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Comparative study by using three different type of Infectious bursal disease vaccine in broiler chicken .

دراسة مقارنة باستخدام ثلاثة انواع مختلفة من لقاح مرض التهاب غدة فابريشيا في افراخ دجاج اللحم

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Abstract

This study was carried out to compare the immunological response produced by using three different types of IBD vaccine .This study was conduct in the Kerbala city by using one hundred and forty broiler chicks of Ross breed were used in this study ,twenty chicks were sacrificed randomly for ELISA test to detect of maternal antibody of IBD at one day of age ,While the other one hundred and twenty chicks were divided in to four equal groups vaccinated by live attenuated vaccine against IBD at 14 days age by drinking water method as followed : Group G1: 30 birds were vaccinated with a hot vaccine live attenuated ((Intervet 228E vaccine)) in the drinking water at, 14 days of age. Group G2: 30 birds were vaccinated with a intermediate plus live attenuated (Ceva IBD-L) in the drinking water at 14 days of age. Group G3: 30 birds were vaccinated with a intermediate live attenuated (Intervet D78 vaccine) in the drinking water at 14 days of age. Group G4: 30 birds were not vaccinated (control). ELISA test were conducted to determine antibody titer at one, 21, 32 days of age for all groups in addition to histology of bursa of fabricious were done in present study. The results showed significant differences at ($p<0.01$)among vaccinated groups ,the antibody titer was higher in groups vaccinated with intermediate plus and intermediate strain than that virulent strain. And also the results of histological examination of bursa of Fabricius showed that intermediate plus and intermediate strain did not causes harm effects on chicken immune system . This study showed that vaccination by intermediate plus IBD vaccine strain gave a batter immune response and safety than virulent and intermediate vaccine in case of high maternal immunity.

المستخلص

صممت هذه الدراسة لغرض مقارنة وتقييم الاستجابة المناعية الناتجة من استخدام ثلاثة انواع مختلفة من اللقاحات الحية المضعفة لمرض الكمبوروا . اجريت الدراسة في مدينة كربلاء المقدسة وشملت استخدام 140 فرخة من دجاج اللحم نوع روز قسمت على اربعة مجاميع 30 طيرا لكل مجموعة فيما اخذ 20 طيرا لغرض قياس المناعة الامية تم تلقيح المجاميع الثلاثة الاولى بعمر14 يوم بطريقة التلقيح بماء الشرب وكما يلي : المجموعة الأولى لقحت باللقاح الحي المضعف لمرض الكمبوروا عترة Intervet 228E vaccine و لقحت المجموعة الثانية باللقاح الحي المضعف لمرض الكمبوروا عترة Ceva IBD-L أما المجموعة الثالثة فقد لقحت باللقاح الحي المضعف لمرض الكمبوروا عترة Intervet D78 vaccine فيما تركت المجموعة الرابعة بدون لقاح كمجموعة سيطرة و تم قياس معيار الاضداد باستخدام اختبار الاليزا بعمر يوم واحد و21 يوما" و32 يوما"لتقييم الاستجابة المناعية ،وقد بينت النتائج ان المجاميع الملقحة تفوقت و بشكل معنوي ($p<0.01$) على مجموعة السيطرة كما أظهرت النتائج تفوق المجموعة الثانية وبشكل معنوي على بقية المجاميع بعمر 32 يوم ، كما بينت نتائج الفحص النسيجي لغدة فابريشا ان استخدام العترتينس Ceva IBD-L و Intervet D78 vaccine ءأمن ولم يحدث ضمور او تنكس في الخلايا اللمفاوية عكس ما احدثته عترةIntervet 228E vaccine.

Introduction

Infectious bursal disease (IBD) a highly contagious and acute immunosuppressive viral disease that infect of young chickens, caused by virus belonging to the family Birnaviridae of the genus Avibirnavirus (1). Subclinical infection may occur in chickens less than two weeks of age leading usually to immunosuppression (2). But chickens of age from 3 to 6 weeks are most susceptible to clinical infection (3). The mortality rate are 5-10% but also can reach to 30-40% (4). The outcome of IBD is largely dependant on the strain and the amount of the virus, age and breed of birds, the route of inoculation, the presence or absence of neutralizing antibodies, intercurrent primary and secondary pathogens and environmental and managemental factors (5). The disease is characterized by the destruction of the lymphoid organs, in particular the bursa of Fabricius (BF), where IBDV infects the actively dividing and differentiating lymphocytes of the B-cells lineage, resulting in lymphopenia (immunosuppression) and secondary infection of the infected birds (6). Therefore, increases susceptibility to opportunistic pathogens such as Newcastle disease, Marek's disease and infectious bronchitis, and lowers responsiveness to vaccination (7) Besides the loss due to mortality and morbidity, immunosuppression is a very important problem associated with IBD infection (9). IBD virus destroys lymphocytes and macrophages as a result of cripples immune system with marked immunosuppressive effect leading to vaccination failures and concurrent infections .Immunization is the principle method that used for the control of IBD in chickens. There are many available live vaccines based on virulence, such as intermediate virulence and highly attenuated strains, while virulent vaccine not available commercially till now. The vaccine must be safe, pure and efficient (8). Despite vaccination tool in place for prevention of IBD, In addition, conventional live vaccines can be inhibited by maternal antibodies, making the timing of vaccination difficult (9). A specific immune response in birds could be generated via two ways. First, passive immunity is achieved when antibodies are transferred to the individual either maternal or orally/through injection. Second, active immunity is the production of antibodies by individuals against a pathogen as a result of vaccine application or recovery from disease, which eventually leads to the generation of memory cells. Birds lack lymph nodes, but they have an avian-specific primary lymphoid organ, the bursa of Fabricius (BF), which is the organ for development of their B-cell repertoire (10). This study was carried out with an aim to detect the best type of vaccine which induce high immune response and possibility protection.

Material and methods

one hundred and forty commercial broiler chicks were selected in this study reared in experimental house in kerbala city at one day of age. Twenty chicks were sacrificed randomly for detection of maternal derived antibodies against IBDV in their serum at one-day age, by using indirect ELISA technique the rest chicks (120 chicks) were divided into four equal groups, thirty chicks for each. Three groups were considering as treated groups numbered as G1and G2 while the last group (G3) was considered as a control group as follows: Group G1: 30 birds were vaccinated with a live attenuated (Intervet 228E vaccine) in the drinking water at, 14 days of age. Group G2: 30 birds were vaccinated with a live attenuated (Ceva IBD-L) in the drinking water at 14 days of age. Group G3: 30 birds were vaccinated with a live attenuated (Intervet D78 vaccine) in the drinking water at 14 days of age. Group G4: 30 birds were not vaccinated (control).

Blood sampling:

Blood was collected from 20 birds to measure the maternal derived antibody (MDA) at the first day of age, as well as at 21st and 32th days post-vaccination to measure antibody response. It was taken from the main brachial wing vein or by heart puncture using 5ml disposable syringes. Three ml of blood from bird was collect from ten randomly selected birds of each group was collected in clean, dry and sterile tubes. The tubes were stoppered and left in slant position for one hour at room temperature and then left for another one hour at 4°C then centrifuged at 3000 rpm for 15 minutes.

Serum samples were carefully separated in a small Eppendorf tube, labeled (11) and heat inactivated and subjected to ELISA test (12).

Histopathological Examination

Samples of bursa of Fabricius were fixed in 10% buffered formalin, then trimmed to thickness of 5mm in size, fixed and dehydrated in a series of alcohol concentration, and embedded in paraffin wax using an automatic tissue processor. Sectioning of tissue was done to thickness of 4 micrometer on a microtome. Then it was mounted on glass slides, dewaxed and stained with Haematoxylin and Eosin (H&E), and examined using x10, and x40 objectives for histological changes (13).

Results and discussion :

The results showed that there is a high maternal derived antibody (MDA)(7014 ± 1146.6)at one day old post hatching and this may retrain to the parent flock were previously vaccinated by oily vaccine or exposure to an infection prior production .This gives chicks' good protection from early challenge caused by classical serotype which belongs to the first type I (14).

Table -1- IBD antibody titer of different vaccines at different times

Day / Group	21 day	32 day
GI	4474.8 ± 521.4 Aa	2600.2 ± 238 Cb
G2	4025.5 ± 642.8 Aa	4867.2 ± 593.9 Ab
G3	2940.5 ± 384.5 Ba	3369 ± 184.1 Bb
G4	582 ± 261 Ca	0 ± 0 Db

G1,G2,G3,G4 (means animal groups of study)
Values are mean± SE. Values fallowed by different letters (Capitals letters = vertically and small letters= horizontally) on the table are significantly Different ($p<0.01$) in comparison within groups.

Innate immune system seems to be fully functional in the newly hatched chickens whereas the optimal adaptive immune responses only develop following the first weeks after hatch. Consequently, they are not able to develop a fast immune response, which makes them susceptible to various diseases in early days. To compensate this deficiency, they receive maternal antibodies via the egg (15).

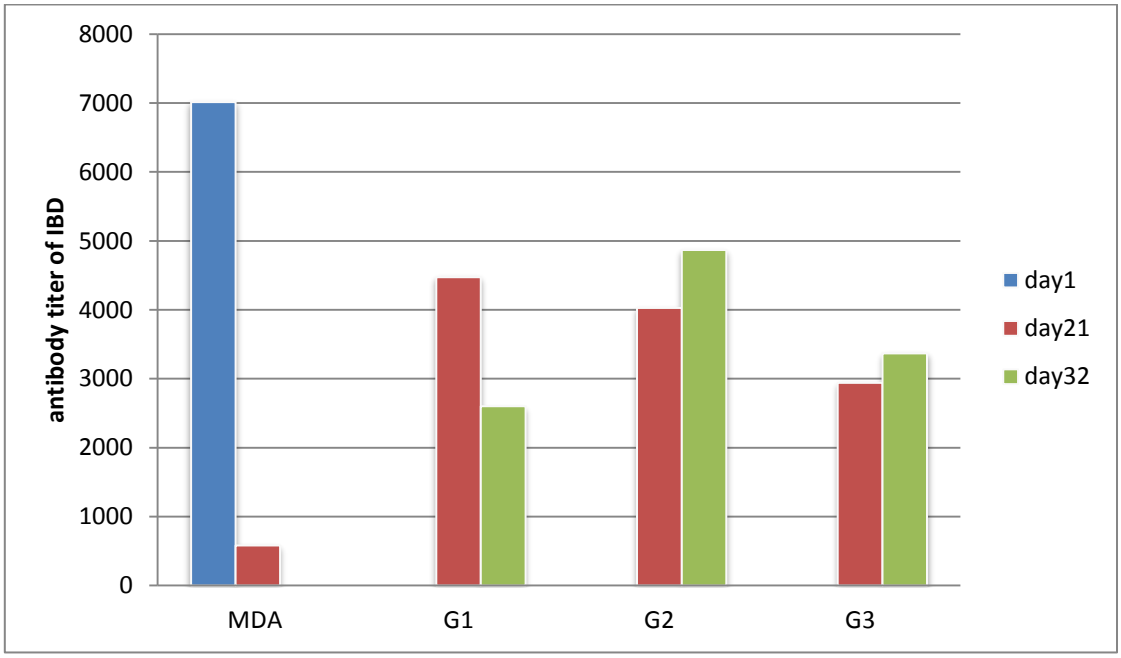


Figure (1) represent the IBD antibody titer of different vaccines at different times.
G1,G2,G3,G4 (means animal groups of study),
MDA (means maternal derived antibody)

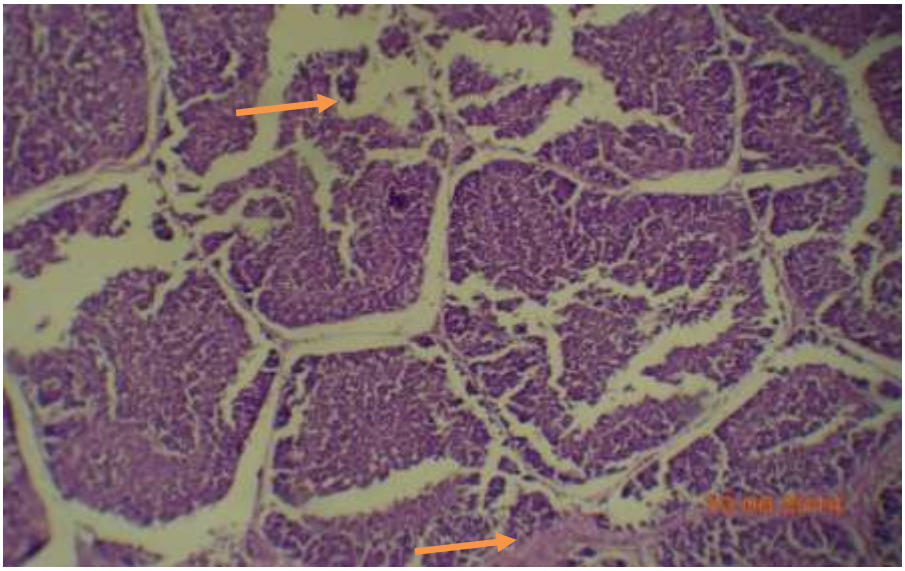
These antibodies are specific against all pathogens that the mother has encountered or has been vaccinated against. This antibody reservoir lifetime is long enough to donate immunity until the chicken's immune system becomes active. Knowing the maternal antibody level is critical for managing vaccination schedules.If chickens receive a vaccine at the time when the maternal antibody level is still high, the vaccine may be inactivated violently by maternal antibodies, which leads to a weak immune response. In contrast, if vaccination takes place long after the decline of maternal antibody levels, chickens would be susceptible to diseases prior to be immunized (16). in our experiment the level of maternal derived antibody categorized as high maternal antibody according to the Kreider et al.(17) how's divided the ELISA titer of the MDA of 1-day-old chickens into 3 level; the low level (<3,000), intermediate level (3,000- 5,000) and high level (>6,000).

However the results of Indirect ELISA test demonstrated that is a significant difference ($P<0.01$) in the level of antibody titer between vaccinated groups from one side and control group from the other side on different ages 21 and 32 days consequently. This means the vaccinated group's at 13 days by different type of vaccines can evoke an immune response during the vaccination. chicken IgG is detectable after 5 days following exposure, peaks at 3 to 3.5 weeks, and then slowly decreases .

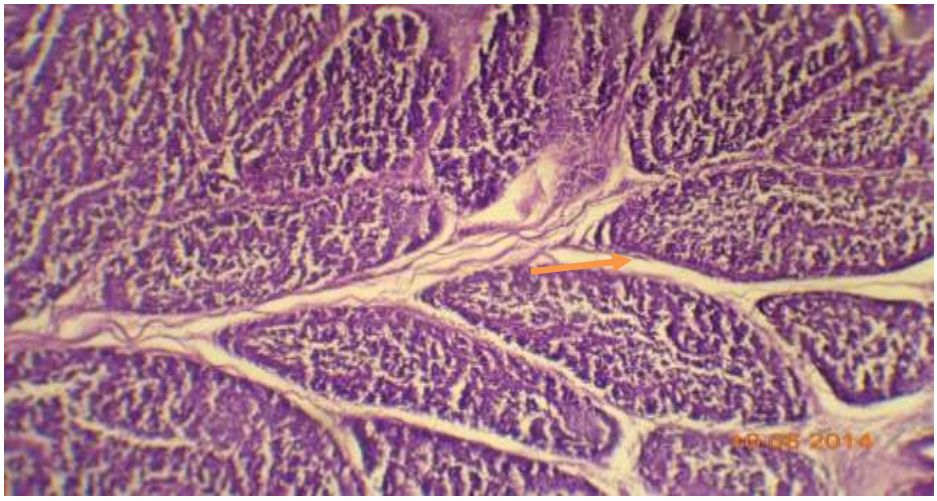
There's no significant difference between G1 and G2, as it will be the highest in G1, then in G2 and both groups are significantly increased($P<0.01$) on G3 in 21 days and this may be retrain to used of different virulence of different vaccine IBD strain as flowed in G1 virulent strain and in G2 intermediate plus strain while in G3 intermediate strain and this finding agree with Silke et al., (18) who confirmed that the virulent of vaccine gives the average of antibody titer when measuring by Indirect ELISA test, than the mild and intermediates vaccines and gives good immunity against Gumboro disease. So The efficacy of IBD vaccine to induce the immune response was related to the level of MDA in the chickens and this agree with Chansiripornchai and Wanasawaeng,(19) how's recorded The MDA of chickens can impede the virus in vaccine infected to the target cells and also reduce the ability of virus in vaccine to stimulate the chicken's immune system .

In 32day old of chicken the results of Indirect ELISA test revealed a significantly increased ($P<0.01$)of G2 on the other vaccinated groups followed by G3and finally G1 on G4(control group) , G2 and G3 of vaccinated groups appear to continuous increased in immune response at 32 days than21 days significantly($P<0.01$) and this may be return to fully development of chicken immune system with advanced age , while in G1 were significantly decreased($P<0.01$) at 32 day than 21 days and this may be due to virulent of vaccine strain which causes immunosuppration due to the ability of IBD virus to infected of avian bursa and attack B-lymphocyte which is considered as the target cell for the virus led to decreased the number of B-lymphocyte which responsible for humeral immune response of the chicken and these led to decreased of antibody titer and this level of antibody titer did not protected from field infection According to (20) GMT ELISA titer infectious bursal disease viruses isolated from commercial must be not less than 3000 and protection % equal or more than 90% , our results agree with Bengelsdorff and Bernhardt (21) compared antibody responses to a high virulent IBD vaccine with those of intermediate vaccines, the "hot 512 vaccine" produced less antibody responses than the intermediate vaccines.

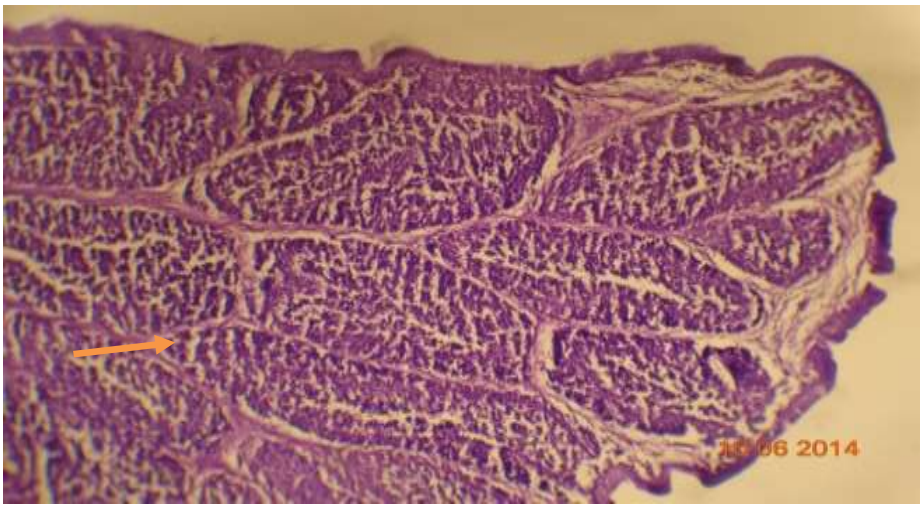
Histological examination of bursa of fabricious in group 2and group3 revealed that mild to moderate lymphocyte depletion and interfollicular spaces also mild hyperplasia of interfollicular septa and this agreement with (22) how's recorded that these intermediate vaccine strains may produce moderate to severe bursal lesions and immu-nesuppression in vaccinated chickens as reported by many researchers . while in group one showed sever lymphocyte depletion and increased interfollicular space and cyst formation due to necrosis and degeneration of B-cell in the lymphoid follicles and infiltration of hetrophile and these results agree with Winterfield andThacker (23) tested immunogenicity and virulence of 8 intermediate vaccination strains against IBD and found considerable differences among the strains. Two of the strains were highly virulent, produced clinical symptoms, caused damage to BF and even death of birds . Indeed , our results of Histological examination of bursa of fabricious in group one which used hot strain might explain why we are observing occur of infection or immunosuppration even in vaccinated flock. So the important point these results confirmed the results of Indirect ELISA test in our experiment .



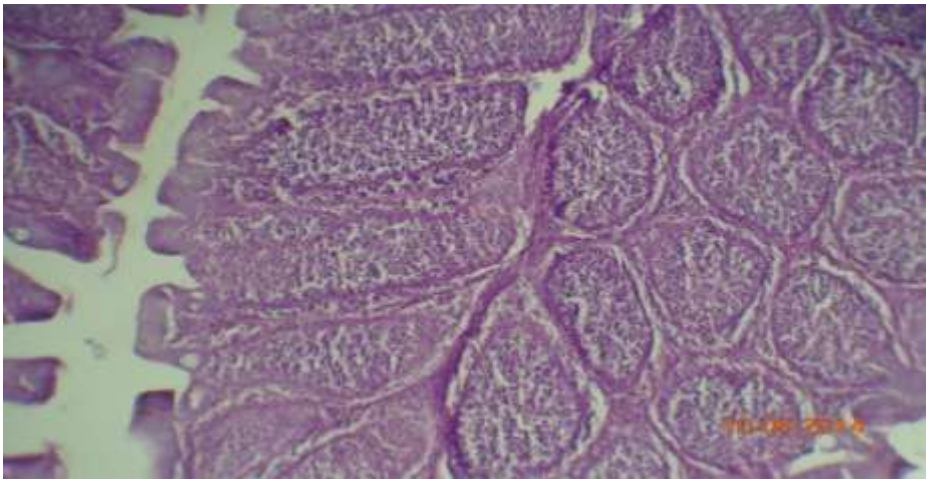
(Fig 1) Histological cross section of bursa of fabricious of (group 1) showed sever lymphocyte depletion and increased interfollicular space and cyst formation due to necrosis and infiltration of hetrophile.H&E stain.10X.



(Fig 2) Histological cross section of bursa of fabricious of (group 2) showed mild to moderate lymphocyte depletion and interfollicular spaces .H&E stain .10



(Fig 3) Histological cross section of bursa of fabricious of (group 3) showed mild lymphocyte depletion and increased interfollicular space.H&E stain.10X.



(Fig 4) Histological cross section of bursa of fabricious of (group 4) normal structure of tissue .H&E stain.10X.

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