Chapter 4

Vectorial Control Based on the General Characteristics of Phlebotomine Sand Flies

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Abstract

Sand flies (Diptera: Psychodidae: Phlebotominae) are vectors insects of zoonotic diseases, caused by protozoa such as *Leishmania* spp. Leishmaniases are diseases of great global epidemiological importance, endemic in tropical and subtropical areas of the Americas, where they constitute a significant public health problem. In this chapter we will discuss the vector control measures based on the general characteristics of phlebotomine sand flies.

Keywords: sand fly; leishmaniasis; vector control.

1. Introduction

Sand flies (Diptera: Psychodidae: Phlebotominae) are vectors insects of zoonotic diseases, or even anthropogenic diseases (in the Old World), caused by protozoa such as *Leishmania* spp. [1], bacteria, for example *Bartonella bacilliformis* or viruses belonging to three different genera: (i) the Phlebovirus [2] including sandfly fever Sicilian virus, sandfly fever Naples virus, Toscana virus and Punta Toro virus; (ii) the vesiculovirus including Chandipura virus and (iii) the orbivirus including Changuinola virus.

The Phlebovirus virus belonged to the family Phenuiviridae, order Bunyavirales and transmitted by sand flies are widely distributed in the Mediterranean region, Africa, Indian subcontinent, Middle East and Central Asia. Being that the major viruses that are pathogenic to human health, the Sicilian Fever Virus (SFSV) and the Naples Fever Virus (SFNV) are the causative agents of transient febrile illness in humans [4], while the Toscana Virus (TOSV) can cause central nervous system infections, as occurred during the hot season in Italy, then in other Mediterranean countries (Spain, France, Portugal, Greece, Turkey, Tunisia), thus presenting an emerging health problem [5].

Pathogenesis has been reported since the early 20th century and likely new cases will continue to be observed within the local populations where phleboviruses are known. In addition, the increasing movement of humans, animals and their belongings may introduce this virus by displacing its vector, so all regions where sand flies are present should be considered as a potential risk for phlebovirus [4].

Estimates indicate the existence of 988 phlebotomine species and subspecies on all continents, except Antarctica. Among them, two genera are important carriers of human leishmaniasis, the genus Phlebotomus, found in Europe, Africa and Asia, and Lutzomyia in South and Central America [6].

Leishmaniases are endemic in tropical and subtropical areas of the Americas, where it represents a significant public health problem [7]. These parasitoses present zoonotic character and affect men and diverse species of wild and domestic animals, that can harbor several species of parasites [8].

Leishmaniasis manifests itself in three forms: visceral (kala-azar), cutaneous and mucocutaneous. Approximately one million new cases of these diseases are reported and 30,000 deaths occur annually [9].

Globally every year, 50 000 to 90 000 new cases of visceral leishmaniasis are recorded. In 2015, 90% of cases reported to WHO (World Health Organization) were observed in seven countries: Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan. However, cutaneous leishmaniasis (CL) is the most common form of leishmaniasis, causing dermatopathies. About 95% of CL cases are found in the Americas, the Mediterranean basin, the Middle East and Central Asia [6].

Thus, in this chapter vector control measures based on the general characteristics of phlebotomine sand flies will be approached.

2. Sand Flies

2.1. General Features

Sand flies are small insects, measuring from two to three millimeters, that have in their body intense pilosity and long and thin legs [10]. They are also known for their painful bites that may cause allergic reactions [11].

The period of activity of these insects begins at dusk and during the day they remain in their shelter. Their attraction to hosts is related to temperature and body odor, being eclectic in relation to their food preference [10].

2.2. Biological Cycle

Sand flies are holometabolous insects, with the phases from egg to adult varying with the food supply and climatic conditions. In most species, in the first in star larvae, a single pair of caudal bristles can be noticed, being that the others exhibit two pairs [10]. Immature stages develop in relatively humid places, with low light and are protected from severe climatic changes by layers of decaying organic materials [12].

The development time of the larvae of some species is 18 days, but this period may be prolonged for months depending on the environmental conditions [13].

After the adult emerges from the pupa, in 24 hours, the external genitalia of the males undergoes a rotation of 180º and from this point, they will be ready for copulation [10]. Adults of both sexes need carbohydrates (sugars) as a source of energy for mating, posture and infectivity of *Leishmania* spp. in their digestive tract [11]. In addition to sugars, females need blood from vertebrates for the maturation of their eggs. Some species feed only once between the postures, while others need several repasts for one oviposition cycle [10], presenting gonotrophic discordance and increasing the chance of protozoal transmission.

The longevity of adult insects in the natural environment is unknown, however, laboratory studies have shown that sand flies can survive between 20 to 30 days [10]. Regarding their dispersion distances under natural conditions, some species can reach from 243m in urban areas [14] up to 700m in a rural community [15], such is the case of *Lutzomya longipalpis.*

2.3. Habitat

The natural habitat of sand flies is characterized by a small variation of temperature and humidity, which favors their presence, since they are sensitive to desiccation. The minimum modification of these factors in microhabitats is enough to alter the population dynamics of these insects [16].

Some species reproduce in peridomestic situations and shelter in human habitation, such as the *Lutzomyia* genus that is seen in and around households and reproduces in organic waste, including manure, animal feces (chickens, rodents), garbage, rodent holes, cracks and fissures in walls with high temperature and humidity [17].

Phlebotomine larvae have already been found in samples of soil and organic matter in different microhabitats, including bases of trees, ground of open forest, soil from under fallen logs, soil of roots and bases of palm trees [18], animal burrows and shelters of domestic animals [19].

2.4. Vector Competence

The vectorial competence of a phlebotomine sand fly is observed by its capacity to become infected with the protozoan of the species of *Leishmania* spp. and transmit it to a susceptible host. This proof is an evidence that a particular insect is a vector of a specific species of *Leishmania*. This parameter is one of the criteria to be evaluated in vector capacity studies, in which the interaction between vectors-parasite-host that are involved in the eco epidemiology of leishmaniasis is searched [20].

Ecological associations and transmission dynamics are also related to the success or failure of transmission of *Leishmania* spp. [21]. So for the characterization of the vectorial competence, its essential to identify the location of the different forms of the protozoan in specific parts of the bowel of the sand flies by microscopy [19], mainly demonstrate the presence of infectious metacyclic forms of the parasite in the anterior midgut of the vector and the experimental transmission [6].

2.5. Control

2.5.1. Mechanical and Chemical Control

Some of the control strategies directed to sand flies are the internal residual spraying (IRS), the treatment of mosquito nets with insecticides, synthetic sex pheromones and environmental management.

The application of insecticides to the walls and roofs of households (IRS) [23] and in animal shelters (eg poultry houses and stockyard) is considered effective in reducing the population of sand flies [24]. Environmental concerns and the risk to human health surrounding the use of organochlorides and other chemical groups have gradually led to their replacement with synthetic pyrethroids (α-cypermethrin, cypermethrin, deltamethrin and λ-cyhalothrin), which are currently used by heads of public health agencies in several countries [25]. On the other hand, in the north of Morocco, cutaneous leishmaniasis has been reduced with house spraying with α-cypermethrin [26].

However, the IRS can be useful in particular situations when a high density of these insects is found near or in human habitations, as well as in poultry, stockyard and dog shelters that may represent a "natural attraction" to the vectors [27-28].

Mosquito nets impregnated with slow release insecticide are also used in the intervention against leishmaniasis, and their use provides a significant reduction in the incidence of the disease in endemic areas [29]. Through intervention with mosquito nets in Bangladesh, a decrease of 70-80% of the density of *Phlebotomus argentipes* has been noted [30].

In studies in the Indian subcontinent, using mosquito net treated with insecticides, a reduction of 25% in the sand fly density in the internal area has been verified [31].

Another control strategy is the use of synthetic pheromone ((S) -9-methylgermacrene-B) of *Lu. longipalpis* species to improve the effectiveness of sand fly control programs, when used with an effective insecticide. The combination of pheromone and insecticide has as a mechanism the action the attraction and death of insects of both sexes, preventing the females from looking for the hosts in the transmission of leishmaniasis and the males in establishing the mating in other places [32].

The feasibility of using synthetic pheromone attraction to attract *Lu. longipalpis* for a long time has been proven and therefore can be used in the control of sand flies and in the prevention of visceral leishmaniasis [33].

It should also be recommended the management of the environment followed by application of insecticide of the group of pyrethroids [34] used so far, or another that is more effective. This management of the environment is fundamental in urban areas to reduce the density of vectors that proliferate very close to the population, in their wooded backyards.

2.5.2. Microbial Control

Strategies are being made through paratrangenesis, which consists of the use of genetically modified symbiotic bacteria that secrete effector molecules that kill infectious agents. This process has been viable for controlling the transmission of pathogens by arthropod vectors [35].In underdeveloped countries, new vector insect control programs are being deployed in an interesting way, as is the case of sand flies [36].

However, the knowledge of the symbiosis of insects can show new ways to control insects vectors of important diseases, through the directed manipulation of the symbionts or host-symbiont associations [37].

In the two main known genera of phlebotomines, *Lutzomyia* represented 57% of the bacteria belonging to *Proteobacteria phylum* (Gram-negative bacteria), while *Phlebotomus* expressed (47%) *Proteobacteria* and (40%) *Firmicutes*. Such a difference in the composition of the intestinal microbiota can be justified by several factors, including the diversity between the evolution of the two subgenera [38].

For the development of a paratransgenic platform to control the transmission of leishmaniasis, a non-pathogenic strain of *Bacillus* (*Bacillus subtilis*) isolated from phlebotomine *Phlebotomus papatasi* was used as a strong candidate for paratransgenic. Even though this bacterium has advantages such as being of genetic manipulation and easy to culture, not pathogenic, its use for the paratransgenic control of *Leishmania* may be challenging because of its ability to establish colonies in the intestines of several species of phlebotomine. For these reasons, it will be of great epidemiological importance to expand a regional strategy for each endemic area with different bacterial isolates [36].

2.6. Sand Flies Saliva, Leishmania Infectivity, and Vaccination

Phlebotomines infected with *Leishmania* spp. inoculate the parasites when making the blood replast in a vertebrate host. In this process, the vector saliva is inserted along with the protozoa into the skin of the host. This saliva is composed of molecules that trigger haemostatic, inflammatory and immunological responses of the host [39].Some of these molecules are immunogenic and develop strong immune responses in animals, including humans [40]. The humoral response against saliva from sand flies has been suggested as a possible epidemiological marker for exposure to vectors in endemic areas of leishmaniasis [41].

Continuous exposure to uninfected phlebotomine bites or immunization with salivary proteins are known to induce cellular and humoral immune responses [42-43].

A study with positive children in the delayed hypersensitivity test (DHT) showed that they were protected from infection because of their ability to expand the effective immune response against *Leishmania* spp. antigens. Although this group offers high concentration of anti-saliva antibodies to *Lutzomyia longipalpis*, its role in protecting against infection formation is not yet clear. Probably, the antibodies are capable of damaging the action of the salivary products, decreasing their functioning on the macrophages, however, favoring a greater activities of the antigens and the amplification of the cellular immunity. Therefore, it is not yet known whether the reduction of parasitic load may induce immune response [44].

Recently, a salivary protein of phlebotomine of the species *Lu. longipalpis*, LJM11, was identified as an immunogenic molecule for humans, dogs and mice exposed to bites of this vector [45].This protein belongs to the family of “yellow” proteins that are present in the salivary gland of phlebotomines of the genera *Lutzomyia* and *Phlebotomus*. Its function was instituted as a high affinity binders of proinflammatory biogenic amines [46].It has already been observed that this molecule is competent to cause a cellular immune response in vertebrates and an additional protection against *Leishmania*. It is important to emphasize, that the immunization with the LJM11 protein led to a protection against *Leishmania* major infection transmitted by Lu. longipalpis, thus highlighting the coverage capacity of this salivary molecule [47].

3. General Considerations

The main concern of this chapter is the vector control measures based on the general characteristics of the sand flies, which were described above and showing that one of the main strategies for control of sand flies in the world are spray and mosquito nets treated with insecticide, but these insects are showing resistance to dichlorodiphenyltrichloroethane (DDT) and deltamethrin [32-33], highlighting the importance of new insect control techniques.

The text also describes a new artifice of the use of synthetic pheromone as an insecticide, that its action is positively proven in the control of sand flies [30], being of great interest its viability and commercialization as a product for the use of vector control.

The role of health education in implementing vector control programs for leishmaniasis should be recognized. The outcome of an effective program can be compromised unless the people involved understand the needs of an intervention and are proactive in maintaining vigilance for the prevention of this zoonosis.

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