GISLENE FÁTIMA DA SILVA ROCHA FOURNIER

Epidemiological aspects of semi-domiciled domestic dogs present in the Brazilian Atlantic Rainforest Area of Caraguatatuba City and the correlation between the genetic variability of dogs' ticks and the wild environment

Aspectos epidemiológicos dos cães domésticos semidomiciliados presentes na área de Mata Atlântica do Município de Caraguatatuba e a correlação entre a variabilidade genética dos carrapatos dos cães e do ambiente silvestre

São Paulo
2017

GISLENE FÁTIMA DA SILVA ROCHA FOURNIER

Epidemiological aspects of semi-domiciled domestic dogs present in the Brazilian Atlantic Rainforest Area of Caraguatatuba City and the correlation between the genetic variability of dogs' ticks and the wild environment

Aspectos epidemiológicos dos cães domésticos semidomiciliados presentes na área de Mata Atlântica do Município de Caraguatatuba e a correlação entre a variabilidade genética dos carrapatos dos cães e do ambiente silvestre

Thesis submitted to the Postgraduate Program in Experimental Epidemiology Applied to Zoonoses of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences

Department: Preventive Veterinary Medicine and Animal Health

Area: Experimental Epidemiology Applied to Zoonoses

Advisor: Prof. Ricardo Augusto Dias Ph. D

São Paulo
2017

Total or partial reproduction of this work is permitted for academic purposes with the proper attribution of authorship and ownership of the rights.

Autorizo a reprodução parcial ou total desta obra, para fins acadêmicos, desde que citada a fonte.

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virginie Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

T. 3593
FMVZ

Foumier, Gislene Fátima da Silva Rocha
Epidemiological aspects of semi-domiciled domestic dogs present in the Brazilian Atlantic Rainforest Area of Caraguatatuba City and the correlation between the genetic variability of dogs' ticks and the wild environment / Gislene Fátima da Silva Rocha Foumier. -- 2017.
115 f. : il.

Título traduzido: Aspectos epidemiológicos dos cães domésticos semidomiciliados presentes na área de Mata Atlântica do Município de Caraguatatuba e a correlação entre a variabilidade genética dos carrapatos dos cães e do ambiente silvestre.

Tese (Doutorado) - Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Medicina Veterinária Preventiva e Saúde Animal, São Paulo, 2018.

Programa de Pós-Graduação: Epidemiologia Experimental Aplicada às Zoonoses.
Área de concentração: Epidemiologia Experimental Aplicada às Zoonoses.
Orientador: Prof. Dr. Ricardo Augusto Dias.

1. *Amblyomma ovale*. 2. Cães domésticos. 3. Mata Atlântica. 4. *Rickettsia parkeri*. 5. *Rangelia vitalii*. I. Título.

Ficha catalográfica elaborada pela bibliotecária Camila Molgara Gamba, CRB 7070-8, da FMVZ/USP.

CERTIFICADO

Certificamos que o Projeto intitulado "Estudo sobre impactos causados pelos cães domésticos semidomiciliados presentes na área de Mata Atlântica do município de Caraguatatuba e a correlação entre a variabilidade genética dos carrapatos dos cães e do ambiente silvestre.", protocolado sob o CEUA nº 6441270114, sob a responsabilidade de **Ricardo Augusto Dias** *e equipe; GISLENE FATIMA DA SILVA ROCHA FOURNIER* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei 11.794, de 8 de outubro de 2008, com o Decreto 6.899, de 15 de julho de 2009, com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovado** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMZV) em reunião de 17/12/2014.

We certify that the proposal "titulo em inglÃªs", utilizing 54 Dogs (54 males), 100 Brazilian wild species (100 males), protocol number CEUA 6441270114, under the responsibility of **Ricardo Augusto Dias** *and team; GISLENE FATIMA DA SILVA ROCHA FOURNIER* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes (or teaching) - it's in accordance with Law 11.794, of October 8 2008, Decree 6899, of July 15, 2009, with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science of São Paulo University (CEUA/FMZV) in the meeting of 12/17/2014.

Vigência da Proposta: de 06/2014 a 06/2016 Área: Departamento De Medicina Veterinária Preventiva E Saúde Animal

Procedência:	Parque Estadual Serra do Mar - Núcleo Caraguatatuba						
Espécie:	Espécie silvestre brasileira	Gênero:	Machos	idade:	sem idade definida	N:	100
Linhagem:	animais silvestres			Peso:	mais de 7g		
Procedência:	Município de Caraguatatuba						
Espécie:	Cão	Gênero:	Machos	idade:	1 ano ou mais	N:	54
Linhagem:	Sem raça definida			Peso:	aproximadamente 10kg		

Dados complementares para animais silvestres: As capturas de pequenos mamíferos silvestres serão realizadas durante 4 noites consecutivas com 10 armadilhas tipo Shermman® (12 x 14 x 45 cm) iscadas com uma mistura de pasta de amendoim, pedaços de milho, banana e óleo de sardinha em cada um dos nove transecto totalizando 90 armadilhas. As armadilhas serão colocadas ao longo de cada transecto distanciadas entre si por dez metros, sendo assim cada transecto terá uma extensão linear de 100m de comprimento, aproximadamente. Todos os animais, após a identificação específica, determinação do sexo e colocação de uma anilha contendo um código de identificação.

São Paulo, 27 de outubro de 2015

Profa. Dra. Denise Tabacchi Fantoni
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia
Universidade de São Paulo

EVALUATION FORM

Author: FOURNIER Gislene Fátima da Silva Rocha

Title: **Epidemiological aspects of semi-domiciled domestic dogs present in the Brazilian Atlantic Rainforest Area of Caraguatatuba City and the correlation between the genetic variability of dogs' ticks and the wild environment**

Thesis submitted to the Postgraduate Program in Experimental Epidemiology Applied to Zoonoses of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences

Date: ____/____/______

Committee Members:

Prof:____

Institution:__________________________________ Decision:______________

Prof:____

Institution:__________________________________ Decision:______________

Prof:____

Institution:__________________________________ Decision:______________

Prof:____

Institution:__________________________________ Decision: ______________

Prof:____

Institution:__________________________________ Decision:______________

AGRADECIMENTOS

Aprendi que algumas vezes “Seres especiais” cruzam nosso caminho por acaso, porém, nunca entram na nossa história sem nenhuma razão. E o convívio com estes “Seres especiais” é o que me deu a sustentação necessária para alcançar este objetivo. Dentre estes Seres, está Jean-François, coautor da minha mais preciosa e amada criação: Arthur Danilo. Vocês dois mudaram minha vida!

Nestes quatro longos anos admito que eu não me fiz sozinha, sempre precisei de um olhar de apoio, uma palavra de incentivo e, claramente, uma chance para começar. Isso tudo me foi dado pelo meu orientador Prof. Dr. Ricardo Augusto Dias a quem eu sempre serei grata. Prof. Dr. Ricardo, juntamente com o Prof. Dr. Adriano Pinter e Dra. Karen McCoy, me deram de mãos abertas um pouco da mais valiosa de todas as coisas: o conhecimento.

Não poderia também deixar de agradecer aos meus pais, Maria Auxiliadora e Cleber Marques por serem responsáveis pela minha existência. Vocês acompanharam meu crescimento e trabalharam dobrado, sacrificando seus sonhos em favor dos meus. Vocês me deram os melhores irmãos, e estes, me deram os melhores sobrinhos, cunhados e cunhada.

Dos Seres que cruzaram meu caminho por acaso, alguns se tornaram impossíveis de serem esquecidos: Danilo S., Marie-Jeanne, Jany Fournier, Jean-Marie Fournier, Davina Fournier, Maria O., Lorena M., Vinícius P., Vinícius E., Filipe C., Ronan P., Barbara E., Leonardo M., Thales, Rodrigo C., Bernard, Gabriela M., Gabriela C, Mariana S., Marilda S., Paulo S., Alessandra L. e Tatiana vocês, especiais por essência, formam uma das maiores razões para este instante de vitória.

Agradecer é admitir que houve um momento em que se precisou de alguém; é reconhecer que o homem jamais poderá lograr para si o dom de ser autossuficiente. Alguns nunca me faltaram durante todo esse período: Dra. Amália Barbieri, Dr. Marcos Gomes, Dr. Diego R, Dra. Marlene Drupaz (França), Prof. Dr. Francisco C., Dr. Felipe K., Prof. Dra. Andrea C., Dra. Grazielle P; Giovanni (Maranhão), Prof. Dr. Jonas Filho, Dr. Thiago Martins, Dra Fernanda, Prof. Dr. Arlei, Prof. Dr. Herbert Soares, Juliana, Dra. Leia, Karinne, Dr.

Sebastian M. (Chile), Luciana Colácio, Paulo (Bilão), Ryan Emiliano, Silvia (Colômbia), Giulia (Itália), Washington obrigada por toda ajuda durante estes anos.

Durante este período, me deparei com diversos profissionais talentosos, pacientes e atenciosos. Para representá-los cito alguns nomes: Prof. Dr. Marcelo B. Labruna; Gisele (SUÍNOS), Sheila (LABMAS), Dra Suely, Danival, Sandrinha, Dra. Hilda Pena, Pedrinho, Cristiane Palhuca (PESM), Stella (SUCEN).

Recebi muito apoio em todos os lugares que trabalhei e agradeço em especial à equipe o Parque Estadual Serra do Mar – Núcleo Caraguatatuba, A equipe da SUCEN, a equipe de zoonoses de Caraguatatuba e à equipe do IRD - Maladies infectieuses et vecteurs: écologie, génétique, évolution et contrôle qui m'a accueilli à bras ouverts. Surtout à Céline Toty, qui n'a pas perdu patience avec moi une minute !

Agradeço especialmente aos colegas de BH que me ajudaram muito nas estatísticas e bioinformática: Dra. Renata S. Buzatti e André Carneiro Muniz do Laboratório de Genética de Populações no Departamento de Biologia Geral, Instituto de Ciência Biológicas na UFMG.

Nestes anos, eu tive as minhas maiores alegrias e as minhas maiores tristezas. Muitas vezes eu pensei em desistir, nestes momentos, o que me segurou foi a certeza de que seres incríveis me amavam e que não importava o quão triste eu estava, eu sou uma pessoa de sorte! Eu tenho a chance de conviver com estes seresikati.

Aquele que se foi no meio da minha jornada fez muita falta e fará por toda minha vida. As saudades embalam as lembranças dos momentos que vivemos e que agora existem em um espaço do passado chamado de lembranças. Estas lembranças me incentivam nas horas de desânimo, me consola nos momentos de tristeza e me alegram ainda mais nesta minha vitória. Ofereço ao meu irmão Danilo Gonçalves Saraiva este trabalho e toda minha gratidão.

Este trabalho não seria possível sem o apoio financeiro da FAPESP a partir dos processos 2014/648-3 e 2016/5355-0 (BEPE)

DEDICATÓRIA

Ao meu irmão Danilo Gonçalves Saraiva que marcou a minha vida, viveu e morreu na minha história.

"O saber deve ser como um rio, cujas águas doces, grossas, copiosas, transbordem do indivíduo, e se espraiem, estancando a sede dos outros. Sem um fim social, o saber será a maior das futilidades."

Gilberto Freyre, em discurso de "Adeus ao Colégio", novembro de 1917.

FOURNIER, G. F. S. R. **Aspectos epidemiológicos dos cães domésticos semidomiciliados presentes na área de Mata Atlântica do Município de Caraguatatuba e a correlação entre a variabilidade genética dos carrapatos dos cães e do Ambiente Silvestre.** 2017. 115 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2018.

Considerado o carnívoro mais abundante do mundo, o cão doméstico pode causar impactos negativos quando presente em áreas florestadas. Cães podem desenvolver diferentes papéis no ambiente em que vivem desde caçador, pastor, guardião, companheiro e até mesmo relações mais afetivas em que o cão é visto como um membro da família. Estes papéis estão intimamente ligados ao modo de vida de seus proprietários. Em Caraguatatuba, cães que vivem no entorno da Serra do Mar, uma importante Unidade de Conservação da Mata Atlântica, são comumente parasitados por *Amblyomma ovale*. A partir de dados obtidos através de três anos de monitoramento em três diferentes áreas do entorno do Parque Estadual Serra do Mar – Núcleo Caraguatatuba, foi possível gerar resultados que auxiliam no entendimento das causas e consequências da presença do cão no entorno da floresta e sua participação na epidemiologia da riquetsiose e rangeliose. Comparando o estilo de vida dos proprietários e o papel do cão no ambiente em que ele está inserido, foi possível estimar o risco de transmição de *Rickettsia parkeri* para estes cães. Além disso, através da análise molecular dos indivíduos de *A. ovale* coletados em cães e também coletados em vida livre, foi possível inferir sobre a estrutura e genética populacional deste parasita na Serra do Mar em Caraguatatuba, importante corredor ecológico para a fauna silvestre da Mata Atlântica.

Palavras-chave: 1. *Amblyomma ovale.* 2. Cães domésticos. 3. Mata Atlântica. 4. *Rickettsia parkeri*. 5. *Rangelia vitalii*.

ABSTRACT

FOURNIER, G. F. S. R. **Epidemiological aspects of semi-domiciled domestic dogs present in the Brazilian Atlantic Rainforest Area of Caraguatatuba City and the correlation between the genetic variability of dogs' ticks and the wild environment.** 2017. 115 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2018.

Considered the most abundant carnivore in the world, the domestic dog can cause negative impacts when he is present in forested areas. Dogs can develop different roles in the environment in which they live as hunter, shepherd, guardian, companion and even more affective relationships in which the dog is seen like a family member. These roles are closely linked to the way of life of their owners. In Caraguatatuba City, dogs that live in the vicinity of Serra do Mar, an important Atlantic Rainforest Conservation Unit, are commonly parasitized by *Amblyomma ovale*. From data obtained through three years of monitoring in three different areas around the Serra do Mar State Park – Caraguatatuba City, it has been possible to generate results that help in understanding the causes and consequences of the dog presence in the surroundings of the forest as well as its participation in the epidemiology of riquetsiosis and rangeliosis. Comparing the lifestyle of the owners and the role of their dogs in the environment in which they are inserted, it has been possible to estimate the risk of transmission of *Rickettsia parkeri* to these dogs. In addition, through the molecular analysis of the *A. ovale* individuals collected on dogs and also in free life, it has been possible to infer about the population structure and genetics of this parasite in Serra do Mar, which is an important ecological corridor for the Atlantic Rainforest wild fauna.

Keywords: 1. *Amblyomma ovale.* 2. Domestic dog. 3. Atlantic rainforest. 4. *Rickettsia parkeri*. 5. *Rangelia vitalii*.

SUMMARY

1. INTRODUCTION

The presence of the most common canid of the world, *Canis lupus familiaris*, in forested areas, is being recorded before in several parts of the world (UDELL; WYNNE, 2008; TORRES; PRADO, 2010; MARTINEZ et al., 2013; LESSA et al., 2016; DOHERTY et al., 2017a).

In the last decade, researchers have been describing reports of invasion, and also, in how and in which situations the invasion can be more aggressive (GALETTI; SAZIMA, 2006; FRIGERI; CASSANO; PARDINI, 2014).

When abandoned in forests, these animals may undergo behavioral changes after one, two or more generations, forming groups of feral animals that end up occupying the role of mesocarnivores in the food chain and causing irreparable disturbances in populations of wild species (GALETTI; SAZIMA, 2006; PRUGH et al., 2009; LESSA et al., 2016).

It is well known that the abilities of domestic dogs as mesocarnivores modify fragmented forest environments (PRUGH et al., 2009; FRIGERI; CASSANO; PARDINI, 2014).

The most pertinent discussion is about changes in human-dog relationships and the purpose of these animals on rural and urban properties.

Dogs can perform different roles in the environment in which they live, from hunter, shepherd, guardian, companion animal and even develop more affective relationships in which the dog is seen as a member of the family (DOHERTY et al., 2017b).

In the old world, modifications in the interactions between dogs and humans occurred during and after the industrial revolution, when rural populations moved to live in cities, and no longer needed the dog as a shepherd or a hunter (MOREY, 1994; MURPHY JASON, 2015). To the extent that the cities grew, in rural areas, economic and social relations improved and the replacement of human labor and technology to increase production contributed to an increasingly intense rural exodus (MOREY, 1994; MURPHY JASON, 2015; UWCT, 2017).

In Brazil, only in the late XIX and early XX centuries these changes began to emerge (VIZEU, 2011; MOURET, 2017). The adhesion of the Industrial Revolution and its mass production was initially made by the coffee growers of São Paulo who, in order to survive the coffee crisis, began to invest in the industrial sector in Brazil (VIZEU, 2011). During this period, small industries of food processing and industries were created. São Paulo City became the biggest industrial center of the country and, gradually, these companies began to spread to the Brazilian Southeast Region (MOURET, 2017).

Surrounded by Brazilian Atlantic rainforest, São Paulo City grew in a disorderly way, pressing more and more the coastal forest. From a continuous forest, in a few decades, the Brazilian Atlantic rainforest has been transformed into discontinuous fragments that are incapable of sustaining the native populations of fauna and flora (MELLO-THÉRY, 2011; OGRZEWALSKA et al., 2011).

During this period, dogs went along with humans to the city and began to share smaller spaces with these. The new mutualistic relationship between these two species of mammals in Brazilian cities is relatively young and still undergoes an adaptive process in relation to the countries that joined the Industrial Revolution a century earlier (MOREY, 1994; MURPHY JASON, 2015; UWCT, 2017).

New socioeconomic patterns influence the relationship between humans and dogs in rural and urban areas around the world (MARTINEZ et al., 2013). However, in Brazil, it is still difficult to understand the role of dogs in these environments and in the same context, the dog can develop different roles when living with humans (FRIGERI, 2013; VIEIRA et al., 2013; SCINACHI et al., 2017).

A dog can be found on a low-income property sharing the same space, feeding from the remains of the family food or even receiving specific food for dogs, but without being seen as a guardian or as a companion dog (UDELL; WYNNE, 2008). Often, these animals hardly receive an affective gesture from the people with who they live and rarely or never receive hygiene or veterinary care (UDELL; WYNNE, 2008). Sometimes even, people do not know how to explain why they live with these animals at home.

In Caraguatatuba City, São Paulo State, where the Brazilian Atlantic rainforest predominates on the slopes that surround the city, human habitations in forested areas are gradually appearing illegally (GIGLIOTTI; SANTOS, 2013).

In general, small houses are built in a few days on the slopes and are shelters to families who live precariously in these areas. However, in the same region, mansions are also erected by people who believe they will not suffer any consequence, and are looking for a privileged view of the ocean. In this socioeconomic gradient of the city, the role of dogs is not well understood.

The new functional role of the dog in this region may be a consequence of new laws that regulate the deforestation and exploitation of natural resources, along with the restrictive rules imposed on hunting in the last decades (VERDADE; CAMPOS, 2004; MARTINEZ et al., 2013). Hunting dogs, for example, were restricted to a secondary role and were replaced by guardian or companion dogs (TORRES; PRADO, 2010; FRIGERI; CASSANO; PARDINI, 2014).

To save the remains of the local biome, the Serra do Mar State Park in Caraguatatuba City was created in 1977. This park is still a refuge for many species of wildlife, including vulnerable or threatened animals (ROCHA-MENDES et al., 2015).

The proximity of humans to forested protected areas of the country facilitates the entry of dogs that chase, injure and even kill wildlife (MARKS; DUNCAN, 2009). Another serious factor is that the invasion of dogs increases the risk of transmission of infectious diseases to humans and local fauna (DANTAS-TORRES, 2007; LABRUNA et al., 2007; PINTER et al., 2008; VIEIRA et al., 2013; GOTTLIEB et al., 2016).

In Caraguatatuba City, the dog does not yet have a well-defined role in the human-dog relationship. However, dogs contribute to the maintenance of pathogens and have an important role as zoonosis reservoirs (SABATINI et al., 2010; BARBIERI et al., 2015). An example of this has been demonstrated in studies on some wild life and scarcely studied diseases, as rangeliosis (GOTTLIEB et al., 2016; FREDO et al., 2017; LEMOS et al., 2017). This disease is caused by *Rangelia vitalii*, a parasite found naturally in the wild in populations of wild canids and that has as possible vectors *Amblyomma ovale, Amblyomma aureolatum, Amblyomma tigrinum,* and *Amblyomma sculptum* (GUGLIELMONE et al., 2003a; LABRUNA et al., 2005).

Recent studies have shown that domestic dogs are susceptible to this disease when inhabiting rural areas or near forested areas where possible vectors are present (GOTTLIEB et al., 2016; FREDO et al., 2017; LEMOS et al., 2017).

Dogs are excellent hosts of wild carnivore ticks, as the *A. ovale,* which is also a vector of the Brazilian Spotted Fever – BSF (MARTINS; MOURA; LABRUNA, 2012; BARBIERI et al., 2015a). The BSF is caused by a bacteria of the genus *Rickettsia* that belongs to the Spotted Fever Group – SFG composed of more than 20 species and subspecies of pathogenic bacteria (WEINERT et al., 2009).

In the last decade in Brazil, five species were described: *Rickettsia rickettsii; Rickettsia riphicephali, Rickettsia amblyommi, Rickettsia felis and Rickettsia parkeri* (PADDOCK et al., 2004; LABRUNA et al., 2007; PINTER et al., 2008; MONJE et al., 2016). Of these, the species that are related to clinical symptoms in humans are *R. rickettsii* and *R.parkeri* (LABRUNA et al., 2001).

The species of ticks described as transmitters of *R. parkeri* strain Atlantic rainforest are *A. aureolatum, A. ovale* and *Rhipicephalus sanguineus*. The last one of these species does not transmit this pathogen to humans (PAROLA et al., 2013; LONDOÑO et al., 2014; LOPES et al., 2016; MONJE et al., 2016).

Amblyomma ovale is considered today, the main specie responsible of the dissemination of the *R. parkeri* strain Atlantic rainforest on the north coast of São Paulo State (MEDEIROS et al., 2011; SZABÓ; PINTER; LABRUNA, 2013). In the cases of this disease reported in the State of São Paulo, dogs were closely related to the transportation of ticks into the houses and, consequently, transmitting these diseases to humans (OGRZEWALSKA et al., 2014). Due to the socioeconomic situation and the ecological changes in the areas around the

Serra do Mar State Park, the role of the dog is a factor that should be studied so that strategies for environmental health protection can be concretized.

The strict work role of the dog, such as shepherding, hunting or guarding rural areas may be shifting to a pet role or a nonspecific function, nonetheless its presence at the edges of the woods and in the interior of the house becomes a problem for the environmental balance and public health.

To understand the real situation of these animals in the surroundings of the park, we investigated through interviews, collection of biological material, geolocation data, photographic records and collection of ectoparasites during three years in three different areas of Caraguatatuba City in the State of São Paulo. Based on these data, we investigated the risk of infection of rangeliosis and rickettsiosis in dogs in three different communities, and the genetic variability of *A. ovale* populations present in these areas was assessed.

1.1. Domestic Dogs

The *Canis lupus familiaris* belongs to the family Canidae, and diverged for more than ten million years from its ancestor (WAYNE et al., 1997; OSTRANDER; WAYNE, 2005).

In recent years, interest in the evolution of dog domestication has resulted in analyzes based on morphology (BERTA, 1987; ZRZAVÝ; ŘIČÁNKOVÁ, 2004; LYRAS, 2009), molecular data (WAYNE et al., 1997; ZRZAVÝ; ŘIČÁNKOVÁ, 2004; OSTRANDER; WAYNE, 2005) and in DNA sequencing (WAYNE et al., 1997; ZRZAVÝ; ŘIČÁNKOVÁ, 2004; TCHAICKA et al., 2016). These and other studies show that dogs live with humans for at least 14 million years.

Fossils of the first dogs from various archaeological sites around the world indicate that at the end of the Pleistocene, dogs already accompanied humans in hunting events (MOREY, 1994). These first dogs, known as wolves, were identified through reliable methods as *Canis lupus*. This assertion is based on an increasing number of molecular data and is reinforced comparing the physiological and behavioral similarities between species (MOREY, 1994).

Some gaps of this story are still being filled, one of them being to understand whether the ancestor of the current domestic dog has benefited from domestication (MURPHY, 2015). Some authors suggest that in comparison with the wolf's ancestors, the domestic dog were benefited (MOREY, 1994; UDELL; WYNNE, 2008; MURPHY JASON, 2015).

Ancestral wolves have been extirpated from most of their vast territories and many subspecies are now extinct (MOREY, 1994). From a Darwinian perspective, wolves that settled with people a few thousand years ago made a smart move from a current point of view (MOREY, 1994; MURPHY JASON, 2015;

SULLIVAN; BIRD; PERRY, 2017). However, some reports show that the human has for a long time used the dog for several types of work that did not seem to benefit both these animals (UWCT, 2017).

Only up to 150 years ago, the domestic dog started to be seen as a friend of humans (UDELL; WYNNE, 2008; UWCT, 2017). Dogs were raised to work in agricultural areas by pulling plows, herding or hunting for humans (MARSHALL-PESCINI; KAMINSKI, 2014; UWCT, 2017). On the other hand, some dogs were bred to perform bizarre functions such as turning the meat on a wood stove (Figure 1).

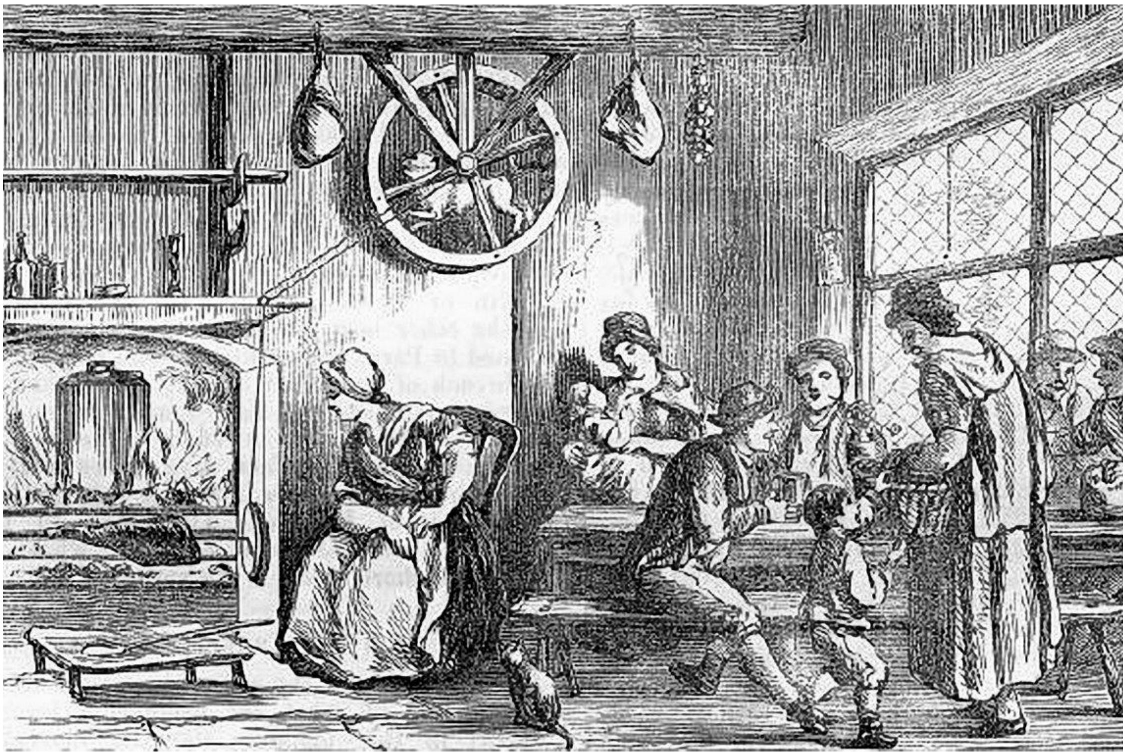

Figure 1 – Turnspit dog working (1799). Illustration, taken from *Remarks on a Tour to North and South Wales*, published in 1800, showing a dog at work. Source: Wikipedia/public domain

The earlier breeds of domestic dogs appeared about 150 years ago during the Victorian era in England. In those humble beginnings, to humans, the breed of a dog was predominantly what this breed use and not its appearance (RANGE;

VIRÁNYI, 2014). The change of this concept began in the mid-nineteenth century.

As the population of London grew exponentially during the 19th century due to the Industrial Revolution and Britain's expanding global empire, efforts to sanitize cities drastically reduced farm animals in more populated areas (MARSHALL-PESCINI; KAMINSKI, 2014). But while pigs, sheep and cattle were removed from the cities, people kept their dogs and probably at this time the way dogs were seen by people changed drastically.

The social system began to change rapidly during the Industrial Revolution and the form of food and labor also went together with this process. Suddenly, dogs began to live in private homes, where they were given the role of pets (UDELL; WYNNE, 2008). The modern world began designing dogs as a hobby of the middle and upper social classes (RANGE; VIRÁNYI, 2014). The dogs began to be shaped and new breeds began to appear constantly (DRISCOLL; MACDONALD, 2010).

Over decades, dogs and human beings began to be inseparable everywhere in the world. In Brazil, current data indicate that *C. lupus familiaris* was already present before the caravels of Cabral (GUEDES MILHEIRA et al., 2017). However, in Brazil, the domestication of the dog seems to be even more complicated to understand than its domestication in the old world.

Cultural diversity and the discrepancy between social classes in the same region caused dogs to develop different roles that are closely linked to their owners way of living (BUTLER; BINGHAM, 2000; ACOSTA-JAMETT et al., 2010).

As in Europe, with the rural exodus, changes in economic and social relations established in Brazilian urban areas contributed to the changes of the farmer dog to that transformed to an urban dog (MARTINEZ et al., 2013). However, some regions demonstrated peculiarities that make the understanding of this human-dog relationship even more difficult.

In Brazil, the proximity between urban and forested areas means that humans, domestic animals and wild animals live in the same area and compete for the same resources offered by forests in different ways (OGRZEWALSKA et al., 2009; TORRES; PRADO, 2010; FRIGERI; CASSANO; PARDINI, 2014; LESSA et al., 2016).

Domestic animals, especially the dog, that inhabit forested areas increase the risk of disease transmission and zoonosis (PINTER et al., 2008; OGRZEWALSKA et al., 2012a; FÁTIMA et al., 2013; MARTINEZ et al., 2013; SZABÓ; PINTER; LABRUNA, 2013; SCINACHI et al., 2017). There are few studies that cover the risks of this proximity in Brazil (SCINACHI et al., 2017). These effects are further aggravated by the fact that owners are unable to care for and monitor their dogs (MARTINEZ et al., 2013). Many animals do not receive adequate food and much less health and hygiene care (MARTINEZ et al., 2013).

Today, dogs in forested and rural areas are seen as a potential risk to public health and biodiversity conservation (UDELL; WYNNE, 2008; OGRZEWALSKA et al., 2011, 2012b; BARBIERI et al., 2015; SCINACHI et al., 2017). In this lengthy period of coexistence with humans, it is not possible to discover if the dog benefited from the action of anthropization. But today, it is possible to confirm that domestic dogs have been selected to serve various functions for humans and even in the absence of a specific role, dogs are tolerated by humans and viewed worldwide as man's best friend.

1.3. Amblyomma ovale – Biology and Geographical Distribution

Amblyomma ovale was first described in 1844 by Carl Ludwig Koch and, for many years, it raised questions and created divergences between researchers (ARAGÃO et al., 1961).

In 1961, Aragão and Fonseca analyzed samples collected in *Tapirus terrestris, Pantera onca* and domestic hunter dogs and concluded that, in all the analyzed material, there were two distinct species that parasitized these animals, *A. ovale* and *A. aureolatum*. Due to the morphological similarities between these two species at this moment, these researchers classify the species as *ovale* Complex. Later, analyzes of DNA sequences proved the validity of both species and overturned the *ovale* Complex given also by morphological taxonomy (GUGLIELMONE et al., 2003).

Amblyomma ovale has been described in all Brazilian biomes and is distributed throughout the Americas, being therefore, a neotropical species (ARAGÃO et al., 1961; GUGLIELMONE et al., 2003a; KRAWCZAK et al., 2016a; MARTINS et al., 2016).

Specimens have been collected from central-northern Argentina to northern Mexico and in Brazil it has beeen collected in Amazonas, Ceará, Goiás, Minas Gerais, Mato Grosso do Sul, Pará, Paraná, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina and São Paulo states (GRACIELLE et al., ; GUGLIELMONE et al., 2003a; BARROS-BATTESTI; ARZUA; BECHARA., 2006).

In São Paulo State, *A. ovale* and *A. aureolatum* can occur in sympatry, however, a study described that these two species present differences in occurrence depending upon altitude and that *A. ovale* would only occur up to

300m above sea level (BARBIERI et al., 2015). The differences between the occurrence areas of these two species may be related to many factors, among them, the range of vertebrate hosts and the occurrence of these hosts in the region (SABATINI et al., 2010; BARBIERI et al., 2015).

Both species were recorded by Sabatini et al. (2010) in the Serra do Mar State Park, from environment and also parasitizing domestic dogs, what may point that dogs have a significant role in the life cycle of these ectoparasites and the pathogens they carry in Serra do Mar.

According to literature, *A. ovale* is associated with wild environments and is rarely found in urban areas. When found in urban areas, the ticks would generally be parasitizing dogs, which had access to forested areas, where infestation might have occurred (ARAGÃO et al., 1961; BARBIERI et al., 2014).

Considering all studies to date, in adult stage, this species has a higher prevalence in carnivores (BARBIERI et al., 2015). However, it was also observed that adults parasitizing species of the order Perissodactyla (GUGLIELMONE et al., 2003a; LABRUNA et al., 2005). What makes this ixodid a threat to human health is the fact that males and females of this tick have been recorded parasitizing humans (GUGLIELMONE et al., 2003a, 2006). In addition, *A. ovale* is associated with the transmission of pathological agents causing Rickettsiosis, Hepatozoonosis, Ehrlichiosis, and Borreliosis (MARTINS; MOURA; LABRUNA, 2012; SZABÓ et al., 2013; LONDOÑO et al., 2014; KRAWCZAK et al., 2016a; LEMOS et al., 2017).

In the immature stages, this species was observed parasitizing procyonid species such as *Nasua nasua* (Linnaues 1766), rodents like *Euryoryzomys russatus* (Wagner 1848), *Proechimys semispinosus* (Tomes 1860) and

Zygodontomys brevicauda (Allen e Chapman 1893) and also some birds (GUGLIELMONE et al., 2003b; OGRZEWALSKA et al., 2011; SZABÓ et al., 2013; MATURANO et al., 2015).

In Brazil, at low altitudes, *A. ovale* is an important domestic dog parasite and closely related to the transmission of *R. parkeri* strain Atlantic rainforest which has already been identified in different locations in the Americas associated with this tick (GRACIELLE et al., ; GUGLIELMONE et al., 2003b; BARBIERI et al., 2015; KRAWCZAK et al., 2016a).

The transstadial and transovarian transmissions of *R. parkeri* strain Atlantic rainforest indicate that this ixodid tick must be the reservoir of this bacteria in the wildlife (KRAWCZAK et al., 2016b). Deleterious effects on populations of *A. ovale,* experimentally infected with *Rickettsia* sp. strain Atlantic rainforest, were observed by Krawczak et al. (2016a), which demonstrates that over successive generations, the infection by these bacteria could disappear from the populations of the *A. ovale*, unless new infected tick strains are generated by horizontal transmission through amplified vertebrate hosts.

An important fact is that, in the current literature, this tick is not always associated with *Rickettsia* infection. Probably because it is a species of broad geographic distribution, some variations such as speciation and vector capacity might interfere with the pathogen-host relationship (MCLAIN et al., 1995). Another factor to be considered is that the genetic distance between populations and the forest fragmentation that prevents the flow of wild vertebrate hosts may result in genetic variations that, among other factors, increase or decrease the potential carrying of pathogens, influencing the formation of outbreaks or risks of infection (MCLAIN et al., 1995; MCLAIN; LI; OLIVER, 2001).

The relevance of *A. ovale* to public health is notorious. However, few studies have investigated the intraspecific variations associated with the pathogen-host relationship between *A. ovale* and the various diseases associated. Studies that analyze the genetic diversity of this tick in different socio-environmental and forest contexts can contribute to the understanding of transmission form and vector capacity in well-known areas of these diseases, especially in cases of BSF in the country.

1.4. Tick-borne-diseases

1.4.1. Rickettsia parkeri strain Atlantic rainforest

In 2009, in Brazil, it was reported that a patient presented a febrile-exanthematous disease ten days after being parasitized by ticks in the Brazilian Atlantic rainforest reserve area, in the south coast of São Paulo State. This patient also had papules, typical of tick parasitism known as “inoculation sores”, which is common in rickettsiosis of the SFG, but rare in cases of *R. rickettsii*.

Skin samples from the bite local were used to identify the presence of a new bacterium from the group of rickettsiae that cause Spotted Fever through isolation, amplification and DNA sequencing (SPOLIDORIO et al., 2010b). Furthermore, through molecular phylogenetic analyzes, it was possible to observe that this new strain was positioned in the group composed by *Rickettsia africae*, *R.parkeri* and *Rickettsia sibirica* (SPOLIDORIO et al., 2010b). In this way, a new human rickettsial disease of the SFG was diagnosed, described and denominated Spoted Fever of the Atlantic rainforest and the bacterium denominated by many as *Rickettsia* sp. strain Atlantic rainforest.

After the description of this new zoonosis, efforts were made by many researchers, mainly Brazilians, to diagnose, describe and evaluate the hosts and vectors involved, as well as the frequency, prevalence and epidemiology of the new strain (KRAWCZAK et al., 2016b, 2016c; OLIVEIRA et al., 2017).

Later on, through a serological, molecular and phylogenetic survey using five concatenated genes of the genus *Rickettsia,* researchers grouped *Rickettsia* sp. strain Atlantic rainforest and *Rickettsia* sp. Black Gap strain, described in

Texas, in a distinct clade and well supported with *R. parkeri* (PADDOCK et al., 2017). Thus, to date, these two strains are believed to form a single lineage of *R. parkeri* associated predominantly or exclusively with ticks of the genus *Amblyomma*. From research, the appropriate denomination for the Brazilian strain is *Rickettsia parkeri* strain Atlantic rainforest (PADDOCK et al., 2017).

In 2010, Sabatini and collaborators (2010) detected the presence of *Rickettsia* sp. strain Atlantic rainforest in 13% of *A. ovale* collected in Serra do Mar State Park, in Cubatão City, suggesting that this tick could be an important vector. In all the coast of São Paulo State, the *A. ovale* species is widely distributed in reserved areas of the Brazilian Atlantic rainforest and its anthropophilic capacity is well known in the adult stages (LABRUNA et al., 2001; GUGLIELMONE et al., 2003b; LONDOÑO et al., 2014; KRAWCZAK et al., 2016a).

A study published by Krawczak et al. (2016) showed that *A. ovale* can perform transovarian and transstadial transmissions of the *R. parkeri* strain Atlantic rainforest for at least two generations. This study strongly supports the clinical and epidemiological data that implicates the *A. ovale* tick as the main vector of *R. parkeri* strain Atlantic rainforest in Brazil up to this moment (SZABÓ; PINTER; LABRUNA, 2013; BARBIERI et al., 2015, 2015c).

It is known that engorged and infected females with *R. parkeri* strain Atlantic forest have a relatively high mortality rate and lower reproductive performance compared to other species of the genus *Rickettsia* and other species of ticks (BURGDORFER, 1975; LABRUNA et al., 2011; KRAWCZAK et al., 2016a; SCHUMACHER; SNELLGROVE; LEVIN, 2016). Thus, possibly very low rates of *Rickettsia* infection may be related to the expressiveness of infected females that survive (LABRUNA et al., 2011).

In the case of the *R. parkeri* strain Atlantic rainforest, the infection rate of *A. ovale* in areas of Brazilian Atlantic rainforest is generally about 10%, which is relatively high in relation to *R. rickettsii* in endemic areas (<1%) (SABATINI et al., 2010; SZABÓ; PINTER; LABRUNA, 2013). Thus, it is possible that the horizontal transmission of *R. parkeri* strain Atlantic rainforest is important in sustaining this pathogen in its endemic areas, as well as, the presence of a vertebrate host that plays the role of amplifier of the disease (KRAWCZAK et al., 2016a; MARTINS et al., 2016).

It is known that *A. ovale*, in the stages of nymph and larva, are found parasitizing small mammals and birds (MARTINS; MOURA; LABRUNA, 2012; OGRZEWALSKA et al., 2011). However, there are no studies that demonstrate which group of animals or which species could be performing the role of amplifier of this pathogen.

Currently, there are a large number of researchers developing studies that include epidemiological aspects, endemic areas, genetic variability and rates of infection of the *Rickettsia* genus of the SFG. However, few data are available on *R. parkeri* strain Atlantic rainforest (PADDOCK et al., 2004; BARBIERI et al., 2015; KRAWCZAK et al., 2016a; MONJE et al., 2016). Therefore, studies directed to the understanding of this pathogen and its epidemiology are extremely important.

1.4.2. Rangelia vitalii

The protozoan *R. vitalii* is still a poorly studied parasite and the taxonomic position of this hemoparasite is, to date, described as a species belonging to the family Babesidae because it is genetically and morphologically related to the hemoprotozoa of this family (SOARES, 2014; SOARES et al., 2014)

The first description of *R. vitalii* is dated early in the twentieth century by Carini (1908), when observing a hemorrhagic and febrile disease in dogs with no specific etiology. Two years after this first report, Pestana (1910) isolated the parasite and called it *Piroplasma vitalii*. This nomenclature remained for four years until Carini and Maciel (1914), relying on the developmental cycle of the parasite, renamed it *Rangelia vitalii*.

After years of controversy about the nomenclature, biology and pathogenesis of this parasite, the validity of the *R. vitalii* species was confirmed in a study of molecular analyzes (SOARES et al., 2011).

In Brazil, only three species of piroplasmids that parasitize dogs can be detected by PCR: *Babesia canis vogeli* (PASSOS et al., 2005) *Babesia gibsoni* (TRAPP et al., 2006) and *R. vitalii* (SOARES et al., 2011). Of these three, *R. vitalii* is characterized by infecting red blood cells, leukocytes and vascular endometrial cells of the host causing severe hemorrhages in dogs. This disease popularly known as Nambyuvú, a Brazilian indigenous word that means "ear that bleeds" because it is a clinical sign in natural infections (PESTANA. BR, 1910; SOARES et al., 2011).

There are very few studies regarding the presence of this parasite in canine populations and their prevalences (LEMOS et al., 2017). Possibly, this disease is well distributed in the South and Southeast regions of the country and clinical cases can often be confused with *Babesia canis* (CARINI A., 1908; PESTANA. BR, 1910; SOARES et al., 2011).

What is known until now is that *R. vitalii* is a naturally occurring parasite found in the wild populations of canids and has as possible vectors *A. ovale, A. aureolatum, A. tigrinum* and *A. sculptum* (GUGLIELMONE et al., 2003a; LABRUNA et al., 2005).

To date, this parasite has been described only in individuals of the family Canidae, manly in domestic dogs that have access to forested areas (LORETTI; BARROS, 2004; GOTTLIEB et al., 2016; LEMOS et al., 2017). Possibly, this fact is related, among other factors, to the disease vectors. The species *A. aureolatum, A. ovale* and *R. sanguineus,* have been found in canines infected by *R. vitalii* (LORETTI; BARROS, 2004). However, a study on *A. aureolatum* and *R. sanguineus*, demonstrated that only *A. aureolatum* was able to infect dogs with the protozoan *R. vitalii* (SOARES et al., 2012).

The geographical distribution of this disease is associated with places where these wild tick species are present. And wild canids may be involved in the maintenance of the protozoan as natural reservoirs (SOARES et al., 2012). Some authors believe that dogs that recover from the infection are able to become asymptomatic carriers of this pathogen and, in these conditions, these animals to be reservoirs of *R. vitalii* for several months (CARINNI, MACIEL, 1914).

A clinically cured dog from a *R. vitalii* infection, once taken to an unscathed region, can infect ticks and create a new focus of disease (BRAGA, 1935). Therefore, in the case of tick-borne-diseases, when a pathogen causes acute disease in a vertebrate host and there is no persistent infection, the reservoir of the disease-causing agent will be the tick that will perpetuate the pathogen from generation to generation through transovarian transmission. On the other hand, when the pathogen causes a chronic disease in a vertebrate host and the infection is prolonged, the reservoir of the pathogen will usually be the infected animal itself (LABRUNA et al., 2011; SOARES et al., 2012).

It is necessary to carry on more studies of this parasite, focused on that include a review of all the diagnoses and on molecular biology, in order to determine the real prevalence and the true importance of the *R. vitalii* on the health of dogs and wildlife.

1.5. Genetic of Populations of the genus *Amblyomma*

Evaluating the genetic variability of a vector is an important step towards the creation of methods to control zoonoses (DURON; CREMASCHI; MCCOY, 2016; MONZÓN et al., 2016). Studies on population genetics of vectors can provide basic information about the ecological properties of the species, such as genetic diversity and the potential of adaptation, the structure and spatial extent of populations, and the mechanisms of dispersion and gene flow (MCCOY, 2008; MONZÓN et al., 2016).

Knowing the genetic variation of a vector can help enrich the knowledge of the transmission patterns of its pathogenic microorganisms in a large geographic scale (GOODING, 1996). This information can help to predict the dynamics of the disease and the development of molecular methods to control the transmission of these diseases.

In any genetic study, an essential step is choosing the genes to be analyzed. They should have a mutation rate appropriate to the evolutionary scale, target of the study (DUPRAZ et al., 2016).

Today, in phylogenetic analyzes and genetic studies of tick populations, the genes encoding the 16s rDNA fragment are favored (NADOLNY et al., 2015; DUPRAZ et al., 2016; DANTAS-TORRES et al., 2017).

The mitochondrial rDNA (12S and 16S) have often been used in phylogenetic studies of ticks because the genes in this region evolve faster than nuclear genes. Because of their reduced size and abundance of mitochondria in the cells, this makes it easier to sequence than the nuclear genome (SHAO et al., 2012; NADOLNY et al., 2015; DUPRAZ et al., 2016; DURON; CREMASCHI; MCCOY, 2016; DANTAS-TORRES et al., 2017).

In the analyzes of tick populations of the genera *Ixodes* and *Amblyomma*, these genes are useful to distinguish closely related tick species and are also suitable to identify distinct populations within the same species (NORRIS et al., 1996; MCCOY, 2008).

Guglielmone et al. (2003) used this fragment to differentiate *A. aureolatum* from *A. ovale* and showed a 13.1% difference between them. The 5' region of Cytochrome Oxidase I (COI) is also a standard marker for identification of tick species (LV et al., 2014). However, some authors describe a limited use in the identification of some species, because for some taxa, the coding sequence is not efficiently amplified by PCR (DIETRICH et al., 2014; LV et al., 2014; DUPRAZ et al., 2016). Due to its rapid evolution, this gene has been frequently used for genetic studies of populations of some species of ticks (DUPRAZ et al., 2016; MUÑOZ-LEAL et al., 2017).

Among other available methods, microsatellites and Single Nucleotide Polymorphisms –SNPs can also be used as an effective tool for studies of the genetic structure of populations, gene flow and to quantify the effects of habitat fragmentation (JEHAN; LAKHANPAUL, 2006; DE MEEUS, 2012; DE WIT; PESPENI; PALUMBI, 2015).

SNPs is currently one of the most revolutionary ways to study parasitic organisms through genetic markers (JEHAN; LAKHANPAUL, 2006). SNPs are small changes or variations that may occur in a DNA sequence in a significant portion (more than 1%) of a population (DE WIT; PESPENI; PALUMBI, 2015). Therefore, SNPs are the most frequent forms of genetic variation.

The pioneering study for population analysis of *A. americanum* through SNPs was carried out by Monzón et al. (2016). In this study, the results suggest that climate change can have effects on the expansion and displacement of *A. americanum* which diverged to adapt itself to new environments. Such factor associated with the modifications of the soil by the anthropic action can influence the dispersion of this parasite, as well as the pathogens that it carries. This study evidenced the importance of the use of molecular techniques of the latest generation for the ecological and epidemiological study of tick species of that may put human health at risk.

In Brazil, until now, only one study has scoped of the genetics of tick populations using microsatellites performed by Ogrzewalska and collaborators (2014). This study suggested that the low genetic structure among the populations of *A. aureolatum* in the metropolitan region of São Paulo City could be attributed to the migrations between them. Considering that the studied areas were more than 20 km apart, it was possible to infer that the gene flow among the populations of *A. aureolatum* in the region occurs due to the dislocation of the hosts, in this case, birds.

Even if the number of migrations per generation is low in the tick populations, this is enough to avoid structuring between populations (OGRZEWALSKA et al., 2014). In this context, the distribution and the chances of colonization of new areas depend strictly on the mobility of hosts and, consequently, on the level of connectivity of the landscape (MADHAV et al., 2004).

Studies of the gene structure of tick populations made by Lampo et al. (1998) also show that host dispersion is the most crucial factor in the determination of gene variation in *Amblyomma dissimile* populations in

Venezuela. In addition, genetically diverse populations among the *Amblyomma* species can have distinct vector competences (MCCOY, 2008).

Considering that genetic variability and phylogenetic variation in ticks may be associated with their ability to acquire, maintain and transmit pathogens (MCCOY, 2008), the study of the genus *Amblyomma* is extremely important in Brazil, in this molecular context.

1.6. OBJECTIVES

1.5.1 General objective

The objective of this study was to analyze the role of dogs in different areas around the Serra do Mar State Park in Caraguatatuba City as dispersers of tick-borne-zoonotic agents and to correlate the genetic variability of *A. ovale* species with the capacity of dogs as hosts.

1.5.2. Specific objectives

- To investigate the genetic variability and population structure of ticks collected in dogs living near the Serra do Mar State Park in Caraguatatuba City;
- Investigate the presence of pathogens of the families Rickettsiaceae and Babesiidae in blood samples and ticks collected from dogs;
- To evaluate the area of use of dogs in the surroundings of the Serra do Mar State Park through telemetry data and photographic records.

1.7. Studied Area

Caraguatatuba City is located in the north coast of São Paulo State and at the east of São Paulo City, about 180km (Latitude: 23° 37' 21'' South; Longitude: 45° 24' 43'' West). The city occupies an area of 485,097 km², with a population of around 116,786 habitants, resulting in a population density of 207.88 inhabitants/km² (IBGE, 2017). The average annual temperature of the city is 25ºC, and the primal vegetation is the Brazilian Atlantic rainforest.

The Human Development Index (HDI) of Caraguatatuba City is considered high in relation to the country (0,759). However, the city presents many problems related to the invasion of forested areas by illegal settlements and, consequently, deforestation (IBGE, 2017). In 1770, Caraguatatuba City was considered a village due to the settlement growth with the arrival of Europeans who settled in the region of Fazenda dos Ingleses, and properly established in the region since 1927 (FONSECA, 2010).

The city grew around the Serra do Mar, an area of native Brazilian Atlantic rainforest. With tectonic geomorphologic formation of the Tertiary period, the region has sedimentary plains cut by flowing rivers and small streams, being in those plains where the human occupations that gradually occupy the slopes of the mountain were developed. In 1967, the city was the scene of one of the biggest natural catastrophe in Brazil, when a flood of water felt from the mountain and caused the slopes to slip across the city causing many deaths (FONSECA, 2010).

In 1977, to preserve the slopes of the native Brazilian Atlantic rainforest, the Serra do Mar State Park has been created. Today, the park has an area of 332.000 hectares, with lands in several municipalities of São Paulo State and with ten Administrative Units (Bertioga, Caraguatatuba, Cunha, Curucutu, Itutinga-Pilões, Itarirú, Picinguaba, Padre Dória, Santa Virgínia and São Sebastião).

References:

CARINNI, A.; MACIEL, J. Sobre a molestia dos cães, chamada nambiuvú,. **An Paul Med Cir**, v. 3, n. 1, p. 65–71, 1914.

ACOSTA-JAMETT, G.; CLEAVELAND, S.; CUNNINGHAM, A. A.; BRONSVOORT, B. M. D. C. Demography of domestic dogs in rural and urban areas of the Coquimbo region of Chile and implications for disease transmission. **Preventive Veterinary Medicine**, v. 94, n. 3–4, p. 272–281.

ARAGÃO, H.; FONSECA, F. da. Notas de ixodologia: VIII. Lista e chave para os representantes da fauna ixodológica brasileira: notas de ixolodologia. **Memórias do Instituto Oswaldo Cruz**, v. 59, n. 2, p. 115–129, jul. 1961.

BARBIERI, A.; FILHO, M. J.; NIERI-BASTOS, F. A.; SOUZA JR, J.C.; SZABÓ, M. P. J.; LABRUNA, M. B. Epidemiology of Rickettsia sp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil. **Ticks and tick-borne diseases,** v.5, n.6., p. 848-53, 2014.

BARBIERI, J. M.; DA ROCHA, C. M. B. M.; BRUHN, F. R. P.; CARDOSO, D. L.; PINTER, A.; LABRUNA, M. B. Altitudinal Assessment of *Amblyomma aureolatum* and *Amblyomma ovale* (Acari: Ixodidae), Vectors of Spotted Fever Group Rickettsiosis in the State of São Paulo, Brazil. **Journal of Medical Entomology**, v. 52, n. 5, p. 1170–1174, 1 set. 2015.

BARROS-BATTESTI, D. M.; ARZUA, M.; BECHARA., G. H. **Carrapatos de importância médico veterinária da região neotropical: um guia ilustrado para identificação de espécies.** 1. ed. Vox, ICTTD-3(Eds): Butantã, 223p., 2006.

BERTA, A. Origin, diversification, and zoogeography of the South American Canidae,. In: TIMM, P. & R. M. (Ed.). **Studies in neotropical mammalogy: essays in honor of Philip Hershkovitz.** vol. 39, Patterson & R.M. Timm (Eds) 39p. 506p.,1987.

BRAGA, A. Contribuição ao estudo experimental das piroplasmoses dos cães. **Bol Vet Exército**, v. 3, n. 1, p. 1–16., 1935.

BURGDORFER, W. A review of Rocky Mountain spotted fever (tick-borne typhus), its agent, and its tick vectors in the United States. **Journal of medical entomology**, v. 12, n. 3, p. 269–78, 25 set. 1975.

BUTLER, J. R.; BINGHAM, J. Demography and dog-human relationships of the dog population in Zimbabwean communal lands. **The Veterinary record**, v. 147, n. 16, p. 442–6, 14 out. 2000.

CARINI A. Notícias sobre zoonoses observadas no Brasil. **Rev Med São Paulo**, v. 22, p. 459–462, 1908.

CHIGAGURE, N. N.; BAXTER, G. D.; BARKER, S. C. Microsatellite loci of the cattle tick Boophilus microplus (Acari: Ixodidae). **Experimental & applied**

acarology, v. 24, n. 12, p. 951–6, 2000.

DANTAS-TORRES, F. The role of dogs as reservoirs of Leishmania parasites, with emphasis on *Leishmania* (*Leishmania*) *infantum* and *Leishmania* (*Viannia*) *braziliensis*. **Veterinary Parasitology**, v. 149, n. 3–4, p. 139–146, 2007.

DANTAS-TORRES, F.; MAIA, C.; LATROFA, M. S.; ANNOSCIA, G.; CARDOSO, L.; OTRANTO, D. Genetic characterization of *Rhipicephalus sanguineus* (sensu lato) ticks from dogs in Portugal. **Parasites & Vectors**, v. 10, n. 1, p. 133, 2017.

DE MEEUS, T. **Initiation à la génétique des populations naturelles applications aux parasites et à leurs vecteurs**. IRD Ed: Marseille, 336p, 2012.

DE WIT, P.; PESPENI, M. H.; PALUMBI, S. R. SNP genotyping and population genomics from expressed sequences - Current advances and future possibilities. **Molecular Ecology**, v. 24, n. 10, p. 2310–2323, 2015.

DELAYE, C.; AESCHLIMANN, A.; RENAUD, F.; ROSENTHAL, B.; DE MEEÛS, T. Isolation and characterization of microsatellite markers in the *Ixodes ricinus* complex (Acari: Ixodidae). **Molecular ecology**, v. 7, n. 3, p. 360–1, mar. 1998.

DIETRICH, M.; KEMPF, F.; BOULINIER, T.; MCCOY, K. D. Tracing the colonization and diversification of the worldwide seabird ectoparasite Ixodes uriae. **Molecular Ecology**, v. 23, n. 13, p. 3292–3305, 2014.

DOHERTY, T. S.; DICKMAN, C. R.; GLEN, A. S.; NEWSOME, T. M.; NIMMO, D. G.; RITCHIE, E. G.; VANAK, A. T.; WIRSING, A. J. The global impacts of domestic dogs on threatened vertebrates. **Biological Conservation**, v. 210, n. April, p. 56–59, 2017a.

DRISCOLL, C. A.; MACDONALD, D. W. Top dogs: wolf domestication and wealth. **Journal of Biology**, v. 9, n. 2, p. 10, 2010.

DUPRAZ, M.; TOTY, C.; NOËL, V.; ESTRADA-PEŇA, A.; GONZÁLEZ-SOLÍS, J.; BOULINIER, T.; DUJARDIN, J. P.; MCCOY, K. D. Linking morphometric and genetic divergence with host use in the tick complex, *Ornithodoros capensis* sensu lato. **Infection, Genetics and Evolution**, v. 46, p. 12–22, 2016.

DURON, O.; CREMASCHI, J.; MCCOY, K. D. The High Diversity and Global Distribution of the Intracellular Bacterium Rickettsiella in the Polar Seabird Tick Ixodes uriae. **Microbial Ecology**, v. 71, n. 3, p. 761–770, 2016.

FOURNIER, G.F.S.R.; DA SILVA, J.I.G.; Cabral, A. D.; Pena, H.F.J.; Gennari, S.M *Toxoplasma gondii* : evaluation of immunofluorescence assay using eterologous secondary antibody in experimentally infected wild small rodents. **Brazilian Journal of Veterinary Research and Animal Science**, v. 50, n. 5, p. 353–358, 2013.

FREDO, G.; LEITE-FILHO, R. V.; PIETZSCH, C. D. Á.; ANDRADE, C. P. de; DUDA, N. C. B.; NASCIMENTO, L. C. do; VALLE, S. de F.; SOARES, J. F.; SONNE, L.; FREDO, G.; LEITE-FILHO, R. V.; PIETZSCH, C. D. Á.; ANDRADE, C. P. de; DUDA, N. C. B.; NASCIMENTO, L. C. do; VALLE, S. de F.; SOARES,

J. F.; SONNE, L. Rangeliosis: histopathological analysis, hematology and molecular detection of canine *Rangelia vitalii* in Rio Grande do Sul, Brazil. **Ciência Rural**, v. 47, n. 10, 2017.

FRIGERI, E. Invasão por cães domésticos (Canis lupus familiaris) na Mata Atlântica: efeitos da perda de habitat e da intensificação agrícola. **Dissertação De Mestrado**, 2013.

FRIGERI, E.; CASSANO, C. R.; PARDINI, R. Domestic dog invasion in an agroforestry mosaic in southern Bahia , Brazil. **Tropical Conservation Science**, v. 7, n. 3, p. 508–528, 2014.

GALETTI, M.; SAZIMA, I. Impact of feral dogs in an urban Atlantic forest fragment in southeastern Brazil. **Natureza & Conservação**, v. 4, n. 1, p. 146–151, 2006.

GIGLIOTTI, C.; SANTOS, M. J. A EXPANSÃO URBANA DE CARAGUATATUBA CITY (1950-2010): UMA ANÁLISE DAS TRANSFORMAÇÕES SÓCIO ESPACIAIS. **Caminhos de Geografia**, v. 14, n. 46, 2013.

GOODING, R. H. Genetic variation in arthropod vectors of disease-causing organisms: obstacles and opportunities. **Clinical microbiology reviews**, v. 9, n. 3, p. 301–20, 1996.

GOTTLIEB, J.; ANDRÉ, M. R.; SOARES, J. F.; GONÇALVES, L. R.; TONIAL DE OLIVEIRA, M.; COSTA, M. M.; LABRUNA, M. B.; BORTOLINI, C. E.; MACHADO, R. Z.; VIEIRA, M. I. B. *Rangelia vitalii*, *Babesia* spp. and *Ehrlichia* spp. in dogs in Passo Fundo, state of Rio Grande do Sul, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 25, n. 2, p. 172–178, 2016.

FERREIRA, G.C.T.; BEZERRA, A.C.D.S.; CARVALHO, O.V.; ALMEIDA, M.R; MAFRA, C. First occurrence of *Amblyomma ovale* in the State of Rio Grande do Norte, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 22, n. 1, p. 167-170, 2013.

GUEDES MILHEIRA, R.; LOPONTE, D M; GARCÍA ESPONDA, C.; ACOSTA, A.; ULGUIM, P. The First Record of a Pre-Columbian Domestic Dog (*Canis lupus familiaris*) in Brazil. **International Journal of Osteoarchaeology**, v. 27, n. 3, p. 488–494, 2017.

GUGLIELMONE, A. A.; BEATI, L.; BARROS-BATTESTI, D. M.; LABRUNA, M. B.; NAVA, S.; VENZAL, J. M.; MANGOLD, A. J.; SZABÓ, M. P. J.; MARTINS, J. R.; GONZÁLEZ-ACUÑA, D.; ESTRADA-PEÑA, A. Ticks (Ixodidae) on humans in South America. **Experimental and Applied Acarology**, v. 40, n. 2, p. 83–100, 2006.

GUGLIELMONE, A. A.; ESTRADA-PEÑA, A.; KEIRANS, J. E.; ROBBINS, R. G. Ticks (Acari: Ixodida) of the Neotropical Zoogeographic Region . (International Consortium on Ticks and Tickborne Diseases (ICTTD-2), Ed.) In: International Consortium on Ticks and Tickborne Diseases (ICTTD-2), Atalanta, Houten, The Netherlands,. **Anais international Consortium on Ticks and Tickborne Diseases (ICTTD-2)** Atalanta, Houten, The Netherlands,: 2003a.

GUGLIELMONE, A. A.; ESTRADA-PEÑA, A.; MANGOLD, A. J.; BARROS-BATTESTI, D. M.; LABRUNA, M. B.; MARTINS, J. R.; VENZAL, J. M.; ARZUA, M.; KEIRANS, J. E. *Amblyomma* aureolatum (Pallas, 1772) and *Amblyomma ovale* Koch, 1844 (Acari: Ixodidae): Hosts, distribution and 16S rDNA sequences. **Veterinary Parasitology**, v. 113, n. 3–4, p. 273–288, 2003b.

GUZINSKI, J.; BULL, C. M.; DONNELLAN, S. C.; GARDNER, M. G. Molecular genetic data provide support for a model of transmission dynamics in an Australian reptile tick, *Bothriocroton hydrosauri*. **Molecular Ecology**, v. 18, n. 2, p. 227–234, 1 jan. 2009.

IBGE. **IBGE | Brasil em Síntese | São Paulo | Caraguatatuba City | Panorama**. Disponível em: <https://cidades.ibge.gov.br/brasil/sp/Caraguatatuba City/panorama>. Acesso em: 7 out. 2017.

JEHAN, T.; LAKHANPAUL, S. Single nucleotide polymorphism (SNP) - methods and applications in plant genetics: A review. **Indian Journal of Biotechnology**, v. 5, n. 4, p. 435–459, 2006.

KANDUMA, E. G.; MWACHARO, J. M.; SUNTER, J. D.; NZUKI, I.; MWAURA, S.; KINYANJUI, P. W.; KIBE, M.; HEYNE, H.; HANOTTE, O.; SKILTON, R. A.; BISHOP, R. P. Micro- and minisatellite-expressed sequence tag (EST) markers discriminate between populations of *Rhipicephalus appendiculatus*. **Ticks and Tick-borne Diseases**, v. 3, n. 3, p. 128–136, jun. 2012.

KRAWCZAK, F. S.; AGOSTINHO, W. C.; POLO, G.; MORAES-FILHO, J.; LABRUNA, M. B. Comparative evaluation of *Amblyomma* ovale ticks infected and noninfected by Rickettsia sp. strain Atlantic rainforest, the agent of an emerging rickettsiosis in Brazil. **Ticks and Tick-borne Diseases**, v. 7, n. 3, p. 502–507, 2016a.

KRAWCZAK, F. S.; BINDER, L. C.; OLIVEIRA, C. S.; COSTA, F. B.; MORAES-FILHO, J.; MARTINS, T. F.; SPONCHIADO, J.; MELO, G. L.; GREGORI, F.; POLO, G.; OLIVEIRA, S. V.; LABRUNA, M. B. Ecology of a tick-borne spotted fever in southern Brazil. **Experimental and Applied Acarology**, v. 70, n. 2, p. 219–229, 8 out. 2016b.

KRAWCZAK, F. S.; MUNOZ-LEAL, S.; GUZTZAZKY, A. C.; OLIVEIRA, S. V.; SANTOS, F. C. P.; ANGERAMI, R. N.; MORAES-FILHO, J.; DE SOUZA, J. C.; LABRUNA, M. B. *Rickettsia* sp. Strain Atlantic Rainforest Infection in a Patient from a Spotted Fever-Endemic Area in Southern Brazil. **American Journal of Tropical Medicine and Hygiene**, v. 95, n. 3, p. 551–553, 7 set. 2016c.

LABRUNA, M. B.; CAMARGO, L. M. A.; TERRASSINI, F. A.; FERREIRA, F.; SCHUMAKER, T. T. S.; CAMARGO, E. P. Ticks (Acari: Ixodidae) from the state of Rondônia, western Amazon, Brazil. **Systematic and Applied Acarology**, v. 10, n. (1), p. 17–32, 2005.

LABRUNA, M. B.; HORTA, M. C.; AGUIAR, D. M.; CAVALCANTE, G. T.; PINTER, A.; GENNARI, S. M.; CAMARGO, L. M. a. Prevalence of *Rickettsia* infection in dogs from the urban and rural areas of Monte Negro municipality, western Amazon, Brazil. **Vector borne and zoonotic diseases (Larchmont, N.Y.)**, v. 7, n. 2, p. 249–255, 2007.

LABRUNA, M. B.; OGRZEWALSKA, M.; SOARES, J. F.; MARTINS, T. F.; SOARES, H. S.; MORAES-FILHO, J.; NIERI-BASTOS, F. A.; ALMEIDA, A. P.; PINTER, A. Experimental Infection of *Amblyomma aureolatum* Ticks with *Rickettsia rickettsii*. **Emerging Infectious Diseases**, v. 17, n. 5, p. 829–834, 2011.

LABRUNA, M. B.; SOUZA, S. L. P.; GUIMARÃES, J. S.; PACHECO, R. C.; PINTER, A.; GENNARI, S. M. Prevalência de carrapatos em cães de áreas rurais da região norte do Estado do Paraná. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 53, n. 5, p. 553–556, 2001.

LEMOS, T. D.; TOMA, H. K.; ASSAD, R. Q.; VIEIRA, A.; GOMES, R.; CORRÊA, B.; REGINA, N.; ALMOSNY, P. Clinical and hematological evaluation of *Rangelia vitalii*- naturally infected dogs in southeastern Brazil. **Jornal Brasileiro de Parasitologia,** v. 2961, p. 307–313, 2017.

LESSA, I.; CORRÊA SEABRA GUIMARÃES, T.; DE GODOY BERGALLO, H.; CUNHA, A.; M. VIEIRA, E. Domestic dogs in protected areas: a threat to Brazilian mammals? **Natureza e Conservacao**, v. 14, n. 2, p. 46–56, 2016.

LI, S.; HARTEMINK, N.; SPEYBROECK, N.; VANWAMBEKE, S. O. Consequences of landscape fragmentation on Lyme disease risk: a cellular automata approach. **PloS one**, v. 7, n. 6, p. e39612, 2012.

LONDOÑO, A. F.; DÍAZ, F. J.; VALBUENA, G.; GAZI, M.; LABRUNA, M. B.; HIDALGO, M.; MATTAR, S.; CONTRERAS, V.; RODAS, J. D. Infection of *Amblyomma ovale* by *Rickettsia* sp. strain Atlantic rainforest, Colombia. **Ticks and Tick-borne Diseases**, v. 5, n. 6, p. 672–675, 2014.

LOPES, M. G.; JUNIOR, J. M.; FOSTER, R. J.; HARMSEN, B. J.; SANCHEZ, E.; MARTINS, T. F.; QUIGLEY, H.; MARCILI, A.; LABRUNA, M. B. Ticks and rickettsiae from wildlife in Belize, Central America. **Parasites & Vectors**, v. 9, n. 1, p. 62, 2016.

LORETTI, A. P.; BARROS, S. S. Parasitismo Por *Rangelia Vitalii* Em Cães (“ Nambiuvú ”, " Peste De Sangue)- Uma Revisão Crítica Sobre O Assunto. **Arquivos do Instituto de Biologia**, v. 71, n. 1, p. 101–131, 2004.

LV, J.; WU, S.; ZHANG, Y.; CHEN, Y.; FENG, C.; YUAN, X.; JIA, G.; DENG, J.; WANG, C.; WANG, Q.; MEI, L.; LIN, X. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). **Parasites & vectors**, v. 7, p. 93, 3 mar. 2014.

LYRAS, G. A. The evolution of the brain in Canidae (Mammalia: Carnivora). v. 139, n. 48, p. 1–93, 2009.

MADHAV, N. K.; BROWNSTEIN, J. S.; TSAO, J. I.; FISH, D. A dispersal model for the range expansion of blacklegged tick (Acari: Ixodidae). **Journal of medical entomology**, v. 41, n. 5, p. 842–52, set. 2004.

MARKS, B. K.; DUNCAN, R. S. Use of Forest Edges by Free-ranging Cats and Dogs in an Urban Forest Fragment. **Naturalist**, v. 8, n. 3, p. 427–436, 2009.

MARSHALL-PESCINI, S.; KAMINSKI, J. The Social Dog: History and Evolution. **The Social Dog**. 32p, 2014. Disponível em: http://dx.doi.org/10.1016/B978-0-12-407818-5.00001-2, Acessado em 20 de outubro de 2017.

MARTINEZ, E.; CESARIO, C.; SILVA, I. D. E.; BOERE, V. Domestic dogs in rural area of fragmented Atlantic Forest: potential threats to wild animals. **Ciencia Rural**, v. 43, n. 11, p. 1998–2003, 2013.

MARTINS, T. F.; MOURA, M. M.; LABRUNA, M. B. Life-cycle and host preference of *Amblyomma* ovale (Acari: Ixodidae) under laboratory conditions. **Experimental and Applied Acarology**, v. 56, n. 2, p. 151–158, 2012.

MARTINS, T. F.; PERES, M. G.; COSTA, F. B.; BACCHIEGA, T. S.; APPOLINARIO, C. M.; ANTUNES, J. M. A. de P.; VICENTE, A. F.; MEGID, J.; LABRUNA, M. B.; MARTINS, T. F.; PERES, M. G.; COSTA, F. B.; BACCHIEGA, T. S.; APPOLINARIO, C. M.; ANTUNES, J. M. A. de P.; VICENTE, A. F.; MEGID, J.; LABRUNA, M. B. Ticks infesting wild small rodents in three areas of the state of São Paulo, Brazil. **Ciência Rural**, v. 46, n. 5, p. 871–875, 2016.

MASEL, J. Rethinking Hardy-Weinberg and genetic drift in undergraduate biology. **BioEssays**, v. 34, n. 8, p. 701–710, ago. 2012.

MATURANO, R.; FACCINI, J. H.; DAEMON, E.; FAZZA, P. C.; BASTOS, R. Additional information about tick parasitism in Passeriformes birds in an Atlantic Forest in southeastern Brazil. **Parasitology Research**, v. 114, p. 4181–4193, 2015.

MCCOY, K. D. The population genetic structure of vectors and our understanding of disease epidemiology. **Parasite**, v. 15, n. 3, p. 444–448, 15 set. 2008.

MCCOY, K. D.; TIRARD, C. Isolation and characterization of microsatellites in the seabird ectoparasite Ixodes uriae. **Molecular ecology**, v. 9, n. 12, p. 2213–4, 2000.

MCLAIN, D. K.; LI, J.; OLIVER, J. H. Interspecific and geographical variation in the sequence of rDNA expansion segment D3 of Ixodes ticks (Acari: Ixodidae). **Heredity**, v. 86, n. Pt 2, p. 234–42, 2001.

MCLAIN, D. K.; WESSON, D. M.; COLLINS, F. H.; OLIVER, J. H. Evolution of the rDNA spacer, ITS 2, in the ticks Ixodes scapularis and I. pacificus (Acari: Ixodidae). **Heredity**, v. 75 (Pt 3), p. 303–19, 1995.

MEDEIROS, A. P.; SOUZA, A. P. de; MOURA, A. B. de; LAVINA, M. S.; BELLATO, V.; SARTOR, A. A.; NIERI-BASTOS, F. A.; RICHTZENHAIN, L. J.; LABRUNA, M. B. Spotted fever group Rickettsia infecting ticks (Acari: Ixodidae) in the state of Santa Catarina, Brazil. **Memorias do Instituto Oswaldo Cruz**, v. 106, n. 8, p. 926–930, 2011.

MELLO-THÉRY, N. A. de. Conservação de áreas naturais em São Paulo.

Estudos Avançados, v. 25, n. 71, p. 175–188, 2011.

MONJE, L. D.; COSTA, F. B.; COLOMBO, V. C.; LABRUNA, M. B.; ANTONIAZZI, L. R.; GAMIETEA, I.; NAVA, S.; BELDOMENICO, P. M. Dynamics of Exposure to *Rickettsia parkeri* in Cattle in the Paraná River Delta, Argentina. **Journal of Medical Entomology**, v. 53, n. 3, p. 660–665, 1 maio 2016.

MONZÓN, J. D.; ATKINSON, E. G.; HENN, B. M.; BENACH, J. L. Population and Evolutionary Genomics of *Amblyomma* americanum, an Expanding Arthropod Disease Vector. **Genome biology and evolution**, v. 8, n. 5, p. 1351–60, 2016.

MOREY, D. F. Animal Evolution of the Domestic Dog can also be described as an evolutionary process. **American Scientist**, v. 82, n. 4, p. 336–347, 1994.

MOURET STEFANIE. **Revolução Industrial no Brasil - Início, incentivo e consequências**. Disponível em: <https://www.estudopratico.com.br/revolucao-industrial-no-brasil/>. Acesso em: 7 out. 2017.

MUÑOZ-LEAL, S.; DIAS, R. A.; ABRAHÃO, C. R.; LABRUNA, M. B. The Ornithodoros capensis group (Acari: Argasidae): a morphological diagnosis and molecular characterization of O. capensis sensu stricto from Queimada Grande Island, Brazil. **Systematic and Applied Acarology**, v. 22, n. 1, p. 28, 2 jan. 2017.

MURPHY JASON. **The Origin and Consequences of Canine Eugenics | Spring 2015 | Washington State University**. Disponível em: <https://history.libraries.wsu.edu/spring2015/2015/01/20/the-origin-and-consequences-of-canine-eugenics/>. Acesso em: 7 out. 2017.

NADOLNY, R.; GAFF, H.; CARLSSON, J.; GAUTHIER, D. Comparative population genetics of two invading ticks: Evidence of the ecological mechanisms underlying tick range expansions. **Infection, Genetics and Evolution**, v. 35, p. 153–162, 2015.

NORRIS, D. E.; KLOMPEN, J. S.; KEIRANS, J. E.; BLACK, W. C. Population genetics of Ixodes scapularis (Acari: Ixodidae) based on mitochondrial 16S and 12S genes. **Journal of medical entomology**, v. 33, n. 1, p. 78–89, 1996.

OGRZEWALSKA, M.; BAJAY, M. M.; SCHWARCZ, K.; BAJAY, S. K.; TELLES, M. P. C.; PINHEIRO, J. B.; ZUCCHI, M. I.; PINTER, A.; LABRUNA, M. B. Isolation and characterization of microsatellite loci from the tick *Amblyomma* aureolatum (Acari: Ixodidae). **Genetics and Molecular Research**, v. 13, n. 4, p. 9622–9627, 2014.

OGRZEWALSKA, M.; PACHECO, R. C.; UEZU, A.; RICHTZENHAIN, L. J.; FERREIRA, F.; LABRUNA, M. B. Ticks (Acari: Ixodidae) infesting birds in an Atlantic rain forest region of Brazil. **Journal of Medical Entomology**, v. 46, n. 5, p. 1225–1229, 2009.

OGRZEWALSKA, M.; SARAIVA, D. G.; MORAES-FILHO, J.; MARTINS, T. F.; COSTA, F. B.; PINTER, A.; LABRUNA, M. B. Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. **Parasitology**, v. 139, n. 10, p. 1283–1300, 2012.

OGRZEWALSKA, M.; UEZU, A.; JENKINS, C. N.; LABRUNA, M. B. Effect of forest fragmentation on tick infestations of birds and tick infection rates by Rickettsia in the Atlantic Forest of Brazil. **EcoHealth**, v. 8, n. 3, p. 320–331, 2011.

OLIVEIRA, C. S.; BRAUNIG, P.; KRAWCZAK, F.; LABRUNA, M. B.; BOTTON, S. A.; VOGEL, F. S. F.; SANGIONI, L. A. Detecção de proteínas imunorreativas de *Rickettsia* sp. Cepa Mata Atlântica. **Pesquisa Veterinaria Brasileira**, v. 37, n. 1, p. 52–57, 2017.

OSTRANDER, E. A.; WAYNE, R. K. The canine genome. **Genome Research**, v. 15, n. 12, p. 1706–1716, 2005.

PADDOCK, c d; SUMNER, w j; COMER, j a; ZARI, s r; GOLDSMITH, c s; GODDARD, J.; MCLELLAN, s l f; TAMMINGA, C. l; OHL, C. A. *Rickettsia parkeri*:a newly recognized cause of spotted fever rickettsiosis in the United States. **Clinical Infectious Disease**, v. 38, n. 6, p. 805–811, 2004.

PADDOCK, C. D.; ALLERDICE, M. E. J.; KARPATHY, S. E.; NICHOLSON, W. L.; LEVIN, M. L.; SMITH, T. C.; BECKER, T.; DELPH, R. J.; KNIGHT, R. N.; RITTER, J. M.; SANDERS, J. H.; GODDARD, J. Unique Strain of Rickettsia parkeri Associated with the Hard Tick Dermacentor parumapertus Neumann in the Western United States. **Applied and Environmental Microbiology**, v. 83, n. 9, p. 03463-16, 2017.

PARKER, R.; KOHLS, G.; COX, G. W.; DAVIS, G. E. Observations of an infectious agent from *Amblyomma maculatum.* **Public Health Reports**, v. 54, n. 32, p. 1482–1484, 1939.

PAROLA, P.; PADDOCK, C. D.; SOCOLOVSCHI, C.; LABRUNA, M. B.; MEDIANNIKOV, O.; KERNIF, T.; ABDAD, M. Y.; STENOS, J.; BITAM, I.; FOURNIER, P.-E.; RAOULT, D. Update on tick-borne rickettsioses around the world: a geographic approach. **Clinical microbiology reviews**, v. 26, n. 4, p. 657–702, 2013.

PASSOS, L. M. F.; GEIGER, S. M.; RIBEIRO, M. F. B.; PFISTER, K.; ZAHLER-RINDER, M. First molecular detection of Babesia vogeli in dogs from Brazil. **Veterinary Parasitology**, v. 127, n. 1, p. 81–85, 4 jan. 2005.

PESTANA. BR. O nambiuvú. **Revista Médica de São Paulo**, v. 22, p. 423–426, 1910.

PINTER, A.; HORTA, M. C.; PACHECO, R. C.; MORAES-FILHO, J.; LABRUNA, M. B. Serosurvey of Rickettsia spp. in dogs and humans from an endemic area for Brazilian spotted fever in the State of São Paulo, Brazil. **Cadernos de Saúde Pública**, v. 24, n. 2, p. 247–252, 2008.

PRUGH, L. R.; STONER, C. J.; EPPS, C. W.; BEAN, W. T.; RIPPLE, W. J.; LALIBERTE, A. S.; BRASHARES, J. S. The Rise of the Mesopredator. **BioScience**, 2009.

RANGE, F.; VIRÁNYI, Z. Tracking the evolutionary origins of dog-human cooperation: The “Canine Cooperation Hypothesis”. **Frontiers in Psychology**,

v. 5, n. OCT, p. 1–10, 2014.

ROCHA-MENDES, F.; NEVES, C. L.; NOBRE, R. de A.; MARQUES, R. M.; BIANCONI, G. V.; GALETTI, M.; ROCHA-MENDES, F.; NEVES, C. L.; NOBRE, R. de A.; MARQUES, R. M.; BIANCONI, G. V.; GALETTI, M. Non-volant mammals from Núcleo Santa Virgínia, Serra do Mar State Park, São Paulo, Brazil. **Biota Neotropica**, v. 15, n. 1, 2015.

SABATINI, G. S.; PINTER, A.; NIERI-BASTOS, F. A.; MARCILI, A.; LABRUNA, M. B. Survey of ticks (Acari: Ixodidae) and their rickettsia in an Atlantic rain forest reserve in the State of São Paulo, Brazil. **Journal of medical entomology**, v. 47, n. 5, p. 913–6, 2010.

SANDRO, A.; FONSECA, S. Caiçaras frente à mudança : transformações na Comunidade de Camaroeiro em Caraguatatuba City / SP (1950-2010). **Cadernos do Ceom**, v. 27, n. 40, p. 1–19, 2010.

SARAIVA, D. G.; SOARES, H. S.; SOARES, J. F.; LABRUNA, M. B. Feeding Period Required by *Amblyomma aureolatum* Ticks for Transmission of *Rickettsia rickettsii* to Vertebrate Hosts. **Emerging Infectious Diseases**, v. 20, n. 9, p. 1504–1510, 2014.

SCHUMACHER, L.; SNELLGROVE, A.; LEVIN, M. L. Effect of *Rickettsia rickettsii* (Rickettsiales: Rickettsiaceae) Infection on the Biological Parameters and Survival of Its Tick Vector— *Dermacentor variabilis* (Acari: Ixodidae). **Journal of Medical Entomology**, v. 53, n. 1, p. 172–176, 2016.

SCINACHI, claudia A.; TAKEDA, gabriela A. C. G.; MUCCI, L. F.; PINTER, A. Association of the occurrence of Brazilian spotted fever and Atlantic rain forest fragmentation in the São Paulo metropolitan region, Brazil. **Acta Tropica**, v. 166, p. 225–233, 2017.

SHAO, R.; ZHU, X.-Q.; BARKER, S. C.; HERD, K. Evolution of Extensively Fragmented Mitochondrial Genomes in the Lice of Humans. **Genome Biology and Evolution**, v. 4, n. 11, p. 1088–1101, 2012.

SOARES, J. F.; DALL'AGNOL, B.; COSTA, F. B.; KRAWCZAK, F. S.; COMERLATO, A. T.; ROSSATO, B. C. D.; LINCK, C. M.; SIGAHI, E.K.O.; TEIXEIRA, R.H.F.; SONNE, L.; HAGIWARA, M. K.; GREGORI, F.; VIEIRA, M.I.B.; MARTINS, J. R.; RECK, J.; LABRUNA, M.B. Natural infection of the wild canid, Cerdocyon thous, with the piroplasmid Rangelia vitalii in Brazil. **Veterinary Parasitology**, v. 202, n. 3–4, p. 156–163, 28 maio 2014.

SOARES, J. F. História natural da rangeliose. **Tese de Doutorado**, p. 121, 2014.

SOARES, J. F.; GIROTTO, A.; BRANDÃO, P. E.; DA SILVA, A. S.; FRANÇA, R. T.; LOPES, S. T. A.; LABRUNA, M. B. Detection and molecular characterization of a canine piroplasm from Brazil. **Veterinary Parasitology**, v. 180, n. 3–4, p. 203–208, 2011.

SOARES, J. F.; SOARES, H. S.; BARBIERI, A. M.; LABRUNA, M. B. Experimental infection of the tick *Amblyomma* cajennense, Cayenne tick, with

Rickettsia rickettsii, the agent of Rocky Mountain spotted fever. **Medical and Veterinary Entomology**, v. 26, n. 2, p. 139–151, 2012.

SPOLIDORIO, M. G.; LABRUNA, M. B.; MACHADO, R. Z.; MORAES-FILHO, J.; ZAGO, A. M.; DONATELE, D. M.; PINHEIRO, S. R.; SILVEIRA, I.; CALIARI, K. M.; YOSHINARI, N. H. Survey for tick-borne zoonoses in the State of Espirito Santo, Southeastern Brazil. **American Journal of Tropical Medicine and Hygiene**, v. 83, n. 1, p. 201–206, 2010a.

SPOLIDORIO, M. G.; LABRUNA, M. B.; MANTOVANI, E.; BRANDÃO, P. E.; RICHTZENHAIN, L. J.; YOSHINARI, N. H. Novel Spotted Fever Group Rickettsiosis, Brazil. **Emerging Infectious Diseases**, v. 16, n. 3, p. 521–523, mar. 2010b.

SULLIVAN, A. P.; BIRD, D. W.; PERRY, G. H. Human behaviour as a long-term ecological driver of non-human evolution. **Nature Ecology & Evolution**, v. 1, n. 3, p. 65, 2017.

SZABÓ, M. P. J.; NIERI-BASTOS, F. A.; SPOLIDORIO, M. G.; MARTINS, T. F.; BARBIERI, A. M.; LABRUNA, M. B. In vitro isolation from *Amblyomma* ovale (Acari: Ixodidae) and ecological aspects of the Atlantic rainforest Rickettsia, the causative agent of a novel spotted fever rickettsiosis in Brazil. **Parasitology**, v. 140, n. 6, p. 719–728, 2013.

SZABÓ, M. P. J.; PINTER, A.; LABRUNA, M. B. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. **Frontiers in cellular and infection microbiology**, v. 3, p. 27, 2013.

TCHAICKA, L.; FREITAS, T. R. O. de; BAGER, A.; VIDAL, S. L.; LUCHERINI, M.; IRIARTE, A.; NOVARO, A.; GEFFEN, E.; GARCEZ, F. S.; JOHNSON, W. E.; WAYNE, R. K.; EIZIRIK, E.; TCHAICKA, L.; FREITAS, T. R. O. de; BAGER, A.; VIDAL, S. L.; LUCHERINI, M.; IRIARTE, A.; NOVARO, A.; GEFFEN, E.; GARCEZ, F. S.; JOHNSON, W. E.; WAYNE, R. K.; EIZIRIK, E. Molecular assessment of the phylogeny and biogeography of a recently diversified endemic group of South American canids (Mammalia: Carnivora: Canidae). **Genetics and Molecular Biology**, v. 39, n. 3, p. 442–451, set. 2016.

TORRES, P.; PRADO, P. Domestic dogs in a fragmented landscape in the Brazilian Atlantic Forest: abundance, habitat use and caring by owners. **Brazilian Journal of Biology**, v. 70, n. 4, p. 987–994, nov. 2010.

TRAPP, S. M.; DAGNONE, A. S.; VIDOTTO, O.; FREIRE, R. L.; AMUDE, A. M.; DE MORAIS, H. S. A. Seroepidemiology of canine babesiosis and ehrlichiosis in a hospital population. **Veterinary Parasitology**, v.140; n.4, p. 223-230, 2006. .

UDELL, M. A. .; WYNNE, C. D. . A Review of Domestic Dogs' (Canis Familiaris) Human-Like Behaviors: Or Why Behavior Analysts Should Stop Worrying and Love Their Dogs. **Journal of the Experimental Analysis of Behavior**, v. 89, n. 2, p. 247–261, 2008.

UWCT. **Domestication : the Evolution of the Dog**. Disponível em: <https://ukwct.org.uk/files/domestication.pdf> acessado em 20 de julho de 2017.

VERDADE, L. M.; CAMPOS, C. B. How much is a puma worth?: economic compensation as an alternative for the conflict between wildlife conservation and livestock production in Brazil. **Biota Neotropica**, v. 4, n. 2, p. 1–4, 2004.

VIEIRA, T. S.; VIEIRA, R. F.; NASCIMENTO, D. A.; TAMEKUNI, K.; TOLEDO, R. o. S.; CHANDRASHEKAR, R.; MARCONDES, M.; BIONDO, A. W.; VIDOTTO, O. Serosurvey of tick-borne pathogens in dogs from urban and rural areas from Parana State, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 22, n. 1, p. 104–109, 2013.

VIZEU, F. Rural heritage of early Brazilian industrialists: Its impact on managerial Orientation. **BAR - Brazilian Administration Review**, v. 8, n. 1, p. 68–85, 2011.

WAYNE, R. K.; GEFFEN, E.; GIRMAN, D. J.; KOEPFLI, K. P.; LAU, L. M.; MARSHALL, C. R. Molecular systematics of the Canidae. **Systematic biology**, v. 46, n. 4, p. 622–53, 1997.

WEINERT, L. A.; WERREN, J. H.; AEBI, A.; STONE, G. N.; JIGGINS, F. M. Evolution and diversity of Rickettsia bacteria. **BMC Biology**, v. 7, n. 1, p. 6, 2009. Disponível em: <http://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-7-6>.

YANG, Z.; NIELSEN, R. Estimating Synonymous and Nonsynonymous Substitution Rates Under Realistic Evolutionary Models. **Molecular Biology and Evolution**, v. 17, n. 1, p. 32–43, 1 jan. 2000.

ZRZAVÝ, J.; ŘIČÁNKOVÁ, V. Phylogeny of Recent Canidae (Mammalia, Carnivora): relative reliability and utility of morphological and molecular datasets. **Zoologica Scripta**, v. 33, n. 4, p. 311–333, 1 jul. 2004.

CHAPTER 1

Role of the domestic dog in the epidemiology of *Rickettsia parkeri* strain Atlantic rainforest e *Rangelia vitalii* in the Brazilian Atlantic rainforest area of São Paulo State, Brazil

Role of the domestic dog in the epidemiology of *Rickettsia parkeri* strain Atlantic rainforest e *Rangelia vitalii* in the Brazilian Atlantic rainforest area of São Paulo State, Brazil

Gislene F. S. R. Fournier[1]; Adriano Pintér[2]; Jonas Morais-Filho[1]; Thiago Fernandes Martins[1]; Marcos Gomes Lopes[1]; Luciana Colácio[3]; Cayo R. S. Môra[4]; Marcelo Bahia Labruna[1]; Ricardo A. Dias[1].

Department, Institution, City, State, Country:

1 - Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia Universidade de São Paulo, São Paulo, Brazil

2- Superintendência de Controle de Endemias de São Paulo, São Paulo, Brazil

3 - *COLLIS Laboratório* de Análise Clínica, Caraguatatuba City, São Paulo, Brazil

4 - Parque Estadual Serra do Mar- Núcleo Caraguatatuba, Caraguatatuba City, São Paulo, Brazil

***** Corresponding author:
E-mail: gislene.fatima@gmail.com

Abstract

We assessed the risk factors related to the presence of domestic dogs in forest areas and the relationship between dogs and their owners. Our study aims to determine the presence of Tick-Borne-Diseases (TBDs) in three different areas around the Serra do Mar State Park, in Caraguatatuba City. We evaluated the aspects between the way of life of the owners and their relationship with their dogs. A total of 61 dogs have been monitored for three years through blood and

tick collections, interviews, radio transmitters and camera traps. In this study, we observed that companion dogs had an infection risk by *R. parkeri* strain Atlantic rainforest 5.4 times higher than those not considered as companions (p=0.009). We also showed that dogs that had at least one *A. ovale* individual collected during the campaigns had a risk of *R. parkeri* strain Atlantic rainforest 10.0 times higher than those who did not have *A. ovale* collected (p= 0.009). Moreover, we identified a positive dog for *R. vitalii* through real-time TaqMan PCR (qPCR) that was parasitized by *A. ovale* in three of the six monitoring campaigns. In addition, we sequenced all positive ticks by conventional PCR for the OmpA2 gene. The positive DNA samples for the OmpaA2 gene that have been sequenced gave 100% of identities of *Rickettsia* sp. strain Atlantic rainforest clone As106 deposited in the GenBank. In summary, our findings demonstrate that dogs that have access to the forest are subject to *A. ovale* infestation and zoonoses related to this parasite. In this context, the control or eradication of domestic dogs in forests and the reduction of zoonoses risks depends strictly on human intervention.

Introduction

The presence of the most common canid in the world, the domestic dog *Canis lupus familiaris*, in forested areas has been registered in several parts of the world [1–4]. In the last decade researchers described not only the dog invasion, but also, how and in which situations the invasion is more deleterious [5,6].

Dogs can play different roles in human society from hunter, shepherd, guardian or companion, to even more affective relationships in which the dog is considered as a family member [7,8]. The epidemiological importance of the dog is highly dependent not only on the role that this animal plays on the life cycles of parasites and pathogenic agents, but also in which environment it lives [9–11].

In urban-forest interfaces, generally in low-income areas of Brazil, dogs can be found sharing the same space, feeding with leftover food of the family or even receiving industrialized dog food, but rarely as a guardian dog or as a pet animal [7]. These animals are not often treated with affection from their owners and rarely or never receive veterinary care [7]. In general, these dog owners are unable to explain why they keep their animals.

Dogs are competent hosts of wild ticks involved in ricketsiosis and rangeliosis, two important tick-borne-diseases (TBDs) typical of wild animals that are present today in anthropic areas [12–14]. In forested environments, ticks of the *Amblyomma* genus are commonly found parasitizing the domestic dog [11,15–18]. In areas of the Atlantic Forest, domestic dogs have been described as hosts of the species *Amblyomma ovale*, vector of an agent causing Spotted Fever (SF) [19–21]. The SF is caused by bacteria of the *Rickettsia* genus belonging to the Spotted Fever Group (SFG), composed of more than 20 species and subspecies of pathogenic bacteria [22].

In Brazil, five species have been described in the last decade: *Rickettsia rickettsii*, *Rickettsia riphicephali, Rickettsia amblyommatis, Rickettsia felis* and *Rickettsia parkeri* [16,23–25]. Among these, *R. rickettsii* and *R parkeri* are the major causes of symptomatic disease in humans [22,26]. *R. parkeri* has been first isolated in *Amblyomma maculatum* on the Gulf Coast of the United States of America in 1973, being considered non-pathogenic [27]. However, in 2004 it has been considered as a mild or moderate pathogen, similar to the Rock Mountain Spotted Fever in some countries of the American continent [28].

In 2010, in São Paulo State, a clinical condition similar to that caused by *R. parkeri* has been described in humans and a new strain has been isolated and called *Rickettsia* sp. strain Atlantic rainforest due to its phylogenetic similarity with the species *R. parkeri, Rickettsia africae* e *Rickettsia sibirica* [29].

Later on, through a serological, molecular and phylogenetic survey using five concatenated genes of the genus *Rickettsia,* researchers grouped *Rickettsia* sp. strain Atlantic rainforest and *Rickettsia* sp. Black Gap strain, described in Texas, in a distinct clade and well supported with *R. parkeri* (PADDOCK et al., 2017). Thus, to date, these two strains are believed to form a single lineage of *R. parkeri* associated predominantly or exclusively with ticks of the genus *Amblyomma*. From research, the appropriate denomination for the Brazilian strain is *Rickettsia parkeri* strain Atlantic rainforest (PADDOCK et al., 2017).

The species of ticks described so far naturally infected with *Rickettsia parkeri* strain Atlantic rainforest are *Amblyomma aureolatum*, *A. ovale* and *Rhipicephalus sanguineus*, the latter not being responsible for transmission to humans [14,21,23,26].

Currently, *A. ovale* is considered the main vector of *R. parkeri* strain Atlantic rainforest in São Paulo State [30,31]. In majority of cases of the ricketsiosis registered in São Paulo City, dogs are closely related to the transport of ticks from the forest into the houses and, consequently, transmitting the pathogenic agent to humans [32].

Rangeliosis is another neglected disease described in several areas of Southern and Southeast Brazil [33–36]. This is caused by the protozoan *Rangelia vitalii*, which still lacks proper taxonomic position and, still today, is described as a species belonging to the Babesidae family because of its genetically and morphologically relation to the hemoprotozoa of this family. Until the time of writing, few studies about the presence of this parasite in canine populations and their prevalence have been published [34,35]. Possibly, this disease is well distributed in the South and Southeast regions of Brazil and its clinical signs may have been confused with *Babesia canis* infections [37–39].

Currently, *R. vitalii* is considered to be naturally maintained by wild canids having as possible vectors *A. ovale, A. aureolatum, Amblyomma tigrinum* and *Amblyomma sculptum*. All these species are described as wild species [33,35].

Considering that today, the role of labor dogs is shifting to companion dogs and, if they gain access to both forest and the interior of the houses, they would become an issue for environmental balance and public health.

Shortly, dogs may share pathogens with wildlife, especially if neglected by their owners. To understand the epidemiological participation of these animals in the surroundings of a Brazilian Atlantic rainforest protected area, we conducted an investigation through interviews, blood sampling, telemetry data, photographic records and active search for ticks in the forest for a three years period in three different areas of Caraguatatuba City seaside, located in São Paulo State, Brazil.

Methods

Study area

Caraguatatuba City is located in the north seacoast of São Paulo State, at about 180km eastward from São Paulo City (Latitude: 23° 37' 21'' Sul, Longitude: 45° 24' 43'' W), the most populous Brazilian city. The invasion of protected forest areas by illegal dwellings and, consequently, deforestation is very common in the area [40,41].

Historically, the city grew around an area of native rain forest called Serra do Mar [41]. With tectonic geomorphological formation of the Tertiary period, the region has sedimentary plains cut by flowing rivers and small streams [42]. Initially occupying these plains, human households gradually started to occupy the slopes of the mountain range.

In 1967, Caraguatatuba City suffered one of the biggest natural catastrophe in Brazil, when a massive storm caused the slopes to slip across the city, destroying it almost completely [41].

Few years after the tragedy, the population reestablished and irregular dwellings on the slopes of the mountains restarted. In order to preserve the slopes of the native Brazilian Atlantic rainforest, the Serra do Mar State Park has been created in 1977, with an area of 332.000 ha, covering several municipalities of São Paulo State. However, the illegal settlement in the protected areas still exists, caused both all types of socio-economical population [43].

Study areas selection - The geographical limits of the Serra do Mar State Park were obtained with the Brazilian Ministry of Environment, and a thematic map was produced using QuantumGIS software. Three distinct study areas were selected according to the following criteria: topographic gradient (from sedimentary plains to mild and high slope mountain) and accessible roads. After this stage, a preliminary campaign has been carried out in July 2014 to evaluate the following factors:

- Area history (expropriation, cultivation, irregular and regular housing, social class of residents);

- Presence of dogs in four conditions: supervised (owned and restricted), partially supervised (owned and let roam freely), unsupervised (without defined owner) or stray dogs.

Sampling

Sampling of Dogs

During the first campaign, in June 2015, a qualitative questionnaire has been applied to the dog owners in order to define the profile of each dog and its role in the human community. To achieve this, the following questions were made:

a. Does the dog never/sometimes/always stay at home?
b. Does the dog have access to the forest when it is free roam?
c. Does the dog have access to the interior of the house?
d. Was the dog vaccinated against rabies?
e. Was the dog sterilized (spayed or neutered) or receives contraceptive drugs?
f. Does the dog wear a collar or any method to eliminate ectoparasites?
g. Is the dog used as a guardian of the house?
h. Is the dog used as a hunter dog?
i. Is the dog considered as a companion?

As inclusion criteria, the dogs should be aged at least one year, born and raised in the region, as previously described [25]. In each study area a cohort of 20 dogs have been sampled approximately every 2 months, totalizing six campaigns until April 2016. During each visit, the dogs were evaluated with the presence of ticks and a blood sample was collected for molecular tests on blood clots for *R. vitalii*.

Serum was extracted and used in the Indirect Immunofluorescence Assay (IIFA) aimed antibodies anti-*R.parkeri*.

Telemetry Data

The SMS trackers GPS-TK102B (CU-TEK®) is an equipment containing GPS and GSM modules, which collect information from the satellites and sending to a cell phone the current position through latitudes and longitudes.

Six GPS Tracker attached to collars were installed on dogs from the three study areas, being two equipment per area. Only dogs that had access to the forest were selected (free dogs). These dogs have been monitored weekly during the months of October and December 2015.

Each dog remained with the tracker for a maximum of seven days and after that period, the tracker was removed and installed on another dog previously selected to receive the device. In this way, four dogs from each area were monitored.

During the monitoring period, the devices have been programmed to send a message with the geographical coordinates every 30 minutes with the exact position of each monitored animal from 6pm to 8am on the following day.

Photographic Records

From December 2015 to August 2016, four cameras traps HD Auto-Focus Nature View(BUSHNELL®) have been used to record the movements of wild and domestic animals in the trails used by the tracker-monitored dogs. In areas 1 and 3, a camera has been installed at the edge of the forest (acronym E) and a second one distanced from the first by 300 meters inside the forest (acronym I) in the trails used by the dogs.

Every 25 days, the batteries were replaced and the recorded videos were downloaded and analyzed. Photographic records made by two cameras traps, also installed on the edge and inside the forest, have been also included by other researchers working in the area in the period of 2016-2017 (unpublished data from Mora et. Al., 2017).

Indirect Immunofluorescence Assay (IIFA)

Dog serum samples were tested against antigens of *R. parkeri* (strain At24) to detect reactive antibodies by the Indirect Immunofluorescence Assay (IIFA), as previously described [44]. Briefly, serums were diluted in phosphate buffered saline (PBS) and screened at a dilution of 1:64 on 12-well antigen slides.

The slides were incubated, washed, then incubated with fluorescein isothiocyanate-labeled conjugate with anti-dog immunoglobulin G (IgG) (Sigma Diagnostics, St. Luis, MO, USA) and washed again, mounted with buffered glycerin, and read using an ultraviolet microscope (BX60; Olympus Corp., Tokyo, Japan) at 400× magnification. In each slide, it has been used a dog serum previously tested to be non-reactive as a negative control and a known reactive dog serum as a positive control.

Serum samples reacting at the screening dilution were tested in twofold serial dilutions to determine the end point titer.

Molecular tests

Extration - The DNA was extracted from blood clots of samples collected from each dog and from the ticks collected on these animals using a commercial DNA extraction kit (QIAamp DNA Mini Kit- tissue and blond®) according to the manufacturer’s instructions (Qiagem) and eluted in 50 μL of buffer that comes with the extraction kit.

Molecular tests on Blood clots - Research of *R. vitalii.* To detect the *R. vitalii* DNA, it has been used a TaqMan real-time PCR system (qPCR) targeting a portion of the gene corresponding to a 179pb fragment amplification of the hsp 70 gene of *R. vitalii*. The selected primers were: Sense Rv751-770 and Antisense Rv930-911 and the specific probe of *R.vitalii* in a concentration of 25 µmol / L, as previously described [37].

For all PCR assays, positive controls were included (blood sample dog experimentally infected and previously tested) and negatives (Autoclaved and DNA-free Milli-Q water).

Molecular tests on collected ticks - Research of *Rickettsia* spp. For *Rickettsia* spp. analysis, each DNA sample was tested by PCR using a pair of primers (CS-78 and CS-323) that amplify a fragment of 401 base pairs (bp) of the gltA gene which is present in all species of genus *Rickettsia* [45].

Positive samples for this gene were tested for a second PCR using a pair of primers (Rr190.70F and Rr190.701R) that amplify a 632bp fragment of the ompA gene, present only in *Rickettsia* ssp. of the SFG as previously described [46,47]. Negative controls were used for each reaction (Autoclaved and DNA-free Milli-Q water) and positive (infected dog serum by *R.parkeri* strain Atlantic rainforest).

Statistical analysis

The nonparametric Kruskal-Wallis test was used to compare the antibodies anti-*R. parkeri* titers between the study areas per campaign. The nonparametric Mann-Whitney test was used to test the heterogeneity among the following variables: dog's role; collection area; and parasitism by *A. ovale* per campaign.

The possible risk factors to the *R. parkeri* infection in dogs were obtained through an univariate analysis using qui-square test, implemented in function chiqs.test() of R. After that, a multivariate analysis was performed using the “enter" method through the glm() function of R. Correlation between variable categories was tested and those with higher association with the independent variable in the univariate analysis were submitted to the multivariate analysis.

Results

Landscape Analysis

The study areas were different in relation to the relief and human occupation. Area 1 was characterized by steep relief, up to 190 meters above sea level. This configuration gives considerable complexity to this area, creating restrictions on land use and occupation. Area 2 was located in the public visitation area of the Park and was classified as a coastal plain, about twelve meters above sea level. Area 3 was represented by a coastal plain of the park occupied by a middle-class condominium of holiday homes. The maximum altitude of this area was twenty meters above sea level.

The human occupation in each area is different. Area 1 presented an irregular occupation, where density diminishes with the slope increase, and with a low-income population occupying the whole area. Lacks of basic infrastructure, disordered occupation, construction of new houses without permission and without inspection from the responsible public agencies. The presence of illegal households inside the perimeter of the State Park has been observed.

Most of the residents have informal jobs or perform subsistence agriculture and cattle breeding. The presence of small plantations at the edge of the forest is frequently observed. No sewer system is available, and most of the houses had a badly planned septic tank. The drinking water comes from small springs without any sanitary treatment.

Area 2 is equally unorganized. Although, it has received some urban interventions such as paved streets and retaining walls and drainage. This was the most affected area during the 1960's landslide catastrophe. After that period, a rapid reoccupation around the Serra do Mar State Park took place. The residents went to the region in search of housing and jobs generated by tourism. Most of the houses are around a river, which receives the sewage without any previous treatment.

Unlike the other two areas, Area 3 presents dwellings in flat land with proper infrastructure. Even though the houses have been built on the edge of the forest, there are walls or fences delimiting the land. There is a private concierge with security guards and all residents must pay a condominium fee. There is garbage collection, access pavement, retaining walls (when necessary) and division of terrains ranging from 180 to 1,000 m^2.

Profile of monitored dogs and their owners in each area

Dogs from each area exhibited three different roles in the community: hunting, guarding and company in different proportions. These three roles were associated with the socioeconomic and cultural profile of the owners. All the dog owners of Area 1 perform extractive activities in the forest and have small plantations on the edge of it. In Area 2, only one dog owner used the forest edge for plantation. In Area 3, no dog owners used the forest except for leisure (Table 1).

Table 1 – Results obtained through the questionnaire applied to dog owners, where the values for the positive responses are represented. It was considered 26 dogs for area 1, 25 for Area 2 and 27 for Area 3. Caraguatatuba City – SP, Brazil.

Dog owner response	Area 1 (n = 26)	Area 2 (n = 25)	Area 3 (n = 27)
Dog gets loose (off the property) all the time.	24 (92%)	9 (36%)	7 (26%)
Dog gets loose only at certain times of the day.	0 (0%)	11 (44%)	11 (40,7%)
The dog never gets loose.	2 (7,7%)	3 (12%)	6 (22,2%)
The dog has access to the forest during the period in which he is loose.	24 (92%)	18 (72%)	16 (59,3%)
The dog has access to the interior of the house.	10 (38,5%)	13 (52%)	16 (59,3%)
The dog was vaccinated during the monitoring year.	15 (57,7%)	23 (92%)	26 (96,3%)
The dog is neutered or uses contraceptive drugs.	0 (0%)	5 (20%)	13 (48,1%)
The dog uses a collar or some method to eliminate ectoparasites.	0 (0%)	2 (8%)	20 (74,0%)
The dog is used as guardian of the house.	19 (73,1%)	21 (84%)	10 (37,1%)
The dog is used as a hunter.	17 (65,4%)	0 (0%)	0 (0%)
The dog is considered as a companion dog.	6 (23,0%)	60%(15)	19 (70,4%)

Telemetry data

Four dogs from each area were monitored through this methodology. One dog from the Area 1 and one from Area 3 were registered up to 300 meters from the edge, inside the forest, where camera traps were subsequently installed (Figure 1). The edge of the forest overlays the protected area limits. The other two dogs were registered close to urban areas. In certain regions, the SMS tracker was unable to return with the result of its location.

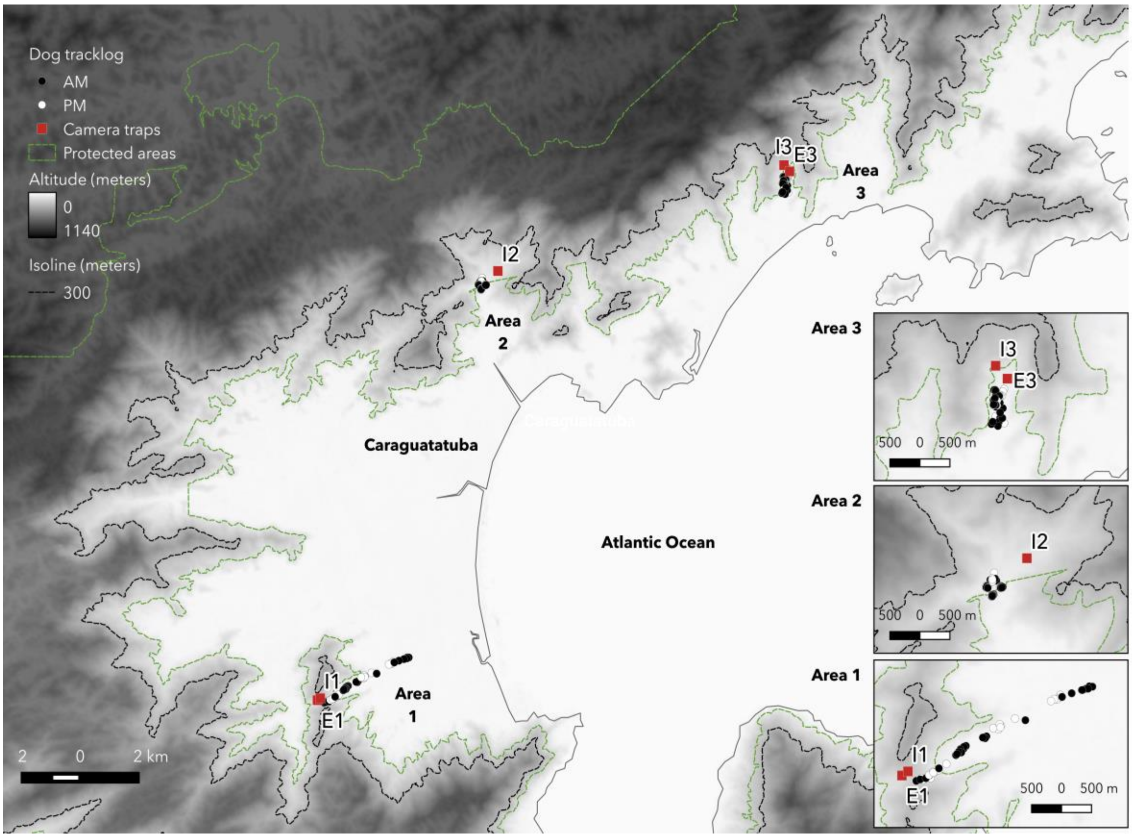

Figure 1. Maps of monitored areas with tracking locations of dogs monitored by GPS collar during the period of monitoring and location of the cameras in 2015 and 2016. Caraguatatuba City – SP, Brazil

Photographic Records

During the study period, 2,855 photos and 156 videos have been recorded by the camera traps. Among these, 38 records were considered as being of medium and large mammals (≥1,0 kg), 22 of which were carnivorous: *Eira barbara*, *Nasua nasua*, *Leopardus pardalis*, *Leopardus* sp., *Puma concolor* and *C. lupus familiaris*.

People were also registered walking in the woods along with their dogs in Area 1 and Area 3. In all areas, records of domestic dogs were only obtained by cameras positioned close to the edge of the forest (camera trap acronyms E1 and E3). However, records of dogs were obtained only in Area 1 by the camera positioned inside the forest (camera acronym I1, Figure 1). All records of dogs alongside humans in Area 1 occurred during the day (AM, Figure 1). On the other hand, dogs registered in Area 3 were recorded at weekends or holidays alongside people, who were probably hiking (Table 2).

Table 2. Dogs registered through camera trap in each area during the monitoring period (2015 to 2016), Caraguatatuba City – SP, Brazil

Area	Animal	Day of the week	Time	Trap 1 (forest edge)	Trap 2 (forest interior)	Humans presence
Area 1	dog 33	Tuesday	14:58		X	
	dog 33	Thursday	13:53	X		
	dog 33	Thursday	18:10	X		
	dog 33	Thursday	6:57	X		
	dog 33	Friday	12:47	X		
	dog 33	Saturday	11:11	X		X
Area 2	dog 42	Friday	14:43	X		
Area 3	dog 7	holidays	13:37	X		X
	dog 7	Saturday	11:20	X		X

Collection and identification of ticks

In total, 61 dogs were investigated (19 in Area 1, 24 in Area 2 and 18 in Area 3). A total of 155 ticks *A. ovale* were collected in Area 1, 15 individuals in Area 2 and 12 in Area 3 throughout the study period. (Figure 2).

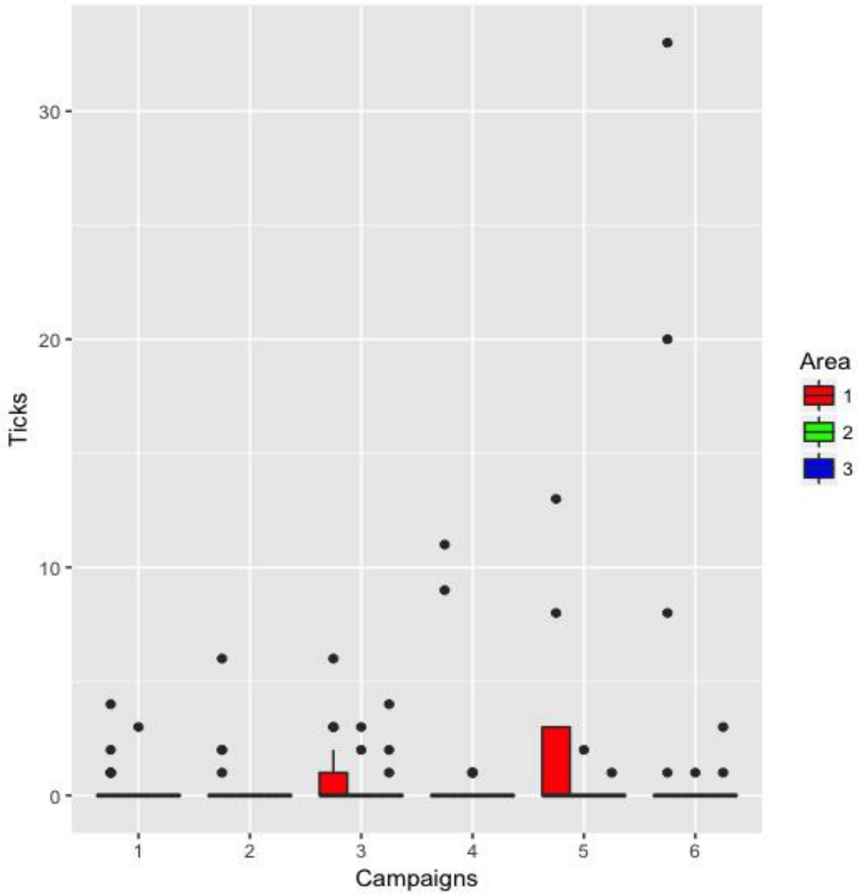

Figure 2 - Distribution of the tick *A.* ovale counts collected in the dogs, by campaign and by area between 2015 and 2016 in Caraguatatuba City - SP, Brazil.

IIFA results for *Rickettsia* sp. in dogs

In total, 61 dogs were selected, being 19 in Area 1, 24 in Area 2 and 18 in Area 3. The dogs were monitored in six different campaigns that occurred in June 2015 (campaign 1), August 2015 (campaign 2), October 2015 (campaign 3), December 2015 (campaign 4), February 2016 (campaign 5) and April 2016 (campaign 6). These dogs were visited in all campaigns and when a dog was withdrawn from the cohort for any reason, whenever possible, a dog with the same profile was included in the study (Table 3).

Table 3. Dogs tested for anti-*Rickettsia* sp. antibody detection during 12 months of monitoring around the Serra do Mar State Park (2015-2016), Caraguatatuba City – SP, Brazil.

Season			Dogs evaluated by campaign	Dogs parasitized by *A. ovale*	Ticks collected	Positive dogs (p≥64)	Medians of the IIFA	Comparison of Titers by area in each campaign
Dry season	campaign 1 (61 dogs)	A1	19	3	3	10	64	P =0.03
		A2	24	1	3	3	0	
		A3	18	0	0	5	0	
	campaign 2 (58 dogs)	A1	17	3	5	14	256	P =0.01
		A2	20	0	0	8	0	
		A3	21	0	0	9	0	
	campaign 3 (54 dogs)	A1	16	7	15	15	384	P =0.00
		A2	20	2	5	12	64	
		A3	18	3	7	6	0	
Wet season	campaign 4 (49 dogs)	A1	11	2	20	10	512	P =0.01
		A2	18	4	4	11	64	
		A3	20	0	0	9	0	
	campaign 5 (51 dogs)	A1	16	7	32	15	2048	P =0.00
		A2	16	1	1	15	256	
		A3	18	1	1	13	64	
	campaign 6 (57 dogs)	A1	19	3	54	18	1024	P =0.00
		A2	19	1	1	11	64	
		A3	19	2	4	9	0	

Antibodies anti-*Rickettsia sp.* were detected (titers ≥ 64) in 91,3% (21/23) of the Area 1 dogs; 72,2% (13/18) of the Area 2 dogs and in 73,9% (17/23) of the Area 3 dogs. A total of 29 dogs became seropositive at some point during the study and 12 remained with titers below 64. The area that presented the highest number of seropositive dogs was Area 1, followed respectively by Areas 3 and 2. Area 1 showed a difference in antibody titers from samples considered positive (≥64), using the Kruskal-Wallis test, in relation to the other areas in each campaign (Figure 3).

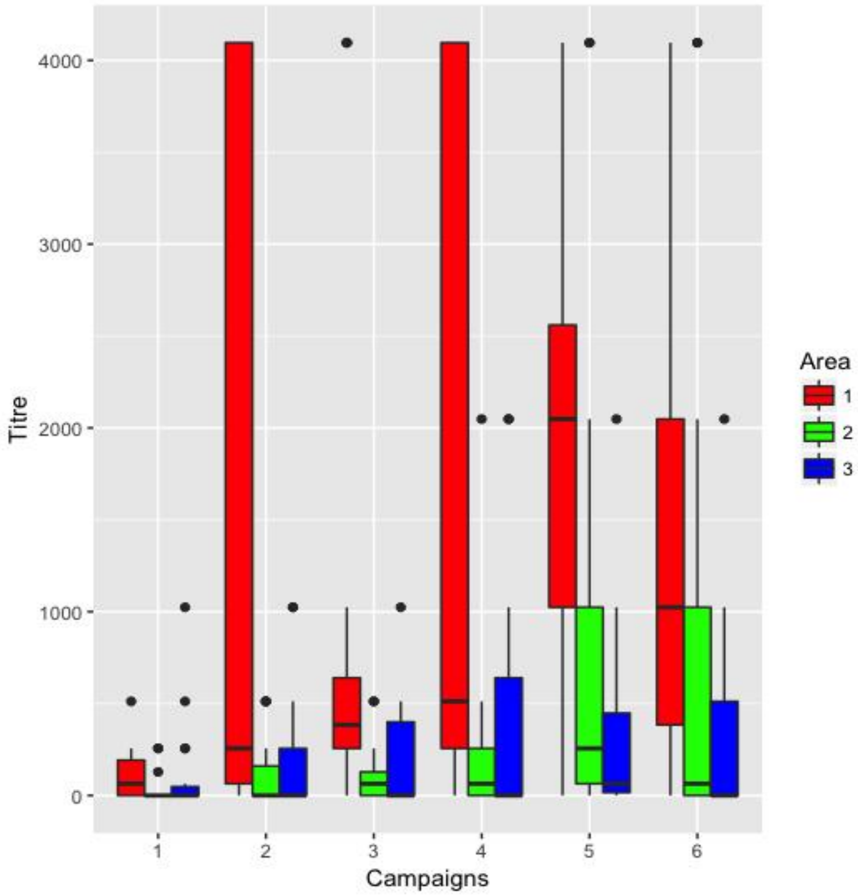

Figure 3 – Distribution of antibody anti-*Rickettsia* sp. titers by IIFA by campaign (2015-2016) and by collection areas in Caraguatatuba City – SP, Brazil.

Dogs that had at least one individual of *A. ovale* tick collected during the campaigns had the risk of infection by *Rickettsia* ten times greater than those who did not have this tick species collected on (p=0.009). It was also observed that companion dogs had a risk of *R*ickettsia sp. infection 5.4 times higher than non-companion dogs (p=0.009) (Table 4).

Table 4. Statistical analyzes in relation to results obtained through the IIFA showing results of the Univariate analysis, Multivariate analysis and Odds Ratio found.

Variables	Univariate analysis Mann-Whitney test (p)	Multivariate analysis glm() (p)	Odds Ratio and confidence interval (95%)
Collection area (A1,A2 e A3)	0.117		-
Collection of ticks in at least one campaign	0.007	0.183	10,05(2;10;76;47)
Guard dog	0.222	0.794	-

Companion dog	0.079	0.043	5,39 (1,61,20,32)
Dog with access to the forest	0.002	0.312	-
Dog without restriction of movement (free)	0.008	0.289	3,09 (0,88,12,01)
Dog occasionally free (stuck at some point of the day)	1	-	-

Molecular tests on blood clots - search of *Rangelia vitalii*

To perform the molecular tests for *R. vitalii*, the clots from the collected blood samples were submitted to the IIFA for *Rickettsia* sp. Only one dog from Area 1 has been controlled as positive for *R. vitalii* during campaign 3 and 6. This animal was also positive for IIFA for *Rickettsia* sp. and was parasitized by *A. ovale* in three monitoring campaigns. This dog had free access to the forest and was classified as hunter and as companion. The owner of this dog cultivated small crops at the edge of the forest and carried out extractive activities in the forest for subsistence.

Molecular examinations of dogs ticks

It has been obtained 35 copies of *A. ovale* amplified fragments by PCR analyzes for the gltA gene and these copies were subjected to a new PCR for the *omp*A *Rickettsia* gene. Ten ticks collected in seven different dogs (five dogs from Area 1 and two from Area 3) had amplified fragments through PCR analyzes for the *omp*A gene (Table 5). These ten samples have been sequenced and all of them were 100% identical with other *R. parkeri* strain Atlantic rainforest clone Ad106 outer membrane clones.

Table 5 - Dogs that had positive ticks for the gltA and ompA genes in each area per campaign in the monitoring period (2015-2016), Caraguatatuba City – SP, Brazil.

Dog ID	IFA	Area	Number of positive ticks per campaign						ompA	Total
			C1	C2	C3	C4	C5	C6		
0039	Positive	1	0	0	0	0	3	0	amplified	3
2954	Positive	1	2	0	0	2	3	0	amplified	7
0036	Positive	1	0	1	0	0	0	0	-	1
2968	Positive	1	0	0	5	0	0	0	amplified	5
2966	Positive	1	0	0	1	0	0	0	-	1
2967	-	1	0	0	3	0	0	0	amplified	3
2960	Positive	1	0	0	0	0	3	0	amplified	3
0042	Positive	1	0	0	0	0	2	0	-	2
0048	Positive	1	0	1	0	0	0	1	-	2
3090	Positive	2	1	0	0	0	0	0	-	1
7087	Positive	2	0	0	0	1	0	0	-	1
3008	Positive	3	1	0	0	0	0	0	amplified	1
3108	Positive	3	1	0	2	0	0	0	amplified	3
2964	Positive	3	0	0	2	0	0	0	-	2
Total positive ticks			**5**	**2**	**13**	**3**	**11**	**1**	**-**	**35**

Discussion

The harms caused by dogs in the forests have been much discussed [1,2,4,48,49]. Several authors have placed the dog as an animal highly adaptable to wildlife and that can easily develop feral habits [6,50,50]. In our results, we observed that the relation of the dog to the wild environments is strongly correlated to the owners' way of life. In this scenario, not necessarily, a dog that has free access to the forest will develop wild habits. We observed that dogs, when living freely, tend to walk into the forest only through the same area used by their owners, spatially and temporally.

In Area 1, where forest use is more intense because of nearby villagers, who develop small crops and use the forest for extractivism, the presence of *Rickettsia* sp. seropositive dogs is greater than in the other two areas (Area 1, n = 91.3%, Area 2, n = 72,2% e Area 3, n = 73,9%). In Area 1, dogs, in their huge majority (n =92%), live freely and are able to go into the forest at any time. The human presence inside the forest was associated with the way of life of the people who inhabit this environment.

Accordingly, with the camera trap data and through our observations in the field, dogs tend to walk alongside their owners into the forest and to stay close to their owners through time.

In a recent study conducted in areas of Brazilian Atlantic rainforest, it was observed that visit of dogs in agroforestry areas were associated with the time and days in which humans were active in the same site [6]. In our results, this association also appears to occur for Area 1, where dogs go along with their owners during moments of hunting or extractive activities and culture on the forest edges.

Even though they were a few telemetry recorders installed on the dogs, it was possible to observe that dogs tend to stay at home with their owners at night. However, in certain regions where the operator signal was weak or the GPS signal was not strong enough, the tracker was unable to communicate its location. Therefore, the GPS Tracker is a functional product, but totally dependent on the signal of the operators and the GPS signal. In this study, in some areas, the SMS request was sent to the location of the device and the feedback SMS was received, but without the geographical coordinates. This occurred in areas where there was no GPS signal, so it was not possible to confirm the position of the dogs monitored. Within the forest, the GPS signal is weak. Hence, it is possible that the dogs could be inside the forest.

It would be expected that dogs raised free with full access to the forest, such as guardian dogs would be exposed to forest ticks more than those dogs raised as companions, but it has been detected a significant association that companion dogs were at infection risk of SFG *Rickettsia* 5.4 times higher than those not considered as companions (p=0,009). It is noteworthy that in Area 1, the same dogs that played the role of hunter was also classified by some of its owners as companion dogs, unlike Area 2 and Area 3 where no dogs were classified as hunters. In Area 2, dogs play mainly a guardian role (84%) and, thus, rarely follow their owners on walks in the forest. In this case, other dogs or other wild mammals might be responsible for bringing the ticks to the backyards of these houses. Since, the houses are located in surrounding areas or even in areas pertaining to the park.

In Area 3, we observed that most owners (70.4%) considered their dogs as companion dogs. In this area, we observed that the owners tend to use the forest as a leisure area for walks and baths in waterfalls, and often would take dogs alongside when hiking into the forest patches.

It is very likely that dogs followed their owners during these walks, which allowed the contact to the tick *A. ovale*. Thus, even for the dogs staying most of the time at home with their owners, the hiking in the forest exposed them to infestation by ticks. This result is supported by the high number of seropositive animals that did not have a compatible profile which would include to have access to the forest, but they were considered as companion dogs in Area 3.

In laboratory tests, the tick *A. aureolatum* in the adult phase, infected with *R. rickettsii* and in its virulent state (reactivated) is able to transmit the pathogen to the host within ten minutes after being fixed [10]. Possibly, this association is also valid for *A. ovale*, a tick that shares very close genetic inheritances with *A. aureolatum* and very similar life habits for the adult stage [15,51]. This fact leads us to deduce that only a single contact with an infected tick in these conditions may be sufficient for dogs to demonstrate positive results in IIFA for riquetosis in the Brazilian Atlantic rainforest.

In our results, we demonstrated that dogs that had at least one individual of *A. ovale* collected during the campaigns had a risk of infection by SFG *Rickettsia* ten times bigger than those who did not have any *A. ovale* collected (p= 0,009). Also, in our results, we observed that in Area 1, where dogs had more frequent contacts with the forest and higher abundance of *A. ovale* parasitism, the IFAT seropositivity titers for SFG *Rickettsia* showed differences in relation to the other two areas. These results reinforce the hypotheses that dogs play an important role in maintaining the population of the tick *A. ovale* and perhaps the bacterium *R. parkeri* strain Atlantic rainforest and its vectors.

A study published by Krawczak et al. (2016) has shown that *A. ovale* is capable of performing transovarial transmission and transstadial perpetuation of *R. parkeri* strain Atlantic rainforest for at least two generations. This study strongly supports the clinical and epidemiological data implicating the *A. ovale* ticks as the main vector of *R. parkeri* strain Atlantic rainforest in the endemic areas observed in Brazil up to date [23,26,29].

Engorged *A. ovale* females infected with *Rickettsia* sp. strain Atlantic rainforest have a relatively high mortality rate and lower reproductive performance, as well as for other species of the genus *Rickettsia* and other tick species [31,52–54]. Thus, possibly very low rates of *Rickettsia* infection may be related to the expressiveness of infected females that survive [53].

In the case of *R. parkeri* strain Atlantic rainforest, the rate of infection of *A. ovale* ticks in Atlantic Forest areas is generally about 10%, which is relatively high in relation to *R. rickettsii* in endemic areas (<1%) [30,55]. Thus, it is possible that the horizontal transmission of *R. parkeri* strain Atlantic rainforest among immature *A. ovale* ticks plays an important role in sustaining this pathogen in its endemic areas. Furthermore, the fact that vertebrate species must play the role of amplifier of the bacterium is strongly defended [31,53].

Obviously, these facts are not valid for *R. vitalii*, also analyzed in this study. Even so, the presence of a positive domestic dog for *R. vitalii* infection is an interesting data because it is a disease that has *A. ovale* as a possible vector in the wild forest [33,36,39,56]. It is noteworthy that the dog that presented infection by *R. vitalii* was also positive for IIFA by *R. parkeri*. This dog has been monitored throughout the study and had free access to the forest. Its owner also frequented the forest to make collections, hunts and crops for his survival. *Amblyomma ovale* specimens have been collected on this dog in three of the six monitoring campaigns.

The large infestation observed in this dog may be associated with the owner's livelihood depending on the forest to survive. Cultural diversity and the discrepancy between social classes around the Serra do Mar State Park in Caraguatatuba City shows that dogs develop different habits and that these are closely linked to the way of life of their owners. Such factors are associated to the occurrence of outbreaks of some diseases and to abundance of wild ticks parasitism, as demonstrated by other authors [10,12,32,57,58].

The changes in economic and social relationships established in Brazilian urban areas contributed to the role change from farm dogs to urban dogs [5]. However, peculiarities of this human-dog relationship, such as the relation of companion, open a discussion on in what point and in what locations dogs should be allowed to follow their owner into the protected forest patches. It is known that human beings can be parasitized by adult ticks of the species *A. ovale* and *A. aureolatum*, but in a very small intensity, generally, by just only one tick. [10,23]. However, large infestations of these ticks can be observed on dogs [15,32,59,60].

In our study, more than 30 individuals of *A. ovale* were collected from a single dog in Area 1. In the Brazilian Atlantic rainforest, dogs can transport these ticks, which may be infected, into households and increase the risk of transmission of *R. parkeri* strain Atlantic rainforest to the people with close contact with them [10,61]. In this context, it is important to develop awareness for the owners of domestic dogs from any social class and that their dogs should be kept out of the forest areas, at home, for the health of the families that live with them and to preserve the ecological balance of the forest.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) in process: 2014/00648-3. The contributions of the Superintendência de Estadual de Controle de Endemias de São Paulo, team of Park Serra do Mar and André Carneiro Muniz (Laboratory of Population Genetics, Institute of Biological Science at UFMG) was fundamental.

References

1. Srbek-Araujo AC, Chiarello AG. Domestic dogs in Atlantic forest preserves of south-eastern Brazil: a camera-trapping study on patterns of entrance and site occupancy rates. Braz J Biol. 2008; 68: 771–779. doi:10.1590/S1519-69842008000400011

2. Torres PC, Prado PI. Domestic dogs in a fragmented landscape in the Brazilian Atlantic Forest: abundance, habitat use and caring by owners. Braz J Biol. 2010; 70: 987–94.

3. Lessa I, Guimarães TCS, Bergalho GH, Cunha A, Vieira EM. Domestic dogs in protected areas: a threat to Brazilian mammals? Nat e Conserv. Associação Brasileira de Ciência Ecológica e Conservação; 2016;14: 46–56. doi:10.1016/j.ncon.2016.05.001

4. Doherty TS, Dickman CR, Glen AS, Newsome TM, Nimmo DG, Ritchie EG. The global impacts of domestic dogs on threatened vertebrates. Biol Conserv. Elsevier; 2017;210: 56–59. doi:10.1016/j.biocon.2017.04.007

5. Martinez E, Cesario C, Silva IDE, Boere V. Domestic dogs in rural area of fragmented Atlantic Forest: potential threats to wild animals. Cienc Rural. 2013;43: 1998–2003. doi:10.1590/S0103-84782013001100013

6. Frigeri E, Cassano CR, Pardini R. Domestic dog invasion in an agroforestry mosaic in southern Bahia, Brazil. Trop Conserv Sci. 2014;7: 508–528. doi:10.1177/194008291400700310

7. Udell MA, Wynne CD. A Review of Domestic Dogs' (*Canis Familiaris*) Human-Like Behaviors: Or Why Behavior Analysts Should Stop Worrying and Love Their Dogs. J Exp Anal Behav. 2008; 89: 247–261. doi:10.1901/jeab.2008.89-247

8. Chapman A, Bakken JS, Folk SM, Paddock CD, Bloch KC, Krusell A, Sexton DJ, Buckingham SC, Marshall GS, Storch GA, Dasch GA, McQuiston H, Swerdlow DL, Dumler JS, Nicholson WL, Walker DH, Eremeeva ME, Christopher A. Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever, Ehrlichioses, and

Anaplasmosis - United States. Centers Dis Control Prev. 2006; 65: 1–48. doi:10.15585/mmwr.rr6502a1

9. Ogrzewalska MA, Bajay MM, Schwarcz K, Bajay SK, Telles MPC, Pinheiro JB, et al. Isolation and characterization of microsatellite loci from the tick *Amblyomma aureolatum* (Acari: Ixodidae). Genet Mol Res. 2014;13: 9622–9627. doi:10.4238/2014.November.14.6

10. Saraiva DG, Soares HS, Soares JF, Labruna MB. Feeding Period Required by *Amblyomma aureolatum* Ticks for Transmission of *Rickettsia rickettsii* to Vertebrate Hosts. Emerg Infect Dis. 2014; 20: 1504–1510. doi:10.3201/eid2009.140189

11. Cunha-filho NA, Pacheco RC, Ferreira F, Pappen FG, Farias NAR, Larsson CE, et al. Canine Infection by *Rickettsia*e and Ehrlichiae in Southern Brazil and *Rickettsia rhipicephali* infecting *Haemaphysalis*] uxtako- Cross-reactivity marily for another project on the epidemiology of neosporosis. 2008; 79: 102–108.

12. Labruna MB, Souza SLP, Guimarães JS, Pacheco RC, Pinter A, Gennari SM. Prevalência de carrapatos em cães de áreas rurais da região norte do Estado do Paraná. Arq Bras Med Vet e Zootec. 2001; 53: 553–556. doi:10.1590/S0102-09352001000500007

13. Martins TF, Arrais RC, Rocha FL, Santos JP, Azevedo FC De, Labruna MB. Carrapatos (Acari : Ixodidae) em mamíferos silvestres do Parque Nacional da Serra da Canastra e arredores , Minas Gerais , Brasil. Ciência Rural. 2015; 45: 288–291. doi:10.1590/0103-8478cr20140734

14. Barbieri JM, Rocha CMBM Da, Bruhn FRP, Cardoso DL, Pinter A, Labruna MB. Altitudinal Assessment of *Amblyomma aureolatum* and *Amblyomma ovale* (Acari: Ixodidae), Vectors of Spotted Fever Group Rickettsiosis in the State of São Paulo, Brazil. J Med Entomol. 2015; 52: 1170–1174. doi:10.1093/jme/tjv073

15. Guglielmone AA, Estrada-Peña A, Mangold AJ, Barros-Battesti DM, Labruna MB, Martins JR, et al. *Amblyomma aureolatum* (Pallas, 1772) and *Amblyomma* ovale Koch, 1844 (Acari: Ixodidae): Hosts, distribution and 16S rDNA sequences. Vet Parasitol. 2003; 113: 273–288. doi:10.1016/S0304-4017(03)00083-9

16. Ogrzewalska M, Nieri-Bastos FA, Marcili A, Nava S, González-Acuña D, Muñoz-Leal S, et al. A novel spotted fever group *Rickettsia* infecting *Amblyomma parvitarsum* (Acari: Ixodidae) in highlands of Argentina and Chile. Ticks Tick Borne Dis. 2016; 7: 439–442. doi:10.1016/j.ttbdis.2016.01.003

17. Krawczak FS, Binder LC, Oliveira CS, Costa FB, Moraes-Filho J, Martins TF, et al. Ecology of a tick-borne spotted fever in southern Brazil. Exp Appl Acarol. Springer International Publishing; 2016; 70: 219–229. doi:10.1007/s10493-016-0070-1

18. Szabó MPJ, Pinter A, Labruna MB. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front Cell Infect Microbiol. Frontiers Media SA; 2013; 3: 27. doi:10.3389/fcimb.2013.00027

19. Martins TF, Moura MM, Labruna MB. Life-cycle and host preference of *Amblyomma ovale* (Acari: Ixodidae) under laboratory conditions. Exp Appl Acarol. 2012; 56: 151–158. doi:10.1007/s10493-011-9506-9

20. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155: 945–959. doi:10.1111/j.1471-8286.2007.01758.x

21. Londoño AF, Díaz FJ, Valbuena G, Gazi M, Labruna MB, Hidalgo M, et al. Infection of *Amblyomma ovale* by *Rickettsia* sp. strain Atlantic rainforest, Colombia. Ticks Tick Borne Dis. 2014; 5: 672–675. doi:10.1016/j.ttbdis.2014.04.018

22. Medeiros AP, Souza AP de, Moura AB de, Lavina MS, Bellato V, Sartor AA, et al. Spotted fever group *Rickettsia* infecting ticks (Acari: Ixodidae) in the state of Santa Catarina, Brazil. Mem Inst Oswaldo Cruz. 2011;106: 926–930. doi:http://dx.doi.org/10.1590/S0074-02762011000800005

23. Barbieri A, Filho MJ, Nieri-Bastos FA, Souza Jr.C, Szabó MPJ, Labruna MB. Epidemiology of *Rickettsia* sp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil. Ticks tick-borne …. 2015; Available: http://www.sciencedirect.com/science/article/pii/S1877959X14001472

24. Labruna MB, Pacheco RC, Nava S, Brandão PE, Richtzenhain LJ, Guglielmone AA. Infection by *Rickettsia bellii* and Candidatus "*Rickettsia amblyommii*" in *Amblyomma neumanni* Ticks from Argentina. Microb Ecol. Springer-Verlag; 2007; 54: 126–133. doi:10.1007/s00248-006-9180-3

25. Pinter A, Horta MC, Pacheco RC, Moraes-Filho J, Labruna MB. Serosurvey of *Rickettsia* spp. in dogs and humans from an endemic area for Brazilian spotted fever in the State of São Paulo, Brazil. Cad Saude Publica. 2008;24: 247–252. doi:10.1590/S0102-311X2008000200003

26. Krawczak FS, Munoz-Leal S, Guztzazky AC, Oliveira S V., Santos FCP, Angerami RN, et al. *Rickettsia* sp. Strain Atlantic Rainforest Infection in a Patient from a Spotted Fever-Endemic Area in Southern Brazil. Am J Trop Med Hyg. The American Society of Tropical Medicine and Hygiene; 2016;95: 551–553. doi:10.4269/ajtmh.16-0192

27. Parker R, Kohls G, Cox GW, Davis GE. Observations of an infectious agent from *Amblyomma maculatum*. 1939;54: 1482–1484.

28. Paddock C D, Sumner W J, Comer J A, Zari S R, Goldsmith C S, Goddard J, et al. *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. Clin Infect Dis. 2004;38: 805–811.

29. Spolidorio MG, Labruna MB, Mantovani E, Brandão PE, Richtzenhain LJ, Yoshinari NH. Novel Spotted Fever Group Rickettsiosis, Brazil. Emerg

Infect Dis. 2010;16: 521–523. doi:10.3201/eid1603.091338

30. Szabó MPJ, Nieri-Bastos FA, Spolidorio MG, Martins TF, Barbieri AM, Labruna MB. In vitro isolation from *Amblyomma ovale* (Acari: Ixodidae) and ecological aspects of the Atlantic rainforest *Rickettsia*, the causative agent of a novel spotted fever rickettsiosis in Brazil. Parasitology. Cambridge University Press; 2013;140: 719–728. doi:10.1017/S0031182012002065

31. Krawczak FS, Agostinho WC, Polo G, Moraes-Filho J, Labruna MB. Comparative evaluation of *Amblyomma ovale* ticks infected and noninfected by *Rickettsia* sp. strain Atlantic rainforest, the agent of an emerging rickettsiosis in Brazil. Ticks Tick Borne Dis. Elsevier GmbH.; 2016;7: 502–507. doi:10.1016/j.ttbdis.2016.02.007

32. Ogrzewalska M, Saraiva DG, Moraes-Filho J, Martins TF, Costa FB, Pinter A, et al. Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. Parasitology. Cambridge University Press; 2012;139: 1283–1300. doi:10.1017/S0031182012000546

33. Soares JF, Dall'agnol B, Costa FB, Krawczak FS, Comerlato AT, Rossato BCD, et al. Natural infection of the wild canid, *Cerdocyon thous*, with the piroplasmid *Rangelia vitalii* in Brazil. Vet Parasitol. Elsevier; 2014;202: 156–163. doi:10.1016/J.VETPAR.2014.02.058

34. Soares JF, Girotto A, Brandão PE, Da Silva AS, França RT, Lopes STA, et al. Detection and molecular characterization of a canine piroplasm from Brazil. Vet Parasitol. 2011;180: 203–208. doi:10.1016/j.vetpar.2011.03.024

35. Gottlieb J, André MR, Soares JF, Gonçalves LR, Tonial de Oliveira M, Costa MM, et al. *Rangelia vitalii*, *Babesia* spp. and *Ehrlichia* spp. in dogs in Passo Fundo, state of Rio Grande do Sul, Brazil. Rev Bras Parasitol Veterinária. 2016;25: 172–178. doi:10.1590/S1984-29612016041

36. Loretti AP, Barros SS. Parasitismo por *Rangelia vitalii* em Cães (" Nambiuvú ", " Peste De Sangue)- Uma Revisão Crítica Sobre O Assunto. Arq Inst Biol. 2004; 71: 101–131. Available: http://www.biologico.sp.gov.br/docs/arq/V71_1/loretti.pdf

37. Carini A. Notícias sobre zoonoses observadas no Brasil. Rev Med São Paulo. 1908; 22: 459–462.

38. Pestana. BR. O nambiuvú. Rev Med São Paulo. 1910; 22: 423–426.

39. Fredo G, Leite-Filho RV, Pietzsch CDÁ, Andrade CP de, Duda NCB, Nascimento LC do, et al. Rangeliosis: histopathological analysis, hematology and molecular detection of canine *Rangelia vitalii* in Rio Grande do Sul, Brazil. Ciência Rural. Universidade Federal de Santa Maria; 2017;47. doi:10.1590/0103-8478cr20161121

40. Geografia CDE, Macedo C, Mestre CG, Regional D, Jos M, Doutor S, et al. A expansão urbana de Caraguatatuba City (1950-2010): uma análise das 1 transformações sócio espaciais. Caminhos Geogr. 2012;14: 150–

159.

41. Gigliotti C, Santos MJ. A Expansão Urbana de Caraguatatuba City (1950-2010): Uma Análise das Transformações Sócio Espaciais. Caminhos Geogr. 2013;14. Available: http://www.seer.ufu.br/index.php/caminhosdegeografia/article/view/17794

42. Cerri RI, Reis FAGV, Gramani MF, Giordano LC, Zaine JE, Cerri RI, et al. Landslides Zonation Hazard: relation between geological structures and landslides occurrence in hilly tropical regions of Brazil. An Acad Bras Cienc. Academia Brasileira de Ciências; 2017; 0–0. doi:10.1590/0001-3765201720170224

43. IBGE. IBGE | Brasil em Síntese | São Paulo | Caraguatatuba City | Panorama [Internet]. 2017 [cited 7 Oct 2017]. Available: https://cidades.ibge.gov.br/brasil/sp/Caraguatatuba City/panorama

44. Labruna MB, Horta MC, Aguiar DM, Cavalcante GT, Pinter A, Gennari SM, et al. Prevalence of *Rickettsia* infection in dogs from the urban and rural areas of Monte Negro municipality, western Amazon, Brazil. Vector Borne Zoonotic Dis. 2007;7: 249–255. doi:10.1089/vbz.2006.0621

45. Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, et al. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J Clin Microbiol. 2004;42: 90–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/14715737

46. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of *Rickettsia*e and estimation of intraspecies sequence divergence for portions of two *Rickettsia*l genes. J Bacteriol. American Society for Microbiology (ASM); 1991;173: 1576–89. Available: http://www.ncbi.nlm.nih.gov/pubmed/1671856

47. Pacheco RC, Horta MC, Moraes-Filho J, Ataliba AC, Pinter A, Labruna MB. *Rickettsia*l infection in capybaras (*Hydrochoerus hydrochaeris*) from São Paulo, Brazil: serological evidence for infection by *Rickettsia bellii* and *Rickettsia parkeri*. Biomédica. 2007; 27(3):364-71. Doi:10.7705/issn.0120-4157

48. Lopes GM. Epidemiological aspects of tick-borne diseases in wild and domestic animals of two environmental protection areas in the Natal City, Rio Grande do Norte State , Brazil. 2016;

49. Acosta-jamett G, Cleaveland S, Cunningham AA, Bronsvoort BMDC. Demography of domestic dogs in rural and urban areas of the Coquimbo region of Chile and implications for disease transmission. Prev Vet Med. Elsevier; 2010;94: 272–281. doi:10.1016/J.PREVETMED.2010.01.002

50. Galetti M, Sazima I. Impact of feral dogs in an urban Atlantic forest fragment in southeastern Brazil. Nat Conserv. 2006;4: 146–151. Available: http://www.rc.unesp.br/ib/ecologia/labic2012/Mauro pdf/Galetti papers

pdf/Galetti_Sazima_FeralDogs.pdf

51. Aragão H, Fonseca F da, Aragão H, Fonseca F da. Notas de ixodologia: IX. O complexo ovale do gênero *Amblyomma*. Mem Inst Oswaldo Cruz. Fundação Oswaldo Cruz; 1961;59: 131–148. doi:10.1590/S0074-02761961000200002

52. Burgdorfer W, Brinton LP. Mechanisms of transovarial infection of spotted fever *Rickettsia*e in ticks. Ann N Y Acad Sci. 1975;266: 61–72. Available: http://www.ncbi.nlm.nih.gov/pubmed/829476

53. Labruna MB, Ogrzewalska M, Soares JF, Martins TF, Soares HS, Moraes-Filho J, et al. Experimental Infection of *Amblyomma aureolatum* Ticks with *Rickettsia rickettsii*. Emerg Infect Dis. 2011;17: 829–834. doi:10.3201/eid1705.101524

54. Schumacher L, Snellgrove A, Levin ML. Effect of *Rickettsia* rickettsii (*Rickettsia*les: *Rickettsia*ceae) Infection on the Biological Parameters and Survival of Its Tick Vector— *Dermacentor variabilis* (Acari: Ixodidae). J Med Entomol. 2016;53: 172–176. doi:10.1093/jme/tjv166

55. Sabatini GS, Pinter A, Nieri-Bastos FA, Marcili A, Labruna MB. Survey of ticks (Acari: Ixodidae) and their *Rickettsia* in an Atlantic rain forest reserve in the State of São Paulo, Brazil. J Med Entomol. 2010;47: 913–6. Available: http://www.ncbi.nlm.nih.gov/pubmed/20939390

56. Molecular detection of *Rangelia vitalii* in domestic dogs from Uruguay. Vet Parasitol. Elsevier; 2015; 210: 98–101. doi:10.1016/J.VETPAR.2015.03.013

57. Passos LMF, Geiger SM, Ribeiro MFB, Pfister K, Zahler-Rinder M. First molecular detection of *Babesia vogeli* in dogs from Brazil. Vet Parasitol. 2005;127: 81–85. doi:10.1016/j.vetpar.2004.07.028

58. Vieira TS, Vieira RF, Nascimento DA, Tamekuni K, Toledo R o. S, Chandrashekar R, et al. Serosurvey of tick-borne pathogens in dogs from urban and rural areas from Parana State, Brazil. Rev Bras Parasitol Vet. 2013;22: 104–109.

59. Maturano R, Faccini JH, Daemon E, Fazza PC, Bastos R. Additional information about tick parasitism in Passeriformes birds in an Atlantic Forest in southeastern Brazil. Parasitol Res. 2015;114: 4181–4193.

60. Martins TF, Peres MG, Costa FB, Bacchiega TS, Appolinario CM, Antunes JMA de P, et al. Ticks infesting wild small rodents in three areas of the state of São Paulo, Brazil. Ciência Rural. Universidade Federal de Santa Maria; 2016;46: 871–875. doi:10.1590/0103-8478cr20150671

61. Ogrzewalska MA, Saraiva DG, Moraes-Filho J, Martins TF, Costa FB, Pinter A, et al. Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. Parasitology. 2012;139: 1283–1300. doi:10.1017/S0031182012000546

CAPÍTULO 2

High gene flow in *Amblyomma ovale* along the edge of the Brazilian Atlantic rainforest.

High gene flow in *Amblyomma ovale* along the edge of the Brazilian Atlantic rainforest.

Gislene F. S. R. Fournier[1]; Adriano Pinter[2]; Renata Santiago[3]; Thiago Fernandes Martins[1]; Marcos Gomes Lopes[1]; Karen D McCoy[4]; Celine Toty[4] Mauricio C. Horta[5]; Marcelo Bahia Labruna[1]; Ricardo A. Dias[1].

1 - Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia Universidade de São Paulo, São Paulo, Brazil

2- Superintendência de Controle de Endemias de São Paulo, São Paulo, Brazil
3 - Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
4 - MIVEGEC UMR 5290 CNRS-IRD-University of Montpellier, Centre IRD, Montpellier, France
5 – Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, Brazil

Abstract

This work analyzed *A. ovale* individuals from five distinct areas of the Brazilian Atlantic rainforest through DNA sequencing to evaluate the gene structure of the population and the association with the presence of dogs at the edge of the forest as hosts of this species. Ticks were collected from domestic dogs and from the environment from 2015 to 2017. Four of these areas are located around and inside the Serra do Mar State Park – São Paulo State (Latitude: 23° 37' 21'' S, Longitude: 45° 24' 43'' W), where dogs were monitored during two years through bimonthly visits, camera traps and GPS/GSM trackers. Ticks collected from dogs living around the Atlantic rainforest area in Serra Bela Park – Ceará State (Latitude: 4°15'40.01"S, Longitude: 38°55'54.51"O), were also included in this study as an external group. The 16s and COI mitochondrial genes were selected for the molecular analyses. A total of 39 haplotypes were observed, 27 of which were sampled from areas inside the Serra do Mar State Park. No haplotype

sharing between the Serra do Mar State Park and Serra Bela Park State has been observed. Although three different haplotype lineages of *A. ovale* occurred in the Serra do Mar State Park, haplotypes were distributed homogeneously across edge areas, demonstrating high gene flow in this species across a spatial scale of 45km. Monitoring data from domestic dogs and wild carnivores showed that they share the same areas at the forest edge, but not in the fragment core. Wild carnivores are therefore likely important actors in the dispersal of *A. ovale* in the Brazilian Atlantic rainforest.

Introduction

The interaction between parasites and their hosts depends on the mutual adaptation process, and the resulting compatibility ensures the survival of both species. However, the host is sometimes responsible for most of the ecological requirement of the parasite [1].

Each parasite requirement is specific, even if they are metabolic or not, needing a single host species, a group of species or different and variable genus to fulfill them [2].

The dependency of a host demands specific adaptations that eventually lead to population divergence and even speciation [1–3]. However, not only the divergence associated to the host requires the outcome of morphological adaptations in the parasite [4]. Molecular techniques are becoming indispensable to measure genetic isolation levels. Even so, identification of the factors associated with these divergences is still difficult to understand, particularly in parasite populations that use several hosts in their life cycles [3,5,6].

In general, ticks of the genus *Amblyomma* need two or more hosts for the development of the larvae, nymph and adult phases [7–9]. Such life cycle gives these ectoparasites the opportunity to maintain their populations and to diversify [6,7,10].

In the *Amblyomma* genus, the *Amblyomma ovale* species is a widely discussed species in decades and, periodically, its phylogenic classification is revised in the same speed as new analytic and molecular tools are used [11,12]. This is due to its classification by some authors as polymorphic and polyphyletic, previously classified as *A. ovale* complex, in which the *Amblyomma aureolatum* species used to be included. [11].

This tick lifecycle still has some knowledge gaps related to its hosts in its three life stages [7,9]. At the time of writing, small rodents shown an important role in *A. ovale* ticks life history, being considered the main hosts for larvae and nymphs in natural conditions [7]. In laboratory conditions, *Gallus gallus* was a moderately adequate host for immature stages [9]. Larvae and nymphs of *A. ovale* were observed parasitizing birds in Southwest Brazil, suggesting that birds may also have some role in dispersion of these ticks in different geographic locations [13,14].

In adult stage, *A. ovale* is considered to have preference for wild carnivores and dogs as hosts, especially if the dogs are in contact with forest habitats [9,12,15]. Considering the intense habitat fragmentation in Brazil, domestic dogs could potentially be an adequate host for adult stages of *A. ovale* [9]. Associated with the high potential capacity of pathogen transmission of ixodid ticks, *A. ovale* probably has a high importance in human and animal health [16,17].

In the last decade, several authors demonstrated that *A. ovale* is probably the primary vector of an emerging rickettsiosis in Brazil, caused by the *Rickettsia* *parkeri* strain Atlantic rainforest, associated with the rain forest habitats in the eastern parts of Brazil [17–19]. In these forest fragments, the invasive domestic dog is associated with the emergence of human cases of this disease [15,17,19]. In that sense, dogs that live in close contact with the wilderness could partially or fully replace wild carnivores as hosts for the adult stage of *A. ovale* in the rain forest.

A possible explanation to this hypothesis is the deforestation or perturbation of natural rain forest habitat, which causes fragmentation and diminishes the support capacity to sustain wild carnivore populations, favoring the access to dogs into the forest and their contact with wildlife [20,21].

The large carnivore species of Brazil use large home range areas and must disperse long distances to maintain viable populations. For example, young male cougars must disperse away from their progenitor range [22] and this dispersion is responsible for regrouping new males and some females in different local populations [22]. As the dispersion distances for large carnivores can be extensive, these animals are less protected in small reserves [23,24]. For this reason, the interaction of body size, life history and size of protected areas may contribute to the high vulnerability of the Brazilian carnivores [25].

Since the tick *A. ovale* may be specialized to feed on carnivore hosts in its adult stage [9], it becomes highly dependent on the presence of these hosts in its natural habitat to maintain viable populations [6,9,12]. Considering that birds do not seem to be the favorite host of *A. ovale* and that wild carnivores capable of transporting these parasites over long distances are absent, speciation due to geographic isolation may occur [1]. Although it is not exclusive of geographic isolation, so far, this is the most parsimonious explanation to the described divergences of *A. ovale* populations broad wide [1,3].

Through genetic DNA sequencing, the present study analyzed *A. ovale* specimens collected on dogs and directly from the environment in five different areas of rain forest remaining areas between 2015 and 2017. Four of these areas are located on the borders of the Serra do Mar State Park, São Paulo State, where dogs were monitored bimonthly, also through camera traps and GPS/GSM trackers. As an external group, it was added a sample of ticks collected on dogs that live on around Serra Bela Park – Ceará State, which also is an area of Brazilian Atlantic rainforest.

Methods

Study area

The Brazilian Atlantic rainforest is considered a biodiversity hotspot, with one of the highest richness of species worldwide [26]. It is also one of the most currently threatened environments. The original range of this biome stretched throughout the Eastern Brazilian coast, covering 131,546,000 hectares [20,26].

Currently, only 8.5% still remains, if one considers forest fragments above 100 hectares in size [27]. If forest patches above three hectares are considered, the proportion increases to 12.5% [27]. The original composition of the Brazilian Atlantic rainforest was a mosaic of open and mixed dense ombrophilous forest, deciduous and semi-deciduous seasonal forest, altitude meadow, mangrove and restinga forest [27,28].

Seventy percent of the Brazilian population currently lives among the remaining forest patches of this biome [29]. To help conserving the remaining native areas, several legal preserved areas have been created, the first being in

1937. One of these is the Serra do Mar State Park (IUCN category II), located on the seacoast of São Paulo State (Latitude: 23° 37' 21'' S, Longitude: 45° 24' 43'' W), with 332,000 hectares spanning across and around 25 cities [30]. Currently, this is the biggest continuous remaining fragment of the Brazilian Atlantic rainforest.

The study areas of the present work consist of a section of the Park located in Caraguatatuba City, in the Northern Coast of São Paulo State, 180 km from the State capital. The city is 48,510 hectares wide, with a population of 116,786 inhabitants [29] living between the coast and the forest. Social and economic inequality is significant and the use of the forest by the local population reflects this situation.

Study area selection

Four different areas inside the Serra do Mar State Park were selected. Three of them (Area 1 to 3) were selected in the surroundings of the Park, where people and dogs lived in close contact with the forest. Area 1 is a poor community characterized by extraction activities as a collection of fruits, vegetables, subsistence plantations and hunting. Area 2 is a low-income community, but without economic use of the forest. Area 3 is a middle-income residential zone, where residents use the forest for leisure. Area 4 is located inside the legal limits of the park, in an area where dogs supposedly have no access, and only the human residents are Park staff.

As the external group, we included ticks collected in Serra Bela Park, located in a Brazilian Atlantic rainforest fragment in Ceará State (Latitude: 4°15'40.01"S, Longitude: 38°55'54.51"O), in the Northeast Brazil (Area 5). The straight line distance between Areas 1-4 and Area 5 is 2,325 km.

Dog sampling

Only dogs older than one year, born and raised in the region were included in the study cohort of 20 subjects as described elsewhere [31]. In Areas 1 to 3, 20 dogs were selected and sampled every two months, for a total of 60 dogs visited on six occasions from June 2015 to April 2016. During each visit, dogs were captured and the presence of ticks were evaluated. All adult ticks were collected and preserved in 70% ethanol. Before starting, the present study was submitted to and approved by the Research Ethics Committee of the Universidade de São Paulo.

Collection of free-living ticks

Free-living ticks were collected in Area 4, inside the Serra do Mar State Park, where dogs had no official access. To reach this area, we walked a 3 km trail inwards towards the forest core several times in April 2017. Ticks found in the vegetation were collected along the way and preserved in 70% ethanol.

Telemetry data

Six SMS GPS-TK102B (CU-TEK®) trackers were used to track dog movements, being two in each area (1 to 3). The trackers were placed on the collars of dogs that could move freely and were thought to use the forest (unrestricted dogs). Each device remained attached to the dog during a one-week period. After that time, the devices were installed on other selected dogs from the same area.

The devices were monitored weekly from October to December 2015. During the monitoring period, the devices were programmed to send an SMS with the geographic coordinates of the dog every 30 minutes, from 6 pm to 8 am. This time was selected because, for the dogs designated, this was the time when they are free to move around without restriction of space and possibly walk in the forest.

Camera trap records

From December 2015 to August 2016, six HD Auto-Focus Nature View camera traps (BUSHNELL®) were used to record animal movements along trails of the Serra do Mar State Park that had been previously identified using the data from the tagged dogs. Two cameras were installed in each area (1 and 3), one on the forest edge and another at least 300 meters away, towards the core of the forest fragment.

The batteries were changed every 25 days and, then, videos and photographs were downloaded. Additionally, photographic records made by two camera traps installed in Area 2 were provided by other researchers working in the same area in the same period (unpublished data by Mora et al., 2017).

Analyses

DNA isolation and PCR amplification

DNA extractions were performed using the DNeasy Tissue kit, following manufacturer's instructions (Qiagen, Valencia, CA, EUA). DNA fragments were amplified by PCR targeting the 16S and COI mitochondrial genes (Table 1) [32,33]. Amplification products were sequenced in both directions using Sanger method by Eurofins Genomics (Courtaboeuf, France) and in Brazil under the same conditions. This procedure was carried out at the Laboratory of Parasitic Diseases of the Department of Preventive Veterinary Medicine and Animal Health of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo.

Table 1 – Primers used in the amplification of the partial sequences of the *Amblyomma ovale* genes and the mitochondrial region.

Gene/Region	Primer pairs	Nucleotide sequences	Approximate size of the amplified product	Reference
16s/CYB	16s+1 16s-1	5'- CTGCTCAATGATTTTTTAAATTGC-3' 5'-CCGGTCTGAACTCAGATCATGTA-3'	460pb	Black and Piesman, 1994
COI/D-loop	Dloop3-1x Dloop4-1x	5'-GGAGGATTTGGAAATTGATTAGTTCC-3' 5'-ACTGTAAATATATGATGAGCTCA-3	600pb	Simon et al.,1994

Genetic and statistical analyses

Initially, DNA sequences were identified by similarity through the comparative analyses with sequences deposited in GenBank using BLASTN (Basic Local Alignment Search Tool - Nucleotide). The sequences were then aligned using the MEGA (version 6.0.5), the same procedure was used to concatenate the sequences of both genes [34].

For each gene fragment, files containing the alignments with the sequences of each population (Areas 1 to 5) were created. Using the Tamura-Nei model [35], the evolutionary divergence of sequence pairs and the molecular phylogenetic analysis by maximum likelihood were calculated.

DNAsp (version 5.10.01) [36] was used to calculate the number of haplotypes (H) and the haplotype diversity (Hd) [37] within each study site. This procedure was performed in the ARLEQUIN software (version 3.5.1.2.) [38].

Phylogenies were then produced for each marker and for the concatenated sequences using NetWork (version 5.0.0.0) using maximum likelihood [39].

To test for genetic neutrality and population equilibrium, the gene sequences from each *A. ovale* population were analyzed using the Tajima neutrality test D [40] and F*s* of Fu [41] implemented in ARLEQUIN. Tests for significance were based on 1,000 random permutations of samples.

To evaluate population structure, Analysis of MOlecular VAriance (AMOVA) was performed to determine whether significant differentiation occurred among tick populations of the different study areas [42]. This analysis was also performed using ARLEQUIN.

A Spatial Analysis of MOlecular VAriance (SAMOVA) was also performed to detect *K* spatial clusters based on the maximized inter group genetic variance (F*ct* index). This analysis was performed to identify the spatial scale of a genetically homogeneous population [43].

Results

Tick sampling

Several dogs were monitored in Areas 1 to 3, being respectively 19, 24, and 18. A total of 190 *A. ovale* specimens were collected directly from these dogs: 155 in Area 1, 18 in Area 2 and 17 in Area 3. All ticks from Areas 2 and 3 and a subset of 20 adult ticks of Area 1 were submitted to molecular analyses. A total of 13 adult *A. ovale* individuals collected in free living, inside the forest were sampled in Area 4.

A total of 32 *A. ovale* individuals collected from dogs living in the surroundings of Serra Bela Park - Ceará State, were included in this study in order to understand the genetic structure of the *A. ovale* species locally. In this way, Serra Bela ticks were included as an external group. These samples were collected in 2015 being, therefore, in the same period as the collection of ticks carried out in Caraguatatuba City.

The ticks collected in the five areas were taxonomically identified using the dichotomous key described by Barros-Battesti et al. 2006 [44].

Telemetry data

A total of four dogs were monitored in Areas 1 to 3. Only one dog from Area 1 and one from Area 3 were observed inside the forest, where camera traps were installed (Figure 1). The other dogs were observed roaming around the urban areas. At times, when dogs were in GSM shaded areas, it was not possible to register the position of the dogs. The telemetry devices were tested on the inside and edge of the forest and it was observed that the telemetry devices produced this fault in both areas.

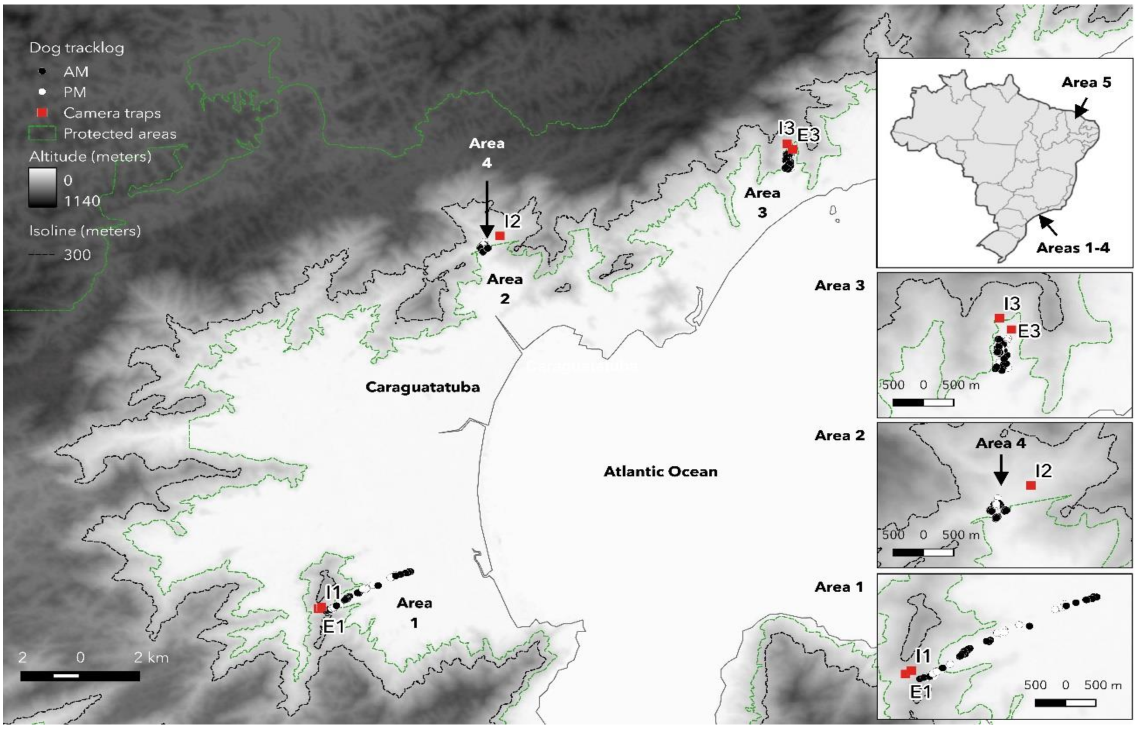

Figure 1 - Maps of monitored areas with tracking locations of dogs monitored by GPS collar during the period of monitoring and location of cameras traps from 2015 to 2016. Caraguatatuba City – SP, Brazil

Photographic Records

During the monitoring period, a total of 2,855 photos and 156 videos were recorded by camera traps. Among these, 38 records were taken of medium or large mammals (≥1 kg), 22 of which were carnivores: *Eira barbara, Nasua nasua, Leopardus pardalis, Leopardus* sp.*, Puma concolor* and *Canis lupus familiaris*. People were also recorded walking in the forest alongside their dogs in Areas 1 and 3. In all study areas, dog records were obtained from the cameras installed at the edge of the forest (cameras E1 and E3, Figure 1).

Dog records were also obtained only in Area 1 by the camera installed inside the forest (camera I1, Figure 1). All records of humans with their dogs obtained in Area 1 were made on the morning (AM acronym, Figure 1) and the dog records obtained in Area 3 were made during the weekends or holidays with their owners.

Leopardus pardalis, Leopardus sp.*,* and *Eira barbara* were recorded by the camera trap installed (camera E3, Figure 1) only 100 meters away from houses, where dogs were also recorded, demonstrating the range sharing, but at different periods of the day. *Puma concolor* was recorded in Areas 1 and 2 by cameras installed inside the forest (cameras I1 and I2, Figure 1).

Genetic and statistical analyses

A total of 97 adult *A. ovale* ticks were submitted to molecular analysis, from which we obtained 87 sequences of the 16S gene (275 bp), 78 of the COI gene (436 bp), and 70 sequences for both genes (711 bp) (Table 2).

Table 2 - *Amblyomma ovale* populations used in this study, with collection localities, population abbreviations, host species and the number of ticks used in genetic (16S and COI genes) analyses. F: female; M: male.

Area	State/UC	Host	Number of male-female ticks	Number of 16s sequences	Number of COI sequences	Number of concatenated sequences
A1	SP/Pq. Serra do Mar	19 dogs	10M-10F	17	13	13
A2	SP/Pq. Serra do Mar	24 dogs	11M-7F	18	13	9
A3	SP/Pq. Serra do Mar	18 dogs	8M-6F	14	13	12
A4	SP/Pq. Serra do Mar	Free living	5M-8F	9	10	9
A5	CE/Pq. Serra Bela	dogs	9M-23F	29	29	27
	Total		97	87	78	70

The external group (Area 5) was isolated from the other populations (Areas 1 to 4). The tick populations of Areas 1 to 4 were closely related, composing a single group without accentuated genetic divergences.

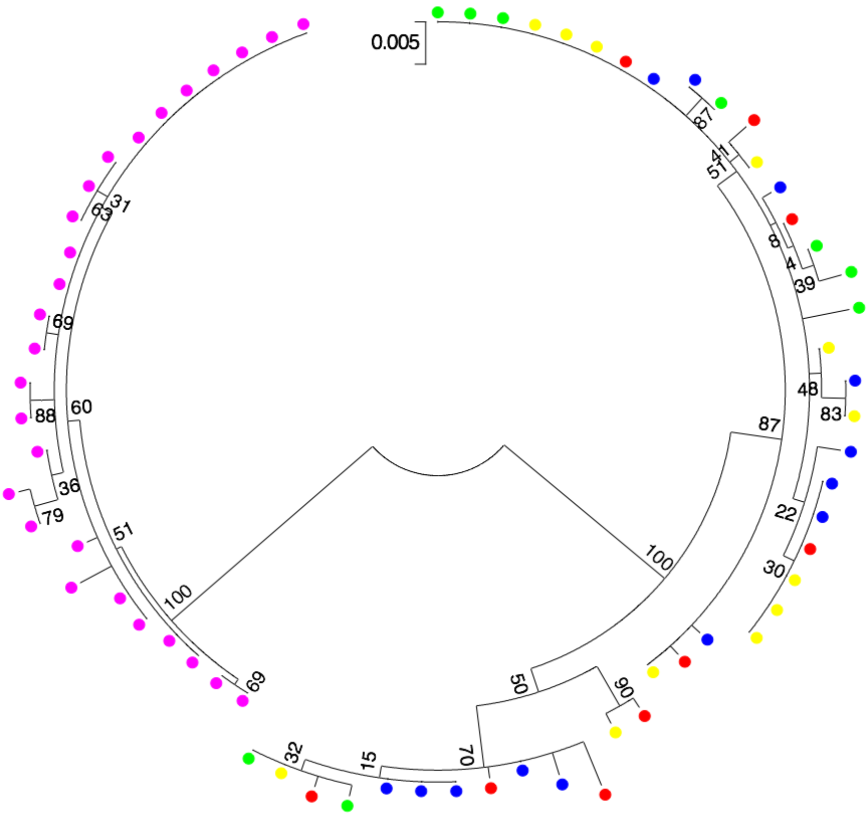

Figure 2 - Phylogenetic tree based in *Amblyomma ovale* mitochondrial genes 16S and COI. Area 5 represents the external group. Blue = Area 1; Red = Area 2; Yellow = Area 3; Green = Area 4 and Pink = Area 5.

No significant structure was apparent among populations from areas 1 to 4, but all comparisons between areas 1 to 4 and area 5 were significantly different (Figure 4).

16s	1	2	3	4	5
A1		0.004	0.004	0.004	0.009
A2	0.013		0.004	0.004	0.010
A3	0.013	0.011		0.003	0.010
A4	0.013	0.011	0.011		0.010
A5	0.033	0.034	0.034	0.035	

COI	1	2	3	4	5
A1		0.003	0.003	0.003	0.015
A2	0.011		0.003	0.003	0.015
A3	0.009	0.009		0.002	0.015
A4	0.011	0.011	0.008		0.015
A5	0.090	0.089	0.088	0.088	

16s+COI	1	2	3	4	5
A1		0.003	0.002	0.003	0.010
A2	0.012		0.002	0.003	0.010
A3	0.011	0.012		0.002	0.010
A4	0.012	0.012	0.009		0.010
A5	0.069	0.068	0.067	0.068	

Figure 3 - Evolutionary divergence of the sequence pairs between *Amblyomma ovale* populations.

As expected, the COI gene fragment yielded higher polymorphism than the 16S gene, with 54 and 22 variable sites, respectively (Table 3). The haplotype and nucleotide diversity were high among tick populations.

Table 3: Genetic indexes of *Amblyomma ovale* population diversity for the 16S, COI and concatenated gene fragments.

	r16s				COI				**r16s+COI**			
Pop.	NS	H	Hd±SD	k	NS	H	Hd±SD	k	NS	H	Hd+SD	k
A1	11	9	0.882±0.003	3.7059	10	6	0.769±0.010	4.6923	21	10	0.949±0.002	8.6026
A2	10	11	0.918±0.002	3.1169	13	7	0.731±0.017	5.1282	22	9	1.000±0.002	9.6389
A3	10	10	0.933±0.002	2.8286	12	4	0.423±0.027	2.5897	22	8	0.909±0.004	5.6061
A4	9	7	0.867±0.011	3.2000	14	3	0.511±0.027	4.3556	23	7	0.917±0.008	8.1111
A5	17	9	0.653±0.007	1.9793	13	8	0.687±0.008	4.1453	13	12	0.875±0.002	2.3077
Total	22	28	0.910±0.00025	5.4865	54	20	0.809±0.001	0.0430	71	39	0.962±0.001	25.044

NS: number of variable sites; H: number of haplotypes; Hd ± SD: Haplotype diversity and standard deviation; k: number of nucleotide differences.

A total of 39 haplotypes were observed based on the two mitochondrial gene sequences. Of these, 27 were observed in samples from Areas 1 to 4. In Area 5, 12 haplotypes were observed; there were no haplotypes shared with the samples from Caraguatatuba City (Areas 1 to 4). However, haplotype sharing was observed among the Caraguatatuba City populations (Areas 1 to 4). These samples comprise three different *A. ovale* haplotype lineages and the external group, a single haplotype lineage, distant from Caraguatatuba City samples by more than 40 mutational steps (Figure 2).

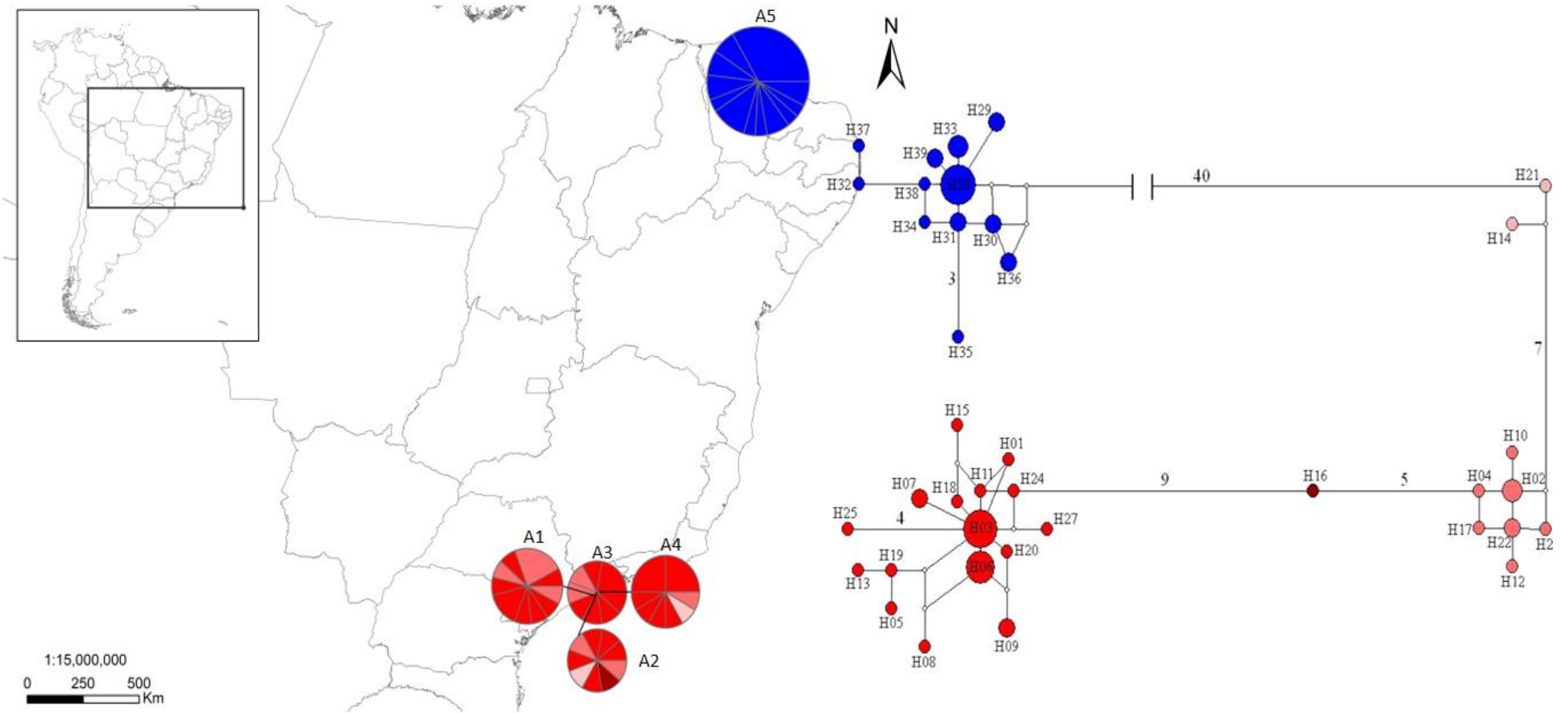

Figure 4 – Geographic distribution of *Amblyomma ovale* haplotypes (H1-39) and the haplotype network indicating the mutational steps of the different haplotype lineages.

No deviation from neutrality was found at the two genes examined for either Tajima's D or Fu's F*s* test (p > 0.05), suggesting that the sampled *A. ovale* populations are in genetic equilibrium and that these genes are not submitted to selection (Table 4).

Table 4: Selective neutrality of *Amblyomma ovale* involving 70 nucleotide sequences (16s+COI) and a total of 708 positions.

Pop.	S	Ps	ϴ	π±SD		D	Fs
A1	21	0.029619	0.009545	0.012100+/-	0.006845	1.164245	-1.27792
A2	22	0.031030	0.011417	0.013595+/-	0.007931	0.943172	-2.90406
A3	22	0.031030	0.010275	0.007907+/-	0.004662	-1.018854	-0.74595
A4	23	0.032394	0.011919	0.011424+/-	0.006758	-0.205787	-1.49974
A5	13	0.018336	0.004757	0.003255+/-	0.002055	-1.066686	-5.10633
Total	71	0.100282	0.020812	0.035374+/-	0.018372	2.358450	-2.08980

Pop = Population per area, S = number of segregating sites, Ps = S/n, ϴ = p_s/a_1, π±SD = nucleotide diversity and standard deviation, D = the Tajima test statistic and F*s* = Fu's F*s* test.

The AMOVA results for all samples showed 85.95% of molecular variation, attributed to differences between Caraguatatuba City and Serra Bela Park populations. For Areas 1 to 4, only 0.17% of the variation could be attributed to the inter-population level and so 99.83% of intra-population molecular variation was observed; no barrier to gene flow seems to occur among these populations (Table 5).

Table 5: AMOVA result, intra and inter- *Amblyomma ovale* populations mitochondrial genes 16S and COI variation.

Source of variation	d.f	Sum of square	Components of variation	Percentage of variation
Among populations (A1 –A5)	4	682.150	12.70751 Va	81.95
Within populations (A1-A5)	65	181.893	2.79836 Vb	18.05
Total	69	864.043	15.50586	
Among populations (A1 –A4)	3	11.898	0.00668Va	0.17
Within populations (A1-A4)	39	151.893	3.89470Vb	99.83
Total	42	163.791	3.90138	

d.f. = degrees of freedom.

The SAMOVA also indicated geographic isolation between Caraguatatuba City (Areas 1 to 4) and Serra Bela Park (Area 5) samples (F*ct* = 0.87335), coinciding with AMOVA results.

Discussion

Parasites specialized in certain host species or groups have a better ability to cope with their immune response and, thus, being more effective in exploitation [1,3]. However, for ticks, the population dynamics is highly dependent upon the host mobility in its evolutionary trajectory [2].

Amblyomma ovale has a unique life history, since it has a small range of hosts and, in subtropical areas, they are not found above 300 meters of altitude. [45]. These traits make the *A. ovale* tick highly dependent on its hosts to achieve a viable population genetic flow.

In the present study, it was possible to demonstrate that in Caraguatatuba City, the *A. ovale* populations have high haplotype diversity with high genetic flow between them. Moreover, 16S and COI genes were able to demonstrate the geographic isolation between *A. ovale* populations from Serra Bela Park to Caraguatatuba City. However, the evolutionary distances were small among *A. ovale* populations from Caraguatatuba City, where the study areas (1 to 4) are less than 15 km apart from each other, despite the significant differences of the life habits of human populations.

Through monitoring data of these dogs (telemetry and camera trap records), it was possible to verify that dogs were not reported to invade the inner parts of large forest fragments, differently from what has been reported by many authors [46,47]. These animals tend to be restricted to the edges of the forest and to accompany their owner's routine. Camera trap records, for instance, categorically demonstrated the presence of wild carnivores using anthropized areas, including those same areas where dogs were reported present as well. Thus, *A. ovale* present in the borders of the forest probably need wild carnivore and rodent mobility to disperse, being subjected to an evolutionary process dependent on their host range. This is the most parsimonious explanation to the high genetic difference between Caraguatatuba City and external group populations. Therefore, considering the Brazilian Atlantic rainforest intense fragmentation intensified in the last 200 years, carnivore populations may be geographically isolated and, consequently, *A. ovale* populations too.

Molecular divergences between Caraguatatuba City and Serra Bela Park populations suggest that the possible role of birds in the dispersion of larvae and nymphs of *A. ovale* should be irrelevant, differently of the descriptions for other *Amblyomma* species [48–50]. However, this hypothesis should be confirmed in a large scale observational study, with higher number of samples and complementary molecular techniques.

Due to the steep relief and relatively narrow coastal plains, Caraguatatuba City was forgotten during the Brazilian economic cycles and, for that reasons, it still has significantly preserved Brazilian Atlantic rainforest fragments [51]. Thus, Serra do Mar State Park is currently one of the most important ecological corridors to the native fauna. This factor, associated with the high dispersion capacity of the wild carnivores, may be the explanation to the high haplotype diversity of *A. ovale* in the region. Differences between the population from the area 1 to 4 may not be discarded until more advanced molecular techniques like Single-Nucleotide Polymorphism (SNP) is employed. This is explained by the fact that there is an enormous diversity of factors that can alter the genetic composition of tick populations. The effect of these factors on the genetic frequency of *A. ovale* depends on the adaptability that the species presents, both for survival and reproduction in the environment, which is closely related to its range of hosts [1–3].

Neutral mutations may persist for many generations, but genetic variability is a consequence of the accumulation of several mutations. Other evolution factors only rearrange variability and do not create anything new [1]. However, a factor that may influence the *A. ovale* gene flow would be the absence of migration. If the migratory rate were very low, it could affect the genetic frequency, and works as the primary factor of evolution by geographic isolation [3]. Considering the distance in the genetic structure between the *A. ovale* individuals collected in Caraguatatuba City – São Paulo State, and the ones collected in Serra Bela Park – Ceará State, it can be suggested that the geographic isolation between these species is a factor that can lead to the creation of different species in future generations.

References

1. McCoy KD, Léger E, Dietrich M. Host specialization in ticks and transmission of tick-borne diseases: a review. Front Cell Infect Microbiol. 2013;3: 1–12. doi:10.3389/fcimb.2013.00057

2. De Meeus T. Initiation à la génétique des populations naturelles applications aux parasites et à leurs vecteurs [Internet]. 2012. Available: http://www.documentation.ird.fr/hor/fdi:010058052

3. Dupraz M, Toty C, Noël V, Estrada-Peña A, González-Solís J, Boulinier T, et al. Linking morphometric and genetic divergence with host use in the tick complex, Ornithodoros capensis sensu lato. Infect Genet Evol. 2016;46: 12–22. doi:10.1016/j.meegid.2016.10.005

4. Bickford D, Lohman D, Sodhi N, Ng P, Meier R, Winker K, et al. Cryptic species as a window on diversity and conservation. Trends Ecol Evol. Elsevier Current Trends; 2007;22: 148–155. doi:10.1016/J.TREE.2006.11.004

5. Nadolny R, Gaff H, Carlsson J, Gauthier D. Comparative population genetics of two invading ticks: Evidence of the ecological mechanisms underlying tick range expansions. Infect Genet Evol. 2015;35: 153–162. doi:10.1016/j.meegid.2015.08.009

6. Mazé-Guilmo E, Blanchet S, Mccoy KD, Loot G. Host dispersal as the driver of parasite genetic structure: A paradigm lost? Ecol Lett. 2016;19: 336–347. doi:10.1111/ele.12564

7. Krawczak FS, Agostinho WC, Polo G, Moraes-Filho J, Labruna MB. Comparative evaluation of *Amblyomma* ovale ticks infected and noninfected by Rickettsia sp. strain Atlantic rainforest, the agent of an emerging rickettsiosis in Brazil. Ticks Tick Borne Dis. Elsevier GmbH.; 2016;7: 502–507. doi:10.1016/j.ttbdis.2016.02.007

8. OGRZEWALSKA M, SARAIVA DG, MORAES-FILHO J, MARTINS TF, COSTA FB, PINTER A, et al. Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. Parasitology. Cambridge University Press; 2012;139: 1283–1300. doi:10.1017/S0031182012000546

9. Martins TF, Moura MM, Labruna MB. Life-cycle and host preference of *Amblyomma* ovale (Acari: Ixodidae) under laboratory conditions. Exp Appl Acarol. 2012;56: 151–158. doi:10.1007/s10493-011-9506-9

10. McCoy KD. The population genetic structure of vectors and our understanding of disease epidemiology. Parasite. 2008;15: 444–448. doi:10.1051/parasite/2008153444

11. Aragão H, Fonseca F da, Aragão H, Fonseca F da. Notas de ixodologia: IX. O complexo ovale do gênero *Amblyomma*. Mem Inst Oswaldo Cruz. Fundação Oswaldo Cruz; 1961;59: 131–148. doi:10.1590/S0074-02761961000200002

12. Murgas IL, Castro AM, Bermúdez SE. Current status of *Amblyomma* ovale

(Acari: Ixodidae) in Panama. Ticks Tick Borne Dis. Elsevier GmbH.; 2013;4: 164–166. doi:10.1016/j.ttbdis.2012.09.002

13. Ogrzewalska M, Pacheco RC, Uezu A, Richtzenhain LJ, Ferreira F, Labruna MB. Ticks (Acari: Ixodidae) infesting birds in an Atlantic rain forest region of Brazil. J Med Entomol. 2009;46: 1225–1229. doi:10.1603/033.046.0534

14. Maturano R, Faccini JH, Daemon E, Fazza PC, Bastos R. Additional information about tick parasitism in Passeriformes birds in an Atlantic Forest in southeastern Brazil. Parasitol Res. 2015;114: 4181–4193.

15. SZABÓ MPJ, NIERI-BASTOS FA, SPOLIDORIO MG, MARTINS TF, BARBIERI AM, LABRUNA MB. In vitro isolation from *Amblyomma* ovale (Acari: Ixodidae) and ecological aspects of the Atlantic rainforest Rickettsia, the causative agent of a novel spotted fever rickettsiosis in Brazil. Parasitology. Cambridge University Press; 2013;140: 719–728. doi:10.1017/S0031182012002065

16. Londoño AF, Díaz FJ, Valbuena G, Gazi M, Labruna MB, Hidalgo M, et al. Infection of *Amblyomma* ovale by Rickettsia sp. strain Atlantic rainforest, Colombia. Ticks Tick Borne Dis. 2014;5: 672–675. doi:10.1016/j.ttbdis.2014.04.018

17. Spolidorio MG, Labruna MB, Mantovani E, Brandão PE, Richtzenhain LJ, Yoshinari NH. Novel Spotted Fever Group Rickettsiosis, Brazil. Emerg Infect Dis. 2010;16: 521–523. doi:10.3201/eid1603.091338

18. Paddock CD, Allerdice MEJ, Karpathy SE, Nicholson WL, Levin ML, Smith TC, et al. Unique strain of Rickettsia parkeri associated with the hard tick Dermacentor parumapertus Neumann in the western United States. Stabb E V., editor. Appl Environ Microbiol. 2017;83: e03463-16. doi:10.1128/AEM.03463-16

19. Sabatini GS, Pinter A, Nieri-Bastos FA, Marcili A, Labruna MB. Survey of ticks (Acari: Ixodidae) and their rickettsia in an Atlantic rain forest reserve in the State of São Paulo, Brazil. J Med Entomol. 2010;47: 913–6. Available: http://www.ncbi.nlm.nih.gov/pubmed/20939390

20. Frigeri E, Cassano CR, Pardini R. Domestic dog invasion in an agroforestry mosaic in southern Bahia , Brazil. Trop Conserv Sci. 2014;7: 508–528. doi:10.1177/194008291400700310

21. Lessa I, Guimarães TCS, Bergallo HG, Cunha A, Vieira EM. Domestic dogs in protected areas: a threat to Brazilian mammals? Nat e Conserv. Associação Brasileira de Ciência Ecológica e Conservação; 2016;14: 46–56. doi:10.1016/j.ncon.2016.05.001

22. Weaver J, Paquet P, Ruggiero L. Resilience and conservation of large

carnivores in the Rocky Mountains. Conserv Biol. 1996;10: 964–976.

23. Verdade LM, Campos CB. How much is a puma worth?: economic compensation as an alternative for the conflict between wildlife conservation and livestock production in Brazil. Biota Neotrop. Programa BIOTA/FAPESP; 2004;4: 1–4. doi:10.1590/S1676-06032004000200014

24. Castilho CS, Marins-Sá LG, Benedet RC, Freitas TRO. Genetic structure and conservation of Mountain Lions in the South-Brazilian Atlantic Rain Forest. Available: http://www.scielo.br/pdf/gmb/v35n1/2011-079.pdf

25. Paviolo A, De Angelo C, Ferraz KMPMB, Morato RG, Martinez Pardo J, Srbek-Araujo AC, et al. A biodiversity hotspot losing its top predator: The challenge of jaguar conservation in the Atlantic Forest of South America. Sci Rep. Nature Publishing Group; 2016;6: 37147. doi:10.1038/srep37147

26. Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol Conserv. 2009;142: 1141–1153. doi:10.1016/j.biocon.2009.02.021

27. Schnell JK, Harris GM, Pimm SL, Russell GJ. Quantitative Analysis of Forest Fragmentation in the Atlantic Forest Reveals More Threatened Bird Species than the Current Red List. Boyce MS, editor. PLoS One. 2013;8: e65357. doi:10.1371/journal.pone.0065357

28. Bieber AGD, Silva PSD, Sendoya SF, Oliveira PS. Assessing the impact of deforestation of the Atlantic rainforest on ant-fruit interactions: a field experiment using synthetic fruits. PLoS One. Public Library of Science; 2014;9: e90369. doi:10.1371/journal.pone.0090369

29. IBGE. IBGE | Brasil em Síntese | São Paulo | Caraguatatuba City | Panorama [Internet]. 2017 [cited 7 Oct 2017]. Available: https://cidades.ibge.gov.br/brasil/sp/Caraguatatuba City/panorama

30. Gigliotti C, Santos MJ. A EXPANSÃO URBANA DE CARAGUATATUBA CITY (1950-2010): UMA ANÁLISE DAS TRANSFORMAÇÕES SÓCIO ESPACIAIS. Caminhos Geogr. 2013;14. Available: http://www.seer.ufu.br/index.php/caminhosdegeografia/article/view/17794

31. Pinter A, Horta MC, Pacheco RC, Moraes-Filho J, Labruna MB. Serosurvey of Rickettsia spp. in dogs and humans from an endemic area for Brazilian spotted fever in the State of São Paulo, Brazil. Cad Saude Publica. 2008;24: 247–252. doi:10.1590/S0102-311X2008000200003

32. Black WC, Piesman J. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proc Natl Acad Sci U S A. 1994;91: 10034–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/7937832

33. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Ann Entomol Soc Am. Oxford University Press; 1994;87: 651–701. doi:10.1093/aesa/87.6.651

34. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. Oxford University Press; 2013;30: 2725–9. doi:10.1093/molbev/mst197

35. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 1993;10: 512–26. Available: http://www.ncbi.nlm.nih.gov/pubmed/8336541

36. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009;25: 1451–1452. doi:10.1093/bioinformatics/btp187

37. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci U S A. 1973;70: 3321–3. Available: http://www.ncbi.nlm.nih.gov/pubmed/4519626

38. EXCOFFIER L, LISCHER HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010;10: 564–567. doi:10.1111/j.1755-0998.2010.02847.x

39. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 1999;16: 37–48. Available: http://www.ncbi.nlm.nih.gov/pubmed/10331250

40. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989;123: 585–95. Available: http://www.ncbi.nlm.nih.gov/pubmed/2513255

41. Fu YX. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 1997;147: 915–25. Available: http://www.ncbi.nlm.nih.gov/pubmed/9335623

42. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992;131: 479–91. Available: http://www.ncbi.nlm.nih.gov/pubmed/1644282

43. Dupanloup I, Schneider S, Excoffier L. A simulated annealing approach to define the genetic structure of populations. Mol Ecol. 2002;11: 2571–2581. doi:10.1046/j.1365-294X.2002.01650.x

44. Barros-Battesti DM, Arzua M, Bechara. GH. Carrapatos de importância médico veterinária da região neotropical: um guia ilustrado para identificação de espécies. 1st ed. Vox, ICTTD-3 B, editor. São Paulo; 2006.

45. Barbieri JM, Rocha CMBM Da, Bruhn FRP, Cardoso DL, Pinter A, Labruna MB. Altitudinal Assessment of *Amblyomma aureolatum* and *Amblyomma* ovale (Acari: Ixodidae), Vectors of Spotted Fever Group Rickettsiosis in the State of São Paulo, Brazil. J Med Entomol. 2015;52: 1170–1174. doi:10.1093/jme/tjv073

46. Galetti M, Sazima I. Impact of feral dogs in an urban Atlantic forest fragment in southeastern Brazil. Nat Conserv. 2006;4: 146–151. Available: http://www.rc.unesp.br/ib/ecologia/labic2012/Mauro pdf/Galetti papers pdf/Galetti_Sazima_FeralDogs.pdf

47. Butler JR, Bingham J. Demography and dog-human relationships of the dog population in Zimbabwean communal lands. Vet Rec. British Medical Journal Publishing Group; 2000;147: 442–6. doi:10.1136/VR.147.16.442

48. OGRZEWALSKA M, SARAIVA DG, MORAES-FILHO J, MARTINS TF, COSTA FB, PINTER A, et al. Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. Parasitology. 2012;139: 1283–1300. doi:10.1017/S0031182012000546

49. Ogrzewalska M, Uezu A, Jenkins CN, Labruna MB. Effect of forest fragmentation on tick infestations of birds and tick infection rates by Rickettsia in the Atlantic Forest of Brazil. Ecohealth. 2011;8: 320–331. doi:10.1007/s10393-011-0726-6

50. Ogrzewalska M, Bajay MM, Schwarcz K, Bajay SK, Telles MPC, Pinheiro JB, et al. Isolation and characterization of microsatellite loci from the tick *Amblyomma* aureolatum (Acari: Ixodidae). Genet Mol Res. 2014;13: 9622–9627. doi:10.4238/2014.November.14.6

51. Mouret S. Revolução Industrial no Brasil - Início, incentivo e consequências [Internet]. 2017 [cited 7 Oct 2017]. Available: https://www.estudopratico.com.br/revolucao-industrial-no-brasil/

FINAL CONSIDERATION

This study contributes to a better understanding of the role of the domestic dog in the cycle of the *A. ovale* tick, and the epidemiology of rickettsiosis and rangeliosis in the Brazilian Atlantic rainforest. Some factors associated with biological invasion through human presence related to sociocultural and economic aspects in the Serra do Mar State Park, Caraguatatuba City, were also discussed.

The obtained results provided information that can help in developing actions that aim to minimize the risks of zoonoses in these areas and the impacts that domestic dogs can cause to wildlife.

Furthermore, Information was updated regarding the epidemiology of the rickettsiosis caused by bacterium *R. parkeri* strain Atlantic rainforest in the studied area through the monitoring of different communities around the park. Additionally, it was evidenced that dogs considered as companions are five times more likely to be infected since they enter in the forest with their owners. This factor is independent of the sociocultural or economic aspect of the owners.

The approximation of the dog to the forest can be a serious threat to the health of the local human population, as these animals can carry infected ticks into houses, exposing at risk not only the people who go to the forest, but also those who live with the dog (OGRZEWALSKA et al., 2012; SARAIVA et al., 2014). Another factor that aggravates the risk of an endemic disease, evidenced in this study, was that dogs that were found with at least one *A. ovale* tick had a risk of infection by *R. parkeri* strain Atlantic rainforest ten times higher than dogs that were not found parasitized by *A. ovale* ticks.

Regarding rangeliosis, this study evidenced that this disease is present in this region. Being the first confirmed record of infected domestic dog in the Serra do Mar State Park in Caraguatatuba City. Whereas rangeliosis is naturally described for wild canines (SOARES et al., 2014), such record may show that domestic dogs are occupying the space of wild dogs in this pathogen cycle.

This study also contributed to the understanding of the gene flow of the *A. ovale* species. Thus, it was observed that the *A. ovale* populations are structured according to the geographical distances. Nevertheless, in the four studied areas of Serra do Mar State Park, the *A. ovale* populations presented a high haplotype diversity with high genetic flow between them. Moreover, 16S and COI gene analyses were able to demonstrate the geographic isolation between *A. ovale* populations from Serra Bela Park to Caraguatatuba City. However, the evolutionary distances were small among *A. ovale* populations of Caraguatatuba City, where the study areas (1 to 4) are distanced by less than 15 km apart from each other, despite the significant differences of the life habits of each local human population.

Finally, corroborating with other authors, this study demonstrates that dogs' invasion of the Brazilian Atlantic rainforest is favored by the way of breeding as well as the relationship between this animal and its owner. The animals that have been raised loose with access to the forest and also to the house present a potential risk to human and animal health. (FRIGERI; CASSANO; PARDINI, 2014). Therefore, adequate management of dogs, including veterinary care and restriction of the spatial use, is fundamental to minimize the risks and impacts of this species in forested areas.