The Veterinary Journal 202 (2014) 659–662

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short Communication

Characterization of pantropic canine coronavirus from Brazil

Luciane D. Pinto [a], Iracema N. Barros [b], Renata F. Budaszewski [a], Matheus N. Weber [a], Helena Mata [a], Jéssica R. Antunes [a], Fabiana M. Boabaid [c], Angélica T.B. Wouters [c], David Driemeier [c], Paulo E. Brandão [b], Cláudio W. Canal [a,*]

[a] Laboratório de Virologia, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9090, CEP 91540-000, Porto Alegre, RS, Brazil
[b] Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, CEP 05508-270, São Paulo, SP, Brazil
[c] Setor de Patologia, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9090, CEP 91540-000, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Accepted 7 September 2014

Keywords:
Dog
Canine coronavirus
Canine parvovirus
Pantropic
Sequencing

ABSTRACT

Characterization of canine coronavirus (CCoV) strains currently in circulation is essential for understanding viral evolution. The aim of this study was to determine the presence of pantropic CCoV type IIa in tissue samples from five puppies that died in Southern Brazil as a result of severe gastroenteritis. Reverse-transcriptase PCR was used to generate amplicons for sequence analysis. Phylogenetic analysis of the CCoV-IIa strains indicated that they were similar to those found in other countries, suggesting a common ancestor of these Brazilian isolates. This is the first report of pantropic CCoV-II in puppies from Latin America and our findings highlight that CCoV should be included as a differential diagnosis when dogs present with clinical signs and lesions typically seen with canine parvovirus infection.

Canine coronavirus (CCoV) infection is usually characterized by high morbidity and low mortality, predominantly affecting dogs in kennels and rescue shelters. CCoV replication is mainly limited to the gastrointestinal tract and can eventually lead to death, particularly when co-infection with canine parvovirus (CPV-2), canine adenovirus type 1 (CAV-1) or canine distemper virus (CDV) are present. CCoV can be grouped into two genotypes: CCoV-I and CCoV-II (Decaro and Buonavoglia, 2008, 2011). Recently, CCoV-II has been subdivided into CCoV-IIa (which derives from recombination with a feline coronavirus; FCoV-II) and CCoV-IIb (which derives from recombination with porcine transmissible gastroenteritis virus; TGEV), but the latter has not been shown to be associated with clinical disease in dogs (Decaro and Buonavoglia, 2011).

Following emergence of the more virulent pantropic CCoV-IIa strain (Decaro et al., 2012), detection, identification and monitoring of these new coronaviruses has become more important, when dogs are presented with severe gastroenteritis that would previously have been attributed to CPV-2 infection. The aim of the present study was to characterize the phylogeny of coronaviruses isolated from dogs in Brazil that had died from gastroenteritis.

Five dogs (aged 1 to 6 months) that died at the Clinical Veterinary Hospital of Universidade Federal do Rio Grande do Sul, Southern Brazil, between June and September 2011, were necropsied. Total RNA was extracted from tissue biopsies and fecal samples, obtained from each dog, using TRIzol LS (Life Technologies) and cDNA synthesized using the Superscript III Reverse Transcriptase Kit (Life Technologies). DNA was extracted from the supernatant of the fecal suspension, using a commercial kit (Simbios Biotecnologia). CCoV-I and CCoV-II primer pairs were used to amplify a region of the M gene, as described by Herrewegh et al. (1998). EL1F, EL1R and S5, S6 primer pairs were used to differentiate between CCoV-I and CCoV-II as described by Pratelli et al. (2004). Reactions specific to CCoV-IIa and CCoV-IIb were performed, using CCoV-IIaF/CCoV-IIaR and CCoV-IIbF/CCoV-IIbR primer pairs in two different reactions, which amplified fragments of the 5′ end region of the S gene (Decaro et al., 2012). Fecal samples were also analyzed by PCR for the presence of other gastrointestinal viruses, namely CPV-2 (Buonavoglia et al., 2001), CAV-1 and CAV-2 (Hu et al., 2001), CDV (Frisk et al., 1999) and canine rotavirus (CRV) (Gouvea et al., 1990). Platinum Taq DNA Polymerase (Life Technologies) was used for PCR, according to the manufacturer's recommendations.

CCoV amplicons were purified using the NucleoSpin Extract II Kit (Macherey-Nagel) and sequenced with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) using a BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems). The maximum likelihood (ML) trees were constructed using PhyML version 3.0.[1]

* Corresponding author. Tel.: +55 513 308 6926.
E-mail address: claudio.canal@ufrgs.br (C.W. Canal).

[1] See: http://atgc.lirmm.fr/phyml/.

http://dx.doi.org/10.1016/j.tvjl.2014.09.006
1090-0233/

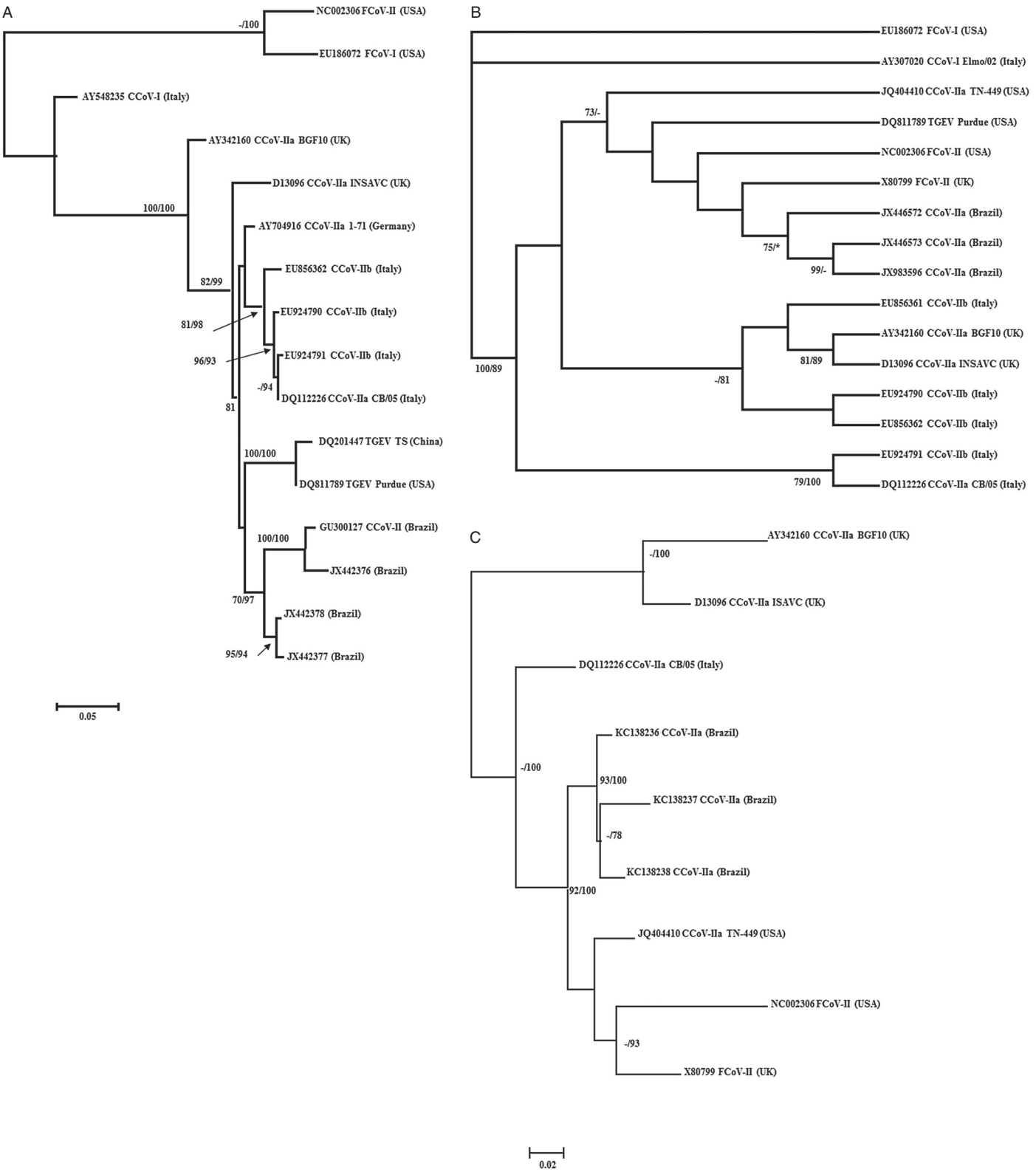

Fig. 1. Maximum likelihood (ML) tree showing the relationships between CCoV strains (JX442376, JX442377 and JX442378 identified in the present study) based on (A) a 332 base pair (bp) fragment of the M gene, (B) a 569 bp fragment from the 3′end region of the S gene and (C) a 938 bp fragment of the S gene (5′end plus 3′end combined dataset), using the TIM2+G model. Nodal support values (<0.7 posterior probability or <70% bootstrap not shown) are indicated for ML and Bayesian (BI) methods, respectively. (–) indicates values <70%, while (*) stands for different resolution in BI analysis.

Table 1
Amino acid differences between Brazilian and reference samples for the CCoV M gene.

Samples[a]	Amino acid position						
	123	127	187	212	223	243	246
D13096 CCoV-IIa (UK)	**Thr**	Ile	**Cys**	**Val**	Lys	Tyr	Asp
AY342160 CCoV-IIa (UK)	Ile	Ile	Tyr	Ser	**Gln**	Tyr	Asp
DQ112226 CCoV-IIa (Italy)	Ile	Ile	Tyr	Ser	Lys	Tyr	Asp
EU924790 CCoV-IIb (Italy)	Ile	Ile	Tyr	Ser	Lys	Tyr	Asp
GU300127 (Brazil)	Ile	**Val**	Tyr	Ser	**Gln**		
JX442376* (Brazil)	Ile	**Val**	Tyr	Ser	**Gln**	**Asn**	**Glu**
JX442377* (Brazil)	Ile	**Val**	Tyr	Ser	Lys	**Asn**	**Glu**
JX442378* (Brazil)	Ile	**Val**	Tyr	Ser	Lys	**Asn**	**Glu**
NC002306 FCoV-II (USA)	**Val**	**Ala**	Tyr	Ser	**Gln**		
Recombitek		Ile	Tyr	Ser	Lys		

[a]GenBank accession numbers are shown with country of origin in parentheses. Recombitek is a commercial vaccine. Amino acid differences are highlighted in bold text. *Identified in the present study.

Table 2
Amino acid differences between Brazilian and reference samples for the 5′ region of the CCoV S gene.

Samples[a]	Amino acid position								
	5	20	21	34	38	54	72	78	125
DQ112226 CCoV-IIa (Italy)	**Leu**	Thr	Thr	**Asp**	**Asn**	Val	Ala	Glu	**Asn**
JQ404410 CCoV-IIa (USA)	Val	Thr	**Ala**	Pro	Tyr	Val	Ala	Gln	Asp
KC138236* (Brazil)	Val	**Met**	**Ala**	Pro	Tyr	Val	Ala	Glu	Asp
KC138237* (Brazil)	Val	Thr	Thr	Pro	Tyr	Val	**Arg**	Glu	Asp
KC138238* (Brazil)	**Glu**	Thr	Thr	Pro	Tyr	**Glu**	Ala	Glu	Asp
NC002306 FCoV-II (USA)	Val	Thr	Thr	**Ala**	**Asn**	Val	Ala	**Gln**	Asp

[a]GenBank accession numbers are shown with country of origin in parentheses. Amino acid differences are highlighted in bold text. *Identified in the present study.

The best substitution models were estimated using JModeltestv.0.1.1[2] for the nucleotide dataset and ProtTest version 2.4[3] for the amino acid dataset, both with the Akaike information criterion (AIC). For Bayesian inference, we selected substitution models according to the AIC using MrModeltest.[4]

The dogs presented with hemorrhagic gastroenteritis, vomiting, fever, loss of appetite and collapse. At necropsy, similar lesions were seen, consisting of thickening and roughening of the small intestinal mucosa, submucosal edema and enlargement of the Peyer's patches and mesenteric lymph nodes. Histopathology revealed necrotic enteritis, with atrophy and fusion of microvilli, edema and congestion of mesenteric lymph nodes and mild to moderate edema and diffuse congestion in the lungs. There was reduced bone marrow content and evidence of necrosis in four of the five dogs.

Molecular analysis of samples from the five dogs revealed that three were positive for CCoV-II and three were positive for CPV-2, with two dogs co-infected by both of these viruses (further details in Appendix: Supplementary material). One dog was negative for all viruses tested and all five dogs were negative for CDV, CRV, CAV-1 and CAV-2. Amplification and sequencing of the 5′ region of the S gene (CCoV-II) and of the VP2 gene (CPV-2) allowed further characterization that these were pantropic CCoV-IIa and CPV-2c. Phylogenetic trees, obtained using different datasets and estimated by different methods, indicated a close relationship between the CCoV identified and the CCoV-II related group (Fig. 1). The phylogenetic analyses, based on the nucleotide sequence of the M gene, grouped the Brazilian pantropic samples from Rio Grande do Sul state (Southern Brazil) with GU300127, identified from a dog in São Paulo state (Southeastern Brazil) in both maximum likelihood (ML) and Bayesian (BI) analyses with moderate to high statistical support (Fig. 1A).

The phylogenic analysis, based on the 3′end region of the S gene, grouped the Brazilian samples into a principal group with CCoV-IIa, FCoV-II and TGEV with moderate to high support (Fig. 1B). However, the relationships within this group are not fully resolved. Analysis using the concatenated fragment of the S gene (5′end plus 3′end) supported this principal group and clustered the Brazilian samples into a highly supported monophyletic clade (Fig. 1C). The phylogeny also suggests a sister–group relationship between the Brazilian samples and the group composed of JQ404410, X80799, and NC002306. Amino acid differences between Brazilian and reference samples of the partial M gene and the 5′ region of the S gene are shown in Tables 1 and 2. BLAST searches[5] using the partial M gene sequences as the query revealed greatest identity with sequence GU300127 (95.5–98.6% identity) and similarity with reference samples AY704916 (Germany) (94.4–96.7% nucleotide identity) and DQ112226 (Italy) (93.7–95.2% nucleotide identity).

This is the first report of pantropic CCoV in dogs from Latin America. Fecal samples and several organs from three dogs affected with gastroenteritis were positive for CCoV, corroborating the data published previously (Decaro et al., 2012; Zicola et al., 2012). Phylogenetic analysis of the CCoV strains showed that these were similar to those found in other countries. The Brazilian samples tended to group into a single clade, suggesting a common ancestor. The results of this study indicate that pantropic CCoV should be considered in puppies, presenting with hemorrhagic gastroenteritis, which would usually be attributed to CPV-2. Inclusion of diagnostic testing for CoCV should be considered in such cases, to differentiate between these two pathogens and to identify animals with co-infections.

[2] See: http://jmodeltest.sharewarejunction.com/.
[3] See: http://darwin.uvigo.es/software/prottest2_server.html.
[4] See: https://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html.
[5] See: http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organization that could inappropriately influence or bias the content of the paper.

Acknowledgments

We are thankful to Simbios Biotecnologia Ltd. for kindly supplying the DNA extraction kits. We thank the veterinarians who collected samples from the dogs and the undergraduate and graduate students of the Laboratory of Virology for their collaboration in this work. Financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant number 475223/2013-6, Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Propesq/UFRGS.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2014.09.006.

References

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., Carmichael, L., 2001. Evidence for evolution of canine parvovirus type 2 in Italy. Journal of General Virology 82, 3021–3025.

Decaro, N., Buonavoglia, C., 2008. An update on canine coronaviruses: Viral evolution and pathobiology. Veterinary Microbiology 132, 221–234.

Decaro, N., Buonavoglia, C., 2011. Canine coronavirus: Not only an enteric pathogen. Veterinary Clinics of North America: Small Animal Practice 41, 1121–1132.

Decaro, N., Mari, V., Reitzenstein, M., Lucente, M.S., Cirone, F., Elia, G., Martella, V., King, V.L., Di Bello, A., Varello, K., et al., 2012. A pantropic canine coronavirus genetically related to the prototype isolate CB/05. Veterinary Microbiology 159, 239–244.

Frisk, A.L., König, M., Moritz, A., Baumgärtner, W., 1999. Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. Journal of Clinical Microbiology 37, 3634–3643.

Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B., Fang, Z.Y., 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. Journal of Clinical Microbiology 28, 276–282.

Herrewegh, A.A.P.M., Smeenk, I., Horzinek, M.C., Rottier, P.J.M., Groot, R.J., 1998. Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. Journal of Virology 72, 4508–4514.

Hu, R.L., Huang, G., Qiu, W., Zhong, Z.H., Xia, X.Z., Yin, Z., 2001. Detection and differentiation of CAV-1 and CAV-2 by polymerase chain reaction. Veterinary Research Communications 25, 77–84.

Pratelli, A., Decaro, N., Tinelli, A., Martella, V., Elia, G., Tempesta, M., Cirone, F., Buonavoglia, C., 2004. Two genotypes of canine coronavirus simultaneously detected in fecal samples of dogs with diarrhea. Journal of Clinical Microbiology 42, 1797–1799.

Zicola, A., Jolly, S., Mathijs, E., Ziant, D., Decaro, N., Mari, V., Thiry, E., 2012. Fatal outbreaks in dogs associated with pantropic canine coronavirus in France and Belgium. Journal of Small Animal Practice 53, 297–300.