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Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: A clinical and laboratory diagnostic dilemma

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Received 10 August 2006; received in revised form 22 September 2006; accepted 12 October 2006
Available online 25 October 2006

Abstract

A total of 29 faecal samples collected from dogs with diarrhoea following canine parvovirus (CPV) vaccination were tested by minor groove binder (MGB) probe assays for discrimination between CPV vaccine and field strains and by diagnostic tests for detection of other canine pathogens. Fifteen samples tested positive only for CPV field strains; however, both vaccine and field strains were detected in three samples. Eleven samples were found to contain only the vaccine strain, although eight of them tested positive for other pathogens of dogs. Only three samples were found to contain the vaccine strain without evidence of canine pathogens. The present study confirms that most cases of parvovirus-like disease occurring shortly after vaccination are related to infection with field strains of canine parvovirus type 2 (CPV-2) rather than to reversion to virulence of the modified live virus contained in the vaccine.

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Keywords: Canine parvovirosis; Vaccination; MGB probe assays

1. Introduction

Canine parvovirus type 2 (CPV-2) causes haemorrhagic gastroenteritis primarily in 2–6-month-old pups [1]. CPV-2, first identified in the late 1970s, was replaced a few years after its emergence by two antigenic variants, CPV-2a and CPV-2b. Those two types are now distributed worldwide [2]. More recently, a third antigenic variant, CPV-2c, was first reported in Italy [3]. Subsequently, it was reported in Vietnam [4], Spain [5], Germany and the United Kingdom (Decaro, unpublished data). This new variant appears to be replacing CPV-2b in the Italian dog population [6–11]. The CPV variants differ in amino acid changes occurring at residue 426 of the capsid protein, with types 2a–2c displaying amino acids Asn, Asp and Glu, respectively [2,3]. This residue is located in a major antigenic site close to epitope B, i.e., over the shoulder region of the capsid in a region considered to affect viral immunogenicity. Concerns have been expressed that the antigenic differences between CPV type 2 and its variants may decrease the effectiveness of the CPV-2-based vaccines [12–14]; however, type 2b vaccines are now licensed in several countries and some investigators recommend their extensive use [15].

Characterisation of the CPV variants was controversial until minor groove binder (MGB) probe technology was applied to obtain rapid and unambiguous identification of the viral type [10,11]. MGB probes are short TaqMan probes conjugated with molecules that form hyper-stabilised duplexes with complementary DNA, allowing reduction in length of the probe and an increase in specificity [16]. MGB probes are, therefore, an attractive tool for revealing single nucleotide polymorphisms in the capsid protein gene between types 2a and 2b and types 2b and 2c. Since the assays differentiating types 2a/2b and type 2b/2c did not discriminate between vaccine and field strains, additional MGB probe assays were developed.

We had previously developed an assay to differentiate field strains from type 2-based vaccines that react as CPV-2a, using the type 2a/2b assay [17]; in addition, two assays were
developed to discriminate between type 2b vaccine and field strains [18]. Such assays could be of practical help, since gastroenteritis in pups within 1 week after CPV vaccination occurs frequently in practice (Buonavoglia, personal observation). In such cases, conventional diagnostic tests are able to detect a CPV strain in the faeces of vaccinated dogs, although it is frequently uncertain whether the virus is a vaccine or field strain.

In the present study, faecal samples from cases of parvovirus gastroenteritis that occurred 1–7 days after CPV vaccination were analysed by the novel MGB assay in order to determine whether the puppy illnesses were associated with virulent or vaccinal CPV types. In such cases, it was especially important to attempt to rule out any involvement of CPV vaccines.

2. Materials and methods

2.1. Samples

A total of 29 faecal samples from dogs that had clinical signs typical of parvovirus gastroenteritis during the first week after CPV vaccination were collected between 2004 and 2006 and tested subsequently. Twenty-two samples were from dogs administered monovalent or multivalent CPV-2 vaccines and four samples were from dogs vaccinated with CPV-2b. The types/brands of CPV vaccine used in the remaining three pups were not reported. Those samples included a unique specimen collected in Scotland, UK (97/06-3, courtesy of Christopher Davies, Institute of Comparative Medicine, University of Glasgow) and specimens collected from two Italian dogs (326/04 and 206/05). As regards the Scottish dog, the vaccine used and the day of vaccination were unknown; the two exceptional Italian dogs also received vaccines that were uncertain. Six of the dogs had been imported to Italy from Poland (n = 3) or Hungary (n = 3), but vaccine had been administered in Italy. In some cases, disputes between dog owners and veterinarians were in progress because of concerns regarding the possible relationship between CPV vaccinations and the subsequent enteric illness.

In order to evaluate the post-vaccinal virus shedding in healthy dogs, 16 faecal samples were also tested that had been collected from non-diarrhoeic dogs between days 3 and 7 after CPV vaccination. The specimens were from dogs inoculated with CPV-2 (n = 12) or CPV-2b (n = 4) vaccines.

2.2. MGB probe assays for characterisation of field strains of CPV-2

Detection and characterisation of CPV-2 was obtained using two real-time PCR assays with the MGB probes described previously [11]. Types 2a/2b and 2b/2c assays were employed to discriminate between types 2a and 2b, and between types 2b and 2c. The MGB probe assays were carried out in a 25-µl reaction containing 10 µl of template or standard DNA (both in duplicates), 12.5 µl of IQ™ Supermix (Bio-Rad Laboratories Srl, Milan, Italy), 900 nM of primers and 200 nM of probes. The thermal protocol was done as follows: activation of iTaq DNA polymerase at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 30 s and primer annealing-extension at 60 °C for 1 min. All reactions were conducted in an i-Cycler IQ™ Real-Time Detection System (Bio-Rad Laboratories Srl) and the data were analysed with the appropriate software (Version 3.0). Specificity, sequence and position of real-time PCR primers and MGB probes are shown in Table 1.

2.3. MGB probe assays for discrimination between vaccine and field strains of CPV-2

Discrimination between vaccine and field strains of CPV-2 was carried out using additional MGB probe assays [17,18]. The type 2/variants assay is able to differentiate CPV-2 (vaccine) from the field variants; the SAH/field assay also discriminates between CPV-2b field strains and vaccine strain SAH contained in vaccines Duramune® DAPPI + LC (Fort Dodge Animal Health) and Procyon® Dog DA2PPi/CvL (Schering-Plough Animal Health, Welwyn Garden City, Hertfordshire, UK). The 39/field assay discriminates between CPV-2b field strains and vaccine strain CPV-39 contained in vaccine Virbagen® Puppy 2b (Virbac Tierarzneimittel GmbH, Bad Oldesloe, Germany). Reactions were carried out following the same protocols used for detection and characterisation of CPV-2 and the oligonucleotides reported in Table 1.

2.4. Screening of faecal samples for other canine pathogens

Molecular methods were used for detection of other viral pathogens of dogs, including mammalian reoviruses (MRV) [19,20], rotaviruses [21], caliciviruses [22,23], canine adenoviruses [24], canine distemper virus (CDV) [25], canine herpesvirus [26] and canine coronavirus (CCoV) [27,28]. Specimens that tested positive for CPV vaccine strains and negative for viral pathogens were examined for bacterial and parasitic pathogens by standardised methods. For bacterial screening the faecal samples were plated onto MacConkey’s agar (Oxoid S.p.A., Garbagnate Milanese, Italy), whereas detection of the most common enteric parasites was achieved using zinc sulphate flotation. The Ziehl Nielsen staining was also performed for detection of Cryptosporidium spp.

3. Results

Results of the diagnostic tests carried out on the 29 post-vaccinal faecal samples from diarrhoeic dogs are reported in Table 2. CPV field strains (virulent virus) were identified alone in 15 samples—12 were type 2a and 3 were type 2c
| Table 1                                                                 |
|------------------------------------------------------------------------|
| **Sequence, position and specificiy of the oligonucleotides used in the** |
| **study**                                                              |
| **Assay** | **Primer/probe** | **Sequence 5′–3′** | **Polarity** | **Specificity** | **Position** | **Amplicon size** |
| **Type 2/variants MGB probe assay**                                      | CPV2/v-For          | GCAGTTAACGGAAACATGGCTTTAG | +          | All types     | 3057–3081d; 772–796e | 68 bp |
|                                                        | CPV2/v-Rev          | TCAACCAATGACCAAGGTTTACAA  | –          | Type 2 (old type) | 3100–3124d; 815–839e | 85 bp |
|                                                        | CPV2-Pb             | FAM-TGTGCAATGATATCAT-MGB  | +          | Field strains | 979–714e | 85 bp |
|                                                        | CPVs-Pb             | VIC-TTGTGCAATGATATCAT-MGB | +          | Field strains | 979–714e | 85 bp |
| **SAH/field assay**                                                     | SAH/f-For           | CAACAAGATAAAAAGACGTTGTAACCT | +          | All types     | 3711–3738d; 1426–1453f | 85 bp |
|                                                        | SAH/f-Rev           | CAACCTCAAGCTGGTCTACTAAGT  | –          | Strain SAH    | 1454–1471f | 85 bp |
|                                                        | SAH-Pb              | VIC-AAATGGGAAAAACAAACT-MGB | +          | Field strains | 1454–1471f | 85 bp |
|                                                        | CPVf1-Pb            | FAM-AAATGGGAATACAAACT-MGB  | +          | Field strains | 1454–1471f | 85 bp |
| **39/field assay**                                                     | CPV39/f-For         | GCATTGGGCTTACCACATTTCATA  | +          | All types     | 3636–3660d; 1351–1375f | 95 bp |
|                                                        | CPV39/f-Rev         | CCACGTCTTTTACCTTGTGAACCTCTATA | –          | Strain CPV-39 | 1379–1395f | 95 bp |
|                                                        | CPV39-Pb            | VIC-CCTTGCTCAATCTGAA-MGB   | +          | Field strains | 1380–1395f | 95 bp |
|                                                        | CPVf2-Pb            | FAM-CCTTGCTCAAGCTGAA-MGB   | +          | Field strains | 1380–1395f | 95 bp |
| **Type 2a/2b assay**                                                   | CPVa/b-For          | AGGAAGATATCCAGAAGAGATTGGA | +          | All types     | 1719–1744f; 1285–1295f | 93 bp |
|                                                        | CPVa/b-Rev          | CCAATGATCTTGTTGTTAAGATACA | –          | Type 2a       | 1765–1783f | 93 bp |
|                                                        | CPVa-Pb             | VIC-CCTTGCTAACAATGATA-MGB  | +          | Type 2b       | 1765–1783f | 93 bp |
|                                                        | CPVb1-Pb            | FAM-CCTTGCTGATAGATGATA-MGB | +          | Type 2b       | 1765–1783f | 93 bp |
| **Type 2b/2c assay**                                                   | CPVb/c-For          | GAAGATATCCAGAAGAGATTGATTCA | +          | All types     | 1721–1748f; 1285–1295f | 150 bp |
|                                                        | CPVb/c-Rev          | ATGCAGTAAAGGACCATAAGTATTTA | –          | Type 2b       | 1823–1870f; 1257–1304f | 150 bp |
|                                                        | CPVb2-Pb            | FAM-CCTTGTAAGCAGTGATA-MGB  | +          | Type 2b       | 1768–1785f | 150 bp |
|                                                        | CPVb-Pb             | VIC-CCTTGTAACAGAAGATA-MGB   | +          | Type 2c       | 1202–1219f | 150 bp |

*a* [17].

*b* [18].

*c* [11].

*d* Oligonucleotide positions are referred to the sequence of CPV-2 (old type) strain CPV-b (accession no. M38245).

*e* Oligonucleotide positions are referred to the sequence of CPV-2a strain CPV-15 (accession no. M24003).

*f* Oligonucleotide positions are referred to the sequences of CPV-2b strain CPV-39 (accession no. M74849).

*g* Oligonucleotide positions are referred to the sequences of CPV-2c strain 56/00 (accession no. AY380577).
Table 2

Results of the diagnostic tests carried out on faecal samples of dogs displaying diarrhoea after CPV vaccination

| Protocol no. | Origin       | Vaccine          | Company  | D.p.v. | CPV vaccine strain | CPV field strain | Other pathogens |
|------------|-------------|------------------|----------|--------|-------------------|------------------|-----------------|
| 120/04     | Hungary     | Vanguard 7a      | Pfizer   | 3      | ND                | 2a               | ND              |
| 306/04     | Italy, Apulia | Vanguard 7a     | Pfizer   | 2      | ND                | 2a               | ND              |
| 326/04     | Italy, Apulia | ?              | ?        | 4      | 2                | 2a               | ND              |
| 49/05-C    | Italy, Piemonte | Nobivac PUPPY CPa | Intervet | 5      | ND                | 2a               | ND              |
| 112/05     | Italy, Lazio | Tetradog-CHPLa  | Merial   | 7      | ND                | 2c               | ND              |
| 121/05-A   | Poland      | Duramune DA2LP + Pv a | Fort Dodge | 2      | ND                | 2a               | CCoV I, II     |
| 121/05-B   | Poland      | Duramune DA2LP + Pv a | Fort Dodge | 2      | ND                | 2a               | CCoV I, II     |
| 121/05-C   | Poland      | Duramune DA2LP + Pv a | Fort Dodge | 2      | ND                | 2a               | CCoV I, II     |
| 128/05     | Hungary     | Nobivac PUPPY CPa | Intervet | 2      | ND                | 2a               | MRV             |
| 159/05-C   | Italy, E. Romagna | Nobivac PUPPY CPa | Intervet | 7      | ND                | 2a               | ND              |
| 160/05     | Italy, Apulia | Canigen CEPPi/La | Virbac   | 4      | 2                | ND               | CCoV I, II     |
| 176/05-A   | Italy, Piemonte | Nobivac PUPPY CPa | Intervet | 4      | 2                | ND               | CCoV I         |
| 202/05     | Italy, Lombardia | Primodoga       | Merial   | 3      | 2                | 2a               | ND              |
| 206/05     | Italy, Apulia | ?              | ?        | 3      | ND                | 2a               | CDV, MRV        |
| 220/05     | Italy, Veneto | Nobivac CEPPi a | Intervet | 3      | 2                | ND               | CCoV I, II     |
| 280/05     | Italy, Apulia | Duramune DA2LP + LC a | Fort Dodge | 3      | ND                | 2a               | CCoV II        |
| 327/05     | Italy, Campania | Vanguard 7a    | Pfizer   | 2      | ND                | 2c               | ND              |
| 338/05-2   | Italy, Lombardia | Duramune DA2LP + LC a | Fort Dodge | 6      | 2b SAH            | ND               | ND              |
| 338/05-4   | Italy, Lombardia | Duramune DA2LP + LC a | Fort Dodge | 7      | 2b SAH            | ND               | ND              |
| 343/05-1   | Italy, Piemonte | Nobivac PARVO-ca | Intervet | 4      | ND                | 2a               | ND              |
| 343/05-8   | Italy, Piemonte | Nobivac PARVO-ca | Intervet | 7      | 2                | ND               | ND              |
| 343/05-9   | Italy, Piemonte | Nobivac PARVO-ca | Intervet | 4      | ND                | 2a               | ND              |
| 97/06-3    | United Kingdom | ?             | ?        | ?      | 2                | ND               | CCoV I          |
| 174/06     | Hungary     | Nobivacb PUPPY CPa | Intervet | 6      | 2                | ND               | CCoV II         |
| 254/06-11  | Italy, Apulia | Primodoga       | Merial   | 7      | 2                | ND               | Isospora canis |
| 254/06-12  | Italy, Apulia | Primodoga       | Merial   | 7      | 2                | ND               | Isospora canis |
| 269/06     | Italy, Apulia | Tetradog-CHPLa  | Merial   | 3      | ND                | 2c               | CCoV II         |
| 291/06     | Italy, Piemonte | Nobivac PUPPY CPa | Intervet | 3      | 2                | 2a               | ND              |

D.p.v., days after vaccination in which the onset of diarrhoea was observed; ND, not detected; ?, unknown.
a Type 2-based vaccines.
b Type 2b-based vaccines.

Viruses. Thirteen samples were from dogs vaccinated with CPV-2, one sample was from a dog vaccinated with CPV-2b and one sample from a dog vaccinated with an unknown formulation. A CPV-2 vaccine strain and a CPV-2a field strain were detected simultaneously in the other three samples; two were from dogs administered a type-2-based vaccine and one from a dog given an unknown vaccine. Eleven samples, including seven and three samples collected from dogs vaccinated with CPV-2 and CPV-2b, respectively, and one sample from a dog administered an unknown vaccine, were found to contain only the vaccine virus. Other canine pathogens were detected in 8 of 11 samples, including CCoV type I, CCoV type II and Isospora canis. The remaining three samples contained only vaccine strains: CPV-2 (one sample); CPV-2b strain SAH (two samples). There was no evidence of other viral, bacterial or parasitic pathogens. In the two samples positive for CPV-2a (128/05; 206/05), the co-presence of MRVs strains was found that gave a signal only in the nested PCR assay. The MRVs could not be characterised by type-specific RT-PCR assays [20]. One sample (206/05) was found positive for CDV by a real-time RT-PCR assay [25]. In order to rule out the vaccine origin of the CDV strain, partial sequences of the haemagglutinin gene were obtained by RT-PCR amplification and subsequent sequence analysis showed that it clustered with field strains of the European lineage [29].

Eleven out of the 16 post-vaccinal faecal samples collected from healthy dogs were found to contain the vaccine virus. Eight samples were from dogs vaccinated with CPV-2 and three samples were from dogs administered a CPV-2b vaccine (data not shown).

4. Discussion

The onset of clinical signs similar to those of canine parvovirosis is a frequent finding in veterinary practice. Often pups become infected with field strains of CPV-2 shortly before or after vaccination; however, diarrhoea may be a consequence of other viral or bacterial infections, parasitosis or poor management. Nevertheless, many veterinary practitioners and dog owners erroneously believe that enteric illness subsequent to the administration of a CPV vaccine results from reversion to virulence of the modified live vaccine (MLV) virus.

Previously, there were few opportunities to address this issue since vaccinated dogs may shed the MLV vaccine virus
in their faeces alone, or concurrently with a virulent field strain. In both instances, conventional diagnostic tests could not provide definitive results. In fact, virus isolation from faecal samples in cell cultures is poorly sensitive [8], especially in mixed infections; furthermore, it may allow the isolation of only the most adapted (vaccine) or most abundant (field) strain. Unless sequencing of several clones is carried out, PCR may selectively, or more efficiently, amplify either of the viruses, so that the other one remains undetected by subsequent sequence analysis. Moreover, the use of conventional tests, e.g., haemagglutination, immunochromatographic test, virus isolation, or PCR, may misdiagnose diarrhoeic dogs as CPV-infected due to the presence of a MLV strain in the faeces. On the other hand, the novel MGB probe assays represent an effective tool for rapid discrimination between vaccine and field strains of CPV-2 since they are able to detect vaccine and field strains that occur simultaneously in the faeces of vaccinated dogs, even when low titres of vaccine strains are shed [17,18].

Reversion of virulence of CPV MLV has been frequently postulated, but never demonstrated, as the attenuation of virulence has proved to be highly stable [30–32]. In the present study, analysis of faecal specimens collected from dogs that developed gastroenteritis shortly after CPV vaccination confirmed that the diarrhoeas observed were most commonly related to infection with field strains of CPV-2.

When a vaccine strain was detected in a diarrhoeic faecal sample, it was generally present together with a CPV-2 field strain, or with other pathogens commonly associated with enteritis in dogs. Only three vaccinated dogs lacking field strain, or with other pathogens commonly associated with enteritis in dogs, however, were detected in the samples tested. Another noteworthy finding was the detection of a CDV strain of European lineage in the faeces of one dog that had been infected simultaneously with CPV-2a and MRV. Since the vaccine formulation (monovalent or multivalent) administered to this dog was not known, the vaccine origin of this strain could not be ascertained. However, sequence analysis of the haemagglutinin gene revealed that the CDV strain had diverged from the Onderstepoort, Rockborn or Snyder Hill CDV vaccine strains, and it clustered with field strains of the European lineage, as reported by Martella et al. [29].

In conclusion, since reversion to virulence of the CPV MLV vaccine strains has not been shown to occur, the present study demonstrates that most cases of gastroenteritis subsequent to vaccination are related to infection with CPV field strains shortly before or after the vaccine administration.

Acknowledgements

We thank Donato Narcisi, Carlo Armenise and Arturo Gentile for their excellent technical assistance. This work was supported by grants from University of Bari, Italy: project ex 60% 2006, “Caratterizzazione delle varianti di campo del parvovirus del cane mediante real-time PCR con sonde minor groove binding (MGB)”.

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