Seroprevalence of Canine Coronavirus in Kars Shepherd Dogs in Kars Province, Turkey

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ABSTRACT

Canine Corona virus (CCoV) is responsible for acute diarrhea, vomiting, appetite loss, lethargy and bronchopneumonia in dogs. This study is a serological investigation of the CCoV in Kars Shepherd Dogs owned by small-scale family farming enterprises in the Kars province in Northeast Anatolia, Turkey. Blood was randomly collected from a total of 102 clinically healthy Kars Shepherd Dogs, 53 females and 49 males older than 1 year of age, unvaccinated for CCoV. The samples were analyzed for CCoV-specific IgG antibodies using indirect enzyme-linked immunosorbent assay (I-ELISA). The seroprevalence for the CCoV antibodies in Kars Shepherd Dogs was 75.49% (77/102), with no significant differences among the genders and age groups. This is the first serological survey for CCoV antibodies in Kars Shepherd Dogs in the Kars province.

Keywords: ELISA; Canine coronavirus; IgG; Turkey; Kars Shepherd Dog.

INTRODUCTION

Canine Coronavirus (CCoV) is an important, highly contagious virus of the coronaviridae that causes gastroenteritis in dogs. The enveloped virus has a pleomorphic structure, and has a diameter of 60-220 nm. It consists of a single ssRNA (positive sense) molecule of 27-32 kb. CCoV has two gentypes, types I and II. CCoV type II is divided into subtypes IIa and IIb (1). CCoV can mutate, resulting in more virulent strains and corresponding increased severity of enteric illness. Highly virulent pantropic CCoV strains belong to subtype IIa. A pantropic CCoV strain (CB/05) has been recently associated to a fatal outbreak of systemic disease in young dogs, including hemorrhagic gastroenteritis and lymphopenia. These signs would be difficult to distinguish from those of parvovirus infection (2). The virus was first reported in dogs with gastroenteritis in 1971 in Germany, and later in mixed infections with canine parvovirus (CPV) infections (3, 4). CCoV infections in dogs vary clinically. Infection is asymptomatic or mild with a quick recovery in adult dogs (5). The infection causes high mortality in newborn puppies, with a poor prognosis. Secondary bacterial, parasitic or viral infection may complicate the disease in puppies and may cause death (6). Clinical signs of CCoV infection may include weight loss, depression, anorexia, acute bloody watery diarrhea, vomiting and dehydration. Death, mostly due to dehydration, may occur within 24-36 hours (7, 8). Pathologically, marked duodenal and jejunal villous degeneration with intestinal mucosal and msenteric lymph node, edema and congestion are noted (9). Infected dogs shed CCoV for 6-9 days in their feces. The primary transmission mode of CCoV among dogs is feco-oral (10).

CCoV infection clinically resembles several other viral infections in dogs, so a definitive diagnosis requires laboratory confirmation and several diagnostic methods, including enzyme linked immunosorbent assay (ELISA) (11, 12, 13, 14), polymerase chain reaction (PCR) analysis (1, 15, 16),

serum neutralization (SN) (17) and immunofluorescence (18, 19) may be used. Indirect ELISA is one of the most sensitive, reliable and quick methods (20).

Kars Shepherd Dogs are bred in the Northeast part of Turkey, especially in the Kars region. This region of Turkey neighbors other Caucasian countries. Kars Shepherd Dogs are characteristically similar to the Caucasian Ovcharka, present in Georgia, Armenia, Azerbaijan and Iran. Kars Shepherd Dogs are specific Turkish livestock-guarding breed gathered under the general rubric of Turkish shepherd dogs (21). Kars Shepherd Dogs were only reported for the first time by Nelsonin 1996 (22).

Kars Shepherd Dogs are strongly muscled and well boned. The head is large and mastiff and often has a dark fascial mask. Coat color is dark or light agouti gray, and it is lighter (light gray or yellowish) towards the tail and legs. Coat length can be long, medium or short. Ears are triangular and hang tight to the head. Eyes are brown or dark brown, medium-sized and oval (23).

In the present study, a serological survey for CCoV was performed on Kars Shepherd Dogs, owned by small-scale family farming enterprises in the Kars province, using indirect ELISA.

MATERIALS AND METHODS

Ethics Statement

This research was conducted after the approval of Kafkas University Animal Testing Local Ethics Council (Approval Number: KAU- HADYEK-2016-064).

Study Area and sampling

This study was conducted in the Kars province, north-eastern Turkey (43.05° E and 40. 36° N), and its vicinity. It is mountainous and has a cold climate and neighbors other Caucasian countries (Figure 1). In this study, whole blood was collected from 102 unvaccinated, clinically healthy, Kars Shepherd Dogs of above one year of age, owned by small-scale family farming operations in the Kars region. None of the dogs were vaccinated against CCoV. Definite health records of the sampled dogs were not obtained so history of diseases was not available. Blood samples were collected between June to

Figure 1: Geographical positioning of the Kars province in which the study was performed.

October 2015. Whole blood samples were collected from the saphenous vein into sterile vacotainer tubes containing no anticoagulant, brought to the laboratory, centrifuged (3000 rpm for 20 min) and havested sera were stored at -20°C pending analysis.

Indirect enzyme linked immunosorbent assay (I-ELISA)

Commercial ELISA (EVL/European Veterinary Laboratory, Woerden Netherlands, Cat No: D1005-AB01) used for the detection of IgG antibodies against CCoV was carried out according to the manufacturer's instructions. Briefly, 100 µL of tested serum, diluted at 1:50 in ELISA buffer, were added to wells, and incubated for one hour at 37°C and then washed with wash solution (diluted in deionized water). Then, 100 µL of Horseradish peroxidase (HRPO) conjugated anti-dog antibodies conjugate were added to each well and incubated for 60 min at 37°C. Unbound conjugate was removed by washing, and 100 µL of substrate solution were added to the wells, and incubated at room temperature for 20 min, and then the enzymatic reaction was stopped by adding 50 µL of stop solution. ELISA results were analyzed with an automated ELISA reader (Epoch, BIO-TEK, USA) at 450 nm. According to the kit procedure: A sample was scored positive if the OD is ≥ 2.5 x OD of the negative control. It was deemed negative if the OD if the sample was < 30 and suspect if it was between 90 and 270.

Statistical Analysis

The chi-square ($\chi 2$) test was used to compare the proportion of positive CCoV serological results among different genders and age groups (Minitab 14.0 Inc., State College, PA, USA). Significance level was set at $P < 0.05$.

RESULTS

This study included 102 Kars Shepherd Dogs, 53 females and 49 males, aged ≥ 1 year, owned by small-scale family farming enterprises (1 to 7 dogs per operation) in the Kars region, not vaccinated for CCoV. The CCoV seroprevalence was 75.5% (77/102). The gender-based seropositivity distribution rates were 79.2% (42/53) for females and 71.4% (35/49) for males, with no statistically significant difference between genders. Animals were grouped according to the ages, the highest seropositive ratio (78.2%) was found in 4-7 year-old dogs. Of the 37 animals aged 1-2 years, 27 (72.9%); of the 33 animals aged 2-4 years, 25 (75.7%) and of the 32 animals aged 4-7 years, 25 (78.2%) were found to be positive. The numbers of the CCoV antibody positive dogs were found to increase by the age, however there was no statistically significant difference between age groups.

DISCUSSION

The Kars Shepherd Dogs, raised throughout the Kars province, guard herds in the high mountains and valleys of northeast Turkey, and are adapted to local climatic conditions. They have characteristics that are similar to the Caucasian Ovcharkain (Caucasian Mountain Dog) of the Caucasus countries bordering the Kars region (21). These dogs have a highly developed instinct for protection and protect herds, homes as well as businesses, and therefore are important for the people in this region. In order to ensure the survival of this local breed, which is present in small numbers over a limited geographical area of Turkey, important diseases must be studied and the necessary measures must be taken against these diseases.

This study investigated the presence of CCoV serologically in Kars Shepherd Dog breed in Kars province, where the main income is animal husbandry and rearing, therefore majority farmer has a shepherd dog. These animals have mostly been used for shepherd dogs in small-medium scale private goat and sheep breeding enterprises, so number of the dogs per farm was among 1 to 7. The dogs are native pure breed dogs in Northeast Anatolia but they have no pedigree papers. Definite health records of the sampled dogs were not obtained, so any clinical symptoms were known. Because vaccination does not apply to these dogs in the region, none of these dogs were previously vaccinated against any viruses. Furthermore, Canine Adenovirus (CAV) was only reported for the first time by Yildirim *et al.*(21) in Kars Shepherd Dogs. There has been no further extensive investigation in Kars Shepherd Dogs to detect viral diseases in Turkey. Yildirim *et al.* (21) investigated the presence of Canine Adenovirus (CAV) serologically in Kars Shepherd Dogs in the same region where this study was conducted and detected seropositivity of 65.95%.

In this study, the seropositivity rate for anti-CCoV antibody in apparently healthy dogs in Kars Shepherd Dogs in the Kars region was 75.5% (77/102 dogs), which is similar

to previous studies (24, 25). The seroprevalence of CCoV antibodies varies between countries, ranging from 15.8% to 95.1% (6, 12, 17, 20, 24, 26, 27). In a study in Italy using the ELISA method by Pratelli *et al.* (20), 109 dogs were tested , and 80 (90.8%) were positive for CCoV-specific antibody. In Australia, Naylor *et al.* (26) reported that the CCoV seropositivity rate in dogs was 15.8% using the ELISA method. Yesilbag *et al.* (25) tested serum collected from 179 clinically healthy dogs from Bursa, Çanakkale, Yalova, Balıkesir, Urfa, Adana and Antalya provinces of Turkey from private owners, municipality shelters, a breeding kennel and stray dogs were tested using serum neutralization and ELISA methods and seropositivity was 62.5% and 74.3%, respectively in Turkish dogs. In a serological survey by Gür *et al.* (12) in apparently healthy, purebred Akbaş Dogs, Turkish greyhounds and the Kangal Dogs in the provinces of Sivrihisar (Eskişehir) and Konya using the ELISA technique, seropositivity was 94.7% in the Eskişehir region, 100% (27/27) in Kangal dogs in Konya and 95.1% in Turkish greyhounds. In another study, Avcı *et al.* (24) conducted a serological and virological investigation of CCoV infection in dogs in animal shelters in the province of Adana, including serum and stool samples from 121 dogs, using the ELISA and rapid test kits. The seroprevalence and viroprevalence were 75.2% and 14.87%, respectively.

From the results presented CCoV is common in Kars Shepherd Dogs. CCoV is highly contagious and spreads rapidly through susceptible dogs. CCoV is shed in the feces of infected dogs for week to months or longer, fecal contamination of the environment is the important source for virus transmission via ingestion (2). Because CCoV is transmitted feco-orally, it has been determined that the spread of the disease and seroprevalence rate are particularly high in environments with a large number of dogs in close contact (e.g. dog breeding farms, shelters, etc.) (28). In agreement with these, in the present study, the seropositivity rate was high in family operations, in which seveal dogs are kept together in close contact, and, with stray dogs roaming free.

The I-ELISA test for anti-CCoV antibody was chosen in this study because it is quick, practical and specific, and is therefore widely used today for antibody screening. Additionally, some studies have shown that the I-ELISA technique is more sensitive and reliable than the virus neutralization (VN) technique in identifying CCoV-specific antibodies, while the VN method may yield misleading results in epidemiological studies (20, 25).

In conclusion, the seroprevalence of anti-CCoV antibodies, in a local dog breed of Turkish dogs, Kars Shepherd Dogs in the Kars region, Turkey is reported here in for the first time, and is relatively high, indicating that CCoV is common among these dogs, and suggesting that preventive measures, such as vaccination, should be performed to decrease CCoV-associated morbidity and mortality. Further, epidemiological studies of the CCoV type are warranted.

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