ORIGINAL ARTICLE **Mirror of Research in Veterinary Sciences and Animals MRVSA/ Open Access DOAJ**

Cellular and humoral immune response of three chicken strains of broilers to avian infectious bronchitis vaccines

Mohammed S. Abdul-Aziz[1], Latif I. kadhim*[1]
[1]Department of Pathology and poultry diseases, Faculty of Veterinary Medicine, University of Baghdad.

ARTICLE INFO
Received: 30.04.2013
Revised: 30. 05.2013
Accepted: 10.06.2013
Publish online: 25. 06.2013

***Corresponding author:**
Email address:
latibr63@gmail.com

Abstract

The aim of this study was to know, which of broiler strains have the best cellular and humoral immune response against infectious bronchitis virus vaccines (IBVv). Differences in genetic immune response and susceptibility are known for many of the major viral pathogens of poultry. Consequently, the study was done. An increase in the level of humoral and cellular immunity provides a possible means of enhancing protection of flocks against IBVv. One-day-old consist of three chicken strain of broilers (Cobb 500, Ross 308 and Hubbard F-15) were assigned into six equal groups of 25 bird as fallow G1, G2 and G3 were vaccinated with IBV Ma5 strain at day 8 and with 4/91 strain at day 21, while the last three groups G4, G5 and G6 did not vaccinated with IB vaccine. All groups were vaccinated with Newcastle disease (ND) vaccine. After completing the immune tests Enzyme Linked Immunosorbent Assay (ELISA), Phytohemagglotnin (PHA)-skin test and lymphoid organs indices it turned out that the Hubbard chicken strain had the lowest and slowest immune response in comparison with Ross and Cobb chicken strains.

To cite this article: Mohammed S. Abdul-Aziz, Latif I. kadhim. (2013.) Cellular and humoral immune response of three chicken strains of broilers to avian infectious bronchitis vaccines. Mirror of Research in Veterinary Sciences and animals. MRSVA 2 (3), 1-10. DOI: 10.22428/mrvsa. 2307-8073.2013. 00231.x

Keywords: cellular and humoral immune response, different chicken strains, infectious bronchitis virus vaccines.

Introduction

Infectious bronchitis virus (IBV) is a very dynamic and evolving virus in most parts of the world where poultry are reared and it is able to spread very rapidly in non-protected birds causing major economic losses to the global poultry industry (Cavanagh and Gelb, 2008; Ababneh *et al.,* 2012). The main method of protecting poultry from infectious bronchitis (IB) is the administration of live or killed vaccines. However, IB continues to cause economic losses in the poultry industry despite intensive vaccination programs in many countries (Lim *et al.*, 2011). This made more difficult to achieve because IBV exists

in the form of many different antigenic or genotypic types, commonly referred to as variants (Cavanagh and Gelb, 2008).

Over the years there has been much emphasis on growth improvement that is negatively associated with some aspects of immunological performance of poultry as reported by Yunis *et al.,* (2000); Cheema *et al.,* (2003). These and other study (Qureshi and Miller, 1991: Cheema*. et al.,* 2003) has established that commercial broiler lines differ in several baselines immune function parameters. Limited information is available relates to the effects of particular genes markers with particular immune response parameters in commercial broiler lines (Yonash *et al.,* 2001).

Variation in immune responsiveness and diseases resistance or susceptibility in chickens has been demonstrated for a variety of antigens and pathogens and for various experimental chicken lines and populations (Parmentier *et al*., 1996). This variation in genetic susceptibility is documented for important viral pathogens of poultry including infectious bronchitis virus, Marek's disease, leukosis virus and infectious bursal disease virus (Bumstead, 1993).

In recent years, the flocks of the Ross 308, Cobb 500, Hubbard F-15 and Hubbard Flex sets have been reared most frequently. The research performed on these birds line concerned mainly the evaluation of basic production indices (Janocha *et al*., 2008). The purposes of this study were to determine the effects of live attenuated IB vaccines on cellular and humoral immune response in different strains of broiler. To assess the immunity, ELISA, PHA-skin test and lymphoid organs indices were employed.

Materials and Methods

The following vaccines were used live attenuated IBV Ma5 strain and IBV 4/91vaccine strain (Intervet – Holland). Live attenuated ND LaSota (Intervet – Holland).
Phytohemagglutinin (PHA-p) (gibco®- USA).

Method of vaccination

Eye drop route (intraocular method) was used for vaccination with IBV Ma5 and NDV (LaSota) strains at day 8 and IBV 4/91 and NDV (LaSota) strains at day 21 of age (Koopman, 2008).

Experimental animals and protocol

One hundred sixty-five- day- old broiler chicks consist from three strains (Ross 308, Cobb 500, and Hubbard F-15) purchased from local hatchery. Upon arrival fifteen chicks were sacrificed and blood samples were collected for indirect ELISA test in order to measure the derived maternal antibody titer against IBV in their sera. The rest 150 chicks were weighed and divided randomly into six equal groups kept at the experimental animal house of College of Veterinary Medicine, Baghdad University. Half of these groups were vaccinated with IBV Ma5 and 4/91 strains at day 8 and 21 respectively and according to the material antibodies titer which was done by ELISA test. The IB vaccination was done by eye drop method. The other half groups not vaccinated. All groups were vaccinated with ND vaccine at day 8 and 21 respectively. Blood samples were randomly collected

from five birds from each group at 18 and 31 days for ELISA test to determine the antibody titer of IBV (Hanson, 1980). Then after five birds from each group was taken after ten day from vaccination at days 18 and 31 for PHA-skin test.

Phytohemagglutinin (PHA-skin test)

The feathers were plucked from one side of each wing web, clearing an area minimally large enough to accommodate the contacts of the calipers. The bare skin was swabbed with alcohol and 0.1 ml of PHA-p (gibco® USA) solution as a T-cell mitogen stimulation was injected intra-dermally (26-gauge intra-dermal needle) into the patagium (wing-web). The subsequent swelling was measured as an assay of *in vivo* T-cell mediated immune responsiveness (Cheng and Lamont, 1988). Following the recommendations of (Martin *et al.,* 2006), thickness measurement of the wing-web in the right wing was made to the nearest 0.05 mm with a micrometer (Electronic digital calipers- Germany) immediately before and 24 h and 48 h after injection.

Lymphoid organ indices

Chickens were individually weighed from each group to determine their body weight. Five birds from each group at day 18 were sacrificed via cervical dislocation. Following a thorough visual appraisal, the thymus, bursa Fabricious and spleen were immediately removed, dry and individually weighed. Since substantial lymphoid organ weight change was anticipated, their indices were calculated (Sellers *et al*., 2007).

Using these formula: Organ index = organ weight (g)/BW (g) x 100.

The results were presented as mean ± standard deviation (SD) and data were subjected to ANOVA. Differences between means were determined using Tukey's test in which the significance level was designated at ($P < 0.05$.) All data were checked for normality and homogeneity of variances using SPSS Inc. (2007) program.

Results

Humoral immunity by ELISA

The Ab titers against IB is shown in Table 1, at day zero high Ab titers was showed in all groups with no significant ($P < 0.05$) differences present between all strains. At these instants, Ab titer in the Ross V+ was significantly ($P < 0.05$) higher than other groups at days 18 and 31 of experiment. Nevertheless, the Hubbard strain was the lowest ($P<0.05$) Ab titer at day 18 than other experimental groups.
After 10 days post vaccination with Ma5 and 4/91IB vaccines, all vaccinated groups have a higher ($P < 0.05$) Ab titer than non- vaccinated groups as well as, Ross v+ had the highest ($P < 0.05$) Ab titration in compare with Cobb v+ and Hubbard v+ as well as, the Hubbard +ve had the lowest ($P < 0.05$) Ab titration in compare with other immunized groups.

Table 1: The effects of different strains of broilers vaccinated with infectious bronchitis on Ab titers during the experimental period (mean ±SD)

Groups	Ab d 0	Ab d 18	Ab d 31
Ross V+	2218±706[a]	1.593±181[a]	2.346±393[a]
Ross V-	2218±706[a]	180±36.7[d]	104±45[c]
Cobb V+	1706±536[a]	946±201[b]	1.470±371[b]
Cobb V-	1706±536[a]	120±49.4[d]	100±35[c]
Hubbard V+	2348±451[a]	679±47.2[c]	1.190±129[b]
Hubbard V-	2348±451[a]	130±34.6[d]	124±25[c]

[a,b,c,d] Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

Phytohemagglutinin-skin test

The data of PHA-skin test during the first vaccination are presented in Table 2. After 10 days post first vaccination with Ma5 IBV, At 24 and 48 hours after injection with PHA-p in wing web accrue swelling, this swelling in vaccinated groups appear higher ($P < 0.05$) than non-vaccinated groups. The Ross vaccinated group has the highest skin thickness but not significant after 24 and 48 h in compare with all vaccinated groups.

Table 2: The effect of different strains of broilers vaccinated with infectious bronchitis on the Phytohaemagglutinin (PHA) skin test after first vaccination of broilers during the experimental period (mean ±SD)

Groups	Before 24 h	After 24 h	Difference	After 48 h	Difference
Ross V+	0.930±0.103[a]	1.880±0.090[a]	0.950±0.093[a]	1.790±0.074[a]	0.860±0.167[a]
Ross V-	0.910±0.089[a]	1.570±0.067[b]	0.660±0.151[bc]	1.410±0.065[c]	0.500±0.154[bc]
Cobb V+	0.920±0.083[a]	1.870±0.075[a]	0.950±0.079[a]	1.760±0.041[a]	0.840±0.065[a]
Cobb V-	0.920±0.057[a]	1.550±0.050[b]	0.630±0.075[c]	1.430±0.083[c]	0.510±0.108[bc]
Hubbard V+	0.950±0.100[a]	1.780±0.103[a]	0.830±0.083[ab]	1.620±0.090[b]	0.670±0.083[ab]
Hubbard V-	0.940±0.108[a]	1.510±0.065[b]	0.570±0.083[c]	1.320±0.057[c]	0.380±0.083[c]

[a, b, c] Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

Similarly, the results of PHA skin after 10 days post second vaccination with 4/91 IBV (Table 3). The vaccinated groups have higher skin thickness ($P < 0.05$) than non-vaccinated groups in all strains. On the other hand, the PHA skin thickness values remained comparable between all vaccinated groups.

Lymphoid organs indices

The lymphoid organs indices are shown in Table 4. However, higher values appear in the vaccinated groups in compare with non- vaccinated groups at day 18. At these instant, the Cobb v+ groups have highest ($P < 0.05$) lymphoid organs indices.

Table 3: The effect of different strains of broilers vaccinated with infectious bronchitis on the Phytohaemagglutinin (PHA) skin test after second vaccination of broilers during the experimental period (mean ±SD)

Groups	Before 24 h	After 24 h	Difference	After 48 h	Difference
Ross V+	0.920±0.083^{a}	1.960±0.082^{a}	1.040±0.119^{a}	1.798±0.057^{ab}	0.878±0.056^{a}
Ross V-	0.920±0.075^{a}	1.600±0.061^{c}	0.680±0.115^{b}	1.420±0.057^{c}	0.500±0.035^{b}
Cobb V+	0.868±0.077^{a}	1.966±0.047^{a}	1.098±0.083^{a}	1.840±0.082^{a}	0.972±0.109^{a}
Cobb V-	0.932±0.063^{a}	1.560±0.065^{c}	0.628±0.113^{b}	1.400±0.079^{c}	0.468±0.120^{b}
HubbardV+	0.880±0.029^{a}	1.818±0.053^{b}	0.938±0.067^{a}	1.680±0.090^{b}	0.800±0.110^{a}
Hubbard V-	0.968±0.101^{a}	1.510±0.065^{c}	0.542±0.077^{b}	1.360±0.041^{c}	0.392±0.069^{b}

$^{a, b,c}$ Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

Table 4: The effects of different strains of broilers vaccinated with infectious bronchitis on lymphoid organs indices during the experimental period (mean ±SD)

Groups	Thymus index d 18	Bursa index d 18	Spleen index d 18
Ross V+	0.535±0.028^{ab}	0.212±0.012^{ab}	0.173±0.022^{ab}
Ross V-	0.486±0.016^{bc}	0.155±0.020^{b}	0.149±0.014^{ab}
Cobb V+	0.558±0.018^{a}	0.236±0.031^{a}	0.178±0.025^{a}
Cobb V-	0.468±0.023^{c}	0.158±0.018^{b}	0.137±0.011^{b}
Hubbard V+	0.556±0.017^{a}	0.211±0.036^{ab}	0.169±0.012^{ab}
Hubbard V-	0.493±0.037^{bc}	0.161±0.041^{b}	0.135±0.011^{b}

^{a,b,c} Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

Similarly, higher values appear in the vaccinated groups in compare with non -vaccinated groups at day 31 after 10 days post immunization with 4/91 IBV (Table 5). However, the lymphoid organs indices values remained comparable between all vaccinated groups.

Table 5: The effects of different strains of broilers vaccinated with infectious bronchitis on lymphoid organs indices during the experimental period (mean ±SD)

Groups	Thymus index d 31	Bursa index d 31	Spleen index d 31
Ross V+	0.235±0.008^{a}	0.096±0.002^{a}	0.066±0.002^{a}
Ross V-	0.185±0.014^{c}	0.080±0.004^{bc}	0.053±0.002^{bc}
Cobb V+	0.230±0.020^{ab}	0.094±0.006^{a}	0.066±0.004^{a}
Cobb V-	0.187±0.013^{c}	0.080±0.004^{bc}	0.047±0.006^{c}
Hubbard V+	0.215±0.020^{abc}	0.090±0.005^{ab}	0.061±0.001^{ab}
Hubbard V-	0.195±0.013^{bc}	0.070±0.007^{c}	0.053±0.003^{bc}

^{a,b,c} Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

Discussion

Humoral immunity by ELISA

The results of Ab at day zero can be explain that there are numerous serotypes of IBV reached the reproductive system of chickens (hens vaccinated by IB attenuated vaccine at early breeding stages followed by oil emulsion at 18 weeks) resulted in activation of the mucosal immunity in the reproductive tract causing direct secretion of IBV antibodies into the eggs (Hamal *et al*., 2006).This result agree with Gharaibeh *et al*., (2008) who found that the IB have second highest material antibody titer after chicken infectious anemia disease that is number one at 37 weeks of stocks breeder.

The serum sample were collected at 10-day post vaccination with IBV according to Da Silva *et al.,* (1991) and proved that at 10 to 14 days IgG, is the predominant antibody, maintaining its levels for a considerable time. This antibody isotype is detected by most routinely used IBV diagnostic tests ELISA. The second vaccination with IB 4/91 strain showed higher Ab titers than those of first vaccination with IBV Ma5 because most of the produced antibodies were induced by a large number of dominant epitopes in the S1 protein in IB 4/91.

These results agree with Cavanagh *et al*., (2005) previous studies on the degree of variation exhibited within the S1 gene nucleotide sequences of IBV 4/91 serotype isolates reported maximum nucleotide and amino acid differences, the IB 4/91 express a large number of dominant epitopes in the S1 protein, hence, a large number of amino acid changes in these regions being sufficient to change the serotype.

In point of view, due to found some or low degree of cross-protection between Ma5 and 4/91 vaccine, I conclude that 4/91 share some antigenic properties with Ma5 vaccine this antigenic properties that stimulate the memory cell to produce high Abs amount in secondary immune response, this point of view agreed with Davison *et al*., (2008) who found that the large amount of memory cells were sensitized to produce additional Abs as rapid as possible after second vaccination with the same antigen in first vaccination. Also, Cowen and Hitchner, (1975) prove that some or low degree of cross protection observed among IBV serotypes.

The strain of Ross vaccinated group at 18 and 31 had the highest ($P < 0.05$) Abs titer then Cobb vaccinated and Hubbard vaccinated groups respectively. This is may be due to the difference in the strain genotypes of Ross breed in comparison with Cobb and Hubbard breeds or due to the accumulation of the genes of Ross strain that result from massive of genetic selection to disease resistance breeding by the primary breeders, this genes are carried on major histocompatibility complex (MHC) genes responsible for the difference in immune response between chicken breeds, this agree with Biozzi *et al.*, (1979) who explain that the production of antibodies in the humoral immune response is controlled by many genes responsible for genetic resistance. In meat-type chickens, the MHC Class IV region had significant effects in lines that had been selected for high or low early antibody response to *Escherichia coli* vaccination at 10 d of age (Uni *et al*., 1993).

Phytohemagglutinin-skin test

After injection of the PHA-p (Table 2 and 3) the wing web can induce swelling response, this swelling due to influx of inflammatory cells and proliferation of T lymphocyte, this swelling had some differences in thickness between different chicken strains, in addition

Hubbard strain is the lowest, genetic background or different selection criteria might be the reason for the observed differences in the results of this study, this result agreed with Sundaresan *et al*., (2005) who proved that chicken lines have been shown to differ in swelling intensity in reaction to PHA-p, demonstrating significant genetic variation for this response. Since MHC molecules are not only important in foreign antigen detection but also serve key roles in the activation and proliferation of T lymphocytes (Gur *et al*., 1999). The PHA-induced skin swelling test is classically used *in vivo* in immunoecological studies and provokes the infiltration and/or proliferation of several types of immune cells (Martin *et al*., 2006).

Another important finding is that the slower spatial peak PHA skin response in the non-vaccinated groups (Table 2 and 3) may possibly be due to low immune response as compared with vaccinated groups that have more active immune response which have highest ($P < 0.05$) change in PHA skin response. The cytotoxic T lymphocyte (CTL) response of chicks to IBV infection plays a critical role in impeding the injurious effects of virus during acute infection activation (Viret and Janeway, 1999).

Lymphoid organs indices

The lymphoid organ weight and their indices are useful indicators of immunological status (Sellers *et al*., 2007) and indicate on the animals' ability to carry infection and the provision of lymphoid cells during an immune response (Heckert *et al*., 2002).Our results showed the vaccinated birds have higher lymphoid organ indices than non- vaccinated birds, this result agree with Bailey *et al*., (2004) who proved that the lower lymphoid organs indices in non-vaccinated groups indicates on low protection. Ubosi *et al.,* (1985) that explain a high antibody response to SRBC has been associated with a larger bursa size in White Leghorn chicken strains.

In other results found variation in lymphoid organ weight and indices between strains groups in Hubbard strain exactly that had lower lymphoid organ indices and weight, this difference is compatible with low antibody titration, low WBC and lymphocyte count of Hubbard strain, since it is the lymphoid organs are responsible for generation of Abs and effective lymphocytes against IBV (Cheema *et al.,* 2007). The generation of Abs and effective T lymphocyte against IBV are controlled by MHC genes and that have differences between breeds of poultry that differ in resistant of diseases (Biozzi *et al.*, 1979; Janse *et al*., 1994).In this study revealed that, Hubbard chicken strain had the lowest and slowest immune response in comparison with Ross and Cobb chicken strains.

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