Determination of Optimal Time of Vaccination Against Infectious Bursal Disease (Gumboro) and Molecular Diagnosis of Clinical Cases in Central Ethiopia

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ABSTRACT

Aim: The aim of this study was to determine optimal time of vaccination against infectious bursal disease (gumboro) and molecular diagnosis of clinical cases in central ethiopia.

Method and Materials: The **s**tudy was conducted on exotic breed chickens kept under semi-intensive and intensive poultry farms selected randomly by multistage sampling technique in Addis Ababa, Bishoftu, and Adama areas. It was a type of prospective longitudinal study where chickens were followed for a defined period of time until they reached 6 weeks of age for clinical cases. A total of 11 samples collected from clinical cases of infectious bursal diease in chickens (5 samples from Bishoftu, 3 samples from Adama and 3 samples from Addis Ababa) were analyzed with RT-PCR. For vaccine experimental study, One hundred eighty, day-old Lohman brawn chicks were reared and used for this purpose. The chicks were divided into three groups A, B, C. Groups A were vaccinated via drinking water route at 7th day whereas B were vaccinated at 14th day of age. Group C was acted as control. Blood samples were collected from wing vein of individual chicken at day 1, 7, 14, 21, 28, 40 and serum was harvested. Indirect Enzyme Linked Immunosorbent Assay (IELISA) was employed to measure Antibody titration.

Results: The proportion of chicks in the unvaccinated group with S/P ratio greater than the protective level continuously fall from 0.90 on day 1 to 0.0 on day 14. At day 21 of age after hatching, the time IBD commonly occurred, 55% of the chicks in group A had protective antibody level with average antibody titre of 1064.61± 748.1621; whereas only 5% of the chicks in group B had protective antibody level with average antibody titre of 123.2321 ± 212.0105.

Conclusion: It was concluded that in chickens with low MDA, the 1st vaccination should be given between 7th and 14th days and repeat after one week. The effect of vaccination programs on the immune response to IBD vaccine in the farms should be further investigated.

Keywords: Commercial farms, Ethiopia, IBD, MDA, PCR, Poultry, Vaccine.

Introduction

Chicken are important livestock resources with the potential to break the vicious cycle of poverty and malnutrition in developing countries. They are considered to be cheap sources of quality animal protein. As a result Ministry of Agriculture has identified poultry production as key sector to deal with food security issues [1]. As the demand for food rises due to a growing population, it will be critically important to develop methods to produce more food with greater efficiency, while lowering the prevalence of infectious diseases that inflicts significant losses in poultry industry. The poultry flocks of Ethiopia are featured by lower inflow of flock than its outflow. Mortality contributed up to 56% of the number of chicken that moved out of flock, implying that disease control is a top priority intervention area. Seventy percent of the mortality is caused by diseases whereas only limited households (6%) have been using vaccines and drugs [2]. Researchers and development workers rate infectious bursal disease as one of the infectious diseases constraining efficiency of poultry production throughout the country.

Infectious bursal disease (IBD) is one of the highly contagious disease affecting mostly young chickens. Infected chickens do not properly respond to vaccination, develop strong post vaccinal reactions, and become susceptible to concurrent infections [3,4]. Moreover, highly virulent IBDV can cause high mortality in unprotected flocks. In all poultry producing regions of the world, infectious bursal disease virus (IBDV) continues to be a major constraint for poultry farmers. The characteristic

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consequences of immune-suppression associated with IBDV are vaccine failure and susceptibility of chickens to opportunistic pathogens. This means that IBDV-infected chicken may become a good propagator for other viral and bacterial pathogens. For example, low pathogenic duck adapted avian influenza virus becomes more virulent when serially passaged in IBDV-infected chickens [5]. This has important implications for zoonotic infections such as E. coli and Salmonella and could contribute to their occurrence, which in turn could impair public health and poultry business.

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 [6].

In Ethiopia IBD is prevalent in various areas [7] causing high mortality ranging from 49.89% to 72% in chicken [8,9]. Its control is constrained by lack of epidemiological information, absence of effective administration schedule and shortage of molecular diagnostic tools. Elsewhere in the world the use of molecular techniques to detect and identify IBDV strains has increased in recent years. RT-PCR has been used to amplify sections of the VP2 gene, which encodes for the major protective epitopes, contains determinants for pathogenicity, and is highly variable among strains [10]. Control through effective vaccines requires knowledge of the occurrence and distribution of the strains of IBDV. At present IBD vaccines are widely used in attempts made to control IBD in Ethiopia. However, there have been several reports of post-vaccination outbreaks. Maternally derived antibody is one of the hypothesized reasons for the occurrence of outbreaks after vaccination. Two different vaccination schedules are used in central Ethiopia, both of which are not supported by systematic evaluation of the effectiveness of the schedules. Therefore, a need was felt for epidemiological assessment of the efficacy of the schedules.

Methods and Materials

Study Areas

This study has two components. The molecular study was conducted in semi-intensive and intensive poultry farms found in Addis Ababa, Bishoftu, and Adama (Fig 1). The experimental study was conducted in Bishoftu. These areas are selected due to the presence of many commercial poultry farms.

Study Population

The study population for the molecular diagnosis consists of exotic layer chickens kept under semi-intensive and intensive production systems in the study areas. However, special attention was given to young chickens aged between 3-6 weeks. For on farm vaccine experimental study, One-day-old Lohman Brawn chicks (Lohmann, Germany) were used in this study. The parent flock was raised at the Debre Zeit agricultural research Center, Poultry farm Station.

Study Design and Sampling Method

List of poultry farms in each town were obtained from respective agricultural offices and poultry producers' associations. Twenty poultry farms for the study were selected from each study area. The study was a type of prospective longitudinal study where chickens on the selected farms were followed until they reached two months of age in a dribbling way from Nov, 2016 - Apr, 2017 for cases of IBD. Samples of bursa and spleen from IBD suspected chickens were collected for molecular diagnosis of the cases. Samples were placed in sterile and labeled universal bottles and transported to National Veterinary Institute, Bishoftu for molecular diagnosis. The samples were processed in the laboratory immediately after submission. Whenever immediate processing was not possible, they were stored at -80oC until processing [11].

For the vaccine effectiveness experimental study, at day one the chicks were randomly assigned to one of three experimental groups consisting of 60 chickens per group. One group of chickens were vaccinated on day 7 with CEVAC@ Gumbo L and day 14 using CEVAC@ IBDL Winter field 2512 G-61 strain. This group was designated group A. These two vaccines were obtained from Alema Poultry farms, Bishoftu, Ethiopia and the vaccination schedule was based on the recommendation of the provider. One group of chickens was vaccinated

with Bursitis virus strain LC75, produced at National Veterinary Institute (NVI), Bishoftu Ethiopia. This group was designated as group B and chickens were vaccinated on day 7 and 14 by drinking ground water taken from Maranata poultry farm. The third group served as unvaccinated control. Blood samples were collected on day 0, 7, 14, 21, 28 and 40 from the wing vein by using sterile disposable 3 ml syringe with 23 gage needle. After leting the blood to clot in the syringe for 8 hours, the serum samples harvested from blood samples were stored at -200C until tested [11].

Study Methodology

Observation of clinical manifestation and post mortem examination

The selected chicken farms were followed for clinical and post-mortem examination. Signs of infection in a flock were the tendency for some birds to pick at their own vents, soiled vent feathers, whitish or watery diarrhea, anorexia, depression, ruffled feathers, trembling, severe prostration, and finally death. Affected birds became dehydrated and, in terminal stages of the disease, had a subnormal temperature. Post-mortem lesions include: dehydration of the muscles with numerous ecchymotic haemorrhages, enlargement and discoloration of the kidneys, with urates in the tubules. Confirmation of clinical disease or detection of subclinical disease was done by using Molecular technique from the bursa.

Sample collection, transportation and preparation

Two types of samples were collected accordingly to meet the two study designs. The bursa and spleen samples from IBD suspected chickens were collected for IBD outbreak investigation started from Nov, 2016 to April, 2017. On the other hand, for IBDV vaccine immunogenicity experimental study, blood samples were collected and the serum was harvested. The serum samples were preserved at -20 oc until processed [11].

Molecular Analysis of the Samples

Extraction of the genetic materials and detection of RNA of infectious bursal disease RNA in the clinical samples collected was carried out RNeasy® Mini Kit (cat. nos. 74104 and 74106) at the Molecular Biology Laboratory of the National Veterinary Institute. Reverse transcription PCR was used to RNA of IBDV from 10 % (w/v) tissue sample suspensions and/or cell culture homogenates using PureLinKTM RNA Mini Kit Cat no.12183-018A, based on the manufacturer protocols. Briefly, 400µL tissue suspension was transferred in to 1.5 mL micro centrifuge tube and 400µL lysis buffer with 2-mercaptoethanole was added to each tube and mixed by votexing and incubated at 56oC for 30 minutes (until the cell is dispersed and appear lyzed). The lysate was transferred in to a clean homogenization tube, and homogenized at maximum speed for 45 seconds.

Amplification of the genetic material was done using Primers:

IBD3-Fow -
5'TGTAAAACGACGGCCAGTGCATGCGGTATGTGACGCTTGGTCAC-3'

IBD3-REV-
5'CAGGAAACAGCTATGACCGAATTCGATCCTGTTGCCACTCTTTC-3'. The amplification was done using First-strand cDNA synthsis kit Catalog no-18080-051. A two step RT-PCR (touchdown PCR) was done for cDNA synthisis (for one reaction). Visualization of the amplification products was done using 1.5% agarose gel. Positive control (RNA from IBD vaccine virus) and negative control (PCR water) were used during the analysis.

On-farm Experimental Study and Determination of Antibody Titres

Ethical statement

All experiments were performed in animal facilities according to Addis Ababa University College of Veterinary Medicine and Agriculture Home Office ethical guidelines on animal welfare.

Experimental animal and management

One hundred and eighty day old chicks from unvaccinated Lohman Brown parent stock were obtained from Debre Zeit Agricultural Research Center (DZARC) for determination of optimal time vaccination. The chicks were placed into separate rearing cages at Dembi site, Bishoftu and provided with standard feed and management. The parent stock (from day-old) was vaccinated Live attenuated Gumboro vaccine but not vaccinated the inactivated vaccine against IBD at pullet age. Progenies hatched from this parent stock were used for all the experiments. Two types of poultry feed (Starter and Grower) were bought from Alema koudjis feed processing company, bishoftu and Starter feed was given in the first month and Grower feed was given in the second month. Feed and water were provided ad

libitum with equal number of drinkers and feeders in each group until the end of the experiment. The spacing in each group was 10 chicks/m3. All experiments were performed in animal facilities following international ethical guidelines on animal welfare.

The IBD vaccines used for the study

Three commercially available live attenuated infectious bursal disease vaccines (IBDV vaccines) were used. Namely: Intermediate: CEVACR GumboL contains LIBDV strain, Intermediate Bursal Disease Vaccine, freeze dried form and Batch No. IBD 04/14 Hungary; CEVACR IBDL Winterfield 2512 G-61 strain, Intermediate plus Bursal Disease Vaccine; freeze dried form, Batch No.3506N, with SPF embryonated hen eggs origin, manufactured by Lohmann Animal Health GmbH, Germany, and Bursitis virus strain LC75 Bursal Disease Vaccine, freeze dried form, Batch No. D037411 Manufactured by U.S.A. LOHMANN ANIMAL HEALTH INT, LTD, with cell culture origin which is actively produced at National Veterinary Institute (NVI) and used in Ethiopia.

Determination of Antibody Titres

The titre of antibody in the serum samples collected during this was assayed using enzyme linked immunosorbent assay (ELISA) kit (ID Screen®, IBD Indirect) supplied by ID.Vet, 310, rue Louis Pasteur – France. All the reagents were allowed to come to room temperature before use and homogenized by inversion and vortex. The samples were pre-diluted 1: 500 in Dilution Buffer and 10µL of the pre-diluted samples were added to 90 µL of Dilution Buffer in the ELISA microplate. The plate was covered and incubated for 30 minutes. The wells were emptied and washed 3 times with 300 µL of Wash Solution. One hundred µL of 1x Conjugate was added to each well and incubated for 30minutes. The wells were emptied and washed 3 times with 300 µL of Wash Solution to each well. One hundred µL Substrate Solution was added to each well and the plates incubated for 15 minutes. One hundred µL of Stop Solution was added to each well. The results of the reactions were read at 450nm using ELISA reader. The presence or absence of antibody to IBDV was determined by relating the A (450 nm) value of the unknown to the positive control mean. The positive control had previously been standardized and represented significant antibody levels to IBD in chicken serum. The relative level of antibody in the unknown was determined by calculating the sample to positive (S/P) ratio. The equation for calculation provided in the ELISA kit was used in calculating the antibody titre as follows:

a) Positive Control Mean (NCX)=

$$\frac{\text{Well A1(450nm)} + \text{Well A2(450nm)}}{2} = OD_{PCX}$$

b) Negative Control Mean (NCX)=

$$\frac{\text{Well A3(450nm)} + \text{Well A4(450nm)}}{2} = OD_{NCX}$$

Interpretation of the results

For each sample the S/P ratio was calculated as described by the manufacture of the kit as:

$$S/P = \frac{\text{OD sample} - \text{OD NC}}{\text{OD PC} - \text{OD NC}}$$

Determination of antibody titre from the S/P ratio is as follows

$$\textbf{Log}_{10}\textbf{(titre)} = 0.97 * \log_{10}(S/P) + 3.449$$

$$\textbf{Titre} = 10^{\log10(titre)}$$

Finally the results of status of infection with IBD are determined as given the table below based on the recommendations of the manufacture.

Table 1. Interpretation of results of ELISA

S/P ratio	ELISA antibody titre	IBD immune status
S/P ≤ 0.3	Titre ≤ 875	Negative
S/P > 0.3	**Titre > 875**	**Positive**

Serum samples with S/P ratios of less than or equal to 0.3 were considered negative. S/P ratios greater than 0.3 (titres greater than 875) were considered positive and indicated either vaccination or exposure to IBD virus.

2.5. Data Management and Statistical Analysis

Data collected in this study were stored in Microsoft excel and the mean ±s.d of the antibody titres among the experimental groups was computed and compared using STATA version 13.

Results

Clinical and Postmortem findings

During the study period, 26 flocks showed very high morbidity (90%) with severe depression in most chickens lasting for 5–7 days. Mortality was risen (80%) sharply for 2 days then declined

rapidly over the next 2–3 days. The incubation period was very short, and clinical signs of the disease were seen within 2-3 days after exposure. Signs of infection in a flock was the tendency for some birds to pick at their own vents, soiled vent feathers, whitish or watery diarrhea, anorexia, depression, ruffled feathers, trembling, severe prostration, and finally, death. Affected birds became dehydrated and, in terminal stages of the disease, had a subnormal temperature.

Results of Molecular Diagnosis

A total of 11 samples collected from clinical cases of infectious bursal diease in chickens (5 samples from Bishoftu, 3 samples from Adama and 3 samples from Addis Ababa) were analyzed with RT-PCR. Of these 8 samples (72.73%; CI: 39.03 – 93.98) were positive for infectious bursal disease virus RNA (Figure1). The RNA of IBDV was detected in 40 of 5 (80%) of samples from Bishoftu and 2 of 3 (66.67%) of samples from each of Adama and Addis Ababa (Table 3).

Table 2. Results of RT-PCR on samples collected from clinical cases of IBD

Sample ID	Location	RT-PCR Result
Sample 1	Bishoftu	negative
Sample 2	Adama	negative
Sample 3	Addis Ababa	negative
Sample 4	Bishoftu	**positive**
Sample 5	Bishoftu	**positive**
Sample 6	Bishoftu	**positive**
Sample 7	Adama	**positive**
Sample 8	Adama	**positive**
Sample 9	Addis Ababa	**positive**
Sample 10	Addis Ababa	**positive**
Sample 11	Bishoftu	**positive**

Results of Experimental study

The results of ELISA tests for MDA performed on the blood of day old chicks (Table 4) from unvaccinated parent flock revealed that antibody titre was on average 2329.045± 898.9366. Ninety percent of the chicks had an S/P ratio greater than the protective level (>0.3). For the unvaccinated chicks the titre was highest on day 1 and it decreased continuously from day 1 to day 21. The MDA falls sharply after day 7. The proportion of chicks in the unvaccinated group with S/P ratio greater than the protective level continuously fall from 0.90 on day 1 to 0.0 on day 14.

Table 3. Maternally derived antibody in unvaccinated chicks titres determined by enzyme linked immunosorbent assay

Unvaccinated chicks	Percent > 0.3	Amount MDA (mean±sd)	S/P ratio
Day 1	90	2329.045± 898.9366	.8255± .326
Day 7	70	1229.387± 791.6917	.42855± .285
Day 14	**0**	**197.0042± 138.2761**	**.0617± .052**
Day 21	**0**	**50.96496 ± 88.91306**	**.00315± .0322**
Day 28	10	265.2102± 302.7019	.108± .083

In group A (those chicks vaccinated with GumboL and IBDL) on day 7 and 14 the antibody titre dropped from 1374.283±884.9499 on day 7 (first vaccination) when 70 % of the chicks had protective antibody level to 344.632± 480.8263 on day 14 (booster vaccination) (Table 5). At this age none of the chicks had protective antibody level and none of the chicks had protective antibody level. Two weeks after the first vaccination (day 21) the antibody titre remains low (1064.61± 748.1621) with only 30 % of the chicks having antibody titre greater than or equal to the protective level. About two weeks after the booster vaccination (day 28 of age) 100 % the chicks had antibody titre greater than the protective level and the mean anti body titre was 3168.555± 1124.5183.

In Group B (those chicks vaccinated with NVI LC75) on day 14 and day 21 the antibody titre was 282.5035± 188.6627 on the day of first vaccination. At this time none of the chicks had protective antibody level. The antibody remains low (123.2321 ± 212.0105) when the chicks receive booster vaccination on day 21. About two weeks after the booster vaccination (day 40 of age) 100 % the chicks had antibody titre greater than the protective level and the mean anti body titre was 4349.256±1097.636.

Table 4. Mean antibody titres and proportion of chicken with antibody greater than protective level

Group	S/P ratio ± s.d	antibody titre (mean± s.d)	%with protective antibody level	Age in days
Group A	.12525± .165	344.632± 480.8263	15	14
Group A	.37025± .266	1064.61± 748.1621	55	21
Group A	1.72285± .464	3168.555± 1124.5183	100	28
Group A	1.72285±.464	4761.077±1246.093	100	40
Group B	.0223± .029	123.2321 ± 212.0105	5	21
Group B	1.52135± .360	1064.61± 748.1621	55	28
Group B	1.56925±.408	4349.256±1097.636	100	40

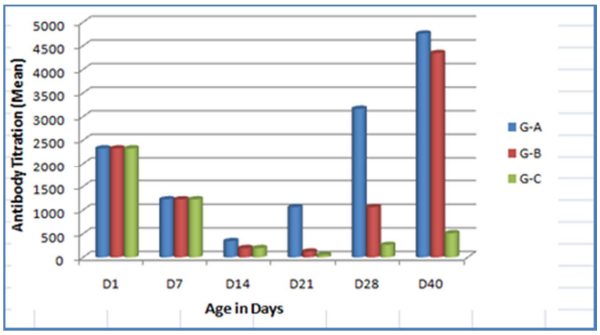

Fig. 1. A bar graph which shows the antibody titration of the three groups in weeks

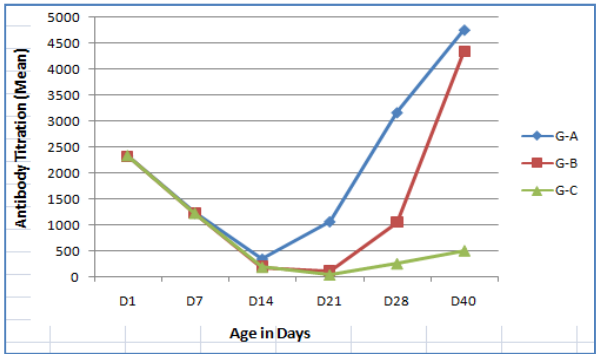

Fig 2. A line graph showing the antibody titration in the three groups

Discussion

Infectious bursal disease is one of the important diseases of poultry with huge loss in young flock. It continues to be a major problem of chickens in poultry rearing areas. It has been documented in Ethiopia before one decade and considered to be endemic in many parts of the country. This confirmed by the results of molecular investigation made in this study. The gene of infectious bursal disease virus was detected in samples collected from all sites. This shows that the disease is widespread at least in central Ethiopia. In this study 72.27% of the samples gave positive signals for IBDV. This is quite in agreement with previous reports made on infectious bursal disease in Ethiopia [12]. However, the proportion of samples yielding positive results in this study is higher than the reports of [13]. It is clear that infectious bursal disease is one of the standing problems of poultry farmers.

The PCR analysis is successfully amplified the virus gene and revealed that the chickens were infected with infectious bursal disease virus by producing the expected band size on agarose gelelectrophoresis analysis. This PCR finding is in agreement with the previous report of [14] that the same PCR product band size obtained using the same amplification primers.

The level of MDA is high for the first week for chicks derived from unvaccinated immune stock, but it decreases rapidly after day 7. The assay of the antibody collected on the second day of hatching showed that 90% of the chicks have protective antibody level and 10% of them were susceptible. That means in chicks hatched from unvaccinated parent flock the optimal time of vaccination is when they are day old. The World Organization for Animal Health recommends that chicks need to be vaccinated at day old with intermediate IBD vaccine and booster dose to be given when 10% of the chicks become susceptible [11]. After two weeks of age low levels of antibodies were present in the blood of the chicks but the proportion of chicks with protective antibody level fell sharply to zero on day 14. That means when the booster vaccination was given 85% of the chicks were susceptible. Thus, in group of chickens vaccinated on day 7 and 14 (Group A), the antibody fell initially and two weeks after booster vaccination was given it jumped sharply concurrent with rise in the proportion of chicks with protective antibody level to 100%. This implies that administration of Gumbo L, which is intermediate type of IBD vaccine on day 7, followed by vaccination with IBDL (intermediate plus) on day 14 provided sufficient level of antibody but the schedule, has to be reconsidered. There has been contrasting results regarding the time of vaccination in chicks that hatch from unvaccinated parent flocks. One study showed that an intermediate vaccine given at 7 days of age did not provide protection to vaccinated chicks in the face of virulent challenge with field strain. However, when given at 14 days of age, the same vaccine fully protected the chicken [15]. In contrast vaccination of chicks at 7 days of with intermediate plus vaccine elicited protective antibody level against virulent challenge in Ghana [16]. Although we did not carry out artificial challenge with virulent infectious bursal disease virus, absence of clinical cases throughout the study period in endemic areas where several outbreaks caused significant loss in chickens suggests that the vaccination schedule works well.

Data from this study revealed that the change in antibody to IBDV is variable. This may be explained by the influence on the half-life of MDA of the vaccine type and its time of

application [17]. When offspring of different parent flocks are raised together, this may result in different levels of MDA and compartment alisation of the herd into individuals with low or high susceptibility to virulent IBDV [18]. Early vaccine failed to stimulate the immune system in the chicks because maternal antibody reacts with live vaccine virus and becomes neutralised or interferes with MDA [19]. Several studies under laboratory conditions have indicated that high MDA at the time of IBDV vaccination might interfere with the vaccine response, neutralises the vaccine virus and delays or even prevents the induction of humoral immunity [6,15]. This means that the vaccination in the first days may not offer the chick any protection against disease. Nevertheless, an increase in titre was observed when vaccination was performed at 14 days [20], as also observed by [21] using a quantitative agar gel precipitation test. As shown in Tables 2 and 3 the MDA to IBDV was high before vaccination (day 7) but decreased below the protective level after first vaccination. This shows that the MDA neutralized the vaccine and when booster vaccine was given the antibody level remained low. Three weeks after the first vaccination (two weeks after booster vaccination) the antibody level rose to above protective level in 100% of the chicks. That is, booster vaccination is must since the first vaccine is neutralized to elicit protective antibody level.

In the group of chickens vaccinated at 14 and 21 days of age using NVI LC75 (intermediate plus) type of vaccine (Group B) the antibody level was nearly nil during the first vaccination. It remained remarkably low during the booster vaccination (on day 21). It is not clear why the antibody remain low since the vaccine was given when the MDA has wane. Similar to the chicks in group A chicks in group B mounted antibody two weeks after the first vaccination but still 45 % of the chicks were susceptible. After two weeks of the booster vaccination the antibody level reached protective level in 100% of the chicks. Previously [22] recommended vaccinating chickens at an age of 2 weeks with intermediate strains of IBD and boosting them with the 'hot' vaccine at an age of 3 weeks. It was reported estimated optimal vaccination timings against IBDV of each flock at the three sampling time points between 16 and 24 days of age [23]. In this study we intermediate plus at both ages and this may be the reason for the failure lower protective antibody titre observed but this need to be elucidated in the future. The appropriate time to implement active vaccination without risk of vaccine failure or incidence of infection seems to vary according the vaccine used.).

In this study, the MDA of control group was decreased from the 1st to the 28th day by half every week. The antibody titer of group B was decreased in a way similar to that of the control group, but a significant ($p<0.05$) difference was present between them at 14th day and thereafter, whereas in group A it was decreased from the 1st-14th day and then increased at 21st 28th of age. Although single dose at the 7th day old vaccination could induce slight increase of IBD antibody in comparison to that of the control, vaccination at the 14th day of age induced high and protective level of IBD antibody titer after one week. These may be due to the ability of vaccine at each time of vaccination to neutralize different levels of MDA. single dose vaccination at the 7th day in chickens with high MDA was ineffective and could not use in chicks so that booster dose should be given at 14th day. It was concluded that in chickens with low MDA, the 1st vaccination should be given between 7th and 14th days and repeat after one week. Early single vaccine failed to stimulate the immune system in the chicks because maternal antibody reacts with live vaccine virus and becomes neutralised or interferes with MDA [19]. Several studies under laboratory conditions have indicated that high MDA at the time of IBDV vaccination might interfere with the vaccine response, neutralises the vaccine virus and delays or even prevents the induction of humoral immunity [6,15,24]. It means that the vaccination in the first days failed to offer the chick any protection against disease. Nevertheless, an increase in titre was observed when booster vaccination was performed at 14 days [20], as also observed by [21] using a quantitative agar gel precipitation test. Under field conditions, however, the decay pattern of IBDV-specific MDA proved to be more complex, as it depends largely on initial antibody levels, which may vary between batches and also within a batch, making it difficult to predict the optimal time for vaccination [25].

In the present study the half-life of MDA to IBDV is between 5 and 7 days. Similarly, other studies reported that the rate of decline was by about half every 5 days [17,26] and between 4 and 5 days [27]. Others have reported that the

half-life MDA to IBD in chicks was 3.46 days [28] and decreased every 4 days [29]. In newly hatched layer-type chicks, MDA exhibits a linear or curvilinear decline with a mean half-life of 5 to 6 days [3]. It was reported [30] a half-life of 6.7 days for IBDV-specific MDA. It is generally thought that the half-life of MDA in broiler lines is much shorter, approximately 3 days [31]. Data from this study revealed that the decrease of MDA to IBDV is variable during the growing period. This divergence may be explained by the influence on the half-life of MDA of the vaccine type, its time of application in hens [17] and probably the immune status of the hen [32].

Despite the time of vaccination, the present study agree with [22] who recommended vaccinating chickens at an age of 2 weeks with intermediate strains of IBD and boosting them with the 'hot' vaccine at an age of 3 weeks in a closed system. [23] reported estimated optimal vaccination timings against IBDV of each flock at the three sampling time points between 16 and 24 days of age. Similarly, [31] indicated that the optimal vaccination time was between 17 and 23 days post-hatch based on the Deventer formula, whilst [20] suggested that broiler chicks vaccinated at days 8, 15 and 23 with live attenuated vaccine or live attenuated vaccine followed by inactivated vaccine at days 8 and 21 could be adequately protected against the virulent form of IBDV. Furthermore, it was observed that chickens vaccinated at 10 or 18 days showed better immune response to IBDV vaccination [24]. In practice however, if a (sub-clinical) infection has occurred during the life of a hen, the antibody titre of the hen will rise, particularly in hens that have not been re-vaccinated with a killed vaccine. The progeny of these hens will need to be vaccinated later than normally expected. On the other hand, one doesn't want to wait too long before vaccinating as this will leave the flock unprotected against early challenge.

The present study clearly shows that a high level of maternally derived antibody at day 1 in the chicks hatched from unvaccinated parent flock. This may be due to subclinical infection in the parent flock. The MDA in the chicks interferes with the vaccine, resulting in no immune response after the 1st vaccination in both groups. However, re-vaccination induced an immune response, particularly when carried out at days 14 and 21. Therefore, two vaccinations should be recommended to achieve good protection against infection by bursal disease virus in a flock.

Conclusion

Infectious Bursal Disease (IBD) is an acute and highly contagious disease affecting young chickens from 3-6 weeks of age. The disease causes immunosuppression in chickens and rendering them vulnerable to a variety of other infections. Early subclinical infections are the most important form of the disease because of economic losses. They cause severe, long-lasting immunosuppression due to destruction of immature lymphocytes in the bursa of Fabricius, thymus, and spleen. The more frequent reason for outbreaks in vaccinated flocks is incorrect application of the vaccine. The present study clearly shows that infectious bursal disease is causing problem in poultry farms. Results of gross and molecular diagnosis of BF and evaluation of Ab titers demonstrated that IBD was indeed a subclinical disease problem on the poultry farms. It is revealed that the vaccination schedules used in central Ethiopia need to be revised as the maternally derived antibody interferes with the vaccines. It seems that two vaccinations starting at 7th day old are necessary for chicks hatched from unvaccinated parent flock. Clear time of vaccination could not be determined in this study.

Therefore, the following recommendations are forwarded:

-Further evaluation of optimal time of vaccine delivery in vaccinated parent flock is needed

-Large scale evaluation of different commercially available vaccine need to be carried out

-Molecular epidemiology of IBDV virus should be studied with a planned interval to assess the antigenic diversity of the IBDV virus.

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