

A Serologic Investigation for Canine Corona Virus Infection in Individually Reared Dogs in Central Anatolia

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Summary: Canine Corona virus (CCoV) is responsible for acute diarrhea, vomiting, appetite loss, lethargy and bronchopneumonia in dogs. In this study, a serologic investigation was conducted for CCoV in individually reared dogs. Total of 87 blood serum samples were collected from healthy and unvaccinated for CCoV, pure breed Akbaş dogs, Turkish greyhounds and Kangal dogs in Sivrisihar, Eskişehir and Konya provinces. According to indirect ELISA results; out of 19 Akbaş dogs samples, 18 (94.7%) were found to be positive in Eskişehir province. The other samples were obtained from Konya province, all of the 27 Kangal dog samples and 95.1% of the 41 Turkish greyhound samples were determined as seropositive. In total, out of 87 dogs, 84 (96.5%) were found to be seropositive for CCoV specific antibodies. These high proportions are notified to be the first data serologically indicated the presence of CCoV infection in Akbaş dogs, Turkish greyhounds and Kangal dogs in Central Anatolia.

Key Words: Canine corona virus, dog, Kangal, serology.

İç Anadolu'da Bireysel Olarak Yetiştirilen Köpeklerde Canine Corona Virus Enfeksiyonuna Karşı Serolojik Bir Araştırma

Özet: Canine Corona virus (CCoV); köpeklerde akut diyare, kusma, iştah kaybı, letharji ve bronkopnömoniden sorumlu tutulmaktadır. Bu çalışmada bireysel olarak yetiştirilen köpeklerde CCoV varlığına yönelik serolojik bir araştırma yürütüldü. Sivrihisar (Eskişehir) ve Konya illerinde, sağlıklı görünen ve CCoV virusuna karşı aşılanmamış saf ırk Akbaş köpeği, Türk tazısı ve Kangal köpeklerinden toplam 87 kan serumu örneği toplandı. Yapılan indirekt ELISA sonucunda Eskişehir bölgesinden toplanan 19 Akbaş köpek örneğinin 18' inin (%94,7) CCoV virüsüne karşı pozitif olduğu bulundu. Konya bölgesinden toplanan diğer örneklerde; 27 Kangal ırkı köpek örneğinin hepsi seropozitif bulunurken, 41 Türk tazısından alınan serum örneklerinin %95,1' i pozitif olarak belirlendi. Toplamda 87 köpek örneğinin 84' ü (%96,5) spesifik CCoV antikorları bakımından seropozitif olarak bulundu. Bu yüksek oranlar İç Anadolu'da Akbaş köpeği, Türk tazısı ve Kangal köpeklerinde CCoV enfeksiyonunun serolojik olarak varlığını gösteren ilk verilerdir.

Anahtar Kelimeler: Canine corona virus, Kangal, köpek, seroloji.

Introduction

Coronaviridae is a large virus family of human and domestic animals and effects both respiratory and enteric systems. Corona viruses have single stranded positive-sense genomic RNA 27-32 kb in size, belonged in the order of Nidovirales (5). The viruses in the Coronaviridae family were classified into three groups considering the host spectrum, the nucleotide sequence and the serological features. Group I includes Human Respiratory Corona virus (HCoV-229E), Porcine Transmissible Gastroenteritis Virus (TGEV), Feline Enteric Corona virus (FECoV), Feline Infectious Peritonitis Virus (FIPV), Rabbit Corona virus (RbCoV) and Canine Corona virus (CCoV) (9, 18, 19, 22).

CCoV was first described by Binn et al., (3). The agent has known as one of the most important

causes for severe enteritis in dogs (1, 12, 13) beside Canine Parvovirus, Canine Rotavirus and Canine Distemper Virus.

Main route for the infection is oral-fecal way. General clinical findings are acute diarrhea, lasts about two weeks, vomiting, appetite loss, and lethargy. Bronchopneumonia may be developed in some of the animals (2). The severity and the duration of signs may be increased in the presence of stress and the secondary parasitic, bacterial and viral infections. CCoV infects dogs in every breed and age groups but newborn puppies are much more sensitive, they can be severely affected and mortality may occur within 1-3 days (4). Adult dogs are generally possessed the infection as silent or mild.

In the present study, a serological investigation for CCoV was performed in individually reared pure breed dogs in Central Anatolia using indirect ELISA, to determine the presence and proportion of the infection.

Material and Methods

The sampled animals

Total of 87 samples were collected from individually breeding pure Akbaş, Turkish greyhounds and Kangal dogs in Sivrisihar, Eskişehir and Konya provinces. The number of breeding animals in per farm was 1 to 6, but only one adult dog was sampled in per farm to perform a healthier survey. Ages of dogs were from 8 months to 7 years, the average was 2.5 years old.

The blood specimens were drawn from *Vena saphena* and transferred to the laboratory in proper conditions. After the centrifugation in 3000 g for 10 minutes, serum samples were separated into stock tubes and kept in -20°C until to the test.

ELISA

A commercially available Canine Corona virus indirect ELISA test Kit (EVL, The Netherlands) was applied for detection of CCoV specific antibodies. The test was performed according to the producer's description and assessed using ELISA reader in 450nm filter. The obtained data and OD values were evaluated using positive and negative control wells.

The statistical analysis

Distribution of positive values according to the breeds and age groups were compared by using Khi-square test in multiple designs.

Results

According to indirect ELISA results; out of 19 Akbaş dogs samples, 18 (94.7%) were found to be positive in Eskişehir province. The other samples were obtained from Konya province; all of the 27 Kangal breed dog samples and 95.1% of the Turkish greyhound samples (n: 41) were detected as seropositive for CCoV. In total, 96.5% positivity was determined (84/87). The infection proportion was found to be similar in these two neighbor provinces.

The sampled dogs were grouped according to the age. Ages of the two negative dogs were under 1 year old, one of them was 9 months old and the other one was 10.5 months old. The third dog was in the second group with 17 month-olds. As could be seen in the Table 2, the numbers of the CCoV antibody positive dogs have been increasing by the age.

Table 1. The number of sampled dog and test results for Canine Corona virus.

Provinces	Breed	No of sampled	CCoV	
			Ab (+)	(%)
Eskişehir	Akbaş	19	18	94.7
Konya	Greyhound	41	39	95.1
Konya	Kangal	27	27	100
Total		87	84	96.5

Ab: Antibody, ($\chi^2=1.404$, $p=0.589$)

Table 2. Distribution of CCoV positivity according to the age groups.

Ages	No of the dogs	CCoV	
		Ab (+)	(%)
1≤	13	11	84.6
1-2	51	50	98
2-4	16	16	100
4-7	7	7	100
	87	84	96.5

($\chi^2=6.724$, $p=0.087$)

The statistical analysis

As a result of Chi-square test in multiple designs, significant differences have not been determined among different breeds ($\chi^2 = 1.404$, $p=0.589$) and age groups ($\chi^2 = 6.724$, $p=0.087$).

Discussion

In this study, a serologic investigation was conducted to prevent the divergences related to population dynamics in individually reared dogs. The number of breeding dogs was among 1 to 6 in the studied farms. The samples were obtained from pure breed Akbaş, Turkish greyhound, and Kangal dogs in private farms that have been breeding sheep or cattle in Konya and Eskişehir provinces. Mentioned dogs are native pure breed dogs in Anatolia. Kangal and Akbaş breeds are excellent shepherd dogs, and Turkish greyhounds have been used in hunting. Vaccination status is known and unvaccinated ones have been used in this study.

Various test systems can be used for antibody detection, but in routine Virus neutralization (VNT) (10) and ELISA (17, 21) tests have been widely used for CCoV. Pratelli et al., (15) assessed ELISA and VNT comparatively; out of 109 dog serum samples, 80 samples were determined as positive in both tests but only 17 samples were reported as positive by ELISA. In another study (23), positivity proportion was detected as 62.5% by VNT and 74.3% by ELISA. In this study, ELISA test was preferred for serological diagnosis of CCoV due to the fact that it is more sensitive and specific than VNT, but antibody titer was not determined. Therefore, the exact time of viral exposure could not be predicted with the available information. However, it is obvious that out of 87 dogs, 84 were exposed to the virus (96.5%) (Table 1). This ratio was found to be higher than expected for individual breeding but this is the first study in Central Anatolia. Therefore, the regional differences would be a factor.

Canine enteric corona virus (CECoV) infection is well known to be widespread in the whole world. Tennant et al., (20) detected sero-prevalence ranging from 76% to 100% in a commercial breeding colony in UK.

There were few studies for CCoV in Turkey. A CCoV outbreak was reported in a kennel with very high mortality in pups in Bursa province; sero-prevalence was detected as 94.5% (8). In a sero-survey (23), sero-positivity proportion for CCoV was detected as 94.2%, 71.4% and 68.7% in

kennelled, strayed and privately owned dogs respectively. We know that, intensive breeding induces transmission (14), and the sub-clinical sero-conversion is a frequent event in breeding colonies (20). The longest period of virus shedding in faeces has been detected as 150 days in post infection period using nested-PCR in naturally infected pups (14). The incidence of infection could be increased in a very short time especially in kennels (6). Similarly, Naylor et al., (11) performed serological study in Australia, and detected 15.8% in open population and 40.8% in kennelled dogs. Even though individually reared dogs were used in this study, obtained proportion values were found to be high from expected.

Corona virus infection is rarely associated with high mortality rates especially in adults, but newborn pups could be severely affected; fatal outbreaks had been reported previously (7, 13). The age of animals and the sub-types of virus are main factors determining the pathogenesis. In addition, as well as the presence of infected dogs, the presumptive factors such as crowding, every kind of stress, poor hygiene and management conditions are important for prognosis.

The animals used in this study have normal/good management conditions due to individual breeding; it was thought that the probability of contamination would be lesser, but as it can be seen in the test result, antibody proportion was above 95%. The animals were breeding freely in the farm and especially Kangal and Akbaş breed dogs have been used as shepherd dogs; possibly the dogs were contaminated by other dogs in the field.

Animals were grouped according to the ages (Table 2), the number of antibody positivity is being increased in all two-year-old dogs. The animals may be infected via contact with animals which are in acute phase or in contaminated environment with excrements.

Due to difficulties for virus isolation from the cell culture, PCR analysis is necessary for the direct diagnosis (13, 16). All of the sampled dogs were healthy adults in this study and only serological examination was performed to determine the prevalence of infection. Considering the sub-clinical adult infections, it is possible to say that, newborn puppies are at risk in the region and the vaccination must be performed for all animals especially in kennels.

Studied breed were pure and the number of pure breed dogs have been diminished over the years. Therefore, the occurrence of mortality in newborn-young pup should be prevented. Definitive diagnosis of CCoV is difficult because it can easily be mixed with viral, bacterial and parasitic infections along with poisons and non-infectious causes of enteritis, but rapid diagnosis and the application of vaccination are essential for the control of epidemics.

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