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Role of Maternal Antibodies in Protection Against Infectious Bursal Disease in Commercial Broilers

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Abstract: Chicks from broiler breeders with known Infectious Bursal Disease Virus (IBDV) vaccination history were examined for residual maternal and experimentally induced antibodies and their role in protection against IBDV. Maternal antibodies in unvaccinated chickens persisted in chicks up to 21 days as determined by ELISA with complete decay by 28 and 35 days. Experimental challenge with live IBDV protected chicks up to 14 days and resulted in 10 to 20% mortality thereafter. However, a group vaccinated with Bursine-2 and challenged with IBDV exhibited mortality in the first week post hatch and followed by complete protection until 8 weeks. These studies suggest that while maternal antibodies against IBDV persist and may protect chicks during the first 14 days of age, they may also interfere with earlier vaccine-mediated protection. Depending upon the flock IBDV antibody status, a customized vaccination regimen may be crucial for complete protection.

Key words: IBDV, maternal antibodies, vaccination

Introduction

A disease of broiler chickens appeared in the Delmarva area around 1956 and was named as "Gumboro Disease". In 1962, Winter field and Hitcher reported on the virus nature of the disease they termed as *nephritis nephrosis syndrome* of chickens. Two viruses were identified, namely, Holte and Gray, which induced a mild disease reminiscent of infectious bronchitis but which was accompanied by renal alterations. Clinicians not making a distinction on etiologic grounds collectively referred to all these maladies as Gumboro disease. However, the Winter field group soon recognized distinct differences and suggested infectious bronchitis variant viruses for the Holte and Gray viruses and infectious bursal agent for a disease which clinicians presently designate as infectious bursal disease (Helmboldt and Garner, 1964).

Infectious bursal disease virus (IBDV) is a non-enveloped, icosahedral virus that is now endemic in most poultry producing areas of the world (Rosales *et al.*, 1989; Sharma *et al.*, 2000). Infectious bursal disease in poultry is characterized by ruffled feathers, watery diarrhea, trembling and severe prostration. Clinical signs occur in 10-20% of the affected flock and in an affected flock mortality ranges from 10-20% (Ley *et al.*, 1983). Chicken is the only avian species known to be susceptible to clinical disease and characteristic lesions caused by IBDV. Turkeys, ducks and ostriches are susceptible to infection with IBDV but are resistant to clinical disease (McNulty *et al.*, 1979). The disease affects the lymphoid organs of the chicken, primarily the bursa of Fabricius. Clinically, IBD is most commonly

recognized in 3-6 weeks old chicks. In both field outbreaks and experimentally produced infections, newly hatched chicks seldom show any clinical signs or experience significant mortality (Skeeles *et al.*, 1978). Infectious bursal disease is important to the poultry industry because of its direct pathological and immunosuppressive effects (Sharma *et al.*, 2000). Once established in a poultry-rearing area, this virus infection may recur in subsequent flock if proper disinfection and husbandry conditions are not observed (Ley *et al.*, 1983). Currently, hyper immunizing dams with live and inactivated IBD vaccines achieve protection against IBD. The dams develop a high level of antibodies, which are transmitted to the progeny chicks in the form of maternal antibodies (Sharma *et al.*, 1989). The duration of the parental immunity is variable in progeny and outbreaks have been reported at different development stages of progeny (Khan *et al.*, 1988). In practice, different vaccination schedules have been recommended and used. Despite these vaccination schedules, outbreaks are still recorded throughout Pakistan. The present study was conducted to investigate the role of maternal antibodies in protection against IBD in commercial broiler chickens.

Materials and Methods

Experimental chicks: One hundred and eighty-day-old Hubbards broiler chicks were obtained from a commercial source in Rawalpindi, Pakistan. The broiler breeder parent flocks of these chicks were vaccinated orally against a live IBD virus vaccine at 2, 4 and 10 weeks of age. At 24 weeks of age these parent flock

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birds had received a killed IBD virus booster via the oral route. The progeny chicks were housed at the Animal Sciences Institute, National Agricultural Research Center, Islamabad, Pakistan, poultry housing facilities under the standard management. The chicks were given the Newcastle disease vaccine (obtained from Poultry Research Institute, Rawalpindi) at day seven of age. A commercial broiler starter and finisher feed (Punjab Feeds Ltd., Lahore) was made available for *ad libitum* consumption.

Quantification of maternal antibody levels in progeny chicks:

First experiment was conducted to assess the levels of maternal anti-IBDV antibodies in the progeny chicks used in this study. Out of the total 180 chicks, 60 chicks were assigned into a group designated as non-vaccinated and non-challenged subgroup. These chicks were then further subdivided into 6 groups of 10 chicks each. At 1, 7, 14, 21, 28 and 35 days of age 10 chicks from a subgroup were bled for serum collection and then euthanized for histopathology of bursa of Fabricius. Serum samples were stored at -20 °C until analyzed. Anti-IBDV antibodies were quantitated by using an indirect ELISA (Enzyme Linked Immunosorbant Assay) as described by Voller *et al.* (1989). A commercial live infectious bursal disease virus vaccine Bursine-2 (Solvay, USA) was used as an antigen for ELISA. It was reconstituted with carbonate bicarbonate buffer (pH 9.6). To determine the working dilutions of the IBD antigen and conjugate, checkerboard titration were carried out. The optimum concentrations for the each were found to be 1:150 and 1:500 for antigen and conjugate respectively (data not shown). All samples were tested in duplicate using flat-bottom micro titer plates. Micro titer plates wells were coated with 100 µl/well of a 1:150 dilution of the IBDV antigen in the carbonate bicarbonate buffer (pH 9.6). The plates were incubated overnight at 4 °C in a humid chamber, then emptied and washed five times (3 minutes each time) manually with phosphate buffer saline (PBS) containing 0.05% Tween 20. The final pH of PBS-Tween 20 preparation was adjusted to 7.4. Serial dilutions of the test sera were made in PBS-Tween 20 and added to the appropriate wells in 100 µl/well volumes. The plates were incubated for 1hr at 37 °C and then the wells were washed five times (3 min each time) manually with PBS-Tween 20. Then 100 µl of rabbit anti-chicken IgG conjugate (Cappel Labs, USA) diluted 1:500, was added to all the wells and incubated for 1hr at 37 °C. Afterwards, the wells were washed with PBS-Tween 20 as described earlier. Finally, 100 µl of freshly prepared enzyme substrate (Orthophenylenediamine 40mg /100ml of citric buffer, pH 5 and 40µl of 30% hydrogen per oxide) was added to each well and incubated for 30 minutes at 37 °C in the dark. The color reaction in each well was stopped with 50µl of 2M sulfuric acid. Positive and negative controls

were run simultaneously in each plate. The plates were read in an ELISA reader (Titertek Multiskan MCC/340, Karl Kolb, West Germany) at 492 nm.

Quantification of protective efficacy of maternal antibodies in progeny chicks against experimental Infectious Bursal Disease Virus challenge: Second experiment was conducted to determine the level of protection, which the maternal anti-IBDV antibodies may provide to the chicks upon experimental challenge with the IBDV. For this purpose, 60 chicks were randomly assigned to 6 subgroups of 10 chicks each.

Preparation of inoculum and bird challenge: The challenge IBDV virus inoculum was prepared from bursae of the IBDV-infected chicks maintained at the Institute. The inoculum was prepared as described by Khan *et al.* (1988). Briefly, bursae from infected birds were collected and 5g of the bursal tissue was mixed with 10g of sterile sand and minced in a Pestle and Mortar. To this, 45 ml of Phosphate Buffer Saline (pH 7.4) was added. This bursal homogenate was then centrifuged at 2500 rpm (800g) for 20 min. The supernatant fraction was collected and filtered through a sterile Whatman No. 1 filter. The filtrate was again centrifuged at 2500 rpm for 20 minutes and the final supernatant fraction was collected in a sterile bottle. Penicillin (1000 IU/ml and streptomycin 10mg/ml) was added to prevent bacterial growth. Ten birds from each of the six subgroups were injected with this inoculum starting at one day of age at seven days interval until the age of 35 days. These chicks were given a single injection of 1mL of viral inoculum per chick subcutaneously under the left wing. The birds were observed up to four days post-challenge. Any mortality was recorded daily. At 96 hours post-challenge, the birds were bled and the serum samples collected and stored at -20 °C till analyzed by ELISA.

Quantification of protective efficacy of a commercial Infectious Bursal Disease Vaccine in progeny chicks with known maternal anti-IBDV antibodies: Third experiment was conducted to determine the level of protection in progeny chicks with maternal anti-IBDV antibodies after IBDV commercial vaccination and experimental challenge. For this purpose, 60 chicks were randomly assigned to 6 subgroups of 10 chicks each. Each of the six subgroups was vaccinated once with Bursine 2 (Solvay Laboratories, USA) given via the drinking water. For this purpose, the drinking water was withheld for three hours prior to administering the vaccine-added water so that all birds within a group could drink and be exposed to the vaccine. Subgroups of 10 chicks each were exposed in such a way starting at one day of age up until 35 days of age with 7-days intervals between each subgroup. At 15 days post

vaccination chicks in each of the six subgroups were challenged with 1 mL of IBDV inoculum as described earlier. In addition to monitoring the birds for any clinical signs or mortality, the birds were bled at 96 hours post-challenge, serum collected and saved at -20 C for further analysis. After bleeding, the birds were euthanised and necropsied. The bursal tissue was collected from each group for histology. The bursae were fixed in 10% formalin, embedded in paraffin, sectioned at 6µm using Cambridge microtome (Cambridge Instruments, Inc. Buffalo, NY) and stained for microscopic examination using hematoxylin and eosin staining technique (McManus and Robert, 1964).

Data analysis: The ELISA data are presented as S/P ratio of the samples where S represents the absorbance value of the test serum divided by the absorbance value of the positive control (P) serum.

Results and Discussion

The important question addressed in this study was to determine the role of maternal anti-IBDV antibodies in protecting the progeny chicks from IBDV infection at earlier ages. For this purpose, a group of broiler breeder hens was selected with a known history of hyper immunization against IBD vaccine as stated in the methods section. The first series of experiments were conducted to determine the levels of anti-IBDV antibodies in the progeny chicks from this breeder flock. As shown in Fig. 1, the levels of passively transferred anti-IBDV antibodies in chicks at day of hatch were fairly high (S/P ratio of 0.56). This level was maintained up to 7 days of age and then declined subsequently. By day 28 of age, the antibody levels in the experimental chicks were almost equal to the negative control chicks and by day 35, the antibody levels had completely declined. The line of best fit to this decay rate of the maternal antibodies to IBD was calculated to be $Y=0.643-0.053$ (week) with a coefficient of determination (r^2) = 0.904. These data clearly show that the amount of maternal antibody against IBDV is fairly significant in the progeny chicks provided the broiler breeder hens are hyper immunized. By implication, these higher levels of antibodies will provide significant protection to the chicks at earlier age when they are more likely to be affected by the IBDV. These data further suggest that the levels of passively transferred, maternally-derived anti-IBDV antibodies must be considered or tested while implementing the IBDV vaccine regimen in the progeny chicks. The assumptions being that if the levels of maternally-derived antibodies are high enough within the first few days of hatch, any active vaccine may not be as effective in that flock of chicks and in fact be neutralized by the circulating maternally-derived antibodies and rendered ineffective.

It has been reported that the maternal antibody titers in

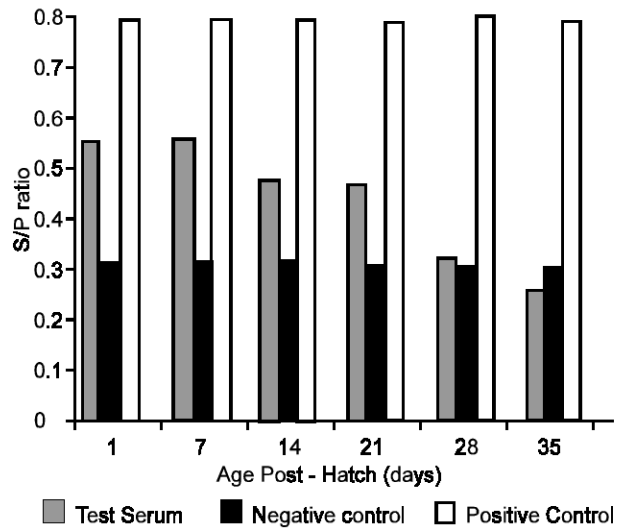


Fig. 1: The levels of maternal anti-Infectious Bursal Disease antibodies in progeny chicks at various ages post hatch. S/P ratio indicates the amount of antibodies as determined by ELISA in experimental chicks relative to the levels in positive control sample.

progeny chicks are demonstrable up to 4th week of age. However, the protective limit of these antibody levels expires by the second week. While Kenzevic *et al.* (1987) reported that the progeny antibodies persisted up to 6 weeks of age, Rosales *et al.* (1989) reported an IBDV infection at 15th day of age in the presence of maternal antibodies. The observed difference in the results of our study and the above mentioned two studies could be due to the difference in the initial titer of IBD in chicks which is a direct reflection of the immune status against IBD in the parental flock.

The second series of experiments were conducted to determine the protective efficacy of the maternally-derived IBDV antibodies to the experimental IBDV challenge. The assumptions for these experiments were that the presence of IBDV antibodies in the progeny chicks would protect chicks from IBDV challenge-induced disease. This was shown to be true. As shown in Table 1, mortality after the experimental challenge varied from 0 - 20% between day 14 and 35. However, no mortality was observed when the chicks were challenged at 1, 7 and 14 days of age. Again, the implications of these findings suggest a clear protective effect of maternal antibodies of IBDV on progeny chick challenge. From day 21, the maternal antibody levels degrade whereas the seroconversion against the challenge virus occurs as shown in Fig. 2. It is however clear from the mortality data that the seroconversion levels were not 100% protective since the mortality was still observed from 10 to 20% despite the increase in challenged-induced antibody titers. This protective level of maternally-derived antibodies observed in our study

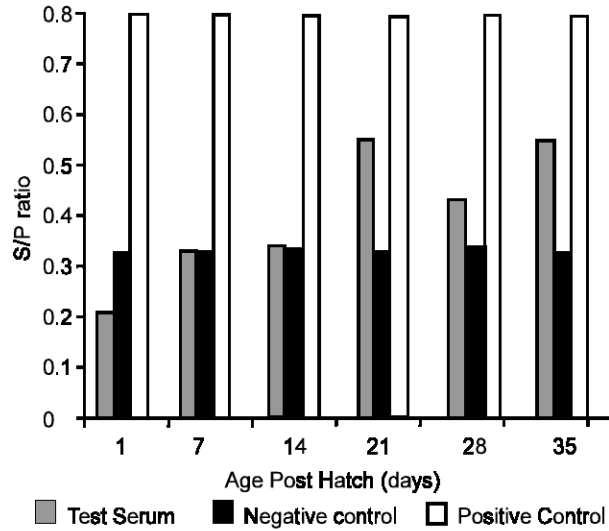


Fig. 2: Protective effects of maternally-derived IBDV antibodies against experimental IBDV challenge. As shown, the ELISA titers decline in test samples upon challenge, but increase after maternal antibodies degrade around day 14 of age.

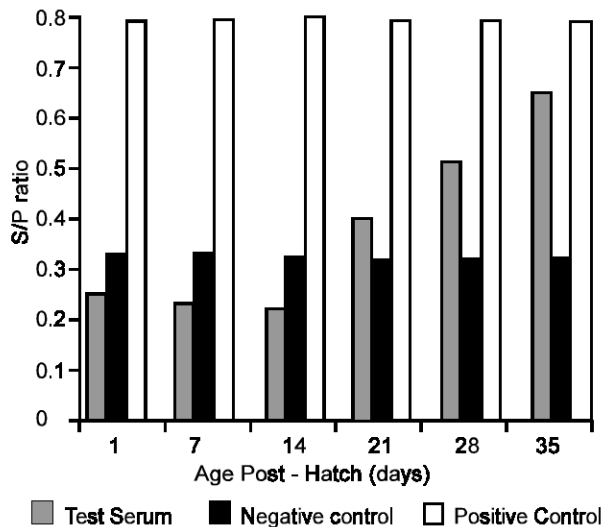


Fig. 3: Seroconversion of progeny chicks after vaccination with a commercial IBDV vaccine. The vaccination regimen is provided in the material and methods section.

were similar to those reported by Chettle and Wyeth (1989) and Van Den Berg and Meulemans (1991). They reported that the broilers were susceptible to IBDV challenge at the age of 38 days with maximum mortality rates between 20-25%. The age at which the broilers were most susceptible to IBDV infection in our study was 35th day of age. This is probably because of the low level

Table 1: Maternally-derived infectious bursal disease virus antibodies protect progeny chicks from the IBDV challenge post-hatch

Age at challenge	Chicks Died/Challenged #	Mortality (%)
1	0/10	0
7	0/10	0
14	0/10	0
21	1/10	10
28	1/10	10

Experimental chicks were obtained from a breeder flock which was hyper immunized against IBDV. The progeny chicks were challenged with the virulent IBDV strain at the indicated age.

Table 2: Mortality (three days post-challenge) in progeny chicks vaccinated against IBDV at various ages and challenged with virulent IBDV

Age at IBDV vaccination (days)	Age at IBDV challenge (days)	Chicks died/total #	Mortality (%)
1	16	1/10	10
7	22	2/10	20
14	29	0/10	0
21	36	0/10	0
28	43	0/10	0
35	50	0/10	0

of maternally derived antibodies at this age. Indeed our experiment showed clearly that even after intensive live vaccination and inactivated booster of parent hens, it was not possible to protect the progeny during the whole growing period. These studies therefore suggest that there is a window of susceptibility in the progeny chicks when the maternally-derived antibody levels decline and the titers against the challenge virus have not yet induced to be protective. This seems to be the window between day 21 to day 35 of age. These studies therefore suggest that perhaps progeny vaccination at day 14 of age may be appropriate. This possibility was tested in subsequent experiments where the progeny chicks with the maternally-derived antibodies were vaccinated against IBDV and challenged at various ages post-vaccine.

The third series of experiments were conducted to determine the level of protection mediated via a live commercially available IBDV vaccine. Chicks were vaccinated at weekly intervals and challenged on the 15th day post vaccination with the virulent IBDV and the antibody titers monitored. The mean weekly ELISA antibody titers of this group of chicks are shown in Fig. 3. The titers were comparable between the experimental and the control chick samples from 1 to 14 days of age suggesting that the vaccine response was poor and presumably neutralized by the circulating maternally-derived IBDV antibodies. However, by day 21 an increase in antibody titers was clearly evident in the vaccinated chicks which lasted up to day 35 of age as

well. The weekly mortality at 96hrs after experimental IBDV challenge of birds vaccinated at various age intervals is shown in Table 2. All birds vaccinated at 14, 21, 28 and 35 days of age were protected. The mortality varied from 10% in birds vaccinated on day 1 up to 20% by day 7. The results of resistance against IBDV experimental challenge after vaccinations at various ages confirmed that the maternally derived antibodies interfered with the IBD vaccination as previously reported by others (Solano *et al.*, 1985, 1986; Wyeth, 1986; Mousa *et al.*, 1988). Our study indicated that the birds vaccinated with live IBDV intermediate vaccine, Bursine II, at 7th day of age were not protected. On the other hand all the birds vaccinated at 14th day till 35th day of age with the same vaccine were protected. The reason for that is probably neutralization of the live vaccine by the maternally derived antibody till 7th day of age. The level of maternally-derived antibodies decreased with the increase in age, which was insufficient to neutralize the vaccine. The mortality at the 1st day (10%) and 7th day (20%) of age suggested that a too early vaccination with this vaccine reduced the protective effect of maternal antibodies significantly.

In the presence of maternal antibodies to IBDV, live virus vaccine reduced the severity of the bursal lesions when administered at 14, 21, 28 and 35th days of age. Bursal lesions were observed in birds vaccinated and challenged with IBDV at 1 and 7 days of age. Multiple hemorrhages were observed at thigh and breast muscles along with edematous bursa, pale and swollen kidneys, enlargement of the spleen and dehydration of the carcass. Histopathologically, the bursal epithelium appeared hyperplastic, folded and thickened. The undifferentiated epithelial cell layer between the cortex and medulla was also visible.

In conclusion, the present study indicates that the maternal antibodies against IBDV do carry over to the progeny. These antibodies lasted at variable levels till 4 – 5 weeks under our experimental conditions. It is also clear that the birds are most susceptible to IBDV around 30 to 35 days of age, especially in the absence of the maternally-derived antibodies. Since maternally-derived antibodies can potentially neutralize the vaccine if done at a very younger age, the findings of our study suggest that the first vaccination may be most effective if done at 14th day via the drinking water. This would allow the maternal levels to degrade such that the vaccine would induce an effective protective immune response. A booster will then be required around day 28 of age for carrying the flock through adult period of production.

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