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Short communication

Diagnostic performance of a rapid in-clinic test for the detection of *Canine Parvovirus* under different storage conditions and vaccination status

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A B S T R A C T

Canine parvovirus (CPV) is one of the most common causes of acute haemorrhagic enteritis in young dogs, while clinical diagnosis is often indecisive. The aim of our study was to evaluate the diagnostic accuracy of an in-clinic rapid test in the detection of *CPV* infection in dogs. To this end, we compared the Rapid Diagnostic Kit of *Canine Parvovirus*, *Coronavirus* and *Rotavirus* antigen (Quicking®) to PCR, which is considered as the most reliable diagnostic method. A total of 78 duplicated faecal samples were collected from diarrhoeic dogs. Vaccination history within a month prior to the onset of diarrhoea was reported for 12 of the sampled dogs. The rapid diagnostic test was performed in 23 of the faecal samples directly, while the rest were placed into a sterile cotton tipped swab suitable for collection and transportation of viruses (Sigma Σ-VCM®) and stored at −20 °C. The sensitivity of the Quicking rapid diagnostic test compared to PCR in the total number of samples, in samples from non-vaccinated dogs and in samples tested directly after collection were 22.22% (95% CI: 13.27–33.57%), 26.67% (95% CI: 16.08–39.66%) and 76.47% (95% CI: 50.10–93.04%) respectively, while the specificity of the test was 100% in any case. In conclusion, negative results do not exclude parvoenteritis from the differential diagnosis, especially in dogs with early vaccination history, but a positive result almost certainly indicates *CPV* infection. An improved sensitivity may be expected when the test is performed immediately.

Canine parvovirus (*CPV*) of the family *Parvoviridae* is one of the main enteric pathogens in dogs, especially puppies between 4 and 12 weeks old. *CPV-2* is a small, non-enveloped icosahedral single-stranded DNA virus (Strassheim et al., 1994), related to other parvoviruses that infect carnivores, such as *Feline Panleukopenia Virus* (*FPLV*), *Mink Enteritis Virus* (*MEV*) and *Racoon Parvovirus* (*RPV*). *CPV* 2 has undergone mutations, resulting in recognition of subtypes 2a and 2b, while a new strain has also been detected in Italy (Martella et al., 2004). This variant (*CPV-2c*) now co-circulates with other *CPV* types in Vietnam (Nakamura et al., 2004), Spain (Decaro et al., 2006), Germany, France (Decaro et al., 2011), Portugal (João Vieira et al., 2008), USA (Gates et al., 2014; Hong et al., 2007), Brazil (Pinto et al., 2012) and Greece (Ntafis et al., 2010).

The gastroenteric-associated lymphoid tissues and intestinal crypts represent the target tissues for viral replication of *CPV*. This results in haemorrhagic diarrhoea, the most characteristic form of clinical disease. Its duration and severity strongly correlate to the titres of maternally derived antibodies at the time of infection. Virus is transmitted via the faecal-oral route through contact with faeces, soil or fomites that carry the virus. It is shed in the faeces of infected dogs within 4–5 days from exposure, throughout the period of clinical disease, and for up to ten days after recovery (Decaro et al., 2005b).

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Table 1
Results of the immunochromatographic test and PCR per group tested.

Samples	Immunochromatographic test		PCR	
	Positive	Negative	Positive	Negative
Total of samples	16	62	72	6
Vaccinated animals	0	12	12	0
Unvaccinated animals	16	50	60	6
Directly examined	13	10	17	6

Table 2
Sensitivity along with the negative likelihood ratio values for each separate group and comparison of proportions.

Samples	SE	95% CI	NLR	95% CI
Total of samples	22.22%[a]	13.27–33.57%	0.78	0.69–0.88
Vaccinated animals	0[b]	–	–	–
Unvaccinated animals	26.67%[a]	16.08–39.66%	0.73	0.63–0.85
Directly examined	76.47%[c]	50.1–93.04%	0.24	75.12–100%

[a,b,c] Figures with different superscripts differ significantly ($P < 0.005$).

Clinical diagnosis of enteritis induced by *CPV-2* is difficult and often indefinite. Therefore, it should be rapidly confirmed by reliable laboratory methods in order to begin therapeutic efforts as soon as possible. Rapid, in-clinic immunochromatographic assays are available for the diagnosis of *CPV* infection (Schmitz et al., 2009). Apart from that, faeces from clinically ill dogs can be tested using haemagglutination, virus isolation (Desario et al., 2005) and molecular methods (PCR or Real Time PCR) (Decaro et al., 2005a). However, in-clinic tests are still the most frequently used diagnostic tool in everyday veterinary practice, as the procedure is simple, inexpensive and timely.

The aim of the present study is to evaluate the diagnostic accuracy of Rapid Diagnostic Kit of *Canine Parvovirus*, *Coronavirus* and *Rotavirus* antigen (Quicking®) in the detection of *CPV* infection in dogs compared to PCR, which is considered as the most reliable diagnostic method. The diagnostic performance of this commercial rapid test was also assessed after examining samples under different storage conditions and samples collected from animals with different vaccination status.

A total number of 78 duplicated samples were collected from dogs with symptoms compatible with parvoenteritis, such as: lethargy, loss of appetite, fever, vomiting, haemorrhagic small-bowel diarrhoea, and dehydration. Specimens were collected following clinical examination and detailed recording of the medical and vaccination history of each animal. In 23 of the faecal samples, the Quicking Rapid Test was performed immediately after sample collection according to the manufacturer's instructions. The rest of the samples were placed into a sterile cotton tipped swab suitable for collection and transportation of viruses (Sigma Σ-VCM) and they were stored at −20 °C pending analysis. All samples were examined both with the Quicking Rapid Test and by PCR. The tests were conducted independently and the readers of PCR were blinded for the result of the other method.

The Quicking Rapid Test is a combined cassette used to differentially diagnose the presence of antigens from the three enteric viruses. The test is based on a sandwich lateral flow immunochromatographic assay. A visible T band in the corresponding testing window denotes the presence of any of the three pathogens in the sample. Regardless of the collection method, the wet swab was inserted in the included buffer tube and was stirred to ensure good sample extraction, as per manufacturer's instructions. Afterwards, three drops were placed in the sample holes of the cassette of the kit. The results were read within 5–10 min and were classified as positive or negative. No invalid results were observed.

To extract the viral DNA, the faecal specimens were homogenized in phosphate buffered saline (PBS) at a percentage of 10% w/v. After a brief centrifugation at high speed, 200 μL of the supernatant of each specimen were used for nucleic acid purification. The aliquots were incubated at 65 °C for 10 min to inactivate PCR inhibitors and then they were chilled on ice (Uwatoko et al., 1995). A commercial DNA Purification kit (Thermoscientific Genomic DNA Purification Kit) was used to complete extraction from the specimens according to the manufacturer's protocol.

Conventional PCR was performed using the primer pair Hfor/Hrev that amplifies a fragment of the capsid protein-encoding gene *CPV-2* according to Decaro et al. (2005a) with slight modifications. These primers yield a product of 630 base pairs. Each 50 μL reaction mixture contained PCR buffer 1× (KCl 50 mM, Tris–HCl 10 mM, pH 8.3), $MgCl_2$ 2 mM, 200 mM of each deoxynucleotide, 1 μM of each primer, 2 U of DNA Polymerase (Thermoscientific Maxima Hot Start *Taq* DNA polymerase) and 10 mL of template DNA. The thermal conditions of this protocol initially indicate an activation of Hot Start *Taq* DNA polymerase at 94 °C for10 min. Following this step, 40 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 1 min and polymerization at 72 °C for 1 min, and finally an extension at 72 °C for 10 min. Following PCR, electrophoresis was performed using 8 μL of the PCR products in a 2% Tris acetate–EDTA–agarose gel. Product sizes were determined using a 100 bp molecular weight ladder.

The sensitivity, specificity and negative likelihood ratio (NLR) as well as the significance of the differences between sensitivities obtained among groups were calculated using commercial software (Calc v. 12.3.0.0 – MedCalc Software, Ostend, Belgium). Also the Kappa statistic was estimated to determine the agreement between the two methods. Kappa value of 1 indicates absolute agreement, whereas a value of 0 indicates that agreement occurs due to chance agreement. In general, Kappa values higher than 0.6 indicate a good level of agreement. In this study, *k*-values were calculated using commercial software (Graph Pad Prism v.6-Graph Pad Inc., San Diego, CA).

The results of both methods used per group are analytically presented in Table 1. All samples were also negative for the other two pathogens of the rapid diagnostic test. The sensitivities and the NLR of the rapid diagnostic test in the total number of samples, in samples of non-vaccinated dogs and in samples tested directly after collection, are presented in Table 2. The specificity of the test was 100% in any case. (95% CI: 0.69–0.88%). The Kappa value between the methods in the total number of samples, in different vaccination statuses and under different collection methods are presented in Table 3.

Canine Parvovirus still represents a major cause of morbidity and mortality in puppies, despite widespread vaccination. A rapid and definitive diagnosis of *CPV-2* infection is crucial, especially in spaces overcrowded with dogs (kennels, shelters, veterinary hospitals) in order to isolate infected animals, start treatment and prevent further spread of the virus. Commercial in-clinic rapid tests are the only assays that allow a quick and low-cost diagnosis of *CPV* in faeces of dogs. According to recent studies, these test seem to also detect the most novel *CPV-2c* variant (Decaro et al., 2010; Markovich et al., 2012; Decaro et al., 2013).

Table 3
The *k*-value estimation between the in-clinic assay (Quicking®) and the established PCR method as well as the strength of agreement corresponding to each calculation for the three groups.

Samples	Agreement	
	k-Value	Strength of agreement
Total number of samples	0.028	Poor
Unvaccinated animals	0.038	Poor
Samples examined directly	0.203	Fair