Genetic characterization of the haemagglutinin gene in canine distemper virus strains from naturally infected dogs in Brazil

Caracterização genética do gene da hemaglutinina em vírus da cinomose caninca de cães naturalmente infectados no Brasil

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Abstract

Canine distemper is one of the major infectious diseases in dogs and wild animals, resulting in high morbidity and mortality. The H gene has the greatest genetic variability among the genes encoded by the canine distemper virus (CDV) genome, and it has been used to characterise field samples, allowing the identification of specific lineages. Variation in the H gene can allow the virus to evade recognition by vaccine-induced antibodies, resulting in vaccine failure. The purpose of this study was to characterise H gene in CDV strains from naturally infected dogs in the state of São Paulo. The phylogenetic analysis revealed that Brazilian CDV strains were genetically related to the circulating CDV strains in Uruguay, Argentina, and Europe. We found no evidence of South America 2 and 3 CDV lineages circulating in Brazilian dogs. The degree of genetic divergence between wild Brazilian CDV strains and vaccine strains may suggest the possibility of vaccine failures and consequently the occurrence of canine distemper outbreaks.
Keywords: Brazil. South America. Canine distemper virus. Haemagglutinin (H) gene. Phylogenetic analysis.

Resumo

A cinomose canina é uma das principais doenças infecciosas em cães e animais selvagens, resultando em alta morbidade e mortalidade. O gene H tem uma das maiores variabilidades genéticas entre os genes codificados pelo vírus da cinomose canina (CDV), e tem sido utilizado para caracterizar as estirpes de CDV, permitindo a identificação de linhagens específicas. A variação no gene H pode permitir que o vírus evite o reconhecimento por anticorpos induzidos pela vacina, resultando em falha vacinal. O objetivo deste estudo foi caracterizar o gene H em estirpes de CDV de cães infectados naturalmente no estado de São Paulo. A análise filogenética revelou que as estirpes de CDV brasileiras estão geneticamente relacionadas as estirpes circulantes no Uruguai, na Argentina e na Europa. Não foi encontrada nenhuma evidência da circulação no estado de São Paulo das linhagens América do Sul 2 e 3. O grau de divergência genética entre linhagens selvagens de CDV brasileiras e as estirpes vacinais podem sugerir a possibilidade de falhas vacinais e consequentemente a ocorrência de surtos de cinomose canina.
Palavras-chave: Brasil. América do Sul. Vírus da cinomose canina. Gene da hemaglutinina. Análise filogenética.

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Received: 01/06/2017
Approved: 27/07/2017

Canine distemper is one of the major infectious diseases in dogs and wild animals, resulting in high morbidity and mortality. The canine distemper virus (CDV) belongs to the genus *Morbillivirus* of the *Paramyxoviridae* family. CDV is an enveloped negative single-stranded RNA virus, and its genome encodes six structural proteins; the Haemagglutinin (H) glycoprotein has been used to characterize field strains (MARTELLA

DOI: 10.11606/issn.1678-4456.bjvras.2017.133315

et al., 2006; PANZERA et al., 2012; NEGRÃO et al., 2013; BUDASZEWSKI et al., 2014). CDV is mainly controlled by vaccination using attenuated vaccines, but reports of the disease in vaccinated animals suggest that there are antigenic differences between wild CDV and the strains used in the vaccines that primarily occur in the haemagglutinin glycoprotein (BAE et al., 2013).

The H gene has the greatest genetic variability among the genes encoded by the CDV genome, and it has been used to characterise field samples, allowing the identification of specific lineages according to geographic location (ROTA et al., 1992). Strains with a genetic divergence greater than 4% can be considered as new viral strains (MARTELLA et al., 2006). Variation in the H gene can allow the virus to evade recognition by vaccine-induced antibodies, resulting in vaccine failure and the consequent appearance of outbreaks (IWATSUKI et al., 2000).

Since canine distemper (CD) is a major cause of mortality in dogs in Brazil (BENTUBO et al., 2007) and because few studies have investigated the molecular epidemiology of CDV in this country, the aim of this study was to characterise haemagglutinin gene in canine distemper virus strains from naturally infected dogs in the state of São Paulo, Brazil.

Brain samples obtained in 2007 from eight dogs with systemic and neurological signs and positive for canine distemper virus either by direct immunofluorescence or by RT-PCR were sequenced for the gene H. The animals were from the region of Botucatu, São Paulo, and four of them had been previously vaccinated against canine distemper.

Total RNA extracted from the brain samples and reverse transcription were performed according to the manufacturer's instructions. The amplification of gene H was performed according to Pardo et al. (2005) with modifications (Table 1). The primers were designed based on the genome of strain A75/17 (AF164967).

Table 1 – Primers used for the amplification and sequencing of the gene H

Reaction	Sense Anti-Sense	Primer 5′ – 3′	Gene Position	Length in base pair	Reference
Reaction 1	1F	TAAGGTCGATCCGACATT	6856-6874	634	Present study
	1R	AGTGGAGATCGCGGAAGT	7372-7490		Pardo et al. (2005)
Reaction 2	2F	GTCCTTCTCATCCTACTGG	7199-7217	562	Pardo et al. (2005)
	2R	ACACTCCGTCTGAGAATAGC	7742-7760		Pardo et al. (2005)
Reaction 3	3F	ACTTCCGCGATCTCCACT	7472-7489	396	Pardo et al. (2005)
	3R	GCATGTCATTCAGCCACC	7851-7868		Pardo et al. (2005)
Reaction 4	4F	TCTCAGACGGAGTGTATG	7746-7763	731	Pardo et al. (2005)
	4R	GTGAATTGGTCTCCTCTA	8465-8478		Present study
Reaction 5	5F	ACCCTTTGGAGGAGGACAGT	8266-8285	698	Present study
	5R	AAGGAATTTCTCACCACAGTCA	8942-8963		Present study

Products with the expected size were purified using the Illustra™ GFXTM PCR DNA and Gel Band Purification (GE Healthcare) kit and sequenced in both directions using the BigDye® Terminator Kit (Applied Biosystems) in an ABI PRISM® 3100 automated sequencer (Applied Biosystems) according to the manufacturer's instructions. The sequences were edited and aligned using the program BIOEDIT v7.2.5 (http://www.mbio.ncsu.edu/bioedit/bioedit.html; HALL, 1999).

Phylogenetic reconstruction was performed using the Bayesian method with the assistance of the program MrBayes 3.2 (http://mrbayes.sourceforge.net/; RONQUIST et al., 2012) with the following parameters: evolutionary model GTR+G; gammashape = 0.9230 and 100.000 generations (jModel Test – http://darwin.uvigo.es/software/index.html). Determination of the genetic distance between clades formed in the phylogenetic analysis was performed using the program Mega5 (TAMURA et al., 2011) with the following parameters: evolutionary model Maximum Composite likelihood and d = transitions + transversions.

The sequences used for comparison were obtained from GenBank: America 1 (Vaccines lineage) (AF259552, Z35493, AF378705); Artic (X84998, Z47760, AF172411); Asia 2 (AY297453, AY297454, AB212730); Asia 1 (AB212963, AB212964, DQ191767); America 2 (AY649446, AY498692, AY438597); South America 2 (FJ392651, KC257463, KC257464); Europe 2 (European wildlife lineage) (GQ214369, Z47759, X84999); South America 3 (KF835423, KF835424, KF835425); Europe 1 (Z77672, Z77673, DQ494317, DQ494318); South America

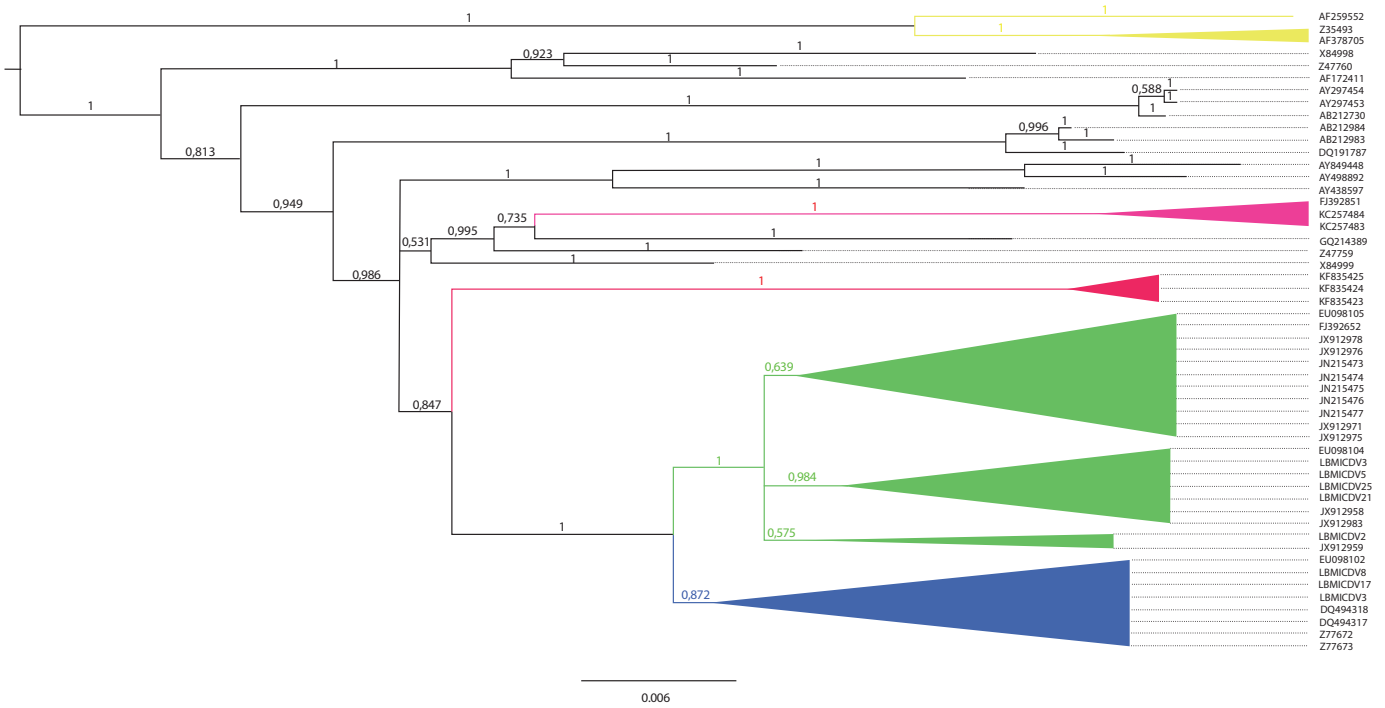

Figure 1 – Phylogenetic trees based on a partial CDV H gene (1122 bp). The Bayesian method tree was generated by using the program MrBayes 3.2. The evolutionary model GTR+G; gammashape = 0.9230 was selected using jModel Test, with 100.000 generations. The clades in blue correspond to the lineage Europe 1; Green to South America 1; Red South America 3; Pink to South America 2; and yellow to America 1 (vaccine)

1 (JN215473, JN215474, JN215475, JN215476, JN215477, FJ392652); Brazilian CDV (EU098102, EU098104, EU098105, JX912958, JX912959, JX912963, JX912971, JX912975, JX912976, JX912978) (Figure 1).

Eight Brazilian partial CDV-H genes (LBMICDV2 KP202308, LBMICDV3 KP202309, LBMICDV5 KP202310, LBMICDV8 KP202311, LBMICDV17 KP202312, LBMICDV21 KP202313, LBMICDV25 KP202314, LBMICDV33 KP202315) (Figure 1) are scattered within the cluster of the Europe 1/South America 1 lineage, with high probability values. This finding is corroborated by reports from Panzera et al. (2012), Negrão et al. (2013), Budaszewski et al. (2014), and Espinal et al. (2014) who worked with South American and Brazilian CDVs.

Genetic differences of partial CDV H gene between different lineages of CDV show that the amino acid sequences of strains from the Brazilian, South America 1, and Europe 1 lineages differed by less than 4%, indicating that they belong to the same lineage. However, the Brazilian CDVs varied over 4% compared to the strains belonging to South America 2 (PANZERA et al., 2012) and South America 3 lineages (ESPINAL et al., 2014), demonstrating that these CDVs are grouped with strains from different lineages. This finding is consistent with the report by Budaszewski et al. (2014) and Panzera et al. (2015), who considered the South America 1 and Europe 1 belonging to same clade and the same lineage. Panzera et al. (2012, 2015) claim that these strains originated from the South America 2 subgroup, are related to Europe 2 lineage (European wildlife lineage), and were most likely transferred from dogs to wild animals.

CDVs from the region of Botucatu, São Paulo, as well as strains from Londrina and Maringá, in the state of Paraná, form two subclades, while the CDVs from Rio Grande do Sul are grouped preferentially with CDVs from Argentina and Uruguay. Given that the distance between Botucatu and Londrina is approximately 400 km and the distance between Londrina and Porto Alegre is around 1000 km, the data indicate that these two sub lineages are circulating in different regions (Figure 1). In addition to the geographical distance another possible factor may be the commercial relationship between these two regions, which could favour the emergence of new variants (PARDO et al., 2005; MARTELLA et al., 2006; PANZERA et al., 2015).

Sequence analysis of the H gene demonstrated that the vaccine CDV strains formed a clade that is completely distinct from other wild strains, including the Brazilian CDV. This result is consistent with studies conducted throughout the world and in Brazil (MARTELLA et al., 2006; PANZERA et al., 2012; NEGRÃO et al., 2013), indicating that the H gene of CDV has evolved differently in wild and vaccine strains. This

observation reflects the differences in the selective pressures experienced by different viral populations, which could lead to differences between wild and vaccine CDV strains.

Our study indicates that Brazilian CDVs exhibit the European/South America 1 genotype, which is distributed throughout the clade. The Brazilian CDVs studied here are genetically related to strains circulating in Uruguay, Argentina, and Europe, and we found no circulation of the South American 2 and 3 lineages of CDV. Furthermore, 50% of strains were from vaccinated animals and presented considerable genetic divergence with the vaccine CDV, which may ultimately lead to vaccine failure and outbreaks of canine distemper.

Conflict of interest statement

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

Acknowledgements

The authors are extremely grateful to Prof. J. P. Araujo Jr. for his essential technical assistance in designing the primers. JM (304630/2013-6) and MBH (306434/2013-0) are indebted to CNPq for the fellowships. This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – Fapesp (06/04924-9).

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