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Genotyping of canine distemper virus strains circulating in Brazil from 2008 to 2012

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ABSTRACT

Canine distemper virus (CDV) is a major pathogen of dogs and represents a serious threat to both unvaccinated and vaccinated animals. This study surveyed dogs with or without clinical signs related to canine distemper from different regions of Brazil from 2008 to 2012. A total of 155 out of 386 animals were found to be CDV positive by RT-PCR; 37 (23.8%) dogs were asymptomatic at the time of sampling, and 90 (58%) displayed clinical signs suggestive of distemper. Nineteen (12.2%) dogs had a record of complete vaccination, 15 (9.6%) had an incomplete vaccination protocol, and 76 (49%) had no vaccination record. Based on the sequence analysis of the complete hemagglutinin gene of 13 samples, 12 of the strains were characterized as Genotype South America-I/Europe. Considering criteria of at least 95% nucleotide identity to define a genotype and 98% to define a subgenotype, South America-I/Europe sequences segregated into eight different phylogenetically well-defined clusters that circulated or co-circulated in distinct geographical areas. Together, these findings highlight the relevance of CDV infection in Brazilian dogs, demonstrate the predominance of one genotype in Brazil and support the need to intensify the current control measures.

1. Introduction

Canine distemper virus (CDV) is a major pathogen of domestic dogs and wild carnivores. CDV infection is relevant worldwide and is associated with high morbidity and mortality. CDV-infected dogs may develop respiratory, gastrointestinal, dermatologic, ophthalmic or neurological disorders (Greene and Appel, 2006). Taxonomically, CDV is a member of the genus *Morbillivirus* within the family *Paramyxoviridae* (King et al., 2011). The genomic RNA encodes six structural proteins: fusion (F), hemagglutinin (H), envelope-associated matrix (M), phosphoprotein (P), large polymerase (L) and nucleocapsid (N) protein (Appel, 1987; Rima, 1983).

Like measles virus, CDV is a monotypic virus, as defined by polyclonal antisera. In general, the introduction and extensive use of live attenuated CDV vaccines in the 1950s has drastically reduced the incidence of canine distemper in dogs. However, CDV infection and disease in immunized dogs have been observed on several occasions (Ek-Kommonen et al., 1997; Józwik and Frymus, 2002; Si et al., 2010; Simon-Martínez et al., 2008). This raises the question of whether the current vaccines effectively protect against wild-type CDV strains that are genetically divergent from the vaccinal viruses. Furthermore, a number of studies have shown antigenic differences between vaccine strains and wild-type isolates (Hashimoto et al., 2001; Si et al., 2010).

Of the six structural proteins, the H gene (Blixenkrone-Møller et al., 1993) has the greatest genetic variation and is therefore a suitable target for investigating the polymorphism of the CDV isolates and for molecular epidemiological studies (Haas et al., 1997; Hashimoto et al., 2001; Von Messling et al., 2001). Based on the variability in the H gene, CDV strains segregate into at least nine major geographically related genetic lineages: America-I, America-II, Asia-I, Asia-II, Europe Wildlife, Arctic, South Africa, South America-I/Europe and South America-II (Calderón et al., 2007; Haas et al., 1997; Martella et al., 2006; Mochizuki et al.,

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Fig. 1. Map of South America. The map indicates the origin of the strains and the distribution of genotypes and subgenotypes within the Brazilian states. Letters indicate the genetic lineages circulating in these regions, including strains from Uruguay and Argentina. Numbers within parentheses indicate the number of positive animals per number of samples analyzed. The states are MT: Mato Grosso; PR: Paraná; RJ: Rio de Janeiro; RO: Rondônia; RS: Rio Grande do Sul; SC: Santa Catarina; SP: São Paulo. SA-I: South America-I/Europe; SA-II: South America-II; RL: Rockborn-like. Letters A-G indicate the subgenotypes.

1999; Panzera et al., 2012; Woma et al., 2010). Classical vaccine CDV strains are derived from America-I genotype viruses, with the exception of the Rockborn vaccine strain (Martella et al., 2011). Rockborn-like strains have been identified from dogs and wild animals as well as in some vaccines currently available on the market (Martella et al., 2011).

While canine distemper is endemic in Brazil (Silva et al., 2007), information on the molecular epidemiology of this viral infection is scarce (Castilho et al., 2007; Negrão et al., 2013; Rosa et al., 2012). To fill this gap, we surveyed the CDV infection burden in seven Brazilian states during a 4-year period and molecularly characterized a representative selection of CDV strains.

2. Materials and methods

2.1. Samples

The study was comprised of a total of 386 rectal swab samples collected from dogs between April 2008 and June 2012, selected by convenience sampling. The samples were collected from animals with or without clinical signs suggestive of CDV infection in seven Brazilian states: Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Rondônia and Mato Grosso (Fig. 1). Information about the animals, such as age, gender, breed, clinical signs and vaccination status was collected. Animals not presenting clinical signs at the time of sampling were considered asymptomatic, although there was no monitoring of the dogs after sampling date. Samples were diluted to 20% (w/v) in phosphate-buffered saline (PBS, pH 7.4) and stored at −80 °C for further analysis.

2.2. RNA extraction

RNA was extracted from rectal swab suspensions using TRIzol LS Reagent® (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The attenuated live vaccine Vanguard Plus® (Pfizer, New York, NY, USA) was used as a positive control, and ultrapure water and rectal swabs from five healthy adult dogs were used as negative controls.

2.3. Primers

The primers used to amplify fragments of the N and H genes were synthesized as described in previous reports (An et al., 2008; Castilho et al., 2007; Frisk et al., 1999; Harder et al., 1996; Hashimoto et al., 2001), with minor modifications, allowing the detection of a larger number of local isolates. The N primers were used for the detection of CDV by nested RT-PCR. CDV-1F and CDV-2R were used for the first round of amplification, and CDV-3F and CDV-4R were used for the second round of amplification.