African Journal of Biotechnology Vol. 11(17), pp. 3949-3957, 28 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1655
ISSN 1684ï 5315 © 2011 Academic Journals

Full Length Research Paper

Detection of avian nephritis virus and chicken astrovirus in Nigerian indigenous chickens

Daniel Oladimeji Oluwayelu[1]*, Victoria Smyth[2] and Daniel Todd[2]

[1]Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.
[2]Agri-Food & Biosciences Institute, Stoney Road, Stormont, Belfast BT4 3SD, United Kingdom.

Accepted 18 August, 2011

Avian nephritis virus (ANV) and chicken astrovirus (CAstV) are widely distributed in poultry flocks worldwide, causing growth retardation. However, these avian astroviruses have not been previously diagnosed in poultry species in Nigeria. Real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) and reverse transcriptase-polymerase chain reaction (RT-PCR) were used for detecting ANV and CAstV in gut contents of apparently healthy Nigerian indigenous chickens. The rRT-PCR assay detected ANV and CAstV in 12 of 13 (92.3%) and 7 of 13 (53.9%) gut content pools, respectively. Using two different primer pairs which amplified approximately 2500 base pairs (bp) and 560 bp fragments in the capsid and polymerase genes of ANV and CAstV, respectively, the RT-PCR assay detected specific ANV and CAstV gene sequences in 4 of 5 (80%) and 2 of 3 (66.7%) rRT-PCR-positive samples in that order. The detection of ANV and CAstV in 8 to 20 week-old Nigerian indigenous chickens indicates that these viruses are probably endemic in the flocks or the environment. This study not only represents the first detection of ANV and CAstV in Nigerian poultry, it is the first report establishing the presence of these two viruses in indigenous chickens.

Key words: Avian nephritis virus, Chicken astrovirus, rRT-PCR, RT-PCR, Nigerian indigenous chickens.

INTRODUCTION

The family Astroviridae is divided into two genera: *Mamastrovirus* (mammalian astroviruses) and *Avastrovirus* (avian astroviruses) (Matsui and Greenberg, 2001). Astroviruses are small, spherical, non-enveloped, positive-sense RNA viruses 25 to 35 nm in diameter with a star-like morphology (Reynolds and Schultz-Cherry, 2008). Although, they were first identified in diarrhoeic stools of children with gastroenteritis (Madeley and Cosgrove, 1975), *astroviruses* have been reported to cause acute gastroenteritis in humans, cattle, swine, sheep, cats, dogs, deer, mice, turkeys, guinea fowl and ducks (Reynolds and Schultz-Cherry, 2008). Diseases have been described in poultry (Matsui and Greenberg, 2001) in which astroviruses have been recognized as a problem in turkeys, and have been shown to cause diarrhea and an increased mortality (McNulty et al., 1980; Yu et al., 2000). In chickens, two astrovirus species, which are antigenically and genetically distinct, have been detected. Avian nephritis virus (ANV), originally considered to be a picornavirus, was later characterized as an avian astrovirus on the basis of its nucleotide sequence (Imada et al., 2000) while Baxendale and Mebatsion (2004) reported the isolation of a different astrovirus, named chicken astrovirus (CAstV), from broiler chickens affected by runting syndrome. ANV acts as an etiological agent of growth retardation of young chickens by causing interstitial nephritis (Imada et al., 1979). It was first isolated from rectal contents of apparently normal broiler chicks in Japan (Yamaguchi et al., 1979). Antibodies against ANV have been found in chicken and turkey flocks in the UK and Japan, suggesting a broad distribution (Nicholas et al., 1988; Takase et al., 2000). Different degrees of pathogenicity in chickens are exhibited by field strains of ANV,

*Corresponding author. E-mail: ogloryus@yahoo.com, do.oluwayelu@mail.ui.edu.ng.

Abbreviations: ANV, Avian nephritis virus; **CAstV,** chicken astrovirus; **rRT-PCR,** real-time reverse transcriptase-polymerase chain reaction; **RT-PCR,** reverse transcriptase-polymerase chain reaction.

Table 1. History of samples examined in the present study.

Flock	Age	Pool or sample number	Comment
Flock 1 (Molete)	10 - 20 weeks	Pool 1 ï 5	Indigenous chickens from live bird market; kept in cages and fed grains.
Flock 2 (Moniya)	10 - 20 weeks	Pool 6 ï 10	Indigenous chickens from village households; kept on free range to scavenge for food and occasionally fed grains.
Flock 3 (University of Ibadan)	8 - 20 weeks	Pool 11 ï 13	Indigenous chickens from a household on the University campus; fed grains and food leftovers.

producing results ranging from subclinical infection to death, and there are at least two serotypes (Frazier et al., 1990; Shirai et al., 1991). CAstVs have been associated with enteritis and growth depression in chickens. In a recent study in the United States (Pantin-Jackwood et al., 2006), CAstVs were detected in gut and faecal samples from healthy broiler chickens and from flocks affected by enteritis and growth problems. CAstVs have also been detected in gut content and cloacal swab samples from UK and German broiler flocks with enteritis and growth retardation problems (Smyth et al., 2009). In addition, antigenically distinct CAstVs, originally recognized as enterovirus-like viruses and shown to cause varying degrees of growth retardation following experimental infections of 1-day-old specific-pathogen-free (SPF) or broiler chicks (McNulty et al., 1991; McNeilly et al., 1994), have recently been characterized (Todd et al., 2009a). CAstV was detected by serology in broiler flocks in the United Kingdom, the Netherlands, Spain, Australia, and the United States (Baxendale and Mebastion, 2004). Moreover, the seroprevalences of CAstV infections have been determined for broiler, broiler parent, grandparent and great-grandparent flocks from within the UK, and breeder flocks from other European countries and some turkey flocks (Todd et al., 2009b).

Although, ANV and CAstV have been implicated in growth depression including õuneven growthö and õstunting-stunting syndromeö the nature and extent of the disease problems that they may be causing remain unknown due to the absence of convenient diagnostic tests (Smyth et al., 2010). Both viruses grow poorly in cell culture, making virus isolation difficult, although ANV grows well in embryonated chicken eggs (Takase et al., 1994). Moreover, virus-specific antibodies are not commonly available for use in diagnostic tests such as immunohistochemistry (Smyth et al., 2010). However, the development of nucleic acid-based tests such as RT-PCR has made definitive diagnosis of these avian astroviruses possible. Several workers have successfully used the RT-PCR method to detect ANV and CAstV in field samples (Mandoki et al., 2006; Pantin-Jackwood et al., 2006; Todd et al., 2009a, 2010; Smyth et al., 2009). In addition, degenerate primers were recently used in a multiplex RT-PCR test to more specifically detect and differentiate avian astroviruses, including ANV and CAstV (Day et al., 2007).

Indigenous chickens (*Gallus gallus* domesticus) are known for traits such as small body size, slow growth rate, late maturity and high degree of adaptability to prevailing climatic conditions (El-Yuguda et al., 2005). In Nigeria, they serve as an important source of animal protein to the rural poor who keep them mostly in free-range systems where they scavenge for food. In addition, they are believed to act as potential reservoirs of infection to themselves and the commercial poultry (Adene et al., 1985; Emikpe et al., 2003). However, no infectious agent has been associated with the cases of growth retardation and ñrunting-stuntingò observed in indigenous chicken flocks in Nigeria. In particular, there has been no report of the detection of avian astroviruses in both indigenous and commercial chickens and turkeys in Nigeria. The use of rRT-PCR and RT-PCR assays to specifically detect two avian astroviruses, ANV and CAstV, in growth retarded Nigerian indigenous chickens is reported in the present study.

MATERIALS AND METHODS

Sample origin and processing

Gut contents and kidneys were collected from apparently healthy Nigerian indigenous chickens aged 8 to 20 weeks from three sources in Ibadan, Oyo State, southwestern Nigeria between June and August, 2010 (Table 1). The locations are Molete (n = 10) which is a market where different poultry species including chickens, ducks, pigeons and doves are sold; Moniya village (n = 10) where indigenous chickens are reared as scavenging backyard flocks by peasant farmers; and the University of Ibadan (UI) campus (n = 5) where some staff rear them in small numbers in their compounds. The collected samples were stored at -20°C until shipped over ice to the Agri-Food and Biosciences Institute, Belfast, United Kingdom where they were immediately transferred to -80°C freezer. The gut contents and kidneys from two birds each were pooled such that for Molete there were 5 pools (1 to 5), Moniya 5 pools (6 to 10) and UI 3 pools (11 to 13). A 10% suspension of each pool was prepared by adding 1 g gut contents to 9 ml chilled phosphate-buffered saline (PBS) containing Penicillin (1 000 units/ml), Streptomycin (1 000 µg/ml) and Amphotericin-B (2.5

Table 2. Primer and probe sequences used in ANV and CAstV rRT-PCR assay.

Parameter	ANV	CAstV
Forward primer	GTAAACCACTGGYTGGCTGACT	GCYGCTGCTGAAGAWATACAG
Probe	6-FAM-CAGCAACTGACTTTC-MGB	6-FAM-CAGAAGTCGGGCCC-MGB
Reverse primer	TACTCGCCGTGGCCTCG	CATCCCTCTACCAGATTTTCTGAAA

ANV, Avian nephritis virus; CastV, chicken astrovirus.

µg/ml) and then vortexing briefly with glass beads to disrupt the solid material. Suspensions of kidneys were made by grinding 1g of the sample with sterile sand in 9 ml PBS. The suspensions were centrifuged at 3 000 rpm for 30 min at 4°C, and the supernatants transferred to fresh tubes for storage at -80°C until required.

RNA extraction

Viral RNA was extracted from 140 µl of each supernatant using the QIAamp Viral RNA Mini Kit (Qiagen, Crawley, UK) according to the manufacturerôs instructions. Each RNA was eluted in 30 µl RNase-free water.

Real-time RT-PCR (rRT-PCR)

The rRT-PCR assay used in this study had been shown to be highly sensitive, specific and efficient (Smyth et al., 2010). Briefly, the rRT-PCR reactions were set up in duplicate per sample with a total volume of 20 µl per replicate reaction. Each reaction comprised 10 µl AgPath-ID™ One-Step RT-PCR 2x buffer (Applied Biosystems), 0.8 µl AgPath-ID™ One-Step RT-PCR enzymes (Applied Biosystems), primers to a final concentration of 400 nM, probe to a final concentration of 120 nM, 2 µl sample or positive control RNA and nuclease-free distilled water (dH_2O) to 20 µl. The 2 µl sample RNA were replaced in the PCR negative control by 2 µl dH_2O or 2 µl negative extraction controls. The reactions were conducted in a 7500 Real-Time PCR System (Applied Biosystems) starting with a reverse transcription stage at 45°C for 10 min, then an initial denaturation stage at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, and then primer annealing and template amplification at 60°C for 45 s. Fluorescence readings were taken during the amplification stage. During post-PCR analysis, cycle thresholds were set while the reactions were in true exponential phase prior to the linear phase. The ANV assay amplified 56 bp within the 3ô untranslated region of the ANV genome while the CAstV assay amplified a 70 bp fragment within the pre-capsid region. The sequences of the assay components are presented in Table 2. The logarithm (to the base 10) of the viral RNA copy number for each sample was determined as previously described (Smyth et al., 2010). Since duplicate reactions were set up per sample, mean log values were calculated. Information relating to the source, propagation history and production of the G4260 serotype 1 ANV isolate (ANV-1) and the 11672 isolate of CAstV used as positive controls in this study was previously described by Smyth et al. (2009). Samples containing medium or high virus RNA levels (Smyth et al., 2010) were selected for subsequent RT-PCR amplification of genomic DNA.

Conventional RT-PCR

A fragment of approximately 2 500 base pair (bp), containing the capsid gene of ANV, was amplified with the forward primer, 5ô ACCTTGAATCCCTGTGGGGCA-3ô and the reverse primer, 5ô AAAAGTTAGCCAATTCAAAATTAATTC-3ôutilizing a One-Step RT-PCR involving the use of SuperScript III One-Step RT-PCR System with the Platinum® Taq DNA Polymerase kit (Invitrogen, Paisley, UK) as described previously (Todd et al., 2011). Each reaction contained 1 x reaction buffer, 1 µM each primer, 1 µl enzyme mix, 2.5 µl RNA and diethyl pyrocarbonate-treated H_2O to 25 µl. Amplification was performed in a Veriti thermocycler (Applied Biosystems, Warrington, UK) and the incubation steps included 45°C for 30 min (reverse transcription), an initial denaturing step of 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 15 sec, annealing at 56°C for 30 sec and extension at 68°C for 2.5 min. There was a final extension step at 68°C for 5 min. For CAstV, a One-Step RT-PCR was similarly performed using a primer pair designed to amplify a 560 bp fragment in the polymerase gene (Smyth et al., 2010). The sequence of the forward primer (CAstV3-for) was 5ôGACGCGGAAAACAAGGAGCTGTT-3ôwhile that of the reverse primer (CAstV3-rev) was 5ôCGTCGGGATCTGC ATTCTCACAC-3ô Amplification started with a reverse transcription step of 45°C for 30 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 50°C for 15 s and extension at 68°C for 40 s. There was a final extension step at 68°C for 7 min. All PCR products were electrophoresed at 100 V for 40 min on a 1% agarose gel in 1 × Tris-acetate ethylenediamine tetra-acetic acid buffer and were visualized by ethidium bromide staining and ultraviolet transillumination. Amplicons of the appropriate sizes were subsequently excised from the gel and extracted with the QIAquick gel extraction kit (Qiagen Inc.).

Restriction endonuclease analysis

Purified 2.5 kb ANV amplicons from the four positive samples (Pools 1, 7, 8 and 10) were treated with restriction enzymes *Pst* I and *Sac* I (New England Biolabs), and *Eco* RI (Invitrogen) in 20 µl reaction volumes. The restriction digests were then analyzed by electrophoresis in 1% agarose gels stained with ethidium bromide and the profiles produced compared with those of six reference ANV strains (Table 4).

RESULTS

Real-time RT-PCR

The ANV assay detected the presence of virus RNA in all 13 (100%) gut content samples tested, with a log value range of 1.91 - 7.17 (Table 3). Samples 2 to 6, 9, 11 and 13 were considered to have low levels of virus RNA (log values < 3.99) whereas samples 1, 7, 8, 10 and 12 had medium (log values 4.00 to 5.99) or high (log values > 5.99) levels. For the CAstV assay, 11 of 13 (84.6%) gut content samples were positive with a mean log value range of 0.67 to 6.10. Samples 1 to 6, 9 and 11 were

Table 3. Real-time RT-PCR detection of ANV and CAstV RNAs in gut contents of Nigerian indigenous chickens

Sample	ANV Mean log value*	CAstV Mean log value
Molete		
1	4.78	2.01
2	3.78	1.63
3	3.52	2.10
4	3.12	1.19
5	3.07	2.28
Moniya		
6	3.41	2.13
7	7.17	4.70
8	5.31	5.43
9	2.01	1.09
10	5.86	6.10
UI		
11	1.91	0.67
12	4.34	Negative
13	2.46	Negative

*Log values relate to the virus RNA copy numbers expressed as logarithmic values (to the base 10).

Table 4. Predicted fragment sizes of the six reference ANV strains using three restriction enzymes.

Reference virus	*Pst* I	*Sac* I	*Eco* RI	Size of original amplicon (bp)
ANV-1	0 cuts	0 cuts	0 cuts	2549
ANV-2	1132 & 1414bp	543 & 1993 bp	0 cuts	2536
VF04-1/2	652 & 1878bp	0 cuts	0 cuts	2430
VF07-13/7	649, 671 & 1205bp	0 cuts	0 cuts	2534
VF08-3a	649, 717 & 1150bp	0 cuts	0 cuts	2518
VF08-65	667 & 1848bp	0 cuts	0 cuts	2515

considered to have low virus RNA levels while samples 7, 8 and 10 had medium or high levels, and samples 12 and 13 were negative. Samples from the Moniya source contained higher proportions of positive samples and higher levels of ANV and CAstV RNA than samples from the Molete and UI sources. Of the kidney pools tested, only samples 7, 8 and 10 from Moniya were positive for ANV with low levels of virus RNA (mean log value range of 2.08 to 2.81) while none was positive for CAstV.

Conventional RT-PCR

Conventional RT-PCR reactions were undertaken for ANV (2.5 kb amplicon) and CAstV (560 bp amplicon) using samples that were shown to contain medium or high virus RNA levels by the rRT-PCR. Analysis of the reaction products by agarose gel electrophoresisis followed by ethidium bromide staining and ultraviolet transillumination showed that amplicons of approximately 2 500 bp were clearly visible in 4 of the 5 gut content samples assayed for ANV DNA by RT-PCR (Figure 1). Of the 3 samples tested for CAstV by RT-PCR, only 2 were positive, producing a single band of 560 bp (Figure 2).

Restriction endonuclease analysis

This analysis was undertaken to provide a preliminary indication of the genetic diversity exhibited by 4 of the ANVs detected in Nigerian samples. *Pst* I cleaved the purified DNA of only sample 8 into two fragments of estimated sizes 1 800 bp and 700 bp (Figure 3), similar to the profile produced by two reference ANVs, VF08-65

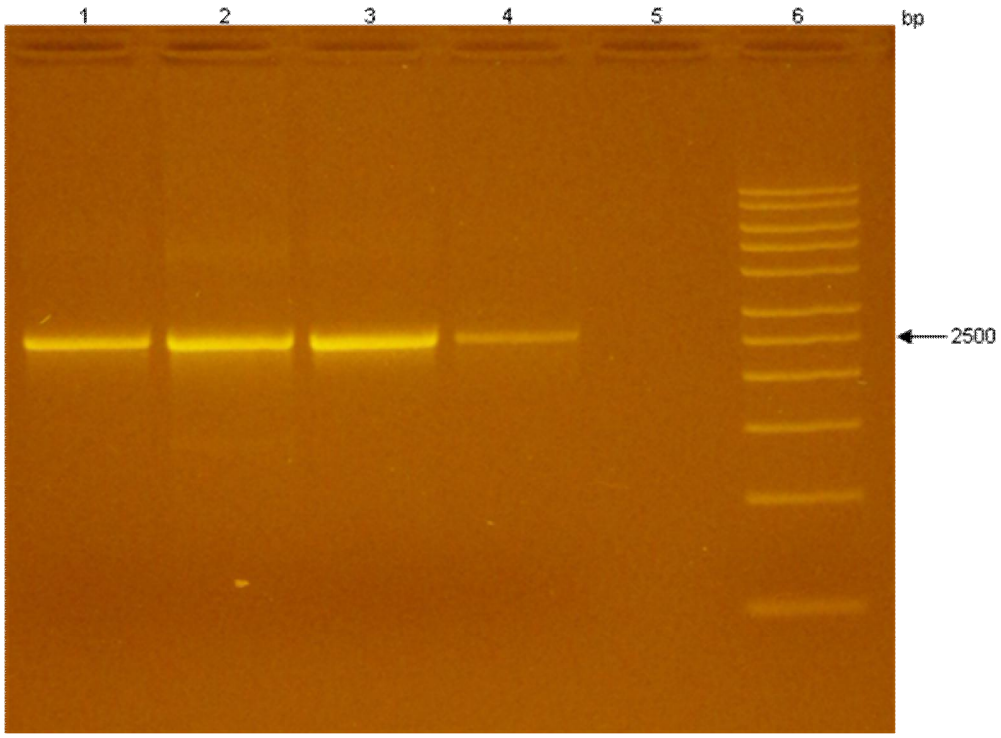

Figure 1. RT-PCR detection of ANV-specific nucleic acid in 4 of the Nigerian indigenous chicken samples tested. Lane 1, Sample 7; lane 2, Sample 8; lane 3, Sample 10; lane 4, Sample 1; lane 5, Sample 12; lane 6, 1 kb DNA ladder (Sigma, USA).

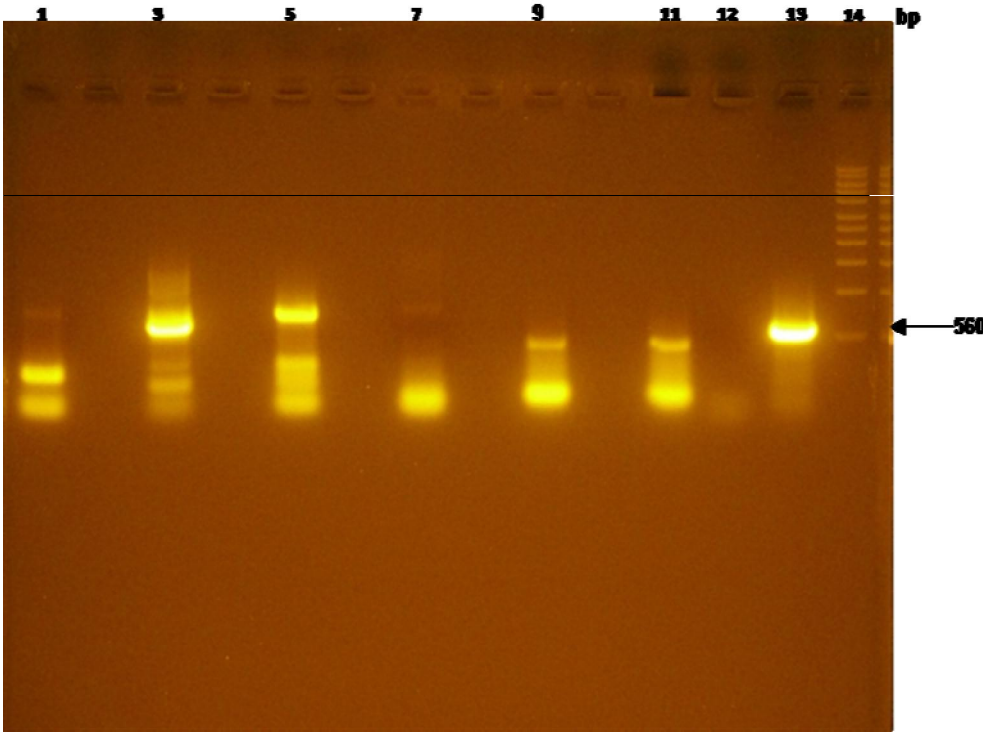

Figure 2. RT-PCR of CAstV-specific nucleic acid in 2 of the Nigerian indigenous chicken samples tested. Lane 1, Sample 7; lane 3, Sample 8; lane 5, Sample 10; lanes 7, 9 and 11, samples obtained from infected UK chickens; lane 12, negative control (nuclease-free water); lane 13, positive control (CAstV 11672); lane 14, 1 kb DNA ladder (Sigma, USA).

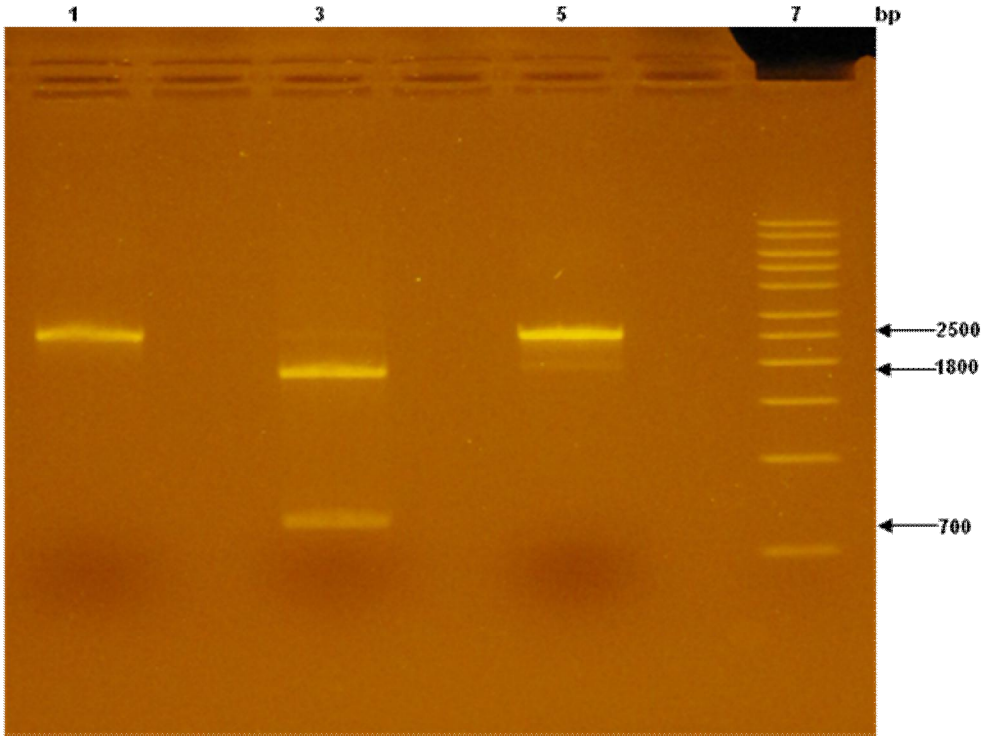

Figure 3. Agarose gel electrophoretic profiles generated following *Pst* I digestion of purified DNAs of Samples 7 (lane 1), 8 (lane 3) and 10 (lane 5) and 1 kb DNA ladder (lane 7).

and VF04-1/2 (Table 4). Sample 7, which was cleaved by *Eco* RI into two fragments of estimated sizes 2 200 bp and 300 bp (Figure 4), was different from the six reference strains in that none of these were cleaved by *Eco* RI. On the contrary, none of the four Nigerian samples produced the same profile as ANV-2 since this virus was cleaved by *Sac* I (Table 4).

DISCUSSION

Avian nephritis virus and chicken astrovirus are feel to infect poultry flocks worldwide but no confirmed cases of infections have been reported in Nigeria although retarded and uneven growth, ótunting-stuntingô and lowered productivity have been observed for many years in Nigerian poultry, especially among indigenous chickens. The study not only represents the first detection of ANV and CAstV in Nigerian poultry, it is also the first report confirming the presence of these two viruses in indigenous chickens.

Virus isolation procedures for detecting ANV and CAstV in clinical samples are complicated, time-consuming and require special cells and expertise, while identification of the isolates is based on serological methods that require ANV- and CAstV-specific antisera (Smyth et al., 2009; Todd et al., 2010). Due to these difficulties, the detection of ANV and CAstV by molecular assays that are more specific and sensitive, and ensure rapid and accurate diagnoses becomes imperative. Real-time RT-PCR and conventional RT-PCR have been used in detecting turkey astroviruses, ANVs and CAstVs in gut contents and faeces of turkeys and chickens (Koci et al., 2000; Spackman et al., 2005; Pantin-Jackwood et al., 2006; Smyth et al., 2009; Smyth et al., 2010; Todd et al., 2010).

In the present study, a rRT-PCR test which has been shown to work for ANVs and CAstVs from the UK, the USA and a number of European countries (Smyth et al., 2010; Todd, unpublished results), was used for preliminary screening of all gut content and kidney samples tested and those with mean log values greater than 4.00 were selected for subsequent conventional RT-PCR testing. 80% (4/5) and 66.7% (2/3) of the gut contents were positive by follow-up RT-PCR for ANV and CAstV, respectively. This detection of ANV and CAstV gene sequences in gut contents of Nigerian indigenous chickens is consistent with recent reports of the RT-PCR detection of ANV (Mandoki et al., 2006; Day et al., 2007; Hewson et al., 2010; Todd et al., 2010) and CAstV (Baxendale and Mebatsion 2004; Pantin-Jackwood et al., 2006; Smyth et al., 2009) in chickens. The RT-PCR and rRT-PCR tests used for this work have been found to be highly sensitive and specific for detection of both ANV and CAstV (Smyth et al., 2009, 2010; Todd et al., 2010). However, since none of the positive kidney samples had mean log value above 4.00, they were not tested further. For the ANV assay, the detection levels were highest in

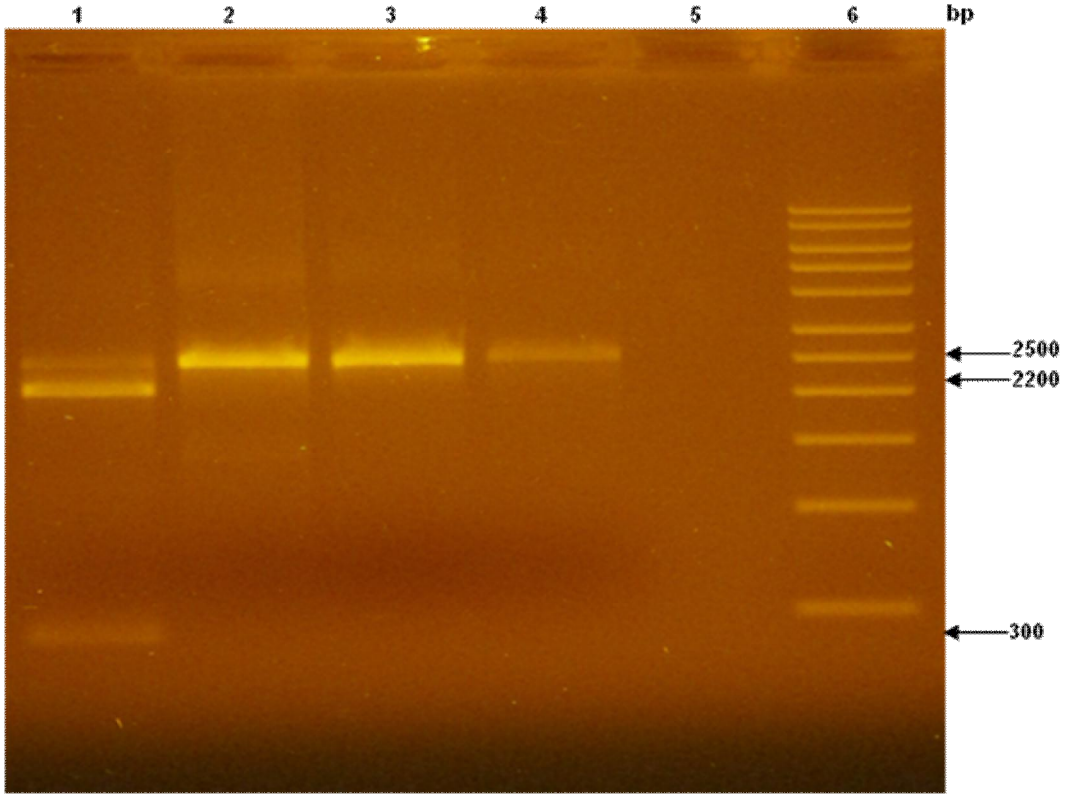

Figure 4. Agarose gel electrophoretic profiles generated following *Eco* RI digestion of purified DNAs of Samples 7 (lane 1), 8 (lane 2), 10 (lane 3) and 14 (lane 4); negative control, lane 5; 1 kb DNA ladder, lane 6.

gut content samples from the Moniya source as 3 of the 5 pools from this location had medium or high virus RNA levels compared to the Molete and UI sources that had only one sample each with medium RNA levels. A similar pattern was observed for the CAstV assay in which rRT-PCR detected medium to high virus RNA levels in 3 Moniya samples, low RNA levels in all Molete samples and negligible to no virus RNA in the UI samples. It is noteworthy that the highest levels of ANV and CAstV RNA were detected in the same samples (7, 8 and 10 from Moniya). This could be due to the fact that these birds were reared under the traditional free-range system of management which allows them to scavenge for food and predisposes them more to contact with infectious pathogens compared to the Molete birds that were kept in cages and fed grains until they were sold, or the UI birds that were kept within enclosures in the residences of staff where they were fed grains and food leftovers. A similar co-infection with the two viruses was observed by Pantin-Jackwood et al. (2008) and Smyth et al. (2010) in commercial chickens.

Furthermore, CAstV RNA was either undetectable or detected in generally low levels in indigenous chickens from the 3 locations in the present study compared to the previous report of Smyth et al. (2010) in which higher incidence and levels of CAstV RNA were detected in a longitudinal study of broiler chickens exhibiting growth problems, and experimentally infected chickens. This disparity could be attributable to the higher (8 to 20 weeks) age range of the indigenous chickens compared to the broiler chickens in which highest virus RNA levels were attained early in the lives of the birds (day 5 or 7) and had waned or become undetectable by day 28 or 35. Astrovirus infections are known to typically occur within the first 4 weeks of life (Reynolds et al., 1987). Moreover, lower virus RNA levels were detected in kidney samples than gut contents for both ANV and CAstV in this study. This is consistent with the findings of Smyth et al. (2010) and suggests that astroviruses have a higher tropism for the gut than the kidneys.

Although, rRT-PCR tests can be advantageous in terms of speed, sensitivity and specificity, the amplicons generated are too small to facilitate meaningful sequence comparison studies. In this investigation, we have used conventional RT-PCR to generate larger amplicons with which sequence-based molecular epidemiology can be undertaken. Samples with medium and high ANV and/or CAstV RNA levels were selected for conventional RT-PCR because our previous experience indicated that positive signals were often not achieved with low RNA

levels as detected by rRT-PCR. Sequencing of the ORF 1b 560 bp amplicon generated with the CAstV samples can be used to assign the CAstVs to one of 2 major groupings while the amplification of approximately 2.5 kbp ANV-specific fragments can be used to generate the sequence of the complete ANV capsid protein. We have recently shown that the capsid proteins of ANV can be assigned to one of six different groups with representative ANVs from each group probably belonging to different serotypes (Todd et al., 2011). Preliminary findings using restriction endonuclease digestion support the view that there are at least 3 different ANVs in the four samples tested and indicate that the Nigerian indigenous chickens probably harbour a mixed population of ANV strains that differ genetically. Cloning and sequencing of the RT-PCR products is therefore necessary in order to appreciate the extent of genetic diversity that exists among the ANVs that infect Nigerian indigenous chickens.

Since neither vaccination nor other preventive measures are practiced in rearing of indigenous chickens in Nigeria, the detection of ANV and CAstV in the gut contents of apparently healthy, 8 to 20 week old indigenous chickens in this study indicates subclinical infection and suggests that they shed the viruses in their faeces. Additionally, the low level detection of ANV RNA in kidney samples by rRT-PCR is consistent with a subclinical infection. It is likely that these viruses are endemic in the flocks or the environment throughout the life of the birds as astroviruses are extremely stable in the environment. The findings of this study indicate the detection of two additional viral pathogens in Nigerian indigenous chickens, the presence of which may affect the dynamics of health and disease among Nigerian poultry flocks. It can be concluded therefore that lower productivity and poor performance generally associated with Nigerian indigenous chickens, among other factors, may also be related to ANV and CAstV infections. Consequently, infections with these astroviruses should be considered as differential diagnoses in cases that present with enteritis, retarded growth, ótunting-stuntingô and poor productivity.

REFERENCES

Adene DF, Oyejide A, Owoade AA (1985). Studies on the possible role of naturally infected Nigerian local chickens and vaccine virus in the epidemiology of IBD. Rev. dÕElev. Med. Vet. Des Pays Trop. 38: 122-126.

El-Yuguda AD, Dokas UM, Baba SS (2005). The effects of Newcastle disease and infectious bursal disease vaccines, climate and other factors on the village chicken population in northeastern Nigeria. Intern. J. Food Agric. Environ. 3(1): 55-57.

Baxendale W, Mebatsion T (2004). The isolation and characterization of astro viruses from chickens. Avian Pathol. 33: 364-370.

Day JM, Spackman E, Pantin-Jackwood M (2007). A multiplex RT-PCR test for the differential identification of turkey astrovirus type 1, turkey astrovirus type 2, chicken astrovirus, avian nephritis virus, and avian rotavirus. Avian Dis. 51: 681-684.

Emikpe BO, Ohore OG, Oluwayelu DO, Oladele OA, Ockiya MA, Eniola SO (2003). Seroprevalence of antibodies to infectious bronchitis virus in Nigerian indigenous chickens in Ibadan. Nig. Vet. J. 24: 9-12.

Frazier JA, Howes K, Reece RL, Kidd AW, Cavanagh D (1990). Isolation of non-cytopathic viruses implicated in the aetiology of nephritis and baby chick nephropathy and serologically related to avian nephritis virus. Avian Pathol. 19: 139-160.

Hewson KA, O'Rourke D, Noormohammadi AH (2010). Detection of avian nephritis virus in Australian chicken flocks. Avian Dis. 54: 990-993.

Imada T, Yamaguchi S, Kawamura H (1979). Pathogenicity for baby chicks of the G-4260 strain of the picornavirus avian nephritis virus. Avian Dis. 23: 582-588.

Imada T, Yamaguchi S, Mase M, Tsukamoto K, Kubo M, Morooka A (2000). Avian nephritis virus (ANV) as a new member of the family astroviridae and construction of infectious ANV cDNA. J. Virol. 74: 8487-8493.

Koci MD, Seal BS, Schultz-Cherry S (2000). Development of an RT-PCR diagnostic test for an avian astrovirus. J. Virol. Methods 90: 79-83.

Madeley CR, Cosgrove BP (1975). Letter 28 nm particles in faeces in infantile gastroenteritis. Lancet, 2: 451-452.

Mandoki M, Dobos-Kovacs M, Bakonyi T, Rusvai M (2006). Molecular diagnosis of avian nephritis preliminary report. Acta Vet. Hung. 54: 51-60.

Matsui SM, Greenberg HB (2001). Astroviruses. In Knipe DM, Howley PM (eds). Fields Virology 4th edn. Lippincott, Williams & Wilkins, Baltimore, MD. 1: 875-893.

McNeilly F, Connor TJ, Calvert VM, Smyth JA, Curran WL, Morley AJ, Thompson D, Singh S, McFerran JB, Adair BM, McNulty MS (1994). Studies on a new enterovirus-like virus isolated from chickens. Avian Pathol. 23: 313-327.

McNulty MS, Curran WL, McFerran JB (1980). Detection of astroviruses in turkey faeces by direct electron microscopy. Vet. Rec. 106: p. 561.

McNulty MS, Connor TJ, McNeilly F, McFerran JB (1991). Biological characterisation of avian enteroviruses and enterovirus like viruses. Avian Pathol. 19: 75-87.

Nicholas RA, Goddard RD, Luff PR (1988). Prevalence of avian nephritis virus in England. Vet. Rec. 123: 398.

Pantin-Jackwood MJ, Spackman E, Woolcock PR (2006). Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States implications for diagnostics. Avian Dis. 50: 397-404.

Pantin-Jackwood MJ, Day JM, Jackwood MW, Spackman E (2008). Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis. 52: 235ï 244.

Reynolds DL, Saif YM, Theil KW (1987). Enteric viral infections of turkey poults incidence of infection. Avian Dis. 31: 272ï 276.

Reynolds DL, Schultz-Cherry SL (2008). Astrovirus infections. In Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE (eds). Diseases of Poultry 12th edn. Blackwell Pub. Ames, Iowa. pp. 351-355.

Shirai J, Nakamura K, Shinohara K, Kawamura H (1991). Pathogenicity and antigenicity of avian nephritis isolates. Avian Dis. 35: 49ï 54.

Smyth VJ, Jewhurst HL, Adair BM, Todd D (2009). Detection of chicken astrovirus by reverse transcription polymerase chain reaction. Avian Pathol. 38: 293-299.

Smyth VJ, Jewhurst HL, Wilkinson DS, Adair BM, Gordon AW, Todd D (2010). Development and evaluation of real time TaqMan® RT-PCR assays for the detection of avian nephritis virus and chicken astrovirus in chickens. Avian Pathol. 39: 467-474.

Spackman E, Kapczynski D, Sellers H (2005). Multiplex real time RT-PCR for the detection of three viruses associated with poult enteritis complex: turkey astrovirus, turkey coronavirus, and turkey reovirus. Avian Dis. 49: 86-91.

Takase K, Uchimura T, Yamamoto M, Yamada S (1994). Susceptibility of embryos and chicks, derived from immunized breeding hens, to avian nephritis virus. Avian Pathol. 23: 117-125.

Takase K, Murakawa Y, Ariyoshi R, Eriguchi S, Sugimura T, Fujikawa H (2000). Serological monitoring on layer farms with specific pathogen free chickens. J. Vet. Med. Sci. 62: 1327-1329.

Todd D, Smyth VJ, Ball NW, Donnelly BM, Wylie M, Knowles NJ, Adair

BM (2009a). Identification of chicken enterovirus like viruses, duck hepatitis virus type 2 and duck hepatitis virus type 3 as astroviruses. Avian Pathol. 38: 21-29.
Todd D, Wilkinson DS, Jewhurst HL, Wylie M, Gordon AW, Adair BM (2009b). A seroprevalence investigation of chicken astrovirus infections. Avian Pathol. 38: 301-309.
Todd D, Trudgett J, McNeilly F, McBride N, Donnelly B, Smyth VJ, Jewhurst HL, Adair BM (2010). Development and application of an RT-PCR test for detecting avian nephritis virus. Avian Pathol. 39: 207-213.
Todd D, Trudgett J, Smyth VJ, Donnelly B, McBride N, Welsh MD (2011). Capsid protein sequence diversity of avian nephritis virus. Avian Pathol. (in press).
Yamaguchi S, Imada T, Kawamura H (1979). Characterization of a picornavirus isolated from broiler chicks. Avian Dis. 23: 571-581.
Yu M, Ismail MM, Qureshi MA, Dearth RN, Barnes HJ, Saif YM (2000). Viral agents associated with poult enteritis and mortality syndrome the role of a small round virus and a turkey coronavirus. Avian Dis. 44: 297-304.