NELSON HENRIQUE DE ALMEIDA CURI

CÃES DOMÉSTICOS COMO ESPÉCIE INVASORA NA MATA ATLÂNTICA:
SENTINELAS DE SAÚDE ECOLÓGICA

LAVRAS – MG

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Tese apresentada a Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Monitoramento de Ecossistemas Sob Interferência Antrópica, para a obtenção do título de Doutor.

Orientador

Dr. Marcelo Passamani

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Dra. Solange Maria Gennari USP

Dra. Joziana Muniz de Paiva Barçante UFLA

Dr. Adriano Garcia Chiarello USP

Dr. Paulo dos Santos Pompeu UFLA

Dr. Marcelo Passamani
Orientador

LAVRAS – MG
2014

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RESUMO GERAL

A importância da saúde e das doenças para a conservação da biodiversidade é reconhecida mundialmente há décadas. Contudo, no Brasil, apenas recentemente esta preocupação permeou a comunidade científica e conservacionista. Apesar da falta de dados sobre o real impacto das doenças na fauna do país, algumas espécies apresentam características ecológicas e epidemiológicas que podem torná-las boas 'sentinelas' de saúde em certos cenários, sendo também alvos para prevenção de surtos ou mortalidade induzida por doenças em populações ameaçadas. Cães domésticos (*Canis lupus familiaris*) são considerados uma espécie invasora com alto potencial de impactos negativos sobre a vida silvestre. Eles agem como mesopredadores eficientes, interferem competitivamente e são os principais reservatórios de patógenos e doenças para carnívoros silvestres. São também importantes fontes de zoonoses, e estudos recentes mostram que existe forte presença deles no interior de áreas protegidas Brasileiras. Porém, pouco se sabe sobre seu potencial como reservatório de doenças para humanos e animais silvestres em áreas de interface entre humanos, animais domésticos e animais silvestres no país. Menos conhecidos ainda são os fatores associados a este potencial. Assim, os objetivos do estudo foram avaliar a ocorrência e a prevalência de agentes infecciosos e parasitas importantes para a conservação animal, particularmente de mamíferos carnívoros, e para a saúde humana, nas populações de cães domésticos do entorno de reservas de Mata Atlântica, e também levantar fatores de risco associados a estas doenças. Estes podem ser, por fim, manejáveis para a proteção da saúde de animais e humanos nessas áreas. Utilizamos uma abordagem epidemiológica seccional para realizar um levantamento sorológico dos cães domésticos para doenças como leishmaniose, cinomose, parvovirose, adenovirose, coronavirose e parasitas gastrintestinais, e testamos associações entre a soropositividade e vários fatores individuais e ambientais que podem interferir na transmissão destas doenças entre animais domésticos, humanos e animais silvestres. Para tal, utilizamos ferramentas estatísticas como regressões logísticas e modelos lineares generalizados mistos, dependendo do tipo de patógeno. Os fatores ecológicos associados à presença de doenças foram então listados, e medidas de manejo preventivo caso-a-caso foram sugeridas. Entre eles estão os hábitos de vida livre e a falta de manejo adequado dos cães. Estes resultado são importantes para a proteção da saúde humana nestes cenários. E principalmente, fornecem diretrizes para a ação conservacionista visando a minimização de um importante e negligenciado fator de extinção e ameaça para os carnívoros silvestres brasileiros: as doenças introduzidas e mantidas por populações ubíquas de cães domésticos. Esperamos que os resultados estimulem práticas, políticas públicas e legislações que objetivem reduzir o impacto ecológico e epidemiológico dos cães em áreas ricas em biodiversidade.

Palavras-chave: Cães domésticos. Carnívoros silvestres. Conservação. Doenças. Fatores de risco. Mata Atlântica. Medicina da Conservação. Parasitas. Saúde. Zoonoses.

GENERAL ABSTRACT

The importance of health and diseases for biodiversity conservation is worldwide recognized since decades ago. However, in Brazil, only recently this concern has entered the scientific and conservationist community. Despite the lack of data on the real impact of diseases over the Brazilian wildlife, some species shows ecological and epidemiological traits that may make them good health sentinels in certain scenarios, being also targets for prevention of outbreaks or disease-induced mortality in threatened populations. Domestic dogs (*Canis lupus familiaris*) are considered an invasive species with high negative impact over wildlife. They act as efficient mesopredators, competitively interfere and are the main reservoirs of pathogens to wild carnivores. They are also an important source of zoonosis, and recent studies demonstrate that they are strongly present inside Brazilian protected areas. However, little is known about their potential as disease reservoirs for humans and animals in wildlife/domestic animal/human interface zones in the country. Even less is known about the factors associated with this potential. With this background in mind, the aims of this study were to assess the occurrence and prevalence of infectious agents and parasites important for conservation (especially of mammal carnivores) and for human health in rural dog populations living around and near Atlantic Forest fragments, and also to raise disease-related risk factors. Such factors can be ultimately manageable to protect human and animal health in these areas. We used a cross-sectional epidemiological approach to perform a serologic inquiry of dogs for several diseases, such as leishmaniasis, canine distemper, parvovirosis, adenovirosis, coronavirosis and gastrointestinal parasites, and tested associations between seropositivity versus individual and environmental features involved with disease transmission between domestic animals, humans and wildlife. For this end, we used statistical tools such as logistic regressions and generalized linear mixed models, depending on pathogen type. We then listed the factors associated with disease presence, and suggested preventive measures in a case basis. Free-roaming behavior and poor management practices were among them. These results are important for human health protection in these scenarios. And, principally, provide guidelines for conservation action targeting a reduction of an important but neglected cause of extinction and threatening of wild carnivores in Brazil: diseases introduced and maintained by ubiquitous domestic dog populations. We hope the results stimulate practices, public policies and legislation to reduce the ecological and epidemiological impact of domestic dogs in biodiversity-rich areas.

Keywords: Domestic dog. Wild carnivore. Conservation. Disease. risk factor. Atlantic Forest. Parasites. Health. Zoonosis. Conservation Medicine.

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A presente tese foi realizada como parte do projeto de pesquisa "Cães na Mata Atlântica", que tem como objetivo estimar o impacto causado pelos cães domésticos sobre a fauna nativa de fragmentos deste bioma. O projeto inclui outras duas teses de doutorado, extraídas do mesmo trabalho de campo, e relacionadas a modelos de ocupação e estimativas de abundância e densidade dos cães domésticos e mamíferos nativos no interior das áreas protegidas estudadas. A parte do trabalho que originou esta tese teve como objetivo avaliar o estado de saúde dos cães rurais que vivem no entorno de (e adentram) fragmentos de Mata Atlântica, e seu potencial como transmissores de doenças para os animais silvestres e humanos. Além de evidenciar fatores de risco para as doenças que podem ser alvo de futuros programas de manejo com a finalidade de promover a saúde e evitar perda de biodiversidade por doenças introduzidas nesse bioma já extremamente ameaçado por outras pressões antrópicas.

Foram realizadas visitas a propriedades rurais no entorno de seis áreas protegidas de Minas Gerais (Figura 1). Os cães domésticos foram examinados clinicamente e amostras biológicas foram coletadas (Figura 2a). Questionários padronizados relativos ao manejo e à saúde dos animais domésticos foram aplicados aos proprietários (Figura 2b e Anexo 1).

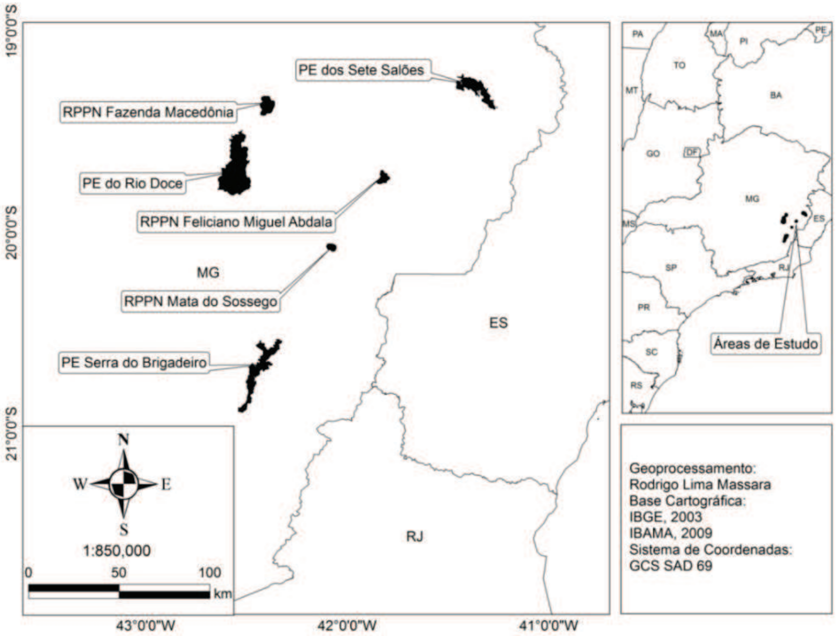

Figura 1 Áreas protegidas de Mata Atlântica no estado de Minas Gerais, Brasil, usadas como áreas de estudo.

No primeiro capítulo, uma abordagem soro-epidemiológica seccional foi usada para avaliar a prevalência de leishmaniose visceral canina, uma importante zoonose que foi pouco estudada em contextos como este, e fatores de risco associados aos animais e ao ambiente referentes a esta doença. Estes resultados são importantes para subsidiar programas de manejo e para a proteção da saúde humana, de animais silvestres e domésticos nessas áreas de interface entre estes diferentes hospedeiros.

A

B

Figura 2 Coleta de materiais biológicos dos cães (a) e entrevista em propriedade no entorno de área protegida (b).

O segundo capítulo usa uma abordagem semelhante, porém para avaliar doenças virais caninas, que são as mais letais para carnívoros silvestres, de acordo com a literatura científica. Vale ressaltar que não encontramos na literatura nenhum estudo que avaliou fatores de risco para doenças caninas em áreas de interface de animais domésticos e silvestres, e este conhecimento é muito importante para o manejo preventivo e para reduzir a transmissão de doenças de cães domésticos para carnívoros silvestres.

O terceiro capítulo descreve a frequência de helmintos e protozoários (parasitas gastrintestinais) nos cães domésticos rurais e fatores associados às infecções específicas e mistas, através de uma análise ecológica e epidemiológica que também visa, por fim, analisar o potencial de transmissão destes agentes para a fauna silvestre e para humanos simpátricos, já que alguns dos helmintos de cães têm potencial zoonótico.

Existe grande carência de informações sobre a saúde de componentes de comunidades ecológicas. No Brasil, poucos estudos foram realizados, porém eles conseguiram provar que a ameaça da 'poluição por patógenos' existe, e que os cães domésticos que vivem livres em interfaces entre humanos e fauna silvestre tem grande potencial como reservatórios e bioacumuladores de patógenos importantes para a saúde de espécies silvestres. Contudo, os fatores de risco associados a infecções nesta espécie ainda são desconhecidos. Os dados levantados nesta tese podem auxiliar no direcionamento de ações e na alocação de recursos para programas que objetivem a promoção da saúde de humanos, animais domésticos e silvestres na Mata Atlântica e em outros biomas que sofrem silenciosamente com os impactos das espécies invasoras.

2 REFERENCIAL TEÓRICO

Parasitas são importantes componentes de ecossistemas, porém, isto é reconhecido apenas há poucas décadas pelos ecólogos. Os trabalhos de Roy Anderson e Robert May (ANDERSON; MAY, 1979; MAY; ANDERSON, 1979) mostraram, através de modelos matemáticos, que os parasitas têm grande potencial de regulação das populações de hospedeiros. Estudos empíricos confirmaram esta hipótese, mostrando que parasitas gastrintestinais são responsáveis pelos ciclos populacionais de lagópodes escoceses (*Lagopus lagopus*) (HUDSON; DOBSON; NEWBORN, 1998). Esta regulação acontece também em mamíferos, interativamente com a disponibilidade de alimentos, como foi demonstrado em roedores (PEDERSEN; GREIVES, 2008) e mesmo em casos de espécies hospedeiras mais longevas como tartarugas e parasitas que causam doenças crônicas (PEREZ-HEYDRICH; OLI; BROWN, 2012). Os parasitas também formam um grupo muito diverso, possuindo mais espécies que grupos de vida livre, e muito importante para o funcionamento dos ecossistemas, apesar de ainda serem negligenciados na educação ecológica e conservacionista (NICHOLS; GÓMEZ, 2011). Parasitas são ubíquos e suas assembleias constituem forças potentes que moldam a estrutura de comunidades através de interações como a competição aparente, ou competição mediada por parasitas, e pela regulação cíclica de populações (HUDSON; GREENMAN, 1998; MARCOGLIESE, 2004). Além da riqueza de espécies, a biomassa de parasitas constitui grande parte da biodiversidade, e as relações parasita-hospedeiro são funcional e proporcionalmente muito importantes em cadeias tróficas (HUDSON et al., 2006).

Hoje existe o consenso de que ecossistemas naturalmente ricos em parasitas são mais saudáveis e resilientes (MARCOGLIESE, 2004; HUDSON et al., 2006), e que episódios de mortalidade induzida por doenças ocorrem normalmente em populações selvagens (YOUNG, 1994). Porém nas últimas décadas a incidência de doenças emergentes e reemergentes vem aumentando em animais silvestres, domésticos e humanos do mundo todo, sendo atualmente consideradas como ameaças importantes para a conservação da biodiversidade (DASZAK et al., 2000; AGUIRRE et al., 2002; 2012; SMITH et al., 2009). A emergência de doenças em humanos, animais domésticos e silvestres e o impacto das doenças na conservação da biodiversidade estão entre os focos de pesquisa da Medicina da Conservação (AGUIRRE et al., 2002; 2012).

De fato, os parasitas e agentes infecciosos receberam bastante atenção nas últimas décadas como agentes de ameaça e causas de extinção de espécies (DASZAK et al., 2000). Das mais de 800 extinções de espécies animais conhecidas nos últimos 500 anos, uma proporção considerável (~4%) foi causada por doenças, incluindo várias espécies de pássaros havaianos (afetadas pela malária aviária) e o marsupial carnívoro tilacino (*Thylacinus cynocephalus*) que pode ter sido extinto, pelo menos em parte, pela introdução de uma doença similar à cinomose canina (SMITH et al., 2006; 2009). Atualmente, uma grande quantidade de espécies está ameaçada por doenças, que agem sinergicamente com outros fatores de extinção (PEDERSEN et al., 2007; SMITH et al., 2009). Entre elas podemos citar o diabo-da-tasmânia (*Sarcophilus harrisii*), ameaçado por um tumor transmissível, e vários gêneros de anfíbios ameaçados na Austrália e nas Américas pelo fungo *Batrachochytrium dendrobatidis* (CARNAVAL et al., 2006; POUNDS et al., 2006; McCALLUM, 2012).

Na maioria das vezes, o aumento da ocorrência de doenças na vida silvestre está associado a atividades antropogênicas que resultam em parasitas atravessando fronteiras evolucionárias, como separações geográficas ou ecológicas (DOBSON; FOUFOPOULOS, 2001; CUNNINGHAM et al., 2003): movimentos e translocações de hospedeiros e parasitas, poluição, mudanças climáticas, redução de habitat e a crescente proximidade e contato entre humanos, animais domésticos e silvestres (DASZAK et al., 2000; CLEAVELAND et al., 2001; HARVELL et al., 2002).

A ordem Carnivora está entre os táxons mais ameaçados do planeta (GITTLEMAN et al., 2001; BOITANI e POWELL, 2012). Os motivos vão desde a alta posição trófica de boa parte de suas espécies, baixas densidades, grandes áreas de vida e mortalidade induzida por caça e doenças. As doenças são um dos fatores mais prejudiciais para a conservação de carnívoros, figurando em todas as listas que citam ameaças e merecendo a atenção de várias publicações, inclusive capítulos inteiros em livros sobre conservação de carnívoros (e.g. MURRAY, 1999; PEDERSEN et al., 2007; FUNK et al., 2001; WENGERT et al., 2012). Os carnívoros são também citados frequentemente em publicações sobre doenças em animais silvestres (e.g. HUDSON et al., 2002; DELAHAY et al., 2009).

Entre os primeiros eventos e estudos que chamaram a atenção para o problema na conservação de mamíferos carnívoros, se destacam os surtos de cinomose em leões (*Panthera leo*) na África (ROELKE-PARKER et al., 1996), em furões-de-patas-negras (*Mustela nigripes*) na América do Norte (THORNE; WILLIAMS, 1988), surtos de raiva em lobos etíopes (*Canis simensis*) (LAURENSON et al., 1998), e em cães selvagens africanos (*Lycaon pictus*) (KAT et al., 1995). Vários levantamentos posteriores mostraram que doenças caninas bem conhecidas como cinomose, raiva e parvovirose estão disseminadas em várias populações de mamíferos carnívoros de vida livre, e que as populações de cães domésticos (*Canis lupus familiaris*) são as principais responsáveis pela introdução e manutenção destes patógenos em comunidades de carnívoros no mundo todo, e o contato com cães realmente influencia a exposição a patógenos em carnívoros silvestres (LAURENSON et al., 1998; CLEAVELAND et al., 2000; ACOSTA-JAMETT et al., 2011; PRAGER et al., 2012; WOODROFFE et al., 2012; KNOBEL et al., 2014) .

O cão doméstico está entre as principais espécies invasoras, e é a espécie de mamífero carnívoro mais abundante e onipresente no mundo devido a sua relação estreita e sua dependência com humanos (GOMPPER 2014). A dependência de alimento, abrigo e proteção é tão forte que a condição de saúde dos cães domésticos está diretamente associada às condições econômicas de seus donos (FUNG et al., 2014). O comportamento humano, no que diz respeito ao manejo de animais domésticos, também influencia a transmissão de doenças na interface entre animais domésticos e silvestres, como foi demonstrado em um estudo recente sobre transmissão de doenças entre cães e canídeos africanos (ALEXANDER e McNUTT, 2010). O grau de cuidado dispensado aos cães domésticos (principalmente quanto a nutrição) determina a intensidade das interações destes com a fauna silvestre (SILVA-RODRÍGUEZ; SIEVING , 2011; SEPÚLVEDA et al., 2014).

Estudos recentes mostram que os cães domésticos estão ocupando em taxas preocupantes e se tornando abundantes em algumas áreas protegidas no Brasil (LACERDA et al., 2009; PASCHOAL et al., 2012). A presença dos cães tem fortes impactos negativos sobre a fauna nativa. Estes impactos vão desde alterações comportamentais para evitar a presença dos cães (VANAK et al., 2009), interferência na distribuição espacial de espécies nativas por competição e perseguição/perturbação (VANAK; GOMPPER, 2010; SILVA-RODRÍGUEZ; SIEVING, 2012), predação e transmissão de doenças (que pode ser traduzida por competição aparente mediada por parasitas) (VANAK; GOMPPER, 2009).

De acordo, em uma revisão recente sobre as interações entre cães e a vida silvestre, não foi mencionado nenhum tipo de impacto positivo dos cães sobre comunidades biológicas (HUGHES; MACDONALD, 2013).

Por outro lado, os cães apresentam características ecológicas, fisiológicas e comportamentais que os tornam uma boa espécie sentinela de doenças para humanos e animais silvestres (CLEAVELAND et al., 2006;

HALLIDAY et al., 2007). Eles convivem com humanos e também adentram áreas ricas em espécies silvestres. Eles são reservatórios competentes para várias doenças comuns a humanos e outros animais e vivem em densidades geralmente altas devido à proteção e subsídios fornecidos pelo homem (CLEAVELAND et al., 2001; YOUNG et al., 2011; GOMPPER 2014, KNOBEL et al., 2014). Esse grau de submissão ao homem também permite relativa facilidade de manuseio e amostragem. Portanto, os cães domésticos que vivem perto de áreas ricas em fauna podem ser utilizados como sentinelas ou indicadores de saúde, e de risco de doenças para a conservação, principalmente de espécies da ordem Carnivora.

No Brasil, estudos sorológicos demostraram a presença, em certos casos, de alta prevalência de patógenos caninos como o vírus da cinomose e o parvovírus em populações de carnívoros silvestres e também de cães domésticos simpátricos (e.g. WHITEMAN et al., 2007; NAVA et al., 2008; CURI et al., 2010; 2012), apesar de os fatores epidemiológicos associados a estas prevalências não terem sido levantados. Casos de mortalidade por doenças infecciosas (e.g. infecções por vírus da cinomose filogeneticamente idêntico ao de cães) também foram relatados em carnívoros silvestres (MEGID et al., 2009; 2010). Portanto, se existem as populações de cães (reservatórios) e carnívoros silvestres (hospedeiros com imunidade provavelmente baixa ou ausente), os patógenos e a possibilidade do contato entre eles, a transmissão de doenças é garantida e as populações de espécies nativas estão indubitavelmente ameaçadas de mortalidade induzida por doenças. De fato, a probabilidade de extinções causadas pela introdução de doenças é maior quando o tamanho das populações é reduzido (como parece ser o caso dos carnívoros da Mata Atlântica, que vivem em pequenos fragmentos) e existem populações simpátricas de reservatórios (como os cães rurais que vivem soltos no entorno dos fragmentos) (DE CASTRO; BOLKER, 2005). Ainda existe a possibilidade de alguns patógenos, mesmo sendo introduzidos via cães domésticos, se perpetuarem isoladamente nas populações de carnívoros silvestres (PRAGER et al., 2012).

Com isto em mente, a ação conservacionista deve ocorrer mesmo que ainda não se saiba o exato impacto das doenças sobre as populações silvestres. A conservação tem o destino de lidar com a incerteza e a prevenção é fundamental para a manutenção da saúde ecológica. Por exemplo, estudos na África mostram benefícios bilaterais da intervenção em populações de cães para a saúde de humanos e animais silvestres (CLEAVELAND et al., 2006). Portanto, não é necessário esperar que ocorram epidemias, surtos e extinções locais em populações de espécies nativas antes de se iniciar medidas de manejo preventivo no Brasil.

Na maioria das zonas rurais do entorno de fragmentos da Floresta Atlântica predominam pequenos produtores de subsistência com baixa renda, em cujas propriedades existem cães que adentram áreas protegidas (Paschoal et al., dados não publicados). Apesar disso, o potencial dos cães como transmissores de doenças importantes para humanos e carnívoros silvestres nestes cenários ainda é desconhecido. Menos conhecidos ainda são os fatores de risco associados a tais patógenos.

Este trabalho tem como finalidade levantar dados que subsidiem o manejo de saúde das populações de cães domésticos no entorno de áreas protegidas. Áreas e fatores de risco associados a doenças caninas em interfaces humanos / animais domésticos / animais silvestres não foram estudados no mundo e tampouco no Brasil, apesar de serem extremamente importantes para o direcionamento de recursos e ações conservacionistas, e também para a proteção da saúde humana nessas áreas.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1

Fatores associados a soroprevalência de leishmaniose em cães do entorno de fragmentos da Floresta Atlântica

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Nelson Henrique de Almeida Curi[1], Ana Maria de Oliveira Paschoal[2], Rodrigo Lima Massara[2], Andreza Pain Marcelino[3], Adriana Aparecida Ribeiro[3], Marcelo Passamani[1], Guilherme Ramos Demétrio[1], Adriano Garcia Chiarello[4]

[1] Postgraduate program in Applied Ecology, Department of Biology, Federal University of Lavras, Lavras, Brazil nelsoncuri@hotmail.com

[2] Postgraduate program in Ecology, Conservation and Management of Wildlife, Department of Biology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

[3] Laboratory of Leishmaniasis, Ezequiel Dias Foundation-FUNED, Belo Horizonte, Brazil

[4] Department of Biology, University of São Paulo, Ribeirão Preto, Brazil

Abstract

Canine visceral leishmaniasis is an important zoonosis in Brazil. However, infection patterns are unknown in some scenarios such as rural settlements around Atlantic Forest fragments. Additionally, controversy remains over risk factors, and most identified patterns of infection in dogs have been found in urban areas. We conducted a cross-sectional epidemiological survey to assess the prevalence of leishmaniasis in dogs through three different serological tests, and interviews with owners to assess features of dogs and households around five Atlantic Forest remnants in southeastern Brazil. We used Generalized Linear Mixed Models and Chi-square tests to detect associations between prevalence and variables that might influence *Leishmania* infection, and a nearest neighbor dispersion analysis to assess clustering in the spatial distribution of seropositive dogs. Our findings showed an average prevalence of 20% (ranging from 10 to 32%) in dogs. Nearly 40% (ranging from 22 to 55%) of households had at least one seropositive dog. Some individual traits of dogs (height, sterilization, long fur, age class) were found to positively influence the prevalence, while some had negative influence (weight, body score, presence of ectoparasites). Environmental and management features (number of cats in the households, dogs with free-ranging behavior) also entered models as negative associations with seropositivity. Strong and consistent negative (protective) influences of the presence of chickens and pigs in dog seropositivity were detected. Spatial clustering of cases was detected in only one of the five study sites. The results showed that different risk factors than those found in urban areas may drive the prevalence of canine leishmaniasis in farm/forest interfaces, and that humans and wildlife risk infection in these areas. Domestic dog population limitation by gonadectomy, legal restriction of dog numbers per household and owner education are of the greatest importance for the control of visceral leishmaniasis in rural zones near forest fragments.

Keywords: visceral leishmaniasis, *Leishmania*, dogs, rural health, serologic tests, risk factors, prevalence, Brazil

Introduction

Landscape changes such as urbanization and human encroachment are among the main drivers of the alteration of disease dynamics, e.g., the increased or altered prevalence and incidence of disease in humans, domestic animals, and wildlife [1-4]. The introduction of exotic domestic species often accompanies human movements during such changes and poses a threat to both wildlife and human health. Since their domestication, pet animals have been closely associated with humans, and dogs (*Canis familiaris*) are the most common and distributed companion animal worldwide [5-6]. Unfortunately, this ubiquitous human-dog bond also brings many host species into contact with their pathogens because dogs occupy both natural and human-modified areas and may therefore enhance disease transmission and persistence in humans and wildlife [7]. But because of this close bilateral interaction, domestic dogs may also be used as sentinels of disease for both human and wildlife populations [8-10].

In Brazil, there are about 40 million dogs, of which five million are represented by rural dogs. Most of these live unrestricted, exhibiting free-ranging behavior, and move in both urban and natural areas [6]. Accordingly, recent studies have shown that the domestic dog has become increasingly common in several Brazilian protected areas [11-12], but the ecological and epidemiological impact of this invasion generally remains unknown. In a study conducted in India, Vanak and Gompper [13] have shown that dogs interfere with the spatial distribution of sympatric native carnivore species. Therefore, they also disturb the spatial distribution of hosts and parasites, affecting disease dynamics and the resulting impact on wildlife and human populations that have contact with these dogs. The contact events and the presence of parasites in domestic dogs indeed increase the risk of disease for both humans and wildlife [7,14-15] and must be investigated if the aim is to minimize risk and to understand the dynamics of the systems into which dogs are introduced and with which they interfere. Human behavior also has the potential to alter parasite dynamics in wildlife-human-domestic animal interfaces [16]. For instance, wild carnivores are more exposed to pathogens in places where they face more frequently their domestic counterparts [15], and dog ownership is itself an important risk factor for human leishmaniasis [14,17].

Visceral leishmaniasis is a dangerous systemic disease among the most significant zoonosis in Brazil, affecting both dogs and humans. Brazil holds the higher number of cases in South America and is one of the six most affected countries worldwide. The disease is caused by parasites of the species *Leishmania infantum*, whose vectors are phlebotomine sand flies of the genus *Lutzomyia* (Psychodidae) [18-20]. The main reservoir of *L. infantum* is the domestic dog, although the possible participation of asymptomatic infected persons is currently been suggested [21-23]. Other wild mammal species may be infected and may develop clinical signs, but their role as reservoirs remains to be clarified [22,24-26]. One of the few well studied species is the widely distributed and relatively abundant South American wild canid crab-eating fox *Cerdocyon thous*, a host with low infectiveness unable to sustain *Leishmania* cycles without the presence of sympatric dogs [21].

Recent studies have considered the surrounding environment and its relation to the epidemiology of human and canine visceral leishmaniasis (CVL). Their results are mixed, although several interesting patterns have arisen, e.g., the influence of other domestic animals as attractors for the vector, which ultimately produces an increased risk of infection in dogs and humans [27-30].

Furthermore, according to a topical review, there is still controversy over risk factors associated with infection in dogs, and surveillance and information is scarce in some areas in Brazil [31]. A recently published paper has identified peridomestic risk factors for both canine and human cutaneous leishmaniasis in an agricultural area of southern Brazil [32].

Visceral leishmaniasis affects mostly poor communities in remote rural areas [19]. However, for CVL, many areas and contexts such as rural settlements around forest fragments and other human-wildlife-domestic animal interface zones have been poorly evaluated. The control and elimination of leishmaniasis is far from realistic in Latin America because it is a zoonosis with a very large domestic reservoir and probably a substantial sylvatic reservoir (though this is a point which still needs further investigation), and the existence of gaps in knowledge and surveillance along with a lack of political involvement [33]. Thus, the goals of this study are to evaluate the seroprevalence of CVL, a neglected but important zoonosis in Brazil, in areas of unknown epidemiological status in the Atlantic Forest domain and to correlate this presence with dog individual traits, animal management and environmental factors. In this way, the patterns of infection detected here can ultimately be targeted or managed by programs for the control of the disease.

Materials and methods

Ethics statement

Sampling and interviewing were performed under consent obtained from the household head or other responsible individual. Licenses from the State Forest Institute – IEF (UC: 080/10, 081/10 and 082/10) and approval from the Ethics Commission on the Use of Animals of the Pontiphical Catholic University of Minas Gerais (CEUA, PUC Minas 037/2010) were obtained prior to the initiation of the field work. Regarding the collection of data from human participants, our project was examined by the Ethics Research Committee (Comitê de Ética em Pesquisa) of the Pontiphical Catholic University of Minas Gerais (PUC-Minas). We did collect some information on the number of people inhabiting the house with the approved consent of the household head. A Consent Term about the confidential character of the records was read to every interviewed person. Animal manipulation procedures adhered to the recommendations from the COBEA (Brazilian College of Animal Experimentation) and the Animal Ethics Committee of FIOCRUZ (Oswaldo Cruz Institute Foundation) of the Brazilian Ministry of Health.

Study sites

Rural settlements surrounding five protected areas in the Atlantic Forest domain of the state of Minas Gerais, southeastern Brazil, were selected for this study. These areas comprise two state parks, Serra do Brigadeiro (PESB, municipality of Araponga) and Sete Salões (PESS, municipality of Santa Rita do Itueto), and three private reserves, Fazenda Macedônia (RPPNFM, municipality of Ipaba), Feliciano Miguel Abdala (RPPNFMA, municipality of Caratinga), and Mata do Sossego (RPPNMS, municipality of Simonésia) (Figure 1, table 1). All of the areas had humans living in their vicinity and various degrees of domestic dog occupancy recorded within their borders [12, Paschoal et al. unpublished data]. The landscapes around the protected areas are mostly composed of a mosaic of forest borders, small rural properties, their legal reserves and small human settlements. Households were mostly located near forests, water bodies, and had vegetation in their vicinities (Figure 2), which are considered risk factors for *Leishmania* infection [31]. According to the official Brazilian health services, these areas are characterized by an absence of recorded human leishmaniasis cases except for Ipaba municipality, where a few records have been obtained in recent years (table 1). Several species of the genus *Lutzomyia* occurs at the Atlantic Forest in both peridomiciliary and forest environments [34-35]. All households were located near potential breeding sites for the vectors (forested areas, water bodies, peridomiciliary microhabitats and plantations). Sand flies are indeed abundant in human-disturbed open areas such as plantations and secondary forest and homesteads with the presence of dogs [36]. Thus, our sampling sites located in rural/forest interfaces are likely not free of the presence of vector species.

Figure 1. Study areas location in the Atlantic Forest domain, Minas Gerais state, southeastern Brazil.

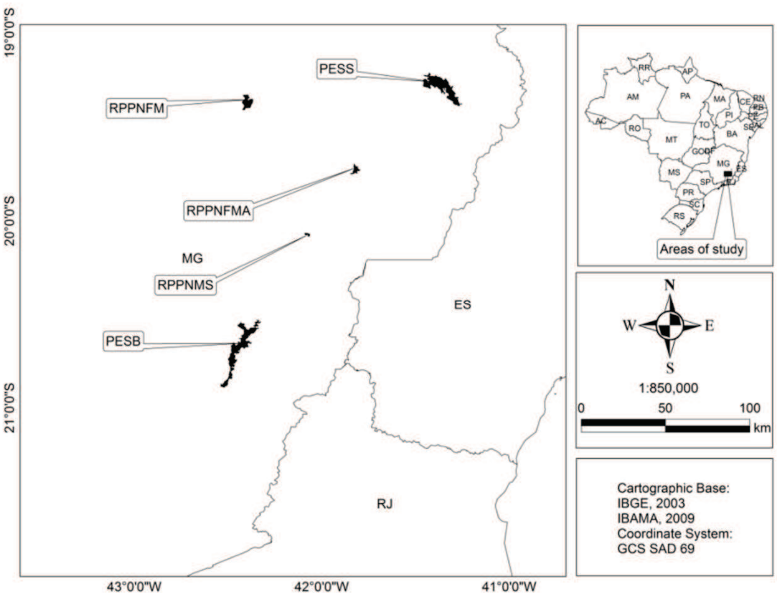

Table 1. Epidemiological features of five protected areas in the Atlantic Forest of the state of Minas Gerais, southeastern Brazil.

Study site	Distance from nearest city (km)	Altitude (m)	Area size (ha)	Transmission status[1]	Human cases[1] / population[2]	Human:dog ratio
RPPNFM	0.3	320	3,343	Sporadic	2 / 16,708	1.2
PESB	3.3	1,437	15,015	Silent	0 / 8,152	1.9
PESS	4.7	687	13,370	Silent	0 / 5,697	1.8
RPPNFMA	10.5	430	1,312	Silent	0 / 22,242	1.1
RPPNMS	7.7	1,340	392	Silent	0 / 18,298	2.9
Total	-	-	-	-	2 / 71,097	1.8 (0.2-8)

[1]Data from 2010-2012 (Brazilian Ministry of Health)

[2]Data from the 2010 census (Brazilian Institute of Geography and Statistics, www.ibge.gov.br)

Figures 2 a and b. Typical households and peridomestic scenarios of rural areas surrounding Atlantic Forest fragments in Minas Gerais State, southeast Brazil.

Sampling

The study was conducted between January 2011 and August 2012. Overall, 291 dogs older than two months were sampled in 124 rural households located up to two kilometers from protected area boundaries around the study areas, and this was the sole eligibility criteria used for this study. After physical restraint, blood was collected from the jugular vein and a complete clinical examination of the dogs was performed (focusing on clinical alterations of visceral leishmaniasis such as weight loss, skin lesions, nail overgrowth and increased volume of the liver and spleen). A standardized questionnaire survey was administered to the owners. Factors related to animal management and behavior (number of dogs, mobility of dogs, access of dogs to the forest and villages, observed interactions between dogs and wildlife, ectoparasite treatment), the presence of vector attractors in peridomestic dwellings (i.e., other domestic species), number of people and geographic coordinates were recorded for each household. The individual and clinical features of the dogs (sex, age, height, weight, fur type, breed, sterilization, body condition, clinical alterations, and the presence of ectoparasites such as fleas and ticks) were recorded in individual files. Weight was measured with a precision scale (Pesola®, 50 kg capacity), and height was measured from the footpad to the top of the scapulae of standing dogs. Body condition of dogs was scored from 0 (extreme emaciation) to 5 (extreme obesity). Refusals to the survey occurred in four cases because the responsible were absent from the households at the time of collection. There were no other refusals, and we believe that the houses that were not surveyed did not affect the overall results.

Laboratory analysis

Blood samples were allowed to clot for 4 h at room temperature and then centrifuged for serum extraction. Serum samples were initially stored at -20ºC, and sent later to be stored at -80°C at Fundação Ezequiel Dias, Belo Horizonte, prior to analysis. Immune enzyme assays (ELISA), indirect immunofluorescence reaction (IFI), and dual path platform immunochromatographic rapid test (DPP) analyses were performed using Biomanguinhos® kits (Fiocruz, Manguinhos, Rio de Janeiro, Brazil). These tests are currently used for the diagnosis of CVL in endemic areas by the laboratories of public health [37-39]. IFI tests were performed with a cut-off point at the dilution of 1:40. The ELISA results are expressed in absorbance values and the DPP test provide visual interpretation of seropositivity.

Statistical analysis

Spearman correlation matrices were built in order to test correlations and assess the level of agreement between the three serological tests, as well as to assess correlation between the ectoparasite presence and previous insecticide treatment in dogs. Dog individual traits, ecological (presence of animals attractive for the sand flies), and management factors (level of dog's restriction, access to forest and urban areas, and ectoparasite treatment) that may be linked to CVL transmission according to previous literature [see 31 and related papers] were used as explanatory or independent variables for different scenarios of seropositivity (positives for at least one test, ELISA, IFI, and DPP positives, and paired tests) for *Leishmania* in dogs, the binary response (dependent) variables. Households were considered positive if they had at least one seropositive dog. At the individual level, sex, age class (younger or older than 12 months), fur type (fur less than 3 cm long was considered short), sterilization, breed (purebred and mixed bred), and the presence of ectoparasites were used as the independent binary variables. Age, weight, height and body condition were included as quantitative variables. For the households, the continuous variables were the numbers of dogs, people, and cats. The presence of chickens, livestock mammals (cattle, horses and pigs), small pets (e.g. rabbits and birds), whether dogs were kept free or not, the access of dogs to the nearest cities and to the protected areas, whether owners observed interactions with wildlife, and ectoparasite treatment, were included as binary factors. Generalized linear mixed models (GLMMs) adjusted with a binomial distribution for the response data and controlling for households and areas as random effects (all other variables were set as fixed effects in the models), were used to select the most important factors or combinations of factors associated with seropositivity. This type of model is considered suitable for cross-sectional epidemiological studies [40]. The variables were subsequently removed from the complete model (significantly different from a null model) by a backward stepwise approach according to their level of significance, until the difference between subsequent models was significant ($p < 0.05$). Comparisons of prevalence ratios among the study areas, and for binary variables of dog individual traits (gender, sterilization, age class: young (<1 yr) versus adult (>1 yr), pure breed versus mixed breed, short fur versus long fur dogs, presence of ectoparasites), and management and environmental features (mobility, access to forests and villages, presence of other domestic animal species, interactions with wildlife and previous ectoparasitic treatment) were performed with multiple and two proportion Yates-corrected Chi-square tests. We did not applied Chi-square or similar tests with the prevalence ratios of continuous variables to avoid unnecessary data categorization and redundancy with the GLMM tests. A threshold of $p<0.05$ was used to determine statistical significance. The GLMM tests were run in package lme4 of R software, and the other analyses were performed in BioEstat 5.0 [41-42]. To assess spatial clustering of seropositive dogs, we used a nearest neighbor dispersion analysis of dog locations with the software BIOTAS version 2.0a 3.8. We based on the STROBE statement [43] as a guide for the reporting of our observational results.

Results

The sex ratio of the dogs was 2:1 (193 males: 98 females), the average age of the dogs was 3.3 yr (ranging from 3 months to 18 yr), and adult dogs (> 1 yr old) represented 78% of the total (227/291). Only 8.6% (25/291) of the dogs had long fur, and purebred dogs represented 15.8% (46/291). The mean body condition score was 2.2 (ranging from 0.5 to 3.5). Low body scores (up to 2) were detected in 170 (58.4%) dogs. Ectoparasites (fleas or ticks) were found in 86% of the dogs, and 77% (226/291) were submitted to previous ectoparasite treatment, and infestation were inversely but weakly correlated to previous treatment ($r = -0.12$; $p = 0.032$). Only 19 dogs (6.5%) had been sterilized. The mean number of dogs per household was 2.8 (including dogs that could not be sampled, maximum number = 15). Ninety-five percent (278/291) of dogs were kept without space restriction. The mean number of people was 3.6 per household, with a maximum of eight. Average human to dog ratio was approximately 2:1. In 80% of the households, the dogs had access to the forest, and they had access to the nearest cities in 36.5% of the households. Chickens were present in 90%, cattle in 55%, horses in 46%, pigs in 38%, cats in 48%, and small pets (rabbits and cage birds) in 14.5% of the households.

There was low correlations between the serological tests used ($r = 0.42$, $p<0.0001$ for IFI and ELISA; $r = 0.23$, $p<0.0001$ for IFI and DDP; $r = 0.05$, p=0.3131 for ELISA and DPP). The ELISA test revealed 13.7% (40/291) of positive samples (39% of positive samples had absorbance values above the cut-off point, including those from symptomatic dogs). Only 9.6% (28/291) of the dogs were seropositive for *Leishmania* sp. according to the IFI test. In the DPP test, eleven samples (3.8%) were positive. When tests were combined, 5.5% of the samples (16/291) were positive for ELISA and IFI. Three samples (1%) were positive for ELISA and DPP. Five samples (1.7%) tested positive for IFI and DPP, and only three samples (1%) were positive for all tests. Because of the low level of agreement among the diagnostic methods used, we calculated prevalence data based on the number of dogs seropositive for at least one test.

Overall seropositivity was 19.9% (58/291). Ten of the 58 positive dogs (17%) were symptomatic for leishmaniasis, showing clinical signs such as weight loss, skin lesions, and nail overgrowth. Forty eight of 124 (38.7%) households had at least one seropositive dog. If the protected areas were considered separately, seroprevalence ranged from 10 to 32% in dogs and from 22 to 55% in households, with significant differences in the prevalence between the areas. Dog and household prevalence were significantly higher in PESB and RPPNMS (Table 2). Differences in prevalence ratios regarding binary variables were detected by the Chi-square tests for the cohabitation of dogs with chickens and pigs (Table 3).

Table 2. Seroprevalence of canine leishmaniasis in rural dogs sampled around five protected areas of the Atlantic Forest.

Study site	Number of dogs	Number sampled (%)	Dogs/house	Dog prevalence (%)	P value	Household prevalence (%)	P value
RPPNFM	98	84 (85)	3.9	13.1 (11/84)	<0.0001	40 (10/25)	0.4233
PESB	86	67 (77)	2.7	32.8 (22/67)	0.0072	54.8 (17/31)	0.4723
PESS	53	48 (90)	2.1	14.6 (7/48)	<0.0001	24 (6/25)	0.0163
RPPNFMA	60	50 (83)	3.3	10 (5/50)	<0.0001	22.2 (4/18)	0.0184
RPPNMS	49	42 (85)	1.9	30.9 (13/42)	0.0136	44 (11/25)	0.6889
Total	346	291 (84)	2.8 (1-15)	19.9 (58/291)	<0.0001	38.7 (48/124)	0.0270

Table 3. Prevalence ratios for *Leishmania* seropositive dogs (for at least one test) in rural areas around Atlantic Forest fragments, and Chi-square tests results for binary variables.

Variable	Category	Number	Positives	Prevalence ratio (%)	Z	P value
Gender	Males	193	37	19.2		
	Females	98	21	21.4	0.45	0.64
Sterilized	Yes	19	6	31.6		
	No	272	52	19.1	1.32	0.18
Breed	Mixed bred	245	51	20.8		
	Purebred	46	7	15.2	0.87	0.38
Hair	Short	266	52	19.5		
	Long	25	6	24.0	0.53	0.59
Age class	Young	64	9	14.1		

	Adult	227	49	21.6	-1.33	0.18
Ectoparasites	Yes	255	50	19.6		
	No	36	8	22.2	0.36	0.71
Mobility	Free	278	54	19.4		
	Restrained	13	4	30.8	1	0.31
Access to forest	Yes	239	46	19.2		
	No	52	12	23.1	0.62	0.53
Access to villages	Yes	75	11	14.7		
	No	216	47	21.8	-1.32	0.18
Presence of chickens	Yes	271	46	17.0		
	No	20	12	60.0	4.64	< 0.0001
Presence of cattle	Yes	180	30	16.7		
	No	111	28	25.2	1.77	0.07
Presence of horses	Yes	153	27	17.6		
	No	138	31	22.5	-1.02	0.3
Presence of pigs	Yes	155	19	12.3		
	No	136	39	28.7	-3.49	0.0005
Presence of small pets*	Yes	59	9	15.3		
	No	232	49	21.1	1	0.31
Interaction with wildlife	Yes	161	32	19.9		
	No	130	26	20.0	-0.02	0.97
Ectoparasite treatment	Yes	226	41	18.1		
	No	65	17	26.2	-1.42	0.15

*Rabbits and cage birds.

The results of the GLMM modeling are summarized in table 4. Models for four of eight possible scenarios (DPP, DPP + IFI, DPP + ELISA, DPP + ELISA + IFI) could not be built due to the small number of positive outputs. In the four viable final models, eleven of 23 entered variables remained in at least one model. The presence of pigs entered all models as a negative association, while the presence of chickens featured in three models, also negatively associated with prevalence. Weight and body score entered two models with negative relationships to infection. The presence of ectoparasites, number of cats per household and mobility of dogs figured in one of the four final models showing negative relationships with seropositivity.

Height of dogs appeared in all models as a positive association with CVL. Sterilization was positively associated with infection in three of four scenarios. Long fur entered one model with a positive association. Age class was positively associated with infection in one model. The correlation matrices provided contained no value above 0.6, thus no colinearity was found that would have prevented the variables to be included in the same model.

Table 4. Best supported GLMMs analyzing associations for leishmaniasis-seropositive rural dogs living around Atlantic Forest fragments.

Scenario / Variables	Estimate (SE)	Z	P value
+ in at least one test			
Sterilized	1.196 (0.569)	2.1	0.03558
Weight	-0.130 (0.044)	-2.9	0.00341
Height	0.139 (0.036)	3.7	0.00016

Presence of chickens	-1.778 (0.530)	-3.3	0.00079
Presence of pigs	-1.084(0.347)	-3.1	0.001804
+ ELISA			
Height	0.043 (0.020)	2.09	0.03663
Presence of chickens	-1.411 (0.557)	-2.5	0.01136
Presence of pigs	-1.144 (0.417)	-2.7	0.00616
+ IFI			
Sterilized	2.294 (0.766)	2.9	0.002739
Body score	-1.132 (0.501)	-2.2	0.024009
Weight	-0.205 (0.083)	-2.4	0.013545
Height	0.142 (0.052)	2.7	0.006397
Presence of ectoparasites	-1.582 (0.659)	-2.4	0.016469
Number of cats	-0.453 (0.218)	-2.07	0.038373
Mobility of dogs	-2.976 (0.823)	-3.6	0.000301
Presence of pigs	-0.992 (0.480)	-2.06	0.039026
+ ELISA / + IFI			
Sterilized	1.307 (0.618)	2.1	0.034550
Long fur	1.375 (0.574)	2.4	0.016681
Age class	1.130 (0.597)	1.89	0.058377
Body score	-0.824 (0.344)	-2.4	0.016719
Height	0.048 (0.020)	2.4	0.015223
Presence of chickens	-1.919 (0.546)	-3.5	0.000442
Presence of pigs	-1.343 (0.384)	-3.5	0.000481

Spatial clustering of seropositive dogs was detected only in PESB (Table 5), and seropositive dogs were randomly or uniformly distributed in the other four sites.

Table 5. Nearest neighbor dispersion analysis results for leishmaniasis seropositive rural dogs around five protected fragments of the Atlantic Forest in the State of Minas Gerais, Brazil.

Study site	Mean distance between seropositive dogs (m)	Distance standard deviation	Z score	Spatial pattern
RPPNFM	951.4	166.2	0.12	Random
PESB	160.8	47.5	-4.48	Clustered
PESS	1351.7	162.5	3.91	Uniform
RPPNFMA	874.3	112.4	4.04	Uniform
RPPNMS	298.6	64.7	-1.38	Random

Discussion

Because the dog is the primary reservoir and the infection in dogs generally precedes human cases [22], more attention should be given to the disease in dogs wherever they occur, i.e., all human-occupied areas. Even though relatively few humans live in our study areas and have access to these dogs, and the ecological impact of leishmaniasis may be greater than the public health impact, rural families' welfare should never be neglected. Additionally, there is ecotourism activity inside and around parks, and human encroachment is ongoing at these sites. Consequently, dogs may be useful as sentinels for zoonotic leishmaniasis in areas with uncertain epidemiological status, and efforts to reveal their patterns of infection are of the highest importance for control and prevention.

We acknowledge that the low accuracy of the serological tests used is a limitation of our study and without a molecular test is not possible to rule out cross-reactions with other protozoans, such as *Trypanosoma* sp., in a proportion of dogs sampled. The same serum samples were tested for *Babesia canis* (Curi et al., unpublished data), and only four (1.3%) were positive for both *Leishmania* and *Babesia*. Therefore, the occurrence of this cross reaction may be considered low or nonexistent in this study. Instead, coinfection by both agents is possible. Our results show a low level of agreement between the serological tests used which may be related, among other factors, to the relatively low indirectly estimated (through ELISA) antibody concentrations detected in most samples. Other studies have reported discrepancies in serologic tests, such as differences in sensitivity and specificity [e.g. 37]. This is of great concern because tests such as ELISA and DPP are currently employed for epidemiological screening and control of CVL in Brazil [38-39], and such inconsistency may hamper any research or control efforts. Therefore, our strategy to use concomitantly different serologic tests is recommended, preferably along with molecular diagnostic methods [44].

Many studies have identified risk factors for zoonotic human and CVL. However, most studies on dogs were primarily concerned with urban zones [e.g. 28-32,44-45,47]. In our study, seven individual traits of dogs were associated with seropositivity. Height was positively associated with seropositivity in all four models. This factor is possibly linked to a target size effect or differences in heat and CO_2 irradiation between small and large sized dogs, enhancing the finding of larger hosts by the vectors. Weight and body score were negatively associated in two scenarios of seropositivity, and this can be explained by the fact that low body condition animals may have impaired immune function and higher susceptibility to infection. However, dog size was not associated with infection in previous studies [31].

The literature shows that ectoparasites may be positively, negatively or neutrally associated with dog infection [31]. However, despite some controversy, other authors claim that ticks may be able to transmit the parasite [22,46]. In our study, the presence of ectoparasites in dogs has entered one final model, but with a negative association with seropositivity. This finding do not corroborate with studies from urban areas [31], but the work of Dantas-Torres and colleagues [45] with dogs from a rural community in northeastern Brazil have showed that ticks are not relevant as vectors of *Leishmania*. Our analysis revealed a weak negative correlation between the presence of ectoparasites and previous ectoparasite treatment, meaning that this intervention has been ineffectively performed in the study areas, and is probably either ineffective against sand flies.

Surprisingly, long fur was positively associated with dog seropositivity in our study by one of the models, because, according to the literature, short fur is considered as a strong predictor of canine leishmaniasis infection in Brazilian cities [28,31,47]. However this relationship did not hold in our data set. Possibly, the lower densities of rural dogs when compared to urban dogs [6] balance the detectability of shorthaired and longhaired dogs by sand flies. Thus, control measures in rural zones should not target any particular dog phenotype, contrary to the focus on shorthaired dogs proposed for urban populations [31].

Dogs older than one year were more likely to be infected, according to one GLMM scenario. Conversely, age did not enter the models and there was no difference in prevalence between young and adult dogs according to the Chi-square tests. Thus, we believe that age is not a strong predictor for *Leishmania* infection and dogs of all ages may be reservoirs in the study areas, and this is in general agreement with previous literature [31].

Sterilized dogs were found to be seropositive more frequently according to three scenarios. This is expected since gonadectomized dogs tend to roam or escape less and spend more time quiet [48-49] being more easily found by the vectors. Conversely, this would depend very much on sand fly density at different sites and peak times of sand fly feeding and of canine resting habits, since sand flies could easily feed on immobile dogs whether they sometimes roam or not.

Four other significant variables linked to dog management (dogs kept free) and vector attractiveness (presence of chickens, pigs and number of cats) entered final models as negatively associated with seropositivity. In the same way as aforementioned about gonadectomized dogs, free-roaming dogs are less sedentary and more difficult targets to vectors, whilst dogs living in restrict spaces spend more time quiet being more easily found, bitten and infected in these rural scenarios. Additionally, the negative association with dog mobility in one of the models indicates that being kept near a human dwelling is associated with increased risk for dog infection. However, in the review of Belo and coauthors [31] is mentioned that the general relationship is the inverse. Perhaps the detectability of dogs by the sand flies varies in some ways between cities and rural areas. Moreover, a purely peridomestic cycle of CVL may be happening in these scenarios, and warrants interesting future investigation.

Negative associations of dog seropositivity and the presence of chickens and pigs were revealed both by the GLMM models and the Chi-square tests. The strongly negative association between positive dogs and the presence of pigs in the households do not agree with most of the past findings. Previous studies have highlighted the presence of large domestic mammals as a positive influence on infection rates in dogs and humans [28-29,31,50]. Our data show that in these rural sites, the presence of large mammalian livestock (cattle and horses) did not influenced *Leishmania* seroprevalence in dogs, but the presence of pigs may be diverting sand fly bites away from dogs, and then reducing their infection rates. The pig is one of the preferred species as blood sources for the phlebotomines

[51], but is apparently an incompetent reservoir [52]. This may facilitate the pig's zooprophylactic effect against CVL in rural zones, what seemingly happened in our case.

The negative association between the presence of chickens and seroprevalence reveals another evidence of the protective effect of some domestic species against leishmaniasis. This result is also quite controversial because some studies have also identified chickens as attractors for sand flies, implying that the presence of chickens ultimately produces increased infection rates in dogs and humans [27,28]. Nonetheless, a recent review of risk factors for visceral leishmaniasis in Brazil shows both positive and negative associations of chickens for canine infection [31]. Our results are pointed at the same direction that those aforementioned for pigs. Because chickens are the preferred vertebrate target for the vectors [27,53] but not suitable hosts for *Leishmania* parasites [54], they also divert the attention of the vectors from the dogs, thus reducing the bite rates and, consequently, the infection rates in dogs. The role of chickens as food sources, vector attractors, and zooprophylactic agents for leishmaniasis has previously been discussed [27,54-55], but only in the context of human infection. The number of cats followed the same pattern, being negatively associated with dog seropositivity (more cats per household are associated with less positive dogs). Cats have been found to be infected with *Leishmania*, can infect sand flies, but do not seem to develop high parasite burdens [22], and may also turn infection away from dogs when in high numbers and densities. Animal sheds and animals on which sand flies feed can increase sand fly density [36] but may also decrease infection prevalence and feeding on dogs and humans, so that the net impact on VL transmission depends on the balance of these outcomes. In our rural context, the balance appears to be favoring a zooprophylactic function of domestic fowl, swine and cats against CVL.

Since there was weak evidence of spatial clustering of seropositive dogs (exclusively for one study site), we believe that the disease is not being maintained in focal points throughout the study areas. Thus, control efforts must be equally employed and cover all properties in these scenarios. One possible explanation for the clustering at PESB is that its higher altitude and the steeper topography drives most human settlements to be located at some of the few valleys and flat areas in the region, resulting in spatial aggregation of households, and consequently, of their dogs.

The Atlantic Forest is a highly diverse and fragmented ecosystem located at the most developed region in Brazil [56]. Therefore, a strong presence of drivers of the dynamic alterations of disease, such as anthropogenic environmental change and increased contact between humans, wildlife, and domestic animals, is expected [4]. However, although governmental prevention programs exist for rural areas, interface areas such as rural zones around forest fragments have received little scientific or government attention in terms of health issues. Our findings show that the study areas should be considered endemic for canine leishmaniasis and that despite the recent trend toward urbanization of the disease [57], it is advisable that government health agencies return to look at rural zones beyond Brazilian urban areas if the aim is to widely control zoonotic leishmaniasis and other tropical diseases. Specifically, in our case, the study areas deserve more attention and thorough investigation through surveys of leishmaniasis in humans, reservoir dogs, wildlife and vectors. Additionally, higher prevalence areas such as PESB and RPPNMS should be prioritized by control programs. The Brazilian visceral leishmaniasis control program should expand the focus to embrace rural and ecosystem health in a holistic view of the problem, and the data presented here should be used as a reference for research and intervention in Brazilian human/wildlife interface areas.

Habitat loss and fragmentation and the subsequent decrease in biodiversity may cause, among many other effects, alterations in parasite ecology that result in increased rates of infection in wildlife [58-60]. Although we have no data on wildlife prevalence, the scenario of infected dogs living around and actually entering important biodiversity sites such as Atlantic Forest remnants [12] raises concerns about possible transmission to and from wild animals. Wild mammals can develop clinical signs of leishmaniasis, especially in stressful situations such as captivity [25], and the prevalence of the disease in many captive and free-ranging populations has been reported [22,24-26]. Therefore, the presence of infected reservoir dog populations around small forest fragments under strong human pressure may warrant persistence, circulation, and the possible, yet unknown, deleterious effects of leishmaniasis on the health and fitness of wild animals. Control programs should primarily involve a reduction in the dog population size and density, e.g., by sterilization (not culling), owner education, and legally limiting the number of dogs per rural household in settlements close to wildlife refuges and by restricting the access of dogs to protected areas, thus reducing the probability of disease transmission to and from humans and wildlife. Other measures that reduce attractiveness for sand flies, e.g. application of insecticides and keeping zooprophylactic species such as pigs or chickens around the house may be also recommendable in rural areas. Of course, the latter needs more investigation to detect general patterns before being adopted. Cats are especially not recommended because they cause great damage to wildlife species [61].

Finally, the results presented here suggest another important reason for controlling and monitoring dog populations around protected areas: the risk of visceral leishmaniasis for humans and wildlife. Our findings also highlight the need for additional surveys to detect epidemiological patterns of leishmaniasis in Brazilian rural zones, especially around wildlife-rich protected areas. Another noteworthy aspect of the results is the difference between the profile of risk factors and the results of most previous studies from urban areas. These differences are crucial for planning thoughtful and effective management initiatives that will protect the interdependent health of humans, domestic animals, and wildlife.

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ARTIGO 2

Identificando fatores de risco para a exposição a patógenos virais importantes para a conservação de carnívoros em cães rurais na Floresta Atlântica

Identifying risk factors for the exposure to carnivore conservation-concern viral pathogens in rural dogs from the Atlantic Forest

Preparado de acordo com as normas da Revista Biological Conservation

Abstract

Despite the crucial role of domestic dogs as reservoirs for some of the most threatening diseases for wild carnivores, such as distemper and parvovirosis, little is known about the epidemiological features and the risk factors involved in pathogen exposure of dogs that live in human/wildlife interfaces and actually have contact with wildlife. Through a cross-sectional serological approach and generalized linear mixed models, we assessed the prevalence along with dog and environmental characteristics associated with seropositivity and antibody titers for four important viral diseases of rural dogs living in households around Atlantic Forest fragments. Analyses detected widespread exposure to canine parvovirus (97%), canine distemper virus (15%) and canine adenovirus (27%), but never for canine coronavirus. According to identified associations, inhibiting dog's free-roaming behaviors and access to nearby forests and villages through restraining, improving veterinary assistance and vaccination, and promoting dog population control through sterilization are required measures. A list of viral

pathogen exposure-associated risk factors is revealed for the first time, supporting preventive management actions to protect the health of rural dogs, and consequently, of Atlantic Forest's wild carnivores.

Keywords: domestic dog, disease, virus, risk factor, carnivore, conservation, domestic/wildlife interface, seroprevalence

Introduction

The domestic dog (*Canis familiaris*) is beyond question one of the man's closest animal species, and consequently the most abundant and widespread carnivore species in the world (Young et al. 2011, Gompper 2014). Notwithstanding the direct negative impacts on wildlife such as predation, competition and harassment (Vanak and Gompper 2012, Hughes and Macdonald 2013), dogs are also the most important reservoirs of diseases relevant for the conservation particularly of wild carnivore species, such as those called by Knobel et al. (2014) as "The Big Three": rabies, distemper and parvovirosis. Infectious disease-driven mortality is a major cause of population decline and extinction of wild mammal carnivores worldwide, because several species are already endangered, populations are mostly small or declining due to habitat loss and fragmentation, and many diseases are shared or continuously acquired from sympatric man-subsidized dog populations (Murray et al. 1999, Pedersen et al. 2007, Knobel et al. 2014).

Through a recent point of view, the scientific community has acknowledged the evident usefulness of dogs as sentinels of human health (Rabinowitz et al. 2005, Cleaveland et al. 2006). Additionally, dogs have been proposed to be included in surveillance strategies to improve pathogen detection in wild populations because they are more easily sampling “bioaccumulators of pathogen exposure” (Cleaveland et al. 2006). Epidemiological patterns in dog populations would, therefore, be of great value for the directing of disease prevention or control efforts for both humans and wildlife.

In South America, similarly to what occur in other parts of the world, recent case reports and studies revealed that dogs are sources of dangerous infectious agents such as distemper virus to wild carnivores (Megid et al. 2009, 2010, Acosta-Jamett et al. 2011), and that several wild carnivore populations have already been exposed to canine pathogens (e.g. Fiorello et al. 2007, Nava et al. 2008, Curi et al. 2010, 2012). Fortunately, some studies were also concerned with the detection and estimation of pathogen prevalence in sympatric domestic dog populations in a conservation context (i.e. those living around protected areas) (Fiorello et al. 2004, 2006, Whiteman et al. 2007, Bronson et al. 2008, Curi et al. 2010, 2012, Santos et al. 2012). However, to our knowledge, the assessment of risk factors and epidemiological parameters related to viral pathogen prevalence has never been performed in South American dog populations, particularly in human/wildlife interfaces and areas relevant for conservation. These aspects are of great importance for disease preventive or control management because the information acquired from dogs is useful to guide and focus limited resources and actions for the promotion of human, domestic and wild animal and hence of ecosystem health.

Therefore, the goals of our study are to detect not only the presence and prevalence, but also risk factors associated with the exposure to viral agents relevant to carnivore conservation in populations of domestic dogs living in rural landscapes around remnants of the Atlantic Forest. We present for the first time a list of dog and environmental features associated with previous exposure to viral pathogens that may be managed for the improvement of health of dog populations and the urgent prevention of disease-induced mortality of the already threatened Atlantic Forest’s wild carnivores.

Methods

Ethics statement

Sampling was performed under consent from the household head or other responsible person. Required licenses were obtained from the State Forest Institute – IEF (UC: 080/10, 081/10 and 082/10). The study was approved by the Ethics Commission on the Use of Animals of the Pontiphical Catholic University of Minas Gerais (CEUA, PUC Minas 037/2010). Regarding the collection of data from humans and households, our project was examined by the Ethics Research Committee (Comitê de Ética em Pesquisa) of the Pontiphical Catholic University of Minas Gerais (PUC-Minas). A term about the confidential character of the records was read in every household. Animal manipulation procedures adhered to the guidelines from the COBEA (Brazilian College of Animal Experimentation) and the Animal Ethics Committee of FIOCRUZ (Oswaldo Cruz Institute Foundation).

Study sites

We selected rural households located at less than two km from borders of six protected areas in the remnant Atlantic Forest of the state of Minas Gerais, southeastern Brazil. These areas comprise three state parks (larger areas): Serra do Brigadeiro (PESB, municipality of Araponga) and Sete Salões (PESS, municipality of Santa Rita do Itueto), Rio Doce (PERD, municipality of Dionísio), and three smaller private reserves: Fazenda Macedônia (RPPNFM, municipality of Ipaba), Feliciano Miguel Abdala (RPPNFMA, municipality of

Caratinga), and Mata do Sossego (RPPNMS, municipality of Simonésia) (see Figure 1and Table 1). Several wild carnivore species were recorded in the areas, including wild canids such as the crab-eating fox (*Cerdocyon thous*) and the maned wolf (*Chrysocyon brachyurus*), felids such as the puma (*Puma concolor*) and small wild felids (*Leopardus* spp.), mustelids (*Eira barbara*, *Gallictis* sp.), and procyonids (*Nasua nasua*, *Procyon cancrivorous*). According to a concomitant camera-trap study, free-roaming domestic dogs, mostly those living in surrounding rural properties, are frequently visiting and actually occupying the interior of these areas (Paschoal et al. 2012, and unpublished data).

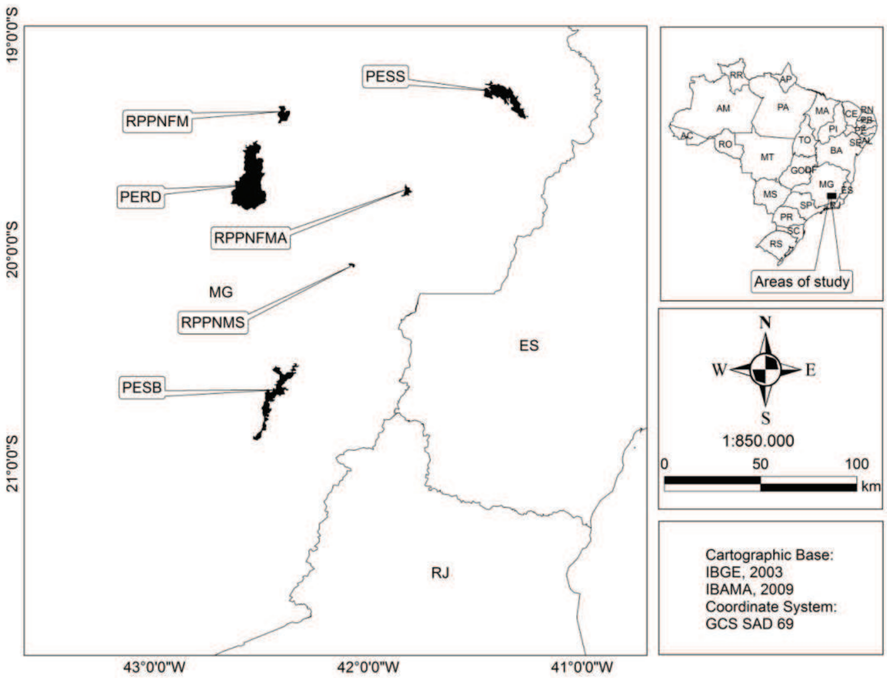


Figure 1. Location of the study areas.

Table 1. Human and dog demographic characteristics of rural settlements around six protected areas in the Atlantic Forest of Minas Gerais State, southeast Brazil.

Area (size in ha)	Distance from city (km)	Houses	Humans	Dogs	Dog:human ratio	Dogs per household
RPPNFM (3,343)	0.3	25	89	98	1.101	3.920
PESB (15,015)	3.3	31	125	86	0.688	2.774
PESS (13,370)	4.7	25	82	53	0.646	2.120
RPPNFMA (1,312)	10.5	18	53	60	1.132	3.333
RPPNMS (392)	7.7	25	102	49	0.480	1.960
PERD (36,100)	11	20	87	34	0.390	1.700
Total	-	144	538	380	0.706	2.638

Sampling

Visits to the households, owner interviews and dog sampling were performed between January 2011 and August 2012. 320 dogs older than two months were sampled in 144 rural households. Blood was collected under physical restraint from the jugular vein and a complete clinical examination of the dogs was performed. A questionnaire survey was administered to each owner. Factors related to animal management and behavior that might be directly or indirectly associated with the exposure to viral agents (number of dogs and cats, mobility of dogs, access of dogs to the forest and villages, observed interactions between dogs and wildlife, recent dog disease or death, previous anti-rabies and multiple vaccination, veterinary assistance, presence of pigs, and the number of people) were recorded for each household. Individual and clinical features of the dogs (sex, age, age class, height, weight, breed, sterilization, body condition, clinical alterations) may influence behavioral patterns and immune function (and thus pathogen exposure and antibody response), and were recorded in individual files. Weight was measured with a precision scale (Pesola®, 50 kg capacity), and height was measured from the footpad to the top of the scapulae of standing dogs. Body condition of dogs was scored from 0 (extreme emaciation) to 5 (extreme obesity). Refusals to the survey happened in six households because the responsible were absent.

Serologic testing

Serum was extracted after centrifugation, and stored at -20°C until sent to the laboratory for antibody detection and titration through serologic testing for canine parvovirus (CPV, hemagglutination inhibition, 1:20 dilution as cut-off point), canine distemper virus (CDV, serum neutralization, 1:8 dilution as cut-off point), canine coronavirus (CCV, serum neutralization, 1:2 dilution as cut-off point), and canine adenovirus type-2 (CAV, serum neutralization, 1:16 dilution as cut-off point). Cut-off points were set according to previous literature, and aimed to maximize the sensitivity of the tests (Appel and Robson 1973, Appel et al. 1975, Senda et al. 1986, Mochizuki et al. 1987). Seroprevalence or prevalence is referred henceforth as the proportion of animals with detectable antibodies for each pathogen and considered as an indicator of previous pathogen exposure in dogs. Positive dogs are those animals for which exposure resulted in infection, but the animal survived, and an immune response was developed. Titers are expressed here as the inverse of the highest positive dilution. Higher antibody titers may reflect more recent infections, larger antigenic burdens (i.e. exposure to higher viral loads) or re-exposition, but also stronger individual immune responses to exposure, which are dependent on many factors including nutrition, stress and genetics.

Statistical analysis

Prevalence proportion rations among areas, between grouped small (private reserves) and large areas (state parks), and for all binary variables were compared through Yates-corrected chi square tests. Generalized linear mixed models (GLMM's) using the areas and households as random effects and all other variables cited above as binary or continuous fixed effects were built for both seroprevalence (adjusted for binomial distribution) and antibody titers (adjusted for Poisson distribution) for each pathogen detected. A backward stepwise approach based on the level of significance (threshold $p<0.05$) of individual variables was then used to eliminate less significant variables and to compose a list of the most strongly exposure-associated factors for seroprevalence and level of antibody titers against each pathogen detected. The presence of pigs was included only in CPV-related statistics, because of the possible exposure and antibody response of dogs to swine parvoviruses. Multiple-agent vaccinated animals were included in the analyses to verify the efficacy of the procedure through expected positive associations with seroprevalence and antibody titers. The GLMM tests were run in package lme4 of R software. Descriptive statistics and chi square tests were performed in BioEstat 5.0. We used the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (von Elm et al. 2007) as a guideline to report our data.

Results

Relevant characteristics of sites, dog and human populations and the number of households sampled in each study area are described in table 1. Males comprised

63.5% (209/320) of dogs, thus sex ratio was male biased (1.88 males for each female). Only 21 dogs (6.5%) were sterilized. Mixed bred dogs comprised 79.3% (254/320) of the samples. Most dogs (78.4%) were adult, with mean age of 3.3 years (39 months; range 3-216; mean 39.9±36.05). Body scores were low in general (range 0.5-4, mean 2.1±0.57), and dogs weighed 13.5±8.6 kilograms in average (range 1.2-80). Height of dogs ranged from 13 to 79 centimeters (mean 44.2±10.4).

Most dogs are allowed to roam freely, and only 10% (33/320) live on restricted spaces as fenced or leashed dogs. Most dogs were reported to access near forests (249/320 or 77.8%), and 30% (96/320) had access to villages or small urban centers. Almost half of the sampled dogs (47.8%) live in households with the presence of domestic swine. Dog mortality or clinical disease in previous two years was reported by owners of 43% and 31% of dogs, respectively, but only four dogs had clinical symptoms compatible with viral disease (diarrhea and ocular secretion) at the time of collection. Anti-rabies vaccination was performed in 85% (261/320) of dogs, but multiple-disease vaccines (protective for the pathogens studied here) were applied in only 6% (19/320) of dogs. Most dogs (53%; 170/320) sampled were reported as having interacted with some wildlife species. Only 25 dogs (8%) received veterinary assistance throughout their lives. Most owners (63%) feed their dogs with human leftovers, which were mostly protein-poor mixtures. Commercial dog food is provided in 39%, and milk alone in 6% of houses. In some households, combinations of commercial dog food plus milk (3.4%) or leftovers (14%) are used to feed dogs. Other items reported include milk whey and minced corn.

Seroprevalence per study area is summarized in table 2. Almost 85% (320 of 380) of resident dogs were sampled. Antibodies against CPV, CDV and CAV were detected with a prevalence of 97%, 15%, and 27.8%, respectively.

Antibodies against CCV were not detected in our samples. The three former agents were detected in all six areas, except for CDV antibodies that were absent in dogs from RPPNFMA. Otherwise, prevalence did not varied significantly among sites, except for dogs from PERD which were proportionally less exposed to CPV ($p \leq 0.007$), but more exposed to CAV ($p \leq 0.01$). When areas were separated by size, CPV was more prevalent in smaller areas (173/174, 99%) than in larger areas (138/146, 94%) ($p = 0.004$). Accordingly, more CDV positive dogs were present in smaller private reserves than in state parks (33/174 or 19%, and 15/146 or 10%, respectively; $p = 0.015$). CAV prevalence did not differ between large and smaller areas (46/174 or 26%, and 43/146 or 29%, respectively; $p = 0.27$).

Table 2. Seroprevalence for canine parvovirus (CPV), canine distemper virus (CDV), and canine adenovirus (CAV) in rural dogs living around Atlantic Forest protected areas in the state of Minas Gerais, southeast Brazil.

Area	Dogs	Sampled	% sampled	CPV +	%	CDV +	%	CAV+	%
RPPNFM	98	84	85.7	83	98.8	25	29.7	28	33.3
PESB	86	67	77.9	65	97.0	11	16.4	15	22.4
PESS	53	47	88.6	47	100	2	4.2	9	19.1
RPPNFMA	60	49	81.6	49	100	0	0	11	22.4
RPPNMS	49	41	83.6	41	100	8	19.5	7	17.1
PERD	34	32	94.1	26	81.2	2	6.2	19	59.3
Total	380	320	84.2	311	97.2	48	15	89	27.8

Prevalence proportion rates and results of chi-square tests for binary variables are shown in table 3. Higher proportions of CPV-seropositivity were detected among unrestricted, (multiple disease) unvaccinated and unassisted dogs, and in houses where other dogs died in the last two years. For CDV, more seropositive dogs were found among adults, unrestricted dogs, rabies-vaccinated dogs and dogs with access to forested areas. For CAV, more seropositives were found among males, adults, dogs with access to villages, and dogs vaccinated with both anti-rabies and multiple vaccines.

Table 3. Prevalence ratios for CPV, CDV and CAV seropositive dogs from rural areas around Atlantic Forest fragments, and Chi-square tests results for binary variables analyzed. Significant differences between categories ($p<0.05$) are shown in black.

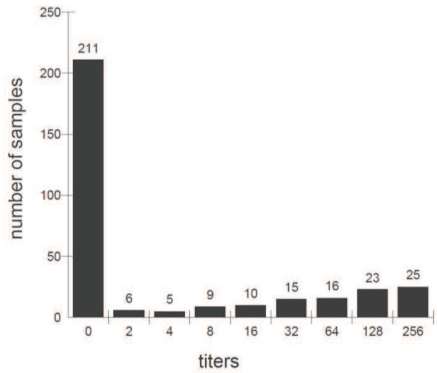
Pathogen / Variable	Category	Number	Positives	%	Z	P value
CPV						
Gender	Males	209	205	98.1	-1.33	0.091
	Females	111	106	95.5		
Sterilized	Yes	21	21	100	-0.80	0.210
	No	299	290	96.9		
Breed	Mixed bred	254	247	97.2	0.12	0.452
	Purebred	66	64	96.9		
Age class	Young	69	66	95.6	-0.87	0.191
	Adults	251	245	97.6		
Mobility	Restricted	33	27	81.8	-5.63	**<0.0001**
	Unrestricted	287	284	98.9		
Access to forest	Yes	249	245	97.9	-1.63	0.103
	No	71	67	94.3		
Access to villages	Yes	96	91	94.7	-1.69	0.080
	No	224	220	98.2		
Cohabitation with pigs	Yes	153	151	98.6	-1.55	0.112

	No	167	160	95.8		
Recent dog mortality	Yes	138	137	99.2	-1.96	**0.049**
	No	182	174	95.6		
Recent dog disease	Yes	101	96	95.5	1.57	0.116
	No	219	215	98.1		
Anti-rabies vaccination	Yes	261	253	96.9	0.57	0.565
	No	59	58	98.3		
Multiple vaccination	Yes	19	17	89.4	2.09	**0.036**
	No	301	294	97.6		
Interaction with wildlife	Yes	170	166	97.6	-0.52	0.596
	No	150	145	96.6		
Veterinary assistance	Yes	25	20	80	5.41	**<0.0001**
	No	295	291	98.6		
CDV						
Gender	Males	209	27	12.9	1.39	0.162
	Females	111	21	18.7		
Sterilized	Yes	21	4	19	-0.54	0.586
	No	299	44	14.6		
Breed	Mixed bred	254	38	14.9	-0.05	0.959
	Purebred	66	10	15.1		
Age class	Young	69	4	5.8	-2.40	**0.016**
	Adults	251	44	17.4		
Mobility	Restricted	33	1	3	-2.02	**0.042**
	Unrestricted	287	47	16.3		
Access to forest	Yes	249	43	17.2	-2.11	**0.034**
	No	71	5	7		
Access to villages	Yes	96	15	15.6	-0.22	0.825
	No	224	33	14.6		
Recent dog mortality	Yes	138	24	13.1	-1.01	0.309
	No	182	24	17.2		
Recent dog disease	Yes	101	19	18.8	-1.31	0.189
	No	219	29	13.1		
Anti-rabies vaccination	Yes	261	46	17.6	-2.79	**0.005**
	No	59	2	3.3		
Multiple vaccination	Yes	19	3	15.7	-0.10	0.916
	No	301	45	14.9		

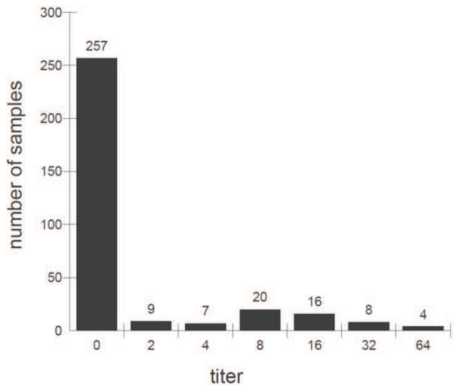
Interaction with wildlife	Yes	170	28	16.4	-0.80	0.418
	No	150	20	13.2		
Veterinary assistance	Yes	25	3	12	0.43	0.666
	No	295	45	15.2		
CAV						
Gender	Males	209	23	11	2.74	**0.006**
	Females	111	25	22.5		
Sterilized	Yes	21	6	28.5	-0.08	0.936
	No	299	83	27.7		
Breed	Mixed bred	254	65	25.5	-1.74	0.081
	Purebred	66	24	36.3		
Age class	Young	69	9	13	-3.09	**0.002**
	Adults	251	80	31.8		
Mobility	Restricted	33	13	39.3	1.56	0.116
	Unrestricted	287	76	26.4		
Access to forest	Yes	249	71	28.5	-0.52	0.599
	No	71	18	25.3		
Access to villages	Yes	96	45	46.8	-4.98	**<0.0001**
	No	224	44	19.6		
Recent dog mortality	Yes	138	42	30.4	-0.91	0.362
	No	182	47	25.8		
Recent dog disease	Yes	101	25	24.7	0.82	0.406
	No	219	64	29.2		
Anti-rabies vaccination	Yes	261	79	30.2	-2.06	**0.039**
	No	59	10	16.9		
Multiple vaccination	Yes	19	10	52.6	-2.48	**0.012**
	No	301	79	26.2		
Interaction with wildlife	Yes	170	42	24.7	1.32	0.186
	No	150	47	31.3		
Veterinary assistance	Yes	25	10	40	-1.41	0.156
	No	295	79	26.7		

Titer frequency distributions for the pathogens detected are shown in figure 2. Most samples had high antibody titers for CPV (>160) and for CAV (>64), but for CDV few positive samples had titers above twenty.

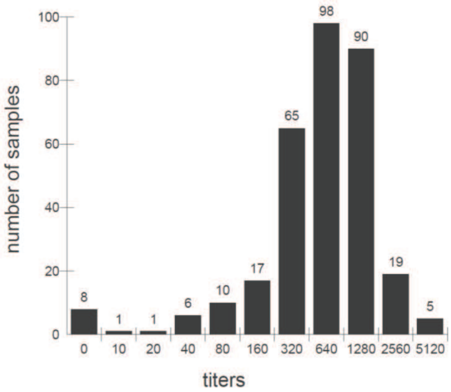
Figure 2. Titer frequency distributions for (a) CAV (cut-off point at 16), (b) CDV (cut-off point at 8) and (c) CPV (cut-off point at 20) in domestic dogs living around protected areas of the Atlantic Forest in Minas Gerais, Brazil (2011 to 2012).



a



b



c

From the six GLMM models ran (seropositivity and titers for CPV, CDV and CAV), four models were successfully built. Results of GLMM modeling, direction and strength of associations are summarized in table 4. Fourteen of eighteen initially entered variables remained in at least one model. Age entered all four models, but with positive relationships with CAV and CDV seropositivity, and negative relationships with CAV and CPV titers. Age class (adult) was negatively influencing CAV titers, but was positively associated with CPV titers. Male sex was positively associated with CAV and CPV titers. Weight of dogs entered three models being positively associated with CAV seropositivity and CPV titers, and negatively associated with CAV titers. Body score and height were positively associated with CAV titers, but negatively associated with CPV titers. Dog sterilization was negatively associated with both CAV and CPV titers. Purebred dogs were negatively associated with CPV titers. The number of people in the households entered models for CAV titers, and CAV and CDV seropositivity with positive associations. Access of dogs to near villages was a positive factor in the models for CAV seropositivity and titers. Unrestricted and veterinary-assisted dogs were negatively associated with CPV titers. Multiple-agent vaccination was positively associated only for CAV positivity and titers. Anti-rabies vaccination was positively influencing CAV titers and CDV seropositivity. The correlation matrices provided indicate no values determining the exclusion of any variable from the models.

Table 4. Best supported GLMM's for seropositivity and antibody titers against adenovirus, parvovirus and distemper in rural dogs living around Atlantic Forest fragments in the state of Minas Gerais, southeast Brazil.

Model /Variables	Estimate	Standard error	Z value	P
Adenovirus seropositivity				
Age	0.013715	0.003979	3.446	0.000568
Weight	0.044507	0.015903	2.799	0.005130
Number of people	0.260044	0.111293	2.337	0.019462
Access to villages	1.589657	0.344836	4.610	4.03e-06
Multiple vaccination	1.552365	0.666499	2.329	0.019852

Adenovirus titers				
Males	0.3795643	0.0305374	12.429	< 2e-16
Sterilized	-0.3625942	0.0574938	-6.307	2.85e-10
Adult (>1 yr)	-0.6085442	0.0353502	-17.215	< 2e-16
Age	-0.0011869	0.0003946	-3.008	0.00263
Body score	0.2334992	0.0271225	8.609	< 2e-16
Weight	-0.0234562	0.0033595	-6.982	2.91e-12
Height	0.0724642	0.0027945	25.931	< 2e-16
Number of people	0.5445616	0.2140479	2.544	0.01096
Access to villages	3.1918031	0.6967334	4.581	4.63e-06
Rabies vaccination	2.8029063	0.9206438	3.045	0.00233
Multiple vaccination	3.4982548	1.3213066	2.648	0.00811
Distemper seropositivity				
Age	0.009777	0.004287	2.281	0.02256
Number of people	0.330997	0.118622	2.790	0.00527
Rabies vaccination	1.478594	0.787822	1.877	0.06054
Parvovirus titers				
Males	6.028e-02	5.599e-03	10.77	<2e-16
Sterilized	-2.422e-01	1.123e-02	-21.58	<2e-16
Purebred	-1.300e-01	7.922e-03	-16.41	<2e-16
Adult (>1 yr)	2.885e-01	9.077e-03	31.78	<2e-16
Age	-1.268e-03	8.007e-05	-15.83	<2e-16
Body score	-1.349e-01	6.572e-03	-20.53	<2e-16
Weight	7.688e-03	6.432e-04	11.95	<2e-16
Height	-1.264e-02	4.887e-04	-25.87	<2e-16
Unrestricted	-5.342e-01	4.827e-02	-11.07	<2e-16
Veterinary assistance	-9.913e-01	4.207e-01	-2.36	0.0185

Discussion

The frequent contact with domestic dogs increases the exposure and disease risk for wild carnivores (Alexander and McNutt 2010, Prager et al. 2012, Woodroffe et al. 2012). Therefore, local pet management practices allowing dog's predominantly free-roaming habits, poor veterinary assistance, along with recent dog death and disease reports and the low multiple vaccination coverage detected *per se* place the wild carnivores at the study sites in a potentially dangerous scenario of disease spillover (or spillback) from dogs.

Exposure to most pathogens tested was widespread throughout the study sites except for CCV, which exposure was not detected in any dog, and antibodies for CDV that were absent from one of the sites, RPPNFMA. However, for all other sites in the case of CDV, and for the other pathogens detected, seroprevalence was widespread and moderate to high, particularly for CPV. In PERD, the largest state park, there was relatively less evidence of exposure to CPV, but more exposed dogs were found for CAV. This can be explained because most dogs around this park lived in a small urban center, and most of them were space-restricted. Therefore, they might have had fewer opportunities for the exposure to environment-resistant CPV, whereas CAV is a more density-dependent contact-transmitted virus (Buonavoglia and Martella 2007), which is probably more frequently concentrated in urban denser dog populations (Gompper, 2014).

Proportionally more CDV and CPV-exposed dogs were found in smaller areas (private reserves). Perhaps the smaller perimeter of these areas allows less space between properties and households, and ensures higher contact rates and exposure (including environmental) to these agents. Therefore, small areas and fragments should, anyway, be prioritized in health improvement efforts for rural dogs aiming also to prevent wildlife disease-associated mortality in the Atlantic Forest, which is currently mostly composed by lesser remnants (Ribeiro et al. 2009).

Higher prevalence proportion ratios for CPV seropositivity indicate that dog's free-roaming habits are favoring the exposure to environmental resistant viral particles, which is the case for this virus (Steinel et al. 2001). According to the lack of association between multiple vaccinated dogs with seroprevalence and titers, the observed low vaccination coverage seems not to be enough for the protection of dog population against CPV (and also CDV), what is probably associated with the lack of veterinary assistance. The higher seropositivity in houses reporting dog deaths indicates that CPV may be a significant cause of mortality in these rural dog populations. Moreover, the high levels of circulation indicated by the high prevalence of exposure indicate that this is perhaps the most dangerous agent in this scenario, because CPV is one of the most commonly reported viral agents in South American wild canids (e.g. Maia and Gouveia 2002, Fiorello et al. 2007, Martino et al. 2004, Curi et al. 2010, 2012), and it is capable to cause serious population impacts, for instance, in gray wolves (*Canis lupus*) (Mech et al. 1993, 2008).

Canine distemper is a systemic highly fatal disease, representing a major conservation concern in many parts of the world (Knobel et al. 2014). Evidence of infection in dogs is widespread in and around South American protected areas (Whiteman et al. 2007, Bronson et al. 2008, Curi et al. 2010). Antibodies against distemper were already found in Brazilian wild canids (Curi et al. 2012) and felids (Nava et al. 2008), and there are reports of distemper-induced mortality in two Brazilian fox species, the crab-eating fox *C. thous* and the hoary fox *Lycalopex vetulus* (Megid et al. 2009, 2010). In Chile, domestic dogs have proven blamed for the transmission of CDV to wild canids (Acosta-Jamett et al. 2011). In our rural settings, adult and unrestricted dogs were more exposed to CDV, a highly infectious virus transmitted mainly by contact or aerosols (Deem et al. 2000). These dogs probably had more time and more opportunities for contact and exposure to CDV. We cannot find plausible explanations for the association of CDV exposure and anti-rabies vaccination, except for the possibility of the vaccination personnel acting as fomites and spreading the virus through the areas. Dogs with access to forested areas were also more exposed to CDV. Of course this is related to the lack of restriction, but it also raises the possibility of acquisition or spillback events from wildlife, since CDV transmission may be, in some cases, predominantly maintained by wild reservoirs (Prager et al 2012, Woodroffe et al. 2012).

Although the impact of CAV in wildlife is still unknown, this directly transmitted virus may cause severe respiratory disease being of widespread concern for domestic dog health, and evidence of exposure was found in many wild species (Buonavoglia and Martella 2007), including in South American wild carnivores and sympatric dogs from Bolivia (Fiorello et al. 2007, Bronson et al. 2008) and Brazil (Curi et al. 2010, 2012). In our study, exposure to CAV were more prevalent in male adult dogs (their high testosterone levels may lead to more social contacts) which visits villages (where CAV transmission is enhanced by dog density), but also in anti-rabies and multiple vaccinated dogs. Again, we could not find a plausible explanation for the relationship between rabies vaccination and antibody response for CAV.

Regarding antibody titer frequency and duration of immunity, mostly high levels of antibodies against CPV and CAV were found. The duration of antibodies to the viral agents studied here is longer than two years, and such titers indicate that the exposure to these agents is mostly recent (Mouzin et al. 2004, Schultz 2006), and that preventive measures to reduce the circulation of these agents are immediately needed if the aim is to avoid transmission between dogs and to wildlife. However, our cross-sectional serologic approach does not permit inferences about temporal differences in exposure nor detects punctual disease introduction events.

Although we did not test the samples against rabies, our survey revealed that despite the apparently good previous vaccination coverage (more than 80%), several owners reported the total absence or the periodic lack of visits of health agencies promoting vaccination against rabies in their households in some years. Canine-mediated rabies is a multi-species highly fatal disease, representing a major problem for carnivore conservation particularly in Africa (Cleaveland et al. 2007). In Brazil there are animal rabies control programs through vaccination ongoing since the 1980's, and no reports of wildlife mortality have been attributed to the disease. However, serological evidence of exposure was already found in Brazilian mammals, including carnivore species (Almeida et al. 2001, Jorge et al. 2010). Therefore, more attention should be given to rabies in wildlife-domestic animal interfaces, and the vaccination program should be improved so as to continuously warrant good coverage in these areas.

The GLMM models revealed both positive and negative associations between seropositivity and titers for CDV, CPV and CAV and several variables. Age was positively related to CAV and CDV exposure, and this is expected since older animals tend to have more opportunities of infection. However, as antibodies decay with time, age of dog was a good predictor of lower antibody titers against CAV and CPV. Conversely, adult dogs were associated with higher CPV titers. This indicates that exposure to CPV is happening continuously throughout dog's life in these areas, and adult dogs should be prioritized in prevention programs. Males had higher CAV and CPV titers, probably because of their more active behavior that increases opportunities of infection. Weight, height and body score entered some of the models. However, their relationship with pathogen exposure is complicated by many confounding factors and, in our opinion, should not be used as reliable predictors of exposure for prevention or control efforts.

Sterilized dogs had lesser antibodies for CAV and CPV, what means that the decrease in dog activity and movement patterns after gonadectomy (Maarschalkerweerd et al. 1997, Spain et al. 2004) may prevent exposure to such virus. Less frequent direct contacts may prevent the exposure to CAV, and reduced roaming behavior may avoid exposure to CPV particles in the environment. Conversely, but following the same direction, dogs with free-roaming behavior were associated with higher CPV titers. Purebred dogs are, in the study areas, mostly those indoor-raised pets, and were associated with low CPV titers. The space restriction is thus probably preventing the exposure and the development of high antibody titers for CPV. The number of people cohabiting households was positively associated with CAV titers and positivity, and also for CDV seropositivity. People may be acting as fomites, increasing the exposure events for these agents in the households. For CAV positivity and titers, the access to villages was a strongly influent factor, and this freedom of dog movement must be prevented in order to reduce exposure and infection by this density-dependent virus. Although mostly unstudied (Knobel et al. 2014), CAV is capable to cause damage to wildlife populations (Murray et al. 1999). Thus, such free-ranging behavior of dogs must be inhibited through restraining in order to reduce contact rates and opportunities for CAV, CDV and CPV exposure and transmission.

Multiple-vaccinated dogs were again associated with CAV positivity and titers, in accordance with the chi-square tests. Seemingly CAV is the only agent for which multiple vaccination is ensuring antibody persistence and maybe some level of protection in dogs. This means that multiple vaccination have to be reinforced, perhaps with a more flexible viable interval in these areas (Schultz 2006), if the aim is to warrant protective herd immunity against dangerous pathogens such as CPV and CDV in dogs from wildlife-rich areas. Vaccination schemes for dogs around protected areas, aiming to protect wild carnivore and human welfare has proven successful (Cleaveland et al. 2006b), even using low coverage vaccination in wildlife species (see Haydon et al. 2006). In our case, the proportion of the dog population living in proximity of protected area borders should be targeted in comprehensive multiple-agent vaccination schemes. Health monitoring should, afterwards, be continuously performed in both domestic and wildlife species, to assess the efficacy of the proposed measures.

As conclusions, this study represents the first attempt to detect pathogens of concern for carnivore conservation in reservoir dogs living in rural settlements around Atlantic forest fragments, and to reveal associated factors that can be managed to improve domestic dog's health and consequently protect wild carnivores from disease-induced population declines in these areas. Restriction of dog movement, control of the reservoir population through sterilization, and proper vaccination programs are among required measures for the purpose.

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ARTIGO 3

Estudo epidemiológico de parasitas gastrintestinais em cães no entorno de áreas protegidas da Floresta Atlântica: implicações para a saúde humana e de animais silvestres

Epidemiological survey of gastrointestinal parasites of dogs living around protected areas of the Atlantic Forest: implications for wildlife and human health

Preparado de acordo com as normas da Revista Veterinary Parasitology

Abstract

Despite the ubiquity of domestic dogs, their strong role as zoonotic reservoirs and the relative large number of studies concerning parasites in dogs, rural areas in Brazil, especially those at the wildlife-domestic animal-human interface, have received little attention from scientists, conservationists and public health managers. This paper reports a cross-sectional epidemiological survey of gastrointestinal parasites of rural dogs living in farms around Atlantic Forest fragments. Through standard parasitological methods, we found 13 parasite taxa (eleven helminths and two protozoans) in feces samples from dogs. The most prevalent was the zoonotic nematode *Ancylostoma* sp. (47%) followed by *Toxocara canis* (18%) and *Trichuris vulpis* (8%). Mixed infections were found in 36% of samples, mostly by *Ancylostoma* and *Toxocara*. Previous deworming had no association with the infections, meaning that this preventive measure is being incorrectly performed by owners. Regarding risk factors, dogs younger than one year were more likely to be infected with *Toxocara*, and purebred dogs with *Trichuris*. The number of cats in the households was positively associated with *Trichuris* infection, while male dogs and low body scores were associated with mixed infections. Our results highlight the risk of zoonotic and wildlife infections from dogs in these scenarios and the need for monitoring and control of parasites of dogs in human-wildlife interface areas.

Keywords: domestic dogs, endoparasites, domestic animal-human-wildlife interface, risk factors, zoonosis, protected areas

Introduction

Domestic dogs (*Canis familiaris*) are the most ubiquitous pets and the most abundant carnivore species worldwide (Young et al., 2011; Gompper, 2014). Of the almost one billion dogs living in the world today, more than a half is considered as rural dogs, i.e. free-ranging dogs that live in farms and small human settlements. These dogs are subsidized with food and shelter by humans, but frequently enter natural areas where they interact with wildlife, mostly with negative outcomes for the latter (Gompper, 2014). In Brazil, estimates shows that there are about five million rural dogs (Gompper, 2014). Recent studies indicated that these dogs are occupying protected areas at an alarming rate (Lacerda et al., 2009; Paschoal et al., 2012), and the vast array of ecological impacts of rural dogs to native communities, such as predation, interference competition and disease transmission (Butler et al., 2004; Vanak and Gompper, 2010; Gompper, 2014) are surely accompanying them.

Such free-ranging behavior actually enhances parasite transmission between dogs, humans and wildlife (Knobel et al., 2014). There are about 360 pathogens that may infect dogs, many of them are zoonotic (Cleaveland et al., 2001), and almost half of the agents are shared with wildlife (Knobel et al., 2014). Moreover, dogs are frequently blamed for the maintenance and transmission of conservation-concern diseases to wild carnivores (Cleaveland et al., 2000;

Acosta-Jamett et al., 2011; Woodroffe et al., 2012). Finally, because of their ubiquity, behavior and competency as hosts, dogs have been opportunely used as 'sentinels' of infections for humans and wildlife around the world (Cleaveland et al., 2006; Salb et al., 2008).

Regarding gastrointestinal parasites, dogs are hosts for several species, including widespread parasites that affect humans such as the helminths *Ancylostoma caninum* and *Toxocara canis* (Dantas-Torres and Otranto, 2014). Hookworms (*Ancylostoma* sp.) causes cutaneous larva migrans and eosinophilic enteritis, and toxocariasis is a major health problem because infections often result in multisystemic disease by visceral larva migrans that may affect important organs, such as the eyes, liver and brain (McCarthy and Moore, 2000; Despommier, 2003). However, studies reporting prevalence and associated risk factors of gastrointestinal parasites of dogs in Brazil are mostly restricted to urban areas (e.g. Oliveira-Sequeira et al., 2002; Balassiano et al., 2009; Klimpel et al., 2010; Heukelbach et al., 2012). Rural dogs, particularly those living around protected areas, i.e. domestic animal-human-wildlife interfaces, have received little scientific attention despite their potential role as reservoirs and sentinels for infections in these scenarios.

Here we report the prevalence of gastrointestinal parasites of rural dogs from six protected areas in the Atlantic Forest domain and assess risk factors for the most prevalent and/or zoonotic parasite taxa and mixed infections. Finally, we discuss our results in the context of the zoonotic potential, and the conservation implications regarding the possibility of transmission to and from wildlife.

Materials and methods

Households with dogs, located at less than 2 km of the borders of six protected areas in the Atlantic Forest domain of the state of Minas Gerais, southeastern Brazil, were aimed for this study. These areas comprise three state parks, Serra do Brigadeiro (PESB), Rio Doce (PERD) and Sete Salões (PESS), and three private reserves, Fazenda Macedônia (RPPNFM), Feliciano Miguel Abdala (RPPNFMA), and Mata do Sossego (RPPNMS) (Figure 1). All of the areas had humans living in their vicinity and various degrees of domestic dog occupancy recorded within their borders (Paschoal et al., 2012; and unpublished data). The landscapes around the protected areas are composed of forest borders, small rural properties, their legal reserves and small human settlements. Households were apparently belonging to low and median income families. Their dogs and cats are fed with human food leftovers and occasionally milk, meat, viscera, milk serum and commercial pet food. Most domestic animals owned do not receive any veterinary care.

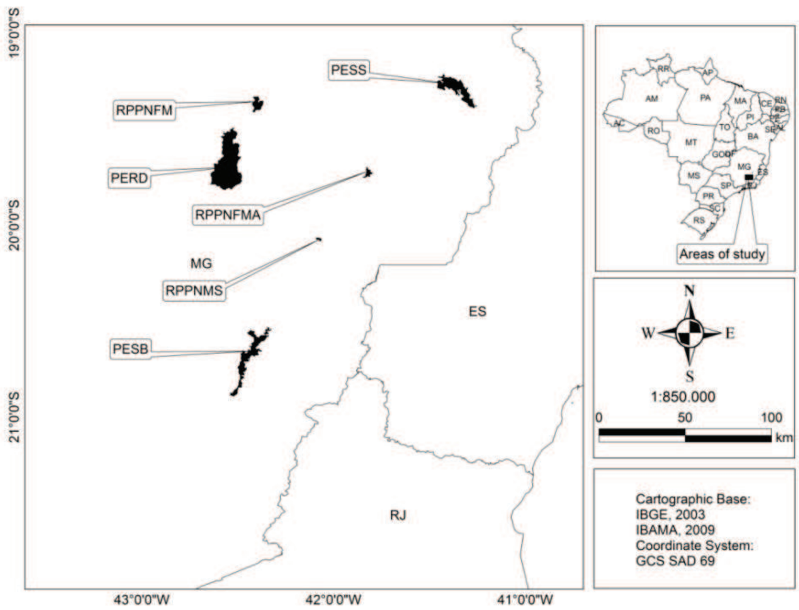

Figure 1. Location of the study areas in the Atlantic Forest domain of Minas Gerais state, Brazil.

Licenses from the State Forest Institute – IEF (UC: 080/10, 081/10 and 082/10) and approval from the Ethics Commission on Animal Use of Pontifícia Universidade Católica de Minas Gerais (CEUA PUC Minas 037/2010) were obtained prior to the field work. Animal manipulation procedures adhered to the recommendations from the COBEA (Brazilian College of Animal Experimentation) and the Animal Ethics Committee of FIOCRUZ (Oswaldo Cruz Institute Foundation) of the Brazilian Ministry of Health. After obtaining verbal approval of the house responsible for interviews and data collection, dogs were physically restrained and examined. Dog characteristics such as sex, age, age class (younger or older than one year), breed, weight, body score (from zero when extremely thin to five when extremely obese), sterilization and deworming status, and clinical alterations were recorded in individual files for the dogs. For the households, the number of people, dogs (including those not sampled) and cats, whether dogs were kept free or not, and if they had access to adjacent forest or nearest villages, were recorded.

Feces samples were collected when present from the rectum of the examined dogs, stored in plastic tubes, identified, cooled up to five days, and sent to coproparasitological analysis. In the lab, samples were submitted to flotation and sedimentation methods. Samples were considered positive when at least one of the methods detected parasite eggs or protozoans. Parasite eggs or larvae were identified to the lower taxonomic level possible.

Prevalence comparisons were performed only for the most common parasite species. To assess differences in prevalence among the study areas, we used multiple Yates-corrected chi square tests. Differences in prevalence between genders, mixed breed and purebred, adults and puppies, and previously dewormed and untreated dogs were evaluated through binomial Yates-corrected chi square tests.

Multivariate logistic regressions were used to assess risk factors associated with infection by the most common parasite taxa and also for mixed infections (i.e. samples with two or more parasite taxa). Dog traits entered as binary (sex, breed, sterilization and deworming status, age class and the presence of ectoparasites) and continuous (age, body score and weight) explanatory variables. Household related explanatory variables were the numbers of people, dogs and cats (continuous), and mobility of dogs, access to forest and cities (binary). The level of significance was set at 95% ($p<0.05$) for all tests used. The Spearman's test was used to detect correlation between significant variables.

Results

We sampled 129 dogs (those which had feces in the rectum at the time of collection; other 194 dogs had no samples collectable) from 88 households. Households had from one to 15 dogs (average 3.4±2.8 dogs per household). Sex ratio was 2:1 (87 males and 42 females), 84.5% of dogs were mixed-breed, and 78.2% were adults (average age 35 months). Average weight was 13.4 kg (ranging from two to 81), and body score ranged from one to four, with an average of 2.1. Only 3.8% were sterilized, and 42.6% of dogs were not previously dewormed. Most dogs (84.5%) were kept without any space restriction, and 43% of dogs (in 44% of the households) share the peridomiciliary space with cats (numbers ranged from 0 to 18, with an average of one cat per household).

Thirteen parasite taxa (11 helminths and two protozoans) were found to be infecting 75 dogs, with an overall prevalence of 58% (ranging from 44 to 89.5%). The most prevalent parasites were *Ancylostoma* sp., *Toxocara canis* and *Trichuris vulpis* (all with zoonotic potential) (Table 1, figure 2). Mixed infections were detected in 27 (36%) of positive samples. Of these, one sample had six parasite taxa (3.7%), two with four taxa (7.4%), five with three taxa (18.5%), and 19 with two taxa (70.4%). Associations between *Ancylostoma* and *Toxocara* occurred in 18 of the 27 mixed-infected dogs (66.6%), between *Ancylostoma* and *Trichuris* in six (22.2%), *Ancylostoma*, *Toxocara* and *Trichuris* in four (14,8%), and between *Toxocara* and *Trichuris* in the same last four cases.

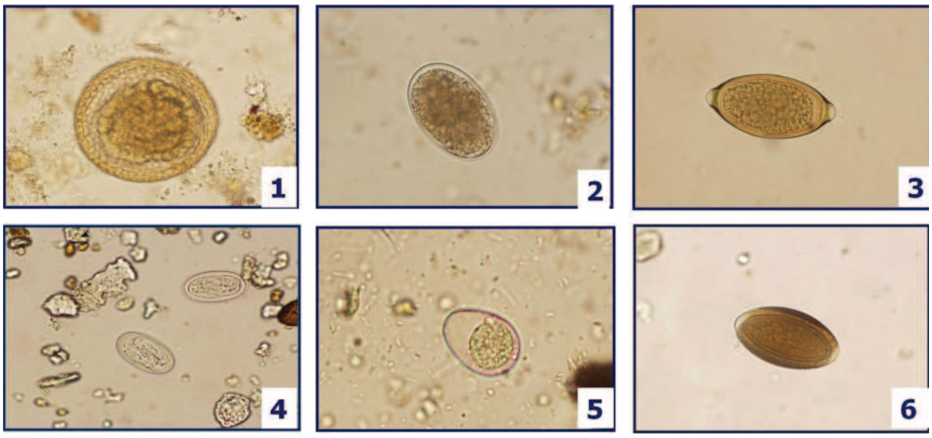

Figure 2. Some of the parasite eggs and oocists found in feces samples of rural dogs in the surroundings of Atlantic Forest fragments: 1.*Toxocara canis*, 2. *Ancylostoma* spp., 3.*Trichuris vulpis*, 4. *Spirocerca lupi*, 5. *Cystoisospora* sp., and 6. Acanthocephala.

Table 1. Prevalence of gastrointestinal parasites in dogs living around six Atlantic Forest fragments, Minas Gerais, Brazil.

Area	*Ancylostoma*	*T. canis*	*T. vulpis*	*Capillaria*	*Ascaridia*	*Spirocerca*	Taeniidae	Acantocephala	*Ascaris*	*Dipylidi Umcaninum*	*Toxascaris*	*Cystoisospora*	*Eimeria*	Prevalence	Taxa
RPPNFM	11	3	2	1	1	1	1	0	0	0	0	0	1	70.6%	8
PESB	10	4	0	0	0	0	0	1	3	0	0	1	0	61.1%	5
PESS	10	5	2	0	0	0	0	0	0	1	0	1	0	44.1%	5
RPPNFMA	9	0	3	0	0	1	0	0	0	1	1	1	0	48.1%	6
RPPNMS	16	10	2	0	0	0	1	1	0	1	0	0	0	89.5%	6
PERD	5	2	1	0	0	0	0	0	0	0	0	0	0	50.0%	3
Total (%)	61 (47)	24 (18)	10 (8)	1(0.7)	1(0.7)	2(1.5)	2(1.5)	2(1.5)	3(2)	3(2)	1(0.7)	3(2)	1(0.7)	58.14	13

Prevalence for *Ancylostoma* sp., *T. canis* and *T. vulpis* were higher in RPPNFM and RPPNMS ($p<0.05$). No differences were found for the prevalence of any of the three parasites regarding sex or previous deworming ($p>0.05$). Differences in prevalence of *T. canis* were detected between puppies and adults: younger dogs were more frequently infected ($p=0.001$). For *T. vulpis*, purebred dogs were found to be more frequently infected ($p=0.04$).

The multivariate logistic regressions were significant for *T. canis* ($p=0.050$; $R^2=24.8$), *T. vulpis* ($p<0.0001$; $R^2=49.6$) and for mixed infections ($p=0.012$; $R^2=29.9$). Factors significantly associated with these infections (five from 15 variables entered the models) are listed in table 2. There was autocorrelation solely between two variables included in the model for *T. vulpis*: purebred and body score (Spearman coefficient=0.1947, p=0.0269). However we retained both variables in the model because of the low Spearman coefficient, lack of biological meaning, and because even when the less significant variable (body score) was removed from the model, the results were consistent for the other variables.

Table 2. Significant factors for gastrointestinal parasitic infections of dogs living around six Atlantic Forest fragments, according to multivariate logistic regressions.

Risk factors	Coefficient	SE	Z	P value	Odds ratio	CI 95%
Toxocara canis						
Age class	-2.54	0.76	-3.34	0.0008	0.0788	0.02-0.35
Trichuris vulpis						
Purebred	5.51	2.44	2.26	0.0238	248.92	2.08-29,749.90
Number of cats	1.64	0.55	2.97	0.0030	5.16	1.75-15.25
Body score	-3.67	1.65	-2.22	0.0262	0.025	0.00-0.65
Mixed infection						
Sex (Males)	1.34	0.62	2.16	0.0307	3.83	1.13-12.95
Body score	-1.42	0.64	-2.21	0.0269	0.24	0.07-0.85

Discussion

The most prevalent dog parasite taxa found here (*Ancylostoma* sp., *T. canis* and *T. vulpis*) did not differ from other studies from urban areas in Brazil (Oliveira-Sequeira et al., 2002; Balassiano et al., 2009; Klimpel et al., 2010; Heukelbach et al., 2012). However, other ten helminths and protozoan parasite taxa were found (Table 1). These less common rural dog parasites are quite different than those from Brazilian urban areas, probably due to the proximity with other host species, whether wild or domestic animals. The latter case is exemplified here by the finding of *Eimeria*, *Ascaridia* and *Ascaris*, which are typical parasite genus of chickens and pigs (Vicente et al., 1997). Hence, these are probably accidental infections, since dogs frequently ingest viscera and feces of chickens, pigs and cats in these scenarios (author's personal observation).

The species richness found (13 taxa) is, for instance, similar to findings from urban and rural environments in Argentina (Fontanarrosa et al., 2006: 11 taxa; Soriano et al., 2010: 13 taxa), but is slightly higher than what was found in Brazilian urban zones (Katagiri and Oliveira Sequeira, 2008; Balassiano et al., 2009: eight taxa; Klimpel et al., 2010: five taxa) and rural areas (Santos et al., 2012: ten taxa). More profound studies on dog parasite communities are necessary to reveal differences in species distribution, richness and abundance patterns among urban, rural, and wildlife-rich areas in South America.

The high and widespread prevalence of gastrointestinal parasites, particularly the helminths *Ancylostoma* sp. and *T. canis* reveals a lack of preventive control of these dog populations, and that the risk of zoonotic diseases from dogs is strongly present in the study areas. The causes may be explained based on some factors cited in the papers of Katagiri and Oliveira-Sequeira (2008) and Balassiano et al. (2009): (1) unawareness of owners about the risks of acquiring zoonosis from their dogs and about prophylactic measures; (2) insufficient interactions between veterinarians and the rural people; (3) lack of governmental programs related to health and zoonosis in rural areas; (4) high environmental contamination due to the free-ranging behavior of dogs, and bad hygiene practices in some households leading to the accumulation of feces, organic matter, and maintenance and spread of parasite eggs. Contaminated soil is an important source of infections by gastrointestinal parasites, notably for *Toxocara* sp. (Tiyo et al., 2008).

The lack of influence of gender on infection and the significantly high proportion of young dogs infected with *T. canis* are in accordance with other studies (e.g. Fontanarrosa et al., 2006; Katagiri and Oliveira-Sequeira, 2008; Balassiano et al., 2009), but disagrees with the results of Heukelbach et al. (2012), who found that males were more infected by *Toxocara*. Purebred dogs are more likely to be infected by *T. vulpis*, according to both chi-square tests and logistic regressions, what is contrary to previous findings (Fontanarrosa et al., 2006; Balassiano et al., 2009). This may be linked to interactions between nutritional/immune status and the supposedly higher genetic variability and resistance to infection of mixed breed dogs, which may be more influent in rural environments, in our case, particularly for *T. vulpis* infection.

Previous deworming was also non-influent in our data set, contrary to findings from urban areas (e.g. Balassiano et al., 2009). This means that deworming is been incorrectly performed in these areas, and that intervention measures with anti-helminthic provision and orientation to owners are urgently needed.

According to the multivariate logistic regression analysis results, age class was negatively correlated to *T. canis* infection, meaning that younger dogs are at higher risk of infection by this parasite, or that their developing immune system is not yet able to control or eliminate this parasite. This pattern confirmed our results of chi-square tests, and seems consistent throughout studies from South America (Oliveira-Sequeira et al., 2002; Fontanarrosa et al., 2006; Katagiri and Oliveira-Sequeira, 2008; Balassiano et al. 2009).

The number of cats influenced positively the presence of *T. vulpis*. This parasite infects both cats and dogs (Dantas-Torres and Otranto, 2014). Therefore the more cats in a house, the more parasites are transmitted to dogs. The latter are probably contaminated via ingestion of cat feces.

Males were more likely to harbor more than one parasite species, and this may be caused by the vagrant behavior of male dogs. To the best of our knowledge, this observation has not been recorded in previous studies. Body scores influenced *T. vulpis* and mixed infections, but in a negative way. Lower body scores are more associated with multiple infections, probably because of nutritional status-immune system interactions. Variables associated with dog movements (space restriction, access to forests and cities) had no influence on parasitic infections, thus, contamination is probably happening at the peridomiciliary environment itself rather than during incursions of dogs to urban or forested areas.

Dogs are able to transmit macroparasites to wildlife (Knobel et al., 2014), and some of the parasite taxa reported here are able to infect wild mammals, especially carnivores. Maned wolves (*Chrysocyon brachyurus*), crab-eating foxes (*Cerdocyon thous*), and sympatric domestic dogs were found to be infected by Ancylostomidae, Trichuridae and *Toxocara* sp. in southeast Brazil (Curi et al., 2010; 2012; Santos et al., 2012). *T. canis* and hookworms may infect and cause disease in wild carnivores (Dunbar et al., 1994; Vieira et al., 2008). Vulnerable rare species of carnivores may also be affected. For instance, the small wild felid *Leopardus tigrinus* may be a host for *Trichuris* sp. (Muniz-Pereira et al., 2009). Although we have no data on parasites of wildlife in these areas, we believe that the strong presence of these free-ranging dogs inside forests (Paschoal et al., 2012; and unpublished data) is enough to warrant environmental fecal contamination and transmission to wildlife. Despite the high prevalence in all areas, attention and control measures should be prioritized on two areas: RPPNFM and RPPNMS, because people in surrounding rural settings and wildlife inside these small reserves are under risk of infection by these dog parasites.

Concluding, our results highlight the need for more investigation and the implementation of disease control measures in dogs from rural areas around forest fragments. These dogs may transmit parasites to humans and wildlife as well, and as sentinels, they are showing us that the risk of animal diseases and zoonosis is high in these areas. Risk factors detected in this study are somehow different from those studies in urban zones, showing that different control strategies should be applied to these environments. Finally, control measures directed to reservoir hosts may be effective to protect against wildlife diseases (Carter et al., 2009), and of course against zoonosis. Culling dogs is not recommended because it is almost always ineffective or creates unexpected (mostly bad) outcomes, and raises serious animal and human welfare issues (Carter et al., 2009; Knobel et al., 2014). Interventions with endoparasitic treatment must be accompanied by population control of hosts (i.e., dogs and cats), or population increases by increased fitness and reduced disease-related mortality may result in the emergence or persistence of other pathogens (Knobel et al., 2014), as well as in other negative effects of the presence of domestic animals in wildlife-rich areas such as the Atlantic Forest remnants.

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CONCLUSÕES GERAIS

Assim como o projeto "Cães na Mata Atlântica" objetivou levantar fatores que favorecem a ocupação e a permanência dos cães em unidades de conservação, esta tese alcançou os objetivos de revelar a presença e a prevalência de patógenos importantes para a conservação e para a saúde pública nesta população de hospedeiros importante, mas até então negligenciada, principalmente em um contexto conservacionista. Além disso, revelamos fatores associados à exposição aos patógenos, que podem ser utilizados tanto para incrementar o conhecimento da ecologia destes parasitas quanto para serem explorados e alvejados em programas de manejo preventivo e de controle de doenças nos cães, que objetivem tanto a conservação de espécies silvestres quanto a saúde geral dos componentes das interfaces entre humanos, animais domésticos e silvestres.

Entre os resultados, destaca-se o perfil de fatores de risco associados à leishmaniose notavelmente diferente de estudos anteriores, principalmente os realizados em áreas urbanas. A forte associação negativa entre a presença de espécies domésticas e a presença de cães infectados revela um possível uso de suínos e aves domésticas como agentes "zooprofiláticos", ou preventivos contra a doença em zonas rurais.

Fatores associados a infecções por helmintos gastrintestinais, inclusive agentes zoonóticos, foram levantados pela primeira vez em cães de interfaces rurais/silvestres, e revelam que esforços de controle (por exemplo, através de vermifugação) são necessários para a proteção de humanos e animais silvestres contra estas infecções.

Por fim, mas não menos importante, agentes infecciosos perigosos circulam nas populações de cães que vivem no entorno de fragmentos da Mata Atlântica. Com relação a doenças virais transmissíveis para carnívoros silvestres, o estudo de fatores de risco é apresentado pela primeira vez, e entre os fatores associados a exposição a patógenos está o estilo de vida livre dos cães e a baixa cobertura vacinal, o que demonstra claramente a necessidade de incentivo a leis de manejo de saúde dos cães no entorno de unidades de conservação.

Nossos resultados demonstram que os movimentos dos cães precisam ser restringidos, sua população controlada e sua saúde bem manejada, para que sua presença não ameace a saúde de humanos e animais silvestres em fragmentos florestais cada vez menores e mais pressionados pela ocupação humana e sua perturbações ambientais inerentes.

ANEXO 1 Ficha de entrevista e coleta de materiais utilizada

Projeto Cães na floresta Atlântica

Ficha de campo – entrevistas com proprietários e amostragem de cães domésticos

Proprietário: n°

Propriedade:

Endereço:

Coordenadas: dimensões: ha ou m²

Obs.:

Contato (fone/email):

Atividade principal: _ leite _ corte _ lavoura _outros:

Uso da terra: _ pasto (%) _ lavoura (%) _ construções (%) _ reservas(%) _ outros: (%)

Confrontantes / limites / vizinhos:

Obs.:

Espécies criadas:_ bovinos _ equinos _ suínos _ caprinos _ ovinos _ aves _ outros ( )

Quantidades:

Finalidade: _ leite _ corte _ pele / lã _ trabalho _ ovos _ outros ( )

Presença de animais domésticos ("pets"): __ sim __ não

Espécies: _ caninos _ felinos _ aves _outros ( )

Quantidades:

Finalidade / função: _ companhia _ guarda / vigia _ pastoreio _ caça _ controle de pragas

Mobilidade: _ presos com coleira/corrente _ presos em canil _ cercados na propr. _ livres

Possibilidade dos animais adentrarem áreas preservadas: _sim _não

Possibilidade dos animais adentrarem áreas urbanas próximas: _ sim _ não

Visualizações / sinais da presença de animais silvestres na propr.: _ sim _não

Espécies:

Obs.:

Manejo dos "pets":

Acompanhamento veterinário: _ sim _ não frequência ( )

Profissional/contato:

Vermifugação: frequência ( ) medicamento( ) dose ( )

Obs.:

Vacinação: _anti-rábica _ múltipla canina _ giardia _ tosse dos canis _ outras ( )

Frequência: ( ) marca da vacina ( )

Tratamento contra ectoparasitas: frequência ( ) produto / dose ( )

Obs.:

Animais esterilizados: _ sim _ não quantidade:

Histórico de saúde:

Óbitos / espécies / quantidades:

Animais doentes nos últimos anos: _ sim _ não espécies/quantidades:

Doença / suspeita:

Diagnóstico veterinário: _ sim _ não ( )

Tratamento / resultado:

Nutrição: _comida _ração _leite/soro _carne _lavagem _caça _outros ( )

Quantidade estimada:

Obs.:

Percepção sobre os impactos de seus animais sobre a fauna silvestre:

Exame clínico:

Animal (nome / número):

Marcas / identificação:

Aspecto geral / inspeção visual / escore corporal:

Pelagem / pele:	cavidade oral:
T (°C):	grau de hidratação:
Mucosas:	linfonodos superf.:
Auscultação torácica:	Palpação abdominal:

Obs.:

Coletas:

Animal / n° Sangue em EDTA soro Esfregaço sg. periférico fezes urina pelos outros