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Marek's Disease Virus: Molecular Detection From Chickens Feather In Central Ethiopia

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Abstract: On the basis of an outbreak report, the current investigation was undertaken in 2019 to detect Marek's disease virus from outbreak samples using Real time PCR from four purposively selected sites (Addis Ababa, Bishoftu, Sebeta, and Ambo). A total of 200 feather samples were obtained from chickens over the whole study area. The DNA of virus was extracted from feather tissue using a Qiagen® DNeasy Mini kit. Using Real time PCR, five pooled samples (2 from Bishoftu, 2 from Addis Abeba, and 1 from Ambo) were proven to be MDV. As a result of this research, it is advised that more research be done on the isolation and molecular characterization of chicken Marek's disease virus in all part of the country.

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Key words: Marek's Disease, Real Time PCR, Feather, Central Ethiopia

INTRODUCTION

Poultry have vital financial, nutritional, plus sociocultural role in the lives of less privileged rural households in low income countries. In Ethiopia the total number of chickens is supposed to be 43 million, with 97% of them being village chickens[1] .Even though its role in increasing incomes and reduction poverty in Ethiopian poultry farm owners, chicken farm is constrained through a number of issues, including infectious diseases including Newcastle disease, Infectious Bursal Disease, and Mareks disease. The most common ones are Mycoplasmosis, Pasteurellosis, and Salmonellosis [2]. Marek's disease in chickens is a extremely infectious lymphoprolifative illness.

Quail and turkeys can be naturally or experimentally infected, although chickens are more susceptible to the disease since they are the most significant natural host for the Marek's disease virus (MDV) [3,4]. The disease is a member of the Mardivirus genus, which is part of the Alpha-herpesvirinae subfamily of the Herpesviridae family [5]. In addition, the herpesvirus of turkeys (HVT), which belongs to the Mardivirus genus, includes two unique MDV species: MDV type 1 (MDV1), also known as Gallid herpesvirus 2 (GaHV-2), and MDV-2 (GaHV-3). MDV-1 is developed from MDV and contains all pathogenic strains as well as certain vaccine strains, whereas MDV-2 contains pathogenic strains that were originally obtained from apparently normal chickens [6]. MDVis spread mostly by feather dander (the white layer that surrounds emerging feathers), although it can also be spread through feces, poultry house dust, litter, blood, and saliva [5,7]. Anorexia, weight loss, paralysis of the legs, wings, and neck, grey eye, vision impairment, blindness, skin lesions, and poor performance are all symptoms of MDV infection. Clinical symptoms and gross or microscopic lesions are used to make a diagnosis. The disease (tumor) must be diagnosed, not the infection, for a definitive diagnosis.

Chickens can be infected with MDV for a long time without showing symptoms. MDV infection is detected through virus culture and the detection of viral nucleic acid, antigen, or antibodies. MDV is distributed all throughout the world, especially in Ethiopia. Several chicken farms have been infected with diseases of various etiologies as a result of the entrance of foreign breeds into the nation. The most serious health problems include viruses like Marek's disease (MD) and infectious bursal disease (IBD), which cause major losses.[8, 9] . In central Ethiopia, Lobago and Woldemeskel conducted a survey on a Marek's disease epidemic at a commercial chicken farm and found a 46% mortality rate[8]. Despite the fact that MDV is Ethiopia's most severe chicken illness, causing significant economic losses, viral isolation and information available on the virus strains circulating in the country is quite restricted. Therefore, the goal of this study was to detect MDV in Central Ethiopia using Real-Time PCR.

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MATERIALS AND METHODS

Study area

In 2019, samples were taken from chicken in the following locations: Addis Ababa, Bishoftu, Ambo, and Sebeta.

Sample collection

A total of 200(in pool of 5) feather chickens were sampled from clinically diseased chickens. The study included chickens of the entire ages and breeds managed in semi-intensive and intensive production and management systems. Each study site collected 40 (pool of five) feather follicles aseptically from MDV suspected clinically unwell chickens for nucleic acid detection. As a result, Addis Abeba has ten pools of five, Ambo has ten pools of five, Bishoftu has ten pools of five and Sebeta has ten pool of five. Samples were collected and delivered via cold chain to NAHDIC's diagnostic laboratory, where they were held in refrigerator at 20 °C until processing.

Ethics statement

In order to confirm the disease samples were gathered from infected chickens during the outbreak investigation. There was no animal experiment done. The National Animal Health Diagnostic and Investigation Center approved the collection of samples and their usage (NAHDIC). During data gathering in mareks disease assumed outbreak site, every effort was made to minimize animal suffering. The owners of the animals gave their permission for a veterinarian to collect Feather tissue samples.

Polymerase Chain Reaction test

Real-time PCR testing were used to examine forty Feather Samples (pool of five). The Qiagen® DNeasy Mini kit was used to extract viral DNA from Feather Samples in accordance with manufacturer's instructions. At the National Animal Health Diagnostic Center in Sebeta, real-time PCR tests were carried out using an Applied Biosystems 7500 Fast Real-Time PCR thermal cycler. The sequence of primer and probe used in this study was showed in the table 1 below. PCR was conducted in a final reaction volume of 20 µL containing Pilatnum PCR mix (6 µL), Primer-f (0.6 µL) ,Primer-R (0.6 µL) , Probe (0.8 µL), BSA (1µL) and RNase free Water (2µL) and 5 µL template DNA. The following cycling condition was used: an initial denaturation at 95°C for 15 min, followed by 45 cycles of denaturation at 95°C for 15s, annealing at 60°C for 15 s and extension at 72 °C for 25 s.

Table 1: Sequence of primer and probes used in this study

Primer and Probe used	primer Sequence	probe sequence
MDV-1F	5'-GGA-GCC-GGA-GAG-GCT-TTA-TC-3'	5'-CGT-CTT-ACC-GAG-GAT- CCC-GAA-CAG-G-3'
MDV-1R	5'-ATC-TGG-CCC-GAA-TAC-AAG-GAA-3'	

RESULTS

Detection of MDV by Real time PCR

Real Time PCR was performed for detection of *Mareks* disease virus from forty pool of five feather samples (figure1). All feather samples were subjected to DNA extraction before performing PCR and Real-time PCR assays revealed that 5 of the 40 pooled samples tested positive for MDV (Table1).

Table 1: Real time PCR test result from all the study area

Study Area	Number examined	Number Positive	Prevalence in %
Addis Abeba	10(in pool of five)	2	20% (2/10)
Bishoftu	10(in pool of five)	2	20% (2/10)
Ambo	10(in pool of five)	1	10% (1/10)
Sebeta	10(in pool of five)	-	0% (0/10)
Total	**40 (in pool of five)**	**5**	**12.5% (5/40)**

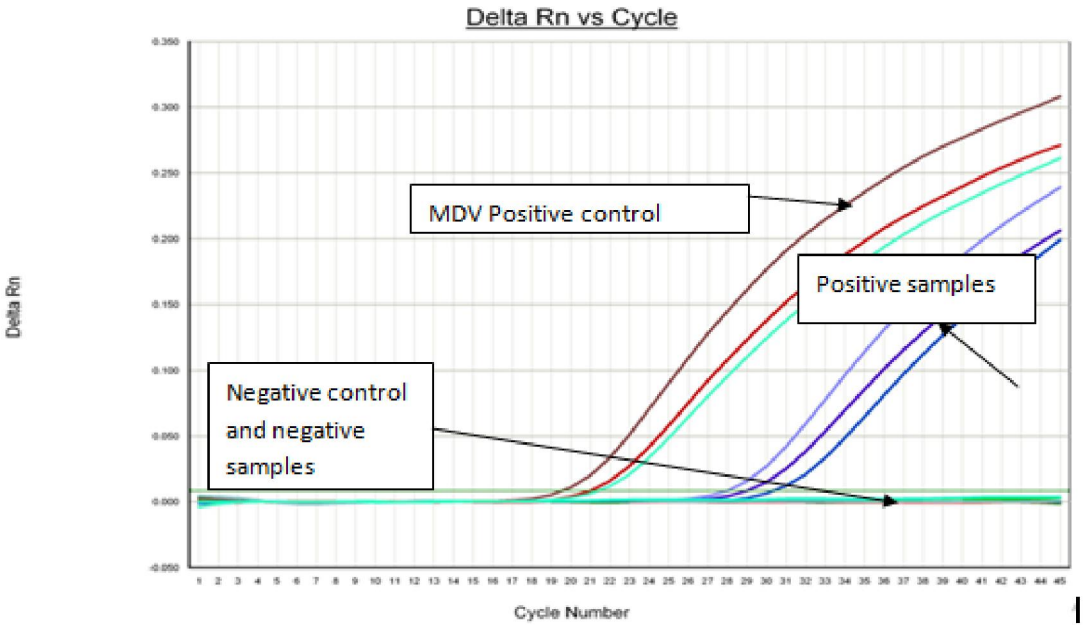

Figure 1: Real time PCR result

DISCUSSION

In this study, Marek's disease virus was detected using Real time PCR in clinically diseased kept under varied production procedures in central Ethiopia. MDV-1 was discovered in 12.5 % (5/40) of the samples examined from pooled feathers of chickens in this study, and the overall prevalence is lower than some of the MD prevalence studies completed in poultry farms elsewhere Demeke [9] found MDV in 91.66 % of the samples they gathered from 12 pooled tissue and feather samples. Yilmaz [10] reported 93.3 % (11/12) and Ayo [11] reported 83.33 % (5/6) higher rates of MD prevalence in chicken farms using conventional PCR. Other investigations found greater rates of MD prevalence in feather tip extracts when using PCR. for instance López-Osorio report 70%(25/35) [12] , Raman report 70% (7/10) [13], Saravanajayan report 20% (12/60) [14] and Davidson and Borenshtain report 100% (11/11) [15]. On the other hand Temam report lower rates of prevalence of MDV 9.76% (8/82) [16] from the feather tissue in the country.

This is the first time a molecular detection of Marek's disease virus has been undertaken in the Ambo area, to our knowledge.

CONCLUSION AND RECOMMENDATIONS

Finally, the current investigation discovered that the disease was circulating in chicken chicken farm in Ethiopia's Central (Addis Ababa, Bishoftu, Sebeta, and Ambo) districts. To make a decision on a harmless and defensive vaccination strain, more research on the culture and molecular characterization of chicken Marek's disease virus in all part of the country, as well as full genome sequencing of the current isolates, is needed. As a result, the Ministry of Livestock and Fisheries, as well as poultry farm owners, should pay special attention to the avoidance and control of Marek's disease, which has become a severe health problem in Ethiopia's poultry business.

AUTHORS' CONTRIBUTIONS:

BS, AA, MS, developed the study design, participated in sample collection, laboratory analysis, data analysis, manuscript writing and editing.

AVAILABILITY OF DATA AND MATERIALS

The manuscript contains all of the data that was collected and evaluated during the research. However, upon reasonable request, the raw data might be obtained from the respective author.

CONCENT FOR PUBLICATION

Not applicable.

COMPETING INTEREST

The authors declare that they have no competing interest.

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