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 prevents 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
time to be analyzed by a specialized laboratory. Immunochromatography assay is the most common rapid field diagnostic method used in clinical practice. Specificity 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).

<table>
<thead>
<tr>
<th>Breeds</th>
<th>German shepherd</th>
<th>Terrier</th>
<th>Doberman pinscher</th>
<th>Mixed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of studied dogs</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>58</td>
</tr>
<tr>
<td>Number of infected dogs</td>
<td>2 (14.28%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Age (&lt; 6 months)</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Age (&gt; 6 months)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dead dogs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
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≤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 specificity 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.

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

Fig.2- Negative sample of rapid CCV Ag test.
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 kenneled 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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References
Antigenic detection of canine coronavirus in...


شناسایی آنتی‌زنیکی کرناوئر وس در سگ های اسپالی اهواز

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چکیده

هدف از انجام این مطالعه، ارزیابی میزان شیوع کرناوئر وس در سگ های اسپالی منطقه اهواز بود. نمونه های مذبور، ۸۸ سگ اسپالی در طول سال ۱۳۸۴-۱۳۸۵ جمع آوری شدند. سگ های تحت مطالعه براساس سن (کمتر یا بالای ۳ سال)، جنس، نژاد، منطقه جغرافیایی (شهری و روستایی) و نوع اسپالی (هموارازیک و غیر هموارازیک) طبقه بندی شده بودند. نتایج نشان دهنده ۳۴ نفر از مجموعه ۳۹۲ نفر به طور کلی از مبتلا به کرناوئر وس بودند. این نتایج نشان می‌دهد که در این بیماری، سگ‌های شهری با بیشترین نرخ بیماری در نظر گرفته شده‌اند. در این مطالعه، سگ‌های شهری چهار برابر سگ‌های روستایی بیماری شده‌اند. کنترل این بیماری برای بهبود دهنده کنترل می‌باشد. تحقیقات بیشتری نیز باید در این زمینه انجام شود.