Antigenic detection of *Canine Coronavirus* in diarrheic dogs in Ahvaz

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Abstract: **The Purpose of this study was to evaluate the prevalence of *Canine Coronavirus* (CCV) in diarrheic dogs of Ahvaz district. Fecal samples were collected from 58 diarrheic dogs between 2005 and 2006. Dogs were grouped by age (less than and above 6 months), sex, breed, geographical location (urban and rural) and quality of diarrhea (hemorrhagic and non-hemorrhagic) to determine whether these factors were associated with *coronavirus* infection, using Fischer's exact test. Prevalence to CCV antigens in these dogs was 3.45% (2 of 58) by means of immunochromatography assay (IC) indicating that this virus is present in the environment. Though the infection had more prevalence in dogs less than 6 months, rural dogs and German shepherds, but there were no significant differences between different sexes, age groups, breeds, location and quality of diarrhea ($p>0.05$). CBC in 2 dogs that were affected with *coronavirus* infection was normal. This study that is the first report on prevalence of CCV in Iran showed that CCV infection in the Ahvaz dogs may be attributed as a cause of viral diarrhea in dogs. A vaccine is recommended, especially for open population and kennel dogs that are at a high risk for contacting the virus.**

Key words: *Canine Coronavirus*, Immunochromatography assay, dog, diarrhea.

Introduction

Canine Coronavirus (CCV) is highly contagious and spreads rapidly through groups of susceptible dogs. CCV is shed in the feces of infected dogs for 2 weeks or longer and can infect dogs of any breed, age, and sex. In young puppies, sometimes in combination with other enteropathogens such as Clostridium perfringens, Campylobacter spp., Helicobacter spp., and Salmonella Spp., CCV infection may cause severe diarrhea, vomiting, dehydration, loss of appetite, and, occasionally, death. Feces are characteristically orange in color, very malodorous, and infrequently contain blood. The dogs generally recover spontaneously 7-10 days after infection, but the diarrhea may persist for more than 2 weeks. Death may occur 1-3 days after the onset of disease, especially in young puppies (Buonavoglia *et al*., 2006, Evermann *et al*., 2005; Hoskins *et al*., 1998). Also dogs can have CCV and canine parvovirus (CPV) infections simultaneously. Some studies suggest that CCV infection makes CPV infection more severe (Pratelli *et al*., 1999). Rapid diagnosis of CCV infection is especially important in kennels and shelters in order to isolate infected dogs and prevent secondary infections of susceptible contact animals (Decaro *et al*., 2004; Squires, 2003). Since a clinical diagnosis is not definitive, several laboratory methods have been developed to detect CCV in the feces of infected dogs such as PCR, HA, ELISA, IFA, Immunoperoxidase and MAbs. Though these tests are more sensitive, specific and more reproducible, but these tests can be expensive and generally take

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Table 1: Distribution of *canine coronavirus* infection based on breed and age in diarrheic dogs of Ahvaz by immunochromatography assay.

Breeds	German shepherd	Terrier	Doberman pinscher	Mixed	Total
Number of studied dogs	14	15	14	15	58
Number of infected dogs	2 (14.28%)	-	-	-	2
Age (< 6 months)	2	-	-	-	2
Age (> 6 months)	-	-	-	-	-
Dead dogs	-	-	-	-	-

time to be analyzed by a specialized laboratory. Immunochromatography assay is the most common rapid field diagnostic method used in clinical practice. Specifity and sensitivity for kits of Rapid CCV Ag Test were found to be 98.8% and 100% respectively (Finlaison, 1995; Esfandiari and Klingeborn, 2000; Pratelli, 2002; Soma *et al*., 2001). In our study, Immunochromatography (IC) was used to investigate the presence of *Canine Coronavirus* antigens in diarrheic dogs in Ahvaz. The present study is the first report on prevalence of CCV in dogs in Ahvaz.

Materials and Methods

Sample collection and preparation: Fecal samples were collected from 58 diarrheic dogs, from Ahvaz, between 2005 and 2006. Number of dogs that had hemorrhagic and non-hemorrhagic diarrhea were 31 and 27 respectively. These dogs were grouped by age (less than and above 6 months), sex, breed, location (urban and rural dogs) and quality of diarrhea (hemorrhagic or non-hemorrhagic) to determine whether these factors were associated with *coronavirus* infection, using Fischer's exact test. The dog breeds were grouped as: Terriers, German Shepherds, Doberman pinschers, and Mixed (German Shepherds + Doberman Pinschers). They had age of 2-11 months old. Diarrheic samples were studied for canine parvovirus infection also. A complete blood count (CBC) was used as a broad screening test to check for such disorders such as anemia, infection and many other diseases.

Immunochromatography assay: The test was carried out with a commercial rapid CCV Ag test kit (Manufactured by Anigen, Animal genetics, Inc., Korea), following the manufacturer's instructions. *Coronavirus* antigen is detected qualitative in canine feces with a chromatographic immunoassay (Esfandiari and Klingeborn, 2000).

Procedure of the test: Swabs were prepared directly from the rectum and mixed with the assay diluents. The samples left for a short time and four drops of supernatant from extracted sample was added into the sample hole. As the test had begun to work, we saw purple color move across the result window in the center of the test device. Interpretation test results took about 5-10 minutes (Esfandiari and Klingeborn, 2000).

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 (Fig. 1). The presence of two color bands (T and C) within the result window, no matter which band appears first, indicates a positive result (Fig. 2). If the purple color band is not visible within the result window after performing the test, the result is considered invalid (Esfandiari and Klingeborn, 2000).

Treatment: Finally treatment of *coronavirus* was directed at correcting the dehydration that accompanied the diarrhea with intravenous fluids (lactated ringers solution). We also gave antiemetic drugs such as metoclopramide (Reglan, 0.5mg/kg three times a day). Besides this, the dogs were placed on antibiotics to prevent secondary bacterial infection (Cefazoline, 20mg/kg three times a day with Gentamicin, 2 mg/kg two times a day) (Hoskins, 1998).

Fig.1- Positive sample of rapid CCV Ag test.

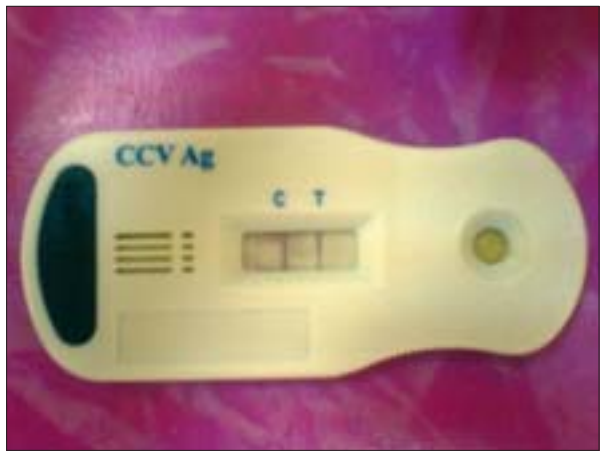

Fig.2- Negative sample of rapid CCV Ag test.

Statistical analysis: Test results and potential association with age, sex, breed, location, CBC and quality of diarrhea were analyzed using SPSS 10.0 for windows and by use of Fishers exact test. Differences were considered significant at $p \leq 0.05$.

Results

Prevalence to CCV antigens in these dogs was 3.45% (2 of 58) by immunochromatography assay, indicating that this virus is present in the environment. Though the infection had more prevalence in less than 6 months dogs and rural areas (6.89%; 2 of 29) as compared with above 6 months dogs and urban areas (0%; 0 of 29), but there was no significant difference ($p>0.05$). The most involved breeds were Germanshepherds (14.28%; 2 of 14) and they were about 2 and 3 months old. Moreover differences were not significant between different sexes and breeds ($p>0.05$). Also, infection had higher prevalence in non-hemorrhagic diarrheic dogs, because 2 affected dogs had diarrhea without blood (7.41%; 2 of 27), nevertheless difference was not significant ($p>0.05$). CBC in the patients was normal, because WBC was between 6000-17000 cells/microl. They were referred to Small Animal Hospital of Ahvaz with signs sudden onset of diarrhea, vomiting, Lethargy and anorexia. Feces were yellowish-orange in color, malodorous and without blood. Fever was not seen and dehydration was estimated above 5%. Prevalence canine parvovirus (2a and 2b) in the studied dogs population was (17.24%; 10 of 58), nevertheless two affected cases were negative against CPV. In our study, there was no death from infection. Affected dogs recovered after 4 - 5 days of supportive treatment. Results are summarized in table 1:

Discussion

The present study that is the first report on prevalence of CCV in dogs in Iran using immunochromatography revealed that 3.45% of Ahvaz area dogs were affected to CCV without evidence of concurrent canine parvovirus (CPV-2) disease. The high sensitivity and specifity of IC assay is a simple and practical method for veterinarians, so we used this method for evaluation of *coronavirus* infection. The results indicated that not all cases of diarrhea (hemorrhagic or non-hemorrhagic) are caused by *coronavirus* and many sick puppies are misdiagnosed as having corona.' In fact the only way to know if a dog has *coronavirus* is through a positive diagnostic test (Sokolow *et al*., 2005).

Knowledge of the prevalence of CCV in diarrheic dogs in Ahvaz is important because this infection 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, CCVs can remain infectious for longer period's outdoors (Carmichael and Binn, 1981, Hoskins, 1998). Our cases emphasize the importance of pursuing a diagnosis of CCV in young puppies when CPV-2 disease has been ruled out.

The true importance of CCV as a cause of infectious enteritis in dogs is the time agree with other pathogen particular CPV. Many reports have emphasized on fatal *coronavirus* infection following CPV infection (Pratelli *et al*., 1999). Of course, in our study we had not any concurrent infections, because the samples were tested for CPV and were negative. Prevalence to CPV (2a and 2b) antigens was detected (17.24%; 10 of 58) in diarrheic dogs of Ahvaz.

In 1971, CCV was first isolated from fecal specimens of American military dogs with diarrhea disease (Hoskins, 1998). In a study in USA, 2 cases were associated with *Canine Coronavirus*, case 1 involved a 7-week-old, Chihuahua, the puppy died within 12 hours of admission and was submitted for diagnostic workup. Case 2 was an 8-week-old, Shih Tzu, the referring veterinarian euthanized the puppy, and the entire body was submitted for diagnostic evaluation (Evermann *et al*., 2005). Our cases that were two German shepherds with age 2 and 3 months, survived with supportive treatments.

The proportions of the new antigenic types of CCV vary in different countries. In a study on the 898 canine serum samples, 160 (17.8%) were positive for anti-CCV antibodies in Japan. The antibody titers ranged from 1:5 to more than 1:640, with 1:160 being the most frequent (Kaneshima *et al*., 2006). In similar study in this country, on the 174 samples collected from naturally infected dogs, 38 were positive for both genotypes CCV (Decaro *et al*., 2005). Other study in China showed that 21 out of 48 diarrhea feces from pet dogs were positive for CCV (Wang *et al*., 2006).

In south of Italy, ELISA was used to detect antibodies against CCV. Out of a total of 109 dog serum samples, 80 were positive (Pratelli *et al*., 2002). In Turkish dog population, of 179 dogs tested for CCV antibodies, 112 (62.5%) were positive by serum neutralization test. The highest prevalence was detected in kennel dogs (Yesilbag *et al*., 2004). In Australian dogs, serum samples (1396) collected from 1984 to 1998 were tested for the presence of IgG antibodies to CCV. Samples were divided into two categories (open and kenneled dogs) on the basis of the number of dogs housed together. Sera from 15.8% of the open population and 40.8% of kennelled dogs were positive for CCV antibodies (Naylor *et al*., 2001).

It is reported that young puppies have an increased risk (Hoskins, 1998). Our study showed that the prevalence of infection was more in age of less than 6 months, though difference was not significant. We did not see any dogs were affected to *coronavirus* above 6 months; presumably it is due to natural resistance to the effects of *coronavirus*. Prevalence of CCV did not differ between sexes and breeds too ($p>0.05$).

Two affected dogs were rural dogs that lived in open population and were in contact with other animals. We did not see any companion dogs that affected to CCV. This study emphasis that the prevalence of CCV is higher in kennelled or open populations. Recently, a vaccine (inactivated and MLV) is available for *Canine Coronavirus* in the world (Zwijnenberg, 2003). *Coronavirus* is not transmitted to human (Vabret *et al*., 1998) but we highly recommend vaccination of dogs living in kennelled or open populations that are at a high risk for contacting the virus.

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شناسایی آنتی ژنیکی کروناویروس در سگ های اسهالی اهواز

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چکیده

هدف از انجام این مطالعه، ارزیابی میزان شیوع کروناویروس در سگ های اسهالی منطقه اهواز بود. نمونه های مدفوع، از ۵۸سگ اسهالی در طول سال ۸۵- ۱۳۸۴جمع آوری شدند. سگ های تحت مطالعه بر اساس سن (کمتر و بالای ۶ماه)، جنس، نژاد، منطقه جغرافیایی (شهری و روستایی) و نوع اسهال (هموراژیک و غیر هموراژیک) طبقه بندی شده بودند تا مشخص گردد که آیا این فاکتورها در ارتباط با عفونت کروناویروسی می باشند یا خیر (با استفاده از آزمون دقیق فیشر). میزان شیوع عفونت ناشی از کروناویروس، در این سگ ها با استفاده از روش ایمونوکروماتوگرافی ۳/۴۵درصد (۲تا از ۵۸مورد) بدست آمد که نشان می دهد ویروس در اکوسیستم محیط موجود است. هر چند میزان شیوع عفونت در سگ های کمتر از ۶ماه، سگ های روستایی و نژاد ژرمن شفرد بالاتر بود اما از نظر آماری تفاوت معنی داری ما بین جنس، سن، نژاد، منطقه و نوع اسهال دیده نشد ($p>0/05$). تابلوی خونی در ۲قلاده سگی که مبتلا به عفونت ناشی از کروناویروس بودند طبیعی بود. این مطالعه که اولین گزارش در مورد شیوع کروناویروس در ایران می باشد نشان داد که عفونت ناشی از کروناویروس می تواند به عنوان یکی از علل اسهال ویروسی در سگ های منطقه اهواز مطرح باشد. واکسیناسیون، به ویژه برای جمعیت های باز و یا سگ هایی که در یک محل نگهداری می شوند و در معرض خطر برای تماس با ویروس هستند توصیه می شود.

واژه‌های کلیدی: کروناویروس، ایمونوکروماتوگرافی، سگ، اسهال.

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