

Investigation of Newcastle Disease Virus Using Reverse Transcription Polymerase Chain Reaction in Selected Districts of Eastern Shewa, Ethiopia

Yemsrach Miressa¹, Yoseph Cherinet^{2*}, Abrha Bsrat³ and Fanos Tadesse²

¹Akaki Woreda Livestock and Fisheries Development, Ethiopia

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

³College of Veterinary Medicine, Mekele University, Ethiopia

Abstract

In Ethiopia Newcastle disease virus is the most important cause of loss in village-dwelling as well as commercially raised chickens. The disease occurs almost at any time of a year throughout the country including East Shewa Zone. A cross sectional study was therefore conducted from December 2014 to May 2015 to determine the prevalence of Newcastle disease in market and village chicken in selected districts of East Shewa zone of Ethiopia. Molecular diagnostic technique, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was employed to detect the viruses during the study. A total of 300 swab samples was collected using simple random sampling technique in Adama and Bishoftu Districts. An overall prevalence of 26.7% (40/300) was recorded during this study from the collected swab samples using real time PCR. The result of this study indicated that village chicken flock are endemically infected with Newcastle disease virus which could pose a threat to commercial poultry farms. Attention should therefore be given for regular monitoring of Newcastle disease virus in village chickens and wild birds and measures to prevent this infection should be taken.

Keywords: East Shewa; Ethiopia; Newcastle disease; RT-PCR

Introduction

Poultry play an important economic, nutritional and socio-cultural role in the livelihoods of poor rural households in developing countries, including Ethiopia. The total poultry population in Ethiopia is estimated at 56.87 million, 95.86% of which are village chickens [1]. In developing countries, chickens are most commonly owned by rural families. Many of these families have scarce resources and many may be headed by women. Increasing the productivity of their chickens would make a significant contribution towards increasing their food security and their ability to have secure livelihoods [2].

Despite this fact the contribution of poultry production to farm household and national income is not proportional to their population numbers due to various constraints like low inputs of feeding, poor management, the presence of diseases of various natures and lack of appropriate selection and breeding practices [3-5]. Poultry production is hampered by wide arrays of constraints among which infectious diseases including Newcastle disease (ND) [6]. ND is one of the major problems in village chickens in most parts of Ethiopia and the disease has become endemic in poultry population and recurs every year inflicting heavy losses [7-9].

ND induces up to 100% mortality as well as a decrease in egg production and poor egg quality. It is also highly contagious, septicemic, fatal and destructive disease which attacks chiefly chickens and turkeys usually in an acute, sometimes in sub-acute or even chronic form. Occasionally human being and even wild birds may be also infected with the virus [10]. It is present in endemic form with frequent out break indifferent parts of the country and it remains as a constant threat to the backyard poultry [11]. It disease occurs almost at any time of the year and widely distributed throughout the country for marked economic losses across the country despite the fact that, this has not been substantiated with laboratory investigations. Therefore,

the objective of this study was to determine the prevalence of ND in Village chicken in selected Eastern Shewa zone of Oromia region, Ethiopia.

Materials and Methods

Study design and area

A cross sectional study was conducted in December 2014 to May 2015 in selected districts of East Shewa namely Bishoftu and Adama Zones of Oromia Regional State which are located south east of Addis Ababa at 45 and 99 km. The total area coverage of the zone is 11,607 km² while the altitude of zone is ranging from 1000-3100 meter above sea levels. The mean annual rainfall of the zone is 500-1200 mm and the daily mean maximum and minimum temperatures were 30°C-42°C and 10°C-28°C respectively [1].

Study population

The population of this study was all backyard unvaccinated chickens in the two selected Districts. The study participants included all unvaccinated backyard chickens managed under traditional free ranging system randomly selected from the live bird market and

***Corresponding author:** Yoseph Cherinet, College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia, Tel: +251911804383; E-mail: yoseph.cherinet@aau.edu.et

Received August 24, 2016; **Accepted** September 14, 2016; **Published** September 19, 2016

Citation: Miressa Y, Cherinet Y, Bsrat A, Tadesse F (2016) Investigation of Newcastle Disease Virus Using Reverse Transcription Polymerase Chain Reaction in Selected Districts of Eastern Shewa, Ethiopia. J Vet Sci Technol 7: 384. doi: 10.4172/2157-7579.1000384

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village chicken population located within 10 kms area coverage from migratory birds' movement area.

Sample size determination

The Sample size was determined according to the formula given by Thrusfield with a 50% expected prevalence (considering no previous study was undertaken), 95% confidence level and 5% precision, sample size was calculated to be 384 [12]. However due to logistic reasons 300 samples were collected using simple random sampling technique.

Sample collection

The samples (tracheal and cloacal) were collected from live chickens by inserting sterile cotton tipped swab into the trachea gently swabbed its wall and cloacal swab deeply into the vent and gently swabbing the wall of the vent. Both the tracheal and cloacal swab samples were placed in sterile separate cryovial containing 2 ml of freshly prepared viral transport media (VTM). After sampling, the specimens were labeled, placed in ice pack and transported to the molecular laboratory at National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia. Sample collection and transportation were conducted according to the standard techniques recommended by OIE [13].

Sample analysis

RNA extraction: Viral RNA extraction from 300 tracheal and cloacal swabs were conducted using QIAamp viral RNA extraction kit according to manufacturer's instruction. The sample was centrifuged briefly in order to get cell free supernatant. The supernatant was lysed by adding 560 µl of prepared buffer AVL containing carrier RNA in to 1.5 micro centrifuge tube and 140 µl of sample was added to the buffer AVL carrier RNA in the micro centrifuge tube. The solutions were then mixed by pulse-vortexing and incubated at room temperature (15-25°C) for 10 minutes. The tube was then briefly centrifuged to remove drops from inside the lid. Then equal 560 µl of 96% ethanol was added to filtrate and mixed thoroughly and washed with 500 µl washing 1 and 2 (500 µl buffer AW 1 and 500 µl AW 2), any unwanted protein and DNA were removed. Then, 60 µl of elute solution (AVE) was added to collect the RNA. Finally, the eluted RNA was kept at -20°C (Qiagen, Inc., Gaithersburg, MD, USA).

Real-Time Polymerase Chain Reaction (RT-PCR): After RNA was extracted the Master Mix preparations were carried out in Ice bath. The reaction mixes were prepared by pipetting all the components of the Master Mixture in to 2 ml PCR tube according to the kit protocol. The reaction components were template RNA, primer solutions, dNTP Mix, and 5x QIAGEN One Step RT-PCR buffer, RNase-free water, probe and enzyme mix. Total RNA was extracted by scraping cells with RLT buffer and isolated according to manufacturers' instructions using the RNeasy Mini Kit (QIAGEN). The control that we used were NDV vaccine strain La Sotaa and RNase free water as positive and negative controls, respectively. Then Applied Bio-systems 7500 Fast Real-Time PCR thermo cycler were used for amplification of the extracted RNA. A primer probe combination from a conserved region of the M gene APMVI F M+4100 5'-AGT GAT GTG CTC GGA CCT TC-3', APMV-I R M- 4220 5'-CCT GAG GAG AGG CATTG CTA-3' and Probe APMV-1M+4169 5'-FAM TTCTCT AGC AGT GGG ACA GCC TGC TAMRA -3' was used to amplify all NDV isolates (Qiagen, Inc., Gaithersburg, MD, USA).

Data management and analysis

All the data collected were entered in Microsoft excel spread sheet and coded appropriately. The coded data was transferred in to SPSS version 20 software for analysis descriptive statics was used.

Results

Out of 300 tracheal and cloacal swabs samples, 40 (26.7%) were found positive for ND using RT-PCR analysis with a distribution of 16.7% and 10% in Adama and Bishoftu respectively (Table 1).

Discussion

The current study indicated that ND was observed as endemic disease in the study districts with the overall prevalence of 26.7% using RT-PCR. The highest prevalence in this finding was obtained from Adama Districts (16.7%) followed by Bishoftu (10%). The result has found in accordance with the previous report in Eastern Shewa who reported a prevalence of 24.2% and 14.2% in dry and wet seasons, respectively [6]. However, the finding was lower than previous report in Debre Berhan (28.7%) and Sebeta/ Nazaret areas (38.33%) [14]. ND prevalence of 6% and 5.9% in the wet and dry seasons respectively findings had reported from Eastern Shewa zone [6]. On the other hand, higher report (43.68%) was also recorded in central Ethiopia among local scavenging chickens kept under traditional management system [4]. Furthermore, the finding of the current study found higher than previous study result in southern Ethiopia of 19.78% [15] and in rift valley area of Ethiopia (11%) [16].

The overall variation of the findings might be due to variation on management system that may serve as a stress factor and favor infection. Poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies, absence of vaccination in traditionally managed chickens, and contact of chickens of one village with those in other villages may facilitate the spread of NCD [17].

Furthermore, the free range management system of poultry in the study areas might also allow the uninterrupted circulation of infection among the poultry flocks. Chicken could also prone to acquire infection from wild birds and local open markets where huge numbers of chicken are gathered which might also serve as continuous foci of infection [15].

Conclusion and Recommendations

The current study indicated that ND is endemic in village chicken of Eastern Shewa of Oromia region. Furthermore, important information has been provided by the study on epidemiological distribution of the disease in the area which alerts to look existence of the disease in other parts of the country and then to development effective prevention and control measures. This may indicate importance of implementing surveillances and bio-security practices in live poultry markets.

Based on the above concluding remarks the following recommendations were forwarded. Further studies should be conducted to understand and identify the circulating strains and pathos-types of ND virus with their dynamics in the study area. Strategic control and

District	No. of sample	M gene Positive	M gene Negative	Prevalence %
Adama	150	25	125	16.7%
Bishoftu	150	15	135	10%
Total	300	40	260	26.7%

Table 1: Prevalence of Newcastle disease using real time RT-PCR.

prevention measures should be implemented to reduce the impact of this disease on poultry production in the area. Extension work should be conducted to create awareness in flock owners about the transmission and prevention measure of the disease.

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