Short Communication

Genetic Characterization of Avian Rotavirus Groups F and G in Brazilian Farms

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Abstract | Rotaviruses are one the causes of enteritis and diarrhea in avian species, resulting in negative impact on egg and poultry production. The objective of this work was to detect and characterize Rotavirus F (RVF) and G (RVG) found in Brazilian commercial avian farms. A total of 97 intestinal contents was collected between 2008 and 2015 from 10 Brazilian states, and submitted to two different RT-PCR, targeting a fragment of viral VP6-coding nucleotide sequence of each viral group. Amplicons were further submitted to genetic sequencing and analyzed by phylogenetic analysis. There were 5 RVF positive samples (5.15%) in 2 broilers, 1 laying hen and 2 breeders. Only one RVG sample (1.03%) was detected in laying hen, in coinfection with RVF. This data contributes to a better understanding of rotavirus distribution in Brazil.

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Introduction

Rotaviruses (RVs) are one the causes of enteritis and diarrhea in avian species, resulting in negative impact on egg and poultry production (Islam et al., 2010; Dhama et al., 2015).

Belonging to *Reoviridae* Family, RVs are triple-shelled non-enveloped particles with an icosahedral morphology. Their genome consists of 11 double-stranded RNA segments, encoding one or two proteins each, resulting in six structural proteins (viral proteins VP1-VP4, VP6 and VP7) and six nonstructural proteins (NSP1-NSP6). They are classified in eight major groups named A to H according to the antigenic properties and nucleotide sequences of the VP6 internal capsid viral protein (Dhama et al., 2015; Mihalov-Kovács et al., 2015), and two new group candidates (I and J) have been proposed more recently (Bányai et al., 2017; Phan et al., 2017).

Rotavirus F (RVF) and Rotavirus G (RVG) have only been detected in avian species so far (Kindler et al., 2013; Dhama et al., 2015). Data on the occurrence of these RV groups in Brazil are scarce, despite the relevance of the avian industry in this country and the

significant economic losses that this infection may result.

In this study, we report the occurrence of RVF and RVG in avian farms, based on the amplification of the VP6 gene, broadening the knowledge about the circulation of these viruses in different Brazilian regions.

Materials and Methods

Samples

A total of 97 intestinal contents was used in this study, collected between the years 2008 and 2015. Farms were located in ten different Brazilian states (Ceará, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Pernambuco, Santa Catarina, São Paulo, Rio Grande do Sul), including broilers (43.29%; 42/97), layers (41.23%; 40/97), and breeders (15.46%; 15/97). There is no information on clinical signs, nor the age of sampled animals.

Reverse transcriptase-PCR (RT-PCR)

Samples were prepared as 50% (w/v) suspensions in diethylpyrocarbonate-treated water and centrifuged at 12,000 g for 15 min at 4ºC; the resulting supernatants were used in the RNA extraction. Total RNA was extracted using TRIzol reagent (Invitrogen™), and cDNA was synthesized using random primers (Invitrogen™) and M-MLV reverse transcriptase (Invitrogen™), as described by the manufacturer. The samples were screened by PCR targeting a fragment of VP6 gene followed by nucleotide sequencing of the detected amplicons, using a group F primer pair (forward primer VP6AVF10 5'-AAGTCAATCAGTCGCAATG-3' and reverse primer VP6AVF891 5'-GGTCTAACTATTCGTAGCCTAA-3') generating a 903-bp fragment (Beserra and Gregori, 2014). For RVG detection, a PCR primer pair was designed (forward primer VP6AVG26 5'-CAGCATGGATCTCATCGA-3' and reverse primer VP6AVG1095 5'-TCACGAACGAATCTTGAGT-3'), targeting the VP6 gene, based on the nucleotide sequence of avian RVG strain 03V0567 (accession number HQ403604) (Johne et al., 2011), generating a fragment of 1070-bp.

For both reactions, 5µL of cDNA was added to the PCR mix, which consisted of 1X PCR buffer (Invitrogen™), 0.2mM of each DNTP, 0.5mM of each primer, 2mM $MgCl_2$, 1.25U Taq DNA polymerase (Invitrogen™), and ultrapure water up to 25 µL, and submitted to the following thermocycling conditions: initial denaturation at 94ºC for 3 min; 30 amplification cycles (94ºC for 45 sec, 50ºC for 30 sec, and 72ºC for 45 sec); with a final extension at 72ºC for 10 min. The products were resolved in a 1.5% agarose gel stained with 3µL of SYBR Safe (Invitrogen™).

DNA sequencing

VP6 PCR amplicons were purified with EXOSAP-it (USB Products®) reagent, submitted to bidirectional DNA sequencing using BigDye 3.1 (Applied Biosystems™), and resolved in an ABI-3500 Genetic Analyzer (Applied Biosystems™), according to the manufacturer's instructions. The nucleotide sequences from each sample were aligned with other rotavirus groups retrieved from GenBank using CLUSTAL/W v. 2.1 (Larkin et al., 2007). A phylogenetic tree was generated with the neighbor-joining distance algorithm and the maximum composite likelihood substitution model with 1000 bootstrap replicates using MEGA v.7.0.26 (Kumar et al., 2016) from a 615-nucleotide-long VP6 partial fragment.

Accession numbers

The RVF sequences were deposited in GenBank under the accession numbers MF361095 to MF361099, and RVG under the accession number MF361100.

Results

Frequency of rotavirus F ang G

Through the RT-PCR and sequencing reactions, there were 5 RVF positive samples (5.15%; 5/97), from Paraná state (n=2/broiler); Mato Grosso do Sul state (n=1/layer); Rio Grande do Sul state (n=1/breeder) and Goiás state (n=1/breeder). Regarding RVG, this study detected only one positive sample (1.03%; 1/97) in a layer hen from Mato Grosso do Sul state. Moreover, this sample presented coinfection with RVF.

Phylogenetic tree

The phylogenetic tree confirmed the PCR results, and the 5 RVF sequenced samples clustered together with rotavirus F representatives. Regarding RVG, the sample detected herein was more related to HK18 (accession number KC876014) RVG strain isolated from pigeon, than the previously described in broilers from Brazilian Amazon. The tree topology could segregate all RV (A, B, C, D, F, G, H and I) clades, supported by high bootstrap values, as shown in Figure 1.

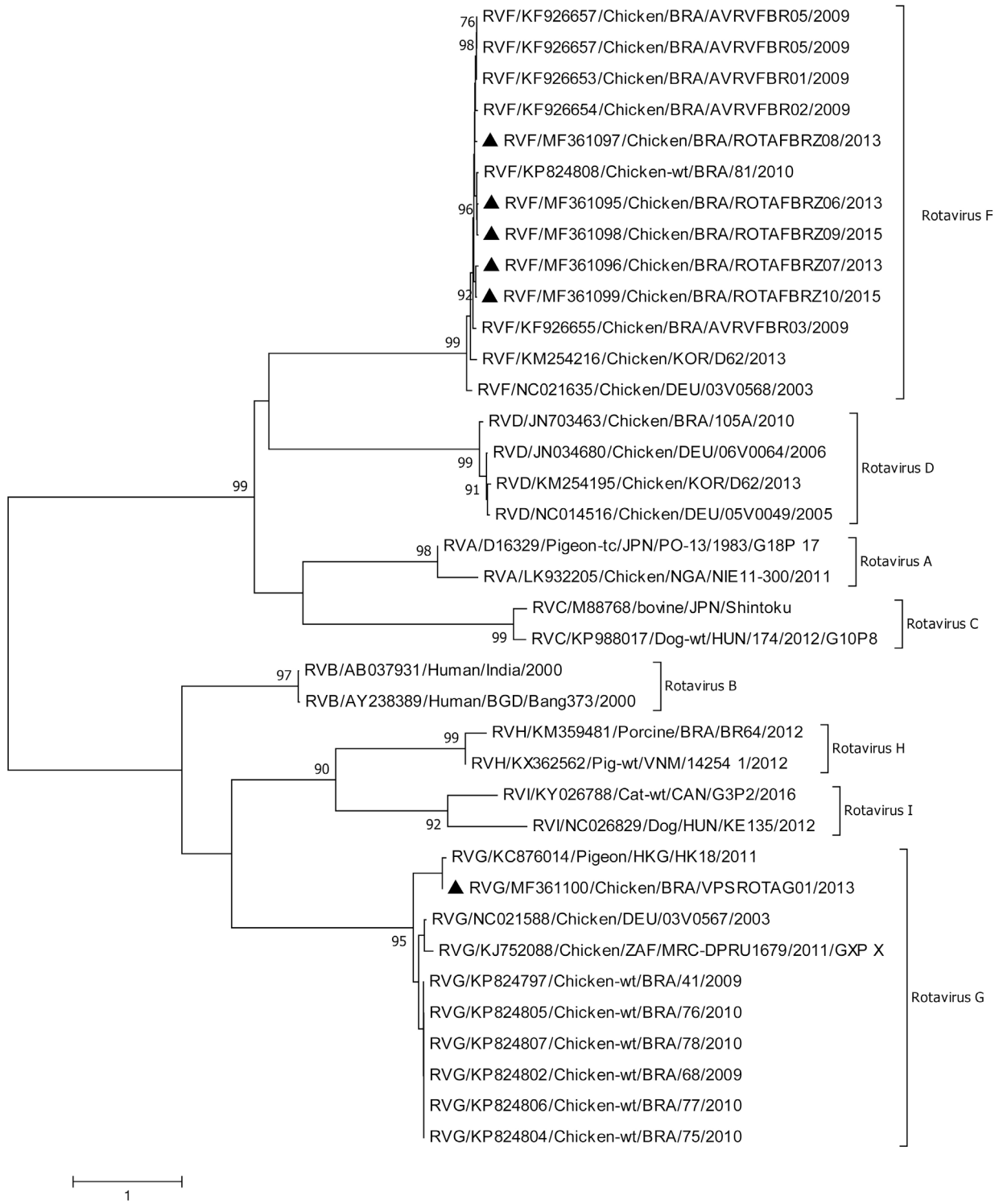

Figure 1: *Nucleotide neighbor-joining distance tree (maximum composite likelihood substitution model) of the partial VP6 rotavirus gene (615-nt), according to RV groups. Strains detected in this study are preceded by black triangles. The numbers at each node are bootstrap values greater than 70% from 1,000 bootstrap replicates, and the scale bar represents the number of substitutions per site.*

Discussion

It was found a relatively low frequency of RVs, with values of 5.15% for RVF and 1.03% for RVG, among different Brazilian States and types of avian farming. These results are similar to those found in Brazilian Amazon, where incidences of 9.4% (8/85) and 10.6% (9/85) were shown for RVF and RVG, respectively, in broiler chickens (Mascarenhas et al., 2016). Similarly, RVF presented a frequency of 9.43% (5/53) in broilers farms from two different Brazilian States (Beserra and Gregori, 2014), which were distant from Ama-

zon region. A low frequency of RVF and RVG were already described in Germany and United Kingdom (Otto et al., 2012).

One of the samples detected in this study demonstrated a coinfection between RVF and RVG. Double rotaviral infections have been detected previously (Mcnulty, 2008, Otto et al., 2012; Beserra and Gregori, 2014) and they may lead to the generation of reassortants. These findings show the importance of performing a diagnosis covering different avian rotavirus groups, otherwise the infections may be underdiagnosed and, consequently, fewer measures could be taken to control the disease.

Even though only partial VP6 gene sequences have been used, the phylogenetic tree depicted a distinction among the rotavirus groups, supported by high bootstrap values (greater than 90%). Figure 1 shows a clade including RVF, RVA, RVD and RVC, and another one with RVB, RVG, RVH and RVI, in agreement with previous studies (Johne et al., 2011; Kindler et al., 2013; Beserra and Gregori, 2014; Mascarenhas et al., 2016).

Considering previous descriptions of RVF and data generated in this study, this agent was diagnosed in all Brazilian geographical regions, and hence a wide distribution in poultry farms, but with relatively low genetic variability (Figure 1).

The positive sample detected in Mato Grosso do Sul is the first RVG description in this state and probably has a different origin from those previously detected in Amazon region (Mascarenhas et al., 2016), given the tree topology, with two different clades (Figure 1). Interestingly, RVG sample was closely related to a pigeon strain detected in Hong-Kong (Phan et al., 2013), forming a separated clade. Additional sequencing of remaining genes may elucidate this finding and provide useful data on epidemiological aspects of this reservoir to the avian industry, once cross-species transmission may occur when different species are mixed (Pauly et al., 2017).

Prophylactic measures that promote biosafety should be strengthened, since there is no vaccine commercially available and there is a limited knowledge about the RV pathogenicity in birds.

Author's Contributions

All the authors contributed equally and approved the manuscript for submission

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article

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