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Molecular Detection and Antibody Level Determination of Infectious Bursal Disease Virus in Chicken Raised in Diredawa and Harar Town, Eastern Ethiopia

[1]*Fethu Lemma,* [1]*Tesfahiwot Zerihun,* [2]*Dirsha Demam,* [1]*Nateneal Tamerat and* [1]*Serkalem Midaye*

[1]Haramaya University, College of Veterinary Medicine, Ethiopia
[2]Wolkite University, Department of Animal Science, Ethiopia

Abstract: Various biological and socio-economic aspects are increased for the decrement of poultry population in Ethiopia. An infectious bursal disease is among the serious diseases of poultry. It causes a great loss in backyard chickens. This study was conducted with the aim of detection of infectious bursal disease viruses from unvaccinated chickens and determination of the antibody level from vaccinated chickens in Harar, DireDawa towns Ethiopia. A total of 384 serums; and 80 bursal tissue samples were collected and tested using enzyme linked immnosorbent assay for antibody level determination; and RT PCR tests for detection of the virus. The result showed that the overall population with protective antibody level of vaccinated chicken optical density value >= 0.306 was 57.3% ranging from 55.8% and 42.6 % in Harar and DireDawa town respectively and the significant difference between the study areas was found ($p < 0.05$) ($x^2 = 14.67$). Out of 80 bursa tissues diagnosed, 81% were detected by RT-PCR. Therefore, this study showed that the overall population with protective antibody level of vaccinated chicken was unsatisfactory as almost half of the birds had antibody titer below the standard level and because of this, the overall percentage was not met 70% and above, this indicate that the chicken were relatively susceptibility to the infection. However, in unvaccinated chickens higher percentage of virus was detected, this indicated that the disease is widely distributed in the study areas. Finally, based on the findings of the study, important conclusion was made to tackle the problems resulting from the diseases in the study areas.

Key word: Antibody Level · Chicken · Enzyme Linked Immune Sorbent Assay · Infectious Bursal Disease · RT PCR

INTRODUCTION

Infectious bursal disease (IBD) is caused by IBD virus which especially elicits a highly contagious infection of young chickens. This virus primarily targets lymphoid tissue and results in extreme kidney damage in birds that are infected [1]. Currently IBDV has a worldwide distribution, occur in all major poultry producing areas. During 63th general session of the world organization for animal health, it was estimated that IBD has considerable socio economic importance at the international level as the disease is present in greater than 95% of the member country and the occurrence of acute clinical cases very virulent IBD (vvIBDV) was reported in 80% of the country [2, 3].

Many biological and socio-economical factors are incriminated for the decrement of poultry population in Ethiopia, of which, diseases are the most important responsible factors. IBD and Newcastle diseases ND are among the different diseases causing damage in the poultry production in the country [4]. Although the diseases are the major health constraints responsible for marked economic losses in a country, the dynamicity and the status of the disease in chickens in the study areas have not been yet studied to full extent and not well documented. Therefore, the objectives of this study were to isolate IBDV from unvaccinated chickens and to determine the level of specific antibody against IBDV from vaccinated chickens in the study areas.

MATERIALS AND METHODS

Detection of the virus and determination of the level of antibody in unvaccinated and vaccinated chickens of

Corresponding Author: Fethu Lemma, Haramaya University, College of Veterinary Medicine, Ethiopia.
Cell: +251-933438991.

infectious bursal diseases virus of chicken in Harar and DireDawa towns was conducted during the period from October 2012 to March 2014 in the National Veterinary Institute Laboratory Ethiopia.

Study Design: A cross-sectional study was conducted from October, 2012 to March, 2014 to detect IBDV from unvaccinated chickens back yard and the antibody levels determination in vaccinated chickens reared in small scale poultry production system and taking into consideration of their age, breed, vaccination status and location.

Sample Size Determination and Sampling Method: Since there was no prior similar research work conducted in the study area, the expected presence antibody level of 50% was assumed to get the maximum number of sample size required to determine the prevalence. The absolute precisions were decided to be 5% at 95% confidence level, thus for sample size estimation the formula described by Thrusfield [5] is used as shown below:

$$n = \frac{1.96^2 \times P_{exp}\left(1 - P_{exp}\right)}{d^2}$$

Where,
n=required sample size,
P_{exp}= expected prevalence,
d^2= desired absolute precision

Accordingly, a sample size of 384 samples was used to perform the ELISA test. However, 80 samples were additionally collected for virus detection so that a total sample to be 464.

Selection of sample was made using a deliberate unbiased process. So, multistage cluster sampling procedure was followed to get sampled birds. This was conducted by dividing the study population into exclusive groups and then number of sampling units selected from each stratum. Study sites were selected based on the existing epidemiological situations such as poultry density, commercial poultry farming activities and following the route of poultry dissemination from multiplication centers.

Blood Collection and *Elisa* Test, Validity and Interpretation

Blood Collection: Four ml of blood were collected using sterile syringe. Sera were prepared and the clarified sera were then stored at -20°C until tested [1, 6].

ELISA Test, Validity and Interpretation: Briefly, the serum samples and the antigen reagents that preserved at -20°C and 4°C respectively were incubated in room temperature at 22° C for 30 minutes prior to the test. All the serum samples were heat inactivated at 56°C for 30 minutes in a water bath [7]. The serum samples were then processed and tested according to manufacturer instruction using commercial ELISA infectious bursal disease virus antibody test kit at the National Veterinary Institute (NVI).

Validity: IBD ELISA result was obtained when the average optical density (OD) value of the normal control serum was less than 0.250 and that of the corrected positive control value range was between 0.250 and 0.900. If either of these values was out of range, the IBD test result was considered as invalid and the samples were retested. OD value range of normal control serum was between 0.07-0.250 and for positive control serum 0.296-0.82. For interpretation of the test results, a sample to positive ratio (S/P) of each test serum was required. Under IBD ELISA titer, the Sample to positive ratio was calculated using the following formula as recommended by the manufacturer:

$$Sp = \frac{\text{Sample absorbance - Average normal control absorbance}}{\text{Corrected positive control absorbance}}$$

Where, corrected positive control absorbance = Positive control mean – Negative control mean

Samples Collection and Preparation of Total RNA Extraction

Samples Collection: A total of 80 bursal tissues from sick and dead chickens aged between 8 - 12 and 20- 24 weeks were collected from study areas for successful isolation and identification of viruses. The sample was put into labeled universal bottles containing phosphate buffered saline (PH 7.0-7.4) containing penicillin and Gentamycine.

Preparation and Total RNA Extraction: The bursa of fabricius was removed aseptically from the chickens and was chopped using two scalpels, later 100mg bursa tissue was mixed with small amount of peptone broth containing penicillin and streptomycin (1000 microgramm/ml each) and 500µl of TRIzol reagent and then the mixture was homogenized in a tissue blender. The remaining process and test was conducted according to guideline instruction at the National Veterinary Institute (NVI).

Table 1: Primers sequences used for IBDV PCR

Directions	Sequences	Nucleotide positions
Forward	5'-TGTAAAACGACGGCCAGTGCATGCGGTATGTGAGGCTTGGTGAC3'	(587- 604)
Reverse	5' CAGGAAACAGCTATGACCGAATTCGATCCTGTTGCCACTCTTTC- 3'	(1212-1229)

Reverse T- Polymerase Chain Reaction: The total RNA extracted was subjected to reverse transcription using 100ng random hexamer primers (Table 1), 50ng heat denatured viral RNA, 50 units RNAase inhibitor, 2µl of 0.1M DTT, 1µl of 10mM dNTPs mix, 4µl of 5X RT buffer and 200 units Superscript II reverse transcriptase. The 20 µL reaction mixture was incubated at 25°C for 10min and then at 42°Cfor 50min. Reverse transcriptase was inactivated by heating at 70°C for 15min. The oligonucleotide primers were used for the amplification of 604 bp amplicons corresponding to very variable region of the VP2 gene of IBDV. For the amplification, 6µl of cDNA was incubated in total volume of 50µl reaction mix containing 5µl 10X PCR buffer, 20pmol each of the forward and reverse primers, 1µl of 10mM dNTPs mix, 3U of *Taq* DNA polymerase (Bangalore Genei, India). The incubation temperature and duration of each cycle of the PCR were 1min at 94°C for denaturation, 1min at 52°C for annealing and 1min at 72°C for extension [8, 9].

Data Management and Analysis: Data collected from questionnaire survey and results of laboratory assays were appropriately described. Laboratory results were entered and managed using Microsoft Excel (2010, Duxbury Press). Detection and antibody level determination were using the formula described by Thrusfield [5] as the total number of positive samples by the total number of sample tested. Descriptive statistics were employed using SPSS version 16.0. Chi-square test was used to see the association between prevalence and explanatory variables such as sex, age, breed. P-value <0.05 were considered as significant in all statistics.

RESULTS

Indirectly ELISA Test for Antibody Titer: The optical density readings ranged from 0.0100-0.760 and the OD value of positive sera of sample to positive ratio were greater than or equal to 0.306 considered as protective. The overall population with protective antibody level of vaccinated chicken OD value >= 0.306 was 57.3% (220/384) ranging from 55.8% (116/208) and 42.6 % (75/176) in Harar and DireDawa town respectively. It showed that the significant difference between the study areas was found ($p < 0.05$) ($x^2 = 14.67$). Conversely, the IBD vaccination antibody level in local breeds 60.5% (130/215) was lower than that of exotic breeds 63.9%; (108/169) ($p > 0.05$) ($x^2 = 14.67$) and the age of 8 -12 weeks 62.8% (103/164) and 20-24week 63.6% (140/220) ($p > 0.05$) ($x^2 = 12.54$). Therefore, in both cases, there was no significant difference observed between the study areas (Table 2).

The percentage population of antibody level was below 70%, which indicated that most of vaccinated chicken have not enough protective immunity against IBD. This is in line with the epidemic theory which suggests that if 70% of the population is immune, the disease outbreak is unlikely to occur because there are not enough susceptible to propagate diseases [10, 11]. According to Butcher, Yeganni [12] the difference in antibody level in areas might be sorts of factors in poultry production systems like: administration and handling of the vaccine, stress, timing, immunosuppression, management practices and vaccine strain. Vaccination failures like, live attenuated vaccine virus potency and poor management system has been also reported by Muller *et al.* [13]. However, similar findings on IBD vaccinated chickens and low antibody titers have been made by Maduike *et al.* [14] which was, out of 483 IBD outbreaks in broiler chickens investigated in India, 334 were among vaccinated flocks while unvaccinated flocks had only 149 outbreaks. This report, therefore, suggests that vaccination only could not be a guarantee for chickens unless with strong management system. As a result, most of the vaccinated chickens do not produce enough immunity to protect the infection and that they remain susceptible and challenge with the same infections regardless of all risk factors of, age, breed and locality in the study areas.

RT-PCR for Detection Infectious Bursal Disease Virus: Infectious bursal disease virus was extracted from bursal samples and detected the virus genome by the highly sensitive technique of nucleic acid based detection tests (RT-PCR) for the detection of the virus using IBDV specific primers (table 1) on clinical field samples. The PCR amplicons yielded a specific, clear and distinct band of RT-PCR product was appeared at the position of 604bp on ethidium bromide stained with the standard 100 bp

Table 2: Distribution of the levels of ELISA antibody titers of IBDV in vaccinated chickens by, age, breed and locality\

			S/P ratio ≥ 0.306 O.D value			
Risk factors		No. of Sample examined	n	%	x^2 test	P-value
Age (in weeks)	8 -12	164	103	62.8	12.54	0.082
	20-24	220	140	63.6		
Breed	Local	215	130	60.5	14.67	0.070
	Exotic	169	108	63.9		
Locality	Harar	208	116	55.8	14.67	0.028
	DireDawa	176	75	42.6		

Table 3: Detection of the IBDV from the study areas through RT-PCR

Study areas	No. of samples	Type of samples	No. of Positive samples (RT-PCR)	Percentage (%)
Harar	40	Bursa of fabricius	36	90
DireDawa	40	Bursa of fabricius	29	72.5
Total	80		65	81

DNA ladder passed through 1.5% Agarose gel electrophoresis. However, there was no amplification in the negative control after RT PCR [9, 15]. Extracted RNA of all 65 field samples, a total of 55 (85%) bursal samples were isolated. 65% (19/29) from DireDawa and 72% (26/36) from Harar (table 3) were found to be positive for IBDV of variable region of VP2 gene. This study results partially agreed with the findings of Banda [16]; Lee *et al*., [17]. The reason of high percentage of detection might be due to the local husbandry practices where different species of wild birds are raised together in the same open range environment encourage infection between birds [18,19]. This cross infection and maintenance of the virus in the environment would also serve as a source of the virus that circulation in the areas.

CONCLUSIONS

IBD are affecting the livelihood of the farmers in study areas, as the majority of the poultry population is found in the extensive backyard production system. These diseases introduced to the different poultry areas by workers, since most of farms have poultry in their house so they serve as a link between diseases and chickens. In the traditional practices systems where different species of wild birds are raised together in the same environment encourage infection between birds. As a recommendation: firstly, there should be fully characterize and identify the strains of viruses through sequencing and phylogenetics analysis of the circulating viruses and proper production of exact strains of vaccines. Secondly, the application of RT-PCR techniques on more numbers of samples followed by further studies using restriction enzyme analysis and sequencing will be helpful in generating epidemiological information in order to formulate a vaccination strategy for effective control of the disease. Additionally, attention should be given to vaccine quality which can be the result of lack of adequate storage facilities, application of expired vaccine batches, faulty application and vaccine handling during transportation from market to farm or due to the electricity failure. Furthermore, restriction of movement of backyard chickens from long distance for scavenging feed since the diseases maintained in wild birds, effective biosecurity should be kept in the commercial chickens since the viruses easily disseminate. Finally, continuous surveillance should be implemented for better understanding of the epidemiology of the diseases maintained in wild birds and their relation to the domestic chickens.

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