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Original article

Simple test kit for rapid detection of the presence of canine parvovirus antigen from dog's feces

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ABSTRACT

Canine parvovirus (CPV) is one of the most important viral infection diseases infects all the dogs worldwide. The management and preventive of this disease is such important target. SensPERT® CPV antigens tests are available to detect parvovirus among dogs, and presumptively to predict protective status. The aim of this study was to determine the diagnostic accuracy of the test to detect the virus by using fecal samples collected from dogs housed at experimental laboratory animals, with or without gastroenteritis sings from Riyadh, Saudi Arabia. The dogs were divided into two groups on the basis of age, vaccination and clinical signs using SensPERT test kits. Prevalence to CPV infection in these dogs was significantly higher in hemorrhagic diarrheic dogs less than 3 months (84%; 21 positive of 25) by means of immunochromatography assay, followed by puppies without signs (40%; 4 positive of 10).Nevertheless, there were no infections in adult vaccinated dogs (100%; 15/15). SensPERT One-rapid test kit was demonstrated to be a useful, simple and very quick diagnostic tool for determining CPV status in dogs populations and it will giving a good idea regarding dogs vaccination importance to prevent and protects other animals against infection.

1. Introduction

Canine parvovirus infection (CPV) is one of the most frequent causes of death in the dogs worldwide; it was first reported in the late 1970's. The virus belongs to a member of the genus parvovirus of the family parvoviridae and contains negative single stranded DNA (Appel et al., 1979; Decaro et al., 2005). A previous observation have demonstrated that CPV is highly contagious dog's virus disease, because it is transmitted from dog to others via oral-fecal route as a result of contact with contaminated feces or vomits from infected dogs and showing hemorrhagic enteritis including vomiting, bloody diarrhea, leukopenia, nausea and often death in dogs particularly in young puppies (McCaw and Hoskins, 2006). Moreover, the infectious virus is relatively resistant to the most disinfectants because it is non enveloped virus survives for at least one year in the dogs' environment (Saknimit et al., 1988; Greene and Schultz, 2006). On the other hand, the mortality rate between infected dogs reaches 91%. However, according to the speed of diagnosis and aggressive treatment of the parvovirus in dog populations, the mortality rates may varies and survival rates may approach 80-95% (Prittie, 2004). Whoever, the treatment usually involves extensive hospitalization, and treatment of infected dog consists of supportive care to correct dehydration and electrolyte abnormalities, fluid losses, control vomiting and prevent secondary infections. Thus, treatment ideally requires Intravenous (IV) fluids, antiemetic and antibiotic drugs (Savigny and Macintire, 2010). Although, despite widespread vaccination, CPV remain the major causes of highly rates of morbidity and mortality, particularly in unvaccinated dogs, in all pet shops, and in animal shelters where several animals are affected simultaneously (Goddard and Leisewitz, 2010; Steneroden, 2011). Therefore, the disease is best prevented by ensuring that vaccinations are carried out appropriately, supplemented by quickly diagnostic tools to detect the virus.

In daily veterinary practices, a confident technique; safe, economical, and rapid diagnostic tests with minimum training of the personnel, will encourage timely use of the test, based on the quick detect of the diseases and help manage outbreaks in canine populations. Therefore, there are several tests and methods to diagnose CPV infection including immunochromatography (IC) tests, virus isolation (VI), hemagglutination (HA) and molecular methods (PCR) (Sakulwira et al., 2003). The most commonly diagnostic tool had been used recently is IC tests due to its rapid result, user-friendly format, and relatively low cost in comparison with other tests. On the other hand, other tests in spite of their sensitivity and specificity are still time consuming, labor-intensive, and need the expertise of specialists (Pereira et al., 2000). To date, in Saudi Arabia, the data available on CPV infection are limited, in addition due to the fact that early diagnosis and treatment of the disease can profoundly affect the outcome. Thus, the aim of the present study is to evaluate the prevalence of CPV by using one step SensPERT® in fecal specimens derived from Beagle dogs in experimental animal facility. Also, we decided to find out the reliability of this test results. Furthermore, to our knowledge this is the first time in Saudi Arabia; the ability of IC test to detect CPV infections was studied.

2. Materials and methods

2.1. Animals

Fifty Beagle dogs (Canis familiaris) of both sexes (18 males and 32 females), were used for experimental purpose-bred, ages were varies between 45 days up to 2 years- old, in the current study. All dogs were housed indoors at the Experimental laboratory affiliated to the Department of Animal Research Center, College of Dentistry, King Saud University, Riyadh, Kingdom of Saudi Arabia. The housing conditions for all animals in this study, were in accordance with the Guide for the Care and Use of Laboratory Animals1, and were managed following the Animal Welfare Regulations prescriptions2. Dogs were housed, throughout the study, in stainless steel cages in each unit. Flooring in the facility was a concrete floor inside and outside with a removable wood shaves to absorbable dropping inside the cages. Dogs caging in the facility were washed in a rack washer that used acid and neutralizer cycles with a water rinse. In addition, Animals were fed once per day with a standard

commercial dogs food at the recommended rates, with supplemented food treats offered once a week, while tap water is available in stainless steels drinking troughs during the day.

2.2. Selectivity

All the fecal samples that had been collected during this study were collected from only one facility where the clinical signs were observed. Thus, the dog's specimen had been selected from healthy adult dogs ages were (10-24 m), and young puppies' ages between 45 days up to 2 month old with and/or without a cute onset of gastroenteritis clinical signs. Most animals were suspected to have CPV infection regarding the signs occurs and only evaluated and the test was performed on site, the first two puppies had a history of complete loss of appetite, severe bloody diarrhea (with a very bad smells), nausea, vomiting and pain or discomfort. Even though, because the CPV infection status of these fecal samples collected for the tests were not clear yet and/or confirmed the diagnosis of the infection need time. Wherefore, the other beagle groups were housed in a separate unit based on conventional preventive steps.

2.3. Clinical samples

Fecal samples from Beagle dogs (*n* =50) with/without the signs of severe gastroenteritis in only One facility located in experimental laboratory animals rather than the other facilities remained in the same area have been selected only for this study. All the fecal samples were collected directly from the beagle dogs inside their separated cages, for detecting the presence of CPV antigens by one step rapid tests, to find out the causative etiologic agent of these clinical signs. These samples were prepared in accordance with the sample preparation procedure. Each sample test continues for at least 6-8 min. Four hours after the start of the experiment, pups were classified as infected and/or healthy, and all animals with positive results were removed outside the colony.

2.4. Sample preparation

To detect the CPV antigens from the beagle dogs specimen. First, each dog had been managed very well manually before the samples taken, and almost about 05/10g of the fresh fecal samples were collected with sterilized swap directly from the dogs, whoever, the fecal samples were kept at 23o C in a sterile tube specific for fecal and subjected to short-term storage (30 min) until sample analysis. After initial feces collection, we used with each sample the specific sterilized swap to take (~30 mg) of the feces from the tube into the test sample tube which containing (1 ml) of the diluents' (buffer solution), then swirl the buffer with the swap and waiting until the feces particles sink to the bottom, the sample was mixed almost for five times, then drop about 4-5 drops of the mixed specimen into each well of the test kit, within 5-10 minutes we considered the test results.

2.5. Chromatographic system

For the detection of canine parvovirus antigens from fecal specimen based on the IC test technique, we used this test which developed by VetAll Laboratories, Kyunggi-Do, Korea. SensPERT® CPV (Fig. 1). It is one of the tests used for primary screening. It contains two molecules containing double bonds in their structure, being possible their detection the antigen of the CPV in canine feces. The test results can appear on Control (C) and Test (T) lines, where the principles of IC test are applied, and a purple band should always appear regardless of the virus antigens. SensPERT test kit structure containing: a test, 1 ml diluents (buffer), as well as, disposable dropper, and swab for sample collection. The test kits should be stored at temperature between (2~30o C). However, expiration date had been checked before used.

2.6. Method validation

Control line (C) in CPV test kit should always appear regardless of the presence of the antigen of CPV in the fecal sample. If (C) line only appears the test should consider negative (n-CPV). If this line does not appear, the test should be considered invalid and should be tested again with another kit. Test line (T) in test kit should be appearing with (C) line and the test should be considered positive (p-CPV), if (T) line was only appearing the test should be considered invalid and should be tested again with another kit. In case both (T) line and (C) lines do not appear, the test should be considered invalid and should be tested again with another kit. One-step rapid test of CPV antigens results between 5~10 min, otherwise consider the test results as invalid after 10 min (Fig. 2).

2.7. Experimental design

Fifty Beagle dogs (n=50) were obtained from experimental animal facility, with different ages were selected to the SensPERT® CPV Ag test. The dogs were allocated in three separate facilities for two groups regarding the ages of the dogs and the clinical signs: Group A (Facility1), fifteen of the beagle dogs (n=15), aged between 10 months and 2 years without any signs, that were allocated to a vaccinated group and were housed together in 3 different cages. Group B1 (Facility 2), ten of beagle puppies (n=10), aged almost 45 days without clinical sigs except only one puppy with loss of appetite and vomition, this puppies were allocated to a non vaccinated group and were housed individually in isolation cages for the duration of the test period. Group B (Facility3), twenty-five of beagle puppies (n=25), aged between 50 days to 8 weeks. Almost 4 puppies in this facility with severe clinical sigs of gastroenteritis disease, this puppies were allocated to a non vaccinated group and were housed individually in isolation cages for the duration of the test period

During March to December 2009, blood smear samples and also ticks on the bodies of 300 horse and ass were randomly collected from Meyaneh area.

The blood smears were stained by Gimsa and the ticks were examines by proper diagnostic keys (Tenter et al., 1986). T.Test, One Way Anovaand also non-parametric tests Mann-Whitney and Kruskal-Wallis were used for analyzing statistical association between the data results.

3. Results

A total of 50 fecal samples were collected from 50 beagle dogs, including 15 specimens from adult vaccinated healthy dogs; 10 specimens from unvaccinated puppies looking healthy and 25 specimens from unvaccinated puppies suspected to infect by CPV due to the clinical symptoms. Results obtained using the SensPERT® CPV test kits, as described previously. The method was initially demonstrated by determining CPV Antigen concentrations in fecal sample, after the administration of 30 mg/feces to buffer solution container. As shown in (Table. 1) the majority of CPV infection seen in the dogs could be classified as one of the following:

Vaccinated dogs; 15 out of 15 (100% n-CPV) all fecal samples examined via SensPERT® test kit, in this group were negative characterized by a purple band appear regardless of the virus antigens in the control line only. Whoever, all dogs in group A were healthy and survived.

Unvaccinated dogs with no signs; (05/10) 50% fecal samples examined were negative to test; in (04/10) 40% pups (only one with symptoms) were all positive for CPV antigen test. Whoever, the remaining sample (01/10) 10% was invalid characterized by waiting for more than 12 minutes and both (T) line and (C) line do not appear, the same sample retested again with another kit and the test result was negative. Only 60% of pups showing negative results first were survive and 40% others were died.

Unvaccinated dogs with severe gastroenteritis; in particular, 4 cases were showing severe signs of the disease including bloody diarrhea, vomiting and completely loss of appetite during last night, when fecal samples examined 20 puppies out of 25 (80%) in this group were positive for CPV; in (4/25) 16% puppies specimen were negative and the remaining sample (01/25) 4% was invalid, when repeat the test it was positive for parvovirus, whoever, all puppies in this facility were died.

By virus isolation, the presence of parvovirus antigen was detected in the specimen of unvaccinated puppies' in the second and third facilities which confirmed the diagnosis of the infection. The highest detection of CPV antigen was 84% in facility-3 with gastroenteritis signs (21/25 p-CPV) were positive, and the remaining 4 puppies from this group were showing negative results at first were all died during 2-7 days from the onset of clinical signs. Also, in facility-2 (40%) of pups were positive and died, whoever, the remaining 60% (06/10 n-CPV) were still survived and vaccinated to prevent the infection, these dogs (n=6) retested again after one week and they were already negative. The adult vaccinated dogs in facility-1 were all survived. Our results showed that only 5 (14.3%) out of 35 animals among those unvaccinated puppies in group B with clinical signs were positive for CPV. Whoever, test kit detecting almost 20/35 (57.2%) of fecal samples were positive for CPV antigens with sub clinical signs and they looking healthy (n=3 from F2) and (n=17 from F3) respectively, whereas, the total number of mortality between unvaccinated puppies were 29/35 (82.9%) (Table 2).

[1]To reduce risk for infection outbreaks between young puppies in (Group B), we segregate the dogs into two facilities 2 and 3 respectively, and both were assigned to a non vaccinated group.

Table 1
Prevalence of Canine parvovirus infections in Beagle dogs based of ages and clinical signs (Vomiting, bloody diarrhea) in Riyadh, Saudi Arabia.

Parameters	Animals (dogs) group		
	Vaccinated group	Unvaccinated group	
	F.1	With signs F.2	without signs F.3
***Outcomes of testing using SensPERT CPV Ag.**	-(15/15) =100%	-(05/10) = 50% +(04/10) = 40% !(01/10) = 10%	+(20/25) = 80% -(04/25) = 16% !(01/25) = 04%
Ages and total No. for the dogs	10- 24 months 15- dogs	45.Days 10 pups	50.Days-2m 25 pups
Clinical signs & Duration of sickness	No clinical signs	Almost No clinical signs accept one puppy with loss of appetite.	Vomiting, bloody diarrhea, and loss of appetite/ 2-4 days
Prognosis	survived	6 dogs survive others died	All dogs died
Breed	Beagle	Beagle	Beagle

*Outcomes of rapid test: (- Negative); (+ Positive) and (! Invalid) test results, F= Facility.

Table 2
SensPERT® test kit results of the fecal samples of dogs in different facilities for detection of canine parvovirus (CPV).

No		Facility 1	Facility 2	Facility 3
1	**Age**			
	50 d-2 m	00	00	25
	45 days	00	10	00
	10-24 m	15	00	00
2	**CPV detection**			
	CPV positive (%)	00 (00)	04 (40)	21 (84)
	CPV negative (%)	15 (100)	06 (60)	04 (16)
3	**Clinical signs**			
	Dogs with signs	00	01	04
	Suspected dogs	00	03	06
	healthy dog	15	06	15
4	**Mortality rates (%)**	00 (00)	04 (40)	25 (100)
5	**Total**	**15**	**10**	**25**

4. Discussion

CPV is represented by clinical signs such as hemorrhagic enteritis signs including vomiting and *mucoid or* bloody diarrhea, and the infection by this virus is generally restricted to young pups (6–12) week-old, more than adult dogs (Decaro et al., 2004; Kapil et al., 2007; Zhao et al., 2013). In comparison between dog's facilities in our study, the most detection of CPV infection and mortality rates were found to be highly occurrence 100% in unvaccinated puppies in F.3 (n=25/25) and ages were between 50 days to 2 months-old. In addition, a lower detection was found in F.2 with 40% (n=4/10) and ages were 45 days, both in the same group B, even though, there was no detect of the virus 100% between adults dogs in F.1 (n=15) in group A. It seems to be because of the different levels of MDA supposed to protect the young puppies at initially ages at the moment of infection and/or due to incubation period of the disease in infected animals which varies between 3–7 days, with mortality rates

less than 1% in adult dogs, and more than 70% in pups (Desario et al., 2005a; Decaro and Buonavoglia, 2012). Although, an Adult dogs seems to be more resistant to the infection due to the initial vaccination regimen which helped develop canine immunity or previous infections at earlier ages (Decaro and Buonavoglia, 2012). Moreover, the test results in vaccinated adult dogs showing 100% negative detection of CPV antigens. Whoever, some studies confirmed the persistence of immunity conferred by commercial multivalent modified-live virus vaccine (Duramune® Max 5 Fort Dodge Animal Health) by the continued detection of antibody and protective immunity for viral diseases including CPV in vaccinated dogs (Gill et al., 2004). In this study, all adult dogs in group A were 100% negative of CPV antigens. Since prophylaxis of this viral infection relies mainly on extensive vaccination. Thus, we regularly used the modified live virus vaccine to immunize the dogs. These findings were confirmed by studies conducted in dogs found that, this type of vaccine is considered one of the highly effective vaccines, and able to protect dogs against CPV disease as well as infection, and almost completely safe with very rarely post vaccination reaction (Spibey et al., 2008; Decaro and Buonavoglia, 2012). Taking into account the concern that the failure of CPV vaccines may occur in dogs regularly vaccinated (Siedek et al., 2011). Furthermore, the subclinical and inapparent infections of CPV, mainly in young dogs with intermediate MDA titers and in adult dogs are frequently detected (Decaro et al., 2005).

In the present study, it was interesting that SensPERT test kit, successfully detected CPV positive antigens from almost 71% of puppies' specimens in group B. Nevertheless, none of our (3/10) 30% in F2 fecal samples, and (17/25) 68% in F3 respectively, were from diarrhic dogs, but latter all these pups were died because of parvo. Whoever, the infected dogs with CPV were able to shed the virus for approximately 8–12 days post-infection (Pollock, 1982); whereas, virus shedding amount may varies very early or late in the course of the disease (Decaro and Buonavoglia, 2012). That could mean that most CPV infections in this group were shedding in feces from infected dogs to other animals, or they were in the early of incubation period during the test. Moreover, there was no evidence of CPV among the healthy dogs in our study. However, these findings were based on limited population area studied. Therefore, further studies are needed to evaluate these findings in other parts of Saudi.

Several other pathogens including parvovirus may cause vomiting and diarrhea in dogs. Whoever, only clinical diagnosis of CPV is not definitive and almost the main characteristic signs of the disease are common to other enteric diseases (de Castro et al., 2007). Therefore, a rapid diagnosis of CPV tests is especially such important in dogs population to confirmed the disease, whoever, to prevent infections of susceptible contact dogs and/or to isolate the infected dogs. Thus, routinely feces from diarrheic infected dogs are based on different techniques to detect CPV such as IC tests, screened using ELISA, PCR and VI or HA tests, but all these techniques are affected by relatively problems related to tests (Uwatoko et al., 1995; Esfandiari and Klingeborn, 2000). The molecular assays, such as PCR diagnostic techniques, were demonstrated to be more sensitive, and more specific in detecting and quantification of CPV within few hours (Firoozjaii et al., 2011). The main disadvantage of this tool, however, it requires expensive equipment, reagents and specialized personnel; thus, their use as tests for the veterinary practice is not possible always due to highly costs and needs (Desario et al., 2005). On the other hand, VI testing is sensitive, but it is too labor-intensive and time-consuming for routine diagnostic testing (Mochizuki et al., 1993). Nevertheless, VI requires specialized personnel and laboratories with highly efficiency regarding the cell culture. Moreover, detection of the viral antigens by this method is time-consuming; it requires a long incubation period at least to 5 days (Desario et al., 2005). Although, HA can be carried out only by using fresh good quality erythrocytes collected from other animal species which are expensive. In addition to the difficulties occurs in the management the housing system of donor animals and/or in the quantities required for the test (Desario et al., 2005). Furthermore, CPV strains lacking HA activity have been reported (Cavalli et al., 2001). Although, the identification of the disease is challenging when detecting CPV infection by using IC tools such as SensPERT test. However, some results were not interpretable because of the insufficient visibility of potential bands, and IC good results require a large amount of CPV antigen to produce a clearly visible band in the test results which is very common (Desario et al., 2005). Nevertheless, because of inconsistent results of several population and comparative studies, it was concluded that none of the methods was 100% reliable and therefore combined testing methods (Esfandiari and Klingeborn, 2000). Recently, a study from Iran designed to compare the ability of IC test in diagnosis of CPV infection with molecular method of PCR in fecal samples found that, the sensitivity of IC test in PCR positive samples was 84% (42 out of 50) (Jamshidi et al., 2013). Whoever, in our study we found that, SensPERT® true test result was:100% in adults, whoever, 84% and 40%, respectively in young puppies, which playing a big rule in protect 5 other puppies facility in the same area of CPV risks due to rapid detecting of infection in these previous facilities in group B. Thus, in concrete situations when diagnosing potentially affected dogs, additive costs, time

and identification of CPV is challenging for personnel with little experience have to be considered in case of combined testing methods. The IC test such as SensPERT® test remained the most common rapid field diagnostic technique used in clinical practice, because of the simplicity of the test, it is instant (real time), sensitive, and generic for all the CPV types, and can be performed by veterinarian as well by all dogs' owners and could be able often to do it in home in minutes (Esfandiari and Klingeborn, 2000), possibly further development of SensPERT test may increase its usefulness in practice compare to expensive ones not common to all veterinary clinics.

Finally, since its emergence in 1978, new different types 2c of CPV have been reported from many countries all around the world (Kapil et al., 2007; Buonavoglia et al., 2001); with respect to classical type 2a and 2b CPVs respectively (Truyen, 2006). In fact, PCR on feces and/or DNA sequence analysis of CPV is the only way to determine the types of this virus. Nevertheless, there is no real advantage afforded by determining which strain has infected dogs since the IC testing kits such as SensPERT® is often inconclusive, and could be able to detecting all CPVs strains that infected dogs including the newly emerged (Decaro et al., 2010). Therefore, in this study differentiation between the strains is not performed with respect to importance of the continually monitor for the emergence of CPV different types, because this types of test are not available in animal experimental units in Saudi Arabia, in addition, almost clinical signs, available vaccine, IC test accuracy, management strategies for all CPVs types are work and similar, and we are looking only for the ability and rapidity of this test to detects virus from dogs feces, whoever, survival rate depends on how quickly CPV is diagnosed.

5. Conclusion

The present work concluded that practical methods such as this easy one step rapid test may help to providing insights into the perfect mechanisms driving the prevention and controlling the virus. Furthermore, the assay performance results indicate that this test kit is precise and accurate enough for the routine determination of CPV infection in canine populations. Thus, if this test becomes a permanently and/or essential protocol in pets clinics during the routine tests examination, facilities, and dogs kennels to detect the disease, it will help to elucidate whether this parvovirus spreading among the dogs population worldwide. Therefore, continued epidemiological surveillance of the distribution and types of the canine viral diseases is such important issue.

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