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# Evaluation of two different infectious bronchitis vaccination programs in broiler breeder chickens

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**Abstract**

The objective of this study was to investigate the serological differences of two different infectious bronchitis (IB) vaccination programs in broiler breeder farms. Ninety broiler breeder chickens was selected and randomly distributed in three groups (each group with three repetitions). In two experimental group two different vaccination programs was used and group three was as a control group and the IB vaccine was not used. On days 1, 110, and 145 blood samples were collected and examined with ELISA test. For Data analyzing One-way ANOVA statistical method was used for compare antibody titers against IB disease and statistical software was PASW SPSS 18th edition. Results of ELISA test showed that mean of antibody titers was higher in the groups that IB inactivated vaccines was used, and also ELISA antibody titer in group that vaccines was used statistically different from the control group ($p<0.05$). Because of economical losses causes by IB disease in both broiler breeder flocks and their progeny, it is necessary to applying exact vaccination programs in broiler breeder flocks and observes of biosecurity to decrease economical losses.

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## Introduction

Infectious bronchitis (IB) is an acute, highly contagious and economically important viral disease. It occurred at all ages of commercial chickens which is caused by infectious bronchitis virus (Cavanagh and Gelb, 2008). Infectious bronchitis virus (IBV) is a member of *Coronaviridae* family and genus *Gammacoronavirus*, with more than 26 serotypes (Enjuanes*et al.*, 2000- King*et al.*, 2012). Infectious bronchitis was first reported in the USA in 1931 as a respiratory disease (Schalk and Hawn, 1931). Some strains of IBV also infect non-respiratory tissues including reproductive tissues (Farzinpour*et al.*, 2009- Van Roekel*et al.*, 1951), kidney (Cumming, 1962- Cumming, 1963- Winterfield and Hitchner, 1962), and alimentary tract (Yu*et al.*, 2001). Whereas the 793/B serotype was first identified in Britain in 1990-1991, it was subsequently confirmed that the virus had been present in France since 1985 (Gough*et al.*, 1992- Parsons*et al.*, 1992- Picault*et al.*, 1995). The first IB serotype to be described was Massachusetts (Schalk and Hawn, 1931). This was followed in the mid-1950s by the Connecticut serotype (Jungherr*et al.*, 1956). Since that time, new IB serotypes have continued to be reported from the US (Gelb*et al.*, 1991), Europe (Davelaar*et al.*, 1984) and many other parts of the world (Cubillos*et al.*, 1991). As a result of molecular studies, it is now known that it is the S1 part of the IB virus that is responsible for determining its serotype. Furthermore, a new IB virus serotype can arise as a result of only a very few changes in the amino acid composition of the S1 part of the spike protein (Cavanagh*et al.*, 1992- Cavanagh*et al.*, 1992), with the majority of the virus genome remaining unchanged.

The first isolation of IBV in Iranian chicken flocks was reported in 1994 (Aghakhan*et al.*, 1994). Later, several Iranian researchers identified the 793/B serotype. (Momayez*et al.*, 2002- Seify Abad Shapouri*et al.*, 2002- Vasfi Marandi and Bozorgmehrifard, 2000). This serotype turned out to be one of the predominant circulating types of IBV in Iran (Nouri*et al.*, 2003- Shoushtari*et al.*, 2008).

Good quality vaccines have been available to control IB infections since the 1950s. However, despite their careful use, IB continues to be a major problem. One reason for this is the large number of antigenic types of IB that are known to exist worldwide. Because many antigens are shared, this might therefore, suggest that the currently available vaccines should be able to provide protection against challenge with IB viruses belonging to different serotypes from the vaccine itself.

The aim of present study was to evaluation the efficacy of two different vaccination programs against infectious bronchitis in broiler breeders by ELISA method.

## Materials and methods

*Study Design*

Ninety broiler breeder chickens was selected and randomly distributed in three groups (each group with three repetitions). In two experimental group two different vaccination programs was used and group three was as a control group and the reovirus vaccine was not used. On days 1, 110, and 145 blood samples were collected and following serum isolation, the samples undergoes ELISA (IDEXX) test and antibody titers obtained from each of vaccines were evaluated.

A vaccination program in two experimental groups was indicated in table 1, and in control group vaccine was not used.

*Statistical Analyzing*

For Data analyzing One-way ANOVA statistical method was used for compare antibody titers against Newcastle disease and statistical software was PASW SPSS 18th edition.

## Results and discussion

The results of study showed that the antibody titer was not different significantly between studied groups in day old chickens because all chickens was purchased from same grandparent farms. At 110 day there was very significant difference between groups

and in control group antibody titers was decreased and the titer was different statistically in comparison to group1 and 2 ($p<0.01$). Also on day 145 there was very significant differences between two vaccinated groups ($p<0.01$), and in groups that inactivated vaccine was used on 126 day old titer was increased significantly in comparison to group 1 ($p<0.05$). The Results of flocks antibody monitoring was demonstrated in table 2.

**Table 1.**vaccination programs in experimental groups.

| Day of vaccine administration | Group 1 | Group 2 | Control Group |
|---|---|---|---|
| 1 | H120 (Spray) | H120 (Spray) | ---- |
| 12 | IB88 (DW*) | IB88 (DW) | ---- |
| 42 | H120 (DW) | H120 (DW) | ---- |
| 105 | H120 (DW) | H120 (DW) | ---- |
| 126 | ---- | IB (inactivated) | ---- |

*Drinking water.

Our results indicated in the control group, which any IB vaccine was not used, antibody titers were decreased and the unvaccinated chickens susceptible to disease. In group 1, only we used live vaccines, the antibody titers was reach to 2651.15±237.77 on 110-day old and finally reached to 3543.75±319.42 on day 145.

In group 2, the antibody titers was reach to 2565.90±235.71 on 110-day old and because inactivated vaccine was used on 126 day old, antibody titers was reach to 4450.50±349.06 on 145-day old. Our results indicated that vaccination with inactivated vaccines was better for protection of breeders and also theirs progenies.

The increasing incidence of respiratory problems in broiler chicks and production problems in laying hens including broiler breeders necessitated screening the vaccination programs. It was demonstrated the chicks that had been experimentally infected with IBV at 1 day of age re-excreted virus at around 19 weeks of age (Jones and Ambali, 1987).

The humoral immune response to IBV vaccination has mostly been investigated by measuring antibody levels in serum, using enzyme-linked immunosorbent assay (ELISA), VN or haemagglutination-inhibition (HAI) tests. (IBV haemagglutinates very poorly until treated with enzyme preparations containing neuraminidases (Alexander*et al.*, 1983- Schultz*eet al.*, 1992). However, there have also been a few studies of IBV antibodies in the nose and trachea. It should be stated at the outset that many studies have shown that the presence or absence or titre of serum antibody to IBV does not correlate with protection; that is, vaccinated chickens may be protected against respiratory disease IBV irrespective of the titre of serum antibody (Ignjatovic and Galli, 1994).

**Table 2.** The results of antibody titers evaluation on days 1, 110, and 145.

| Group | Day | | |
|---|---|---|---|
| | 1 | 110 | 145 |
| 1 | 7011.55±250.19 | 2651.15±237.77$^{*b}$ | 3543.75±319.42$^{b}$ |
| 2 | 6694.35±226.57 | 2565.90±235.71$^{b}$ | 4450.50±349.06$^{c}$ |
| Control | 6632.85±221.16 | 820.60±119.79$^{a}$ | 282.70±26.79$^{a}$ |
| Sig. | 0.438 | 0.001 | 0.001 |

* Different letter in each column, indicated statistical difference between groups.

The profile of the serum antibody response depends on the method used to detect it. Following infection of chickens with a virulent strain of IBV, specific antibody was first detected by ELISA (plates coated with IBV), a majority of the birds having detectable antibody by 6 days after infection, with titres maximal within 21 days and tending to decline shortly afterwards (Mockett and Darbyshire, 1981).

VN antibody was delayed in comparison with ELISA-detected antibody, being first detected in the period 9 to 21 days after infection, peaking within 35 days and remaining level for the remainder of the sampling period (63 days after inoculation), although in 25% (2/8) of the birds the VN antibody was still increasing at 63 days, when sampling ended. The HAI antibodies were first detected at 9 days, peaking at 14 to 17 days and then declining, although in one bird the HAI titre did not peak until much later (as with the VN titre in the same bird). Mockett (1985) also compared ELISAs using purified S and M proteins with the conventional virus-coated ELISA; the primary and secondary antibody response profiles were very similar, VN antibody again later than ELISA antibody. Following infection with a live IBV vaccinal strain there was a good primary immunoglobulin IgM response (Martins*et al.*, 1991- Mockett and Cook, 1986).

As expected, the primary IgM response peaked, and declined, before that of the IgG response (Martins*et al.*, 1991). The secondary IgM response (i.e. in response to a second (challenge) infection) peaked at the same time as that of IgG, but declined faster. Researchers suggested that measurement specifically of serum IgM would be useful in defining recent infection (Mockett and Cook, 1986). In contrast to the response after IBV infection, vaccination with inactivated virus produced almost no IgM response, as well as a poor IgG response (Martins*et al.*, 1991). A single and double vaccination with inactivated IBV resulted in HAI titres of 1/28 and 1/219, respectively, 4 and 2 weeks after the respective vaccinations (Gough*et al.*, 1977). A single inoculation of 3 mg purified IBV induced maximum titres of HAI and ELISA antibody of 6 log2 and 12 log2, respectively, by 3 to 4 weeks after vaccination (Cavanagh*et al.*, 1986). VN antibodies peaked 1 week later, at 5 log2. Titres decreased eight-fold within 4 weeks of peaking.

The poultry industry prefers to use live vaccines rather than inactivated ones. The former arecheaper to make and buy, and easier/cheaper to apply.

In a number of studies, two vaccinations with inactivated IBV produced protective immunity in >85% of chickens. That said, in some studies even >2 inoculations of high doses of inactivated IBV gave protection in only 50% or so of chickens. It might be that the criterion of protection used in these studies-non-re-isolation of challenge virus-might be too stringent. That is, some amelioration of the clinical effects of infection might be obtained even if there is some detectable replication of the challenge virus. Experimental IBV subunit vaccines, in the form of the S1 protein, were efficacious, but, as expected, not more so than inactivated virus. In two studies, protection of the trachea was achieved after three vaccinations in 70% and 59% of chickens, respectively. Protection of the kidney was less efficient (10% and 25%, respectively).

**Conclusions**

As previously reported uses of inactivated vaccines in chickens partly protects chickens but it could not be completely protect various organs infection. Our results also indicated that uses of inactivated vaccine increases antibody titers against IB and it could be protect partly birds from IB infection and decreasing of egg production and its quality.

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