



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

F.A. Isihak<sup>1</sup>, H.Kh. Ismail<sup>2</sup> and A.A. Wahid<sup>3</sup>

<sup>1</sup>Department of Microbiology, <sup>2</sup>Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Mosul, <sup>3</sup>General Manager, Yarabarz Company for Poultry Feeding and Veterinary Services, Erbil, Iraq

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#### Correspondence:

F.A. Isihak  
[fanar1976@uomosul.edu.iq](mailto:fanar1976@uomosul.edu.iq)

### 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  $7810 \pm 858$  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.

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### 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 humeral 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 used 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 2<sup>®</sup>) (Lukert-intermediate strain) (Zoetis-USA) at 7 and 21 days of age via drinking water, group B was vaccinated with Immune-Complex vaccine (Icx) 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 -20°C 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 titers 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/ ProfFLOK /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 1<sup>st</sup> and 2<sup>nd</sup> 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	Mean of antibodies titer against IBD (age /days)					
	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 IBD (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±226b	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±73.4b	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 sever fibrosis and hemorrhage with infiltration of mononuclear inflammatory cells in the inter lobular and inter follicular space (Figure 1a), while the bursal sections at 28 dpv showed more sever histological changes in comparison with 14 dpv and discriminated by medullary vacuolation, depletion of B-lymphocytes, sever degeneration, inter follicular fibrosis and necrosis of follicular lymphocytes (lymphoid follicles) (Figure 1b).

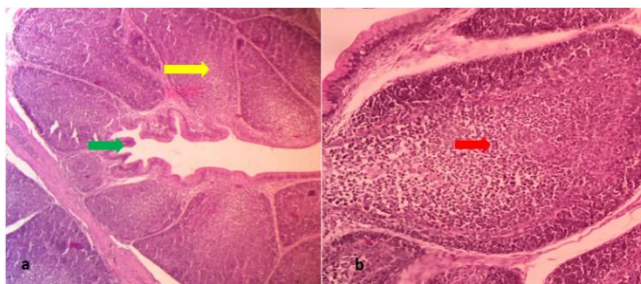


Figure 1: a- Bursal tissue 14 day post vaccination explain degeneration and necrosis (arrow) of follicular lymphocytes with folding and hyperplasia (arrow) of the epithelial cells in basement membrane. H&E. 40x. b- bursal sections at 28 dpv showed sever histological changes and discriminated by medullary depletion of B-lymphocytes (arrow). H&E. 100x.

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

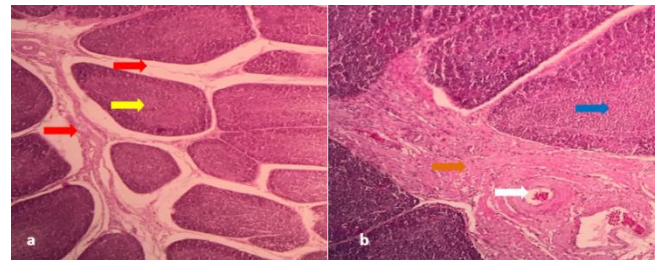


Figure 2: a-examined sections in group B at 14 dpv with distinct oedema (arrow) between the lymphoid follicles associated with lymphatic depletion (arrow). H&E. 40x. b- degeneration and necrosis (arrow) of medullary lymphocytes, thickening (arrow) and congestion (white arrow) of blood vessels in the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

The bursal sections of group C at 14 dpv showed hemorrhage in the inter follicular 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 inter follicular space and hyperplasia of epithelial cells (Figure 3b).

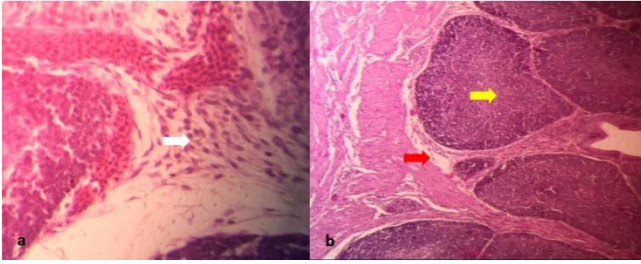


Figure 3: a- bursal sections of group C at 14 dpv showed hemorrhage (**white arrow**) in the inter follicular space with loss of demarcation between cortex and medulla. H&E. 100x. b- 28 dpv of the examined sections revealed mild depletion (**arrow**) in lymphoid follicles, distention (**arrow**) of inter follicular space and hyperplasia of epithelial cells. 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).

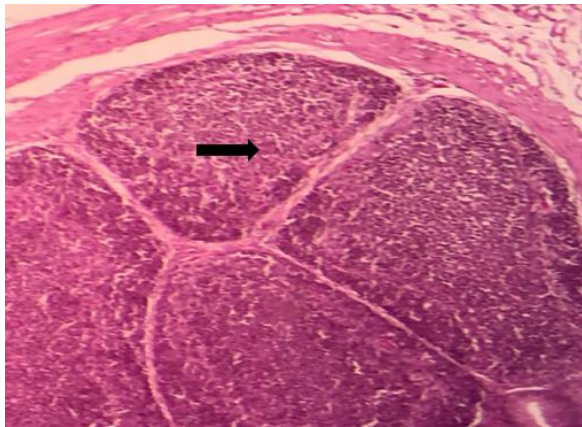


Figure 4: Normal architecture of bursal tissue which characterized by presence of intact lymphoid follicles of variable size and separated by thin connective tissue (**arrow**). H&E. 100x.

## 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 these Abs from yolk sac in the first 3 days of age and then these MABs 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 Bughio *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



character of Abs resulted to regular decline of MDABs against ND in group D (negative control) with age.

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 فعالية اللقاح المناعي المعقد واللقاح الحي التقليدي ضد مرض الكمبورو في فروج اللحم

فنان ابلح اسحق<sup>١</sup>، هناء خليل إسماعيل<sup>٢</sup> وعبدالواحد عبدالحكيم واحد<sup>٣</sup>

<sup>١</sup> فرع الأحياء المجهرية، <sup>٢</sup> فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة الموصل، الموصل، <sup>٣</sup> المدير العام، شركة يارابرز للدواجن والخدمات البيطرية، أربيل، العراق

### الخلاصة

هدفت هذه الدراسة إلى تقييم الاستجابة المناعية والتغيرات النسجية المترافقة مع استخدام نوعين مختلفين من اللقاحات ضد مرض التهاب جراب فابريشيا الخمجي. تم استخدام ٢٠٠ فروج اللحم بعمر يوم واحد، قسمت إلى ٤ مجاميع. تم تحصين المجموعة أ بلقاح حي مضعف بعمر ٧ و ٢١ يوم، وتم تحصين المجموعة ب بلقاح مناعي معقد بعمر يوم واحد، بعدها تم تحصين الأفراخ في المجاميع أ و ب و ج بلقاح مرض النيوكاسل الحي بعمر ١٠ و ٢٤ يوم، بينما تركت أفراخ المجموعة د كسيطرة سالبة. تم جمع الدم عند ١، ٧، ١٤، ٢١، ٢٨، ٣٥ يوم للحصول على المصل واستخدامه في اختبار الاليزا. تم اخذ عينات من جراب فابريشيا ومن المجاميع كافة بعمر ١٤ و ٢٨ يوم للفحص النسجي. أظهرت نتائج التحصين في المجموعة أ ارتفاعاً في معيار الأضداد بعد الجرعة الأولى والثانية من التحصين بعمر ٧ و ٢١ يوماً. وفي المجموعة ب ارتفع هذا المعيار بعمر ٢١ يوماً وبلغ أعلى مستوى له عند ٣٥ يوماً، بينما انخفض هذا المعيار تدريجياً في كل من المجموعة ج و د. تفاوتت نتائج الفحص النسجي لجراب فابريشيا في المجموعة أ و ب بعد التلقيح بعمر ١٤ و ٢٨ يوماً تمثلت بظهور تنكس وتخر الخلايا الليمفاوية الجريبية بمستويات مختلفة. استنتج من هذه الدراسة بأن التحصين بلقاح مناعي معقد يحسن من الاستجابة المناعية مقارنة بلقاح مرض التهاب جراب فابريشيا الحي المضعف.