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*Mini Review*

# Chicken Astrovirus Infection: Minireview and Preliminary Serologic Evidence of Antigenically and Genetically Distinct Chicken Astroviruses in Nigerian Indigenous Chickens

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**ABSTRACT:** Poultry have gradually assumed a very important role in the economy of many industrialized and developing countries. However, with the continued discovery of new viral agents affecting the poultry industry worldwide, it is important that stakeholders in the industry in Nigeria be updated about these emerging viral threats in order to better understand the dynamics of health and disease that affect the industry. This knowledge will engender research into the prevalence, distribution, pathogenicity and economic losses caused by these diseases, and ultimately lead to the design of prevention strategies and formulation of policies for effective control. This paper includes a review on chicken astrovirus (CAstV), a recently emerged virus that causes enteritis, retarded growth and poor productivity in chickens, with particular emphasis on the history, economic importance, epidemiology, diagnosis and control. In addition, the result of a preliminary serological survey of CAstV antibodies in Nigerian indigenous chickens in Ibadan, Oyo State is reported. Using the indirect immunofluorescence test with CAstV 612- and CAstV 11672-infected cells respectively, only 4% and 8% of the tested sera were positive for CAstV antibodies. Our findings provide the first serologic evidence of CAstV infection in Nigeria and indicate the circulation of, at least, two antigenically and genetically distinct CAstVs in the Nigerian poultry population. We conclude that the runting-stunting, retarded growth and poor productivity commonly seen in Nigerian indigenous chickens could be due, among other factors, to CAstV infections.

**Keywords:** Chicken astrovirus, growth retardation, indirect immunofluorescence, indigenous chickens, Nigeria

## INTRODUCTION

The livestock sector currently accounts for 9.4% of Nigeria's agricultural gross domestic product (FAO, 2005), with the poultry sub-sector contributing significantly to this figure. However, growth of the Nigerian poultry industry is greatly limited by the scourge of infectious diseases which decimate poultry populations nationwide resulting in reduced productivity and severe economic losses. Recently, the incidence of exotic and emerging viral diseases of poultry such as avian influenza, Newcastle disease, avian metapneumovirus and enteric viral diseases have increased with negative consequences for the poultry industry worldwide. It is therefore pertinent that stakeholders in the industry in Nigeria be informed about these emerging viral threats in order to better understand the dynamics of health and disease that affect the industry. This paper provides relevant information on one of these emerging viral threats to the Nigerian poultry industry, chicken astrovirus.

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### Description of Astroviruses

Astroviruses are small round, non-enveloped, single-stranded RNA viruses 25-35 nm in diameter with a

star-like morphology, typically spread via the fecal-oral route (Reynolds and Schultz-Cherry, 2008). They usually cause a mild gastroenteritis in most species although several reports indicate the association of astroviruses with acute disease in the young of multiple species and more severe diseases in poultry have also been ascribed to astroviruses (Matsui and Greenberg, 2001). In chickens, two astrovirus species which are antigenically and genetically distinct have been described: avian nephritis virus (ANV), originally considered to be a picornavirus, but later characterized as an avian astrovirus on the basis of its nucleotide sequence (Imada *et al.*, 2000), and a novel astrovirus named chicken astrovirus (CAstV) isolated from broiler chicks (Baxendale and Mebatsion, 2004). CAstV has been associated with growth depression problems in chickens, including runting-stunting syndrome. Smyth *et al.* (2009) detected CAstV in very high (96%) proportion of gut content samples from growth-retarded broiler flocks, and also in pooled gut content samples collected in longitudinal surveys of four broiler flocks with below-average performance.

**History**

Astroviruses belong to the family *Astroviridae* which is divided into two genera: *Mamastrovir*us (mammalian astroviruses) and *Avastrovirus* (avian astroviruses) (Matsui and Greenberg, 2001). The name astrovirus comes from 'astron' (Greek for star) describing the characteristic 5- or 6-pointed star-like surface projections detected by negative-stained electron microscopy (Madeley and Cosgrove, 1975). Although they were first identified in diarrhoeic stools of children with gastroenteritis (Madeley and Cosgrove, 1975), outbreaks of astrovirus diarrhoea have also been described in the elderly (Gray *et al.*, 1987). Apart from the infection in humans, astroviruses are known to cause acute gastroenteritis in cattle, swine, sheep, cats, dogs, deer, mice, turkeys, guinea fowl and ducks (Reynolds and Schultz-Cherry, 2008). They have been reported to cause outbreaks of gastroenteritis with growth depression and increased mortality in turkeys (McNulty *et al.*, 1980; Yu *et al.*, 2000) while recent studies revealed the presence of astroviruses in numerous poorly performing and healthy chicken and turkey flocks in the United States (Day *et al.*, 2007).

**Economic importance**

The overall impact of astroviruses on animal health and economics is not fully understood (Matsui and Greenberg, 2001). In particular, the economic impact of astrovirus infections on the poultry industry is yet to be determined (Reynolds and Schultz-Cherry, 2008). Additionally, the cost-benefit of countermeasures against CAstV infections is not known. However, although there is no information on the economic losses caused by CAstV to the poultry industry, it has been implicated in growth depression including uneven growth and runting-stunting syndrome which result in financial losses due to increased culling, poor feed conversion and lower uniformity at slaughter.

**Epidemiology**

Chicken astrovirus infections are geographically widespread. Baxendale and Mebatsion (2004) detected CAstV antibodies in field sera from broiler flocks in the United Kingdom, the Netherlands, Spain, Australia and the United States. They observed that there was no correlation between the presence of antibody and uneven growth as some flocks that displayed the runting and stunting syndrome had no CAstV antibodies, while other flocks in which the chicks grew normally had CAstV antibodies. Recently, CAstV was detected in gut and faecal samples from healthy broiler chickens and from flocks affected by enteritis and growth problems in the United States (Pantin-Jackwood *et al.*, 2006). The virus has 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). Moreover, serological investigations with CAstV (Todd *et al.*, 2009b) showed that infections with this virus are ubiquitous in broiler chicken flocks. They obtained high seroprevalences of CAstV infections in broiler and broiler parent flocks but substantially lower seropositivities in grandparent and great-grandparent flocks from within the UK. They also observed high seroprevalences in breeder flocks from eight other European countries and some turkey flocks.

Antigenically distinct CAstVs, originally regarded as enterovirus-like viruses (ELVs) (McNulty *et al.*, 1990; McNeilly *et al.*, 1994), have also been recently described (Todd *et al.*, 2009a). One of these CAstVs, designated ''FP3'', was isolated in the UK from dead-in-shell chicks as part of an investigation into early broiler mortality (Spackman *et al.*, 1984). A second CAstV known as ''612'' was isolated in South Africa from broilers with respiratory problem (McNeilly *et al.*, 1994). Apart from this CAstV 612 obtained in South Africa, there are no reports of CAstV detection in Africa.

**Diagnosis**

The nature and extent of the disease problems caused by CAstV are not known due to the absence of convenient diagnostic tests. Although electron microscopy is one of the principal means of demonstrating avian astroviruses in diagnostic samples,

this method relies on observing the star-like morphology (Madeley and Cosgrove, 1975) and lacks adequate specificity and sensitivity (Pantin-Jackwood *et al.*, 2006). Baxendale and Mebatsion (2004) showed that field sera positive for CAstV by the serum neutralisation test also had gel precipitating antibodies and suggested that the gel diffusion test may be of value as an inexpensive, technically simple flock test for CAstV. Antigen detection tests including fluorescent antibody tests performed with cryostat tissue sections or tissue impression smears, and antigen capture enzyme-linked immunosorbent assay have not been developed for CAstVs due to the absence of virus-specific antisera (Smyth *et al.*, 2009). In addition, CAstV grows poorly in cell culture making virus isolation difficult (Smyth *et al.*, 2010).

However, the development of nucleic acid-based tests such as reverse transcriptase-polymerase chain reaction (RT-PCR) has made definitive diagnosis of CAstV possible. Pantin-Jackwood *et al.* (2006) successfully used the RT-PCR method to detect CAstV in field samples from across the USA while Day *et al.* (2007) reported the use of degenerate primers in a multiplex RT-PCR test to more specifically detect and differentiate avian astroviruses, including CAstV. Recently, Todd *et al.* (2009a) used a novel degenerate primer-based RT-PCR combined with sequencing to show genetically that previously characterized ELVs of chickens (McNulty *et al.*, 1990; McNeilly *et al.*, 1994) were isolates of CAstV. Also, Smyth *et al.* (2009) described the development of a highly sensitive RT-PCR test that was capable of detecting two genetic groups of CAstVs when applied to field samples from broiler flocks with enteritis and growth retardation problems, and from longitudinal surveys of commercial flocks. The test was shown to be an improvement on the one reported earlier by Day *et al.* (2007) that failed to detect CAstVs in 22/52 (42%) field samples and swabs, 50 of which were positive using the newly described test. Furthermore, a real-time RT-PCR test has now been developed for quantitative detection of CAstV RNAs in field samples (Smyth *et al.*, 2010). The test detected high CAstV RNA levels ($>10^{5.99}$ viral copies) in gut content samples from growth-retarded broiler flocks and revealed that CAstV RNA levels were higher in the gut contents than in the kidneys. Essentially, molecular methods for detecting enteric viruses (including CAstV) offer several additional advantages over traditional methods such as detection of multiple viruses in one sample, no need for virus propagation, the ability to test a large number of samples quickly, and reduced cost of the assays. In addition, molecular techniques would identify more infected flocks and the identity of the viruses could be confirmed and further characterized through sequencing (Pantin-Jackwood *et al.*, 2008).

**Prevention and control**

There are currently no efficacious vaccines or chemotherapeutics for the control and/or prevention of astrovirus infections (Reynolds and Schultz-Cherry, 2008). Hence, strict containment is the only known method of preventing and controlling infections with any of the known astroviruses. Infected flocks, especially those that exhibit severe loss in viability and production, need to be treated with the utmost concern for biosecurity; complete sanitation of all materials and restricted access to facilities by personnel is required to contain the outbreak to the affected farm (Koci and Schultz-Cherry, 2002). To eliminate astrovirus infections, contaminated farms should be thoroughly disinfected. All the litter and manure should be removed and disposed of in a manner that ensures runoff does not contaminate the driveways or entrances to poultry houses. The floors, walls, fans, feeders, watering systems and all equipment should then be adequately scrubbed and disinfected using compounds and procedures proven useful at eliminating highly stable small round viruses (Koci and Schultz-Cherry, 2002).

**Astrovirus infections in Nigeria**

While there have been reports of astrovirus prevalence in different parts of the world (Imada *et al.*, 2000; Baxendale and Mebatsion, 2004; Pantin-Jackwood *et al.*, 2006; Day *et al.*, 2007; Todd *et al.*, 2009a), there are no reports among both indigenous and commercial poultry in Nigeria. 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 and are believed to act as potential reservoirs of infection to themselves and the commercial poultry (Emikpe *et al.*, 2003). As part of efforts towards improving these chickens for enhanced productivity, there is a need for focused research to identify the infectious agents that contribute to the retarded growth and 'runting-stunting' commonly associated with them in order to design effective prevention and control strategies.

In addition to the minireview, this paper presents a preliminary report on the use of indirect immunofluorescence assay to specifically detect CAstV antibodies in a limited number of growth-retarded Nigerian indigenous chickens in Ibadan, Oyo State, Nigeria.

## MATERIALS AND METHODS

### Sample collection

Blood samples were collected from 25 apparently healthy but growth retarded Nigerian indigenous chickens. These birds aged 8–20 weeks were obtained from three sources in Ibadan, Oyo State, southwestern Nigeria between June and August, 2010. The locations are Molete (n=10) which is a live bird 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. Harvested sera were stored at
-20$^{o}$C until shipped over ice to the Agri-Food and Biosciences Institute, Belfast, United Kingdom where they were immediately transferred to -80$^{o}$C freezer until tested.

### Indirect immunofluorescence test

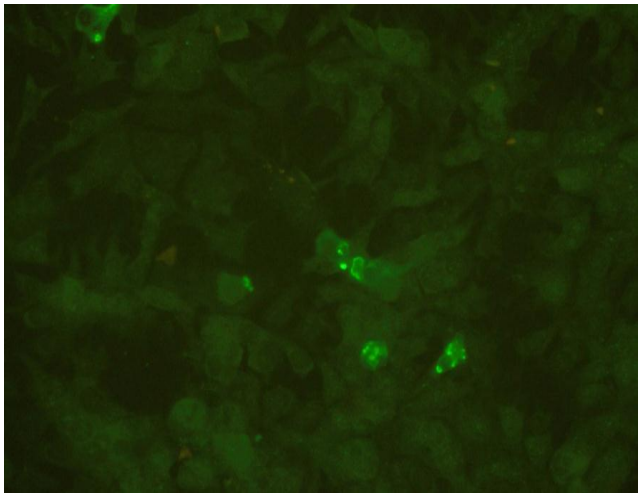
Indirect immunofluorescence (IIF) test was performed as previously described (Todd *et al*., 2009a) on the 25 Nigerian indigenous chicken sera using coverslip cultures of LMH cells (Kawaguchi *et al*., 1987), a chicken hepatocellular carcinoma cell line, infected with two antigenically and genetically distinct chicken astroviruses, CAstV 612 and CAstV 11672. Briefly, acetone-fixed cultures were incubated for 1 hour at 37$^{o}$C with 30µl of 1:100 dilution of each chicken serum. After washing, bound chicken antibody was reacted with 30µl of a 1:80 dilution of fluorescein isothiocyanate-conjugated rabbit anti-chicken immunoglobulin (Nordic Immunologicals, the Netherlands) for 1 hour at 37$^{o}$C. CAstV 612- and CAstV 11672-positive sera were used as controls and immunofluorescent staining was detected using an ultraviolet microscope. Samples showing specific intracytoplasmic inclusions were considered positive.

## RESULTS

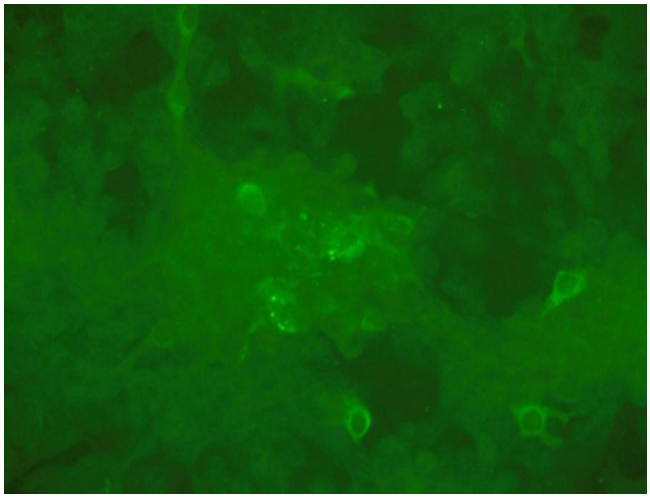
Of the 25 sera tested, only one (4%) and two (8%) were positive for CAstV antibodies after showing intracytoplasmic immunofluorescent staining with CAstV 612- and CAstV 11672-infected cells respectively (Figure 1). All the three positive sera were from indigenous chickens obtained from the University of Ibadan while samples from Molete and Moniya chickens were negative

## DISCUSSION

The detection of virus-specific antibodies in serum samples provides a convenient way of determining the extent of virus infection within animal populations (Todd *et al.*, 2009b).



CAstV 612-infected cells



CAstV 11672-infected cells

**Figure 1:**
IIF detection of CAstV-specific antibodies in Nigerian indigenous chicken sera using CAstV 612- and CAstV 11672-infected cells

Previous field surveys showed that CAstV antibodies are widespread among broiler, parent, grandparent and great grandparent flocks in the United Kingdom, Europe, Australia and the United States (Baxendale and Mebatsion, 2004; Todd *et al*., 2009b). However, despite these reports of widespread presence of CAstV in poultry flocks worldwide, the disease has not been described in Nigeria. This paper, which describes the detection of antibodies to two antigenically and genetically distinct CAstVs (Todd *et al.*, 2009a), is the first report on serologic evidence of CAstV infection in the Nigerian poultry population.

Considering that there is no vaccine currently available against CAstV and that Nigerian indigenous chickens are generally not vaccinated against any poultry disease, our detection of antibodies to CAstV 612 and CAstV 11672 in these chickens, albeit at low prevalence rates (4% and 8% respectively), indicates that the birds had been naturally exposed to the virus. This detection of CAstV-specific antibodies in the sera of apparently healthy 8-20 week-old indigenous chickens suggests subclinical infection with the virus since astrovirus infections are known to typically occur within the first 4 weeks of life (Reynolds *et al.*, 1987). Moreover, the presence of virus-specific antibodies against two antigenically and genetically distinct CAstVs in this study suggests the circulation of, at least, two CAstV strains in the Nigerian poultry population. It is possible therefore that the runting-stunting, retarded growth and poor productivity commonly seen in Nigerian indigenous chickens are due, among other factors, to CAstV infections. Also, the scavenging nature of these indigenous chickens predisposes them to CAstV infections since astroviruses are reported to be excreted in large numbers in faeces with relatively high level of resistance to inactivation (Guy *et al.*, 2008).

Interestingly, the positive samples in this study were from indigenous chickens in the University of Ibadan while the Molete and Moniya samples were negative. This is surprising because, compared to the University of Ibadan, Molete live bird market and Moniya village are rural communities where infectious disease agents are expected to circulate relatively freely among the poultry population. The limited sample size of the chickens tested could have contributed to the zero seropositivity obtained for these two locations. It is also possible that the birds that were seropositive in the University of Ibadan were obtained from CAstV-infected flocks elsewhere.

The serologic detection of CAstV infection in indigenous chickens in this pilot study is an indication that the disease merits further investigation in Nigeria. Since indigenous chickens are believed to serve as potential reservoirs of important poultry diseases, and considering that most CAstV infections are likely to occur as a result of horizontal transmission involving the faecal-oral route, we conclude that CAstV may be circulating among Nigerian commercial chickens. Therefore, broad-based seroepidemiological studies involving larger populations of indigenous and commercial chickens should be conducted to determine the true prevalence, distribution and impact of CAstV infection in Nigeria. Furthermore, the IIF test described in this paper is not suitable for flock screening involving large sample numbers because it is labour-intensive, time-consuming and requires expertise to read the stained slides. Thus, tests such as the ELISA will be required for large-scale flock testing. Additionally, efforts should be made to isolate and characterize CAstV from Nigerian chickens in order to identify potential vaccine candidate strains.

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