Triatoma sanguisuga Blood Meals and Potential for Chagas Disease, Louisiana, USA

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To evaluate human risk for Chagas disease, we molecularly identified blood meal sources and prevalence of *Trypanosoma cruzi* infection among 49 *Triatoma sanguisuga* kissing bugs in Louisiana, USA. Humans accounted for the second most frequent blood source. Of the bugs that fed on humans, ≈40% were infected with *T. cruzi*, revealing transmission potential.

Chagas disease, caused by the parasite *Trypanosoma cruzi*, is mainly transmitted to humans and other mammals by blood-sucking insects called triatomines (also known as kissing bugs). In the United States, 24 species of wild mammals have been found to be naturally infected with *T. cruzi*, but only a few (<25) autochthonous cases of vectorial transmission to humans have been described (*1,2*). This number is probably an underestimate, and there is concern that vectorial transmission to humans in the United States may increase because of the following factors: 1) loss of sylvan blood sources because of habitat destruction, forcing the bugs to seek other (possibly human) blood sources; 2) climate change that could extend the range of the vectors northward; and 3) introduction of parasites by migrants from disease-endemic countries (*3–5*). Among the 11 triatomine species in the United States, the most widely distributed and the only 2 found in Louisiana are *Triatoma lecticularia* and *T. sanguisuga* (*5*). Bugs of the species *T. sanguisuga* are responsible for the first described autochthonous case of *T. cruzi* transmission in Louisiana (*6*), but little is known about their feeding habits in natural conditions. To evaluate the risk for Chagas disease (based on human/vector/parasite contact) and determine the feeding behavior of the species *T. sanguisuga*, we molecularly identified the blood meal sources and *T. cruzi* infection in *T. sanguisuga* kissing bugs.

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The Study

In 2007, at the site of the first autochthonous case of transmission of Chagas disease in Louisiana, 49 *T. sanguisuga* (16 male and 33 female) bugs were collected and identified (*6–8*). DNA was isolated from the abdomen of each bug by using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA), and presence of *T. cruzi* was assessed by PCR (*9*). *T. cruzi* infection was found in 27 (55.1%) bugs; prevalence did not differ significantly between males (50.0%, 8/16) and females (57.6%, 19/33) (Fisher exact test; p = 0.76) (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/20/12/13-1576-Techapp1.pdf).

Blood meals were detected by using PCR with universal vertebrate primers targeting the 12S ribosomal RNA gene (*10*). The PCR products were purified and cloned to enable detection of multiple blood sources in a single bug. For cloning, the p-GEM-T Easy Vector System (Promega, Madison, WI, USA) was used; for the ligation step, the DNA-to-vector ratio was 3:1. After transformation, up to 8 transformants per bug were randomly selected and sequenced. Blood meals were detected in 45 (92%) of 49 bugs, and 43 (96%) of the 45 detected blood meals were successfully cloned (online Technical Appendix Table).

Blood meal sources were inferred by using BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi); ≥97% identity was considered a match. From the 43 bugs, 201 vertebrate 12S sequences were obtained. In all, 8 vertebrate blood-source species were identified. Multiple blood source species were identified in 21 (48.8%) of 43 bugs; the maximum number of blood meal sources was 4 (online Technical Appendix Table), confirming the ability of the cloning approach to identify multiple blood meals. The average number of blood source species detected per bug was 1.6. The predominant blood source was the American green tree frog (*Hyla cinerea*), found in 53.5% of triatomines; the second most predominant was the human (*Homo sapiens*), found in 48.8%, followed by the raccoon (*Procyon lotor*), found in nearly 30% of triatomines (Figure). Less prevalent blood sources included cow (*Bos taurus*), dog/wolf (*Canis lupus*), squirrel (*Sciurus carolinensis*), cat (*Felis domesticus*), and woodrat (*Neotoma floridana*), each found in <15% of bugs.

In total, 33 different vertebrate 12S haplotypes were found at an average of 2.1 per bug (online Technical Appendix Table, Figure). We found 8 human haplotypes, indicating that bugs had fed on at least 8 persons, assuming that multiple haplotypes did not result from heteroplasmy. More female than male bugs had fed on frogs (Fisher exact test, p = 0.005). Neither the average number of blood

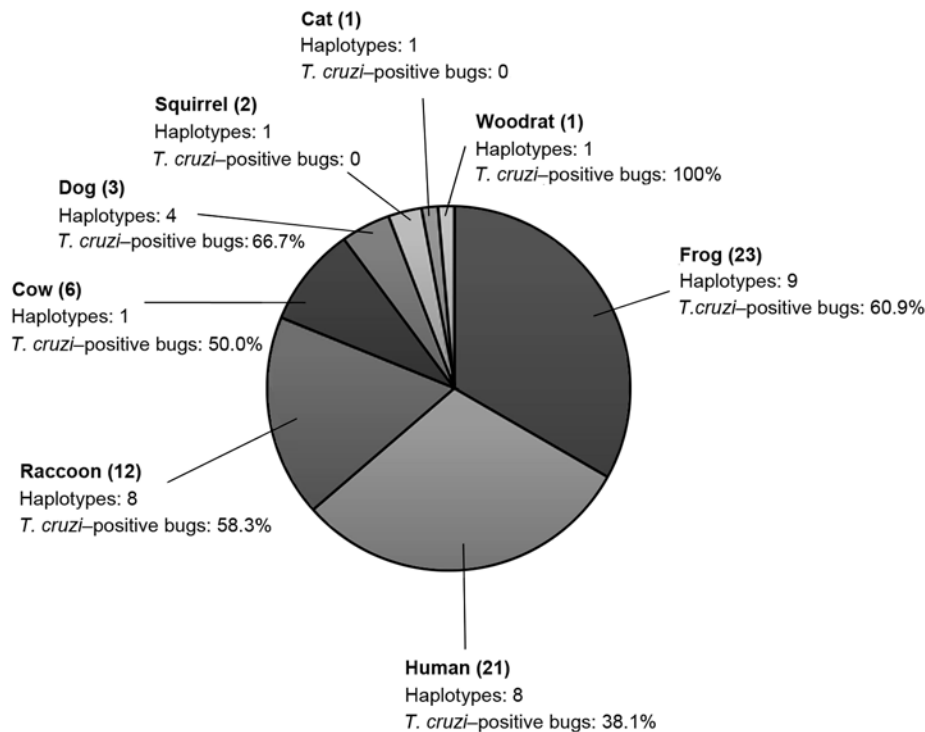[1]Current affiliation: Universidad Autónoma de Yucatán, Mérida, México

Figure. Vertebrate blood meal sources of *Triatoma sanguisuga* kissing bugs detected by 12S rDNA assay (*10*). The numbers of triatomines containing each vertebrate blood source are indicated in parenthesis. The numbers of haplotypes of each vertebrate source and the *Trypanosoma cruzi* infection prevalence in the triatomines containing this vertebrate blood source are indicated.

sources detected (females 1.7 ± 0.8 vs. males 1.4 ± 0.6, *t*-test; p = 0.23) nor the average number of vertebrate haplotypes found (females 2.0 ± 0.9 vs. males 2.1 ± 0.9, *t*-test; p = 0.75) differed significantly between bugs of each sex.

Of the 55% of bugs that were infected with *T. cruzi*, 61.9% had fed on frogs (incompetent *T. cruzi* host) and 38.1% on humans. We found 3 human haplotypes in *T. cruzi*–infected bugs, suggesting, in the absence of heteroplasmy, that at least 3 persons were bitten by an infected bug (online Technical Appendix Table). Because only 2 persons lived at the location sampled, some bugs may have fed on visiting persons or migrated from nearby houses. No significant association was found between infection and a particular blood source, even after we removed from analysis all insects that had fed on at least 1 frog or had fed on frogs only.

Conclusions

Our results indicate that *T. sanguisuga* kissing bugs pose an epidemiologic threat to humans and animals in Louisiana. Human/vector/*T. cruzi* contact is frequent; 55% of bugs were infected, of which nearly 40% had fed on humans.

In addition to humans, the bugs fed on a wide variety of vertebrates; multiple blood sources were detected in about half of the bugs. These observations support catholic and opportunistic feeding habits for *T. sanguisuga*, which probably feed on any available animal. The high occurrence of the American green tree frog as a blood source

is not surprising because these frogs are abundant in this region (*11*). Although amphibians are incompetent *T. cruzi* hosts, frogs contribute to the epidemiology because as a blood source, they help maintain large populations of bugs near human dwellings. Further investigation could determine whether frogs also control the bug population by eating them, as do *Neotoma* spp. rats, the traditional hosts of kissing bugs in North America (*5*). Raccoons serve as sylvatic *T. cruzi* reservoirs in the southeastern United States and play an epidemiologic role because they are frequently found close to humans and, as do woodrats, they link the sylvatic cycle of the parasite with a domestic cycle (*5*). Dogs also serve as reservoirs and are at high risk for Chagas disease; many dogs in the southern United States die of this disease (*1,12,13*). In addition to the loss of companion animals, Chagas disease in animals has an evident economic effect (*13*).

Results of our study, as well as those of Stevens et al. (*10*) and Kjos et al. (*14*), reject the assertion that kissing bugs in North America prefer blood from wild animals, which has been one explanation for the low prevalence of Chagas disease in the United States. Our study provides evidence of frequent vector/human/*T. cruzi* contact in Louisiana and reveals the potential for transmission in the United States. Even if this finding does not apply to all localities (e.g., human blood has rarely been detected in triatomines from Texas [*15*]), the risk for vectorial transmission of *T. cruzi* in the United States may increase because of expansion of human settlements into formerly sylvatic

areas. Moreover, the low number of Chagas disease cases reported in the United States is probably caused, at least in part, by a lack of awareness (*4,5*). Because knowledge of the feeding behavior of triatomines is critical for the implementation of efficient control measures, more studies of the blood sources of triatomines in North America are needed. In addition, awareness of Chagas disease and surveillance of insect vectors and human disease should be improved.

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Triatoma sanguisuga Blood Meals and Potential for Chagas Disease, Louisiana, USA

Technical Appendix

Technical Appendix Table. Blood meal sources of *Triatoma sanguisuga* kissing bugs, determined by 12S rDNA assay (*1*)*

T. sanguisuga bug no.	Sex	Infection with *T. cruzi*†	Blood meal presence (vertebrate 12S amplification)†	No. clones selected and sequenced after 12S reamplification	*Hyla cinerea* (American green tree frog)	*Homo sapiens* (Human)	*Procyon lotor* (Raccoon)	*Bos taurus* (Cow)	*Canis lupus* (Dog/Wolf)	*Sciurus carolinensis* (Squirrel)	*Felis domesticus* (Cat)	*Neotoma floridana* (Eastern woodrat)	No. species detected	No. haplotypes detected
1	F	−	−	NA			No blood meal detected							
2	F	+	−	NA			No blood meal detected							
3	F	+	+	8	A							A	2	2
4	F	−	+	7	B						A		2	2
5	F	+	+	1	A								1	1
6	F	−	+	7	A	A	A, B						3	4
7	F	−	+	1		B							1	1
8	M	+	+	3	B	A							2	2
9	F	−	+	7		A							1	1
10	F	+	+	7	C	A							2	2
11	M	−	+	3		A							1	1
12	M	−	+	4			A, C, D						1	3
13	F	−	+	6	A								1	1
14	F	+	+	3	D	A							2	2
15	F	+	+	5	A		A, E						2	3
16	M	−	+	2		A, C							1	2
17	F	+	−	NA			No blood meal detected							
18	F	+	+	2	A								1	1
19	F	+	+	3	A, E	D							2	3
20	M	+	+	7	A		A						2	2
21	F	+	+	6	A, F								1	2
22	M	+	+	6			A, F						1	2
23	F	+	+	6			A						1	1
24	F	−	+	8	A		A						2	2
25	F	+	+	5	B		A	A	A				4	4
26	M	−	+	8	A,G	A							2	3
27	F	−	+	7	A	A, E							2	3
28	F	+	+	0			No transformants obtained							
29	F	+	+	3	B, H								1	2
30	F	−	+	4	A		A						2	2

| T. sanguisuga bug no. | Sex | Infection with T. cruzi† | Blood meal presence (vertebrate 12S amplification)† | No. clones selected and sequenced after 12S reamplification | Vertebrate blood meal source detected‡ | | | | | | | | No. species detected | No. haplotypes detected |
| | | | | | Taxa and haplotypes detected | | | | | | | | | |
					Hyla cinerea (American green tree frog)	*Homo sapiens* (Human)	*Procyon lotor* (Raccoon)	*Bos taurus* (Cow)	*Canis lupus* (Dog/Wolf)	*Sciurus carolinensis* (Squirrel)	*Felis domesticus* (Cat)	*Neotoma floridana* (Eastern woodrat)		
31	F	+	+	3	A								1	1
32	F	−	+	6	A, I								1	2
33	M	+	+	6					A, B, C				1	3
34	F	+	+	7		A		A					2	2
35	M	−	+	5					A, D				1	2
36	F	+	−	NA				No blood meal detected						
37	M	−	+	2		A							1	1
38	F	+	+	0				No transformants obtained						
39	M	−	+	1						A			1	1
40	F	+	+	5			A, G						1	2
41	F	−	+	5	A	A	H						3	3
42	F	−	+	6		A, F							1	2
43	F	−	+	8		A		A					2	2
44	M	+	+	5		A							1	1
45	M	+	+	8		A, G		A					2	3
46	M	+	+	7		A	A						2	2
47	M	−	+	8		A, H		A		A			3	4
48	M	+	+	8	A, B								1	2
49	F	−	+	8		A		A					2	2
Total		**27/49 (55.1%)**	**45/49 (92%)**	**227**										
Average				5.04 clones/bug (n = 45)									1.6 species/ bug (n = 43)	2.1 haplotypes/ bug (N = 43)
Range				0–8 clones/bug (n = 45)									1–4 species/ bug (n = 43)	1–4 haplotypes/ bug (n = 43)

*NA, not applicable
†To rule out PCR inhibition, the samples for which no vertebrate or parasite DNA was detected were re-amplified after adding 100 ng of positive control DNA to the PCR mixture. For the only sample with these results (bug #1), PCR inhibition was ruled out as the added DNA was amplified.
‡Blood meal sources were inferred by using BLAST with ≥ 97% identity as the criterion for a match.

Nucleotide position

species	Haplotypes	10	20	30	40	50	60	70	80	90	100	110	120	130	140

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                                    1         2         3         4         5         6         7         8         9         1         1         1         1         1
                                    0         0         0         0         0         0         0         0         0         0         1         2         3         4
                                                                                                                              0         0         0         0         0

Felis domesticus    A    GAGATGGTGAGGTATATCGGGGTTTATCGATTATAGAACAGGCTCCTCTAGAGGGATGTAAAGCACCGCCAAGTCCTTTGAGTTTTAAGCTATTGCTAGTAGTTCTCTGGCGGATAGTTTTGTTTAGGGTAACTATCTAAG

                    A    ---------....T..G.....A.........C..G.........C.......GT....A.GGG.T....T...........G.........-.....C.......C....---------------------------------
                    B    --------....T..G.....A.........C..G.....C.......GT....A.GGG.T....T...........G.........-.....C.......C....---------------------------------
                    C    ---------....T..G.....A.........C..G.....C.......GT....A.GGG.T....T...........G.........-.....C....C.C....---------------------------------
                    D    ---------....T..G.....A.........C..G.....C.......GT....A.GGGAT....T...........GA........-.....C.......C....---------------------------------
Hyla cinerea        E    ---------....T..G..A..A.........C..G.............GT....A.GGG.T....T...........G.........-.....C.......C....---------------------------------
                    F    ---------....T..G.....A.........C..G.............GT....A.GGG.T....T...........G......G...-.....C.......C....---------------------------------
                    G    ---------....T..G.....A.........C..G.............GT....A.GGG.T....T...........G.........-..C...C.......C....---------------------------------
                    H    ---------....T..G.....A.........C..G.....C.......GT....A.GGG.T....T...........G.........-C....C.......CT...---------------------------------
                    I    ---------....T..G.....A.........C..G.............GT....A.GGG.T....TA..........G.........-.....C.......C....---------------------------------

                    A    ------------.TG...............C................A.G..........G...............G.G...C.....GT.......AGC-------------------
                    B    ------------.TG..A............C................A.G..........G...............G.G...C.....GT......AGC-------------------
                    C    ------------.TG...............C................A.G..........G...............G.G...C.....G.......AGC-------------------
Homo sapiens        D    ------------..A..T............C.....T..........A............G...T....GT.....A.C-------------------
                    E    ------------.TG.........C..C...................A.G..........G...............G.G...C.....GT......AGC-------------------
                    F    ------------.TG...............C................A.G.......GG..................G.G...C.....GT......AGC-------------------
                    G    ------------.TG...............C................A.G..........G..............CG.G...C.....GT......AGC-------------------
                    H    ------------.TG...............C................A.G..........G...............G.G...C.....GT.....AAGC-------------------

                    A    --------....T..............................................G.................AA...A.......AC---------------
                    B    --------....T..............................................G...........CT.....AA...A.......AC---------------
                    C    --------....T.......C......................................G.................AA...A.......AC---------------
Procyon lotor       D    --------..A..T..............G..............................G.................AA...A.......AC---------------
                    E    --------....T..............................................GC................AA...A.....A.AC---------------
                    F    --------....T...A..........................................G.................AA...A.......AC---------------
                    G    --------....T..............................................G.................A...A.......AC---------------
                    H    --------....T..............................................G.................AA...A.......CAC---------------

Bos taurus          A    ------....T...................................A...A.......................G...........A...A...A.......---------------

                    A    ------....T...................................A..........................C.....A..TA.......GTAAAATTA.-------
Canis lupus         B    ------....T...................................A...........A..............C.....A..TA.......GTAAAATTA.-------
                    C    ......T..G.....A.......C..G........A..............G.......C.....A..TA.......GTAAAATTA.-------
                    D    ------....T...................................A..................C.......C.....A..TA.......GTAAAATTA.-------

Sciurus carolinensis A    -GAG.........T..A....................G.A...................AG.G................A...T.C.....A.TT.A.TA.-------

Neotoma floridina   A    -GAG....A.......A................T....A.....T.....................G.G..C..........G...AA...A.......A-TT.A.T---------
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Technical Appendix Figure. Alignment of the vertebrate 12S haplotypes detected in *Triatoma sanguisuga* kissing bug abdomens. Dots indicate identity with nucleotide in first sequence. Haplotypes were defined on alignments of 100 bp (American green tree frog [*Hyla cinerea*]), 103 bp (human [*Homo sapiens*]), 118 bp (raccoon [*Procyon lotorl*]), 119 bp (cow [*Bos Taurus*]), 127 bp (dog/wolf [*Canis lupus*]), 133 bp (squirrel [*Scirus carolinensis*]), 141 bp (cat [*Felis domesticus*]), or 130 bp (woodrat [*Neotoma floridana*]). The number of haplotypes is the minimum number of specimens fed on, in the absence of heteroplasmy, because different individuals may share 12S haplotypes and only a subset of clones were sequenced.

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