CROSS-CANADA DISEASE REPORT

RAPPORT DES MALADIES DIAGNOSTIQUÉES AU CANADA

Canine parvovirus type 2b is the most prevalent genomic variant strain found in parvovirus antigen positive diarrheic dog feces samples across Canada.

Carl A. Gagnon[a,b,c,*], Véronique Allard[c], Guillaume Cloutier[d].

[a]Groupe de recherche sur les maladies infectieuses du porc (GREMIP), [b]Swine and Poultry Infectious Diseases Research Center (CRIPA) and [c]Molecular diagnostic laboratory, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Quebec, Canada.

[d]Boehringer Ingelheim (Canada) Ltd, Burlington, Ontario, Canada.

*Corresponding author: Mailing address: Faculté de médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, Saint-Hyacinthe, Québec, Canada, J2S 7C6. Phone: 450-773-8521 (8681). Fax: 450-778-8108. Email: carl.a.gagnon@umontreal.ca .

In 1978 an emerging virus was identified in dog population, the canine parvovirus (CPV) which was subsequently named CPV type 2 (CPV-2). The latter was distinct from known parvovirus; the canine minute virus (CnMV), formerly known as canine parvovirus type 1 (1). CPV-2 was inducing hemorrhagic enteritis, severe diarrhea, vomiting, leukopenia associated with a high mortality in infected dogs and had spread into non-immune dog populations worldwide causing a pandemic. Furthermore, CPV-2 was responsible for myocarditis in puppies. Today, the prevalence of CPV antibodies positive adult dogs is high due to vaccination and/or natural infection causing a high protective immunity status. Nevertheless, young non-immune animals of 6 weeks to 6 months old are susceptible to CPV infection when passive protection from maternal derived antibodies (MDA), which are able to protect puppies against myocarditis, have been lost. (2). The CPV-2 belongs to the virus family *Parvoviridae*, subfamilly *Parvovirinae* (which infect vertebrates) and genus *Parvovirus* (3). CPV-2 is a small non-enveloped single-stranded DNA virus of around 25 nm in size (1). The CPV-2 genome length is 5.2 kilo bases (kb) and possesses at least two major open reading frames (ORFs) (4). In the conventional orientation, the right-hand ORF encodes the viral capsid proteins VP1 and VP2, which are the main antigens that induce protective antibodies (5-7). The left-hand ORF encodes the non-structural proteins NS1 and NS2 (8). Since PCV-2 emergence, several viral genomic variants have emerged due to a surprisingly high substitution rate in CPV genome similar to those of RNA viruses (1, 9). At least four genomic variants have been described and officially recognized: the initial emerging CPV (named afterward CPV-2), followed shortly after by the CPV-2a (in 1979), the CPV-2b (in 1984) and CPV-2c (in 2000) (1). These CPV variants are differentiated through single nucleotide

47 polymorphism (SNP) located at the VP2 gene (10). The prevalence of CPV variants are
48 geographically restricted and have evolved over time (1, 10). Unfortunately, to our
49 knowledge, the prevalence of CPV genomic variants in Canada is not known. Thus, the
50 main objective of the present study was to determine the prevalence of CPV genomic
51 variants within Canadian diarrheic dog population.
52
53 To fulfill this objective, Canadian veterinarian volunteers have submitted feces from
54 diarrheic animals that were previously confirmed to be positive for CPV antigen after
55 local testing by any commercially available capture antigen assay (such as SNAP® Parvo
56 Test, Idexx Laboratories). The CPV vaccination status of diarrheic animals was
57 requested and when they were known to be recently vaccinated, i.e. less than 30 days
58 after the last vaccination, the feces samples of those animals were excluded from the
59 study. It has been recently reported that animals that are vaccinated with a live attenuated
60 vaccine may shed the vaccine strain for up to 21 days post-vaccination, as established by
61 CPV real-time PCR positive results (11). Thus, a delay of 30 days post-vaccination for
62 CPV genotyping was sufficient to circumvent the effects of vaccine strains on the results.
63 Overall, samples from 49 cases were included in this study. These samples were
64 submitted over a three year period (from September 27th 2012 until August 21st 2015) and
65 originated from seven different provinces (see Table 1). Genotyping of CPV variants
66 was performed by the concomitant analysis of three multiplex real-time PCR (qPCR)
67 assays using minor groove binder (MGB) probes technology as previously described (12,
68 13) but with minor modifications. These MGB probes allow SNP discrimination of the
69 ORF encoding the VP2 protein and therefore allow strains genotyping. Briefly, about 1

gram of feces was resuspended in 5 mL of phosphate buffer saline (PBS). Nucleic acid extraction was performed on supernatants or directly on vaccine formulation (Duramune®Max Pv and Nobivac®Canine 3-DAPv which contains the CPV-2b and CPV-2 variants, respectively, as positive controls) with either the QIAamp cador Pathogen Mini Kit (Qiagen) or the BioSprint 96 One-For-All vet kit (Qiagen) according to manufacturer's instructions. Thereafter, the three multiplex qPCR assays were carried out in a 25 µL volume containing 5 µL template or control DNA, 12.5 µL of QuantiTect Probe PCR buffer 2x (Qiagen), 3 pmol of TET-labeled probe, and 1.25 µL of a 20X mixture consisting of forward and reverse primer and the FAM-labeled probe. The qPCR were either performed on a SmartCycler (Cepheid) or RotorGene (Qiagen). The first conducted multiplex qPCR assay was targeting the change from adenine to guanine at position 4062 (SNP A4062G) in Genebank reference strain M38245 between CPV-2a and 2b variants, while the second multiplex qPCR assay was targeting the thymine to adenine change at position 4064 (SNP T4064A) between CPV-2b and 2c variants. As previously reported, the first multiplex qPCR assay could not properly discriminate CPV-2 and 2a variants. Thus, a third multiplex qPCR assay targeting SNP T3088C was developed to ensure discrimination between CPV-2 variant from all three other variants. Taken together, these three multiplex qPCR assays allow reliable and fast CPV genotyping results.

As shown in Table 1, the CPV-2b variant was the most prevalent in all the seven provinces from which the samples were obtained. The prevalence of CPV-2b between the different provinces was varying from 85.7 to 100%. Overall, the Canadian CPV-2b

prevalence was 89.8%. This finding was surprising because since 2007, CPV-2c was reported to be the most prevalent variant into the United States (US), the Canada neighboring country, varying between 48.1 to 73.5% (14, 15). In contrast, only one sample originating from Ontario was positive for CPV-2c variant in the current study (Table 1). Consequently, CPV-2c has an overall Canadian prevalence of 2%, which is much lower as compared to US prevalence. Four samples were positive for CPV-2a variant and they were originating from three provinces: Alberta, British Columbia and Quebec. Thus, CPV-2a has an overall Canadian prevalence of 8.2% (Table 1). As expected, no CPV-2 variant was detected as it is considered to be extinct (Table 1). Because vaccination could significantly affect the genotyping of CPV as attenuated vaccine strains could be shed in feces (11), information in regards to vaccination status was requested with the submitted samples. Unfortunately, the vaccination status of seven animals (i.e. 14.3% of the feces samples) was unknown (data not shown). Thus, even if those seven cases are excluded from our analyses, the CPV-2b variant remained the most prevalent variant found in diarrheic dogs with a value of 90.5% (data not shown). Interestingly, 19 diarrheic animals have been reported by veterinarians to be vaccinated and at least three different commercial vaccines have been used to vaccinate animals (data not shown). Noteworthy, only 1 of these animals (5.3%) seems to have been vaccinated according to the equivalent guidelines of the American Animal Hospital Association (AAHA) and the World Small Animal Veterinary Association (WSAVA). These guidelines are that: *"Puppies with poor MDA may be vulnerable (and capable of responding to vaccination) at an earlier age, while others may possess MDA at such high titres that they are incapable of responding to vaccination until ≥12 weeks of age. No*

single primary vaccination policy will therefore cover all possible situations. Thus, puppies should be vaccinated every 3–4 weeks between the ages of 6 and 16 weeks (e.g., at 6, 10, and 14 weeks, or 8, 12, and 16 weeks). To minimize the risk of MDA interference with vaccination, the final dose of the initial series should be administered between 14 and 16 weeks of age, regardless of the product used" (16, 17). Thus, at least 83.7% (n=41) of the diarrheic animals of this study were not or were improperly vaccinated (data not shown) because they received only one or two doses or the last puppy booster was given prior to 14 weeks of age. Noteworthy, three of the vaccinated animals received three vaccine shots as recommended by AAHA and WSAVA but surprisingly, the last booster shot was given prior to 14 weeks of age (data not shown). Consequently, Canadian veterinarians should encourage their pet owners to vaccinate their puppies following the AAHA and WSAVA guidelines. Several reports have demonstrated a very good efficacy of CPV vaccines in the context of homologous and heterologous challenges (10, 17). Our results may have indicated a vaccination failure in one vaccinated animal but it might be explain by errors included in the clinical data that were reported by the owner and/or the veterinarian. Moreover, other co-infecting microorganisms (bacteria, parasites, viruses) could have contributed to the diarrhea in the clinical cases that were submitted (18). Unfortunately, the intestinal microbiota of diarrheic animals was not part of this study and thus, it was not investigated. In conclusion, by far the most prevalent CPV variant found in feces of Canadian diarrheic dogs is CPV-2b.

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Table 1. Prevalence of canine parvovirus type 2 genomic variants in feces of diarrheic dogs

Province	Total of submitted feces samples[a]	Genomic variants % (n)							
		CPV-2		CPV-2a		CPV-2b		CPV-2c	
Nova Scotia	3	0,0	(0)	0,0	(0)	100,0	(3)	0,0	(0)
New Brunswick	1	0,0	(0)	0,0	(0)	100,0	(1)	0,0	(0)
Quebec	10	0,0	(0)	10,0	(1)	90,0	(9)	0,0	(0)
Ontario	13	0,0	(0)	0,0	(0)	92,3	(12)	7,7	(1)
Saskatchewan	1	0,0	(0)	0,0	(0)	100,0	(1)	0,0	(0)
Alberta	7	0,0	(0)	14,3	(1)	85,7	(6)	0,0	(0)
British Columbia	14	0,0	(0)	14,3	(2)	85,7	(12)	0,0	(0)
Total:	49	0,0	(0)	8,2	(4)	89,8	(44)	2,0	(1)

201 [a]Samples were submitted from CPV antigen positive diarrheic animals starting 2012/09/27 until 2015/08/21.