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LETTER TO THE EDITOR

Coronaviruses Detected in Brazilian Wild Birds Reveal Close Evolutionary Relationships with Beta- and Deltacoronaviruses Isolated From Mammals

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Abstract This study showed that the most of the coronaviruses (CoVs) detected in Brazilian wild birds clustered with the mouse hepatitis virus A59 strain, belonging to the *Beta*CoV group. Furthermore, CoV detected in two different bird species, *Amazona vinacea* and *Brotogeris tirica,* clustered with a CoV isolated from Sparrow (SpaCoV HKU17) belonging to a monophyletic group related with the CoVs isolated from swines (PorCoV HKU15), both belonging to the *Delta*CoV genus, previously unreported in South America. Considering the risk of inter-species host switching and further adaptation to new hosts, detection in bird species of CoVs closely related to mammal CoVs should warn for the potential emergence of new threatening viruses.

Keywords Coronavirus · Betacoronavirus · Deltacoronavirus · Wild birds · Evolution

Coronaviruses (CoVs), belonging to the order *Nidovirales*, family *Coronaviridae*, and subfamily *Coronavirinae*, are grouped into the *Alpha*CoV, *Beta*CoV, *Gamma*CoV, and *Delta*CoV genera (Woo et al. 2011). *Alpha*- and *Beta*CoVs infect or cause disease in mammals, while *Gamma*CoV are almost exclusively found in birds, except for the CoV detected in beluga whale and bottlenose dolphin (Mihindukulasuriya et al. 2008; Woo et al. 2010). The fourth genus (*Delta*CoV) was described recently and has already been identified in pigs and birds (Woo et al. 2011).

In this research, 368 cloacal and tracheal swabs from 33 bird species were collected in different regions belonging to the States of São Paulo (Bosque dos Jequitibás, Campinas-SP; Rio Claro-SP; Rehabilitation center [CRAS] Parque Ecológico do Tietê, São Paulo-SP) and Mato Grosso do Sul (CRAS Campo Grande-MS), Brazil. The viral RNA was extracted (QIAmp Viral RNA Mini kit, QIAGEN, Hilden, Germany), and the cDNA synthesis was performed following the manufacturer's protocol (Applied Biosystems, Foster City, USA).

A PanCoV PCR selective essay was performed targeting a 440 bp of *RNA-dependent RNA polymerase* (RdRp) gene (Chu et al. 2011). Nineteen samples (5.2 %) distributed in 11 bird species were positive for CoV. The positive CoV species are as follows: *Amazona vinacea*, *Ara ararauna*, *Asio clamator*, *Brotogeris tirica*, *Colaptes campestris*, *Columba livia*, *Coragyps atratus*, *Megascops choliba*, *Pitangus sulphuratus*, *Pyroderus scutatus*, and *Rupornis magnirostris* (See Supplementary Table S1).

Different CoV sequences were used to represent the four CoV genera of the family *Coronaviridae*. Phylogenetic inference was reconstructed and implemented in FastTree 2.1.7 software (Price et al. 2010) using the Maximum-Likelihood (ML) method with the General Time-Reversible (GTR) model of nucleotide evolution with 20 rates of gamma distribution and 1.000 Shimodaira–Hasegawa-like (SH-like) values.

Our results showed that most of the samples clustered with the mouse hepatitis virus A59 strain (MHV A59)

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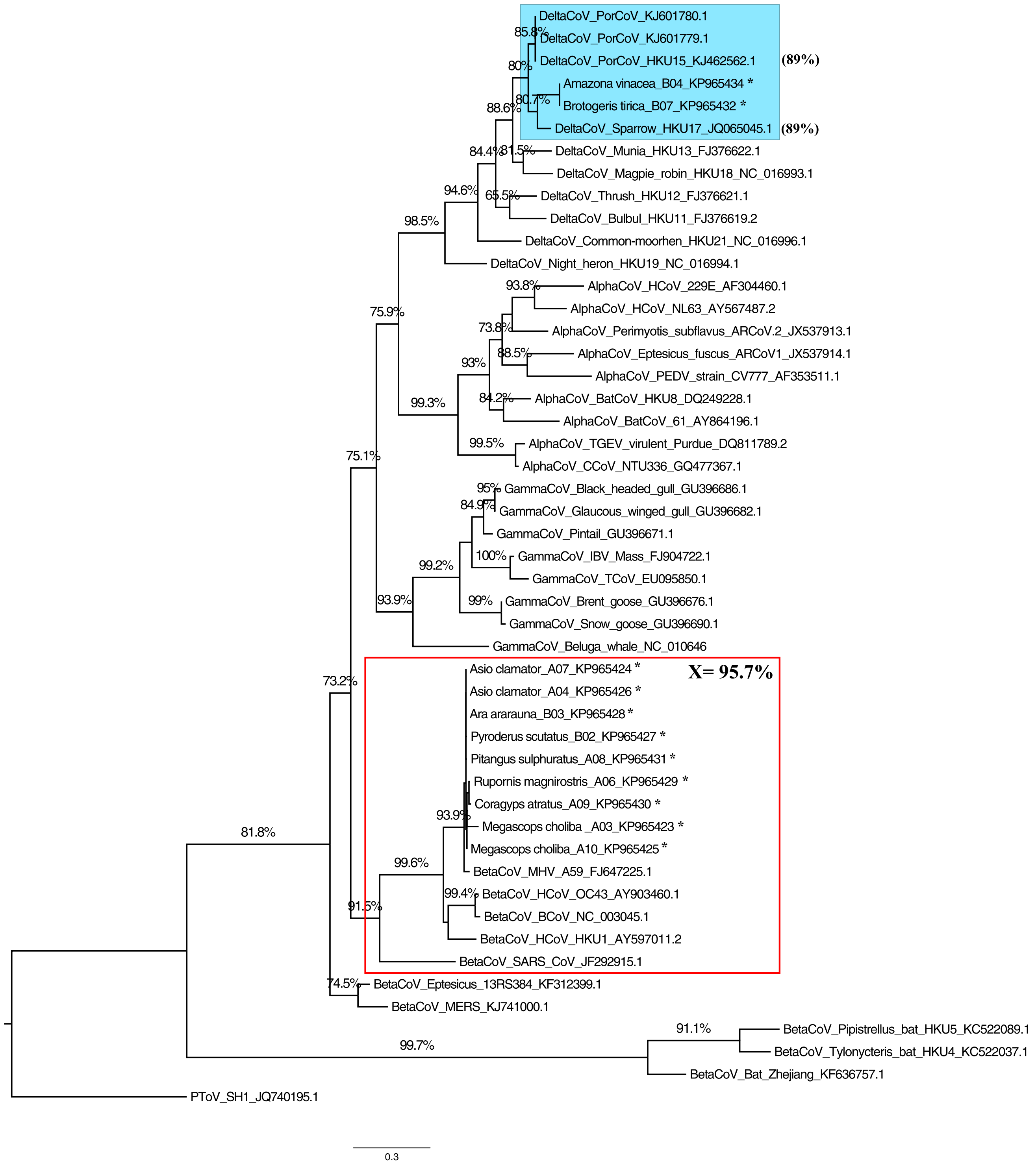

belonging to the *Beta*CoV group. Mice are the natural hosts of MHV A59, which is associated to hepatotropic and neurotropic diseases (Eriksson et al. 2008). Besides, two of our samples that were, respectively, detected in *A. vinacea* (Parrot-breasted-purple) and *B. tirica* (Plain Parakeet) clustered with CoV isolated from Sparrow (SpaCoV HKU17) belonging to a monophyletic group related with the CoVs isolated from swines (PorCoV HKU15), both belonging to the *Delta*CoV genus (Fig. 1), previously unreported in South America. PorCoV HKU15 was detected in April 2014 in the USA (Wang et al. 2014), in 2015, which was directly associated to play a key role in

◀**Fig. 1** Molecular phylogenetic inference with *Alpha*-, *Beta*-, *Gamma*-, and *Delta*CoV groups by ML method under GTR evolutionary model for the partial RdRp gene (267 bp). The *colors* represent CoVs detected in wild birds grouped with *Beta*CoV (*red*) and *Delta*CoV (highlighted in *blue*) groups. The *asterisk* represents the bird samples from which the DNA sequences were obtained. The *number within parenthesis* shows the nucleotide identities of PorCoV HKU15 and SpaCoV HKU17 (both *Delta*CoVs) with the CoV detected in *Amazona vinacea*. The *letter X* represents the arithmetic mean of nucleotide identities between the CoVs detected in the study and the MHV A59. The GenBank accession numbers of sequences deposited (from study) and retrieved from the database are shown in the tree. *PorCoV* porcine coronavirus; *HCoV* human coronavirus; *PEDV* porcine epidemic diarrhea virus; *BatCoV* bat coronavirus; *TGEV* transmissible gastroenteritis coronavirus; *CCoV* canine coronavirus; *IBV* infectious bronchitis virus; *TCoV* turkey coronavirus; *MHV* mouse hepatitis virus; *BCoV* bovine coronavirus; *SARS* severe acute respiratory syndrome; *MERS* middle east respiratory syndrome coronavirus; *PToV (outgroup)* porcine torovirus. The *numbers above the branches* represent the SH-like support values of 1000 replicates. Only values greater than or equal to 70 % are presented. Online version in color

diarrheic disease in swine; no treatment or vaccines are currently available (Hu et al. 2015; Ma et al. 2015).

Finally, we highlighted in this study the evolutionary proximity of CoVs detected in wild birds with *Beta*- and *Delta*CoVs, both isolated from mammal and involved in the pathogenicity of their hosts. We believe that these findings may be useful for the understanding of CoVs' evolutionary dynamics. Considering the risk of inter-species host switching and further adaptation to new hosts, detection in bird species of CoVs closely related to mammal CoVs should warn for the potential emergence of new threatening viruses.

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References

Chu DKW, Leung CYH, Gilbert M, Joyner PH, Ng EM, Tse TM, Guan Y, Peiris JS, Poon LL (2011) Avian coronavirus in wild aquatic birds. J Virol 85:12815–12820. doi:10.1128/JVI.05838-11

Eriksson KK, Cervantes-Barraga L, Ludewig B, Thiel V (2008) Mouse hepatitis virus liver pathology is dependent on ADP-ribose-1-phosphatase, a viral function conserved in the alpha-like supergroup. J Virol 82:12325–12334. doi:10.1128/JVI.02082-08

Hu H, Jung K, Vlasova AN, Chepngeno J, Lu Z, Wang Q, Saif LJ (2015) Isolation and characterization of porcine deltacoronavirus from pigs with diarrhea in the United States. J Clin Microbiol 53:1537–1548. doi:10.1128/JCM.00031-15

Ma Y, Zhang Y, Liang X, Lou F, Oglesbee M, Krakowka S, Li J (2015) Origin, evolution, and virulence of porcine deltacoronaviruses in the United States. mBio 6:e00064-15. doi:10.1128/mBio.00064-15

Mihindukulasuriya KA, Wu G, St Leger J, Nordhausen RW, Wang D (2008) Identification of a novel coronavirus from a beluga whale by using a panviral microarray. J Virol 82:5084–5088. doi:10.1128/JVI.02722-07

Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490. doi:10.1371/journal.pone.0009490

Wang L, Byrum B, Zhang Y (2014) Porcine coronavirus HKU15 detected in 9 US States, 2014. Emerg Infect Dis 20:1594–1595. doi:10.3201/eid2009.140756

Woo PCY, Huang Y, Lau SKP, Yuen KY (2010) Coronavirus genomics and bioinformatics analysis. Viruses 2:1804–1820. doi:10.3390/v2081803

Woo PCY, Lau SKP, Lam CSF, Lau CCY, Tsang AKL, Lau JHN, Bai R, Teng JL, Tsang CC, Wang M, Zheng BJ, Chan KH, Yuen KY (2011) Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol 86:3995–4008. doi:10.1128/JVI.06540-11