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Short Communication

Antigenic detection of *Canine Parainfluenza virus* in urban dogs with respiratory disease in Ahvaz area, southwestern Iran

Mosallanejad[*1], B., Avizeh [1], R., Seyfiabad Shapouri[2], M.R., Ramesh[1], B.

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

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ABSTRACT

Canine parainfluenza virus (CPiV) is one of the most common organisms isolated from dogs with signs of infectious tracheobronchitis (ITB). Distribution is apparently worldwide. Although CPiV may cause mild clinical infections, clinical diseases is expected to be more severe in dogs co-infected with *bordetella bronchiseptica* than with any these agents alone. The present study was conducted to determine the prevalence of *Canine parainfluenza virus* infection in urban dogs of Ahvaz area, southwestern Iran. The urban dogs were selected between referred dogs (companion) to Veterinary Hospital of Ahvaz University. Sample of respiratory secretions was collected randomly from 76 affected dogs between June 2008 and May 2009. The studied dogs were divided into two age groups (<6 months and >6 months) and based of environment into two groups (close and open) also. The results were analyzed by using Chi-square analysis and Fischer's exact test. Prevalence to *Canine parainfluenza virus* antigens was 5.3% (4 of 76) by means of immunochromatography indicating that this antigen is present in the ecosystem. The infection had more prevalence in those dogs that were in open environment (17.65%; 3 of 17) in compared with close environment (1.69%; 1 of 59) and the difference was significant between different groups (P= 0.033). Prevalence was more in dogs less than 6 months (6.82%; 3 of 44) in compared with dogs above 6 months (3.12%; 1 of 32), but the difference was not significant between two groups (P= 0.436). Prevalence of infection was 4.44% (2 of 45) in male dogs and 6.45% (2 of 31) in female dogs. Prevalence was 6.06% (2 of 33) in Mixed breed, 5.55% (1 of 18) in Germanshepherds and 11.11% (1 0f 9) in Doberman pinchers. There was no significant difference between different sexes and breeds also (P>0.05). This study showed that *Canine parainfluenza virus* can be as a risk factor particularly for those dogs are in contact together in open environment and kennel dogs in Ahvaz area.

Keywords: *Canine parainfluenza virus*, immunochromatography, dog, Ahvaz, Iran

INTRODUCTION

Canine parainfluenza virus (CPiV-2) is a single-stranded RNA virus belonging to the family Paramyxoviridae and is closely related to simian virus 5 (SV-5). CPiV-2 is very contagious. Symptoms of the infection are a dry and hacking cough. Puppy vaccinations and boosters can help protect dogs from the virus. CPiV-2 and *bordetella*

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

bronchiseptica have been reported as the most common organisms isolated from the respiratory tract of dogs with infectious tracheobronchitis (ITB) synonyms: kennel cough. Several other viruses (such as adenovirus type 2 and to a lesser extent adenovirus type 1, herpesvirus and reovirus) and bacteria (such as mycoplasma, Pasteurella, pseudomonas and streptococcus) are known to influence the clinical course and outcome of infection. Compared with adults, puppies appear more vulnerable to life-threatening pneumonia. Infection generally results in self-limiting, short-lived cough, with minimal systemic effects (Richard 2006, Buonavoglia & Martella 2007). *Canine parainfluenza virus* was first isolated by Binn *et al* in 1967 as a Simian-5 (SV5) like virus from laboratory dogs with respiratory disease. Subsequently Crandell *et al* (1968), Appel *et al* (1970), Rosenberg *et al* (1971) Cornwell *et al* (1976), Mc Candlish *et al* (1978) and many other researchers has been reported on it (Ajiki *et al* 1982). The *canine influenza virus* is not the same as the *canine parainfluenza virus*. Both are respiratory diseases that involve dry, or unproductive, coughing and nasal discharge. Both diseases can be mistaken for tracheobronchitis, or kennel cough. The *canine influenza virus* is thought to have been first discovered in greyhounds at a Florida racing track in 2004. The disease began spreading to other greyhound racing tracks throughout the United States. The *canine influenza virus* is similar to the *equine influenza virus* and may even be a mutation of it. Clinical diagnosis is not definitive. Routine hematology and biochemistry profiles are not diagnostic also, so several laboratory methods have been developed to detect CPiV in the respiratory secretions or serum of infected dogs such as PCR, ELISA, Immunoperoxidase staining and monoclonal antibodies. 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. Specifity and sensitivity for kits of Rapid CPiV Ag Test were found to be highly 98.8% and 100% respectively (Esfandiari & Klingeborn 2000, Etienne 2007). In our study, Immunochromatography (IC) technique was used to investigate the presence of *Canine parainfluenza virus* antigens in urban dogs with respiratory disease in Ahvaz district, southwestern Iran. The present article that describes the first report about the survey of CPiV in Iran, provides preliminary information in Ahvaz district.

MATERIAL AND METHODS

Study area and sample population. This study was performed in Ahvaz area, southwestern Iran that is located at an elevation of 12 meters above sea level and the climate is hot-humid. A total of 76 specimens of the respiratory secretions were obtained randomly from individually urban affected dogs in Ahvaz district, between June 2008 and May 2009. The urban dogs were selected between referred dogs (companion) to Veterinary Hospital of Ahvaz University. The studied dogs were divided into two age groups (<6 months, and >6 months). The dogs under study were from 6 breeds: Terriers, German shepherds, Doberman pinschers, Spitz, Pekingese and Mixed. Signs of upper respiratory problems were included conjunctivitis (irritated eyes), rhinitis (runny nose), sneezing, cough, low-grade Fever, difficulty breathing, purulent nasal discharge, loss of appetite and depression.

Laboratory methods. The samples were collected from conjunctiva and nasal discharges using the sample collection swab pre-wetted with saline solution. Then the swabs were inserted into the specimen tube containing assay diluents. The swab samples were mixed with assay diluents to extract well removed the test device from the foil pouch

and placed it on a flat and dry surface. Respectively, we added four drops of the mixed sample into the 2 sample holes using the dropper, drop by drop and slowly. As the test began to work, we saw purple color move across the result window in the center of the test device. If the migration was not appeared after 1 minute, was added one more drop of the mixed sample to the sample well. Finally, test results were interpreted at 5-10 minutes. The animals' identification, age, sex, breed, health status, geographic area and the date of collection were recorded. All data were entered and stored in a computerized database. Age was estimated by dental formulary (Esfandiari & Klingeborn 2000).

Immunochromatography assay. The test was carried out with a commercial rapid CIRD-3 Ag test kit (Manufactured by Anigen, Animal genetics, Inc., Korea), following the manufacturer's instructions. *Parainfluenza* antigen was detected qualitative in canine respiratory secretions with a chromatographic immunoassay. Sensitivity and specifity of these kits were above 95% (Esfandiari & 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. The presence of two color bands (T and C) within the result window, no matter which band appears first, indicates a positive result. If the purple color band is not visible within the result window after performing the test, the result is considered invalid (Esfandiari & Klingeborn 2000).

Treatment. Finally treatment of *parainfluenza* was directed for prevention of secondary or concurrent infections with broad-spectrum antibiotics (Amoxicillin-clavulanate 25 mg/kg PO for 10 days), Antitussives (Butorphanol 0.55 mg/kg PO as needed) and Bronchodilators (Aminophylline 10 mg/kg PO as needed) (Richard 2006).

Statistical analysis. Dogs were grouped by age, sex, breed and geographic area (open and close environment) to determine whether these factors were associated with *canine parainfluenza virus* infection, using fisher's exact test and chi-square analysis. Statistical comparisons were carried out using SPSS 16.0 statistical software. Differences were considered significant when $P< 0.05$.

RESULTS AND DISCUSSION

Among the 76 affected companion dogs at the veterinary hospital, the most common clinical signs were low-grade fever, purulent nasal discharge and cough. Prevalence to *Canine parainfluenza* antigens in the studied dogs was 5.3% (4 of 76) by means of immunochromatography assay, indicating that this virus is present in the ecosystem. The infection had more prevalence in those dogs that were in open environment (17.65%; 3 of 17) in compared with close environment (1.69%; 1 of 59) and the difference was significant between different groups ($P= 0.033$). Prevalence was more in dogs less than 6 months (6.82%; 3 of 44) in compared with dogs above 6 months (3.12%; 1 of 32), but the difference was not significant between two age groups ($P= 0.436$). Prevalence of infection was 4.44% (2 of 45) in male dogs and 6.45% (2 of 31) in female dogs. Prevalence was 6.06% (2 of 33) in Mixed breed, 5.55% (1 of 18) in Germanshepherds, and 11.11% (1 0f 9) in Doberman pinchers. Terriers (11), Pekingese (3) and Spitz (2) were negative. There was no significant difference between different sexes and breeds also ($P>0.05$). Results are summarized in table 1. The present study that is the first report on prevalence of CPiV in dogs in Iran using immunochromatography assay revealed that 5.3% of companion dogs in Ahvaz area were affected to this virus. The

high sensitivity and specifity (above 95%) of IC assay is a simple and practical method for veterinarians, so we used this method for evaluation of *parainfluenza infection*. The only way to know if a dog has *parainfluenza virus* is through a positive diagnostic test. Knowledge of the prevalence of CPiV in dogs in Ahvaz area 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 (Richard 2006, Rance 2005).

Table 1: Prevalence of *Canine parainfluenza virus* infections in urban dogs of different age and sex in Ahvaz district, Iran by immunochromatography, 2008-2009.

Age	< 6 months		> 6 months	
Sex	**Neg.**	**Pos.**	**Neg.**	**Pos.**
Male	24	2	18	1
Female	17	1	13	0
Total = 76	41	3	31	1

The true importance of CPiV as a cause of respiratory infectious in dogs is the times agree with other pathogen particular *bordetella bronchiseptica* and adenovirus type 2. Many reports have emphasized on severe respiratory infection following above pathogen agents. Depending on the design, the sample population and the laboratory methodology implemented, prevalence has been found to vary considerably between studies. The reported results confirm that prevalence of CPiV infection in dogs is different not only between countries but also between different areas of each country. Ranges for prevalence of CPiV in various populations, have been 1 – 10% in dogs (Richard 2006). Ajiki *et al* (1982) isolated a cytopathogenic agent (parainfluenza virus) in dog kidney cell cultures from pneumonic lung tissue of a three-month-old dog that died at an outbreak of respiratory disease. The disease was characterized by anorexia, fever, purulent nasal discharge, dyspnea and diarrhea. In another epidemiological study for diagnosis of parainfluenza in dogs in England, it was showed that only 14% (14 out of 100) of dog sera were positive for parainfluenza by haemagglutination inhibition (HI) (Cuadrado 1965). Study on 68 household dogs with clinical signs of respiratory infection showed that 5 dogs (7.4%) were found to be positive for *canine parainfluenza virus*. Among the viruses examined, CPiV was detected with the highest frequency. Other pathogens included *bordetella bronchiseptica* (10.3%), *canine adenovirus-type-2* (2.9%), *canine respiratory coronavirus* (1.5%), and canine distemper virus (1.5%) (Mochizuki *et al* 2008). In another investigation in England, 19.4% of tracheal and 10.4% of lung samples were positive for CPiV by PCR. Their study showed that CPiV is present at kennels despite vaccination. In addition, other agents such as *canine herpesvirus* and *coronavirus* play a role in the pathogenesis of canine respiratory diseases (Erles *et al* 2004). Levy *et al* 2008, with study on 95 serum samples in the Galapagos showed that antibodies were present in dogs against *parainfluenza virus* (100%). In the absence of vaccination, a reservoir of susceptible animals remains vulnerable to new disease introductions.

The prevalence of antibodies against CPiV-2 was investigated in a population of 302 pet dogs in Sweden. Sera were analyzed for CPiV-2 specific neutralising antibody by means of a haemagglutination inhibition test. CPiV-2 had a seroprevalence of 28 percent. (Englund *et al* 2003). In another survey, blood samples were collected from 64 wild North American river otters from Newyork State and analyzed for serologic evidence of exposure to CPiV. No clinical signs of disease nor lesions suggestive of prior viral exposure were seen. Titers were not detected for antibodies against *canine parainfluenza virus* (Kimber *et al* 2000). Paired serum samples and bacterial swabs

were collected from 52 dogs with clinical signs of infectious tracheobronchitis in three districts of Norway. The results revealed a fourfold or greater rise in the titer of antibodies against *canine parainfluenza virus* in 79 per cent of the cases, strongly suggesting that the virus was an importance etiological agent in the outbreak of disease. *Bordetella bronchiseptica* was not isolated from the diseased dogs, and they showed no rise in the titres of antibodies against *influenza virus*, reovirus or adenovirus (Ueland 1990). The lungs of 35 dogs that died in Mexico from acute or subacute pneumonia were examined immunohistochemically for *canine parainfluenza virus*, CDV and CAV to determine their frequency, occurrence and possible associations. CPiV was identified in 18 (51%) of cases. The most frequent dual association was that between CDV and CpiV (five cases; 14%)(Damian *et al* 2005). A total of 398 blood serums of dogs of various breeds and age categories, in Bohemia and Slovakia, were examined for the content of haemagglutination-inhibiting (HI) antibodies to CPiV-2. Out of this total number, 115 serums (28.9%) reacted against CPiV-2 in titres from 1:2 to 1:256 (Zuffa & Krobot 1987). The crowding of dogs together is significantly associated with the seroprevalence of CPiV-2. The dogs' age, gender and breed group affiliation is not correlated with the seroprevalence of infection. Affected dogs are more likely to have a history of recent stays in a pet shop, boarding facility, kennel or shelter. Although dogs are affected most commonly during summer and fall, the high density of dogs living in shelters or kennels can be expected to result in infections at any time of year (Englund *et al* 2003). It is reported that young puppies less than 6 months of age are in increased risk (Richard 2006). Our study showed that the prevalence of infection was more in age of less than 6 months, though difference was no significant. Prevalence of CPiV did not differ between sexes and breeds too (P>0.05), of course the number of infected dogs in this study were too small. In the present study, infection had more prevalence in the dogs that were in open environment compared with close environment and the difference was significant between different groups (P=0.033). Three out of four the affected dogs had lived in open environment and were in contact with other animals. This study emphasis that the prevalence of CPiV is higher in kennel or open populations. We propose vaccination for the protection of dogs against respiratory signs of infection with *parainfluenza virus* of canine origin. *Canine parainfluenza virus* vaccine (live) is a preparation of a suitable attenuated strain for dogs. A trivalent vaccine containing *Bordetella bronchiseptica*, *canine parainfluenza*, and *canine adenovirus-type-2* is administered as intranasal at 3 weeks of age. Although natural infection may result in detectable serum antibody, titer to CPiV-2 dose not correlates well with protection from clinical disease. CPiV vaccines (DHPPiL) are commonly available and are recommended for all dogs (Rance 2005). The vaccine was 71.2% and 81.8% effective in decreasing the incidence of coughing during the winter and summer trials, respectively. No adverse reactions were observed with any of the intranasal vaccines (Glickman & Appel 1981). *Canine parainfluenza* is not transmitted to human. We highly recommend vaccination of dogs living in kennel or open populations that are at a high risk for contacting the virus (Richard 2006, Tilley & Smith 2005). In conclusion, the results of this study indicate that further biological studies are required to fully determine the public health significance of the CPiV strains, as well as other genotypes and species for immunocompromised host's prevalence, transmission and species status of the CPiV.

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