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Research paper

Molecular epidemiology of canine parvovirus in Morocco

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ABSTRACT

Since it first emergence in the mid-1970's, canine parvovirus 2 (CPV-2) has evolved giving rise to new antigenic variants termed CPV-2a, CPV-2b and CPV-2c, which have completely replaced the original strain and had been variously distributed worldwide. In Africa limited data are available on epidemiological prevalence of these new types. Hence, the aim of the present study was to determine circulating variants in Morocco. Through TaqMan-based real-time PCR assay, 91 samples, collected from symptomatic dogs originating from various cities between 2011 and 2015, were diagnosed. Positive specimens were characterised by means of minor groove binder (MGB) probe PCR. The results showed that all samples but one (98.9%) were CPV positive, of which 1 (1.1%) was characterised as CPV-2a, 43 (47.7%) as CPV-2b and 39 (43.3%) as CPV-2c. Interestingly, a co-infection with CPV-2b and CPV-2c was detected in 4 (4.4%) samples and 3 (3.3%) samples were not characterised. Sequencing of the full VP2 gene revealed these 3 uncharacterised strains as CPV-2c, displaying a change G4068A responsible for the replacement of aspartic acid with asparagine at residue 427, impacting the MGB probe binding. In this work we provide a better understanding of the current status of prevailing CPV strains in northern Africa.

1. Introduction

Canine parvovirus (CPV) is the most dreadful enteropathogen causative of fatal disease in puppies and adult dogs worldwide (Decaro and Buonavoglia, 2012). CPV is a non-enveloped, single-stranded DNA virus that has been recently included in the same viral species, *Carnivore protoparvovirus 1*, along with feline panleukopenia virus (FPLV) and other parvoviruses of wild carnivores (Cotmore et al., 2014).

Since its emergence in the mid-1970s likely as a host variant of FPLV through adaptation to an intermediate wild-carnivore host, CPV has further evolved giving rise to three antigenic variants, namely CPV-2a, CPV-2b (Hoelzer and Parrish, 2010) and the most recent CPV-2c (Buonavoglia et al., 2001). These variants show a high pathogenic potential and the ability to replicate in cats (Decaro and Buonavoglia, 2012).

The geographic distribution of CPV antigenic variants is heterogeneous, with a prevalence of CPV-2b in United States, followed by CPV-2c and CPV-2a (Hong et al., 2007). In Mexico, only CPV-2c was typed (Pedroza-roldán et al., 2015). The three variants are circulating in South America with a higher frequency of CPV-2c in Ecuador (Aldaz et al., 2013), Argentina (Gallo et al., 2015), Brazil (Pinto et al., 2012) and Uruguay. Interestingly, the occurrence of CPV-2a was gradually reported and increased since 2011 being the main strain in Uruguay (Maya et al., 2013).

The variant CPV-2a was the predominant in New Zealand with a prevalence of 98.5% beside the original strain CPV-2 detected at a small proportion (1.5%) (Ohneiser et al., 2015).

In Japan, CPV-2b was the most prevailing strain followed by CPV-2a at a lesser frequency (Soma et al., 2013). In Taiwan a co-circulation of CPV-2a and CPV-2b at the same rate was reported (Lin et al., 2014). In China and Korea CPV-2a was the predominant variant followed by CPV-2b (Jeoung et al., 2008; Yi et al., 2014) and a recent circulation of CPV-2c was identified in northeast China (Geng et al., 2015). In India the three variants were identified with predominance of CPV-2b (Nandi et al., 2010).Whereas, in a recent study CPV-2c was not found (Mittal et al., 2014).

Predominance of CPV-2a in European countries, Bulgaria (Filipov et al., 2011), Italy, UK, Spain, Germany, France, Belgium and Hungary, followed by CPV-2c and CPV-2b was reported (Decaro et al., 2007). Whereas, in Portugal, CPV-2c was the dominant variant, followed by CPV-2b and CPV-2a at a smaller proportion (Miranda et al., 2016).

In Africa, there are only few reports elucidating the CPV type distribution in the continent. While both CPV-2a and 2b were detected

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in South Africa and Namibia (Dogonyaro et al., 2013; Steinel et al., 1998) and only CPV-2a was reported in Nigeria (Dogonyaro et al., 2013). A recent study from Tunisia reported a high prevalence of CPV-2c in this country (Touihri et al., 2009). However, there is no other study that was carried out in northern Africa.

In Morocco, during the last years, a high number of suspected outbreaks of CPV infection were reported by breeders and veterinarians in vaccinated and unvaccinated dogs with lethargy, diarrhoea and/or vomiting, but only few cases were confirmed by a laboratory diagnosis, which was based on hemagglutination (HA) or virus isolation (VI) assays (unpublished results). These assays have been recognised to be poorly sensitive, resulting in a high proportion of false-negative results (Decaro et al., 2005b; Desario et al., 2005). Molecular assays, based on the detection of the viral nucleic acid, represent the gold standard for a sensitive diagnosis of CPV infection (Desario et al., 2005). In addition to gel-based PCR tests, real-time PCR (qPCR) assays were developed for a more sensitive, specific and reproducible diagnosis (Decaro et al., 2005b; Wilkes et al., 2015). These assays were successfully used for rapid prediction of the antigenic type by means of type-specific minor groove binder (MGB) probes labelled with different fluorophores (Decaro et al., 2006b). MGB probe assays were also set up for discrimination between vaccine and field viruses (Decaro et al., 2006a, 2006c), which could be useful in the case of occurrence of post-vaccinal gastroenteritis.

The aim of the present paper is to report the results of an epidemiological investigation for CPV carried out on dogs with diarrhoea in Morocco using traditional and molecular tools.

2. Material and methods

2.1. Sampling

A total of 91 specimens (86 faecal samples and 5 pools of organs, consisting of liver, spleen and myocardium) were collected from sick and/or autopsied dogs of different breeds received at the university veterinary clinic in Rabat or at private veterinary clinics localised in Khemisset, Machraa Belakssiri, Rabat, Sale, Temara, Casablanca, Settat and Marrakech during years 2011–2015. These animals, had displayed pronounced clinical signs compatible with CPV infection, including haemorrhagic diarrhoea. Details about nature and year of collection of the tested samples, as well as about age, breed, sex, origin and vaccination status of the sampled dogs are reported in Table 1.

Clinical samples were homogenized in phosphate buffered-saline (PBS, pH 7.4) in a 10% *w/v* proportion and then clarified by brief centrifugation. The supernatants were stored at −80 °C until use.

2.2. HA testing

HA screening was conducted according to the method described by Carmichael et al. (1980) using a 1% PBS suspension of freshly prepared porcine erythrocytes. Serial dilutions of each specimen were tested twice in order to ensure accuracy. The viral titre was expressed as the highest sample dilution giving complete hemagglutination.

2.3. DNA preparation

DNA extraction from faecal samples was carried out by boiling 200 μl of homogenate supernatants for 10 min, chilling and dilution at 1:10 in water, as useful treatment to ensure inactivation of Taq DNA polymerase inhibitors (Schunck et al., 1995). DNA extraction from organs was achieved using a commercial kit (DNeasy® Kit, QIAGEN), following the manufacturer's instructions.

2.4. TaqMan real-time PCR

Real time PCR was conducted according to the method developed by Decaro et al. (2005b), using primers and probe which allows for detection of a 93-bp amplicon (Table 2). The 25-μl final PCR mixture consisted of 12.5 μl of Master mix (BioRad Laboratories), 600 nM of each CPV primer 555For and 555Rev, 200 nM of probe CPV-Pb and 10 μl of template DNA, and nuclease-free water. The thermal cycling was performed in a CFX96™ BioRad (BioRad laboratories) and consisted of activation of iTaq DNA polymerase at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 10 s and primer annealing and extension at 60 °C for 30 s. Data were analysed by the BioRad CFX Manager Software (BioRad Laboratories).

2.5. MGB probe assays for CPV characterisation

CPV positive samples were characterised by real-time PCR assays using primers and fluorescently labelled MGB probes specific for types 2a/2b and 2b/2c, following the method developed by Decaro et al. (2006b). Briefly, each positive DNA template was tested in two reaction mixtures, containing primers and probes specific to detect type 2a/2b or 2b/2c (Table 2). The reaction was carried out to obtain a final mixture of 25 μl consisting of 12.5 μl of Master Mix (BioRad Laboratories), 900 nM of specific primers, 200 nM of probe, 10 μl of template DNA, and free-nuclease water. Reactions were cycled under the same thermal conditions as previously detailed for generic real-time PCR.

Table 1
Nature of samples, year of collection, age, breed, sex, and vaccination status of tested dogs.

Code	Sample	Year	Age(month)	Breed	Sex	Vaccination	Origin	Strain
Alg-11	I	2011	NA	NA	NA	NA	Marrakech	NC
S10-11	I	2011	2	R	F	UV	Rabat	2c
Dark11	S	2011	NA	NA	NA	NA	NA	2c
Parvo 3	I	2012	1	R	F	UV	Rabat	2c
Parvo 4	I	2012	NA	NA	NA	NA	Rabat	2a
P01-13	RS	2013	3	P	F	V	Rabat	2c
P02-13	RS	2013	4	LR	M	V	Rabat	2c
P03-13	I	2013	6	LR	F	V	Rabat	2c
P04-13	RS	2013	6	GR	F	V	Rabat	2c
P05-13	Mc,S	2013	3	MB	F	V	Rabat	2c
P06-13	RS	2013	2	P	M	UV	Rabat	2c
P09-13	S	2013	6	MB	NA	UV	Rabat	2c
P1-13	I	2013	4	MB	M	UV	Khemisset	2c
P2-13	I	2013	3	MB	M	UV	Khemisset	2c
P3-13	RS	2013	3	MB	F	UV	Khemisset	2b
P4-13	I	2013	3	MB	F	UV	Khemisset	2c
P5-13	I	2013	3	MB	M	UV	Khemisset	2c
P6-13	I	2013	3	MB	M	UV	Khemisset	2c
P7-13	I	2013	3	MB	M	UV	Khemisset	2c