Comparison of immunochromatographic rapid test with molecular method in diagnosis of canine parvovirus

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Abstract:

BACKGROUND: Canine parvovirus (CPV) infection is one of the most common causes of infectious gastroenteritis in dogs and is a highly contagious, often fatal disease. The original virus (CPV type 2) has had some mutations since its emergence and new variants (CPV-2a, 2b and 2c) have been reported from many countries all around the world. Early diagnosis and treatment can profoundly affect the disease outcome. **OBJECTIVES:** To compare the ability of Immunochromatographic (IC) test to detect CPV infection in 50 PCR positive samples (n=50) with regard to virus strains. **METHODS:** 50 rectal swabs (n=50) were prepared from suspicious dogs and subjected to PCR and IC test respectively. **RESULTS:** The sensitivity of IC test in PCR positive samples was 84% (42 out of 50 samples) and the positive predictive value of the test was 100%. Using PCR, CPV strains in our study were 2a (18/50, 36%) and 2b (32/50, 64%) with the predominance of 2b strain. IC test was also able to diagnose 15/18 (83.3%) of CPV-2a and 27/32 (84.3%) CPV-2b strain positive samples, which means IC test can detect CPV infections caused by both virus strains (2a and 2b), without significant difference. **CONCLUSIONS:** This study shows that IC test results are relatively reliable for diagnosing CPV infection in daily veterinary practice and the test is able to diagnose both CPV-2a and CPV-2b which are prevalent strains in Iran.

Introduction

Canine Parvovirus (CPV) was first reported in 1978 and soon after became a major cause of fatal gastroenteritis in young dogs (Kelly,1978; Appel et al., 1979; Burtonboy et al., 1979; Decaro et al., 2005a). The virus belongs to feline parvovirus subgroup and the genus *parvovirus* (Decaro et al., 2005a). Parvoviral enteritis is caused by canine parvovirus type 2 (CPV-2) which is a small, non-enveloped, single stranded DNA virus (Nakamura et al., 2004). Soon after the emergence of CPV-2, two new variants were reported and named CPV-2a and

CPV-2b and in 2001 the newest variant was reported and named CPV-2c (Parrish et al., 1985; Decaro et al., 2005b; Buonavoglia et al., 2001). The virus is transmitted via oral-fecal route and infects rapidly proliferating cells such as intestinal epithelium, lymphatic tissues and bone marrow (Prittie, 2004). Cytopathic effects of the virus lead to cellular death and occurence of severe gastroenteritis and immuno-suppression. Severe vomiting and diarrhea (which is the result of villus atrophy) lead to loss of huge amounts of body fluids and proteins which can cause hypovulemic shock, septicemia, endotoxemia and death (Goddard et al., 2010). The mortality rate of the

disease was reported to be variable in previous studies and is about 25% to 35% (Brinke et al., 2010). Early diagnosis and treatment of the disease can influence the mortality rate. In patients who have not been treated, mortality rate reaches 91%. However, with early diagnosis and aggressive treatment 80-95% of patients can survive (Prittie, 2004). There are several tests to diagnose parvoviral infections such as immunochromatography (IC), hemagglutination (HA), virus isolation (VI) and molecular methods (PCR). The most commonly used test in daily veterinary practice is immunochormatographic test due to its rapid result, user-friendly format, and relatively low cost in comparison with other tests. On the other hand, molecular tests, in spite of their sensitivity and specificity are still time consuming, labourintensive, and need the expertise of specialists (Pereira et al., 2000). Since early diagnosis and treatment of the disease can profoundly affect the outcome, we decided to compare the most practical (IC test) and most sensitive tests (PCR) with each other to find out the reliability of IC test results in daily veterinary practice. Furthermore, for the first time in Iran, the ability of IC test to detect parvoviral infections based on the virus strain was studied.

Materials and Methods

Clinical specimens: Samples were obtained from 50 (n=50) young dogs (less than 2 years old) with clinical signs of acute gastroenteritis referred to Small Animal Teaching Hospital of University of Tehran.

Fecal specimens were collected for PCR and immunochromatographic antigen test kit using sterile cotton swabs soaked in sterile water. All fecal samples were subjected to PCR test. Samples with positive PCR results were subjected to canine fecal antigen test kit in order to compare the efficacy of these two tests for detecting parvoviral infection with each other. A commercial vaccine (Biocan,Czech Republic) was also used as control positive.

PCR Reaction: Genomic DNA was extracted from fecal specimens and commercial vaccine using AccuPrep stool DNA extraction kit (Bioneer Co, Korea) based on manufacturer's instructions.

Three different pairs of primers were used in this study. Primer pairs P2 and Pb which detect CPV2 and CPV-b respectively, were designed by Pereira (Pereira et al., 2000). Third primer pair (Pab) which detect CPV-2a and CPV-2b was designed by Senda (Senda et al., 1995). These primers were selected from different regions of VP2 gene which codes virus capsid protein. The sequences of primer pairs were as follows: Pb sense: 5'_CTTTAACCTTCCTGTA-ACAG_3', Pb antisense: 5'_CATAGTTAAATTGGT TATCTAC_ 3', P2 sense: 5'_GAAGAGTGGTTGTA AATAATA_ 3', P2 antisense: 5'_CCTATATCAC CAAAGTTAGT AG_3' and Pab sense: 5'_GAAGA GTGGTTGTAAATAATT_3', Pab antisense: 5'_C CTATATAACCAAAGTTAGTAC_3'. Primer pairs Pb amplify the region between nucleotides 4043 and 4470, and the length of PCR product is 427 bp. Primer pairs P2 amplify the region between nucleotides 3025 and 3706, and the length of PCR product is 681 bp. Primer pairs Pab amplify the same region as primer pairs Pb and the difference between these 2 primers is restricted to one base at the 3´ end of each primer. These three pairs of primers were provided by Cinnagen Co, Iran. These 3 pairs of primers can differentiate parvovirus strains from each other. Original CPV-2 virus could be recognized by primer pair P2, while CPV-2a could be recognized only by primer pair Pab and CPV-2b with primer pairs Pab and Pb (Shoorijeh et al., 2011).

PCR amplification was performed using eppendorf thermocycler with an initial denaturation step at 95°C for 5min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1min, and extension at 72°C for 1min. A final extension step was performed at 72°C for 5min (La Torre et al., 2009).

PCR products were electrophoresed on 1.2% agarose gel and then were stained with ethidium bromide and visualized by Gel Doc.

Immunochromatographic reaction (Canine fecal antigen test kit).

Immunochromatographic assays, also known as lateral flow test, were first developed in 1956 (Singer et al., 1956). These kinds of tests are user friendly, rapid and relatively inexpensive. Figure 1 shows a schematic view of an IC test.

Free antigen (parvovirus) that had been captured by rectal swab was diluted with a buffer and then 4 drops of supernatant was fluid placed on the sample pad. Conjugated pad contained dyed microspheres

which attach to the antigen and migrated through test line where the specific antibodies were located to capture the antigen-dyed microsphere complex. If the test was positive, then a colored line would appear in the test line. Species-specific antibody which was able to capture the reagent particles was located on the control line and confirmed that the test was complete.

All PCR positive specimens (50 samples) were subjected to parvovirus fecal antigen test kit (SensPERT, VetAll Laboratories, Korea) based on manufacturer's instructions. All IC tests were blinded to the strains of parvovirus. The number of positive results was used to compare the performance of these two tests.

Results

Virus strains: Three pairs of primers which can differentiate parvovirus strains from each other were used in this study. PCR results showed that 18 out of 50 (36%) samples were positive for CPV-2a and 32 (64%) were positive for CPV-2b. Vaccine strain was also positive for CPV-2 which is the original type (Figure 2).

Comparison of PCR and Immunochromatographic test: PCR test result was positive for all 50 fecal samples with the previously mentioned primer pairs. 42 of 50 (84%) PCR positive fecal samples had positive results with canine fecal IC test kit too. The sensitivity of IC test in PCR positive specimens was 84% and the positive predictive value of IC test was 100%. The IC test was able to detect 15/18 (83.3%) of CPV-2a strain and 27/32 (84.3%) CPV-2b ones (Table 1).

Discussion

Parvoviral enteritis is one of the most contagious and fatal causes for gastroenteritis in puppies. There are several diagnostic methods to detect the disease including IC tests, slide agglutination test, immunoassay procedures, molecular methods and serologic tests. Parvoviruses can cause agglutination of porcine erythrocytes. This test has been used to detect CPV infection but it is slightly more sensitive than IC test and poorly specific due to the presence of iso-aglutinins in fecal samples or other hemagglutinating viruses, and it also needs constant access to fresh
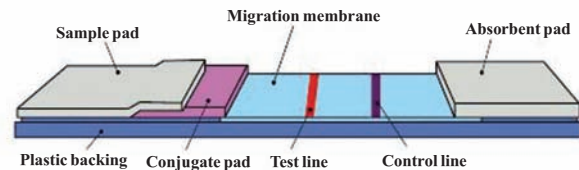

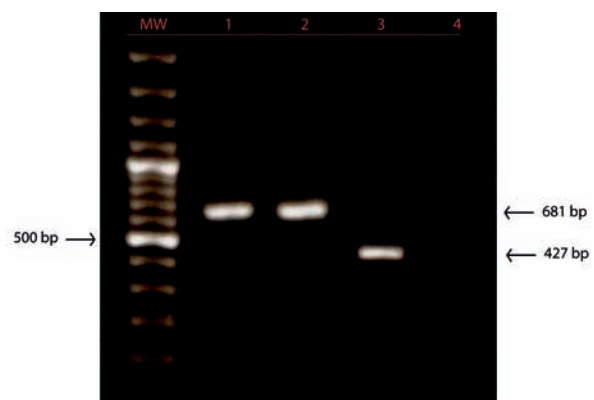Figure 1. Schematic view of rapid Immunochromatographic test.

Figure 2. PCR-product of different genomic DNA of infected dogs and control positive samples using different primers as mentioned in materials and methods. MW is molecular weight. Lane 1 is PCR-product 681bp using primer pairs of Pab, Lane 2 is PCR-product 681bp using primer pairs of P2 (positive control, commercial vaccine), Lane 3 is PCR-product 427bp using primer pairs of pb, Lane 4 is negative control.

erythrocyte (Desario et al., 2005). It is possible to detect CPV using tissue culture systems and immunochemical procedures if performed in the early stages of disease because most virions will be coated and cleared by antibodies in later stages. CPV causes intranuclear inclusions in most tissues, which are possible to detect by microscope. These procedures are time consuming and need special equipment which is not available in many veterinary practices. Serologic tests have been used to diagnose CPV but these tests have some disadvantages. Most dogs are vaccinated against CPV or have been exposed to the virus before. Therefore, it is not of clinical relevance to detect CPV specific antibody in patient's serum. However, presence of high titre of hemagglutination

Table 1. Percentage of positive results with IC test in PCR positive fecal samples based on virus strain.

Virus strain	PCR test result	IC test results in PCR positive samples
CPV-2a	18.50 (36%)	15.18 (83.3%)
CPV-2b	32.50 (64%)	27.32 (84.3%)

Table 2. Number of parvovirus strains reported in various countries (From Greene, Infectious diseases of dog and cat, 4th edition, 2012).

Continent and country	CPV-2a	CPV-2b	CPV-2c
EUROPE			
Italy	56	6	62
Portugal	0	16	15
Spain	3	1	9
France	0	9	7
United Kingdom	52	97	1
Belgium	17	0	9
Germany	13	18	21
Greece	81	1	2
Switzerland	1	0	0
Czech Republic	1	1	0
Romania	2	0	0
Hungary	27	0	0
Bulgaria	1	0	0
Slovenia	1	0	0
AFRICA			
Tunisia	15	21	14
NORTH AMERICA			
United states	1	36	30
SOUTH AMERICA			
Uruguay	1	0	24
Argentina	9	4	14
ASIA			
India	23	4	0
Taiwan	2	34	0
Korea	119	7	0
Japan	4	21	0
China	27	5	0
Thailand	19	7	0
OCEANIA			
Australia	41	1	0

inhibition (HI) in an unvaccinated dog's serum with at least 3 days of clinical illness is diagnostic for CPV infection. Another serologic procedure is to compare two serum samples (one immediately and the other one after 10 to 14 days post infection) to detect a rise in antibody titers (Greene, 2012), which is time consuming and not suitable for diagnosing an acute disease like CPV infection. PCR test has been proven to be highly sensitive in diagnosis of parvoviral infections but this method needs relatively expensive equipment which is not available in most daily veterinary practices. The most common method for diagnosing parvoviral infections in practice is immunochromatographic based canine fecal antigen test kit which is rapid and can be done with minimal costs. In this study an attempt was made to compare the result obtained with canine parvovirus fecal antigen test kit with PCR positive fecal samples.

Based on the result of this study, 32 of 50 (64%) samples were positive for CPV-2b and this strain was the predominant strain in our sample population. There are also several studies from many countries which report the distribution pattern of parvovirus strains (Table 2). In South American countries, Europe and North Africa all three strains (2a,2b and 2c) have been reported. 2b and 2c were predominant strains in North American countries. However, in Asia and isolated islands which have import limitations (e.g, Britain, Japan, Australia) CPV-2a and 2b were predominant strains among dog populations (Greene, 2012). There is no report regarding the distribution pattern of parvovirus strains from Iran's neighbours. According to one study conducted in southern Iran, CPV-2b was the predominant strain in parvovirus infected dogs (89% of CPV positive samples), which is compatible with our result (Shoorijeh et al., 2011). However, another study shows these differential primers are not able to differentiate new strains (CPV-2c) from older ones (CPV-2a and 2b) and sequence analysis is needed in order to determine the virus strain (La Torre et al., 2009). Since sequence analysis was not performed in our study, we cannot exclude the presence of CPV-2c in Iran and this issue should be studied in future.

IC test was able to diagnose 42 of 50 positive samples which shows 84% sensitivity in PCR positive samples. The positive predictive value of the test was 100%. Detection rates of CPV infection based on virus strain were 83.3% and 84.3% for CPV-2a and CPV-2b, respectively. Based on statistical analysis (using χ^2 square test), the rate of detection was not statistically different between virus strains ($p<0.05$). A previous study which was done with a different commercial fecal antigen test kit (SNAP Canine Antigen Test, IDEXX laboratories), showed 80.4% and 78% detection rates for CPV-2a and CPV-2b, respectively (Decaro et al., 2009). The quantity of viral particles can affect the IC test results which is one disadvantage of this test. It is proved that samples with viral load more than 109 DNA copies/mg faeces were generally detected by in-house assay (Decaro et al., 2009). As the virus load in our samples was not measured, it should be evaluated later.

Our study showed immunochromatography based office-use test kits are sensitive in comparison with molecular methods to diagnose CPV infections

and the result of these tests are reliable in daily practice. This study also shows IC test is able to detect both parvovirus strains (2a and 2b), which are prevalent in Iran.

Acknowledgments

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مقایسه روش سریع ایمونوکروماتوگرافی با روش مولکولی در تشخیص پارووویروس سگ‌ها

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چکیده

زمینه مطالعه: پارووویروس سگ‌سانان یکی از شایع‌ترین علل بروز گاستروآنتریت عفونی در سگ‌ها است. این بیماری بسیار مسری و غالباً کشنده در اثر ابتلا به پارووویروس سگ‌سانان تیپ ۲ (CPV-۲) ایجاد می‌شود. ویروس عامل بیماری از زمان پیدایش تاکنون چندین بار دچار جهش و تغییر شده است و سویه های جدیدی از آن (شامل سویه‌های ۲a,۲b,۲c) در بسیاری از کشورهای جهان گزارش شده است. تشخیص سریع و درمان به موقع بیماری نقشی تاثیر گذار در بهبودی بیمار دارد. **هدف:** مقایسه توانایی تشخیص بیماری با استفاده از تست سریع ایمونوکروماتوگرافی در ۵۰ نمونه PCR مثبت (n=۵۰) بر اساس سویه ویروس. **روش کار:** برای انجام این تحقیق ۵۰ سوآب (n=۵۰) آغشته به مدفوع ناحیه رکتوم سگ های مشکوک به بیماری، ابتدا با استفاده از تست PCR وسپس با استفاده از تست سریع ایمونوکروماتوگرافی مورد آزمایش قرار گرفتند. **نتایج:** بر اساس نتایج تجزیه و تحلیل آماری، حساسیت تست سریع ایمونوکروماتوگرافی در نمونه‌های PCR مثبت ۸۴٪ (۴۲ نمونه از ۵۰ نمونه) و ارزش اخباری مثبت این تست ۱۰۰٪ محاسبه شد. با توجه به نتایج تست PCR، ۱۸ نمونه از ۵۰ نمونه (۳۶٪) سویه ۲a و ۳۲ نمونه از ۵۰ نمونه (۶۴٪) سویه ۲b بودند و سویه ۲b از شیوع بیشتری برخوردار بود. تست سریع ایمونوکروماتوگرافی در ۱۵ نمونه از ۱۸ نمونه (۸۳/۳٪) سویه ۲a و ۲۷ نمونه از ۳۲ نمونه (۸۴/۳٪) سویه ۲b قادر به تشخیص آلودگی بود. **نتیجه‌گیری نهایی:** این تحقیق نشان می‌دهد که نتایج حاصل از تست سریع ایمونوکروماتوگرافی تا حد زیادی قابل اطمینان بوده و تست مناسبی جهت تشخیص بیماری در امور درمانی می‌باشد. همچنین این تست قادر به تشخیص هر دو سویه پارووویروس شایع در ایران (۲a,۲b) می‌باشد.

واژه‌های کلیدی: پارووویروس سگ‌ها، روش‌های تشخیصی، واکنش زنجیره‌ای پلیمراز، ایمونوکروماتوگرافی

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