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PREDICTORS OF *TRYPANOSOMA LEWISI* IN *RATTUS NORVEGICUS* FROM DURBAN, SOUTH AFRICA

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ABSTRACT: This study investigated associations between *Trypanosoma lewisi* and *Xenopsylla cheopis*, a common cyclical vector of *T. lewisi*; *Polyplax spinulosa*, a reported mechanical vector; and *Laelaps echidnina* and *Laelaps lamborni*, 2 rodent mites of *Rattus norvegicus* in Durban, South Africa. In total, 379 *R. norvegicus* individuals were live-trapped at 48 sites in 4 locality types around Durban during a 1-yr period. Rats were euthanized, cardiac blood was taken to check for hemoparasites, and ectoparasites were removed for identification. Parasite species richness was higher in pups (2.11) and juveniles (1.02) than adults (0.87). Most rats in the study harbored 1 or 2 of the 5 parasites surveyed. Rats with trypanosomes and fleas were more prevalent in the city center and harbor, where juveniles were most affected. Rats with lice were more prevalent in informal settlements and urban/peri-urban areas, where pups had the highest infestations. There was a significant positive association between rats with fleas and trypanosomes and a negative association between rats with lice and trypanosomes. Location and rat age were significant predictors of *T. lewisi*, *X. cheopis*, and *P. spinulosa*. Mites showed no strong association with trypanosomes. Ectoparasite associations are possibly habitat and life-cycle related. We conclude that Durban's city center, which offers rats harborage, an unsanitary environment, and availability of food, is a high-transmission area for fleas and trypanosomes, and consequently a potential public health risk.

Rodents are reservoirs of a number of zoonoses (e.g., plague, murine typhus, leptospirosis, angiostrongyliasis, and toxoplasmosis) that can be transmitted to humans directly or indirectly via vectors such as ectoparasites (Begon, 2003; Meerburg et al., 2009). Common ectoparasites of rats belong to the following orders: Siphonaptera (fleas), Phthiraptera (lice), Mesostigmata (mites), and Acarina (ticks) (Paramasvaran et al., 2009). Ixodid ticks, mites, and fleas are temporary obligate parasites, whereas lice (both adults and nymphs) are permanent parasites (Askew, 1971; Service, 1980). Life stages of ticks, some mites, and lice, as well as adult fleas, are hematophagous (Noble and Noble, 1976). Although these arthropods can transmit bacterial and viral diseases, only fleas are vectors of the helminths *Hymenolepis diminuta* and *Hymenolepis nana*, and the protozoan *Trypanosoma lewisi* (Beaver et al., 1994). Lice (*Polyplax spinulosa*) have been implicated (in laboratory studies) as mechanical vectors of rodent trypanosomiasis (Mac Neal, 1904; Nuttall, 1908); however, other experiments with lice, ticks, mites, and bugs did not produce infection with *T. lewisi* (Strickland and Swellengrebel, 1910).

Trypanosoma lewisi is a blood flagellate of the sub-genus, *Herpetosoma* (stercoraria section) that parasitizes *Rattus* spp. Fleas are cyclical vectors of *T. lewisi*, and the most common species are *Xenopsylla cheopis* in tropical and sub-tropical areas and *Nosopsyllus fasciatus* in temperate regions (Hoare, 1972). Transmission to the mammalian host is via ingestion of the vector's moist feces or the vector itself (Minchin and Thomson, 1915). In 1845, Chaussat found trypanosomes in the blood of *Rattus rattus*, and it was Lewis' work in 1878 on *T. lewisi* in wild rats in India that highlighted trypanosomiasis in mammals (Laveran and Mesnil, 1907). Plummer (1913) reported *T. lewisi* as a highly host-specific parasite exclusively found in *Rattus* spp. However, this has since been disproved, as the trypanosome has been isolated and genetically confirmed from humans (Howie et al., 2006; Shah et al., 2011; Verma et al., 2011), captive monkeys (da Silva et al., 2010), and *Bandicota* rodent species in Thailand (Jittapalapong et al., 2008). More recently, in Southeast Asia, mice, shrews, and rats of the genera *Bandicota*, *Berylmys*, *Niviventer*, *Moxomys*, and *Rattus* were found to be infected with *T. lewisi* and *Trypanosoma evansi* (Pumhom et al., 2015).

Prevalence of *T. lewisi* in *Rattus* spp. on most continents ranges from 1.5% (Siti Shafiyyah et al., 2012) to 82.3% (Laha et al., 1997). Data on *T. lewisi* prevalence on the African continent are relatively scant; however, a prevalence of 75.7% was recorded in *R. rattus* in Nigeria (Akinboade et al., 1981). Few studies have statistically examined the influence of extrinsic (location, season) or intrinsic (age and gender) factors, or the prevalence of ectoparasite infestations, on *T. lewisi* prevalence. One example found no significant effect of rodent habitat (described as rice fields, upland fields, secondary forests, and domestic habitats) on *T. lewisi* infection in 3 of the 12 rodent species trapped, namely, *Rattus exulans*, *Bandicota savilei*, and *Bandicota indica*, in Thailand (Jittapalapong et al., 2008).

There are also few studies on other *Trypanosoma* species of indigenous rodents and their corresponding flea vectors. One comprehensive study on the interactive effects of extrinsic and intrinsic factors on hemoparasite and ectoparasite infections in indigenous spiny mice (*Acomys dimidiatus*) in Egypt found an overall prevalence of 17.9% for fleas (*Parapulex chephrensis* and *Xenopsylla dipodilli*), 17.5% for trypanosomes (*Trypanosoma acomys*), and 32.1% for 2 *Polyplax* species of lice (Bajer et al., 2006). Fleas and trypanosomes were aggregated in 2 of the 4 wadis (rivers or valleys), and abundance of both these parasites on

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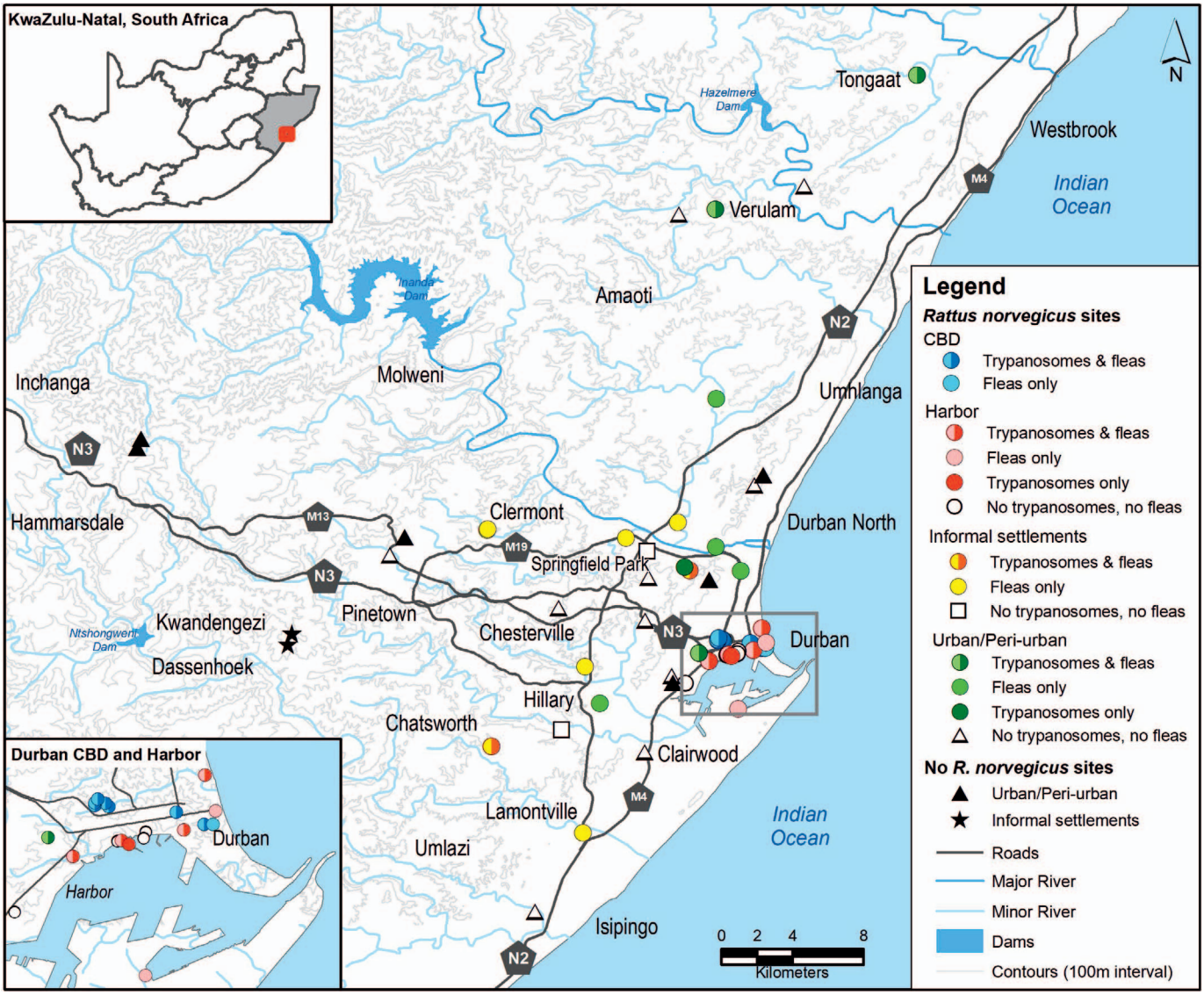

FIGURE 1. Map of eThekwini municipality (Durban) showing the 56 trapping sites, 48 where *Rattus norvegicus* were trapped, and 8 where only by-catches of *R. rattus* and *Mastomys natalensis* were trapped. Inset in top-left corner shows Durban (black or red square) in relation to KwaZulu-Natal province (gray), within South Africa. Inset at bottom-left corner is an expanded view of the harbor and central business district (CBD), to show the separate sites. Color version available online.

spiny mice was significantly affected by site (Bajer et al., 2006). However, studies on factors driving *T. lewisi* infection in *Rattus* spp. within cities in developing countries are lacking.

Building on our comprehensive database of endoparasites of wild rats sampled in 2009 in the port city of Durban, South Africa (Archer et al., 2017), we report here on *T. lewisi*, one of its common cyclical vectors, *X. cheopis*, and its potential arthropod mechanical vectors, including *P. spinulosa*, *Laelaps lamborni*, and *Laelaps echidnina* in *Rattus norvegicus*, the most common rodent captured in Durban. The aim of our study was to investigate the effects of abiotic (location and season) and biotic (rat age, gender, and abundance of 4 ectoparasites) factors on the prevalence and intensity of *T. lewisi* infection in *R. norvegicus*. We predicted that the prevalence and abundance of *T. lewisi* and the arthropod vector(s) in the rats would be interdependent.

MATERIALS AND METHODS

Study locations and seasons

The study area, located in the eThekwini metropolitan area commonly known as Durban, South Africa, was divided into 4 locations: central business district (CBD), harbor (HBR), informal settlements/slums (IS), and urban/peri-urban (U/PU) (Fig. 1). The CBD and HBR form a typical metropolis of high-rise buildings that is densely populated, with an abundant food trade and consequent litter. The IS consist of densely populated shacks and low-cost houses, and U/PU includes formal residences, food shops, markets, a wildlife facility, parks, and waste-water treatment stations. The study spanned 1 yr (2009) and included both wet and dry seasons, with mean temperature/rainfall of 21.8 C/121 mm and 19.1 C/30.9 mm, respectively. For 75% of 2009, humidity was ≥70%. Climate data were provided by weather-

station number 461, Mount Edgecombe, 29°42′0″S, 31°2′0″E, at 96 m above sea level (South African Weather Services).

Sampling of rodents

The Animal Ethics Committee of the University of KwaZulu-Natal (Ref. 031/09/Animal) approved this study, providing that euthanasia was performed according to international ethical guidelines (Gannon et al., 2007). Custom-made Monarch-like live-traps were baited with food scraps, including meat, cereal-based foods, and vegetables, and were set up and collected by eThekwini Health Department's Vector Control Division. This was for safety and logistic reasons, because traps needed to be placed where they would not be stolen, and high crime rates make entering settlements dangerous for researchers. Trapping of rodents was carried out at 56 sites in total; however, *R. norvegicus* individuals were trapped at only 48 of these, with by-catches of *R. rattus* and *Mastomys natalensis* captured at the other 8 sites (Fig. 1). The Durban Natural Science Museum, as part of their own research, identified the rodents from our study using detailed morphometric measurements and genetic confirmation where necessary.

Euthanasia was performed using chloroform, followed by cardiac puncture to obtain blood samples. To prevent carry-over of ectoparasites from one rat to the next, a different brush and comb were used for each animal processed on one day. Afterwards, all implements were thoroughly washed and dried before the next day's cohort of rats was processed.

Each rat was thoroughly dry-combed and brushed to dislodge and remove ectoparasites from the fur. This was done over a tray lined with a white paper towel to easily see ectoparasites. The surface of each body region was systematically and meticulously inspected so as to avoid missing any ectoparasites, and those still clinging to the skin or fur were removed with forceps or by further brushing. Skin lesions were excised using a scalpel. All containers and surfaces where the euthanized rat had been placed were also checked for ectoparasites that may have left the dying host. Brushings from the paper towel were inspected using a stereomicroscope. All ectoparasites collected were placed directly into appropriately labelled 1.5-ml Eppendorf tubes containing 70% ethanol for preservation. Fleas, mites, and lice were separated and stored in appropriately labelled Eppendorf tubes and then prepared and mounted on microscope slides using a modification of the Canada balsam technique (Palma, 1978). This involved cleaning specimens in 10% sodium hydroxide without damaging the exoskeletons, gradual dehydration in increasing strengths of ethanol for 1 hr per concentration, clearing in clove oil, mounting in Canada balsam, and allowing time to dry and set. Sample slides of each parasite were sent to various South African experts for confirmation of identifications.

Thin and thick blood smears were made immediately from blood drawn by cardiac puncture, and serum was harvested and frozen for use by the National Institute of Communicable Diseases (NICD) in Johannesburg, South Africa. Thin blood smears were fixed in 100% methanol, and thick smears were air-dried for 1 hr. The former were stained with May-Grünewald/Giemsa, and the latter were stained with Giemsa (Lynch et al., 1969); samples were then allowed to dry, stored in wooden slide boxes, and examined later using a compound light microscope and 100× oil-immersion objective.

Rodents were weighed, gender and breeding status were recorded, and selected body parameters were measured (Archer et al., 2017), and this information was used to age the rodents. The rats were then dissected; all internal organs were removed, and parasites were harvested for further studies. Feces were collected from the rectum and preserved in 10% formal saline for further examination.

Aging of rodents

Given that the patency period of most of the helminth fauna of rodents is 4–6 wk, we decided to use the age tables of Hirata and Nass (1974) to differentiate among un-weaned pups, weaned juveniles, and fully mature rats. Pups included those <5 wk (females <70 g, males <77 g); juveniles were approximately 5–10 wk, probably weaned and some sexually mature (females <142 g, males <222 g); and adults were all sexually mature and >10 wk old (females >113 g, males >164 g). There was an overlap in mass ranges between each week of age. Thus, at the age of 5 wk, where we separated pups from juveniles, and at 10–11 wk (separation of juveniles from adults), total body length and sexual and breeding status were also used to allocate them to age groups.

Morphological identification of parasites

Trypanosoma lewisi was morphologically identified according to Hoare (1972). Thick and thin smears were examined, and if positive, the degree of infection was recorded based on a scale of 1–4: 1 = light, 2 = moderate, 3 = heavy, and 4 = severe. (However, to simplify reporting, this infection intensity of *T. lewisi* will be termed 'abundance,' as for the ectoparasites.) All thin and thick smears were meticulously examined to ensure no light *T. lewisi* infections were missed and to check for other hemoparasites.

Mounted ectoparasites were identified to species level, counted, and differentiated by gender. Fleas, mites, and lice were identified according to Haeselbarth et al. (1966), Matthee and Ueckermann (2009), and Ledger (1980), respectively. The prevalences of other ectoparasites were ≤6.9% and were thus excluded from this study, because they would not have had any statistical significance.

Statistical analyses

Statistical tests were run in IBM SPSS Statistics for Windows (version 24.0; IBM Corp., Armonk, New York) and R (v. 3.4.2). First, differences in the number of rats caught between locations, seasons, and in age classes and genders were assessed using 2-way ANOVAs and Tukey's post-hoc tests. To assess the associations between parasites and trapping locations, cross-tabulations were run on the prevalence of *T. lewisi* and each of the 4 ectoparasites among the 4 locations, and a Pearson's chi-square test was included to test the null hypothesis that the parasite infections were not dependent on location.

Binary logistic regression (BLR) was used to test the prevalence data. BLR1 consisted of 5 models (a–e) to examine the abiotic (location and season) and biotic (rodent age and gender) factors as predictors of the prevalence of each of the following parasites: (a) *T. lewisi*, (b) *X. cheopis*, (c) *P. spinulosa*, (d) *L. lamborni*, and (e) *L. echidnina*. BLR2 consisted of 2 models, (a) a full model that tested all the abiotic and biotic (including the 4 ectoparasites)

predictor variables on the prevalence of *T. lewisi* as the dependent variable, and (b) the best sub-model that was identified based on the Akaike information criterion (AIC) using the package glmulti (Calcagno, 2013) in R.

Due to the large number of absolute zeros in our database, the count outcome variables were over-dispersed, and so negative binomial regression (NBR) was used to test the abundance data. Instead of using the default dispersion parameter of 1 in SPSS, we chose the estimate option, which allows SPSS to estimate this value. AIC values indicated that the latter option produced better models than the former. NBR1(a) tested the effects of location, season, and rat age and gender, and the abundance of each of the 4 ectoparasites on the abundance of *T. lewisi*, and NBR1(b) included only the significant variables from NBR1(a). NBR2 examined the effects of location, season, and rat age and gender on parasite species richness.

RESULTS

Rodents trapped per location and season

The number of rodents trapped at each location (CBD = 101, HBR = 93, IS = 88, U/PU = 97) and season (wet = 137, dry = 242) were significantly different (2-way ANOVA: $F_{7,378} = 22.136$; $P < 0.001$). A post-hoc Tukey's test showed that significantly more *R. norvegicus* individuals were trapped at CBD than IS ($P < 0.001$) and U/PU ($P = 0.001$), and significantly more rats were trapped during the dry months (n = 242 in 7 mo) than wet months (n = 137 in 5 mo) ($P < 0.001$). One rat (pup) was excluded from the analyses because we were unable to draw blood from it.

Examination of the data

The cross-tabulations showed that 50.6% of *T. lewisi*–positive rats were from CBD, 20% were from HBR, 20% were from U/PU, and 9.4% were from IS. The chi-square test showed that there were significant differences in *T. lewisi* prevalence among locations ($\chi^2 = 35.515$, df = 3, $P < 0.001$). We found that 51.3% of the rats infested with *X. cheopis* were from CBD, 21.9% were from HBR, 16.9% were from IS, and 10% were from U/PU. There were significant differences in the prevalence of fleas among locations ($\chi^2 = 94.791$, df = 3, $P < 0.001$). The percentages of rats with trypanosomiasis and fleas were both predominant in the CBD.

A different picture emerged with the prevalence of *P. spinulosa*. There were significant differences in lice prevalence among locations (chi-square test: $\chi^2 = 42.995$, df = 3, $P < 0.001$), and most were found from IS (46.3%), with much lower prevalence at HBR (25.6%), U/PU (23.2%), and CBD (4.9%).

There were significant differences among locations for *L. lamborni* and *L. echidnina* (chi-square test: $\chi^2 = 25.167$, df = 3, $P < 0.001$ and $\chi^2 = 14.76$, df = 3, $P = 0.002$, respectively). The prevalence of *L. lamborni* was 29.4% at CBD, 26.4% at IS, 23.4% at HBR, and 20.8% at U/PU, whereas *L. echidnina* prevalence was 32.2% at HBR, 32.2% at IS, 22.2% at CBD, and 13.3% at U/PU.

Chi-square results confirmed a strong association between the parasites and location and supported our decision not to run location as a random factor, but rather as an independent, categorical variable in the BLRs.

Prevalence and mean intensity/abundance of parasites

Table I shows the prevalence for each parasite, mean intensity of *T. lewisi*, mean abundance of each ectoparasite, and mean species richness, overall, and for each location, season, rat age, and rat gender. For both *T. lewisi* and its cyclical vector, *X. cheopis*, the highest prevalence had correspondingly higher mean abundance per location, season, age, and gender, except for fleas on pups and juveniles. This pattern was not found for the remaining ectoparasites (Table I).

An assessment of trypanosome- and flea-infected and -uninfected rats showed the following: 51.6% (n = 195/378) of rats had no fleas and no trypanosomes; 25.9% (n = 98/378) of rats had fleas but no trypanosomes; 15.6% of rats (n = 59/378) had both fleas and trypanosomes; and 6.9% (n = 26/378) of rats had trypanosomes but no fleas.

Statistical analyses of prevalence and abundance data

The 5 individual BLR1 models that examined location, season, rat age, and rat gender as predictors for (a) *T. lewisi*, (b) *X. cheopis*, (c) *P. spinulosa*, (d) *L. lamborni*, and (e) *L. echidnina* were all significant. The Hosmer-Lemeshow test showed that the models for *T. lewisi*, *L. lamborni* and *L. echidnina* were a good fit ($P > 0.05$), while that for *X. cheopis* was acceptable, and that for *P. spinulosa* was not a good fit (Tables II, III).

Location had a significant effect on the prevalence of all parasites, except *L. echidnina* ($P = 0.072$; Table II). Odds of CBD rats having trypanosomiasis as opposed to HBR rats were 3.4 (1/0.294), as opposed to IS rats were 6.5 (1/0.155) and as opposed to U/PU rats were 3.6 (1/0.281). Odds of CBD rats harboring fleas were 7.9 times that of HBR rats, 11.9 times that of IS rats, and 23.3 times that of U/PU rats. Odds of HBR rats harboring lice were 8.3 times that of CBD rats, and odds of U/PU rats having lice were 8.9 times that of CBD rats. The prevalence of lice was highest at IS (46.3%), and the odds of these rats harboring lice were 20.8 times that of CBD rats, 2.5 times that of HBR rats, and 2.3 times that of U/PU rats. Odds of rats with *L. lamborni* mites were greater at CBD and IS than HBR and U/PU. Age was significant for *T. lewisi*, *X. cheopis*, and *L. echidnina* models, where odds of having trypanosomiasis, fleas, and *L. echidnina* mites were between 2 to 3 times greater for both pups and juveniles than for adults. Odds of *P. spinulosa* and *L. echidnina* on rats in the wet rather than the dry season were 2.2 and 1.8 times, respectively. These were the only parasites significantly affected by season, and none of the parasites displayed any prevalence associations with gender (Table II).

The best sub-model, BLR2(b), had a lower AIC value than the full model BLR2(a), and in both models, only rat age, *X. cheopis*, and *P. spinulosa* were significant predictors. The goodness of fit statistics for BLR2(b) were: Hosmer-Lemeshow test $\chi^2(8) = 4.852$, $P = 0.773$, AIC = 334.9; cases correctly predicted = 77.5%. The odds of CBD rats having trypanosomes compared to HBR and IS rats were 2.1 times ($P = 0.057$) and 2.4 times ($P = 0.075$), respectively (Table IV).

NBR models showed that *P. spinulosa* abundance had no effect on *T. lewisi* abundance, and there were no significant associations between the abundance of mites and trypanosomiasis. However, location was a significant predictor of trypanosome abundance. The significant results for NBR1(a), NBR1(b), and NBR2 are presented in Table V. Coefficients (B) for each of the predictor

TABLE I. Prevalence (Prev.) and mean infection intensity/abundance (Mean) data for *Trypanosoma lewisi*, *Xenopsylla cheopis*, *Polyplax spinulosa*, *Laelaps lamborni*, and *Laelaps echidnina*; and mean species richness at 4 locations: central business district (CBD), harbor (HBR), informal settlements (IS), and urban/peri-urban (U/PU). These data are also given for season, rodent age, and rodent gender.

	T. lewisi		*X. cheopis*		*P. spinulosa*		*L. lamborni*		*L. echidnina*		Parasite
Variables	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	species richness, mean ± SD
Overall	22.5	0.60 ± 1.25	42.2	3.26 ± 7.83	21.6	1.83 ± 6.99	79.9	13.25 ± 21.22	23.7	0.69 ± 2.07	1.89 ± 1.05
Location											
CBD	43.0	1.20 ± 1.59	81.2	7.19 ± 10.68	4.0	0.06 ± 0.34	88.1	19.15 ± 29.13	19.8	0.32 ± 0.71	2.35 ± 0.85
HBR	18.3	0.56 ± 1.28	37.6	4.06 ± 9.51	22.6	0.94 ± 2.47	76.3	12.08 ± 17.59	31.2	1.49 ± 3.61	1.86 ± 1.01
IS	9.1	0.24 ± 0.83	30.7	1.05 ± 2.27	43.2	4.22 ± 8.31	90.9	16.28 ± 20.76	33.0	0.81 ± 1.65	2.07 ± 1.16
U/PU	17.5	0.36 ± 0.89	16.5	0.41 ± 1.23	19.6	2.35 ± 10.68	64.9	5.47 ± 9.68	12.4	0.20 ± 0.62	1.29 ± 0.88
Season											
Wet	16.8	0.44 ± 1.08	42.3	3.37 ± 8.41	32.1	3.58 ± 10.31	83.9	11.99 ± 16.80	33.6	1.24 ± 3.01	2.07 ± 1.09
Dry	25.7	0.70 ± 1.33	42.1	3.20 ± 7.50	15.7	0.83 ± 3.72	77.7	13.96 ± 23.35	18.2	0.38 ± 1.16	1.79 ± 1.02
Age											
Pups	18.1	0.53 ± 1.26	50.0	3.42 ± 6.48	31.1	2.90 ± 7.44	81.1	13.83 ± 17.50	31.1	0.70 ± 1.56	2.11 ± 1.17
Juveniles	30.9	0.88 ± 1.45	47.1	4.70 ± 9.82	16.9	1.90 ± 9.17	81.6	11.85 ± 21.82	25.7	1.05 ± 3.02	2.01 ± 1.02
Adults	14.6	0.30 ± 0.83	28.5	1.73 ± 6.45	20.3	1.02 ± 2.93	75.6	11.98 ± 21.09	16.3	0.31 ± 0.84	1.54 ± 0.87
Gender											
Females	22.5	0.61 ± 1.25	41.0	3.15 ± 7.92	20.2	1.26 ± 3.69	78.2	10.15 ± 17.29	25.0	0.68 ± 1.98	1.86 ± 1.04
Males	20.9	0.55 ± 1.22	42.4	3.51 ± 8.02	24.3	2.56 ± 9.45	80.8	14.92 ± 22.99	23.2	0.72 ± 2.23	1.90 ± 1.06

variables (including dummy variables) were all positive, except for *P. spinulosa*. Results for predictors with dummy variables, e.g., location, are interpreted as follows. NBR1(a): Compared to HBR, the expected log count of CBD increased by 1.136 (B-value); compared to adults, the expected log count of juveniles increased by 1.025; and for the continuous scale result for the covariate *X. cheopis*, for each 1-unit increase in *X. cheopis* mean abundance, the expected log count of the abundance of *T. lewisi* increased by 0.045. NBR2 produced a negative B-value for *P. spinulosa*; i.e., for each 1-unit increase in the abundance of *P. spinulosa*, the expected log count of *T. lewisi* abundance decreased by 0.079.

The incident rate ratios (IRRs) for the same examples showed that the incident rate of a higher abundance of *T. lewisi* at CBD was 3.1 times that for the reference group (HBR), holding all other variables constant; the incident rate of a higher abundance of *T. lewisi* for juveniles was 2.8 times that for the reference group (adults). Each positive variable's contribution to the model can be read in the same way, and for *P. spinulosa*, the IRR is interpreted as follows: The percent change in abundance of *T. lewisi* is a 0.9% decrease for every 1-unit increase in *P. spinulosa* abundance. Rats at CBD, HBR, and IS had higher incidence rates of the greatest number of parasites (parasite species richness), as did pups and juveniles compared to adult rats. All the significant parameters are shown in Table V and can be interpreted as described above.

DISCUSSION

We found that in the port city of Durban, *T. lewisi* prevalence in *R. norvegicus* had a significant positive association with *X. cheopis*, a negative association with *P. spinulosa*, as well as a significant association with rat age, with younger rats more likely to be infected with *T. lewisi* (and *X. cheopis*). Further, *X. cheopis* abundance, rat age, and location were significant predictors of trypanosome abundance. A similar situation was reported in Egyptian spiny mice (*A. dimidiatus*), where trypanosome infections were 3–4 times higher in flea-infested than non-infested mice. Prevalence of fleas peaked in mice from age class 2 (which corresponds to our juveniles) and then decreased in the oldest age group (Bajer et al., 2006). To the best of our knowledge, there are no comprehensive studies on *T. lewisi* and its flea vectors in *Rattus* spp. across varying habitat types within large cities. However, there are studies that incorporated season, rodent age, and rodent gender. Perhaps the most comparative study in this regard was in Brazil, where *R. norvegicus* individuals were trapped at dumps in the Belo Horizonte municipality (Linardi and Botelho, 2002). Consistent with our results, overall *T. lewisi* prevalence was 21.7% (n = 93/429; cf. 22.5% in Durban), and the highest prevalence of *T. lewisi* coincided with the greatest *X. cheopis* infestations. Further, there were significantly more infected young (29.3%) and immature (27.1%) rats than adults (8.8%), similar to our study (pups 18.1%, juveniles 30.9%, and adults 14.6%). Significantly more rats were infected in the cooler, dry months than the rainy, wet months, and significantly more males than females were infected (Linardi and Botelho, 2002), whereas we found no significant differences in seasons and genders. Conversely, *T. lewisi* prevalence in *R. norvegicus* in Memphis, Tennessee, was higher in winter than summer, yet, similar to Durban rats, prevalence was significantly higher in rats <200 g, with very few rats >300 g infected (Eyles, 1952). A recent Egyptian study on associations between fleas and trypanosomes in *Rattus* spp. on farms and houses in rural areas reported an overall prevalence of 24.7%, no significant difference in infection between genders, and also a significant inverse correlation between parasite load and host age (Danesh and Mikhail, 2016). This common trend, where *T. lewisi* declines as the rat ages, is due to the development of immunity when the host

TABLE II. Significant results of binary logistic regressions (BLRs) for each parasite as dependent variable (BLR1a–BLR1e), with location, season, and rat age as predictor variables. Odds ratios (OR), 95% confidence intervals (CI), and *P*-values are given. Abbreviations: central business district (CBD), harbor (HBR), informal settlements (IS), and urban/peri-urban (U/PU), reference category (ref.).

			95% CI for OR		
BLR; Parasite	Significant variable	OR	Lower	Upper	*P* value
1(a) *Trypanosoma lewisi*	Location CBD (ref.)	1			
	HBR	0.294	0.143	0.602	0.001
	IS	0.155	0.066	0.367	<0.001
	U/PU	0.281	0.140	0.564	<0.001
	Rat age Juveniles (ref.)	1			
	Adults	0.352	0.178	0.694	0.003
1(b) *Xenopsylla cheopis*	Location CBD (ref.)	1			
	HBR	0.126	0.062	0.257	<0.001
	IS	0.084	0.040	0.177	<0.001
	U/PU	0.043	0.020	0.094	<0.001
	HBR (ref.)	1			
	U/PU	0.341	0.166	0.702	0.004
	Rat age Adults (ref.)	1			
	Pups	2.774	1.410	5.458	0.003
	Juveniles	2.625	1.398	4.929	0.003
1(c) *Polyplax spinulosa*	Location CBD (ref.)	1			
	HBR	8.293	2.326	29.564	0.001
	IS	20.749	6.010	71.637	<0.001
	U/PU	8.914	2.493	8.914	0.001
	IS (ref.)	1			
	HBR	0.400	0.199	0.803	0.010
	U/PU	0.430	0.212	0.872	0.019
	Season Wet (ref.)	1			
	Dry	0.456	0.257	0.809	0.007
1(d) *Laelaps lamborni*	Location CBD (ref.)	1			
	HBR	0.398	0.176	0.898	0.026
	U/PU	0.265	0.123	0.574	0.001
	IS (ref.)	1			
	HBR	0.285	0.116	0.699	0.006
	U/PU	0.190	0.079	0.457	<0.001
1(e) *Laelaps echidnina*	Season Wet (ref.)	1			
	Dry	0.556	0.331	0.934	0.027
	Rat age Adults (ref.)	1			
	Pups	2.260	1.143	4.470	0.019
	Juveniles	1.921	1.004	3.676	0.049

TABLE III. Goodness-of-fit statistics for binary logistic regressions (BLR1a–BLR1e) in Table II. Table also shows Akaike information criterion (AIC).

		Hosmer-Lemeshow test			
BLR1; Parasite	AIC	χ^2 (8)	*P*	Cases correctly classified (%)	*P* value of model
(a) *Trypanosoma lewisi*	353.07	6.204	0.624	78.6	<0.001
(b) *Xenopsylla cheopis*	400.59	13.468	0.097	74.5	<0.001
(c) *Polyplax spinulosa*	340.43	27.382	0.001	83.0	<0.001
(d) *Laelaps lamborni*	358.64	2.335	0.969	79.2	<0.001
(e) *Laelaps echidnina*	390.97	8.709	0.367	75.9	<0.001

TABLE IV. Binary logistic regressions BLR2(b), with location, rat age, *Xenopsylla cheopis*, and *Polyplax spinulosa* as predictors of *Trypanosoma lewisi* in rats. Abbreviations: central business district (CBD), harbor (HBR), informal settlements (IS), odds ratio (OR), confidence intervals (CI), reference category (ref.).

			95% CI for OR		
BLR2(b)	Significant variable	OR	Lower	Upper	*P* value
T. lewisi	Location CBD (ref.)	1			
	HBR	0.480	0.225	1.023	0.057
	IS	0.421	0.162	1.092	0.075
	Rat Age Juveniles (ref.)	1			
	Pups	0.488	0.244	0.976	0.043
	Adults	0.446	0.228	0.871	0.018
	Absence of fleas (ref.)	1			
	Xenopsylla cheopis	3.022	1.566	5.830	0.001
	Presence of lice (ref.)	1			
	Polyplax spinulosa	4.719	1.580	14.096	0.005

produces IgM antibodies to surface antigens on the trypanosomes, resulting in lysis of the flagellates in the blood of the mature host, with consequent immunity to challenge infections (Linardi and Botelho, 2002).

This study is the first to investigate whether lice or mites act as mechanical vectors in wild *Rattus* spp. We found no statistical associations between mites and the prevalence or abundance of *T. lewisi* in rats. Conversely, there was a significant negative association between rats with trypanosomes and lice (the odds of louse-free rats having *T. lewisi* were 4.7 times that of louse-infected rats), and this association was strongly related to location, as the odds of rats at IS with this ectoparasite were 20.8 times that of rats at CBD (where fleas and trypanosomes were most prevalent), and it was also strongly related to age, with lice more abundant on pups.

Taken together, the ecologies of *R. norvegicus* and its ectoparasites may explain the findings of our study. *Rattus norvegicus* is known to be synanthropic (Tufty, 1966) and more common in areas where poor communities reside, buildings are poorly constructed or in disrepair, and the environment is unsanitary (Donaldson, 1925; Jassat et al., 2013). In support, large numbers of rats were frequently trapped where these conditions existed, particularly at the CBD (Archer et al., 2017). *Xenopsylla cheopis* is also more common in commercial than residential areas, especially where foods like cereals are handled or stored (Cole and Koepke, 1946; Pollitzer, 1954; this study). Only adult fleas feed on blood, and they leave the host to breed, which they often do in rats' burrows (Briscoe, 1956), and in suitable feeding sites like cereal and grain stores, where cereal debris provides an ideal substrate for the development of their young (Pollitzer, 1954). This could explain why juvenile rats, which are likely the most active age group, may be at higher risk to become infested with fleas while foraging. Further, *T. lewisi* infection is often relatively common in rodents inhabiting highly built-up human settlements such as the CBD (Pumhom et al., 2013; this study). Given that the home ranges of synanthropic rats are relatively small (Davis et al., 1948), and trapping sites at the CBD and HBR were more closely set together than trapping sites at U/PU and IS (Fig. 1), CBD rats were probably more likely to interact and infect other CBD and HBR rats with *T. lewisi* and *X. cheopis* than rats at U/PU and IS. By contrast, lice are obligatory ectoparasites that live, breed, and feed on their hosts, and they spread by direct contact (Ledger, 1980). We propose that this probably explains why pups, which have close contact with their own mothers, as well as other nursing females in the nest, had the highest abundance of lice in this study. However, the inverse relationship between lice and *T. lewisi* has not been previously reported, and it remains a question that requires further research.

We concede that the standard approach to identify parasite and vector inter-relationships, would be to dissect each arthropod and

TABLE V. Significant results from negative binomial regression models (NBRs), with *Trypanosoma lewisi* abundance (NBR1) and parasite species richness (NBR2) as dependent variables, and the following as predictors: NBR1(a)—location, season, rat age, and rat gender, abundance of 4 ectoparasites; NBR1(b)—location, rat age, abundance of *Xenopsylla cheopis*; and NBR2—location, season, rat age, and rat gender. Abbreviations: Akaike information criterion (AIC); the coefficient estimate of the model (B); incidence rate ratio (IRR); lower to upper confidence intervals (CI [l–u]); juveniles (Juv.); central business district (CBD); harbor (HBR); informal settlements (IS); urban/peri-urban areas (U/PU). Significant variables: first dummy variable is reference category, e.g., within location, CBD is compared with HBR, and written 'Location HBR (ref.)/CBD.'

Model no. and parasite (AIC)	Significant variables	B	IRR	95% CI for IRR (l–u)	*P* value
1(a). *T. lewisi* (666.20)	Location HBR (ref.)/CBD	1.136	3.113	1.436–6.749	0.004
	Location IS (ref.)/CBD	1.323	3.755	1.634–8.627	0.002
	Location U/PU (ref.)/CBD	0.884	2.421	1.079–5.432	0.032
	Rat age Adults (ref.)/Juv.	1.025	2.788	1.398–5.556	0.004
	X. cheopis abundance	0.045	1.047	1.002–1.093	0.038
1(b). *T. lewisi* (662.95)	Location HBR (ref.)/CBD	1.031	2.804	1.353–5.812	0.006
	Location IS (ref.)/CBD	1.488	4.430	2.018–9.726	<0.001
	Location U/PU (ref.)/CBD	0.947	2.577	1.217–5.461	0.013
	Rat age Pups (ref.)/Juv.	0.661	1.937	0.993–3.778	0.052
	Rat age Adults (ref.)/Juv.	1.051	2.860	1.480–5.525	0.002
2. Parasite species richness (1083.22)	Location U/PU (ref.)/CBD	0.554	1.740	1.393–2.174	<0.001
	Location U/PU (ref.)/HBR	0.290	1.337	1.052–1.698	0.017
	Location U/PU (ref.)/IS	0.401	1.493	1.176–1.897	0.001
	Location HBR (ref.)/CBD	0.264	1.302	1.058–1.602	0.013
	Rat age Adults (ref.)/Pups.	0.248	1.281	1.043–1.573	0.018
	Rat age Adults (ref.)/Juv.	0.272	1.313	1.083–1.591	0.006

examine for the presence of *T. lewisi* life stages. Unfortunately, we could not investigate this because the ectoparasites were used for a separate study by Hope (2011). Moreover, the unambiguous identification of endo- and ectoparasites of rats has significant implications for parasitologists and vector biologists. Thus, identification based on both morphology and genetic markers should be an integral part of future studies investigating the pathogens and their vectors carried by urban rats.

To conclude, this study highlights the inter-dependence of the well-established trypanosome-flea vector cycle parasitizing *R. norvegicus*, particularly at the CBD of Durban. There are no reports of *T. lewisi* from humans in South Africa. However, there is 1 report from Gambia, North Africa (Howie et al., 2006), as well as a number of documented cases in India and Asia (Truc et al., 2013). It is important to note that the flea vector *X. cheopis* is also the vector of plague (Bitam et al., 2010) and intermediate host for the tapeworm *H. diminuta* (Smit, 1973). Durban and other densely populated African cities all experience the same problems of harborage, unsanitary environments, and plentiful rodent food, conditions that are highly favorable for synanthropic rats (Taylor et al., 2008). If vigorous steps are not taken to clean up the city and rid it of invasive *Rattus* spp., the result could pose a threat to public health (Jassat et al., 2013).

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