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Canine coronavirus infection in dogs in Turkey: Virological and serological evidence

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ABSTRACT

In the present study, virological and serological investigations were performed to determine the presence and prevalence of Canine corona virus (CCoV) infection in dog population in Turkey. Sera samples were analyzed for specific antibodies against CCoV by indirect enzyme linked immunosorbent assay (i-ELISA) while leukocyte samples were inoculated onto monolayers of Madin Darby Canine Kidney permanent cell culture. The cells were examined for viral antigen by direct immunofluorescence (IF) test after third passage. CCoV seropositivity was found in 46 (24.46%) of 188 dogs by indirect ELISA while only one leukocyte sample (0.53%) was detected as antigen positive by IF. Seropositive and antigen identification results were considered as indication of infection. From the results of this study it can be concluded that CCoV infection is widespread in the Turkish dog population and the virus may be attributed to be one of the important viral agents in dogs. In conclusion diagnosis of CCoV is difficult because it can easily be mixed with respiratory, enteric and generalized infections by other viral, bacterial and parasitic agents, but diagnosis and the vaccine application are essential for the control and prevention of CCoV infections.

Key words: CCoV, Dog, Immunofluorescence, i-ELISA, Sera.

INTRODUCTION

The emergence of human severe acute respiratory syndrome encouraged renewed interest in animal coronaviruses as potential agents of direct and indirect zoonosis and has new attention focused on coronaviruses. Coronaviruses are large viruses that cause respiratory, enteric and generalized disease in humans and domestic animals (Chinese SARS Molecular Epidemiology Consortium 2004, Slingenbergh *et al.*, 2004; Lau *et al*., 2005). Coronaviruses, Nidovirales order, have single stranded positive-sense genomic RNA (De Vries *et al.*, 1997; Decaro and Buonavoglia 2008).

Canine coronavirus (CCoV) belongs to one of the major antigenic groups of coronaviruses (Lai and Holmes 2001; Pratelli, 2011; Costa *et al*., 2014). CCoV, first described by Binn *et al*. (1974), can affect both domestic and wild dogs worldwide (Decaro and Buonavoglia, 2008; Priestnall *et al.*, 2014). Mild disease or asymptomatic carriages are probably in many cases common outcomes of infection (Graham and Baric 2010). To date, two different genotypes of CCoV are known, CCoV type I (CCoV-I) and CCoV type II (CCoV-II). CCoV type II is divided in two subtypes, CCoV-IIa (classical strains) and CCoV-IIb, with CCoV-IIb emerging as a result of a putative recombination between CCoV-IIa and transmissible gastroenteritis virus (TGEV) (Ntafis *et al.*, 2013).

Different serologic (indirect ELISA, micro-neutralization) (Mochizuki *et al.*, 1987; Tuchiya *et al.*, 1991; Elia *et al.*, 2003; Buonavoglia *et al.*, 2006; Le Poder, 2011) and virologic (direct ELISA, cell culture, direct immunofluorescence) diagnostic methods are widely used to detect the genotypes of CCoV (Costa *et al.*, 2014).

In the present study, it has been hypothesized that CCoV infection which reported from different region of Turkey may affect dogs in Konya, Isparta and Burdur. The aim of this study was to determine the serological and virological status of CCoV infection in mentioned provinces.

MATERIALS AND METHODS

Animals: Blood samples were collected from 111 dogs showing clinical symptoms which were admitted to the Internal Medicine Clinic of Faculty of Veterinary Medicine, Selcuk University, and from 77 unvaccinated dogs, which were housed at dog shelters in Isparta (n: 49) and Burdur (n: 28) provinces and were randomly sampled irrespective of their displaying clinical signs.

Indirect ELISA tests: Serum samples were tested for specific antibodies against CCoV by commercially available indirect enzyme linked immunosorbent assay (EVL/ European Veterinary Laboratory, Catalog no: D1005-AB01, Netherlands) kit. The test was performed according to the manufacturer's instructions and assessed using automatic ELISA plate reader (Rayto RT-2100C, China) 450nm filter.

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Results were analyzed on the basis of the optical densities values of samples using (%) positive and negative control wells.

Cell culture: Madin Darby Canine Kidney (MDCK) permanent cells were grown in Dulbecco's Minimum Essential Medium (DMEM, Biological Industries, Israel) supplemented with 100 U of penicillin/mL, 100 µg of streptomycin/mL and 5% fetal calf serum (Biological Industries, Israel) for CCoV isolation. The cells were incubated in an atmosphere of 5% CO_2 incubator (Nuve, Turkey) at 37°C.

Blood samples: Blood samples were drawn into sterile tubes with EDTA. Leukocyte samples were prepared by a standard method (Bas and Demet, 1992) and were kept on -20ºC until being used. Leukocyte samples were analyzed for CCoV antigens by direct immunofluorescence (IF) test after third blind passage on monolayers of MDCK permanent cell culture system.

Direct immunofluorescence: For the IF test, the leukocyte samples inoculated into the 24-well micro plates were retrieved from -20ºC and thawed in a water bath at 37°C. The third blind passages of the leukocyte samples grown in MDCK cell culture were made in 24-well micro plates. Each well of the Lab-Tek chamber slides procured from Thermo Fisher Scientific (USA, Catalogue no. 178599) was inoculated with 200 µL of MDCK cells. Next, the slides were incubated at 37°C for 24 hours. Later, 20 µL of the third-passage fluid of each leukocyte sample was inoculated into two wells. After adsorption period (24hour), the cell surfaces were rinsed with phosphate buffer solution (PBS) and 200 µL of medium without serum was added to each well. At the end of the third day, the medium was removed and the ready-to-use CCoV conjugate procured from VMRD (USA, Catalogue no. CJ-F-CCV-10 mL) was added to all wells, and the slides were incubated in humid chamber at 37°C for 30 min. After incubation period, the conjugate was removed from the micro plates wells, and the cells were rinsed with FA Rinse Buffer, pH 9.0 (VMRD catalogue no. 210-90-RB) for three times and allowed 10 min for drying. 50 µL of 90% glycerol solution was added to each well. Finally, the wells were examined under a fluorescence microscope (Olympus Bx51, Japan).

Statistical analysis: Results of this study amongst provinces, sex, and breeds were calculated by using chi-square test (Minitab 14.0 Inc., State College, PA, USA). Difference were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

A total of 46 (24.46%) from 188 dogs were detected as seropositive for CCoV antibodies by indirect ELISA (Table 1). Only one leukocyte sample (0.53%) from 2 years old male dog from Burdur province, was detected as antigen positive by IF (Fig. 1, Fig. 2) while antibody against CCoV was negative. There was no statistical significance when CCoV antigen was determined between female (31/67) and male (15/65), whereas existence of CCoV antigen in street dog (46/111) was higher ($P<0.05$) than owned dogs (0/31).

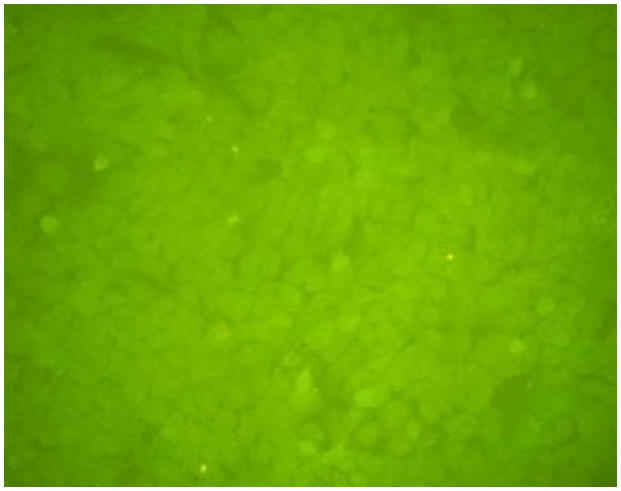

Fig 1: MDCK cell control (X40).

Table 1: Prevalence of CCoV according to province, breed, and sex.

Provinces	Breed	No of samples	Sex Male/Female		Total +/-
Konya	Siberian Husky	2	-	2	13/98[a](n:111)
	Cross breed with Pitbull	2	1	1	
	Akbas dog	1	-	1	
	Kars Shepherd dog	1	1	-	
	Crossbreed with Kangal	21	6	15	
	Spanish cocker	1	1	-	
	Pointer	1	-	1	
	Terrier	1	-	1	
	Beagle	1	-	1	
	Street dog	80	37	43	
Isparta	Street dog	49	19	30	18/31[b](n:49)
Burdur*	Street dog	28	15	13	15/13[b](n:28)
Total		188	80	108	188

[a, b:] Different letters in same column are statistically significant ($P<0.001$)

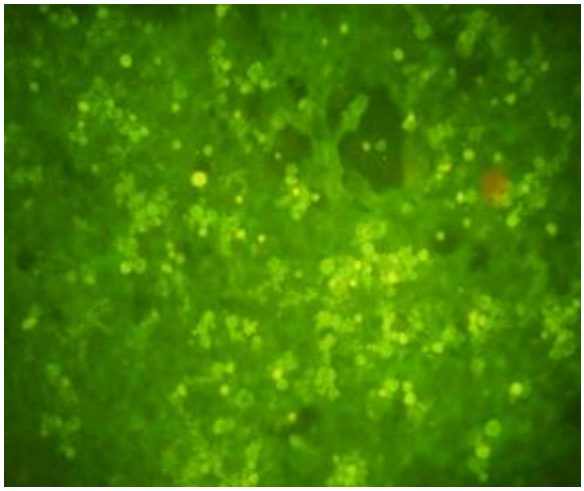

Fig 2: Antigen positive sample by IF (X40).

Canine Coronavirus is generally reported from dogs with gastroenteritis but mix infections by canine parvovirus (CPV) have been detected in the recent years (Evermann *et al.*, 2005; Bodewes and Egberink, 2009). Different laboratory diagnostic tests can be used for specific antibody detection, but generally two tests [ELISA (Tuchiya *et al.*, 1991; Gur *et al.*, 2008; Ellis *et al.*, 2011) and Virus Neutralization (Mochizuki *et al.*, 1987)] have been widely used for detection of CCoV. Serum neutralization tests (Huxsoll *et al.*, 2006) and direct florescence antibody test (dFAT) (Hansa *et al.*, 2012) can also be used for definitive diagnosis of CCoV.

The results in the present study are the first evidence for CCV infection on dogs in Isparta and Burdur provinces. Serologic diagnosis of CCV in Kangal dogs in Konya have been reported earlier by Gur *et al*. (2008) but in the present study, only street dogs were determined as seropositive for CCV infection in Konya, Isparta and Burdur. All of owned dogs were negative by both serologic and virologic tests. When provinces were compared there was no statistical difference in CCV infections likely to be seen in dogs between Isparta and Burdur while the lowest serologic results were obtained from Konya ($P<0.001$). Also, there was no statistical differences between sex in CCV infection in dogs ($P>0.05$).

CCoV infection have been reported from different countries Turkey (Gur *et al.*, 2008), Italy (Decaro *et al.*, 2012), Greece (Ntafis *et al.*, 2012), Belgium (Zicola *et al.*, 2012), and detection has been reported from different specimens such as intestinal system (Keenan *et al.*, 1976), feces (Battersby and Harvey, 2006; Ntafis *et al.*, 2013; Costa *et al.*, 2014), and internal organs like liver, lung, mesenteric lymph node and thymus (Ntafis *et al.*, 2013). CCoV viral RNA detection from blood sample was reported by Decaro *et al*. (2010) from one experimentally infected by CB/05 on dog (Decaro and Buonavoglia, 2011) during viremia. Leukocyte positive result obtained from this research is in accordance with the results of other researchers (Decaro and Buonavoglia, 2011). Positive dog had been evaluated on viremia phase of infection. Hence, as per knowledge CCoV detection from leukocyte was reported for the first time.

According to the seropositive (indicated as natural infection because unvaccinated street dogs were used) and antigen positive results of this study, it can be concluded that CCoV infection in Turkish street dog population is widespread and it may be attributed to be one of important viral pathogens in dogs. Findings obtained from this research showed that all owned animals were antigen negative. These results indicated that vaccination for CCV could be the most useful programme for prevention from CCV infection. In conclusion, CCoV infections can easily occur as mix infections with other pathogens such as viral, bacterial or parasitic agents. Hence, rapid diagnosis and the vaccination application as soon as possible are essential for the control of CCoV epidemics.

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