International Journal for Parasitology: Parasites and Wildlife 6 (2017) 59–68

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Temporal and demographic blood parasite dynamics in two free-ranging neotropical primates

Gideon A. Erkenswick [a, b, *], Mrinalini Watsa [b, c], Alfonso S. Gozalo [d], Nicole Dmytryk [a], Patricia G. Parker [a, e]

[a] Department of Biology and Whitney R. Harris World Ecology Center, University of Missouri-St. Louis, One University Blvd., Saint Louis, MO 63121, USA
[b] Field Projects International, 7331 Murdoch Ave, Saint Louis, MO 63119, USA
[c] Department of Anthropology, Washington University in St. Louis, One Brookings Drive, Saint Louis, MO 63130, USA
[d] Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[e] WildCare Institute, Saint Louis Zoo, One Government Dr., Saint Louis, MO 63110, USA

ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form
8 March 2017
Accepted 10 March 2017

Keywords:
Co-infection
Blood parasites
Cooperative breeding
Longitudinal sampling
Callitrichidae

ABSTRACT

Parasite-host relationships are influenced by several factors intrinsic to hosts, such as social standing, group membership, sex, and age. However, in wild populations, temporal variation in parasite distributions and concomitant infections can alter these patterns. We used microscopy and molecular methods to screen for naturally occurring haemoparasitic infections in two Neotropical primate host populations, the saddleback (*Leontocebus weddelli*) and emperor (*Saguinus imperator*) tamarin, in the lowland tropical rainforests of southeastern Peru. Repeat sampling was conducted from known individuals over a three-year period to test for parasite-host and parasite-parasite associations. Three parasites were detected in *L. weddelli* including *Trypanosoma minasense*, *Mansonella mariae*, *and Dipetalonema* spp., while *S. imperator* only hosted the latter two. Temporal variation in prevalence was observed in *T. minasense* and *Dipetalonema* spp., confirming the necessity of a multi-year study to evaluate parasite-host relationships in this system. Although callitrichids display a distinct reproductive dominance hierarchy, characterized by single breeding females that typically mate polyandrously and can suppress the reproduction of subdominant females, logistic models did not identify sex or breeding status as determining factors in the presence of these parasites. However, age class had a positive effect on infection with *M. mariae* and *T. minasense*, and adults demonstrated higher parasite species richness than juveniles or sub-adults across both species. Body weight had a positive effect on the presence of *Dipetalonema* spp. The inclusion of co-infection variables in statistical models of parasite presence/absence data improved model fit for two of three parasites. This study verifies the importance and need for broad spectrum and long-term screening of parasite assemblages of natural host populations.

1. Introduction

The surveillance of parasites and pathogens in wildlife populations has received international attention, since wildlife conservation outcomes can be affected by parasitic infections (van Riper et al., 1986; Levin et al., 2013), and since wildlife are increasingly found to host pathogens that can infect humans (Guberti et al., 2014). Parasite-host associations are dictated by characteristics intrinsic to the host, the parasite, and the environment. Although associations can vary, parasitism is frequently correlated with host density (Poulin, 2004; Fernandes et al., 2012), age (Sol et al., 2003; Clough et al., 2010; Parr et al., 2013; Leclaire and Faulkner, 2014) sex (Poulin, 1996; Schall et al., 2000; Clough et al., 2010; MacIntosh et al., 2010), and dominance status (Muehlenbein and Watts, 2010). Meta-analyses across species indicate that parasitism positively correlates with group size (Vitone et al., 2004; Rifkin et al., 2012), but this is modulated by the mode of transmission and mobility of the parasites in question (Cote and Poulin, 1995). In addition to these host-specific factors, when longitudinal data are available, studies of parasite prevalence in diverse taxa demonstrate temporal effects of season (Huffman et al., 1997; Raharivololona and Ganzhorn, 2010) and year

* Corresponding author. Department of Biology, University of Missouri-St. Louis, R223 Research Building, One University Blvd., St. Louis, MO 63121, USA.
E-mail address: gaet4b@umsl.edu (G.A. Erkenswick).

http://dx.doi.org/10.1016/j.ijppaw.2017.03.004

(Bakuza and Nkwengulila, 2009; Clough et al., 2010; Moreno et al., 2013).

In nature, animals almost always exhibit infections by several different parasites at the same time and in succession (Pedersen and Fenton, 2007; Telfer et al., 2008, 2010). Since parasites can bring about distinct changes to host hematology, body condition and immune investment (Budischak et al., 2012; van Wyk et al., 2014), it follows that even disparate parasites can boost (Monteiro et al., 2007b; Knowles, 2011; Thumbi et al., 2014) or suppress one another (Moreno et al., 2013) via their influences on host immune function (Cox, 2001; Ulrich and Schmid-Hempel, 2012). Although the logistics and economics of collecting long-term, individual-based infection data are challenging, these data are critical to study the effects of age, social structure, life history, time, seasonal variation, and co-infection on disease dynamics (Clutton-Brock and Sheldon, 2010). For example, with repeat sampling we can assess how particular parasites influence host susceptibility to other parasites (Telfer et al., 2008), and if concomitant parasite infections reduce or increase host fitness overall (Balmer et al., 2009). While long-term studies on human populations are numerous (Weil et al., 1999; Bloch et al., 2011), comparable monitoring of wild animal populations are rare (Telfer et al., 2008; Tompkins et al., 2010); however, such studies are critical in the case of long-lived hosts, such as the primates, with complex social organization.

A minority of primates exhibit social systems in which non-biological parents care for the offspring of dominant individuals in a group in a process known as alloparenting (Riedman, 1982; Sussman and Kinzey, 1984). These cooperative breeders, primarily tamarin and marmoset genera within the Callitrichidae, may exhibit greater amounts of parasitism than solitary or pair-bonded breeders due to elevated levels of sociality (Burkart, 2015), which will influence density- and frequency dependent parasite-host relationships (Anderson and May 1978; Altizer et al., 2003; Poulin, 2004; Patterson and Ruckstuhl, 2013). However, if sociality affords an overall reduction in group energy expenditure, then cooperative breeding could instead decrease parasitism by allowing improved individual host immune function (Spottiswoode, 2007; Lutermann et al., 2013). Here, we present novel haemoparasite infection data from a longitudinal study of two free-ranging sympatric populations of cooperatively breeding primate species - the saddleback tamarin (*Leontocebus weddelli,* formerly *Saguinus fuscicollis wedelli*) and the emperor tamarin (*S. imperator*) (Matauschek et al., 2011; Buckner et al., 2015). This study explores the potential influences of intrinsic host factors, co-infection, and temporal variation on parasite prevalence via a mark-recapture program that allowed us to track the parasite infection status of individual animals across multiple years. This enabled us to control for biases due to temporal, environmental and protocol-related changes, which have been rarely addressed in studies of these species to date (Lisboa et al., 2000; Phillips et al., 2004; Wenz et al., 2009; West et al., 2013), but see (Monteiro et al., 2007a, 2007b).

We predicted four patterns of parasite prevalence would occur within this study system. Due to our observations of consistent host social group sizes through an annual mark-recapture program from 2009 to 2015 (Watsa et al., 2015), we assumed stable host populations and that parasite prevalence would exhibit only minor fluctuations between years due to random stochastic variation in the environment (Schall et al., 2000; Knowles et al., 2013). Second, although sex-biased parasitism is a topic of long debate across taxonomic orders (Morales-Montor et al., 2004) with a tendency to assign greater parasite risk to males (Poulin, 1996; Klein, 2004; Muehlenbein, 2005; Muehlenbein and Watts, 2010) we predict the opposite trend in this host system. Callitrichid sociality is characterized by stark competition among females for breeding opportunities, with primary breeding females in return suppressing the reproduction of subdominant females behaviorally or through physiological stress (Ziegler et al., 1987; Beehner and Lu, 2013). Conversely, callitrichine males share mate access with little to no overt antagonism, and do not invest in potentially costly secondary sexual characteristics or extensive mate-guarding rituals (Hamilton and Zuk, 1982; Setchell et al., 2009). Callitrichids exhibit unusually high rates of twin offspring among primates (>80% of births) with groups usually consisting of a single female that reproduces, while all other adults assist in rearing her offspring (Sussman and Kinzey, 1984; Terborgh and Goldizen, 1985). While absolute male-female sex ratios are not skewed in this population (Watsa, 2013), operational sex ratios are biased towards males, since typically a single female reproduces in each group. Thus, if there is a parasite risk associated with maintaining social status we predict that it should be borne predominantly by the primary breeding female in a group. Third, while immunosuppression in juveniles can lead to a preponderance of infections in younger age classes (Sol et al., 2003), immunosenescence in aging adults intensifies the accumulation of parasites over a lifespan, resulting in high incidences of infection among older age classes (Shanley et al., 2009). However, although callitrichids can live up to 20 years in captivity, higher predation risks in the wild result in lower lifespan maximums of around 9–11 years (Goldizen, 1996). We therefore predict a lower parasite prevalence in adults vs. subadults or juveniles in this study system. Finally, we predict significant associations between co-infecting parasites that present ecological overlap in infection sites, host resource use, or arthropod vectors, since they are more likely to interact directly or indirectly via the host immune response (Cox, 2001).

2. Material and methods

2.1. Study area and sample collection

Sample collection took place at the Estación Biológica Rio Los Amigos (EBLA) in the Madre de Dios Department of southeastern Peru (12°34′07″S, 70°05′57″W)). The 900-hectare tropical rainforest research station is located at the confluence of the Los Amigos and Madre de Dios Rivers, and is contiguous with the much larger Los Amigos Conservation Concession (~1400 km^2) that lies within the buffer zone of Manu National Park (Watsa, 2013). Samples were collected annually during the dry season between May and August from 2012 to 2014. All of the primate social groups in this study inhabit a uniform area of forest with similar access to terra firme and várzea habitat. A safe animal mark-recapture program ongoing since 2009, based on the Peruvian trap model (Savage et al., 1993), was optimized to minimize the risk of harm to animals (Watsa et al., 2015). Animals were given permanent identification tags via subcutaneous microchips (Avid, Home Again©). Blood samples (<300 μl) drawn from the femoral vein under ketamine hydrochloride anesthesia (Ketalar, Pfizer Inc., New York, USA) were stored dry on Whatman FTA Micro Elute Cards, and 2 to 3 blood smears were prepared with fresh blood from each animal. All sampling protocols adhere to guidelines outlined by the American Society of Mammalogists (Sikes and Gannon, 2011) and were approved by the Institutional Animal Care and Use Committee at the University of Missouri-St. Louis (317006-2, 733363-2) and the Directorate of Forest and Wildlife Management (DGFFS) of Peru annually.

2.2. Age class and breeding status determination

Among callitrichids, age and sexual maturity can become desynchronized, particularly among females, as a result of

reproductive suppression of all but one dominant breeding female per group (Ziegler et al., 1987; Saltzman et al., 1998). Reproductive suppression has not been confirmed among males; however they do exhibit significant variation in testicular volume and body size within a group, suggestive of a loose dominance hierarchy (Garber et al., 1996; Watsa, 2013). Therefore, we used separate methods to classify age-sex classes and breeding status for both sexes.

We used three broad age classes based on dental eruption patterns (Watsa, 2013). Juveniles were defined as individuals whose adult teeth were absent or not fully erupted (<11 mo). Sub-adults were animals with adult teeth, but that were juveniles in the preceding year. All remaining individuals were assigned to the adult age class. Older adults could not be distinguished based on tooth eruption patterns alone and were thus pooled within the adult age-class.

Breeding status assignments were based on individual weight measurements and reproductive morphology following Watsa et al. (2016). Briefly, body size and suprapubic gland area for both sexes, testes volume in males, and average nipple length and vulva index in females were combined in a principal component analysis (PCA). Coordinate values from the first two dimensions, accounting for >80% of variation in the dataset, were applied to a linear discriminant function analysis that assigned all individuals of unconfirmed breeding status to one of three categories: primary breeder, secondary breeder, or non-breeder.

2.3. Parasite detection and identification by microscopy

Immediately after blood draw, blood smears were made on standard microscope slides and air-dried. All smears were fixed for five minutes in 100% methanol within six hours. They were stained in Giemsa's solution following Valkiūnas et al. (2008) within three weeks of fixation, and observed at 400× magnification using light microscopy for the presence of parasites. Small extracellular and intracellular blood parasites were recorded while conducting a total leukocyte count estimation (enumeration of leukocytes in 10 non-overlapping fields of view in the smears' monolayer at 400× magnification) and differential (classification of 200 leukocytes in the monolayer at 1000× magnification) using COUNT, an open access cell counting tool (http://erktime.github.io/count/); each slide examination took less than 30 min. Examinations were carried out in a systematic direction to avoid overlapping fields of view, and damaged sections, where leukocytes and parasites were too distorted to identify, were excluded. To rule out the possibility of false negatives for trypanosome infection, blood smears from individuals that tested negative from two targeted PCR screenings were scanned a second time for 15 min at 1000× magnification, including areas of the smear not in the monolayer. Representative micrographs of each parasite were recorded under oil-immersion at 400× and 1000× magnification from the blood smears of at least ten separate individuals per host species. Measurements of length and width and the location of key anatomical features were made using printed photographs, which were compared to reference values from the literature to confirm species identification (Eberhard et al., 1979; Petit et al., 1985; Bain et al., 1986; Sato et al., 2008).

2.4. Molecular detection and sequencing

DNA was isolated from a 3 mm diameter hole punch from the blood stored on Whatman FTA Micro Elute Cards into 30 μl of ddH_20 according to manufacturer instructions (GE Health Care Life Sciences, Pittsburgh USA). First and second elutions of DNA were obtained from each hole punch and both effectively amplified parasite DNA from infected animals; we used the first elution for consistency across animals for this study. Two genes were targeted to detect and identify filarial nematodes. The internal transcribed spacer 1 (ITS1) was amplified using forward primer S.r.ITSI-NC5/F1 and reverse primer NC13R following the protocol of Sato et al. (2008). Also, mitochondrial cytochrome oxidase I (COI) was amplified using forward primer COIintF and reverse primer COIintR following the protocol of Casiraghi et al. (2001). Both targets produced a single solid band on agarose gels for infected individuals that did not differentiate the two nematode parasites. The additional information from thin blood smears was used to assign single or concomitant infection status. If parasite infections remained unclear after viewing multiple blood smears for each animal, Sanger Sequencing of COI (rather than ITSI) clearly differentiated the parasites by sequence, or resulted in chromatograms with double-peaks in the case of co-infection (Supplementary Fig. S4). A universal nested PCR reaction targeting the ssrRNA gene was used to detect and identify *Trypanosoma* spp. in all samples. The outer reaction used forward primer TRY927F and reverse primer TRY927R, and the inner reaction used forward primer SSU561F and reverse primer SSU561R following the protocol of Noyes et al. (1999), but see primer clarifications in (Noyes et al., 2000). For all parasites we concluded that a sample was positive for an infection if it appeared in a blood smear or if it could be successfully amplified by PCR. Conversely, to be considered negative for a given parasite, a sample had to be blood smear negative and PCR negative across a minimum of three replicate reactions.

For parasite classification, a subset of the positive PCR products were purified using Machery-Nagel PCR Clean-up Kits (Bethlehem USA) and sent to EuroFin Genomics (Louisville USA) for Sanger Sequencing using forward primers S.r.ITSI-NC5/F1 and COIintF for microfilariae, and SSU561F for *Trypanosoma* spp.

2.5. Statistical analyses

To test for significant changes in parasite prevalence across the study period while controlling for repeat measures of individual animals, we implemented randomized Z-tests of proportions. Each individual in our study was selected at random only one time for 1000 iterations of the test, thereby removing concerns of non-independence. If greater than or equal to 95% of the iterations resulted in p-values < 0.05, a difference in prevalence was considered significant. If a significant difference across the entire study period (2012–2014) was detected, then similar pairwise tests between all combinations of years were carried out but with p-values adjusted using the Holm-Bonferonni procedure (Holm, 1979).

The presence/absence of each parasite was modeled using generalized linear mixed effect models (GLMMs) with logit link functions and binomial errors (Bates et al., 2014). Fixed factor model terms included ‘species’, ‘sex’, ‘age class’, and ‘breeding status’, while ‘group’, ‘individual identity’, and ‘year’ factors were incorporated as random effects when they showed any impact on model outcomes (Zuur et al., 2007). If convergence errors occurred during model selection, individual identity was excluded from the most saturated fixed-factor models, as there were only ~1.7 captures/individual in the study. However, all random effects were reinstated following the first one or two rounds of model selection (Telfer et al., 2008). Model selection was carried out by stepwise term deletion and comparison of nested models with likelihood ratio tests, and we confirmed that the removal of all minimal model terms increased the Akaike Information Criterion (AIC) by at least two units (Akaike, 1974). All statistical analyses were performed using R software v.3.2.2 (R Development Core Team, 2015).

To discern the influences of host intrinsic factors from other concomitant parasite infections, a nested modeling approach was

used (Telfer et al., 2008). Concomitant parasite infection data were added to models of each parasite response variable with its optimal host factor structure. This enabled us to determine whether co-infections generally improved model fit or strengthened or weakened associations with host factors. Additionally, we constructed a GLMM with Poisson errors to model parasite species richness. For this analysis, factors were the same as in other models, except 'group size' was inserted as additional fixed factor and 'species' was converted to a random effect since one of the three parasites discovered did not infect *S. imperator*.

3. Results

3.1. Blood parasite detection and identification

In total, we collected 186 blood samples (120 *Leontocebus weddelli*, 66 *Saguinus imperator*) from 111 individuals (74 *L. weddelli*, 37 *S. imperator*) (Table 1). Three blood parasites were identified by microscopy and targeted PCR screening: filarial nematodes *Mansonella mariae* and *Dipetalonema* spp. and kinetoplastid *Trypanosoma minasense*. 90% of animals infected with a filarial nematode were detected by PCR alone; however, the remaining (8/76 and 10/94 for each nematode, respectively) infections were only detected by microscopy. We relied primarily on PCR to detect infections of *T. minasense* since low numbers of parasites (1–3 parasites/blood smear/infected animal) led to frequent false negatives; however, ~20% (25/127) of our positives came from blood smears for which corresponding PCR screening was repeatedly negative.

Our morphological description and measurements of *Mansonella mariae* and *Trypanosoma minasense* (Supplementary Table 3S) were consistent with previously published references for these parasites (Petit et al., 1985; Sato et al., 2008). Also, our partial sequences of ITS1 (N = 7) for *M. mariae* and ssurRNA gene (N = 10) for *T. minasense*, were both 99% identical (100% coverage) to records already on GenBank. *Dipetalonema* spp. did not sequence cleanly using ITS1; instead, we amplified CO1. Our sequences (N = 8) matched with *Dipetalonema* spp. on GenBank (95% identify, 100% coverage), and did not differentiate between three congeners, *D. gracile*, *D. graciliformis*, and *D. robini*. Morphological measurements from thin blood smears suggested mixed infections of *D. gracile,* and *D. graciliformis* present in both hosts based on reference values from the literature (Table 3S) (Eberhard et al., 1979; Bain et al., 1986). Sequences of all three parasites have been deposited on GenBank (accession nos. for *Dipetalonema* KX932481, KX932482; for *M. mariae* KX932483, KX932484; for *T. minasense* KX932485, KX932486, KX932487, KX932488, KX932489, KX932490).

3.2. Infection prevalence over time

Across the study period the prevalence of *M. mariae* remained stable (0.54–0.67) (Fig. 1). Although Fig. 1 suggests that there should be significant increases in *Dipetalonema* spp. infection across the study period, after controlling for repeated measures, the upward trend approached significance for *L.weddelli* alone (mean $\chi^2 = 2.41$, df = 2, mean $P = 0.074$, but $P < 0.05$ 63.4% of the time) (Supplementary Table 4S). Differences in prevalence for *T. minasense* across the study period were significant considering just *S. imperator* and both host species combined ($P < 0.05$ 96.7% and 100% of the time, respectively), and approached significance for *L. weddelli* ($P < 0.05$ 92% of the time) (Table 4S). By graphing changes in infection status per individual per parasite across the entire study period, the sources of annual variation in prevalence could be tracked (Fig. 2). A large spike in the presence of *T. minasense* in 2014 was the result of previously uninfected individuals acquiring infections, with no previously infected individuals losing infection – this was different from 2012 to 2013 when equal numbers of individuals gained and lost infection. A small number of previously uninfected individuals acquired new infections of *Dipetalonema* spp. each year, and we observed only one instance for which a previously infected individual was not found infected with *Dipetalonema* spp. in the subsequent year.

In any given year, assuming independence, a simple expectation for the rate of co-infection can be obtained by multiplying the prevalence of two parasites together. All co-infection rates met these expectations except the rate of co-infection of *Dipetalonema* spp. and *M. mariae*, which was significantly higher in 2013 ($\chi^2 = 10.2$, df = 1, $P < 0.001$) (Fig. 1).

Table 1
Host sampling stratification by year, sex, age class, and breeding status.

Year		2012	2013	2014
L. weddelli		35	49	36
Sex	Male	18	30	19
	Female	17	19	17
Age	Juvenile	8	10	4
	Sub-adult	4	6	1
	Adult	23	33	31
Breeding Status	Non-breeder	11	12	4
	Secondary Breeder	5	15	18
	Primary Breeder	19	22	14
S. imperator		21	24	21
Sex	Male	10	15	10
	Female	11	9	11
Age	Juvenile	6	3	4
	Sub-adult	2	3	1
	Adult	13	18	16
Breeding Status	Non-breeder	8	4	5
	Secondary Breeder	2	6	4
	Primary Breeder	11	14	12
Mean captures per individual '12 – '14			*L. weddelli*	1.6 (1–3)
			S. imperator	1.8 (1–3)
Median captures per individual '12 –' 14			*L. weddelli*	1
			S. imperator	2

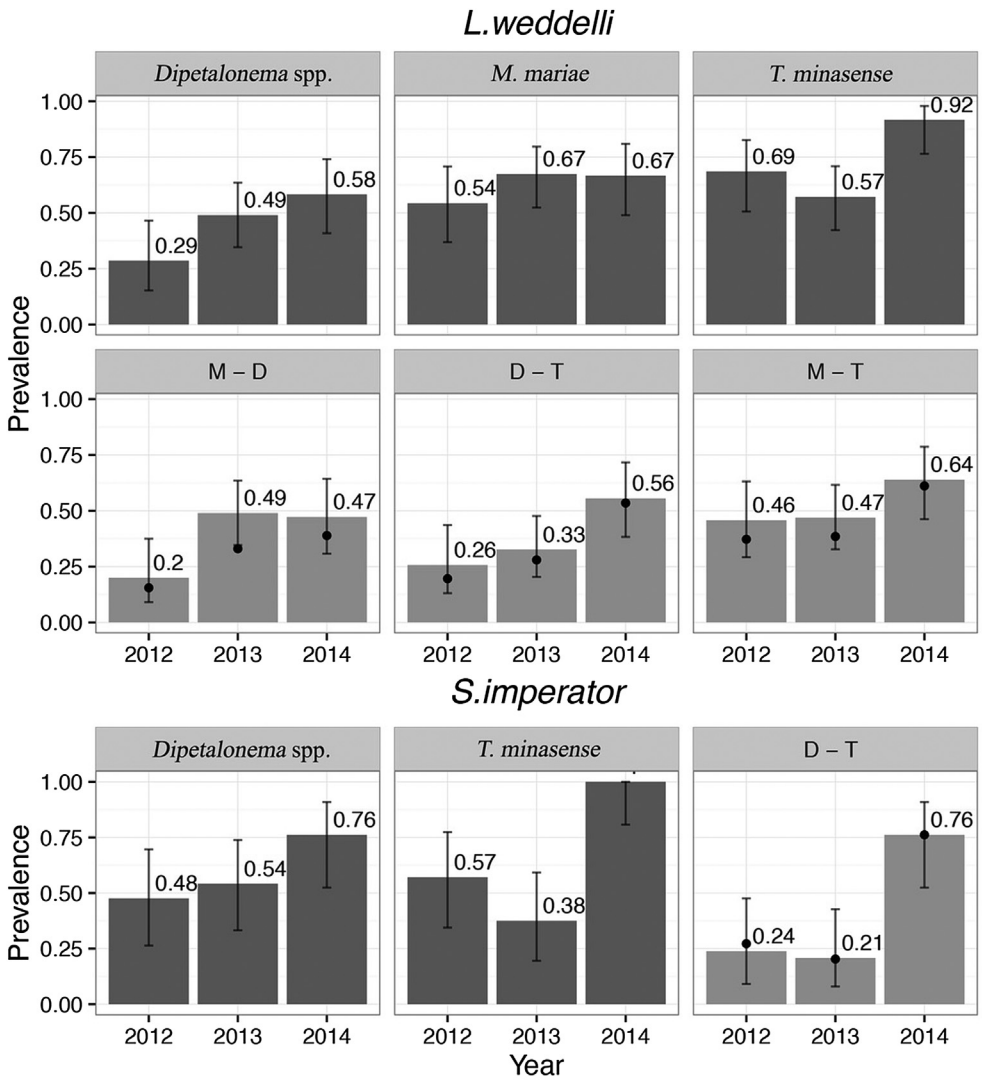

Fig. 1. Annual prevalence of single- and co-infections by species. Prevalence indicated for each parasite (dark gray), and each pairwise combination of parasites (light gray). Numbers near the top of each bar show the exact prevalence; black lines indicate 95% confidence intervals; dots indicate expected levels of co-infection (refer to Section 3.2). M-D is co-occurrence *of M. mariae* and *Dipetalonema* spp., D-T is *Dipetalonema* spp. and *T. minasense*, and M-T is *M. mariae* and *T. minasense*.

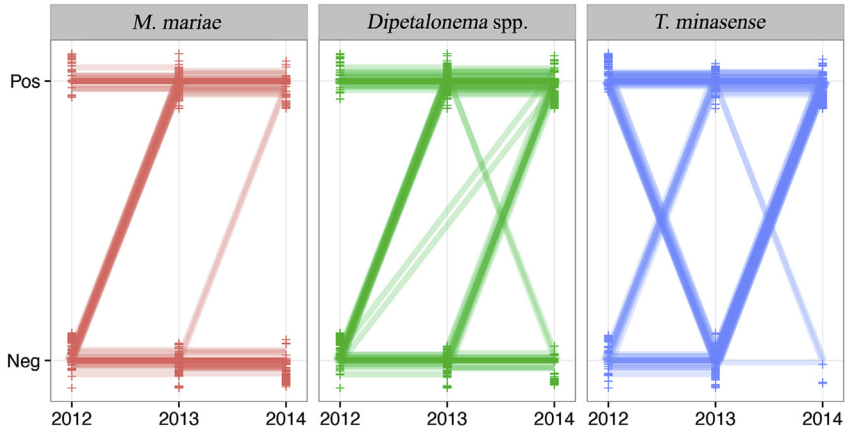

Fig. 2. Individual infection status by parasite by year. Strength and thickness of lines are scaled to the number of individuals that took a given infection trajectory from one year to the next. Two diagonal lines span 2012–2014 because those individuals were not sampled in 2013. The + symbols represent every infection or non-infection found across all individuals in the study.

3.3. Modeling outcomes

To explore parasite-host and parasite-parasite associations we constructed a series of nested GLMMs. The goal of the first model was to identify those host variables that best explained the presence or absence of each infection, and subsequent models incorporated co-infection explanatory variables to observe if model fit improved, or whether significant predictors remained the same. Infection by *Dipetalonema* spp. was positively associated with body weight and model fit was improved by adding of co-infection with *M. mariae* ($x^2 = 5.032$, df = 1, $P = 0.0249$), although the GLMM estimate for *M. mariae* only approached significance (Table 2). Minimal models of prevalence of *M. mariae* included either age class or breeding status, and not both, as these factors were negatively correlated (Fisher's Exact Test, $p < 0.001$). When co-infection variables were introduced, the presence of *Dipetalonema* spp. also had a significant positive effect on *M. mariae*. *T. minasense* infection was predicted by an animal's age class or breeding status (again, correlation required factor reduction), and the addition of co-infection with *M. mariae* significantly improved model fit (Table 2). Regarding individual parasite species richness (PSR), younger age classes exhibited significantly fewer unique parasite infections (Fig. 3, Table 2).

Table 2
Model outcomes for each parasite response variable and parasite species richness.

Dipetalonema spp.					
	estimate	Std. Err	Wald (x^2)	Df	P-value
W/out co-infection: n = 186, df = 181, AIC = 178.0					
(intercept)	−0.79	1.80			
Body weight	5.66	2.37	5.70	1	0.017
W/co-infection: n = 186, df = 181, AIC = 175.0					
(intercept)	−1.81	1.60			
Body weight	5.10	2.23	5.24	1	0.022
M.mariae	2.91	1.66	3.07	1	0.080
Mansonella mariae					
	estimate	**Std. Err**	**Wald (x^2)**	**Df**	**P-value**
W/out co-infection: n = 120, df = 116, AIC = 94.5					
(Intercept)	−4.34	2.12			
Br_Primary	7.76	3.51	5.12	2	0.077*
Br_Secondary	5.19	2.63			
W/co-infection: n = 120, df = 114, AIC = 88.7					
(Intercept)	−4.89	1.99			
Br_Primary	7.14	2.79	6.59	2	0.037
Br_Secondary	4.42	1.96			
Dipetalonema spp.	2.56	1.13	5.18	1	0.023
Trypanosoma minasense					
	estimate	**Std. Err**	**Wald (x^2)**	**Df**	**P-value**
W/out co-infection: n = 186, df = 180, AIC = 205.0					
(Intercept)	1.66	0.81			
Age_Juvenile	−1.72	0.54	10.01	2	0.007
Age_Sub-adult	−0.56	0.63			
W/co-infection: n = 186, df = 179, AIC = 202.2					
(Intercept)	1.19	0.88			
Age_Juvenile	−1.16	0.54	4.57	2	0.102*
Age_Sub-adult	−0.45	0.64			
M. mariae	1.07	0.50	4.69	1	0.030
Parasite Species Richness					
	estimate	**Std. Err**	**Wald (x^2)**	**Df**	**P-value**
n = 186, df = 181, AIC = 496.7					
(Intercept)	0.61	0.15			
Age_Juvenile	−1.43	0.26	34.89	2	<0.001
Age_Sub-adult	−0.59	0.25			

Minimal models shown above. Saturated models included fixed factors 'species', 'sex', 'body weight', 'breeding status', and 'age class'. Co-infection models began with terms from minimal host infection models and other parasites as fixed factors. Parasite species richness was modeled using only host factors. All tests included random effects 'animal identity', 'group', and 'year' when they evidenced any discernable effect on model outcomes. The * symbol indicates factors where only one of two levels was significant, and therefore a combined x^2 statistic for all levels of those factors is not significant.

4. Discussion

The majority of studies on parasite distributions from natural populations are constrained by sampling that takes place at a single time point on unknown host individuals. This limits our ability to interpret data due to the normal fluctuations in parasite prevalence and uncertainty regarding an animal's future or past parasite infection status. In our study, we were able to sample from consecutive years at the same site, thus minimizing confounding variables such as animal disappearances or sampling from different individuals between years. The use of combined microscopy and molecular methods to screen for haemoparasitic infections improved our confidence of the infection status of each individual. We encountered slight discordance in microfilariae infection status, ~10% of blood smear positive samples were PCR negative. We think that this disparity is most likely attributable to parasitemia levels that are below the sensitivity of the PCR assay, poor sample quality, or a combination of both, as both of our assays worked 90% of the time and each primer set has been used on a broad range of nematodes in other studies (Casiraghi et al., 2001; Sato et al., 2006, 2008; Merkel et al., 2007). Discordance for *T. minasense* was greater, ~20% of blood smear positive samples were PCR negative. This level of parasite under-detection is unsurprising, as chronically infected humans and animals with low levels of parasitemia are known to occur with *Trypanosoma* spp. (Piron et al., 2007). Qvarnstrom et al. (2012) observed that different genotypes of *Trypanosoma cruzi* are differentially detected by real-time PCR assays, and therefore recommend the use of multiple protocols that target different genes. It is not known whether similar challenges are associated with detection of *T. minasense*, but if so, we would expect false-negatives to be equally represented across the study period. Instead, we had zero false-negatives in 2014, suggesting again that low parasitemia or poor sample quality were the likely culprits of

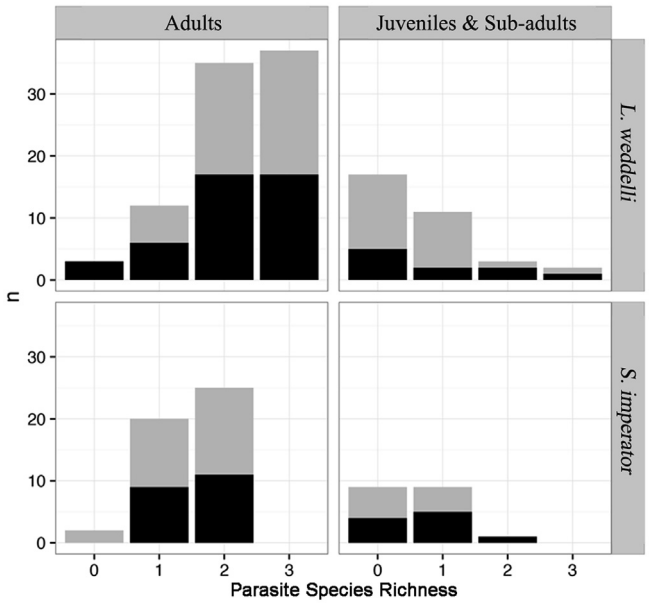

Fig. 3. Parasite species richness by species, age class and sex. Colors represent females (black) and males (gray).

false negatives. Nested PCR reactions, which we employed in this study, are the current gold standard for detection of active Trypanosome infections (Ndao et al., 2004; Aguiar et al., 2012).

We predicted that haemoparasite prevalences would remain constant over the study period, reflecting stable numbers of host individuals with average group sizes varying from 4.3 to 4.9 individuals over a range of 13–15 social groups per year. While prevalence remained stable for *M. mariae*, we cautiously report annual increases for *Dipetalonema* spp.; increases approached significance among *L. weddelli* after controlling for animal repeated measures through randomization tests (significant 63.4% of the time in 1000 iterations). *Mansonella mariae*, like other filarial nematodes such as the human-infecting *Wuchereria bancrofti*, likely exhibits circadian migrations from deeper tissues to peripheral blood where it is picked up by hematophagus vectors (Barrozo et al., 2004). We reliably detected continued *M. mariae* infection in all animals harboring infections the previous year, so temporal sampling bias would not explain differences in prevalence from *Dipetalonema* spp. Given the relatively stable climate between years and that both parasites are vectored by ceratopogonid biting midges (Shelley and Coscarón, 2001; Lefoulon et al., 2015), we suspect that within-host dynamics are responsible for the variation observed between these two filarids, although the physiological consequences of these species on wild hosts remain unknown (Strait et al., 2012). It is worth mentioning that *Dipetalonema* spp. likely represents two species, *D. gracile* and *D. graciliformis*, and our study does not differentiate whether one or both are responsible for the increase in prevalence. Phylogenetic relationships within the *Dipetalonema* clade of the Onchocercidae are an area of active research that is beyond the scope of this study (Lefoulon et al., 2015), and since mixed infections are common in nature (Sato et al., 2008; Strait et al., 2012), we do not know if parasite-host relationships would vary at sub-genus levels. The prevalence of *T. minasense* did change significantly across the study period, and most dramatic was the 2014 spike to 100% of *S. imperator* individuals and close to 100% among *L. weddelli*. Like *M. mariae*, circadian patterns of parasitemia could contribute to varying prevalence when sample collection occurs at different timings (Deane and Da Silva, 1974), however, in this study sample collection consistently took place between 6 am and noon. Instead, a new environmental stressor on the host populations, or changes in vector populations, in 2014 might explain the spike in prevalence, but follow-up studies are needed to confirm this.

In addition, multi-host, multi-parasite systems are common in nature but often unacknowledged, particularly in areas of high species density (Hopkins and Nunn, 2007). In Kibale National Park in Uganda, a long-term study of the sympatric black-and-white (*C. guereza)* and red (*Pilocolobus tephrosceles)* colobus monkeys by Chapman et al. (2005) found that forest disturbances precipitated a population increase in *P. tephrosceles* but a decrease in *C. guereza*. Simultaneously, the authors observed that a shared roundworm parasite, *Trichuris* sp., decreased in prevalence and intensity in *P. tephrosceles* while increasing in *C. guereza*. Although *Trichuris* sp. is typically asymptomatic at low levels, it can cause pathologies at higher intensities (Gillespie and Chapman, 2008; Gachinmath et al., 2014), and may very well have contributed to the decline of *C. guereza* through altered parasite-host dynamics initiated by forest clearings. Our data on haemoparasites establish a baseline that will facilitate similar research in the event of future climate or landscape disturbances. That L. *weddelli* and *S. imperator* form mixed-species associations (Watsa, 2013), possess broadly overlapping diets (*pers. obs.*), and exhibit similar social structures and reproductive strategies (Goldizen, 1996), but only share two of the three parasites is surprising. We did not expect that *M. mariae* would be absent from *S. imperator* given that it was previously documented in a more distantly related primate, the common squirrel monkey (*Saimiri sciureus*) (Sato et al., 2008). Nevertheless, as *M. mariae* evidenced a positive association with *Dipetalonema* spp., which are shared by both hosts, its potential for indirectly regulating parasitism in *S. imperator* should not be discounted.

Contrary to our hypothesis, and to a recent study on parasite distributions from a cooperatively breeding meerkat population (Smyth and Drea, 2015), we found no evidence that dominant individuals (in this case primary breeding females) were more or less parasitized than others across both host species. Our results are consistent with Viljoen et al. (2011) who found no clear relationship between parasitism and female reproductive dominance in the highveld mole rat (*Cryptomys hottentotus pretoriae*). One explanation for this negative finding is methodological. Since effects on host health and fitness are often parasitemia dependent, our reliance on parasite presence-absence, rather than densities, decreases our ability to detect small associations between parasites and host factors. Hence, we can challenge the existence of a strong relationship between blood parasites and sex or breeding status, but not entirely rule it out. Another explanation might be that cooperative breeding behavior produces group-wide energy conservation benefits that offset any extra burden that would be experienced by dominant breeding individuals (Lutermann et al., 2013). It will also be worth considering the effects of social rank from the perspective of gastrointestinal parasites in this same population. Parasites found in the gastrointestinal tract versus peripheral blood generally differ in their modes of transmission (usually direct and indirect versus arthropod-vectored, respectively) and this can precipitate fundamentally different associations with host populations. For example, the encounter-dilution effect predicts a negative association between parasitism and social group size for arthropod-vectored parasites (Mooring and Hart, 1992; Cote and Poulin, 1995). Our data showed no relationships between group size and parasite species richness; however group size only ranges from 3 to 8 in this system (Watsa et al., 2015), which may not be sufficiently variable to test this hypothesis (Patterson and Ruckstuhl, 2013).

Across all of our models, breeding status and age class were too tightly correlated to be included as separate explanatory variables, and future studies might avoid this limitation by developing fine scale measures to differentiate the adult population. That parasite species richness was significantly elevated among adult individuals and breeders, as opposed to juveniles or sub-adults, suggests that more time for parasite exposure, and not immune status, is responsible for age-biased parasite distributions. We do not think this result was influenced by parasite prepatent periods, that can last months for filarial nematodes (Wong et al., 1969), since the trend included both juveniles and sub-adults ranging from 4 to 18 months of age. Additionally, positive relationships between prevalence and intensity of filarial infections and host age are well-documented from studies on human populations (Vivas-Martínez et al., 2000; Terhell et al., 2001; Opara and Fagbemi, 2008). We also established a small but significant positive association between body weight and prevalence of *Dipetalonema* spp. Generally, the potential effects of host body size are considered with respect to parasite species richness in interspecific comparisons (Hubbell, 1997; Vitone et al., 2004), and not intraspecific prevalence. In this study, *S. imperator* is about 25% larger than *L. weddelli* (Watsa et al., 2015), and yet our models did not detect an interspecific difference in microfilariae infection, after controlling for concomitant infections. We suspect that body size covaries with other factors, such as age class, which we have shown to effect prevalence.

Controlled experimental studies on co-infection dynamics (Cox, 2001; Knowles, 2011) and studies from wild populations (Pedersen and Fenton, 2007; Monteiro et al., 2007b; Telfer et al., 2008)

consistently show that relationships between parasites should not be ignored when evaluating parasite distributions in a single host (Christensen et al., 1987). This study provides additional evidence for that conclusion; specifically, the nested modeling approach uncovered two instances in which the addition of co-infection variables improved model fit. The patterns of co-infection in this study also raise questions. *Trypanosoma minasense* and *Dipetalonema* spp. (at least marginally) exhibited increases in prevalence, and we might expect an association between the two that is mediated by the immune system. Instead, co-infection between *M. mariae* and *Dipetalonema* spp. exhibited a positive association, and *M. mariae* alone best predicts the presence of *T. minasense*.

These findings demonstrate that longitudinal sampling from known individuals provides valuable insight into limiting confounding variables and unraveling complicated relationships between parasites and wild host populations. They also reemphasize the importance of factoring co-occurring parasites into analyses on parasite distributions. Challenges associated with conducting repeat sampling of blood from wild mammalian hosts have skewed prior research towards noninvasive gastrointestinal parasite monitoring from fecal samples, yet it is important to detect deviations in these patterns due to differences in parasite life cycles. Particularly when attempting to understand parasite distributions and individual risks among hosts with complex social organization, longitudinal sampling protocols that incorporate multiple hosts can be indispensable. Here, we have established that two very similar cooperatively breeding hosts in sympatric association differ in their blood parasite assemblages, and further, that individual differences engendered by callitrichid cooperative breeding dynamics do not appear to influence blood parasite prevalence. We recommend that future work incorporate measures of individual immune status alongside blood parasite data, and also include analyses of gastrointestinal parasites.

Acknowledgements

We wish to thank researchers from Field Projects International who aided in the collection of biological samples, in particular Frances Humes. We want to thank Cindee Retke for her assistance with wet laboratory analyses. We express our gratitude to the following agencies and organizations that provided non-monetary support to this investigation: Centro de Ecologia y Biodiversidad, Amazon Conservation Association, el Estación Biológica Río Los Amigos, Dirección General Forestal y de Fauna Silvestre (Perú).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2017.03.004.

Funding

This work was supported by Field Projects International, the American Society of Mammalogists, University of Missouri Trans World Airlines Scholarship Program, Whitney R. Harris World Ecology Center, Sigma Xi Society, Idea Wild, and the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Comparative Medicine Branch.

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