Journal of Virological Methods xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Short communication

Resurgence of canine parvovirus 2a strain in the domestic dog population from Argentina

Q1 Marina Gallo Calderón[a,*], Carina Romanutti[a], Maximiliano Wilda[a], Alejandra D' Antuono[a], Leticia Keller[b], Mónica N. Giacomodonato[c], Nora Mattion[a], Q2 José La Torre[a]

[a] Instituto de Ciencia y Tecnología Dr. Cesar Milstein, CONICET, Saladillo 2468, C1440FFX Ciudad Autónoma de Buenos Aires, Argentina
[b] Fundación de Estudios en Virología Animal (FEVAN), Saladillo 2468, C1440FFX Ciudad Autónoma de Buenos Aires, Argentina
[c] Instituto de Investigaciones en Microbiología y Parasitología Médica (UBA-CONICET), Facultad de Medicina, Paraguay 2155, p12, C1121ABG Ciudad Autónoma de Buenos Aires, Argentina

Article history:
Received 17 May 2015
Received in revised form 19 June 2015
Accepted 22 June 2015
Available online xxx

Keywords:
Canine parvovirus
Sequence analysis
Virus evolution
VP2 gene

A B S T R A C T

Ninety-three rectal swab samples were taken, from dogs suspected of canine parvovirus (CPV) infection and analyzed by PCR. A fragment of the VP2 gene, was amplified in 41 (44%) of them, resulting CPV positive samples. Sequencing analysis of these PCR products showed that 37 samples (90.2%) belonged to the CPV2c type, whereas four samples (9.8%) were identified as CPV2a, which has not been found since 2008.

It was also found that 24 out of 37 CPV2c samples (65%), carried the mutation Thr440Ala, whereas this mutation was absent in the four CPV2a strains reported herein.

Using phylogenetic analysis of the full length VP2 gene, which was amplified by PCR in six local samples, it was seen that CPV2a Argentine strains reported in this study, were genetically closer to a previous local CPV2a isolate (year 2003) and to a South African CPV2a strain, than to any of the recently reported Uruguayan CPV2a strains.

The results obtained in this work, together with those reported previously in Uruguay strongly suggest that, in spite of the geographical proximity, wild type CPV strains undergo different evolutive pathways in each country, resulting in the prevalence of different strains in related dog populations.

Further extensive epidemiological studies are needed in order to improve the understanding of CPV evolution.

Canine parvovirus (CPV) is a 26 nm-diameter, non-enveloped virus carrying a single stranded DNA genome of approximately 5200 nucleotides (Reed et al., 1988). It belongs to the *Parvoviridae* family and is responsible for acute, sometimes fatal, gastroenteritis in dogs (Decaro and Buonavoglia, 2012).

Canine parvovirus 2 (CPV2) was first recognized in 1978 as a new pathogen of dogs; shortly after its emergence, CPV2 became endemic in the global dog population. The original CPV2 strain continued to evolve, and was subsequently replaced by three different but closely related antigenic variants, designated as CPV2a, CPV2b and CPV2c, which now coexist in different proportions in the worldwide dog population (Mochizuki et al., 1993; de Ybanez et al., 1995; Greenwood et al., 1996; Truyen et al., 1996; Steinel et al., 1998; Sagazio et al., 1998; Truyen et al., 2000; Buonavoglia et al., 2000; Pereira et al., 2000). The newest variant CPV2c, often associated to severe disease in adult dogs including those that have completed the vaccination protocols, it is becoming prevalent in different geographic regions, and this is the case in Argentina (Martella et al., 2004; Nakamura et al., 2004; Kapil et al., 2007; Decaro et al., 2007, 2009a,b, 2013; Hong et al., 2007; Perez et al., 2007; Joao Vieira et al., 2008; Calderon et al., 2011, 2009; Nandi et al., 2010; Ntafis et al., 2010; McElligott et al., 2011; Filipov et al., 2014; Cavalli et al., 2014).

In Uruguay, a neighbor country, CPV2c was the predominant variant during 2006–2009. However, an epidemiological change occurred in 2010, with the emergence of a novel CPV2a strain, which was detected with an increasing frequency up to 85% in 2011, and continues nowadays (Perez et al., 2012; Maya et al., 2013).

In the present study, an update in the identification and molecular characterization of different CPV strains in Argentina is reported.

* Corresponding author. Tel.: +54 11 4105 4127.
E-mail address: marinagallocalderon@yahoo.com.ar (M.G. Calderón).

http://dx.doi.org/10.1016/j.jviromet.2015.06.012
0166-0934/

A total of 93 rectal swabs samples obtained from domestic dogs from different regions of Argentina between October 2010 and October 2013, were received in the laboratory for diagnostic purposes.

CPV genomic DNA was extracted directly from the samples and a fragment of the VP2 gene (nucleotides 4003–4585) was amplified by PCR using a protocol previously described (Buonavoglia et al., 2001). A fragment of 583 bp was amplified in 41 out of 93 (44%) analyzed samples, resulting positives for CPV. Forty of the positive samples (97.6%) belonged to animals that had shown clinical symptoms compatible with CPV, while the remaining animal was asymptomatic. Interestingly, 33 out of 41 positive samples (80.5%), were obtained from puppies which had been vaccinated against CPV at least once, whereas four samples (9.7%) were recovered from unvaccinated dogs and the vaccination status of the remaining four dogs was unknown. Moreover, it must be highlighted that most of the positive samples (92.7%), were recovered from 1 to 5 months old puppies (Table 1).

The amplified DNA fragments were directly sequenced by Macrogen Inc. (Korea). Sequence analysis showed that 90.2% of the samples carried the amino acid (aa) Glu at the position 426, characteristic of CPV2c strains, and 65% of these CPV2c strains showed the substitution Thr440Ala (Table 1). The presence of this substitution was slightly higher than that previously reported in 2009 (58%) and 2010 (50%) (Calderon et al., 2011).

The high prevalence of CPV2c strains in Argentina seen in the last years, continues nowadays (Calderon et al., 2011). No CPV2b strains have been detected among local samples since 2009. However, unexpectedly four CPV2a samples were found during the year 2012.

In order to evaluate any other change in those CPV2a strains, the full length VP2 gene was amplified by PCR and cloned in the pGEM®-T Easy Vector, from those four samples characterized as CPV2a. Moreover, two representative strains which had been characterized as CPV2c were subjected to full length VP2 amplification and cloning.

The 11 critical amino acid positions in which changes were most frequently reported (Desario et al., 2005) are shown in Table 2 for CPV2a strains. Analysis of the VP2 aa sequences, showed that Uruguayan CPV2a and four out of five Argentine strains shared typical aa changes with respect to the CPV2 strain (Met87Leu, Ile101Thr, Ala300Gly, Asp305Tyr, Asn375Asp), also present in the old CPV2a reference strain (1984). This strain is the one which retains the mutation Val555Ile.

Three CPV2a Argentine strains (Arg 9, Arg 91 and Arg 98) presented the substitution Ser297Asn which has already been reported in other Argentine strains (Gallo Calderon et al., 2011) and in a South African CPV2a strain. In contrast, it has been reported that Uruguayan strains displayed in this position the mutation Ser297Ala, typical of the CPV2a/2b related isolates in Asia, Italy and Germany (Nakamura et al., 2004).

None of the recently reported changes in Uruguayan CPV2a strains, Phe267Tyr; Thr440Ala and Tyr324Ile (Perez et al., 2012) were found in local CPV2a strains; except for this last mentioned change which is present only in Arg 98 strain.

It is worth to mentioning that Arg 101 strain shares 10 out of 11 critical aa of the CPV2 strain, with the exception being the Ala300Asp change, which has been previously suggested to cause the loss of canine host range and to alter the antigenic properties of the virus (Llamas-Saiz et al., 1996).

Finally, a phylogenetic tree was built using 74 full-length VP2 sequences (six sequences determined in this study and 68 from the GenBank database).

As shown in Fig. 1, wild type viruses from Argentina and the rest of the world are phylogenetically separated from older CPV2 strains (which is only found in commercial live attenuated vaccines), with

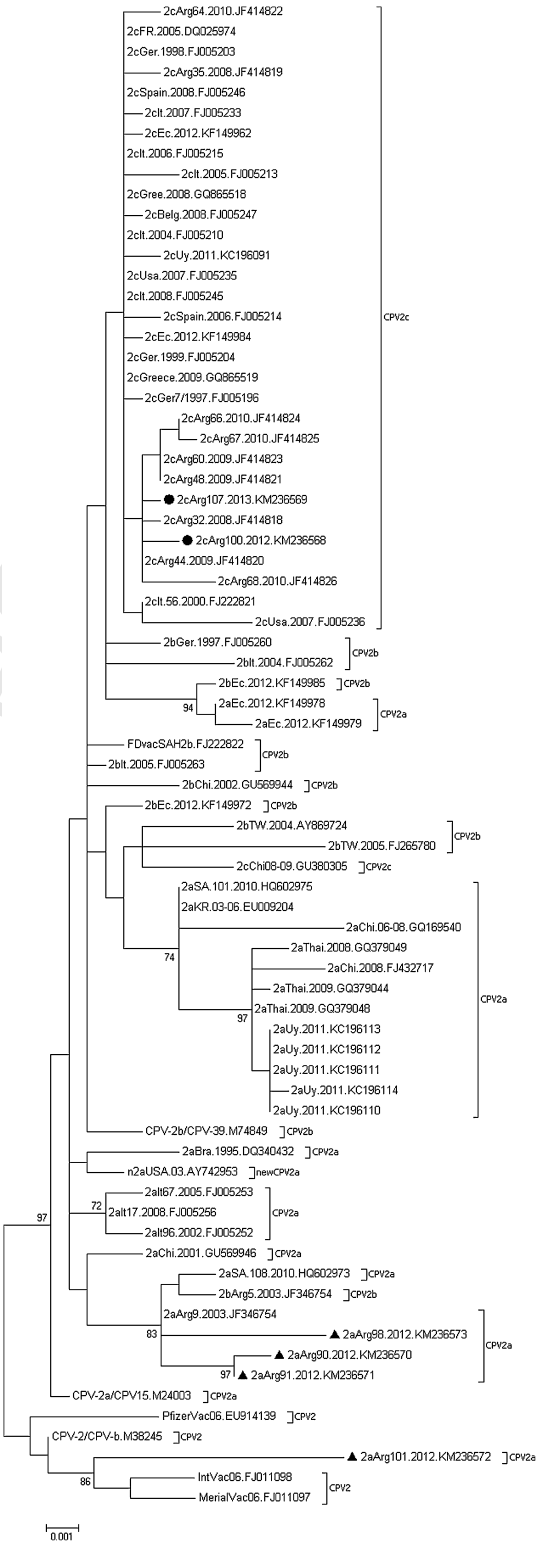

Fig. 1. Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura and Nei (1993) model. The tree with the highest log likelihood (−3916.1249) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search was obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 74 nucleotide sequences. There were a total of 1755 positions in the final dataset corresponding to full-length VP2 genes of local and worldwide strains of CPV. Evolutionary analysis was conducted in MEGA6 (Tamura et al., 2013).