Determining Optimum Time for Administration of Live Intermediate Vaccine of Infectious Bursal Disease to Chickens at Mekelle Farm

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Abstract

The study was conducted from December 2013 to April 2014 to predict the proper age for vaccination of chickens against infectious bursal disease (IBD) and calculate the maternal antibody transfer rate. A total of 99 chickens: 80-day-old chickens and 19 parent chickens' were randomly selected from Mekelle Farms. Blood samples were collected from heart and wing vein of individual chicken and serum was harvested. Indirect Enzyme Linked Immunosorbent Assay (IELISA) was employed to measure the Maternally Derived Antibodies (MDAs) from chickens of day 2, 18, 21 and antibody titers from parents. Samples from 2 day old chicken were having MDA titer in the range of 805.095-2821.239 with a mean titer of 1677.5. Antibody titers from 19 parents were also measured and the antibody titer showed that it falls within the range of 1825.94-4856.87 with a mean titer of 2964.602 and maternal antibody titer transfer rate from parents to 2 day old chickens was 56.57%. Other MDA titers were measured from 38 chickens of 21 days age of which 19 were vaccinated at 18th day of age and 19 them were non-vaccinated. Titers from vaccinated and non-vaccinated chickens were also compared using paired independent T- test and there was a significant difference with p-value of p=0.00. The present study showed that the proper time for administration of live intermediate IBD vaccine is 18 days instead of 21 days with the management conditions in place at the farm. Therefore, IBD vaccine should not be given to chickens whose parents had taken IBD vaccine without determining MDA titer and age for vaccination.

Keywords: Infectious bursal disease; ELISA; MDA; Vaccine

Introduction

Poultry industry comprises one of the most rapidly growing food producing sectors in the world and keeps expanding with an increase in population. The production and consumption of eggs and poultry meat has been increasing worldwide over the last three decades as the consumption of eggs has doubled and that of chicken meat has tripled [1].

Poultry production in its general term includes the production of domesticated birds such as chickens, turkeys, ducks, geese and others, which are mainly kept for production of egg and meat. Among these, chickens are the most important species, adapted globally to different ecological conditions where human beings live and contribute a significant role in supplying animal origin protein to improve human nutrition [2].

Chicken production has an important economic, social and cultural benefit and plays a significant role in family nutrition in the developing countries. The proportional contribution of poultry to the total animal protein production of the world by the year 2020 is believed to increase to 40%, the major increase being in the developing world [3]. It has been estimated that 80% of the poultry population in Africa is found in traditional scavenging systems [4]. In most tropical countries it is based mainly on scavenging production system, which makes substantial contributions to household food security throughout the developing world [5].

Small and large scale chicken farms are rapidly growing in Ethiopia. The chicken strains imported are temperate breeds that are less adapted to the heat stress and disease challenges in the country. Accompanying intensification of poultry farming, there is occurrence of epidemics of newly introduced diseases and/or epidemics of endemic diseases. One of the diseases that is of growing concern in poultry is Infectious Bursal disease (Gumboro disease) [6].

Infectious Bursal Disease (IBD) or Gumboro disease is caused by a small, non-enveloped double stranded RNA virus, belonging to the *Birnaviridae* family. Although it was first recognized more than 50 years ago, in 1962 in Gumboro, Delaware, USA, the disease still causes significant economic losses in the poultry industry worldwide. It is highly contagious viral infection of the young chick (3 to 6 weeks age) which targets and destroys the bursa of Fabricius, the organ where B lymphocytes are produced in chickens. It causes 30 to 40% mortality and 100% morbidity in affected flocks. Its indirect effect is associated with immunosuppression causing increased susceptibility to other diseases and decreased response to subsequent vaccination, and culminates into poor performance and/or death of birds [7].

The characteristic gross lesions of the disease include dehydration of the muscles with ecchymotic hemorrhages, enlargement and orange discoloration of kidneys. The bursa of fabricius shows the main diagnostic lesions in birds that die at the peak of the disease. It becomes enlarged and shows pale yellow discoloration. Intra-follicular hemorrhages may be found and Pin point hemorrhages on the skeletal muscles are usually prominent. Clinical signs include ruffled feathers, chicks' reluctance to move, anorexia, watery diarrhea, trembling and severe prostration [8].

In addition to the direct economic losses of the clinical disease,

the damage caused to the immune system (bursa of fabricius) causes severe immunosuppression by destroying B-lymphocyte precursors found within the bursa of Fabricius that impairs the chickens' ability to develop antibodies hence, lowered resistance to other infectious agents and a poor response to commonly used vaccines. Immunosuppressed flocks have poor performance that results in reduced economic return [9].

The main means to control the disease is by restricted biosecurity and vaccination with a suitable vaccine and at a proper age. Live vaccines are administered to achieve active immunity but interference of maternally derived antibody (MDA) is the crucial problem in determining a successful live IBDV vaccination schedule. Vaccinating chickens in the presence of high levels of MDA results in vaccine virus neutralization and no immunity [10].

In order to have chickens protected from IBDV field challenge, it is crucial to determine the optimal timing for IBD vaccine delivery [11]. The optimal timing is often predicted based on serological data following detection of IBDV MDA by an ELISA system during the first week post hatch [12]. The "Deventer formula" was developed to estimate the optimal vaccination time based on the half-life time of the MDA, the age of the chicken at sampling, genetic background, breakthrough titer of the vaccine, and the requested percentage of the flock having antibody levels below the breakthrough titer of the vaccine at the time of administration [13].

The present research was planned on the background as 22000 chickens were died at Mekelle Farms due to IBD after they were vaccinated at 21st day of age. This led to the assumption that the maternal antibody decrease significantly before 21 days age enhancing the susceptibility of the chicks to the disease. Hence, the objective of this research is to evaluate the levels of maternally derived antibody in day old chickens and to find the proper time for vaccination against IBD.

Materials and Methods

Study area

The study was conducted in Mekelle poultry farm located 783 km north of the capital city, Addis Ababa, Ethiopia. It is located at latitude of 30° 29' N and longitude of 39°28' E with elevation of 2084 meters above sea level [14].

Animals and sampling procedure

A simple random sampling procedure was adopted to get sampled birds. Mekelle poultry farm was selected to run the study. A total of 99 apparently healthy day old and parent chickens were randomly selected and blood samples were taken from each chicken at day 2, 18, 21 and at 38 weeks of parent's age.

Study design and sample processing

An experimental study was conducted on 99 chickens to determine the antibody titer of each chicken. Chickens were categorized randomly as group A, B, C and D with 23, 19, 19, and 19 chickens in each group respectively. Sample from chickens' of group A was taken at 2 day's old of their age to predict the optimal time for IBD vaccination. Sample from group B was taken at 18th day of their age to check the predicted titer from 2 day's old chickens. Chickens in-group C were vaccinated at their 18th day of age with live intermediate IBD vaccine and samples were taken at 21 days age to check their response for the vaccine. Chickens in group D were control groups and their samples were taken

at 21 days age to check the decrease of MDA below the protective level. Another 19 samples were taken from parent chickens at 38 weeks age to determine the maternally derived antibody titer transfer rate to their offspring. The serum samples were tested at National Veterinary Institute (NVI), Debre Zeit, Ethiopia, using Indirect Enzyme-Linked Immunosorbant Assay (IELISA).

Blood sample collection

Two milliliters of blood was collected from heart of 23 apparently healthy two day old chickens using 5ml sterile disposable syringe of 22 gauge and 11/4 needle size to estimate the vaccination age for IBD vaccination. The same size syringe was used to collect blood from the brachial (wing) vein of 19 chickens at the age of 18th day to check whether the maternal antibody had significantly reduced to which the titer cannot protect the chickens from IBD or not after the vaccination prediction time was calculated using Deventer formula from the maternal antibody titer of 2 day's old chicks. Samples were also taken from 38 chickens of 21 day aged, of which 19 were vaccinated with live intermediate IBD vaccine for IBD at their 18th day by eye drop and the other 19 samples were collected from non-vaccinated chickens to check the non-protective titer. Another blood sample from 19 parents was collected to determine by how much their antibody was transferred to their off springs via the egg [13].

These collected blood samples were transferred to plain vacutainer tubes at their respective time. The vacutainer tubes were placed at 45° and allowed to clot for 24 hours for serum separation. Separated serum was transferred to labeled sterile cryovial tubes and kept at deep freeze (-20°C) for 8 days until shipped to NVI and tested there. The cryovials containing serum samples were transported with ice box [13].

Vaccination of chickens with IBD vaccine

Chickens in group C were vaccinated with live intermediate IBD vaccine with breakthrough titer of 125 by eye drop to determine the chicks' response for the vaccine they take, whereas chickens in group D were left non vaccinated to check the decrease of maternal antibody titer below the protective level.

Determination of vaccination age

The optimal vaccination time for IBD was calculated by the Deventer Formula [13] as follows:

Vaccination age=$\{($log$_2$ IBDV antibody titer of the bird (%) - log$_2$ ELISA breakthrough titer of the vaccine) x t ½$\}$+age at sampling+correcting Value 0-4 in which

Bird (%): titer of the bird (at sampling) that represents certain Percentage of the flock (in this study: 75%) was used. The highest 25% was rejected and the highest titer from the remaining was used.

Breakthrough: breakthrough (ELISA) titer of the vaccine to be used (live intermediate IBD vaccine with breakthrough titer of 125 was used).

T1/2: half life time of the antibodies (ELISA titer) in the type of chickens that were sampled (dual purpose breeds: 3.5 days)

Age at sampling: age of the birds at sampling. It was 2.

Correcting value 0-4: extra days when the sampling was done at 0-4 days post hatch [2]

Results

Maternally derived antibody titers from chickens at day 18 were

reduced to a mean titer of 201.6489 which was just under two times than the expected titer (125) and titers from 21 days age were reduced to mean titer of 93.65334 which was lower than the expected titer. Titers from chickens of 21 days age which were vaccinated with intermediate IBD vaccine at 18 days of age show increased level with mean titer of 790.7799. Titers from parents were higher than the 2 day's old chickens and the antibody titer transfer rate was 56.57%. The antibody titers of chickens sampled at days 2, 18, 21 and parents is summarized in the Table 1.

Discussion

Infectious bursal disease is an acute highly infectious viral disease of young chickens preferably 3 to 6 weeks of age. The virus affects the bursa of fabricus which plays a crucial role in programming and maturing immature lymphocytes so as to produce antibodies. The main way to prevent this disease is through vaccination at a proper age. The MDA titers of 2 day's old chickens fall in the range of 805.095-2821.238. From these titers, the proper age for IBD vaccination was calculated and found to be 18 days post hatch. This result was different from Block et al. [15] who predicted the vaccination age to be 21 days. This is due to differences in administration route of the vaccine and vaccination frequency in parents, where he used the vaccine with drinking water which resulted in a high level of MDAs in their offspring's that protects them from IBD for 21 days post hatch whereas in the current study the 2 day's old chicken were vaccinated by eye drop. This was in agreement with Babiker and Tawfeeg [16] who reported chickens vaccinated with live intermediate IBD vaccine with drinking water were having higher level of maternally derived antibody titer in their off springs whereas chickens vaccinated with the same vaccine by subcutaneous route, eye drop and spray had less maternally derived antibody titers than the former one. With regard to the frequency of vaccination parent chickens used by Block et al. [15] were vaccinated twice whereas parent

chickens in this study were vaccinated only once which yields less MDA titers.

In contrary to the present finding Suzuki et al. [17] reported 15th days of chickens' age as optimal vaccination time for gumboro that was 3 days earlier than the present study. This difference might be due to variation in type of vaccine used as he has used live intermediate plus IBD vaccine whereas in the current study was used intermediate IBD vaccine only. This was supported by De Wit [13] who clearly stated that chickens vaccinated using live intermediate IBD vaccine has a capacity to breakthrough maternally derived antibody titer of 125 whereas chickens vaccinated using intermediate plus IBD has the capacity to break through maternal antibody titer of 500.

Previously, live intermediate IBD vaccine was given at 21 days of age of chickens in the farm that leads them to remain without protective immunity for 4 days (18-21 days of age). Antibody titers of the parents were in the range of 1825.938-4856.867 with a mean titer of 2964.602. These titers were slightly lower than Bart and Arts [18]. This arises from differences in vaccination frequency as mentioned earlier. Vaccination was done on broiler parent chickens twice at the predicted date and at pre layer stage in Bart and Arts [18] that yielded high levels of maternally derived antibody titer in the off springs whereas parent chickens which were used in this study were vaccinated only once. In this study, 56.57% of parents' titer was passed to offspring's which was almost similar with the reports of Bart and Arts [18] 57.7% in the same age of parents (38weeks).

There was significant difference (p=0.00) in titers taken from non-vaccinated chickens at day 2,18 and 21 when compared by one way analysis of variance (ANOVA) and this shows that the MDA titers were reduced from day to day and reach a level (93.65334) to which it cannot protect the chickens . This finding agrees with the reports of Alam et al. [19] in that the maternally derived antibody had declined to unproductive level at the interval of 15-20 days of chickens' age with a mean titer of 390 in intermediate plus IBD vaccine vaccinated chickens. It also agrees with Azab et al. [20] who reported the maternally derived antibody titer declined to unproductive level at 18th day of chickens' age.

Titers from vaccinated and non-vaccinated chickens of 21 days age was also compared by paired independent t-test and there was a significant difference with p-value of 0.00. This indicates that there was a rise in titer in vaccinated chickens as it was administered at the appropriate age but declined in non-vaccinated chickens.

Conclusion and Recommendations

The present study showed that the proper time for administration of the vaccine is 18 days post hatch with the management conditions in place at the farm. This shows that there is difference with the results of the present work and the vaccination practice in place at the farm which is 21 days. In light of the present finding, the following points were recommended.

- IBD vaccine should not be given to chickens whose parents had taken IBD vaccine without determining MDA titer and age for vaccination.

- Chickens should be boosted with IBD vaccine at pre layer stage to pass more antibodies to their offspring's which help the offspring's protect themselves from IBD for longer period of time until vaccinated.

Ser No	Day 2(A)	Day 18(B)	day18 vacc. and day 21 sampled (C)	Day21(D)	Parents
1	805.095	523.472	941.72	107.695	1825.94
2	1009.09	184.462	1156.81	97.455	3030.15
3	1099.56	190.214	684.581	102.62	3168.31
4	1264.77	125.142	990.215	114.462	2317.75
5	1341.23	108.284	708.284	85.027	2022.19
6	1414.35	101.445	892.729	101.284	2022.19
7	1484.58	127.691	918.724	85.685	3030.15
8	1484.58	218.727	484.581	68.535	3168.31
9	1484.58	296.783	1021.49	107.695	2022.19
10	1484.58	225.142	778.692	85.0955	3168.31
11	1552.27	301.442	846.585	77.453	4856.87
12	1552.27	190.212	679.752	65.462	3674.9
13	1681.06	125.231	673.095	123.432	2946.56
14	1742.59	405.652	508.284	81.011	4657.56
15	1742.59	125.142	392.729	93.861	3452.63
16	1860.75	103.732	664.342	99.496	2874.5
17	1860.75	100.453	900.564	84.342	2668.45
18	1860.75	275.243	990.857	107.851	2856.26
19	1860.75	102.861	567.956	90.952	2564.24
20	2132.96				
21	2518.77				
22	2518.77				
23	2821.24				
Mean titer	1677.3	201.6489	790.7799	93.65334	2964.602

Table 1: Antibody titers from chickens of days 2, 18, 21 and parents.

Acknowledgments

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