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Evaluation of the Immunogenisity of Immune Complex Infectious Bursal Disease Vaccine Delivered *In ovo* to Embryonated Eggs or Subcutaneously to Day-Old Chickens

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Abstract: Immune complex vaccine of infectious bursal disease (IBD) were applied *in ovo* to embryonated eggs and subcutaneously to newly-hatched chickens in the hatchery, while the other group of chickens received a conventional IBD vaccine at days 12, 17 and 22 of age. At day 28 of age, ten chickens from each group were challenged with the field strain of IBD virus. Hatchability of eggs, survival of chicks, antibodies titres against Newcastle disease (ND) and IBD viruses were determined. Bursal index of post-challenged chickens were also measured. The present data indicates that *in ovo* IBD vaccination did not affect the hatchability of eggs or survival of hatched chicks. Vaccination with immune complex vaccine did not interfere with the degree of protection induced by ND vaccines. Moreover, this finding demonstrates that immune complex vaccine similar to that of conventional vaccine is able to provoke active immunity of birds and seem to protect chickens sufficiently from the IBD.

Key words: Chickens, infectious bursal disease, *in ovo*, immune complex vaccine

Introduction

Infectious bursal disease (IBD) is a contagious disease of fowl caused by double stranded RNA virus. Infectious bursal disease virus (IBDV) is of major importance in all poultry producing regions of the world. It is highly infectious in young chickens and causes severe damage to the bursa, resulting in suppression of the immune system as well as the possibility of significant morbidity and mortality (Van Den Wijngaard *et al*., 2001). It is suggested that passive immunity is of critical importance because chicks have to be protected throughout the early period of life, when they are more susceptible to the immunosuppressive effects of IBDV. However, high maternal antibody levels in the chickens seldom protect broilers to the age of slaughter (Coletti *et al*., 2001, Van Den Wijngaard *et al*., 2001).

During the last decade, very virulent IBDV has caused outbreaks of disease with high mortality in Europe and some other parts in the world. The current vaccination programs failed to protect chicks sufficiently. Vaccination failures were mainly due to the inability of the intermediate vaccines to protect the birds before they became susceptible to challenge with virulent field virus. However, when progeny are vaccinated at an early age with a mild or highly attenuated live vaccine, high levels of maternal antibody may interfere with the development of active immunity (Skelees *et al*., 1979, Van Den Berg and Meulemans, 1991). Because of maternal antibody interference associated with lack of uniform antibody titers in progeny (Winterfield and Thacker, 1978), repeated vaccinations are needed until maternal antibody wanes (Coletti *et al*., 2001). Unfortunately, this practice often does not prevent virulent of IBDV responsible for field outbreaks (Chettle *et al*., 1989).

Recently, an immune complex vaccine is made by mixing a live intermediate plus IBD virus with bursal disease antibody contained in whole hyperimmune serum (Chettle and Wyeth, 1994; Avakian *et al*., 1994; Haddad *et al*., 1997). The vaccine is applied either *in ovo* to 18-days-old embryonated eggs or by injection subcutaneously to 1-day-old chickens which had maternally derived antibodies. The reason to mix antibodies with intermediate plus IBDV vaccines to form complex vaccines is to reduce the virulence of the IBD-viral strains used when applied *in ovo* or to young chickens (Whitfill *et al*., 1992). The second reason is to stimulate an early immunoresponse in chickens, when they have high levels of maternal antibody (Coletti *et al*., 2001).

The present study was designed to compare the IBDV immune complex vaccinations with conventional vaccine in an experimental and in a commercial broilers flock raised under field conditions. Therefore, the potential for immune complex vaccine to interfere with the hatchability of the eggs and with the immunoresponse to attenuated Newcastle disease (ND) vaccine were tested. Vaccinated chickens were also examined for the antibody response against IBDV, challenge with field virus and bursal / body weight ratios (bursal index).

Materials and Methods

Vaccination and challenge groups: The present study was performed at same time under field (F) and an experimental (E) condition. Forty-five thousand

Table 1: Infectious bursal disease vaccination programmes in a field (F) and an experimental (E) conditions

Groups	Vaccination programmes		
	In ovo vaccination	Day-old vaccination	Conventional Vaccination
AF	+	-	-
BF	-	+	-
CF	-	-	+
AE	+	-	-
BE	-	+	-
CE	-	-	+
UE	-	-	-

AF: Chickens received a single dose of IBDV complex vaccine *in ovo* at day 18 of incubation and kept in the field.
BF: Chickens received a single dose of IBDV complex vaccine subcutaneously at day 1 of age and kept in the field.
CF: Chickens received a conventional vaccine via drinking water in the field at days 12, 17 and 22 of age.
AE: Chickens received a single dose of IBDV complex vaccine *in ovo* at day 18 of incubation and kept in laboratory room
BE: Chickens received a single dose of IBDV complex vaccine subcutaneously at day 1 of age and kept in the laboratory room.
CE: Chickens received a conventional vaccine via drinking water in the laboratory room at days 12, 17 and 22 of age.
UE: Unvaccinated IBDV vaccines or control chickens.

embryonated eggs from one commercial breeder broiler flock were randomly divided into 3 equal (A, B and C) groups and incubated in a standard incubator under similar condition until chicks hatched.

In group A, fifteen thousand embryonated eggs were vaccinated *in ovo* by IBDV immune complex vaccine at day 18 of incubation (Table 1) according to a standard protocol (Whitfill *et al.,* 1992). Ten thousand of these hatched-chickens were placed in poultry house (AF) while 150 of those birds were kept in a clean laboratory room (AE) for following study.

In group B, newly-hatched chicks were vaccinated a single dose of IBDV immune complex vaccine subcutaneously in the hatchery. Ten thousand of these chickens located in second broiler house (BF), in same area and under similar condition of group A and 150 of these birds were reared in the laboratory room (BE). These two groups (A and B) birds did not receive other vaccines.

In group C, ten thousand of day-old chicks were placed in the third broiler house (CF) in same area while 150 of those were kept in third laboratory room (CE). These two groups chickens were vaccinated with a conventional IBD intermediate vaccine at days 12, 17 and 22 of age via drinking water.

Groups UE: Unvaccinated IBD vaccines (control) chickens that was reared experimentally.

The procedure for ND vaccination was similar in all groups. Hitchner B_1 was given as a single eye-drop at day 8 and was followed by LaSota ND vaccine via drinking water, plus 0.5 ml of inactivated ND vaccine by intra-muscular injection at day 18 of age. All birds had *ad libitum* access to commercial broiler food and drinking water.

At day 28 of age, ten chickens from each vaccinated and control groups were randomly chosen, marked and placed in a clean room for challenge. Each chicken was inoculated orally with 1 ml of a bursal homogenate containing of IBD field strain virus that was taken from a natural field outbreak. At day 35 of growing period, 10 control birds (UE) and all challenged birds were weighed, euthanised and autopsied for macroscopic lesions. The bursa index was calculated as follows: the bursa of Fabrisius weight (g) / body weight (g) x 1000 (Coletti *et al.,* 2001).

Hatchability and serological assays: For determination of hatchability, final hatching of *in ovo* vaccinated and unvaccinated eggs were recorded. Blood samples were taken from 30 newly hatched chicks per group by cardiac puncture in the hatchery and were followed on days 21, 28 and 35 of the growing period from the wing veins of 30 chickens of each group. Blood samples were allowed to clot, sera was separated and stored at -20°C until antibodies titres determination against ND and IBD viruses using of haemagglutination inhibition (HI) and Elisa tests (Allan and Gough, 1974; Van Den Berg and Meulemans, 1991).

Statistical analysis: Statistical analysis variance was performed using the "General linear model procedure" (SAS 1998). If a significant overall effect ($p < 0.05$) was found, treatment means were compared by using the Scheffe and T tests.

Results and Discussion

The hatchability rates of the eggs vaccinated *in ovo* at day 18 of incubation and unvaccinated eggs were 84.5% and 84.8% respectively. This result revealed that inoculation of embryonated chicken eggs with IBD vaccine virus did not affect hatchability of eggs or the survival of hatched chicks confirming the previous studies (Sharma, 1985; Coletti *et al.,* 2001).

The results of antibody titres against ND virus in differently IBD vaccinated and control chickens that were kept in laboratory rooms decreased until day 21 of age and then increased similarly up to the end of experiment (Fig. 1). No significant differences were found between the HI antibody titres of the different groups at days 1, 21, 28 and 35 of ages. These results indicated that vaccination by immune complex vaccine applied either via *in ovo* or subcutaneously did not interfere with the protection to Newcastle disease. Furthermore, the similar levels of HI antibody titres in intermediate plus

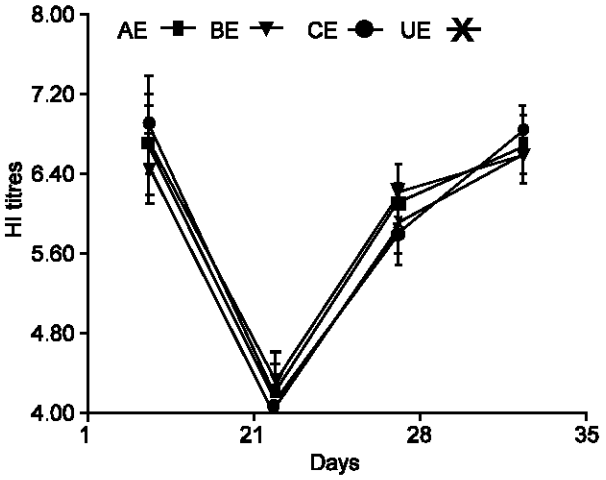

Fig. 1: Mean HI antibody titres against ND virus in different groups of broiler chickens that were reared under experimental condition.
AE: Experimental reared and in ovo vaccinated
BE: Experimental reared and subcutaneous vaccinated
CE: Experimental treated and conventional vaccinated
UE: Unvaccinated IBDV vaccine

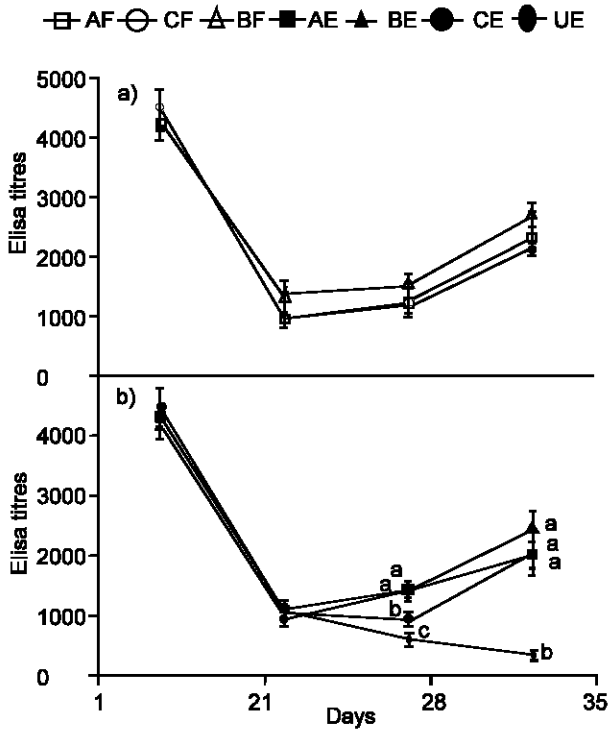

Fig. 2: Mean antibody titres against IBD virus in different groups of broiler chickens that was reared under field (a) and experimental (b) conditions. A, b, c, with in same age, values having different letters are significantly different (P<0.05)
AF: Field reared and *in ovo* vaccinated
BF: Field reared and subcutaneous vaccinated
CF: Field reared and conventional vaccinated
AE: Experimental reared and in ovo vaccinated
BE: Experimental reared and subcutaneous vaccinated
CE: Experimental reared and conventional vaccinated
UE: Unvaccinated IBDV vaccine

vaccinated chickens with the control chickens indicated, the protective immunity and antibody response to ND vaccination program were not impaired by immune complex vaccination (Avakian *et al.*, 2004).

Sharma (1985); Van Den Berg and Meulemans (1991) reported that maternal IBDV antibody interferes with protection from vaccinal virus. Additionally, immune complex vaccine is not neutralized by maternal antibodies existing in the chick, allowing the vaccine virus in the virus-antibody complex to exert its immunizing effect as maternal immunity to IBD declines (Van Den Wijngaard *et al.*, 2001). In the present work, a decrease of antibody titre was evident in all field and experimental groups at days 1 and 21 of age. This illustrated a progressive decrease of maternal antibody during the first 21 days of age (Fig. 2a,b). However, from day 21 onwards, vaccinated birds located either in poultry houses or in laboratory rooms had significantly (P<0.0001) higher antibody response to the different vaccination programs when compared with the control birds (Fig. 2a,b). These results confirming the previous reports indicate that immune complex vaccine begin to immunize the broiler as soon as maternal immunity drops to low levels (Haddad *et al.*, 1997; Coletti *et al.*, 2001). Furthermore, this finding demonstrates that immune complex vaccine similar to that of conventional vaccine were able to provoke active immunity of birds and seem to protect chickens sufficiently from IBD (Fig. 3a). The raising of antibody levels in field-reared chickens was more pronounced than to the chickens reared in laboratory rooms, however the difference was not significant (Fig. 3a). This could explain the contamination of birds to the field IBD virus that might persist in poultry houses. Such involvement to field virus can help to immune system of birds for synergistically provoking of active immunity.

In post mortem examinations, only unvaccinated- and challenged-chickens (EU-ch) showed mild form of infection bursal disease, e.g. moderate haemorrhages in the muscles and/or oedema in the bursa of Fabricius. All challenged groups showed bursal atrophy compared to the control (EU) chickens, consequently, bursal index of challenged-chickens were significantly (P<0.0001) lower when compared with the control chickens at day 35 of age (Fig. 3b). The bursal atrophy of post challenged chickens compared to control birds shows the activity of vaccinal and / or challenge viruses. However, the mild form of IBD observed in post challenged unvaccinated- and challenged-chickens could indicate a low virulence of the challenged virus strains. The absence of lesions in post challenged

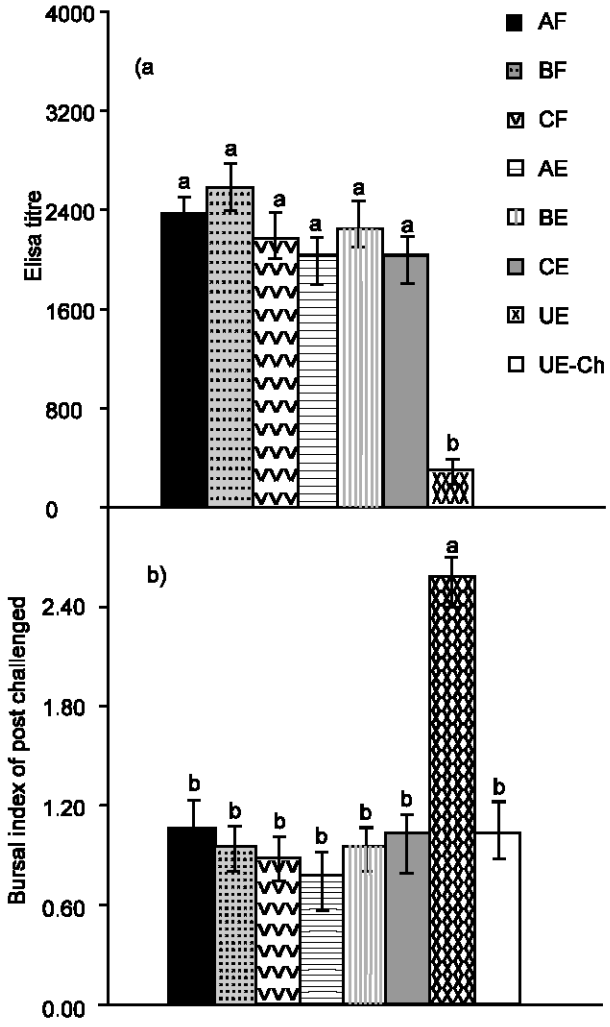

Fig. 3: Mean antibody titres against IBD virus (a) and bursal index (b) in different groups of 35 days old broiler chickens that were reared under field and experimental conditions. a, b, with in groups, values having different letters are significantly different (P<0.05)

AF: Field reared and in ovo vaccinated
BF: Field reared and subcutaneous vaccinated
CF: Field reared and conventional vaccinated
AE: Experimental reared and in ovo vaccinated
BE: Experimental reared and subcutaneous vaccinated
CE: Experimental reared and conventional vaccinated
UE: Unvaccinated IBDV vaccine
UE-ch: Unvaccinated IBDV vaccine and challenged

vaccinated birds can be due to protection evoked by IBD vaccination.

It is known in advance that it is impossible to immunize 100% of birds by IBD vaccination via water or spray. Since a number of chickens will either not receive vaccine at all, or receive an insufficient dose. Moreover, it is difficult to estimate the right time of vaccination due to lack of uniform maternal antibody levels in each flock (Van Den Berg and Meulemans, 1991). Therefore, the challenge concerning IBD prevention and control is increased, hence extensive vaccinations of breeders and progeny still cause economic losses.

As observed, the IBDV antibody complex vaccine was shown to be safe in the field trials and to be protective in many studies. The complexing of virus with the correct ratio of antibody allows for safe *in ovo* administration because viral replication in the bursa of birds is delayed until several days after hatch. Additionally, even high levels of maternal antibody did not interfere with the immune complex vaccines' ability to immunize broilers (Van Den Wijngaard *et al.*, 2001).

Our study supports the previous reports that immune complex vaccine which applied either *in ovo* or subcutaneously is able to induce humoral antibody, IBD protection and hence could be comparable with results of 3 conservative vaccinations with conventional live IBD vaccines. More studies should be done for the better understanding of the exact mechanisms of immune complex vaccine which is applied *in ovo* to embryonated eggs or subcutaneously to young chickens.

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