UNIVERSIDADE FEDERAL DO PARANÁ

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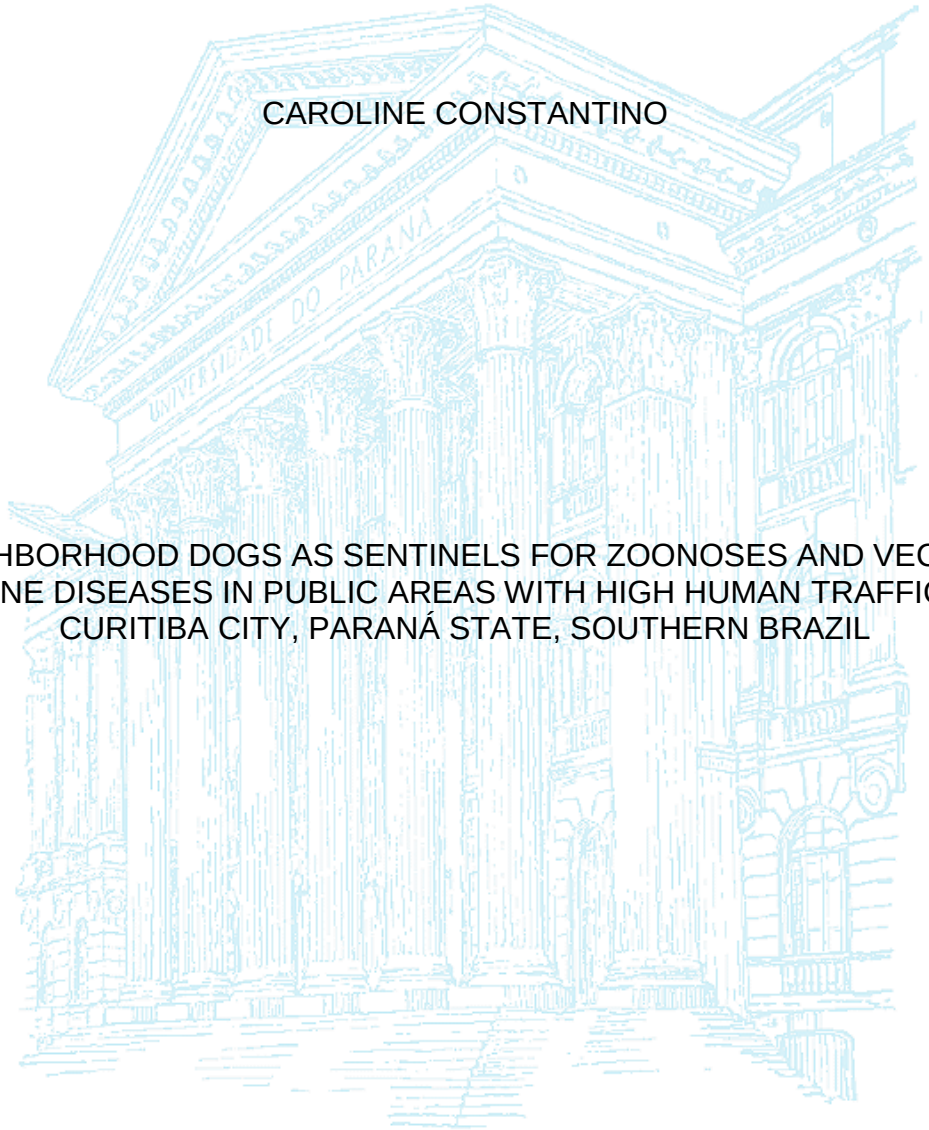

NEIGHBORHOOD DOGS AS SENTINELS FOR ZOONOSES AND VECTOR-BORNE DISEASES IN PUBLIC AREAS WITH HIGH HUMAN TRAFFIC OF CURITIBA CITY, PARANÁ STATE, SOUTHERN BRAZIL

CURITIBA
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Dissertation presented as partial requirement for the degree of Master of Veterinary Science, the Postgraduate Course in Veterinary Science.

Professor advisor: Dr. Alexander Welker Biondo.

Steering Committee: Dr. Elizabeth Santin and Dr. Rafael Felipe da Costa Vieira.

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PARECER

A Comissão Examinadora da Defesa da Dissertação intitulada **"NEIGHBORHOOD DOGS AS SENTINELS FOR ZOONOSES AND VECTOR-BORNE DISEASES IN PUBLIC AREAS WITH HIGH HUMAN TRAFFIC OF CURITIBA CITY, PARANÁ STATE, SOUTHERN BRAZIL"** apresentada pela Mestranda **CAROLINE CONSTANTINO** declara ante os méritos demonstrados pela Candidata, e de acordo com o Art. 79 da Resolução nº 65/09–CEPE/UFPR, que considerou a candidata **APTA** para receber o Título de Mestre em Ciências Veterinárias, na Área de Concentração em Ciências Veterinárias.

Curitiba, 29 de fevereiro de 2016

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I offer this work to my parents Benedito and Carmen, my sister Leticia and my niece Sarah who built me more this dream, supporting me and giving strength to face all my challenges. I dedicate also to all the animals that have had their health and well-being somehow I competed in my personal and professional life.

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“In a world where no creatures are truly isolated and diseases spread as fast as jets can fly, we are all canaries and the entire planet is our coal mine. Any species can be a sentinel of danger – but only if the widest array of health-care professionals is paying attention.”

Natterson-Horowitz B. & Bowers K., 2012

ABSTRACT

Dogs may serve as environmental sentinels for vector-borne pathogens and their vectors transmission, acting as species-specific diseases bio-indicators, particularly zoonoses. These animals may be suitable sentinels for public health due to their close interaction with human beings, sharing the same environmental, and food and water sources. Dogs with outdoors life and free roaming habits may be higher exposed to vector-borne pathogens and their vectors, as well as protozoan infections, then owned dogs. However, dogs also may serve as a possible source of vector infection, and can carry infected ectoparasites. Thus, while the dogs act as sentinels they may play a role in disease maintenance and environmental spreading. The twenty-two bus stations of Curitiba, southern Brazil, have been 1.1 million daily users, while it shelters neighborhood dogs with unknown health status. Neighborhood dogs are defined by the World Health Organization semi-restricted or free-range animals with semi-dependence on one or more families for food and shelter. In Curitiba City, dogs with this profile have been in daily contact with the population, and may act as disseminators and/or sentinel for vector-borne pathogens and environmental zoonoses in urban areas due to outdoor life and daily movement. From February to August 2014, 27 neighborhood dogs living in 10/22 bus stations and two public parks of city of Curitiba have blood samples taken by Animal Service of Curitiba city for laboratorial analyses. In 2015, the biological samples are screened for vector-borne diseases and environmental zoonoses for this research, with Environment Office of Curitiba permission. Accordingly, the general aim of the present study was to reveal the health status of neighborhood dogs from public areas with highly human traffic (bus stations and public parks) of city of Curitiba, southern Brazil, and evaluate them as disseminators and/or sentinels of vector-borne diseases and environmental zoonoses. In the first study, whole blood and serum samples of 21 neighborhood dogs have been screened by serological and molecular methods for vector-borne disease. This study has revealed 7/21 (33.3%) dogs with *Mycoplasma haemocanis* infection, 9/21 (42.8%) with '*Candidatus* Mycoplasma haematoparvum' and 1/21 (4.7%) dog seroreactive for *Borrelia burgdorferi* sensu stricto. In the second study, 26 serum samples of neighborhood dogs have screened by indirect immunofluorescent antibody test for four different protozoan and reveled a total of 8/26 (30.7%) seroreactive dogs to *Toxoplasma gondii* and 3/26 (11.5%) to *Neospora caninum*. Despite their daily exposure in public areas with highly human movement, neighborhood dogs have shown low vector-borne disease and protozoan zoonoses prevalence, revealing low environmental risk and consequently low risk of spreading. Since the neighborhood dogs are in daily contact with stray dogs and thousands of people in public areas of Curitiba, these animals should be continuously monitored, making them sentinels of environmental risk for vector-borne diseases and protozoan zoonoses in public areas with highly human traffic.

Key-words: sentinel animals, spreaders animals, health profile, bus stations, public parks.

RESUMO

Cães podem ser utilizados como sentinelas da presença de patógenos e vetores no ambiente, servindo como bioindicadores de doenças espécie-específicas e, sobretudo, de zoonoses. Estes animais são excelentes sentinelas em saúde pública devido a sua estreita relação com os seres humanos, muitas vezes compartilhando o mesmo ambiente e utilizando as mesmas fontes de água e alimento. Cães com vida ao ar livre e livre circulação podem estar mais expostos a vetores e a patógenos por eles transmitidos, além de infecções por protozoários, do que cães domiciliados. Entretanto, cães também podem atuar como fonte de infecção para os vetores e carrear artrópodes infectados. Assim, podem atuar como sentinelas, mas também como mantenedores e disseminadores ambientais de patógenos. Os 22 terminais de ônibus urbanos de Curitiba, sul do Brasil, contam com 1,1 milhão de usuários diariamente, ao mesmo tempo em que abriga cães comunitários com perfil sanitário desconhecido. Cães comunitários são definidos pela Organização Mundial da Saúde como animais de circulação semirrestrita ou totalmente livre e que dependem de uma ou mais famílias para alimentação e abrigo. Em Curitiba, cães com este perfil estão em contato diário com a população e podem atuar como disseminadores e/ou sentinelas de doenças transmitidas por vetores e zoonoses ambientais em áreas urbanas por causa da sua vida ao ar livre e seu tráfego diário. Portanto, o objetivo geral desta pesquisa foi revelar o perfil sanitário de cães comunitários de áreas públicas com grande circulação de pessoas (terminais de ônibus urbanos e parques públicos) de Curitiba, sul do Brasil, e avalia-los como disseminadores e/ou sentinelas para doenças transmitidas por vetores e zoonoses ambientais. Entre fevereiro e abril de 2014, 27 cães comunitários que viviam em 10/22 terminais de ônibus urbanos e dois parques públicos de Curitiba tiveram amostras de sangue e soro coletadas pela Agência de Proteção Animal para exames laboratoriais. Em 2015, as amostras biológicas foram testadas para patógenos transmitidos por vetores e para zoonoses ambientais nesta pesquisa, com a permissão da Secretaria de Meio Ambiente de Curitiba. No primeiro trabalho, amostras de sangue e soro de 21 cães comunitários foram testadas por métodos moleculares e sorológicos para doenças transmitidas por vetores. Este estudo revelou 7/21 (33,3%) cães infectados por *Mycoplasma haemocanis*, 9/21 (42,8%) por '*Candidatus* Mycoplasma haematoparvum' e 1/21 (4,7%) cão sororreagente para *Borrelia burgdorferi* sensu stricto. No segundo trabalho, 26 amostras de soro de cães comunitários foram avaliadas pela reação de imunofluorescência indireta para quatro diferentes protozoários, que revelou um total de 8/26 (30,7%) cães sorreagentes para *Toxoplasma gondii* e 3/26 (11,5%) para *Neospora caninum*. Apesar da sua exposição diária em áreas públicas com grande circulação de pessoas, os cães comunitários apresentaram uma baixa prevalência de doenças transmitidas por vetores e de zoonoses por protozoários, revelando um baixo risco ambiental e consequente baixa disseminação de patógenos. Por estarem em contato com milhares de pessoas em áreas públicas de Curitiba, os cães comunitários podem ser continuamente monitorados para atuarem como sentinelas para o risco ambiental de doenças transmitidas por vetores e de zoonoses por protozoários em áreas públicas com grande circulação de pessoas.

Palavras-chave: animal sentinela, animal disseminador, perfil sanitário, terminais de ônibus urbanos, parques públicos.

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SUMMARY

1. PRESENTATION

The use of animals as environmental health monitoring has been historically reported (SCHIMIDT, 2009). Dogs may be used as sentinels for pathogens and vectors presence in the environment, acting as diseases biomarkers, particularly, zoonoses (BACKER et al., 2001; BRYAN et al., 2011; CLEAVELAND; MESLIN; BREIMAN, 2006; SALB et al., 2008). These animals may be act as suitable sentinels for public health due to your close relation with human beings, sharing environment and the same food and water sources (BACKER et al., 2001).

However, dogs also may act as reservoirs and point serving as source for vector infection, posing risk public health (SCHIMIDT, 2009). Thus occurs in visceral leishmaniasis, wherein the dog is the main source for sandfly infection in peridomiciliary environment, maintains the infection and precedes human cases (OLIVEIRA et al., 2001; OTRANTO; DANTAS-TORRES; BREITSCHWERDT, 2009).

In this context, other canine vector-borne diseases with zoonotic risk need special attention because dog may act as a possible source for vector infection and as infected ectoparasites spreading (Dantas-Torres, 2008; Schmidt, 2009). Moreover, when the dogs are lake of veterinary support, such as vaccination and parasites control, become more vulnerable to disease and may act as bioaccumulators pathogens in the environment (SALB et al., 2008).

Thus, while dogs may act as sentinels, they may also contribute to the zoonoses and species-specific diseases maintenance and spreading in environment (OTRANTO; DANTAS-TORRES; BREITSCHWERDT, 2009; SALB et al., 2008; SCHIMIDT, 2009).

The 22 bus stations of city of Curitiba, state of Paraná, have been 1.1 daily users of Curitiba and neighboring cities, some bus stations have a circulation of over 100,000 users daily, at the same time housing neighborhood dogs with health status unknown (Table 1). Neighborhood dogs are defined by the World Health Organization (1990) as semi-restricted or free roaming animals with semi-dependence on one or more individuals for food and shelter. In Curitiba, dogs with this profile have been identified in bus stations and public parks. These animals are in daily contact with the population and may act as disseminators and/or sentinels for vector-borne pathogens and environmental zoonoses in urban areas due to their

outdoors life and daily movement (AZZAG et al., 2015; COLLANTES-FERNÁNDEZ et al., 2008; MEIRELES et al., 2004; NAZIR et al., 2014).

TABLE 1 – NEIGHBORHOOD DOGS DISTRIBUTION BETWEEN FEBRUARY AND AUGUST 2014 IN BUS STATIONS AND PUBLIC PARKS, AND ITS DAILLY HUMAN MOVEMENT IN CITY OF CURITIBA, STATE OF PARANÁ, BRAZIL, 2016.

Local	Daily human traffic	Number of identified neighborhood dogs
BS Bairro Alto	18,834	2
BS Barreirinha	28,425	6
BS Campo Comprido	46,598	2
BS Capão Raso	121,928	1
BS Centenário	26,486	2
BS Fazendinha	55,299	2
BS Pinheirinho	130,310	5
BS Portão	67,348	2
BS Santa Felicidade	37,218	2
BS Sítio Cercado	57,330	1
PP Atuba	Not controlled	1
PP Peladeiro	Not controlled	1
TOTAL	589,776	27

FONTE**:** Animal Protection Service of City of Curitiba and Urbanization of City of Curitiba, 2014.
BS: Bus station; PP: Public park

The “Neighborhood Dog Program” was established in Curitiba in February 2013, an initiative of Animal Protection Service/Environment Secretary of City of Curitiba, in partnership with the Universidade Federal do Paraná, with aim to develop an alternative to the population management of street dogs in public areas of high human traffic. The project was financed by the Araucaria Support Foundation for Scientific and Technological Development of the state of Paraná (grant number 292/13). Dogs with neighborhood dog profile that housed in public areas with high human traffic (bus stations and public parks) were identified and registered.

Between February 2013 and December 2015, 51 neighborhood dogs were registered in 13/22 bus stations and two public parks. The neighborhood dogs and their corresponding keeper were identified in bus stations on a daily active search by Animal Protection Service staff and undergraduates of veterinary medicine. In public parks, the neighborhood dogs and their corresponding keeper were registered by population demand by means of city Hall Curitiba phone number 156. After the visit to identify the dogs with neighborhood dog profile, the registration was effected.

In the first approach, the neighborhood dogs were captured and physically restrained to physical examination (SUPPLEMENT 1), microchipping, deworming (Vermivet Plus®, Bio-Vet S / A, Vargem Grande Paulista, São Paulo, Brazil) and vaccination against rabies (Rabisin-i ®, Merial Inc., Lyon, France) and canine species-specific diseases (Recombitek® C6 / CV, Merial Inc., Athens, Georgia, USA). On clinical history form by physical examination were the vital parameters and evaluation of cutaneous, cardiovascular, respiratory, digestive, genitourinary, lymphatic and nervous, among other information. An epidemiological questionnaire was also applied to corresponding keepers with information about the physical and behavioral characteristics of each neighborhood dog, housing environment, food and health (SUPPLEMENT 2). Subsequently to these procedures the animals were neutered/spayed and after surgical recovery returned to local of capture.

The neighborhood dogs were biweekly monitored in the local registry about the hygiene, animal welfare and health. Although we observed the installation of new dogs in these locals and recorded the adoption, disappearance or death of neighborhood dogs already registered. The description of the “Neighborhood Dog Program” in Curitiba was published in the Veterinary Clinic Journal, year XIX, edition number 113, November/December 2014 (APPENDIX A).

Between February and August 2014, only 27 animals remained in the monitored areas, 25 (92.6%) dogs distributed in 10/22 bus stations and two (7.4%) in 2 public parks (TABLE 1). Blood samples from these animals were taken by Animal Protection Service staff for complete blood cell count and biochemistry profile, in order to verify the health status of the monitored animals. Aliquots of blood and serum were stored at -20 ° C in the Zoonoses and Molecular Epidemiology Laboratory of the Veterinary Hospital of the Universidade Federal do Paraná for future analysis.

The Environment Secretary of the city of Curitiba authorized in 2015 the use of these stored samples for research of vector-borne pathogens and environmental zoonoses carried out in this master's dissertation (SUPPLEMENT 3).

Considering the need to know the health status of these neighborhood dogs that sharing public areas with thousands of people and other animals, including stray dogs, the general aim of this research was to reveal the health status of neighborhood dogs housed in bus stations and public parks of city of Curitiba and

evaluate them as spreaders and/or sentinels for vector-borne diseases and environmental zoonoses.

In the first chapter of this work is a literature review about the role of dogs as sentinels for public health. The second chapter presents the health status of 21 neighborhood dogs by molecular and serological techniques for diagnosis, with a focus on vector-borne diseases and the potential of these animals as environmental sentinels in urban areas. The third chapter discusses the neighborhood dogs as fsentinels for environmental zoonoses and potential sentinels for non-endemic vector-borne zoonoses in Curitiba, and expose the serology result of 26 neighborhood dogs for four different protozoa with public health importance. This study was approved by the Ethics Committee for Animal Experimentation and Animal Welfare of the Universidade Federal do Paraná, state of Paraná, Brazil by protocol number 027/2015 (SUPPLEMENT 4).

Part of the literature review of this research, worked for the discipline of Postgraduate Veterinary Science Program of "One Health and Veterinarian", with the professor Dr. Rita de Cássia Maria Garcia, were submitted as an abstract to 22th IUHPE World Conference on Health Promotion to be held in May 2016, which was selected for oral presentation (SUPPLEMENT 5). Partial results of second chapter were presented in poster format at the First Brazilian Meeting of Veterinary Protozoology (APPENDIX B). Chapter II was produced an expanded abstract (APPENDIX C) and a poster (APPENDIX D) presented at the 42nd Brazilian Congress of Veterinary Medicine. Also a lecture talk (SUPPLEMENT 5) and a minicourse (SUPPLEMENT 6) about the “One Health and living with animals”, addressing the neighborhood dogs as sentinels for one health, were held in the First Metropolitan Seminary on Environmental Education, promoted by the city of Pinhais in partnership with the Universidade Tecnológica Federal do Paraná (UTFPR).

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2. A REVIEW THE DOGS AS SENTINELS FOR PUBLIC HEALTH

ABSTRACT

Natural sentinels are animals or animal populations which may be more susceptible for a disease than the target population. Dogs have been identified as early disease indicators due to earlier clinical onset and similar or analogical premature physiological responses when compared to human beings. Furthermore, dogs may be suitable sentinels for human health due to their close relation, overlapping environment and sharing of food and water sources. Accordingly, the aim of this descriptive review was to analyze the reportedly role and suitability of dogs as sentinels for potential hazards for public health on environmental health, food safety, infectious diseases and human criminal behavior. For the research data were used the Pubmed and Google Scholar databases, and articles published from January 1980 to January 2016. Due to their highly exposure, soil contact, cohabitation with human beings and shorter latency between exposure and disease, domestic dogs have been used to indicate environmental contamination of chronic exposure to contaminants with low doses and to identify bioterrorism attacks in course. Since domestic dogs are at the top of food chain and may share the same food sources with human beings, pathogen bio-accumulation may post them as suitable food safety sentinels. Moreover, dogs may be important sentinels for several vector-borne diseases due to available source of blood meal for vectors. Finally, dogs may act as sentinels for human behavior, mainly related to domestic and criminal violence. In summary, dogs may be used as environmental, food and water contamination biomarkers, evidencing infectious, non-infectious diseases and bioterrorist attacks, acting as sentinels for human, animal and environment health (one health). The multidisciplinary and inter-sectoral involving veterinarians and other public health professionals may collaborate to health hazards monitoring, optimizing diagnosis and interventions to prevent the infection spread in the target population.

Key-words: companion animals, bio-indicators, environmental contamination, food safety, infectious diseases, domestic violence.

REVISÃO: OS CÃES COMO SENTINELAS DA SAÚDE PÚBLICA

RESUMO

Sentinelas naturais são animais ou populações de animais suscetíveis mais suscetíveis a uma doença do que a população-alvo. O cão tem sido reconhecido como indicador precoce de doença devido a manifestação clínica e resposta fisiológica prematuras semelhantes ou iguais, quando comparado com os seres humanos. Além disso, os cães podem ser sentinelas adequados para a saúde humana devido à sua estreita relação, e por compartilhar o mesmo ambiente e fontes de alimento e água com seres humanos. Portanto, o objetivo desta revisão descritiva foi analisar o papel e a adequação dos cães como sentinelas para os potenciais perigos para a saúde pública em saúde ambiental, segurança alimentar, doenças infecciosas e comportamento humano. Para o levantamento dos dados utilizou-se as bases Pubmed e Google Acadêmico, e artigos publicados entre janeiro de 1980 a janeiro de 2016. Devido à sua grande exposição, contato com o solo, a coabitação com os seres humanos e menor período de latência entre a exposição e a doença, cães domésticos têm sido usados para indicar a contaminação ambiental e exposição crônica a contaminantes com doses baixas e identificar ataques bioterroristas em curso. Cães domésticos estão no topo da cadeia alimentar e podem compartilhar as mesmas fontes de alimento com seres humanos, a bioacumulação de patógenos pode fazer destes animais adequados sentinelas da segurança alimentar. Além disso, os cães podem ser sentinelas importantes para várias doenças transmitidas por vetores por serem a principal fonte para o repasto sanguíneo de alguns vetores. Finalmente, os cães podem agir como sentinelas para o comportamento humano, principalmente relacionado à violência doméstica e criminal. Em resumo, os cães podem ser utilizados como bioindicadores de contaminação ambiental, de alimentos e da água, além de evidenciar doenças infecciosas, não infecciosas e ataques bioterroristas, atuando como sentinelas da saúde humana, animal e ambiental (saúde única). Equipes multidisciplinares e intersetoriais envolvendo médicos veterinários e outros profissionais da saúde pública podem colaborar para o monitoramento de riscos para a saúde, otimizando o diagnóstico e intervenções para prevenir que a afecção se dissemine na população-alvo.

Palavras-chave: animais de companhia, bioindicadores, contaminação ambiental, doenças infecciosas, violência doméstica.

2.1 Introduction

Sentinel animals have been considered as susceptible animals or animal populations naturally more susceptible to disease than the target population (SCHMIDT, 2009). In such scenario, ideal animal sentinels may produce quick and determinable immune response, present clinical signs even with absence on target species, representing low risk of zoonotic transmission and not acting as reservoirs or hosts. These features may allow early disease detection, thus enabling development of control measures to prevent the spreading over target species.

Animals may act as accidental or experimental sentinels as well (HALLIDAY et al., 2007). They may be accidental sentinels with naturally environmental occurrence with potential risk of disease (BRYAN et al., 2011) or as experimental sentinels when intentionally introduced in such scenarios to determine local occurrence (SCHMIDT, 2009). Moreover, both a single individual as an entire herd may act as grouping level of sentinels for health hazards (HALLIDAY et al., 2007).

The use of animals as environmental sentinel has been historically reported, from the early 1900s with canaries were taken inside the coal mines to signal the carbon monoxide concentration, a toxic gas also for human beings (NATIONAL RESEARCH COUNCIL, 1991). From that point on, a wide range of animal species have been shown as environmental hazard indicators such as sea turtles and fish species for contaminants in aquatic disasters (AGUIRRE; LUTZ, 2004; BÜCKER; CARVALHO; ALVES-GOMES, 2006), also birds and sea turtles population dynamic changes (PRINCÉ; ZUCKERBERG, 2015; QUINTERO-HERRERA et al., 2015; TOMILLO et al., 2015) and vector-borne diseases outbreaks indicating global climatic changes (ANYAMBA et al., 2012; CHAVES et al., 2014; FRANCHINI; MANNUCCI, 2015).

Accordingly, the aim of this review was to analyze the reportedly role and suitability of dogs as sentinels for potential hazards for public health as environmental health, food safety, infectious diseases and human criminal behavior.

2.2 Development

2.2.1 Dogs as sentinels for environmental health

Domestic dogs may act as sentinels for human and animal health when sharing the same environmental spaces (BACKER et al., 2001; BRYAN et al, 2011; SALB et al., 2008; SCHMIDT, 2009). Similar or analogical but relatively premature physiological responses when compared to human beings, may indicate dogs as early disease indicator due to potential shorter latency period (BACKER et al., 2001). Sharing environment with human beings may expose dogs to several common risks and may indicated the environmental health hazards before causing injuries to human beings (REIFF, 2011; SLIZOVSKIY et al., 2015).

Dogs have been used to indicate environmental contamination of chronic exposure to contaminants with low doses. Their exposure to herbicides, arsenic, lead and asbestos, have been described as similar to exposure in humans (BACKER et al., 2001; BERNY CÔTÉ; BUCK, 1995; CASTRO et al., 2013; GLICKMAN et al., 1983).

A study performed in an herbicide contaminated area has shown owned dogs as suitable environmental sentinels in the United States, with herbicide genotoxic effects from contaminated air and water detected in dogs by micronucleus assay (BACKER et al., 2001). Another dog survey has revealed hair of owned dogs as biomarker of chronic arsenic exposure in Argentina, in which dog hair reflected the human cohabitants exposure (CASTRO et al., 2013). In this last study, female dogs were considered better sentinels due to their higher sensitivity to arsenic exposure.

Furthermore, indoor owned dogs have been considered suitable sentinels for environmental lead exposure in human, particularly children, due to their soil proximity and catch on and eat stuff from the floor, similar to the children behavior (BERNY; CÔTÉ; BUCK, 1995). Consequently, the screening in children should be considered when dogs have lead intoxication clinical signs (DOWSETT; SHANNON, 1994).

Since bioterrorism agents have been mostly from zoonotic origin, dogs may play a role as sentinel to identified bioterrorism attacks in course due to your highly exposure, soil contact and proximity of human beings. The clinical signs or

syndromes can be identified early on animals before the injury affect humans (RABINOWITZ et al., 2006).

Likewise, environmental pollutants, as pesticides, dioxins and organic solvents, as well as housed in industrial areas, have been increased risk for canine lymphoma (GAVAZZA et al., 2001; HAYES et al., 1991), bladder cancer (HAYES; HOOVER; TARONE, 1981), and mesothelioma (GLICKMAN et al., 1983) correlated factor with the same diseases in humans. Due to your cohabitation with humans and the shorter latency between exposure and cancer onset, they may be a valuable environmental sentinel for carcinogenic compounds (GAVAZZA et al., 2001; GLICKMAN et al., 1983).

2.2.2 Dogs as sentinels for food safety

Dogs may be suitable food safety sentinels since they share the same food sources with human beings (ARAÚJO et al., 2011; BURNS, 2007; HARLEY et al., 2016; SALB et al., 2008). A recent study in the Artic region has shown mercury bio-accumulation in marine fish populations by monitoring gene expression changes in sled dogs, which have eaten contaminated fish species along human beings, as environmental sentinels for exposure to food threats (HARLEY et al., 2016).

A food contamination episode with melamine has shown owned dogs as good sentinels for food safety in the United States (BROWN et al., 2007; SCHMIDT, 2009). In this report, corn gluten imported from China was used in pet food manufacture and many dogs fed with this feed developed acute renal failure and deaths. Also humans have been exposed to melamine contamination when consumed contaminated animal food supply (BURNS, 2007). Dogs that have intake the pet food contaminated in 2007 developed melamine crystals in renal tubes with particular characteristics (BROWN et al., 2007; LEWIN-SMITH et al., 2009). Therefore, the urine analysis or renal pathology monitoring in dogs may indicate a melamine food contamination (LEWIN-SMITH et al., 2009), since untimely illness and death of pets has led to increased monitoring of imported food supplies for both pets and people (BURNS, 2007; SCHMIDT, 2009).

2.2.3 Dogs as sentinels for vector-borne and other infectious diseases

Domestic dogs may act as sentinels for evidencing the pathogen and vector occurrence (BRYAN et al, 2011; CLEAVELAND; MESLIN; BREIMAN, 2006; SCHMIDT, 2009) due to their infection susceptibility, exposure to infected arthropods, immune-specific response capacity and similar clinical signs in human beings (AZZAG et al., 2015; DINIZ et al., 2007).

Dogs may actively play a role in disease cycle and therefore may be a source for arthropod infection in several vector-borne diseases with zoonotic concern, so the use of dogs as experimental sentinels for diseases such as leishmaniasis should be avoided (SCHMIDT, 2009). On the other hand, in non-endemic areas for *Leishmania* spp. and *Trypanosoma cruzi*, dogs may be used as accidental sentinels due to infection in dogs precedes the disease in human beings (OLIVEIRA et al., 2001; SCHMIDT, 2009). Moreover, although dogs may carry infect ticks to peridomiciliary areas (SCHMIDT, 2009), they may act as accidental sentinels for spotted fever and Lyme disease due to environmental overlapping to infected ticks without providing direct transfer to human beings (GOOSSENS; VAN DEN BOGAARD; NOHLMANS, 2001).

The surveillance of sentinel dogs has been reportedly effective (HALLIDAY et al., 2007; LÓPEZ et al., 2009; SCHURER et al., 2014), particularly when associated with rabies vaccination campaigns for proprietary research authorization, besides for low cost, large number of sampled animals and the easy access and ethical handling of these animals for wild canids, increasing surveillance and understanding the disease spread (BRYAN et al., 2011; CLEAVELAND; MESLIN; BREIMAN, 2006; NEYRA et al., 2015). However, the sentinel animal surveillance only may be effective when all sectors of health care, whether human, animal or environmental health, are alert to the warnings that these animals give (HALLIDAY et al., 2007; LÓPEZ et al., 2009; RABINOWITZ et al., 2005).

Since dogs are at the top of the food chain, they may ingest infected tissues and act as pathogen and pollutant bio-accumulators, allowing the agents identification with low prevalence in animal reservoirs or in wildlife with difficult access for research (CLEAVELAND; MESLIN; BREIMAN, 2006; HARLEY et al., 2016; SALB et al., 2008).

Dogs with outdoors life and free-roaming habits in urban areas may have a higher risk of exposure to vectors and pathogens when compared to owned dogs (AZEVEDO et al., 2005; AZZAG et al., 2015; CARDOSO; MENDÃO; DE CARVALHO, 2012; COLLANTES-FERNÁNDEZ et al., 2008; MAIA et al., 2015; MOURA et al., 2009; NAZIR et al., 2014; VIEIRA et al., 2013). Moreover, in several vector-borne diseases, such as Chagas diseases, leishmaniasis and West Nile fever, dogs can be an important sentinel due to may be a favorite source of blood meal for vectors (GÜRTLER et al., 2009; HALLIDAY et al, 2007; HASSAN et al., 2009; MOLAEI et al., 2007). In addition, the zoonoses surveillance in different dog populations has been proven to be an effective measure for diseases monitoring in a particular region, as well as the effectiveness of control measures adopting (CABEZÓN et al., 2010; SANTI et al., 2014; SCHURER et al., 2012; TENNEY et al., 2014; VERA et al., 2014).

Because your close relation with humans and early measurable immune response (DAVOUST et al., 2014), besides being the most common *Culex* mosquitoes host (MOLAEI et al., 2007), dogs may act as sentinels for human infection by West Nile virus (GAUNT; WALDNER; TAYLOR, 2015; HALLIDAY et al., 2007), particularly juvenile (RESNICK et al., 2008) and stray dogs (KILE et al., 2005). In addition, dogs may have briefly viremia and have been attracted infected mosquitoes to itself, which could reduce the human infection risk by mosquito bites (DAVOUST et al., 2014).

In non-endemic areas for *Leishmania* spp. and *Trypanosoma cruzi*, as well as in endemic areas for West Nile virus, dogs may be used as sentinels due to infection in dogs precedes the disease in human beings (KILE et al., 2005; OLIVEIRA et al., 2001; RESNICK et al., 2008; SCHMIDT, 2009). Moreover, although dogs may carry infect ticks to peridomicilary areas (SCHMIDT, 2009), they may be act as accidental sentinels for spotted fever and Lyme diseases due to sharing environmental to infected ticks without provide direct transfer to human beings (GOOSSENS; VAN DEN BOGAARD; NOHLMANS, 2001).

A study has been indicated dogs as potential sentinel to indicate the re-emerging of *T. cruzi* transmission after treatment with insecticides for the triatomine vectors elimination (NEYRA et al., 2015).

The survey of antibodies anti-*Yersinia pestis* in dogs has been performed as part of the plague surveillance (ARAGÃO et al., 2002). Since dogs may be act as

mechanical vector for plague-infected fleas, veterinarians have been the opportunity to early recognizing this illness in this sentinel population and prevent the propagation in human beings (NICHOLS et al., 2014).

Likewise, in other zoonoses dogs may develop a similar clinical signs or syndrome similar to that developed by humans, allowing early identification of the illness in this species. This has occurred to surveillance of dogs with acute respiratory disease that may provide an early detection influenza virus occurrence (PRATELLI; COLAO, 2014), and to *Bartonella* spp. infection wherein has been observed cardiac and arthritis illness (CHOMEL et al., 2006).

Also, dogs may be a suitable sentinel for human *Toxoplasma gondii* infection because these animals are exposed to similar infection risk due to common transmission routes, either by common contaminated environment by oocysts, or by water or food contaminated consumption (ARAÚJO et al., 2011). Moreover, the low seroprevalence for *Toxoplasma gondii* in dogs may indicate a low environmental oocyst contamination (MEIRELES et al., 2004).

2.2.4 Dogs as sentinels for human behavior

Dogs may act as sentinels for human behavior, particularly domestic violence and criminal, where the animal is a victim of the cycle of violence, being a tool of control and intimidation of children and women who suffer abuse (ASCIONE, 2007; BELL, 2001; TIPLADY; WALSH; PHILLIPS, 2012), the first or one of the victims of aggressive criminals or as a problems behavioral sign in children (ARLUKE; MADFIS, 2014; MELLOR et al., 2015).

People who practice school massacres have been showed a tendency to hurt dogs and cats in previous circumstances, using upclose and personal abuse methods, and choosing unknown victims, expressing the desire to exercise power and control over the lives of others and dominance over another being, albeit a non-human animal (ARLUKE; MADFIS, 2014).

Since the domestic violence may be linked with level of care and the prevalence of negative interactions with dogs (FIELDING, 2010), veterinarian professionals may be able to recognizing animal abuse and that the human members of an abused animal’s family may be at risk for further violence and report these situations to authorities (THIEL, 2002; WILLIAMS et al., 2008).

2.3 Conclusion

Due to their close relation with human beings, sharing environment, food and water sources, domestic dogs may be suitable environmental sentinels for several public health hazards.

Dogs may act as environmental, food and water contamination biomarkers, may show the vector and pathogen occurrence, warn to human behavior deviations and alert for exposure risk to non-infectious diseases and bioterrorist attacks current, making them sentinels for human, animal and environment health (one health).

The multidisciplinary and inter-sectoral involving veterinarians and other public health professionals may collaborate to health hazards monitoring, optimizing diagnosis and interventions to prevent the infection spread in the target population.

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3. VECTOR-BORNE DISEASE SURVEY IN NEIGHBORHOOD DOGS OF SOUTHERN BRAZIL

ABSTRACT

Neighborhood dogs may act as environmental reservoirs and spreaders of vector-borne diseases in urban areas. Accordingly, this study has aimed to access clinical status and canine vector-borne disease (CVBD) pathogens in neighborhood dogs of public areas with highly human traffic from Curitiba City, southern Brazil. Blood samples from 21 neighborhood dogs identified in 9/22 bus stations and 2 public parks were submitted for complete blood cell (CBC) count, biochemistry profile, commercial ELISA rapid test and real-time PCR vector-borne panel (*Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Leishmania* spp., *Neorickettsia risticii*, *Bartonella* spp., *Mycoplasma haemocanis*, '*Candidatus* Mycoplasma haematoparvum'). The CBC count and biochemistry profiles were within normal range and only 1/21 (4.7%) dog was seroreactive for *Borrelia burgdorferi* sensu stricto. Real time PCR has revealed 7/21 (33.3%) dogs with *M. haemocanis* infection, 9/21 (42.8%) with '*Ca*. M. haematoparvum' and 4/21 (19.0%) with both. Association between infected animals and changes in clinical examination, laboratory tests, or ectoparasite presence were not significant ($p>0.05$). In conclusion, neighborhood dogs have revealed low prevalence of CVBD, satisfactory animal welfare and may be used as sentinels of environmental vector-borne disease exposure.

Key words: tick-borne diseases, community dogs, *Borrelia burgdorferi*, hemoplasmas, sentinel animals.

PESQUISA DE DOENÇAS TRANSMITIDAS POR VETORES EM CÃES COMUNITÁRIOS DO SUL DO BRASIL

RESUMO

Cães comunitários podem atuar como reservatórios e disseminadores ambientais de doenças transmitidas por vetores em áreas urbanas. Portanto, o objetivo deste estudo foi verificar o *status* clínico e a ocorrência de doenças transmitidas por vetores (DTVs) em cães comunitários residentes em áreas públicas com grande circulação de pessoas do município de Curitiba, sul do Brasil. Amostras de sangue de 21 cães comunitários identificados em 9/22 terminais de ônibus urbanos e 2 parques públicos foram submetidas a testes de hemograma completo, perfil bioquímico sérico, teste rápido comercial de ensaio imunoenzimático e painel comercial de PCR em tempo real para doenças transmitidas por vetores (*Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Leishmania* spp., *Neorickettsia risticii*, *Bartonella* spp., *Mycoplasma haemocanis*, '*Candidatus* Mycoplasma haematoparvum'). A média dos resultados dos testes de hemograma completo e perfil bioquímico sérico estavam dentro dos intervalos normais para a espécie canina e apenas 1/21 (4,7%) cães foram sororreativos para *Borrelia burgdorferi* sensu stricto. O PCR em tempo real revelou 7/21 (33,3%) cães infectados por *M. haemocanis*, 9/21 (42,8%) por '*Ca.* M. haematoparvum' e 4/21 (19,0%) co-infectados por ambos. Não houve associação significativa ($p > 0,05$) entre infecção por estes patógenos e alterações no exame físico ou nos testes laboratoriais, ou presença de ectoparasitas. Em conclusão, cães comunitários apresentaram baixa prevalência de DTVs e bem-estar satisfatório, e podem atuar como sentinelas ambientais para exposição a doenças transmitidas por vetores.

Palavras-chave: doenças transmitidas por carrapatos, cães de vizinhança, *Borrelia burgdorferi*, hemoplasmas, animais sentinela.

3.1 Introduction

Dogs have been reportedly indicated as potential public health sentinels due to intimate interaction with human beings, particularly owners, associated to common infectious agents and vectors (BACKER et al., 2001; SCHMIDT, 2009; SCHURER et al., 2012). Whereas true sentinels should reveal disease without harboring, dogs may become reservoirs for certain diseases such as visceral leishmaniasis (DANTAS-TORRES, 2009). Besides, dogs may act as potential spreading source for invertebrate vectors and their zoonosis transmission (SCHMIDT, 2009).

Although neighborhood dogs have been defined for more than two decades as semi-restricted or free-range animals with semi-dependence on one or more families for food and shelter (WHO/WSPA, 1990), health status and role on maintenance and spreading of zoonotic diseases has remained to be fully established. In addition, dogs lacking health care such as regular vaccination, deworming and ectoparasite control may have negative impact in animal welfare and more susceptibility to diseases and environmental bioaccumulation (SALB et al., 2008).

Owned dogs of southern Brazil have already shown a higher exposure to tick-borne pathogens in urban areas than in rural settings (VIEIRA et al., 2013b). Moreover, dogs may serve as potential source for transmission of canine vector-borne diseases (CVBDs) with zoonotic risk (DINIZ et al., 2007; EREMEEVA; DASCH, 2015; OTRANTO et al., 2009a, 2009b; VIEIRA et al., 2013a). In such scenario, neighborhood dogs may be expected to have higher infection risk due to urban roaming and vector exposure.

Curitiba City, capital of Parana State, is currently the eighth biggest city and the ninth biggest metropolitan area of Brazil with approximately 3.5 million inhabitants (IBGE, 2014). Although commonly found in low-income neighborhoods of urban areas, neighborhood dogs may find sufficient food and shelter in bus stations due to infrastructure and highly human traffic. Since Curitiba City has no subway, 1.1 million daily users rely on ground transportation interconnected by 22 main bus stations. Accordingly, the aim of the present study was to survey CVBDs pathogens in neighborhood dogs of public areas with highly and daily human traffic (bus stations and public parks) of Curitiba City, southern Brazil.

3.2 Material and Methods

The present study has been approved by the Ethics Committee in Animal Experimentation and Animal Welfare of the Universidade Federal do Paraná, Paraná State, southern Brazil (protocol number 027/2015).

3.2.1 Cross-sectional study and sampling

All 22 bus stations and two public parks of Curitiba City (25°25'40" S and 49°16'23" W), southern Brazil were included in this study (FIGURE 1). Curitiba had at the time of survey an estimated population of 1,864,416 inhabitants distributed within 435,036 km^2, located at 934.6 meters above sea level, under humid subtropical climate, with rainfall rate of 1,434 mm/year and an average winter and summer temperatures of 10 °C and 22 °C respectively (IBGE, 2014).

Dog inclusion criteria was applied according to the World Health Organization (WHO) definition of neighborhood dogs (WHO/WSPA, 1990) as follows: semi restricted or entirely free to wander and established a food and shelter dependency with users and workers at the stations and parks.

A total of 21 neighborhood dogs, 19 from 9/22 bus stations (40.9%) and two from each public park, were identified from February to April of 2014 (FIGURE 1). Dogs were all mixed breed and aged ≥ 1 years, 8/21 (38.1%) were females and 13/21 (61.9%) males. Animals were physically restrained, underwent physical examination and blood samples collected by venipuncture of the jugular vein using commercial sterile vacuum tubes with and without EDTA (BD Vacutainer®, Franklin Lakes, NJ, USA). Thereafter, complete blood cell (CBC) count and biochemistry profile were performed, with the remaining aliquots stored at -20 °C for further molecular and serological analysis.

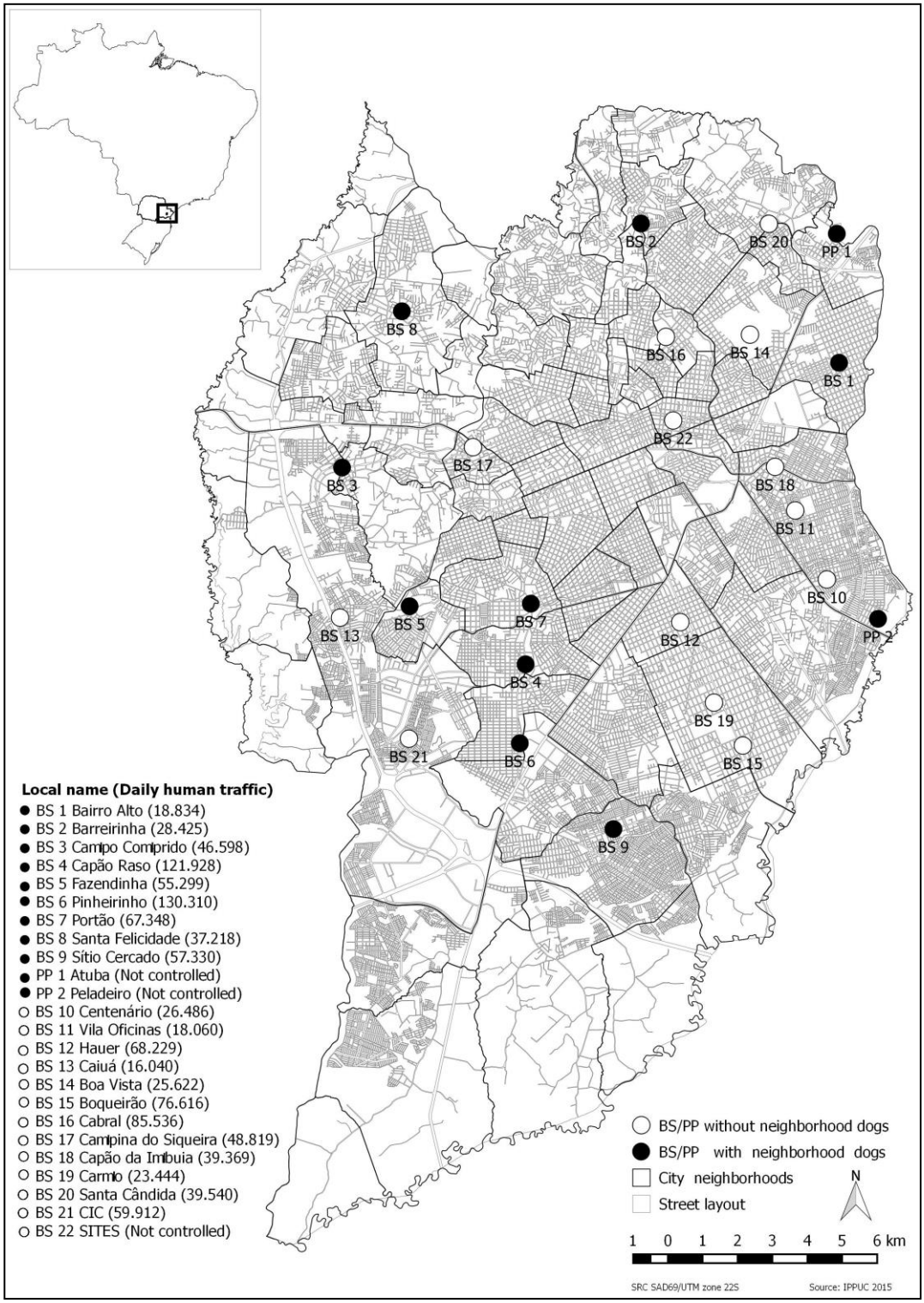

Figure 1. Map of Brazil showing the location of state of Paraná and city of Curitiba. Enlarged map shows location and daily human traffic of bus stations (BS) and public parks (PP) included in the study, distinguishing those with sampled neighborhood dogs. City of Curitiba, State of Paraná, Brazil, 2016.
BS: bus station; PP: public park.

3.2.2 Laboratory tests

Complete blood cell count and biochemistry profile were performed in all samples. The CBC count was performed in a commercial automated hematologic analyzer (CC-550, Celm Ltd, São Paulo, Brazil). Blood smears were made from fresh blood samples, dried, stained by a commercial stain (Diff-Quick - Panótico Rápido LB, Laborclin, São Paulo, Brazil) and the leukocyte differential count and cells morphology analysis performed by light microscopy. Alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and creatinine concentrations were determined by kinetic method, urea concentrations by enzymatic method and total protein and fractions by colorimetric method by a commercial semiautomatic analyzer (Bio-2000lL, Bioplus, São Paulo, Brazil).

Serum samples were tested for *Dirofilaria immitis*, *Ehrlichia* spp. (*E. canis*, *E. chaffeensis* and *E. ewingii*), *Borrelia burgdorferi* sensu stricto (s.s.) and *Anaplasma* spp. (*A. phagocytophilum* and *A. platys*) using a commercial ELISA rapid test (SNAP® 4Dx® Plus, IDEXX Laboratories Inc., Westbrook, ME, USA), according to the manufacturers' instructions.

Whole EDTA-blood samples were tested for *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Leishmania* spp., *Neorickettsia risticii*, *Bartonella* spp., *Mycoplasma haemocanis* and '*Candidatus* Mycoplasma haematoparvum', by a commercial real-time PCR vector-borne panel (IDEXX Laboratories, Westbrook, ME, USA). DNA was extracted from the blood samples using standard protocols on a commercial platform (Corbett XTractor-Gene, Qiagen, Valencia, CA, USA). A housekeeping gene (18S rRNA) was used to determine DNA content and quality. Both the primers for the housekeeping gene and for the PCR test were based on IDEXX's proprietary real-time PCR oligonucleotides (IDEXX Laboratories). Real-time PCR was performed using default-cycling conditions on a commercial instrument (Roche LC480 in the 384-well plate configuration, Roche Applied Science, Indianapolis, IN, USA).

3.2.3 Statistical analysis

Data were stored in electronics spreadsheets (Microsoft Excel® 2010) and later analyzed with statistical software SPSS (version 17.0). Descriptive analyses were performed with frequency distributions and in order to verify bivariate

associations, nonparametric Fisher's exact tests were conducted, at the 5% significance level. Besides, to measure the relationship between daily human traffic and number of neighborhood dogs in the study locations, the Pearson correlation coefficient was calculated.

3.3 Results

All 21 neighborhood dogs were considered clinically healthy and with CBC count (TABLE 2) and biochemistry profile (TABLE 3) means within the normal range for the species, except for eosinophils, wherein 12/21 (57.1%) dogs have eosinophilia (APPENDIX E). Only 1/21 (4.8%) dog was seroreactive for *Borrelia burgdorferi* s.s. by the commercial ELISA rapid test. The commercial real-time PCR panel revealed 7/21 (33.3%) positive dogs for *M. haemocanis*, 9/21 (42.8%) for '*Ca.* M. haematoparvum', and 4/21 (19%) infected by both (TABLE 4). All 21 neighborhood dogs tested negative for the remaining CVBDs pathogens by both real-time PCR and ELISA rapid test.

No significant association was found between gender (p = 0.337) or presence of ectoparasites (p = 0.638) and infection by *M. haemocanis*, neither between gender (p = 0.642) or presence of ectoparasites (p = 0.642) and infection by '*Ca.* M. haematoparvum'. Number of neighborhood dogs was not correlated with daily human traffic of bus stations (*Pearson correlation coefficient = 0,347, p = 0,123*).

TABLE 2. HEMATOLOGIC POPULATION CHARACTERISTICS: COMPLETE BLOOD CELL COUNT OF 21 NEIGHBORHOOD DOGS FROM 9 BUS STATIONS AND 2 PUBLIC PARKS OF CITY OF CURITIBA, STATE OF PARANÁ, BRAZIL, 2016.

Parameter	Normal range in dogs*	Mean ± Standard deviation	Minimum and maximum value
RBC (M/ml)	5.5 – 8.5	7.10 ± 1.04	7.10 – 10.00
HGB (g/dl)	12 – 18	16.86 ± 1.65	13.00 – 20.00
HCT (%)	37 – 55	50.24 ± 6.11	38.00 – 68.00
MCV (fL)	60 – 77	70.81 ± 6.12	52.50 – 80.20
MCH (pg)	19 – 23	23.91 ± 1.98	19.40 – 27.10
MCHC (g/dl)	32 – 36	33.36 ± 3.35	21.40 – 36.90
PP (g/dl)	6 – 8	7.45 ± 0.90	6.20 – 10.40
WBC (No./ml)	6,000 – 17,000	10.65 ± 3.12	6.00 – 18.10
Neutrophils (No./ml)	3,000 – 11,500	5929.19 ± 2266.29	3240.00 – 10557.00
Lymphocytes (No./ml)	1,000 – 4,800	2490.33 ± 1472.60	744.0 – 6878.00
Monocytes (No./ml)	150 – 1,350	480.62 ± 292.63	101.00 – 1060.00
Eosinophils (No./ml)	120 – 1,700	1721.67 ± 1074.57	550.00 – 4092.00
Basophils (No./ml)	0 – 340	0 ± 0	0

RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PP: plasmatic protein; WBC: white blood cells.
*Weiss; Wardrop; Schalm, 2010.

TABLE 3. HEMATOLOGIC POPULATION CHARACTERISTICS: BIOCHEMISTRY PROFILE OF 21 NEIGHBORHOOD DOGS FROM 9 BUS STATIONS AND 2 PUBLIC PARKS OF CITY OF CURITIBA, STATE OF PARANÁ, BRAZIL, 2016.

Parameter	Normal range in dogs*	Mean ± Standard deviation	Minimum and maximum value
ALT (U/l)	21 – 102	30.33 ± 9.32	15.00 – 57.00
ALP (U/l)	20 – 156	25.48 ± 14.91	8.00 – 58.00
Urea (mg/dl)	21 – 60	37.33 ± 13.07	15.00 – 64.00
Creatinine (mg/dl)	0.5 – 1.5	1.02± 0.16	0.70 – 1.30
TP (d/dl)	5.4 – 7.1	7.21 ± 0.91	5.60 – 9.90
Albumine (g/dl)	2.6 – 3.3	3.09 ± 0.34	2.40 – 3.80
Globuline (g/dl)	2.7 – 4.4	4.12 ± 0.84	3.20 – 6.50

ALT: alanine aminotransferase; ALP: alkaline phosphatase; TP: total protein.
* Kaneko; Harvey; Bruss, 2008.

TABLE 4. LOCATION AND RESULTS OF MOLECULAR AND SEROLOGICAL TESTS PERFORMED ON BLOOD SAMPLES OF 21 NEIGHBORHOOD DOGS OF CITY OF CURITIBA, STATE OF PARANÁ, BRAZIL, 2016.

Neighborhood dog		Commercial real-time PCR vector-borne panel				Commercial ELISA rapid test	
		Micoplasma haemocanis		'*Candidatus* Micoplasma haematoparvum'		*Borrelia burgdorferi*	
Location	Number	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
BS 1	1	0/1 (0)	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)	1/1 (100)
BS 2	5	1/5 (20)	4/5 (80)	1/5 (20)	4/5 (80)	0/5 (0)	5/5 (100)
BS 3	1	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)
BS 4	1	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	1/1 (100)
BS 5	2	1/2 (50)	1/2 (50)	2/2 (100)	0/2 (0)	0/2 (0)	2/2 (100)
BS 6	5	2/5 (40)	3/5 (60)	1/5 (20)	4/5 (80)	0/5 (0)	5/5 (100)
BS 7	2	0/2 (0)	2/2 (100)	2/2 (100)	0/2 (0)	1/2 (50)	1/2 (50)
BS 8	1	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (50)
BS 9	1	1/1 (100)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (50)
PP 1	1	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (50)
PP 2	1	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	1/1 (50)
Total	21	7/21 (33.3)	14/21 (66.6)	9/21 (42.8)	12/21 (57.1)	1/21 (4.8)	20/21 (95.2)

BS: bus station; PP: public park

3.4 Discussion

In this cross-sectional study**,** neighborhood dogs have been screened for CVBD pathogens by serological and molecular methods. Despite WHO definition published 25 years ago, to the best of authors´ knowledge, this is the first study to survey health status and CVBD pathogens in neighborhood dogs. Since these dogs may have continuously spent outdoor and roaming habits, exposure to vectors and pathogens has been expected to be relatively higher when compared to owned dogs (AZZAG et al., 2015).

Surprisingly, dogs were healthy on clinical examination, with CBC count and biochemistry profile means within the normal range, and mostly tested negative for CVBDs. In Brazil, previous studies have shown that prevalence of CVBD pathogens in dogs from urban areas has varied from absent to 91.2% (LASTA et al., 2013; SPOLIDORIO et al., 2013; VIEIRA et al., 2013b). Despite neighborhood dogs have comparatively presented lower CVBD prevalence (AZZAG et al., 2015; VIEIRA et al., 2013b) in the present study, such widely differences in CVBD prevalence may be explained by the population studied, lifestyle, environmental occurrence of vector and disease, diagnostic test used, and vector competence of ticks from the *Rhipicephalus sanguineus* group (BALAKRISHNAN et al., 2014; MAIA et al., 2015; MORAES-FILHO et al., 2015).

As observed herein, infection by CVBD pathogens may occur in the absence of clinical signs (AZZAG et al., 2015; JOPPERT et al., 2001; MAGGI et al., 2014) and hematological changes (BALAKRISHNAN et al., 2014; MORAES-FILHO et al., 2015; NOVACCO et al., 2010). A previous study in owned dogs have shown no significant associations between hemoplasma infection and anemia, ectoparasite infestation, gender and clinical status (TENNANT et al., 2011), corroborating with the findings in neighborhood dogs.

Although neighborhood dogs with outdoors life in urban areas may have a higher risk of exposure to tick-borne pathogens (CARDOSO; MENDÃO; DE CARVALHO, 2012; MAIA et al., 2015; VIEIRA et al., 2013b), only 1/7 (14.3%) dog infected by *M. haemocanis* and 1/9 (11.1%) by '*Ca.* M. haematoparvum' were infested by ticks. No association was found between the presence of ectoparasites and positivity to at least one *Mycoplasma* spp. (p = 0.397).

Although *Mycoplasma* spp. infection has been previously reported in dogs (RAMOS et al., 2010; VALLE et al., 2014; VIEIRA et al., 2015) and associated to exposure to ticks and fleas in Brazil (VALLE et al., 2014), our findings have corroborated with other reports from Brazil, Greece and Africa, which found no association between hemoplasma infection and ticks (BARKER et al., 2010; TENNANT et al., 2011; VIEIRA et al., 2015). As clinical examinations were limited to samplings, dogs may have acquired and eliminated ticks before sampling occasions.

Despite the low hemoplasma prevalence by *M. haemocanis* and '*Ca*. M. haematoparvum' in the neighborhood dogs, these pathogens have been previously reported infecting humans (MAGGI et al., 2013a; MAGGI et al., 2013b; KALLICK, 2010). Since neighborhood dogs may play a role as environmental CVBDs reservoirs and/or spreaders, zoonotic potential should be always considered, particularly due to the daily close contact with users at bus stations. Thus, these animals should be continuously monitored as environmental sentinels for hemoplasmas and other CVBD pathogens.

Although positive serology has been already reported in dogs, human beings and horses in Brazil, no molecular evidence of *B. burgdorferi* infection has been found to date (JOPPERT et al., 2001; LABARTHE et al., 2003; MONTANDON et al., 2014; SPOLIDORIO et al., 2010), making the seropositive neighborhood dog for *B. burgdorferi* identified in the present study a potential false positive. This result may be due to high sensitivity (94.1%) and good specificity (96.2%) for specific antibodies to the C6 synthetic peptide to *B. burgdorferi* s.s. detected by the commercial ELISA rapid test used herein**.**

Alternatively, our findings may be consequence of low rate of infected ticks (SPOLIDORIO et al., 2010) or a conceivable different *B. burgdorferi* strain or like-species in Brazil (JOPPERT et al., 2001; MONTANDON et al., 2014). Regardless, further studies should be performed to fully establish a potential association of antibody presence and the zoonotic Brazilian Baggio-Yoshinari Syndrome.

Since direct transfer of infected ticks between dogs and human beings have been considered as a minor transmission factor (GOOSSENS et al., 2001), dogs may not be important pathogen spreaders. As previously shown, dogs may be suitable sentinels to evaluate environmental risk of human exposure to *B. burgdorferi* (DUNCAN et al., 2005). Moreover, as dogs may be more exposed to ticks,

particularly in endemic areas, monitoring of antibodies anti-*B. burgdorferi* in neighborhood dogs may indicate an environmental risk of human exposure.

Despite relatively low number of individuals, neighborhood dogs were randomly distributed throughout the city (FIGURE 1). In addition, high result repetition has been observed since all dogs were considered with adequate health status and within normal range for CBC count and biochemistry profile for the species, with low prevalence of CVBDs.

Finally, adequate clinical and laboratorial health status of neighborhood dogs may have implied that neighborhood dogs of Curitiba City have been living under good animal sanitary and welfare conditions, particularly since some of bus stations have a human traffic over than 100,000 daily users (FIGURE 1). Besides, since neighborhood dogs have received water, food and shelter at the bus stations, individual CBC count and clinical biochemistry all together may indicate animal welfare and an overall non-harmful environment.

3.5 Conclusion

Despite lacking traditional ownership, neighborhood dogs have shown low of CVBD prevalence, revealing low environmental risk and consequently low risk of spreading, particularly in bus stations. Moreover, clinically healthy dogs with CBC count and biochemistry profile within normal range may represent low environmental toxicity and satisfactory animal welfare. In conclusion, neighborhood dogs should be continuously monitored and may serve as sentinels of environmental risk such as vector-borne diseases in public areas with highly human traffic.

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4. SPATIAL DISTRIBUTION OF SEROSURVEY FOR *LEISHMANIA* SPP., *TOXOPLASMA GONDII*, *TRYPANOSOMA CRUZI* AND *NEOSPORA CANINUM* IN NEIGHBORHOOD DOGS IN SOUTHERN BRAZIL

ABSTRACT

Neighborhood dogs may act as reservoirs for several zoonotic protozoan infections, particularly in urban areas, thus constituting a potential public health threat. Accordingly, the aim of the present study was to evaluate the exposure of neighborhood dogs to four protozoan pathogens in public areas with high levels of human movement in Curitiba, southern Brazil. Blood samples from 26 neighborhood dogs were screened by means of the indirect immunofluorescent antibody test (IFAT) for *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum*, and a questionnaire was answered by the respective keeper. A total of 8/26 dogs (30.7%) seroreactive to *T. gondii*, 3/26 (11.5%) to *N. caninum* and 2/26 (7.7%) to both were identified. All the samples were seronegative for *T. cruzi* and *Leishmania* spp. Pathogen seroreactivity was not associated with the daily human movements or other epidemiological variables investigated ($p > 0.05$). In conclusion, despite daily exposure in public areas with high levels of human movement, the low protozoan seroprevalence may have indicated low environmental and food risk for animal infection. Moreover, neighborhood dogs may be used as environmental sentinels for the presence of protozoan pathogens and their vectors.

Key-words: community dogs, urban area, sentinel animals, toxoplasmosis, neosporosis, IFAT.

DISTRIBUIÇÃO ESPACIAL DE INQUÉRITO SOROLÓGICO PARA *LEISHMANIA* SPP., *TOXOPLASMA GONDII*, *TRYPANOSOMA CRUZI* E *NEOSPORA CANINUM* EM CÃES COMUNITÁRIOS DO SUL DO BRASIL

RESUMO

Cães comunitários podem atuar como reservatórios para algumas zoonoses causadas por protozoários, principalmente em áreas urbanas, constituindo potencial ameaça à saúde pública. Portanto, o objetivo do presente estudo foi avaliar a exposição de cães comunitários a quatro protozoários em áreas públicas com alta circulação de pessoas em Curitiba, sul do Brasil. Amostras de sangue de 26 cães comunitários foram testadas pela reação de imunofluorescência indireta (RIFI) para *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* e *Neospora caninum*, e um questionário foi respondido pelo respectivo mantenedor. Um total de 8/26 (30,7%) foram sororeagentes para *T. gondii*, 3/26 (11,5%) para *N. caninum* e 2/26 (7,7%) para ambos. Todas as amostras foram soronegativas para *T. cruzi* e *Leishmania* spp. Não houve associação entre sororeatividade para os patógenos pesquisados e o tráfego diário de pessoas e outras variáveis epidemiológicas analisadas ($p > 0,05$). Concluindo, apesar de sua exposição diária em áreas públicas de grande circulação de pessoas, a baixa soroprevalência para protozoários pode ter indicado baixo risco ambiental e alimentar para a infecção dos animais. Além disso, os cães comunitários podem atuar como sentinelas ambientais quanto à presença de protozoários e seus vetores.

Palavras-chave: cães comunitários, área urbana, animais sentinela, toxoplasmose, neosporose, RIFI.

4.1 Introduction

Neighborhood dogs (WHO/WSPA, 1990) may act as reservoirs or sentinels for several zoonotic and protozoan infections, particularly in urban areas, thus constituting a potential threat to animal and public health (CABEZÓN et al., 2010; MEIRELES et al., 2004; ROBERTSON et al., 2000; SALB et al., 2008). Although protozoan infection has previously been assessed in different canine populations (AZZAG et al., 2015; COLLANTES-FERNÁNDEZ et al., 2008; LANGONI et al., 2013), the health status of neighborhood dogs remains to be fully established.

Because of the outdoor and free-roaming habits of neighborhood dogs, these dogs may present greater exposure to environmental and vector-borne diseases such as *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum* (CABEZÓN et al., 2010; SEABRA et al., 2015). Accordingly, the aim of the present study was to evaluate the exposure of neighborhood dogs to four protozoan pathogens in public areas with highly levels of human movement in the city of Curitiba, the eighth biggest city in Brazil.

4.2 Material and Methods

This study was approved by the Ethics Committee for Animal Experimentation and Animal Welfare of the Federal University of Paraná, state of Paraná, Brazil (protocol number 027/2015).

A total of 26 neighborhood dogs were identified in 10/22 bus stations (45.5%) and two public parks in the city of Curitiba (25°25’40” S; 49°16’23” W), state of Paraná, southern Brazil, between February and August 2014. These dogs were all of mixed breed, aged ≥ 1 year, and comprised 9/26 females (34.1%) and 17/26 males (65.4%). An epidemiological questionnaire was applied to corresponding keeper about the dog’s environmental conditions and habits such as organic material accumulation, raw meat intake and hunting practices.

Whole blood samples (5 mL) were collected by means of venipuncture of the jugular vein using sterile vacuum tubes containing serum separator gel (BD Vacutainer, Franklin Lakes, NJ, USA) and were stored at room temperature (25 °C) until visible clot retraction. The samples were centrifuged at 1500 g for 5 minutes, serum separated and stored at –20 °C until serological analysis. The serum samples

were tested for specific IgG antibodies against *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum* by means of the indirect immunofluorescent antibody test (IFAT), as previously described (CAMARGO, 1974). Samples were considered reactive when antibody titers ≥ 40 for *Leishmania* spp., ≥ 16 for *T. gondii*, ≥ 20 for *T. cruzi* and ≥ 25 for *N. caninum* were found. The titers were determined to the largest dilution at which fluorescence was viewed around the protozoa (endpoint titers). Fisher's exact test was used to determine differences relating to whether individual factors were associated with seroreactivity through IFAT. Moreover, the Spearman correlation coefficient was calculated to verify the correlation between daily human movement and seroreactivity. Results were considered to be statistically significant when $p < 0.05$. Data were stored and analyzed using commercially available software (Epi Info, version 7.1.5.2, CDC, Atlanta, USA).

4.3 Results

In total, 3/26 neighborhood dogs (11.5%; 95% CI: 2.35-29.16%) were seroreactive to *N. caninum*, with antibody titers ranging from 25 to 200; 8/26 (30.7%; 95% CI: 14.33-51.79%) were seroreactive to *T. gondii*, with antibodies titers ranging from 16 to 64; and only 2/26 (7.7%) were seroreactive to both (FIGURE 2). All the dogs were seronegative to *Leishmania* spp. and *T. cruzi*. No significant associations were found between gender, diet, raw meat intake or hunting practices and seroreactivity to *N. caninum* and/or *T. gondii*. The seroprevalence results for *N. caninum* and *T. gondii* among the neighborhood dogs and the respective variables are presented in Table 5. Likewise, no correlation was found between daily human movement and seroreactivity to *N. caninum* (Spearman correlation coefficient = -0.174; $p = 0.631$) and/or *T. gondii* (Spearman correlation coefficient = 0.302; $p = 0.397$).

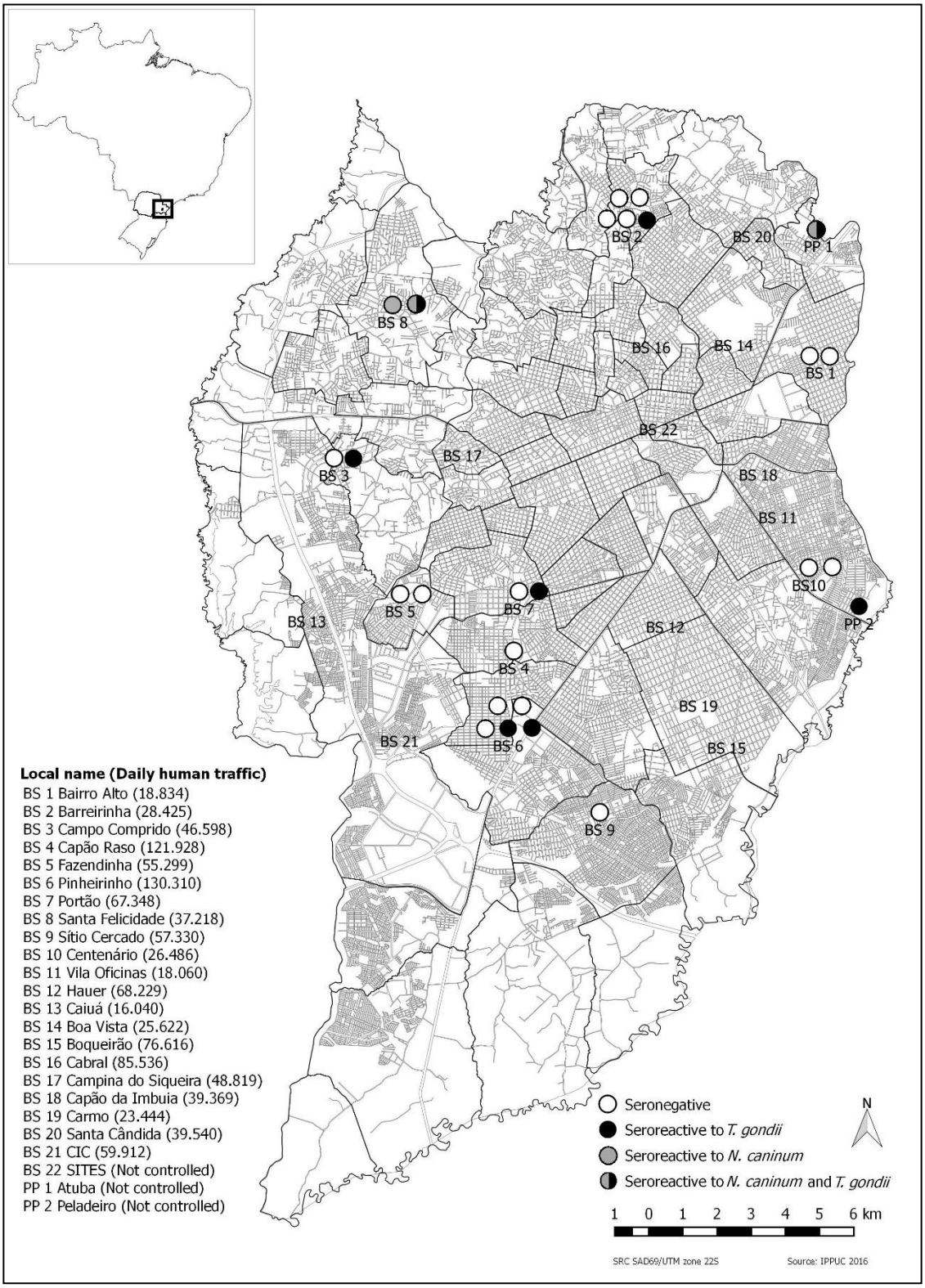

FIGURE 2. Map of Brazil showing the location of the state of Paraná and city of Curitiba. Enlarged map shows the spatial distribution of neighborhood dogs according to blood test results, in relation to *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum*, and daily human movements in bus stations (BS) and public parks (PP) included in the study, city of Curitiba, state of Paraná, southern Brazil, 2016.
BS: bus stations; PP: public parks.

TABLE 5. SEROPREVALENCE FOR NEOSPORA CANINUM AND TOXOPLASMA GONDII AMONG NEIGHBORHOOD DOGS ACCORDING TO IFAT, IN RELATION TO EACH VARIABLE STUDIED, CITY OF CURITIBA, STATE OF PARANÁ, SOUTHERN BRAZIL, 2016.

	Neospora caninum				*Toxoplasma gondii*			
Variable	+/N (%)	OR	95% CI	*p*-value	+/N (%)	OR	95% CI	*p*-value
Gender								
Male	1/17 (5.9%)	0.50	0.01 – 44.10	1.0000	3/17 (17.6%)	0.17	0.02 - 1.42	0.0781
Female	2/9 (22.2%)	Ref.			5/9 (55.6%)	Ref.		
Hunting practice								
Yes	1/8 (12.5%)	1.14	0.02 – 25.42	1.0000	2/8 (25.0%)	0.66	0.05 – 5.56	1.0000
No	2/18 (11.1%)	Ref.			6/18 (33.3%)	Ref.		
Raw meat intake								
Yes	1/5 (20.0%)	2.4	0.03 – 55.07	0.9769	1/5 (20.0%)	0.50	0.09 - 6.63	1.0000
No	2/21 (9.5%)	Ref.			7/21 (33.3%)	Ref.		

+: number of positive animals; N: number of samples per variable; OR: odds ratio; 95% CI: 95% confidence interval; Ref.: variable used as a reference value; -: undetermined.

4.4 Discussion

Surprisingly, to the best of our knowledge, this study was the first serosurvey on this dog population worldwide. These neighborhood dogs have spent mostly of their free-roaming lives in densely occupied public areas, such as bus stations and public parks, thus sharing their environment with around 1.1 million daily bus users in the city of Curitiba.

Despite the outdoor and free-roaming habits of neighborhood dogs, which may increase their exposure to protozoan infections (AZEVEDO et al., 2005; COLLANTES-FERNÁNDEZ et al., 2008; MOURA et al., 2009; NAZIR et al., 2014), they presented in this study a low seroprevalence for *T. gondii* and *N. caninum* compared with literature results (MEIRELES et al., 2004; SEABRA et al., 2015; VALADAS et al., 2010), which may indicate that there was a situation of low environmental oocyst contamination (MEIRELES et al., 2004).

Since dogs play a secondary role in the toxoplasmosis cycle, neighborhood dogs may be considered to be true environmental sentinels for *T. gondii*, particularly when sharing a contaminated urban environment and contaminated food and water (MEIRELES et al., 2004). In contrast, as the definitive hosts of *N. caninum*, dogs may play an important role in spreading this disease (NAZIR et al., 2014).

As expected, all the neighborhood dogs tested negative for *Leishmania* spp. and *T. cruzi* in the present study. In fact, the city of Curitiba has been considered to be a non-endemic area for both of these diseases and, thus, these results may reflect the historical absence of vectors and pathogens. However, canine visceral leishmaniasis was previously detected in 1/364 stray dogs (0.0027%) from a neighboring city (FREHSE et al., 2010), and triatomine (*T. cruzi* vector) infestation was detected in 7/9 cities (77.8%) in the northern part of the state of Paraná (FALAVIGNA-GUILHERME et al., 2004). Moreover, the first autochthonous case of human visceral leishmaniasis in the state of Paraná was registered in 2015, and the first death due to this disease was reported in January 2016 (PARANÁ STATE SERVICE, 2016). Since infection of dogs by *Leishmania* spp. may precede human cases (OLIVEIRA et al., 2001), periodic serosurveys among neighborhood dogs may be used as a means of active surveillance in non-endemic areas such as the city of Curitiba.

4.5 Conclusion

Despite daily exposure of these neighborhood dogs to risk of infection, in public areas with high levels of human movement, the protozoan seroprevalence was within the general population range and may indicate that there was a situation of low environmental and food risk with regard to animal infection. Moreover, neighborhood dogs may be used as environmental sentinels for the presence of these protozoan pathogens and their transmission vectors in non-endemic areas.

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5. GENERAL CONCLUSIONS

This research allowed to show the neighborhood dogs health status and their minor role on maintenance and spreading of vector-borne and zoonotic diseases in public areas of city of Curitiba.

The adequate CBC count and biochemistry profile associated with clinical status, may have implied that neighborhood dogs have been living under good animal sanitary and welfare conditions. Besides, since neighborhood dogs have received water, food and shelter at the bus stations, their clinically healthy may indicate an overall non-harmful environment.

Moreover, despite their daily exposure in public areas with highly human movement, neighborhood dogs have shown low vector-borne disease and protozoan zoonoses prevalence, revealing low environmental risk and consequently low risk of spreading.

Neighborhood dogs may have spent your entirely time in the same place, at the same time that may have even high exposure for vector-borne diseases, protozoan diseases and their vectors due to their outdoors life and free roaming habits. Since the neighborhood dogs are in daily contact with stray dogs and thousands of people in public areas of city of Curitiba, these animals should be continuously monitored as part of monitoring of zoonoses service, making them sentinels of environmental risk for vector-borne diseases and protozoan zoonoses in public areas with highly human movement.

APPENDICES

APPENDIX A

Published article: “Neighborhood dogs as animal management public policy”

Cães comunitários como política pública de manejo animal

Ao andar pelas ruas dos grandes centros urbanos do Brasil (e do mundo), podemos notar uma grande quantidade de animais em situação semidomiciliar ou mesmo de abandono. A falta de políticas públicas efetivas e de conhecimento sobre guarda responsável e sobre o manejo populacional de animais domésticos tem contribuído para esse desequilíbrio nas ruas, sobretudo de cães, gerando discussão e polêmica na sociedade e tornando-se um grande desafio para os gestores.

Muitos cães escolhem locais específicos para se instalar, como, por exemplo, os cães comunitários – termo utilizado pela Organização Mundial de Saúde (OMS) para definir animais de circulação semirrestrita ou totalmente livre e que são semidependentes de uma ou mais famílias quanto à alimentação e ao abrigo. A lei n. 12.916/2008 do estado de São Paulo define o cão comunitário como aquele que estabelece vínculos de dependência e manutenção com a comunidade onde vive, embora não haja um responsável definido por ele. Geralmente esses animais contam com mantenedores, ou seja, munícipes que fornecem alimento e abrigo de forma continuada.

Segundo estudos da Universidade Estadual Paulista (Unesp) de Araçatuba, a eliminação maciça de cães adultos, ainda promovida pelos Centros de Controle de Zoonoses em alguns estados brasileiros, pode gerar um aumento da população jovem, com implicações epidemiológicas tais como aumento da suscetibilidade a doenças, diminuição da resposta à vacinação e aumento da prolificidade reprodutiva. Desde o início da década de 1990 a Organização Mundial da Saúde não recomenda mais a eutanásia (ou extermínio) como método isolado de controle populacional de cães, pois, quando um cão é removido de determinado local, as condições ambientais permanecem favoráveis para que outro animal não monitorado se instale ali. Além disso, a eutanásia de animais sadios e para fins de manejo populacional já é proibida por lei em alguns estados brasileiros como São Paulo (Lei Estadual n. 12.916/08), Paraná (Lei Estadual n. 17.422/12) e Rio Grande do Sul (Lei Estadual n. 13.193/09), gerando assim a necessidade de desenvolver medidas alternativas para prevenir a superpopulação de cães de rua.

Estudos da Universidade Federal do Paraná (UFPR) mostram que o fluxo de animais tende a diminuir no local onde vive um cão comunitário, visto que este possui um comportamento de dominância territorial natural, evitando que outros cães se instalem na mesma área. Quando vacinados, castrados e desverminados regularmente, esses cães também contribuem como barreira sanitária e reprodutiva. Outros países em desenvolvimento, como a Índia, também comprovaram a diminuição da incidência de raiva na população humana em locais onde havia cães comunitários vacinados contra o vírus rábico.

“No município de Curitiba, capital paranaense, o projeto Cão Comunitário, parceria entre a Prefeitura Municipal de Curitiba e a UFPR, financiado pela Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná (FAP), foi implantado no primeiro semestre de 2013 como estratégia de manejo populacional de cães em terminais de ônibus e alguns parques da cidade (Figuras 1 e 2).

Figura 1 - Equipe da Prefeitura de Curitiba vacinando, microchipando e agendando a castração de cão comunitário em um dos terminais de ônibus de Curitiba

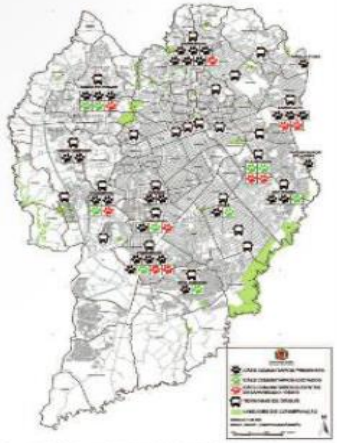

Figura 2 - Mapa dos terminais de ônibus e das unidades de conservação do Município de Curitiba com cães comunitários registrados no período de março de 2013 a outubro de 2014 (Fonte: adaptado de Urbanização de Curitiba S/A – URBS)

Segundo a Organização Mundial da Saúde Animal, a dinâmica populacional de cães está relacionada às atividades humanas, portanto o manejo populacional de cães é afetado por qualquer mudança no comportamento humano. A inserção de políticas públicas nessa esfera é complexa, envolvendo diversos fatores e exigindo postura da população e da administração pública perante essa problemática. Ao passo que, ao se optar por implantar o programa Cão Comunitário, deve-se atentar para uma série de medidas e para o papel de cada setor, para só então estabelecer atitudes conjuntas.

Para implantar o programa no município de Curitiba, houve a interação dos gestores com a comunidade para que as seguintes práticas fossem adotadas:

1 – Definição dos locais: isso é feito de acordo com a demanda existente, sendo os principais critérios para a seleção a quantidade de animais presentes, a relação destes com a comunidade (mantenedores) e a frequência de pessoas nesses locais. Terminais de ônibus, por exemplo, favorecem o comportamento de dominância territorial, por serem locais mais delimitados. No entanto, parques, praças e *campi* universitários não são lugares de circulação adequados, principalmente pelo potencial risco de agravos (mordeduras) e por colocarem em risco a fauna nativa local;

2 – Pesquisa de opinião da população: o fato de a comunidade aceitar manter os cães comunitários no local foi importante para o bom andamento do programa. Geralmente, grande parte da população é a favor de alimentar e abrigar animais de rua; entretanto, muitos não concordam com isso, pelo risco a que esses animais podem estar expostos, bem como pelo risco de agressão a pessoas e pela degradação ambiental. No caso de Curitiba, 75% dos telespectadores na chamada do jornal do almoço da maior rede de televisão estadual ligaram aprovando a iniciativa;

3 – Identificação dos cães com perfil de cães comunitários: Feita por meio de estudos observacionais de comportamento do animal e fundamental para se evitarem agravos e riscos para o animal e para os usuários do local público. Observa-se sua relação com a comunidade, o estabelecimento de vínculos afetivos e de dependência alimentar e possíveis comportamentos agonísticos;

To be continued.

Figura 3 - Cães comunitários vacinados, microchipados, castrados, desverminados e identificados com coleiras verdes circulam tranquilamente entre os usuários dos tubos de um dos terminais de ônibus de Curitiba

4 – Identificação dos mantenedores: o mantenedor é quem assume compromissos como alimentação e abrigo, e é responsável por comunicar ao órgão público a necessidade de assistência médico-veterinária, além de informar o poder público sobre casos de abandono de cães;

5 – Avaliação da saúde física e comportamental e procedimentos básicos: médicos veterinários, zootecnistas e biólogos estão envolvidos no programa de avaliação da saúde física e comportamental, bem como na realização de procedimentos básicos. A vacinação e a desverminação regulares, assim como a esterilização cirúrgica, são realizadas para tornar os cães comunitários barreiras sanitárias a doenças espécie-específicas e zoonoses, e também barreiras reprodutivas. Os animais foram todos identificados por método permanente (microchipagem) e receberam também coleiras verdes com uma placa identificando o nome do cão e um telefone de contato da Rede de Proteção Animal de Curitiba. Além disso, amostras de sangue e soro são coletadas periodicamente para a realização de hemograma, perfil bioquímico e testes sorológicos e moleculares de patógenos (sendo portanto utilizados como sentinelas de zoonoses urbanas);

6 – Monitoramento periódico dos animais e do fluxo de cães no local: realizado pelos mantenedores e pelo órgão público envolvido no programa para o estabelecimento da saúde única local. São realizados exames periódicos para verificação da sanidade e detecção de comportamentos agonísticos dos animais, que podem ocorrer ao longo do programa;

7 – Educação da população: realizada simultaneamente às outras etapas. O programa tem forte papel educativo e de responsabilidade pelo bem-estar animal, atingindo pessoas de diversas idades, com informações sobre guarda responsável, maus-tratos e o papel do cão comunitário na saúde única.

Ao implantar o programa Cão Comunitário no município, percebeu-se a redução do fluxo de animais nos locais selecionados, devido ao comportamento territorialista dos cães e à esterilização, que impede a reprodução desordenada. Os mantenedores se tornaram monitores de casos de abandono e serviram de multiplicadores para alertar os órgãos públicos envolvidos na adoção de medidas preventivas e punitivas.

Em conclusão, os cães comunitários podem ser integrados ao convívio social em locais adequados, mesmo que esses lugares sejam de grande circulação de usuários, como terminais de ônibus e rodoviárias (Figura 3). Esses cães são geralmente territorialistas, e quando castrados, vacinados, desverminados e microchipados, promovem uma contenção da capacidade de suporte do ambiente, tornando-se barreiras de manejo populacional e sanitário. Os profissionais da medicina veterinária do coletivo (*shelter medicine*) são os mais capacitados para realizar pesquisas de demanda social para melhorar a qualidade de vida de animais em centros urbanos, particularmente da fauna doméstica em risco, semi ou não domiciliada. O grande desafio da recente especialidade é oferecer respaldo científico para que as políticas públicas de manejo animal sejam implementadas de forma correta, eficaz e continuada.

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Conclusion.

APPENDIX B

Presented poster in I Encontro Brasileiro de Protozoologia Veterinária

Cães comunitários como sentinelas de doenças transmitidas por vetores no sul do Brasil – Dados Preliminares

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INTRODUÇÃO

Cães são considerados sentinelas da saúde pública devido a sua estreita relação com os seres humanos, compartilhando o mesmo ambiente e as mesmas fontes de alimento e água (Schmidt, 2009).

A Organização Mundial da Saúde (OMS) define como cão comunitário o animal com circulação semi restrita ou totalmente livre e que depende de uma ou mais famílias para alimentação e abrigo. Estes animais podem atuar como sentinelas de doenças transmitidas por vetores (DTVs), particularmente de zoonoses, por seu comportamento de livre circulação e maior exposição aos vetores. Não é rara a presença de cães comunitários em bairros periféricos e em locais centrais com grande circulação de pessoas como terminais de ônibus urbanos e parques de Curitiba.

OBJETIVOS

O objetivo deste trabalho foi avaliar o papel dos cães comunitários como sentinelas para DTVs em locais públicos de grande circulação de pessoas em Curitiba, Paraná.

MATERIAL E MÉTODOS

Todos os 22 terminais de ônibus urbanos e 2 parques de Curitiba foram inclusos no estudo e os cães com perfil de comunitários aí presentes foram registrados, identificados e monitorados mensalmente entre fevereiro de 2013 e dezembro de 2014. Foi realizada a coleta de sangue e soro entre fevereiro e abril de 2014 para hemograma, perfil bioquímico sérico, ELISA comercial (SNAP® 4Dx® Plus, IDEXX Laboratories Inc.) e um painel comercial de PCR em tempo real (qPCR) para DTVs que é capaz de detectar DNA de *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Leishmania* spp., *Bartonella* spp., *Mycoplasma haemocanis* e '*Candidatus* Mycoplasma haematoparvum' (RealPCR™, IDEXX Laboratories Inc.).

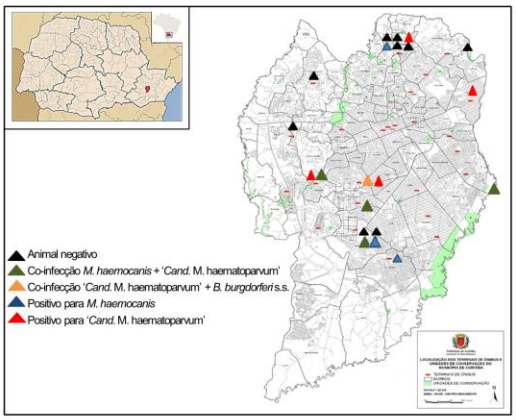
RESULTADOS E DISCUSSÃO

Um total de 51 cães foram identificados em 13 terminais e 2 parques, entretanto foi possível a coleta de sangue/soro e exame físico em apenas 21/51 (41,2%) animais. Todos eram adultos, 8/21 (38,1%) eram fêmeas e 13/21 (61,9%) machos.

Não foram detectadas alterações relevantes no hemograma, perfil bioquímico sérico e no exame físico dos animais. O ELISA comercial detectou anticorpos anti-*Borrelia burgdorferi* sensu stricto (s. s.) em 1/21 (4,7%) cão. O PCR em tempo real para DTVs identificou 7/21 (33,3%) cães infectados por *M. haemocanis* e 9/21 (42,8%) por '*Candidatus* M. haematoparvum', sendo 4/21 (19%) co-infectados por ambos (Figura 1). Todas as amostras foram negativas na qPCR para os demais agentes e não reagentes no ELISA comercial para *Dirofilaria immitis*, *Ehrlichia* spp. (*E. canis*, *E. chaffeensis* e *E. ewingii*), e *Anaplasma* spp. (*A. phagocytophilum* e *A. platys*).

Cães de áreas urbanas do sul do Brasil têm maior risco potencial de infecção por patógenos transmitidos por carrapatos (Vieira et al., 2013). Apesar de cães comunitários apresentarem livre circulação, vivendo em ambientes propícios ao desenvolvimento de carrapatos, apenas dois animais que apresentavam infestação estavam infectados por *M. haemocanis* e '*Cand.* M. haematoparvum'. Estas espécies de micoplasma tem sido relatadas em seres humanos (Kallick, 2010; Maggi et al., 2013), destacando seu potencial zoonótico. Vieira et al. (2015) encontrou cães infectados por estas espécies em um assentamento rural no Paraná, reforçando o papel destes animais como sentinelas para infecção de hemoplasmas em humanos.

Figura 1: Distribuição espacial de cães comunitários de acordo com os resultados do PCR em tempo real e do ELISA comercial para DTVs, Curitiba-PR, Brasil.



A presença de anticorpos anti-*B. burgdorferi* s. s. em um cão sugere a circulação do agente no ambiente. A infecção pelo agente da Doença de Lyme, ou Síndrome Baggio-Yoshinari como é conhecida no Brasil, precede o aparecimento de casos humanos pois estes animais estão mais expostos aos carrapatos, apoiando o conceito de cães como sentinelas adequados também para esta doença emergente (Schurer et al., 2014).

CONCLUSÃO

O adequado estado clínico associado a baixa prevalência de DTVs nos cães comunitários residentes em locais de grande circulação de pessoas de Curitiba, pode indicar um baixo risco ambiental para estes patógenos nestes locais. O monitoramento do status sanitário destes cães pode refletir a exposição humana a DTVs nestes ambientes.

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APPENDIX C

Expanded abstract sent to 42° Congresso Brasileiro de Medicina Veterinária

SANIDADE DE CÃES COMUNITÁRIOS DE CURITIBA/PR

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RESUMO

O projeto "Cão Comunitário" foi implantado em Curitiba/PR no início de 2013. Objetivou-se avaliar a condição sanitária destes animais. Foram coletadas amostras de sangue de 23 animais para exames de hemograma, bioquímico sérico, teste de reação em cadeia da polimerase em tempo real (qPCR), ELISA comercial (SNAP® 4Dx Plus, IDEXX Laboratories Inc.) e reação de imunofluorescência indireta (RIFI). Na análise do sangue, houve algumas alterações características de estresse e infecção parasitária. Só 21 foram submetidas ao qPCR que revelou 6 positivos para *Mycoplasma haemocanis* e 5 para candidatus *Mycoplasma haematoparvum*, 4 apresentando coinfecção. O ELISA detectou anticorpos anti-*Borrelia burgdorferi* em 1 animal. Na RIFI 7 apresentaram anticorpos anti-*Toxoplasma gondii*. Conclui-se que apesar de expostos a diversos riscos, não apresentaram grandes problemas de saúde, entretanto é necessário seu acompanhamento, devido as alterações encontradas e seu contato diário com pessoas.

Palavras-chave: Saúde única, sanidade, zoonoses

To be continued.

SANITY OF COMMUNITY DOGS IN CURITIBA/PR

ABSTRACT

The "Community Dog" project was implemented in Curitiba/PR in 2013. Our purpose was to assess the health condition of these animals. Blood samples were collectedfrom 23 animals for blood count, biochemical tests, real-time polymerase chain reaction (qPCR), commercial ELISA (4DX SNAP® Plus, IDEXX Laboratories Inc.) and indirect immunofluorescence assay (IFA). In blood analysis, there was some stress and parasitic infection characteristics changes. Only 21 were subjected to qPCR, revealed 6 positive for *Mycoplasma haemocanis* and 5 to candidatus *Mycoplasma haematoparvum*, 4 presenting coinfection. The ELISA test detected antibodies anti-*Borrelia burgdorferi* in 1 animal. At IFA 7 showed anti-*Toxoplasma gondii* antibodies. We conclude that although exposed to various risks, showed no large health problems, despite of monitoring is necessary, because the alterations found and their daily contact with people.

Keywords: One Health, sanity, zoonoses

INTRODUÇÃO

O “cão comunitário” ou “cão da comunidade”, é aquele que temos mais de um indivíduo reivindicando direitos sobre o animal (ICAM, 2007). No ano de 2013 a Prefeitura de Curitiba-PR, por meio da Secretária Municipal do Meio Ambiente, começou a cadastrar animais residentes em terminais de ônibus e parques da cidade. Após a identificação desses cães, foram cadastrados seus respectivos mantenedores. Em datas pré-estabelecidas com os mantenedores, foram realizados exames clínicos e coleta de sangue para exames complementares. O objetivo deste estudo foi avaliar a sanidade de cães, residentes em áreas de alta circulação de

To be continued.

pessoas, com perfil de comunitários, por meio de exames clínicos e complementares, tais como hemograma, perfil bioquímico e diagnóstico de algumas doenças infecciosas. A partir dos resultados, buscou-se verificar a susceptibilidade dos animais ou seu possível papel como sentinelas de patógenos relevantes a saúde pública e animal.

MATERIAL E MÉTODOS

Foram cadastrados 49 cães no projeto, sendo 95,92% (47/49) em terminais de ônibus e 4,08% (02/49) em parques. Foi possível realizar os exames completares de 23 destes animais. Os exames laboratoriais realizados foram: Hemograma e perfil bioquímico (FA, ALT, Ureia e Creatinina), Reação em Cadeia da Polimerase em Tempo Real (qPCR) para: *Babesia spp, Anaplasma spp, Ehrlichia spp, Rickettsia spp, Hepatozoon spp, Leishmania spp, Neorickettsia risticii, Bartonella spp, Mycoplasma haemocanis,* candidatus *Mycoplasma haematoparvum, Leptospira spp*e, *Brucella canis*, o Teste de ELISA comercial (SNAP® 4Dx Plus, IDEXX Laboratories Inc.) para: *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi*e, *Anaplasma phagocytophilum*, e a Reação de Imunofluorescência Indireta (RIFI) para: *Trypanosoma cruzi, Neospora canis, Leishmania spp* e *Toxoplasma gondii.*

Os valores de hemograma e perfil bioquímico foram comparados com os valores de referência da espécie canina (SCHALM e JAIN, 1986)(KANEKO, HARVEY e BRUSS, 1997).

RESULTADOS

Nenhum cão apresentou alterações no exame físico. No hemograma apenas um animal (4,35%) apresentou poliglobulia, 2 (8,7%)

To be continued.

policitemia e 5 (21,73%) aumento da concentração de hemoglobina. Dois (8,7%) apresentaram aumento do volume corpuscular médio (VCM), 3 (13,05%) da concentração de hemoglobina corpuscular média (CHCM) e 10 (43,48%) da hemoglobina corpuscular média (HCM), enquanto um (4,35%) animal apresentou baixos níveis para estes parâmetros. Nenhum dos cães apresentou anemia. Um (4,35%) apresentou leucocitose. Nenhum apresentou alteração nas quantidades de bastonetes e segmentados, 13 (56,52%) apresentaram eosinofilia, um (4,35%) basofilia e um (4,35%) monocitopenia. Em 11 amostras foi possível a contagem de plaquetas, todas dentro dos limites aceitáveis. Na análise bioquímica do soro, 5 (21,7%) cães apresentaram baixos níveis de alanina aminotransferase (ALT), 12 (52,17%) de fosfatase alcalina (FA), 2 (8,7%) uremia, 3 (13,05%) hipouremia, 2 (8,7%) hipoalbuminemia, 1 (4,35%) hiperalbuminemia, um (4,35%) hipoglobulinemia, 2 (8,7%) hiperglobulinemia e 11 (47,83%) hiperproteinemia, nenhum apresentou alterações de creatinina. Das 23 amostras, 21 foram submetidas ao qPCR que revelou 6 (28,57%) animais positivos para *Mycoplasma haemocanis* e 5 (23,81%) para *candidatus* Mycoplasma haematoparvum, 4 (19,04%) apresentando coinfecção. O ELISA comercial detectou anticorpos anti-*Borrelia burgdorferi* em um (4,76%) animal. Na RIFI, das 23 amostras analisadas, 7 (30,44%) apresentaram anticorpos anti-*Toxoplasma gondii.*

DISCUSSÃO

As alterações encontradas podem estar relacionadas ao estresse e infecções parasitárias por vermes redondos. A micoplasmose é conhecida por causar anemias graves, associada a quadros assintomáticos, como é o caso desses animais. Mesmo que os cães não passem diretamente a borreliose e nem a toxoplasmose, esse resultado

To be continued.

é importante para definir se há a presença desses patógenos no ambiente, onde temos um fluxo grande de pessoas todos os dias.

CONCLUSÃO

Conclui-se que os cães comunitários, apesar de expostos a diversos riscos, não apresentaram grandes problemas de saúde. Entretanto, é necessário o acompanhamento destes animais devido as alterações encontradas e seu contato diário com usuários destas infraestruturas urbanas, sendo necessário mais estudos sobre o uso deles como possíveis sentinelas.

AGRADECIMENTOS

A Fundação Araucária do Estado do Paraná, Brasil, pelo suporte financeiro.

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Conclusion.

APPENDIX D

Poster presented in 42° Congresso Brasileiro de Medicina Veterinária

SANIDADE DE CÃES COMUNITÁRIOS DE CURITIBA/PR

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Introdução

Diversas estratégias de manejo populacional são empregadas pelo poder público, para combater o crescimento das populações caninas. O projeto "Cão Comunitário" foi implantado em Curitiba/PR no início de 2013, nos terminais de ônibus e parques da cidade, sendo aqueles que estabelecem laços de dependência e manutenção com a comunidade, embora não possua responsável único e definitivo.

Materiais e Métodos

No período de Fev/2013 – Nov/2014 foram cadastrados 49 cães e seus respectivos antenedores. Foi realizado Exame físico e foram coletadas amostras de sangue de 23 animais para exames de hemograma, bioquímico sérico e reação de imunofluorescência indireta (RIFI). E em 21 foi possível realizar o teste de reação em cadeia da polimerase em tempo real (qPCR) e o ELISA comercial (SNAP® 4Dx Plus, IDEXX laboratories Inc.).

Objetivos

Objetivou-se avaliar a condição sanitária destes animais, para possível adoção e seu potencial como sentinelas.

Resultados

Nenhum animal apresentou alteração no exame físico.

No hemograma apenas um animal (4,35%) apresentou poliglobulia, 2 (8,7%) policitemia e 5 (21,73%) aumento da concentração de hemoglobina. Dois (8,7%) apresentaram aumento do volume corpuscular médio (VCM), 3 (13,05%) da concentração de hemoglobina corpuscular média (CHCM) e 10 (43,48%) da hemoglobina corpuscular média (HCM), enquanto um (4,35%) animal apresentou baixos níveis para estes parâmetros. Nenhum dos cães apresentou anemia. Um (4,35%) apresentou leucocitose. Nenhum apresentou alteração nas quantidades de bastonetes e segmentados, Dois (8,7%) apresentaram linfocitose e linfopenia, 13 (56,52%) apresentaram eosinofilia, um (4,35%) basofilia e um (4,35%) monocitopenia. Em 11 amostras foi possível a contagem de plaquetas, todas dentro dos limites aceitáveis. Na análise bioquímica do soro, 5 (21,7%) cães apresentaram baixos níveis de alanina aminotransferase (ALT), 12 (52,17%) de fosfatase alcalina (FA), 2 (8,7%) uremia, 3 (13,05%) hipouremia, 2 (8,7%) hipoalbuminemia, 1 (4,35%) hiperalbuminemia, um (4,35%) hipoglobulinemia, 2 (8,7%) hiperglobulinemia e 11 (47,83%) hiperproteinemia, nenhum apresentou alterações de creatinina.

Os resultados da qPCR, ELISA e RIFI estão expressos na tabela 1. Nenhum cão apresentou anemia, principal sintomas das micoplasmoses, sendo animais assintomáticos. O animal positivo para Borreliose não apresentava nenhum carrapato durante a coleta, nem sintomatologia. Os positivos para *Toxoplasma gondii* podem ter se infectado comendo carnes contendo os cistos ou até diretamente do ambiente, tendo contado com fezes de gatos infectados.

Tabela 1 – Resultados qPCR[1], ELISA [2] e RIFI [3]

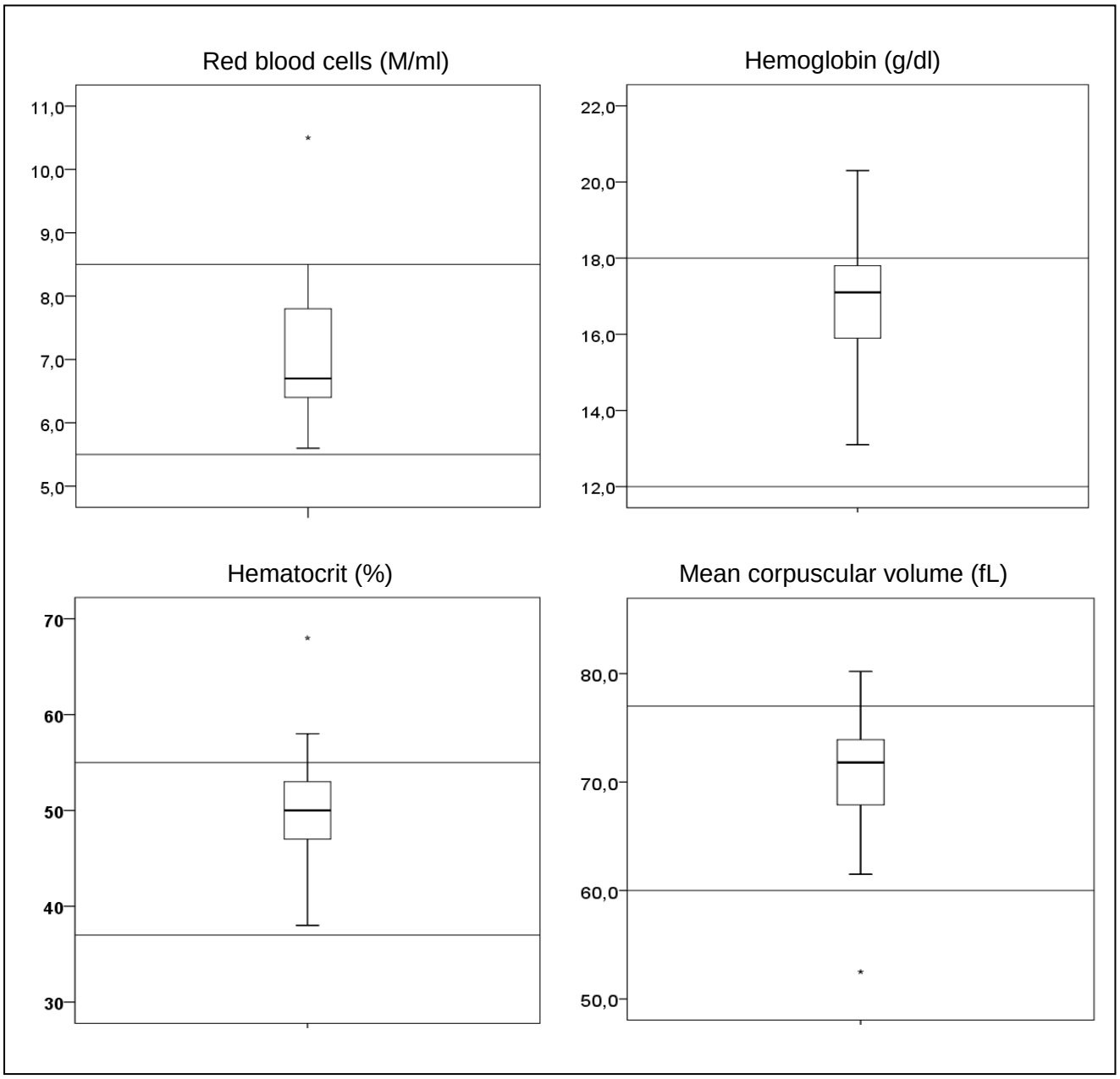
Patógeno	Resultado	Quantidade
[1] Mycoplasma haemocanis	+	6/21
[1] Candidatus Mycoplasma haematoparvum	+	5/21
[2] Borrelia burgdorferi	+	1/21
[3] Toxoplasma gondii	+	7/23

Conclusão

Conclui-se que os cães comunitários, apesar de expostos a diversos riscos, não apresentam grandes problemas de saúde, estando sadios para adoção. As análises hematológicas revelaram algumas alterações características de estresse, reflexo de fuga e infecção parasitária. Mesmo que os cães não passem diretamente a borreliose e nem a toxoplasmose, esse resultado é importante para definir se há a presença desses patógenos no ambiente que esses animais habitam, onde temos um fluxo grande de pessoas todos os dias, sendo necessário mais estudos sobre o uso deles como possíveis sentinelas.

APPENDIX E

Hematological parameters distribution of 21 neighborhood dogs from 9 bus stations and 2 public parks of city of Curitiba, state of Paraná, Brazil, 2016.



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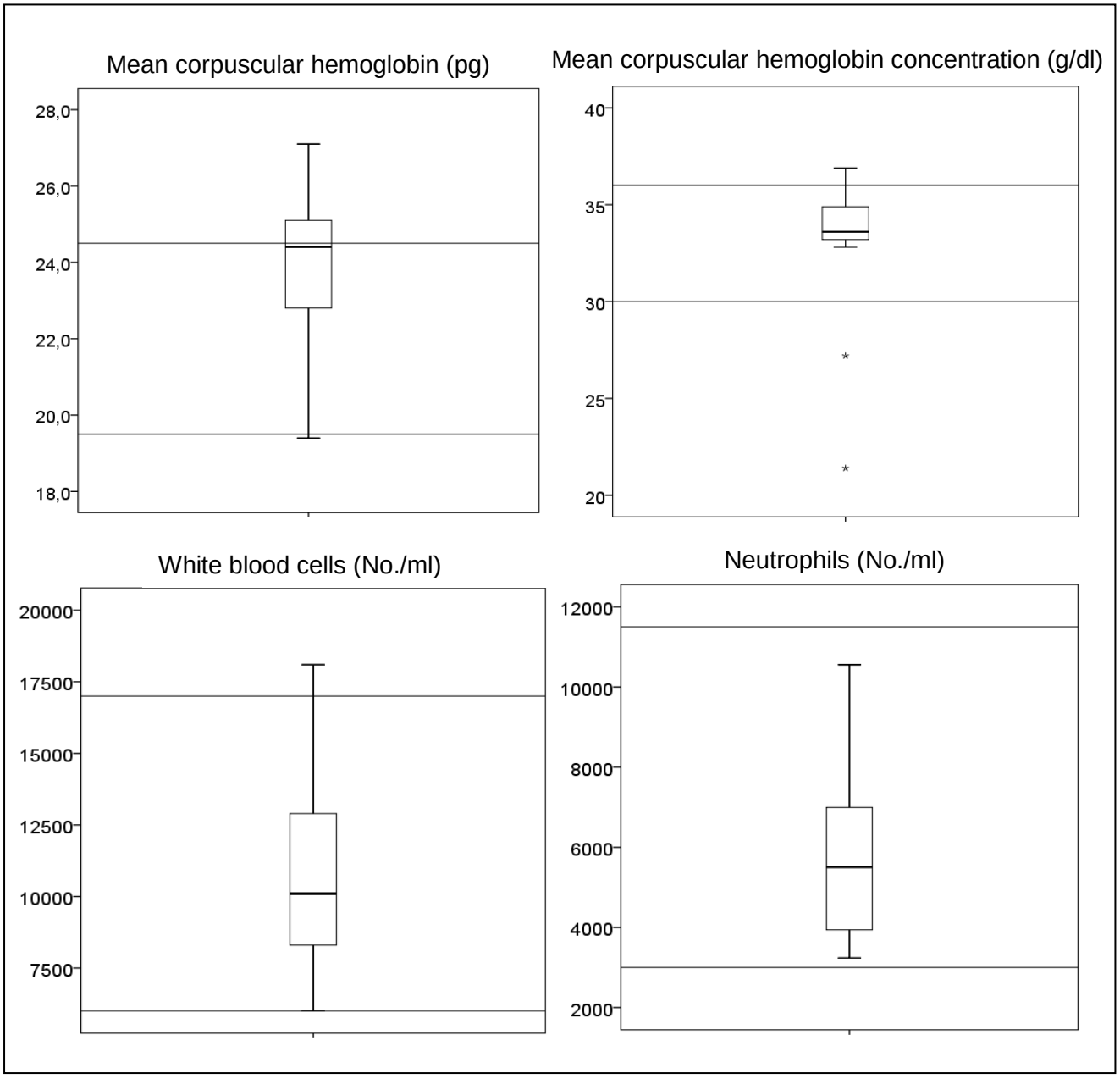

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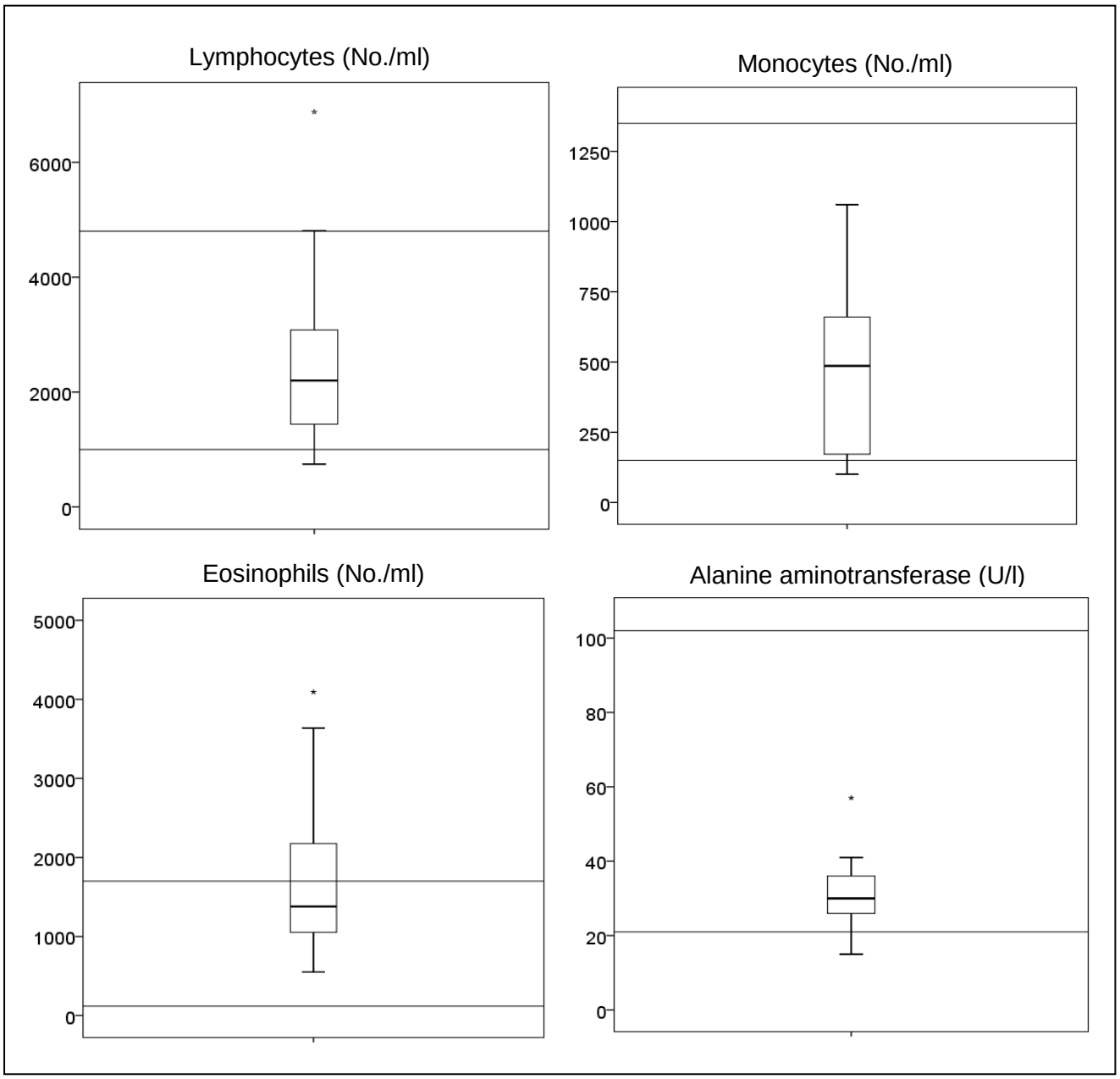

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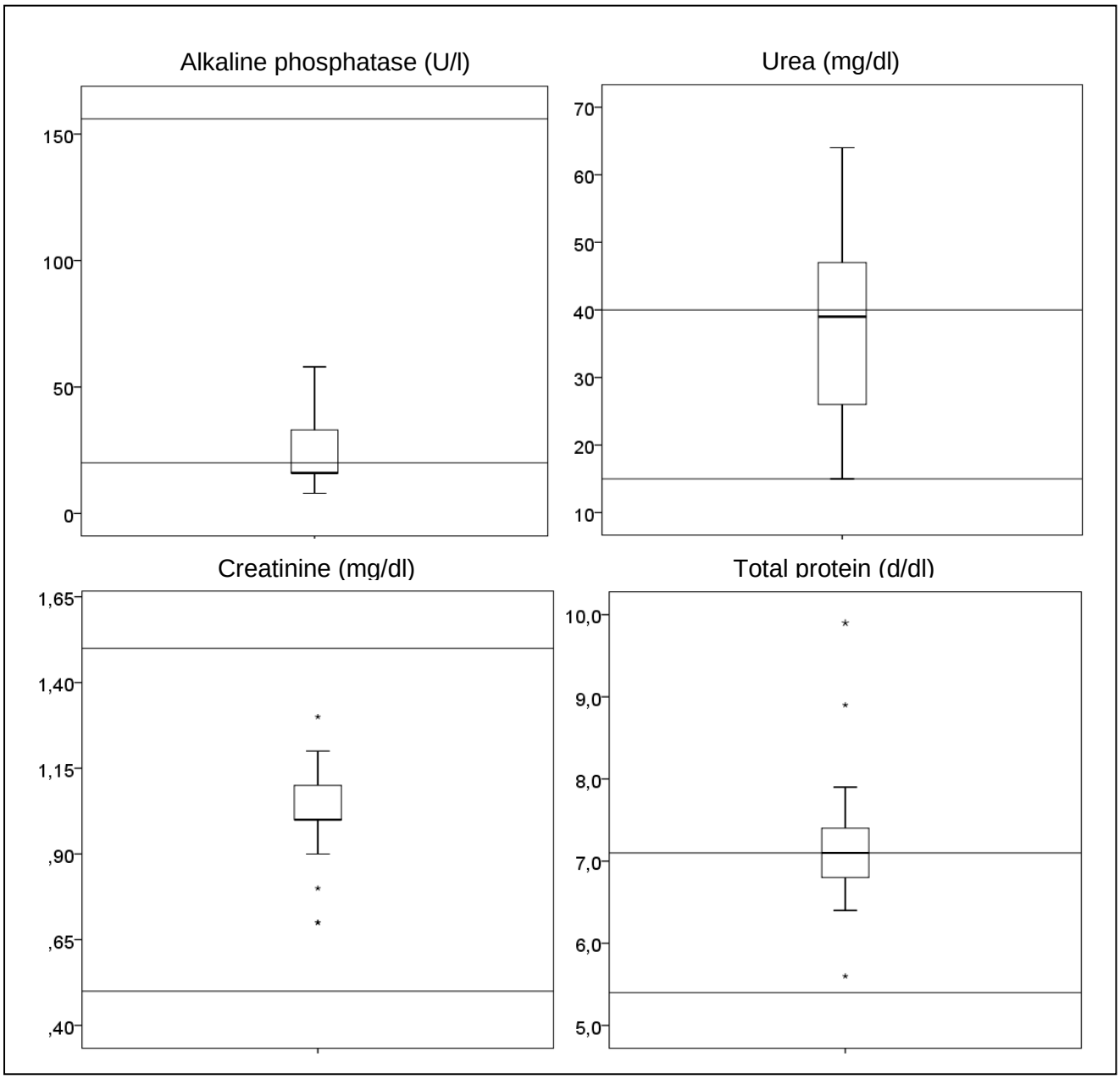
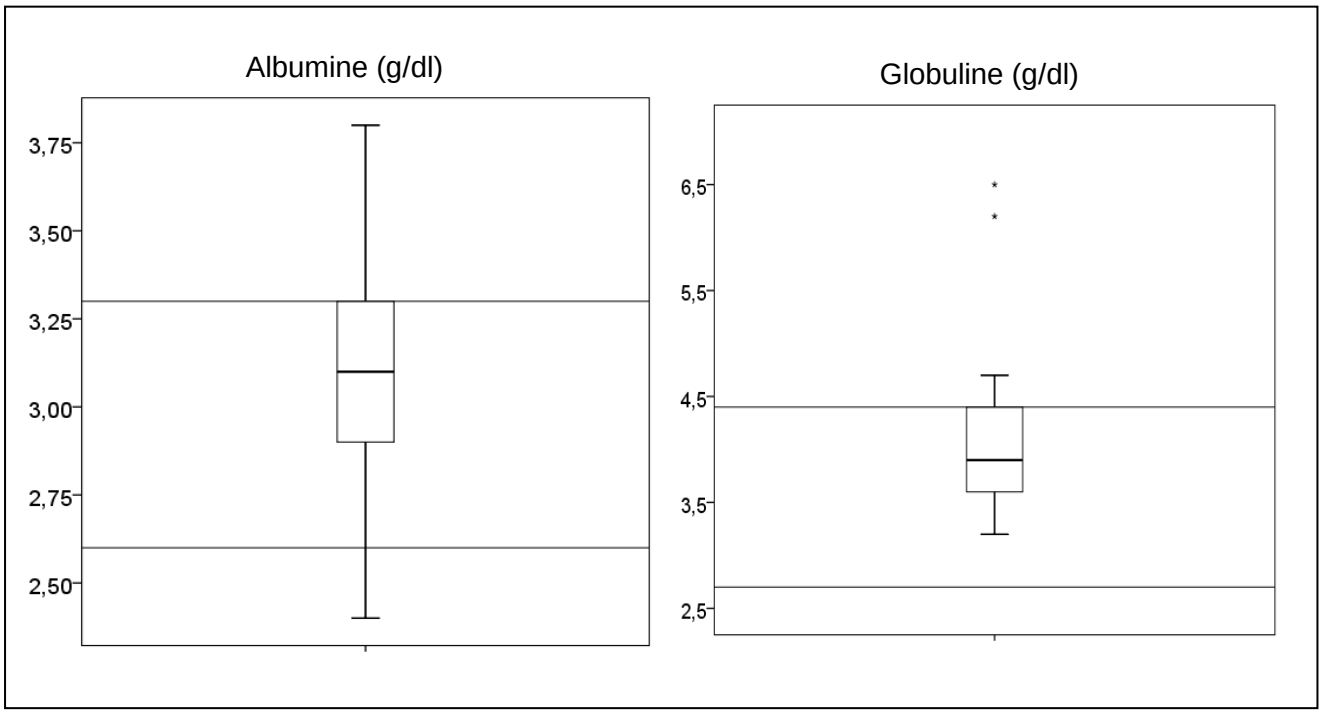


To be continued.


Albumine (g/dl)
3,75
3,50
3,25
3,00
2,75
2,50
Globuline (g/dl)
6,5
5,5
4,5
3,5
2,5

Conclusion.

SUPPLEMENTS

SUPPLEMENT 1 – Neighborhood dogs project clinical history of Curitiba City Service, 2014

CURITIBA

FICHA CLÍNICA PROJETO CÃO COMUNITÁRIO - 2014

NOME DO CÃO:_____ **Número do microchip:**_____

DATA: ____/____/_____

MANTENEDOR: _____

RESPONSÁVL PELO PREENCHIMENTO:_____

Temperamento: ☐ Dócil ☐ Agressivo ☐ Medroso ☐ Inconsistente ☐ Arisco

☐ Secreção nasal:_____ ☐ Secreção ocular:_____

☐ Secreção Vaginal/Prepucial:_____

OBS.:_____

_____

_____

ANAMNESE FISIOLÓGICA:

VACINAÇÃO: PV_____ (__/__/__) AR_____ (__/__/__)

VERMIFUGAÇÃO:_____ (__/__/__)

EXAME CLÍNICO DO ANIMAL:

TR:_____°C FC: _____ bpm FR: _____mpm TPC: _____s MUCOSAS: _____

Desidratação: (☐ Sim / ☐ Não): _____ % PULSO: _____ ESCORE CORPORAL (1-5: _____

LINFONODOS ALTERADOS: Submandibulares: (☐ D/ ☐ E) Pré-Escapulares: (☐ D/ ☐ E) Axilares:(☐ D/ ☐ E)

Inguinais: (☐ D/ ☐ E) Poplíteos: (☐ D/ ☐ E) ☐ LINFONODOS NORMAIS

AVALIAÇÃO DA CAVIDADE ORAL: _____

AVALIAÇÃO OFTALMICA: _____

To be continued.

SUPPLEMENT 1 – Neighborhood dogs project clinical history of Curitiba City Service, 2014

SISTEMA TEGUMANTAR:

☐ Picadas/Escoriações ☐ Ectoparasitas ☐ Icterícia

☐ Lesões : ☐ Generalizadas ☐ Regulares

☐ Irregulares ☐ Exsudativas ☐ Circunscritas

☐ Crostosas ☐ Eritrematosas ☐ Pruriginosa

☐ Apruriginosas ☐ Petéquias ☐ Pápula

☐ Mácula ☐ Placa ☐ Nódulo ☐ Tumor

☐ Pústula ☐ Comedo ☐ Outros:_____

_____

APARELHO CARDIOVASCULAR: ☐ Taquicardia ☐ Bradicardia ☐ Arritmia

☐ Sopro: ☐ Sistólico ☐ Diastólico ☐ Misto_____

SISTEMA RESPIRATÓRIO: ☐ Taquipnéia ☐ Bradpnéia ☐ Aumento Sons BV ☐ Sibilos ☐ Estridor

☐ Estertor ☐ Crepitação ☐ Diminuição Sons B ☐ Dispnéia: ☐ Inspiratória ☐ Expiratória ☐ Mista

_____

APARELHO DIGESTÓRIO: ☐ Dor à Palpação (_____)

☐ Esplenomegalia ☐ Hepatopatomegalia _____

APARELHO URINÁRIO: ☐ Dor à palpação (_____)

☐ Bexiga (_____)/_____

APARELHO REPRODUTIVO:_____

APARELHO LOCOMOTOR: _____

SISTEMA NERVOSO: _____

EXAMES COMPLEMENTARES: ☐ AMOSTRA COM EDTA ☐ AMOSTRA SEM EDTA

Responsável pela coleta:_____

Conclusion.

SUPPLEMENT 2 – Neighborhood dogs project epidemiological questionnaire of Curitiba City Service, 2014

CURITIBA

FICHA EPIDEMIOLÓGICA – PROJETO CÃO COMUNITÁRIO – 2014

NOME DO CÃO:_____ **Número do microchip**
DATA: ____/____/_____
MANTENEDOR: _____
RESPONSÁVEL PELO PREENCHIMENTO: _____

I. DADOS DO ANIMAL

1. IDADE: ( ) menor de 1 ano ( ) entre 1 e 8 anos ( ) maior de 8 anos

2. SEXO: ( ) MACHO ( ) FÊMEA

3. PELAGEM:
3.1 comprimento : ( ) curta ( ) média ( ) longa
3.2 cor:
( ) preto
( ) branco
( ) caramelo
( ) chocolate
( ) cinza
( ) tigrado
( ) tricolor (especificar cor):_____
( ) bicolor (especificar cor):_____
( ) malhado (especificar cor):_____
*alguma marca que o identifique (cicatriz, ausência de um membro,.....): _____

4. PESO: ( ) entre 1 e 5 Kg ( ) entre 5 e 10 Kg ( ) entre 10 e 15 Kg
( ) entre 15 e 20 Kg ( ) entre 20 e 25 Kg ( ) entre 25 e 30Kg

5. ESCORE CORPORAL: ( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5

II. LOCAL DE HABITAÇÃO
6. Local de habitação do animal:
6.1. TERMINAL:

(6.1A)	Bairro Alto	(6.1I)	Capão da Imbuia	(6.1Q)	Portão
(6.1B)	Barreirinha	(6.1J)	Capão Raso	(6.1R)	Santa Cândida
(6.1C)	Boa Vista	(6.1K)	Carmo	(6.1S)	Santa Felicidade
(6.1D)	Boqueirão	(6.1L)	Centenário	(6.1T)	Sites
(6.1E)	Cabral	(6.1M)	CIC	(6.1U)	Sítio Cercado
(6.1F)	Caiuá	(6.1N)	Fazendinha	(6.1V)	Vila Oficinas
(6.1G)	Cmp. do Siqueira	(6.1O)	Hauer		
(6.1H)	Cp. Comprido	(6.1P)	Pinheirinho		

7. Presença de outros cães comunitários no local: ( ) sim ( ) não
8. Local de descanso
( ) Cama ( ) Papelão ( ) Grama
( ) Coberta ( ) Chão
9. Periodicidade da limpeza do local de descanso dos animais
( ) uma vez ao dia ( ) uma vez por semana
( ) mais de uma vez ao dia ( ) uma vez a cada 15 dias
( ) mais de uma vez por semana ( ) superior a 15 dias
10. Presença de materiais que ofereçam risco aos animais no local ( ) sim ( ) não
( ) materiais cortantes ( ) materiais químicos
11. Presença de outros animais domésticos no local ( ) sim ( ) não
Cães não comunitários ( ) sim ( ) não
Gatos ( ) sim ( ) não
Equinos ( )sim ( ) não
12. Presença de roedores no local ( ) sim ( ) não
12.1 Períodos em que são visualizados ( ) durante o dia ( ) durante a noite ( ) dia e noite
13. Presença de pombos no local ( ) sim ( ) não
14. Presença de mosquitos ao redor do local de descanso dos animais ( ) sim ( ) não
14.1 Período de maior aparecimento ( ) manhã ( ) tarde ( ) noite
15. Presença de matas ao redor do local de descanso dos animais ( ) sim ( ) não
16. Acúmulo de matéria orgânica ao redor do local de descanso dos animais ( ) sim ( ) não
17. Presença de terreno baldio ao redor do local de descanso dos animais ( ) sim ( ) não
17.1 Presença de depósito de lixo nesse terreno baldio ( ) sim ( ) não
18. Presença de áreas alagadiças ao redor do local de descanso dos animais ( ) sim ( ) não
18.1 Área alagadiça demora a secar ( ) sim ( ) não

To be continued.

SUPPLEMENT 2 – Neighborhood dogs project epidemiological questionnaire of Curitiba City Service, 2014

III. ALIMENTAÇÃO

19. Tipo de alimento: ( ) Ração ( ) Comida caseira ( ) Mista
    19.1 O animal come carne crua ( )sim ( )não
20. Fornecimento de alimento ( ) uma vez ao dia ( ) mais de uma vez ao dia
21. Disponibilidade do alimento ( ) o dia todo ( ) retirada após a ingestão
22. A água para consumo dos cães é tratada ( )sim ( )não

IV. COMPORTAMENTO

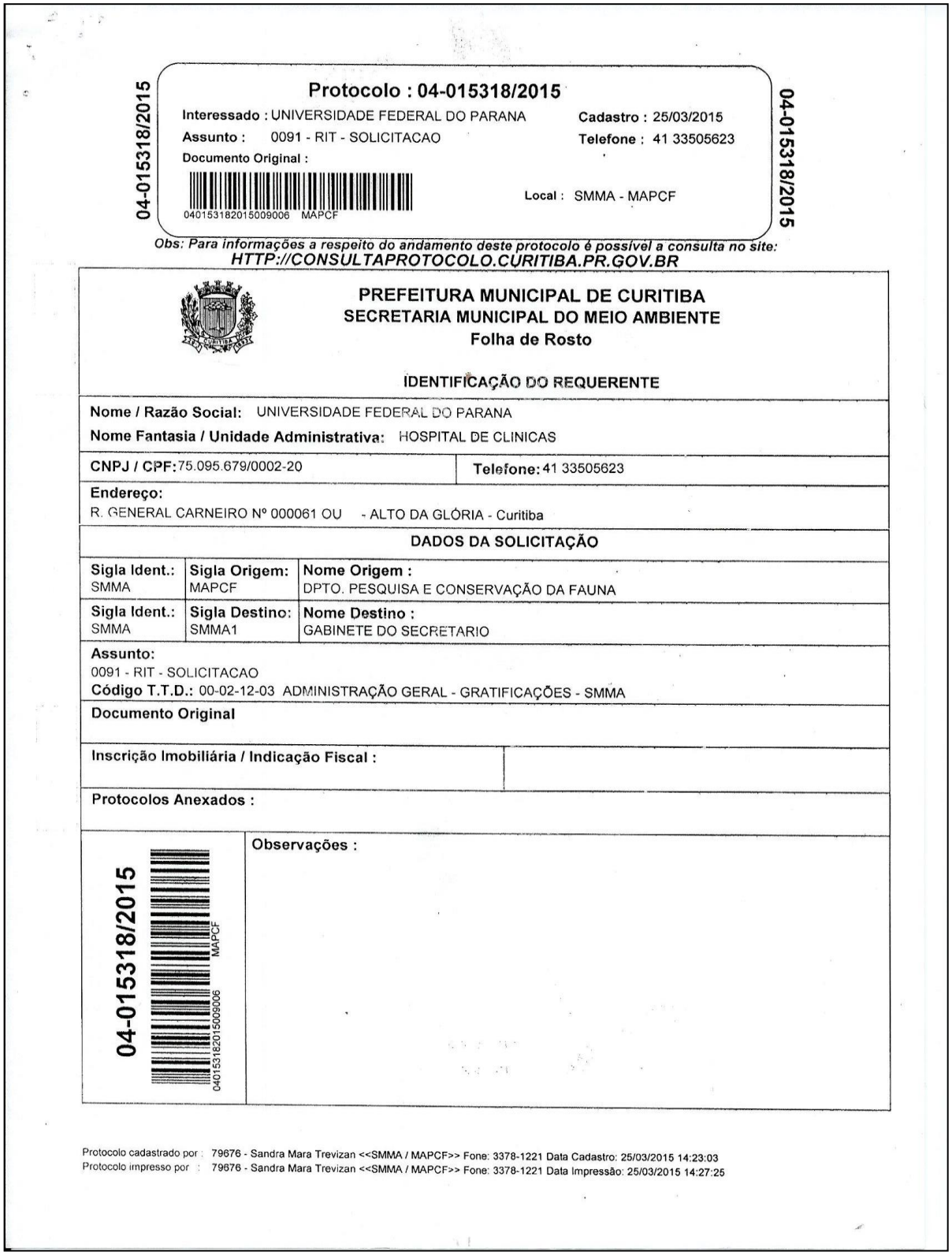
23. Comportamento em relação aos humanos ( ) dócil ( ) agressivo ( ) arisco ( ) indiferente
24. Comportamento em relação aos outros cães comunitários ( ) receptivo ( ) não receptivo
25. Comportamento em relação aos outros cães não comunitários ( ) receptivo ( )não receptivo
26. O animal costuma se ausentar por longos períodos ( )sim ( ) não
27. Há quanto tempo o animal está inserido no grupo ( ) a menos de um ano ( ) a mais de um ano ( ) não soube informar
28. Animal tem comportamento de caça ( )sim ( )não
    28.1 Quais animais caça ( ) pássaros ( ) roedores ( ) insetos ( ) outros
    28.2 Periodicidade da caça ( ) esporádico ( ) frequente
29. Costuma revirar o lixo ( ) sim ( ) não

V. SANIDADE

30. Vacinação ( ) sim ( ) não
    30.1 Polivalente ( ) sim ( ) não Anualmente ( ) sim ( ) não
    30.2 Antirrábica ( ) sim ( ) não Anualmente ( ) sim ( ) não
31. Desverminação ( ) sim ( ) não Semestralmente ( ) sim ( ) não
32. Castrado ( ) sim ( ) não
33. Condição de higiene do cão: ( ) limpo ( ) sujo

Conclusion.

SUPPLEMENT 3 – Environment Service and Animal Research Department of Curitiba City authorization for neighborhood dogs samples use in research



04-015318/2015

Protocolo : 04-015318/2015

Interessado : UNIVERSIDADE FEDERAL DO PARANA
Cadastro : 25/03/2015
Assunto : 0091 - RIT - SOLICITACAO
Telefone : 41 33505623
Documento Original :
040153182015009006 MAPCF
Local : SMMA - MAPCF

04-015318/2015

Obs: Para informações a respeito do andamento deste protocolo é possível a consulta no site:
HTTP://CONSULTAPROTOCOLO.CURITIBA.PR.GOV.BR

PREFEITURA MUNICIPAL DE CURITIBA
SECRETARIA MUNICIPAL DO MEIO AMBIENTE
Folha de Rosto

IDENTIFICAÇÃO DO REQUERENTE

Nome / Razão Social: UNIVERSIDADE FEDERAL DO PARANA
Nome Fantasia / Unidade Administrativa: HOSPITAL DE CLINICAS

CNPJ / CPF: 75.095.679/0002-20 | **Telefone:** 41 33505623

Endereço:
R. GENERAL CARNEIRO Nº 000061 OU - ALTO DA GLÓRIA - Curitiba

DADOS DA SOLICITAÇÃO

Sigla Ident.:	Sigla Origem:	Nome Origem :
SMMA	MAPCF	DPTO. PESQUISA E CONSERVAÇÃO DA FAUNA
Sigla Ident.:	**Sigla Destino:**	**Nome Destino :**
SMMA	SMMA1	GABINETE DO SECRETARIO

Assunto:
0091 - RIT - SOLICITACAO
Código T.T.D.: 00-02-12-03 ADMINISTRAÇÃO GERAL - GRATIFICAÇÕES - SMMA

Documento Original

Inscrição Imobiliária / Indicação Fiscal :

Protocolos Anexados :

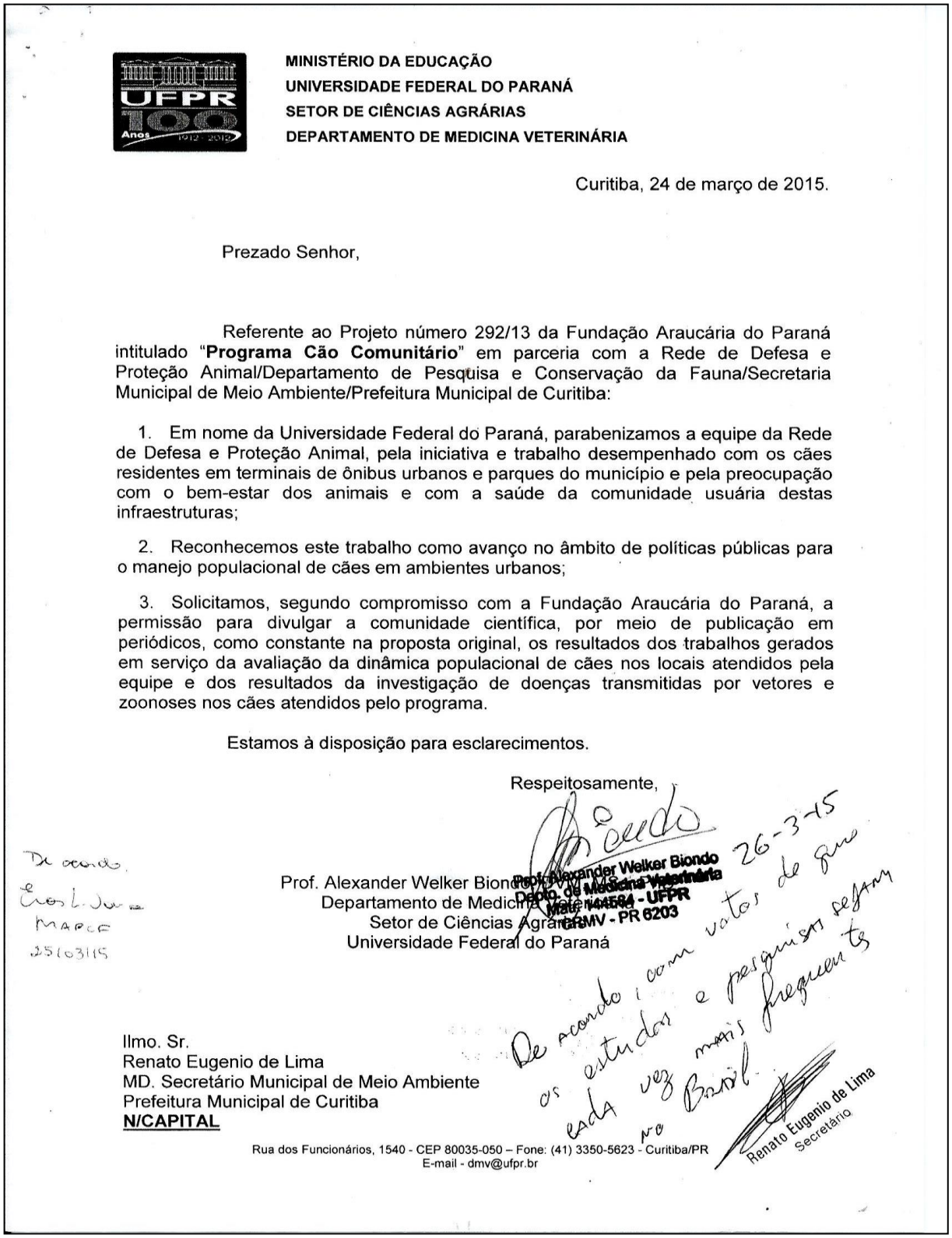
04-015318/2015 MAPCF 040153182015009006

Observações :

Protocolo cadastrado por : 79676 - Sandra Mara Trevizan <<SMMA / MAPCF>> Fone: 3378-1221 Data Cadastro: 25/03/2015 14:23:03
Protocolo impresso por : 79676 - Sandra Mara Trevizan <<SMMA / MAPCF>> Fone: 3378-1221 Data Impressão: 25/03/2015 14:27:25

To be continued.

SUPPLEMENT 3 – Environment Service and Animal Research Department of Curitiba City authorization for neighborhood dogs samples use in research



MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
DEPARTAMENTO DE MEDICINA VETERINÁRIA

Curitiba, 24 de março de 2015.

Prezado Senhor,

Referente ao Projeto número 292/13 da Fundação Araucária do Paraná intitulado "**Programa Cão Comunitário**" em parceria com a Rede de Defesa e Proteção Animal/Departamento de Pesquisa e Conservação da Fauna/Secretaria Municipal de Meio Ambiente/Prefeitura Municipal de Curitiba:

1. Em nome da Universidade Federal do Paraná, parabenizamos a equipe da Rede de Defesa e Proteção Animal, pela iniciativa e trabalho desempenhado com os cães residentes em terminais de ônibus urbanos e parques do município e pela preocupação com o bem-estar dos animais e com a saúde da comunidade usuária destas infraestruturas;

2. Reconhecemos este trabalho como avanço no âmbito de políticas públicas para o manejo populacional de cães em ambientes urbanos;

3. Solicitamos, segundo compromisso com a Fundação Araucária do Paraná, a permissão para divulgar a comunidade científica, por meio de publicação em periódicos, como constante na proposta original, os resultados dos trabalhos gerados em serviço da avaliação da dinâmica populacional de cães nos locais atendidos pela equipe e dos resultados da investigação de doenças transmitidas por vetores e zoonoses nos cães atendidos pelo programa.

Estamos à disposição para esclarecimentos.

Respeitosamente,

Prof. Alexander Welker Biondo
Departamento de Medicina Veterinária
Setor de Ciências Agrárias
Universidade Federal do Paraná

Prof. Alexander Welker Biondo
Depto. de Medicina Veterinária
Mat. 144584 - UFPR
CRMV - PR 6203

De acordo
Cristiano Lima
MAPA
25/03/15

De acordo com vistos de 26-3-15
os estudos e pesquisas sejam
cada vez mais frequentes
no Brasil.

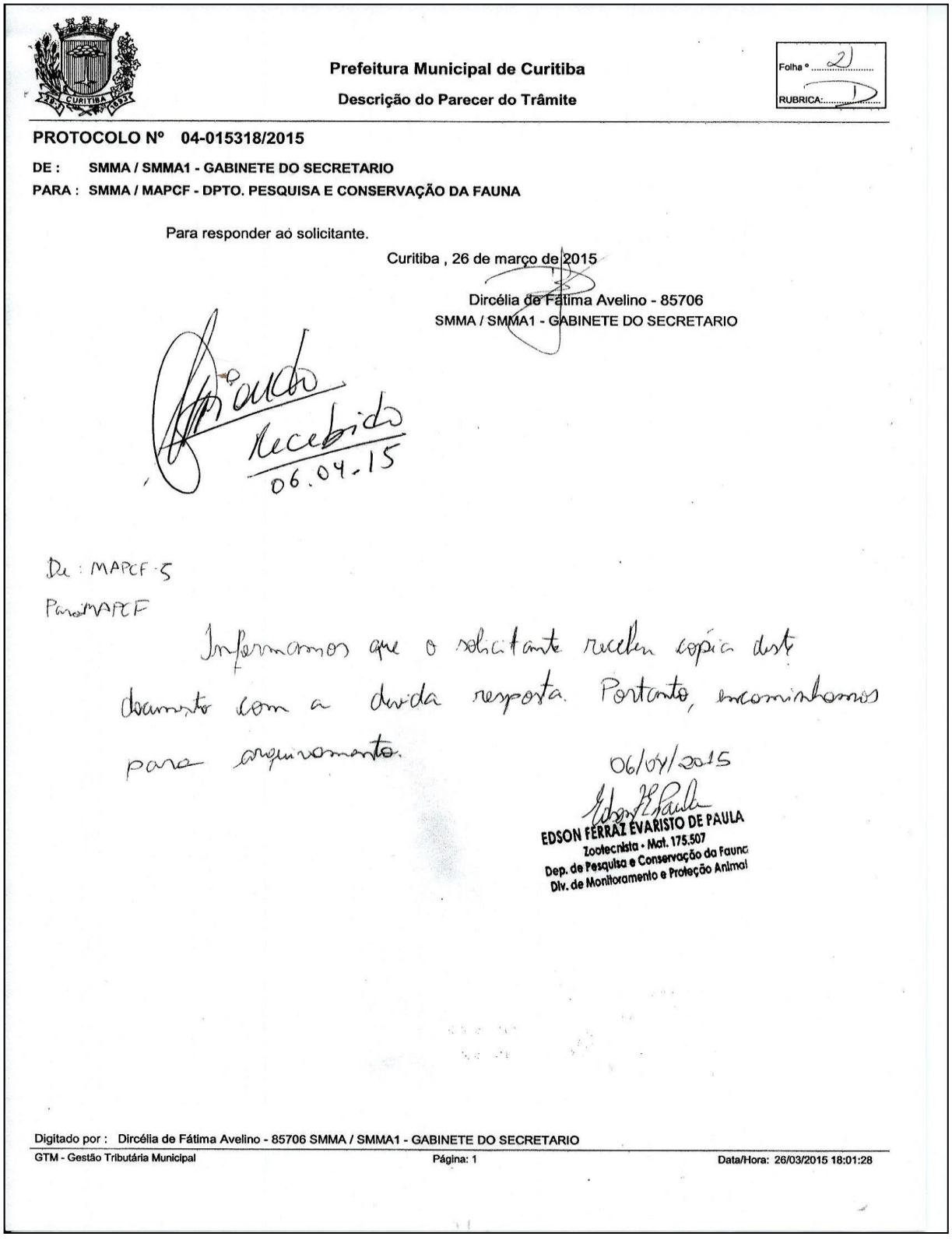
Renato Eugenio de Lima
Secretário

Ilmo. Sr.
Renato Eugenio de Lima
MD. Secretário Municipal de Meio Ambiente
Prefeitura Municipal de Curitiba
N/CAPITAL

Rua dos Funcionários, 1540 - CEP 80035-050 – Fone: (41) 3350-5623 - Curitiba/PR
E-mail - dmv@ufpr.br

To be continued.

SUPPLEMENT 3 – Environment Service and Animal Research Department of Curitiba City authorization for neighborhood dogs samples use in research



Prefeitura Municipal de Curitiba

Descrição do Parecer do Trâmite

Folha º 2

RUBRICA:

PROTOCOLO Nº 04-015318/2015

DE : SMMA / SMMA1 - GABINETE DO SECRETARIO

PARA : SMMA / MAPCF - DPTO. PESQUISA E CONSERVAÇÃO DA FAUNA

Para responder ao solicitante.

Curitiba , 26 de março de 2015

Dircélia de Fátima Avelino - 85706
SMMA / SMMA1 - GABINETE DO SECRETARIO

Recebido
06.04.15

De: MAPCF-5

Para MAPCF

Informamos que o solicitante recebeu cópia deste documento com a devida resposta. Portanto, encaminhamos para arquivamento.

06/04/2015

EDSON FERRAZ EVARISTO DE PAULA
Zootecnista - Mat. 175.507
Dep. de Pesquisa e Conservação da Fauna
Div. de Monitoramento e Proteção Animal

Digitado por : Dircélia de Fátima Avelino - 85706 SMMA / SMMA1 - GABINETE DO SECRETARIO

GTM - Gestão Tributária Municipal Página: 1 Data/Hora: 26/03/2015 18:01:28

Conclusion.

SUPPLEMENT 4 – ANIMAL USE ETHICS COMMITTEE (CERTIFICATE)

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 027/2015, referente ao projeto "Cães como sentinelas de zoonoses e barreira sanitária em locais públicos de grande circulação de pessoas", sob a responsabilidade de Alexander W. Biondo, na forma em que foi apresentado (utilização de amostras de sangue de animais previamente coletadas e como grau A de invasividade), foi aprovado pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias da Universidade Federal do Paraná - Brasil, em reunião realizada dia 22 de Abril de 2015.

CERTIFICATE

We certify that the protocol number 027/2015, regarding the project "Dogs as sentinels zoonoses and sanitary barrier in public places of great movement of persons", under Alexander W. Biondo supervision, in the terms it was presented (use of previously collected blood animals and was classified as grade A of invasiveness), was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of the State of Paraná, Brazil) during session on April 22, 2015.

Curitiba, 22 de Abril de 2015.

Ananda Portella Félix
Presidente CEUA-SCA

Simone Tostes de Oliveira Stedile
Vice-Presidente CEUA-SCA

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR

SUPPLEMENT 5 – 22nd IUHPE WORLD CONFERENCE ON HEALTH PROMOTION – ABSTRACT ADMISSION FOR ORAL PRESENTATION

22nd IUHPE World Conference on Health Promotion

Expo Unimed Teatro Positivo - Curitiba/PR

22 - 26 May 2016

Abstract n. 9296

Author(s): CAROLINE CONSTANTINO; STEFANY MONSALVE BARRERO; RITA DE CASSIA MARIA GARCIA

The abstract **ANIMAIS COMO SENTINELAS DA SAÚDE COLETIVA** was accepted to the **22nd IUHPE World Conference on Health Promotion** as **Oral Presentation**.

Curitiba, 01/12/2015

Scientific Committee - 22nd IUHPE World Conference on Health Promotion

SUPPLEMENT 6 – “SAÚDE ÚNICA E O CONVÍVIO COM OS ANIMAIS” – LECTURE TALK CERTIFICATE

Seminário Metropolitano de Educação Ambiental

Certificamos que **Caroline Constantino** ministrou palestra durante o **I Seminário Metropolitano de Educação Ambiental,** promovido pela Prefeitura Municipal de Pinhais em parceria com a Universidade Tecnológica Federal do Paraná – UTFPR, realizado no dia 25 de Novembro de 2015, com a duração de 01 (uma) hora.

Pinhais, 25 de Novembro de 2015

Ambrósio Struginski
Secretário Municipal de Meio Ambiente

Andreia Francischini
Secretário Municipal de Educação

Cesar Augusto Romano
Diretor Geral da UTFPR
Campus Curitiba

UTFPR Secretaria Municipal de Meio Ambiente Secretaria Municipal de Educação PREFEITURA DE PINHAIS

SUPPLEMENT 7. “SAÚDE ÚNICA E O CONVÍVIO COM OS ANIMAIS” – MINICOURSE TALK CERTIFICATE

VITA

Caroline Constantino is graduated from Veterinary Medicine by Universidade Estadual de Londrina in 2013. She has experience in Zoonoses, Veterinary Public Health and Shelter Medicine, acting on responsible animal ownership, zoonoses prevention and control, canine population management, neighborhood dogs management and vector-borne diseases.

In 2014 was a researcher at the Universidade Federal do Paraná, in the project "Management and population control strategy of dogs residents in public areas with high human and vehicles traffic", working as technical support.

At present she is a master's student in the Veterinary Science Post-Graduate Program at the Universidade Federal do Paraná, with emphasis in One Health.

She was approved in the Multidisciplinary Residency Program of Health Professional Area in the Family Health Program, beginning in March 2016.