UNIVERSIDADE DE BRASÍLIA

FACULDADE DE AGRONOMIA E VETERINÁRIA

PESQUISA DE PIROPLASMÍDEOS EM CAPIVARAS (*HYDROCHOERUS HYDROCHAERIS*) DE VIDA LIVRE DO DISTRITO FEDERAL

LUIZ FERNANDO MARTINS DOS REIS

DISSERTAÇÃO DE MESTRADO EM CIÊNCIAS ANIMAIS

BRASÍLIA

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DISSERTAÇÃO DE MESTRADO SUBMETIDA AO PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS ANIMAIS, COMO PARTE DOS REQUISITOS NECESSÁRIOS A OBTENÇÃO DO GRAU DE MESTRE EM CIÊNCIAS ANIMAIS

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1. Piroplasmídeos. 2. Babesiose. 3. Vetores artrópodes. 4. capivaras.

Nenhuma sociedade que esquece a arte de questionar pode esperar
respostas para os problemas que a afligem.
Zygmunt Bauman

À minha eterna filhotinha Zelda.

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SUMMARY

RESUMO

A presença de capivaras (*Hydrochoerus hydrochaeris)* nos centros urbanos tem aumentado nas últimas décadas e com isso houve também um aumento da preocupação em relação à presença de carrapatos em áreas de lazer de Brasília. No entanto, não existem ainda muitos estudos que avaliem o status sanitário desses animais, sobretudo no que tange a ocorrência da infecção por agentes transmitidos por carrapatos, especialmente piroplasmídeos. Para este estudo, foram colhidas amostras de sangue de 53 capivaras capturadas nas regiões do Zoológico de Brasília, assim como na Fazenda Experimental Sucupira (EMBRAPA) e na orla do Lago Paranoá. As amostras de sangue foram submetidas a hemogramas, testes bioquímicos e analisadas para a presença de piroplasmídeos por meio de diagnóstico molecular (PCR). Um total de 15% (8/53) das amostras das capivaras amplificou o DNA dos hemoparasitas. Não foram observadas alterações laboratoriais entre os animais positivos e negativos. Portanto, neste estudo pode-se verificar a presença de piroplasmídeos em capivaras de vida livre, porém mais estudos precisam ser feitos para identificação e caracterização das espécies de piroplasmídeos infectantes.

Palavras-chave: 1. Babesia. 2. Ordem piroplasmida. 3. Vetores artrópodes. 4. Capivaras.

ABSTRACT

The presence of capybaras (Hydrochoerus hydrochaeris) has increased in recent decades and with them there has also been an increase in concern regarding the presence of ticks in frequently visited recreational areas, such as the Brasilia Zoo Foundation. However, there are still not many studies that assess the health status of these animals, hence the importance of studies like the present one. For this study, blood samples were collected from 53 capybaras captured in the regions of the Brasilia Zoo Foundation, as well as from the Sucupira Experimental Farm (EMBRAPA) and from the shore of Paranoá Lake. The blood samples were submitted to hemograms, biochemical tests and analyzed for the presence of piroplasmids by means of molecular diagnostics (PCR). A total of 15% of the capybara samples amplified the DNA of the hemoparasites. In the hematological and biochemical tests, the only discrepancy between the positive and negative capybaras was for urea nitrogen, which is possibly a consequence of muscle catabolism due to stress. Therefore, in this study we could verify the presence of piroplasmids in free-living capybaras, but more studies need to be done to identify the infecting species.

Key words: 1. babesiosis. 2. piroplasmid order. 3. arthropod vectors. 4. capybaras

LISTA DE SÍMBOLOS E ABREVIAÇÕES

ALT – Alanine Aminotransferase

AST – Aspartate Aminotransferase

CEUA – Comitê de Ética de Uso Animal (Ethics Committee for Animal Use)

DNA – Deoxyribonucleic acid

dNTP – deoxyribonucleoside triphosphates

EDTA – Ethylenediaminetetraacetic acid

FAV - Faculdade de Agronomia e Veterinária

GAPDH – Glyceraldehyde 3-phosphate dehydrogenase

IBAMA/MMA – Brazilian Institute of Environment and Renewable Natural Resources

MCHC - mean corpuscular hemoglobin concentration

MCV - mean corpuscular volume

mM – Millimolar

bp – Base pair

PCR – Polymerase Chain Reaction

pMol – Picomole

TPP –Total plasma proteins

RNA – Ribonucleic Acid

°C – Degree Celsius

μL – Microliters

μMol – Micromol

% - Percentage

CAPÍTULO 1

REVISÃO DE LITERATURA

BRASÍLIA

2022

INTRODUCTION

Capybaras (*Hydrochoerus hydrochaeris*) are the largest rodent species and are present throughout the whole Latin America, excepting Chile (MOREIRA *et al,* 2012). The species plays an important role in the dynamics of multiple diseases with zoonotic potential, such as spotted fever, leptospirosis, leishmaniasis, among others (CHIACCHIO *et al,* 2014; VALADAS *et al,* 2010; DE ALBUQUERQUE *et al,* 2017).

Parasites belonging to the order Piroplasmida cause a multitude of diseases in mammals, including wild and companion animals, as well as humans (SCHNITTGER *et al.,* 2022). One of the most common of these pathogens belong to the genus *Babesia* and they cause a major impact economically on both human and animal medical care (JALOVECKA et al., 2018).

These parasites are transmitted by ticks, and also have a certain specificity in terms of mammal host and vector (SCHNITTGER *et al.*, 2022). In the vertebrate host, the parasite blood cells, generally causing signs such as hemolytic anemia, although subclinical and asymptomatic transitory infections are common (BAJER *et al*., 2022).

Diagnosis involves clinical findings, parasitological diagnosis serological as well as molecular tests (O'DWYER *et al*., 2009). Polymerase chain reaction (PCR) represents a very specific and sensitive test for these hemoparasites, being very sensible to verify the prevalence in studies such as the present one.

Nevertheless, this important group of hemoparasites have not yet been sufficiently studied. Not many studies have been conducted in order to investigate the presence of piroplasmids in capybaras. One single study (GONÇALVES *et al*., 2021) describes the presence of a still uncharacterized piroplasmid species collected from ticks from *R. rattus* and *H. hydrochaeris,* therefore more study needs to be done in order to identify possible unknown species that occur in large rodents.

1.1. General Objective

Assess the presence of agents of the order Piroplasmida in free-living capybaras (*Hydrochoerus hydrochaeris*) from the Federal District.

1.2. Specific Objectives

- Perform Piroplasmid detection by Polymerase Chain Reaction (PCR);
- Compare laboratorial changes between positive and negative animals.

2. Review

2.1. Capybaras (*Hydrochoerus hydrochaeris*)

Capybaras (*Hydrochoerus hydrochaeris*) are considered to be the largest rodents in the world, and can be found throughout Latin America. It is a semi aquatic species that uses rivers and flooded areas to reproduce, regulate its body temperature, and avoid predators. It has anatomical features that allow for greater swimming ability, such as large and adaptable nostrils and interdigital membranes. The presence of water is critical for the existence of the species in the environment (MACDONALD *et al.*, 1984; CRUZ *et al*., 1998; MADELLA *et al.*, 2006; MEIRELES *et al.*, 2007; SINKOC *et al*., 2009) ; AL MEIDA *et al.*, 2013).

Capybaras feature a complex social organization and the groups can range from 2 to more than 50 individuals (MACDONALD, 1981). They are gregarious animals that live in family groups composed of one dominant male adult, a few submissive males, several females, and their nestlings (FERRAZ; VERDADE, 2001).

In terms of diet, capybaras are herbivores that tend to forage near bodies of water and tend to have high dietary plasticity. They are monogastric, generalist herbivores that perform caecal fermentation. During rainy periods in which the availability and quality of food is higher, they tend to choose those with higher levels of protein and fiber (BARRETO; HERRERA, 1998). Deforestation, absence of natural predators and high availability of food has led to an increase of these animals in urban areas (MEIRELES *et al*., 2007; ALMEIDA *et al*., 2013).

When it comes to reproduction, these mammals are highly prolific. Females have on average 5 nestlings per year (FERRAZ; VERDADE, 2001). Even though they might be fertile throughout the year, according to Vargas *et al* (2007) most of the mating occurs during rainy

seasons, which might be due to the fact that during these seasons there is more food availability, which consequently increases the ovulation rates in the fertile females. Finally, sexual dimorphism is not perceptible for the genitalia in males and females is identical; despite this fact, males can be differed from females through the observation of the nasal gland, which is more prominent in males compared to females (MOREIRA; MACDONALD, 1996).

To summarize, capybaras are highly adaptable, very prolific animals that have been increasing their presence in cities due to the fact that they can reproduce very well, dietary items are highly available and also absence of natural predators. Their presence in urban areas might be concerning since they might be hosts to multiple zoonotic diseases, such as spotted fever, leptospirosis, salmonella, among others (LUZ *et al*., 2019; DE ALBUQUERQUE *et al.*, 2017; FARIKOSKI *et al.*, 2019).

2.2. Order Piroplasmida

Parasites belonging to the order Piroplasmida cause multiple diseases in humans, companion animals (dogs and cats) and wildlife (SCHNITTGER *et al*., 2022). The name piroplasmida refers to its pear-shaped (pyriform) intra-erythrocytic stages (VOTÝPKA *et al.,* 2017). These parasites are one of the most common groups of mammalian blood parasites and cause a major impact economically and on veterinary and medical care (JALOVECKA *et al.*, 2018). The order includes two main families: Babesiidae and Theileriidae. Taxonomically they are grouped among the genera *Babesia*, *Cytauxzoon*, and *Theileria,* comprising of dixenous haemoprotozoan parasites which are transmitted by ticks to mammalian, avian or reptilian hosts (SCHNITTGER *et al*., 2022; VOTÝPKA *et al*., 2017).

The genus *Babesia* comprises over 110 species, representing important pathogens in bovines, sheep, goats, horses, pigs, cats, rodents, and humans (UILENBERG, 2006). Parasites from the genus *Theileria* infect mostly ruminants, although multiple non-ruminant species have been described as hosts as well, such as foxes, and horses (SIVAKUMAR *et al*., 2014). The *Cytauxzoon* genus is far less studied compared to the other two genera within the order Piroplasmida, and it has been related to infections in domestic and wild felids (PANAIT *et al*., 2021).

Not many studies have been conducted in order to investigate the presence of piroplasmids in capybaras. One single study (GONÇALVES *et al*., 2021) describes the presence of a still uncharacterized piroplasmid species collected from ticks from *R. rattus* and

H. hydrochaeris, therefore more study needs to be done in order to identify possible unknown species that occur in large rodents.

2.2.1. Transmission

The incidence of tick-borne diseases among humans, domestic as well as wild animals has been increasing in the past decades. This increase might be related to the destruction of preserved areas and to a closer proximity of these locations to urban areas, enabling contact between wild animals and their respective ectoparasites, with domestic animals and humans (ANDRÉ, 2018).

The transmission of piroplasmids is mainly through vectors (ticks) (VOTÝPKA *et al.*, 2017). Their life cycle, as illustrated in Fig.1, consists of sexual multiplication in the definitive host (ticks) and assexual multiplication in the erythrocytes of vertebrates (CHAUVIN *et al*., 2009; FRIEDHOFF, 2018).

The ticks involved in the transmission of piroplasmids belong to the family Ixodidae (ONYICHE *et al*., 2021). Ticks from this family -also known as hard ticks- are obligate hematophagous, feed on a broad range of hosts, and have over 700 species described (GUGLIELMONE *et al*., 2014).

The ticks get infected by feeding on contaminated vertebrate hosts (SCHNITTGER *et al.*, 2022). However, infected females can transmit to their offspring which is called transovarial transmission (WESTBLADE *et al.*, 2017), transstadial transmission might occur, which means that the infection persists from one phase of the development of the tick to another, given that it can prolong up to two ecdyses of the parasite (ASENSI, 2018). Concerning the mammal hosts, besides de tick bites, infections can be transmitted also through blood transfusions from contaminated individuals (VIEIRA *et al.*, 2013).

Not much data concerning the lifecycle of piroplasmids within the vector is available, and parts of it remain unclear (BECKER *et al.,* 2013). Once the tick gets infected, the parasites within the blood cells migrate to the intestinal lumen, and only the ones in the form of gametocytes survive and multiply (gamogony and sporogony) (JALOVECKA *et al.*, 2018). The gametocytes then differentiate into two different types of gametes still in the tick gut (BECKER *et al.,* 2013). The fertilization of the gametes occurs when finger-like structures from one gamete penetrate an opposite gamete, which results in the formation of a zygote (BISHOP *et al*. 2015). The zygote then enters into the epithelial cells of the ticks intestine, where it undergoes meiotic divisions to form kinetes, which are released into the

tick hemolymph, from which they migrate to multiple organs, including the salivary glands, where they develop into the infective stage, known as sporozoites (BECKER *et al.,* 2013).

When it comes to the transmission of the genus *Cytauxzoon,* the tick *Dermacentor variabilis* is generally related to the transmission, although reports of transmission by *Amblyomma americanum* also exist (ZIEMAN *et al.,* 2017; THOMAS *et al*., 2017). As for the genus *Theileria*, ticks from the genus *Amblyomma* and *Rhipicephalus* are generally involved (BISHOP *et al*., 2015). *Babesia* spp. tends to present host specificity, allowing multiple species of *Babesia* to emerge, each one infecting preferably a specific kind of vertebrate organism (CHAUVIN., 2009). Similarly, there is also a certain specificity when it comes to the species of the arthropod vector; for example: *B. vogeli* is transmitted within dogs through the tick *Rhipicephalus sanguineus*, and *B. bovis* transmitted to cattle by *Rhipicephalus microplus* (SCHNITTGER *et al.*, 2012).

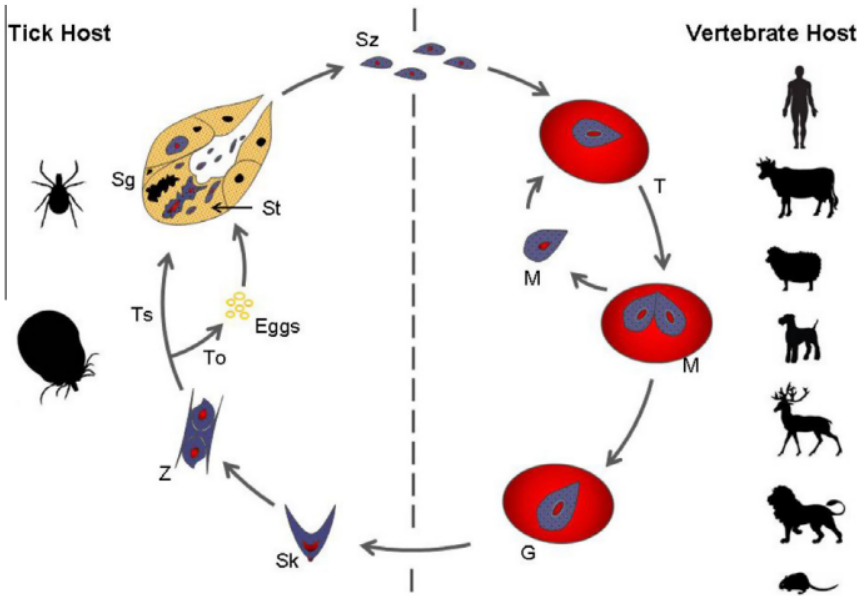

Fig. 1. Generic life-cycle of *Babesia* spp. *Babesia* sporozoites (Sz) are injected into the bloodstream of a vertebrate host with minute amounts of saliva, during the blood meal of an infected tick. After invading erythrocytes they differentiate into trophozoites (T), which divide asexually (merogony) into two or sometimes four merozoites (M). Merozoites exit the erythrocytes and invade new ones, continuing the replicative cycle in the host. A few merozoites stop division and transform into gamonts or pregametocytes (G). Gamogony and sporogony take place in the tick. When gamonts are taken up by a tick feeding on an infected host, they differentiate in the gut into gametes, also

known as ray bodies or Strahlenkörper (Sk), that fuse forming a diploid zygote (Z, gamogony). Zygotes undergo meiosis giving rise to motile haploid kinetes, which multiply by sporogony and access the hemolymph, invading and continuing their replication in several tick organs, including the salivary glands (Sg). Here, a final cycle of differentiation and multiplication takes place, in which kinetes transform into sporozoites that will infect a vertebrate host after the tick has molted into the next stage, i.e. larvae to nymph or nymph to adult (transstadial transmission, Ts). In some *Babesia* spp. (*Babesia* sensu strictu), kinetes also invade the tick ovaries and eggs, and infective sporozoites are formed in the salivary glands of the next generation larvae (transovarial transmission, To) (Font: MEHLHORN; SCHEIN, 1985).

Capybaras (*Hydrochoerus hydrochaeris*) have been reported as the primary host of some species belonging to genus *Amblyoma,* especially the species *A. sculptum* and *A. dubitatum;* these parasites can infest humans and other species, contributing to the spread of multiple diseases, such as babesiosis (LABRUNA *et al*., 2007; BRITES-NETO *et al*., 2018).

2.2.2. Physiopathology, clinical signs and laboratorial changes

The manifestations of babesiosis can differ greatly, ranging from subclinical infections, mild nonspecific symptoms to being potentially fatal, as a multisystemic disease (BAJER *et al*., 2022). After being deposited into the dermis, sporozoites invade red blood cells, then undergo differentiation into trophozoites, then differentiate once again into merozoites, which triggers host cell lysis. As a consequence, usually host animals present anemia, fever, and myalgia (VANNIER *et al*., 2022; ZWART *et al*., 1979).

Capybaras are animals that are very overlooked in terms of scientific studies. As a consequence, studies describing physiopathology and clinical signs still don't exist. Therefore, for this review these aspects will be analyzed based on studies in other species for comparison purposes.

In dogs, the condition may present itself as clinical or subclinical. Considering most cases in Brazil have a subclinical manifestation, it is important to evaluate and cross examine all the clinical findings to actually reach diagnosis (TRAPP *et al*., 2006). The clinical forms might present an acute, hyper acute or chronic onset (KELLY *et al.*, 2015). Multiple factors might influence the severity of the symptoms, such as age, genetics, immune status (SCHNITTGER *et al.*, 2012), as well as the *Babesia* strain involved (KELLY *et al.*, 2015).

The prognosis tends to be worse if the animal is also affected by other pathogens (SCHNITTGER, 2012).

For the clinical form of babesiosis in dogs, common clinical signs include: apathy, regenerative hemolytic anemia, fever, anorexia, vomiting, diarrhea, haemoglobinuria, jaundice (BARBOSA et al., 2020). Hepatopathy is a complication that might occur resulting in signs like bilirubinemia, pigmenturia and jaundice, which is observed even in dogs with only hemolysis due to the erythrocyte parasite (KELLY *et al.*, 2015). Also, a less common complication is acute kidney injury, which might happen as a consequence of anemic hypoxia from erythrocyte destruction, resulting in hemoglobinuria (LOBETTI; JACOBSON, 2001). Nevertheless, it is important to highlight that there is no pathognomonic sign, therefore for an accurate diagnosis it is crucial to associate the clinical signs with the presence of the specific vectors within that region, and according to laboratory tests (OTRANTO *et al*., 2010).

In rodents, experimental infection of *B. microti* in nude mice (*Foxn1nu*) found signs of anemia, and ataxia during parasitemia, however rats that underwent thymectomy were not able to eliminate the parasite (CLARK; ALLISON, 1974). Furthermore, *B. microti* causes transient infection in mice (*Mus musculus*) that is usually resolved within 2-3 weeks of exposure; whereas the animals generally develop anemia, the intensity of this clinical sign is rarely severe (BARNARD *et al*., 1996).

As for cytauxzoonosis, wild felids are natural reservoirs to the parasite, being generally asymptomatic; on the other hand, domestic cats present a myriad of signs, generally of severe intensity, frequently leading to fatalities, which thus makes the cat an accidental, dead-end host (PANAIT et al., 2021). The disease has a tissue and an intraerythrocytic phase which causes symptoms such as severe circulatory impairment and hemolytic anemia (BIRKENHEUER, 2006). Domestic cats might present fever, jaundice, dyspnea, anorexia, and lethargy (SNIDER *et al.,* 2010). A characteristic of this disease is that the parasite undergoes schizogony inside mononuclear cells transforming them into schizonts (Fig.2); these schizonts attach to the endothelium or within the lumen of veins and venules of multiple organs, and after getting enlarged they act as thrombi-like structures causing occlusion (REICHARD *et al*., 2021). The occlusions are directly related to the main clinical signs and beggin 11 to 14 days after infected ticks start feeding (SNIDER *et al.,* 2010). However, recent studies have demonstrated that the circulating strain of *Cytauxzoon* in Brazilian cats are less fatal, being generally asymptomatic, suggesting that different strains affect felids in North America and South America (ANDRÉ *et al*., 2022; DE OLIVEIRA *et al*., 2022). The genus *Theileria* is another important group of piroplasmids that affect a broad range of domestic and

wild ruminants (SIVAKUMAR *et al.*, 2014). Besides ruminants some other species have also been described as hosts to specific *Theileria*, for example *T. anne* in foxes, *T. equi* in horses, and *T. youngi* in woodrat (KJEMTRUP *et al*., 2001; CAMACHO *et al.*, 2001; MEHLHORN; SCHEIN, 1998).

The genus *Theileria* affects lymphocytes, erythrocytes, and macrophages (KILINC, 2018). As a consequence signs such as anemia, lymph node enlargement, hypoxia as well as renal, cardiac and respiratory disorders (RAZAVI, 2015). Acute renal failure might occur as a consequence of hemolysis and subsequent nephropathy induced by hemoglobin as well as from systemic hypoxia (AHMADPOUR *et al.*, 2020). The cardiac tissue damage might be due to the hypoxia as a consequence of the anemia, and also due to endothelial cell injury from homocysteine, an aminoacid resulting from the demethylation of methionine (RAZAVI, 2015). The respiratory consequences are attributed to schizonts enlarging and multiplying within lymphocytes and macrophages, causing the production of complement factors, which end up causing pulmonary congestion and edema (KILINC, 2018; RAZAVI, 2015).

Thrombocytopenia is another common sign for most diseases caused by piroplasmids, although its mechanism is not yet clarified (REDDY *et al.*, 2016). Even without hemorrhages, infected animals might present thrombocytopenia. This might be due to multiple mechanisms such as disseminated intravascular coagulation, immune-mediated platelet destruction, and splenic platelet sequestration (BOOZER; MACINTIRE, 2003)

As for the biochemical changes, an expressive urea elevation might be present, and its possible cause is an increased muscle catabolism (BOOZER; MACINTIRE, 2003). When associated with increased creatinine levels it might indicate acute renal failure (ROY, 2019). ALT values might also be elevated possibly due to hepatic hypoxia as well as the muscle catabolism (LOBETTI, 2012).

2.2.3. Diagnosis

The methods of diagnosing babesiosis include clinical findings, parasitological diagnosis, serological and molecular tests (O'DWYER *et al*., 2009). Also, detection methods have become much more sensitive within the last decades. Detection of biomarkers of infection are classified according to the type of biomarker: antibody, nucleic acid, or antigen. Bead-based methods can be adapted for detection of either nucleic acid or antibody, while ELISA can be used to detect antibody or antigen (MEREDITH *et al*., 2021).

Among the direct methods of demonstration of *Babesia* parasites there are two options: experimental inoculation and blood film microscopy. The first one involves inoculating living animals with blood from suspected subjects into a healthy susceptible host, such as hamsters. The latter consists in identifying the parasite on a Giemsa stained blood film, being a lot less time consuming than the inoculation method. The chance of a false negative can be a problem for these methods (MEREDITH *et al*., 2021).

Detection of biomarkers are generally more sensitive as well as time and cost efficient. Nucleic acid-based assays, antigen detection assays, antibody-based assays can detect infections considerably more efficiently and have been replacing the direct demonstration methods in the last decades (MORITZ *et al.*, 2016).

Molecular methods, such as polymerase chain reaction (PCR), have a higher specificity and sensitivity compared to other methods, such as blood smear testing for the detection of *Babesia* spp. in peripheral blood (VASCONCELOS, 2010). The combination of gene sequence analysis and PCR can increase the information about the subspecies, presenting an advantage in epidemiological studies with molecular methods (INOKUMA *et al.*, 2004).

The molecular revolution created huge improvements in diagnostic essays, which are able to both target genes from the whole order Piroplasmida as well as specific genes and species (CRIADO-FORNELIO, 2007). Most primers target the 18S RNA gene, as it is a well preserved gene, being able to detect most pathogens (ZHU; ALTMANN, 2005). The primers CryptoF/CryptoR amplify about 1700 bp, being sensitive to most parasites belonging to the phylum Apicomplexa, which includes the piroplasmids (HERWALDT *et al.*, 2003). The primers Piro A Piro B amplify about 400 bp, and are efficient in detecting most *Babesia* and *Theileria* species (LAMPEREUR, 2017; CARRET, 1999). Other genes that have similar spectrum are BJ1/BN2 (411 - 452 bp) and BTV4_Fow/BTV4_Rev (411 - 452 bp), and are also sensitive to most piroplasmids (SCHNITTGER *et al*., 2012; LAMPEREUR, 2017).

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CAPÍTULO 2

RESUMO

A presença de capivaras (*Hydrochoerus hydrochaeris)* tem aumentado nos centros urbanos nas últimas décadas e com isso houve também um aumento da preocupação em relação à presença de carrapatos em áreas de lazer de Brasília. No entanto, não existem ainda muitos estudos que avaliem o status sanitário desses animais, sobretudo no que tange a ocorrência da infecção por agentes transmitidos por carrapatos, especialmente piroplasmídeos. Para este estudo, foram colhidas amostras de sangue de 53 capivaras capturadas nas regiões do Zoológico de Brasília, assim como na Fazenda Experimental Sucupira (EMBRAPA) e na orla do Lago Paranoá. As amostras de sangue foram submetidas a hemogramas, testes bioquímicos e analisadas para a presença de piroplasmídeos por meio de diagnóstico molecular (PCR). Um total de 15% (8/53) das amostras das capivaras amplificou o DNA dos hemoparasitas. Não foram observadas alterações laboratoriais entre os animais positivos e negativos. Portanto, neste estudo pode-se verificar a presença de piroplasmídeos em capivaras de vida livre, porém mais estudos precisam ser feitos para identificação e caracterização das espécies de piroplasmídeos infectantes.

Palavras-chave: 1. Babesia. 2. Ordem piroplasmida. 3. Vetores artrópodes. 4. Capivaras.

ABSTRACT

The presence of capybaras (Hydrochoerus hydrochaeris) has increased in recent decades and with them there has also been an increase in concern regarding the presence of ticks in frequently visited recreational areas, such as the Brasilia Zoo Foundation. However, there are still not many studies that assess the health status of these animals, hence the importance of studies like the present one. For this study, blood samples were collected from 53 capybaras captured in the regions of the Brasilia Zoo Foundation, as well as from the Sucupira Experimental Farm (EMBRAPA) and from the shore of Paranoá Lake. The blood samples were submitted to hemograms, biochemical tests and analyzed for the presence of piroplasmids by means of molecular diagnostics (PCR). A total of 15% of the capybara samples amplified the DNA of the hemoparasites. In the hematological and biochemical tests, the only discrepancy between the positive and negative capybaras was for urea nitrogen, which is possibly a consequence of muscle catabolism due to stress. Therefore, in this study we could verify the presence of piroplasmids in free-living capybaras, but more studies need to be done to identify the infecting species.

Key words: 1. babesiosis. 2. piroplasmid order. 3. arthropod vectors. 4. capybaras.

1. INTRODUCTION

The presence of groups of capybaras (*Hydrochoerus hydrochaeris*) have increased in several recreational areas of the Federal District, such as the Zoo of Brasilia and the margins of Paranoa Lake. Commonly, cases of tick infestations in people who frequent these places are reported in the news media (DFTV, 2017; MEDEIROS, 2018). Capybaras (among other wild mammals) are associated to some extent with the maintenance of certain tick populations, as they are considered the primary hosts to these arthropods (HORTA *et al.*, 2004; LABRUNA *et al.*, 2004).

There is a concern about what diseases capybaras might carry, although not many studies have been made. Capybaras, as well as rodents in general, can potentially carry tick-borne diseases. Despite the fact that these diseases, such as babesiosis and Lyme disease, have been emerging continually in the past decade, they still remain under-notified, especially in underdeveloped countries, such as Brazil (KUMAR et al., 2021). In fact, capybaras studies, such as the present one, are important to determine if capybaras might actually play an important role in the transmission of zoonosis, which is crucial for the delineation of efficient public health programs.

Ticks are vectors of multiple pathogens, and for some the relationship between these arthropods and capybaras might be concerning in the sense that the rodents would be carrying and maintaining in urban areas agents with zoonotic potential. One of these possible pathogens are the parasites belonging to the order Piroplasmida, which are one of the most prevalent hemoparasites in mammals, having an important economical impact in veterinary as well as in human medical care (JALOVECKA *et al*., 2018). The most prevalent parasite from this order belongs to the genus *Babesia.* They are intra-erythrocytic parasites that affect mammals in general, it can be asymptomatic or cause blood related dysfunctions, such as anemia, and in some casesit can be potentially deadly (BAJER *et al.*, 2022).

The role that capybaras play in the emergence of diseases is still unclear. Furthermore, with regards to piroplasmids it is still unknown whether they carry pathogens that have zoonotic potential. Gonçalves et al. (2021) describes the presence of an unidentified piroplasmid in a group of sampled capybaras, but further studies have yet to be made to determine which species affect these large rodents.

This study aimed to assess the presence and prevalence of piroplasmids infecting capybaras that live around urban areas from the Federal District, Brazil, as well as correlating that with possible laboratory changes.

2. MATERIALS AND METHODS

2.1. Capture and biological sample collection

The handling of the animals was performed according to the SISBIO license number 43798-1, and with the approval of the Ethics Committee for Animal Use (CEUA) of the University of Brasilia registered with protocol number 20/2019 (Appendix X).

Capybaras were captured from the flooded areas of the Brasilia Zoo Foundation (Figure 1); the Sucupira Experimental Farm, belonging to Embrapa Genetic Resources and Biotechnology (Figure 2), and Paranoá Lake (Figure 3). The animals were conditioned by feeding them with fruits, grains, and foliage, to enter the stalls, where they were submitted to sedation and physical restraint for the collection of blood samples (Figure 4).

The chemical restraint protocol was performed with the use of intramuscular anesthetic darts, using the association of xylazine (0.5 mg/kg) and ketamine (2.0 mg/kg). After the harvests, a dose of yohimbine (0.1 mg/kg), selective antagonist of alpha adrenergic receptors, was applied in order to reverse the action of the applied xylazine and reduce the anesthetic recovery time. To minimize the risk of accidents, the animals were handled individually, monitored, and released only after full recovery from any effect caused by the dissociative drug.

Blood samples were collected by puncture of the cephalic or femoral vein (Figure 5), stored in tubes with anticoagulant (EDTA) and in dry tubes (without anticoagulant). The samples were kept cooled between 4 and 8 ºC until transport to the laboratory. After being analyzed, whole blood samples and blood serum were frozen at -20°C for later DNA extraction for PCR.

2.2. Hematology and Biochemical Tests

Hemograms and biochemical tests on capybara blood samples were performed at the Veterinary Clinical Pathology Laboratory of the College of Agronomy and Veterinary Medicine (FAV) of the University of Brasilia (UnB), within a maximum time of 2 h after harvesting.

Blood cell count was determined by manual counting in a hemocytometer, as previously described (Weiser, 2012). Hemoglobin concentration was measured on a BIO 2000 (BIOPLUS®) semi-automatic spectrophotometer using a commercial kit (Labtest®). The hematocrit was determined using the microhematocrit technique, and the amount of total

plasma proteins (TPP) was determined using the same capillary tube by refractometry. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined by standard calculation. Whole blood smears were prepared and stained with rapid panoptic (Laborclin®) to perform the leukocyte differential, morphological observation of blood cells, hemoparasite search, and platelet estimation.

Blood serum was obtained by centrifuging blood samples without anticoagulant. These samples were used to determine serum concentrations of total proteins, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), urea, creatinine, and alkaline phosphatase in an automatic biochemical analyzer Cobas - C111 (Roche®).

2.3. Molecular assays

The polymerase chain reaction (PCR) tests were performed in the Microbiology and Molecular Pathology Laboratory, from the College of Agronomy and Veterinary Medicine (FAV) of the University of Brasilia – (UnB).

DNA from EDTA blood samples was extracted using the Illustra Blood Genomicprep Kit (GE HealthCare®) according to the manufacturer's guidelines. The extracted DNA samples were subsequently stored at -20ºC until the PCR was performed.

All DNA samples extracted from whole blood were submitted to PCR for confirmation of the gene encoding the enzyme GAPDH (glyceraldehyde-3phosphate dehydrogenase), for the evaluation of the integrity of the extracted DNA and absence of PCR inhibitors (BIRKENHEUER *et al,* 2003). For each sample, a 25 µL mixture was prepared containing: 1.0 µmol/ µL of each oligonucleotide (10 pmol); 1 X Taq polymerase buffer (Invitrogen®); 1.5 mM MgCl2; 0.2 mM dNTP (Invitrogen®); 1 U of Taq polymerase (Invitrogen®); and about 2.0 ng of extracted DNA. The PCR assays were performed according to the following protocol in the thermal cycler: one cycle of initial denaturation (5 min, 95 ºC); followed by 40 cycles of denaturation (1 min, 95 ºC), annealing (1 min, 53°C) and extension (1 min, 72ºC) and; one cycle of final extension (5 min, 72ºC).

The oligonucleotides PIRO A (**5'**AATACCCAATCCTGACACAGGG **3'**) and PIRO B (**5'** TTAAATACGAATGCCCCCAAC **3'**) were used for the amplification of piroplasmid 18S rRNA gene, resulting in a product of approximately 400 base pairs (bp), described by Armstrong (1998). For each sample, a 25 µL mixture was prepared containing: 1.0 µmol/ µL of each oligonucleotide (10 pmol); 1 X Taq polymerase buffer (Invitrogen®); 0.75 mM of

MgCl2; 0.2 mM of dNTP (Invitrogen®); 1 U of Taq polymerase (Invitrogen®); and 2.0 ng of extracted DNA. These PCR assays were performed following the protocol proposed by the same author: initial denaturation at 95°C for 5 minutes, followed by 50 cycles 95° C for 45 seconds, 58°C for 45 seconds, and 72°C for 45 seconds, and final extension at 72°C for 5 minutes. PCR products were subjected to 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light.

All PCR reactions were performed on the C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). Amplification products were separated by 2% agarose gel electrophoresis, stained in ethidium bromide (Vetec Sigma-Aldrich®, St Louis, MO) and visualized in an ultraviolet transilluminator (UV transilluminator®, UVP LLC, Upland,32 CA). All DNA samples were tested in duplicates, and the reactions included positive and negative control samples.

2.4. Statistical analysis

Statistical analysis was performed using the Mann-Whitney test, using the software The jamovi project (Version 2.2, 2021) for Windows. The aim of the test is to evaluate the effects of the infection by comparing hematological, and biochemical results between the positive and negative groups. A significance of 5% was considered for this test.

Figure 1. Georeferencing of the collection points located at the Fundação Zoológico Point A: Lat:15° 50' 48" S, Long: 047° 56' 27" W; Point B: Lat:15° 51' 03" S, Lon: 047° 56' 17" W and Point C: Lat:15° 51' 00" S, Long: 047° 55' 57" W. Available at https://earthexplorer.usgs.gov/. Accessed on 23/08/2022.

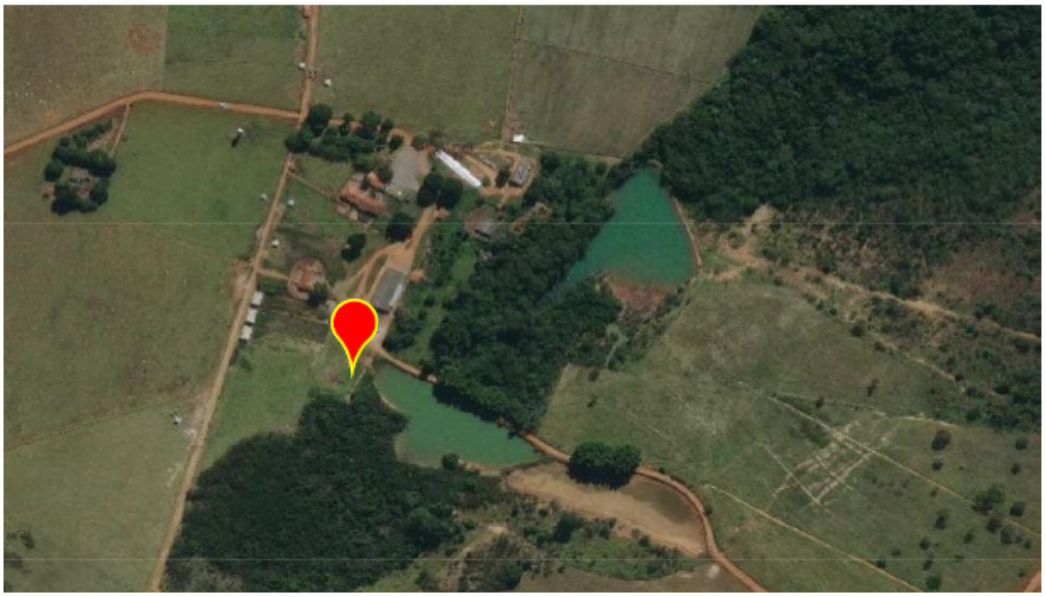


Figure 2. Georeferencing of the collection point at the Fazenda Sucupira Experimental Sucupira experimental field. Lat:15° 55' 31" S, Lon:048° 02' 59" W. Available at https://earthexplorer.usgs.gov/. Accessed on 23/08/2022.

Figure 3. Georeferencing of the collection point in the Paranoá Lake region. Lat:15° 47' 30" S, Long: 047° 47' 49" W . Available at https://earthexplorer.usgs.gov/. Accessed on 23/08/2022.

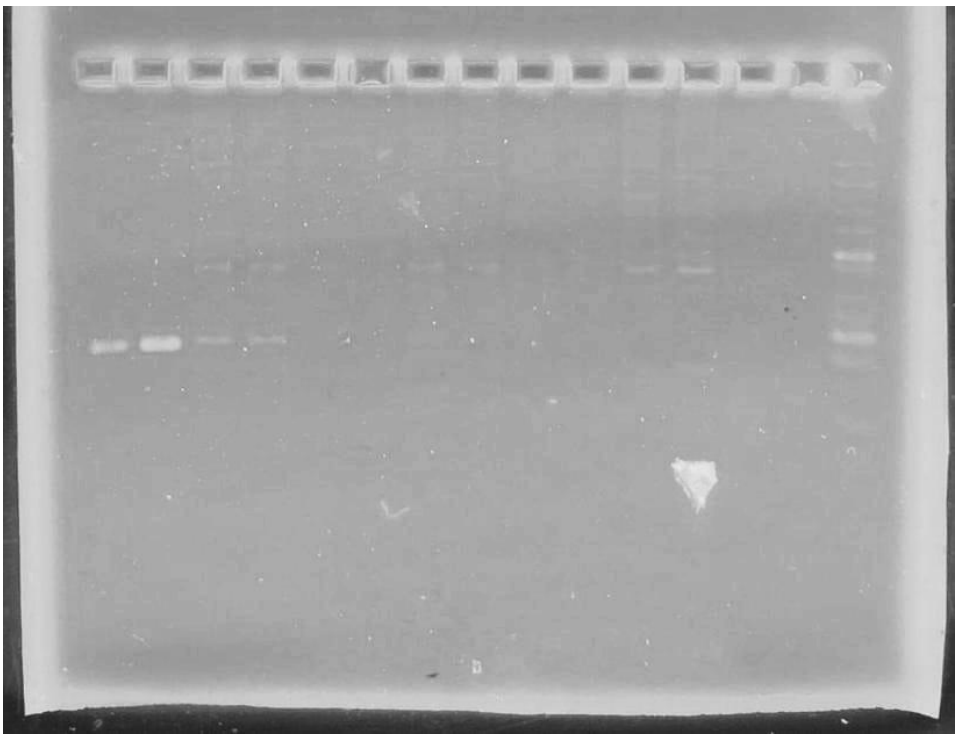
Figure 4. Capybaras restrained in a breech using conditioning by means of feeding.

Figure 5. Capybara blood sampling by femoral vein puncture.

3. RESULTS

A total of 53 free-living capybaras were captured for biological sampling, being 15 males (13 adults and two juveniles) and 40 females (33 adults and seven juveniles). Considering the regions sampled, 13 adult females were captured in the Paranoá Lake region, two adult females at Sucupira Farm, and 40 animals (15 males and 25 females) at the Zoo Foundation.

PCR assays using the oligonucleotides PIRO A and PIRO B were used to identify piroplasmids positive samples. Within the 53 DNA samples extracted from the capybara blood tissue eight (8) samples amplified piroplasmid templates. An example of a positive test is presented in Figure 6. None piroplasmid inclusion was observed in blood smears.



<400bp

Figure 6. PCR results in agarose gel (2%) to piroplasmids from blood samples of capybaras using the oligonucleotides PIRO A/PIRO B. All samples were tested in duplicates.
PC: positive control; NC: Negative control
L: 100 bp Molecular Weight Marker (Biorad®)
01 and 02: Amplified DNA in duplicate
03 to 10: negative PCR samples

A total of six (6) positive tested capybaras were captured at the Brasilia Zoo Foundation, and two (2) were from the Paranoá Lake region. Only two capybaras were captured at Sucupira Experimental Farm, however none of them tested positive (Table 1). Also, three out of the eight positive animals were juveniles, and the 5 others were adults. Four of them were females, two males and two individuals were not identified. The laboratorial results as well as the list of animals and their origins are demonstrated on tables 1 to 3 and on Appendix I to III.

Table 1. Distribution of positive and negative piroplasmid animals according to sample location.

Origin	Positive	Negative	TOTAL
Zoo Foundation	6	32	38
Paranoá Lake	2	11	13
Sucupira Experimental Farm	0	2	2
TOTAL	8	45	53

Table 2. Values obtained in the hemograms of free-living capybaras (*Hydrochoerus hydrochaeris*) from the Federal District that tested positive, and negative for piroplasmids by PCR.

	Mean ± Std deviation		Median	
Erythrogram	**Positive**	**Negative**	**Positive**	**Negative**
Hematocrit (%)	37,75 ± 4,027	37,4 ± 5	37.5	38
Hemoglobin (g/dl)	11,64 ± 2,083	11,65 ± 2,9	12.3	11.4
RBCs (x 106/ µL)	2,963 ± 0,6059	2,972 ± 0,848	3.065	2.82
MCV (fl)	131,2 ± 24,67	131,7 ± 29,34	128.7	133.3
MCHC (g/dl)	30,74 ± 3,664	31,66 ± 8,177	31.95	30.59
Leukogram				
Total leukocytes (x 103/ µL)	7056 ± 1621	7023 ± 2351	6625	6500
Basophils (x 103/ µL)	0	0,1778 ± 0,5347	0	0
Monocytes (x 103/ µL)	873,3 ± 221,2	842,5 ± 524,8	893.5	780
Eosinophiles (x 103/ µL)	817,8 ± 523,1	1059 ± 613,5	786	915
Lymphocytes (x 103/ µL)	2366 ± 1079	1821 ± 936,6	2288	1800
Neutrophils (x 103/ µL)	2999 ± 1983	3279 ± 1529	2438	2958
Platelets (x10³ / µL)				
	213000 ± 64169	190000 ± 56664	215000	190000

RBC: red blood cells; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration.

Even though the erythrogram results didn't present any significant differences between the positive and negative groups, a few disparities can be observed in the leukogram

and in the biochemical tests. However only the urea nitrogen parameter presented a statistical difference ($p<0.05$).

Table 3. Values obtained from serum biochemical tests of free-living capybaras (*Hydrochoerus hydrochaeris*) from the Federal District that tested positive, and negative for piroplasmids by PCR.

Biochemical tests	Mean ± Std deviation		Median	
	Positive	Negative	Positive	Negative
ALT (UI/L)	51,75 ± 31,18	56,29 ± 23,63	57.5	59
AST (UI/L)	47,38 ± 31,13	42,89 ± 21,72	38.5	37
Alkaline phosphatase (UI/L)	156 ± 123,8	143 ± 93,28	132.5	110
Urea (mg/dl)	64 ± 70,99[a]	31,29 ± 11,1[b]	43[c]	31[d]
Creatinine (mg/dl)	1,3 ± 0,3137	1,5 ± 0,4	1.5	1.4
Total protein (g/dl)	6,113 ± 0,60	6,0 ± 0,72	6.2	6
Albumin (g/dl)	2,725 ± 0,3232	2,8 ± 0,6	3	2.9
Globulin (g/dl)	3,388 ± 0,7633	3,242 ± 0,65	3.3	3.3

ALT: alanine aminotransferase; AST: Aspartate aminotransferase;. Numbers in the same line followed by different letters differ ($p<0.05$) by Mann-Whitney test.

4. DISCUSSION

Several groups of capybaras (Hydrochoerus hydrochaeris) circulate freely in recreational areas of the capital of Brazil. The regions surrounding the Paranoá lake and the Brasilia Zoo Foundation, where the biological sampling points for the present study were installed are an important example of their presence in urban areas. In addition to this, every year cases of tick infestations in children and adults that frequent these areas are reported in the news. These cases have been directly associated with the presence of capybaras and there are still few published studies that report the health situation of animals of the species in the region (DFTV, 2017; MEDEIROS, 2018).

A total of eight capybaras out of the 53 sampled amplified piroplasmid DNA, therefore 15% of the sampled animals, which is a considerable amount. This is compatible with the prevalence of piroplamids in dogs within the region (PANTI-MAY; RODRÍGUEZ-VIVAS, 2020). A total of six positive tested capybaras were captured at the Brasilia Zoo Foundation, and two were from the Paranoá Lake region. Taking into consideration that 13 of the animals were from the Paranoá Lake region, the proportion of positive animais per region still remains around 15%, therefore not having a difference between the prevalence of the regions. This is compatible with the prevalence of piroplasmids (*Cytauzoon felis* and *B. vogeli*) found in cats in the Federal District region (DE OLIVEIRA *et al*., 2022). Only two capybaras were captured at Sucupira Experimental Farm, however none of them tested positive. No infection variation pattern concerning age, sex or origin could be observed, also consistent with the findings of De Oliveira *et al.* (2022) in cats.

The species of piroplasmid was not determined. However, taking into consideration that *Babesia* is the genus that causes infection in most rodents, it is possible to infer that possibly for capybaras the same is true. This can be supported by the fact that the region of the Federal district is also considered an endemic area for multiple species of *Babesia* (DUARTE *et al.*, 2011). In dogs, the prevalence of *Babesia* sp. varies widely, but studies show a range from 4 to 26% depending on the region (PANTI-MAY; RODRÍGUEZ-VIVAS, 2020; PAULINO *et al*., 2018; SPOLIDORIO et al., 2010). As for cats of the region, a prevalence of 21.7% was found, from which 11.4% were positive for *Babesia* sp., and 7.2% were positive for *Cytauxzoon* spp (DE OLIVEIRA *et al*., 2022).

The positive animals did not present any clinical and laboratorial signs of infection, except for urea increase. It is important to notice that regarding the hematological and biochemical values obtained, it was not possible to compare the data with other authors due to the absence of reference values for the species in the literature. As for the hematological study, no significant statistical difference can be detected in terms of red blood cell changes. This contrasts with literature which generally correlates parasitemia with significant decrease in red blood count, hemoglobin concentration and packed cell volume in other species of mammals such as dogs, donkeys, bovids, and rodents (AMBAWAT et al., 1999; REDDY et al., 2013; SALEM et al., 2016; PARK et al., 2015). However, domestic cats infected with piroplasmids from the Federal District region also do not seem to manifest laboratorial changes, as demonstrated by De Oliveira (2020). Finally, no discrepancies could be detected in terms of the leukogram of the two groups, differing from previously studies in dogs which demonstrated that *Babesia* positive animals generally present neutropenia, lymphopenia as well as thrombocytopenia (REDDY et al., 2013).

On the other hand, referring to biochemical changes, a significant discrepancy can be noticed in the urea levels. This is consistent with literature, which describes significant increase of those biomarkers in positive animals (ROOPALI et al., 2018). This alteration could be related to either acute kidney injury (SCHOEMAN, 2009) or catabolism of lysed erythrocytes (SCALLY et al., 2004), which can happen in positive animals. Also, according to Lobetti and Jackobson (2001), acute renal injury is a less common complication in dogs, and might be related to the anemic hypoxia derived from erythrocyte destruction, resulting in hemoglobinuria. Another possible cause of elevated urea is an increased muscle catabolism, which might happen as a consequence of the stress that the animals overcome while being handled (HASSELGREN, 2000), as well as possible dehydration (PROSS, 2017). Taking into consideration that none of the animals presented any other alteration, muscle catabolism could be a plausible explanation for their case. Another common finding for positive animals was an elevation in AST levels (appendix II), which corroborates with the possibility of muscle catabolism likely due to stress (EVANS, 2022).

The present study had a few limitations. Firstly, there is a number discrepancy between the positive and negative group. This problem is common when dealing with wildlife animals, as the researcher has no control over the amount or which animals will be set for each group. Another problem is that reference values for capybaras have not been described yet, so it is difficult to determine whether the results obtained are within reference range for the species.

Furthermore, this study opens questions to future studies. Firstly, the role that capybaras play in the maintenance of the infectious agents, such as piroplasmids, is not clarified due to the lack of knowledge of the dynamic of these diseases in wildlife. Secondly, it is not clear whether the piroplasmids that affect capybaras have pathogenic potential to affect other species, such as humans. Therefore, it is important that further studies explore more the dynamic of these pathogens.

5. CONCLUSION

Capybaras from the Federal District (Brazil) have an expressive prevalence of piroplasmid infection. A total of 15% of the subjects tested positive, which is compatible with the average prevalence in dogs and cats in the Federal District region. However, the laboratorial tests did not demonstrate relevant changes. This might suggest the infection presents asymptomatic in capybaras. Finally, further studies are required to determine which species of piroplasmids affect capybaras and if they might also affect humans.

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Appendix

Appendix I. Hemograms of positive capybaras

Capybara	1	5	8	10	16	30	35	42
Erythrogram								
Hematocrit (%)	44	37	40	38	40	30	36	37
Hemoglobin (g/dl)	14.2	11.8	13.1	13.1	12.8	9.2	10.6	8.3
RBCs (x 106/ µL)	3.09	3.76	3.36	3.57	2.45	2.17	3.04	2.26
MCV (fl)	142.39	98.4	119.05	106.44	163.27	138.25	118.42	163.72
MCHC (g/dl)	32.27	31.89	32.75	34.47	32	30.67	29.44	22.43
Leukogram								
Total leukocytes (x 103/ µL)	6650	5200	6400	9800	5200	8500	6600	8100
Basophils (x 103/ µL)	0	0	0	0	0	0	0	0
Monocytes (x 103/ µL)	1130.5	1092	896	980	780	425	792	891
Eosinophiles (x 103/ µL)	1197	676	896	588	416	0	1716	1053
Lymphocytes (x 103/ µL)	1995	2080	2496	4018	1456	680	2640	3564
Neutrophils (x 103/ µL)	2327.5	1352	2112	4214	2548	7395	1452	2592
Platelets (x10³ / µL)								
	162000	156000	192000	238000	324000	132000	238000	262000

Appendix II. Biochemical tests of positive capybaras

	1	5	8	10	16	30	35	42
Biochemical tests								
ALT (UI/L)	68	80	84	78	3	13	41	47
AST (UI/L)	27	44	41	43	34	123	31	36
Alkaline phosphatase (UI/L)	52	117	223	186	416	148	33	74
Urea (mg/dl)	45	60	47	41	36	237	21	25
Creatinine (mg/dl)	1.6	0.9	0.8	1.2	1.6	1.4	1.5	1.5
Total protein (g/dl)	6.7	6.1	6.6	6	6.7	6.3	5.2	5.3
Albumin (g/dl)	2.9	3.7	3.8	3.1	3.1	1.5	1.4	2.3
Globulin (g/dl)	3.8	2.4	2.8	2.9	3.6	4.8	3.8	3

Appendix III. List of animals sampled.

Number	Sex	Age	Origin	Status
1	Male	Adult	Zoo	Positive
2	Female	Juvenile	Zoo	Negative
3	Female	Juvenile	Zoo	Negative
4	Male	Adult	Zoo	Negative
5	Female	Juvenile	Zoo	Positive
6	Male	Juvenile	Zoo	Negative
7	Female	Juvenile	Zoo	Negative
8	Male	Juvenile	Zoo	Positive
9	Male	Adult	Zoo	Negative
10	Female	Juvenile	Zoo	Positive
11	Female	Juvenile	Zoo	Negative
12	Male	Adult	Zoo	Negative
13	Female	Adult	Zoo	Negative
14	Female	Adult	Zoo	Negative
15	Male	Adult	Zoo	Negative
16	Female	Adult	Zoo	Positive
17	Female	Adult	Zoo	Negative
18	Female	Juvenile	Zoo	Negative
19			Zoo	Negative
20	Male	Adult	Zoo	Negative
21	Female	Adult	Zoo	Negative
22			Zoo	Negative
23			Zoo	Negative
24			Zoo	Negative
25			Zoo	Negative
26			Zoo	Negative
27			Zoo	Negative
28	Female	Adult	Zoo	Negative
29			Zoo	Negative
30			Zoo	Positive
31	Female		Paranoá Lake	Negative
32	Female		Paranoá Lake	Negative
33	Female		Paranoá Lake	Negative
34	Female		Paranoá Lake	Negative
35	Female		Paranoá Lake	Positive
36	Female		Paranoá Lake	Negative
37	Female		Paranoá Lake	Negative
38	Female		Paranoá Lake	Negative

39	Female		Paranoá Lake	Negative
40	Female		Paranoá Lake	Negative
41	Female		Paranoá Lake	Negative
42	Female		Paranoá Lake	Positive
43	Femea		Paranoá Lake	Negative
44			Zoo	Negative
45			Zoo	Negative
46			Zoo	Negative
47			Zoo	Negative
48			Zoo	Negative
49			Zoo	Negative
50			Zoo	Negative
51			Zoo	Negative
52			Sucupira	Negative
53			Sucupira	Negative