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SUSCEPTIBILITY OF LABORATORY-REARED FEMALE *LUTZOMYIA LONGIPALPIS* (LUTZ & NEIVA, 1912) TO INFECTION BY DIFFERENT SPECIES AND STRAINS OF *LEISHMANIA* ROSS, 1903

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A study was undertaken to compare the susceptibility of laboratory-reared female Lutzomyia longipalpis *to infection by different species or strains of New World* Leishmania. *The sand flies proved to be highly susceptible to infection by a strain of* Le. guyanensis, *with flagellates developing in all (18/18) of the specimens examined. A lower infection rate of 37% (10/27) was recorded in flies exposed to infection by a strain of* Le. amazonensis. *Flagellates developed in 13% (6/46) of the sand flies that blood fed on dogs in the early stage of experimental infection with an old laboratory strain of* Le. chagasi. *In contrast, promastigotes did not develop in sand flies that blood fed on dogs with naturally acquired* Le. chagasi. *The naturally infected dogs were in an advanced stage of disease. Flagellates developed in 9% (3/32) of the sand flies that blood fed on lesions of hamsters infected with a strain of* Le. braziliensis *and in 9% (3/34) of those that fed on hamsters with lesions due to a parasite of the* mexicana *complex (strain MHOM/BR/73/BH121). Sand flies did not develop flagellate infections after blood feeding on hamsters bearing lesions induced by strain MHOM/BR/71/BR49. Factors influencing the susceptibility of* Lu. longipalpis *to infection by New World species of* Leishmania *are discussed.*

Key words: *Lutzomyia longipalpis* – susceptibility to infection by New World species of *Leishmania*

Lutzomyia (Lutzomyia) longipalpis is one of the most widely distributed species of New World sand flies (Martins et al., 1978). It is one of the few American species that has adapted to domestic and peridomestic conditions, and the only one incriminated as a vector of *Leishmania (Leishmania) chagasi* (Deane, 1956; Deane & Deane, 1962; Lainson, 1983, 1989). *Lu. longipalpis* has also proved to be a species amenable to laboratory maintenance and colonization. Killick-Kendrick et al. (1977a) described methods for the establishment of closed colonies of this sand fly, and several such colonies are now maintained in various laboratories.

The available evidence (Lainson et al., 1984, 1985; Le Pont & Dejeux, 1984; Ryan et al., 1987) suggests that female *Lu. longipalpis* are the natural insect hosts only for *Le. chagasi.* In contrast, laboratory observations have shown that the flies are susceptible to infection with other species of *Leishmania.* All New World *Leishmania* studied by Coelho (1966), with the exception of *Le. enriettii,* developed in *Lu. longipalpis* and two strains of Old World *Leishmania* did so as well. The establishment of closed colonies of the sand flies has enabled studies on the morphology of *Leishmania* within in insect host (Killick-Kendrick et al., 1974; Molyneux et al., 1975; Killick-Kendrick et al., 1977b).

Lainson et al., (1977) suggested that *Lu. longipalpis* is a useful tool to study the developmental pattern of New World *Leishmania* in an insect host. The utility of such studies depends, however, on the assumption that *Lu. longipalpis* is equally susceptible to infection with all strains/species of New World *Leishmania.* Comparative susceptibility trials were undertaken and are reported herein.

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MATERIALS AND METHODS

Sand flies – Female phlebotomines belonging to the 12th – 22nd laboratory generations of two closed colonies of *Lu. longipalpis* were used. Both colonies originated from females collected in the north of the State of Pará and had been maintained, separately but in identical conditions, in the Belo Horizonte sand fly insectary since December 1984.

The one colony was first established at Instituto Evandro Chagas, Belém, from females captured on Ilha de Marajó. Eggs of the third laboratory generation reared in Belém were donated to establish a colony in Belo Horizonte. The other closed colony was established from female *Lu. longipalpis* captured in municipality of Abaetetuba. Eggs laid in the Belém laboratory by wildcaught females were brought to Belo Horizonte to initiate the colony.

For routine maintenance of both colonies, adult females were blood fed on hamsters, larvae received the same diet, and the insectary was maintained at a temperature of 24 ± 1 °C, with an humidity of ± 80% RH. Adult sand flies were also provided with a source of sugar meals, renewed daily to prevent the development of bacterial and fungal contaminants. Female flies exposed to infection by *Leishmania* were maintain in this regime.

At the time that the experiments reported herein were undertaken, it was thought that males of the Marajó and Abaetetuba lines were morphologically similar, with a pair of white spots only on the fourth abdominal tergite. More recently, one of us (PW) re-examined males of both lines belonging to the first generations reared in Belo Horizonte and five years after the specimens had been slide mounted in Berlese fluid. The Marajó line was found to consist only of males with a large pair of spots on the fourth abdominal tergite. Males of the Abaetetuba line were found to have a prominant pair of pale spots on the fourth abdominal tergite and a smaller, somewhat darker, pair of spots on the third. As a result of the retrospective studies on the males of the two closed colonies, differences in the susceptibility of females of the two lines has had to be taken into consideration.

Species and/or strains of Leishmania – Experiments were carried out to determine the susceptibility of laboratory-reared female *Lu. longipalpis* to seven species/strains of New World *Leishmania*.

Using the classification system of *Leishmania* proposed by Lainson & Shaw (1987), and the recommended code (Shaw, 1987), the following stocks were used:

IFLA/BR/67/PH8: *Le. (Leishmania) amazonensis*
MHOM/BR/66/H9 (=LV63): *Le. (Viannia) braziliensis*
MHOM/BR/70/M1176: *Le. (V.) guyanensis*
MHOM/BR/70/BH46: *Le. (Le.) chagasi*
MHOM/BR/73/BH121: *Le. (Le.) sp.*
MHOM/BR/71/BH49: *Le. ? sp.*

Some sand flies blood fed on dogs naturally infected by an uncharacterized strain(s) of *Le. (Le.) chagasi* from the municipality of Montes Claros, Minas Gerais, Brazil.

Stocks PH8, and M1176 have been recommended by WHO as references for the respective species. Stock BH46 was isolated from a human case of visceral leishmaniasis discovered in the municipality of Mantena in Minas Gerais. This is, undoubtedly, *Le. chagasi*. Strain BH121 was isolated from a case of human cutaneous leishmaniasis, with multiple lesions, in the Rio Doce Valley in Minas Gerais. These parasites belong to the subgenus *Leishmania* but cannot be identified with any of the characterized stocks. Strain BH49 was isolated from a case of human cutaneous leishmaniasis, most probably in the State of Goiás, but there is confusion regarding both its origin and subsequent history. The material isolated from naturally infected dogs in Montes Claros is still under study.

Exposure of female sand flies to Leishmania *infection* – Four or five days after emergence, female *Lu. longipalpis* were given the opportunity to blood feed directly on leishmanial lesions on hamsters induced by dermatropic species of *Leishmania* or on the skin of hamsters or dogs infected with *Le. chagasi*. The sand flies were dissected four or more days after exposure to infection. Specimens were dissected and examined by the method described by Johnson et al. (1963). The position of flagellates within the digestive tract of each fly was recorded and numbers within each part of the digestive tube were estimated.

TABLE

Numbers of female *Lutzomyia longipalpis* exposed to infection by different species/strains of *Leishmania*, and the numbers in which flagellates developed

Source of blood meal	Species/strain	No. exposed	No. dissected	No. infected	No. not infected
Hamster	*Le. guyanensis*	32	18	18	–
Hamster	*Le. amazonensis*	90	27	10	17
Hamster	MHOM/BR/73/BH121	90	34	3	31
Hamster	*Le. braziliensis*	81	32	3	29
Hamster	MHOM/BR/71/BH49	108	38	–	38
Dog (experimental infection)	*Le. chagasi*	95	46	6	40
Dog (natural infection)	*Le. chagasi*	20	11	–	11

RESULTS

Preliminary statistical analyses of the susceptibility to infection by the various species/strains of *Leishmania* tested revealed no differences between the females of the Marajó and Abaetetuba lines, even though the males of the two lines differ morphologically. For this reason, the observations made on females of the two closed colonies are combined and summarized in the Table.

The proportions of sand flies that development infections varied considerably, depending on the species/strains of parasite to which they had been exposed. No infections were detected in flies exposed to strain BH49 or those that blood fed on dogs naturally infected with *Le. chagasi*. On the other hand, all flies exposed to *Le. guyannensis* developed infections. Between these two extremes, the proportions of flies that development infections varied from 0.09 to 0.37.

Using χ^2 and Fisher's exact tests, there is a statistically significant difference between the susceptibility to infection by *Le. amazonensis* (0.37) and strain BH121 (0.09). The levels of susceptibility to infection by *Le. amazonensis* also differed significantly from those recorded in flies exposed to *Le. chagasi* (0.13) and *Le. braziliensis* (0.09). The other recorded differences in susceptibility were not statistically significant.

The patterns of development of BH121 and PH8 were similar, with promastigotes recorded in the thoracic and abdominal parts of the stomach. With strain M1176, parasites were observed in all parts of the digestive tract from the head to the ileum; those in the hind-gut were predominantly paramastigotes whereas those in the mid-and fore-gut were promastigotes. Parasites of H9 were confined to the pylorus and ileum. Promastigotes of strain BH46 were detected in the thoracic and abdominal portions of the stomach and also in the pylorus.

DISCUSSION

Female *Lu. longipalpis* originating from Lapinha, municipality of Lagoa Santa, Minas Gerais, have been found to be susceptible to infection by strains of *Le. mexicana* from Belize and Brazil (Coelho et al., 1967), to *Le. amazonensis* (Killick-Kendrick et al., 1974; Deane et al., 1986) and to two strains of the *Le. braziliensis* complex (Killick-Kendrick et al., 1977b).

Lainson et al. (1977) recorded that females of a closed colony of *Lu. longipalpis* originating from Morada Nova, State of Ceará, were highly susceptible to infection by *Le. braziliensis, Le. guyanensis, Le. panamensis, L. mexicana, Le. chagasi* and to three strains of *Leishmania* that, at the time, had not been completely characterized. Females of *Lu. longipalpis* of the Morada Nova colony were poor insect hosts for *Sauroleishmania hoogstraali, Le. hertigi, Le. deanei* and *Le. enriettii.* The latter three species of *Leishmania* are not thought to be infective to man; after initial development in

Lu. longipalpis, the parasites were dispelled. However, a strain of parasite belonging to the *Le. mexicana* complex and isolated in the State of Bahia, also failed to develop in females of the Morada Nova colony of *Lu. longipalpis* (Cuba et al., 1984; Vexanat et al., 1985).

The results of the present preliminary studies revealed that female *Lu. longipalpis,* originating from northern Pará, are not uniform in their susceptibility to infection by the species/strains of *Leishmania* tested. Certain factors, which might influence the development of parasites, can be excluded as explanations of the results obtained.

Leishmania guyanensis developed in all the sand flies exposed and surviving long enough to be dissected. Because sand flies had a 1.00 susceptibility rate to one of the strains tested, it is clear that the methods used to expose sand flies to infection were adequate.

Amastigote density in the mammalian host does not seem to be a feature influencing the subsequent development of parasites in the digestive tract of sand flies. Hamsters infected with *Le. guyanensis* or *Le. braziliensis* have small nodular lesions with macrophages containing scanty, and small, amastigotes (Shaw & Lainson, 1976). In comparison, hamsters infected with *Le. amazonensis* or strain BH121 develop prominent swollen lesions with macrophages containing abundance of relatively large amastigotes. By comparing the susceptibility levels of female *Lu. longipalpis* to these four species/strain *(Le. guyanensis* – 1.00; *Le. amazonensis* – 0.37; *Le. braziliensis* – 0.09; strain BH121 – 0.09), it is clear that sand fly susceptibility was not influenced by amastigote density in lesions.

The age of infection in a mammalian host might be a factor contributing to the subsequent development of parasites in the insect host. This has been examined in a sequence of unpublished experiments in which sand flies of the Abaetetuba line were exposed to infection by *Le. guyanensis.* During the first six weeks after infecting a hamster, female sand flies that blood fed at the inoculation site did not develop infections. From the sixth week after infecting the hamster, flagellates developed in an increasing proportion of flies and 100% infection rates were recorded in sand flies that blood feed 14-15 weeks after inoculating the hamster. A similar observation has been reported by Lainson et al. (1990) when female *Lu. longipalpis* (Marajó colony) blood fed on a fox *(Cerdocyon thous)* experimentally infected with *Le. chagasi.* No infections were found in insects that fed on the 8th week, whereas 7.4% of those that feed in the 15th week developed moderate promastigote infections.

Age of infection, the condition of the skin of the mammalian hosts and their immunological condition, either separately or in combination, could account for the failure to infect sand flies that blood fed on three dogs from Montes Claros, Minas Gerais, with naturally acquired *Le. chagasi* infections. All of the dogs were in an advanced stage of disease and died soon after the attempts were made to infect sand flies. In addition to skin changes induced by *Le. chagasi* infection, the dogs were suffering from scabies. The sand flies were reluctant to blood feed and those that did so did not feed to repletion.

Bearing in mind that *Lu. longipalpis* is the only known natural insect host for *Le. chagasi,* the susceptibility level (0.13) of sand flies that blood fed on experimentally infected dogs was surprisingly low. Both colonies of *Lu. longipalpis* used in the experiments originated from foci of visceral leishmaniasis (Chagas et al., 1937, 1938; Lainson et al., 1985) in northern Pará. The strain of parasite was isolated in 1970 from a patient resident in municipality of Mantena, State of Minas Gerais. It is possible that long laboratory maintenance of the strain, without passage through an insect host, induced changes that reduced the parasite's capability to develop in sand flies. A comparative study of susceptibility of *Lu. longipalpis* to strain BH46 and more recent isolates of *Le. chagasi* is planned.

Inherent characteristics of the species/strain of *Leishmania* could be a determining factor influencing sand fly susceptibility to infection. This is the most likely explanation for the failure to infect female *Lu. longipalpis* with strain BH49. This has been a problematic strain ever since it was received and its antecedents are vague, allegedly having been isolated from a human case of cutaneous leishmaniasis, either in the States of Mato Grosso or Goiás. Melo et al. (1987) showed that strain BH49 develops in NNN, LIT and also in the chemically defined MD29 medium of Melo et al. (1985). Cultured

promastigotes failed to infect laboratory animals until they had undergone 202 passages in medium MD29, when flagellates in the logarithmic stage of culture proved to be infective to hamsters (but not to any of the other laboratory animals tested).

Because of the difficulties experienced in infecting laboratory animals with strain BH49, the failure to infect sand flies with these parasites was not surprising. After prolonged culture in MD29, strain BH49 presumably underwent a mutation that rendered promastigote infective to hamsters. The strains has since been maintained by serial inoculation of hamsters with amastigotes, and it can be concluded that the mutant gene is not expressed in the flagellate phase. Failure to infect female *Lu. longipalpis* with strain BH49 suggests that infectability of *Leishmania* to mammalian and insect hosts is governed by different genes (or sets of genes). The genetic composition within the host, whether mammalian or insectan, is a further influencing factor determining infectability in the passage of the parasite from one host to another.

The strain of *Le. amazonensis* used in the present study cannot be distinguished biologically from strain BH121. Biochemically, the two strains differ in the electrophoretic mobility patterns of at least four isoenzymes: ALAT, PGM, G6PDH and MDH (Melo et al., 1987). The statistically significant difference in the susceptibility of female *Lu. longipalpis* to the two parasites suggests that comparative trials such as those recorded herein could provide an additional means for the biological characterization of New World *Leishmania*.

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