THE PROTECTIVE EFFICACY OF MATERNALLY DERIVED ANTIBODIES AGAINST INFECTIOUS BURSAL DISEASE VIRUS IN BROILER CHICKS

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المستخلص

اجريت هذه الدراسةلتقييم القدرة المناعية للأجسام المضادة المنقولة من الأم ضد مرض القمبورو ومتابعة زمن فقدان فعاليتها.لهذا الغرض تم استخدام 25 كتكوت غير ملقحة ضد مرض القمبورو ولكنها انتجت من امهات تم تلقيحها بواسطة لقاح يحتوى على فيروسات مضَعفة(D78 intermediate strain)
تم استخدام طريقةELISA لقياس مستوى الأجسام المضادة
فى كتاكيت اللاحم غير الملقحة فى فترات مختلفة . اوضحت النتائج فقدان فعالية الأجسام المضادة فى الأسبوع الرابع بالرغم من وجود الأجسام المضادة حتى الأسبوع السادس. على ضوء هذه النتائج يمكن قياس وتقييم فعالية الأجسام المضادة الفعالة خلال نمو الكتاكيت بين عمر 14-20 يوم مع احتمال تمديده لمدة اسبوع آخر قبل اعطاء اللقاح.

Key words: Infectious Bursal Disease Virus (IBDV), Maternal antibodies (MDA), ELISA, Protection
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ABSTRACT

This study was conducted to assess the protective potentials and decaying pattern of the maternally derived antibodies (MDAs) in broiler chicks against infectious bursal disease virus (IBDV). For this purpose, twenty five unvaccinated chicks were used. These chicks were hatched from chickens vaccinated by the live IBDV vaccine containing the chick-embryo propagated, D78 intermediate strain of the virus. The enzyme-linked immunosorbent assay (ELISA) was used to determine the antibody (Ab) levels in the chicks sera at various points of time. The study revealed that the MDAs against IBDV in chicks persisted up to the sixth week of chicks age. However, the protective level of these antibodies expired by the fourth week.. Based on these data, we suggest that, for appropriate vaccination day, the level of MDAs must be evaluated while the chicks are growing at 14 -20 days of age and probably one week later.

INTRODUCTION

Infectious bursal disease (lBD), also known as Gumboro disease, is an acute highly contagious viral infection, particularly important in young chicks (Parkhurst, 1964; Lukert and Saif, 1991). The causative virus of IBD is the infectious bursal disease virus (lBDV), member of the Birnaviridae family and genus avibirna virus (Murphy et al., 1999) and posses two serotypes 1 and 2 (McFerran et al., 1980; Jackwood et al., 1982). Very virulent strains of IBDV were responsible for outbreaks of IBD which lead to high mortality rates and thus resulted in huge economic losses in different parts of the world (Chettle et al., 1989; Hair-Bejo, 1992; Nakamura et al., 1994; Farooq et al., 2003). The diseas raised the attention and concerns of poultry industry specialists due to the reduced productive and reproductive potentials among infected chicks (Shane et al., 1994). The causative virus was also found to have an important immunosuppressive activity (Faragher et al., 1974; Sharma et al., 2000; Ali et al.,2004). Rational vaccination schedules and strict biosecurity measures were indicated in many reports as essential tools for

the control of IBD (Giambrone and Clay, 1986; Wyeth and Chettle, 1990; Whitfill et al., 1995; Haddad et al., 1997; Farooq et al., 2003).
Many previous studies proved the role of the maternally-derived antibodies (MDAs) in protection against IBDV in chicks (Tsukamoto et al., 1995; AI-Natour et al., 2004). The MDAs are acquired by chicks through the passage of IgG from hen's serum to the embryo; remain protective for a certain period of time before starting to decay. The amount and duration of these MDAs were variable in progeny chicks. In practice, different vaccination schedules have been recommended and used but still outbreaks are reported. The MDAs are among many factors including the time of vaccination, type of the vaccine, routes of administration which determine the efficacy of IBD vaccination. They were proved to interfere with the live IBD vaccine virus (intermediate 078) replication though it was recently confirmed that they had no detectable effect on the vector recombinant vaccine taken (A turkey herpes virus, HVT-IBD) as observed by Bublot et al. (2007) and Le Gros et al. (2009). The objective of the present study was, therefore, to determine the decaying pattern and role of maternally derived antibodies in protection against IBD in broiler chicks obtained from chickens vaccinated with the intermediate strain of the virus.

MATERIALS AND METHODS

Experimental chicks: Twenty five, one day old unvaccinated, Hy-line broiler chicks were obtained from EI Gharia Company, Khartoum, Sudan. The broiler breeder parent flocks of these chicks were vaccinated against IBD using the live IBDV vaccine containing the chick-embryo propagated, D78 intermediate strain of the virus.

Housing of chicks: The chicks were reared in isolated rooms of an open system poultry houses at the Faculty of Animal Production, University of Khartoum. All chicks were fed, watered and kept under the same environmental conditions throughout the experiment. Before the start of the experiment, the rooms were thoroughly cleaned, washed, disinfected and left for 4 weeks before being used for the experiment.

Blood collection and serum preparation: Blood was collected from the wing vein of the chicks at different points of time post-hatching. Sterile

disposable syringes with 29 gauge and 1-millimeter length were used for blood collection. An amount of 0.5 ml was collected from each bird. The blood was left for 2 hours at room temperature, and the clot was then loosened from the surface of the syringe, kept overnight at 4°C. The serum was separated and clarified by centrifugation at 2000 revolutions per minute (rpm) for 10 minutes. The serum was stored in test tubes at -20°C till use.

Enzyme-Linked Immunosorbent Assay (ELISA): An indirect ELISA for IBD antibody test kit was obtained from BioCheck B.V. Crabethstaat 38-C 2801 AN (Gouda Holland). The antigen coated plates (coated with the inactivated viral antigen on microtitre plates) and the ELISA kit reagents were adjusted at room temperature prior to the test. The test serum was diluted; five hundred folds (1 :500) prior to the assay with sample diluents provided. 100 ul of diluted serum was then put into each well of the plate. This was followed by addition of 100 ul of undiluted negative control (specific pathogen free serum in phosphate buffer with protein stabilizers and sodium azide preservative (0.1 % w/v). 100 ul of positive control was also added (antibodies specific to IBD in phosphate buffer with protein stabilizers and sodium azide preservative (0.1 % w/v). The plate was then incubated for 30 minutes at room temperature. Each well was then washed 4 times with washing buffer containing 0.05% Tween 20 in powdered phosphate buffered saline (300 ul per well). A 100 ul of conjugate reagent (sheep anti-chicken alkaline phosphatase in Tris buffer with protein stabilizers, inert red dye and sodium azide preservative (0.15 w/v) was added into each well and the plate was incubated at room temperature for 30 minutes.
Each well was washed again 4 times with the washing buffer. 100ul of substrate reagent (p-Nitro phenyl phosphate dissolved in Oiethanolamine buffer with enzyme co-factors) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally 100 ul of stop solution (Sodium Hydroxide in Oiethanolamine buffer) was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 405 nm wavelength using ELISA microtitre Plate reader.

Experimental design: Twenty five, one day old broiler chicks were reared in separation. The chicks were given infectious bronchitis (lB)

vaccine and Newcastle (ND) vaccine (colon 30) as spray at day 3 of age and another dose of Newcastle vaccine (colon 30) spray at day 14 of age for protection from IB and ND but were not vaccinated against infectious bursal disease (IBD). Blood was collected from five chicks randomly selected at days 1, 18, 25, 32, 39 and 45 day old and sera was prepared as described above.

Data analysis: IBD antibody titre was calculated automatically, using soft ware in the computer attached to the spectrophotometer reader. The ELISA data are presented as SIP ratio. SIP ratio of the samples was calculated using the following formula:

$$S/P = \frac{\text{mean of test sample - mean of negative control}}{\text{mean of positive control -mean of negative control}}$$

Where (S) represented the absorbance value of the test serum divided by the absorbance value of the positive control (P) serum.

The following equation relates the S/P of a sample at a 1 :500 dilution to an end point titre

Log 10 Titre = 1.1 x Log (S/P) + 3.361 Antilog = Titre

S/P value	Titre range	Antibody status
0.145 or less	284 or less	negative
0.150 - 0.199	285 - 390	suspect
0.200 or greater	391 or greater	positive

RESULTS

The maternally derived antibody (MDA) against IBDV was observed to decline as the age of chick's progresses. High S/P ratio (5.4) of MDA was obtained at day one. This level was almost stable up to the 7th day with S/P ratio of 5.06 after which the MDA started to decline rapidly. By the 18th day the level reached 0.62.
It continued to decrease progressively reaching 0.04 when the chicks were 45 days old (Figure 1).

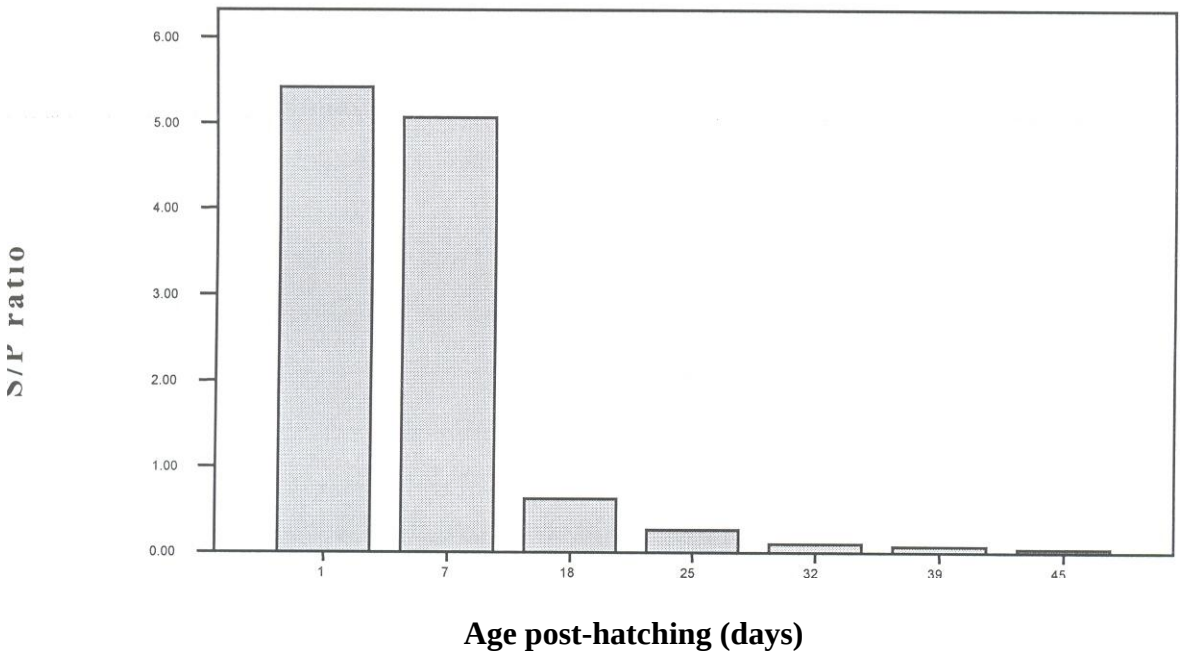

Figure 1: The levels of maternal anti-infectious bursal disease antibodies in progeny chicks at various ages post hatching

DISCUSSION

The results obtained in the present study showed that the level of maternally derived antibodies (MDAs) (i.e. passively transferred antibodies) against infectious bursal disease virus (IBDV) were high at day one of age when they hatched from vaccinated hens. This indicates that, the parent's flocks of these chicks were hyper-immunized when vaccinated with D78 intermediate vaccine of IBDV. The hens transmitted this high level of antibodies in the form of maternal derived antibodies (MDA) to the progeny chicks. Similar results were previously published by Sharma et al. (1989) and Kumar et al. (2000). These high levels of MDAs proved to protect the chicks from infection by IBDV in early ages, but will hinder vaccination against IBD as the vaccine will be neutralized by the circulating MDAs and rendered ineffective (Solano et aI., 1986).

Although the levels of MDAs in this study were observed to decline rapidly, after the first week, they were noted to remain detectable in chicks up to 45 days of age with appreciable magnitude (SP of 0.04). Their protective level expired by the 4th week. While the findings of this study appear to be almost in agreement with those of Kenvic et al. (1987), who reported that the progeny antibodies started to diminish after the first week but persisted up to 6 weeks of age. These results are, however, in disagreement with those of Ahmed and Akhter (2003), who reported that the progeny antibodies persisted up to 4 weeks of age and that their protective limit expired by the second week. This variation in the results could be attributed to the difference in the initial titer of MDAs against ISDV in chicks, which is a direct reflection of the immune status against ISD in the parent flock. This also explains the individual variation of the chicks response to the vaccine

The rapid decline of the MDAs after the first week of age but their detection by the end of the study (45 days) is not surprising since these chicks were not raised on specific pathogen free environment and were perhaps still exposed to low levels of antigenic challenge from external sources. This pattern of MDAs decay was also observed by Ahmed and Akhter (2003). The reason for such a rapid decline at this period of age could be attributed to the proteolytic degradation of antibodies or neutralization because of naturally occurring IBDV challenge. It is clearly evident that the appropriate time for maternally derived antibodies level testing in chicks is the period between day10 and 14 since the rate of decrease in the level of MDA is affected by existence of the pathogen in the environment, metabolism and growth rate of the bird.

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