Canine coronavirus (CCoV) in dogs vaccinated and unvaccinated domiciliated in Pelotas, RS, Brazil

Coronavirus canino (CCoV) em cães vacinados e não vacinados domiciliados em Pelotas, RS

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Abstract

Canine coronavirus (CCoV) has been reported causing enteric disease mainly in young pups. In this study, to investigate immunity and exposure to CCoV, was estimated the frequency of serum antibodies to CCoV in 121 dogs in the Pelotas, South of Brazil, by serum neutralization test (SN): 22 had not been vaccinated, 69 had been vaccinated at least once, and 30 had unknown vaccination history. Antibodies were present in 47,8% (33/69) of the vaccinated dogs, in 45,5% (10/22) of the unvaccinated, and in 43,3% (13/30) of the dogs with unknown historical vaccination. There was no significant relationship between these antibodies and sex, age, habitat, and season of collection. The results proved the circulation of the CCoV among this dog population and indicated that the infection affects a significant group of animals. The large proportion of seronegative vaccinated dogs indicates failure of CCoV vaccine in inducing neutralizing antibodies, suggesting that immunizations to CCoV should be reevaluated. The authors indicate the need for further studies in order to evaluate the impact of the infection caused by CCoV, as well as to propose and evaluate preventive measures.
Key words: Canine coronavirus, antibodies, vaccine, immunity

Resumo

Coronavírus canino (CCoV) foi relatado como causa de doença entérica principalmente em cães jovens. Nesse estudo, para investigar imunidade e exposição ao CCoV, foi estimado a frequência de anticorpos em 121 cães de Pelotas, sul do Brasil, pelo teste de soro-neutralização (SN): 22 não haviam sido vacinados, 69 haviam sido vacinados com pelo menos uma dose, e 30 possuíam histórico de vacinação desconhecido. Foram detectados anticorpos em 47,8% (33/69) dos cães vacinados, em 45,5% (10/22) dos não vacinados, e em 43,3% (13/30) dos cães com histórico de vacinação desconhecido. Não houve associação significativa entre os anticorpos e sexo, idade, habitação e estação da coleta. Os resultados confirmam a circulação do CCoV entre essa população de cães e indicam que a infecção acomete população significativa de animais. A grande proporção de cães vacinados soronegativos indica falha da vacina em indução de anticorpos neutralizantes, sugerindo que as imunizações para CCoV devam ser reavaliadas. Os autores por meio de seus dados indicam ainda a necessidade de posteriores estudos para avaliar o impacto da infecção causada pelo CCoV, bem como para avaliar e propor mediadas preventivas.
Palavras-chave: Coronavírus canino, anticorpos, vacina, imunidade

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Introduction

Canine coronavirus (CCoV) is a large, single-stranded, positive-sense RNA viruses (MURPHY et al., 1999). CCoV infection was first described after isolation of the virus from faecal specimens of American military dogs with diarrhoeal disease (BINN et al., 1974). Currently two CCoV genotypes have been identified, namely CCoV type I and CCoV type II, which are responsible for the occurrence enteritis in dogs and are frequently associated in mixed infections (PRATELLI et al., 2004a; DECARO; BUONAVOGLIA, 2008). Clinical signs of CCoV infection in dogs include diarrhea, vomiting and dehydration (EVERMANN; ABBOTT; HAN, 2005). Mortality rate from CCoV infection in healthy dogs is frequently low (TENNANT; JONES; GASKELL, 1993), however, severe illness as a consequence of mixed infections with canine parvovirus type 2 (CPV-2) (DECARO et al., 2006; DECARO et al., 2007b), canine adenovirus type 1 (PRATELLI et al., 2001; DECARO et al., 2007a) or canine distemper virus (DECARO et al., 2004b) has been observed. Although its tropism is restricted to the gastroenteric tract, CCoV has been recently associated to systemic disease followed by fatal outcome in pups (BUONAVOGLIA et al., 2006; DECARO et al., 2008).

While the immune mechanisms of the protection from CCoV infection are still not clear, studies suggest that effective vaccines which are able to induce truly protective and long-lived immunity to infection should be sought to control the spread of CCoV in high risk dog populations (PRATELLI et al., 2004b). The assessment of antibodies by the virus neutralization assay or by indirect enzyme-linked immunosorbent assay (ELISA) provides an indication of the protective immunity or, in unvaccinated animal, exposure to CCoV (MOCHIZUKI; SUGIURA; AKUZAWA, 1987; PRATELLI et al., 2002; PRATELLI et. al., 2003).

In this study, to investigate immunity and exposure to CCoV, the frequency of serum antibodies to CCoV in vaccinated and unvaccinated dogs in the Pelotas, Rio Grande do Sul, Brazil, was estimated. Next, was determined if there has any relationship between these antibodies and the following host parameters: sex, age, habitat, and season of collection.

Materials and Methods

Cells and virus

CrFK (Crandell feline kidney cells) were cultured in Eagle's minimum essential medium (E-MEM-Cultilab) supplemented with 10 % of foetal bovine serum and 10 mg/ml of enrofloxacin (Bayer).

A cell culture adapted CCoV strain Mav 795 was used throughout this study. The strain was supplied by the Virology Laboratory of Federal University of Santa Maria (UFSM, Brazil).

Clinical Specimens

Serum samples were collected from hundred and twenty one dogs that would be submitted to elective surgeries in the Veterinary Hospital of the Federal University of Pelotas (UFPel) and in five private clinics of the region, from July 2005 to August 2006. The samples were analyzed regarding information about vaccination history, sex, age, habitat that would allow contact with other animals and season of collection. All information was recorded on a special form. Antibodies to CCoV were determined by serum neutralization assay (PRATELLI et al., 2002) with some modifications. Serum samples were initially diluted in 1:5 and then two-fold until 320, and then were added 100 $TCID_{50}$ of the CCoV. After one hour at 37 ^{o}C, a suspension containing 30.000 CrFK cells was added to each well. The microplates were incubated at 37 ^{o}C in atmosphere containing 5 % of CO_2 for up to 5 days. In all the plates, negative and positive control sera were included. Reading of microplates were carried out when reverse titering confirmed the 100 $TCID_{50}$. The titre was expressed as the highest serum dilution neutralizing the virus. Serum samples with titer < 5 were considered negative for CCoV.

Statistical Analysis

Differences in frequency of antibodies were evaluated using Kruskal-Wallis (Statistix®. Statistix for Windows. Tallahassee, FL, USA: Analytical software; 2003), comparing the results between the host parameters (sex, age, habitat, and season of collection). $P<0.05$ was used as the criterion for statistical significance.

Results and Discussion

The results of tests for antibodies to CCoV in serum samples are reported in Table 1. Samples were obtained from dogs aging from 2 months until more than six years. From 121 dogs, 55 (45,5%) were male, 66 (54,5%) female. Regarding age, 27 animals (22,3%) were less than 1 year old, 53 (43,8%) between 1 and 6 years old; 22 (18,2%) were older than 6 years old and 19 dogs (15,7%) were of unknown age. Forty two dogs (34,7%) had limited access to the streets; 45 (37,2%) were housed but with access to the streets; 10 (8,3%) lived in the streets and 24 (19,8%) had no information about habitat.

Sera from 10 (45,5%) of the unvaccinated and 30 (24,8%) of the dogs with unknown vaccination history were positive for antibodies, confirming the circulation of the CCoV among these dog population and indicating that the infection affect a significant group of animals. These results are similar to the ones reported in other studies (RIMMELZWAAN et al., 1991; TENNANT; JONES; GASKELL, 1993; BANDAI et al., 1999; NAYLOR et al., 2001; YEŞILBAĞ et al., 2004; DEZENGRINI; WEIBLEN, FLORES, 2007). The primary means of transmission is via exposure of susceptible hosts to virus shed in feces (CARMICHAEL; BINN, 1981). Infected dogs generally shed CCoV in the feces for 6–9 days post infection, but some naturally infected dogs have shed virus for a period as long as 6 months after clinical signs had ceased (PRATELLI, 2006). In this study, infection in most of the animals may have happened due to environmental exposure. CCoV is highly contagious and once the virus has become established in the environment, the spread of the infection is difficult to control. Avoiding contact with infected dogs and their excretions is the only way to ensure disease prevention (PRATELLI, 2006). There was not a significant association between seropositivity and parameters evaluated (sex, age, habitat that would allow contact with other animals and season of collection), suggesting low but continuing exposure to CCoV in all categories analyzed.

There was previous vaccination history against CCoV, with at least one dose, for 69 dogs (57,8%); 36 vaccinated dogs (52,2%) have no neutralizing antibodies to CCoV. Little is known about the immune mechanisms involved in protection to CCoV enteritis. It appears that local immunity (IgA in the gut mucosa) is considered essential for protection against infection (DECARO et al., 2004a). Studies on the role of serum antibodies as a predictor of susceptibility to coronavirus infection and illness have yielded ambiguous results (VANCOTT et al., 1994). Nevertheless, due to lack of antibodies in the serum of vaccinated dogs seems plausible to think that there was failure in the induction of immunity. Vaccine failure may occur by several factors: conservation of the vaccine with inadequate or intense oscillations of temperature; deficient immune response of the animal; administration on immunocompromised or immunosuppressed animals; administration in animals with passive immunity; use of vaccines with expired shelf-life; or, use of ineffective vaccines, with inadequate ability to produce the expected antigenic stimulation, promoting little or no reaction of the immune system. Considering the large number of vaccinated dogs seronegative, is possible that the quality of commercial vaccines against CCoV, should be reassessed. In a recent study, Pratelli et al. (2003) demonstrated the low efficacy of a widely used inactivated commercial vaccine in reducing faecal shedding after challenge with a field virus. Although the efficacy and the duration of immunity engendered by inactivated vaccines have not been substantiated, only killed CCoV vaccines have been licensed in Brazil. Modified life (ML) vaccines have been licensed in the past, but they often resulted in a high frequency adverse of post-vaccinal reactions (SAIF, 1993; PRATELLI, 2006).

Table 1. Distribution of positive canine sera in Pelotas for coronavirus canino (CCoV) by vaccination, sex, age, location, and season of collection*

Títer	No. of positive sera (%)	Vaccination			Sex		Age (Yr)				Location				Season				
		Yes	No	U	Male	Female	< 1	1 a 6	> 6	U	LS	HAS	Streets	U	Summer	Autumn	Winter	Spring	U
Neg	65 (53,7)	36 (52,2)	12 (54,5)	17 (56,7)	24 (43,6)	41 (62,1)	13 (48,1)	31 (58,5)	12 (54,5)	9 (47,8)	26 (61,9)	24 (53,3)	4 (40)	11 (45,8)	7 (36,8)	11 (57,9)	25 (62,5)	21 (53,8)	1 (25)
5	27 (22,3)	15 (21,7)	6 (27,3)	6 (20)	16 (29,1)	11 (16,7)	8 (29,6)	11 (20,7)	4 (18,2)	4 (21,1)	8 (19,5)	12 (26,3)	3 (30)	4 (16,6)	7 (36,8)	3 (15,8)	7 (17,5)	9 (23,1)	1 (25)
10	19 (15,7)	12 (17,4)	2 (9,1)	5 (16,7)	10 (18,2)	9 (13,6)	4 (14,8)	6 (11,3)	3 (13,6)	6 (31,6)	4 (9,5)	5 (11,1)	3 (30)	7 (29,2)	4 (21,1)	2 (10,5)	6 (15)	5 (12,8)	2 (50)
20	5 (4,1)	3 (4,3)	1 (4,5)	1 (3,3)	2 (3,6)	3 (4,5)	1 (3,7)	2 (3,8)	2 (9,1)	0 (0)	1 (2,4)	3 (6,7)	0 (0)	1 (4,2)	0 (0)	1 (5,3)	2 (5)	2 (5,1)	0 (0)
40	2 (1,6)	0 (0)	1 (4,5)	1 (3,3)	1 (1,8)	1 (1,5)	0 (0)	1 (1,9)	1 (4,5)	0 (0)	0 (0)	1 (2,2)	0 (0)	1 (4,2)	1 (5,3)	0 (0)	0 (0)	1 (2,6)	0 (0)
80	2 (1,6)	2 (2,9)	0 (0%)	0 (0)	2 (3,6)	0 (0)	1 (3,7)	1 (1,9)	0 (0)	0 (0)	2 (4,8)	0 (0)	0 (0)	0 (0)	0 (0)	2 (10,5)	0 (0)	0 (0)	0 (0)
160	0 (0)	0 (0)	0 (0%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
320	1 (0,8)	1 (1,4)	0 (0%)	0 (0)	0 (0)	1 (1,5)	0 (0)	1 (1,9)	0 (0)	0 (0)	1 (2,4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2,6)	0 (0)
Total	121	69 (57,8)	22 (18,2)	30 (24,8)	55 (45,5)	66 (54,5)	27 (22,3)	53 (43,8)	22 (18,2)	19 (15,7)	42 (34,7)	45 (37,2)	10 (8,3)	24 (19,8)	19 (15,7)	19 (15,7)	40 (33,1)	39 (32,2)	4 (3,3)

*Yr: years; Neg: negative; U: unknown; LS: limited access to the streets; HAS: Housed but with access to the street

Conclusion

Serological evidence indicates that infection with CCoV affect a significant portion of dogs in the area studied. The large proportion of seronegative vaccinated dogs indicates failure of CCoV vaccine in inducing neutralizing antibodies, suggesting that immunizations to CCoV should be reevaluated. The data indicates the need for further studies in order to evaluate the impact of the infection caused by CCoV in the dog population, as well as to propose and evaluate new preventive measures.

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