | 2545±952ab | 1388±226ab | 3119±775a | 5940±1685a |
| Group D    | 8062±1023a | 4549±1309a | 1716±435b | 0.0±0c | 0.0±0b | 0.0±0c |

a, b, c The different superscript in each column means statistically different significantly at P<0.05.

Table 3: Mean of body weight (gm) ± SE with two different IBD vaccine

| Groups     | Mean of antibodies titer against IBD (age/days) |
|------------|-----------------------------------------------|
|            | 1     | 7     | 14    | 21    | 28    | 35    |
| Group A    | 41.8±1.1a | 201.9±3.61a | 471.8±11.1b | 912.3±30.1a | 1549.8±73.4ab | 2263.2±153.3ab |
| Group B    | 41.6±1.2a | 197.3±7.4a | 455.7±18.3ab | 986.8±21.8a | 1687.1±100a | 2369±172.4a |
| Group C    | 42.1±1a | 203.3±4.8a | 511.7±15.6a | 982.1±23a | 1755.8±89.1a | 2380±280.2a |
| Group D    | 39.8±1.2a | 202.6±2.6a | 505.4±11.3a | 977.7±23.1a | 1679.8±76a | 2295.2±187.5a |

a, b, c The different superscript in each column means statistically different significantly at P<0.05.

**Histological findings**

The histological examination of bursal sections in group A at 14 days’ post vaccination showed degeneration and necrosis of follicular lymphocytes (lymphoid follicles) associated with folding and hyperplasia of the epithelial cells in basement membrane. Another section showed severe fibrosis and hemorrhage with infiltration of mononuclear inflammatory cells in the interlobular and interfollicular space (Figure 1a), while the bursal sections at 28 dpv showed more severe histological changes in comparison with 14 dpv and discriminated by medullary vacuolation, depletion of B-lymphocytes, severe degeneration, interfollicular fibrosis and necrosis of follicular lymphocytes (lymphoid follicles) (Figure 1b).

The examined sections in group B at 14 dpv revealed a distinct oedema between the lymphoid follicles associated with lymphatic depletion (Figure 2a). Degeneration and necrosis of medullary lymphocytes, thickening and congestion of blood vessels in the interfollicular space and infiltration of mononuclear inflammatory were observed at 28 dpv (Figure 2b).

Figure 1: a- Bursal tissue14 day post vaccination explain degeneration and necrosis (arrow) of follicular lymphocytes with folding and hyperplasia (arrow) of the epithelial cells in basement membrane. H&E. 40x. b- bursal sections at 28 dpv showed severe histological changes and discriminated by medullary depletion of B-lymphocytes (arrow). H&E. 100x.

The bursal sections of group C at 14 dpv showed hemorrhage in the interfollicular space with loss of demarcation between cortex and medulla (Figure 3a), while at 28 dpv the examined sections revealed mild depletion in lymphoid follicles, distention of interfollicular space and hyperplasia of epithelial cells (Figure 3b).

Figure 2: a-examined sections in group B at 14 dpv with distinct oedema (arrow) between the lymphoid follicles associated with lymphatic depletion (arrow). H&E. 40x. b-degeneration and necrosis (arrow) of medullary lymphocytes, thickening (arrow) and congestion (white arrow) of blood vessels in the interfollicular space at 28 dpv. H&E. 40x.

Figure 3: a-examined sections in group C at 14 dpv with hemorrhage (arrow) in the interfollicular space associated with lymphatic depletion (arrow). H&E. 40x. b-examined sections at 28 dpv showed mild depletion in lymphoid follicles with hyperplasia of epithelial cells (arrow). H&E. 40x.
Finally, the sections of group D showed the normal architecture of bursal tissue which characterized by presence of intact lymphoid follicles of variable size and separated by thin connective tissue which contain inter follicular blood vessels (Figure 4).

![Figure 4: Normal architecture of bursal tissue which characterized by presence of intact lymphoid follicles of variable size and separated by thin connective tissue (arrow). H&E. 100x.](image)

**Discussion**

In the poultry industry, immunization against IBD is archived by administration of commercially live, inactivated or recombinant IBD vaccines (13).

Recurrent or regular vaccination of breeder hens produces a prevailing high serum antibodies and offspring chickens have maternal-derived antibodies (MDAbs) from breeder hens via the yolk sac that offer protection for many days after hatching (14).

The ELISA technique is commonly used as a standard serological tool for detection of antibody titers against IBD in poultry farms (15).

The titer of MDAbs is steady and considered as safe to moderate at the first few days of age and declined gradually as a result of the releasing of theseAbs from yolk sac in the first 3 days of age and then these MDAbs undergo catabolism; hence the titer decreased with age (16).

In group A it was observed that two doses of live vaccine at 7 and 21 gave protective Abs titers and this result agreed with Bugnio et al. (17) who mentioned that two doses of vaccine one at 10-12 day of their age either with an intermediate or hot strain and other at 22-24 days of age induced protective antibodies against IBD.

In group B (Icx) vaccine resulted in an early release of viral antigen from captured Abs in this vaccine that exhibited on immune response as the IBD ELISA titer and this result disagreed with Sedeik et al. (18) who mentioned that the Icx vaccine produced a weak immune response at 5 weeks of age which may be explained by the virus in the vaccine being still captured with some virus neutralizing Abs that leading to weak induction of immune response, while there is an agreement with another result of Sedeik et al. (18) when compared the results of vaccination of live and immune complex vaccine at 4 weeks of age and the titer of Abs gave 100% protection against the challenge. The MDAbs titer showed gradual decrease weekly in group C and D (non-vaccinated groups) and became non protective as a result of catabolic degradation of these Abs within the body (19,20).

Finally, Sedeik et al. (18) summarize that the Icx vaccine at one-day old was safer and provided higher protection which is similar to our results.

Thus the type of the vaccine is one of the key aspects that detects and regulates the efficacy of IBD vaccination (21).

This study also was designed to determine the interaction between the common commercial vaccines used to control the endemic diseases in Iraqi poultry farms. In table 2 as a result of comparison of Abs titer of group B and C with group A the significant difference appeared at 14,21 days of age and this result disagreed with finding of (22) which who observed that IBDV infection or vaccination decrease the immune response of guinea fowls to Newcastle disease vaccine “LaSota”, in addition to disagreement with finding by (23) when they cited that vaccination of chicken with ND vaccine “LaSota” which adversely affected by IBD vaccine when administered primarily.

The significant difference between the groups appear at 35 days of age and the high titer of Abs was in group C compared with group A because significant decline of Abs production of chickens against ND vaccine when administered after IBD vaccine of infection (24,25).

While the significant difference between group C and B may be due to the releasing of live intermediate plus strain form Icx vaccine captured Abs and the depletion of B-cell in the bursa of Fabricius was remarkably less severe than after vaccination with Icx vaccine (26). The catabolic...
The main significant differences in body weigh occurred at 28, 35 days between groups B, C and D compared with A and this results disagreed with Okwor et al. (27) when they mentioned that mixed vaccination against ND and IBD using live vaccines did not show alteration the immune response, feed intake and weight gain in healthy broilers.

The B-lymphocytes necrosis and depletion in group A is agreement with Khatri et al. (28) when this depletion was associated with loss of large number of B-lymphocytes post vaccination as a results of targeting of bursal tissue with virus vaccine (29).

In group B the lymphatic depletion, degeneration and necrosis of medullary B- lymphocytes occurred as a results of virus replication in bursa (30) and this lesion is due to the effect of induction of chemical mediators especially cytokines that released by macrophages (28).

While the mild depletion of B-lymphocytes and no alteration of the normal architecture of the bursa is in agreement with Igwe et al. (31).

Conclusions

The Icx vaccine improves the immune response after IBD and ND vaccination and expands body weight in comparison with live IBD vaccine.

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Conflict of interest

The authors declare that there is no conflict of interest.

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دراسة مقارنة بين فاعالية اللقاح المناعي المعقد واللقاح الحي التقليدي ضد مرض الكمبورو في فروج اللحم

فقرر ابحث اسماً، هناء خليل اسماعيل وعبدالواحد عبد الحكيم واحد

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الخلاصة

هدفت هذه الدراسة إلى تقييم الاستجابة المناعية والتغيرات النسجية المتصلة باستخدام نوعين مختلفين من اللقاحات ضد مرض التهاب جراب فابريشيا الخمجي. تم استخدام 200 فرج للحم بعمر يوم واحد فُقدت إلى 4 مجموعات. تم تسمية مجموعة بلقاح حي بعمر يوم 7 و 21 يوم، وتم تسمية مجموعة بلقاح معقد بعمر يوم واحد، بعدها تم تخصيص الأفراد في المجموعات A و B و C و D بلقاح حبيب مرض نيبوكالسيك الحي بعمر 24 و 54 يوم، بينما تركت أفراد أفراد المجموعة D كسيطرة سالبة. تم جمع الدم عند اليوم 14، 21، 28، 35 يوم للحصول على المصل واستخدامه في اختبار الاليزا. تم إخضاع عن طريق جراثم فابريشيا ونوعين من المجاميع كافة بعمر 14 و 28 يوم للفحص النسجي. أظهرت نتائج التحصين في المجموعة A ارتفاعاً في مستويات الفيروكس لل.Reverse transcription polymerase chain reaction (RT-PCR) عند 7 و 21 يومًا. وفي المجموعة B ارتفع هذا العيار بعمر 21 يومًا وبلغ على مستوى 35 يومًا. بينما انخفض هذا العيار تدريجياً في كل من المجموعة C و D. تفاوتت نتائج الفحص النسجي لجراثم فابريشيا في المجموعة A و B بعد التلفيق بعمر 14 و 28 يومًا ملتئم بظهور تنكس وتذكر الخلايا الليفية الجريبية في مستويات مختلفة. استنتجت من هذه الدراسة بأن التحصين بلقاح معقد يحسن من الاستجابة المناعية مقارنة بلقاح مرض التهاب جراب فابريشيا الحي المعضم.