

Review

Genetic Diversity of Canine Distemper Virus in South America

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Abstract | Canine Distemper virus is the etiological agent of “Distemper”, the most significant infectious disease among carnivores worldwide. Herein, we present the state of the art of CDV dynamics in South America, by means of a detailed analysis of the genetic diversity, evolution, and epidemiology of the virus in several countries. Our study allowed us to establish the presence of four genetic lineages in South America, being the continent with more lineages described to date, underscoring the high genetic diversity of the circulating strains.

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The *Morbillivirus* is an antigenically related genus within the *Paramyxoviridae* which includes important mammal pathogens, such as measles virus in humans and canine distemper virus (CDV) in several carnivore species. *Paramyxoviruses* have a non-segmented single-stranded negative RNA genome of 15.7 kb. CDV encodes for the nucleoprotein (N), viral polymerase (L), and phosphoprotein (P), which are associated with the genomic RNA forming the ribonucleoprotein complex; the matrix (M) is a membrane associated protein, whilst the hemagglutinin (H) and fusion (F) proteins are the antigenic determinants anchored at the viral envelope (Lamb & Parks, 2007).

CDV is the etiologic agent of canine distemper, a severe multisystemic and globally distributed disease, which represents the most important infectious threat of domestic dogs (*Canis lupus familiaris*) (Appel & Summers 1999). CDV infection has been reported in all families of terrestrial carnivores and several marine carnivores (Deem et al., 2000; Mamaev et al., 1995). The ability of the virus to jump between carnivores hosts is considered as one of the causes for the success of its worldwide spread. As viral reservoirs, domestic

dogs pose a significant risk to captive and free-ranging wildlife species, because of dog abundance and their ability to travel for long distances (Fiorello et al., 2006). Although live attenuated vaccines have been used since 1950 to control the disease, CDV continues to cause outbreaks in receptive domestic and wild carnivores in several geographical areas (Lednicky et al., 2004; Martella et al., 2010; Woma et al., 2009; Zhao et al., 2010; Panzera et al., 2012). Distemper outbreaks registered since the 1980s in vaccinated and unvaccinated dogs, were consequence of natural infections with field strains not related to the current vaccine strains (Harder & Osterhaus, 1997; Mochizuki et al., 1999, Pardo et al., 2005; Lan et al., 2006; Calderón et al., 2007; Sarute et al., 2011). While cross-neutralization and kinetic-neutralization assays can be used to distinguish between vaccine and field strains (Appel et al., 1994; Harder et al., 1993, 1996), only molecular analyses have revealed differences between field strains.

Sequence analyses of different genes have been performed to characterize the circulating strains (Harder et al., 1996; Lednicky et al., 2004; Pardo et al., 2005;

Headley et al. 2009). The H gene is the most widely employed because it has the highest variability within the CDV genome. Its analysis has led to identify geographic lineages providing important advances to the knowledge of CDV evolution worldwide (Bolt et al., 1997; Martella et al., 2006).

In South America, distemper outbreaks had apparently been known since the XVIII century in Peru, and afterwards the virus spread to Europe according to clinical and historical records (Howell, 1965; Blancou, 2004).

(Fig. 1) (Headley et al., 2012).

In Bolivia, more than the 95% of the dogs had CDV antibodies, which were also detected in wild carnivores (Fig. 1) (Fiorello et al., 2006, 2007; Bronson et al. 2008).

Furthermore, in Galapagos Island, where vaccination and dog importation is forbidden, anti-CDV antibodies were detected in resident dogs (Fig. 1) (Levy et al., 2008).

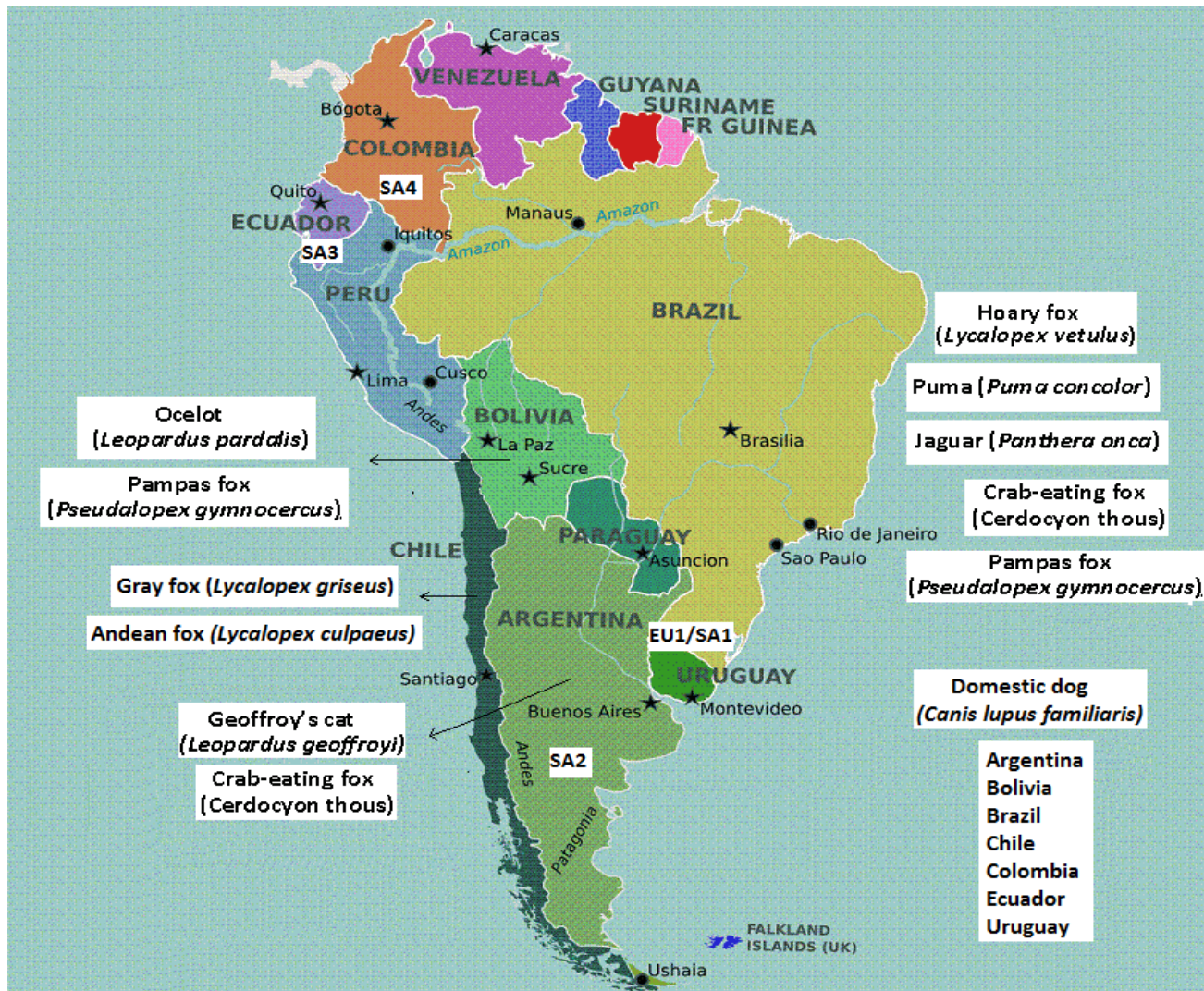


Figure 1. Political map of South America. In boxes are detailed the carnivores species infected with canine Distemper virus and the lineages described for each country.

The first serological reports performed in Brazil detected anti-CDV antibodies in 9% of the Amazon dog populations with no evidence for infections in wild populations (Fig. 1) (Courtenay et al., 2001). In other Brazilian regions seroprevalence surveys in domestic and wild hosts detected high antibodies titers

In Chile, a Distemper outbreak involving two fox species occurred in 2003 (Moreira & Stutzin, 2005; Gonzalez-Acuña et al., 2003; Acosta-Jamett, 2009) and urban dogs were blamed for CDV transmission due to their high seroprevalence (Fig. 1) (Acosta-Jamett et al., 2011).

Phylogenetic analyses based on CDV strains from South America have been performed to establish the evolutionary patterns of the virus in the region. The

first reports from Brazil, Argentina and Uruguay revealed that field strains clearly differed with respect to the vaccine strains (Saito et al., 2006; Calderón et al., 2007; Sarute et al., 2011).

A phylogenetic study based on a partial region of the N gene reported that Brazilian strains isolated from wild and domestic hosts were closely related and suggested that domestic dogs were the source of infection for wild carnivores (Fig. 1) (Megid et al., 2009; 2010). In 2007, Calderón analyzed a partial region of the H gene determining the presence of two genetic variants circulating in Argentina: one variant was related to European strains, while the other was unique and predominant in the country (Calderón et al., 2007). Studies based on strains isolated from free-ranging wild carnivores showed a high percentage of identity with the predominant CDV variant that affect dogs in Argentina (Fig. 1) (Ferreira, et al. 2009).

Until 2012, the global analysis of the complete H gene had permitted the identification of eight lineages according mainly to their geographic origin: three in Europe, two in Asia, two in North America, and one in South Africa (Martella et al., 2006; An et al., 2008; Woma et al., 2009). The analysis of the full-length H gene sequences of field strains from Uruguay and Argentina, and their comparison with strains from Brazil and with strains belonging to the eight lineages, allowed us to identify two co-circulating lineages in South America which different distribution, prevalence and origin. The Uruguayan, Brazilian and one Argentinean strains clustered within a European lineage were renamed as Europe1/South America1 (EU1/SA1). The remaining Argentinean strains formed a new genetic lineage, denoted as South America 2 (SA2), that is exclusively distributed in Argentina according to the previous report of Calderón et al., (2007) (Panzer et al. 2012).

Recent studies have confirmed that all the Brazilian strains characterized from domestic dogs belong to the EU1/SA1 lineage. The absence of the other lineages in Brazil is remarkable considering the broad area of the country (47% of South America) (Negrao et al., 2013; Budaszewski et al., 2014). The predominant EU1/SA1 lineage has been circulating for at least more than a decade in southern South America. Although this lineage was previously described in Europe (Bolt et al., 1997), it is not possible to establish the genetic flow direction between both continents.

Nevertheless, the high genetic homology between the South American and the European strains is indicative of a common ancestor.

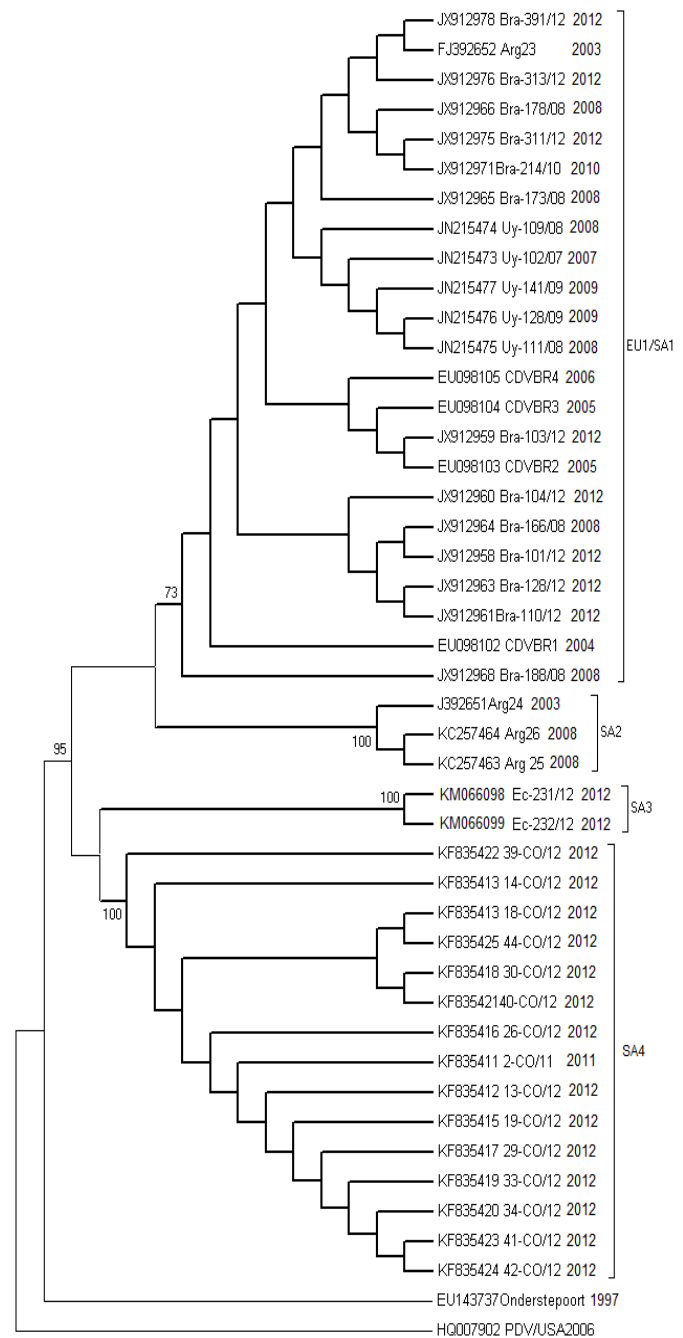


Figure 2. Maximum likelihood tree constructed using the Tamura 3-parameter (I) substitution model inferred through 500 replicates for 44 South American partial sequences (630 bp) of the H gene. GenBank accession number, country, isolates and collection year are detailed. Arg: Argentina, Bra/BR: Brazil, Co: Colombia, Ec: Ecuador, Uy: Uruguay. EU1/SA1: Europe 1/South America 1, SA2: South America 2, SA3: South America 3, SA4: South America 4. A Phocid Distemper virus strain (PDV/USA2006) was used as outgroup.

Even though the lineages are defined by the complete H gene variability, its amplification directly from field samples can be difficult because of its size (1824 bp) and its transcription level which is proportionately lower in comparison to the genes located in the 3' terminal region of the genome. These restrictions could limit its usefulness for characterization purposes. Notably, a strong phylogenetic signal was detected for the fusion protein signal-peptide (Fsp)-coding region by likelihood mapping which was as high as for the H gene. The Fsp coding region consists of only 405 bp and can be easily obtained to achieve a rapid characterization of circulating strains (Sarute et al., 2013).

A novel CDV lineage has been described based on the analysis of the Fsp region of South American strains, represented only by Ecuadorian strains and named South America (SA3), revealing the high diversity of the virus in this continent (Sarute et al., 2014).

Shortly after, phylogenetic evidence revealed a different lineage circulating among Colombian dog populations, clearly separated from the previous lineages described worldwide. Although the characterization of the Colombian lineage was performed using complete H gene sequences, and is therefore not comparable with the Fsp-coding region sequences available for the SA3 lineage; it is possible a genetic similarity between both lineages because they have similar geographic distribution in northern South America (Espinal et al., 2014). To evaluate this hypothesis, here we compared all South American strains characterized to date using a partial region of the H gene (630 bp). We built a dataset including two Ecuadorian sequences (SA3 lineage) recently published and 42 sequences of South American strains, which were employed to infer a phylogenetic tree (Fig. 2). Our finding revealed that there are currently four lineages circulating in South America: EU1/SA1, SA2, SA3 and those formed by Colombian strains, showing a high nucleotide and amino acid divergence. Therefore, the Colombian clade should be more properly denoted as South America 4 (SA4) lineage. The South American lineages have different geographical distribution, and appear spatially structured without apparent events of migration within the continent; underscoring the importance that local diversification has had in CDV dynamics.

In summary, there are currently eleven lineages circulating worldwide: three in Europe, two in Asia, two in North America, one in South Africa, and four in

South America. The presence in South America of tree unique lineages and a four intercontinental one (EU1/SA1) suggest that this continent harbor the highest genetic diversity of CDV strains. The extend of variation may be even greater as only five out of the twelve South American countries have performed CDV characterization studies. This epidemiological scenario encourages additional analyses in domestic and wild species to map the geographical spread and variability of CDV variants in South America and to provide new insights into local viral diversity and evolution.

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