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Review

Evaluation of feeding habits of haematophagous insects, with emphasis on Phlebotominae (Diptera:Psychodidae), vectors of Leishmaniasis - Review

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ABSTRACT

Many countries have been facing the emergence, re-emergence, expansion and urbanization of vector borne-diseases, such as leishmaniasis. The assessment of feeding diet of Phlebotominae provides important information on attraction of vectors to potential reservoirs (wild, synanthropic and domestic mammals) and anthropophilic habits, which can reflect the ability to occupy new areas resulting in changes of transmission profiles. Thus, this review article aims to discuss the available information on feeding habits of insect vectors of human pathogens, based on the used techniques, discussing their application, benefits and limitations. In this context, the discussion focuses on Phlebotominae vectors of Leishmaniasis.

KEYWORDS: *Lutzomyia*, *Phlebotomus*, Phlebotominae, *Aedes*, *Anopheles*, food habits, hematophagous insects

ABBREVIATIONS

PCR - Polymerase Chain Reaction
ELISA - Enzyme-Linked Immunoabsorbent Assay
RFLP-PCR - Restriction Fragment Length Polymorphism - Polymerase Chain Reaction
ACT - American Cutaneous Leishmaniasis
AVL - American Visceral Leishmaniasis
SNDC - National Information System of Diseases of Compulsory Notification
CL - Cutaneous Leishmaniasis
VL - Visceral Leishmaniasis

INTRODUCTION

Phylum Arthropoda - Success of the class Insecta

Arthropods are one of the most important phyla in terms of domination of terrestrial and aquatic ecosystems. It is estimated that arthropods represent more than two thirds of all animals. Their highly adaptive diversity has allowed them to survive in all habitats [1].

The class Insecta is the largest group among the Arthropoda capable of occupying aerial, terrestrial and aquatic environments alike. Insects are by far the best examples of biodiversity, as they form the most diverse group of organisms on the planet. The number of species, diversity of adaptations, their biomass and ecological impact exceed those of all other animal groups [2].

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Insect vectors of human pathogens: Adaptation of mouth apparatus and hematophagy

Insect vectors have been transmitting pathogens to human beings since ancient times, and the mortality rate associated with these infections exceeds thousands of cases every year. These insects can host and carry bacteria, viruses and protozoa that cause diseases such as yellow fever, dengue fever, malaria, leishmaniasis, chagas disease, filariasis, etc.

Insect mouth parts consist of a pair of mandibles and two pairs of maxillae (maxillary palps and labial palps). Mouth appendages suffer morphological changes according to the insect's eating habits. Mouth apparatus can be classified as: (1) chewing, as in cockroaches and grasshoppers; (2) piercing-sucking, present in *Anopheles*, Culicidae, Phlebotominae and Hemiptera; (3) licking, as in house flies. The piercing-sucking type mouth apparatus, found in all hematophagous insects, is characterized by the adaptation of the mouth parts, or some of them, to the function of puncturing the skin; they have become a set of thin, hard styli, either pointed or serrated at their end [3].

The mouth parts of hematophagous insects have evolved in different ways, which influence the feeding mechanism: (1) selenophagy: thin, long styli puncture the skin to make way for thin, tubular parts which reach the capillaries and directly tap the blood. During the draining of the blood, drops of saliva are released from the end of a parallel, thinner tube, the salivary tube, connected to the deferent ducts of the salivary glands. This mode of feeding has several advantages: the host normally does not notice the piercing, and microorganisms are tapped/injected directly into the host's bloodstream; (2) pool feeding: the mouth parts are short and/or wide, which prevents deep penetration and direct piercing of the vessels. As a consequence, these vectors tear the victim's skin and tissues, causing a hemorrhage from which they suck the blood, cells and lymph leaking from the minuscule torn capillaries. This type of feeding rarely goes unnoticed, but it favors the collection of parasites that tend to concentrate in other tissues rather than in blood [4].

Some insect orders are important vectors of pathogens, in particular, the orders Diptera and Hemiptera, which transmit malaria, dengue fever, filariasis, yellow fever, leishmaniasis, bartonellosis, arbovirus diseases, onchocercosis, chagas disease, among others [4].

MATERIALS AND METHODS

For specific bibliography, searches were performed using the following keywords: Feeding Habits, Dengue Fever, Chagas Disease, ELISA/Enzyme-Linked Immunosorbent Assay, Yellow Fever, Leishmaniasis, Malaria, Onchocerciasis, Food Preferences, Phlebotominae/ Psychodidae, Polymerase Chain Reaction, Precipitin Tests, in the site health Sciences Descriptors - http://decs.bvs.br/. Some databases were considered in this review: thesis database - http://www.capes.gov.br/servicos/banco-de-teses, LILACS - http://lilacs.bvsalud.org/, MEDLINE - http://www.ncbi.nlm.nih.gov/pubmed, OMS - www.who.int, SciELO - http://www.scielo.org, Ministério da Saúde www.saude.gov.br.

RESULTS AND DISCUSSION

Classification of hematophagous insects according to their feeding pattern

Insects can be classified as active hunters (those that have a feeding preference and seek the food that attracts them the most) or passive opportunists (opportunist insects that feed on the nearest host, regardless of it being attractive or not) [5]. They may also be simple feeders (a single blood meal) or multiple feeders (more than two feedings) [6, 7].

Importance of alimentary identification in hematophagous insects

Interest in the identification of the food sources of hematophagous insects has increased as new discoveries were made regarding their involvement in disease transmission. Establishing the feeding pattern of hematophagous insects is very important to understand their biology, other than having a fundamental role in terms of Public Health [7]. It is an important piece of information used to outline control strategies for various diseases transmitted by these invertebrates [8, 9, 7].

In entomological surveillance actions, the information obtained about the biology of vectors, in particular about their interaction with reservoir animals, in association with environmental factors, may point towards alterations in the transmission profile of diseases [10]. Thanks to studies on food sources, it is possible to learn about the behavior of hematophagous insects in various circumstances, as it is known that their behavior suffers modifications according to environmental alterations and the food that is available [6, 7]. Attraction to a certain host also suffers variation according to other aspects, such as host size and, as a consequence, the body surface that is exposed [11].

Techniques for the evaluation of food sources in hematophagous insects

In order to properly evaluate feeding habits, it is necessary to know the biology of the insect under scrutiny, the fauna of vertebrate hosts in the area and the environment in which it is found; this information will influence the choice of technique that is to be used, taking into consideration its advantages, disadvantages, sensitivity, specificity, cost, etc. [7].

Some of the most popular techniques are: (1) the use of animals as bait; (2) non-immunological methods (hemoglobin crystallization, histological slides and PCR – molecular analysis); and (3) immunological methods (precipitation tests, immune-enzymatic tests).

The use of animal (and even human) baits to collect insects has brought to light important information about the biology of these vectors, although it is currently admitted that laboratory-processed techniques can give more accurate answers about the feeding habits of insects, complementing the preliminary information collected in the field [12]. In a Technical Note, the Brazilian Ministry of Health allows the capture of *Anopheles* using human baits (a procedure in use since the 60's), because this is considered the only efficient method to target and evaluate the impact of entomology and control actions against malaria vectors, provided that the agents are equipped with personal protection equipment and that capture takes place before the blood meal [13]. It is important to note that the use of human baits with body and face exposure is currently not recommended by the Ethics Committees related to Human Research or by the World Health Organization, as identification techniques for the type of blood ingested by pathogen-transmitting insects are constantly advancing [14].

In the hemoglobin crystallization method, the contents of the stomach are placed in contact with a saturated solution of ammonium oxalate, forming crystals. The controls are known samples of crystallized hemoglobin [15].

The use of histological sections of insect abdomens makes it possible to analyze the digestion process and the formation of the peritrophic membrane, thus making it possible to identify multiple meals [16].

The advent of DNA amplification has made it possible to use this important diagnostic tool to determine the origin of the food source, amplifying genes or small fragments that are later amplified and sequenced. Thanks to these characteristics, this technique is more sensitive and specific than the others.

In studies about the identification of food sources of arthropod vectors [17], the precipitin reaction has contributed to demonstrate the attraction between vectors and probable parasite hosts, in addition to improving the evaluation of the level of anthropophilia. Basically, immune-enzymatic tests are used on ingurgitated specimens or on those with some residual blood meal. According to the size of the insect under study, the evaluation can be carried out by dissecting its abdomen, in the case of small insects such as Phlebotominae, or the material can otherwise be collected on filter paper by squeezing the abdomen, as in the case of Triatominae.

Distinct procedures based on precipitation have been described [18, 19, 20] that can be carried out in liquid or gel [7].

Even though the technical specificity of precipitin was well known, new investments were made in new technologies that presented a higher level of sensitivity. This is how immune-enzymatic techniques (ELISA) came to be used to study the feeding habits of mosquitoes [21], Triatominae [7] and Phlebotominae [11, 22, 23, 24, 25, 26]. This technique is efficient due to its high specificity and sensitivity when compared to other food content evaluation methods [7, 27]. After the standardization of the technique executed by

Burkot [21], other modifications were introduced, such as direct ELISA, sandwich ELISA, competitive ELISA, DOT-ELISA and DOT-PAP [7].

Among the main techniques used to identify the feeding habits of insect vectors, the most widely used are precipitin and ELISA. The precipitin technique offers a few advantages: it is cheap, fast and, most importantly, easy to perform, although not very sensitive. It requires the use of large amounts of antiserum, and, in small insects with small amounts of blood collected, some studies prefer to use a pool of insects. Another drawback is that its reading is visual (without quantification), which makes it less reliable. On the other hand, the immune-enzymatic technique has greater sensitivity, and its specificity can be increased according to the cut off variation and the questions the study intends to address; its reading is more objective, and, as it requires only a small amount of antiserum, more varieties of possible reservoirs can be tested. It is, however, a complex technique that requires specialized training and has high initial costs.

Serologic methods have been criticized due to possible cross reactions and variations in sensitivity and specificity. The PCR technique minimizes these problems by using specific sequences – primers that can specifically identify each target (food source to be investigated). Among the selected targets are the genes used in phylogenetic studies [28], preserved sequences and with numerous copies.

Another advantage of the PCR technique is that, thanks to the use of different primers, it is possible to identify the food source and the presence of a natural infection in the same specimen. This joint identification, in particular in Phlebotominae studies, combines two important pieces of information, crucial for the study of the eco-epidemiology of the species, making it possible to generate important data for studies on the reservoirs of leishmaniasis.

A few studies with vectors of human pathogens

The analysis of *Anopheles gambiae* using polymerase chain reaction (PCR) and specific primers originated from individuals who took part in the study as human bait, showed that 35-39% of the profiles generated were identical to the DNA of the subjects who had participated in the study [29]. The sensitivity of multiplex PCR for the detection of human blood was possible for up to 3 days following the blood meal of *Aedes aegypti*; as the mosquito can maintain its life cycle by feeding from other hosts, the authors suggested that the feeding pattern must be taken into consideration during the implementation of a control strategy [30]. Blood meal identification and the detection of avian malaria parasite from mosquitoes (Diptera: Culicidae) were done by polymerase chain reaction-based methods for field samples collected in coastal areas of Tokyo Bay, Japan. The obtained results gave evidence of direct contact between *Cx. pipiens pallens* and both resident and migratory birds infected with avian malaria parasites in Japan, suggesting this culicidae as the principal vector in transmission of the parasite in Japanese wild bird communities [31].

Microdot-ELISA, a variation of standard ELISA assays, successfully evaluated the “anthropophilic index” of *Anopheles subpicuts*, *Anopheles culcifacies* and *Anopheles anmulares* [32], so that it was no longer necessary to use human bait to collect the insects.

By studying the feeding pattern of *Aedes albopictus*, [9] used the precipitin technique to screening the material and the ELISA technique to confirm the results. The females were fed in the laboratory with human and rabbit blood, and killed at different hours after the meal. The ELISA assay was capable of detecting positive samples in up to 30 hours after the meal. Studies applying a double-antibody enzyme-linked immunosorbent were conducted in order to determine the blood meal sources of adult mosquitoes (Diptera: Culicidae) from encephalitis vector surveillance mosquito traps in Western Australia. Besides, blood meals from a variety of vertebrate hosts were identified in different mosquitoes species and the results suggest that other mosquitoes, apart from *Ae*. *camptorhynchus* and *Cx*. *annulirostris* also may have a role in enzootic and/or epizootic transmission of arboviruese [33].

A few studies have been carried out that revealed important epidemiological aspects of chagas disease. Samples of *Triatoma rubrovaria*, collected at Quaraí (RS, south of Brazil), infected with *Trypanosomatidae* and analyzed with the precipitin test, showed positivity for rodent, goat, pig and human antiserum. These findings confirm the eclectic feeding habits of this species, an epidemiologically important piece of information, and highlight the need for surveillance as this is a wild species that lives near human-inhabited areas, having taken the place of *Triatoma infestans* [34]. Samples of *T. infestans* collected in Bolivia, an area with high levels of human infection, were analyzed using PCR. Results showed that 48% of the samples had fed from more than one source and preferred domestic (dogs) and peridomestic (birds and pigs) animals. From the epidemiological point of view, the results confirmed the importance of domestic and peridomestic animals in the dynamics of infection by *Tripanossoma cruzi*, as well as in the regulation of the dispersion of *T. infestans* in the region. [35].

Evaluating the ecological parameters of *Triatoma brasiliensis* in the Brazilian north-eastern state of Ceará, in dry and rainy seasons alike, ELISA tests found that the main sources of food were birds and armadillos, and were more frequent in the rainy season. This result, together with the lower nutritional pattern, low infection rates and easier capture, resulting in an increased demand for blood meals in the dry season, highlighted the importance of epidemiological interventions and defined the period in which Triatominae tend to invade the peridomestic environment more intensely, in search of food and possibly in an attempt to re-colonize artificial structures [36].

The sandflies and leishmaniasis

Sand flies are natural vectors of some etiological agents of human and animal diseases, such as protozoa of the genus *Leishmania* and other trypanosomatids, bacteria of the genus *Bartonella* and numerous arboviruses [37].

Leishmaniasis are protozoonoses, caused by protozoa flagellate heteroxenos of the genus *Leishmania* (Ross 1903) (Kinetoplastida: Trypanosomatidae). They are transmitted through the bite of sand flies, order Diptera, family Psychodidae and subfamily Phlebotominae, of the genus *Lutzomyia* in the Americas and *Phlebotomus* in the Old World (Young & Duncan 1994). These diseases are major public health problems, affecting men, women and children, lying, overall, among the six most important parasitic diseases on the world; they are also considered neglected diseases [14]. In the New World, are occurring as American Cutaneous Leishmaniasis (ACL) which has a distinct pattern of clinical features (cutaneous, mucosa, mucocutaneous, and anergic diffuse), and American Visceral Leishmaniasis (AVL) with tropism of the parasite to the mononuclear phagocytic system of the spleen and liver, leading to hyperplasia and hypertrophy of these organs [38,39].

In Brazil, leishmaniasis, are included in the National System of Compulsory Notification Diseases/SNDC, by the Ministry of Health, and are present in all the federal units. Over the past twenty years, its incidence has been increased in all Brazilian States [40, 38, 39], clearly showing a process of expanding to major cities [14].

Importance of the evaluation of feeding habits of phlebotominae

Knowing the feeding pattern of these flies is very important in the understanding of the eco-epidemiology of leishmaniasis, as it makes it possible to comprehend the interactions between the links of the epidemiological chain. The result is a significant contribution from the epidemiological point of view and in the research into likely leishmaniasis hosts, other than getting to know the level of anthropophilia of the species.

As Brazil has been facing an expansion of ACL and AVL, the latter having a high mortality rate, it is crucial to acquire knowledge regarding the determining factors of these new epidemiological profiles. The evaluation of the feeding habits of Phlebotominae vectors provides important information regarding their attraction to potential reservoirs, which, of course, would be a part of the enzootic cycle, or others that presumably help maintain the epidemiological chain in the rural and/or urban environments. It is important to highlight that the role of some species of mammals is still under discussion regarding their ability to occupy and establish themselves in new areas, contributing to the alterations in the transmission profile and in its expansion process [38, 39].

About the feeding habits of phlebotominae

Some studies carried out in the Old and New World about food sources for adults should be highlighted, as they have provided important information from the epidemiological point of view.

Precipitin studies carried out in the west of Bengal (India) revealed that *Phlebotomus argentipes*, a vector of visceral leishmaniasis in the country, and originally a zoophilic species, can now be found in households, and prefers to feed on humans, mostly in areas in which no bovines are found. The authors alerted to the fact that the behavioral changes described may influence the occurrence of outbreaks in the region [41].

Phlebotomus perniciosus, one of the species responsible for the transmission of *Leishmania*, were collected in four different places in Spain and submitted to an ELISA test (avidin-biotin method). The species showed an opportunistic behavior, feeding on whatever animals were more readily accessible. However, a few trends were observed: the species did not feed on birds and showed high positivity for canine antiserum, higher than expected, based on the dog-human ratio. The authors pointed out that the composition of the local fauna plays a very important role in the dynamics of the transmission of *Leishmania*; in peri-urban areas the risk of transmission to humans is higher, as humans are the most numerous vertebrate, while in rural areas the *Leishmania*-human transmission is reduced because of the presence of several other mammals, besides a higher number of dogs [22].

Studies were carried out in Kenya, using animal baits to capture *Phlebotomus guggisbergi*, vector of *Leishmania tropica*, causative agent of CL in Laikipia. The purpose was to identify its rural reservoir. Goats and sheep proved to be the most attractive reservoirs, while small mammals such as hamsters and hyraxes were the least attractive. The authors concluded that larger animals were proportionally more attractive to females of this species. Although the study did not reveal non-human reservoirs, it provided evidence for further investigation [42].

In a study conducted in the province of Macerata, in Italy, the ELISA technique detected specimens of *P. perniciosus* which had fed on humans, dogs, horses, sheep and birds; *Phlebotomus perfiliewi* on dogs, horses, sheep and birds; and *Phlebotomus papatasi* on dogs, horses and birds. Ninety-five percent of the specimens had fed on the animals in the shelter where they had been gathered, showing that the choice of food source was related to its availability (size and number) and not to a specific level of attractiveness. The feeding habits of these leishmania vectors have epidemiological implications (in urban and peri-urban areas), as the females can seek other sources of food, such as humans and dogs, whenever other hosts are unavailable [43]. Studies in outbreak sites of CL in Sanliurfa, Turkey, using the ELISA technique, showed the feeding preferences of *Phlebotomus sergenti* and *P. papatasi*. Both species proved to be attracted to birds and mammals, in particular rodents, which are abundant in the households and play an important role in the circulation of the parasite [44].

In a study conducted in Nepal, an endemic area of VL, specimens of *P. argentipes*, the only VL vector described on the Indian subcontinent, were fed with human and hamster blood and submitted to blood testing using ELISA and precipitin techniques. The ELISA assay proved to be more sensitive and specific, with 100% of sensitivity up to 72 hours after the meal, while in the precipitin technique this value drops to 48 hours. The understanding of the feeding behavior of this vector can be used in local intervention programs [45].

By analyzing the species *Phlebotomus perfiliewi transcaucasicus* using the RFLP-PR technique, employing regions of cytochrome B of mitochondrial DNA (Xho I and Hae III), it was possible to differentiate human feeding from that on other vertebrates (cows and dogs), showing their epidemiological role in Iran [46].

Due to its natural shelters, lower flying patterns and low anthropophilic tendency, it is known that *Lutzomyia flaviscutellata* prefers to feed on rodents [47, 48], which allows for a more efficient collection method in which rodents are ideal bait [49, 50, 51] in Disney traps [47]. This kind of trap is recommended for epidemiological studies in situations in which human cases were recorded in areas of transmission of *Leishmania (L.) amazonensis*, [39]. In Mato Grosso do Sul, in central Brazil, modified Disney traps successfully captured *Bichromomyia flaviscutellata* (second most prevalent species, corresponding to 41.4% of the specimens collected) using a rodent as bait (*Mesocricetus auratus*). The finding of vector *B. flaviscutellata* and of *L. (L.) amazonensis* (isolated from sentinel hamsters) can be considered a prediction factor for the occurrence of leishmaniasis in the local population.

Using animal bait, such as marsupials, birds, reptiles and rodents, other than taking into consideration the samples that bit the collectors during studies carried out in the National Park of Serra dos Órgãos, in the southern state of Rio de Janeiro, it was observed that cockerels and men were the most attractive bait for *Lutzomyia ayrozai* and *Lutzomyia hirsuta;* most bites took place near ground level and mostly between 5 p.m. and midnight [52]. These species have been related to the transmission of ACL in other parts of Brazil [53, 54, 55]. On the São Luís Island (in the northern state of Maranhão), Carvalho *et al.* [56] successfully used chicken as bait to collect Phlebotominae; it is known that chicken coops near houses attract Phlebotominae and function as incubators; these factors contribute to closer contact between vectors and humans, as they facilitate vector domiciliation.

In Panama, Tesh *et al.* [57] observed that *Lutzomyia shannoni* is strongly attracted to rodents and toothless mammals, while *Lutzomyia ylephiletor* (or *ylephiletrix*) was only attracted to toothless mammals. They also found in *Lutzomyia vespertilionis* a reaction against the antiserum of bats. In the central Amazonia region, specimens of the *shannoni* group were mostly found to have fed on sloths [58], a fact with high epidemiological significance as sloths are reservoirs of *Leishmania guyanensis* in Panama.

In Peru, Ogosuku *et al.* [59] observed the eclectic feeding habits of *Lutzomyia peruensis* and *Lutzomyia verucarum*, although the former proved to be more anthropophilic; from among the samples with different food sources, most specimens had fed on sources that usually remain inside the house and on a second source located outside the house. Multiple feedings are a consequence of the difficulty faced by some species in obtaining a full meal from a single host. This characteristic is an important transmission factor for leishmaniasis.

Studies conducted in the town of Mandaguari (Paraná, southern Brazil) showed that *Nyssomyia whitmani*, *Pintomyia fischeri*, *Migonemyia migonei*, *Nyssomyia neivai* did not present particular preferences among the available bait (pig, dog, rabbit and chicken), once again confirming the opportunistic character of female Phlebotominae, which probably adjust their feeding habits to the available hosts. This suggests eclectic feeding habits in anthropic environments [60]. In Baturité, in the Brazilian state of Ceará, an outbreak area of ACL, a study proved the hematophagous activity of *Lutzomyia whitmani* and *Lutzomyia migonei*. Although both species had fed on humans, *L. whitmani* was more frequent on this bait, while *L. migonei* was more abundant during collections using animal bait (horses). This hematophagous behavior, together with the presence of naturally infected specimens, suggested that these two species function as ACL vectors in the region [61, 62, 55].

In Axixá, a community with one of the highest rates of human ACL cases in the northern state of Maranhão, specimens of *L. (N.) whitimani*, the main vector of ACL in the state, were collected. Using the precipitation technique, a study was conducted to understand the feeding preferences of this species. The results showed a higher attraction for chickens, rodents and mainly humans, other than domestic and synanthropic animals, which play an important role in the transmission cycle of ACL and explaining the cases of this disease in the city [63].

Specimens of *Lutzomyia umbratilis* collected in the northern state of Amazonas were analyzed using the precipitin method, and were mostly positive for rodent blood, unlike previous reports that showed that this species was attracted to sloths [64].

In the state Rio de Janeiro, Afonso *et al.* [65] while studying *Lutzomyia (N.) intermedia*, a vector of ACL, observed positivity in the domestic and peri-domestic environment for the antiserum of rodents, birds, dogs, horses and humans, a proof of the eclectic feeding habits of this vector. In both environments, higher positivity was found for rodents, showing the species' intense attraction to these animals, suggested as possible primary reservoirs of *Leishmania (Viannia) braziliensis* in the north-eastern state of Pernambuco [66].

In a study conducted in Buriticupu, in the Amazon, an area of ACL transmission, the precipitin test applied resulted in 87.6% of simple reactions, 8% of double reactions and 4.4% of non-reactive tests. The authors believe that the finding of samples containing, at the same time, human blood, blood of synanthropic animals (rodents and mucuca) and domestic animals (dogs and horses) confirms the hypothesis that transmission occurs in this peri-domestic environment, which explains the occurrence of autochthonous human cases of ACL in the region [67].

Morrison *et al.* [68] while working in Colombia, an endemic area for CL, showed the opportunistic character of *Lutzomyia (Lutzomyia) longipalpis*, as well as its low anthropophilic nature and its attraction for dogs, considered to be, intermediate hosts of AVL. Females of *L. (L.) longipalpis* collected in the city of Raposo, in the state of Rio de Janeiro, were analyzed using the precipitin technique. Birds (chickens) were found to be the most reactive animals [69]. Alexander *et al.* [70] described how attractive birds are to male and female specimens of *L. (L.) longipalpis*. Although birds are refractory to *Leishmania*, they function as food sources for Phlebotominae and attract potential reservoirs of *Leishmania (Leishmania) infantum chagasi* closer to residences, making the installation and maintenance of the transmission cycle possible in the rural environment and near city suburbs [71].

Data from research studies in Araçatuba, in the state of São Paulo, regarding the food sources of *L. (L.) longipalpis* analyzed using the precipitin technique, revealed that 91.4% of reactive samples were positive for canine blood. This fact was correlated to the abundance of this mosquito and to the presence of dogs in the peri-domestic environment. The epidemiological role of dogs in the transmission chain of AVL in Araçatuba is very significant, considering that the canine enzootic precedes transmission to humans. It is also important to highlight that this species shows a high level of attraction to dogs [72].

Marassá *et al.* [23] detected the efficacy of the avidin-biotin method in female specimens of *L. (L.) longipalpis*, laboratory fed, between 12 and 24 hrs, after the meal. According to the authors, the test can be successfully used to identify the source of food, as females can be found in nature during the process of blood digestion (an amount of blood similar to the one used in the experiments).

In an analysis made with specimens of *L. (L.) longipalpis* from Campo Grande, an endemic area for AVL, Oliveira *et al.* [73] used the ELISA immune-enzymatic test (avidin-biotin) to show the opportunistic and eclectic character of this mosquito and the factors that maintained the transmission of *L. (L.) infantum chagasi*. They also suggested new investigations regarding the actual role played by dogs in the VL epidemiology in this area, and the possibility that man is a source of infection for mosquitoes, as high levels of positivity were found for human antiserum.

Afonso *et al.* [26], analyzing populations of *L. (L.) longipalpis* from the north-east of Brazil (Jequié, in the state of Bahia; Teresina, capital of the state of Piauí; Sobral and Massapê, in the state of Ceará), used the ELISA technique to verify significant percentages of positivity for bird antiserum. Specimens were found that had fed on humans, dogs (domestic VL reservoirs) and opossums (synanthropic animals, possible reservoirs of *Leishmania (L.) infantum chagasi*). The finding of reactivity for more than one source of blood was common, confirming the eclectic feeding behavior of this species, suggested as an adaptation factor to different habitats. In the same study location, Deane [74] found proof of the role of dogs as important reservoirs of *L. (L.) infantum chagasi* and as a source of infection for *L. (L.) longipalpis*, after carrying out xenodiagnosis in infected dogs. In several VL transmission areas, epidemiological evidence shows that dogs are domestic reservoirs [71]. Silva *et al.* [75], while performing studies on the vectorial competence of *L. (L.) longipalpis* in the area of Bela Vista, in the north-eastern city of Teresina, found 1.1% of infected female specimens, relating these data to the existence of dogs with VL in the surrounding areas.

According to the studies of Afonso *et al.* [26], a significant fact was the finding of females of *L. (L.) longipalpis*, from Jequié (in the state of Bahia), Sobral and Massapê (in the state of Ceará), that were positive for opossum antiserum. This information requires further detailed analysis, as the opossum *Didelphis albiventris* has been suggested as a possible reservoir of *L. (L.) infantum chagasi* according to isolated samples obtained in Jacobina (Bahia), in which 2 animals out of 84 were found to be positive. For some authors, this is not epidemiologically significant [76, 77], although other, later studies reported the natural infection of *Didelphis marsupialis by Leishmania* spp., possibly *L. (L.) infantum chagasi*, therefore taking into consideration the role of these mammals as potential reservoirs for VL [78, 79, 38].

Heouas *et al.* [28] studying the food sources of *Phlebotomus duboscqi,* used a single copy PNOC gene (prepronociceptin gene) and mitochondrial cytochrome B. These genes were selected for their use in phylogenetic studies in different mammalian species and proved to be good targets for studying the origin of the food contents of sucking insects. The analyzes were performed with insects fed on humans and wild mammals, demonstrating that this analysis can generate important information in the study of new reservoirs and allow a better understanding of complex parasite life cycles.

Sant'Anna *et al.* [80], used a multiplex PCR to identify food sources and natural infection by *Leishmania* in specimens of *L. (L.) longipalpis* collected in an endemic area of AVL in Brazil. The results showed a clear attraction of specimens to birds (probably chicken), an important determinant factor in the urbanization process of the disease [70], considering the proximity to chickens as a risk factor of transmission to man [81, 82, 83]. The greater sensitivity of the method reported here means that information can be obtained from specimens that have ingested relatively small amounts of blood [80].

A very recent study of DNA samples isolated from engorged *Psychodopygus lloydi*, using cytochrome b gene (cytB) in polymerase chain reaction (PCR), discussed the importance of using this methodologies as a tool for identifying the food sources of female sand flies [84].

CONCLUSION

Among the techniques mentioned here, the immunological are still the most widely used (Precipitin and ELISA), although the PCR technique, has been employed more often. All these techniques give good results, varying in sensitivity and specificity; however several parameters must be considered (Table 1). The knowledge of feeding habits of insects of medical importance, not only contribute to the increase of information on their habits, but can provide important epidemiological evidences. In the context of leishmaniasis, probably the adaptation of sand fly vector to new habitats, in part is due to feeding plasticity which could be a determinant in the process of expansion and urbanization of the disease and the emergence of new epidemiological profiles.

Table 1. Main characteristics of techniques: Precipitin, ELISA and PCR.

Techniques **Characteristics**	**Precipitin**	**Elisa**	**PCR**
Sensibility	Variable	High	High
Specificity	Variable	High/Variable	High
Processing	Simple	Complex	Complex
Diagnosis	Fast	Fast	Fast/Variable
Cost	Low	High	High
Reading	Subjective	Objective/Quantified	Objetive/Quantified
Amount of antisera	High	Low	Low

REFERENCES

1. Ruppert, E. E. and Barnes, R. D. 1996, São Paulo, Toca (Ed.), 1029.
2. Félix, M., Almeida, C. E., Serra-Freire, N. M. and Costa, J. 2010, Otten (Ed.), Rio de Janeiro, Brasil, 375.
3. Rey, L. 1991, Rio de Janeiro, Ganabara Koogan (Ed.), 731.
4. Lourenço-de-Oliveira, R. 2005, Guanabara Koogan (Ed.), Rio de Janeiro, 2096.
5. Edman, J. D., Weber, L. A. and Kale, H. W. 1972, J. Méd. Entomol., 9, 429-434.
6. Boreham, P. F. L. and Garret, J. C. 1973, Bull Who, 48, 605-614.
7. Duarte, R. 1997, IOC-FIOCRUZ, 103.
8. Ngumbi, P. M., Lawyer, P. G., Johnson, R. N., Kiilu, G. and Assiago, C. 1992, Med. Vet. Entomol., 6. 385-388.
9. Savage, H. M., Niebylski, M. L., Smith, G. C., Mitchell, C. J. and Craig Jr. G. B. 1993, J. Med. Ent., 30, 27-34.
10. Gomes, A. C. 2002, Informe Epidemiológico do SUS, 11, 79-80.
11. Quinnell, R. J., Dye, C. and Shaw, J. J. 1992, Vet. Entom., 6, 195-200.
12. Dias-Sversutti, A., Scodro, R. B., Reinhold-Castro, K. R., Neitzke, H. C. and Teodoro U. 2007, Neotrop. Entomol., 36, 953-959.
13. Brasil Ministério da Saúde, 2006, Brasília, Ministério da Saúde (Ed.), 52.
14. Organização Mundial de Saúde, 2011, http://www.who.int.
15. Washino, R. K. and Else, J. G. 1972, Am. J. Of Trop. Med. Hyg., 21, 120-122.
16. Rosemser, W. S., Edman, J. D., Lornz, L. H. and Scott, T. 1989, Am. J. Trop. Med. Hyg., 41, 737-742.
17. Bull, C. G. and King, W. V. 1923, Am. J. Hyg., 3, 491-496.
18. Siqueira, A. F. 1960, Rev. Inst. Med. Trop., 2, 294-297.
19. Crans, J., Duarte, R. and Msrchon, S. 1995, Mosq. News, 29, 563-566.
20. Tesh, R. B., Chaniotis, B. N., Aranson, M. D. and Jahnson, K. M. 1971, J. Trop. Med. Hyg., 20, 150-156.
21. Burkot, T. R., Goodman, W. G. and Defoliart, G. R. 1981, Am. J. Trop. Hyg., 30, 1336-1341.
22. Colmenares, M., Portús, M., Botet, J., Dobaño, C., Gállego, M., Wolff, M. and Seguí, G. 1995, J. Med. Entomol., 32, 229-233.
23. Marassá, A. M., Consales, C. A. and Galati, E. A. B. 2004, Rev. Soc. Bras. Med. Trop., 37, 441-446.
24. Afonso, M. M. S. 2008, Universidade Federal de Viçosa, 88.
25. Afonso, M. M. S., Duarte, R., Miranda, J. C., Souza, L. C. and Rangel, E. F. 2008, 6th International Symposium ons Phelebotomine Sandflies, Peru.
26. Afonso, M. M., Duarte, R., Miranda, J. C., Caranha, L. and Rangel, E. F. 2012, J. Trop. Med., ID 858657, doi:10.1155/2012/858657.
27. Gomes, L. A. M., Duarte, R., Lima, D. C., Diniz, B. S., Serrão, M. L. and Labarthe, N. 2001, Mem. Inst. Oswaldo Cruz, 96, 693-695.
28. Heouas, N., Pesson, B., Boudabous, R., Dedet, J. P., Babba, H. and Ravel, C. 2007, Am. J. Trop. Med. Hyg., 77, 1054-1059.
29. Gokool, S., Curtis, C. F. and Smith, D. F. 1993, Med. Vet. Entomol., 7, 208-205.
30. Siriyasatien, P., Pengsakul, T., Kittichai, V., Phumee, A., Kaewsaitiam, S., Thavara, U., Tawatsin, A., Asavadachanukorn, P. and Mulla, M. S. 2010, Southeast Asian J. Trop. Med. Public Health, 41, 43-47.
31. Kim, K. S., Tsuda, Y. and Yamada, A. 2009, J. M. Entomol., 46, 1231-1234.
32. Roy, A. and Sharma, V. P. 1991. J. Am. Control. Assoc., 7, 11-14.
33. Johansen, C. A., Power, S. L. and Broom, A. K. 2009, J. of Med. Entomol., 46, 1167-1175.
34. Martins, L. P., Castanho, R. E., Casanova, C., Caravelas, D. T., Frias, G. T., Ruas-Neto, A. L. and Rosa, J. A. 2006, Rev. Soc. Bras. Med. Trop., 39, 198-202.
35. Pizarro, J. C. and Stevens, L. 2008, PLoS One, 3, 3585.
36. Sarquis, O., Carvalho-Costa, F. A., Oliveira, L. S., Duarte, R. D., Andrea, P. S., de Oliveira, T. G. and Lima, M. M. 2010, J. Vector. Ecol., 385-394.
37. Shaw, J., Rosa, A. T. and Souza, A. 2003, FIOCRUZ (Ed.), Rio de Janeiro, Brasil, 337-352.
38. Brasil Ministério da Saúde, 2006, Brasília, Ministério da Saúde (Ed.), 120.

39. Brasil, Ministério da Saúde, 2007, Brasília, Ministério da Saúde (Ed.), 182.
40. Rangel, E. F. 1995. Tropical Disease, Society and the Environment, 2, 103-110.
41. Das, S., Boreham, P. F. and Bhattacharya, N. C. 1976, Indian J. Med. Res., 64, 1307-1313.
42. Johnson, R. N., Ngumbi, P. M., Mwanyumba, J. P. and Roberts, C. R. 1993, Vet. Entomol., 7, 216-8.
43. Bongiorno, G., Habluetzel, A., Khoury, C. and Maroli, M. 2003. Acta. Trop., 88, 109-16.
44. Svobodová, M., Sádlová, J., Chang, K. P. and Volf, P. 2003, Microbes Infect., 5, 361-4.
45. Burniston, I., Roy, L., Picado, A., Das, M., Rijal, S., Rogers, M., Coosemans, M., Boelaert, M., Davies, C. and Cameron, M. 2010, J. Med. Entomol., 47, 902-906.
46. Mohammad, A., Oshaghi, N. M. R., Ezat-Aldin, J., Mehdi, M., Homa, H., Zabiholah, Z., Fatemeh, M. and Yavar, R. 2012, Am. J. Trop. Med. Hyg., 81, 4572-4577.
47. Disney, R. H. L. 1966. Bull. of Entomol. Research, 56, 445-451.
48. Lainson, R. and Shaw, J. J. 1968, Trans. R. Soc. Trop. Med. Hyg., 62, 385-395.
49. Shaw, J. J. and Lainson, R. 1968, Trans. R. Soc. Trop. Med. Hyg. 62, 396-405.
50. Shaw, J. J., Lainson, R. and Ward, R. D. 1972, Trans. of the Royal Society of Tropical Med. and Hyg., 66, 718-723.
51. Rangel, E. F. and Lainson, R. 2003, Rio de Janeiro, FIOCRUZ (Ed.), 291-310.
52. de Aguiar, G. M. and Soucasaux, T. 1984. Mem. Inst. Oswaldo Cruz, 79, 197-209.
53. Arias, J. R., Miles, M. A., Naiff, R. D., Povoa, M. M., de Freitas, R. A., Biancardi, C. B. and Castellon, E. G. 1985, Am. J. Trop. Med. Hyg., 34, 1098-1108.
54. Lainson, R., Shaw, J. J., Silveira, F. T., Braga, R. R. and Ishikawa A. Y. 1990, An. Paras. Hum. Comp., 65, 282-284.
55. Rangel, E. F. and Lainson, R. 2009, Mem. Inst. Oswaldo Cruz., 104, 937-54.
56. Carvalho, M. L., Rebêlo, J. M. M., Araújo, J. C. and Barros, V. L. L. 2000, Entomol. Vectores, 7, 19-32.
57. Tesh, R. B., Chaniotis, B. N., Aranson, M. D. and Jahnson, K. M. 1971, J. Trop. Med. Hyg., 20, 150-156.
58. Christensen, H. A., Alias, J. A., Vasquez, A. M. and Freitas, R. A. 1982. Am. J. Trop. Med. Hyg., 31, 239-242.
59. Ogosuku, E., Perez, J. E., Paz, L., Nieto, E., Monje, J. and Guerra, H. 1994. Ann. Trop. Med. and Parasitol., 88, 329-335.
60. Muniz, L. H. G., Rossi, R. M., Neitzke, H. C., Monteiro, W. M. and Monteiro, U. 2006. Rev. Saúde Pública, 40, 1087-1093.
61. Azevedo, A. C., Rangel, E. F., Costa, E. M., David, J., Vasconcelos, A. W. and Lopes, U. G. 1990. Mem. Inst. Oswaldo Cruz, 85, 251.
62. Azevedo, A. C. and Rangel, E. F. 1991, Mem. Inst. Oswaldo Cruz, 86, 405-10.
63. Fonteles, R. S., Vasconcelos, G. C., Azevêdo, P. C., Lopes, G. N., Moraes, J. L., Lorosa, E. S., Kuppinger, O. and Rebêlo, J. M. 2009. Rev. Soc. Bras. Med. Trop., 42, 647-50.
64. Nery, L. C. R., Lorosa, E. S. and Franco, A. M. R. 2004, Mem. Inst. Oswaldo Cruz, 99, 571-574.
65. Afonso, M. M., Gomes, A. C., Meneses, C. R. and Rangel, E. F. 2005, Cad. Saúde Pública, 21, 1816-1820.
66. Brandão, S. P., Brito, M. E., Carvalho, F. G., Ishikawa, E., Cupolillo, E., Floeter-Winter, L. and Jefferey, J. 2003, Trans. R. Soc. Trop. Med. Hyg., 97, 291-296.
67. Oliveira-Pereira, Y. N., Moraes, J. L. P., Lorosa, E. S. and Rabelo, J. M. M. 2008, Cad. Saúde Pública, 24, 2183-2186.
68. Morrison, A. C., Ferro, C. and Tesh, R. B. 1993, Am. J. Trop. Med. Hyg., 49, 68-75.
69. Dias, F. O., Lorosa, E. S. and Rebêlo, J. M. 2003, Cad. Saúde Pública, 19, 1373-1380.
70. Alexander, B., Carvalho, R. L., McCallum, H. and Pereira, M. H. 2002. Emerg. Infect. Dis., 8, 1480-1485.
71. Lainson, R. and Rangel, E. F. 2005, Mem. Inst. Oswaldo Cruz, 100, 811-827.
72. Camargo-Neves, V. L. F., Rodas, L. A. C. and Gomes, A. C. 2007, Bol. Epidemiológico Paulista, 4, 2-7.
73. Oliveira, G. O., Marassá, A. M., Consales, C. A., Dorval, M. E. C., Fernandes, C. E., Oliveira, G. R., Brazil, R. P. and Galati, E. A. B. 2008, Acta Tropica, 107, 238-241.
74. Deane, L. M. 1956. Brasil, Serviço Nacional de Educação Sanitária, 162.

75. Silva, J. G. D., Werneck, G. L., Cruz, M. S. P., Costa, C. H. N. and Mendonça, I. L. 2007, Cad. Saúde Pública, 23, 1715-1720.
76. Sherlock, I. A., Miranda, J. C., Sadigursky, M. and Grimaldi, Jr. G. 1984, Mem. Inst. Oswaldo Cruz, 7, 511.
77. Sherlock, I. A., Miranda, J. C., Sadigursky, M. and Grimaldi, Jr. G. 1988, Mem. Inst. Oswaldo Cruz, 83,141.
78. Schallig, H. D., da Silva, E. S., Van Der Meide, W. F., Schoone, G. J. and Gontijo, C. M. 2007, Vector Borne Zoonotic Dis., 7, 387-393.
79. Cabrera, M. A. A., Paula, A., Camacho, L. A. B., Marzochi, M. C., Xavier, S., Silva, A. V. M. and Jansen, A. M. 2003, Rev. Inst. Med. Trop. S. Paulo, 45, 79-83.
80. Sant'anna, M. R., Jones, N. G., Hindley, J. A., Mendes-Sousa, A. F., Dillon, R. J., Cavalcante, R. R., Alexander, B. and Bates, P. A. 2008, Acta Trop., 107, 230-237.
81. Lainson, R. 1988, Philos. Trans. R. Soc. Lond. B. Biol. Sci., 31, 389-404.
82. Genaro, O. 1993, Belo Horizonte, UFMG.
83. Caldas, A. J., Costa, J. M., Silva, A. A., Vinhas, V. and Barral, A. Trans. R. 2002. Soc. Trop. Med. Hyg., 96, 21-28.
84. Quaresma, P. F., Carvalho, M. L., Ramos, M. C. N. and Andrade Filho, J. D. 2012, Mem. Inst. Oswaldo Cruz, 107, 480-485.