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## Antibody Titers Against Canine Distemper Virus in Unvaccinated Rural Dogs from Ahvaz, Iran

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**Abstract:** The purpose of this study was to evaluate the prevalence of antibodies to Canine Distemper Virus (CDV) in unvaccinated rural dogs without known immunization status and to assess risk factors for infection by means of indirect immunofluorescent assay (IFA), in Ahvaz district, southwest of Iran. Serum samples were randomly collected from 97 healthy dogs 6 months and older from villages around Ahvaz city between 2004 and 2005. Dogs were grouped by age and sex to determine whether these factors were associated with antibody titers, using Fisher's exact test. Seroprevalence to CDV antibodies in these dogs were 17.52% indicating that this virus is present in the ecosystem. Also there is evidence of previous natural exposure to CDV. Prevalence of antibodies to CDV did not differ between sexes or ages. Rural dogs are abundant in the ecosystem of area and interact with other species of wild carnivores and domestic animals in ways that could encourage disease transmission. Therefore the role of rural dogs in the epizootiology of CDV in both urban dogs and wildlife needs to be further explored.

**Key words:** Canine distemper virus, IFA, dog, measles

### INTRODUCTION

Canine Distemper Virus (CDV) is a member of the genus *Morbillivirus* in the family *Paramyxoviridae* and is closely related to viruses that cause measles, rinderpest and distemper in other animals (Neumeister *et al.*, 2001). Canine distemper has been recorded in domestic dogs for centuries. It is now recognized as a worldwide problem of carnivores and has the second highest fatality rate of any infectious disease, after rabies, in domestic dogs (Deem *et al.*, 2000). CDV infection in dogs generally is transmitted by inhalation of infectious aerosols. Contact between recently infected (subclinical or diseased) animals maintains the virus in a population and a constant supply of puppies helps to provide a susceptible population for infection (Greene and Apple, 1998). CDV has a wide host spectrum and during the past years, distemper has been observed in species that were previously not considered to be susceptible (Harder and Osterhaus, 1997). Practical diagnosis of canine distemper is primarily based on clinical suspicion. Various virologic and serologic methods are used for the diagnosis of the disease. At this time, serum antibodies are detected by various techniques including SN, IFA, ELISA and virus neutralization tests (Tizard and Ni, 1998). A dramatic change was seen in the risk of this disease when modified

live CDV vaccine administered in urban dogs of Ahvaz (a tropical city at the southwest of Iran). In recent years, an increasing number of CDV outbreaks of varying severity among rural dog populations of this geographic region have been occurred (unpublished data). But specific laboratory diagnostic tests are not available to confirm the suspicion of CDV infections and the practicing veterinarians must instead rely on nonspecific findings of routine laboratory procedures or pathologic findings. In this study, IFA was used to investigate the presence of canine distemper antibodies in unvaccinated rural dogs in Ahvaz.

### MATERIALS AND METHODS

**Canine sera:** From 2004 to 2005, a total of 97 blood samples were collected from the dogs aged from 6 months to 10 years. Samples were taken from rural dogs (33 male, 64 female) of villages around Ahvaz city (south west of Iran). After coagulation, blood samples were centrifuged at 2000 rpm for 5 min. Sera were separated and heat inactivated at 56°C, before storing at -20°C.

**Preparation of antigen:** Antigen preparation was performed as described by Waner *et al.* (2003) but with some modifications. Due to unavailability of a reference

strain of CDV for us, we used the Vero cells infected with a vaccine strain of Measles virus (AIK-HDC, Razi institute, Iran), as the substrate antigen. Measles virus and CDV both belong to *Morbillivirus* genus, members of which show antigenic cross reactivity in immunological assays (Stephenson and Meulen, 1979). In brief, Vero cells cultured in a 25 cm<sup>2</sup> flask, were infected by a 1:20 dilution of vaccine in Dulbecco's minimal essential medium (DMEM, Bahar Afshan, Iran) and incubated for 1 h at 37°C on a shaker. The inoculum was then removed, DMEM supplemented with 2% fetal calf serum was added and the cells were incubated at 35°C for 5 days. Virus replication was confirmed by the appearance of syncytium in the cell culture. When a widespread viral cytopathic effect was observed, the cells were trypsinized, washed with PBS and placed on slides in three spots. The slides were air-dried, fixed in cold acetone for 10 min and stored at -85°C.

**Indirect Fluorescent Assay (IFA):** The IFA substrate slides consisted of Vero cells infected with Measles virus. A 1:10 dilution of control and test sera was prepared. Virus-infected cells were incubated with diluted sera in a humidified chamber for 30 min at 37°C. Slides were thoroughly washed and air-dried before incubating with a 1:10 dilution of fluorescein-conjugated, affinity-purified rabbit antidog IgG (Sigma, USA) in a humidified chamber for 30 min at 37°C. Slides were washed again and coverslips of each slide were prepared and mounted, using glycerol-phosphate-buffered saline mounting solution. A non-infected Vero cells control spot was also included for each serum. Sera with a specific cytoplasmic fluorescence in the infected cells but no fluorescence in the control spot were considered as positive.

**Statistical analysis:** Serologic test results and potential association with age and sex were analyzed using SPSS 10.0 for windows and by use of Fisher's exact test. Differences were considered significant at  $p \leq 0.05$  (Cattet *et al.*, 2004).

## RESULTS

The presence of CDV-specific serum antibodies was detected in 17 (17.52%) of the 97 dogs sera by indirect fluorescent antibody test. Prevalence of antibodies against CDV was nonsignificantly higher in females (21.20%; 7 of 33) than males (15.62%; 10 of 64). There was no correlation between the presence of CDV antibodies and ages of seropositive animals. However, the greatest prevalence of antibody to CDV (27.27%; 3 of 11) was observed in sera of dogs, greater than 5 years old (Table 1).

Table 1: Distribution of antibodies against CDV in sera collected from dogs based on various ages by indirect fluorescent antibody test

Age (year)	No. of dogs	IFA positive sera (%)
< 1	12	(2) 16.67
1-3	43	(7) 16.28
3-5	31	(5) 16.13
> 5	11	(3) 27.27
Total	97	(17) 17.52

## DISCUSSION

The present study is the first report on seroprevalence of CDV in dogs in Iran using IFA. Immunofluorescent techniques can facilitate a specific diagnosis of canine distemper (Greene and Apple, 1998). Also, there are several advantages to the indirect technique of IFA and is recently much favored over the direct test (Abbas *et al.*, 1994). The IFA revealed that 17.52% of animals had antibodies to CDV. The results indicate a previous exposure to the wild type virus, because the animals had not been vaccinated in their life. Despite contagious nature of CDV, the prevalence of antibodies among rural dogs of the region seems be low from those previously reported in Nigeria (Ogunkoya *et al.*, 1985), Sweden (Olson *et al.*, 1990) and England (Tennant *et al.*, 1991). This discrepancy may be due to the hot climate of the area and the fact that the virus is unstable outside the host and deteriorates fast (Greene and Apple, 1998; Tilley and Smith, 2000). The detection of lower rate of antibody carrier animals also may reflect the nonexistence or low levels of colostrum intake, which results from chronic infection or lack of vaccination. However, the comparable results have been previously reported by studies on dogs from Turkey (Gencay *et al.*, 2004; Ozkul *et al.*, 2004), Japan (Gemma *et al.*, 1995) and the United States (McCaw *et al.*, 1998), as well as other species in Canada (Cattet *et al.*, 2004) and Alaska (Chomel *et al.*, 1998).

Prevalence of antibody against CDV did not differ between sexes in this study and other previous surveys (Twark and Dodds, 2000; McCaw *et al.*, 1998; Cattet *et al.*, 2004; Zarnke *et al.*, 2004). Age was not significantly associated with serum CDV antibody titers but was higher in older dogs than puppies. Age distribution of dogs with antibody to CDV was not significant in other serologic surveys (Gencay *et al.*, 2004; Twark and Dodds, 2000; McCaw *et al.*, 1998). But other previous studies showed that prevalence of antibody to CDV in adults is more common than in juveniles (Zarnke *et al.*, 2004; Guo *et al.*, 1986).

In conclusion, the presence of CDV in rural dogs could be a source of canine distemper infection for urban dogs with non-specific antibody response to the virus in the region. A possibility of prolonged virus excretion from seropositive animals with mild antibody

titer is also regarded as a critical point for epidemiology of disease in rural dogs. Therefore, on the basis of the importance of rural dogs as guards and shepherds, vaccination of rural dogs is recommended.

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