

## FIELD STUDY ON IMMUNE RESPONSE AND PERFORMANCE OF MATERNALLY IMMUNE COMMERCIAL BROILER CHICKENS TO LIVE INTERMEDIATE INFECTIOUS BURSAL DISEASE VACCINE.

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### SUMMARY

In this study, 8000 Hubbarred 1-day old broiler chicks having maternal antibodies to infectious bursal disease (IBD) were reared on deep litter in a poultry farm. The chicks were received IBD (Bursine-2) intermediate vaccine at the 12<sup>th</sup> ay of life via drinking water to investigate its immunogenicity and its effect on broiler performance. No IBD related clinical signs or mortalities or lesions were observed after vaccination till the end of the breeding period (7 weeks).

The result of serological response to IBDV vaccines using ELISA test showed that the maternal antibody titer to IBD antigen in 1day old chicks was  $5571.1 \pm 1761.8$  and waned to  $1237.7 \pm 915.8$  at 2 weeks (2days after vaccination. At the 3<sup>rd</sup> and 4<sup>th</sup> weeks after vaccination IBD ELISA titres were increased to  $2662.1 \pm 186.3$  and  $3394.1 \pm 768.8$ ; respectively. This result revealed that the used live IBD vaccine was able to induce antibody levels in chickens with maternal

IBD antibodies. The best weekly feed conversion rate (FCR) was recorded at the 4<sup>th</sup> week (1.31) and the 6<sup>th</sup> week (1.71) with total FCR of 1.89. Mortality rate in the susceptible age (2-6 week) was the lowest (0.4-.73%) with total mortality of 3.27 at the end of the 7<sup>th</sup> week.

These fining pointed out that live IBD intermediate Burcin-2 vaccine was save, immunogenic to maternally immune chicks and has no adverse effect on performance of vaccinate chickens.

**Key words:** Live IBD Vaccine, Immune response, broiler chickens and performance.

### INTRODUCTION

Since, the first reporting of Infectious bursal disease (IBD) by Cosegrove (1962) in Gumboro area, Delaware, USA, the disease spread rapidly allover the world (Okoye, 1984 and Saif et al., 2003) with induction of severe economic losses in poultry industry

due to mortalities ( 2-3% or near 100 %) (Bygrave and Fragar, 1970, El-Batrawi, 1990; Van den Berg et al., 1991, Etteradossi et al., 1992 and Amer et al, 2007 ), growth retardation (Mcilroy et al., 1989 and Amer et al, 2007) and increased condemnation rate in broiler at processing (Saif, et al 2003). Chickens aged 3-6 weeks are mostly susceptible with mortalities in classical and very virulent virus infection. While, infection in the 1<sup>st</sup> 10 days can induce immunosuppression (Mazzariegos et al., 1990).

Prevention of IBDV infection in chicken is based mainly on vaccination as established by Saif et al. (2003). There are live attenuated IBD vaccines essentially intended for prevention of infection in young chickens. IBDV live vaccine strains vary in virulence from mild, intermediate to hot and used according to the level of maternal antibodies (Thronton and Pattison, 1975; Naqi et al., 1980; Giambrone and Clay, 1986 et al., Tsukamoto et al. 1995). The intermediate strain vaccines are superior to mild vaccines in induction of immunity in the presence of maternal antibodies (Mazzariegos et al., 1990).

Chicks with high maternal immunity may require hot vaccines to induce active immunity (Winterfield et al., 1980).

In the neonate chick the immune system is not yet fully developed, which makes the chick relatively vulnerable. The degree of this

depends on the immune system kinetics and interactions, and on the genetic aptitudes for the immune system (Pinard-Van der Laan, 2000). An evolutionary attempt to compensate for the immaturity is expressed in a maternal immunity (MI) component consisting of antibody (AB) absorbed from the egg and provided by the dam in a proportionate manner. MI provides early age protection against pathogens, and that it prevents unfavorable development of tolerance to pathogens (Klipper, et al., 2004). Effects are however controversial, as it can also hinder stimulus and activation of the chick's own immune system (the innate and the acquired immunity) (Chu and Rizk, 1975; Tizard, 2000, Jeurissen, et al., 2000; Klipper et al., 2004)).

This work was carried out to investigate the efficacy of live IBDV intermediate vaccines Bursine-2 on the immune response of commercial maternally immune broiler chicks and its effect on chicken performance.

## **MATERIAL AND METHODS**

### Chicken flock:

In this field study 8000 commercial 1-day old broiler Hubbar breed chicks' in house deep litter. These chicks were derived from breeders vaccinated with both live and inactivated IBD vaccines.

### Ration:

The chicks were fed on prepared ration according to the Ross broiler management manual and NRC (1984) requirement for broiler. All housed chickens were given ration ad libitum.

### Serum sampled:

Twenty random blood samples were collected for serum at 1 day (0 week) and weekly 1-7 weeks of life. Samples collected at 1 day were taken from sacrificed chicks while those collected at other times were taken from wing vein. The collected blood was allowed to coagulate and centrifuged at 1500 rpm for 3 min. The separated sera were collected in dry sterile tubes and stored at  $-20\text{ }^{\circ}\text{C}$  till use (Jain, 1986). Only 15 of highest quality serum samples/ time were subjected to ELISA test against IBD antigen coated plates.

### Infectious Bursal disease vaccine:

The used chicks were vaccinated against IBD with live intermediate vaccine Bursine-2 (Solvay, USA). The chicks were vaccinated via drinking water at 12<sup>th</sup> day of age. The vaccine was diluted to give each bird approximately a dose of  $10^{2\text{ EID}}_{50}$ .

### Vaccine titration:

The used vaccine was titrated on the chorioallantoic membrane of 10-11 days old embryonated chicken eggs (Villegas and Purchase, 1989), the titer was expressed as 50% embryo infective dose ( $\text{EID}_{50}$ )/ml, that was calculated according to Reed and Meunch (1938).

### Coating of plates for ELISA test:

A commercial live IBD virus vaccine (Bursine-2) was use as antigen as described by Voller et al (1989). It was reconstituted with carbonate bicarbonate buffer (pH 9.6). To determine the working dilution of antigen and conjugate (checkerboard titration was carried out, where the optimum concentration form each were found to be 1:150 and 1:500; respectively. Flat-bottom micro titre plates wells were coated with 100  $\mu\text{l}$ /well of 1:150 dilution of antigen in carbonate bicarbonate buffer (pH 9.6). The plates were incubated overnight at  $4\text{ }^{\circ}\text{C}$  in a humid chamber, then emptied and washed 5 times; 3 minutes each; with phosphate buffer saline (PBS) containing 0.05% Tween 20 (pH 7.4) . The Serial dilution of the test sera were made in PBS-Tween 20 and added to the appropriate wells in 100 $\mu\text{l}$ /well. The plates were incubated at  $37\text{ }^{\circ}\text{C}$  for 1 hour and then the wells washed 5 times; 3 minutes each; with PBS-Tween 20. The 100 $\mu\text{l}$  of rabbit anti-chicken IgG conjugate (Cappel labs, USA) diluted 1:500 was added to all wells and incubated for 1 hour at  $37\text{ }^{\circ}\text{C}$ . Afterwards, the wells were washed with PBS-Tween 20. A 100 $\mu\text{l}$  of freshly prepared enzyme substrate (Orthophenylenediamine 40 mg/100 l of citric buffer (pH5) and 40 $\mu\text{l}$  of 30% hydrogen peroxide) was to each well and incubated for 30 minutes at  $37\text{ }^{\circ}\text{C}$  in the dark. The color reaction in each well was stopped with 50 $\mu\text{l}$

of 2M sulfuric acid. Positive and negative plate. The plates were read in an ELISA reader (Titertek Multiskan MC/340, Karl Kolb, W- Germany) at 492 nm. The ELISA data are presented as S/P ratio of the samples. Where S represents the absorbance of the test serum divided by the absorbance value of the positive control (P) serum. The obtained results are shown in table (1) and fig (1).

#### Broiler performance parameters:

In this study broiler performance parameters including average weekly mortality rate, body weight gain /gm., Feed intake/gm (CFI/gm), feed conversion rate (FCR) were used and calculated according to Sainsbury (1984). The obtained results are shown in table (2) and fig (2).

## RESULTS AND DISCUSSION

In this study, we directed our work to investigate the immunogenicity of Bursine-2 as one of the most popularly IBD intermediate vaccine used in the presence of maternal antibodies in chicken flocks, in Republic of Yemen. In this study, no IBD related clinical signs or mortalities or lesions was observed after vaccination till the end of the along the whole breeding period (7 weeks). This result may indicate safety of the used living IBDV vaccines as well as its ability to protect birds against the possible field challenge. Similarly Edgar and Cho (1973), Roasales et al.(1989) Thangavelu et al. (1998), Eterradosi et

control sera were run simultaneously in each al.(2004), Sultan et al. (2006) and Amer et al. (2007) found that using of live IBDV vaccines could protect the birds from development of clinical signs and mortalities.

The result of serological response to IBDV vaccines was illustrated in table (1) and fig (1). The result showed that the maternal antibody titer to IBDV in the used day old chicks was  $5571.1 \pm 1761.8$ ; this titer waned to reach  $4776.9 \pm 1764.8$  at 1 week of age and  $1237.7 \pm 915.8$  at 2 weeks (2days after vaccination). At the 3rd and 4th weeks after vaccination IBD ELISA titres were increased to  $2662.1 \pm 186.3$  and  $3394.1 \pm 768.8$ , respectively. There was an elevation in the antibody titers in comparison with the titer just before immunization. This result revealed that the used live IBDV vaccine was able to induce antibody levels in chickens with maternal IBDV antibodies. Such result confirmed by the findings of Marquardt et al., (1980); Briggs et al., (1986); Solano et al., (1986) and Van den Berg and Meulemans, (1991). Also, this result was in accord with this reported by Abdel-Alim and Kawkab (2006) who found that live intermediate plus IBDV vaccines were immunogenic with better immune response in eye drop vaccinated groups. Decreased titres at the 6<sup>th</sup> and 7<sup>th</sup> week of age may indicates absence of field infection.

Concerning results of performance (table 2) including mortality rate, the average weekly

body weight and the feed conversion rate (FCR) in (fig 2). The best weekly FCR was recorded at the 4<sup>th</sup> week followed by that of the 6<sup>th</sup> and the 7<sup>th</sup> as 1.31, 1.71 and 2.02; respectively. The total FCR was 1.89. Also, mortality rate in the susceptible age (2-6 week) was the lowest (0.4-0.73%); while the total mortality rate was 3.27 at the end of the breeding period. These findings clearly pointed out that Burcin-2 vaccine has no adverse effect on performance and may protect chickens against field infection. This result

agree with those reported by Naqi et al.,(1980) and Amer et al. (2007) where there were no differences between the vaccinated groups in the measured performance parameters. In the other hand comparing these results with the farm stander, there was improvement in both total mortality and feed conversion rates. This result supported that the IBD with live intermediate vaccine (Bursine-2) is of value in improving broiler performance, especially in presence of maternal antibodies.

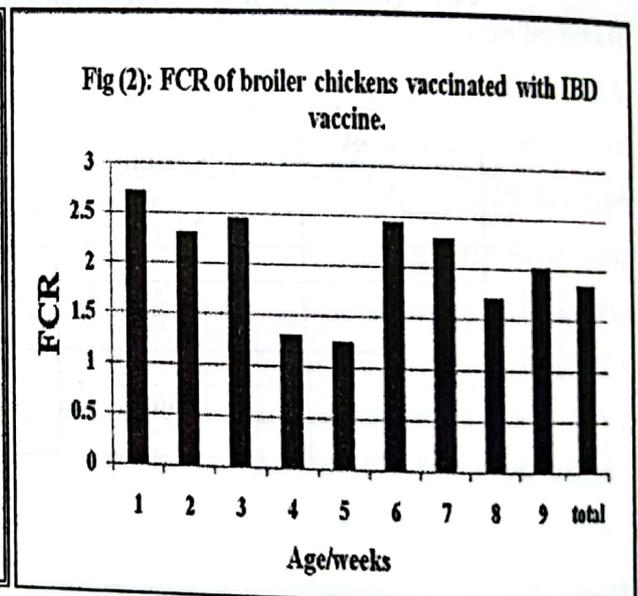
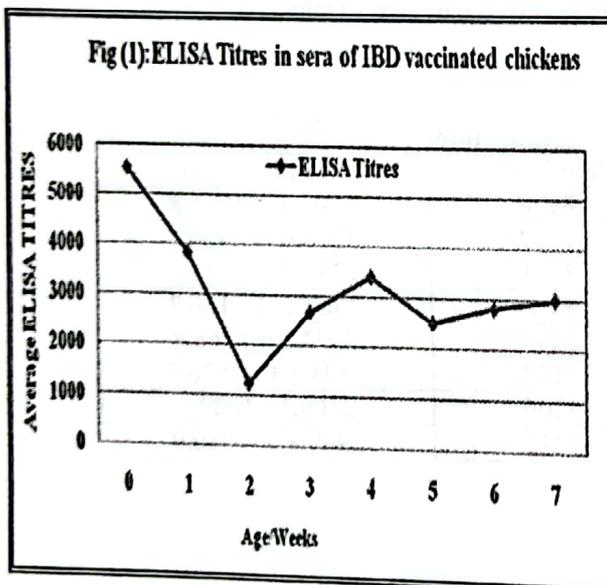
**Table (1): ELISA titres of broiler chickens received IBD live vaccine.**

Age/weeks	ELISA titres		
	Minimum	Maximum	Mean ± SD
0	2477	8428	5571.1 ± 1761.8
1	2715	7237	4776.9 ± 1764.8
2	0	3431	1237.7 ± 915.8
3	0	621	2662.1 ± 186.3
4	0	2243	3394.1 ± 768.8
5	0	4169	2504.3 ± 1526.5
6	1036	5206	3366.1 ± 1022.9
7	1612	5411	2940.8 ± 1050.9

**Table (2):** Mortalities, average body weight gain and feed intake, feed conversion rate

Age/weeks	Mortality		Average weekly		FCR
	NO.	%	Body weight gain/gm	Feed intake /gm	
1	84	1.05	42.7	116.3	2.72
2	32	0.4	121	281.2	2.31
3	43	0.54	226	556.1	2.46
4	30	0.38	320	554.1	1.31
5	15	0.22	400	615.2	2.25
6	53	0.73	350	600	1.71
7	23	0.32	300	600.5	2.02
Total	261	3.27	1759.7	3328.4	1.89

(FCR) of broiler chickens vaccinated with IBD vaccine.



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## دراسة حقلية عن الاستجابة المناعية والانتاجية لدجاج التسمين ذا المناعة الامية لللقاح التهاب غدة فبريشى المعدى المتوسط الحى.

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فى الدراسة تم استخدام ٨٠٠٠ كتكوت تسمين عمر يوم من سلالة الهابرد لديها مناعة امية لفروس التهاب غدة فبريشى المعدى. ربيت فى احد العنابر على الفرشة العميقة وتم تحصنها باللقاح الحى المتوسط من نوع برسین-٢ عند عمر ١٢ يوم فى ماء الشرب لاستبيان قدرات اللقاح المناعة وكذا تأثيره على معدلات الانتاجية للكتاكيت. لم يتم التعرف على اعراض او وفيات او افات تشريحية متخصصة لمرض التهاب غدة فبريشى المعدى على مدى مدة التربية وهى ٧ اسابيع.

اوضحت نتائج الاختبار المصلى بالاليزا ان المناعة الامية عند عمر يوم كانت  $1761.8 \pm 5571.1$  ثم انخفضت الى  $915.8 \pm 1237.7$  عند الاسبوع الثانى (٢ يوم بعد التحصين). بينما كانت النتائج فى ارتفاع الى  $2662.1 \pm 186.3$  و  $768.8 \pm 3394.1$  فى الاسبوع الثالث والرابع على التوالى بعد التحصين على التوالى. مما اوضح قدرة اللقاح على احداث المناعة فى اكتاكيت فى وجود المناعة الامية.

كان افضل معدلات التحويل الغذائى الاسبوعى عند الاسبوع الرابع من العمر (1.31) ثم السادس (1.71) وكان الاجمالي 1.89. كانت معدلات النفوق فى العمر الاكثر حساسية للمرض (٢-٦ اسابيع) اقل المعدلات (0.4-73%) بينما كان المعدل الكلى للنفوق 3.27 فى نهاية الاسبوع السابع.

اوضحت نتائج الدراسة ان لقاح التهاب غدة فبريشى المعدى الحى المتوسط من النوع برسین-٢ امن ونو قدرة على احداث المناعة فى الكتاكيت ذات المناعة الواردة من الأم كما لم تسجل تأثيرات ضارة على الانتاجية.