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Canine parvovirus (CPV-2) variants circulating in Nigerian dogs

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ABSTRACT

Canine parvovirus type 2 (CPV-2) is a highly contagious viral disease with three variants (CPV-2a, CPV-2b and CPV-2c) currently circulating in dogs worldwide. The main aim of this study was to determine the prevalent CPV-2 variant in faecal samples from 53 dogs presenting with acute gastroenteritis suspected to be and consistent with CPV-2 to Nigerian Veterinary Clinics in 2013–2014. Seventy-five per cent of these dogs tested positive for CPV-2 in a commercial antigen test and/or by PCR. Partial sequencing of the VP2 gene of six of these demonstrated them to be CPV-2a. Most of the dogs (60 per cent) were vaccinated, with 74 per cent of them puppies less than six months old.

INTRODUCTION

Canine parvovirus-2 (CPV-2) is a contagious viral disease of dogs characterised by severe diarrhoea with a high mortality rate. Three variants of CPV-2 (CPV-2a, CPV-2b and CPV-2c) are currently reported to be circulating worldwide with regional differences in which variants predominate (Cavalli and others 2008). There are few reports on the genetic characterisation of CPV-2 and its variants in Africa. All three variants were reported to be circulating in Tunisia (Touihri and others 2009) and Morocco (Amrani and others 2016), whereas Dogonyaro and others (2013) reported CPV-2a and CPV-2b in South Africa and CPV-2a only in Nigeria. The main aim of the current study was to determine the variants of CPV-2 currently circulating in Nigeria.

MATERIALS AND METHODS

Samples were collected from 53 dogs presenting with clinical signs of acute gastroenteritis at three different veterinary clinics in Nigeria: Magma and Mokola vet clinics in Ibadan (n=49), Vetworld Veterinary Clinic in Abuja (n=3) and the Veterinary Teaching Hospital in Makurdi (n=1). This research was approved by the University of Nottingham School of Veterinary Medicine and Science Ethical Review Committee. The consent of dog owners was obtained verbally and demographic information was also collected. Samples were tested for CPV-2 at point of care using the SNAP Parvo Antigen Test (IDEXX). In addition, samples were collected onto Whatman FTA cards, which were then stored at room temperature before transport to the University of Nottingham for molecular analysis.

DNA was isolated from the stored faecal samples using the QIAamp DNA stool mini kit (Qiagen, Germany) following the manufacturer's standard protocol for pathogen detection with the following exceptions. Ten punches were removed from the Whatman FTA card into a 2 ml centrifuge tube using a 3-mm punch and matt (Harris Puncher). Beater beads and buffer ASL (Qiagen) were added and incubated at 70°C for three hours in a heating block (Grant Instrument, England). The incubated samples were vortexed (JenconsS) at 30-minute intervals to ensure adequate homogenisation, lysis of CPV-2 virus capsid and recovery of dried faecal samples from the Whatman FTA cards. Sample centrifugation was done at 11,000 rpm in a bench-top centrifuge for 10 minutes before and after addition of inhibitEx tablets (Qiagen). Half of the recommended volume of elution buffer (AE, Qiagen) was used, the elution buffer was heated at 70°C for five minutes before elution and sequential elution was done to ensure maximum DNA yield. Further DNA concentration was performed via vacuum centrifugation (Christ, Germany) set at 30°C for an average of 20 minutes. DNA concentrations were measured using Nanodrop 8000 spectrophotometer (Thermo Scientific) before PCR was performed. Positive control DNA for the CPV-2 VP2 was derived from freeze-dried Novibac parvo vaccine (MSD Animal Health, UK) using the same protocol as for the faecal samples.

PCR was performed on all the samples extracted using two sets of published primers

and protocols to amplify the CPV-2 VP2 gene (Touihri and others 2009), and one set for canine GAPDH amplification (Tarlinton and others 2013a). Details of the primers and amplicons are provided in Table 1. The 50-µl PCR master mix reaction prepared consisted of 100-µM dNTP (BioLabs), 20-pmol forward primer, 20-pmol reverse primer (Eurofins), 10X standard *Taq* (magnesium-free) reaction buffer (BioLabs), 3-µM magnesium chloride, five units of DNA *Taq* polymerase (BioLabs), 31 µl of RNase-free water (BioLabs) and 1 µl of the template. The thermal cycling conditions involved 95°C of initial denaturation for 10 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, 55.5°C annealing temperature for 30 seconds, extension at 72°C for 3 minutes with a final extension at 72°C for 15 minutes using a Techne TC-512 PCR machine. Following gel electrophoresis to confirm amplification, PCR products were purified using the Nucleospin extract II kit (Machery-Nagel). Samples were sequenced by the Sanger method by Source BioScience.

Statistical analysis (chi-square) was performed using IBM Statistical Package for the Social Sciences software V.22, 32 bits edition with a cut-off for statistical significance of 0.05.

RESULTS

The demographic summary of the animals tested with the SNAP Parvo Antigen Test is presented in Table 2. Of dogs presenting with acute gastroenteritis, 74 per cent were puppies less than six months of age, with non-indigenous breeds (Alsatian, Boerboel, bull mastiff, Caucasian shepherd dog, Neapolitan mastiff, Rottweiler and terrier) making up 92 per cent of the cases. The majority (60 per cent) were vaccinated, though in most cases vaccination history was incomplete, confounding further analysis. Sixty-four per cent tested positive for CPV-2 with the SNAP Parvo Antigen Test. No statistically significant associations between CPV-2 test status and demographic characteristics were seen by chi-square.

Twenty samples were positive by PCR using the short VP2 gene primers, but PCR product was only obtained for eight of these with the long VP2 gene primers. This indicates that recovery of DNA from the Whatman FTA cards was an issue. Furthermore, only two samples tested positive for GAPDH, which was included as a quality control reference gene. Only 15 samples produced sequence suitable for genetic analysis (six long and nine short); these have been submitted to GenBank (accession numbers KX192407–KX192421). The short CPV-2 VP2 gene PCR detected 14 (26.4 per cent) of the samples that were positive by the SNAP Parvo Antigen Test and an additional 6 (11.3 per cent) that were not detected by the antigen test. Full details of all the animals and their test results are presented in online supplementary file 1. Poor recovery of DNA from the Whatman FTA cards makes interpretation of those samples that did not amplify in PCR as true negative rather than assay failure difficult; therefore, negative results are not recorded. Analysis of the translated amino acid sequences of the Nigerian samples demonstrated that all of the isolates fell within the CPV-2/2a clade with an asparagine residue at position 426 of the VP2 gene (Table 3). This is the characteristic amino acid distinguishing CPV-2/2a from CPV-2b (aspartic acid) and CPV-2c (glutamic acid) (Cavalli and others 2008). The longer sequences also encompass amino acid 305; the tyrosine present at this position is characteristic of CPV-2a as opposed to CPV-2 (Hoelzer and Parrish 2010).

DISCUSSION

The presence of canine parvovirus was confirmed by SNAP Parvo Antigen Test and/or PCR for most (75 per cent) of the Nigerian dogs that presented with clinical signs of CPV-2 in this study, indicating that a presumptive clinical diagnosis is reasonably reliable. The common assumption that native breed dogs are resistant to CPV-2 infection or disease cannot be determined as only four native breed dogs were present in this data set. This bias may simply reflect a preference for imported breeds by clients who can afford veterinary care for companion animals in Nigeria. This is supported by reports that 66 per cent of the case load of Nigerian clinics are 'non-indigenous' dogs (Otolorin and others 2014).

Although PCR is generally more sensitive than the IDEXX antigen test (Decaro and others 2013), the low

TABLE 1: List of primers used for PCR amplification of canine parvovirus type 2 (CPV-2) VP2 gene and canine GAPDH gene

Primer name	Sequences	Size (bp)	Reference
CPV-2 VP2			Touihri and others (2009)
VP2-long–forward	5′ GAGCATTGGGCTTACCA 3′	1195	
VP2-long–reverse	5′ TTAATATAATTTTCTAGGTGCTAGTTGAGA 3′		
VP2-short–forward	5′ GGTGATCCAAGATATGCATTTGG 3′	430	
VP2-short–reverse	5′ GCAAGATGCATCAGGATC 3′		
GAPDH			Tarlinton and others (2013a)
GAPDH forward	5′-GAGAAAGCTGCCAAATATG-3′	193	
GAPDH reverse	5′-CCAGGAAATGACCTTGACA-3′		

TABLE 2: Demographic data of canine parvovirus type 2 cases based on SNAP Parvo Antigen Test result

Category	Positive		Total	
	No.	%	No.	%
Age (months)				
≤ 6	26	49	39	74
≥6	8	15	14	26
Vaccination status				
Vaccinated	23	43	32	60
Unvaccinated	5	9	11	21
Unknown	6	11	10	19
Breed				
Native dogs	1	2	4	8
Non-native dogs	33	62	49	92
Gender				
Female	18	34	25	47
Male	16	30	28	53
Total	34	64	53	100

TABLE 3: Variations in amino acid residues of the VP2 capsid protein sequence of Nigerian samples compared with the canine parvovirus type 2 (CPV-2a) reference strain EU659118

Amino acid	305	324	426	440	564
Reference	**Y**	Y	**N**	T	S
NG32	.	I	.	A	.
NG33	.	I	.	A	.
NG35	.	I	.	A	.
NG36	.	I	.	A	I
NG39	.	I	.	A	.
NG40	.	I	.	A	.
NG21	ND	ND	.	A	ND
NG22	ND	ND	.	A	ND
NG24	ND	ND	.	A	ND
NG25	ND	ND	.	A	ND
NG26	ND	ND	.	A	ND
NG27	ND	ND	.	A	ND
NG29	ND	ND	.	A	ND
NG37	ND	ND	.	A	ND
NG38	ND	ND	.	A	ND

Amino acids identical to the reference strain are indicated by a '.' Residues that allow CPV-2 variants to be distinguished are highlighted in bold typeface (426 distinguishes CPV-2 and CPV-2a from CPV-2b and CPV-2c, and 305 further distinguishes CPV-2 from CPV-2a)
ND, not done (only shorter sequence available); GenBank accession numbers for these sequences are KX192407–KX192421

DNA recovery evident for these samples has likely resulted in a high degree of PCR failure rather than a true negative test. Hence, the authors have only presented demographic summaries for the IDEXX-confirmed cases where the positive/negative test status is more reliable. Sequence analysis of 15 of the PCR-positive samples demonstrated CPV-2/2a sequences only. Six of these samples, where longer PCR products were obtained, were definitively CPV-2a, the remaining nine for which only short sequences were obtained were CPV-2 or CPV-2a. However, as CPV-2 is not considered to have circulated since its replacement by CPV-2a worldwide in the 1980s and detection of vaccine strains even in dogs recently vaccinated does not commonly occur (Miranda and Thompson 2016a, b), it is very likely that these are CPV-2a. The only other residue that differed from the reference CPV-2a sequence in the longer PCR products (Table 3) has been reported in isolates from other countries previously (Yoon and others 2009). This is consistent with the findings from a previous South African study, which also reported CPV-2a in the only two samples sequenced from Nigerian dogs (Dogonyaro and others 2013). There are reports by Nigerian veterinarians that canine parvovirus is common in vaccinated dogs (Shima and others 2015), which is upheld by these data. The current commercial vaccines used in Nigeria contain live attenuated strains of CPV-2 or CPV-2b, which are reported to provide adequate protection from CPV-2a challenge (Cavalli and others 2008). However, there are both anecdotal and published reports of single-dose vaccine regimens being used in dogs in Nigeria (possibly due to cost concerns for the clients) (Shima and others 2015, Babalola and others 2016). These vaccine regimens are unlikely to provide adequate immunity to CPV-2 in juvenile animals. Interference with CPV-2 vaccine efficacy by prolonged maternal immunity, particularly in environments where dogs are exposed to high levels of viral challenge and bitches are likely to have a high antiviral titre to pass to puppies, is a well-described cause of failure of canine parvovirus vaccination (Ling and others 2012). The young age of most of the animals presented for gastroenteritis in this study, combined with a single-dose vaccination regimen, is the most likely reason for the high number of cases in vaccinated dogs. A further potential caveat is that without knowledge of the sequence of all the CPV-2 vaccines in use in Nigeria (some produced by international firms, some produced locally), we cannot exclude detection of vaccine strains as an explanation for these results. However, recent studies (Miranda and Thompson 2016a) in European dogs would indicate that shedding of CPV-2 virus strain after live virus vaccine administration is rare, with vaccine strains not detected in clinical samples (even from dogs vaccinated within 10 days of developing clinical signs of CPV-2).

Further exploration of vaccination practice by Nigerian veterinarians, sequencing of the vaccine strains in use in Nigeria for comparison with field infections and more detailed information on vaccine timing and status with comparison to the onset of clinical are obvious follow-on studies to explore the underlying reasons for the high level of CPV-2 disease seen in vaccinated dogs.

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Contributors TTA performed the sample collection and experimental work in the study and drafted the manuscript. JMD and RET designed and oversaw the study and critically revised the manuscript.

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Data sharing statement Sequence data are available on the GenBank repository (accession number KX192407-KX192421). Demographic data from the animals included in the study are provided as online supplementary file.

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