

Antigenic detection of *Canine rotavirus* group A in diarrheic dogs in Ahvaz district, Southwestern Iran

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Abstract The aim of the present survey was to evaluate the prevalence of *Canine rotavirus* (CRV) infection in companion dogs in Ahvaz district, Iran. The studied dogs were selected between referred cases to Shahid Chamran University of Ahvaz. Fecal samples were collected from 98 diarrheic dogs between December 2011 and August 2013. The dogs were classified according to age, sex, breed, clinical signs, and season. They were divided into three age groups (<3 months, 3–6 months, and >6 months), five different breeds, and another two groups on the basis of clinical symptoms (hemorrhagic and non-hemorrhagic diarrhea) using Chi-square analysis, Fischer's exact test, and *Z* test. The prevalence of *rotavirus* infection was 16.33 % (16 out of 98) (95 % CI for proportion 9.1–23.6 %) by means of immunochromatography assay (ICA) (Catalog No. RG18-03). The infection had significantly more prevalence in dogs less than 3 months (48.15 %; 13 out of 27) compared with dogs between 3 and 6 months (6.06 %; 2 out of 33) and above 6 months (2.63 %; 1 out of 38) ($p < 0.05$). Prevalence was higher in males (17.54 %; 10 out of 57) than females (14.63 %; 6 out of 41), in winter season (21.74 %; 5 out of 23), and breed of German shepherd (19.05 %; 4 of 21), but the difference was not significant between the prevalence of infection relative to gender, season, and breed ($p > 0.05$). Nevertheless, infection was significantly higher in none-hemorrhagic diarrheic dogs (23.08 %; 15 of 65) ($p < 0.05$). The obtained results provide useful information on the epidemiology of *Canine rotavirus* in this area.

Keywords *Canine Rotavirus* · Diarrhea · Dog · Immunochromatography assay (ICA) · Iran

Introduction

Rotaviruses are recognized as one of the most important neonatal enteric pathogens of many species, including humans and dogs. Currently, *rotaviruses* are classified as distinct members of the family Reoviridae, genus *rotavirus*, comprising five species (A to E) and two tentative species (F and G). *Canine rotavirus* (CRV) is a double-stranded RNA, non-enveloped virus that is approximately 60 to 75 nm in diameter (Kapikian et al. 2001; Hoskins 2006). The virus is transmitted by fecal-oral contamination. *Rotaviruses* affect the mature epithelial cells on the tips of the small intestinal villi, leading to mild to moderate villus atrophy and watery to mucoid diarrhea which typically resolves within 8–10 days. Gastroenteritis is more seen in young puppies less than 12 weeks of age (Yeşilbag et al. 2007). Some cases of severe fatal enteritis associated with CRV have been reported to occur in pups as young as 2 weeks (Fulton et al. 1981). It may contribute to enteric disease in mixed viral infections. A differential diagnosis would include *Canine parvovirus*, *Clostridium perfringens* enterotoxin A, *Escherichia coli*, intestinal nematodes and protozoa, *Coronavirus*, and *Distemper* virus (Iturriza-Gomara et al. 2004; Hoskins et al. 2006). Rotaviral infections in people usually occur in young infants and children. It was discovered in 1973 and accounts for up to 50 % of hospitalizations for severe diarrhea in infants (Kapikian et al. 2001; Hoskins et al. 2006). It is possible that occasional cross-species transmission occur. Documented case reports of rotavirus infection in children can be found in published literature in different areas of the world (Kapikian et al. 2001; Iturriza-Gomara et al. 2004). Therefore, appropriate hygiene precautions should be taken when handling diarrheic feces from dogs. A high prevalence of antibodies has been found in adult animals. The role of dogs as the source of human infections has not been since investigated in Iran. Rapid diagnosis of CRV infection is especially important in kennels and shelters in order to isolate infected

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puppies for preventing of secondary bacterial infections in susceptible contact animals. The clinical diagnosis of CRV is indecisive, since several other pathogens may cause diarrhea in dogs, so several laboratory methods have been developed to detect antigen in the feces of infected dogs. Routine hematology and biochemistry profiles are not diagnostic for infection. Most rotaviruses can be detected by many methods, including such as enzyme-linked immunosorbent assay (ELISA), latex agglutination test (LAT), polymerase chain reaction (PCR), polyacrylamide gel electrophoresis (PAGE), molecular techniques such as genome sequencing, and electronic microscope (Biermann et al. 1991; Hoskins 2006). Though these tests are more sensitive, specific, and more reproducible, they can be expensive and generally take time to be analyzed by a specialized laboratory. Immunochromatography assay (ICA) is one of the most common rapid field diagnostic methods used in clinical practice. According to the manufactures instruction, specificity and sensitivity for kits of Rota Ag Test (Bionote, Inc, Korea) were found to be highly 100 and 92 %, respectively. There is no cross reaction with other pathogen of enteric diseases for this kit. This survey was conducted to provide preliminary information on the seroprevalence of CRV infection among diarrheic dogs found in Ahvaz city. The obtained results are important in control and prevention program. To our knowledge, the present study is the first report of the occurrence of group A *rotaviruses* in dogs in Ahvaz district, Southwestern Iran.

Materials and methods

Study area and sample population This study was performed in the Ahvaz area, southwestern Iran that is located at an elevation of 12 m above sea level and the climate is warm-humid. In the present survey, fecal samples were collected from 98 diarrheic dogs (two per dog) between December 2011 and August 2013 by immunochromatography assay (ICA). The dogs were classified according to age, sex, breed, clinical signs, and season. They were selected between referred cases to the Veterinary Hospital of Shahid Chamran University of Ahvaz, Southwestern Iran. The dogs were divided into three age groups (<3 months, 3–6 months, and >6 months), five different breeds (terriers, German shepherds, Doberman pinschers, Great Dane, and mixed), and another two groups on the basis of clinical symptoms (hemorrhagic and non-hemorrhagic diarrhea). At the time of fecal collection, they were young dogs from 2 weeks to 12 months and showed clinical signs suggestive of diarrhea. The studied dogs were not vaccinated against distemper, adenovirus, parvovirus, parainfluenza, leptospira, and rabies vaccines. Finally, treatment of the affected dogs was directed at correcting the life-threatening dehydration that accompanied the diarrhea with intravenous fluids, antiemetic, and broad-spectrum antibiotics

to prevent secondary bacterial infection. In this study, we evaluated the detection rate of the *rotavirus* infection by using a commercially available ICA kit (Catalog No. RG18-03).

Immunochromatography assay This kit is a chromatographic immunoassay for the qualitative detection of *rotavirus* antigen in canine feces. The test was carried out with a commercial rapid CRV Ag test kit (manufactured by Anigen, Animal genetics, Inc., Korea), following the manufacturer's instructions. Specificity and sensitivity were calculated to be highly 100 and 92 %, respectively for kits of Rota Ag Test (Bionote, Inc, Korea).

Procedure of the test The samples were provided by a swab from the stool. They were then inputted and mixed into the assay diluents. They were left for a short time and finally four drops of supernatant were added into the sample hole. As the test began to work, a purple color was observed moving across the result window in the center of the test device. Interpretation of test results was at 5–10 min.

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. The presence of two color bands (T and C), no matter which band appears first, indicates a positive result (Esfandiari and Klingeborn 2000).

Statistical analysis

To examine whether there were any statistically significant relationships between the prevalence of positive cases and other factors such as age, sex, breed, clinical symptoms, and season, data were examined using chi-square analysis, Fisher's exact test, and Z test with a confidence interval of 95 %. Differences were considered significant when $p < 0.05$.

Results

In a total of 98 diarrheic dogs, 16 (16.33 %) were affected to CRV antigens (95 % CI for proportion, 9.1–23.6 %). The infection had significantly more prevalence in dogs less than 3 months (48.15 %; 13 out of 27) compared with dogs between 3 and 6 months (6.06 %; 2 out of 33) and above 6 months (2.63 %; 1 out of 38) ($p < 0.05$). The difference was not significant between dogs of 3–6 months and above 6 months ($p > 0.05$). Prevalence was higher in male dogs

Table 1 Frequency (%) of *Canine rotavirus* infection in diarrheic dogs of different age and sex in Ahvaz district, Iran by ICA, 2011–2013

Age Sex	<3 months Positive	3–6 months Positive	>6 months Positive
Male	44.44 % (8 out of 18)	55.55 % (1 out of 18)	4.76 % (1 out of 21)
Female	55.55 % (5 out of 9)	6.67 % (1 out of 15)	0 % (0 out of 17)
Total=98	48.15 % (13 out of 27)	6.06 % (2 out of 33)	2.63 (1 out of 38)

95 % CI for proportion, 9.1–23.6 %

(17.54 %; 10 out of 57) than females (14.63 %; 6 out of 41), in the season of winter (21.74 %; 5 out of 23), and breed of German shepherd (19.05 %; 4 of 21), but the difference was not significant between the prevalence of infection relative to host gender, season, and breed ($p>0.05$). Nevertheless, infection was significantly higher in none-hemorrhagic diarrheic dogs (23.08 %; 15 out of 65) ($p<0.05$). Among the positive samples, only one dog (3.03 %; 1 out of 33) had hemorrhagic diarrhea. Prevalence in other seasons (spring, summer, and autumn) was 13.04 % (3 out of 23), 16.67 % (4 out of 24), and 14.28 % (4 out of 28), respectively. These results are summarized in Tables 1 and 2.

Discussion

Our survey is the first published study on *Canine rotavirus* infection in Iran. The obtained results showed that 16.33 % of the diarrheic dogs were positive for *rotavirus* infection in Ahvaz district, suggesting that this virus is present in environment. Our data provide geographic information about this viral enteritis which can be largely overcome by using seroprevalence studies in other regions of the country. Sampling was conducted in the studied dogs for nearly 2 years, so the results can be considered representative for the diarrheic dog's population in this area. On the methodological side of this study, ICA is an easy method for antigenic detection in diarrheic samples; it was used to confirm the positive results. Although the sensitivity and specificity of the other diagnostic tests may be very high, ICA tests are used for the detection of fecal antigens in living animals where laboratory facilities are not easily available.

Rotaviruses had already been detected in feces from diarrheic dogs (Fulton et al. 1981). The obtained results confirm that the prevalence of infection in dogs is different not only between countries but also between different areas within a country. These differences can be explained by diversity in the epidemiology of this infection, the method, number of animals studied, the geographic area, and a consequence of environmental factors, as these can influence the development of *rotavirus* infection in humans and other animals (Hoskins 2006). The present seroprevalence (16.33 %) was higher than the prevalence reported in Germany (7.1 %) (Biermann et al. 1991) and Brazil (3.0 %) (Luchs et al. 2012). Other authors have associated *rotavirus* infection with a fatal neonatal diarrhea in a 3-day-old pup (Martella et al. 2001). Similar results were obtained by those who observed diarrhea in several litters of pups below 1-week-old from the same kennel. In a 2-day-old pup that died from diarrhea, *rotavirus* particles were detected in specimen prepared from the intestinal homogenate (Fulton et al. 1981). The incidence of *rotavirus* infections in dogs in other areas needs to be determined to better assess the risk of infection with these viruses for humans.

Clinical symptoms associated with rotaviruses group A in humans and the widespread prevalence of antibodies to this type of viruses suggests that rotaviruses may be enzootic (Kapikian et al. 2001). Most rotavirus infections run a subclinical course, but they have been associated with severe diarrhea in neonatal dogs. Antibodies to rotavirus have been demonstrated in a high percentage of adult dogs (80 %), suggesting that the majority of infections may be asymptomatic (Hoskins 2006). Due to the lack of pathognomonic clinical signs in *rotavirus* infections, evaluation of stool specimen has become a helpful and widely used tool in diagnostics and epidemiological surveys. In the

Table 2 Frequency (%) of *Canine rotavirus* infection in diarrheic dogs of different age and season in Ahvaz district, Iran by ICA, 2011–2013

Age Season	<3 months Positive	3–6 months Positive	>6 months Positive
Spring	40 % (2 out of 5)	10 % (1 out of 10)	0 % (0 out of 8)
Summer	66.67 % (4 out of 6)	0 % (0 out of 8)	0 % (0 out of 10)
Autumn	60 % (3 out of 5)	0 % (0 out of 9)	7.14 % (1 out of 14)
Winter	36.36 % (4 out of 11)	16.67 % (1 out of 6)	0 % (0 out of 6)
Total	48.15 % (13 out of 27)	6.06 % (2 out of 33)	2.63 % (1 out of 38)

95 % CI for proportion, 9.1–23.6 %

present study, only one of the affected dogs (3.03 %) had hemorrhagic diarrhea and the infection was significantly higher in none-hemorrhagic diarrheic dogs (23.08 %). Abe et al. (2010) reported the characterization of rotaviruses in a Japanese raccoon dog. Their findings showed that wild animals may constitute a potential zoonotic risk of *rotaviruses*. In another research by Otto et al. (1999), three out of nine fecal samples from diarrheic dogs were positive in Germany.

It has been demonstrated that direct interspecies transmission between heterologous strains are key mechanisms in generating rotavirus strain diversity in new hosts (Matthijnsens et al. 2011). These studies indicate that dogs may be infected with rotaviruses from different species and that transmission may occur through contact with other animals. Wu et al. (2012) reported the characterization of the first human rotavirus from East Asia identified in a 2-year-old child in Taiwan. Sequence analysis demonstrated a close genetic relationship between the genes of Taiwanese strain and an Italian canine strain isolated a decade ago, suggesting a canine origin for the Taiwanese strain. Wang et al. (2013) suggested that reassortment may have occurred among feline or canine RVA strains, before being transmitted to humans.

In general, season or breed predisposition has not been reported in texts for *rotavirus* infection in dogs (Hoskins 2006). Similarly, there was no significant difference in the prevalence of positive samples between different sexes and seasons of dogs with infection. In the present study, the prevalence of infection was 17.54 % in males and 14.63 % in female dogs, respectively.

Age is demonstrated by the increasing rate of susceptibility to infection with the decreasing age of the dogs (Hoskins 2006). In our survey, most of the infected dogs had age below 3 months (48.15 %). We saw only three dogs above 3 months that were affected with *rotavirus*; presumably, it is due to natural resistance to the effects of *rotavirus*.

The results of the present survey provide useful information on the epidemiology of CRV infection in Iran, which until now was not well studied, as well as, studies with larger sample sizes among dogs, humans, and other animals in different areas would be beneficial in determining the transmission cycle and the status of this zoonosis. The observations provided in our survey may also be useful in formulating prevention and control measures. Knowledge of the prevalence of CRV in diarrheic dogs in Iran is important, because CRV 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 animals and humans. It should be noted that *rotavirus* is relatively stable and resistant to most environmental conditions (Hoskins 2006). The presence of antigens in fecal specimens of dogs is a public health concern due to the close contact between dogs and human, which provides a link between an environmental factor and humans. Humans should avoid contact with stray dogs. They should be

eliminated by the local animal control officials, particularly in areas where there is likely contact with humans. The occurrence of antibodies in adult dogs suggests that veterinarians should pay attention to this disease and include it within the differential diagnosis for gastroenteritis cases. We hope that, in the near future, this and other similar projects will provide the basis of an epidemiologic surveillance program in diarrheic dogs of Iran, which will establish the basis for prevention and control of these kinds of emerging diseases.

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