Trypanosoma cruzi Infection Does Not Decrease Survival or Reproduction of the Common Bed Bug, *Cimex lectularius*

Jennifer K. Peterson,^{1*}† Renzo Salazar,^{2†} Ricardo Castillo-Neyra,¹ Katty Borrini-Mayori,² Carlos Condori,² Casey Bartow-McKenney,¹ Dylan Tracy,¹ César Náquira,² and Michael Z. Levy¹

¹Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania;

²Facultad de Salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Peru

Abstract. Although not presently implicated as a vector of human pathogens, the common bed bug, *Cimex lectularius*, has been suspected of carrying human pathogens because of its close association with humans and its obligate hematophagy. Recently, we characterized the vectorial competence of *C. lectularius* for the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease. We observed that *C. lectularius* can acquire *T. cruzi* infection when fed on *T. cruzi*-carrying mice, and subsequently transmit *T. cruzi* to uninfected mice. This led us to ask why has *C. lectularius* not been implicated in the transmission of *T. cruzi* outside of the laboratory? We hypothesized that *T. cruzi* reduces *C. lectularius* fitness (i.e., survival and/or reproduction) as an explanation for why *C. lectularius* does not transmit *T. cruzi* in natural settings. We tested this hypothesis by comparing the survival and reproduction of uninfected and *T. cruzi*-infected *C. lectularius*. We observed that *T. cruzi* had a variable effect on *C. lectularius* survival and reproduction. There were negligible differences between treatments in juveniles. Infected adult females tended to live longer and produce more eggs. However, no effect was consistent, and infected bugs showed more variation in survival and reproduction metrics than control bugs. We did not observe any negative effects of *T. cruzi* infection on *C. lectularius* survival or reproduction, suggesting that decreased fitness in *T. cruzi*-infected *C. lectularius* is not why bed bugs have not been observed to transmit *T. cruzi* in natural settings.

INTRODUCTION

The common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), has been a persistent human pest throughout history.^{1,2} Although eliminated from much of the world after the Second World War,³ the species is presently undergoing a global re-emergence, especially in urban areas, resulting in a worldwide public health problem.^{4,5}

Cimex lectularius and other *Cimex* species are associated with a variety of human health issues including skin lesions, anemia, and mental health problems.^{6–10} Although bed bugs are not presently implicated as vectors of human pathogens, at different points throughout the last century, bed bugs have been suspected of carrying more than 40 different human pathogens,^{11,12} including bacteria, fungus, virus, and protozoa species. Indeed, at least under laboratory conditions, bed bugs are able to transmit deadly pathogens. Recently, we published a study on the vectorial competence of *C. lectularius* for the protozoan parasite *Trypanosoma cruzi*, under laboratory conditions.¹³ Transmitted by the triatomine bug (Hemiptera: Reduviidae: Triatominae), *T. cruzi* is the causative agent of Chagas disease, a chronic infection that can lead to serious cardiac and/or gastrointestinal problems.^{14,15}

Although triatomine bugs are the only confirmed vectors of *T. cruzi* in natural settings, we observed that *C. lectularius* can acquire *T. cruzi* infection when fed on *T. cruzi*-carrying mice, and subsequently transmit *T. cruzi* to uninfected mice, which confirmed earlier studies on bed bugs and *T. cruzi* transmission.^{16–18} This led us to ask, why has *C. lectularius* not been implicated as a vector of *T. cruzi*

outside of the laboratory? Is it merely because not enough field-caught bed bugs have been tested for *T. cruzi*, or is there a biological limitation preventing *C. lectularius* from transmitting *T. cruzi* in natural settings?

METHODS

Experimental overview. To investigate the survival and reproduction of *C. lectularius* when infected with *T. cruzi*, we infected a total of 280 *C. lectularius* individuals with *T. cruzi* and observed their survival and reproduction from the 1st juvenile stage (i.e., instar) to death, and compared it with 240 uninfected *C. lectularius*. We carried this out across three experimental replicates each consisting of separate cohorts (diagram visualizing experiment structure in Figure 1). The 1st replicate had three cohorts with 40 bugs each: two cohorts of *T. cruzi*-infected bugs and one cohort of uninfected bugs. The next two replicates had a total of four cohorts of 50 bugs each: two cohorts of infected bed bugs and two control cohorts. A total of 520 bugs were used across the entire experiment.

Ethical statement. The Institutional Animal Care and Use Committee (IACUC) of the Universidad Peruana Cayetano Heredia reviewed and approved the animal-handling protocol used for this study (identification number 61410). The committee is registered with the United States National Institutes of Health (PHS Approved Animal Welfare Assurance Number A5146-01) and adheres to the Animal Welfare Act of 1990.

Bed bug maintenance. *Cimex lectularius* individuals came from a laboratory colony of Arequipa strain CL-1 bugs maintained at the Universidad Peruana Cayetano Heredia/University of Pennsylvania Zoonotic Disease Research Laboratory in Arequipa, Peru. The colony was founded in 2011 from bed bugs captured in a hotel in Arequipa. The colony is maintained at an ambient temperature of 27°C ± 2.5°C, with a relative humidity of 60% ± 10%, and a 12-hour photoperiod.

Bed bug infection. At the beginning of each replicate, we collected unfed 1st instar *C. lectularius* individuals between

* Address correspondence to Jennifer K. Peterson or Michael Z. Levy, Department of Biostatistics and Epidemiology, University of Pennsylvania, 714 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104. Email: jenni.peterson@gmail.com and mzlevy@pennmedicine.upenn.edu

† These authors contributed equally to this work.

FIGURE 1. Diagram of experiment structure. In total, 520 bugs were used. This figure appears in color at www.ajtmh.org.

1 and 2 weeks old from the laboratory colony and fed each bug to engorgement on one of three 1-month-old mice infected with the *T. cruzi* strain Arequipa TC-35¹⁹ (mouse infection details are presented in the following paragraphs). Between 40 and 100 bed bugs fed on each infected mouse once a week for 4 weeks, with one mouse per replicate to control for age and parasitemia, and three used in total throughout the entire experiment. Bugs took multiple infective meals to ensure infection, as the volume of blood ingested by 1st instar bed bugs is generally very small (< 1 µL), and we have found that one infective meal will not always result in an infection.¹³ We specifically chose four feedings to ensure that bugs fed on the mice with peak *T. cruzi* parasitemia, which occurs between 2 and 5 weeks postinoculation.¹³ Control bugs were fed on an uninfected mouse (one mouse per replicate, three in total) following the same schedule as infected bugs. After feeding, we kept the bugs in glass tubes (21 mm in diameter and 7 mm deep) in groups of 40–50 individuals. Tubes contained a small folded piece of paper for shelter and were covered with a piece of fine fabric with a pore size of < 1 mm. Bugs from each tube were considered to be a cohort.

Bed bug feeding. After 4 weeks, we fed all bugs once a week on uninfected 2-month-old female guinea pigs (*Cavia porcellus*) free of *T. cruzi* infection. We did not continue to feed them on mice for ethical reasons, as mice are too small to feed large numbers of *Cimex* in later developmental stages. (We did not infect bugs using *T. cruzi*-infected guinea pigs because we have found in our prior work that *T. cruzi*-infected guinea pigs have highly variable infectiousness to triatomine bugs.¹⁹) We used one individual guinea pig per treatment in each replicate for a total of six guinea pigs used across all replicates. Bugs from each group fed on a different individual to control for animal age. To ensure that the guinea pigs used to feed infected bugs did not become infected with *T. cruzi*, we analyzed two microhematocrit tubes of blood from the saphenous vein of each guinea pig every 2 weeks. No guinea pig became infected in the experiment.

Mouse infection. We obtained mice from the bioterium of the University Catolica Santa Maria in Arequipa, Peru, which is an IACUC approved animal rearing facility. We infected three 1-month-old female BALB/c mice (*Mus musculus*) with 2×10^3 *T. cruzi* trypomastigotes in 100 µL of medical saline solution for injections (sodium chloride 9%; Medifarma) via intraperitoneal injection. Mice were infected 2 weeks before bed bug infection. We evaluated *T. cruzi* blood parasitemia in mice weekly using blood drawn from the tail of each mouse and subsequently transferred into heparinized microhematocrit tubes.^{13,20} Mice retained a parasitemia throughout the course of the experiment (Table 1).

Data collection. After the 1st feeding on a *T. cruzi*-infected mouse, we recorded nymphal development time and mortality of the bugs in each cohort once a week from 1st instar to the adult phase. On reaching adulthood, we separated bugs into male–female pairs and transferred each pair into modified glass tubes (10 mm diameter and 7 mm deep) to measure reproductive parameters.

To investigate reproduction, we recorded the number of eggs laid each week per female and counted the number of weeks in which each female laid eggs. Eggs from each pair were removed weekly and placed into microtubes covered with fine fabric, as described previously, where we recorded the number of hatched eggs per female each week. Females were kept in pairs the entire time; if the male died, another male of the same treatment was placed with the remaining female, as females must mate periodically to remain fertile.^{21,22} For the control and infected groups, respectively, 73% and 78% of females were with one male, 23% and 17% were with two males, 3% and 5% were with three males, and 1% were with four males (one control female; Supplemental Table 5).

Each week, the number of dead bugs in each treatment was counted and recorded and the dead bugs were removed from the cohort. Dead bugs in the infected treatments were examined for *T. cruzi* infection through examination of their feces and intestinal contents for trypanosomes through direct microscopic observation, adapted from the protocol for triatomine bugs.^{13,23} All bugs in the infected treatment were positive for *T. cruzi* infection.

Data analysis. To analyze survival, we carried out stage-specific analyses as opposed to an age-specific analysis because *C. lectularius* have a stage-structured population for which stage-specific survival is more descriptive of an individual's life history than age-specific survival.²⁴ Here, we calculated the survival probability, $l(x)$, and stage-specific survival probability, $g(x)$, for each cohort and replicate.²⁵ Survival probability, $l(x)$, is the probability that an individual in a given cohort survives from the 1st instar to stage x , calculated as:

$$l_x = \frac{n_x}{N_0}$$

TABLE 1
Levels of *Trypanosoma cruzi* parasitemias in mice used to feed bugs in the infected treatments by replicate

	Min	Max	Average
Replicate 1	4.93×10^5	5.44×10^6	2.63×10^6
Replicate 2	1.39×10^6	5.56×10^6	3.84×10^6
Replicate 3	9.14×10^5	3.48×10^6	1.71×10^6

Parasitemias are shown as number of parasites per mL of blood.

where n_x is the number of bugs alive at the beginning of stage x and N_0 is the number of bugs at the beginning of the experiment.

Stage-specific survival probability, $g(x)$, is the probability that an individual survives from stage x to stage $x + 1$, calculated as:

$$g_x = \frac{l_{(x+1)}}{l_x}.$$

Because individual insects within each cohort were not raised independently (i.e., separated into individual jars) until they reached the adult stage, there were not a sufficient number of independent samples to compare survivor function²⁶ or instantaneous hazard rates.²⁷ As adults were reared in independent male–female pairs, we tested for differences in adult longevity between infected and uninfected adults by sex within each cohort and replicate. In addition, we tested for an association between weeks lived as an adult and number of mates in adult females. All statistical tests were carried out in the R statistical computing environment.²⁸ To see if we could pool cohorts of the same treatment within each replicate, we 1st tested for differences between cohorts using a Kruskal–Wallis test followed by a post hoc multiple comparison test if a significant difference was found. The multiple comparison test allowed us to identify which cohorts were different and to control for family-wise error. We carried out the test using the `kruskalmc` function from the “`pgirmess`” package.²⁹ If there were no differences between cohorts of the same treatment in each replicate, data for cohorts of the same treatment were pooled by replicate and a Wilcoxon rank sum test with continuity correction test was carried out to test for a difference between treatments in each replicate.

To examine development, we calculated mean development time per nymphal stage and mean total preadult development period between treatments. As mentioned previously, nymphs were not reared independently, so there were not a sufficient number of independent samples to carry out a statistical analysis of development.

To investigate reproduction, we compared the following reproductive parameters between treatments: total eggs laid, eggs laid per week, number of reproductive weeks (i.e., number of weeks in which eggs were laid), and proportion of oviposited eggs that were viable (i.e., that hatched). We also tested for associations in each treatment between the parameters listed above and the number of males per female. To control for differences in time spent reproducing, we compared the proportion of adult female life span spent reproducing (i.e., number of reproductive weeks/total number of weeks lived as adult) and number of eggs laid per reproductive week. As with adult longevity, we 1st tested for differences between treatments in the same replicate using a Kruskal–Wallis test followed by the multiple comparison test described previously if a difference was found. If there were no differences between cohorts of the same treatments within a replicate, cohorts of the same treatment were pooled and differences between treatments were then tested for in each replicate using a Wilcoxon rank sum test with continuity correction.

Population statistics. To incorporate survival and reproduction, we compared the net reproductive rates, R_0 , and the intrinsic rate of increase, r , between treatments in each

cohort and replicate.²¹ R_0 represents the number of female offspring a given female will contribute to future populations, adjusted for mortality. We calculated R_0 using the formula

$$R_0 = \sum_{k=0}^x l_x m_x,$$

where l_x is the probability of surviving to stage x as described previously and m_x is half the average number of viable eggs laid per female in stage x .

The intrinsic rate of increase, r , represents the average weekly rate of increase per female. This metric controls for differences in life span that may be associated with higher or lower R_0 values. We calculated the intrinsic rate of increase using the following formula

$$r = \frac{\ln R_0}{T},$$

where T represents generation time. We calculated R_0 and r using data from all stages to compare treatments on a population level, where l_x per stage was used, and T was equal to the entire adult life span. For these calculations, we assumed there to be a 50:50 sex ratio as in previous studies^{21,30,31} and mortality was assumed to be similar and constant among males and females in the juvenile stage.

Finally, we also calculated intrinsic rates of increase, r , for only adult females to compare reproduction rates between treatments on a weekly basis. Here, x was measured in weeks lived as an adult, and l_x and m_x were calculated on a weekly basis from the beginning of the adult stage. In this case, T was calculated as

$$T = l_x m_x x.$$

We differentiate the two r calculations with the subscripts “a” (for “adults”) and “p” (for “population”), presented as r_a and r_p in the results.

RESULTS

Survival probability, l_x . The probability of surviving to stage x , l_x , declined with stage in both treatment groups, but nymphs in the control group had higher mean l_x values and lower standard deviation (SD) between cohorts than the infected group in every stage except the 5th instar, in which the control group had a low outlier (cohort 1a; Figure 2A). The largest difference between the treatment groups was the probability of surviving to the adult stage in replicate 1, with the l_x for the control group being less than half that of the infected group (0.22 and 0.45, respectively). Survival probabilities were similar between treatments in the other two replicates (0.80 and 0.63 for control group in replicates 2 and 3, respectively, versus 0.82 and 0.64 for the infected group). Survival results by stage, treatment and replicate are presented in Tables 1–4 of the Supplemental Appendix.

Stage-specific survival probability, g_x . Stage-specific survival probability, g_x (i.e., the probability of surviving from stage x to stage $x + 1$), tended to be higher in the control group in instars 1–3, whereas infected insects tended to have higher stage-specific survival probability in instars 4 and 5 (Figure 2B). Most of the variation came from replicate 1, where

FIGURE 2. Box and whisker plots of survival metrics across instars and between treatments. (A, above) I_x , the probability that a *Cimex lectularius* individual survives from the 1st instar to stage x . (B, below) g_x , the probability of surviving from stage x to stage $x + 1$. In each column, control groups are represented on the left, and infected groups are represented on the right. Dots represent cohorts. Each box contains quartiles 1–3 of the data, the remaining 25% of the data along the whiskers, and min and max values at the whisker tips. Outliers (values ± 1.5 times the upper and lower quartiles, respectively) are indicated by grey dots. All outliers are from replicate 1. Stars represent means. Horizontal lines within each box are medians. This figure appears in color at www.ajtmh.org.

g_x declined by 32% in the control group between stages 3 and 4 (from 0.94 to 0.64), and by 33% between stages 4 and 5 (from 0.64 to 0.43). The infected group experienced a 19% decline in g_x from the 4th to the 5th instar in the same replicate (0.88 to 0.72). There was little variation between treatments or stages in replicates 2 and 3.

Nymphal development time. Nymphal developmental times were similar between infected and control groups across all replicates (Figure 3). In both treatments, nymphal stages 1–3 tended to develop more rapidly and with lower SDs between cohorts than 4th and 5th instars. The 4th instar nymphs in both treatments in replicate 1 had the longest mean development time (4.91 and 3.54 weeks for control and infected groups, respectively; Figure 3). These times were more than twice that of corresponding development times in replicates 2 and 3. In addition, mean total nymphal development time in replicate 1 was about 3 weeks longer in both treatments than in replicates 2 and 3, with a SD over twice that of the other two replicates.

Adult longevity. Adult longevity, measured as weeks lived in the adult stage, was variable between treatments across replicates (Figure 4). Infected females lived significantly longer than the control group in replicate 2 (Wilcoxon, $P < 0.01$), but not in replicates 1 or 3. In addition, infected females with two male partners lived significantly longer than control females with one partner in replicate 2 only (Kruskal–Wallis rank sum test, $P < 0.05$). Infected males lived significantly longer in

replicates 2 and 3 (Wilcoxon rank sum test, $P < 0.01$ in both replicates), a pattern which was also observed when analyzing both sexes together (Wilcoxon rank sum test, $P < 0.01$ in both replicates). It was not possible to test for a difference in males in replicate 1, as only two control males survived to the adult stage in replicate 1.

Total life span. Average total life span (1st instar to death) tended to be longer in infected bugs, with mean lifespan in the infected group being 3.84–6.14 weeks longer than mean lifespan in the control group (Table 2). Infected bug life span was more variable than control bugs, with a SD 3–5 weeks larger than the control group in every replicate (Table 2).

Reproduction. Eighty-nine percent (63/71) of the adult females in the control group laid a total of 2,430 eggs, of which 1,589 hatched (65.4%; Figure 5). Ninety-five percent (60/63) of these females laid at least one viable egg. Of the infected females, 96.5% (83/86) laid a total of 5,894 eggs in which 68.3% of the eggs (4,024) hatched (Figure 5). Of the 83 infected females that laid eggs, 89.1% (74/83) laid at least one viable egg. There was no difference in eclosion rates between treatments (Wilcoxon rank sum test, $P > 0.05$) nor were there any associations between fecundity and number of males per female.

Differences in egg output between treatments were consistent over time (Supplemental Table 6). There were no

FIGURE 3. Mean nymphal development time in weeks for infected and uninfected *Cimex lectularius* across replicates. Nymphal stages progress upward, beginning with the 1st instar (n1) and progressing to the 5th and final juvenile instar (n5). Error bars represent standard deviation from the mean. This figure appears in color at www.ajtmh.org.

FIGURE 4. Adult longevity in weeks by sex and treatment across replicates. In each column, females are represented in the white boxes on the left and males are in the grey boxes on the right. The control group is represented by the blue dots and the infected group is represented by the red dots. Each dot represents an individual bug. Stars represent means. A low and high outlier in the control female group in replicate 1 are represented by grey dots. This figure appears in color at www.ajtmh.org.

significant differences in reproductive output between treatments in replicate 1. Infected females in replicate 3 laid significantly more eggs in total and per week than control females, and they spent a significantly larger proportion of their adult life laying eggs than control females (i.e., number of weeks where eggs were laid/total weeks lived as adult; Wilcoxon rank sum test, $P < 0.03$ for all metrics; Table 3). Infected females in replicate 2 also spent significantly more of their adult life laying eggs than the control group and they laid more eggs per reproductive week than the control group (Wilcoxon rank sum test, $P < 0.03$ for all metrics; Table 3). A reproductive week is defined as a week in which at least one egg is laid, regardless of viability. Control females had faster rates of intrinsic increase, r_a (i.e., the average weekly rate of intrinsic increase per female in her adult life only; Table 3).

Population statistics. Infected females had an average net reproductive rate, R_0 , more than twice that of the control group in all cohorts, meaning their net contribution to future populations was double that of uninfected bugs (Figure 6A). Intrinsic rates of increase, r_p (i.e., the average weekly rate of

intrinsic increase per female in her entire lifetime) were more similar between treatments than net reproductive rates (Figure 6B). Infected bugs increased faster than control bugs in all cohorts except in 2a, but the difference was variable; in cohorts 1a and 3a, infected bugs increased 50% faster per bug per week than control bugs, whereas in cohort 3b, infected bugs increased only 8% faster. In cohort 2a, control bugs increased 13% faster per bug per week than infected bugs.

DISCUSSION

Overview. In this work, we tested the hypothesis that *T. cruzi* decreases the survival and reproduction of *C. lectularius*. Although we observed some life history dissimilarities between infected and control groups, we did not observe any clear or consistent decreases in survival or reproduction in *T. cruzi*-infected *Cimex* in comparison to the control groups. We therefore reject the hypothesis that *T. cruzi* decreases survival and/or reproduction in *C. lectularius*.

Trypanosoma cruzi heterogeneity. The effects of *T. cruzi* infection are known to vary both between and within host species,^{19,32–35} and we observed the *T. cruzi*-infected *C. lectularius* life history response to be heterogeneous as well. This is consistent with the results from previous studies on the effect of *T. cruzi* on its hosts, both vertebrate and invertebrate.³⁶ In triatomine bugs, the known vectors of *T. cruzi*, some studies report that *T. cruzi* retards development time, reduces survival, decreases reproductive rates, and modifies other phenotypes in *T. cruzi*-infected bugs,^{37–40} whereas others have reported no significant effects.^{41–43} Life history

TABLE 2

Mean total life span (1st instar to death) in weeks by treatment and replicate

	Control		Infected	
	Mean total life span (weeks)	SD	Mean total life span (weeks)	SD
Replicate 1	14.28	11.91	18.12	14.95
Replicate 2	12.35	6.73	18.49	12.07
Replicate 3	14.16	10.27	19.55	15.42

SD = standard deviation.

FIGURE 5. Proportion of eggs that hatched by treatment and replicate. Control groups are shown on the left in each column; the infected groups are on the right.

variation in *T. cruzi*-infected triatomines has also been associated with insect age, sex,³⁹ *T. cruzi* strain,⁴⁴ parasite community within the host,^{45,46} and temperature.⁴⁷

Cimex heterogeneity. Bed bug life history varies by several external factors, including feeding frequency, blood meal source, temperature, bed bug strain, presence of males, and number and frequency of copulations.^{21,22,30,48,49} Mean life spans in both treatments of our experiment were shorter than those reported by Barbarin (21.9–33.9 weeks, depending on

strain),^{48,49} but several external conditions in their experiment differed from ours, especially blood meal source. Hosts can possess minute variations, even in a controlled experiment, and it is known that *C. lectularius* is very sensitive to factors pertaining to diet, such as blood regimen and blood source, and even blood type.^{30,31,48,49} If the bugs in our experiment were sensitive to variation in the individual hosts they fed on, it could explain the differences we observed between replicates independent of treatment, as the only factors that differed

TABLE 3
Reproductive output of the control and infected treatment groups by replicate

	Replicate 1		Replicate 2		Replicate 3	
	Control (N = 8)	Infected (N = 16)	Control (N = 35)	Infected (N = 35)	Control (N = 29)	Infected (N = 35)
Eggs/female	64.4	52.8	22.3	47.9	41.4	96.3*
Viable eggs/female	35.4	34.8	15.0	31.5	28.1	67.6**
% Hatched/female	52.7	63.6	54.4	53.5	60.7	66.4
Eggs/female/week	3.5	2.7	2.9	3.4	2.7	4.6***
Viable eggs/female/week	1.8	1.9	2.0	2.2	1.9	3.3**
Weeks as adult	18.3	16.4	7.3	12.2*	15.2	18.5
Weeks laid eggs ("reproductive weeks")	12.2	11.4	3.8	7.4*	8.2	13.1
Weeks laid viable eggs	11.1	10.2	3.3	6.2*	6.8	12.4
Eggs/female/reproductive week	4.7	4.0	4.9	5.2	4.6	6.5***
Viable eggs/female/reproductive week	1.8	3.0	3.7	3.7	3.9	4.8
% Adult life laid eggs	69.3	62.3	45.5	61.4*	52.6	66.6*
% Adult life laid viable eggs	61.0	56.3	40.1	49.4	44.1	61.3*
Intrinsic rate of increase, r_a	0.28	0.25	0.50	0.36	0.47	0.39

All values shown are means unless otherwise stated. Differences were tested for between treatments in each replicate. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

FIGURE 6. Population statistics by treatment. (A, above) Average net reproductive rates, R_0 , by treatment. (B, below) Intrinsic rates of increase, r , by treatment. In both figures, the control groups are represented in blue on the left and the infected groups are represented in red on the right. Dots represent cohorts. Stars represent means. This figure appears in color at www.ajtmh.org.

between replicates were the individual animals used to feed the bugs. (Bugs from each replicate fed on a different set of animals to control for animal age and parasitemia, in the case of infected animals.)

We observed the most variation in reproductive output, both between treatments and between individuals of the same treatment, but the average number of eggs laid per week per female still fell within the range of 2.7–13 eggs/week reported in the literature.^{31,49,50} In addition, although the net reproductive rates, R_0 , of infected bugs were twice that of the control bugs in all replicates, when examining reproduction at

a finer scale, the results were less conclusive. In replicate 2, infected females lived longer as adults than control females, and spent a significantly greater number of weeks laying eggs, including viable eggs, and a significantly larger proportion of their adult life laying eggs (but not viable eggs). However, the increased time dedicated to reproduction of the infected females did not lead to significant differences in number of eggs laid when compared with uninfected females. Indeed, there were no significant differences between treatments in any measure of eggs laid; in total, per week or per reproductive week. Moreover, intrinsic rates of increase in adult females, r_a ,

from the control group were always faster than those of infected adult females. This suggests that infected females in replicate 2 required more time (as adults) to reach a reproductive output comparable to that of the control group.

In replicate 3, infected females spent a larger proportion of their adult life laying eggs, including viable eggs, than bugs from the control group. As opposed to replicate 2, this did lead to differences in the number of eggs laid per female (including viable eggs) between treatments, both in total and per week, with infected bugs laying significantly more eggs. When comparing output only in weeks in which eggs were laid ("reproductive weeks"), infected bugs still laid significantly more eggs per reproductive week, but they did not lay significantly more viable eggs than control bugs. This suggests that in replicate 3, infected bugs were using resources less efficiently than control bugs by producing more unviable eggs.

These results suggest that *T. cruzi* infection may stimulate a response that affects the regulation of *Cimex* reproduction; in replicate 2, insects needed more time to produce a comparable number of eggs, and in replicate 3, insects needed to produce more eggs per week to produce a comparable number of viable eggs. Reproduction in *Cimex* species is costly because of their use of traumatic insemination to copulate, where the male punctures the abdomen of the female. Thus, it is unsurprising that reproduction would be the most variable factor. Although there were no differences in fecundity between females who were with more than one mate over the course of the experiment, in replicate 2 we did observe that *T. cruzi*-infected females that had two partners, (albeit one at a time), lived significantly longer than control females that had just one partner. Perhaps, the immune function in *T. cruzi*-infected bugs is altered in a way that counteracts the negative effects of traumatic insemination. It would be interesting to carry out a follow-up experiment investigating the mechanistic factors underlying this effect, such as endocrinological and immunological differences between infected and uninfected bugs that might affect reproductive processes.

***Cimex lectularius*: an insect of medical importance for Chagas disease?** In spite of potentially negative effects of *T. cruzi* on reproductive regulation, we did observe that infected bugs had higher R_0 values, a metric that is often used as a measure of fitness.²⁵ If *T. cruzi*-infected bugs are in fact more fit than uninfected bugs, it would be concerning from an epidemiological perspective, as bed bugs have several innate characteristics that would facilitate the transmission of *T. cruzi* to humans. These include cohabitation with humans; higher feeding rates than *Triatoma infestans*, a key triatomine bug vector of *T. cruzi* to humans^{22,51,52}; infestation of densities about an order of magnitude higher than that of domestic *T. infestans* infestations (M. Z. Levy, personal communication); and a tendency to defecate on the host during or immediately after taking a blood meal,¹³ which would facilitate the stercoral transmission of *T. cruzi*. In addition, the ability of bed bugs to survive for more than a year without eating⁶ combined with extensive insecticide resistance^{5,53,54} make it quite challenging to eliminate them. Finally, although there are few published studies of bed bugs in Latin America, their distribution continues to increase in *T. cruzi*-endemic regions.⁵ In Arequipa, Peru, reports of bed bug infestations have been increasing considerably since we began studying them 5 years ago. Characterizing bed bug distribution in other *T. cruzi*-endemic areas will be an important area of future research.

When considering the increased fitness we observed in *T. cruzi*-infected bed bugs in combination with their biological characteristics that could facilitate transmission of *T. cruzi* to humans, it becomes all the more critical to be able to confidently discard the possibility that *C. lectularius* could vector *T. cruzi* in natural settings. To do so, more factors that could affect *T. cruzi* transmission by *C. lectularius* must be investigated. In this experiment, we noted three areas to possibly be pursued in follow-up research: 1) bug sensitivity to host variation, including host parasitemia; 2) differences in virulence between *T. cruzi* in culture versus *T. cruzi* in natural settings; and 3) the number of infection events necessary to sustain *T. cruzi* infection in bed bugs over time.

In addition, variation in parasitemia levels between infected hosts, both between individuals and over time, may lead to variable health effects in the bed bugs feeding on them. A human in the chronic indeterminate phase of Chagas disease may have a lower average parasitemia than the recently infected 1-month-old mice we used to infect the bugs, whose parasitemia averaged 2.5×10^6 parasites per mL of blood. *Cimex lectularius* primarily feed on humans, for whom detectable blood parasitemias occur just during the acute phase of Chagas disease,¹⁵ which lasts just a few weeks after initial infection. For the rest of the lifelong disease, infection must be diagnosed from serological tests because of generally undetectable parasitemias.¹⁴ Therefore, a lower average *T. cruzi* parasitemia in humans could be one explanation for why *C. lectularius* is not infected outside the laboratory; perhaps, the *T. cruzi* parasitemia found in humans does not result in a large enough infective dose to establish *T. cruzi* infection in the bug. More testing of bed bugs caught in *T. cruzi*-endemic areas is needed to answer this question.

The final factor that we observed in our experiment that could affect *T. cruzi* transmission by *C. lectularius* is the ability of the parasite to sustain its infection in the bug after just one infection event. We reinfected bugs once a week for the 1st 4 weeks of each replicate, for a total of four infection events, and we do not know if the bugs sustain *T. cruzi* infection for the full duration of their lives from the just one infection event in the first instar. Of course, both single and multiple contacts with *T. cruzi*-infected humans are possible depending on the dynamics of the host population (e.g., permanent residents versus temporary guests).

CONCLUSION

We found no evidence that *T. cruzi* decreases *C. lectularius* survival or reproduction. Given that *C. lectularius* can transmit *T. cruzi* in laboratory settings, it is critical to continue investigating the factors that may prevent *C. lectularius* from playing a role in *T. cruzi* transmission in the field to ensure that *C. lectularius* does not threaten the success of Chagas disease vector control.

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Authors' addresses: Jennifer K. Peterson, Ricardo Castillo-Neyra, Casey Bartow-McKenney, Dylan Tracy, and Michael Z. Levy, Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, E-mails: jenni.peterson@gmail.com, cricardo@upenn.edu, casb@pennmedicine.upenn.edu, tradylan@sas.upenn.edu, and mzlevy@pennmedicine.upenn.edu. Renzo Salazar, Katty Borrini, Carlos Condori, and César Náquira, Universidad Peruana Cayetano Heredia/University of Pennsylvania Zoonotic Disease Research Lab, Urbanización Las Begonias D-11, Distrito José Luis Bustamante y Rivero, Arequipa, Perú, E-mails: rendaths@gmail.com, yttakbm@gmail.com, carlosedcopino@gmail.com, and cesar.naquira@gmail.com.

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