Original Article

Canine distemper virus, *Ehrlichia canis* and *Borrelia* spp. in stray dogs

Virus del distemper canino, *Ehrlichia canis* y *Borrelia* spp. en perros callejeros

https://eqrcode.co/a/DhVUta

Vânia Gomes dos Santos [1] , Rita de Cássia Campbell Machado Botteon [1] ,

Matheus Dias Cordeiro [2] , [iD] Adivaldo Henrique da Fonseca [2] *

[1]Departamento de Medicina e Cirurgia Veterinária do Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro BR 465 Km 07, CEP 23890-000, Seropédica - Rio de Janeiro, Brazil.
[2]Departamento de Epidemiologia e Saúde Pública do Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro BR 465 Km 07, CEP 23890-000, Seropédica - Rio de Janeiro, Brazil.

ABSTRACT: This study is aimed at detecting canine distemper, *Ehrlichia canis* and *Borrelia burgdorferi* sensu lato (s.l.) in stray dogs in the cities of Rio de Janeiro and Seropédica, State of Rio de Janeiro, Brazil. One hundred and fifty-eight canine blood samples from the two cities were collected and analyzed. Of these, 96 from stray dogs located at the Zoonosis Control Center and 62 from free-roaming animals near the *Campus* of the Federal Rural University of Rio de Janeiro. The animals were of both sexes, of different ages, of undefined breed and with an unknown vaccination history. The presence of one animal positive for canine distemper virus (1/38) and the absence of clinical cases indicated the susceptibility of the housed animals to the risk of a possible outbreak of the disease. However, high titers of anti-*E. canis* (35/38) and anti-*B. burgdorferi* s.l. antibodies (85/158) indicated that those hemoparasites were circulating in the study regions.

Key words: Borreliosis, dogs, canine distemper, ehrlichiosis.

RESUMEN: El objetivo del presente trabajo fue detectar el Moquillo canino, *Ehrlichia canis* y *Borrelia burgdorferi* sensu lato (s.l.) en perros callejeros de las ciudades de Río de Janeiro y Seropédica, Estado de Río de Janeiro, Brasil. Se colectaron 158 muestras de sangre de caninos de las dos ciudades y se muestrearon 96 caninos callejeros ubicados en el Centro de Control de Zoonosis y de 62 animales ambulantes de cercanías a los predios de la Universidad Federal Rural de Río de Janeiro. Los animales fueron de ambos sexos, diferentes edades, raza no definida y con historia de vacunación desconocida. La presencia de un animal positivo a virus de distemper canino (1/38) y la ausencia de casos clínicos indican la susceptibilidad de los animales alojados a un riesgo de un posible brote de la enfermedad. Sin embargo, los títulos altos de anticuerpos anti-*E. canis* (35/38) y anti-*B. burgdorferi* s.l. (85/158) indican que hay circulación de estos hemoparásitos en las regiones de estudio.

Palabras clave: Borreliosis, caninos, distemper canino, ehrlichiosis.

INTRODUCTION

Infectious diseases are the leading cause of death among dogs ([1]), and the simultaneous occurrence of more than one disease is common, especially in immunocompromised or predisposed dogs. Although in clinical practice, diagnosis is usually based on the evolution of clinical signs and laboratory findings, many dog diseases present non-specific signs such as apathy, anorexia and occasionally fever, which progress to respiratory, gastrointestinal and neurological manifestations ([2,3]).

These clinical signs are usually related to viruses such as canine distemper and infection by agents of the genus *Ehrlichia*, in addition to the possible presence of other infectious agents such as *Borrelia* spp.

In general, these may begin with general signs of apathy and anorexia, but when they appear with fever and ocular-nasal discharge, they may be associated with respiratory manifestations of canine distemper ([4]), ehrlichiosis ([5]) and borreliosis, which may include neurological manifestations ([6]).

This study is aimed at detecting canine distemper, *Ehrlichia canis* and *Borrelia burgdorferi* sensu lato (s.l.) in stray dogs in the cities of Rio de Janeiro and Seropédica, State of Rio de Janeiro, Brazil

MATERIALS AND METHODS

The study was carried out at the Zoonosis Control Center of Paulo Dacorso Filho (CCZ) located in the Santa Cruz neighborhood, and at the Campus of the Federal Rural University of Rio de Janeiro (UFRRJ), located in Seropédica, both places belong to the State of Rio de Janeiro, Brazil. One hundred and fifty-eight canine blood samples from the two cities were collected and analyzed. Of these, 96 from stray dogs located at CCZ and 62 from free-roaming animals near the *Campus* of UFRRJ. The animals sampled were of different ages, sexes, undefined breeds and with an unknown vaccination history.

Blood samples were obtained by puncture of the jugular or cephalic vein into vacuum tubes with and without anticoagulant. The blood collected without anticoagulant was placed in a tilted support and left at room temperature until clot formation. Then, serum was obtained by centrifugation at 2,600xg for 5 minutes, and it was stored at -20ºC (-4ºF) until use. During collections, canines were clinically evaluated.

To research hemoparasites, peripheral blood thin smears were obtained from the first drop of blood from the capillary vessels of the atrial region. The smears were air-dried, fixed in methanol and stained with Giemsa. These slides were examined under light microscopy using a 100x objective.

The sera were thawed at room temperature to be analyzed by serology and to detect canine distemper virus antigens. Thirty-eight samples from CCZ were tested for anti-*E. canis* antibodies and for the qualitative detection of canine distemper virus antigen with the chromatographic immunoassay test "Antigen Rapid CDV Ag Test Kit," according to manufacturer's recommendation.

For the serological diagnosis of *E. canis*, the Indirect Fluorescent Antibody Test (IFAT) was performed, using the *E. canis* São Paulo reference strain ([7]). For the detection of antibodies of homologous IgG class against *B. burgdorferi* s.l., an indirect ELISA serological test was performed, according to a previously developed methodology ([8]).

RESULTS AND DISCUSSION

The young female dog (1/38), a stray animal that presented apathy, anorexia, fever, and ocular-nasal discharge at the time of collection, was the only animal with a positive CDV antigen test. This animal tested positive for both eye-drainage and serum samples. It should be noted that with the exception of this animal, the other dogs were asymptomatic while living in the same environment. When the similarity of the clinical manifestations of the diseases studied and the possibility of co-infections were confirmed, the animal tested positive for *E. canis* antibodies with a 1:40 titer in IFAT.

With regard to the results of the study by Hartmann *et al.* ([9]), which was carried out on breeding dogs of different ages with an unknown vaccination history and using a neutralising antibody test, the authors concluded that most of the dogs did not have specific CDV antibody titers, indicating that there was no contact with canine distemper virus antigen due to natural infection or previous immunisation ([10]). In addition to this finding, the authors suggested that the absence of CDV neutralizing antibodies in unvaccinated animals may lead to a high risk of exposure to subsequent CDV infection.

In this study, only one animal tested positive among the 38 dogs tested (2.63%) and the rate, although close to that obtained by Headley *et al.* ([11]), was contrary to expectations due to the conditions of the population studied. In an epidemiological study by Borba *et al.* ([12]), an infection rate of 2.07 % was identified among animals in veterinary clinics in Maringa, State of Paraná, between 1998 and 2001. The highest frequency of infection occurred in winter.

Headley *et al*. ([11]) have also reported low infection rates (1.98 %). CDV infection has a pre-patent period of about 20 days, followed by fever peaks and a subsequent asymptomatic state ([3]). The antigen can only be detected in animals with viraemia, which occurs between 3 and 10 days after infection, clinically manifested by fever and apathy, among other signs of systemic involvement, which was not common among the animals in this study despite coexistence with the positive and symptomatic animal.

Although normal cleaning and disinfection procedures are usually effective against the canine distemper virus ([5]), the presence of an infected animal poses a risk for those living in the same environment, especially since it is a chronic, low immunogenic disease involving different organs and systems.

A prevalence of 22.8% (13/158) of *E. canis* was found by the observation of blood smears. Thus, frequencies of 4.22 % (3/62) and 10.42 % (10/96) were observed in UFRRJ and CCZ, respectively. In the serological test for *E. canis*, 92.1 % (35/38) of the animals had antibodies against the antigen of *E. canis* São Paulo reference strain. Most of the seropositive animals had titers equal to or higher than 1:10,240 (23/38), which may suggest a field exposure to the infectious agent. Therefore, these data were above the perspectives reported to those found in the northeastern region of Brazil, where the frequency was 35.6 % ([13]). In a comparative study of 30 dogs treated at the UNESP Veterinary Teaching Hospital in Jaboticabal, São Paulo State, 63.3% of the samples were positive by IFAT, 70% by DOT-ELISA and 53.3% by nPCR. The authors highlighted the importance of clinical and haematological tests for the diagnosis of canine ehrlichiosis ([14]).

In the present study, a large number of asymptomatic positive animals indicate a high prevalence of subclinical infection among the animals tested. According to Harrus *et al*. ([2]), clinical ehrlichiosis should be considered for differential diagnosis in dogs from endemic areas with typical clinical signs and hematological and biochemical abnormalities. Traditional diagnostic techniques, including hematology, cytology, serology, and isolation are important diagnostic tools; however, the definitive diagnosis of the

infection by *E. canis* requires molecular techniques.

Of the 158 sera tested, 85 were positive (53.8 %) with IgG homologous class titers against *B. burgdorferi* s.l. They were closer than those already reported in Brazil, using either the indirect ELISA method used in this study ([8],[15]-[17]) or the ELISA method used in the Snap 3 Dx® test kit (IDEXX Laboratories) ([18]). Antibodies to *Borrelia* spp. were detected in stray dogs at UFRRJ in 48.39 % (30/62) of the animals tested, while at CCZ, the frequency of positives was 57.29 % (55/96).

Using an indirect ELISA test, Jopper *et al*. ([15]) found 9.7 % seropositivity in the city of São Paulo, and Carlos *et al.* ([18]) observed a positivity of only 1 % of the animals studied by an ELISA test in Ilhéus, state of Bahia. In the rural areas of seven municipalities in the State of Rio de Janeiro, O'Dwyer *et al*. ([16]) found 15.85 % of seropositive dogs. Among the dogs from the municipalities of Baixada Fluminense, Rio de Janeiro, Soares *et al.* ([8]) and Alveset *et al.* ([19]) found a positivity of 20 % and 48.25 %, respectively. Cordeiro *et al.* ([17]) found an infection rate of 52.56 % in companion dogs in the metropolitan area of Rio de Janeiro.

In Lyme borreliosis, the dog may act as an epidemiological sentinel, hosting the spirochete, behaving as a reservoir in the domestic environment, and the tick vector to vehicle the pathogen to humans and other animals ([19]). The origin of the animals in this study was unknown, and most animals were captured in urban areas, which enhanced the possibility of them to act as reservoir of infection to both animals and humans.

The animals sheltered in kennels with dividing collective bays were exposed to a stressful situation because of the cohabitation condition. Despite having a good management, disputes over food and water may occur. In addition, the unknown vaccination history situation predisposed susceptible animals to disease and to develop a clinical, sub-clinical or chronic form of disease. These conditions favored a state of immunosuppression that enabled co-infections.

Moretti *et al*. ([20]) reported triple agent co-infection with canine distemper, ehrlichiosis and toxoplasmosis in a dog with a neuropathological

clinical picture. The authors analyzed canine distemper as a primary disease and its association with ehrlichiosis based on clinical and epidemiological data, inadequate immunological prophylactic protocol and the role of these diseases in immunosuppression. Canine distemper and ehrlichiosis were diagnosed on the basis of the epidemiological situation of the region and compatible clinical signs, combined with blood count and cytology results. In this study, animals with good to poor nutritional status, but good mood and active, had antibody titers for more than one disease-causing agent.

The presence of an animal positive to canine distemper virus and without clinical cases indicated the presence of the agent and suggested that dogs were naturally immunized or sick at a stage when it was not possible to detect circulating antigens. The high number of seropositive animals, although asymptomatic to ehrlichiosis, showed a high prevalence of infection among the dogs examined. The presence of anti-*B. burgdorferi* s.l. antibodies also showed the circulation of the spirochete *Borrelia* spp. in the dogs in the studied region.

ACKNOWLEDGMENTS

REFERENCES

1. Bentubo HDL, Tomaz MA, Bondan EF, Lallo MA. Expectativa de vida e causas de morte em cães na área metropolitana de São Paulo (Brasil). Ciênc Rural. 2007;37(4):1021-1026.
2. Harrus S, Waner T. Diagnosis of canine monocytotropic ehrlichiosis (Ehrlichia canis): An overview. Vet J. 2011;187(3):292-296.
3. Greene CE. Infectious diseases of the dog and cat: Elsevier Health Sciences; 2013.
4. Koutinas A, Baumgärtner W, Tontis D, Polizopoulou Z, Saridomichelakis M, Lekkas S. Histopathology and immunohistochemistry of canine distemper virus-induced footpad hyperkeratosis (hard pad disease) in dogs with natural canine distemper. Vet Pathol Online. 2004;41(1):2-9.
5. Feldman EC. Tratado de medicina interna veterinária. 1997.
6. Yoshinari NH, Mantovani E, Bonoldi VLN, Marangoni RG, Gauditano G. Doença de lyme- símile brasileira ou síndrome baggio yoshinari: zoonose exótica e emergente transmitida por carrapatos. Rev Ass Med Brasil. 2010;56(3):363-369.
7. Aguiar DM, Hagiwara MK, Labruna MB. In vitro isolation and molecular characterization of an Ehrlichia canis strain from São Paulo, Brazil. Braz J Microbiol. 2008;39:489-493.
8. Soares C, Fonseca A, Ishikawa M, Manera G, Scofield A, Yoshinari N. Sorologia para borreliose em cães procedentes da Baixada Fluminense, estado do Rio de Janeiro. Rev Bras Med Vet. 1999;21(1):111-114.
9. Hartmann TLS, Batista HBCR, Dezen D, Spilki FR, Franco AC, Roehe PM. Anticorpos neutralizantes contra os vírus da cinomose e da parainfluenza em cães de canis dos municípios de Novo Hamburgo e Porto Alegre, RS, Brasil. Ciência Rural. 2007;37(4):1178-1181.
10. Dezengrini R, Weiblen R, Flores EF. Soroprevalência das infecções por parvovírus, adenovírus, coronavírus canino e pelo vírus da cinomose em cães de Santa Maria, Rio Grande do Sul, Brasil. Ciênc Rural. 2007;37(1):183-189.
11. Headley SA, Graça DL. Canine distemper: epidemiological findings of 250 cases. Braz J Vet Res An Sci. 2000;37(2).
12. Borba TR, Mannigel RC, Fraporti CK, Headley SA, Saito TB. Cinomose: dados epidemiológicos Maringá-PR (1998-2001). Inic Cient Cesumar. 2007;4(1):53-56.
13. Souza BMPdS, Leal DC, Barboza DCPM, Uzêda RS, Alcântara ACD, Ferreira F, et al. Prevalence of ehrlichial infection among dogs and ticks in Northeastern Brazil. Rev Brasil Parasitol Vet. 2010;19:89-93.
14. Nakaghi ACH, Machado RZ, Costa MT, André MR, Baldani CD. Canine ehrlichiosis: clinical, hematological, serological and molecular aspects. Cienc Rural. 2008;38(3):766-7670.

15. Joppert AM, Hagiwara MK, Yoshinari NH. Borrelia burgdorferi antibodies in dogs from Cotia county, São Paulo State, Brazil. Rev Inst Med Trop São Paulo. 2001;43:251-255.

16. O'Dwyer LHO, Soares CA, Massard CL, Souza JCP, Flausino W, Adivaldo HF. Soroprevalência de Borrelia burgdorferi latu sensu associada à presença de carrapatos em cães de áreas rurais do Estado do Rio de Janeiro, Brasil. Ciênc Rural. 2004;34(1):61-4.

17. Cordeiro MD, Meireles GS, Silva JB, Souza MMS, Fonseca AH. Soroprevalência para Borrelia spp. em cães no município de Seropédica, estado do Rio de Janeiro. Rev Brasil Med Vet. 2012;34(3):5.

18. Carlos RSA, Muniz Neta ES, Spagnol FH, Oliveira LLS, Brito RLLd, Albuquerque GR, et al. Freqüência de anticorpos anti-Erhlichia canis, Borrelia burgdorferi e antígenos de Dirofilaria immitis em cães na microrregião Ilhéus-Itabuna, Bahia, Brasil. Rev Brasil Parasitol Vet. 2007;16:117-1120.

19. Alves AdL, Madureira RC, Silva RAd, Corrêa FdN, Botteon RCCM. Freqüência de anticorpos contra Borrelia burgdorferi em cães na região metropolitana do Rio de Janeiro. Pesq Vet Brasil. 2004;24:203-206.

20. Moretti LdA, Silva AVd, Ribeiro MG, Paes AC, Langoni H. Toxoplasma gondii genotyping in a dog co-infected with distemper virus and ehrlichiosis rickettsia. Rev Inst Med Trop São Paulo. 2006;48:359-363.