GENETIC EVIDENCE OF ROTAVIRUS IN CHICKEN FROM TWO LOCALITIES IN SOUTHWESTERN NIGERIA

[1]ONI, Oluwole Oyetunde and [2]OWOADE, Ademola Amubieya
[1]Department of Veterinary Medicine & Surgery, University of Agriculture, Abeokuta,
[2]Department of Veterinary Medicine, University of Ibadan, Nigeria.

Corresponding Author: Oni. O. O. Department of Veterinary Medicine & Surgery, College of Veterinary Medicine, University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria. **Email:** writewole@yahoo.com **Phone:** +234-8033506443

ABSTRACT

The presence of rotavirus in some poultry flocks in Abeokuta and Oyo was evaluated using the reverse transcription–polymerase chain reaction (RT-PCR) technique. A total of 49 fecal samples in form of cloacal swabs were collected from chickens and turkeys with age groups ranging between 5days and 43weeks. Six samples from diarrheic chicken aged 8 and 9 days old were positive for the virus, while others were negative. To our knowledge, this is the first study to report rotavirus infection in Nigerian poultry. It is recommended that epidemiological surveys be carried out to provide more data on rotavirus infection in poultry flocks in Nigeria.

Keywords: Chicken, Rotavirus, RT-PCR

INTRODUCTION

Rotavirus gastroenteritis is a worldwide disease affecting primarily infants, young children and a wide variety of young mammalian and avian species (Estes *et al.,* 1983; McNulty *et al.,* 1984). Rotavirus infection in avian species was first reported by Bergeland *et al.* (1977) who found particles morphologically indistinguishable from rotavirus in intestinal contents of poults with watery droppings and increased mortality. Since then it has become apparent that rotaviruses infect many species of domestic birds. Rotavirus infection in avian species is frequently associated with outbreaks of diarrhoea. The rotaviruses belonging to the family Reoviridae contain a genome of 11 segments of double stranded RNA (dsRNA), which can be separated into distinct bands by electrophoresis. The migration pattern of the 11 genome segments following electrophoresis of the viral RNA in polyacrylamide gel is called the RNA electropherotype (Estes *et al.,* 1984). Rotavirus in birds belongs to groups A, D, F and G (Saif *et al.,* 1985). Detailed studies on the epidemiology of rotavirus associated diarrhoea in poultry have been performed in advanced countries but none has been reported in Nigeria. Genetic evidence of avian rotavirus in Nigeria was reported in this study.

MATERIALS AND METHODS

Collection and Preparation of Samples: Thirteen flocks comprising 5 broiler, 4 local chicken, 2 turkey and 2 exotic chicken from 2 local government areas in southwestern, Nigeria were sampled. Faecal samples in the form of cloacal swabs were collected from diarrheic (26) and non-diarrheic (23) birds. Approximately 3 g of fresh fecal samples were collected from the litter and placed in 500µl of viral transport medium (VTM) containing Hank's balanced salt solution, penicillin, streptomycin and fungizone. The cloacal swabs were similarly placed in VTM and clinical signs for each flock were noted. All samples were iced, transported to the laboratory and stored at -20^{0}C until used.

Isolation and Purification of RNA from Samples: Isolation of Rotavirus RNA from samples and its purification were done using Qiagen RNA minikit (QIAGEN GmbH, Germany). Reverse transcription was done to generate cDNA from the RNA by mixing 5µl of extracted RNA with 8µl of mix 1 (2µl of distilled H_20, 1µl of 10mM dNTPs, and 5µl of 0.03µg/µl random primer) and incubated at 72^0C for 10 minutes after which 7µl of mix 2 (1µl of DTT, 1µl of RNAase inhibitor, 4µl of 5x first strand Buffer and 1 µl of superscript III) was added and then incubated at 50^0C for 1 hour 20 minutes and later at 70^0C for 15 minutes.

The polymerase chain reaction for Rotavirus was carried out by adding 2.5µl of cDNA to 22.5µl of PCR mix containing 17.2µl of distilled water, 2.5µl of 10X PCR buffer, 2.0µl of $MgCl_2$ (50mM), 0.5µl of dNTP (10mM), 0.1µl of forward primer NSP4-F30, 0.1µl of reverse primer NSP4-R660 and 0.1µl of taq polymerase (5µ/µl) (Pantin-Jackwood *et al.,* 2007).

The PCR reactions was carried out using the following cycling conditions; initial denaturation at 94^0C for 5min, 35 cycles of amplification at 94^0C for 30 sec, 50^0C for 30 sec and 72^0C for 1min and final extension at 72^0C for 10min. The PCR product sizes were visualized by UV illumination in 2% agarose gel stained with ethidium bromide as compared to the 1kb+ size (invitrogen). The positive specimens were detected with band at 630bp.

RESULTS AND DISCUSSION

The broiler flocks were apparently healthy, while the turkey, pullet and local chicken flocks showed signs of dullness, emaciation and diarrhea. Four fecal samples from pullet chicks and two from local chickens were positive for Rotavirus out of the 49 analyzed by the RT-PCR technique (Figures 1 and 2). These were from 2 farms from two different local government areas. In this study, rotavirus infected birds were found diarrhoeic, dehydrated, anorectic and with low body weight and increased mortality. These observations were in conformity with the earlier reports of McNulty (2003) and Tamehiro *et al.* (2003) who reported

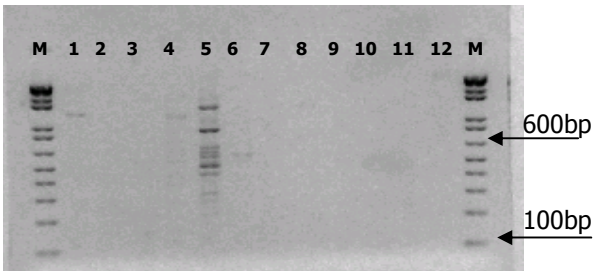

Figure 1: Agarose gel electrophoresis for demonstration of RT-PCR product (630bp). Lane M = DNA Marker, Lanes 5 and 6 are positive samples, Lane 12 Negative control

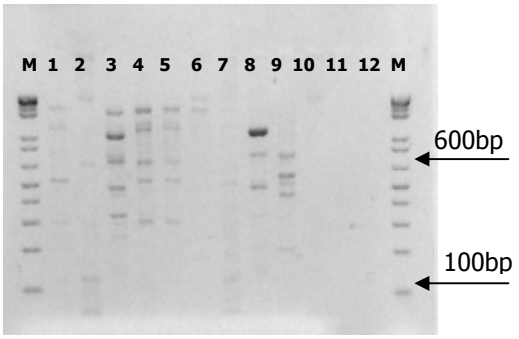

Figure 2: Agarose gel electrophoresis for demonstration of RT-PCR product (630bp). Lane M = DNA Marker, Lanes 3, 4, 8 and 9 are positive samples, Lane 12 Negative control

that in field conditions, rotavirus infections in poultry may induce subclinical manifestations, or they may be associated with enteritis, dehydration, anorexia, unrest, litter ingestion, low weight gain and increased mortality. Cumulatively, all these can lead to huge economic losses to poultry production systems (McNulty 2003; Villarreal *et al.,* 2006). Chickhood mortalities in Nigerian poultry have previously been linked to various factors including disease; none however has been related to rotavirus infection. This study serves as the first report of rotavirus infection of chicken in Nigeria.

Since the potential economic resources of the poultry industry may not be fully utilized until the etiological agent of diseases are recognized and possibly controlled. There is need therefore to conduct epidemiological studies to determine prevalence of rotavirus in

Nigerian poultry with subsequent genetic characterization of the virus.

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