esa

ECOSPHERE

DISEASE ECOLOGY

Coinfection modifies carriage of enzootic and zoonotic parasites in Norway rats from an urban slum

Ticiana S. A. Carvalho-Pereira,[1,2,3,]† Fábio Neves Souza,[1,2] Luana Ribeiro do Nascimento Santos,[2] Gabriel Ghizzi Pedra,[3] Amanda Minter,[3] Thiago Campanharo Bahiense,[1] Mitermayer Galvão Reis,[1,2,4] Albert Icksang Ko,[4] James E. Childs,[4] Eduardo M. da Silva,[1] Federico Costa,[1,2,3,4] and Mike Begon[3]

[1]*Instituto de Biologia, Instituto de Ciências da Saúde, Faculdade de Medicina, Instituto de Saúde Coletiva, Federal University of Bahia (UFBA), Salvador, Brazil*

[2]*Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde, Salvador, Brazil*

[3]*Institute of Integrative Biology, University of Liverpool, Liverpool, UK*

[4]*Department of Epidemiology of Microbial Disease, Yale School of Public Health, New Haven, Connecticut, USA*

Citation: Carvalho-Pereira, T. S. A., F. N. Souza, L. R. N. Santos, G. G. Pedra, A. Minter, T. C. Bahiense, M. G. Reis, A. I. Ko, J. E. Childs, E. M. da Silva, F. Costa, and M. Begon. 2019. Coinfection modifies carriage of enzootic and zoonotic parasites in Norway rats from an urban slum. Ecosphere 10(10):e02887. 10.1002/ecs2.2887

Abstract. We examine coinfection between helminth species and the bacterium *Leptospira interrogans* in a natural population of the Norway rat, *Rattus norvegicus*. We ask whether coinfection can influence the probability and intensity of infection of these enzootic and zoonotic parasites in urban rats, which may affect the loads of parasites excreted into the environment. Rodent sampling was carried out during two seasons in 2014 in a Brazilian urban slum. We sampled rats' feces, kidney imprints, and urine to identify and quantify helminth eggs/larvae and infection by *L. interrogans*. Eleven species/groups of helminths and *L. interrogans* were identified among 299 captured rats. Simple correlation tests and generalized linear models were performed to identify general patterns of coinfection and potential direction of effects, respectively, after controlling for consolidated environmental and host biotic variables. Significant associations were illustrated in an interaction network. Focusing on parasites with the potential to cause zoonoses among humans, we observed that coinfection between *L. interrogans* and the nematode *Angiostrongylus cantonensis* was significantly more frequent than expected. Reduced prevalence of *A. cantonensis* was found in the presence of *Nippostrongylus brasiliensis*, and *N. brasiliensis* intensities (eggs per gram of feces) were increased with greater intensities of both *L. interrogans* and *Strongyloides* sp., the latter of which was, in turn, found to increase the intensities of *A. cantonensis*. A higher probability of finding *L. interrogans* was associated with infection by *Strongyloides* sp. Our study provides a novel perspective on evaluating helminth coinfection profiles in populations of naturally infected urban rats, moving beyond previous studies which have been limited to descriptions of co-occurrence. Noticeably, infection risk was dependent on coinfection and this should be accounted for when targeting the control of zoonotic pathogens in natural populations.

Key words: *Angiostrongylus cantonensis;* coinfection; helminths; interaction network; *Leptospira interrogans; Nippostrongylus brasiliensis;* Norway rats; *Rattus norvegicus; Strongyloides* sp.; urban slums; zoonotic diseases.

Received 22 March 2019; revised 6 August 2019; accepted 7 August 2019. Corresponding Editor: Leah R. Johnson.

† **E-mail:** carvalhopereira.tsa@hotmail.com

Introduction

In the past, communities of helminths in natural host populations were rarely considered to be more than randomly constructed assemblages (Behnke et al. 2001, Poulin 2001). However, after Lello et al. (2004) showed patterns of association among helminths in a cross-sectional study of a natural population of rabbits, interest increased in the ecological concepts needed to understand the communities of parasites within a host (Pedersen and Fenton 2007, Graham 2008, Fenton et al. 2010). This growing interest reflects a need for fundamental knowledge about the complexity of ecological interactions between parasites within natural host populations (Beldomenico and Begon 2010, Telfer et al. 2010). In addition, understanding parasites' coinfection profiles can potentially improve understanding of disease dynamics, since coinfection can, in particular cases, reduce the potential of a host to transmit a parasite or create super-shedders (Jolles et al. 2008, Lass et al. 2012). Understanding patterns of coinfection is particularly important when considering zoonotic pathogens, since there may be implications for human risk of infection. Simple correlation analyses can detect positive or negative association between parasites, irrespective of a direction of effect. However, further statistical analyses are generally necessary, which can suggest the occurrence and direction of the interaction with much greater confidence, while accounting for possible confounders (Fenton et al. 2010).

Here, we examine coinfection in a natural population of the Norway rat, *Rattus norvegicus* Berkenhout (1769). It is one of the major reservoir hosts for the bacteria *Leptospira*, the etiological agent of leptospirosis. Globally, leptospirosis affects more than 1,000,000 people per year, with approximately 60,000 deaths (Costa et al. 2015*a*, Torgerson et al. 2015). Norway rats are ubiquitous worldwide (Singleton et al. 2003); furthermore, urban slum areas of developing countries present favorable environments for resources and shelter (Costa et al. 2014*a*). Due to rats' peridomestic habit, humans, pets, and sylvatic species are at risk for spillover infection, given that *Leptospira* bacteria are shed through rats' urine into the environment. According to Costa et al. (2015*b*), an urban slum population of around 100 rats can shed more than a billion *Leptospira* bacteria per day, and rats in poor body condition (assessed by the number of wounds) carry significantly higher concentrations of *Leptospira* in their urine.

Moreover, Norway rats can harbor several other human pathogens, in isolation or concomitantly, including helminths (Alicata 1965, Hancke et al. 2011, Costa et al. 2014*b*, Carvalho-Pereira et al. 2018). Among the helminth community of Norway rat populations, nematodes account for the highest number of species (Kataranovski et al. 2010, Hancke et al. 2011, Simões et al. 2016). Generally, nematode species have whole blood and epithelial cells as their source of nutrition (Weinstein and Jones 1956, Archer et al. 2011, Klementowicz et al. 2012, Viney and Kikuchi 2017) and can reduce rat body condition through anemia and tissue damage (Garcia et al. 2014, Viney and Kikuchi 2017). More specific body condition loss can also occur. For example, laboratory rats were found to be anorexic or to present allergenic-inflammatory-related processes after infection by *Nippostrongylus brasiliensis* and *Strongyloides venezuelensis*, respectively (Mercer et al. 2000, Silveira et al. 2002). Thus, by reducing body condition and, further, affecting the immune system, helminth species can potentially facilitate or hamper the occurrence of other species. One question, then, arises: Does coinfection with different parasite species influence the load (occurrence and intensity) of pathogens, including zoonotic pathogens, in wild populations of rats?

In an urban slum of Brazil, we evaluated whether coinfection of Norway rats with helminth species and *Leptospira interrogans* is associated with the probability and intensity of infection of individually assessed parasite species. Specifically, we asked (1) whether helminth species themselves are associated with the presence and intensity of infection by other helminth species in rats, which may influence the excretion load of zoonotic helminths into the environment; and (2) whether helminth species alter the load of *L. interrogans* in rats which could affect the urinary load of leptospires shed into the environment. We investigate these associations by building an interaction network of probability and intensity of species infections, thus moving

beyond previous studies in rats which have been limited to descriptions of co-occurrence (Kataranovski et al. 2010, Hancke et al. 2011, Simões et al. 2016). We believe that an integrative approach, which accounts for environmental and rat demographic and body condition variables to define patterns of helminth and *L. interrogans* coinfection among Norway rats, is necessary for improving our ability to predict parasitic associations in the field and, consequently, for providing tools for parasite management practices.

Materials and Methods

Sampling design and data collection

The sampling design has been reported previously (Panti-May et al. 2016, Carvalho-Pereira et al. 2018). Briefly, it consisted of 101 trapping points randomly selected in an area located in the suburban neighborhood of Pau da Lima (13°32′53.47″ S; 38°43′51.10″ W) in the city of Salvador (BA, Brazil). The area is 0.17 km^2 in extent and a large, densely packed human population resides within the three valleys which constitute this favela community. Common qualities of favelas or slums include a high density of urban residents, substandard housing conditions (e.g., temporary constructions, often with tin roofs and dirt floors), and inadequate sanitary infrastructure (e.g., open sewers and lack of garbage collection; Reis et al. 2008, IBGE 2010). This area has a high annual incidence of severe leptospirosis cases (19.8 per 100,000 pop.) and asymptomatic or mild human *Leptospira* infection, as determined by serosurveys (35.4 per 1,000 pop.; Felzemburgh et al. 2014, Hagan et al. 2016). Residents most often acquire leptospirosis from exposures to environments contaminated with spirochetes shed in the urine from infected Norway rats. These same environments harbor pathogenic helminths excreted in rat feces.

Norway rats were trapped using Tomahawk cages (45 × 16 × 16 cm) placed at each of the 101 points over two periods in 2014, one in the rainy and one in the dry season, with a sampling effort of 2318 and 1494 trap-nights, respectively (Carvalho-Pereira et al. 2018). Animal handling and the collection of environmental and biotic variables (including demographic and body condition variables of rats) followed protocols previously described and validated (Glass et al. 1988, Mills et al. 1995, Costa et al. 2014*a*, *b*, 2015*b*; see Appendix S1: Table S1). The demographic features of the Pau da Lima rat population have been described by Carvalho-Pereira et al. (2018).

Rat feces were collected directly from the intestines at necropsy and placed in 10% formalin for subsequent identification and quantification of helminth species eggs, applying Hoffman et al.'s (1934) sedimentation and Gordon and Whitlock's (1939) flotation techniques (using $ZnSO_4$ saline solution with density of 1.2 g/cm^3). Urine was collected to identify and quantify *L. interrogans* by quantitative real-time PCR (qPCR; Costa et al. 2015*b*). In cases of an absence of enough urine (<400 μL), immunofluorescence staining of kidney impressions—longitudinal cut surface of a kidney printed upon a poly-L-lysine-coated glass slide—was used, following procedures fully described in Chagas-Junior et al. (2009). Hence, results were obtained for the presence of helminth species and *L. interrogans*, and for their intensity, measured as reproductive potential of pathogens, as determined by the enumeration of eggs per gram of feces (EPG) for helminths and genome equivalents (GEq) for leptospires. This study was approved by the Ethical Committee of the Animal Use (CEUA) protocol 003/2012 of the CPqGM–Oswaldo Cruz Foundation (Fiocruz).

Statistics

We sought, first, to approach the relationship between parasite species by simple correlation tests, to link our work to numerous purely correlational studies in the literature (Hancke et al. 2011, Simões et al. 2016) and highlight the contrast. In a second approach, we pursued the identification of potential causal effects between species' probabilities of occurrence and intensities of infection using statistical generalized linear models (GLM). A flowchart of both approaches, which are briefly detailed below, can be found in Fig. 1.

To assess whether a pair of parasite species co-occurred more often than expected, we compared observed with expected frequencies of coinfection by contingency chi-squared tests (Fig. 1A). To further verify simple relationships between intensities of infections, we used *t* tests and additional Pearson's correlation tests, to first identify whether the mean intensities were different in cases of co-occurrence and, if significant,

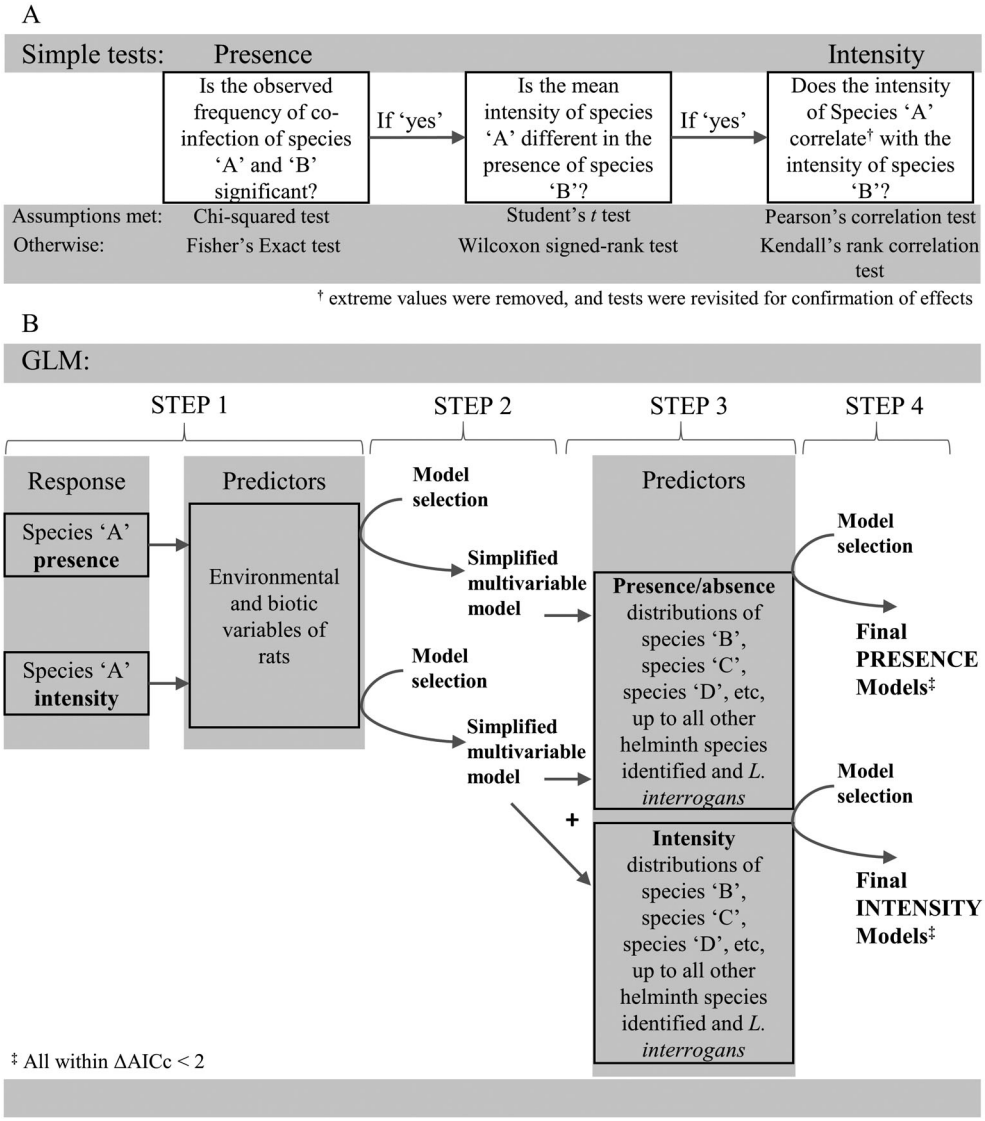

Fig. 1. Flowcharts of the statistical approaches. (A) Steps for identification of simple associations of presence and intensity among helminth species and *Leptospira interrogans*. (B) Diagram of generalized linear model steps for identification of additional coinfection effects on the probability and intensity of infection of a parasite species —helminths or *L. interrogans*—modeled individually.

identify the nature (positive or negative) of the simple association, respectively. Nonparametric tests were used if test assumptions were not met. For ease of interpretation, species intensity EPG and GEq data were log2-transformed for the simple correlation tests and for all the subsequent GLM analyses. Results of the correlation tests did not condition the inclusion of any parasite species as a predictor in the GLMs.

To assess potential causal effects between species' probabilities of occurrence and intensities of infection, we used GLMs, with helminth species or *L. interrogans* probability or intensity of infection, individually, as response variables. After investigating the effects of environmental and host biotic variables, optimizing models in stages (for full explanation, see Carvalho-Pereira et al. 2018), we determined whether coinfection would

contribute additional independent explanatory power to the models (Fig. 1B).

To investigate the risk factors associated with the probability (dependent binary variable) and intensity (dependent continuous variable with only positive values) of infection of helminth species individually, GLMs with binomial or gamma errors, respectively, were applied (Crawley 2007). The same was done for *L. interrogans* probability of occurrence and intensity of infection but using a linear regression model in the intensity analysis, due to the nature of the GEq distribution. Among the environmental and biotic variables of rats (see Appendix S1: Table S1), variables with a P value of below 0.1 in univariable analyses were added to the multivariable analyses, subsequently performed (Fig. 1B: STEP 1). Model simplifications were conducted using a threshold Akaike information criterion value of 2, corrected for small samples (AIC_c) (Hurvich and Tsai 1989; Fig. 1B: STEP 2). These steps generated simplified multivariable models, which potentially included environmental predictors and demographic and body condition features of the rats. Then, coinfection variables were added to the simplified models of each helminth species and *L. interrogans* probability or intensity of infection (Fig. 1B: STEP 3), to check for model improvement. Model selections were re-conducted (Fig. 1B: STEP 4), and we discuss all the models for each species' probability and intensity of infection which were within $\Delta AIC_c < 2$ compared to the final best-AIC_c models (indistinguishable explanatory power), using the function model.sel() of the R package MuMIn. We used the ratios of Akaike's weights, and so created conditional probabilities for each model, allowing us to determine the strength of support for one model over another and ensure robustness of conclusions (Wagenmakers and Farrell 2004). We illustrate the odds ratios (ORs) and the rates (antilog2 of the beta [β] coefficients for helminths' intensity models) or simply β coefficients (for the *Leptospira* intensity model) of the coinfection associations present in the most plausible ($\Delta AIC_c < 2$) binomial and gamma or lm models, respectively, in an interaction network. Observations with missing values for any of the variables under evaluation were excluded in all the models performed here. Analyses were performed in R 3.3.1 (R Development Core Team 2011), for which we applied a significance level of $P < 0.05$.

Results

We caught 299 rats from which we identified 11 species/groups of enzootic and zoonotic helminths (Carvalho-Pereira et al. 2018). The total prevalence of *L. interrogans* was high (71%). Of the possible simple associations, observed coinfection between *L. interrogans* and the nematode *Angiostrongylus cantonensis,* another human pathogen, was significantly more frequent than expected ($\chi^2 = 7.06$, df = 1, $P < 0.01$), whereas that between *A. cantonensis* and *N. brasiliensis* was less frequent, although this association was marginal ($\chi^2 = 3.05$, df = 1, $P = 0.08$). However, there was no difference in the mean intensities of *A. cantonensis* when *L. interrogans* was present or absent and vice versa. Neither were there differences among mean intensities when considering the marginal simple association between *A. cantonensis* and *N. brasiliensis*. Because *Strongyloides* sp. was present in 97% of the rats (Carvalho-Pereira et al. 2018), we investigated whether its mean intensity varied in the presence or absence of other parasite species, regardless of the simple association tests for presence. We found that the intensity of *Strongyloides* sp. was positively correlated with the intensity of *N. brasiliensis* ($\tau = 20$, $P < 0.01$, Kendall's rank correlation), both soil-transmitted helminth species.

The summaries of model selections for the probabilities and intensities of infection of each helminth species and of *L. interrogans* are provided in Table 1. Generalized linear models for *Hymenolepis* spp., helminths of the family Trichuridae, and *Gongylonema neoplasticum* did not include any significant coinfection variables and were not pursued further (but see Carvalho-Pereira et al. 2018). It is noteworthy that we could only perform qPCR for the urine samples of 162 out of the 299 animals, and, therefore, *L. interrogans* intensity associations, when significant, were considered for smaller sample sizes in the models. The details of final models, including variables of coinfection, are available in Appendix S1: Table S2, with main predicted effects shown in Figs. 2, 3.

After accounting for other factors (environmental and biotic variables of the rats), the

Table 1. Summary of model performances in explaining the probability and intensity of infection of each helminth species and *Leptospira interrogans*.

Parasite†	Models	AIC_c	ΔAIC_c	wi
Angiostrongylus cantonensis‡	**i. *y* ~ Valley + Sex + Maturity + Smi + *N. brasiliensis* infection + *Hymenolepis* spp. infection + Sex:Valley + Sex:*Hymenolepis* spp. infection**	**355.14**	**0.00**	**0.95**
	ii. $y \sim$ Valley + Sex + Maturity + Smi + Sex:Valley	361.09	5.96	0.05
	iii. $y \sim 1$	392.67	37.53	0.00
Nippostrongylus brasiliensis‡	**i. *y* ~ Season + Trails + Sex + Age(d) + Age^2(d) + Sex:Trails + Sex:Age(d)**	**368.59**	**0.00**	**0.79**
	ii. $y \sim$ Season + Trails + Sex + Age(d) + Sex:Trails + Sex:Age(d)	371.24	2.65	0.21
	iii. $y \sim$ Season + Trails + Sex + Sex:Trails	378.95	10.37	0.00
	iv. $y \sim 1$	403.5	34.91	0.00
Strongyloides sp.‡	**i. *y* ~ Burrows (*n*) + Sewer + Sex + Smi + Age(d) + Age^2(d) + *L. interrogans* infection + Sex:Sewer**	**79.57**	**0.00**	**0.67**
	ii. *y* ~ Burrows (*n*) + Sewer + Sex + Smi + Age(d) + Age^2(d) + Sex:Sewer	**80.99**	**1.41**	**0.33**
	iii. $y \sim 1$	89.63	10.06	0.00
Leptospira interrogans‡	**i. *y* ~ Age(d) + *Strongyloides* sp. infection**	**281.50**	**0.00**	**0.76**
	ii. $y \sim$ Age(d)	283.76	2.26	0.24
	iii. $y \sim 1$	360.14	78.65	0.00
A. cantonensis§	**i. log2(*y*) ~ Cumulative rain + Tree (*n*) + Burrows (*n*) + Sex + Smi + *Strongyloides* sp. intensity + Sex:Cumulative rain + Sex:Burrows (*n*)**	**493.80**	**0.00**	**0.69**
	ii. log2(*y*) ~ Cumulative rain + Tree (*n*) + Burrows (*n*) + Sex + Smi + Sex:Cumulative rain + Sex:Burrows (*n*)	**495.42**	**1.62**	**0.31**
	iii. $\log2(y) \sim 1$	502.96	9.16	0.00
N. brasiliensis§¶	**i. log2(*y*) ~ Rat faeces + Valley + Interval-sampling-analysis + Fat presence + *Strongyloides* sp. intensity + *A. cantonensis* intensity**	**408.30**	**0.00**	**0.47**
	ii. log2(*y*) ~ Rat faeces + Valley + Interval-sampling-analysis + Fat presence + *Strongyloides* sp. intensity	**409.14**	**0.85**	**0.31**
	iii. $\log2(y) \sim$ Rat faeces + Valley + Interval-sampling-analysis + Fat presence	409.83	1.54	0.22
	iv. $\log2(y) \sim 1$	434.99	26.70	0.00
	i. log2(*y*) ~ Rat faeces + Valley + Interval-sampling-analysis + Fat presence + Sex + *L. interrogans* intensity	**256.62**	**0.00**	**0.53**
	ii. log2(*y*) ~ Rat faeces + Valley + Interval-sampling-analysis + Fat presence	**257.24**	**0.63**	**0.39**
	iii. $\log2(y) \sim$ Rat faeces + Valley + Interval-sampling-analysis + Fat presence + *Strongyloides* sp. intensity + *A. cantonensis* intensity	261.62	5.00	0.04
	iv. $\log2(y) \sim 1$	262.15	5.53	0.03
Strongyloides sp.§	**i. log2(*y*) ~ Season + Cumulative rain + Ground + Burrows (*n*) + Sex + Age (d) + *G. neoplasticum* infection + *N. brasiliensis* intensity + *G. neoplasticum* intensity + Sex:Ground + Sex:Age(d) + Sex:*G. neoplasticum* infection**	**956.74**	**0.00**	**0.93**
	ii. $\log2(y) \sim$ Season + Cumulative rain + Ground + Burrows (n) + Sex + Age (d) + *G. neoplasticum* infection + *N. brasiliensis* infection + Sex:Ground + Sex:Age(d) + Sex:*G. neoplasticum* infection	962.04	5.30	0.07
	iii. $\log2(y) \sim$ Season + Cumulative rain + Ground + Burrows (n) + Age (d) + Sex + Sex:Ground + Sex:Age (d)	975.22	18.48	0.00
	iv. $\log2(y) \sim 1$	1032.13	75.39	0.00
L. interrogans§	**i. log2(*y*) ~ Season + Sex + Maturity + Trichuridae infection + Sex:Maturity**	**781.68**	**0.00**	**0.58**
	ii. log2(*y*) ~ Season + Sex + Maturity + Sex:Maturity	**782.34**	**0.66**	**0.42**
	i. $\log2(y) \sim 1$	870.16	88.49	0.00

Note: AIC_c, corrected Akaike's information criterion; ΔAIC_c, difference between AIC_c score and lowest AIC_c score; wi, Akaike's model weight. All the plausible models are shown in bold.

† Generalized linear models of *Hymenolepis* spp., Trichuridae, and *G. neoplasticum* did not present any significant coinfection variable, and, therefore, these models can be found elsewhere (Carvalho-Pereira et al. 2018).

‡ Models for infection.

§ Models for intensity of infection.

¶ *Nippostrongylus brasiliensis* intensity models were developed for two sets of data, for, when models included significant *L. interrogans* intensity of infection, the number of observations was reduced (in this case, $n = 54$).

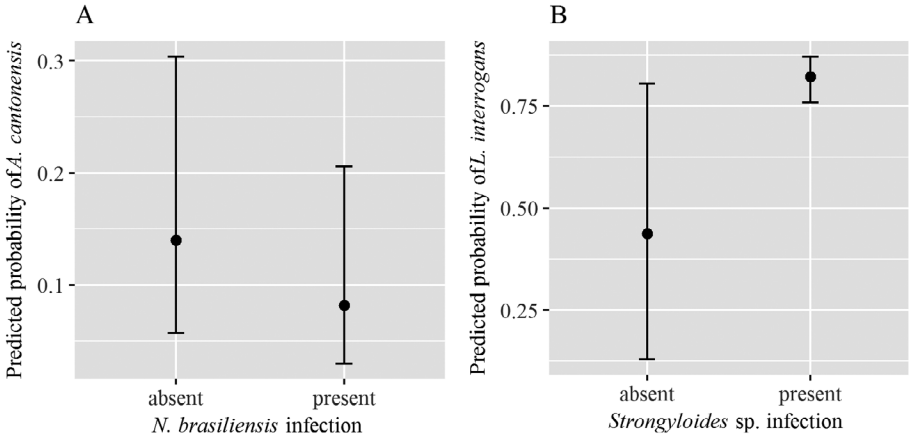

Fig. 2. Predicted coinfection effects on the probability of infection of *Angiostrongylus cantonensis* (A) and *Leptospira interrogans* (B).

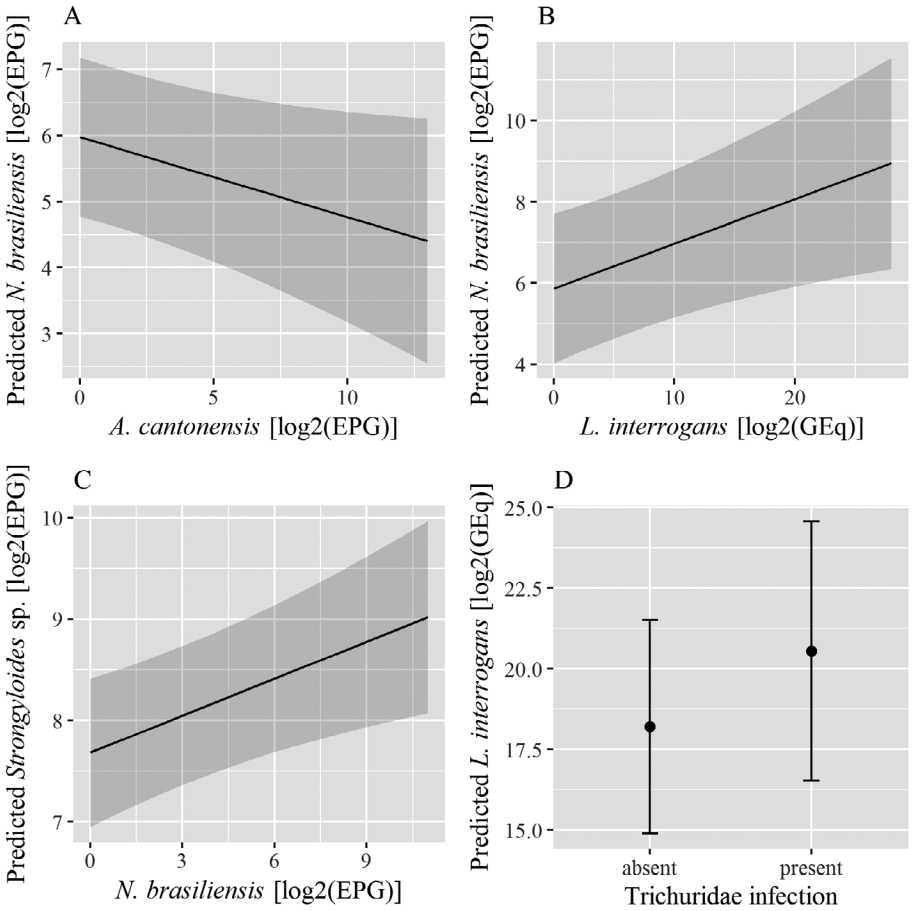

Fig. 3. Predicted coinfection effects on the intensity of infection of *Nippostrongylus brasiliensis* (A, B), *Strongyloides* sp. (C), and *Leptospira interrogans* (D).

chance of occurrence of *A. cantonensis* was lower in the presence of infection with *N. brasiliensis* (OR 0.55; 95% confidence interval, CI 0.31–0.95; Fig. 2A) and in the presence of infection with *Hymenolepis* spp., but only for male rats (OR 0.17; 95% CI 0.03–0.91). The infection model including coinfection presented an AIC weight of 0.95 compared to the other models (Table 1). By contrast, the intensities of *A. cantonensis* infections increased when coinfected with *Strongyloides* sp.: A doubling in intensity was associated with a 1.17-fold increase in the intensity of *A. cantonensis*. Although this interaction was only bordering significance (95% CI 1.00–1.35), the intensity model including coinfection received twice as much support from the data as the model without it (see AIC weights in Table 1).

The models provided no support for any coinfection variable in explaining the probability of infection by *N. brasiliensis*. However, once present, although only marginally significant, *N. brasiliensis* intensity was lower at higher intensities of *A. cantonensis* (Rate 0.92; 95% CI 0.85–1.01; Fig. 3A) and higher at higher intensities of *Strongyloides* sp. (Rate 1.14; 95% CI 1.00–1.30), strengthening the simple correlation patterns. In a reduced dataset, *L. interrogans* intensity was, too, positively associated with increased *N. brasiliensis* intensities. A doubling in the intensity of *L. interrogans* was associated with a 1.08-fold increase in the intensity of *N. brasiliensis* (95% CI 1.01–1.15; Fig. 3B), with this model having 13 times more support (AIC weight 0.53) from the data than the model including the intensities of *A. cantonensis* and *Strongyloides* sp. (AIC weight 0.04).

The chance of finding a rat infected by *Strongyloides* sp., due to its high prevalence, was only marginally associated with infection by *L. interrogans*. However, once present, a doubling in the intensity of *N. brasiliensis* was associated with a 1.09-fold increase in the intensity of *Strongyloides* sp. (95% CI 1.03–1.15; Fig. 3C) and, for male rats, the presence of *G. neoplasticum* was associated with a 7.36-fold increase in the intensity of *Strongyloides* sp. (95% CI 1.89–31.64; AIC weight 0.93).

Leptospira interrogans probability of infection was, in turn, significantly higher in the presence of *Strongyloides* sp. (OR 5.93; 95% CI 1.10–35.06; Fig. 2B), with the coinfection model having three times more support than the one including only age. The intensity models of *L. interrogans* only provided support for an increased log2 intensity of infection in the presence of parasites of the family Trichuridae, and this was only marginally significant (β 2.34; 95% CI −0.40 to 5.09; Fig. 3D).

The interaction network including the summary of associations of probabilities and intensities of infection between helminth species and *L. interrogans,* presented in the final selected models, is illustrated in Fig. 4.

Discussion

The helminth community of urban Norway rats in Salvador comprises a complex interaction network, where, of the possible connections between species, many exist. Coinfection with other parasites was significantly associated with a modification in the carriage of pathogens, including human pathogens, in the rat population. Associations represented by simple correlation tests in Fig. 1 were in most instances supported by GLM analyses, which in each case suggested (but could not definitively prove) a putative direction of effect between pairs of parasites, lending strong support to the idea that helminth communities within hosts are more than just random assemblages. We acknowledge that higher order interactions may exist and should be pursued by in studies where larger sample sizes are possible, that would avoid serious loss in statistical power. We further acknowledge the limitations of cross-sectional GLM in reliably detecting interactions between parasites (Fenton et al. 2014), but longitudinal (repeated capture) methods are rarely possible with pest species such as urban rats, though Childs et al. (1987) is an exception. By controlling for possible confounders more thoroughly than has been typical (Mohd Zain et al. 2012, Fenton et al. 2014, Simões et al. 2016), and identifying coinfection variables that add independent explanatory power to the models, we have sought to maximize support for these putative causal effects.

Of the parasites studied here with zoonotic potential, the observed coinfection between *L. interrogans* and *A. cantonensis* suggests that rats which shed *L. interrogans* in their urine are probably shedding *A. cantonensis* larvae in their feces as well, exposing humans to multiple pathogens (Wang et al. 2012, Costa et al. 2015*b*). Moreover,

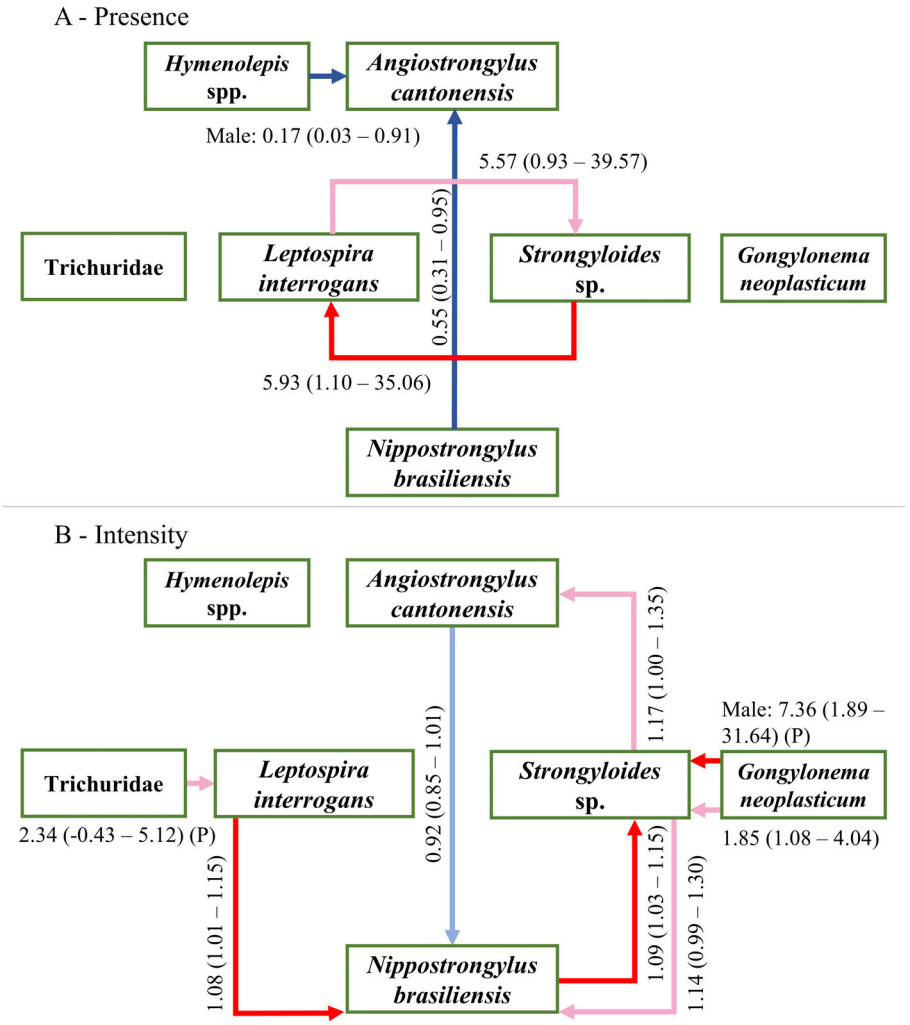

Fig. 4. Figurative representation of the positive (red) and negative (blue) associations between helminth species and *Leptospira interrogans* represented as an interaction network. Arrows indicate the direction of effects in the generalized linear model, with lighter colors assigned to interactions between $0.05 < P < 0.10$ that are nonetheless present in the most plausible models ($\Delta AIC_c < 2$). All the other interactions were statistically significant ($P < 0.05$). (A) Coinfection associations related to the probabilities of infection, with effects represented by the odds ratio and 95% confidence intervals (CIs; in brackets). (B) Coinfection associations related to the intensity of infection, with effects represented by the rates (antilog2 of the β coefficients for the helminths' models) or simply β coefficients (for the *Leptospira* model) and 95% CIs (in brackets). Where the estimates contain a letter "P" in brackets, this means that there was an effect of the presence of infection of one parasite species on the intensity of another.

infection risk is, noticeably, dependent on coinfection. Focusing on *A. cantonensis*, the risk of infection by this parasite was shown to be lower in the presence of infection by the equally frequent *N. brasiliensis* and by *Hymenolepis* spp. However, once present, the intensity of *A. cantonensis* infection was shown to be higher at higher intensities of the most prevalent helminth found in the rat population, *Strongyloides* sp. The most common asexual reproduction strategy of *Strongyloides* sp, which leads directly to the development of infective larvae (Viney and

Lok 2007), probably led to the infection success in the rat population. This makes the interaction between this species and *A. cantonensis* very concerning, since the latter is of public health importance (Wang et al. 2012, Morassutti et al. 2014). *Strongyloides* sp. was also positively associated with increased risk of infection by *L. interrogans* —a relationship that was likely to be reciprocal. Because the same environment (e.g., exposed moist soil or puddles) is favorable for both parasite species in allowing skin/mucosal penetration (Abadie 1963, Ko et al. 2009), it is likely that an infrastructure intervention directed at reducing the presence of one parasite, such as by paving pathways and household entrances/backyards, will reduce infection by the other. We note, too, that higher intensities of infection with *L. interrogans* were associated with coinfection with trichurids.

Turning to the possible mechanisms underlying the observed interactions, the negative association between *A. cantonensis* and *N. brasiliensis* may plausibly be attributed to competition for shared resources. After migrating to the central nervous system of rats and molting to the subadult (L5) stage, *A. cantonensis* infective third-stage larvae establish in the pulmonary arteries, where they become adult and reproduce (Alicata 1965). Eggs hatch inside the lungs, and the first-stage (L1) larva migrates up to the trachea to be swallowed, ultimately being shed in the feces (Alicata 1965). *N. brasiliensis* larvae also use the lungs as a route of migration, where they feed and molt, before establishing, in the adult form, in the small intestine (Schwartz and Alicata 1934, Haley 1962). Both nematode species feed on whole blood (Weinstein and Jones 1956, Archer et al. 2011). Hence, it is plausible that larvae of *A. cantonensis* and *N. brasiliensis* compete for resources during their common phase in the lungs. Alternatively, there is evidence that *N. brasiliensis* causes anorexia in rats during larval migration (Mercer et al. 2000), and thus, the negative effect on *A. cantonensis* may be caused by resource depletion on a whole organism scale. On the other hand, cross-effective immune response may play a role in negative associations between parasites (Telfer et al. 2010)—something that could be investigated further between *A. cantonensis* and both *N. brasiliensis* and *Hymenolepis* spp.

Among the positive associations reported, the only pair of parasites that could be expected to interact directly was *N. brasiliensis* and *Strongyloides* sp., due to their common routes of exposure and similar migration routes inside the host (Yokogawa 1922, Abadie 1963). In the gut, both *N. brasiliensis* and *Strongyloides* sp. are found to establish in the small intestine (preferred habitat), but also in the large intestine (Shintoku et al. 2005). However, adults of *Strongyloides* species usually embed in the small intestine mucosa or even invade and establish in a host's epithelial layer, whereas *N. brasiliensis* adults physically attach to the small intestine wall by their anterior end, feeding on traumatized tissue cells, blood, and intestine contents (Weinstein and Jones 1956, Dawkins et al. 1983, Viney and Lok 2007). Hence, these species tend to use different niches within the same habitat. Their reciprocal positive influences on one another's intensities may therefore reflect the trauma each inflicts on the gut environment. Alternatively, this and the other positive interactions between helminth species identified in this study may find foundation through indirect interactions, including host-immune-mediated effects.

Although helminth species are often known to elicit hosts' Th2 immune response, which could create hostile conditions for another helminth coinfecting species (Cox 2001), and hence negative associations between them, low concentrations of *Strongyloides* sp. can trigger a Th1 immune response within the host (Bleay et al. 2007). Similarly, some helminth species, such as *Trichuris muris*, can immunomodulate the Th2-Th1 equilibrium by eliciting the production of a Th1-associated cytokine, which down-regulates Th2 activity (Grencis et al. 2014). This may therefore create favorable conditions for the success of other helminth coinfecting species inside the host (Behnke et al. 2001). In the present study, *Strongyloides* sp., despite its high prevalence, was found with a median intensity of 150 EPG (interquartile range, IQR 27.8–450.0 EPG), which may corroborate this idea. However, if the Th1 arm of the immune response was active in low intensities of *Strongyloides* sp. and Trichuridae infections, it is unlikely that this affected the probability of infection of *L. interrogans*, given the positive association between these two parasites. It either suggests that *L. interrogans* is not

affected by the *R. norvegicus* immune system or that the Th1 *Strongyloides*-related response is tissue-specific.

Inside the host, bacteria are known to elicit Th1 activity. To our knowledge, the immune response of wild Norway rats to *L. interrogans* infection has never been assessed, but in susceptible species in the laboratory, *Leptospira* do elicit a Th1 immune response (Zuerner et al. 2011). If this is also true of Norway rats, this could explain the increased intensities found for *N. brasiliensis*, by down-regulating Th2 response. Likewise, the complex immune activities associated with trichurid infection (Grencis et al. 2014) may have led to the marginal increase in the intensity of *L. interrogans* infection in coinfected animals.

In summary, the complex interaction network presented here supports strongly the idea that risk of infection by a parasite species is not independent of coinfection with other parasites. The majority of the associations found in our study are suggestive of indirect interactions, which should be evaluated at finer scales (e.g., in revealing cross-immunity or identifying specific mechanisms of down-regulation). By applying previously established methods, our study provides, in particular, a novel perspective on evaluating helminth coinfection profiles in populations of naturally infected urban rats, after environmental and host biotic factors are taken into account. Apart from adding to our (still sparse) knowledge of coinfection patterns in natural populations, the results are important, first, in providing a realistic assessment of the overall risks from all parasites posed by infected rats when only one or a very few parasites are monitored. Observations on one parasite may imply greater or lower risks or intensities of infection with other parasites, including zoonotic parasites. In addition, the results emphasize that parasite-specific interventions, or even rodent control, will have implications for a range of parasites and may influence patterns of zoonotic species maintenance: potentially either adding value to the control program or counteracting it if other parasites, such as life-threatening leptospires, are released from competition (Lello et al. 2004, Telfer et al. 2010, Thumbi et al. 2014), as is also the case with human coinfection (Lello et al. 2013, Griffiths et al. 2014).

Acknowledgments

The authors are grateful to the Fundação de Amparo à Pesquisa do Estado da Bahia—Fapesb and the Brazilian Federal Agency for Support and Evaluation of Graduate Education—Capes, which granted Mrs. Carvalho-Pereira scholarships, making this work possible. We would like to thank the staff of the Center for Control of Zoonosis from Salvador for their assistance in conducting the study. This work could not be accomplished without the joint collaborative effort of the resident associations, community leaders, and residents. This work was supported by the Oswaldo Cruz Foundation and Secretariat of Health Surveillance, Brazilian Ministry of Health, by the Fapesb (grant 10206/2015), by the Brazilian Research Council–CNPq (465767/2014-1, and 421305/2017-7), by the National Institutes of Health of the United States (grants U01 AI088752, R01 AI121207, R01 TW009504, and R25 TW009338), by the Wellcome Trust (102330/Z/13/Z), and by the Medical Research Council (MR/P024084/1) of the UK.

Literature Cited

Abadie, S. H. 1963. The life cycle of *Strongyloides ratti*. Journal of Parasitology 49:241–248.

Alicata, J. E. 1965. Biology and distribution of the rat lungworm, *Angiostrongylus cantonensis*, and its relationship to eosinophilic meningoencephalitis and other neurological disorders of man and animals. Advances in Parasitology 3:223–248.

Archer, C. E., C. C. Appleton, S. Mukaratirwa, and K. J. Hope. 2011. The rat lung-worm *Angiostrongylus cantonensis*: a first report in South Africa. South African Medical Journal 101:174–175.

Behnke, J. M., A. Bajer, E. Sinski, and D. Wakelin. 2001. Interactions involving intestinal nematodes of rodents: experimental and field studies. Parasitology 122:S39–S49.

Beldomenico, P. M., and M. Begon. 2010. Disease spread, susceptibility and infection intensity: vicious circles? Trends in Ecology & Evolution 25:21–27.

Bleay, C., C. P. Wilkes, S. Paterson, and M. E. Viney. 2007. Density-dependent immune responses against the gastrointestinal nematode *Strongyloides ratti*. International Journal for Parasitology 37:1501–1509.

Carvalho-Pereira, T., et al. 2018. The helminth community of a population of *Rattus norvegicus* from an urban Brazilian slum and the threat of zoonotic diseases. Parasitology 145:797–806.

Chagas-Junior, A. D., A. J. McBride, D. A. Athanazio, C. P. Figueira, M. A. Medeiros, M. G. Reis, A. I. Ko, and F. W. McBride. 2009. An imprint method for detecting leptospires in the hamster model of

vaccine-mediated immunity for leptospirosis. Journal of Medical Microbiology 58:1632–1637.

Childs, J. E., G. E. Glass, G. W. Korch, and J. W. LeDuc. 1987. Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, Maryland. American Journal of Tropical Medicine and Hygiene 37:648–662.

Costa, F., J. E. Hagan, J. Calcagno, M. Kane, P. Torgerson, M. S. Martinez-Silveira, C. Stein, B. Abela-Ridder, and A. I. Ko. 2015*a*. Global morbidity and mortality of leptospirosis: a systematic review. PLoS Neglected Tropical Diseases 9:e0003898.

Costa, F., E. A. Wunder Jr., D. De Oliveira, V. Bisht, G. Rodrigues, M. G. Reis, A. I. Ko, M. Begon, and J. E. Childs. 2015*b*. Patterns in *Leptospira* shedding in Norway rats (*Rattus norvegicus*) from Brazilian slum communities at high risk of disease transmission. PLoS Neglected Tropical Diseases 9:e0003819.

Costa, F., et al. 2014*a*. Influence of household rat infestation on leptospira transmission in the urban slum environment. PLoS Neglected Tropical Diseases 8: e3338.

Costa, F., et al. 2014*b*. Infections by *Leptospira interrogans*, Seoul virus, and *Bartonella* spp. among Norway rats (*Rattus norvegicus*) from the urban slum environment in Brazil. Vector-Borne and Zoonotic Diseases 14:33–40.

Cox, F. E. G. 2001. Concomitant infections, parasites and immune responses. Parasitology 122:S23–S38.

Crawley, M. J. 2007. The R book. First edition. John Wiley & Sons, Chichester, UK.

Dawkins, H. J. S., T. A. Robertson, J. M. Papadimitriou, and D. I. Grove. 1983. Light and electron microscopical studies of the location of *Strongyloides ratti* in the mouse intestine. Zeitschrift fur Parasitenkunde-Parasitology Research 69:357–370.

Felzemburgh, R. D. M., et al. 2014. Prospective study of leptospirosis transmission in an urban slum community: role of poor environment in repeated exposures to the *Leptospira* agent. PLoS Neglected Tropical Diseases 8:e2927.

Fenton, A., S. C. L. Knowles, O. L. Petchey, and A. B. Pedersen. 2014. The reliability of observational approaches for detecting interspecific parasite interactions: comparison with experimental results. International Journal for Parasitology 44:437–445.

Fenton, A., M. E. Viney, and J. Lello. 2010. Detecting interspecific macroparasite interactions from ecological data: patterns and process. Ecology Letters 13:606–615.

Garcia, J. S., et al. 2014. Hematological and histopathological changes in *Rattus norvegicus* (Wistar) experimentally infected by *Angiostrongylus cantonensis* (Chen, 1935). Parasitology International 63:631–637.

Glass, G. E., J. E. Childs, G. W. Korch, and J. W. LeDuc. 1988. Association of intraspecific wounding with hantaviral infection in wild rats (*Rattus norvegicus*). Epidemiology & Infection 101:459–472.

Gordon, H. Mc. L., and H. V. Whitlock. 1939. A new technique four counting nematode eggs in sheep faeces. Journal of Scientific & Industrial Research 12:50–52.

Graham, A. L. 2008. Ecological rules governing helminth microparasite coinfection. Proceedings of the National Academy of Sciences USA 105:566–570.

Grencis, R. K., N. E. Humphreys, and A. J. Bancroft. 2014. Immunity to gastrointestinal nematodes: mechanisms and myths. Immunological Reviews 260:183–205.

Griffiths, E. C., A. B. Pedersen, A. Fenton, and O. L. Petchey. 2014. Analysis of a summary network of co-infection in humans reveals that parasites interact most via shared resources. Proceedings of the Royal Society B 281:20132286.

Hagan, J. E., et al. 2016. Spatiotemporal determinants of urban leptospirosis transmission: four-year prospective cohort study of slum residents in Brazil. PLoS Neglected Tropical Diseases 10:e0004275.

Haley, A. J. 1962. Biology of the rat nematode, *Nippostrongylus brasiliensis* (Travassos, 1914). II. Preparasitic stages and development in the laboratory rat. Journal of Parasitology 48:13–23.

Hancke, D., G. T. Navone, and O. V. Suarez. 2011. Endoparasite community of *Rattus norvegicus* captured in a shantytown of Buenos Aires City. Helminthologia 48:167–173.

Hoffman, W. A., J. A. Pons, and J. L. Janer. 1934. Sedimentation concentration method in *Schistosomiasis mansoni*. Puerto Rico Journal of Public Health and Tropical Medicine 9:283–298.

Hurvich, C. M., and C.-L. Tsai. 1989. Regression and time series model selection in small samples. Biometrika 76:297–307.

IBGE (Instituto Brasileiro de Geografia e Estatística). 2010. http://www.censo2010.ibge.gov.br/sinopsepo rsetores/?nivel=st

Jolles, A. E., V. O. Ezenwa, R. S. Etienne, W. C. Turner, and H. Olff. 2008. Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo. Ecology 89:2239–2250.

Kataranovski, D., M. Kataranovski, and I. Deljanin. 2010. Helminth fauna of *Rattus norvegicus* Berkenhout, 1769 from the Belgrade area, Serbia. Archives of Biological Sciences 62:1091–1099.

Klementowicz, J. E., M. A. Travis, and R. K. Grencis. 2012. *Trichuris muris*: a model of gastrointestinal parasite infection. Seminars in Immunopathology 34:815–828.

Ko, A. I., C. Goarant, and M. Picardeau. 2009. *Leptospira*: the dawn of the molecular genetics era for

an emerging zoonotic pathogen. Nature Reviews Microbiology 7:736–747.

Lass, S., P. J. Hudson, J. Thakar, J. Saric, E. Harvill, R. Albert, and S. E. Perkins. 2012. Generating supershedders: Co-infection increases bacterial load and egg production of a gastrointestinal helminth. Journal of the Royal Society Interface 10:20120588.

Lello, J., B. Boag, A. Fenton, I. R. Stevenson, and P. J. Hudson. 2004. Competition and mutualism among the gut helminths of a mammalian host. Nature 428:20–24.

Lello, J., S. Knopp, K. A. Mohammed, I. S. Khamis, J. Utzinger, and M. E. Viney. 2013. The relative contribution of co-infection to focal infection risk in children. Proceedings of the Royal Society B 280:20122813.

Mercer, J. G., P. I. Mitchell, K. M. Moar, A. Bissett, S. Geissler, K. Bruce, and L. H. Chappell. 2000. Anorexia in rats infected with the nematode, *Nippostrongylus brasiliensis*: experimental manipulations. Parasitology 120:641–647.

Mills, J. N., J. E. Childs, T. G. Ksiazek, C. J. Peters, and W. M. Velleca. 1995. Methods for trapping and sampling small mammals for virologic testing. U.S. Department of Health & Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Mohd Zain, S. N., J. M. Behnke, and J. W. Lewis. 2012. Helminth communities from two urban rat populations in Kuala Lumpur, Malaysia. Parasites & Vectors 5:1–23.

Morassutti, A. L., S. C. Thiengo, M. Fernandez, K. Sawanyawisuth, and C. Graeff-Teixeira. 2014. Eosinophilic meningitis caused by *Angiostrongylus cantonensis*: an emergent disease in Brazil. Memórias do Instituto Oswaldo Cruz 109:399–407.

Panti-May, J. A., et al. 2016. A two-year ecological study of Norway rats (*Rattus norvegicus*) in a Brazilian urban slum. PLOS ONE 11:e0152511.

Pedersen, A. B., and A. Fenton. 2007. Emphasizing the ecology in parasite community ecology. Trends in Ecology and Evolution 22:133–139.

Poulin, R. 2001. Interactions between species and the structure of helminth communities. Parasitology 122:S3–S11.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reis, R. B., et al. 2008. Impact of environment and social gradient on *Leptospira* infection in urban slums. PLoS Neglected Tropical Diseases 2:e228.

Schwartz, B., and J. E. Alicata. 1934. The development of the Trichostrongyle, *Nippostrongylus muris*, in rats following ingestion of larvae. Journal of the Washington Academy of Sciences 24:334–338.

Shintoku, Y., E. Kimura, T. Kadosaka, H. Hasegawa, S. Kondo, M. Itoh, and M. Z. Islam. 2005. *Strongyloides ratti* infection in the large intestine of wild rats, *Rattus norvegicus*. Journal of Parasitology 91:1116–1121.

Silveira, M. R., K. P. Nunes, D. C. Cara, D. G. Souza, A. Corrêa Jr., M. M. Teixeira, and D. Negrão-Corrêa. 2002. Infection with *Strongyloides venezuelensis* induces transient airway eosinophilic inflammation, an increase in immunoglobulin E, and hyperresponsiveness in rats. Infection and Immunity 70:6263–6272.

Simões, R. O., J. L. Luque, R. Gentile, M. C. S. Rosa, S. Costa-Neto, and A. Maldonado Jr. 2016. Biotic and abiotic effects on the intestinal helminth community of the brown rat *Rattus norvegicus* from Rio de Janeiro, Brazil. Journal of Helminthology 90:21–27.

Singleton, G. R., L. A. Hinds, C. J. Krebs, and D. M. Spratt. 2003. Rats, mice and people: rodent biology and management. Monograph No. 96. ACIAR, Canberra, Australian Capital Territory, Australia.

Telfer, S., X. Lambin, R. Birtles, P. Beldomenico, S. Burthe, S. Paterson, and M. Begon. 2010. Species interactions in a parasite community drive infection risk in a wildlife population. Science 330:243–247.

Thumbi, S. M., et al. 2014. Parasite co-infections and their impact on survival of indigenous cattle. PLOS ONE 9:e76324.

Torgerson, P. R., J. E. Hagan, F. Costa, J. Calcagno, M. Kane, M. S. Martinez-Silveira, M. G. Goris, C. Stein, A. I. Ko, and B. Abela-Ridder. 2015. Global burden of leptospirosis: estimated in terms of disability adjusted life years. PLoS Neglected Tropical Diseases 9:e0004122.

Viney, M., and T. Kikuchi. 2017. *Strongyloides ratti* and *S. venezuelensis* – rodent models of *Strongyloides* infection. Parasitology 144:285–294.

Viney, M. E., and J. B. Lok. 2007. *Strongyloides* spp. *In* The C. elegans Research Community. WormBook. https://dx.doi.org/10.1895/wormbook.1.141.1

Wagenmakers, E. J., and S. Farrell. 2004. AIC model selection using Akaike weights. Psychonomic Bulletin & Review 11:192–196.

Wang, Q.-P., Z.-D. Wu, J. Wei, R. L. Owen, and Z.-R. Lun. 2012. Human *Angiostrongylus cantonensis*: an update. European Journal of Clinical Microbiology & Infectious Diseases 31:389–395.

Weinstein, P. P., and M. F. Jones. 1956. The in vitro cultivation of *Nippostrongylus muris* to the adult stage. Journal of Parasitology 42:215–236.

Yokogawa, S. 1922. The development of *Heligmosomum muris* Yokogawa, a nematode from the intestine of the wild rat. Parasitology 14:127–166.

Zuerner, R. L., D. P. Alt, M. V. Palmer, T. C. Thacker, and S. C. Olsen. 2011. A *Leptospira borgpetersenii* serovar hardjo vaccine induces a Th1 response, activates NK cells, and reduces renal colonization. Clinical and Vaccine Immunology 18:684–691.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2887/full