Feeding Patterns of *Haemagogus janthinomys* (Diptera: Culicidae) in Different Regions of Brazil

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ABSTRACT New data on the feeding patterns of *Haemagogus* (*Haemagogus*) *janthinomys* Dyar from different geographical regions of Brazil, by using the precipitin test as the bloodmeal-identifying tool, are presented. The following antisera were used: bird, dog, human, rodent, cattle, horse, and opossum. The origins of 287 bloodmeals were identified, whereas 33 specimens were negative to the antiserums tested. Among the reactive specimens, 174 (60.6%) fed on only one food source, of which 35.1% originated from birds, 19.5% from rodents, 12.6% from humans, 10.3% from cattle, 10.3% from opossums, 7.5% from dogs, and 4.6% from horses. One hundred six (37.0%) mosquitoes fed on two sources, of which the most common combinations were bird + rodent (16.0%), bird + human (10.4%), and horse + human (9.4%). Seven (2.4%) mosquitoes fed on three different hosts. Our results suggest that *Hg. janthinomys* is more eclectic and opportunist than previously known in relation to its hosts and that such patterns are probably highly adaptive to a temporally and spatially variable environment.

KEY WORDS food habitats, mosquito, insect vectors, *Haemagogus janthinomys*

Haemagogus janthinomys Dyar is a forest-dwelling mosquito with diurnal activity during the hottest hours of the day. Although it is acrodendrophilous, it is capable of feeding on blood at ground level in deforested areas. However, this behavioral pattern may vary in different regions. In French Guiana, for example, it has been observed that activity at ground level may occur at different times over the course of the year (Pajot et al. 1985). Among the species already identified as potential transmitters of the virus that causes forest yellow fever, *Hg. janthinomys* is the principal vector in the Americas (Vasconcelos et al. 1992, Dégallier et al. 1998). Its geographical distribution coincides with the areas where yellow fever is endemic. Its vectorial competence also is demonstrated by its high susceptibility to experimental infections, becoming infected even with low viral titers. In periods of epidemics in the natural environment, samples from the crowns of trees are frequently found with high rates of infection (Vasconcelos 2003). Strong consideration also must be given to the report by Mondet et al. (2002) of transovarian transmission in *Hg. janthinomys*, which had only previously been observed for *Haemagogus equinus* Theobald, under experimental conditions.

Knowledge of the blood-feeding patterns of mosquitoes, through use of the precipitin test, provides important support when estimating the risk of resurgence of forest yellow fever in regions of Brazil that are presently considered to be unaffected.

Materials and Methods

The mosquitoes analyzed came from four different geographical regions of Brazil. For the southeastern region, samples came from Rio de Janeiro, municipality of Duque de Caxias (22° 47′ S and 43° 18′ W); and from Minas Gerais, municipalities of Ituiutaba (18° 58′ S and 49° 27′ W), Patrocínio (18° 56′ S and 46° 59′ W), Uberaba (19° 44′ S and 47° 55′ W), Monte Carmelo (18° 43′ S and 47° 29′ W), and Além Paraíba (21° 53′ S and 42° 42′ W). For the northeastern region, samples came from Alagoas, municipality of Atalaia (9° 30′ S and 36° 01′ W); and from Bahia, municipalities of Canavieiras (15° 40′ S and 38° 56′ W) and Ilhéus (14° 27′ S and 39° 02′ W). For the west central region, samples came from Goiás, municipality of Niquelândia (14° 28′ S and 48° 27′ W). For the northern region, samples came from Tocantins, municipality of Pedro Afonso (8° 58′ S and 48° 10′ W) (Fig. 1). The specimens of *Hg. janthinomys* from the municipalities of Duque de Caxias, Ituiutaba, Patrocínio, Uberaba, Monte Carmelo, Além Paraíba (southeastern region), Atalaia, and Canavieiras (northeastern region) and Pedro Afonso (northern region) that were used came from dried, pinned specimens in the entomological

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Fig. 1. Location of the collection sites in Brazil.

collection of the Department of Entomology of the Oswaldo Cruz Institute (Rio de Janeiro, Brazil). The other specimens had recently been collected from the natural environment.

The regions studied are natural habitats, consisting of areas of dense forests, riverbank forests, and savanna. In the southeastern and northeastern regions, in well-preserved Atlantic Forest environments, we found two well-defined tree strata. The forest is formed by large-sized pioneer trees, which are adorned with epiphytes and bromeliads, thereby giving a dense appearance to the plant cover. The ground is covered by a thick layer of leaf litter and burrows made by rodents and marsupials are widespread. In regions that have many rivers and waterfalls, the characteristics of riverbank forests are frequently found, as remainders of the native forest, with trees of up to 20 m in height on which there is also significant presence of bromeliads. In the west central region, the vegetation of the savanna biome is very diversified and ranges from open field types to relatively dense forest types known as dense savanna. In general, we can identify two strata in the vegetation that is typical of the savanna: the woody stratum, made up of trees and bushes, and the herbaceous stratum, made up of grasses and small bushes. The northern region is practically all made up of typical Amazon forest. The tree settings can be divided into three principal forest types: swamp, floodplain, and terra firma forests. These areas are normally delimited by the amount of rainfall and the regime of regional flooding. Thus, swamp forest covers the areas that are permanently covered with water, floodplain forest covers the areas that are seasonally flooded, and terra firma forest is characterized by dense vegetation and large-sized trees, generally not flooded.

The mosquitoes were caught using an entomological net with an opening of 30 cm in diameter, a length of 60 cm, and a short handle. The specimens were packed in polyethylene cages, labeled according to locality, and stored in cool isothermic boxes for live transportation to the laboratory. Subsequently, the mosquitoes were anesthetized by exposure to chloroform vapor and kept in a freezer at −4°C, to interrupt the digestive process. In the laboratory, the specimens were identified using the key of Arnell (1973) and then triturated in test tubes containing saline solution (0.85% NaCl), for the application of the precipitin technique following Siqueira (1960), with some modifications according to Lorosa et al. (1998b). The preparation of the antisera and the evaluation of the titration and specificity also were according to Siqueira (1960). The following antisera, with their respective titers, were used: bird 1:10,000 (*Gallus*), dog 1:15,000

Table 1. Results of precipitin tests of *Hg. janthinomys* females, from different regions of Brazil

Food source location	Bird		Rodent		Human		Opossum		Cattle		Dog		Horse		No reaction		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Atalaia	9	22.5	7	17.5	7	17.5	3	7.5	4	10.0	1	2.5	4	10.0	5	12.5	40	9.2
Canavieiras	10	27.8	6	16.7	7	19.4	5	13.9	2	5.6	2	5.6			4	11.1	36	8.3
Ilhéus	52	25.4	36	17.6	32	15.6	16	7.8	18	8.8	21	10.2	17	8.3	13	6.3	205	47.3
Niquelândia	4	15.4	5	19.2	6	23.1	3	11.5	2	7.7	4	15.4			2	7.7	26	6.0
Além Paraiba	3	30.0	2	20.0	2	20.0	2	20.0					1	10.0			10	2.3
Ituiutaba	6	33.3	4	22.2	3	16.7	1	5.6	1	5.6			2	11.1	1	5.6	18	4.2
Monte Carmelo	2	33.3	2	33.3			1	16.7	1	16.7							6	1.4
Patrocínio	1	5.6	4	22.2	1	5.6	4	22.2	2	11.1	2	11.1	1	5.6	3	16.7	18	4.2
Uberaba	6	28.6	5	23.8	2	9.5	2	9.5	3	14.3	1	4.8	1	4.8	1	4.8	21	4.8
Duque de Caxias	10	25.6	7	17.9	7	17.9	4	10.3	3	7.7	2	5.1	3	7.7	3	7.7	39	9.0
Pedro Afonso	4	28.6	3	21.4	1	7.1	2	14.3	1	7.1	1	7.1	1	7.1	1	7.1	14	3.2
Total	107	24.7	81	18.7	68	15.7	43	9.9	37	8.5	34	7.9	30	6.9	33	7.6	433	100.0

n is number of specimens; % is percentage of the total number of specimens analyzed.

(*Canis*), human 1:10,000 (*Homo*), rodent 1:15,000 (*Rattus*), cattle 1:15,000 (*Bos*), horse 1:15,000 (*Equus*), and opossum 1:15,000 (*Didelphis*).

The feeding patterns of *Hg. janthinomys* were analyzed by means of the following statistical tests: analysis of variance (ANOVA), Tukey test (minimum significant difference), and Duncan test (minimum significant amplitude). In these analyses, the following variables were taken into account: types of host, regions sampled, and localities studied. The ANOVA was based on breaking down the total variation found within the series of observations into known factors relating to the biology and behavior of *Hg. janthinomys*, and one factor that was unknown (the host's feeding preferences) or that could not be controlled under natural conditions.

Our hypothesis was that *Hg. janthinomys* was primarily anthropophilic. Because the numbers of repetitions differed among the treatments, the data were transformed by squaring the totals for each treatment and dividing the result by the corresponding number of repetitions before summing, according to the following formula:

$SQ = (T_1^2/n_1 + T_2^2/n_2 + .. T_K^2) - FC$, where SQ is squared total, T is treatment, n is number of cases, k is last case, and FC is correction factor (Pimentel 2002).

Results

Among the stomach contents of 320 adult females of *Hg. janthinomys*, 287 (89.7%) reacted to the precipitin test and 33 (10.3%) did not react to any antiserum. The results obtained are presented in Table 1. Among the positive specimens, 174 (60.6%) reacted to one source of blood: 61 (35.1%) to bird, 34 (19.5%) to rodent, 22 (12.6%) to human, 18 (18.0%) to cattle, 18 (10.3%) to opossum, and 13 (12.0%) to dog blood. Among the mixed reactions, most were positive to two sources (106 specimens; 36.9%), and seven specimens (2.4%) were positive to three sources (Table 2). The most frequent combinations were bird + rodent (17 cases; 16.0%), followed by opossum + rodent (14 cases; 13.2%), bird + human (11 cases; 10.4%), horse + human (10 cases; 9.4%), and cattle + human (seven cases; 6.6%). None of the mixed samples reacted to the dog + opossum antiserum (Table 2). Among the populations of *Hg. janthinomys* tested, the sample from the municipality of Ituiutaba was the one that presented the greatest positivity to bird antiserum, with 33.3%. However, the reactive specimens from the municipality of Niquelândia had the greatest positivity to human blood, with an anthropophily rate of 23.1% of the total examined, followed by 19.2% positivity to rodent blood (Table 1). The mosquitoes from the municipality of Monte Carmelo showed a high degree of zoophilia: bird (33.3%), rodent (33.3%), cattle (16.7%), and opossum (16.7%), as did those from the municipality of Patrocínio: rodent (22.2%), opossum (22.2%), cattle (11.1%), dog (11.1%), bird (5.6%), horse (5.6%),

Table 2. Double and triple reactions to food sources of *Hg. janthinomys* females from different regions of Brazil

Food source	*n*	%
Bird + cattle	4	3.8
Bird + dog	6	5.7
Bird + horse	1	0.9
Bird +opossum	4	3.8
Bird + human	11	10.4
Bird + rodent	17	16.0
Cattle + dog	2	1.9
Cattle + horse	4	3.8
Cattle + opossum	1	0.9
Cattle + human	7	6.6
Cattle + rodent	4	3.8
Dog + horse	1	0.9
Dog + human	5	4.7
Dog + rodent	2	1.9
Horse + opossum	2	1.9
Horse + human	10	9.4
Horse + rodent	2	1.9
Opossum + human	4	3.8
Opossum + rodent	14	13.2
Human + rodent	5	4.7
Bird + dog + human	1	14.3
Bird + dog + rodent	1	14.3
Bird + opossum + rodent	1	14.3
Cattle + dog + human	1	14.3
Cattle + dog + rodent	1	14.3
Cattle + horse + human	1	14.3
Dog + horse + human	1	14.3

and human (5.6%). From the mosquitoes collected in the municipality of Monte Carmelo, no positive reaction to human antiserum was registered.

In all the samples from the localities studied, horse antiserum presented a positive reaction, with the exceptions of the municipalities of Monte Carmelo, Canavieiras, and Niquelândia. A positive reaction to human antiserum was frequently found in most samples, except for those from the municipality of Monte Carmelo, which presented greater rates for bird antiserum (33.3%) and rodent antiserum (33.3%). Among the samples collected in the municipality of Patrocínio, the greatest positivity was for rodent antiserum, with a positive reaction rate of 22.2% (Table 1).

The statistical analyses that we performed brought up some important points. There was no manifest food preference; the greater frequency of positivity to bird antiserum was by chance. The greatest blood-feeding activity took place in the municipality of Ilhéus. Excluding this locality, the differences in feeding patterns were shown with three groupings: birds, rodents + humans + cattle, and dogs + opossums. As expected, the horse host was found to be a chance food source. We did not observe any significant differences in blood-feeding activities for *Hg. janthinomys* between the states of Alagoas, Goiás, Minas Gerais, Rio de Janeiro, and Tocantins. However, this group differed from the observations in Bahia State. Nonetheless, if the municipality of Ilhéus is again excluded, the differences in blood-feeding activity were not significant between the six states investigated. The population of *Hg. janthinomys* in the municipality of Ilhéus presented a differentiated blood-feeding behavior, with birds as the primary host, rodents as the secondary hosts, and the group of humans + dogs + cattle as the tertiary host, whereas horses and opossums were used separately as chance or alternative hosts.

Discussion

Hg. janthinomys is frequently defined as an acrodendrophilous species that feeds mainly on primates. However, in the current study, we observed feeding behavior that was opportunistic and eclectic in relation to food sources, with significant variation in host types. Dajoz (1983) reported that the food regime for a species is rarely constant over the whole year in all locations and that this variation may be influenced by the environmental characteristics.

In a study carried out by Guimarães et al. (1987), in areas of the Atlantic Forest in the Serra dos Órgãos National Park, Rio de Janeiro State, most captured specimens of *Hg. capricornii* were anthropophilic, thereby indicating behavior similar to that of other cogeneric species that have been previously confused with *Hg. janthinomys.* In areas close to this national park, Davis (1945) found that *Hemagogus* was feeding preferentially on bird blood. Forattini (1965), in areas of the Atlantic Forest in São Paulo state, caught *Hg. capricornii* feeding on mammalian, especially human blood, and blood from birds. This trend also was observed by Guimarães et al. (1987), in samples from the same national park. Our data corroborate these authors' findings, both in relation to the eclecticism and a partial tendency toward preferential feeding on birds, because 26.8% of the samples analyzed in the current study reacted to bird antiserum. In almost all the populations studied, greater positivity to bird antiserum was found, except in the specimens from the municipality of Patrocínio (5.6%), which differed in host preference: opossums (22.2%), rodents (22.2%), cattle (11.1%), and dogs (11.1%).

Hervé et al. (1985) observed that bloodmeal-seeking *Haemagogus* species move vertically between the ground and the crowns of the trees. Pajot et al. (1985), in French Guiana, found that such a behavior is variable and observed that 96.5% of the specimens were captured at ground level, suggesting that aggressive behavior was more prolonged at ground level than at the crowns of the trees. In our observations, if we consider the positivity rates for the antisera against rodents (81 cases; 18.7%), primates (68; 15.7%), opossums (43; 9.9%), cattle (37; 8.5%), dogs (34; 7.9%), and horses (30; 6.9%), it seems that there is a significant possibility of movement between the ground and crown of the trees during host seeking.

The above-mentioned authors also found that 13 of 21 specimens of bloodmeals, obtained from wild females of *Hg. janthinomys*, were positive to cattle antiserum. Because cattle were not being raised in that region, these authors speculated that the blood samples might have come from wild ruminants (*Mazama gouazoubira* Azara or *Mazama americana* Azara, known locally as "biche" or "cariacou"). Forattini et al. (1989) reported that mosquitoes sought out humans for completing their bloodmeals, after feeding earlier on other hosts. We observed that this may be taking place in the regions we studied, considering that, of the 113 cases of multiple food sources (39.3%), 46 were positive to human antiserum, of which 42 had two food sources and four had three food sources.

The mosquitoes from the municipality of Niquelândia had the greatest frequency of reaction to human antiserum, with positivity of 23.1%, followed by rodent (19.2%) and bird (15.4%). The high anthropophily of this sample corroborates the data obtained by Guimarães et al. (1987), who found that *Hg. capricornii* showed the greatest predilection for human blood. According to Hervé et al. (1986), *Hg. janthinomys* remains in close contact with monkeys at the crowns of the trees, where it shows high preference for feeding on primates.

Vasconcelos et al. (1997) observed that *Hg. janthinomys* and other vectors of forest yellow fever only descended to ground level impelled by the presence of humans, who are often there because of wood extraction activities, or when the numbers of monkeys are small. Martinez (1950) caught *Hg. capricornii* feeding on various mammals, birds, and especially humans. Neves (1972), in a study in the Mangabeiras Park, Minas Gerais state, found this species feeding in highest numbers on humans and lowest on horses.

We have seen in this study that the positivity to domestic cattle and horses may be related to these animals often going into the forest during the day and being retrieved at night. We conclude from our data that *Hg. janthinomys* is not primarily anthropophilic as previously inferred, but rather eclectic, being able to shift among various wild or domestic animal hosts according to their local availability.

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