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Evaluation of an in-clinic assay for the diagnosis of canine parvovirus

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ABSTRACT

The results of a study designed to evaluate the performance of an in-clinic test for the detection of canine parvovirus (CPV) are reported. A total of 150 faecal samples collected from dogs with acute diarrhoea were tested using the in-clinic test, a haemagglutination assay (HA) and a real-time PCR assay for CPV detection, quantification and characterisation.

CPV was detected in 66, 73, and 101 faecal samples by in-clinic, HA and PCR testing, respectively. The relative sensitivity and specificity of the in-clinic test were 86.3% and 96.1%, respectively, when the test was compared to HA, and 65.3% and 100%, respectively, when compared to real-time PCR. The sample distribution according to the virus type was CPV-2a, $n = 44$; CPV-2b, $n = 11$; CPV-2c, $n = 44$, CPV-2, $n = 2$, as determined by minor groove binder probe assays and/or sequence analysis. The percentage of positive in-clinic tests was 70.5% for CPV-2a, 72.7% for CPV-2b and 75.0% for CPV-2c ($P > 0.05$). Using real-time PCR as the reference standard for CPV detection, the in-clinic test was more specific than HA and had comparable sensitivity to HA, demonstrating detection rates similar to those previously observed for other rapid in-clinic tests. The in-clinic test was also able to detect all CPV types at equivalent rates.

Introduction

Canine parvovirus (CPV) is one of the most common causes of acute gastroenteritis in young dogs. The virus is included in a unique species, *Feline panleukopenia virus*, of the genus *Parvovirus* (family *Parvoviridae*), along with feline parvovirus and other parvoviruses of domestic and wild carnivores (Decaro and Buonavoglia, 2012). The original strain CPV-2 emerged in late 1970s and was replaced by three antigenic variants, CPV-2a, CPV-2b and CPV-2c, that arose consecutively. These variants are variously distributed in the world and CPV-2c strains are becoming the predominant variants in many countries (Decaro et al., 2006b, 2007, 2011; Hong et al., 2007; Calderón et al., 2011).

Several methods are currently available for the diagnosis of CPV infection, including in-clinic assays (Schmitz et al., 2009; Decaro et al., 2010), haemagglutination (HA), virus isolation (Desario et al., 2005), and molecular methods (Decaro et al., 2005b). DNA-based assays are the most sensitive, representing the reference standard. These assays are also able to characterise the viral strains (Decaro et al., 2005a, 2006b) and discriminate between vaccine and field viruses (Decaro et al., 2006a, 2006c). In-clinic and other laboratory tests are less sensitive than molecular assays, due to the sequestration of CPV particles by gut antibodies (Desario et al., 2005). Concerns have been expressed regarding the ability of in-clinic tests to detect the new variant CPV-2c with the same accuracy as CPV-2a/2b (Kapil et al., 2007). However, a recent study showed that an in-clinic assay detected CPV-2c with the same accuracy as other variants (Decaro et al., 2010).

The aim of this study was to compare the performance of an in-clinic test for the detection of CPV (Witness Parvo test, Synbiotics Corporation) with HA and a real-time PCR assay.

Materials and methods

Clinical specimens

A total of 150 faecal samples were collected from dogs with acute diarrhoea and other clinical signs suggestive of CPV (leukopenia, vomiting). Most of the dogs were recruited from animal shelters, breeding kennels, and academic or private veterinary clinics in Italy ($n = 137$), but a few specimens also came from Spain ($n = 3$) and Albania ($n = 10$), so that the test validation was not restricted to CPV strains of Italian origin. Dogs were aged from 1 month to 14 years (mean ± standard deviation [SD], 7.5 ± 19.2 months). Sixty-eight dogs (45.3%) were mixed-breed and the purebred animals (82/150, 54.7%) included Labradors ($n = 11$), Beagles ($n = 9$), Bernese mountain dogs ($n = 8$), German shepherds ($n = 8$), Yorkshire terriers

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(n = 8), Chihuahuas (n = 7), American cocker spaniels (n = 6), Boxers (n = 6), Dachshunds (n = 5) and dogs of other breeds (n = 14). Twelve specimens (8%) were from dogs that had been recently vaccinated against CPV (<10 days before sample collection).

Each sample was submitted masked for in-clinic, haemagglutination and real-time PCR testing.

In-clinic test

Faecal material was collected on a test kit swab following the manufacturer's instructions. The extraction buffer/conjugate was dispensed into the sample tube via the kit swab. The sample swab was then inserted into the tube containing the liquid and placed in a vortex. The extracted faecal material/conjugate liquid was transferred to the in-clinic test device using the swab pipette for the test kit as per manufacturer's instructions.

Haemagglutination (HA) assay

Twofold dilutions of the supernatant of each faecal homogenate were made in phosphate buffered saline (pH 7.2) starting from a 1:2 dilution (Desario et al., 2005). Tests were carried out in 96-well V-plates (50 μL of sample dilution per well); equal amounts of a suspension containing 0.8% pig erythrocytes and 1% fetal calf serum (FCS) were added to each dilution. Results were read after 4 h at 4 °C and expressed as the reciprocal of the highest sample dilutions able to produce HA.

Real-time PCR assays for CPV detection, quantification and characterisation

Specimens were homogenized (10% w/v) in Dulbecco's modified Eagle's medium and subsequently clarified by centrifuging at 2500 *g* for 10 min. Viral DNA was extracted from the supernatants of faecal homogenates by boiling for 10 min and chilling on ice. To reduce residual inhibitors of DNA polymerase activity to ineffective concentrations, the DNA extract was diluted 1:10 in distilled water (Decaro et al., 2005b, 2006b). CPV DNA was detected by real-time PCR using a conventional TaqMan probe (Decaro et al., 2005b), whereas virus characterisation was obtained by a panel of minor groove binder (MGB) probe assays able to predict the viral type (Decaro et al., 2005a, 2006b) and to discriminate between vaccine and field strains of CPV (Decaro et al., 2006a, 2006c).

Sequence analysis

The CPV strains that were not characterised by the MGB probe assays were submitted for sequence analysis of the VP2 protein gene, as previously described (Decaro et al., 2008, 2009). Briefly, sequence reactions were carried out using BigDye 3.1 Ready reaction mix (Applied Biosystems) according to the manufacturer's instructions. The sequenced products were separated with a 3130 Genetic Analyzer (Applied Biosystems). Sequences were imported and assembled with Bionumerics 5.0 software (Applied Maths). The VP2 sequences obtained were compared to those of reference strains retrieved from the GenBank database.

Data analysis

Data were analysed using a web-based software program (R version 2.8.1).[1] An experiment critical value alpha of 0.05 was used to set statistical significance. Sensitivity and specificity were calculated as follows (Altman and Bland, 1994):

$$\text{Sensitivity} = \text{true positives} \div (\text{true positives} + \text{false negatives})$$

$$\text{Specificity} = \text{true negatives} \div (\text{true negatives} + \text{false positives})$$

The proportion of positive samples by viral load (DNA copies/mg of faeces) and CPV type was evaluated using a Pearson's Chi-squared test. Student's t test was used to compare the average viral loads associated with the CPV types.

Results

Performance of the in-clinic test

The results of the three tests used for CPV detection in the faeces of dogs with clinical signs of parvovirus are summarised in Fig. 1. The in-clinic test was able to detect CPV antigen in 66/150 (44%) of analysed samples. Using HA, 73 (48.7%) samples tested positive and 77 (51.3%) were negative. The highest detection rates (101/150, 67.3%) were obtained using real-time PCR. The relative sensitivity and specificity of the in-clinic test were 86.3% (95% confidence interval [CI] 76.6–92.4%) and 96.1% (CI 89.2–98.7%), respectively, when the test was compared to HA (Fig. 1a), and 65.3% (CI 55.7–73.4%) and 100% (CI 92.7–100%), respectively, when compared to real-time PCR (Fig. 1b).

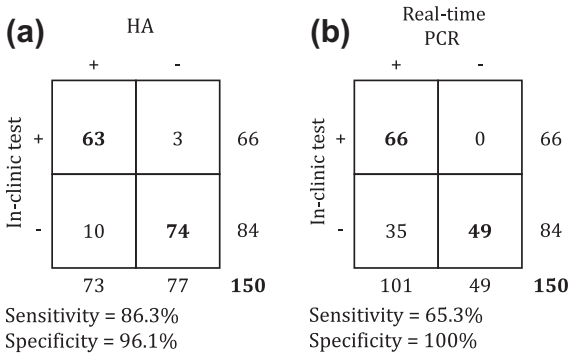

Fig. 1. Comparison of the in-clinic test (Witness Parvo test, Synbiotics Corporation) with haemagglutination (a) and real-time PCR (b). Numbers indicate the samples positive (+) or negative (−) for canine parvovirus (CPV). Results obtained by comparing different techniques are bolded. The overall agreement, sensitivity and specificity of the in-clinic testing compared with the other assays were calculated and are indicated.

CPV type distribution, detection rates using the in-clinic test and viral loads

Using MGB probe assays, the CPV strains detected by real-time PCR were characterised as CPV-2a (n = 44), CPV-2b (n = 11), CPV-2c (n = 36) or CPV-2 (n = 2), and eight strains were not typed. When sequence analysis of the VP2 protein gene was performed, the uncharacterised strains were confirmed as CPV-2c, since they displayed the non-coding mutation A4061G in the CPV-2c-probe binding region (Decaro et al., 2009, 2013). Thus, combining the results of MGB probe assays and sequence analysis, the sample distribution according to virus type was CPV-2a, n = 44 (43.6%); CPV-2b, n = 11 (10.9%); CPV-2c, n = 44 (43.6%); CPV-2, n = 2 (1.9%).

Both CPV-2 strains were detected in dogs that had been recently given CPV-2 based vaccines. None of the detected CPV-2b strains were characterised as vaccine virus. Additionally, 10 dogs that had been vaccinated against CPV less than 10 days before sample collection tested negative using in-clinic and HA testing. However, 5/10 dogs tested CPV-positive using real-time PCR, being infected with CPV-2c (n = 2), CPV-2a (n = 2), or CPV-2b (n = 1).

The in-clinic assay was able to detect 31/44 CPV-2a, 8/11 CPV-2b and 27/44 CPV-2c. Consequently, the percentage of positive in-clinic test results was 70.5%, 72.7% and 75.0% for CPV types 2a, 2b and 2c, respectively. Detection rates for the three CPV types were not different when compared statistically ($P > 0.05$). Neither of the samples containing the original CPV-2 strain tested positive using the in-clinic assay, which was expected on the basis of the low viral titres detected in these specimens.

CPV DNA titres calculated by the TaqMan assays ranged from 1.09×10^3 to 1.03×10^{10} DNA copies/mg faeces. The average viral loads in the clinical specimens were 1.11×10^9 (SD 1.86×10^9), 1.01×10^9 (SD 9.94×10^8), 1.39×10^9 (SD 2.77×10^9), and 2.51×10^3 (SD 2.00×10^3) for CPV types 2a, 2b, 2c, and 2, respectively. Viral load was not significantly different for CPV-2c type when compared to CPV-2a ($P > 0.05$) and CPV-2b ($P > 0.05$). The in-clinic test gave positive results for specimens containing more than 10^6 DNA copies/mg faeces (Fig. 2).

Comparison of in-clinic test results and viral loads showed that the minimal CPV titre required for a positive result was about 10^6 DNA copies/mg faeces. Specimens with viral loads higher than

[1] See: http://www.r-project.org/ (accessed 25 August 2013).