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Detection of rotavirus VP7 gene in helmeted guinea fowls and Japanese quails in Ogun state, Nigeria

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Abstract

Rotaviruses classified into groups A through H are important etiological agents of gastroenteritis in man and animal. In Nigeria vaccination of infants has continuously been carried out, however the disease is still a burden to the nation which remains one of the countries with the highest cases of rotavirus gastroenteritis. There are several evidence of interspecies cross transmission and reassortment among group A rotaviruses. However, few studies have focused on rotavirus in the avian species, thus the virus has only been reported in chickens from southwest Nigeria. Guinea fowls (*Numidea meleagris*) and Japanese quails (*Coturnix coturnix japonica*) serve as source of income to the rural house-hold where they are raised in backyards. A total of 100 fecal samples from Guinea fowls (50) and Japanese quails (50) from different locations in Ogun state were collected and analyzed using group A specific RT-PCR. Fecal samples were screened for rotavirus using VP7 primers. The virus was detected in pooled diarrheic fecal samples of both Guinea fowls and Japanese quails and also in non-diarrheic feces of Guinea fowls. Due to the close proximity at which different breeds of birds are raised in backyard poultry in Nigeria and the reassortment ability of the virus, there will continuously be an increase in the diversity of the virus. This is not leaving out a zoonotic transmission with subsequent contribution to vaccine failure in man. It is thus important to continually survey for the virus in man and animal. This study provides the first report of rotavirus in helmeted Guinea fowls and Japanese quails in Nigeria.

Keywords: Guinea fowl, Japanese quail, Feces, Detection, Rotavirus, VP7 gene

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Introduction

Rotaviruses have been identified as one of the main aetiological agents of diarrhoea and enteritis in avian species (Bergeland *et al.*, 1977; Pantin-Jackwood *et al.*, 2008). Rotavirus is classified as a genus of the Reoviridae family, having a different morphology that easily allows distinguishing them from other enteric viruses and a characteristic 11-segmented RNA. This virus has been isolated from a wide variety of avian species, including turkeys, chickens, and pheasants (McNulty, 2003; McNulty & Jones, 2008). In Nigeria, avian rotavirus has only been reported in chickens (Oni & Owoade, 2010). Avian rotaviruses

are those designated as groups D, F, and G (Trojnar *et al.*, 2010; Johne *et al.*, 2011), however, avian rotaviruses from group A have already been isolated from the intestinal contents of chickens, turkeys, and other avian species (Sugiyama *et al.*, 2004). The glycoprotein VP7 is the major outer capsid protein and it forms trimers on the viral surface (Estes & Kapikian, 2007). The VP7 protein defines the G serotype and the protease-sensitive VP4 protein defines the P serotypes (Patton, 2012) and are both involved in immunity to infection (Pesavento *et al.*, 2006). Since the first report in avian species by

Bergeland *et al.* (1977), it has become apparent that domesticated bird. In field conditions, rotavirus infections in poultry may induce subclinical manifestations, or they may be associated with enteritis, dehydration, anorexia, low weight gain, and increased mortality (McNulty, 2003; Tamehiro *et al.*, 2003). It may also vary from a mild disease in young chickens to a more severe manifestation in 12 to 21-day-old chickens, characterized by unrest, litter ingestion; watery feces, wet litter, and severe diarrhea (Barnes, 1997). There are several evidence of interspecies cross transmission and reassortment among avian group A rotaviruses (Schumann *et al.*, 2009; Johne *et al.*, 2016). Thus, due to its isolation in various domesticated birds, it is possible that the virus occurs in different avian species in Nigeria because of the system of management of birds. This study was thus carried out to detect rotavirus in Guinea fowls and Japanese quails in southwestern Nigeria.

Materials and Methods

Sample collection

One hundred (100) fecal samples were collected using the simple random technique from adult helmeted guinea fowls (50) and quails (50) in live bird markets and backyard poultry in Ogun state between February and September 2013. Fresh faecal samples were collected from diarrheic and non-diarrheic birds. Samples were collected into microtubes and placed in cooler containing ice packs. Five samples were pooled together to make a total of 20 test samples before RNA extraction. These consisted 12 pooled diarrheic and 8 pooled non-diarrheic test samples.

RNA extraction using QIAamp Viral RNA mini kit

Isolation of Rotavirus RNA from samples and its purification were done using the QIAamp Viral RNA minikit (Buffer AVL, Lysis buffer), Ethanol (96-100%), Buffer AW1 and AW2 (washing buffer), Buffer AE (Elution buffer) and spin columns.

Feces were resuspended in 500µl of Medium 199 (Lonza, Belgium) containing 2000U/ml penicillin, 200mg/ml streptomycin, 2000U/ml polymyxin B, 250mg/ml gentamycin, 60mg/ml ofloxacin, 200mg/ml sulfamethoxazole and 2.5mg/ml

rotavirus infects many species of amphotericin B. Samples were clarified at 800 rpm for 10 min and 140µl of supernatant were used for RNA extraction using QIAamp Viral RNA Mini Kit (Qiagen, Venlo, The Netherlands).

Molecular detection

The sequence and binding positions of the forward and reverse primers used for the detection of the VP7 gene is as shown in table 1. RNA was denaturated at 95°C for 2 min followed by cooling on ice. Rotavirus is a double stranded RNA (dsRNA) and the denaturation at 95°C is necessary to separate the dsRNA strands prior to the reverse transcription step. Omission of the denaturation step decreases the efficiency of reverse transcription and subsequent amplification of the dsRNA (Freeman *et al.*, 2008). Rotavirus detection RT-PCR for group A detection was performed with RT-PCR conditions consisting of 1X Qiagen OneStep RT-PCR kit reaction buffer, 320 µM of each dNTP, 0.6 µM of each primer, and 1 µl of Qiagen RT-PCR enzyme blend and 2.5 µl of extracted RNA, for a total volume of 25 µl.

Amplification was performed with the following incubation steps: 50°C for 30 min, 94°C for 15 min, 40 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 60 sec. The RT-PCR was carried out in a total volume of 25µl. The PCR products were separated on a sybr safe stained 2% agarose gel by electrophoresis.

Results and Discussion

Fecal samples from both the Guinea fowls and Japanese quails were positive for rotavirus (figure 1). The virus was detected in diarrheic feces for both guinea fowls and quails and also in non-diarrheic feces of Guinea fowls as shown in figure 1.

The only genetic evidence of avian rotavirus in Nigeria was reported by Oni & Owoade (2010) in chicken. In this study, fecal samples from Guinea fowls and Japanese quails were screened by laying emphasis on the VP7 of the genome segments. In all, 100 samples were pooled into 20 test samples and screened using the one-step PCR protocol. The VP7 gene segment was detected in both diarrheic and non-diarrheic fecal samples.

Table 1: Primer sequence for detection of avian rotavirus

Name	Gene	Binding position	Sequence	Product size
RotaVP7-587F	VP7	587-606	5'-CAGACYTCAGAGGCTAATAA-3'	416bp
RotaVP7-1003R	VP7	985-1003	5'-TATGTCTAGAAGATCGCGC-3'	

The detection of rotavirus in guinea fowls and quails also affirms its high diversity in infecting different breeds of birds which is evident with the interspecies transmission and zoonotic ability of the virus (Desselberger, 2014). The evidence of interspecies transmission and reassortment among avian group A rotaviruses has also been reported by Schumann *et al.* (2009). Avian rotavirus has been established as the etiological agent of enteritis, replicating in intestinal epithelium, resulting in diarrhea and nutrient malabsorption causing an increase in feed conversion ratio (Pantin-Jackwood *et al.*, 2008). It also causes decreased weight gain, increased morbidity, increased mortality and increased production costs due to poor feed conversions. Cumulatively, all these can lead to huge economic losses in poultry production

systems (McNulty 2003; Villarreal *et al.*, 2006) and the potential economic resources of the poultry industry may not be fully utilized until the etiological agent of diseases are recognized and possibly controlled. Over 70% of Nigeria's population keeps different types of birds as backyard poultry, where they serve as source of protein and income to the owners (Adene & Oguntade, 2006). Some commercial farms consist of different avian species including chickens, turkeys, ducks, guinea fowls and quails. Different types of birds are also sold in the same environment on different live bird market where some of these samples were collected from. The presence of such different breed of birds gives room for the cross transmission of the virus between different bird breeds. Due to such close proximity at which different breeds of birds are raised in Nigeria and the reassortment ability of the virus, there will continuously be an increase in the diversity of the virus. This is not leaving out a zoonotic transmission to man with subsequent contribution to vaccine failure and further increasing the genetic diversity of the virus in man, since the surveillance of circulating rotaviruses in the human population has revealed the presence of several uncommon genotypes (Cook *et al.*, 2004) which may be from animal origin. This would contribute considerably to the spread and also maintenance of the virus in the environment either from sick or apparently healthy birds. This is as demonstrated in this study by the detection of the virus in diarrheic and non-diarrheic feces of birds which would subsequently aid the spread of the

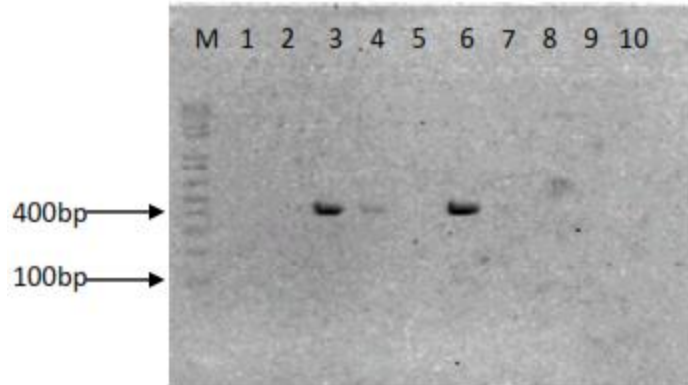


Figure 1: Agarose gel electrophoresis for demonstration of PCR product at 416bp. Lane M = DNA Marker, Lane 1= guinea fowl diarrheic feces, Lane 2= guinea fowl diarrheic feces, Lane 3 = guinea fowl diarrheic feces, lane 4 = quail diarrheic feces Lane 5= quail diarrheic feces, lane 6 = guinea fowl non-diarrheic feces, Lane 7= guinea fowl non-diarrheic feces, Lane 8= quail non-diarrheic feces, Lane 9= quail non-diarrheic feces, Lane 10 = Negative control

virus either as diseased or carrier birds infecting the commercial poultry birds. There have been several reports on the detection of avian rotavirus genotypes similar to those found in humans around the world and in South Africa (Ngoveni *et al.*, 2012) suggesting human to chicken transmission of rotavirus. There are also reports that animal rotaviruses can indeed infect humans and cause disease whenever the chance exists (Midgley *et al.*, 2012a). This is based on the identification of unusual rotavirus types, with properties of strains more commonly found in animals, which were isolated from various cases of human infection (Midgley *et al.*, 2012b). Although it remained unknown if mammalian RVAs could package genome segments from avian RVA strains, Johne *et al.* (2016) recently described avian and mammalian rotaviruses can exchange genome segments, resulting in replication-competent reassortants with new genomic and antigenic features. It is thus important to minimize intermingling of different avian species to prevent cross transmission and reassortment of the virus from different birds. However, further studies are needed to compare rotaviruses from different breeds in different regions of Nigeria to obtain better understanding on the types of rotaviruses circulating in avian flocks in Nigeria. This would help to relate an ancestor for the gene sequences and also be of high interest for local economics, public and animal health. Whole genome sequencing is also necessary to characterize the virus. The detection of

rotavirus in helmeted guinea fowl and quail will also contribute to the huge economic losses associated with diarrheal syndrome in birds, making this a major concern in the poultry industry (Niture *et al.*, 2010). This result has established the genetic

evidence of rotavirus in helmeted guinea fowl and quail, where the virus is rarely investigated and justifies the need to monitor the virus in avian species in Nigeria.

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