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Prevalence of Immune Response of Healthy Equines with Antibodies Anti *Leishmania chagasi* in an Endemic Area of Leishmaniasis

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Abstract: Indirect Immunofluorescence Reaction (RIFI) was used to test employing conjugated anti-equine marked with fluorescein isothiocyanate and titration with point cutoff the dilution 1:40, for sample analysis serum from equines coming from an endemic area of leishmaniasis. The chi-square test were utilized to study the frequencies and prevalences of serum reagents between counties, races, sexes and ages of animals, which naturally made up the groups. The RIFI showed the prevalence of anti-*Leishmania chagasi* (*donovani*) in the sample population of counties - 40% - and sexes-42% of female - studied, confirmed that animals had contact with the parasite of Leishmania protozoonosis.

Key words: Equine · *Leishmania* sp · Leishmaniasis reservoirs · Phlebotomine · Immunofluorescence

INTRODUCTION

Obviously that in the absence of vector there is no transmission of protozoonosis which results in leishmaniasis. it is assumed the importance of analyzing the behavior of vectors in the transmission of this disease mainly infectious to humans, dogs and other domestic animals. There is no complete study for all who engage in blood feeding parasites (bats, ticks and other parasites that survive by biting) and the vector-perpetuating cycle of leishmaniasis. However, in a silvatic environment is important enshrined in the cycle, the sandflies vectors (promastigotes in the vector of cold blood) from Leishmania that feed on blood of mammals (amastigotes the host warm-blooded), establishing the perpetuation of the reservoir, vector and parasite [1-7].

Once broken the hegemony environment especially with the expansion of human civilization, new frontiers for colonization urban rural open to replace the old areas of primary forest reserves (extradomiciliary/sylvatic transmitter) and also migrate along the sandfly vector. New mammalian hosts are visited by the vectors in search of food for breeding. This interface with the domestic environment of peridomiciliary and domiciliary are listed all livestock mammals and birds can be visited by sandflies in search of food. However, there will consider the particularities of each new host that can be asymptomatic carriers or show the natural infection [8-10]. Thus, Zilberstein and Shapira [7] and Rosenzweig *et al.* [6] mentioned in the specific case of birds, although attractive as a source of blood, the role of pH and heat shock in gene regulation and its contribution to the differentiation processes in *Leishmania* is crucial because the physiological differences translated especialy the body temperature of ± 41°C and nucleated red blood cells, flagellated extracellular promastigotes could not differentiate or proliferate aflagellated the intracellular amastigotes since the genetic programming of an obligate intracellular parasite does not reflect the change of biochemical environment in this species. On the other hand, has been reported to break resistance followed by clinical form of visceral Leishmaniasis in animals considered silvatic reservoirs as is the case presented by Sherlock *et al.* [11] in Bahia in describing natural infection in opossum caught in residence in Bahia.

The *Equidae* (horses: *Equus caballus*; donkeys: *Equus asinus* and mules: *Equus caballus* x *Equus asinus*) have been diagnosed carrying natural infection to cutaneous Leishmaniasis [12-15].

Leishmaniasis, widely reported in the literature, it is known that since the evidence described as a disease caused by protozoan Trypanosoma similar to the agent, who by the impression smear of spleen from patients who died and then the Romanowsky's staining method using

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the compound of methylene blue and eosin, which became known the Leishman's stain for diagnosis of "Dum-dum fever", published in the Journal British Medical reporting the consequences, which attributed the discovery to William Boog Leishman [16], medical officer of the british army in India and Charles Donovan [17], in homage to which the parasite systemic at first was called Leishman-Donovan bodies, an infectious parasitic protozoonosis whose etiological agent is not contagious "*Leishmania donovani*" causes Kala azar or visceral leishmaniasis.

At the time, Leishman [16] reported the similarity of the parasite seen in smears of spleen stained with Romanowsky's modified as the agent, looking for trypanosomata - *T. evans*i and *T. lewisi*, causes the disease, the similarity of agents of trypasonomiasis of domestic animals found by other researchers in the African regions of the Gambia and Congo ("bad chair-*T. Evansi*" "dourine"-*T. equiperdum*; "nagana" or " tsetse "-*T. brucei*) and other continents visited and mentioned by several researchers on dates next at 1850 [16, 17].

In Brazil, a considereable epidemic was notable cases of ulcers accompanied by mucosal lesions in the early twentieth century in São Paulo, with the construction of the northwest ailroad, described as "Bauru ulcer", which culminates with the identification agent in 1909 and the vector *Lutzomyia* sp, name assigned to this vector, to honor the outstanding discovery of the brazilian scientist Adolpho Lutz [18] who first reported the existence of the sandfly [18-20].

Leishmaniasis, a disease caused by obligate intracellular, kinetoplastid protozoan of the genus *Leishmania*, is an old but largely unknown disease that afflicts the World's poorest populations. The disease is transmitted by the bites of infected sandflies that belong to the *Phlebotomus* and *Lutzomyia* genera in the Old and the New World, respectively. Neglected by researchers and funding agencies, leishmaniasis is endemic in 88 countries of the World in which 350 million people who are considered at risk of infection live. Cutaneous Leishmaniasis is a public health problem which is distributed in four continents (Americas, Europe, Africa and Asia), with record annual 1 to 1.5 million cases. It is considered by the World Health Organization as one of six major infectious diseases, due to its high detection rate and ability to produce deformities [15, 21-23].

The global burden of leishmaniasis has remained stable for some years, causing a morbidity and mortality loss of 2.4 million disability adjusted life-years and approximately 70,000 deaths, a significantly high rank among communicable diseases. There are two million new cases of leishmaniasis annually and 14 million infected people worldwide. The leishmaniasis are characterized by a spectrum of clinical manifestations: ulcerative skin lesions developing at the site of the sandfly bite (localized cutaneous leishmaniasis); multiple non-ulcerative nodules (diffuse cutaneous leishmaniasis); destructive mucosal inflammation (mucosal leishmaniasis); and disseminated visceral infection (visceral leishmaniasis). The outcome of infection depends on the species of *Leishmania* parasites and the host's specific immune response [24].

In Brazil, from 1985 to 2005, there was an average of 28,568 autochthonous cases recorded and the average detection rate of 18.5 cases/100,000 inhabitants, there being higher weightings in 1994 and 1995, when they reached levels of 22.83 and 22.94 cases/100,000 inhabitants, respectively, which gives them a character hyperendemic [15].

Leishmania is a protozoan belonging to the family *Trypanosomatidae* obligate intracellular parasite of mononuclear phagocytic system cells, with two main forms: a flagellate promastigote or found in the gut of the insect vector and another amastigote or not flagellate observed in tissues of vertebrate hosts [25].

Leishmania infections that cause the American Cutaneous Leishmaniasis (ACL) have been described in several species of silvatic animals, synanthropic and domestic (dogs, cats and horses). Regarding the latter, its role in maintaining the parasite in the environment has not been definitively clarified. Have been recorded as hosts and potential reservoirs some rodent species, marsupials, edentulous and silvatic canids [26-28].

Are numerous cases registered of infection in domestic animals. However, there is no scientific evidence that confirms the role of these animals as reservoirs of *Leishmania* species and are considered accidental hosts of the disease [29-31].

The parasite was isolated from silvatic rodents (*Bolomys lasiurus*, *Nectomys squamipes*) and synanthropic (*Rattus rattus*) in Pernambuco, cats (*Felis catus*) in Rio de Janeiro, dogs (*Canis familiaris*) in Ceara, Bahia, Espirito Santo, Rio de Janeiro and Sao Paulo and *Equidae* (*Equus caballus*, *Equus asinus*) in the states of Ceará, Bahia, Paraná and Rio de Janeiro [32].

Although the role played by these animals in the transmission cycle has not been well defined, evidence indicates, only the wild rodents as primary reservoirs of *Leishmania* probable. The ecoepidemiology of american cutaneous leishmaniasis associated with *Leishmania* (*Viannia*) *braziliensis* has assumed different

characteristics over time in different biomes of the country.

The differential diagnosis is made with paracoccidioidomycosis, squamous cell carcinoma, basal cell carcinoma, lymphomas, rhinophyma, rhinosporidiosis, entomophthoromycosis, leprosy, tertiary syphilis, traumatic septal perforation or by drug use, allergic rhinitis, sinusitis, sarcoidosis, granulomatosis and other rarer diseases [15].

Elapsed more than a century after its first diagnostic description, leishmaniasis still remains one of systemic infectious and parasitic zoonotic endemic in Brazil, with the spread of habitat for the urban jungle along with urbanization in the areas of transition with the urban-rural habitat hosts and vectors in peridomestic areas and households by increasing their occurrence in areas where reservoirs of Cutaneous Leishmaniasis, wild animals, armadillos, dogs, rodents - the bush rat (*Proechymis*) one of the main reservoirs of *Leishmania* (*Leishmania*) *amazonensis* in Brazil and opossum (*Didelphys marsupialis*) and Visceral Leishmaniasis, besides the above, the dog (*Canis familiaris*) mainly, not excluding man as an asymptomatic carrier and all other warm-blooded estimation or domestic animals that may be sources of amastigotes are present as hosts of amastigotes of protozoonosis that through the bite of female sandfly *Phlebotominae hematophagous* vector, *Phlebotomus* in the Old World (Africa, Asia and Europe) and *Lutzomyia* in the New World (Americas) may inoculate the pathogen into healthy hosts susceptible to fall ill. Thus, leishmaniasis, remains of great concern and relevance to public health, side by side with other infectious and parasitic diseases, character fundamentally social, as a result of the marked inequalities that still dominate the world economy in the third millennium, endemic diseases such as malaria, Chagas disease and schistosomiasis [28, 33-39].

Characteristically outbreaks may be, a classic, related to forestry activities and deforestation, with silvatic animals as reservoirs and generally occurs in the form of outbreaks near the pioneer settlement. Another apparently unrelated to the forest, which is usually observed at the periphery of urban centers in areas of old colonization and possibly due to adaptation of parasites and vectors to environmental changes and domestic animals such as new reservoirs [4, 23, 40].

According to Killick-Kendrick [21], approximately 30 species of *Leishmania* have been identified. The global distribution of leishmaniasis includes 97 countries on four continents and affects nearly one million and half people every year. There are about 800 species of sandflies identified worldwide, of which 400 species are found in the New World, but only about 40 species are vectors of leishmaniasis, bartonellosis and arboviruses in the countries of the Old and New World. Demonstrably, only 11 species of the genus *Phlebotomus* (Old World) and eight species of the genus *Lutzomyia* (New World) were recorded with natural infection by *Leishmania* [41].

According to Tolezano [40], some vector species present extradomiciliary and peridomiciliary habits and despite being eclectic as the food, are very anthropophilic. The species *Lutzomyia intermedia* is clearly adapted to the changed environment, ie, attends to dwellings. This adaptation allows reaching a large population and therefore keep new sources of infections among synanthropic and domestic animals (dogs, horses, mules, rodents) and even the man himself [33].

According with Ministério da Saúde of Brazil [15], the disease has opened new frontiers and began to occur in rural areas have almost deforestation and peri-urban areas. Are observed the existence of three epidemiological profiles: a) Silvatic - where transmission occurs in areas of primary vegetation (zoonosis of silvatic animals), b) Occupational or leisure - where transmission is associated with uncontrolled exploitation of forests and felling forests to road building, logging development of agricultural activities ecotourism (anthropozoonosis) and c) Rural or suburban - in areas of colonization (zoonosis of residual forests) or peri-urban, which had vector adaptation to the peridomiciliary (zoonosis residual forests and/ or anthropozoonosis).

According to Vianna [10], exist in the country, associated with various species of sand fly vectors (*Lutzomyia* and others), several species of *Leishmania* that cause human disease. Among them, *Leishmania* (*Leishmania*) *chagasi* and *Leishmania* (*Viannia*) *braziliensis* are those that have greatest potential for urbanization. They cause, respectively, Visceral and Cutaneous Leishmaniasis. In areas of the former Atlantic Forest in the states of Espirito Santo, Rio de Janeiro and Sao Paulo, the transmission of *Leishmania* (*V.*) *braziliensis* is associated with *Lutzomyia intermedia*, among others. Areas of recent deforestation or old, on both coasts and in humid valleys of rivers in rural areas and peridomiciliary areas, population of *Lutzomyia intermedia* is greatly increased. Modified environments where the population of sandflies has increased due to recent or past human activities, which have probably led to the decline of natural enemies of the sand fly, along with large blood supply to food [3].

Thus, evidence epidemiological and parasitological suggest the possibility of *Leishmania chagasi* and *Leishmania braziliensis* keep on introducing new in urbanized areas where there are sand flies in the appropriate density, giving rise to new outbreaks of endemic diseases where, so called clonal expansion, there is, greater genetic homogeneity of the parasites [4].

The jump of the parasite to perpetuate the cycle, necessarily, put to the test, the versatility of this to survive in different classes, kingdoms, in accordance with the classification, below. The scientific classification of: [Protozoan: Kingdom-Protista; Subkingdom - Protozoa; Phylum - Sarcomastigophora; Subphylum - Mastigophora; Class - Zoomastigophora; Order - Kinetoplastida, Suborder - Tripanosomatina; Family - *Trypanosomatidae*; Genres - *Leishmania* and *Trypanosoma*; Subgenres: *Viannia* and *Leishmania*]; [vectors: Kingdom: Animalia; Phylum: Arthropoda; Class: Insecta; Order: Diptera; Family: *Psychodidae*, Subfamily: *Phlebotominae*; Genus: *Phlebotomus* and Genus: *Lutzomyia*]; and [Mammalian hosts: Reino: Animalia; Classe: Mammalia, Linnaeus, 1758] [19].

Noting the classification they belong to different classes and families for whom this subject the protozoan *Leishmania* to change the vector for the different hosts it is believed a masterful genetic jump so you can survive and perpetuate the cycle [7].

Several species of protozoa of the genus *Leishmania* can determine according to their form of expression receives the names of cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis, which can be transmitted by the sting of the female insect vector (sandfly). In the Americas, circulating among humans and several species of domestic animals and silvatic [2].

Lainson and Rangel [39] citing Lainson and Shaw, mentions that the most used classifications follow the current taxonomic model proposed divides the leishmania in subgenera *Viannia* and *Leishmania*. In Brazil, at least seven species of *Leishmania* responsible for human disease, with the cutaneous form caused mainly by *L.* (*V.*) *braziliensis*, *L.* (*V.*) *guyanensis* and *L.* (*L.*) *amazonensis* and, more rarely, by *L.* (*V.*) *lainsoni*, *L.* (*V.*) and *L. naiffi* (V.) shawi, while *L.* (*L.*) *chagasi* is responsible for visceral disease. Each species presents particularities conserning the clinical manifestations, the vectors, epidemiological patterns, geographical distribution and selfsame even to therapeutic response. Six species of *Leishmania* (*Leishmania* (*Viannia*) *braziliensis*, *Leishmania* (*Viannia*) *guyanensis*, *Leishmania* (*Viannia*) *lainsoni*, *Leishmania* (*Viannia*) *shawi*, *Leishmania* (*Viannia*) *naiffi*, *Leishmania* (*Leishmania*) *amazonensis*) are at present known to cause cutaneous and/or mucocutaneous leishmaniasis in Brazil and they are all to be found in the Amazon region of this country. The eco-epidemiology of each is discussed, with the observation that the Amazonian leishmaniases are all zoonoses, with their source in silvatic mammals and Phlebotomine sandfly vectors. With man's destruction of the natural forest in southern Brazil, some sandfly species have survived by adapting to a peridomestic or domiciliary habitat in rural areas. Some domestic animals, such as dogs and equines are seemingly now involved in the epidemiology of the disease. No such process has yet been reported in the Amazon region, but may well take place with the continuing devastation of its forest [22].

In Brazil, visceral leishmaniasis also known as kala azar is caused by *Leishmania chagasi* (*donovani*) and transmitted to humans through the bite of an *Psichodideae* the *Lutzomyia longipalpis*. *Leishmania* (*Leishmania*) *tropica*, *L.* (*L.*) *major* and *L.* (*L.*) *aethiopica*, which produces the so-called "Oriental button" or Cutaneous Leishmaniasis and *Leishmania braziliensis*, causing American Cutaneous Leishmaniasis (ACL) and Mucocutaneous Leishmaniasis. The ACL is endemic in the Amazon ago long time. It is believed that even their existence comes from the days of ancient civilizations. It is commonly called a "brave wound" and is the second major parasitic problem in the Amazon. It is an infection that tends to be more regional focus and due to be intimately related to the breakdown of the ecological environment. It was recently isolated parasites mucosa of patients with muco-cutaneous lesion caused by *Leishmania guyanensis*, what we did not know until last year [23]. Absence of preventive vaccine for leishmaniasis, prophylaxis is solely and exclusively linked to the prevention of contact with the transmitter.

Among the reservoirs, are found wild species and domestic and the dog is considered the main natural reservoir linked to human cases. This host, presents variations in the clinical disease, from apparently healthy animals to oligosymptomatic and may reach severe stages of disease [13].

The female sandfly of the genus *Lutzomyia* is the invertebrate host. During blood feeding in man, dog or horse the amastigotes (2-4 μ) are sucked and move to the sandflies's gut, becoming (,) in promastigotes (14-20 μ)

and then those invading the anterior portions of the stomach and proventriculus and then inoculated by sandfly in a vertebrate host, the promastigotes in man are phagocytosed by tissue macrophages and transform into amastigotes, causing cycles of multiplication in the vertebrate host [19]. In average, the time between inoculation and initial nodule multiplication is 3-4 days, the signs of Kala azar appear in 4-6 months and the cutaneous forms of ulcerative 3-4 weeks. Na infection, occur hyperplasia and hypertrophy of macrophages, infiltration of lymphocytes and plasma cells, the cutaneous form (cutaneous leishmaniasis, mucocutaneous, diffuse cutaneous), occurs ulcer, necrosis and parakeratosis; forms visceral (Kala azar) occurs intermittent fever, adenitis, splenomegaly and hepatic fibrosis of the capsules spleen, heart attack, anemia, ulcers in the gastrointestinal mucosa. The diagnosis is established by isolation in cultures, liver and splenic biopsy and immunological tests (intradermal - proof of Montenegro, immunofluorescence, elisa test and hemagglutination) [9, 15].

L. donovani is a parasite exclusive of the Mononuclear Phagocyte System (SFM), penetrate in the reticulum endothelial cells of the spleen, liver and bone marrow. In Cytoplasm of cells the parasites multiply, distending the cells until rupture. The released parasites are phagocytosed by new reticular cells and this cycle continues indefinitely. The consequences are increased organs rich in cells in SFM, hepatomegaly and splenomegaly [6, 42].

The diversity of agents, reservoirs, vectors, epidemiological situations, coupled with the still insufficient knowledge about these various aspects, highlights the complexity of control. For the selection of appropriate strategies for each geographical region shall be considered the epidemiological analysis of data on: registration of human cases as the clinical presentation, sex, age and origin; entomological studies to define the vector species, their dispersion, degrees anthropophilic and exophilic, natural infection; parasitological study to define the species of etiologic agent in stock focus; ecological studies to determine the animal reservoirs involved; characterization of an epidemic outbreak.

The pathogen is transmitted by the bite of the *Phlebotomine* sand fly *Lutzomyia longipalpis* (Lutz and Neiva, 1912) [36] and although humans infected can be they are believed to be "dead-end" hosts, domestic dogs are the main reservoirs for the parasite.

In areas of ancient rainforest in the states of Espirito Santo, Rio de Janeiro and Sao Paulo, the transmission of *Leishmania* (V.) *braziliensis* is associated with *Lutzomyia Intermedia*, among others. Moreover, in the back and in humid valleys of rivers in rural areas and peridomestic areas, population of *Lutzomyia Intermedia* is greatly increased [3].

Birds showed questionable role in the transmission of leishmaniasis, according to particular aspects of this species, which were considered by some authors involving zooprophylaxis factors precluding chickens and the hosts of *Leishmania*. Chickens have several physiologic characteristics that preclude Them from sustaining Leishmania infections, including their body temperature of 41.0°C. Leishmania infection in birds unable to develop, but also might be eliminated existing infections in sand flies taking a second blood meal from chickens [7]. Enzymatic processes in the sand fly function differently when triggered by different types of blood meal and blood from certain sources may be lethal to *Leishmania* [43].

In the hypothesis of others or new reservoirs by altering the ecosystem, must be taken in account the interpretation of the eco-epidemiology of leishmaniasis, provided all of which are zoonoses, with their source in silvatic mammals and phlebotomine sandfly vectors. With man's destruction of the natural forest in southern Brazil, some sandfly species have survived by adapting to a peridomestic or domiciliary habitat in rural areas. Some domestic animals, such as dogs and equines are seemingly now involved in the epidemiology of the disease in these environments. No such process has yet been reported in the Amazon region, but may well take place with the continuing devastation of its forest [22].

Are the ungulates, family *Equidae*, genus *Equus* (*Equus caballus*, *Equus asinus* and *Equus caballus* versus *Equus asinus*) reservoirs of the genus of protozoonosis Leishmanisis? The role of these animals in the eco-epidemiology of this disease is not determined despite the substantial scientific progress around the sixth biggest disease of global importance. In the states of São Paulo, Rio de Janeiro e Paraná (*Equus caballus*), Ceara, Bahia (*Equus asinus*) were reported cases of cutaneous leishmaniasis in equids [12-14, 32, 44-49]. In Brazil, besides the man and dog, only in an opossum (Marsupialia, Didelphidea: *Didelphis albiventris*) was described in the Kala azar in Bahia [11]. This animal was positive for amastigotes in the tissues of the mononuclear phagocyte system (spleen, liver, bone marrow).

The defense mechanism of immune system for protozoa of leishmaniasis has been shown to be complex. At one stage, some animals not only for potential reservoirs of the disease has been described by presenting the clinical form of disease. Among the wild animals, though rare adductions, some animals considered asymptomatic reservoirs has expressed leishmaniasis. Sherlock *et al.* [11] described that opossums showed clinical form of the disease. Among domestic animals, some citations mention the evident clinical cases in horses that had cutaneous leishmaniasis. According Mancianti [27], calves and horses who could act the accidental reservoirs of leishmania, while sheep appears not to be susceptible to experimental infection. In endemic foci for kala azar in Sudan, cows, goats and donkeys had a high prevalence of specific antibodies. Recently in Europe, sporadic cases of equine leishmaniasis have been reported: *L. infantum* was the causative agent. Equine leishmaniasis appears as a self-healing skin-dwelling disease, with a massive accumulation of parasites. The animals of the often not show detectable specific antibodies and recover without any chemotherapy.

In Portugal, the parasite DNA detection was by real-time PCR using a probe specific for *L. infantum* in a case of leishmaniasis in horse with skin lesion [50].

According to Cerqueira *et al.* [45] after of experimental research with inoculation of amastigotes in donkeys concluded that *Equus* aren't considered an important reservoir in the chain of transmission of visceral leishmaniasis, although they represent a source of blood meal and probable proliferation of vectors.

Follador *et al.* [47] mentions that the focus of leishmaniasis in Bahia involving human prevalence of 5.2% positive, 22% of horses and 8% of dogs had positive serology for the disease whose Phlebotomine surveys showed the vector *Lutzomya intermedia* in 94% of outbreaks in the areas of domiciliary and peri-domiciliary. However, no equine showed skin lesions. Cases positive for skin lesions in horses has been reported in brazilian literature and mentioned equids with as possible reservoirs [12-14]. The man has been described with greater frequency, involvement by leishmaniasis seen the activity that performs in different environments, extra domiciliary in rural areas, semi deforestation, or extraction activities in native forests.

Another relevant aspect is the problem of co-infection with HIV virus in humans [24] and the trypanosome in dog and other domestic animals [51].

Antibody detection against leishmaniasis was performed using a direct agglutination test (DAT) which resulted samples for *Leishmania* screening using DAT were drawn from his family members, 67 neighbors and animals, including 9 dogs, 1 cat and 3 rats. No other active cases were found since all human and animal serum were negative for *Leishmania* antibodies. These negative findings and a history of travel as a truck driver indicate the patient may have acquired this infection from elsewhere, since *L. infantum* leishmaniasis can remain asymptomatic in patients for more than 20 years [26].

This is research, were utilized the establishment of equines in the center-west region of the São Paulo state which has significant populations of these animals, with strong cultural linkages which bring man and horse allies the multiple uses of animals in various practice equine therapy, sport, leisure, work and reproduction, with the objective of study aimed to investigate the different categories that make up the herd of horses on farms, spread over nine counties, the immune response of healthy horses for *Leishmania chagasi* in an endemic area for leishmaniasis in the mesoregion of Bauru, Statistically analyze the frequencies by chi-square of influence of parameters such as race, sex and age on the prevalence of serum reagents.

MATERIAL AND METHODS

In this research, were sampled different ages, sexes and races of equines during the year 2007. Animals examined belonged to endemic areas - Bauru region - or were located in the surroundings of endemic municipalities for the incidence of positive cases in human and dog leishmaniasis. Analyses were conducted in the Laboratory of Rabie and Encephalitis of Instituto Biológico, São Paulo. Were tested 100 serum from equines in the rurals counties of Agudos, Arealva, Bauru, Boracéia, Duartina, Iacanga, Lucianopólis, Paulistânia e Piratininga. Was utilized the Indirect Immunofluorescence Reaction-RIFI, employing conjugated anti-equine marked with fluorescein isothiocyanate and titration with point cutoff the dilution 1:40.

RESULTS

For the nine cities studied, for a hundred serum samples analyzed, sampling revealed that 40% of the animals examined showed antibodies, anti-*L. chagasi*. The higher prevalence of reagents - 35% - was observed in Bauru (Table 1; Figure 1).

Among the five races studied, the major prevalence of serum reagents *L. chagasi* - 57.50% - was observed in the Quarter Horse (Table 2).

Between animals serum reagents, the sexes analyzed, the major prevalence ($P<0,05$) of animals serum reagents for *L. chagasi* - 42.38% - was observed in the group of females (Table 4; Fig. 5).

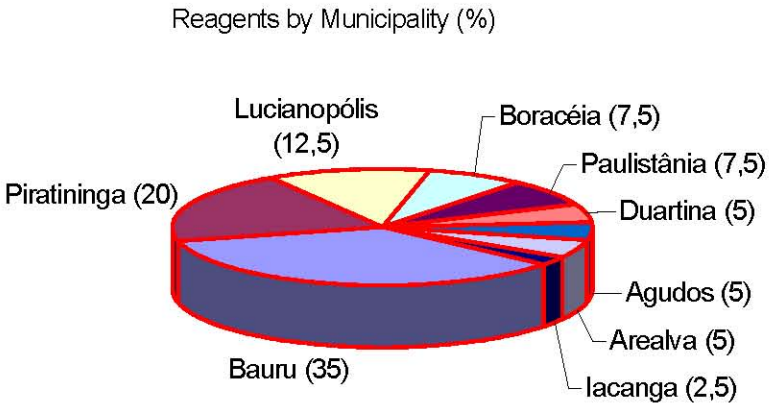

Fig. 1: Distribution of reagents animal of according to the percentage of sero-reagents for the region's municipalities of Bauru

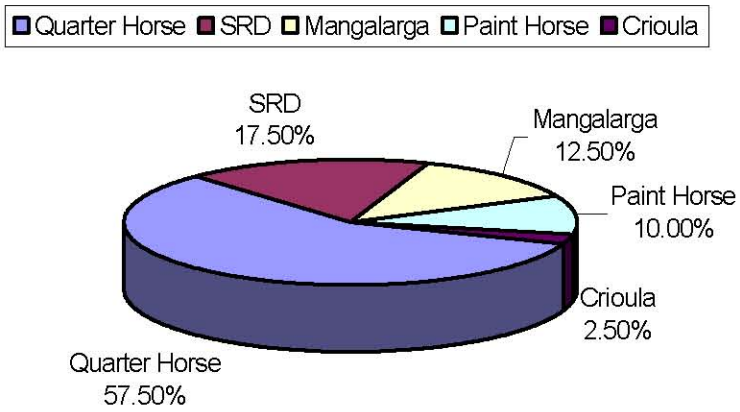

Fig. 2: Sero-reagents (%) for *Leishmania chagasi* according with the race studied °96: ≥96 months

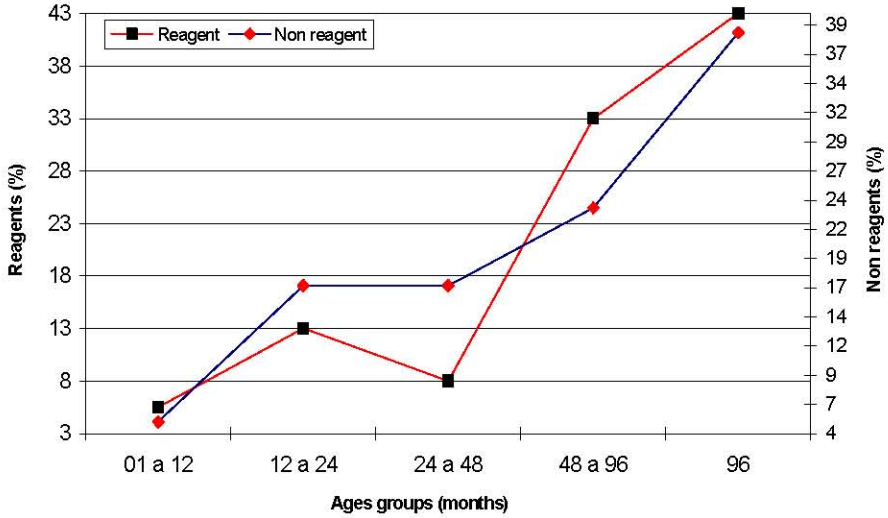

Fig. 3: Reagents animals according to age (months)

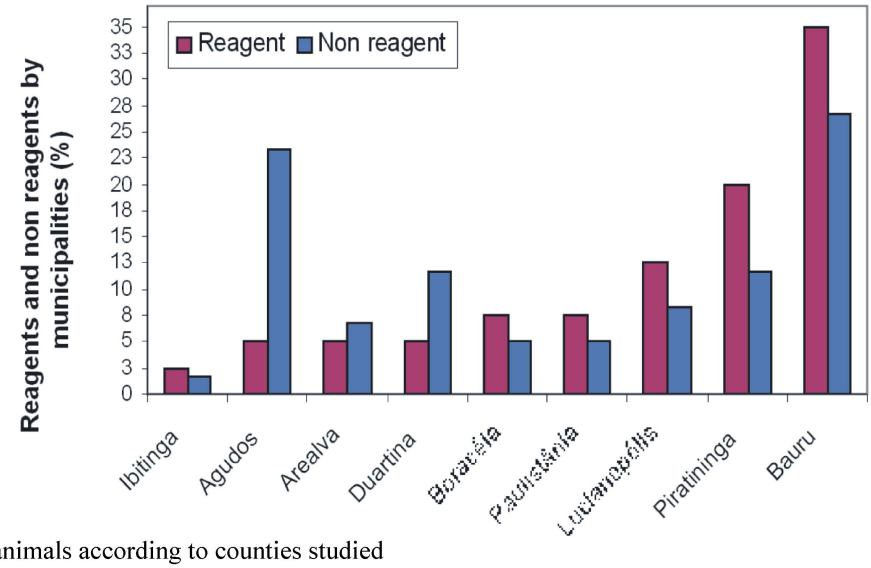

Fig. 4: Reagents animals according to counties studied

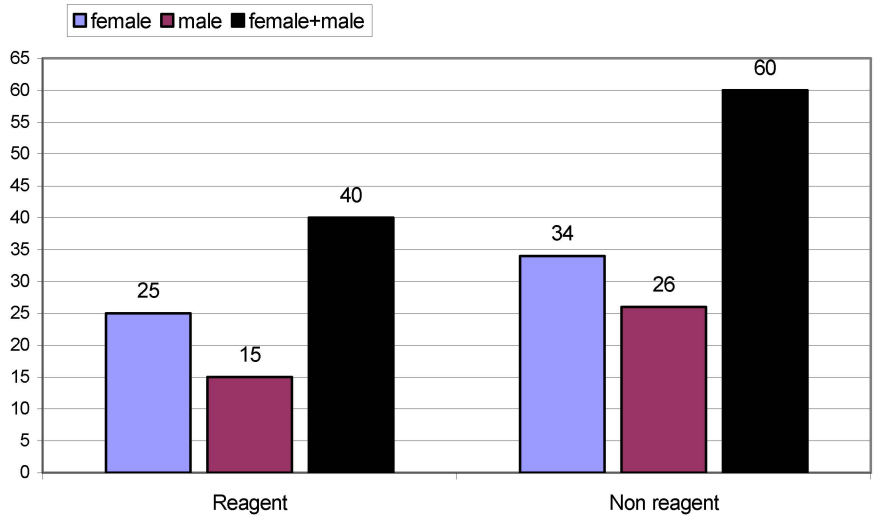

Fig. 5: Reagents animals and non reagents animals at *Leishmania chagasi*, according the sex studied in the region of Bauru

Table 1: Number of reagents and not reagents animals to *Leishmania chagasi* in the municipalities studied in the mesoregion of Bauru

Counties/examined	Reagents	%	Non-reagents	%	Subtotal (Countie)
Agudos	2	5.00	14	23.33	16
Arealva	2	5.00	4	6.67	6
Bauru	14	35.00	16	26.67	30
Boracéia	3	7.50	3	5.00	6
Duartina	2	5.00	7	11.67	9
Iacanga	1	2.50	1	1.67	2
Lucianopólis	5	12.50	5	8.33	10
Paulistânia	3	7.50	3	5.00	6
Piratininga	8	20.00	7	11.67	15
Totals	40	100.00	60	100.00	100

Table 2: Percent of reagents and not reagents animals to *Leishmania chagasi* according with the races studied in the region of Bauru

	Race									
Examined	Quarter Horse	%	Manga- larga	%	Crioula	%	Paint Horse	%	SRD*	%
Reagent	23	57.50	5	12.50	1	2.50	4	10.00	7	17.50
Non-reagent	39	65.00	5	8.33	3	5.00	6	10.00	7	11.67
Totals	62		10		4		10		14	

* SRD-without defined race

Table 3: Percent of reagents and not reagents animals to *Leishmania chagasi* according with the age groups (months) studied in the region of Bauru

	Age (months)									
Examined	01 a 12	%	12 a 24	%	24 a 48	%	48 a 96	%	≥96	%
Reagent*	2	5.00	5	12.50	3	7.50	13	32.50	17	42.50
Non reagent	3	5.00	10	16.67	10	16.67	14	23.33	23	38.33
Total reagents	5		15		13		27		40	

*P<0.05.

Table 4: Percentage of reagent and non-reagent animals to*Leishmania chagasi*, according to the sex studied in the region of Bauru

Examined	Female	Male	Total
Reagent*	25 (42.38%)	15 (36.59%)	40
Non-reagent	34	26	60
Totals	59	41	100

* P<0,05

DISCUSSION

In this research, the prevalence of 40% of equines of all counties sampled (Table 1), regardless of race, sex and age, showed antibodies anti-*Leishmania chagasi* (Tables 2, 3 e 4; Fig. 1 to 5). Similar results, were reported by Vedovello Filho *et al.* [48] in Paraná state when reporting that few studies have been undertaken on the occurrence of the disease in other domestic animals and horses. The authors detected in 55 horses, coming from rural areas of several municipalities, 76.3% showed title to Direct Agglutination Test and 7.1% were positive by polymerase chain reaction (PCR) analyzes led to the detection of *Leishmania* (*Viannia*) DNA, suggests the authors the participation from these animals in the Leishmania transmission cycle since the presence of the anti-*L.* (*V.*) *braziliensis* antibodies and *Leishmania* (*Viannia*) DNA in horses.

However, so overall, there is no rule to characterize given mammal as a natural reservoir or carrier asymptomatic protozoan Leishmania. He must be submissive to a complex of factors and conditions predisposing that we analyze. The vector has, at principle, the wild environment naturally, silvatic reservoirs vectors and mammals are equilibrium and leishmaniasis is not manifested until mammals susceptible to contact with vectors or interfere with this balance [20, 43].

Environmental changes have been associated with changes in eco epidemiological profile of cutaneous leishmaniasis, as evidenced in research differences between seasons and opportunity in the cycle of Leishmania parasite [23, 40]. A higher incidence of vector has been reported in hot and humid months of the year. Among the various strategies for prophylaxis of leishmaniasis, combating vectors, elimination of infected dogs and hospitalization, followed by treatment of humans has been positive the measures recommended in the prophylaxis of infectious and parasitic disease [15].

The role of different reservoirs in the maintenance of the parasite has been the subject of intense study in areas where the leishmaniasis are endemics. By modifying the habitat of sivaltic animals, the vectors have migrated to areas where it previously was not present or minimally found and new habitats peridomiciliary and domiciliary has recorded infestation of sand flies. In these situations, domestic animals are important sources of food of vectors [3, 4]. Many of them are new fronts of human habitation evidence uncertainty policy public basic sanitation for these areas, noting that, the absence or low quality installed equipment and services rendered.

The health and environmental degradation resulting from human settlements and domestic animals in slums has resulted in constant contact with domestic waste, proliferation of insects and rodents, densification and the buildings in the little space and with so many growing dwellers in houses. Accordingly, the containment of

domestic animals in environments inhabited by humans and without basic sanitation can lead to the emergence of endemic zoonosis [10].

At this juncture, 40% prevalence of serum-reactive animals to *Leishmania chagasi* detected in this study mesoregion Bauru endemic, showed similar behavior to that presented by Follador *et al.* [47] what investigated an outbreak of ACL in Bahia, detected by the reaction of Montenegro, positivity in 26 of 29 (89.7%) cases evaluated and suspicious lesion in humans. In this focus, the authors conducted an epidemiological inquiry in domestic animals. Detected 4.8% (104 dogs), 8% (100 dogs) of positive surveys in succession. In the clinical examination and serological survey of 77 horses from the village, no animal had skin lesions suggestive of leishmaniasis and serological test was positive in 17 (22%) animals. According to the authors, there was a predominance of *Lutzomyia intermedia* (94%) in vectors identified besides of the presence of *Lutzomyia migonei* and *Lutzomyia* sp.

However, it should be noted that confirmation of equids infected in outbreaks of leishmaniasis has been described by researchers in Brazil and other countries including Venezuela [12], Portugal [50], Germany [52], Espain [53] and Sudan among others. Thus, Aguillar *et al.* [12, 13] in the municipality of Rio de Janeiro state working in an outbreak of cutaneous leishmania, detected human, canine and equine species parasitized by American Cutaneous Leishmaniasis in an endemic area. The authors recorded five horses (Horses - *Equus caballus*) and five donkeys (mules - *Equus caballus* x *Equus asinus*) with skin lesions, of which four of each species were isolated from the Leishmania protozoan, resulting in 30.80% of the herd parasitized.

Aguilar *et al.* [13] mentioned that outbreaks of cutaneous leishmaniasis in Rio de Janeiro involving dogs and horses and humans have been isolated *Leishmania brasiliensis brasiliensis* in hamsters by inoculation. Since the findings of Aguilar *et al.* [14] of zoonotic cutaneous Leishmaniasis in Venezuela and Brazil and Vexenat *et al.* (1986) in Brazil contributed to understanding of the epidemiology of ACL in certain areas of those countries where apparently dogs as well as equines might be regarded the sources of infection.

Lesions of skin for cutaneous leishmaniasis in horses has been described in some Brazilian states. Vexenat *et al.* [49] in Bahia isolated wound of castration of a donkey (Equus asinus) forms amastigotes of *L. braziliensis braziliensis* in hamsters by inoculation of material from the biopsy of the lesion. The authors cited the importance of investigating the role of horses in the epidemiology of cutaneous leishmaniasis, a time that remains unfinished its importance in the link ecoepidemiology transmission.

Yoshida *et al.* [20] mentioned that the first epidemic disease of ACL in São Paulo coincides with the opening and construction of the railway in the northwestern state in 1908, when many cases of the disease, known as the "Bauru ulcer", patients were described as working in areas felling of the forest where vectors were abundant. The same authors leading studies on leishmaniasis, affirmed the absence of evidence of natural enzootic outbreaks of infection, despite research to the reservoirs. However, cases of leishmaniasis in humans, dogs and horses associated with these same species were detected. They concluded that the ACL due to *L.* (*Viannia*) *brasiliensis* can occur in older established communities in non-forest areas, where the cycle is maintained, involving domestic animals as reservoirs and sandfly vector habitats with peridomiciliary.

According Falqueto *et al.* [29], dogs and horses found have been infected with *L.* (*V.*) *braziliensis* and implicated were the domestic reservoirs of infection for humans in a number of foci [46]. The authors stated that there was a clear correlation between the human disease and the relatively high prevalence and wide distribution of the infection in dogs, information resulting from the molecular characterization is the similarity of the stocks derived from both human and canine cases of ACL. The presence of the same genotypes in humans and dogs in the same area is suggestive that intra-specific transmission cycles, related with *Lutzomya intermedia*, *Lutzomya migonei* and *Lutzomya whitmani*, could be defined using molecular technology.

Contrasting with the above authors, Cerqueira *et al.* [45] reported that inoculation experimental Equus asinus with promastigotes of *Leishmania chagasi*, although challenged with higher numbers of promastigotes, the animals did not develop patent infections and not experimentally infected *Lutzomyia longipalpis*. The results encourage us to believe that, equids are lacking of important reservoir in the chain of transmission of visceral leishmaniasis, though, serve as a good blood supply and proliferation of the vector of *Lutzomyia longipalpis*.

On the hand, Barbosa-Santos *et al.* [44] in the of Rio de Janeiro state, isolated forms amastigotes of *L. braziliensis* of the lesion of a quarter horse mare pregnant with aged of 13 years presenting disseminated cutaneous leishmaniasis. Agreeing with reported by the author above, in this research the highest prevalence of serum reagents to *L. Chagasi* was evidenced in the group of animals over eight years.

The role of the reservoirs have not been well defined for some species. Thus, Alexander *et al.* [35] analyzing the role of domestic fowl in the epidemiology of the disease and inferred that chickens attract male and female sand flies - *Lutzomyia longipalpis* - but are unable to sustain infections with Leishmania and their presence may exercise zooprophylactic effect. The authors analyzed that the spread and increasing prevalence of visceral leishmaniasis in urban areas are linked to human migrations, involving the transportation of infected dogs from visceral leishmaniasis-endemic regions to impoverished urban areas where *Lutzomyia longipalpis* already exists [54]. The authors mentioned also that studies in Colombia showed that chickens attract more vectors than others, animals, domestic and complicating factor, such as, poultry farms, can be attractive to opossums (*Didelphis albiventris*) and black rat (*Rattus rattus*), potential reservoirs of Leishmania. In this research if we analyze this fact, many of the breeding farms of horses and other livestock varieties, have shelters where they store ingredients for rations and other foods that may have an important role in attractiveness, reservoirs and vectors of Leishmania, the which imposes the need of detailed study these farms [28, 34, 55, 56].

Questions about the importance of domestic animals as reservoirs, deserves emphasis, in all studies. As for equids, birds too, has received important considerations. Thus, the role of birds in the cycle of Leishmania was wisely considered, the research developed by Zilberstein and Shapira [7] who reported that chickens characteristics that have several physiologic preclude them from sustaining Leishmania infections, including Their body temperature of 41.0°C [43]. Enzymatic processes in the sand fly function differently when triggered by different types of blood meal and blood from certain sources may be lethal to Leishmania [6]. Turkey blood meals significantly reduced *L. tropica* infections in the Old World sand fly *Phlebotomus papatasi*, even when you were insects infected after digestion of the blood meal, perhaps due to DNAase activity triggered by the presence of nucleated erythrocytes.

Another peculiarity of Leishmania infections in accordance with Rosenzweig *et al.* [6], this in that, in *L. donovani* are the causative agents of kala azar, a fatal disease in humans cycle between phagolysosomes of mammalian macrophages and the alimentary tract of sand flies. In the insect vector, they grow as flagellated extracellular promastigotes, whereas in the mammalian host, they proliferate as aflagellated intracellular amastigotes. Promastigotes are introduced into the host during a blood meal taken by the fly and are subsequently phagocytosed by macrophages, where they differentiate into amastigotes. This process is mimicked *in vitro* by shifting cultured promastigotes (grown at 26°C, pH 7) to a lysosome-like environment (37°C and pH 5.5; differentiation signal). Although axenic amastigotes are not identical to animal-derived amastigotes, they closely resemble them and are the best available model for the study of parasite intracellular development.

Agreeing with these statements, Kima [1] states that is understanding of the mechanisms deployed by *Leishmania* amastigotes to modulate the host cell's response to infection is still rudimentary the amastigote forms of *Leishmania* are experts at exploiting host cell processes to establish infection and persist.

Thus, Guerra *et al.* [38] stated that, conditions ecoepidemiology Brazil are influenced by significant growth, both in magnitude and geographic expansion, observing the coexistence of a dual epidemiologic profile expressed by the maintenance of cases from the old foci or areas close they and the emergence of epidemic outbreaks associated with factors such as the accelerated expansion of agricultural frontiers, the establishment of areas of mining, construction roads and the invasion process in the periphery of cities among several others. Thus, major number of cases are recorded in the period of rainfal (october to may) when it increased the density of vectors.

According to Colla-Jacques *et al.* [36], in São Paulo of state, 66 species are present, of which seven are considered to be epidemiologically important. *Nyssomyia intermedia* (Lutz and Neiva 1912), *Nyssomyia neivai* (Pinto 1926), *Nyssomyia whitmani* (Antunes and Coutinho 1939), *Migonemyia migonei* (França 1920), *Pintomyia pessoai* (Coutinho and Barreto 1940) and *Pintomyia fischeri* (Pinto 1926) have been indicated as probable vectors of *Leishmania (Viannia)braziliensis*, the main causative agent of ACL in the state, *Lutzomyia longipalpis* (Lutz and Neiva 1912) is the main vector of *Leishmania infantum chagasi* (Cunha and Chagas 1937), the ACL causative agent and is now present in all Brazilian regions [31]. Its first occurrence within an urban area of São Paulo was in Araçatuba in 1997 and since then, a clear expansion of its distribution area has been recorded. According the above authors, at present, this species has been registered in the urban area of 107 municipalities, with Espírito Santo do Pinhal being the only one in the northeast area of São Paulo. The high abundance of the vector, coupled with canine transmission, has been indicated as a precursor to

the occurrence of autochthonous human cases in the urban area of SP. From 1999 to 2009, were reported within the state. 1,539 human cases, with 134 cases resulting in death. *L. longipalpis* was first collected in the rural area of Espírito Santo do Pinhal in 1994 and in the urban area in 2000 [36].

In conclusion, the results of this research showed substantial frequency of 40% equines of the region with specific antibodies against *L. chagasi* and suggests that exposure to a parasite is common. The results also suggest that equines living in endemic areas may present silent infection by *L. chagasi*.

The reports in the literature is restricting the dignosis of the ACL form - Cutaneous leishmaniasis in equids (*Equus caballus*, *Equus asinus* and mules: *Equus caballus* x *Equus asinus*). At present literature, there are no reports of visceral leishmaniasis in equids.

In the literature, consulted a variety of silvatic and domestic animals are considered potential reservoirs of leishmniasis, depending on the circumstance ecoepidemiology, chance of biting of the vectors and host immunity. In this complex, is not excluded mammals such as reservoirs or hosts infected including equines, objective of this research. However, more detailed studies for each species need to be developed, including meticulous research of the connection between the amastigotes forms from the equids for the vectors - promastigotes - and the reverse of the cycle route.

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