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Detection and molecular characterization of chicken astrovirus associated with chicks that have an unusual condition known as "white chicks" in Brazil

L. F. N. Nuñez,* S. H. Santander Parra,* C. Carranza,* C. S. Astolfi-Ferreira,* M. R. Buim,† and A. J. Piantino Ferreira*,[1]

*Department of Pathology, School of Veterinary Medicine, University of São Paulo (USP), Av. Prof. Dr. Orlando M. Paiva, 87, CEP 05508–270, São Paulo, SP, Brazil; and †Biological Institute, Av. Gaspar Ricardo, 1700, CEP 17690–000, Bastos, SP, Brazil

ABSTRACT Chicken astrovirus (CAstV) is one of many viruses related to enteric diseases in poultry that are associated with Runting-Stunting Syndrome (RSS), which affects young chickens. CAstV was also recently associated with an unusual condition in chicks called "white chicks." Some hatcheries in certain states of Brazil have reported several incubation problems, mortality, and the presence of chicks with white plumages over the past several months. These chicks were termed locally as "white chicks." The present work investigated 30 chicks with this unusual condition using a multidisciplinary approach. Postmortem examination of each chick showed enlarged livers and intestines that were full of liquid and gas (30/30). The pancreas, kidneys, and spleen were pale (30/30). The other organs did not show any macroscopic alterations. CAstV, chicken parvovirus (ChPV), avian nephritis virus (ANV), avian rotavirus (ARtV), avian reovirus (AReoV), infectious bronchitis virus (IBV), and fowl adenovirus group I (FAdV-1) were tested in the intestines, pancreas, proventriculus, gizzard, liver, spleen, bursa, kidneys, thymus, lung, heart, brain, and yolk sac in each chick. All organs and yolk sacs were positive for CAstV in different titres and negative for the other tested viruses. The partial molecular characterization of the ORF 1b gene of CAstV using 28 sequences revealed a high similarity of the nucleotides and amino acids with sequences of CAstV from North America, Europe, and Asia, and our CAstV sequences clustered into a unique group that was separate from the other sequences. These results demonstrated that CAstV was associated with the white chick condition in Brazil. The virus was distributed in most organs, including the brain and yolk sac. These results suggest that the virus could be transmitted vertically. The molecular characterization also revealed that the CAstV associated with white chick condition was molecularly related to other CAstV sequences found worldwide.

Key words: chicken, astrovirus, white chicks, detection, characterization

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INTRODUCTION

Enteric diseases are of paramount importance to the national and global poultry industry (Jindal et al., 2012). 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, which causes the body weights at slaughter to differ broadly (Otto et al., 2006). Olsen first reported RSS in the broiler industry in 1977 (Olsen, 1977). This syndrome is a transmissible disease of uncertain etiology that affects chickens early in life (Goodwin et al., 1993; Kang et al., 2012b). Enteric viruses, such as CAstV, ChPV, ANV, ARtV, AReoV, and FAdV-1 are related and have been detected in chickens with signs of RSS (Day et al., 2007; Pantin-Jackwood et al., 2008; Pantin-Jackwood et al., 2011; Mettifogo et al., 2014). However, CAstV is recognised as the causal agent of enteritis in chickens, primarily young chicks, and it has been detected in one-day-old chicks (Pantin-Jackwood et al., 2008; Mettifogo et al., 2014). There are actually 2 astroviruses in chickens that are genetically characterised as ANV and CAstV (Imada et al., 2000; Baxendale and Mebatsion, 2004; Todd et al., 2009a). Astroviruses are small, spherical viruses that are 25 to 35 nm in diameter and possess single-stranded, positive-sense RNA genomes approximately 7 kb in length. The genome encodes for 3 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 (Kang et al., 2012a). The genome begins with a 5′ untranslated region (**UTR**), followed by the 3 ORFs, a 3′

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[1]Corresponding author: ajpferr@usp.br

Table 1. Description of samples' characteristics obtained from poultry companies with hatching of white chicks, in Brazil.

Sample designation	Origin of sample	External characteristics of chicks with unusual condition of white chicks		Age of chicks	Brazilian state localization	GenBank accession number
		White plumage	Pale beak and legs/feet			
541–1	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013267
541–2	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013275
541–3	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013276
541–4	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013268
541–5M	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013271
541–6M	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013274
541–7F	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013269
541–8	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013272
541–9	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013270
541–10	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013273
541–11	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013254
541–12	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013255
541–13	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013251
541–14	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013253
541–15	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013249
541–16	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013252
541–17	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013250
541–18	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013256
541–19	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013258
541–20	Broiler chicks	Yes	Yes	1 day	São Paulo	Not performed
541–21	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013262
541–22	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013257
541–23	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013264
541–24	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013259
541–25	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013263
541–26	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013260
541–27	Broiler chicks	Yes	Yes	1 day	São Paulo	Not performed
541–28	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013265
541–29	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013261
541–30	Broiler chicks	Yes	Yes	1 day	São Paulo	KR013266

UTR, and a poly A tail (Koci and Schultz-Cherry, 2002; Nuñez and Piantino Ferreira, 2013a). ORF1a encodes the viral protease and is followed by ORF1b, which encodes the RNA polymerase (RdRp). ORF2 encodes the precursor of the capsid protein, and it is located downstream of ORF1b and prior to the untranslated region of the genome (Kang et al., 2012a). An experimental infection with CAstV in one-day-old chickens showed mild diarrhea and distension of the small intestine (De Wit et al., 2011). Clinical signs, including diarrhea, decreased food consumption, and nervousness, developed between one and 3 wk of age (Moser and Schultz-Cherry, 2005). CAstV has been detected in the United Kingdom, United States, Korea, India, Netherlands, Croatia (Day et al., 2007; Pantin-Jackwood et al., 2007; Todd et al., 2009a,b; Smyth et al., 2010; Kang et al., 2012b; Koo et al., 2013). And, more recently, in Brazil (Mettifogo et al., 2014), which demonstrates a worldwide distribution (De Benedictis et al., 2011). Most of the CAstV reported worldwide was detected in chickens with enteric disease. However, more recent reports have indicated an association of CAstV with a low hatching rate, weakness, and white plumage, which is characterised as an abnormal condition termed white chicks (Smyth et al., 2013).

The molecular characterization of CAstV is very important in understanding the relationship and genetic similarity among the different isolates worldwide. This study examined the viral agent that is associated with the condition of white chicks in Brazil and described the partial molecular characterization of the agent related with chicks with this unusual condition and its presence in Brazilian poultry.

MATERIAL AND METHODS

After 2014, some states of Brazil began reporting problems in their hatcheries of an increase in pipped eggs and chick mortality immediately after hatching. The chicks presented white plumage with discolored and pale beaks and legs. This uncommon condition was termed "white chicks" (Smyth et al., 2013). Thirty chicks with this unusual condition were sent to the Laboratory of Avian Diseases at the School of Veterinary Medicine, University of São Paulo, to determine the agent that is related to this condition (Table 1). The chicks were subjected to postmortem examination and molecular screening for CAstV, ANV, ARtV, AReoV, IBV, FAdV-1, and ChPV.

Postmortem Examination

The chicks were subjected to postmortem examination. Each organ was collected separately, and weighed. The organs analyzed in the present survey were brain, intestines (duodenum, jejunum, ileum, and ceca), pancreas, liver, kidney, heart, lung, thymus, bursa, spleen, gizzard, proventriculus, and yolk sac.

Table 2. Primers sequences used in the PCR and RT-PCR used in the present work, gene target, amplicon generated, and references for each virus.

Virus	Gene target	Primer designation	Sequences	Amplicon	Reference
CAstV	ORF-1b	CAS pol 1F	5′GAYCARCGAATGCGRAGRTTG3′	362 bp	Day et al., 2007
		CAS pol 1R	5′TCAGTGGAAGTGGGKARTCTAC3′		
ANV	ORF-1b	ANV Pol 1F	5′GYTGGGCGCYTCYTTTGAYAC3′	473 bp	Day et al., 2007
		ANV Pol 1R	5′CRTTTGCCCKRTARTCTTTRT3′		
Avian rotavirus	NSP4	NSP4-F30	5′GTGCGGAAAGATGGAGAAC3′	630 bp	Day et al., 2007
		NSP4-R660	5′GTTGGGGTACCAGGGATTAA3′		
Avian reovirus	S4	S4-F13	5′GTGCGTGTTGGAGTTTCCCG3′	1120 bp	Pantin-Jackwood et al., 2008
		S4-R1133	5′TACGCCATCCTAGCTGGA3′		
IBV	UTR	UTR 11	5′ATGTCTATCGCCAGGGAAATGTC3′	179 bp	Cavanagh et al., 2002
		UTR 44	5′GGGCGTCCAAGTGCTGTACCC3′		
		UTR 31	5′GCTCTAACTCTATACTAGCCTA3′		
FAdV-1	Hexon	Hexon A	5′CAARTTCAGRCAGACGGT3′	897 bp	Meulemans et al., 2001
		Hexon B	5′TAGTGATGMCGSGACATCAT3′		
ChPV	NS	PVF1	5′TTCTAATAACGATATCACT3′	561 bp	Zsak et al., 2009
		PVR1	5′TTTGCGCTTGCGGTGAAGTCTGGCTCG3′		

Molecular Detection

Each organ was subjected to the molecular detection of the above-mentioned enteric viruses using PCR and RT-PCR (Table 2). An aliquot of each organ or the entire organ, depending on organ weight, was placed into a 1.5 mL sterile microfuge tube containing 0.1 M phosphate-buffered saline (PBS, pH 7.2), in equivalent volume to the organ weight or the aliquot placed in the microfuge tube. Macerated thymus, pancreas, and spleen were placed into a 1.5 mL sterile microfuge tube with 300 μL of 0.1 M PBS (pH 7.2). An aliquot of yolk (750 μL) was also placed into a 1.5 mL sterile microfuge tube with the same quantity of 0.1 M PBS (pH 7.2). These suspensions were subjected to 3 cycles of freezing to −80°C for 10 min and thawing for one min to 56°C, homogenised, and centrifuged to 12.000 x g for 20 min to 4°C. The supernatants (250 μL) were treated with TriZOL reagent for the extraction of RNA and DNA (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instruction. Ultra-pure water was used as a negative control.

Reverse Transcriptase

The RNA obtained was submitted to a reverse transcription reaction to obtain complementary DNA (cDNA). A volume of 3.5 μL of extracted RNA was denatured at 95°C for 5 min in a 200-μL microtube, and 6.5 μL of a mixture containing 2 μL 5X buffer, one μL dithiothreitol (DTT), 10 mM deoxynuclotides triphosphates (dNTPs), one μL of each forward and reverse primers, and 0.5 μL M-MLV enzyme (Invitrogen Life Technologies, Carlsbad, CA) was added. A reverse transcription reaction was performed under the following conditions: 45°C for 60 min and 72°C for 10 min. The cDNA obtained was submitted to PCR.

Polymerase Chain Reaction

The PCR reaction used 24 μL of a mixture that contained 0.5 μM of each forward and reverse primers, 2.5 μL 10X buffer, 5 mM of each dNTP, 37.5 mM of $MgCl_2$, one U of Platinum DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA), and one μL cDNA. PCR amplification was performed under the following conditions: a cycle of 94°C for 3 min to completely denature the cDNA; 30 cycles of a denaturation temperature at 94°C for one min; hybridisation of primers at 56°C for one min; extension at 72°C for one min; and a final incubation at 72°C for 10 min. The reaction was maintained at 4°C for an undetermined period until storage at 20°C. RT-PCR for CAstV amplification was performed using similar protocols as Day et al. (2007) (Table 2). Chicks with the unusual condition white chicks were tested for other enteric viruses. PCR for ChPV was performed according to Zsak et al. (2009). PCR for FAdV-1 was performed according to Meulemans et al. (2001). RT-PCR for ANV and avian rotavirus was also performed according to Day et al. (2007). RT-PCR/NESTED for IBV was performed according to Cavanagh et al. (2002), and RT-PCR for reovirus was performed according to Pantin-Jackwood et al. (2008). Table 2 describes the primer sequences used. The amplified products (362-bp CAstV, 473-bp ANV, 179-bp IBV, 630-bp ARtV, 1120-bp AReoV, 561-bp ChPV, and 897-bp FAdV-1) were submitted to electrophoresis in a 1.5% agarose gel. The samples were stained with Sybr Safe (Invitrogen by Life Technologies, Carlsbad, CA) and compared with a 100-bp molecular ladder (Invitrogen by Life Technologies, Carlsbad, CA). The results were analyzed on a transilluminator and photographed using an Alpha Imager Mini Analysis System (Alpha Innotech by Protein Symple, Santa Clara, CA).

DNA Sequencing and Phylogenetic Analyses

The amplified products of the ORF1b gene of CAstV from each animal were purified using a GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Piscataway, NJ) as described by the

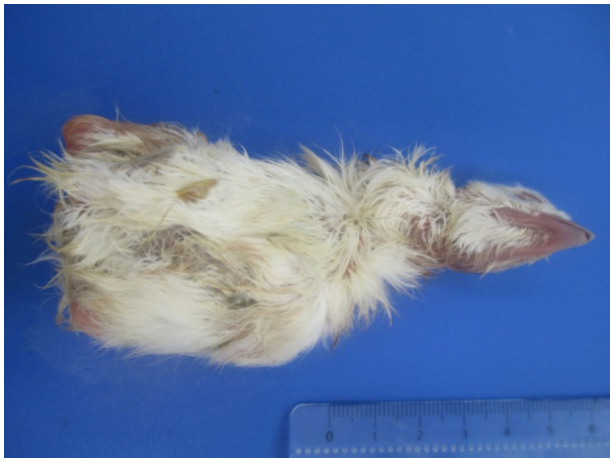

Figure 1. Chicks showing white plumage.

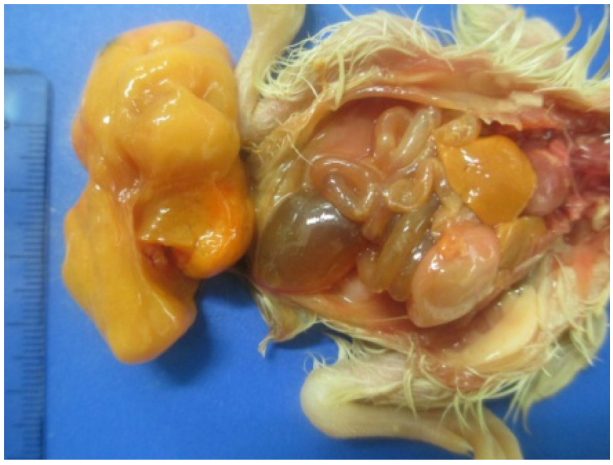

Figure 2. Postmortem examination of white chicks showed enlarged liver, and intestines filled with gas and liquid. Also, chick is presenting white feet.

manufacturer. Each purified product was sequenced in the forward and reverse direction using a BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems by Life Technologies, Carlsbad, CA). Sequencing reactions were run in an ABI 3730 DNA Analyzer (Applied Biosystems by Life Technologies, Carlsbad, CA). Nucleotide sequences were edited using the CLC Main WorkBench 7.5.1 package software (CLC Bio, Qiagen, Waltham, MA) and aligned with other sequences using the CLUSTAL W method available in the ClustalX 2.0.11software package (UCD, Dublin, Ireland). The nucleotide phylogenetic tree was inferred using the neighbour-joining, maximum composite-likelihood method with 1,000 bootstrap replicates that were integrated in the MEGA version 5 software (Tamura et al., 2011).

GenBank Accession Number

The accession number of ORF 1b sequences of CAstV were obtained here are as follows: KR013249 to KR013276. Figure 3 shows the GenBank accession numbers of the sequences used for molecular analyses in the present work.

RESULTS

Postmortem Examination

White chicks exhibited discolored beaks and legs/feet and white plumage (30/30; 100%), as shown in Figure 1. The abdominal organs showed several abnormalities. Livers were enlarged and yellow and surpassed the last ribs by approximately 0.5 cm (30/30; 100%), and the gallbladders were full. Intestines were filled with liquid and gas bubbles (30/30; 100%) with several intestines containing meconium (25/30; 83.33%), yellowish with a yolk-like consistency, and all yolk sacs were filled with yolk (30/30; 100%), as presented in Figure 2. The pancreas and spleen were pale, and the spleen had an oval form (30/30; 100%). Some chicks showed a dilatation of the proventriculus (12/30; 40%). The kidneys were pale with some yellow foci (30/30; 100%). The gizzard was apparently normal without erosions or hemorrhage. The brain, thymus, bursa, heart, and lungs did not exhibit any macroscopic alterations.

Molecular Detection

RT-PCR amplified the ORF1b gene of CAstV and obtained an amplicon of 362 bp. All of the collected organs were subjected to the molecular detection of enteric viruses. At least one organ from each bird was positive for CAstV in this study. The intestines, liver, spleen, thymus, bursa, pancreas, kidneys, heart, and lungs were positive for CAstV, and no other enteric viruses were detected (Table 3). CAstV was primarily detected in samples of the gizzard (96.67%), intestines (93.33%), lungs (93.33%), kidneys (83.33%), pancreas (80%), spleen (80%), and yolk (60%), but the virus was less detected in the liver (13.13%), proventriculus (10%), heart (6.67%), brain (6.67%), and thymus (6.67%), as shown in Table 3. The present study demonstrated that CAstV was distributed in all organs tested and the yolk.

DNA Sequencing and Phylogenetic Tree

The sequences of CAstV from white chicks were edited and compared with other sequences from GenBank using the BLAST tool. The results showed high similarity with other CAstV. A fragment of approximately 360 bp from sequences of the CAstV from white chicks in the present work was compared with other sequences. The Brazilian sequences of CAstV obtained here revealed a high similarity to nucleotide (**nt**) (100 to 99.1%) and amino acid (**aa**) (100 to 98.3%) between the sequences. Comparisons with sequences from other countries showed a high similarity of nt (90.3 to 85.6%) and aa (97.5 to 95%) with sequences of the

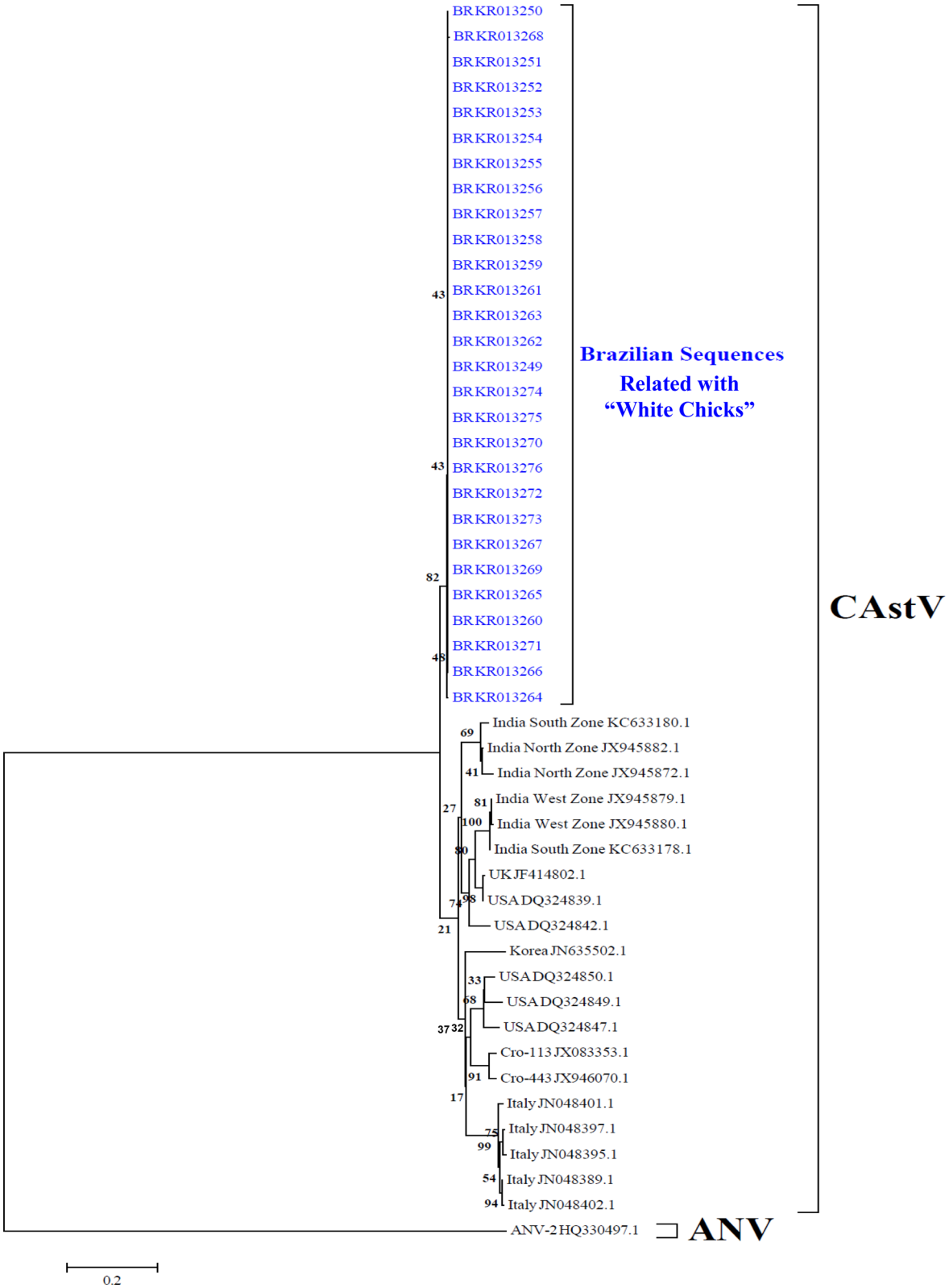

Figure 3. Neighbor-joining tree based on alignments of the partial ORF 1b sequences of CAstV. Phylogenetic relationships of the Brazilian (BR) CAstV sequences from white chicks compared with other sequences from Croatia (CRO), India, Italy, Korea, United Kingdom (UK), and United States (USA). Sequences were aligned using the Clustal W method in ClustalX software. The tree was constructed using MEGA 5 software package. Numbers along the branches refer to bootstrap values for 1,000 replicates. The scale bar represents the number of substitutions per site. ANV-2 sequence was used as the out-group.

Table 3. Molecular detection using RT-PCR of CAstV in the organs from chickens with unusual condition of white chicks.

Sample designation	Proventriculus	Intestine	Pancreas	Spleen	Kidney	Liver	Yolk sac	Lung	Bursa	Thymus	Brain	Heart	Gizzard
541—1	–	+	+	+	+	–	–	+	–	–	–	–	+
541—2	–	+	+	+	+	–	–	+	–	–	+	–	+
541–3	–	+	+	+	–	–	–	–	–	–	–	–	+
541–4	–	+	+	+	+	–	–	+	–	–	–	–	+
541–5M	–	+	–	–	+	–	+	+	–	–	–	–	+
541–6M	–	+	+	+	+	–	+	+	–	–	–	–	+
541–7F	–	+	–	+	+	+	+	+	–	–	–	–	+
541–8	–	+	+	+	+	–	+	+	–	–	–	–	+
541–9	–	–	+	–	+	–	+	+	–	–	–	–	+
541–10	–	+	+	+	+	–	+	+	–	–	–	–	+
541–11	–	+	+	+	+	–	+	+	–	–	–	–	+
541–12	–	+	+	+	+	–	+	+	–	–	–	–	+
541–13	–	–	–	+	+	–	–	+	–	–	–	–	+
541–14	–	+	+	+	+	–	–	+	–	–	–	–	+
541–15	–	+	+	+	–	–	–	+	–	–	–	–	+
541–16	–	+	+	+	+	–	–	+	–	–	–	–	+
541–17	–	+	–	+	+	–	+	+	+	–	–	–	+
541–18	–	+	+	+	+	+	+	+	+	–	–	–	+
541–19	–	+	+	–	+	–	+	+	+	–	–	–	+
541–20	–	+	–	–	–	–	–	+	–	–	–	–	–
541–21	–	+	–	–	+	–	+	+	+	–	–	–	+
541–22	–	+	+	+	+	–	–	+	–	–	–	–	+
541–23	+	+	+	+	–	–	+	+	–	–	+	–	+
541–24	–	+	+	+	+	–	+	+	–	+	+	+	+
541–25	+	+	+	+	+	–	–	+	–	–	–	+	+
541–26	–	+	+	+	+	–	+	+	–	–	–	–	+
541–27	–	+	+	+	+	+	+	+	–	–	–	–	+
541–28	–	+	+	+	–	+	+	+	–	–	–	–	+
541–29	+	+	+	+	+	–	–	+	–	–	–	–	+
541–30	–	+	+	–	+	–	+	–	–	+	–	–	+
Total of positives	3/30 (10%)	28/30 93.33%)	24/30 (80%)	24/30 (80%)	25/30 (83.33%)	4/30 (13.13%)	18/30 (60%)	28/30 (93.33%)	4/30 (13.33%)	2/30 (6.67%)	3/30 (10%)	2/30 (6.67%)	29/30 (96.67%)

United States; a high similarity of nt (88.9 to 88.6%) and aa (96.6 to 95%) with sequences of the India West Zone; a high similarity of nt (89.7 to 88.1%) and aa (97.5 to 93.3%) with sequences of the India North Zone; a high similarity of nt (89.2 to 88.3%) and aa (97.5 to 95.8%) with sequences of the India South Zone; a high similarity of nt (86.7 to 85.3%) and aa (96.6 to 95.8%) with sequences of Italy; a high similarity of nt (87.8 to 87.2%) and aa (98.3 to 96.6%) with sequences of Croatia; a high similarity of nt (86.1 to 85.9%) and aa (94.1 to 93.3%) with sequences of Korea; and a high similarity of nt (90 to 89.7%) and aa (97.5 to 96.6%) with sequences of the United Kingdom. The phylogenetic tree clustered the Brazilian sequences in a separate group with a bootstrap value of 82% and 0.01451 substitutions per site. The sequences of other countries were clustered into another group, with a bootstrap value of 21% and 0.04038 substitutions per site. The sequences of CAstV from other countries clustered accordingly to their geographic origin, except for the sequence from the United Kingdom, which clustered with the sequences of the United States (Figure 3). The grouped sequences from other countries, which contained the sequences from India that belonged to the subgroup Bi of CAstV, were grouped according to capsid sequence diversity. No other sequences from other countries grouped with the Brazilian sequences obtained here.

DISCUSSION

Enteric diseases were reported in mammals, and several outbreaks of diarrhea, were associated with enteric viruses, such as astrovirus (Dai et al., 2010; De Benedictis et al., 2011; Zsak et al., 2013), rotavirus (Rajendran and Kang, 2014; Wu et al., 2014), and coronavirus (Costa et al., 2014; Pinto et al., 2014). Chickens and turkeys are also affected by enteric diseases, and outbreaks of enteric diseases have been described in several parts of the world (McNulty et al., 1980a; Pantin-Jackwood et al., 2008; Guy et al., 2011; Day and Zsak, 2013; Nuñez and Piantino Ferreira, 2013a). Viruses that are associated with mammalian enteric diseases, including ARtV (Spackman et al., 2010; Moura-Alvarez et al., 2013), AReoV (Davis et al., 2013), ChPV (Zsak et al., 2013; Nuñez et al., 2015b), and CAstV (Smyth et al., 2009). CAstV (Baxendale and Mebatsion, 2004), TAstV (Saif et al., 1985), and ANV (Imada et al., 1979; Imada et al., 2000) were identified in poultry, and all of these viruses are associated mainly with enteric problems. CAstV was reported in the United States (Pantin-Jackwood et al., 2008; Smyth et al., 2013), the United Kingdom (Todd et al., 2009a), Korea (Koo et al., 2013), Italy (Canelli et al., 2012), India (Bulbule et al., 2013), and Brazil (Mettifogo et al., 2014; Nuñez et al., 2015c).

The present work reported the presence of CAstV in chicks with white plumage and weakness that died within a few h of life (i.e., white chicks). The presence

of this condition was recently reported by (Smyth et al., 2013), who showed that CAstV was involved in the presentation of this alteration in recently hatched chickens. The first report of white chicks showed that several countries in Europe and North America where experiencing this condition in chicken flocks (Smyth et al., 2013). Over the past several y, there have been many reports of the presence of white chicks in Brazilian hatcheries, and these chicks showed an increase in mortality and hatchability problems. The present study showed that CAstV is involved with this condition in chickens in Brazil.

The present study also suggests that CAstV is vertically transmitted and that the emergence of white chicks may not be a unique condition related with CAstV, but could also be associated with the occurrence of cases of enteric disease (diarrhea) in young chickens where CAstV has already been detected at one d of age (Mettifogo et al., 2014), which confirms the vertical transmission. The present work demonstrates that CAstV was distributed in several organs, principally in the digestive tract, gizzard, and intestine. However, there were fewer proventriculus positive samples. CAstV also was detected in the yolk sac remnant, which suggests that the virus originated there and began to develop in the intestine with continuous propagation in the gizzard. The virus may subsequently move to the proventriculus. However, difficulty in the isolation of CAstV hinders our understanding of the path that the virus uses for propagation in the embryonic organism (Nuñez et al., 2015c). Further, CAstV was described in the accessory glands, such as the pancreas and liver, and in the lymphoid organs, such as the thymus, bursa, and spleen. However, few liver samples were positive for CAstV, which contrasts a Smyth et al. (2013) report in which the liver exhibited more prevalence for CAstV. Moreover, in the present work, CAstV was detected in the brain of analyzed chicks. To the authors' knowledge, there are no reports of CAstV in the brains of chickens. These results suggest that the viremia may reach several organs, including the brain.

Astrovirus in chickens was detected using transmission electronic microscopy in feces, where it was visualized as astrovirus-like particles (McNulty et al., 1980b; McNulty et al., 1990). Molecular tests were developed subsequently to detect CAstV with more accuracy (Pantin-Jackwood et al., 2006; Day et al., 2007; Smyth et al., 2009; Pantin-Jackwood et al., 2011). RT-PCR amplified a specific region of the ORF 1b gene of the CAstV genome in the present work (Day et al., 2007). The sequences obtained here from our chickens with the condition of white chicks showed a high similarity of nucleotides and amino acids with sequences from several parts of the world, which demonstrates that the CAstV related with white chicks in Brazilian flocks is molecularly related with other CAstV. However, the Brazilian CAstV was grouped into a separate cluster. Different molecular analyses showed that CAstV was classified into several groups: group A principally included sequences of the United Kingdom, and group B (Smyth et al., 2012) formed two subgroups, Bi and Bii. The sequences obtained in the present work were grouped into a unique group, and the other sequences were grouped with isolates from India that belonged to Subgroup Bi (Bulbule et al., 2013). However, a high similarity of nt and aa were shared with the Brazilian sequences, which suggests that the sequences obtained here may also belong to the Subgroup Bi. Moreover, the sequences obtained here were compared with sequences from groups A and Bii according to Bulbule et al. (2013), and the results showed a low similarity of nt and aa (data not shown). More studies must be performed to understand the molecular features of CAstV that are related to white chicks and determine whether the complete genome of this virus is completely related to the viruses detected in chickens with enteric diseases to determine its importance in the occurrence of enteric diseases.

CAstV is related principally with enteric disorders, such as RSS (Koci and Schultz-Cherry, 2002; Day et al., 2007; Pantin-Jackwood et al., 2007; Pantin-Jackwood et al., 2011). CAstV was also involved with an outbreak of gout (Bulbule et al., 2013), in which CAstV was related to chicken embryos with discolored feathers and mortality, which are features that are very similar to the characteristics of the chicks analyzed in the present work. Experimental infection with CAstV associated with gout in specific pathogen-free (**SPF**) chicken embryos also resulted in the stunting of embryos, necrosis of the liver, and pale and swollen kidneys (Bulbule et al., 2013). CAstV in the present work was detected in the kidneys and lungs, which demonstrates that these organs are another target for virus replication.

CAstV is circulating in Brazilian chicken flocks, and the actual data show the presence of CAstV in the kidneys of analyzed chicks. These results suggest that CAstV, in addition to causing enteric diseases, may be causing renal alterations, but this hypothesis requires further investigation to determine whether these factors are related also to gout.

Normally, broiler chicks hatch with yellow plumage, and the color is due to a genetic effect (Park et al., 2013) and carotenoid pigments (Perez-Vendrell et al., 2001). Carotenoid pigments are common colorants of egg yolk, feathers, and bare parts (such as the beak and legs) in birds. Carotenoids are supplied in the diet, and they are usually absorbed in different sections of the intestine, principally the ileum. The absorption of lutein occurs in the duodenum and jejunum (Tyczkowski and Hamilton, 1986). White chicks exhibit a white plumage at hatching, from which the name was derived. Hypothetically, this unusual white plumage pigmentation may be due to a lack of carotenoid accumulation and a decrease in carotenoid (lutein) absorption provoked by CAstV replication in the intestinal mucosa.

Enteric diseases are a very significant health problem in the poultry industry. Over the last several years, several outbreaks of enteric diseases were reported in

some states of Brazil, including primary agents, such as CAstV, ChPV, FAdV-1, ARtV, and AReoV (Mettifogo et al., 2014). A compromised enteric tract is a concern for the poultry industry because of the low performance, high production costs, and increase in the use of therapeutic drugs that are required to reduce the impact of associated pathogens.

Detection of CAstV in chicks with clinical signs of white chicks in the present study confirmed that the virus is circulating in Brazilian chicken flocks and that it may be vertically transmitted and causing hatchability problems. The molecular analyses showed that the Brazilian CAstV is similar to CAstV around the world. Further studies must be performed to understand the role of this virus in chicken health.

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