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Evaluation of Antibody Titers in Commercial Chickens Vaccinated by Infectious Bursal Disease Virus LC-75 Vaccine in Sebeta Town, Oromia, Ethiopia

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Abstract

This research assessed the titre of antibodies in response to flocks vaccinated with the infectious bursal disease virus LC-75 vaccine in and around Sebeta town, Ethiopia. 19 flocks with an average of 21 chickens were included in the study, which was conducted from December 2021 to May 2022. After a questionnaire survey of chicken breeders, approximately 2.5 mL of blood was collected from each study chicken and the serum samples were examined by an indirect ELISA at the Animal Health Institute, Sebeta, Ethiopia. The test was conducted according to the manufacturer's protocol. Of the total 399 chicken samples, 398 (99.75%) were found to have a protective level of antibody titre, irrespective of the time they were assessed after the last time of vaccination. There was also a statistically significant association with production stage ($P < 0.05$) and a non-significant association with hygienic condition ($P > 0.05$) according to the study. This suggests that the current antibody titers were due to the infectious bursal disease virus LC-75 vaccination despite the routine vaccination programs, which may be regardless of the vaccination timing and dose and other relevant factors like genetic immunogenicity.

Keywords: Antibody titre, Chickens, ELISA, Infectious bursal disease, Vaccination

1. Introduction

Infectious Bursal Disease (IBD) also called "Gumboro disease" due to the location of the first outbreak in Gumboro, Delaware, USA. IBD is an acute and highly contagious viral disease of growing chickens (*Gallus gallus domesticus*). The etiological agent for IBD is an IBD virus (IBDV), which belongs to the genus *Avibrinavirus* of the family *Birnaviridae. The* primary target of the IBDV is the lymphoid tissue of the bursa of Fabricius. The virus, which primarily affects young chickens aged 3-6 weeks, was initially classified as an avian nephrosis due to kidney damage (Cosgrove, 1962; Van den Berg *et al*., 2000; Quinn *et al*., 2015; Delmas *et al*., 2019),

but was later named as IBD due to varying morphologic and histological changes observed in the lymphoid tissues of the Fabricius bursa (Hitchner, 1970; Chou & Calnek, 1997).

IBD is a very devastating disease of poultry due to mortality and the rendering of chickens susceptible to secondary infections (Kurukulasuriya, 2017). There are two serotypes of the virus: serotype 1 and serotype 2.Serotype 1 IBDV strains are pathogenic to chickens (Muller *et al*., 2003; Van Den Berg *et al*., 2004), whereas serotype 2 strains are non-pathogenic to chickens (Meferran *et al*., 1980). Serotype 1 IBDV isolates comprise the variant, classical virulent and very virulent IBDV (vvIBDV) strains, which widely differ in their pathogenicity to chickens (Muller *et al*., 2003).

In the avian immune system, lymphocyte stem cells mature into immunocompetent B lymphocytes in the bursa of Fabricius, a lymphoid and epithelial organ. This depends on how the bursa develops. It is therefore essential to induce active immunity to the virus as soon as possible after hatching because the first few weeks of a chicken's life are when they are most susceptible to IBDV infections. The immune status of chickens should be monitored periodically with a quantitative serologic test such as ELISA. If antibody levels decrease, hens should be re-vaccinated to maintain adequate immunity in their progeny (Müller *et al*., 2012). However, while innate immune mechanisms appear to be fully functional in the newly hatched chick, optimal adaptive immune responses do not develop until several weeks after hatching, and maternal antibodies help to protect the offspring until the adaptive immune response becomes fully effective. Therefore, maternally derived IBDV-specific antibodies that are transmitted to the offspring via the yolk of embryonated eggs may interfere with early vaccination (Davison *et al*., 2008; Müller *et al*., 2012).

During the first few weeks of life, vaccination is thought to be an essential method of protecting domestic chickens (Camilotti *et al*., 2016). As a result, various types of vaccines are mostly available for the prevention of IBD in different countries. A recombinant IBDV-vp2 protein vaccine (Wu *et al*., 2004), an inactivated oil-emulsion adjuvant vaccine (Rosenberger *et al*., 1987), and a live attenuated vaccine (egg-adapted or tissue culture-adapted) were all created by Schijns *et al*. (2014).Live attenuated Gumboro vaccines comprise a virulent virus whose pathogenicity has been weakened through consecutive cultures in living cells, but the virus maintains its immunogenic antigenicity for stimulating the body's immune response, a process which is commonly known as attenuation (Ganguly *et al*., 2010).

There are different Gumboro disease virus strains used in the vaccines and different levels of attenuation: mild (highly attenuated), intermediate (very-attenuated), intermediate plus (moderately attenuated), and hot vaccines (poorly attenuated) (Hair-Bejo *et al*., 2000). Of these, the mild vaccines do not cause bursal damage in chickens but have poor efficacy in the presence of maternally derived antibodies (MDA) or vvIBDV infection (McMullin, 1985). Vaccines of higher pathogenicity (intermediate, intermediate plus, or hot) may break through the high levels of maternal immunity but may produce bursal lesions, with subsequent immunosuppression leading to a secondary infection. In other words, they may not protect against infection with vvIBDV or antigenic variants (Kumar *et al*., 2000; Rautenschlein *et al*., 2005; Dey *et al*., 2019). Killed vaccines comprise viruses whose pathogenicity has been inactivated through the use of physical and chemical means, but the protein coat structure has been maintained, which acts as immunogenic. The viruses are physically inactivated by the use of ultraviolet radiation and heat, and through chemical means by the use of formalin (Furuya *et al*., 2010).

As a general rule, proper prevention and control strategies have been implemented in the poultry industry using proper disinfection and vaccination at the appropriate time (Gardin *et al*., 2009). However, vaccine failures have been reported globally (Muller *et al*., 2012; Adamu *et al*., 2013), in Kenya, and in Sudan (Kasanga *et al*., 2007;

Babiker *et al.*, 2008; Wanzila, 2016). Even though the disease was first reported in Ethiopia on a farm at Bishoftu in 2002 with a high mortality rate of 50% in the affected 20–25-day-old broiler and layer chickens, vaccination of chickens against IBD by a vaccine prepared from classically virulent IBDVs at the National Veterinary Institute of Ethiopia has been implemented throughout commercial poultry farms since it was first reported in 2005 (Zeleke *et al.*, 2005; Mekuriaw *et al*., 2017).

A nationwide vaccination campaign was started following the use of live vaccine imported from the Netherlands on commercial poultry farms and the first report of the case in Bishoftu town in 2002 (Zeleke *et al*., 2005). Because the nation's vaccinations were either imported or locally made from imported master seed and were thought to be incompatible with the locally circulating strains, this move was made out of concern that they were not totally effective in preventing the illness (Qin& Zheng, 2017); and/or the fact that IBD outbreaks have occurred in flocks that had received vaccinations, however, shows that vaccines do not fully protect against IBDV. Nonetheless, the vaccine is still in use throughout the country (Negash *et al*., 2012; Shegu *et al*., 2020).Therefore, the objective of this research was to evaluate the antibody titre against the IBDV LC-75 vaccine in immunized layer flocks that are currently engaged in egg production and to assess the impact of different risk factors on the development of protective levels of antibody titre from the study flocks in the study area.

2. Materials and Methods

2.1 Study area

A cross-sectional study was conducted from December 2021 - May 2022 in Bovans and Sasso breeds, both from layers and pullets, in Sebeta town, Oromia, Ethiopia. Sebeta is in the Oromia Special Zone, which surrounds Addis Ababa, and is located at 8°55′N latitude and 38°37′E longitude, at an elevation of 2,356 meters above sea level. The town is situated about 25 km southwest of Addis Ababa and has a total area of 102,758 km^2. The annual mean rainfall registered in the area ranges from 860 to 1200 mm. The ambient temperature averages 28°C in the area. The livestock population of the area comprised about 114,880 cattle, 21,225 sheep, 12,696 goats, 3,919 horses, 2,520 mules, 16,340 donkeys, and 67,305 poultry (i.e., 59,276 local and 8,029 exotic breeds) (CSA, 2013).

2.2 Study population

A total of 399 chickens from 19 flocks; 13 from layers and 6 from pullets of Bovans and Sasso breeds, were included in the study. They were randomly selected for post-vaccination antibody titration status evaluation of the infectious bursal disease live vaccine. The flocks were under intensive and semi-intensive management systems.

2.3 Study design

A cross-sectional study was conducted from December 2021 to May 2022 to assess the antibody titration of the IBDV post LC-75vaccination and associated risk factors for variation of the antibody titre in commercial poultry production in Sebeta town (Bedasa *et al*., 2022). Additionally, a semi-structured questionnaire was administered to assess the breed, management strategy, hygienic status, and vitamin supplementation activity of chickens that may determine the degree and duration of immunity imparted by the IBD vaccine.

2.4 Study methodology and data collection

2.4.1 *Questionary survey*

A semi-structured questionnaire was administered to interview selected owners of commercial poultry farms to assess potential risk factors for the difference in IBDV LC-75 vaccine antibody titration, such as: last day of vaccination, production stage, hygienic level of house, age, vitamin supplementation, and farming system in the study area.

2.4.2 Blood sample collection

On average, 21 chickens were selected for blood collection out of 19 flocks as recommended by Siegmann (Siegmann, 2013). After disinfecting the humeral region of the wing vein with 70% alcohol, using a 3 mL volume syringe and 22 gauge needles, about 2.5 mL of blood volumes was collected from each chicken and transported to AHI, Sebeta, Ethiopia. Blood samples within syringes were kept overnight at about 45° horizontally until the blood clotted and separated from the serum. Then, the separated serum was transferred to sterile cryovials, labeled, and kept at -20°C until the indirect ELISA was performed (Bedasa*et al*., 2022) to detect antibodies against the IBDV. The indirect ELISA test was valid if the mean optical density (OD) of the positive control was greater than 0.25 (ODpc > 0.25). The ratio of the mean of OD of positive and negative controls (ODPC and ODNC) is greater than 3. So, if the calculated S/P ratio is > 0.3, serum is taken as sufficient with protective antibody against IBD, and ≤ 0.3 is considered as less protective antibody.On November 12, 2021, the Animal Health Institute in Ethiopia reviewed and approved the study for ethical reasons.

2.4.3 Serological test and procedure

Antibodies against the IBD vaccine were measured from sera that were collected. The mean titre among the flock samples was taken according to the instructions of the kit manufacturer (IDvet, France). The indirect ELISA method was used to detect antibodies in chickens directed against the IBD vaccine. In brief, five hundred-fold (1:500) dilutions were used. About 245µl of dilution buffer 14 was added to each well of the pre-dilution plate except the controls. Then, except for the controls, 5µl of sample was added to each well. 100µl of negative control was added to wells A1 and B1 and 100µl of positive control was added to wells C1 and D1. Then 90µl of dilution buffer 14 and 10µl of the pre-diluted samples were dispersed into appropriate 92-well plates coated with IBDV viral antigen, and the plate was covered and incubated at room temperature for 30 minutes. The plate was washed three times with 300µl of the wash solution 1x at the end of the incubation period, followed by the addition of 100µl horseradish peroxidase conjugate into each well. Then the plate was allowed to incubate at room temperature for 30 minutes and washed three times for the second round before adding 100µl of the substrate solution to each test well, which was then incubated for 15 minutes in a dark place at room temperature. Finally, 100µl of stop solution was added to each well to stop the reaction, and the absorbance was read at 450 nm using a microplate reader (Bedasa *et al*., 2022).

2.5 Data management and statistical analysis

The data collected in this study was stored in MS Excel and analyzed. The mean antibody titer, standard deviation, and coefficient of variation (CV %) have been calculated using Microsoft Office Excel. Furthermore, STATA version 13 (College Station, USA) was used to analyze the data obtained from the study. Specifically, a student t test was performed to compare the mean antibody titer of different groups. The differences were considered statistically significant at the level of $P \leq 0.05$.

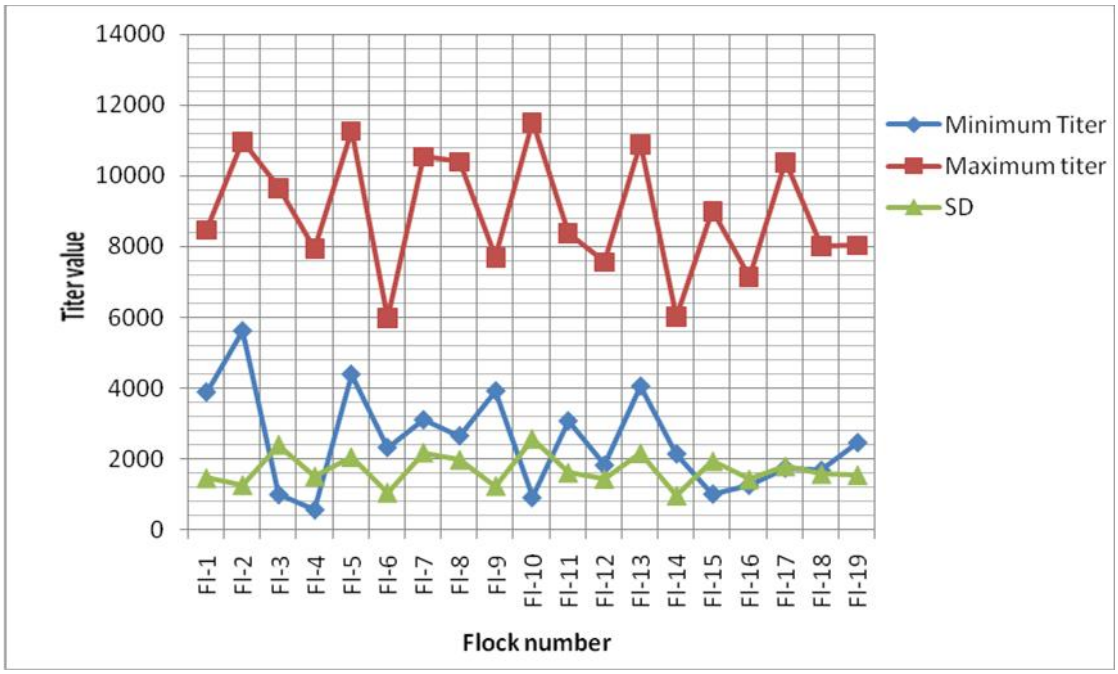
3. Results

From the total number of chickens vaccinated irrespective of the flock and length of time after they are vaccinated, 398 (99.75%) have developed an antibody which was protective as the antibody titrations measured. Among the flock samples from the study, flock number 4 was found with one chick that showed antibody titre below the protective level. In all other flocks, there is a difference in the measure of vaccine titre among chickens sampled from each flock. Accordingly, the highest titre measure of 8245.30 (7650.19, 8840.41) was recorded from flock 2 as compared to the minimum titre of 4129.78 (3670.07, 4589.50), which was measured from flock 6 (**Table 1**). The minimum and maximum vaccine titre measures of each flock were better described in **graph 1** below, and similarly, the vaccine titre range was also depicted.

Though the average number of chickens sampled from each flock was 21, the actual number varied among the flocks included in the study. The study identified differences in antibody titre between flocks by using an equal number of chicken samples from each flock. Accordingly, flocks 1, 4, 10, and 11 were found with different antibody titres despite equal (22 from each) chicken samples being sampled with the highest titre 5963.30 (5312.99, 6613.61) measured from flock 1 (**Table 1**).

Similarly, flocks 2, 7, 8 and 13 and flocks 3, 6, 12, 15 and 17 were also found with different antibody titre measures despite having the same number of chickens included in the study, 20 and 23, respectively. In the first category, the highest titre was measured in flock 20 (8245.30 (7650.19, 8840.41)) and in the second group, from flock 3 (6580.73(5541.18, 7620.29)). The remaining three flocks; 5, 14, and 19, each had a different number of chickens; 11, 24, and 18 were included in the study. The second highest titre measure was recorded from flock 5 from its 11 chickens included in the test, whereas the second lowest titre (4212.02(3800.98, 4623.06)) was measured from flock 14 from the highest number of chickens included in the study (**Table 1**).

The vaccination titre measurement was also assessed by taking different risk factors into account. The production status of chicken was found to be significantly associated (P = 0.0254) with the production of the protective level of antibody. In line with this, layers were found to have developed a higher amount of titre (5961.47+348.35) as compared to the pullets (4610.24+280.544). The hygienic status of the farm was not significantly associated (P = 0.5828) with the development of the protective level of vaccine titre in chickens. All other risk factors: breed of chicken managed, management system, and vitamin supplementation of the farm were evaluated for the existence of statistically significant association with the difference in mean vaccine titre measure as the P value can't be executed because a single flock in one category was compared with a number of flocks in the counterpart (**Table 2**).



Graph 1: Graphic presentation of the minimum and maximum Ab titer for each flock

Table 1: The mean titer value of each flock using the ELISA test and the percentage of chickens with protective antibody titer after being vaccinated with LC-75 vaccine per flock

Flock number	Number of samples	Days since last vaccination	Minimum Titer	Maximum titer	Mean (95%CI) titer	SD	CV%	Percentage of animals per flock with protective titer
1	22	5m	3883.86	8478.16	5963.30 (5312.99, 6613.61)	1466.72	24.60	100 (22/22)
2	20	8m	5609.41	10974.32	8245.30 (7650.19, 8840.41)	1271.56	15.42	100 (20/20)
3	23	11m & 3wks	991.77	9656.45	6580.73(5541.18, 7620.29)	2403.97	36.53	100 (23/23)
4	22	4m	560.79	7948.08	4328.84(3658.74, 4998.94)	1511.36	34.91	95.5 (21/22)
5	11	9m	4385.22	11273.83	7279.13(5898.29, 8659.98)	2055.41	28.24	100 (11/11)
6	23	71dys	2320.93	5971.74	4129.78(3670.07, 4589.50)	1063.10	25.74	100 (23/23)
7	20	11m & 3wks	3102.85	10550.81	7065.60(6045.18, 8086.01)	2180.31	30.86	100 (20/20)
8	20	1yr,11m & 3wks	2648.57	10397.85	5705.34(4778.80, 6631.87)	1979.71	34.70	100 (20/20)
9	21	1yr & 45dys	3917.12	7695.62	5963.72(5397.20, 6530.25)	1244.59	20.87	100 (21/21)
10	22	7m & 2wks	906.57	11522.69	5773.87(4634.69, 6913.05)	2569.33	44.50	100 (22/22)
11	22	4m& 2wks	3070.72	8374.69	5659.43(4942.04, 6376.82)	1618.01	28.59	100 (22/22)
12	23	6m& 2wks	1820.87	7557.25	4285.91(3662.14, 4909.68)	1442.47	33.66	100 (23/23)
13	20	5m& 2wks	4050.98	10903.06	7130.91(6118.14, 8143.68)	2163.97	30.35	100 (20/20)
14	24	6m &2wks	2140.28	6008.14	4212.02(3800.98, 4623.06)	973.42	23.11	100 (24/24)
15	23	15wks	1005.28	9009.85	4275.48(3312.51, 4988.69)	1938.08	45.33	100 (23/23)
16	21	15wks	1254.85	7147.71	4666.15(4015.18, 5317.12)	1430.10	30.65	100 (21/21)
17	23	11m	1740.91	10390.18	4318.70(3542.9, 5094.51)	1794.05	41.54	100 (23/23)
18	21	1yr,11m	1674.58	8013.7	5153.03(4432.17, 5873.90)	1583.64	30.73	100 (21/21)
19	18	1yr & 3m	2456.21	8035.87	4411.33(3638.88, 5183.79)	1553.33	35.21	100 (18/18)

CI=Confidence interval; CV=Coefficient of variation; M=Months;SD=Standard deviation; W=Weeks and Yr= Years

Table 2: Student t-test comparison of mean titers among categories under each variable

Variables	Categories	No. of flocks vaccinated with	Mean ± SE*	T-test	P-value
Breed types	Bovans	18	5603.48±297.95	-	-
	Sasso	1	4285.91		
	Combined	19	5534.14		
	Difference		1317.57		
Management	Intensive	18	5449.05 ± 293.35	-	-
	Semi-intensive	1	7065.598		
	Combined	19	5534.135		
	Difference		1616.544		
Production stage	Layer	13	5961.47± 348.35	2.4500	0.0254
	Pullet	6	4608.24± 280.544		
	Combined	19	5534.14 ± 290.24		
	Difference		1353.23± 552.34		
Hygiene	Good	13	5646.71± 363.14	0.5599	0.5828
	Poor	6	5290.24 ± 507.12		
	Combined	19	5534.14 ± 290.24		
	Difference		356.47± 636.65		
Vitamin supply	Provided	18	5604.06 ±297.80	-	-
	Not provided	1	4275.48		
	Combined	19	5534.14		
	Difference		1328.58		

SE=Standard Error; Dash showed due to a single flock in one category was compared with a number of flocks in the counterpart.

4. Discussion

The current study revealed that chickens vaccinated with the IBDV LC-75 vaccine had a protective level of antibodies for a minimum of two months, as it had been 71 days since the flocks were checked for serological antibody titre during the study. Results obtained from this study showed an average protective antibody titer in 99.75% of chickens tested.

According to the ELISA test results from 19 flocks, nearly all 399 chickens displayed sufficient seroconversion in the flocks, which was higher than the estimated S/P ratio of 0.3. This is because a vaccination's success was not influenced by a number of variables. In contrast to the fact that it was confirmed by various literature reports that vaccine failure can be brought on by the type of chicken (layer or broiler), the immune system's delayed maturation, a poor vaccine's immunogenicity, a chicken's age, a genetic inability to respond to a specific vaccine antigen, and other biological factors, as the virus weakens the humoral and cellular immune responses in chickens (Rautenschlein *et al*., 2002), infection with IBDV may exacerbate prior infections with other infectious agents and decrease the bird's ability to respond to vaccination(Sharma *et al*., 2000; McMullin, 2020).Moreover, dose, route of administration, revaccination, vaccine storage, and number of birds per drinking place (Witter, 2001; Farooq *et al*., 2002;De & Cook, 2014) are known for their immune suppressive effects and may play a role in low vaccine titre development. This can further be supported by the reports of Shrestha *et al* (2003) who said that immunization by vaccination could not give 100% protection against IBD.

Besides this, the measure of the protective level of the vaccine was not consistent with the age of the chick after the vaccination time. Accordingly, some flocks have a high measure of protective mean vaccine titre a short time after the delivery of vaccine and others have a low vaccine mean titre after shot time of vaccine delivery. The same is true in the opposite. Such variation in the mean measure of vaccine titre might be due to variation in the production stage of the chicken involved in the survey and the hygienic status of the farm (Kaufer and Weissi, 2005). Insufficiencies in vitamin supplementation, breeds of chicken and management strategy might have also posed their impact in the difference of titre measure (Dey*et al*., 2019).

Moreover, the difference in the titre measure of vaccine among individual chickens might also have contributed to the discrepancies in the mean measure of the vaccine titre among different flocks. With regards, flocks with high mean titre measure may have many individual chickens with high titre measure and few chickens with low titre measure the mean of which may become very high and the opposite might be true for those with low mean titre measure (Abdullahi *et al*., 2009; Bosha and Nongo, 2012).

The presence of a significant association (P <0.0254) was detected between the production stage and the antibody titre. Interestingly, flocks with a majority of chickens that are pullets (below 5 months) were found with a very low antibody titre measure. For example, flock number 6 (4129.78 (3670.07, 4589.50) (4months since vaccination) and flock number 15 (4275.48 (3312.51, 4988.69) (3.2months since vaccination) have less protective antibody titers based on the ELISA and found to agree with the result report of (Abdullahi *et al*., 2009; Tizard*et al*., 2017). Such low immune titre measure in young age category may be due to less developed immune function of younger chicken but disagreed with result of (Witter, 2001; Jakka *et al*., 2014) who reported that pullets vaccinated with IBD live-vaccine showed a significant IBD antibody level increase at 42 and 71 days post hatch.

Another risk factor assessed for association with the antibody titre measure was the hygienic status of the farm. There was no significant correlation between adequate IBD serum antibody titers and any of the chickens sampled under different hygienic levels (P>0.5828). This finding disagreed with earlier works of Dey *et al* (2019) and Sharma *et al* (2000)who reported that improper cleaning of the beddings will expose

young birds to the virus at an early age which can cause severe, long-lasting suppression of immune system and immunocompromised birds do not respond well to vaccination and are more susceptible to other infections.

5. Conclusion and Recommendations

This study showed that the IBDV LC-75 vaccine induced an adequate protective immunological response. But the titre measures were not consistently uniform in different flocks as compared to the age of the chickens. The findings were measured in chickens with good hygienic status and support the fact that chickens have to be kept in a clean environment so as to avoid any compromising factors contributing to the reduced immunogenicity. Layers older than 5 months have shown a better immune response to the IBDV LC-75 vaccine than pullets. Moreover, the differences in the titre measure among different flocks, irrespective of time elapsed, showed the possible association of vaccination inaccuracies due to factors that can be chicken, the vaccine itself, or vaccination procedure-related. Therefore, based on the above conclusion, the following recommendations were forwarded: (i) the low sero-conversion of some chickens emphasizes that chickens have to be tested regularly to evaluate vaccination failures that can be caused by many factors. (ii) Detailed studies on efficacy testing by challenging the chickens with virulent strains of the virus would suggest the overall protection level of the vaccines, and (iii) further study is also needed to examine the protective efficacy of this and other IBD vaccines being used in Ethiopia.

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Disclosure

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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