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Prevalence of *Canine parvovirus* (CPV) in diarrheic dogs referred to veterinary hospital in Ahvaz

Mosallanejad[1]*, B., Ghorbanpoor Najafabad[2], M., Avizeh[2], R. Ronagh, A.[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

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ABSTRACT

This study was performed to determine the prevalence of *Canine parvovirus* (CPV) in dogs referred to Veterinary Hospital of Ahvaz, Khouzestan province, Iran. Fecal samples were collected from 78 diarrheic dogs between 2005 and 2007. The dogs were divided into two age groups (< 6 months and > 6 months), four different breeds (Terriers, Germanshepherds, Doberman pinschers and Mixed) and another two groups on the basis of clinical signs (hemorrhagic and non-hemorrhagic diarrhea) using Fischer's exact test. Prevalence to CPV (2a or 2b) antigens in these dogs was 16.7% (13 of 78) by means of immunochromatography assay (IC) indicating that this virus is present in ecosystem. The infection had more prevalence in dogs less than 6 months (21.95%; 9 of 41) and in breeds of Terriers (26.31%; 5 of 19) and German Shepherds (21%; 4 of 19), but there were no significant differences between different sexes, age groups and breeds ($P>0.05$). Nevertheless, infection was significantly higher in hemorrhagic diarrheic dogs (41.38%; 12 of 29) ($p<0.05$). CBC showed that most infected dogs had leucopenia, lymphopenia and neutropenia.

Keywords: *Canine parvovirus*, Immunochromatography, Dog, Diarrhea, Ahvaz

INTRODUCTION

Canine parvovirus type 2 (CPV-2) is one of the most common viruses responsible for acute hemorrhagic enteritis and diarrhea in susceptible dogs (Carmichael 1994). CPVs are small, non enveloped, DNA-containing viruses that require rapidly dividing cells for replication. CPV-2 induced disease is observed mainly in 6–12 week-old pups; whereas, younger dogs are generally protected from CPV-2 infection by maternally-derived immunity (Decaro *et al* 2004). CPV-2 spreads from infected to susceptible dogs by the fecal-oral route (Carmichael 1994, Decaro *et al* 2005). Virus is shed for approximately 8–12 days post-infection. The close antigenic and genetic relationships, exist between CPV-2, feline panleukopenia virus (FPV) and mink enteritis virus (MEV) (Truyen *et al* 1994), suggest that CPV-2 may have originated by genetic mutation in a wildlife host receptive to one of the FPV-like parvovirus that infect carnivores (Truyen *et al* 2000). Mechanisms of the evolution of most viruses are unclear; however, it has been speculated that the emergence of CPV-2 antigenic

*Author for correspondence. E-mail:bmosallanejad@scu.ac.ir

variants may have been induced by a process of immune selection by low levels of antibodies (Strassheim *et al* 1994). The enteric form of the disease is a serious problem in breeding kennels, or where vaccination is not widely practiced. (Greenwood *et al* 1996). Rapid diagnosis of CPV infection is especially important in kennels and shelters in order to isolate infected dogs and prevent secondary infections of susceptible contact animals. Since a clinical diagnosis is not definitive, several laboratory techniques have been developed to detect CPV in the feces of infected dogs such as PCR, HA, ELISA, IFA and MAbs. Though these tests are more sensitive, specific and more reproducible, but they can be carried out only in specialized laboratories. On the other hand, the immunochromatography assay is most common rapid field diagnostic method used in clinical practice because the test procedure is simple and rapid, and can be performed by veterinarians as well by owners. The Anigen Rapid CPV Ag Test Kit were found to be highly specific (98.8%) and sensitive (100%) (Esfandiari & Klingeborn 2000). In our study, IC was used to investigate the presence of *canine parvovirus* antigens in companion dogs in Ahvaz. The present study is the first report on prevalence of CPV in dogs in southwest of Iran.

MATERIALS AND METHODS

Sample collection and preparation. Fecal samples were collected from 78 diarrheic referred dogs to Veterinary Hospital, in the Ahvaz area, capital city of the Khoozestan province, which is situated in the southwest of Iran (2005– 2007). Dogs were divided into two age group (< 6 months and > 6 months), four different breeds (Terriers, Germanshepherds, Doberman pinschers and Mixed) and based of clinical signs into two group (hemorrhagic and non-hemorrhagic diarrhea). It was for determination whether these factors were associated with *parvovirus infection*, using Fischer's exact test. They were young dogs from 2 to 11 months. Also, blood samples were collected in all of dogs to characterize CBC. Finally treatment of affected dogs was directed at correcting the life-threatening dehydration that accompanied the diarrhea with intravenous fluids, medicines that relax intestinal spasms and broad-spectrum antibiotics to prevent secondary bacterial infection.

Immunochromatography assay. It was carried out with a commercial rapid CPV Ag test kit (Manufactured by Anigen, Animal genetics, Inc., Korea), following the manufacturer's instructions. This kit is a chromatographic immunoassay for the qualitative detection of *parvovirus* antigen in canine feces. It can detect the pathogenic CPV subtypes CPV2a or CPV2b.

Procedure of the test. First we provided swab the feces from the stool and then it was inputted and mixed the swab into the assay diluents. Latter we left the bottle for a short time and finally add four (4) drops of supernatant from extracted sample into the sample hole. As the test begins to work, we will see purple color move across the result window in the center of the test device. Interpret test results will be at 5-10 minutes.

Interpretation of the test. A color band will appear in the left section of the result window to show that the test is working properly. This band is the control band. The right section of the result window indicates the test results. If another color band appears in the right section of the result window, this band is the test band. The presence of only one band within the result window indicates a negative result (Figure 1). The presence of two color bands (T and C) within the result window, no matter which band appears first, indicates a positive result (Figure 2). If the purple color band is not visible within the result window after performing the test, the result is considered invalid. (Esfandiari & Klingeborn 2000).

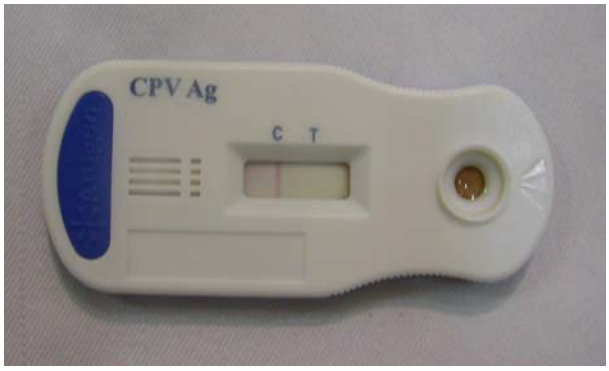

Figure 1. Negative sample of rapid CPV Ag test.

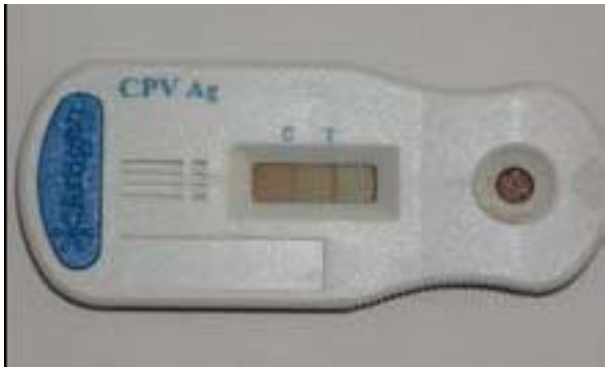

Figure 2. Positive sample of rapid CPV Ag test.

Statistical analysis. Test results and potential association with age, sex, breed, CBC, vaccination situation and clinical signs were analyzed using SPSS 10.0 for windows and by use of Fishers exact test and chi square analysis. Differences were considered significant at $p \leq 0.05$.

RESULTS

A total 78 diarrheic dogs, 13 (16.7%) were affected to CPV antigens (2a or 2b). The infection had more prevalence in dogs less than 6 months (21.95%; 9 of 41) in similar to dogs above 6 months (10.81%; 4 of 37) and in breeds of Terriers (26.31%; 5 of 19) and German Shepherds (21%; 4 of 19), but there were no significant differences between different sexes, age groups and breeds ($P>0.05$). The prevalence of infection in both Doberman pinschers and mixed breeds (Doberman pinschers + German shepherd) was 10% (2 of 20). Number of dogs that had hemorrhagic and non-hemorrhagic diarrhea was 29 and 49 respectively. Clinical signs in ill dogs were as depression, vomiting, hemorrhagic diarrhea, profound dehydration, fever. Infection was significantly higher in hemorrhagic diarrheic dogs (41.38%; 12 of 29) in similar to non-hemorrhagic diarrheic dogs (2%; 1 of 49) ($p<0.05$). In relation to sex, 17.8% (8 of 45) of male and 15.15% (5 of 33) female dogs carried the *parvovirus* infection. CBC in all of dogs that affected to *parvovirus* infection was as leucopenia, lymphopenia and neutropenia. Vaccination was not accomplished in any ill dogs, but in healthy dogs, 29.23% (19 of 65) were vaccinated against parvovirus. The difference between two groups was significant ($P<0.05$). In our study, the death rate from infection was 7.7% (1 of 13), in spite of treatment. The dead dog was

Doberman pinscher. Results are summarized in tables 1 and 2.

Table 1. Prevalence of *Canine parvovirus* infections in dogs of different age and breeds in Ahvaz district, Iran by immunochromatography assay, 2005-2007.

Clinical signs		CBC	
Hemorrhagic diarrhea	non-hemorrhagic diarrhea	leucopenia (WBC < 6000 cells/µl)	Normal (WBC = 6000 – 17000 cells/µl)
12 of 29 (41.38%)*	1 of 49 (2%)	13 of 27 (48.15%)*	0 of 51 (0%)
Total of infected dogs = 13			

DISCUSSION

The present study that is the first report on prevalence of CPV in dogs in Ahvaz using immunochromatography revealed that 16.7% of dogs were affected with CPV (2a or 2b). The

results indicated that not all cases of hemorrhagic diarrhea are caused by parvovirus and many sick puppies are misdiagnosed as having 'parvo.' Yet the only way to know if a dog has *parvovirus* is through a positive diagnostic test.

Table 2. Prevalence of *Canine parvovirus* infections in affected dogs based of clinical signs **(**hemorrhagic and non-hemorrhagic diarrhea) and CBC in Ahvaz district, Iran, 2005-2007.

Breeds	Number of studied dogs	Number of infected dogs (%)	Age(< 6 months)	Age(> 6 months)	Dead dogs
Terrier	19	5 (26.31%)	4	1	-
German shepherd	19	4 (21%)	4	1	-
Doberman pinscher	20	2 (10%)	1	1	1
Mixed	20	2 (10%)	-	1	-
Total	78	13	9	4	1

The most common form of *parvovirus* infection, in our survey, was an acute inflammation of the small intestine (enteric form), because all of ill dogs had hemorrhagic diarrhea. This was characterized by depression, vomiting, hemorrhagic diarrhea, profound dehydration and fever. Our survey showed that vaccination is very important and lack of it, can be as a risk factor for *parvovirus* infection, because all of our ill dogs hadn't any history of vaccination. Our manifestation has showed that hemorrhagic diarrhea is common in dogs of Iran, because vaccination is not complete in population of dogs. Knowledge of the prevalence of CPV in diarrheic dogs in southwest Iran is important because CPV is highly contagious and there are many stray and rural dogs that are not vaccinated. These animals can be concerned in transmission disease to other dogs. Meanwhile, CPVs are extremely stable and resistant to adverse environmental influences. The first report of isolation of *Canine parvovirus* was detected in Tehran University in Iran. In their survey, immunobloating test and MDCK cell line were used for detection of virus. The affected dog was 7 months and was referred with signs vomiting, diarrhea and anorexia (Hemmatzadeh *et al* 2004). The proportions of the new antigenic types of CPV vary in different countries. The first report of CPV-2c has stated in the American continent. Its presence in South American supports the assumption that CPV-2c is reaching a worldwide distribution as occurred with 2a/2b antigenic types (Ruben *et al* 2007). In the Southern Africa, CPV-2b is the predominant virus (Parrish *et al* 1991). Both CPV-2a and 2b are present in the UK, Germany and Spain with similar frequencies of isolation (Ybanez *et al* 1995, Bohm *et al* 2004). Previous studies reported that type 2a virus was the major type in Taiwan (Wang *et al* 2005). In other study, CPV type 2b was the predominant type in Taiwan (Chang *et al* 1996). CPV-2 still causes many cases of acute infectious diarrhea in dogs in Japan (Hirasawa *et al* 1996). In another survey, forty-one of 266 (15.4%) sera had positive titers of 1:40 or higher against the CPV in Japan (Hashimoto *et al* 2001). In the Italy, CPV -2a, 2b and 2c are characterized. Our study showed that there are subtypes 2a or 2b of CPV in Iran, because our kits could detect the pathogenic CPV subtypes CPV2a or CPV2b**.** *Parvovirus* is as one of the most common causes of infectious diarrhea in dogs younger than 6 months. Dogs between 6 weeks and 6 months of age are in increased risk. (Hoskins 1998). In a study in Berlin, the main death causes, were viral infectious diseases (51.76%), especially *parvovirus* infections (26.73%) in dogs younger than 18 months (Walter & Kirchhoff 1995).

Our study showed that the prevalence of infection was more in age of less than 6 months, though difference was no significant. We did not see any dogs that affected to *parvovirus* above 1

year, presumably it is due to natural resistance to the effects of *parvovirus*. Also, prevalence of CPV did not differ between sexes.For unknown reasons, Doberman Pinschers, Rottweilers, Labrador retrievers, American Staffordshire terriers, German shepherds and Alaskan sled dogs seem to have an increased risk (Hoskins 1998). In our study, Terriers (26.31%) and German Shepherds (21%) breeds were more involved. Only one of Doberman pinscher dogs dead, probably due to sensitivity to *parvovirus*. Those that survived five to six days after treatment recovered. The white blood cell count in the surviving dogs showed higher mean total leucocyte, neutrophil and eosinophil counts (3-4 days after onset of treatment) whereas the mean lymphocyte and monocyte counts on the first day of the disease were lower in those dogs which survived than in those ones which died later. In conclusion, we emphasis that vaccination against *parvovirus* and hygienic procedures are as an important protocol for the prevention of CPV infections in situations in dogs population.

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