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Short communication

MT-PCR panel detection of canine parvovirus (CPV-2): Vaccine and wild-type CPV-2 can be difficult to differentiate in canine diagnostic fecal samples

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ABSTRACT

Canine parvovirus (CPV-2) remains an important cause of devastating enteritis in young dogs. It can be successfully prevented with live attenuated CPV-2 vaccines when given at the appropriate age and in the absence of maternal antibody interference. Rapid diagnosis of parvoviral enteritis in young dogs is essential to ensuring suitable barrier nursing protocols within veterinary hospitals. The current diagnostic trend is to use multiplexed PCR panels to detect an array of pathogens commonly responsible for diarrhea in dogs. The multiplexed PCR assays do not distinguish wild from vaccine CPV-2. They are highly sensitive and detect even a low level of virus shedding, such as those caused by the CPV-2 vaccine. The aim of this study was to identify the CPV-2 subtypes detected in diagnostic specimens and rule out occult shedding of CPV-2 vaccine strains. For a total of 21 samples that tested positive for CPV-2 in a small animal fecal pathogens diagnostic multiplexed tandem PCR (MT-PCR) panel during 2014–2016 we partially characterized the VP2 gene of CPV-2. Vaccine CPV-2 strain, wild type CPV-2a subtypes and vaccine-like CPV-2b subtypes were detected. High copy number was indicative of wild-type CPV-2a presence, but presence of vaccine-like CPV-2b had a variable copy number in fecal samples. A yardstick approach to a copy number or C_t-value to discriminate vaccine strain from a wild type virus of CPV-2 can be, in some cases, potentially misleading. Therefore, discriminating vaccine strain from a wild type subtype of CPV-2 remains ambitious.

Canine parvovirus (CPV-2) causes a devastating enteritis and subsequent diarrhea in young and immunonaive dogs, due to viral tropism to rapidly multiplying cells, such as enterocytes in the intestinal crypts [3,11]. Successful vaccination programs using live attenuated CPV-2 vaccines exist, but controversies arise between dog owners, veterinarians and vaccine companies when diarrhea occurs within a few days of CPV-2 vaccine administration [4]. Reversion of the vaccine virus to a virulent CPV-2 virus has not been documented and is considered unlikely [3]. It has been postulated that clinical signs of canine parvovirus a few days after CPV-2 vaccination result from a different disease, or from a wild virulent CPV-2 subtypes incubating at the time of vaccination [3]. Diagnosing parvoviral enteritis in dogs is essential to ensuring suitable barrier nursing protocols within veterinary hospitals. The current diagnostic trend is to use multiplexed PCR panels to detect an array of pathogens that commonly cause diarrhea in dogs [6]. The multiplexed PCR assays do not distinguish wild from vaccine CPV-2. They are highly sensitive and detect even a low level of virus shedding, such as those caused by the CPV-2 vaccine [6]. Interpretation of test results is based on quantitation of the viral load (quantitative PCR) and clinical presentation including commonly found panleucopenia on peripheral blood. In fatal cases structural evidence can be utilized to complement qPCR and hematology to confirm parvoviral enteritis, however the aim is accurate antemortem diagnosis that rapidly differentiates vaccine associated

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viral presence from wild-type parvoviral enteritis.

Canine parvovirus (CPV-2) emerged as a canine pathogen in the late 1970s. In the 1980s, two antigenic variants emerged (CPV-2a and CPV-2b) and eventually completely replaced the original CPV-2 [9]. CPV-2 is still used in many commercial vaccines with some more recently replacing this with CPV-2b. Differentiation of CPV-2 viruses is based on viral capsid protein sequence (VP2). The original CPV-2 differs in five or six amino acid residues from CPV-2a and CPV-2b [2,3], and there are only two amino acid residues differentiating CPV-2a from CPV-2b [11]. A more recently discovered strain (CPV-2c) is defined by a further single nucleotide change [3].

The aim of this study was to identify the CPV-2 subtypes detected in diagnostic specimens presented to The Veterinary Pathology Diagnostic Service at the University of Sydney. Diagnostic dilemmas for clinicians not uncommonly include the possibility of occult shedding of CPV-2 vaccine strains in diarrhea of other etiology, and the possibility of vaccine breakdown in the face of emerging CPV-2 subtypes.

A small animal fecal pathogens diagnostic multiplexed tandem PCR (MT-PCR) panel (Cat. No. 38175 Version 1 through Version 3, AusDiagnostics, Sydney, Australia) was implemented at the University of Sydney Veterinary Pathology Diagnostic Services from 2014. The MT-PCR incorporates a multiplexed initial amplification and a subsequent single-target amplification, using SYBR Green chemistry in a real-time PCR to detect DNA of parvovirus (CPV-2), as well as *Salmonella*, *Campylobacter*, *Giardia, Cryptosporidium, Dientamoeba, Blastocystis, Toxoplasma,* feline coronavirus, *Tritrichomonas,* canine distemper virus, and canine coronavirus and is used for both canine and feline fecal samples [10]. The MT-PCR panel includes amplification of host target DNA to evaluate sample adequacy, and each processed sample is spiked with proprietary DNA and tested for its presence to assess PCR inhibition in the test specimens (http://www.ausdiagnostics.com/, AusDiagnostics Pty. Ltd., Beaconsfield, NSW 2015, Australia). For each fecal sample, 250 mg of feces were first homogenized and disrupted in a FastPrep-24 Homogenisation System equipped with QuickPrep Adapter (MP Biomedicals, Australia) at a speed setting of 6.0 m/s for 40 s prior to DNA isolation. Genomic DNA was then isolated using PowerMag® Microbiome RNA/DNA Isolation Kit (magnetic separation) (Mo-Bio Laboratories; GeneWorks, Thebarton, Australia) optimised for KingFisher® Duo (Thermo-Fisher Scientific, Scoresby, Australia).

A total of 21 samples that tested positive for CPV-2 in the MT-PCR small animal fecal pathogens diagnostic panel during 2014–2016 were included in this study (Table 1; Supplementary Table 1). Using the latest Version 3 of the MT-PCR panel (see above), 76% (16/21) were CPV-2 positive (Table 1). To evaluate if the CPV-2 DNA originated from the vaccine or wild type parvovirus, we partially characterized the VP2 gene of CPV-2 from 43% (9/21) of clinical specimens (eight of which were MT-PCR V3 positive for CPV-2). Two overlapping sets of primers HFor/HRev (residues 3556–4166; **M38245**) and 555For/555Rev (residues 4003–4585; **M38245**) were used, as previously published [2]. Review of CPV-2 genome sequences available in GenBank revealed poor conservation of 555Rev primer site, therefore an alternative primer JS-2 (residues 4799–4818; **M38245**) [8] was used in combination with 555For or Hfor as an alternative combination, improving amplification success for 3 samples previously negative using 555For/555Rev primers (Table 2). The amplified region included the diagnostic residues 297, 300, 305, 426 and 555 for typing of CPV-2, CPV-2a, CPV-2b and CPV-2c [3]. The conventional PCR included MyTaq Red MasterMix (BioLine, Sydney, Australia) and were run on a Verity PCR cycler (Thermo Fisher Scientific, Australia). To match the obtained sequences to vaccine strains of CPV-2, we amplified VP2 from two frequently used live attenuated vaccines (CPV-2b strain, Protech C4, Boehringer Ingelheim, Australia; CPV-2, Canigen $DH_{A2}P$, Virbac, Australia). All direct DNA sequencing with the amplification primers in both directions was done by Macrogen (Seoul, Korea) using amplification primers.

Vaccine strain CPV-2 (Canigen) was detected in four samples, based on 100% match between the vaccine DNA sequence and DNA amplified from canine fecal samples (Table 2). Near identical DNA sequences to vaccine strain CPV-2b (Protech) were detected in three dog feces (Table 2, Fig. 1). The CPV-2b vaccine strain DNA had a single mismatch (nucleotide position 4710, **M38245**) in the non-coding region of the viral DNA from the canine CPV-2b DNA sequences (15–2425, 15–2378, 16–3871; Fig. 1A). This mismatch was only recognized with 555For/JS-2 primers, regions amplified with

Table 1
Small animal fecal pathogens diagnostic multiplexed tandem PCR (MT-PCR) panel during 2014–2016 testing positive for CPV-2.

ID	Breed	Age	Weight (kg)	Outcome	Spike	CPV-2	Sal	Cam	Giar
14–1644	Samoyed	2 months	3.1	Dead	14.40	11.21	24.97		
14–6993	Bullmastiff X	4 years	12.2	Recovered	14.97	24.35	25.18		
14–7931	Miniature Poodle	13 years	4.4	Recovered	15.69	25.14			
15–2378	English Cocker Spaniel	4 months	6.8	Sent home[a]	14.51	<10			19.04
15–2425	Pug X	2 months	0.4	Sent home[a]	16.73	18.87			19.86
15–2429	Daschshund X	2 months	N/A	Healthy	15.95	25.55		11.83	
15–3568	Labradoodle	2 months	N/A	Healthy	14.77	-^		18.36	
15–3702	Staffordshire Bull Terrier	2 years	N/A	N/A	15.04	-^			
15–4390	Greyhound	11 years	N/A	N/A	15.26	-^		18.56	
15–4399	Unknown	Unknown	N/A	N/A	16.31	-^			
15–4401	Staffordshire Bull Terrier	6 months	N/A	N/A	15.33	23.67	24.75	21.68	
15–5505	Unknown	Unknown	N/A	N/A	14.61			25.04	
15–6583	Unknown	Unknown	N/A	N/A	13.19	22.12		24.87	
15–6585	Unknown	Unknown	N/A	N/A	13.15	25.10	24.39	17.80	
15–6588	Unknown	Unknown	N/A	N/A	14.67	20.34			
16–0873	Miniature Pinscher X	9 months	4.0	Euthanasia	13.95	25.24			
16–3871	Golden Retriever	8 months	35.0	Recovered	14.65	17.91		24.93	24.70
16–4759	Miniature Dachshund	10 months	5.6	Recovered	12.96	23.57		19.84	
16–6027	Maltese X	11 months	6.3	Recovered	13.76	25.04			
16–7855	Kelpie X	2 months	7.4	Sent home[a]	14.66	<10			
16–9394	Staffordshire Bull Terrier X	8 months	N/A	Sent home[a]	14.25	23.72	17.14		

-^ Tested positive with previous version of the diagnostic kit, but was negative with Version 3.
Abbreviations: *Sal – Salmonella, Cam – Campylobacter, Giar - Giardia.*
[a] No further information available.