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Research Article
Correlation Analysis of IGY Titer Between Chickens and Parent Broiler Breeder Immunized Against Infectious Bursal Disease Virus

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Abstract

Background and Objective: Infectious bursal disease (IBD) affects younger chickens and it is a threat for development commercial poultry farms worldwide. The aims of this study are to determine maternal antibody titer and its associate with the age of the parent breeder consequently to estimate the proper age for vaccination. **Materials and Methods:** Sera samples were collected from 75 parent breeders and 75 of their progeny (3 days old) at monthly interval for three consecutive months and analyzed using Indirect IDXX IBD ELISA kits. Questionnaire was also used to generate information regarding the application of vaccines against IBD. **Results:** The association between parent and chicks antibody titer were statistically significant ($p = 0.00$) for both farms and also positively correlated at $r = 0.87$ and $r = 0.58$ for Alema and ELFORA, respectively. However, age has no association ($p>0.05$) with the level of antibody titer in chicken and parent breeder in both farms. Optimum date of vaccination was calculated as15th and 19th day and 14th and 19th day post hatch for ELFORA and Alema farms, respectively. The percentage coefficient of variation (CV%) was greater than 30% which indicates poor uniformity in the antibody titer, therefore, booster vaccination was mandatory. Based on questionnaire survey, 75% of medium scale poultry farms use domestic vaccines whereas the reaming uses imported vaccines. However, 100% of large scale commercial poultry farms relay on imported vaccines from European countries. All farms vaccinated there chickens based on the recommendation of manufacturers i.e., none was based on MDA titer. About 70 and 80% of medium scale and commercial farms had IBD outbreak in the last 3 years, respectively, in which 58.3% thought it was due to vaccine failure. **Conclusion:** The level of antibody titer in the progeny was associated with antibody titer in the parent breeder. Therefore, proper vaccination to parent breeder and designing proper vaccination schedules based on the MDA should be encouraged.

Key words: CV (%), IBD, MDA, titer

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry production have a great significance in the development of Ethiopia, where they have an essential role in reducing poverty, providing food security and used as an immediate cash income for the rural communities[1] . Among the multiple health problems, infectious bursal disease (IBD), also known as Gumboro is one of the major health constraints that hamper poultry productivity in Ethiopia[2].

The IBD is caused by a virus of the genus *Avibirnavirus* of the family Birnaviridae. It causes an acute, highly contagious, immunosuppressive disease in chickens[3]. Different pathotypes of IBDV have been classified in increasing order of virulence as mild, intermediate, classical virulent and very virulent. Chickens are highly susceptible to IBDV with the age between 3 and 6 weeks[4]. It is an environmentally stable virus which is known as one of the most economically important diseases that affect chickens worldwide[5,6]. Depending on the virulence of the IBDV strain, the age at the time of infection, the presence of IBDV anti-bodies and the genetic background of the infected chicken, infection with IBDV may induce a temporary or permanent destruction of the bursac localis and other lymphoid tissues[7]. Infection of these lymphoid tissues causes immunosuppressions that predispose chickens to other secondary infections and also have poor performance, which results in reduced economic return[8].

In Ethiopia, diseases such as Newcastle disease, Fowl pox, Fowl typhoid, Infectious Bursal Disease, Mareks and Mycoplasma are the common infectious disease problems existing in poultry farms. The IBD is an emerging disease of chicken that has been speculated to be introduced concurrent with the increased number of commercial state and private poultry farms flourishing in the country. The first report of IBD in Ethiopia was in 2005 involving 20-45 days' old broiler and layer chickens from commercial farms[9]. Since then, frequent and widespread IBDV outbreaks have been reported to the Ministry of Agriculture of Ethiopia from commercial poultry farms located in different parts of Ethiopia. In recent years, there has been an unexpected increase in frequency of IBD outbreaks over time in commercial and backyard poultry production system in Ethiopia[10]. Protection from these disease relay on regular vaccination of chicken using imported vaccines or vaccines produced at the National Veterinary Institute (NVI), Ethiopia and strict biosecurity measures (Personal communications). However, in recent years, IBD is becoming a priority problem in the commercial and backyard poultry production system despite regular vaccination practices using imported vaccine[11].

In the global poultry industry, the control of IBDV is based mainly on the immunization of chickens with live, inactivated, or recombinant vaccines[12]. The parent birds develop a high level of anti-bodies after vaccination, which is transmitted to the progeny chicks in the form of maternal antibodies[13]. Even though live vaccine is administered to achieve active immunity, high levels of MDA results in the vaccine virus neutralization and immunity will not be obtained[14]. This improper age for vaccination is one of the reasons for the occurrence of IBD outbreaks in vaccinated flocks[12,15]. In order to protect chickens from IBD, it is crucial to determine the optimal timing for IBD vaccine delivery[16]. At present, IBD vaccines are widely used to prevent IBD outbreaks in commercial poultry farms of Ethiopia. Most of these vaccines are administered only based on the manufacturers' recommendation without taking into consideration the status of MDA titer in their farm situation. Therefore, the aim of this study is to determine the effect of MDV on the vaccination of commercial broiler and layer chickens.

MATERIALS AND METHODS

Study area: The study was conducted from December, 2017 to February, 2018 in two commercial poultry farms, namely ELFORA and Alema poultry farms located in Bishoftu (Fig. 1). They are the largest commercial farms for broiler and layer chickens in Ethiopia. Bishoftu is located 45 Km southeast of Addis Ababa. The area is situated at 9°N latitude and 40°E longitude at an altitude of 1850 above sea level with annual rainfall of 866 mm of which 84% is in the long rainy season (June to September)[17] and mean annual temperature of and rainfall 27°C and 746.6 mm[18], respectively.

Study population: The study was conducted populations include parent broiler breeds (Ross and Cobb) and their progeny at 3 days old chickens having a history of IBD vaccination. During the start of sampling the age of parent breeder were 35 and 40 weeks of age for ELFORA and Alema poultry farm, respectively.

Study design: In this study, 25 chickens from the breeder parent stock and 25 of their progeny at the age of 3 days were sampled for a consecutive of 3 months. A total of 150 samples, 75 each were sampled per farm.

Sample collection: Two milliliter of blood samples were collected from the parents using disposable 3 mL syringe with a 22-gauge needle from the wing vein. Moreover, blood

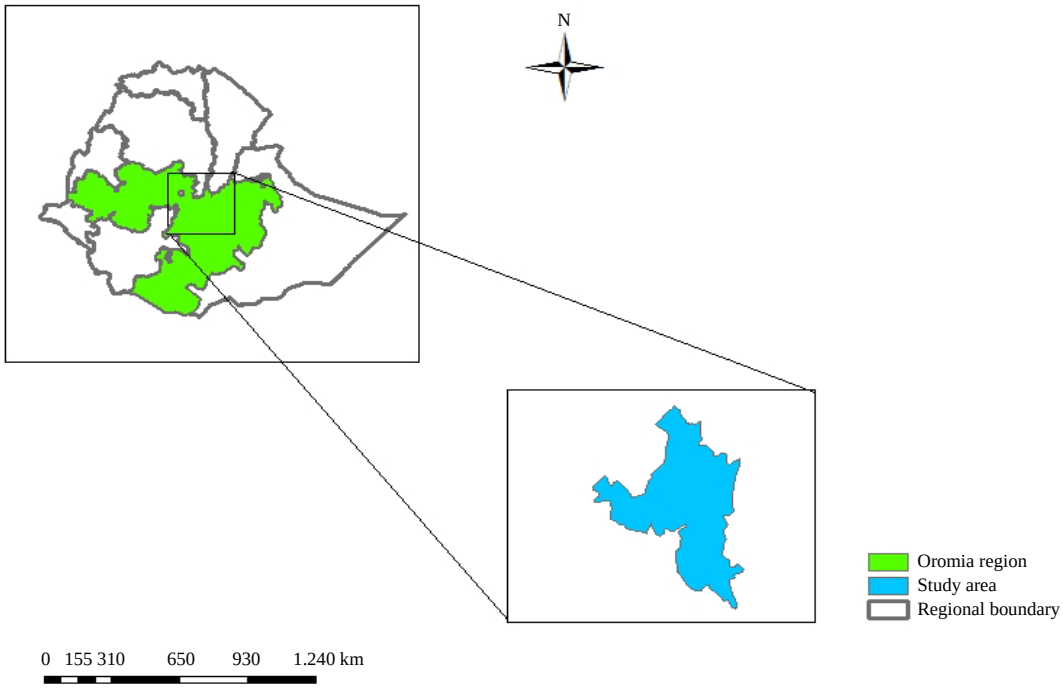

Fig. 1: Location of Bishoftu/Debre Zeit

collection was performed by incising the jugular vein of chicks and pouring the blood directly in to plain vacutainer tube. Blood samples was kept for overnight to facilitate blood clotting for separation of sera and sera samples were harvested into cryovials, labeled and transported using Ice box to NAHDIC. Samples were kept at -20°C until laboratory analysis was conducted.

Questionnaire survey: Five questionnaires were distributed to 5 commercial large-scale poultry farms and hatchery having an overall capacity of over 10,000 birds and 20 more questionnaires were also distributed to interview medium-scale private farm owners having the capacity of 1000-10,000 birds. The questionnaire was distributed to acquire information about IBD vaccine usage, vaccine source incidence of IBD outbreak in a vaccinated flock and about vaccination schedule implemented in the farms.

Laboratory analysis: The IBDV-Indirect-ELISA Kit (IDEXX Laboratories, Inc, Westbrook, Maine 04092, USA) was used according to the manufacturer's instructions. In details, the test samples were added into the coated well, upon incubation anti-body specific to IBDV forms a complex with the coated viral antigens. After washing, conjugate was added which binds to any attached chicken antibody in the wells. Unbound conjugate was washed away and substrate was added. Finally, a stop solution was added and read at the optical density (OD) of 650 nm. The relative level of anti-body in the sample was determined by calculating the sample to positive (S/P) ratio. Endpoint titers were calculated using the following equations:

$$S/P = \frac{\text{Sample mean-NC mean}}{\text{PC mean-NC mean}}$$

$$\text{Log}_{10}^{\text{Titer}} = 1.09\left(\text{Log}_{10}^{S/P}\right)+3.36$$

$$\text{Antibody titer} = 10^{1.09(\text{Log10S/P})+3.36}$$

The test was interpreted as negative if S/P$\leq$0.20 and positive if S/P>20. A positive result (titer >396) indicates vaccination or other exposure to IBDV.

Estimation of optimum date of vaccination: The Deventer formula was used to determine the optimum date of the vaccination. The formula uses an assumption 75% flock immunity level as a default percentage[19].

The equation is as follows:

$$\text{Vaccination age} = \{(\log 2 \text{ titer bird\%} - \log 2 \text{ breakthrough}) \times t\_\} + \text{age at sampling} + \text{correction (0-4)}$$

Where:

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 anti-bodies 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 0-4 days of age

The vaccination date first calculated using highest and lowest antibody titer and if the difference in date was less than four, then it shows good uniformity so vaccination can be done possibly once depending on the epidemiology of the disease. If the difference is greater than 4 days, then it is a sign of poor uniformity which requires two successive vaccinations. Therefore, the formula is adjusted by using 40% and 90% of the flock that can be vaccinated successfully instead of the default percentage.

Data analysis: The mean antibody titer, standard deviation and percentage coefficient of variation (CV%) have been calculated by using Microsoft offices excel. Furthermore, SPSS version 20.0 software was also used to analyze the data obtained from the study. Specifically, one way ANOVA was performed to compare the mean antibody titer and Pearson's correlation was used to correlate chicks and parental antibody titer in both farms. The differences were considered statistically significant when $p<0.05$. The CV (%) is interpreted as <30% Excellent, 30-50% Good, 51-80%, Fair and >90% poor response to challenge or vaccine[20].

RESULTS

Questionnaire survey: According to the questionnaire survey, the source for chicken to medium-scale farms were Alema (40%), Ethio-chicken (25%), SW Poultry farm (15%), Hawassa Genene (10%) and Gerado (10%). About 75% of the medium-scale and 100% of commercial farms vaccinate their flocks for IBD. Most medium-scale farms use vaccines from National Veterinary institute (NVI) (50%) and the remaining use imported vaccines (25%) purchased from Alema poultry farm. On the other hand, 100% of interviewed large-scale commercial farms relay on imported vaccines. Vaccination is carried out under the supervision of veterinarian's in the commercial farms (100%) but only in 35% of medium-scale farms. Vaccination schedules employed differ from farm to farm but none was based on MDA titer in any of these farms. An incidence of IBD outbreak in a vaccinated stock has occurred in the last 3 years in 70% of the medium scale and 80% of the commercial farms. The respondents of the medium-scale (58.3%) perceive IBD outbreaks were due to vaccine failure. Nonetheless, none of the medium-scale farms have attempted to revise their schedule based on MDA titer, while 40% of the respondents from commercial farms have attempted to do so.

Level of MDA: Table 1 showed the mean antibody titer and CV% for the farms. Accordingly, the mean antibody titer for Alema farms (AD) was higher than ELFORA (ED). In case of parent breeder, the parent antibody titer was almost equivalent in both farms. The CV (%) for antibody titer in response to vaccination in both farms was found good in uniformity (CV (%) = 30-50%) (Table 2).

Table 1: Antibody titer in day old chicks

Frequency of sampling	No. of samples	Minimum Ab titer	Maximum Ab titer	Mean Ab titer±SD	CV (%)
ED1	25	1149.7	1725.3	8717.8±4676.2	54
ED2	25	603.0	19393.5	6607.6±4494.6	68
ED3	25	2267.2	14962.5	7583.5±4398	59
AD1	25	574.5	14525.4	15867.8±5879.6	37
AD2	25	381.9	25347.8	13404.2±4609.8	34
AD3	25	250.0	16729.1	14517.1±6014	41

Table 2: Antibody titer in parent breeder stock

Frequency of sampling	No. of samples	Minimum Ab titer	Maximum Ab titer	Mean Ab titer±SD	CV (%)
EP1	25	2937.6	25987.1	15867.6±4398	37
EP2	25	6312.9	21720.0	13404.0±4609.8	34
EP3	25	1764.7	26732.7	14517.1±6014	41
AP1	25	3129.3	22946.0	11551.0±5395.5	47
AP2	25	6390.6	21534.4	15720.3±4321	28
AP3	24	2228.1	27401.7	15081.4±6615.3	51

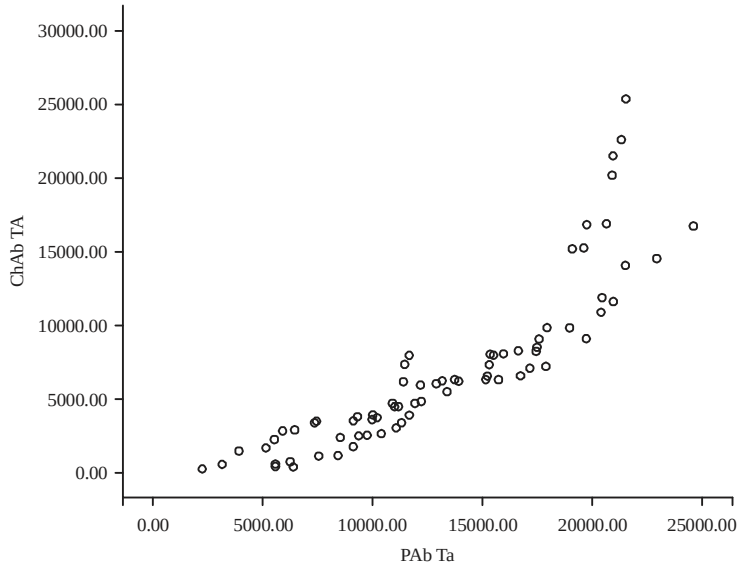

Fig. 2: Chick and parent antibody titer correlation for Alema farms
ChAbTA: Chicken Antibody titer, PAbTA: Parent antibody titer

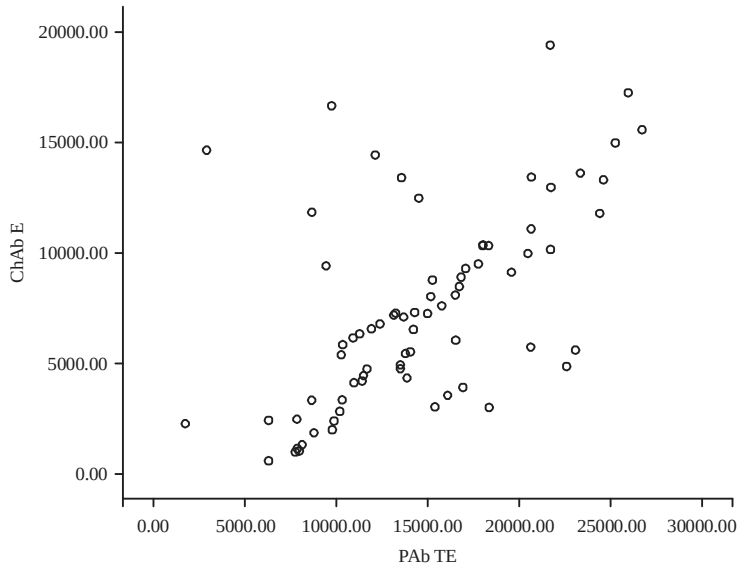

Fig. 3: Chick and parent antibody titer correlation of Alema
PAbTA: Parent antibody titer, ChAbTA: Chick antibody titer

The relationship between the parents age (PageA) and parent antibody titer (PAbTA) and parent age (PageA) and chick antibody titer (ChAbTA) were not significantly different ($p = 0.549$) for Alema farms. Nevertheless, as shown in Fig. 2, there was significant positive correlation between the parent's antibody titer and chicks antibody titer ($r = 0.87$, $p = 0.00$). In case of ELFORA farm, the correlation among the parents age (PageE) and parent antibody titer (PAbTE) and parent age (PageE) and chick antibody titer (ChAbE) were not statistically significant ($p>0.05$). On the other hand, there was positively significant correlation between parent and chick antibody titer ($r = 0.58$, $p = 0.00$) (Fig. 3).

The mean antibody titer from Alema farm showed that there was no association between age of parent breeder and antibody titer ($F = 1.675$, $p = 0.195$). In contrast, the chick antibody titer was significantly associated ($F = 5.912$, $p = 0.04$) with the age of parent breeder. However, there was no statistically significant difference among chicks ($F = 1.39$, $p = 0.255$) and parent antibody titer ($F = 1.24$, $p = 0.29$) for ELFORA poultry farm.

Table 3: Estimated optimum date of vaccination of IBD

Frequency of sampling	No. of samples	Minimum Ab titer	Maximum Ab titer	Mean Ab titer±SD	Age of vaccination based on Ab titer: 75% (Ab. Titer)	40% (Ab. Titer)	90% (Ab. Titer)	Schedule used by framers
Elfora poultry farm								
ED1	25	1149.7	1725.3	8717.8±4676.2	18 days (12465.9)	16 days (6033.2)	20 days (14334.5)	7 and 14 days
ED2	25	603.0	19393.5	6607.6±4494.6	18 days (9494.7)	14 days (5834.2)	18 days (11092.5)	
ED3	25	2267.2	14962.5	7583.5±4398	18 days (9971.9)	15 days (4769.4)	20 days (14962.5)	
*Average					18 days	15 days	19 days	
Alema farms								
AD1	25	574.5	14525.4	15867.8±5879.6	16d (6576.2)	14d (3807.5)	18d (9834.3)	7 and 14 days
AD2	25	381.9	25347.8	13404.2±4609.8	20d (16833.8)	16d (6329.8)	21d (21483.8)	
AD3	24	250.0	16729.1	14517.1±6014	17d (82307.7)	13d (3622.5)	19d (11874.6)	
**Average					18 days	14 days	19 days	

Optimum date for IBD vaccination: The estimated optimum date of vaccination based on the level of MDA titer is 15th and 19th days for ELFORA and 14th and 19th days of age for Alema farms (Table 3).

DISCUSSION

Vaccination for IBD requires developing vaccination schedule based on the level of MDA which decreases as the age of the chickens (progeny) advanced; otherwise, it interferes with efficiency of active immunization. Different studies reported that under laboratory conditions, high MDA at the time of IBDV vaccination interfere with the vaccine response, neutralizes the vaccine virus and delays or even prevents the induction of humoral immunity[21-23]. For this reason, it is imperative that when the MDA level is minimum to avoid its negative effect during application of live vaccines.

In this study, the optimum age of vaccination was almost similar between the ELFORA (15 and 19 days) and Alema farms (14 and 19 days). In both cases chickens had very high antibody titer. In support of these findings, high variation in MDA levels between birds in a flock can make it advisable to vaccinate a broiler flock twice to induce homogeneous protection[24,25]. In addition, Suzuki *et al.*[26] has reported that uniformity of the MDA titer distribution is related to the number of vaccinations required. These were in agreement with the report of Suzuki *et al.*[26], who estimated the optimal age of vaccination was at the 15th day of chicken's age. However, this finding was in the contrary, 21 days of age[27,28] and 17-21 days[29] of vaccination. These substantial differences might be due to the difference in the route of vaccine administration[30] and the amount of antibodies transferred from hen to chick[31-33].

Even though the two farms had different breeds of chickens (Ross and Cobb), the vaccination schedule was almost similar. This study revealed breed of chickens did not affect optimum date of vaccination. Similar study reported that different genetic backgrounds of the broiler flocks have not influence on the outcome of the IBDV vaccine response[28]. Moreover, the level of antibody transfers to day-old-chickens originating from four different breeds was not significantly different[29,34]. A significant difference in the mean antibody ($p<0.05$) titers was observed among 3 days old chickens which was in accordance with Fantay *et al.*[35], who indicated a significant difference ($p = 0.00$) in mean titers taken from chickens at 2 days age.

CONCLUSION

Most farms have no proper vaccination schedule developed according to the MDA level as a result frequent disease outbreaks were recorded. The present study clearly shows that determining the MDA level of chicks is the most important concept in formulating the optimum date of vaccination for better protection in chicks. Accordingly, there was positive correlation between parent and their progeny antibody titer, the estimated date for administration of live attenuated IBD vaccine was 14th and 15th days post hatch and boosted at the 19th days in Alema and ELFORA farms, respectively.

SIGNIFICANCE STATEMENT

This study discovered the effect of maternal antibody on the vaccination schedule against infectious bursal disease virus and its correlation with the age parent breeder. This helps the poultry industries to consider MDA level whenever IBDV vaccines were applied.

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