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Evaluating the Level of Maternally Derived Antibody Level, Post Vaccinal Antibody Titer and Determining the Optimum Date of Vaccination Againstinfectious Bursal Disease/Gumboro for Commercial Poultry Farms

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Abstract: The study was carried out between July to November 2015 to determine flock immunity andlevel of maternally derived antibody titer (MDA) against Infectious bursal disease virus (IBDV). For this purpose 400 sera samples were collected from 3 multiplication centers (6-32weeks age), 1 large scale (58 weeks age) and 2 small scale (16-24 weeks age) poultry farms. Moreover, 165 day old chickens of Sasso (n=65), Ross (n=50) and Lohman brown (n=50) breeds were purchased from two commercial hatchery farms of which Sasso (n=40), Ross (n=25) and Lohman brown (n=25) were scarified to collect blood samples and determine the level of MDA in the sera of chickens and estimate the optimum date of vaccination. The reaming 76 chickens were grouped according to their breeds into three each having five controls to assess post vaccination antibody titer. IBDV IDxx ELISA was used to analyze the level of antibody titer. The level of flock immunity were accounted 83.7%(n=92), 78%(n=78), 100%(n=48), 72%(n=24) and 87%(n=20) for FMC_1, FMC_2, FMC_3, FLC_1, FSC_1 and FSC_2 respectively.There was significant difference (P=0.000) in the mean antibody titer among farms (flocks). MDA level against IBDV accounted that 3469.3±1327.3, 3957.8±1535.4 and 3269.4±1018.2 for Ross, Lohmann brown and Sasso breed respectively. There was no significance difference on the mean MDA level among the flocks in the three farms (P=0.169). Based on the MDA level the estimated optimum date of vaccination against IBD in case of FMC_1 was found 13 and 22 date for broilers and layer breeds respectively whereas 14^{th} date was found for FMC_2. The aforementioned breeds also vaccinated for IBD according the instruction of manufacture, National Veterinary Institute (NVI), Ethiopia and bleed at weekly interval after the date of the last vaccination (21 days) for two times (28 and 35 days). The mean post-vaccination antibody titer for IBD at the first bleeding showed that 645±5089, 746.8±311.1 and 1188±502.1 which was expected to be increased during second bleeding; however, the titer was decreased to 99±97.6, 52±38.2 and 402.6 ±386.8 for Ross, Lohman and Sasso breed respectively with high variation. Considering the results of this study an effort should be made to improve vaccine quality and standardize the vaccination schedule for poultry farms.

Key words: Antibody Titer · IBD · MDA

INTRODUCTION

Ethiopia is a country with huge poultry and livestock resources in Africa because of its diverse agro-ecology. According to the latest Livestock Sample Survey[1], the total poultry population at country level was estimated to be about 50 million. However, a number of challenges and obstacles has been limiting the success and profitability of both backyard and semi-intensive production systems: including infectious diseases, low input of veterinary services, poor housing, poor biosecurity, predators and, the quality and cost of feed [2-8]. Infectious diseases, such as: Newcastle disease, Infectious Bursal Disease, Mycoplasmosis, Pasteurellosis and Salmonellosis, are the major constraints negatively affecting the poultry industry in the country [9].

Infectious bursal disease (IBD) also called Gumboro disease in chicken is caused by infectious bursal disease virus (IBDV). The disease was first reported in 1962, in Southern Delaware, USA [10] and in Ethiopia in 2002[11]. The causative agent of this disease is a dsRNA virus with a bisegmented genome enclosed within an icosahedral,

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non-enveloped capsid of 55-65 nm. It is a member of genus Avibirnavirus of family Birnaviridae [12].The virus has been found to naturally range in virulence from attenuated to very virulent (vvIBDV). The vvIBDV designate is usually reserved from those virulent IBDV that cause high mortality [13]. Very virulent (vv) IBDV strains emerged in Europe in the late 1980s, causing up to 60 % mortality [14,15]. This virus can overcome maternally derived antibodies (MDA) and can cause 80 to 100 percent mortality in susceptible chickens [16]. IBDV is very stable and resistant to many disinfectants and therefore vaccination is considered as the best way to control the disease [17]. Chickens infected with IBDV between 3 and 6 weeks of age develop clinical IBD which may result in death but those infected at less than 3 weeks of age usually have few or no clinical signs. The disease has also been observed in chickens older than 6 weeks, even in up to 20-week-old chickens [18].

A previous study in Ethiopia indicated that mortality rate of IBD range from 45-50%[11]. The overall prevalence of IBD antibody recorded in different part of country and poultry production system reached up to 93.3% [19]. Among infectious diseases, infectious bursal disease (IBD) has been a great concern for the poultry industry worldwide. This study was designed to determine the level of maternally derived antibody and estimate optimum date of vaccination and post-vaccination antibody titer against IBDV in chicken.

MATERIALS AND METHODS

A total of six poultry farms (three poultry multiplication centers, one large and two small scale poultry farms) from Addis Ababa, Bishoftu and Wolkite (Gubre) were investigated between July and November 2015. Farms were coded as FMC (Multiplication center), FSC (Small Scale) and FLC (Large scale) poultry farms. Four hundred (n=292 from multiplication center, n=48 from LSC and n= 60 from SSC farms) were collected and analyzed using indirect IBD ELISA kit (ID screen IBD indirect, ID.vet, France). Moreover, a total of 210 day old chickens of different breeds Ross (n=65), Lohman brown (n=65) and Sasso (n=80) were purchased from Alema and Gubre commercialized poultry farms respectively. This multiplication centers were a major sources of chickens for small scale and/or large scale poultry farms in the country. Of this 75 five day-old chickens (25 chickens from each breed) had been sacrificed and sera samples were collected to assess maternally derived antibody titer. The remaining 135 chickens were divided into three groups each containing 45 chickens based on their breeds to assess post-vaccination antibody titer.

Vaccine Preparation: National Veterinary Institute, Ethiopian produces live Gumboro vaccines using vaccinal strains (D78, LC75). The live vaccines are freeze dried with suitable stabilizer and it need to be reconstituted with proper diluents. Vaccines were reconstituted with saline water and administered through oral rout or eye drop. We were used eye drop method to administer the vaccine at the recommended 14 and 21 days.

Sample Collection: Two milliliter of blood (2ml/chicken) was aseptically collected at day three for determining the maternally derived antibody. In order to measure post-vaccination antibody titer,chickens were bled two times at weekly interval after first vaccination using 3ml syringe with 19G needles. Sera samples were stored at-20°C until it was analyzed.

Laboratory Analysis: An indirectEnzyme Linked Immune-Sorbent Assay (ELISA) diagnostic kit (ID screen IBD indirect, ID.vet, France) was used to detect antibodies directed against the infectious bursal disease (IBD). It is a quantitative test for detection of IBD specific antibodies in chicken sera. Micro-wells are coated with purified IBD antigen. Samples to be tested and controls are added to the wells. Anti- IBD antibodies, if present, form an antigen antibody complex. After washing, an anti-chicken horse radish peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies, forming an antigen-antibody complex. After elimination of the excess conjugate by washing, the substrate solution (TMB) is added. The resulting correlation depends on the quantity of specific antibodies present in the specimen to be tested. In the presence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution. In the absence of antibodies, no coloration appears. The test is valid if the mean OD value of the positive (ODpc) is greater than 0.250 and the ratio of the mean value of the positive and negative controls (OD_{PC} and OD_{NC}) is greater than 3. In order to interpret the result for each sample, calculate the S/P ratio and antibody titer as follows:

$$S/P = \frac{OD_{sample} - OD_{Nc}}{OD_{PC} - OD_{NC}}$$

If S/P=0.3=Tites=875=Negative and S/P>0.3=Titer>875=Positive

Antibody titer was calculated using the formula below
Log10 (titer) = 0.97*log10(S/P) +3.449
Titer= 10^log10 (titer)

Determination of Optimum Date of Vaccination: The Deventer formula is used to determine the optimum date of the vaccination. A minimum of 18 samples from high quality chicken per house is required to obtain a representative sample of the flock. Based on field experience the Deventer formula uses 75% as a default percentageof the flock can be successfully vaccinated (www.enfermdad-gumboro.com). The formula is as follows

Vaccination age = {(log2 titer bird% - log2 breakthrough) x t _} + age at sampling + correction
0-4
In which: Bird% = ELISA titer of the bird representing a certain percentage of the flock breakthrough = breakthrough (ELISA) titer of the vaccine to be used t _ = half-life time (ELISA) of the antibodies in the type of chickens being sampled
Age at sampling = age of the birds at sampling
Correction 0-4 = extra days when the sampling was done at 0 to 4 days of age.

Data Analysis: The mean antibody titer, Standard deviation and % Coefficient of variation were calculated using Microsoft office Excel. Statistical Package for social Sciences (SPSS) version 20.0 software was used to analyze the data of the study. ANOVA was performed to compare the meant antibody titer between farms and breeds. Student t-test was used to compare the mean antibody titer after the first and second vaccination. Percentage coefficient of variation (%CV) interpreted as %CV <30, 30-50, 51-80 and >90 Excellent, Good, Fair and Poor response to challenge or vaccine respectively [20].

RESULTS AND DISCUSSION

Flock Immunity Assessment: A total of 400 serum samples from 3multiplication centers, one large scale and two small scale poultry farms were collected randomly from the flock. The sero-positivity against IBD for each flock was indicated in table-1 below. There was significant difference (P=0.000) in the mean antibody titer among farms (flocks). This might be due to difference in vaccine types and source, sample size, age of parent stock and the overall management systems in the selected farms. The mean antibody titer was found to be enough to protect the flock against IBD (Table 1) which was in agreement with Daniela *et al*. [21] and Ostyina *et al*. [22] who were indicated for the flock to be fully protected, the antibody titer post vaccination should attain =1500 in the sera.

The highest %CV (91%) was found in FMC_1 and the lowest (52.1%) was recorded in FLC_1 which was a large scale poultry farm. Highest %CV indicates lack of uniformity in antibody titer in the flock. This higher variation might be attributed to poor vaccine quality and vaccine usage and the difference in the origin of the flock (Table 1).

Table 1: IBDV Antibody titer and %CV for each poultry farm

	Antibody titer						
Farm code	n	No. positive (%)	Min. titer	Max. titer	Mean titer	SD	%CV
FMC1	92	90(97.8)	22.9	23524.5	7596.14	6915.7	91%
FMC2	100	78(78)	3.29	7553.6	3063.6	1870.3	61.1%
FMC3	100	87(87)	29.35	9178.4	3124.5	1959.9	62.7%
FLC1	48	48(100)	3298.4	23325.9	9016.8	4777.1	52.1%
FSC1	35	24(72)	129.42	10006.8	4318.3	3061.8	70.9%
FSC2	25	20(87)	137	9479.6	4563.2	2580.8	56.6%

n = number sampled

Level of Maternally Derived Antibody: A total of 75 day old chicken (25 chickens per breed) were scarified in order to determine the MDA level in the sera samples. There was no significance difference on the mean MDA level among the flocks in the three farms (P=0.169). Since the %CV lays between 30 and 50 the uniformity of the MDA level was found good. The mean IBD antibody titer for Ross, Lohmann brown and Sasso were accounted 3469.3, 3957.8 and 3269.4 respectively. Therefore, at this titer chickens were protected at early stage from IBDV (Table 2). This finding was in agreement with findings of Hamal *et al.* [23]. The report by Ritu *et al.*[24] was also indicated the level of antibody transfer to day old chickens originated from for different breeds was not significantly different among the breeds which was in congruent with current findings. This might be due to the level of maternally derived antibodies in chickens were depend upon the level of the circulating antibodies in the dam. However, our finding was not in conformity with findings of some earlier workers[25] who have reported detailed variation in 4 native and crossbred chicken lines with respect to the amounts of inherited maternally derived antibodies in both yolk and day-old chicks.

Table 2: MDA level against IBD for different breeds of chickens originated from commercial poultry farms

Breed	Poultry type	No. of chicken	Min. titer	Max. titer	Mean titer ±SD	%CV
Ross	Broiler	25	1473.0	6710.7	3469.3±1327.4	38.3
Lohman Brown	Layer	25	847.0	6504.0	3957.8±1535.4	38.8
Sasso	Dual	25	508.3	4455.7	3269.4±1018.2	31.1

Note: Min=Minimum; Max= Maximum; SD=Standard deviation; CV=Coefficient of variation

Table 3: Post vaccination antibody titer for Infectious Bursal Disease virus (IBDV)

Breed	Vaccine type	Age at 1st vaccination (days)	Age at 2nd vaccination (days)	Age at 1st bleeding (days)	Age at 2nd bleeding (days)	Mean ±SD at first bleeding	Mean ±SD at 2nd bleeding	CV% after 1st vaccination	CV% after 2nd vaccination
Ross	IBD	14	21	28	35	645.1±508.9	99±97.6	78.9	72.6
Lohman brown	IBD	14	21	28	35	746.8±311.1	52.6±38.2	41.7	98.6
Sasso	IBD	14	21	28	35	1188±502.1	402.6±386.8	43.8	96.1

Table 4: The MDA level determined for chickens using ELISA techniques

No.	FMC1(Broiler)	FMC1(Layer)	FMC2(Dual)
1	1472.96	847.04	508.28
2	1481.5	1235.87	894.15
3	1805.22	2596.04	1874.24
4	2059.49	2747.08	2660.35
5	2132.8	2752.66	2693.8
6	2526	2777.8	2840.01
7	2828.08	3001.03	2919.28
8	2844.83	3001.03	2977.65
9	2856	3154.2	3017.23
10	3001	3234.86	3113.01
11	3009.39	3534.74	3125.49
12	3259.89	3750.83	3254.42
13	3321.02	4024.54	3347.9
14	3565.24	4101.85	3352.05
15	3665	4562.03	3660.99
16	3689.92	4606.04	3675.49
17	3844.9	4694.02	3936.08
18	4019	4754.48	3969.13
19	4090.81	4905.53	4004.24
20	4101.9	5264.72	4159.01
21	4250.82	6226.26	4262.1
22	4864.35	6231.71	4315.67
23	4891.8	6479.51	4332.15
24	6710.7	6504	4387.76
25	6710.7	7099.1	4455.69
Min	1473.0	847.0	508.3
Max	6710.7	6504.0	4455.7
Mean	3469.3	3957.8	3269.4
STDEV	1327.3	1535.4	1018.2
%CV	38.3	38.8	31.1

Note: Min=Minimum; Max=Maximum; STDEV=Standard Deviation; %CV= Percentage coefficient of variation

Optimum Date of Vaccination: Based on the level of MDA (Table 4) and using the Deventer formula the estimated date of vaccination for IBD was 13 and 23 days for broilers and layers respectively in FMC1 where as 14 days was found for FMC2. The estimated optimum date of vaccination in this finding was earlier for broilers in FMC1 and FMC2 than indicated by Winterfield*et al.* [26] who reported 18 days of age was appropriate time for vaccination. Jakka*et al.*[27]Found that the optimum date of vaccination for IBD should lie in between 17-21 days which was different from the current findings. Similarly, Naqi, *et al*. [28] also suggested that the chickens should be vaccinated at day 21, as the uniformity of MDA is poor (coefficient of the variation [CV] > 30%) and boosted at day 28.

Post Vaccination Antibody Titer: Out of 135 chickens, 35 chickens from each breed were vaccinated following the prescription made by the vaccine producer, the National Veterinary Institute (NVI), Ethiopia. Ten chickens (n=10) were remained as non-vaccinated for control. Out of 35 vaccinated chickens 25 of them were selected randomly from each breed to assess post vaccinal antibody titer. During the first bleeding (7 days after 1st vaccination), there were detecting antibody against IBDV for 52% (39/75) of samples, the remaining 48 %(36/75) samples were found negative for antibodies against IBD. Since the antibody titer maintained after post vaccination was below the protective level the chickens were at risk to acquire IBDV. The same numbers of chickens were also bleed for the second time (15 days after 2nd vaccination) to measure the antibody titer; however, only 8%(6/75) were positive, where as the remaining chickens were negative for IBDV antibody. The level of antibody that a vaccine can break through depends on the vaccine strain [26]. Mild vaccine strains are efficient only when chickens have no or very low levels of MDA, while the intermediate strains including D78 and the "hot" strains can break through higher levels of MDA titer [27].

Hagazi *et al*. [29] in his finding indicated that there was an increased level of antibody level post vaccination which was in contrary to the current finding. The decrease in antibody titer might be due to high level of maternally

derived antibodies which neutralizes the live vaccine and the poor quality of the vaccines. Besides, the estimated optimum date of vaccination recommended by the manufacturer might be not properly formulated. Moreover, the higher level of MDA was also incriminated as the cause for lack of provoking the active immune system of the chicken [28].

CONCLUSION

In general, maintaining good flock immunity with excellent matching with the filed strain is enables to minimize the risk for IBDV infection. Determining the MDA level in chickens is on the most important concept in formulating the optimum date of vaccination for chickens. Based on current findings the level of MDA was good in uniformity and enough to protect the chickens at the early age of infection; however, post vaccination antibody titer was too low to protect the chicken against IBDV. Therefore, to improve the quality of vaccines and developing vaccination schedule on the bases of the MDA level has paramount significance in order to contain IBDV outbreak.

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