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Morphological investigation of bursa of fabricius of imported broilers and local chicks vaccinated with two types of ibd vaccines

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Abstract

This study was designated to investigate the morphology of bursa of Fabricius (BF) in broilers and local chicks. Eighty Hubbard broilers and one hundred local, one-day-old chicks were reared on litter floor for 35 days. They were divided into six groups, A, B and C for broilers, and D, E and F for local chicks. Two types of Infectious Bursal Disease (IBD) vaccine were used. Chicks of group A, and D were vaccinated with an intermediate type vaccine (Bursine ® -2) at 14th day, whereas group B and E were vaccinated with an intermediate-plus vaccine (Bursine ® plus) at 14th day also (Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Chicks of group C and F were acted as control. Five chicks were sacrificed from A, B, D and E groups at 21st, 28th and 35th day and at 1st, 7th, 14th, 28th and 35th day from C and F control groups. Body weights, bursa weights, diameters and relative weight of bursa were measured. The results showed a non significant difference ($P>0.05$) between group A and C, whereas group B showed a significant reduction ($P<0.05$) of bursal index at 21st day compared with group C. Different levels of significancy of bursal index (BI) were noticed in local chicks. Reduction of bursal weight of vaccinated groups may be associated with the proliferation of the virus vaccine in the bursal tissues. This study also revealed that intermediate-plus vaccine caused severe BF injury in vaccinated local bird. The present study indicated a significant correlation between bursa diameter and the injury produced by the vaccination process in broiler chicks, on the other hand the same trend was also recorded in the local chicks. The present study was also revealed a clinical signs and mortality up to 66.6% due to highly virulent IBD vaccine in group E of local chicks, because of their high susceptibility to this vaccine.

Keywords: Morphology, Bursal Index, Bursa Fabricius, Infectious Bursal Disease.
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دراسة التغيرات الشكليه لجراب فابريشا في افراخ فروج اللحم وللأفراخ المحليه بعد تلقيحها بنوعين من لقاحات التهاب جراب فابريشيا المعدي

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الخلاصه

صمم هذا البحث لدراسة الخصائص الشكليه المتضمنه معرفة دليل وقياس قطر جراب فابريشيا في أفراخ فروج اللحم والدجاج المحلي. أستخدم لغرض أنجاز البحث ثمانون من أفراخ فروج اللحم نوع هابرد ومائة من الافراخ المحلية بعمر يوم واحد. تم تربية الافراخ لمدة ٣٥ يوما. قسمت الافراخ الى ستة مجاميع (A, B و C) لفروج اللحم و (D, E و F) للافراخ المحليه. لقحت أفراخ المجموعتين (A و D) بلقاح حي متوسط الضراوة ولقحت المجموعتين (B و E) بلقاح عالي الضراوة بعمر ١٤ يوما فيما اعتبرت المجموعتان (C و F) مجموعتا سيطرة. تم قتل ٥ أفراخ من كل مجموعه للايام (٢١, ٢٨ و٣٥) بعد التلقيح، اما مجاميع السيطرة فقد تم قتل ٥ طيور منها أبتداء من الأسبوع الأول الى السادس. تم أزالة جراب فابريشيا ووزنه وتم قياس قطره كما تم وزن الطيور

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لأستخراج دليل الجراب من خلال تطبيق المعادلة [وزن الجراب / وزن الجسم×١٠٠]. أظهرت النتائج عدم وجود فروقات معنويه لهذة القياسات في المجموعه A ووجودها في المجموعه B عند المقارنه مع مجموعة السيطرة بعمر٢١، اما في الطيور المحليه فقد ظهرت فروقات معنوية بين المجاميع حسب الأستجابة المناعيه والعمر ونوع اللقاح. أوضحت الدراسة أن هناك فرقا معنويا بين المجاميع الملقحه ومجموعتي السيطرة فيما يتعلق إنخفاض وزن الجراب بسبب التأثير الناتج من تكاثر الفايرس في أنسجة الجراب. بينت الدراسه وجود إرتباط بين قطر الجراب وبين الأذى الناتج عن عملية التلقيح. وأظهرت الدراسة وجود علامات سريرية وهلاكات بنسبة ٦٦,٦ % في المجموعة E للافراخ المحلية الملقحة باللقاح العالي الضراوة للفترة من ٢١–٣٥ يوما بسبب شدة تأثر هذه الأفراخ بهذا النوع من اللقاح.

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Introduction

The bursa of Fabricius (BF) is an immunological organ that plays a primordial role in the poultry immunity. The different aggressions of the environment such as stress, bad hygiene, vaccination and pathologies were influenced on the anatomical and physiological development of BF (1).

The measures of immunity that have been commonly used and assessed in poultry are lymphoid organ weights (2). The evaluation of bursa weight and the determination of bursal index (BI) (bursa weight:body weight ratio) is the most used model to estimate protection rate given by vaccines against IBD (3).

Lymphoid organ weights are easily measured and they reflect the body's ability to provide lymphoid cells during an immune response. Bursal weight was the best indicator of the stress that was related to housing density in which BI gave the most consistent and reliable indication of stress. As density increased, bursa weight and BI decreased significantly. Problems in the barn environment may be the cause of the small bursa since stress can also decrease bursa size (2,4).

Alloui *et al.* (1) found that the evolution of the bursal weight with regard to the body weight gives a positive and significant correlation, which explains that the weight of the bursa increased effectively according to the body weight, but without reaching some compliant values. This variation of the bursal morphology demonstrates the immunodepression state at certain broilers chickens.

Sahar *et al.* (5) reported that the bursal enlargement is more obvious in chicks vaccinated with the hot strain followed by vaccination with the intermediate strain and least in those vaccinated with the intermediate and boostered with the hot one. Kulikova *et al.* (6), indicated that the intermediate vaccine in the group of Leghorn chickens produced the lowest average post-vaccination antibodies titers and statistically insignificant BF index. The most virulent vaccines demonstrated an almost, high post-vaccination antibodies titers and statistically significant bursal atrophy.

Cookson and Giambroneb (7) referred that the bursa to the body weight ratio are the standard method for calculating percent protection in IBD virus. Bolis *et al.* (3) concluded that bursa weight:body weight ratio evaluated before and after challenge with vvIBDV was not enough too consistently and conclusively differentiate or estimate the protection given by the vaccination.

Native or local chicken is a name of a bird that was originated in an area and adapted to its environment. These birds are characterized by their small size, different Plumage colors, different comb shapes, low production rate and small egg sizes. These characteristics have been developed through natural selection (8).

This study was designated to investigate the bursal index and bursal diameter in broiler and local chicks vaccinated with two types of IBD vaccines.

Materials and methods

Eighty Hubbard one-day-old broiler chicks were delivered from Fadak Agriculture Company, and one hundred one-day-old local chicks were obtained from local markets Basrah Government. The chicks were raised under control condition in separated portions in the Animal house at the College of Veterinary Medicine, Basrah University. The birds were supplied with feed and water *ad libitum*. Broiler chicks were divided into A, B and C group, whereas local birds were placed into group D, E and F. The chicks of group A, B, D and E were vaccinated with IBD vaccine as depicted in table 1, whereas chicks of group C and F were served as control. All groups were reared for thirty five days of age (9).

Two commercial vaccine which were available in the local market were used. They were administered according to the manufacturer's recommendations. The vaccines were intermediate vaccine (Bursine ®-2) and intermediate-plus (Bursine ®-plus) (Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Each vial of vaccines contained 1,000 doses.

Each bird was received one dose of IBD vaccine in 0,5 ml distilled water, given intracrop using a syringe and blunted needle to ensure that all birds has been received the vaccine (10).

The body weight (gm) and bursa weight (gm) were recorded for each individual bird, and bursal index (bursa

weight/ body weight × 100) was calculated. The bursal diameters were also measured by digital vernia instead of bursometer which is used in the determination of bursa diameter. The results were expressed as arithmetic mean for each experimental group. Five chicks were sacrificed by decapitation from all groups at different ages as shown in Table 1 for bursa of Fabricius determination (11,12).

Data were analyzed using the statistical software's: Genstat Discovery Edition 3 (Genstat procedures Library Release PL15) 2007 (ANOVA tes), Graphpad Software (t-test) and Med cale software (F-test).

Table 1: Experimental Design.

Group	No. of birds	Breed & Age	IBD Vaccine	Date of vac.	Bursal Index and Bursa Diameter
A	25	Broiler 1 day old	Intermediate (Bursine - 2)	14th day	21st, 28th and 35th days
B	25	Broiler 1 day old	Intermediate plus (Bursine-plus)	14th day	21st, 28th and 35th days
C	30	Broiler 1 day old	Control	–	1st, 7th, 14th, 21st, 28th& 35th days
D	25	Local 1 day old	Intermediate (Bursine - 2)	14th day	21st, 28th and 35th days
E	45	Local 1day old	Intermediate plus (Bursine-plus)	14th day	21st, 28th and 35th days
F	30	Local 1day old	Control	–	1st, 7th, 14th, 21st, 28th& 35th days

Results

Table (2) below, revealed that the difference between bursal index (BI) of group A which was vaccinated with (bursine-2) at 14th day of age and that of control C group in broiler chicks was not significant at different ages. However, a reduction of BI in group B which was vaccinated with Bursine-plus at 14th day, that has been noticed 7 days post-vaccination and in control groups were (0.1156±0.358) and (0.1792±0.0424) respectively. This result was significantly differed at (P<0.05).

Table 2: Mean bursa weight in (grams) to mean body weight in (grams) x 100 (bursal index) (IB) of the experimental groups at different ages.

Age (days)	Experimental groups					
	Broiler			Local		
	A	B	C	D	E	F
1	----------	----------	0.1098±0. 0677	----------	----------	0.1302±0.0223
7	----------	----------	0.1689±0.0523	----------	----------	0. 0892±0.0185
14	----------	----------	0.1584±0.0616	----------	----------	0.0659±0.0212
21	0.1456±0.0505*ab	0.1156±0.0358b	0.1792±0.0424a	0.1082±0.0075b	0.1523±0.0455ab	0.1775±0.0639a
28	0.1242±0.0242	0.1739±0.0546	0.1351± 0.0616	0.0956±0.0235b	0.1513±0.0565ab	0.2427±0.1059a
35	0.1593±0.0391	0.1201±0.0417	0.1337± 0.0400	0.2029±0.0924b	0.1430±0.0320b	0.3153±0.0593a

a, b, ab Means in horizontal rows with different superscripts were significantly different at (P<0.05).
SD: Standard Deviation * Figures are means of five chicks for each group.

The Table also showed that BI of group D which was vaccinated with (Bursine-2) at 14th day and of control F group in local chicks at 21st, 28th and 35th day of age were (0.1082± 0.0075, 0.0956±0.0235 and 0.2029±0.0924) and (0.1775±0.0639, 0.2427±0.1059 and 0.3153+0.0593) respectively. These results were significantly differed at (P<0.05).

Table 2 also indicated that BI in group E of local chicks which was vaccinated with (bursine-plus) at 14th day that has been noticed 21 days post-vaccination and in control group were (0.1430±0.0320) and (0.3153±0.0593) respectively. This result was highly differed significantly at (P<0.05).

The non – IBD vaccinated groups (C and F) did not show any abnormal changes in the BI. Bursal index of group D of local chicks at 21st to 28th and 35th day of age was significantly differed between each other at (P<0.05).

Table 3 which depicted above, expressed that the bursa diameter of the broiler chicks in group A and B was insignificantly differed (P>0.05) from that of group C at different ages.

Table 3 also exhibited that bursa diameter in group D and control group F at 21st day of age were (3.68±0.192) and (6.04±0.963) respectively. This result was significantly differed at (P<0.05).

The Table also showed that bursa diameter of group E compared with group F at 21st day were (3.70±1.051) and (6.04±0.963) respectively, this result was also significantly differed at (P<0.05), where as a significant level at (P<0.05) was recorded between these two groups at 28th day which were (3.52±0.645) and (5.26±1.532) respectively. The bursa diameters of group C of broiler chicks at 21st and 28th days were (6.22±0.396) and (7.30±1.918) respectively (Table.3). This was significantly differed at (P<0.05).

All chicks were observed daily for clinical signs and mortality up to 3 weeks post-vaccination. No adverse clinical manifestation or mortalities were observed during the experiment with the exception of group E of local chicks which vaccinated with (Bursine-plus) at 14th day of age. The birds of this group exhibited 66.6 % mortality from 21st to 35th days of age especially at the last week when the mortality has been obviously increased.

Table 3: Mean bursa diameter in millimeters of the experimental groups at different ages.

Age (days)	Experimental groups					
	Broiler			Local		
	A	B	C	D	E	F
1			3.46±0.618			3.78± 0.661
7			4.56±0.626			3.86± 0.444
14			5.54±0.950			4.02± 0.370
21	6.30±0.696*	5.78± 0.481	6.22± 0.396	3.68± 0.192[b]	3.70±1.051[b]	6.04± 0.963[a]
28	6.64± 0.472	8.54± 0.838	7.30± 1.918	4.00± 0.400	3.52±0.645[b]	5.26± 1.532[a]
35	8.56± 0.726	8.26± 0.502	8.02± 1.810	6.18±1.605	4.72±0.788	5.64± 1.154

[a, b] Means in horizontal rows with different superscripts were significantly different at (P<0.05).
SD: Standard Deviation * Figures are means of five chicks for each group.

Discussion

The bursa functions are half of the bird's immune system and the size of the bursa reflects the bird's overall health status. Sick or stressed birds have small bursa while healthy, productive birds have large bursa. Bursa size is a biological measure of how well flocks are managed and protected from disease. Monitoring bursa size is one way that the birds can tell a producer how they feel about the stresses and challenges on the farm (4). On the other hand (13) corroborates the hypothesis that bursa measurement is a less sensitive method to evaluate vaccination effect when compared to histopathological score.

Bursal index (BI) is one of the most important parameters to evaluate the immunosuppression caused by IBD virus and by IBD vaccine (3).

The bursal index in the present study was presented in Table 2. The results obtained after 7 days post-vaccination had shown insignificant level between group A which was vaccinated with intermediate vaccine at 14th day and the unvaccinated group (C) of broiler chicks, whereas group B which was vaccinated with intermediate-plus vaccine at the same age had shown a significant reduction of bursal index at 21st day of age in comparison with that of control group.

The result of BI in group A was in agreement with that of (14), whereas BI of chicks in group B was in disagreement with that of the same author who found that there was no important reduction in the BI in birds vaccinated with intermediate and those vaccinated with intermediate-plus vaccine. This finding may be attributed to the fact that intermediate vaccine is more attenuated than the intermediate-plus vaccine. Therefore, broiler chicks vaccinated with hot strain vaccines which were more invasive and pathogenic than other resulted in lower BI due to the lower degree of attenuation of this kind of vaccine. Similar observations were also reported by (5) who revealed that the hot vaccine is usually harmful on the broiler chick than the intermediate one.

Kulikova *et al.* (6) also reported that broiler chicks vaccinated with intermediate vaccine expressed higher BI than that which was vaccinated with virulent vaccine. The author stated that the intermediate vaccine caused no major damage to the BF compared with other vaccine at 10th day post-vaccination.

Luengo *et al.* (15) have been evaluated the severity of lesions caused by IBDV infection in birds vaccinated at 4 weeks of age with an intermediate strain and stated the reduction of bursa weight:body weight ratio may be responsible for immunodeficiency.

The insignificant reduction of BI between group A and B recorded in the present study can be explained on the basis of higher degree of attenuation of intermediate vaccine, which was not capable to destroy B-lymphocytes present in the BF and reducing their size compared with

intermediate-plus vaccine which was more pathogenic and less attenuated than intermediate type.

As for the local chicks represented by group D and E, different levels of BI were noticed according to the age and type of vaccine. However, significant reduction (P<0.05) was found including BI for each week in group D administered intermediate vaccine at 14th day of age in comparison with that of control group (F) at the same ages. Group E recorded high significant decrease (P<0.05) of BI in comparison with that of group F at 35th day of age. Accordingly the reduction of BI in these groups could be considered important.

These findings were in agreement with that of (16) who found that five native Egyptian breeds challenged with vvIBDV had a significantly lower bursa body weight ratio (P<0.05), but without bursal lesions.

Abdul Ahad (11) indicated that bursa:body weight ratios calculated after infection with local isolate in rural chicks resulted in the swelling and the hypertrophy of the bursa in the challenged groups at day 3 post-inoculation (PI). Atrophy of bursa was observed at day 10 PI.

Winterfield and Thacker (17) tested the 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, and caused damaged to BF and even the death of birds. Similar results were reported by (18). Muskett *et al.,* (19) who studied the properties of two live IBD vaccines on susceptible chickens and observed major damaged to the BF caused by one of them. According to Edwards *et al.* (20), immunosuppresion may last up to 4 weeks following vaccination.

Interesting results were reported by Mazariegos *et al.* (21) who studied six commercial vaccines against IBD designated as intermediate by their producer. Using the BF index, the authors classified two of the vaccines as highly virulent, two as intermediate and the remaining two as mild.

Sokale and Oyejide (22) indicated that since the reports of IBD outbreaks among indigenous chickens are rare unlike the situation among commercial poultry flocks, it was concluded that local chickens probably act as carrier of IBD virus.

Table 2 also indicated that the control group of both broiler and local chicks exhibited an increase in BI from 21st, 28th and 35th day of age respectively. The result was in agreement with that of (13) who stated that the non IBD vaccinated group did not show any disorder in the BF.

Data in table 3 expressed that the normal development of BF has been shown in the control group C of broiler chicks. The diameter was increased constantly with the age of the bird. The Table also revealed that group D and E of local chicks which were vaccinated with intermediate and intermediate-plus vaccines, respectively showed high significant decrease (P<0.05) of bursa diameter comparing with that of control group F.

The same results has been obtained by (23) who found that the intermediate vaccines were not able significantly (P> 0.05) to reduce bursa size compared to the control group. However, the more pathogenic intermediate and the very virulent vaccines were able to cause noticeable reduction (P<0.05) in bursa size, and were different from the control group as well as from group of birds that were vaccinated with intermediate vaccines.

Table 3 also displayed the normal development of BF in the control group F of the local chicks. The diameter was increased constantly with the age of the bird until the 21st day of age. Thereafter the bursa exhibited an irregular growth pattern. This group followed the same trend as in group C but until 21st day of age. This might be an indication of evolution time of BF in local chicks, otherwise this phenomena is not easy to be explained here.

Group E of local chicks which was vaccinated with Bursine-plus, exhibited 66.6% of mortality at 21st to 35th days of age. This high mortality rate was especially occurred at the last week of the experiment where 30 out of 45 chicks were died until 35th day of age.

The affected (vaccinated) birds exhibited a typical signs and lesions of IBD. Abdul Ahad (11) evaluated the virulence of a local isolate of vvIBDV in vaccinated and non-vaccinated local Sonali chickens against IBVD. The lowest and highest mortality were 13 – 85 % respectively. The susceptible age was 20 – 54 days. Hassan *et al.* (15) investigated the response of vaccinated and unvaccinated chickens of different breeds to infection with vvIBDV. Five-week-old chickens of five Egyptian breeds and foreign white Leghorn pullets were tested. The susceptibility to infection and mortalities of these breeds were ranged from 11 to 85% with typical clinical signs and lesion of IBD.

The present study suggested that local chicks vaccinated with Bursine-plus vaccine at 14th day revealed high degree of susceptibility to this kind of hot strain, which has the ability to overcome the effect of MDA earlier than the intermediate vaccine, resulted in the appearance of clinical signs and lesion of IBD with high rate of mortality especially at the third week post-vaccination. According to (23), higher susceptibility of commercial layers to IBD than broiler breed might be related to the differences in the major histocompatibility complex (MHC) between the two types of birds.

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