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Feeding Preferences of *Lutzomyia longipalpis* (Diptera: Psychodidae), the Sand Fly Vector, for *Leishmania infantum* (Kinetoplastida: Trypanosomatidae)

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Abstract

Leishmania infantum, the causative agent of visceral leishmaniasis (VL) in Brazil, is spread mostly by the bite of the sand fly *Lutzomyia longipalpis* (Lutz & Neiva). We trapped sand flies in endemic neighborhoods near Natal, Brazil, where cases of human and dog VL were documented. Amplification of species-specific cytochrome *b* (*Cyt b*) genes by polymerase chain reaction

revealed that sand flies from rural and periurban areas harbored blood from different sources. The most common source of bloodmeal was human, but blood from dog, chicken, and armadillo was also present. We tested the preference for a source of bloodmeal experimentally by feeding *L. longipalpis* F₁ with blood from different animals. There were significant differences between the proportion of flies engorged and number of eggs laid among flies fed on different sources, varying from 8.4 to 19 ($P < 0.0001$). Blood from guinea pig or horse was best to support sand fly oviposition, but human blood also supported sand fly oviposition well. No sand flies fed on cats, and sand flies feeding on the opossum *Monodelphis domestica* Wagner produced no eggs. These data support the hypothesis that *L. longipalpis* is an eclectic feeder, and humans are an important source of blood for this sand fly species in periurban areas of Brazil.

Keywords

Leishmania infantum; *Lutzomyia longipalpis*; opossum; host preference; sand fly

Lutzomyia longipalpis (Lutz & Neiva) is the major vector involved in the transmission of *Leishmania infantum*, the etiological agent of visceral leishmaniasis (VL) in Latin America (Lainson et al. 1977). Population shift from rural to periurban regions has promoted poor sanitary conditions, creating niches conducive to sand fly replication in settings where humans and dogs reside in proximity (Sutherst 2004). In particular, decomposing organic material in peridomestic animal shelters favors the growth of immature forms of *Lutzomyia* spp., the sand fly that transmits *Leishmania* species to humans (Wijeyaratne et al. 1994). Temperature influences the growth of sand flies (Cross and Hyams 1996), a factor that has gained importance with the increase in global temperature (Hemmer et al. 2007). Overall these changes have resulted in the widening of VL endemic areas in Brazil (Costa et al. 1990, Jeronimo et al. 1994, Marzochi et al. 1994, Nascimento et al. 1996, Albuquerque et al. 2009).

VL was previously acquired predominantly in rural areas, but as Brazil became more urbanized, the disease shifted to perimetropolitan areas of major cities. Currently most Brazilian states report autochthonous cases of VL, which was previously limited to northern and northeastern states, but it has now been reported as far south as northern Argentina (Costa et al. 1990, Jeronimo et al. 1994, Marzochi et al. 1994, Nascimento et al. 1996, Salomon et al. 2008, Albuquerque et al. 2009). The pattern of VL in Natal, Rio Grande do Norte, northeast Brazil, provides an example of the periurbanization of leishmaniasis. An outbreak of VL was first reported in the neighborhoods around the periphery of Natal beginning in early 1990s, and has continued throughout the past two decades (Jeronimo et al. 1994, 2004; Lima et al. 2012). Sand flies capable of transmitting *Leishmania* spp. were found in trapping studies throughout most geographic areas of the state of Rio Grande do Norte (Ximenes et al. 1999).

The incidence of VL cases in the Natal vicinity has occurred in cycles, similar to the pattern of VL observed in other endemic areas of Brazil (Carneiro et al. 2007, Malaviya et al. 2011). This has been attributed to the proximity of humans to infected reservoir hosts and sand fly vectors. Domestic dogs and wild foxes have been cited as the main reservoirs for *L.*

infantum in the peridomestic or sylvatic environments, respectively, in many areas of the world (Lainson and Rangel 2005, Marty et al. 2007). Nonetheless, fluctuations in the *L. longipalpis* population and incidence of VL were associated with the presence of the marsupial *Didelphis albiventris* in peridomestic areas of Jacobina, Bahia, northeast Brazil (Sherlock 1996). Studies conducted in the state of Rio Grande do Norte showed that the risk of human infection with *Leishmania* spp. was associated with proximity to animal shelters and proximity of households to any animals, including dogs (Ximenes et al. 2007). Many of the animals associated with sites of human VL transmission (e.g., horses and chickens) are not known to sustain *Leishmania* spp. infections. Most likely, these serve to attract sand flies to breeding grounds in peridomestic areas (Queiroz et al. 2009). Given that proximity between human dwellings and animal reservoirs is critical to the transmission of leishmaniasis, the feeding preference of sand flies for bloodmeals from different animals is also a critical factor influencing disease spread. The only way to discern the animals that could serve as true reservoirs of disease is to determine the animal source of blood taken up during a sand fly bloodmeal.

The objective of this study was to assess the effects of blood source and environment on the viability and propagation of sand flies collected at sites where human VL is actively transmitted. To pursue this goal, first, we observed sand flies captured in nature for the source of bloodmeal. Second, we examined their preferred source of bloodmeal and the effects of ambient temperature on *L. longipalpis* development. Sand fly development was supported well by several blood sources, including human, cavy, horse, dog, and chicken.

Materials and Methods

Characterization of the Study Areas

The state of Rio Grande do Norte is endemic for both human and dog VL (see map in Fig. 1; Jeronimo et al. 1994). Study areas included the periurban and rural areas in proximity of Natal. Our working definition of a periurban site is an area in which a business district (group of stores) can be reached by walking for less than a kilometer or by public transportation. Thousands of people have recently settled in the periurban areas in the vicinity of Natal, with a current population of 500,000 people. Houses tend to be closer, but still can have yards, often with fruit trees and animals (generally dogs and chickens). Our working definition of a rural location is a region where houses are located in small farms with animal shelters (chicken, pigs, horses, and donkeys) in proximity to the main house. Rural areas are ≈20–30 km from Natal. We showed in previous studies of both periurban and rural areas near Natal that the incidence of *L. infantum* infection in humans and dogs is high (Jeronimo et al. 1994, 2004). The localities were chosen because there was documented history of human and dog VL in the neighborhood.

Capture and Identification of Sand Flies

Sand flies were captured in both the rural and periurban areas of Natal. The study period was from August 2007 to June 2008. Sand flies were captured with light CDC traps (Hausherr's Machine Works, Toms River, NJ), which were set at 5:30 p.m. and removed the next day at 6:00 a.m. The time of sunrise varies from 4:45 to 5:00 a.m., whereas sunset in the area

varies from 5:30 to 6:00 p.m. Traps were placed near the location where horses, armadillo, chickens, and dogs spent the night. The soil from both rural and periurban areas tend to be sandy, as localities were 20–30 km from the Atlantic Ocean. Sand flies were also captured using a manual device from the skin of horses in the vicinity, or to aspirate flies from walls and furniture inside the houses of subjects with VL, $\approx$ 40–60 min was spent inside the household for fly capture. Flies that were captured using the manual device were brought to the laboratory, and the females were potted individually. They were screened daily, but the ones that died were removed from the pot and used for species identification. Male and female sand flies were identified by microscopy according to the Young and Duncan classification (Young and Duncan 1994). Female sand flies were kept in the BOD incubator (model 147, FANEM, São Paulo, Brazil) or immediately submitted for DNA extraction.

Influence of Animal Blood Source on *L. longipalpis* on Oviposition Rate

In a laboratory setting, female sand flies from the F₁ generation were fed by exposure to different animals, which were sedated with ketamine and xylazine, 1:1 ratio. Approximately 100 female *L. longipalpis* were used for each source of animal blood. The oviposition of flies fed on cavy (*Galea spixii*), guinea pig (*Cavia porcellus*), horse (*Equus caballus*), two species of opossum (*Monodelphis domestica* and *Didelphis albiventris*), common marmoset (*Callithrix jacchus*), cat (*Felis catus*), dog (*Canis familiaris*), chicken (*Gallus gallus*), and hamster (*Mesocricetus auratus*) was examined. The oviposition of the flies captured on horse was examined in a similar manner; however, horses lived in the peridomiciliary in a neighborhood in the proximity of Natal. Sixty-six female F₁ sand flies were fed with human blood through an artificial feeder using a chick skin membrane. The feeder is made of a glass, which has a 14-mm body, and a top arm and through tub, which passes a rubber tube, where water circulates at 37°C. The blood (2–4 ml) is kept at 37°C and is drained to the membrane. Individual engorged sand flies were transferred to plastic containers coated with plaster to determine the oviposition rate. The percentage of engorged female sand flies was determined. After exposure, sand flies were examined against light to better assess the distention of the abdomen. Females that died or laid eggs were removed and stored in alcohol for subsequent species identification. The oviposition rate was determined considering the number of eggs laid by the number of engorged sand flies.

Effect of Temperature on Sand Fly Biological Life Cycle

Female *L. longipalpis* sand flies from the F₁ generation after isolation from the field were maintained in a BOD incubator at temperatures 22, 25, 28, 32, or 35°C, under a daily controlled photoperiod of 12:12 (L:D) h cycle and humidity varying between 80 and 90%. Sand flies were fed on anesthetized hamsters and with a sucrose solution (10%).

Determination of Blood Source by Polymerase Chain Reaction

DNA was extracted from individual female sand flies using phenol–chloroform extraction method as described by Michalsky et al. (2002). A mean of 14 µg of DNA was extracted per fly. One hundred nanograms of sand fly DNA was used to amplify the *Cyt b* gene. The *Cyt b* sequences for the different animals were retrieved from GenBank. Primers were designed for dog, chicken, horse, armadillo, and human (Supp. Table 1 [online only]). The reaction

was carried on with 2.5 µl of 10× NEB buffer; 1 µl of 10 × MdNTPs (Bioline USA Inc., Taunton, MA); 0.5 µl of each primer, 0.05 µl of AmpliTaq 5 U/µl (Applied Biosystems, Foster City, CA), 15.45 µl of water, and 5 µl of DNA (80 ng total) to a total 25 µl/reaction. DNA fragments were amplified using an initial denaturing step for 2 min at 94°C, followed by 30 cycles at 94°C for 30 s, 58°C for 30 s, 68°C for 2 min, and a final extension step at 68°C for 5 min. As positive controls for primers, DNA was isolated from the blood of the animals studied (*Homo sapiens*, *Canis familiaris*, *Equus caballus*, *Gallus gallus*, and *Euphractus sexcintus*). Polymerase chain reactions (PCRs) were set up using all primer sets to show that amplification of *Cyt b* occurred only when using primers hybridizing to the sequences from the corresponding animal species.

Statistical Analysis

The maximum likelihood chi-square test (ML- χ^2) was used to test the hypothesis of association between categorical variables. The median days of sand fly development at different temperatures was estimated using the Kruskal–Wallis (KW) test, with the following equation:

$$\text{Days, KW, } H(4, 114) = 97.00, P = 0.0000; F(4, 109) = 218.41, P < 0.0001$$

where KW is the results of the Kruskal–Wallis statistics (comparison of the median with 4 and 114 df), *P* value, and *F* is the Fisher statistic of the ANOVA test of the comparison of the mean, with 4 and 109 df. The mean of the days for each condition was validated by the parametric *F*-ANOVA test. The method of the minimal ordinary squares was used to adjust the polynomials between the development rate and temperature.

Ethical Considerations

The human subjects protocol was reviewed and approved by the Universidade Federal do Rio Grande do Norte Ethical Committee (CEP–UFRN 172-06) and by the Brazilian National Ethical Commission (CONEP 13745). The Ethical approval number is CAAE 0139.0.051.069-06 (www.sisnep.gov.br).

Results

Abundance and Diversity of Sand Fly Species in the Endemic Area for VL

In total, 1,768 sand flies were collected in the periurban and rural sites, with a mean of 16 flies per trap collected from the periurban sites and 14 sand flies from the rural sites. For both sites, sand flies were collected from the peridomicile, in proximity to where animals were housed, and inside the household. Of these, 1,538 were males and 230 were females for an overall ratio of 6:1. Among the males, four different species of the *Lutzomyia* were found; 86.0% were *L. longipalpis*, 10.5% were *Lutzomyia evandroi* (Costa Lima & Antunes), 3.2% were *Lutzomyia lenti* Mangabeira, and 0.3% were *Lutzomyia whitmani* (Antunes&Coutinho) (Table 1). There was a significant difference between the distribution of sand fly species found in the rural and periurban areas ($P < 0.0001$). *L. lenti* was found only in the rural area (Table 1). Among the sand flies collected, the only one known species competent to serve as a vector for *L. infantum* in the region is *L. longipalpis*.

Identification of Sand Fly Bloodmeal Sources by Using PCR for the *Cyt b* Gene

The 230 females captured in a natural habitat included 136 from rural areas and 94 from periurban areas. In all, 35.2% (81 of 230) were visibly engorged with blood. Using each of the five primer sets listed in Supp. Table 1 (online only), we used PCR to determine the host source of the blood. A PCR fragment of 151 bp corresponding to the *Cyt b* gene of the six-banded armadillo *E. sexcintus* was amplified from all of the 69 engorged sand flies collected in the rural region. In contrast, a PCR band corresponding to the *H. sapiens Cyt b* gene was amplified from all 12 of the engorged sand flies collected in the periurban area. DNA from the remaining 149 (unengorged) female sand flies failed to amplify a band corresponding to *Cyt b* using any of the primer sets listed in Supp. Table 1 (online only). Observation of females that were not visibly engorged suggests that these females had not recently taken a bloodmeal. DNA sequencing verified that the amplified PCR fragments corresponded to the sequences of *E. sexcintus* or *H. sapiens Cyt b* genes as predicted (Fig. 2).

Source of Animal Blood Affects the *L. longipalpis* Oviposition Rate

The ability of different vertebrates to serve as a source of blood for sand flies was determined experimentally by offering F₁ generation female sand flies a bloodmeal on anesthetized animals or on human blood across an artificial membrane. The number of flies exposed to animals is indicated in Table 2. The data must be interpreted in light of the fact that all feedings were performed on anesthetized animals with the exception of human blood, in which case feeding occurred through an artificial membrane. Thus, the effects of kairomones, CO₂, and other factors may have led to differences between the numbers of flies feeding on human blood vs. other animals. Even between the animal-fed flies, we observed significant variability in sand fly engorgement and the number of eggs laid (ML- χ^2 , 378.0674; df = 9; $P < 0.0001$; Table 2). For instance, there was no difference between sand fly engorgement in human vs. horse blood ($P < 0.63756$), but there were significant differences between human and cavy blood ($P < 0.0001$), between dog and opossum blood ($P = 0.02614$), and between chicken and marmoset blood ($P < 0.0001$). The mean number of eggs laid per sand fly varied from 8.4 to 19 eggs. Despite feeding through a membrane, human blood supported sand fly oviposition well. Sand flies fed well on most animals tested, although none of the sand flies fed on cats, and there were no eggs observed from sand flies that fed on the opossum *M. domestica*.

Influence of Temperature on *L. longipalpis* Life Cycle

We analyzed the influence of ambient temperature on sand fly colonies initiated from flies collected in endemic settings. At 22°C, the number of eggs laid was 44, larval stage lasted 38 d, and the pupae stage lasted 15 d, and the total cycle duration was 79 d. At 25°C, the number of eggs was 40, egg stage lasted 5–11 d, larvae stage 17–20 d, pupae stage 10 d, with a total cycle duration of 32 to 40 d. At 28°C, the number of eggs was 35, the larval stage lasted 21 d, and the pupae stage lasted 9 d, with a total cycle duration of 51 d. At 32°C, the mean number of eggs was 10 eggs per fly; the larval stage lasted 11 d, the pupae stage lasted 6 d, and the total life cycle duration was 32 d. Eggs were laid at 35°C by sand flies, but no hatching occurred. Statistical comparisons revealed significant association between cycle duration with temperature (Kruskal–Wallis and ANOVA tests), showing a strong

polynomial relationship between the number of days of the complete life cycle of the sand flies and the temperature ($P < 0.0001$; Fig. 3).

Discussion

The distribution of VL in South America has changed substantially over the past 30 yr (Peterson and Shaw 2003). VL has expanded to subtropical countries in South and Central America. *L. longipalpis*, the main insect vector transmitting *L. infantum* in the Americas, is distributed widely throughout South American countries, including Brazil, northern Argentina, and Paraguay (Lainson and Shaw 1972, Rotureau 2006, Salomon et al. 2009). The purpose of this study was to define the relationship between insect vectors and mammalian hosts in settings with anthrophilic transmission of *Leishmania* spp. Such information will yield important information regarding potential reservoirs for human infections with the *Leishmania* species.

Consistent with observations previously made for *L. evandroi* (Ximenes et al. 1999, de Melo Ximenes et al. 2001), the biological life cycle of *L. longipalpis* F₁ generation collected from periurban areas of Natal was 52 d (Ximenes et al. 1999). As expected, the sand fly life cycle was dependent on ambient temperature, with both the length of life cycle stages and the numbers of ova produced being associated with temperature. Previously, similar observations were shown for *P. papatasi* (Chelbi and Zhioua 2007). This demonstrates the critical influence of environment on the disease vector. We previously showed that the *L. longipalpis* population density in Rio Grande do Norte varies with season, variation in temperature, wind flow, and humidity (Ximenes et al. 2006). The current study documents, in a laboratory setting, the effects of ambient temperature on the life cycle of *L. longipalpis* from northeast Brazil (Lainson and Rangel 2005, Afonso et al. 2012, Costa et al. 2013).

As a prelude to defining the reservoirs for *L. infantum*, the ability of blood from different animals to support the sand fly development was examined. Experimental feeding on 9 types of anesthetized animals documented their relative attraction for sand flies taking a bloodmeal. Because sand flies were exposed to human blood through an artificial membrane, a direct comparison could not be made with other animal blood sources. Furthermore, all experimental laboratory models are imperfect models of attraction for sand flies fed in a natural environment. Nonetheless, our data showed that different blood sources were able to support the entire developmental cycle of sand flies. Furthermore, comparisons between feeding behavior on different anesthetized animals raised hypotheses about the relative likelihood of flies to obtain bloodmeals from different animals found in the VL-endemic area. In addition, based on our results, we can state that human blood can sustain the sand fly life cycle. Between the anesthetized hosts that can be directly compared, the animals that best supported sand fly development and fecundity were cavy and *D. albiventris* opossums. Cats and a different species of opossum (*M. domestica*) did not support sand fly development at all, and dogs were intermediate in their abilities to efficiently support the sand fly life cycle. These observations raise hypotheses about animals that could serve as potential reservoir hosts for *Leishmania* species (Humberg et al. 2012). Recent studies have found evidence of VL in cats (Maia and Campino 2011, Vides JP et al. 2011).

Eighty six percent of sand flies captured in natural habitats in the perimetropolitan area of Natal were *L. longipalpis*, and 11% were *L. evandroi*. Consistently, in this study the most highly prevalent sand fly species in houses in the vicinity of Natal was also *L. longipalpis* followed by *L. evandroi*, *L. lenti*, and *L. whitmani*. Although *L. longipalpis* and *L. evandroi* show great similarity in behavior, life cycle, and geographic distribution (Ximenes et al. 1999, 2007), the former transmits *L. infantum*, whereas there is no evidence that *L. evandroi* can serve as a vector for *Leishmania* species either in natural or experimental conditions. In contrast, *L. whitmani*, which was captured in both rural and periurban regions, has been associated with transmission of cutaneous leishmaniasis due to *Leishmania braziliensis* (Bezerra and Teixeira 2001). Sand fly abundance is closely correlated with poor sanitary conditions and with the presence of animals in the peridomestic areas. Animal shelters in peridomestic areas probably favor *L. longipalpis* adaptation in domiciliary environment. Therefore, many domesticated animals have become an important factor favoring VL dissemination in periurban areas of Natal, although not all animal hosts can sustain the sand fly life cycle. Despite the ability of several hosts to support the biological cycle of *L. longipalpis*, PCR studies using species-specific primers for *Cyt b* genes indicated that sand flies captured both inside households and in peridomestic areas had apparently fed primarily on humans and armadillos.

Studies of *Lutzomyia* feeding preference show a wide spectrum of hosts that can sustain their life cycle. *L. longipalpis* captured in a natural environment feed most preferably on birds and rodents, but can also feed on humans, opossums, oxen, horses, and dogs. Therefore, any of these various bloodmeal sources that could sustain a *Leishmania* infection could serve as a reservoir host for *Leishmania* spp. There is some literature available suggesting that humans are the preferred source of sand fly bloodmeal (Quinnell et al. 1992). In Colombia, the marsupial *Didelphis marsupialis* serves as the main wild reservoir for *Leishmania* spp. and other trypanosomatids (Corredor et al. 1989, Yoshida et al. 1979). In experimental conditions, it was shown that opossums were more susceptible to VL than armadillos or ferrets, although any of these animals could sustain *Leishmania* development (Travi et al. 1998). Some authors consider *L. longipalpis* as an anthropophilic species (Quinnell et al. 1992). Our observation that human blood was the exclusive source of bloodmeal for sand flies collected from periurban areas supports this hypothesis.

The most common source of bloodmeal for sand flies collected in periurban sites was human blood, as shown by the amplification of *H. sapiens Cyt b*. *L. longipalpis* is by nature anthropophilic. The high rate of sand fly feeding on humans in periurban regions, and the high rates of *L. infantum* infection in these human-fed flies (data not shown) create a situation optimal for transmission of *L. infantum* in periurban areas of Brazil.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Map of South America indicating Brazil and the state of Rio Grande do Norte.

B.

<i>Euphractus sexcintus</i>	CGTTCATTGACTTACCCACCCCAACAAGCATCTCA	35
<i>Homo sapiens</i>	CATTCATCGACCTCCCCACCCCATCCAACATCTCC	35
<i>Euphractus sexcintus</i>	TCATGATGAAATTTTGGTTCACTACTAGGCATCTG	70
<i>Homo sapiens</i>	GCATGATGAAACTTCGGCTCACTCCTTGGCGCCTG	70
<i>Euphractus sexcintus</i>	CCTAGTTATCCAAATTTTAACAGGCCTATTTCTAG	105
<i>Homo sapiens</i>	CCTGATCCTCCAAATCACCACAGGACTATTCCTAG	105

Fig. 2.

A) Agarose gel electrophoresis of amplified PCR products of *Cyt b*. Lanes 1 and 12, Marker (100-pb ladder); Lanes 2 and 8, Blank; Lane 3, Positive control to gene *Cyt b* from *H. sapiens* (335 pb); Lanes 4 and 11, Sample of DNA from unengorged sand flies; Lanes 5 and 6, PCR product amplified from engorged sand fly with human blood, *Cyt b* from *H. sapiens* (335 pb); Lane 7, Positive control to *Cyt b* gene from *E. sexcintus* (151 pb); Lanes 9 and 10, PCR product amplified from engorged sand fly with armadillo blood, *E. sexcintus* (151 pb).
 B) Alignment of one DNA sequence obtained from the PCR fragment shown in Fig. 2A.

Fig. 3.

The mean number of days for the entire life cycle of *L. longipalpis*, considering from eggs to adult, at different temperatures is represented in a box-plot graph. The temperatures varied from 22 to 35°C. No eggs were laid at 35°C.

Table 1

Species and infection rates of sand flies collected in rural and periurban areas of metropolitan Natal

Sand fly species	Geographic areas (N, 5)		
	Rural	Periurban	Total
<i>L. longipalpis</i>	519 (73.9%)*	805 (96.3%)*	1,324 (86.1%)
<i>L. evandroi</i>	133 (18.9%)*	28 (3.3%)*	161 (10.5%)
<i>L. lenti</i>	49 (7.0%)*	0 (0.0%)*	49 (3.2%)
<i>L. whitmani</i>	1 (0.1%)	3 (0.4%)	4 (0.3%)
Total	702 (100.0%)	836 (100.0%)	1,538 (100.0%)

The proportion of different *Lutzomyia* (sand fly) species collected in rural or periurban regions, identified morphologically, are enumerated. The proportions of DNA samples extracted from *L. longipalpis*, the vector for *L. infantum* infection, from which *Leishmania* spp. DNA was amplified is enumerated, from rural or periurban regions.

* $P < 0.0001$; association test comparing sand fly species in rural versus periurban regions, χ^2 .

Table 2

The percentage of engorged *L. longipalpis* and their oviposition rate when fed on blood from different hosts

Animal bloodmeal source (common name)	<i>L. longipalpis</i> engorgement		Total	No. of experimental exposures	Mean no. of eggs/ engorged female	Days from egg to adult (*) (temp, Celsius)
	N	%				
<i>H. sapiens</i> (human) ^a	65	98.5	66	1	14	40 (25°)
<i>C. porcellus</i> (guinea pig)	97	96.0	101	6	19	28 (25°)
<i>E. caballus</i> (horse)	97	97.0	100	1	19	32 (25°)
<i>C. familiaris</i> (dog)	95	85.6	111	6	10.3	34 (25°)
<i>G. spixii</i> (cavy)	110	80.1	136	6	26	31 (25°)
<i>G. gallus</i> (chicken)	68	71.6	95	4	14	29.2 (25°)
<i>D. albiventris</i> (opossum)	67	71.3	94	1	8.4	25 (25°)
<i>M. domestica</i> (opossum)	44	42.7	103	3	0	N/A ^b (25°)
<i>C. jacchus</i> (common marmoset)	51	36.2	141	2	15	33.8 (25°)
<i>F. catus</i> (cat)	50	0%	50	3	0	0 (25°)

A mean of 997 sand flies were exposed to different blood sources for 1 h each, after which the number of engorged sand flies was counted. Engorged flies were subsequently transferred to plastic containers and the number of eggs laid per sand fly was determined. The number of days required for development from eggs to adult is also listed. The table shows significant differences between the oviposition rates and the numbers of eggs laid with different bloodmeals (ML- χ^2 , 378.0674; df = 9; $P < 0.0001$).

^a An artificial feeder was used to feed sand flies on human blood. Thus, numbers are not directly comparable between this row and other rows of the table, in which flies were fed on anesthetized animals.

^b N/A, not applicable; no eggs were produced.