Antibody Prevalence to Canine Distemper Virus (CDV) in Stray Dogs in Turkey

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RÉSUMÉ

Les échantillons de sérum de 609 chiens sains errants qui vivent dans les provinces turques d’Ankara, Mugla et d’Istanbul ont été collectés entre 2000 et 2002. Une recherche d’anticorps du virus de la maladie de Carré a été effectuée à l’aide d’une technique de neutralisation (VNA). La prévalence des anticorps neutralisants est de 9.03% (n=55) à la dilution de 1/2. Les titres d’anticorps des séums positifs sont compris entre 1/2 et 1/32. Ce résultat suggère que les chiens errants constituent une source potentielle d’infection par le virus de la maladie de Carré pour les animaux sensibles vivant en zone urbaine en Turquie.

MOTS-CLÉS : Virus de la maladie de Carré, séroprévalence, anticorps neutralisants, Turquie.

SUMMARY
In this study, serum samples were collected from 609 clinically healthy stray dogs in the Ankara, Mugla and Istanbul provinces in Turkey between 2000 and 2002. Dog sera were tested for the presence of canine distemper virus (CDV) neutralizing antibodies using Virus Neutralization assay (VNA). The prevalence of antibodies in animals against CDV was found to be 9.03% (n=55) in 1/2 dilution. It was determined that the antibody titers of positive sera were ranged between 1/2 and 1/32. This result suggested that the stray dogs are potential source of CDV infections for susceptible animals living in an urban environment in Turkey.

KEY-WORDS : Canine distemper virus (CDV), seroprevalence, neutralizing antibody, Turkey.

Introduction
Canine distemper virus (CDV), a morbillivirus (Paramyxoviridae family) is the causative agent of a serious infection in dogs and other carnivores [2] and has been shown to be antigenically related to other members of the genus [13]. CDV infection in dogs generally is transmitted by inhalation of infectious aerosols. CDV causes acute generalized infection or chronic localized and persistent infection in the central nervous system of dogs [20] and has received great attention throughout the world in recent years. CDV infection may result in subclinical disease, catarrhal form, which consists in gastrointestinal signs and/or respiratory signs, and systemic form characterized with fever, rhinitis, and respiratory problems frequently associated with central nervous system involvement [1,2]. CDV also induces a highly contagious systemic viral infection and often-fatal disease accompanied by a multifocal demyelinating disease in the central nervous system (CNS) in many species of carnivores [12, 24].

The virus is shed mainly in oronasal secretions but all discharges and excretions can carry the virus [1, 9]. Histologically, acute and subacute non-inflammatory encephalitis and subacute inflammatory and chronic plaques are distinguished [23]. Various virologic and serologic methods are used for the diagnosis of the disease [17]. The serologic diagnosis, such as neutralization assay [3] and ELISA [6] is the diagnostic method of choice. Neutralization of CDV has been used to detect antibodies in dogs and many species of carnivores [9, 15, 19, 21]. Sera from dogs in the acute phase of infection contain infectious CDV-antibody complexes [4]. The majority of puppies acquire CDV antibodies via placenta or colostrum. Control of CDV infection can realistically only be achieved by vaccination [7] but it may be impossible to eradicate the virus because of its global distribution and wide variety of susceptible host species [5]. In this study, the presence of canine distemper infection in stray dogs was investigated in order to dispel suspicions that stray dogs are the main reservoir of the infection in an urban environment as has been previously shown [18].

Material and Methods
CELL CULTURE AND VIRUS
Vero cells were used in the study and were cultivated in Dulbecco’s minimal essential medium (DMEM, Biochrom, Germany) supplemented with 5% heat-inactivated fetal calf serum (FCS, Biochrom, Germany). Vero cells were used for virus propagation, titration and neutralization procedures. Ondersteport strain of CDV was used as a reference virus in the study. Following the propagation in Vero cells, the virus was harvested by one cycle of freezing and thawing. The
resulting supernatant was liquated and stored at -80°C. The stock virus was then titrated according to the method of Ohashi et al., [14] and used in 100 DKID₅₀ dilution in VNA.

**CANINE SERUM SAMPLES**

From 2000 to 2002, a total of 609 serum specimens were sampled from the dogs aged from 3 months to 7 years. Of these, 227 serum samples were collected from dogs in and around Ankara, 118 in Mugla and 264 in Istanbul. Forty-four of these specimens were obtained from various patients in Ankara University Veterinary Faculty clinics and 183 in dog rehabilitation centers (DRC) and kennels in Ankara. The remaining 118 sera were sampled from various DRCs and kennels around Mugla and Istanbul. The list of the serum samples collected during the study is presented in Table 1. Serum specimens in vacuum blood tubes (Venoject, Belgium), were separated by centrifugation at 1000 rpm for 10 minutes. The dog sera were heat-inactivated at 56°C for 30 minutes and stored at -20°C until use.

**VIRUS NEUTRALIZATION ASSAY (VNA)**

The presence of CDV-specific serum neutralizing antibodies was assessed by virus neutralization assay using the CDV Ondersteeport strain in serum samples from 609 dogs. Vero cell suspension containing 2×10⁵ cells/ml were grown in 24-well plates. Two subsets of sera were tested for their neutralizing activity against CDV. Serial twofold serum dilutions starting from 1/2 were prepared in eppendorf tubes. A hundred µl of each serum dilution was mixed with an equal volume of 100 TCID₅₀ diluted (1:750) CDV suspension. After overnight neutralization at 4°C, 24-well plate containing monolayer Vero cells was inoculated in duplicate with 100 µl of each serum-virus mixture. The plates were incubated at 37°C for one hour in a humidified 5% CO₂ atmosphere for adsorption of free virus particles and then wells were washed once with DMEM (Dulbecco’s modified Eagle medium). The inoculated plates were further incubated under 5% CO₂ atmosphere at 37°C for 5 days after adding FCS-free DMEM to the wells. The results were assessed at the end of the 5-day post-inoculation (dpi). The reciprocal of serum dilutions in which at least 50 % of 100 TCID₅₀ virus infectivity was blocked, was accepted as mean antibody titer against CDV strain Ondersteeport.

**Results**

The presence of CDV-specific serum antibodies were detected in 55 (9.03%) of the 609 dogs sera by direct neutralization assay following a 5-day incubation. The greatest prevalence of antibody to CDV was observed in sera of dog in Ankara with 12.77% (29 of 227). CDV neutralizing antibody was less prevalent in dogs in Istanbul (7.19%; 19 of 264) and Mugla (5.93%; 7 of 118). The regional distribution and prevalence values are presented in Table 1. The youngest animal in which antibody response has been detected was 9 months old. In addition, there was no correlation detected between neutralizing titers in the dogs at different ages in seropositive animals. The seroconverted dogs (n=55) had mounted neutralizing antibody titers between 1/2 and 1/32. The highest ratio (45.45%, 25 of 55) of neutralizing antibody was found at 1/4 dilution. Details with regard to the antibody titers are presented in Table 2.

**Discussion**

The present study is the first comprehensive report on seroprevalence of CDV in stray dogs in Turkey between 2000 and 2002. In this study, the presence of neutralizing antibodies against CDV has been demonstrated in dogs. The VNA revealed that 55 (9.03%) out of 609 serum samples had specific neutralizing antibodies to CDV (Table 1). This result obviously showed that distemper virus infection is circulated between stray dogs in urban life. On the other hand, it seems that there was no correlation between the presence of CDV neutralizing antibodies and the ages of dogs sampled.

The overall percentage (9.03%) of seroconversion indicated previous exposure to the wild type virus, because the youngest seroconverted animal detected in the study was 9 months old. Another possibility, however, should be taken into consideration that some of these animals might have been vaccinated at sometime during their life, even though they are stray dogs or are housed in DRCs. On a regional basis, the highest prevalence of CDV-specific antibody was detected in Ankara with 12.77% (29 of 227). Results are fairly low from those previously reported in some countries [14, 16, 22]. However, they are compatible with a previous
study, which was conducted in Ankara province in Turkey [18] and with those obtained from a survey in Japan [10]. The detection of lower rate of antibody carrier animals may reflect the nonexistence or low levels of colostraum intake, which results from chronic infection or failure of vaccination. Moreover, occurrence of low neutralizing activity with Ondersteep strain of CDV may not correspond to real rates and/or titers against the field viruses.

The presence of CDV in housed dogs in Mugla province located in the Aegean coast may raise another serious question, which is the possibility of transmission of the virus to sea mammals. Accidental contacts between virus secreting free-ranging dogs and Mediterranean seals which often visit natural harbors may result in spreading the virus through local marine mammals as described previously [11, 15, 19, 21]. CDV-infected dead stray dogs in seaside are potential virus source for the marine mammals that are also carnivore. Similar situation has been described after CDV epidemics in Caspian seals in 1997. The possible source of CDV in seals was proposed as terrestrial carnivores particularly wolves, which co-exist with seals on the winter ice in the Northern Caspian Sea [8].

The protective antibody titer for CDV that may be acceptable was offered as ≥1/80 in a previous study [17]. The antibody titers of only two sera were determined as 1/32 in this study. The titers of antibody in seropositive animals indicated that even though they had been exposed to the virus in their life, the resulting antibody response is below the efficacious protective level but has some contributory role on the etiological diagnosis of the disease.

In conclusion, stray dogs are accepted as predominant source of canine distemper infection in metropolitan life in Turkey. In addition, a possibility of prolonged virus excretion from seropositive animals with mild antibody titer (unpublished data of authors) is also regarded as a critical point for the disease epidemiology in animals with non-effective antibody response to the virus. Free-ranging dogs should be kept under control in local DRCs in order to protect housed animals not only from CDV but also other critical disease which threaten animal and public health. If these animals are not able to be under control, the alternative vaccination policies against disease (i.e. oral vaccination like in rabies case) should also be considered. Finally, we emphasize that further antigenic and seroepidemiological studies in dogs are needed to clarify the pathogenic and epidemiologic aspects of canine distemper infection in Turkey.

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References