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Jacobs Journal of Veterinary Science and Research

Research Article

Status of Humoral Immunity against Newcastle Disease Virus in Commercial Poultry Farms in Ethiopia

Asamenew Tesfaye[1], Rediet Belayneh[1], Asegedech Sirak[1], Gizat Almaw[1], Tadewos Kassa[1], Hassen Chaka[1] and Teshale S[2].

[1]National Animal Health Diagnostic and Investigation Center (NAHDIC), P. O. Box 04

[2]College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia

**Corresponding author:* Teshale S, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia. e-mail: teshalesori2002@yahoo.com

Received Date: 09-19-2018

Accepted Date: 10-23-2018

Published Date: 10-31-2018

Abstract

Vaccination is the method of choice for control of Newcastle disease in developing countries. A cross-sectional survey was conducted in six commercial poultry farms to assess their immune status against Newcastle disease in Ethiopia. In addition, experimental study was carried out in 210 day old chickens belonging to Rose, Lohmann brown and Sasso breeds to compare their response to HB1 and La Sota vaccines. Blood samples were collected from 75 day-old chickens (25 chickens from each breed) to assess the level of maternally derived antibody titer before vaccine was given. Blood samples were collected fortnightly after the first and booster vaccinations. Haemagglutination inhibition assay was used to assess the level of antibody in serum samples collected. Only two of the six farms investigated maintained good flock immunity (≥85% level of protection) with low percentage geometric coefficient of variation (4.9% -14.4%). The maternally derived antibody titre was significantly higher in Sasso breeds (134.4±1.14) followed by Ross (92.2±1.14) and Lohmann brown (68.6±1.35). The level of anti-Newcastle disease virus antibody after the first vaccination was 24.6 ± 1.2 in Lohmann brown, 29.3±2 in Sasso and 34.5±1.1 in Ross breeds. The antibody titer was 30.9±1.3 in Lohmann brown, 29.4±1.2 in Sasso and 33.1±1.2 in Ross breeds after booster vaccination. In conclusion the maternally derived antibody level was sufficient to protect chickens from infection during early age but the flock immunity and post vaccination antibody level was low suggesting the investigation of alternative vaccination schedules for better prevention and control of Newcastle disease than the current one (day 0, day 7 and day 21).

Keywords: Breed; %GCV; Flock immunity; HI; MDA

Introduction

Infection of chickens with virulent strains of Newcastle disease virus (NDV) causes considerable economic losses as a result of high mortality [1]. In Africa and Asia Newcastle disease (ND) is a major constraint for development of commercial and village poultry production [2]. In Ethiopia it was reported for the first time in 1972 in the then Eretria province from which it spreads to all poultry producing areas [3] causing up to 80% mortality in naive flocks [4]. Newcastle disease is caused by virulent strains of avian avulavirus type 1 (APMV-1) in the genus *Avulavirus* belonging to the family *Paramyxoviridae* [5, 6]. The strains of NDV are genetically highly diversified and are known for their great variation in pathogenicity in chickens [7].

Optimum control of ND relies on appropriate use of safe and efficient vaccines. Live attenuated vaccines (LAV) prepared from lentogenic strain of NDV are commonly used in broiler and layer flocks [8]. It has been shown that circulating and mucosal antibodies in addition to cell-mediated immunity are elicited by LAV-NDV vaccines in chicken. However, live vaccines such as La Sota have been known to induce post-vaccination reactions such as respiratory signs when given during the first few days, or weeks of life [9]. Inactivated NDV vaccines on the other hand are safer and provoke strong circulating antibody response but are less efficient in inducing cell-mediated immunity [10]. Sequential combination of live attenuated and inactivated vaccines has been shown to elicit high level of humoral and cellular responses [11]. In Ethiopia, vaccination of day old chicks with Hitchner B1 followed by booster vaccinations of La Sota at day 7 and 21 is a common practice. Despite intensive vaccination using this vaccination schedule, outbreaks of ND have been reported frequently from Ethiopia. Neutralization of the vaccine antigens by maternally derived antibody might have contributed to the vaccination failure. This study was, therefore, conducted to assess the level of maternally derived antibody and the status of immune responses of chicken after vaccination with Hitchner B1 and La Sota in Ethiopian poultry settings following the manufacturer's recommendation.

Materials and Methods

Study Farms

Six poultry enterprises (three breeding centers, one large scale and two small scale commercial layer farms) were purposively selected from Addis Ababa, Bishoftu and Wolkite, all located in central Ethiopia. The farms were coded as FMC (Multiplication center), SSC (small scale commercial) and LSC (Large scale commercial) poultry farms.

Study design

This study consists of two parts. Firstly, a cross-sectional study was carried out between July and November, 2015, to assess the status of flock immunity in the adult chicken population of the selected poultry farms. Secondly, an experimental immunization study was conducted in 210 day old chicken of different breeds (Ross = 65; Lohman brown = 65 and Sasso = 80) obtained from Alema and Gubre breeding farms to assess their response against Newcastle disease vaccination. Of this, day-old chickens (25 chickens from each breed) were sacrificed and sera were collected to assess the level of maternally derived antibody titer. Another batch of 25 day-old chicks of each breed was vaccinated with Hitchner B1 and La Sota produced at National Veterinary Institute, Ethiopian via eye drop on days 7 and 21, respectively.

Sample collection

Two to three mL of blood were collected from the wing vein of each bird. Blood samples were kept at room temperature overnight and sera were collected into cryovial tubes. The serum samples were transported to National Animal Health Diagnostic and Investigation Center on ice and stored at -20°C until analyzed.

Preparation of chicken red blood cell (RBC) suspension

About 5 mL of RBC were collected from chickens maintained in SPF module (in National Veterinary Institute) and mixed with an equal volume of Alsever's solution. The blood was centrifuged at 1500 g for 15 minutes at +4°C. The plasma and buffy coat component was siphoned with a pipette and discarded. The RBC component was washed three times with PBS and a final concentration of a 1% RBC (v/v) suspension was prepared using PBS.

Haemagglutination inhibition (HI) assay

The HI assay was performed as described by OIE (2012) [5]. Briefly 0.025 mL of PBS were dispensed into each well of a V-bottomed microtiter plate (Nunc). Twofold dilutions of sera samples were prepared in test wells of the plates to which 4 haemaglutination units (HAU) of the antigen prepared from La Sota was added. The plates were left for 30 minutes at room temperature. Then 0.025 mL of a 1% chicken RBC suspension was added to each well and mixed gently. The plates were kept for 40 minutes at room temperature to allow the RBC to settle. Positive and negative controls were added to positive and negative control wells. The results were recorded based on evidence of agglutination. The HI titer of each serum sample was expressed as the reciprocal of the highest serum dilution not showing agglutination [5]. Samples were considered protective to virulent infection when HI titres were ≥ 4 $\log_2$.

Data analysis

Geometric mean antibody titer, standard deviation and % geometric coefficient of variation (% GCV) were computed. A one -way analysis of variance was performed to compare the geometric mean antibody titer between farms and breeds. Student t-test was used to compare the mean antibody titer after the first and second vaccination. The level of protection was expressed as excellent, good, fair and poor when the percentage coefficient of variation (% CV) was <30%, 30-50%, 51-80% and >90%, respectively among chicken within a group. For all tests and comparisons, $P < 0.05$ was considered statistically significant.

Results

Assessment of flock immunity

Table 1 shows the status of flock immunity against NDV in chicken raised on the farms included in this study. Chicken sampled from two multiplication centers ($FMC_1$and FMC_2) maintained good level of flock immunity, with 92% level of protection in both cases. In contrast, poor level of flock immunity was observed in chicken sampled from small scale farms (FSC_1 and FSC_2). Similarly the geometric mean antibody titer (Table 2) was highest in chicken raised in farms with good flock immunity (FMC_2 and FMC_1) and lowest in chicken raised by farms with poor level of flock immunity (FSC_1 and FSC_2). The difference in GM antibody titers among the farms was statistically significant (Table 2). The % GCV for the HI titer was below 30% in all farms suggesting uniform response to vaccination.

Table 1: The results of assessment of flock immunity in chicken raised in different poultry farms.

Farms	Number sampled	Number & percent (%) with protective antibody level
FMC_1	100	92 (**92.0**)
FMC_2	100	92 (**92.0**)
FMC_3	100	46 (**46.0**)
FLS_1	48	32 (**66.7**)
FSC_1	35	11 (**31.4**)
FSC_2	25	6 (**24.0**)
Total	**408**	**279 (68.4)**

Table 2: Geometric mean antibody titer against newcastle disease virus in chicken sampled from different poultry farms.

Farms	Antibody titer														
	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	2^{11}	$>=2^{12}$	Total	GM titer ±SD	%GCV
FMC_1	2*	3	3	**11**	**15**	**5**	**4**	**2**	**7**	**14**	**10**	**24**	100	122.1±1.8	9.6
FMC_2	-	7	1	-	-	-	**1**	**7**	**21**	**35**	**17**	**11**	100	356.2±1.6	4.9
FMC_3	6	21	27	**11**	**7**	**3**	**-**	**1**	**-**	**-**	**-**	**24**	100	6.9±1.6	14.2
FLS_1	1	3	11	**9**	**12**	**4**	**3**	**1**	**-**	**-**	**1**	**3**	48	17.1±1.5	14.4
FSC_1	4	6	12	**4**	**3**	**1**	**-**		**-**	**-**	**-**	**3**	35	6.4±1.6	11.4
FSC_2	2	4	10	**1**	**4**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	25	7.2±1.6	9.3

Note: GM= Geometric mean, SD= Standard deviation, %GCV= percentage coefficient of variation. *=the number of chickens under each titer

Comparison of antibody response in different chicken breeds

The results of maternally derived GM antibody titer in day old chicken of Ross, Lohmann and Sasso breeds were 92.2±1.14, 68.6±1.35, and 134.4±1.14, respectively (Table 3). Since the %GCV ranges from 14.2 to 35, the difference in GM antibody titer was statistically significant. The chicken in the here breeds considered had antibody titer that is sufficient to confer protection against virulent infection (4 log_2). The level of anti-Newcastle disease virus antibody after first vaccination was 24.6 ± 1.2 in Lohmann brown, 29.3±2 in Sasso and 34.5±1.1 in Ross breeds. The antibody titer was 30.9±1.3 in Lohmann brown, 29.4±1.2 in Sasso and 33.1±1.2 in Ross after booster vaccination was provided.

Discussion

Vaccination remains an affordable means of controlling ND in Ethiopia. In this regards, maintaining good level of flock immunity is crucial. For ND a good level of herd (flock) immunity is achieved when a high proportion of chickens (>85%) has higher antibody titre (haemagglutination inhibition titre of ≥ 3 log2) after two vaccinations [12, 13]. In this study, only chicken flocks from two out of three multiplication centers maintained good level of flock immunity (92%) with higher than 3 log2 antibody titre. Since full protection is considered when the anti-NDV antibody titre reaches 4 log2 or higher [10], these multiplication centers maintained flock immunity that is sufficient to provide full protection. The rest of the farms did not maintain good level of flock immunity. One of the multiplication center (FMC3), the large scale farm (FLS) and the two the small scale farms considered (FSC1 and FSC2) had antibody titer lower than the protective level (4 log2). This suggests that chickens raised on these farms are at high risk of acquiring infection with NDV despite vaccination. The difference observed in maintaining flock immunity among the farms studied could be due to variation in the route of vaccine administration, variable vaccination schedules and improper storage of vaccines. Besides, the variable prevalence of immunosuppressive infection could be a reason for variability in flock immunity observed [6, 10]. The vaccination schedules and routes of vaccination varied among the farms studied.

In this study the level of maternally derived antibody titres in day-old chicks was found to be higher and sufficient to provide protection against virulent NDV infection. This suggests that maintaining good flock immunity in breeding chickens is crucial in the prevention of outbreaks of ND during the first days of life. Both systemic and mucosal antibodies have been shown to be sufficiently transferred from hens to their chicks. For instance, 27 % to 40% of

Table 3: Maternally derived anti-NDV antibody titer in chickens of different breeds.

Breed	№ of chicken	Min. titer	Max. titer	GM ±SD	%GCV
Ross	25	32	256	92.2±1.14	14.2
Lohmann	25	4	256	68.6±1.35	35.0
Sasso	25	32	256	134.4±1.14	14.2

Note: Min=Minimum; Max= Maximum; SD=Standard deviation; GCV=Geometric coefficient of variation

NDV specific antibodies of all immunoglobuline classes were shown to be passively transferred from hens to chicks, which are directly proportional to antibody titre in the hens [14]. Therefore, maintenance of good flock immunity in breeding hens can provide sufficient level of antibody in chicks that can protect them during early ages. On the other hand higher level of maternally derived antibody in day-old chicks can interfere with vaccines when given to day old chicks or if administered during the first two weeks of life. In a comparative study carried out on SPF chicks and chicks with high level of maternally derived antibody revealed that protection was delayed by 1 – 2 weeks in chicks with maternally derived antibody [15]. Immunization with live attenuated ND vaccine in chicken with high level of maternally derived antibody failed to provide protection since the vaccine virus was neutralized by the antibody. This could be either by sequestration of the vaccine antigens or restriction of replication of the vaccine viruses [16, 17]. This situation may result in ND outbreaks in chicken vaccinated during early age when the level of maternally derived antibody level is high. Hence, knowledge of the level of maternally derived antibody level is important for chosing an optimum vaccination schedule.

The titre of maternally derived antibody significantly varies among breeds of chicken although all of them had higher than protective level. This suggests that different breeds of chicken respond differently to ND vaccination. This in turn has effect on the level of flock immunity and incidence of ND. However, when vaccinated with Hichner B1 and La Sota on day 7 and 21, respectively the level of anti-NDV antibody in all the three breeds of chicken was lower than the level of maternally derived antibody. Even after booster vaccination was provided the antibody titer was lower than the maternally derived antibody titre. This observation suggests the neutralizing effect of maternally derived antibody on the vaccine virus implying the need to wait until the maternally derived antibody level evades. Some of the chicks had antibody titre that is greater than 1024 (210). Such high antibody titre has been previously reported in sub-clinically infected chicken [18]. In conclusion, this study showed that the level of flock immunity against NDV was low implying the risk of infection with virulent NDV. The level of maternally derived antibody was comparable among the breeds of chicken considered; however, the post-vaccination antibody level was minimal to provide protection.

Acknowledgements

The authors would like to thank Dutch embassy and Addis Ababa Chambers of Commerce in supporting this project work.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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