Iranian Journal of Virology 2021;15 (1): 1-6
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Original Article

Detection and Molecular Characterization of Chicken Astrovirus from Broiler Flocks in Iran: The First Report

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Abstract

Background and Aims: Enteric diseases have had detrimental impacts on commercial poultry through depressing growth rates and caused considerable economic losses to poultry producers. Chicken astrovirus (CAstV) is one of the most common viruses related to enteric diseases in chickens, especially in young chicks.

Materials and Methods: Chicken astroviruses were detected by reverse transcriptase and polymerase chain reaction (RT-PCR) in intestinal contents collected from commercial chickens. RT-PCR test amplified a fragment of 601 base pairs located in conserved regions within the ORF 1b (RNA polymerase) gene.

Results: Astroviruses were detected in birds from 36 poorly performing flocks with signs of enteric disease or retarded growth. In total, chicken astroviruses were discovered in pooled intestinal contents of 16 (44.4%) affected broiler flocks, while twenty flocks were negative (55.6%). Phylogenetic analysis based on a 601-bp segment of the ORF1b gene revealed two subgroups of Iranian astroviruses.

Conclusion: This study discloses the presence of chicken astroviruses in broiler chickens in Iran with enteric problems and stunting syndrome for the first time.

Keywords: Chicken Astrovirus; Broiler; Phylogenetic Study; Iran

Introduction

The Astroviridae family is divided into two genera: Mamastrovirus (mammal astroviruses) and Avastrovirus (avian astroviruses) [1]. Astroviruses are small, spherical, non-enveloped, positive-sense RNA viruses, 28 to 30 nm in diameter, have a star-like morphology. They cause enteric diseases in mammalian and avian species [2].

Their genome, which is 6.8–7.9 Kb in length, encodes three proteins: the non-structural (NS) polyprotein, the RNA-dependent RNA polymerase (RdRp), and the capsid protein. The NS polyprotein and capsid protein are encoded by individual open-reading frames (ORF), ORF1a and ORF2, and RdRP (ORF 1b) acts as a fusion protein to the NS protein [3]. The genome begins with a 5 ´untranslated region (UTR), followed by the 3 ORFs, a 3´UTR, and a poly-A tail. ORF1a encodes the viral protease and is followed by ORF1b, which encodes the RNA polymerase (RdRp). ORF2, located downstream of ORF1b and before the untranslated region of the genome, encodes the precursor of the capsid protein [4].

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Six different avian astroviruses have been identified in poultry: turkey astroviruses 1 and 2 (TAstV-1 and TAstV-2), chicken astrovirus (CAstV), avian nephritis virus (ANV), and duck astrovirus 1 and 2 (DAstV-1 and DAstV-2) [5, 6].

CAstV is associated with growth retardation, nephritis, white chicks hatchery disease, and running stunting syndrome (RSS) in the broiler. However, the pathogenicity of some CAstV strains is in question by the isolation of CAstV from normal birds [6].

Runting-Stunting Syndrome (RSS) is an enteric syndrome with mild clinical signs, such as diarrhea that causes flocks to mature in a non-uniform pattern and consequently causes the body weights at slaughter to differ broadly [7]. Olsen first reported RSS in the broiler industry in 1977 [8]. This syndrome is a transmissible disease of uncertain etiology that affects chickens early in life [9]. CAstV has been detected in the United Kingdom, United States, Korea, India, Netherlands, Croatia [6], and more recently in Brazil [10], demonstrating a worldwide distribution.

Electron microscopy is one of the principal means of demonstrating avian astroviruses in diagnostic samples. However, this method relies on observing the star-like morphology [11], which is not apparent in some types of astrovirus, including ANV. Also, electron microscopy is not suited to high sample throughput and lacks sensitivity [5, 12]. Isolating astroviruses in cell culture presents difficulties as they grow poorly and are often outgrown by reoviruses and adenoviruses, commonly in enteric samples. The molecular characterization of CAstV is very important in understanding the relationship and genetic similarity among the different isolates worldwide. Antigenic characterization of avian astroviruses has been limited due to the difficulty of isolating and growing these viruses [12].

To understand and control enteric disease and take appropriate preventive measures in the broiler, more information on the prevalence and epidemiology of contributing enteric viruses is essential. The objective of this study was to find the prevalence of CAstV in broiler using molecular methods. In addition, phylogenetic analysis of the viruses detected in this study was also performed to characterize these viruses and provide epidemiological data.

Methods

Sample collection: Intestinal samples (n=470) of 36 broiler flocks were collected from chicks with the enteric disease of culled or stunted chicks. Clinical manifestations mostly consisted of diarrhea, dehydration, anorexia, growth retardation, and increased mortality. The age of birds was up to 3 weeks. The intestinal tracts from 10-15 chicks per flocks were removed from the duodenum to vent. The intestinal tracts from birds were cut into small sections (5 cm in length) and were placed in a sterile bag. Then sterile phosphate-buffered saline (PBS) was added (1:5). After homogenizing, the supernatant was used for RNA extraction .

RNA extraction and Reverse Transcriptase: RNA was extracted from 100 µL of each supernatant using the RNX plus™ kit (Sina Clone, Iran) according to the manufacturer's instructions. The RNA was subjected to a reverse transcription reaction to obtain complementary DNA (cDNA).

PCR: The cDNA obtained was submitted to PCR. Chicken astrovirus was detected using a pair of primer TAPG-L1 (TGG TGG TGY TTY CTC AAR A) and TAPG-R1 (GYC KGT CAT CMC CRT ARC A) to amplify a 601 base pair of viral RNA-dependent RNA polymerase (ORF-1b) [13]The PCR reaction used 20µL of a mixture that contained 0.5µM of each forward and reverse primers,2µL 10X buffer, 0.5 µL of 1250dNTPs, one µL of 50 mM MgCl2, one U of Taq DNA polymerase (SinaClone, Iran), and 3 µL cDNA.

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Table 1. Sequence homology matrix for Chicken Astroviruses isolated in this work and different strains based on partial nucleotide sequences of the ORF-1b gene.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	IR/CAstV/H793-31/14																						
2	IR/CAstV/H793-32/14	100.00																					
3	IR/CAstV/H1608-34/15	73.13	73.13																				
4	IR/CAstV/H1608-36/15	73.40	73.40	99.82																			
5	IR/CAstV/H1718-3/15	97.59	97.59	73.43	73.15																		
6	IR/CAstV/H1836-42/15	97.01	97.01	73.10	72.82	98.72																	
7	IR/CAstV/H2036-2/16	97.01	97.01	73.10	72.82	98.72	100.00																
8	IR/CAstV/H2608-3/17	73.13	73.13	100.00	99.82	73.43	73.10	73.10															
9	IR/CAstV/H2608-4/17	73.13	73.13	100.00	99.82	73.43	73.10	73.10	100.00														
10	IR/CAstV/H2708-1/17	73.13	73.13	100.00	99.82	73.43	73.10	73.10	100.00	100.00													
11	IR/CAstV/H2748-2/17	97.40	97.40	73.68	73.40	99.27	99.27	99.27	73.68	73.68	73.68												
12	IR/CAstV/H2748-12/17	97.40	97.40	73.68	73.40	99.27	99.27	99.27	73.68	73.68	73.68	100.00											
13	IR/CAstV/H3718-27/18	96.64	96.64	73.15	72.87	98.53	98.53	98.53	73.15	73.15	73.15	99.27	99.27										
14	IR/CAstV/H3718-2/18	97.21	97.21	73.45	73.18	99.64	98.35	98.35	73.45	73.45	73.45	98.90	98.90	98.53									
15	IR/CAstV/H3778-1/19	97.40	97.40	73.68	73.40	99.45	99.27	99.27	73.68	73.68	73.68	99.82	99.82	99.09	99.09								
16	IR/CAstV/H3778-19/19	97.40	97.40	73.68	73.40	99.45	99.27	99.27	73.68	73.68	73.68	99.82	99.82	99.09	99.09	100.00							
17	PDRC/1804/India_(KC633180)	82.92	82.92	73.40	73.40	83.87	83.68	83.68	73.40	73.40	73.40	83.90	83.90	83.92	84.33	84.14	84.14						
18	GA2011_(JF414802)	85.29	85.29	73.85	74.13	83.33	83.38	83.38	73.85	73.85	73.85	83.85	83.85	83.87	83.36	83.60	83.60	86.75					
19	CC_CkAstV_(KX397575)	84.36	84.36	71.62	71.91	82.37	82.42	82.42	71.62	71.62	71.62	82.89	82.89	82.42	82.39	82.64	82.64	84.92	97.40				
20	CAstV/Poland/G059/2014_(KT886453)	84.52	84.52	71.71	71.71	84.98	84.55	84.55	71.71	71.71	71.71	85.00	85.00	85.46	85.00	84.76	84.76	85.83	86.07	85.39			
21	TAstV-1(Y15936)	41.24	41.24	49.42	49.80	45.04	42.61	42.61	49.42	49.42	49.42	43.84	43.84	42.61	44.26	44.26	44.26	45.50	42.56	43.78	44.88		
22	Avian_Nephritis_Virus_(AB033998)	42.49	42.49	35.79	35.79	42.44	41.25	41.25	35.79	35.79	35.79	42.06	42.06	41.25	41.96	41.63	41.63	40.43	43.74	42.87	41.09	36.25	

PCR amplification was performed under the following conditions: initial denaturation at 94◦C for 3 min; 35 cycles of 94◦C for 10 sec; 56◦C for 20 sec; 72◦C for 30 sec. And a final incubation at 72◦C for 10 min.

Sequencing and Bioinformatics Analysis: PCR products were purified using the AccuPrep PCR purification kit (Bioneer Co., Korea). Sequencing reactions were performed with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Bioneer Co., Korea) in forward and reversed directions. The nucleotide sequences of ORF-1b genes were compared with the chicken astrovirus sequences data available in GenBank, and the phylogenetic relationship was established. All of the sequences were aligned using the CLUSTALW with MEGA7 software[14]. Distance-based neighbor-joining trees were constructed by using the Tamura-Nei model. 1000 bootstrap replicates assessed the robustness of the phylogenetic trees. The sequences of the polymerase gene were submitted to GenBank under the accession nos. MN871899-MN871914.

Results

Detection of chicken astrovirus: RT-PCR test was applied to find astrovirus in clinical samples from 36 broiler flocks. Thirty flocks They were less than 10-day old (83.4%), and the other (16.6%) were between ten and twenty days old.

Chicken astrovirus was found in the gut content of 50% of sampled birds that were less than ten days old (15/30). However, the detection rate of astroviruses was less (16.6%) in birds age between 10-20 days old, and astroviruses was detected in one flock from six sampled flocks. In total, chicken astroviruses were discovered in pooled intestinal contents of 16 (44.4%) of affected broiler flocks, and twenty flocks were negative (55.6%).

Sequence analysis and the phylogenetic tree: All positive samples (16 samples) were sequenced in both forward and reverse directions. The sequence of 16 Iranian astroviruses was compared with the avian astroviruses sequence gene available in GenBank. The neoclutide and predicted amino acid sequences of the ORF1b gene of astrovirus found in this study showed sequence identities ranging from 77.7 to 100 and 90.7 to 100 %, respectively (Table 1). Phylogenetic analysis based on a 601-bp segment of the ORF1b gene revealed two subgroups of Iranian astroviruses (Fig. 1).

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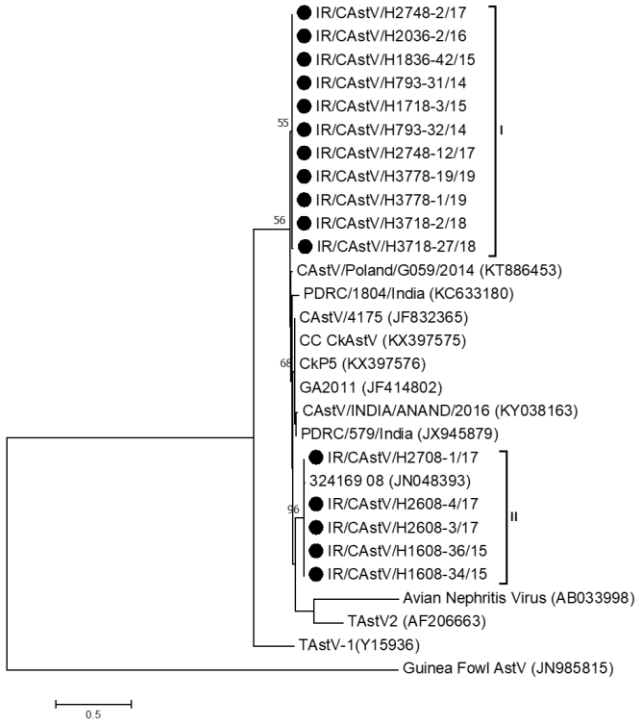

Fig. 1. The neighbor-joining phylogenetic tree of chicken Astroviruses. 1000 bootstrap replicates to assign a confidence level to the branches of the phylogenetic tree. Strains detected in the current study and Iranian previously identified strains marked by a black circle. The vertical lines are for spacing branches and labels. The scale bar represents the distance unit between sequence pairs.

Discussion

Several viruses, namely chicken astrovirus (CAstV), avian nephritis virus (ANV), chicken parvovirus (ChPV), infectious bronchitis virus (IBV), avian rotavirus (AvRV), avian reovirus (ARV), and fowl adenovirus (FAdV), are considered as a potential cause of enteric diseases. Astroviruses (AstVs) are among the most common viruses associated with runting stunting syndrome (RSS) in broilers. They can also be isolated from clinically healthy birds, leading to questions about the role of particular AstV strains in disease pathogenicity [15, 16], which requires further investigation. The study was done to detect the prevalence of chicken astrovirus in broiler flocks with enteric disease by molecular methods. Detection of chicken astrovirus in affected birds may imply the important role of astrovirus in enteric disease.

The capsid protein of astrovirus is related to antigenicity, and it has been found the most variable astrovirus gen. Human and turkey astroviruses are classified into different serotypes based on the gene sequence [17]. An investigation into CAstV strain diversity from historical and circulating field strains was reported in 2012 comparing sequences of ORF 2 (capsid gene) as this is where the most hypervariable regions associated with anti-genicity are located.

Before this study, two distinct serogroups of CAstV had been identified, supported by only a minor degree of cross-reactivity with the heterologous antisera. Antibody against them is reported to be widespread, and the existence of these two serogroups was further supported in the genotyping study by the subsequent clustering of strains into CAstV groups A and B according to a lower level of shared amino acid identity cross ORF 2 (38%–40%).

The group A CAstVs comprised three subgroups, within-subgroup homologies from 77% to 82%. The B group CAstVs comprised two subgroups, B i and B ii, which shared inter-subgroup identities of 84%–85%[6].

Based on partial ORF 1b sequences of CAstVs in the UK, a phylo-genetic analysis of 20 CAstVs indicated the existence of two groups. RT-PCR detected CAstVs in 50/52 (96%) samples. CAstVs were detected between 30% and 72.5% pooled gut content samples from longitudinal surveys of four broiler flocks displaying below-average performances [13].

Phylogenetic analysis based on a 330-bp segment of the ORF1b gene revealed 4 sub-groups of Korean CAstV strains that showed a 12% difference in nt sequences [18].

Great diversities have been seen in ORF1b of Iranian chicken astroviruses, and therefore it is expected to be high nt sequence divergence in the capsid antigenicity and distinct serotypes. Strain et al. (2008) found evidence for recom-bination between astrovirus genomes, so it is possible that different ORF 1b CAstV sequen-ces could be contiguous with very similar ORF 2 capsid protein sequences, or, conversely, that CAstVs with very similar ORF 1b sequences may have different ORF 2 sequences [19].

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It was shown that most birds are infected at very early ages, with a horizontally-acquired virus that was either carried over within the house from earlier broiler flocks or excreted by small numbers of vertically infected chicks. Infections at later time points might be expected to have less deleterious effects on chicken growth than those at early time points [20].

Most of the phylogenetic studies carried out on capsid genes. Recently on molecular characterization of chicken astroviruses in gout-affected commercial broiler chickens in India, Capsid gene-based phylogenetic analysis revealed the clustering of CAstV strains from this study with Indian strains of serogroup Biii suggesting their antigenic relatedness [21].

Given that the polymerase gene is considered quite conserved, two different groups of viruses were detected. The mutation observed might be due to positive selection because of synonymous to nonsynonymous nucleotide [21].

Although not a capsid-based study, considering its association with viruses whose identities have been identified as capsid genes, we can conclude that group 1 may be classified as a group of viruses that cause kidney problems. And group II CAstVs tend to be intestinal. But this is a prediction, and a thorough study should be done on the capsid gene or serology.

The complete genome or ORF-1b of CAstV has not been published, so many questions related to the genome structure and how they relate to other avian astroviruses remain to be answered. As more sequence data become available, a clearer picture will emerge about chicken astroviruses. This will have to be complemented by the serological and biological characterization of these viruses. It is suggested that more accurate identification studies on Iranian CAstVs based on capsid genes or full genome be recommended. The present study was conducted in commercial broiler chickens; further studies are needed to ascertain the status of CAstVs in breeder and layer flocks.

Conclusion

This study underlines the great genetic variability of CAstVs and indicates that there are most likely multiple serotypes of each CAstV circulating in Iranian broiler farms.

Acknowledgment

We Acknowledge from PCR Lab staff for their technical supports.

Conflict of interest

There is no conflict of interest

Funding

Not applicable.

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