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Chicken Astrovirus

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Abstract: Chicken astrovirus (CAstV) has been associated with poor growth of broiler flocks, enteritis and diarrhea and is a candidate pathogen in cases of runting stunting syndrome. More recently CAstV has been implicated in cases of two other diseases of broilers as the sole etiological agent, namely severe kidney disease of young broilers with visceral gout and the "White Chicks" hatchery disease. Examination of the strains of CAstV associated with the two latter diseases reveals they are closely related genetically. This review will discuss the pathogenesis of CAstV in relation to strain diversity and the effects of vertical versus horizontal transmission, virus load, co-infections and age of bird at infection, all factors that may impact upon disease severity.
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1. Introduction

Chicken astrovirus (CAstV) is a recently emerged virus and the most recently identified member of the avian astroviruses. With a shared familial morphology and genomic arrangement, like other astroviruses CAstV is a small, round, nonenveloped virus typically <35 nm in diameter with a positive sensed, single-stranded RNA genome that, being close to 7.5 kb in length **(Kang et al.,2012)** is within the astrovirus family genome size range of 6.2 kb (human) to 7.7 kb (duck**) (Méndez and Arias, 2013)** Astroviruses primarily cause enteric infections and infect many animal species including humans, where they are a leading cause of infant diarrhea. Madeley and Cosgrove termed these viruses astroviruses, from the Greek word astron, meaning "stars", due to the protruding capsid spikes that give the characteristic star-like appearance under electron microscopy **(Madeley and Cosgrove, 1975)**. Astrovirus species infecting mammals are classified into the genus *Mamastrovirus*. The other major group of astroviruses that have been studied are the avian astroviruses, particularly those that infect commercial flocks although they are also detected in wild birds, and are classified in the genus *Avastrovirus*, which along with *Mamastrovirus* make up the two genera of the family *Astroviridae*.

Historically astroviruses have been named according to the species they infect, e.g., turkey astrovirus (TAstV) although species cross-over has been observed for some astroviruses, e.g., astroviruses of chickens have been detected in turkeys **(Pantin-Jackwood et al.,2006).**

Officially, there are three different astrovirus species that currently comprise the *Avastrovirus* genus according to the International Committee on Taxonomy of Viruses, namely *Avastrovirus 1, 2* and *3*, in keeping with the naming of mammalian astroviruses as *Mamastrovirus 1–19*. Although the first report of avian disease caused by astroviruses was in ducklings in 1965 **(Asplin, 1965)** the virus responsible was only recognized as an astrovirus in the mid-1980s by electron microscopy **(Gough et al., 1984)** and is referred to as duck hepatitis virus 2 (DHV-2) in older papers and as duck astrovirus serotype I (DAstV-1) more recently **(Gough et al., 1985).** A second astrovirus of ducks, originally called DHV-3 also causes hepatitis in duckling **(Haider and Calnek 1979)** It is now known as DAstV-2 and is antigenically and genetically distinct from DAstV- 1 **(Todd et al., 2009)**. Two astrovirus species were isolated from turkeys: the first was identified in the UK in 1980 and called TAstV serotype 1 (TAstV-1**) (McNulty et al., 1980)** and the second species, TAstV-2, was reported in 2000 **(Koci et al., 2000).**

There are two astrovirus species that infect chickens and both are associated with growth problems, enteritis and kidney lesions in young chickens. The first, avian nephritis virus (ANV), was isolated from a one week old, normal broiler chick in 1976 **(Yamaguchi et al., 1979)** and was originally thought to be a picornavirus **(Imada et al.,1979)**, but later identified as an astrovirus **(Imada et al.,2000).**

Transmission of Chicken Astrovirus

CAstV is an enteric pathogen and infections often occur very early, either transmitted horizontally by the fecal–oral route, or some CAstV strains can also be vertically transmitted from naive in-lay parent birds, and chicks may hatch shedding high levels of CastV. CAstV is more resistant to disinfection and cleaning than other viruses as it is non-enveloped and may be more persistent in poultry houses where darkling beetles can act as vectors for CAstV **(Rosenberger, 2010).** For instance, CAstV was detected in internal tissues and in washings from the surface of darkling beetles by RT-PCR. **(Smyth et al., 2017)** A recent investigation was carried out into CAstV carryover contamination between broiler crops in commercial broiler houses after the removal of spent litter both before and after cleaning and disinfection using proprietory disinfectants at recommended concentrations (**Smyth et al.,2017**).

Identification and Genomic Structure of Chicken Astrovirus

In 2004 three isolates were cultured from two submissions of broiler chicks with runting stunting syndrome (RSS) and one submission from a flock with uneven growth. The isolates were antigenically identical **(Baxendale and Metbatsion,2004).** Genetic sequencing of a 320 base pair reverse transcription (RT)-PCR amplicon made from RNA extracted from one of the isolates showed the agent was related to TAstV and ANV but was from a separate species also identified as an astrovirus and termed "chicken astrovirus" **(Baxendale and Metbatsion,2004)**. Prior to its molecular identification in 2004, CAstV had been described as an "enterovirus-like virus" (ELV) due to sharing similar characteristics to viruses of the genus *Enterovirus* of the family *Picornaviridae* **(McNeilly and Connor 1994).**

Sequencing of the CAstV genome has shown that it shares a similar genetic organisation to other astroviruses being composed of only three open reading frames (ORF). The first two ORFs, ORF 1a and ORF 1b, code for non-structural proteins including a protease (ORF 1a) and an RNA dependent RNA polymerase (ORF 1b). In keeping with other astroviruses, CAstV contains a conserved heptameric frame shift motif at the 3′ end of ORF 1a **(Jiang et al., 1993)** that is involved in the translation of ORF 1b in a different frame to ORF1a although possibly through a different mechanism to other astroviruses **(Kang et al., 2012).** The third ORF, ORF 2, codes for the capsid protein, the most variable region of the genome, especially in the 3′ half of the ORF which codes for the outer surface of the capsid including the star-like capsid spikes which interact with the host immune system hence variability is desirable. It also contains the start of the conserved s2m motif **(Monceyron et al., 1997),** which continues into the 3' untranslated region (UTR). A short 5′ UTR exists upstream of ORF 1a and a longer 3′ UTR after ORF 2. A polyadenylated polyadenylated tail is located at the extreme 3′ region completing the positive sensed, single stranded RNA genome.

Pathogenesis of Chicken Astrovirus

1. Runting Stunting Syndrome and Uneven Flock Performance

Historically, CAstV has been associated with malabsorption diseases of broiler chickens such as runting stunting syndrome (RSS) and with enteritis and growth problems in flocks **(Kang et al.,2012).** However, CAstV is one of a number of endemic, enteric viruses that have been implicated in RSS but as yet a single etiological agent has not been identified. A runted chick hatches small while a stunted bird exhibits a failure to grow, and often appears to have delayed development, where its overall appearance appears to be that of a much younger chick with down and immature feathering, yellow colouration and small comb and beak. RSS is a production disease that was originally characterised by poor weight gain in young broiler flocks, frequently observed between six and twelve days post hatch but can be evident up to three weeks **(Rosenberger,2012)**. This coincides with the occurrence of intestinal cysts that reduce nutrient absorption along with reduced villus size or altered villus shape. Other common symptoms include enteritis and diarrhea, leg weakness and irregular feathering **(Kouwenhoven et al.,1978).**

Uneven flock performance occurs when the variance in weights at slaughter is larger than expected, potentially causing carcass processing problems, and is a more common, chronic condition than RSS. Many of the same viruses, including CAstV, are present in underperforming flocks but they are often also present in the good performing flocks so the differences in factors that tip the balance of performance are likely to be subtle yet complex and probably involve co-infection with other pathogens especially other viruses, pathogen strain variation, infection timing, virus load and the presence or absence of maternal antibodies. It is also possible that early CAstV and other enteric viral infections may create an abnormal gut environment that facilitates later dysbacteriosis, an imbalance of naturally colonising bacteria, usually occurring between days 20 and 30 post hatch and which could further impair performance due to diminished nutrient digestibility and weakened intestinal barrier protection **(Tierlynck. et al., 2011).**

2. Kidney Disease and Visceral Gout

While CAstV is predominantly an enteric virus contracted through the fecal–oral route it is also known to infect organs outside of the enteric tract

including the liver and kidneys. Severe kidney disease of young broiler chicks with outbreaks of visceral gout and up to 40% mortality were reported in India in 2012 with the causative agent being identified as a group B CAstV **(Bulbule et al.,2013)** This particular strain of CAstV was isolated in embryonated SPF chicken eggs using homogenates from 18 CAstV positive kidney samples resulting in significant embryo stunting, liver necrosis and pale, swollen kidneys. Isolates made from clinical kidney homogenates passed through either SPF chicks or SPF eggs and inoculated into day old SPF chicks and day old broiler chicks resulted in extremely high levels of mortality (67.5%–100%) between days 5 and 10 p.i. for the SPF chicks and days 7 and 10 p.i. for the broilers Post mortem findings showed that the chicks all had diseased kidneys and visceral gout. Molecular testing found the kidneys positive for CAstV and negative for ANV and infectious bronchitis virus **(Bulbule et al.,2013).**

3. White Chicks Hatchery Disease

Recently CAstV has become associated with hatchery diseases, most noticeably "White Chicks", reports of which have come from various Scandanavian countries, North America, Poland and Brazil **(Nuñez et al., 2016),** but also with the "clubbed down" problem, although the latter association is less clear and still to be fully determined. White chicks that hatch have pale plumage, are weak and runted and do not tend to survive very long. The symptoms and lesions observed in white chicks share characteristics with those of RSS including lesions in the kidneys and liver, runting/poor development and weakness, and also abnormal feathering. An increase in mid to late embryo deaths was noted and there is a transient but substantial reduction in hatchability, which in Finland averaged 29% in affected flocks but which reached as high as 68% on one farm, with many dead in shell embryos in which CAstV was detected (**Smyth et al., 2013).** a 4%–5% hatchability decrease was observed for a single breeder flockover a 4-week period when a maximum of 1% of chicks were pale and weak **(Sajewicz-Krukowska et al., 2016).** These observations are indicative of a vertical virus transmission and since it was reported that affected Finnish breeder flocks only experienced the disease once during their lifetime, it seems probable that acquired immunity prevents disease recurrence and further vertical transmission. It was discovered through CAstV quantitative diagnostic testing that many chicks were shedding high loads of CAstV at hatch **(Smyth et al.,2013).**

Immunity, Treatments and Future Developments

Currently there are no medicines to treat RSS, CAstV-associated kidney disease or White Chicks disease nor are there any vaccines to prevent transmission of CAstV to broiler chicks. Hygiene and biosecurity are the only ways in which CAstV infection risk can be minimised. It would be highly advantageous if breeder hens could supply adequate CAstV maternal antibodies to the eggs since this would prevent vertical transmission of CAstV strains and give early protection against horizontal transmission. Although it has yet to be definitively determined, the age of the chick when first infected appears to have a bearing on all of these conditions so early protection is encouraged. Breeder hens that become naturally CAstV seropositive during rear or through the use of a CAstV breeder vaccine is advocated in order to protect embryos and hatched chicks against the range of CAstV strains in circulation and prevent vertically infected hatched chicks from shedding CAstV to infect naive broiler chick housemates. While the involvement of CAstV as a key agent in cases of RSS remains to be fully elucidated, it is clear from recent evidence that certain strains of CAstV are associated with White Chicks and others with severe kidney disease and visceral gout. The development of a commercial vaccine that can protect against the strains causing these two diseases, which are not that far apart genetically and serologically is to be hoped.

The use of wild-type strains of CAstV as breeder vaccine candidates that can be conveniently grown in eggs or cell culture has the advantage of cellular replication to higher titres but may be limited in effectiveness due to serological differences between strains and so a greater understanding of the relationship between circulating strain diversity and disease severity is desirable, particularly in the case of RSS. There is also the concern that an attenuated live vaccine could evolve into a more pathogenic form, although this is unlikely to unduly affect older birds. Alternative vaccine strategies may involve the use of recombinant protein technology to develop non-replicative CAstV capsid precursor proteins. Recombinant CAstV capsids have been produced by two groups using the baculovirus system, to subgroup B ii **(Sellers et al.,2010)** and subgroup B i **(Lee et al.,2013).** The recombinant CAstV B ii vaccine gave partial protection against experimental RSS challenge whereby weight restriction was significantly less pronounced in vaccinated broiler chicks **(Sellers et al.,2010)**. Lee *et al.* demonstrated that the B i recombinant CAstV capsid precursor proteins consistently stimulated virus-specific antibodies in SPF chickens at 3 and 4 weeks p.i. after 2 immunizations **(Lee et al.,2013)**. Given that wild-type infections of breeder birds with the White Chicks strain of CAstV appear to confer lifetime immunity, it is hoped that an effective CAstV vaccine would have

the same duration of effect but this can only be determined empirically.

One of the limitations of working with CAstV has been a lack of convenient diagnostic tools requiring researchers to develop their own in-house tools. The baculovirus expressed recombinant CAstV capsid precursor proteins developed as vaccine candidates have both been used successfully in ELISA (enzyme linked immunosorbent assay) tests to quantify CAstV seroconversion during *in vivo* CAstV experiments (Sellers et al., 2010). The B i recombinant capsid protein has since been used as the basis of a CAstV B group ELISA test **(Skibinska et al., 2015).**

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