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Seasonal effects in gastrointestinal parasite prevalence, richness and intensity in vervet monkeys living in a semi-arid environment

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Abstract

Parasite and pathogen incidence and prevalence is driven by both periodic variation in environmental conditions and host characteristics. Given the increasing risk of zoonotic transmission to humans, and the close phylogenetic relationship between humans and non-human primates, understanding this variation in parasite dynamics is becoming essential for epidemiologists and conservationists alike. The extreme seasonal temperatures coupled with declining annual rainfall and severe periodic drought of the semi-arid Karoo poses distinct challenges to both hosts and pathogens and serves as a window into how animals confront climate change-induced environmental changes. Here we quantified annual variation in gastrointestinal parasite prevalence, intensity and richness in three troops of wild vervet monkeys (*Chlorocebus pygerythrus*) and determined what climatic variables were driving these changes. Further, we assessed whether there is long-term temporal dependence in intra-individual faecal egg counts. We found variation in the prevalence of five genera of helminths identified in the study population, but little variation in parasite richness across the year. Such variation was driven primarily by precipitation and maximum daily temperature. Finally, we found structure in faecal egg counts, suggesting that contrary to previous findings, egg shedding of *Trichostrongylus* sp. and ?*Protospirura* sp. are not stochastic processes and may serve as an indicator of individual levels of infection in our population. Combined, these results provide the first report of seasonal effects in gastrointestinal parasites of vervet monkeys living in an extreme environment.

Introduction

Seasonality in the incidence of pathogens and parasites has been well-documented across several host and parasite genera (Altizer *et al.,* 2006), yet the underlying causes for this variation are often unknown. Seasonality in pathogens may be driven by external environmental factors, host characteristics, or features of the host-pathogen system (Lass & Ebert, 2006). Host environment is a well-documented driver of both species richness and diversity across genera (Barrett *et al.,* 2013) and seasonal climatic changes can generate periodic variation in the biology and behaviour of both pathogens and their hosts (Altizer *et al.,* 2006). Environmental conditions, including rainfall, temperature and humidity, all have an impact on parasite diversity, richness, prevalence as well as, in some cases, transmission dynamics (Harvell *et al.,* 2002; Altizer *et al.,* 2006). This can result from a seasonal influence on the pathogen's ability to survive and proliferate in the environment, presence or absence of invertebrate vectors, or from seasonal changes in host characteristics and behaviour, such as reproductive seasonality or grouping patterns (Gulland, 1995; Benavides *et al.,* 2012).

In addition to the external environment, parasite prevalence and intensity are driven by host characteristics, including age, sex, stress-related and immunosuppressive hormones and behaviour (Altizer *et al.,* 2006; Nunn & Dokey, 2006; MacIntosh, Hernandez & Huffman, 2010). Sex differences are evident in many populations ranging from sexual dimorphism to more complex physiological mechanisms and energetic needs (Key & Ross, 1999), including parasite prevalence and intensity. Sex has been shown to predict helminth infections in both humans and animal models (Monteiro *et al.,* 2007). These sex differences are often an interaction between the effects of sex hormones on immunosuppression, as well as the physiological demands of pregnancy and lactation (Moore & Wilson, 2002; Klein, 2004). In general, it is postulated that female mammals are more resistant to parasitic infections than males are and that this is likely to be due to sex-associated differences in exposure to parasites as well as the immunosuppressive properties of testosterone (Morales-montor *et al.,* 2004). There is mixed evidence for sex bias in parasite prevalence in non-human primates. Against expectation, Benavides *et al*. (2012) found that parasite species richness was higher in female chacma baboons (*Papio ursinus*) than in males, while Wren, Gillespie, Camp, & Remis (2015) and Valenta *et al*. (2017) found no evidence of sex differences in parasite infection in vervet monkeys (*Chlorocebus pygerythrus*). Sex differences have also been shown to vary across age classes (golden lion tamarins, *Leontopithecus rosalia*: Monteiro *et al.,* 2007) and according to reproductive season where males show higher parasite prevalence during the mating season and females harbour more parasites during the birth season (Japanese macaques**,** *Macaca fuscata yakui*: MacIntosh *et al.,* 2010).

Here, we investigate whether variation in gastrointestinal parasites of vervet monkeys living in the semi-arid Karoo biome in South Africa is linked to environmental conditions and sex. Specifically, we (1) assess whether rainfall, temperature, food availability/ground cover (NDVI) and sex predict parasite richness, prevalence and intensity across the annual cycle. We have shown previously that, over the short-term, this population exhibited an unusually high parasite prevalence, with an infection rate of 98% for parasites with an arthropod intermediate host (Blersch *et al.,* 2019). Here we investigate (2) whether this trend, and the trends found for other parasite genera, are sustained across the year. Given that faecal egg counts are often considered to be an unreliable index of adult parasite burden (Coadwell & Ward, 1982; Stear *et al.,* 1995; Roepstorff *et al.,* 1996; Vidya & Sukumar, 2002; Gillespie, 2006), we also (3) investigate whether egg shedding is a purely stochastic process or whether there is evidence of structure in egg shedding across the year.

Materials and methods

Study subjects

Data were collected across 12 consecutive months – April 2017 to March 2018 – from three fully habituated groups (PT, RBM, and RST) of wild vervet monkeys at Samara Private Game Reserve, South Africa (32°22’S, 24°52’E). These monkeys have been the subject of continuous data collection since 2009 and are individually identifiable from natural markings. Data were collected from a subset of 27 adult individuals (PT: 4 males, 6 females from 16 adults; RBM: 2 males, 6 females from 14 adults; RST: 3 males, 6 females from 14 adults), selected to be representative of adult demography and to reflect the full range of dominance ranks.

Study site and environmental conditions

The study area is semi-arid riverine woodland, an area characterized by low rainfall, very hot summers and cold winters (Pasternak *et al.,* 2013; McFarland *et al.,* 2015). The area is under escalating risk from climate change (Jury, 2013) and has a declining annual average rainfall of 386 mm, and average minimum and maximum temperatures of 6.1°C and 21.2°C, respectively (Pasternak *et al.,* 2013). Climate data were recorded at the field site. Information on the daily minimum and maximum temperatures was taken from a centralized weather station at the field site (Hobo U30-NRC, Onset Computer Corporation), while daily measurements of precipitation during the study period were recorded using a standard rain gauge.

For the study period, the daily minimum temperature ranged from −3.9°C to 20°C while daily maximum temperature ranged from 11.8°C to 49.6°C. Monthly rainfall was low except for two distinct peaks in October 2017 (69 mm) and January 2018 (67 mm). Total precipitation for the study period was 317 mm. Mean monthly NDVI ranged from a low of 0.3 in July 2017 to a high of 0.5 in March 2018 (Fig. 1).

Faecal sampling and analysis

Faecal samples were collected by four to five observers spread across the three troops during each of the 234 10h study days. Faecal samples were collected ad libitum and non-invasively twice per month from each of the study subjects ($N = 573$ samples; $\bar{x} = 21$/subject ± 3.1 SD).

For each sample, approximately 1 g of fresh faeces was weighed in the field immediately after defecation and directly placed into 10% neutral, buffered formalin. Individual identity (ID), date, time, troop and exact faecal weight (mean = 1.21 g ± 0.3 SD) were recorded. Samples were stored in a field laboratory before being transported to the University of Lethbridge, Canada, where faecal flotation and sedimentation techniques were used to identify parasites. A zinc sulphate centrifugal flotation technique was used to isolate helminth eggs, after modification to include an additional washing step.

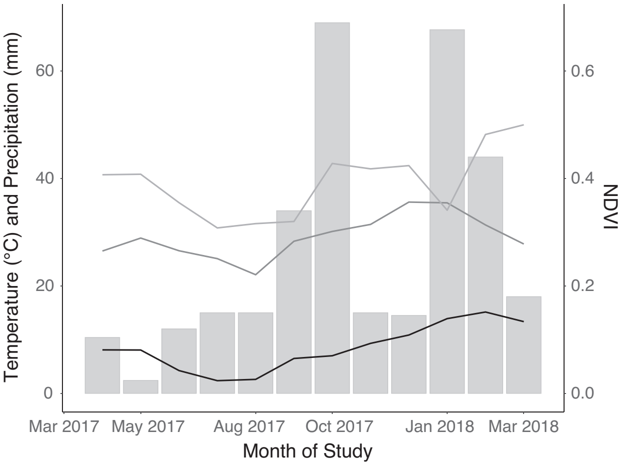

Figure 1 Graph showing overall relationships between average monthly minimum temperature (black line), average monthly maximum temperature (dark grey line), total monthly precipitation (grey bars) and average monthly normalized difference vegetation index (light grey line) across the study period (April 2017–March 2018). The y-axis (left) shows temperature in degrees Celsius and precipitation in millimetres. The y-axis (right) shows NDVI score on a 0–1 scale.

This washing step reduced the specific gravity of the $ZnSO_4$, preventing egg damage and allowing the eggs to deposit. Full methods are provided in Blersch *et al.* (2019).

Ethyl-acetate sedimentation was used to isolate potential protozoans as well as trematodes that were too heavy to float during ZnSO4 flotation using the deposit from the flotation. Following sedimentation, the entire pellet was examined under the microscope (for full methods see Blersch *et al.,* 2019). For both methods, parasite eggs were identified to genus level based on egg shape, shell thickness, colour and contents, and all eggs were counted. Representative eggs were photographed.

Food availability (NDVI)

We quantified the food available in each troop's home range by calculating the Normalized Difference Vegetation Index (NDVI) every 16 days (Dostie, 2020, in progress) from MODIS data collected by Earth Observing System (EOS) satellites Terra (EOS AM-1) and Aqua (EOS PM-1). Using Moderate Resolution Imaging Spectroradiometer MOD13Q1 vegetation indices at a 250-metre resolution (Didan, 2015), NDVI measures the amount of biomass or chlorophyll activity by calculating the difference between the visible red and near-infrared bands divided by their sum. The resultant estimate ranges between -1 and 1, where negative values indicate an absence of vegetation and positive values approaching 1 indicate larger concentrations of green vegetation (Pettorelli *et al.,* 2005). Given the generalist, largely plant-based nature of vervet diet (Pasternak *et al.,* 2013), the synoptic view of NDVI is a reliable measure of food availability in this species (Willems, Barton & Hill, 2009; Jarrett *et al.,* 2020).

Statistical analysis

All statistical analyses were undertaken in a Bayesian framework, using the 'brms' package (Bürkner, 2017, 2018) in R version 3.5.2 (R Core Team, 2018). We constructed hierarchical generalized additive mixed models to allow for non-linear relationships between explanatory and response variables (Pedersen *et al.,* 2019).

We present summary statistics and posterior density plots ('bayesplot' package Gabry *et al.,* 2019) for posterior means, standard errors and 95% credible intervals (CIs) for the main effects, and for individual variance within the random effects. For the smooth terms, we modelled both global and individual-level smooths and present summary statistics of the spline variance parameter ('wiggliness') for the global smooth and each individual's smooth. We conducted prior predictive checks (Gabry *et al.,* 2019) for each model and specified weakly informative priors (normal (0, 1)), unless otherwise indicated. We ran models with 4 chains and 2000 iterations, which provided us with a large enough sampling pool to conduct posterior sampling and achieve model convergence (McElreath, 2016; Bürkner, 2018). Chain convergence was confirmed by $\hat{R}$ values ≤ 1.01, and model goodness-of-fit was assessed using the 'posterior predictive check' (pp_check) function from the 'bayesplot' package (Gabry *et al.,* 2019). We assessed potential collinearity of fixed and random effects visually using pairs plots which produce univariate histograms and bivariate scatterplots for each parameter (mcmc_pairs function: 'bayesplot' package). Collinearity would manifest as narrow bivariate plots, which were not observed between our predictor variables. We used the 'bayes_R2' function to generate conditional R^2 values for each model (Gelman *et al.,* 2019).

Hierarchical generalized additive mixed-effects models

We constructed four generalized additive mixed effects models (GAMMs) to assess whether environmental variables and sex predicted parasite prevalence, richness or intensity across the year. Table 1 provides a summary of the model parameters. For all models, fixed effects and random effects were constant. To assess whether there was non-linearity in parasite prevalence and richness across the year, and to account for samples not being an equal number of days apart, we included a spline on date.

For parasite intensity (egg count), we were interested in individual-level annual variation, in addition to population-level annual variation, and structure in egg count. Thus, we specified the thin plate regression spline (k = 15, m = 1) on date of collection by individual ID as a fixed effect which allows each individual to have its own smoothing parameter and 'wiggliness' (GI model: Pedersen *et al.*, 2019). We also included a global smoother (thin plate regression spline: $k = 12$, $m = 2$) on date to assess population-level variation in parasite intensity across the year (GI model: Pedersen et al., 2019). Given that not all samples were exactly 1g, we included log faecal weight as an offset variable. All continuous predictor variables were mean-centred and standardized by two standard deviations to allow for effect size comparisons across continuous and dichotomous variables (Gelman, 2008).

Parasite prevalence

We could not fit statistical models for three genera, owing to low frequency and resultant small sample size (<5% annual sample prevalence), and we therefore present only descriptive statistics. One genus, where sample prevalence was 98%, could not be modelled given that it was present on all sample collection dates. For the remaining genus with high prevalence (>20% annual sample prevalence), we classified the genus as present or absent in each faecal sample and constructed a Bayesian GAMM with a Bernoulli distribution. We specified presence/absence as the binary response variable and included fixed effects, random effects and individual-level spline (Table 1).

Species richness

To determine whether parasite richness (number of parasite genera) was influenced by environmental factors and sex, we first constructed a GAMM with a Poisson distribution. Given 98% of samples were positive for one of the parasite genera, we used a non-parametric dispersion test using the 'DHARMa' package (Hartig, 2020) to confirm underdispersion (for these

Table 1 Model parameters used in the specification of generalized additive mixed-effects models (GAMMs) to assess the influence of environmental factors and sex on vervet monkey parasite prevalence, richness and intensity. Log of faecal weight was included in all models as an offset variable to account for variation in faecal weight

Measure	Species	Response variable	Fixed effects	Splines (fixed effects)	Random effects	Distribution
Parasite prevalence	*Trichostrongylus* sp.	Presence/absence	Maximum daily temperature Minimum Daily temperature Bi-weekly precipitation NDVI Sex	Date	Individual Troop	Bernoulli
Parasite richness	All (combined)	Count: number of species	Same as above	Date	Same as above	Poisson (hurdle)
Parasite intensity	*Trichostrongylus* sp.	Egg count	Same as above	Date, Date by Individual	Same as above	Poisson (hurdle)
	? Protospirura sp.	Egg count	Same as above	Date, Date by Individual	Same as above	Negative binomial

results, see Appendix S1). To account for the lower than expected variance, we used a hurdle model with a Poisson distribution. This allows for the assumption of fewer (or greater) zeroes than expected for a count distribution (Min & Agresti, 2005; Hilbe, 2017). Our response variable was the number of parasite genera recovered from each faecal sample with the predictors as specified in Table 1.

Parasite intensity and stochasticity in faecal egg counts

This model set served two purposes: to establish if there was annual variation in eggs related to environmental conditions and sex, and to assess whether there was detectable structure in egg shedding. As we have shown that total faecal egg counts between successive individual samples are correlated, we have suggested that egg counts may be a reliable indicator of an underlying infection in these vervet monkeys, rather than reflecting some stochastic process (Blersch *et al.,* 2019). We tested this for the two most prevalent parasite genera across an annual cycle. If egg shedding were not stochastic, we would have expected date of collection to explain variation in egg count.

As with model set 1, we present descriptive statistics only for the three genera with low sample prevalence (<5% sample prevalence) and low egg count, and modelled the genera with higher prevalence (>20%). For the genus with the highest prevalence (>98%), we constructed a GAMM, specifying a negative binomial distribution, with faecal egg count as our response variable. For the remaining genus, we used a GAM hurdle model with a Poisson distribution to account for the large number of zeroes (Table 1). We used prior predictive checks to first assess the suitability of the default priors set for the model parameters (Gabry *et al.,* 2019) and assess whether predicted egg counts using a prior-only model were reasonable. Using a weakly informative prior, normal(0,1), for model parameters resulted in predicted infinity values suggesting unreasonably high egg counts. Thus, we constrained two priors, hu and sds, to normal(0,0.5) for the *Trichostrongylus* sp. model and one prior, sds, for ?*Protospirura* sp. model. Hu is the parameter for the hurdle portion of the model and is the probability that the response variable will be zero, and sds is the variance parameter for the spline which controls the wiggliness of the smooth.

Results

Model Set 1: Do environmental variation and sex predict parasite presence/absence and richness?

We identified 5 helminth taxa, namely, *Trichostrongylus* sp., *Ternidens* sp., *Oesophagastomum* sp., *Subulura* sp., and a spirurid, with 98.6% of samples being positive for one or more parasite genera. All genera were recovered in both the flotation and sedimentation and egg counts reported are combined (for egg morphology, see Appendix S1). The spirurid could not be identified to species- or genus- level based on microscopy alone, as the eggs of *Physaloptera* sp. and *Protospirura* sp. are too similar to differentiate. However, based on the morphological characteristics of the eggs, including their size and the presence of a hyaline substance (Brumpt, 1931; Petrzelkova *et al.,* 2006), we consider it most likely to be *Protospirura* sp. (hereafter referred to as ?*Protospirura* sp.). Given the morphological similarities between strongyle eggs and potential size overlap, there is a degree of uncertainty in distinguishing between *Ternidens* sp. and *Oesophagastomum* sp. based solely on morphology. Molecular analysis is underway to confirm all identified genera. We recovered no protozoa from faecal samples.

We found annual variation in parasite prevalence across all identified genera (Table 2). While host group prevalence is the preferred indication of parasite prevalence in a population (Bush *et al.,* 1997), we present both percentage host group and sample prevalence for comparison to other literature (Table 2).

The most prevalent parasite in our population was ?*Protospirura sp*., with a mean annual sample prevalence of 98.7%, and present in all 27 individuals. Second was *Trichostrongylus* sp. which was recovered from 21 individuals, with a mean annual sample prevalence of 22%. Mean annual sample prevalence for the remaining nematodes was considerably lower: 3.83% for *Subulura* sp. recovered from 12 individuals, 2.42% for *Ternidens* sp., recovered from 13 individuals, and 1.7% for *Oesophagastomum* sp., recovered from 8 individuals.

Generalized additive mixed effects models results

We present model results for *Trichostrongylus* sp. only. Sample size was too small to model *Ternidens* sp., *Subulura* sp. and *Oesophagastomum* sp. and ?*Protospirura* sp. eggs were recovered on every collection date and thus equally probable across the year, regardless of host and environmental characteristics.

Model results showed evidence of non-linear variation in parasite prevalence across the year for *Trichostrongylus* sp. as indicated by the date spline parameter (Table 3: smooth-term). The probability of *Trichostrongylus* sp. occurring was predicted by maximum daily temperature, with occurrence being highest when temperatures were higher (Estimate = 0.74, Estimate error = 0.33, l-CI = 0.09, u-CI = 1.4). No other environmental variables predicted presence or absence of *Trichostrongylus* sp., and no sex differences were found (Table 3). We found evidence of inter-individual variation in the prevalence of *Trichostrongylus* sp. and, while there was evidence of inter-troop differences, credible intervals were wide, suggesting that the estimate was very uncertain (Table 3). The full model explained 41% of variance ($R^2_{\text{conditional}} = 0.41$, Est. Error = 0.03, l-CI = 0.35, u-CI = 0.46).

Parasite richness

While individual species prevalence varied across the year (Table 1), model results showed low annual variation in parasite species richness (Fig. 2).

Neither environmental variables nor sex strongly predicted variation in parasite species richness (Fig. 3). There was weak evidence of a negative relationship between parasite species

Table 2 Monthly sample prevalence (SP: number of samples positive for the parasite genus/number of samples collected) and host group prevalence (HG: number of positive hosts/number of sampled hosts) for identified genera. Austral seasons: summer (December to March), autumn (March - June), winter (June - September) and spring (September to December)

		? *Protospirura* sp.		*Trichostrongylus* sp.		*Subulura* sp.		*Ternidens* sp.		*Oesophagostomum* sp.	
Year	Month	SP (%)	HG (%)	SP (%)	HG (%)	SP (%)	HG (%)	SP (%)	HG (%)	SP (%)	HG (%)
2017	Apr	98.18	100	16.36	22.22	1.82	3.70	14.55	22.22	1.82	3.70
	May	96.83	100	46.03	51.85	0	0	4.76	11.11	0	0
	Jun	100	100	53.85	50.00	0	0	0	0.00	3.85	4.55
	Jul	100	100	43.40	50.00	5.66	11.54	5.66	11.54	1.89	3.85
	Aug	100	100	35.56	38.46	2.22	3.85	2.22	3.85	6.67	11.54
	Sept	100	100	14.81	25.93	1.85	3.70	1.85	3.70	0	0
	Oct	100	100	23.33	26.92	3.33	7.69	0	0	0	0
	Nov	100	100	4.88	8.00	7.72	12.00	0	0	0	0
	Dec	94.34	96.30	9.43	14.81	3.77	7.41	0	0	3.77	7.41
2018	Jan	100	100	14.71	15.79	8.82	10.53	0	0	2.94	5.26
	Feb	97.62	95.65	0	0.00	4.76	8.70	0	0	0	0.00
	Mar	97.87	100	2.13	4.35	6.38	8.70	0	0	0	0.00
Mean		98.74	99.33	22.04	25.69	3.83	6.48	2.42	4.37	1.74	3.03
		(±1.74 SD)	(±1.51 SD)	(± 17.56 SD)	(±17.53 SD)	(±2.71 SD)	(±3.99 SD)	(±4.13 SD)	(±6.77 SD)	(±2.09 SD)	(±3.59 SD)

Table 3 Summary statistics of Bayesian mixed-effects model for parasite prevalence (present/absent) of *Trichostrongylus* sp. in vervet monkeys

	Effect	Estimate	Est. Error	l-95% CI	u-95% CI
Fixed effects					
Population-level	Intercept	−1.99	0.75	−3.45	−0.3
	Precipitation (bi-weekly)	−0.4	0.33	−1.06	0.22
	Minimum daily temperature	−0.51	0.35	−1.2	0.18
	Maximum daily temperature	**0.74**	**0.33**	**0.09**	**1.4**
	NDVI	−0.23	0.5	−1.2	0.76
	Sex (reference: male)	−0.74	0.64	−1.97	0.52
	s(Date)	0.14	1.03	−1.82	2.12
Smooth Terms	sds(Date)	5.17	2.56	1.6	11.56
Random effects					
ID	sds(ID)	1.86	0.4	1.22	2.77
Troop	sds(Troop)	0.79	0.83	0.02	3.01
Family					
	hu	0.78	0.02	0.74	0.81

CI, credible interval; SD, standard deviation. Smooth-term sds(), spline 'wiggliness' (spline variance parameter).
Estimates for fixed effects where credible intervals do not cross zero are in bold.

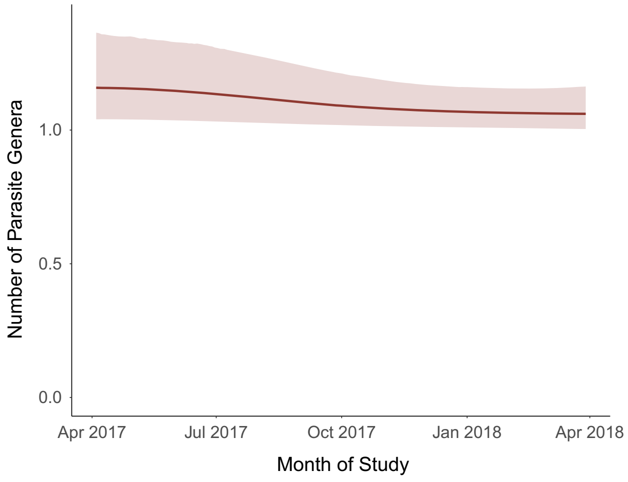

Figure 2 Estimate of parasite species richness across the study period derived from the fitted Bayesian mixed-effects hurdle model with a Poisson distribution. Upper and lower 95% credible intervals (bands) were derived from the fitted model. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

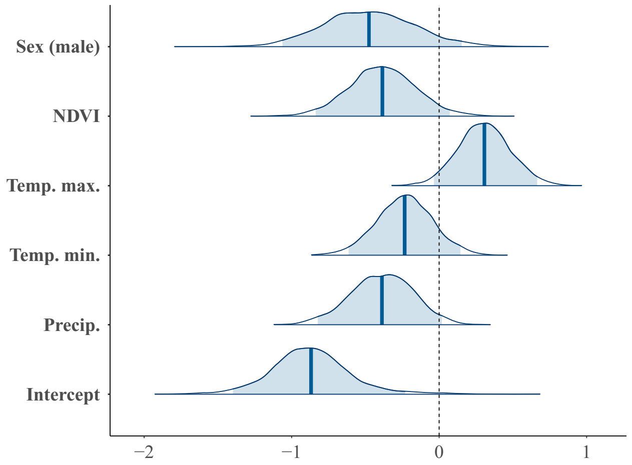

Figure 3 Posterior density plots from the Poisson hurdle GAMM showing the relationships between environmental variables (bi-weekly precipitation, minimum daily temperature, maximum daily temperature, NDVI), host sex and parasite species richness. Vertical lines represent the mean and area under the curves show 95% credible intervals. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

richness and bi-weekly precipitation and NDVI (Fig. 3). However, these estimates were small with high estimate uncertainty and should be interpreted with caution (full table: supplementary material Tables S1-S4). The full model explained 27% of variance ($R^2_{\text{conditional}} = 0.27$, Est. Error = 0.05, l-CI = 0.17, u-CI = 0.38).

Model set 2: Do faecal egg counts vary stochastically across samples?

Overall parasite intensity

There was large variation in parasite intensity (faecal egg count) within and between individuals. For*Protospirura* sp., annual minimum and maximum egg counts from positive samples (ps) were 2 EPG and 5841 EPG respectively ($\text{mean}_{\text{ps}} = 752.22$, ±861.33 SD), while mean annual egg counts for individuals ranged from 93.84 (±90.00 SD) EPG to 1862.94 (±1521.61 SD). There was also evidence of individual variation in ?*Protospirura* sp. egg shedding with some individuals having consistently low egg counts (e.g. 7–400 EPG) and some individuals having consistently high egg counts across the year (e.g. 600–2500 EPG). For *Trichostrongylus* sp., egg counts ranged from 2 to 47 EPG ($\text{mean}_{\text{ps}} = 6.5$, ±5.2). Overall, there was lower variation in egg counts for the other identified genera. For Ternidens sp., egg counts recovered from positive samples ranged from 1 to 8 EPG ($\text{mean}_{ps} = 3.10$, ±2.22 SD), *Oesophagastomum* sp. ranged from 1–17 EPG ($\text{mean}_{ps} = 4.08$, ±2.17 SD) and *Subulura* sp. ranged from 1.92 – 10.3 EPG ($\text{mean}_{ps} = 5.6$, ±2.03 SD).

Using a hierarchical generalized additive model and individual-level splines, we found evidence of temporal dependence, or structure, in egg counts (Fig. 4). If egg shedding were a stochastic process, the spline would fail to capture any pattern in the faecal egg counts. We found inter-individual variation in the magnitude of change (e.g. lowest versus highest egg count), how quickly egg counts changed across an individual's range ('wiggliness') and in overall mean egg counts, with some individuals showing consistently high egg counts and others consistently low egg counts across the year (Fig. 4). Notably, credible intervals varied across individuals, regardless of spline 'wiggliness', suggesting lower certainty in the structure of consecutive egg counts for some individuals (Full results: Appendix S1). That is, while there was a relationship between consecutive egg counts for all individuals, model results suggest it was less structured for some individuals than for others, and this was not a function of egg count being more variable for that individual.

We found evidence for overall annual variation in ?*Protospirura* sp. egg counts where intensity peaked in austral spring (Fig. 4) and individual variation in egg counts (Fig. 4). There was some evidence that parasite intensity was predicted by biweekly precipitation where higher precipitation resulted in lower ?*Protospirura* sp. egg counts (Estimate = −0.19, Estimate error = 0.07, $\text{lower}_c = -0.33$, $\text{upper}_c = -0.05$) however, the estimate was small. No other environmental variables affected parasite intensity ?*Protospirura* sp. and no sex differences were found (Fig. 5). The full model explained 68% of variance ($R^2_{\text{conditional}} = 0.68$, Est. Error = 0.03, l-CI = 0.63, u-CI = 0.73).

Trichostrongylus sp. was both less prevalent and had lower egg counts than ?*Protospirura* and we found some evidence of temporal dependence, or structure, in *Trichostrongylus* sp. egg counts, although uncertainty was high (Fig. 6). Additionally, there was lower inter-individual variation in both spline wiggliness and mean *Trichostrongylus* sp. egg count (Fig. 6) and wider credible intervals (Table S1).

Trichostrongylus sp. egg counts did not vary meaningfully over the year (Fig. 6) and none of the environmental variables included in the model predicted *Trichostrongylus* sp. parasite intensity, and no sex differences were found (Fig. 7). The full model only explained 10% of variance suggesting there are other predictors of *Trichostrongylus* sp. parasite intensity not accounted for in our models ($R^2_{\text{conditional}} = 0.09$, Est. Error = 0.07, l-CI = 0.03, u-CI = 0.31).

Discussion

Our population had a distinctively high annual sample prevalence of 98.74% for their primary parasite genus, ?*Protospirura* sp. This level of infection is in line with previous, short-term research conducted on this population (Blersch *et al*., 2019), and is considerably higher than gastrointestinal parasite infection in other non-human primate populations. The highest infection proportion reported in vervet monkeys in South Africa was a combined *Physaloptera* sp. and *Streptopharagus pigmentatus* host group prevalence of 68% (Wren *et al*., 2015). Given that ?*Protospirura* sp. is transmitted via an

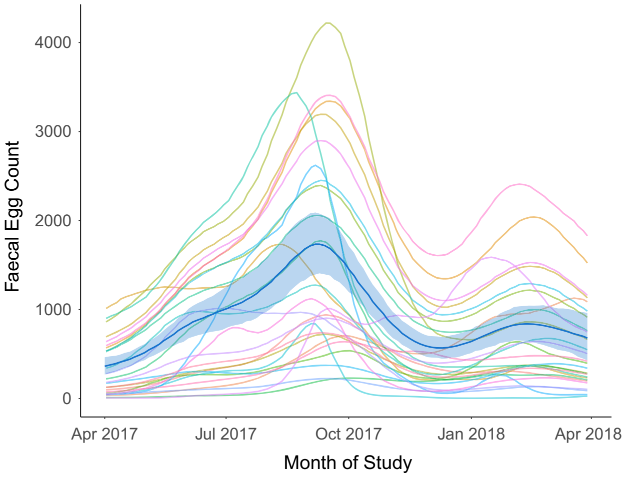

Figure 4 Estimate of mean faecal egg count of ?*Protospirura* sp. across the study period derived from the fitted Bayesian GAMM. The thicker line shows the global smooth for all individuals with upper and 95% credible intervals (bands) derived from the fitted model. Thin lines are the estimates of individual-level faecal egg counts across the study period (individual-level smooths). Individual-level credible intervals are not shown to allow for clarity (see Appendix S1 for CIs). [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

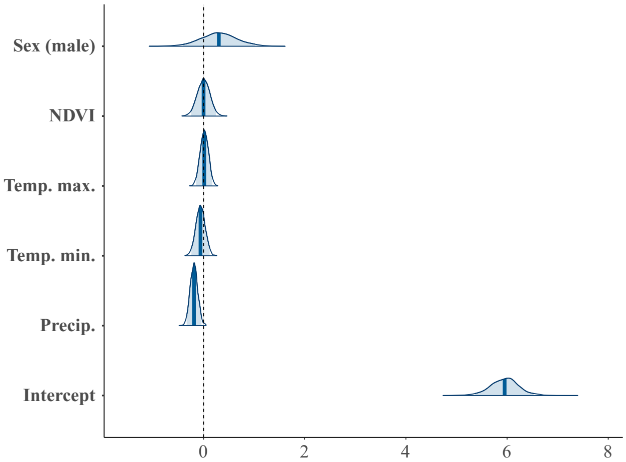

Figure 5 Posterior density plots from the negative binomial GAMM showing the relationships between environmental variables (biweekly precipitation, minimum daily temperature, maximum daily temperature, NDVI), host sex and ?*Protospirura* sp. intensity. Vertical lines represent the mean and area under the curves show 95% credible intervals. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

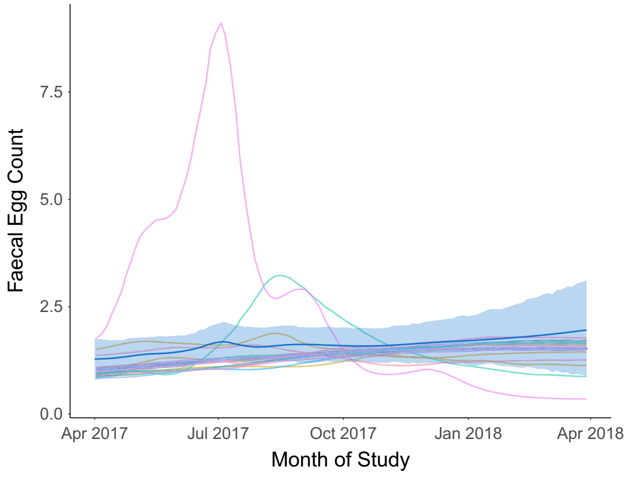

Figure 6 Estimate of mean faecal egg count of *Trichostrongylus* sp. across the study period derived from the fitted Bayesian GAMM. The thicker line shows the global smooth for all individuals with upper and 95% credible intervals (bands) derived from the fitted model. Thin lines are the estimates of individual-level faecal egg counts across the study period (individual-level smooths). Individual-level credible intervals not shown to allow for clarity (see Appendix S1 for CIs). [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

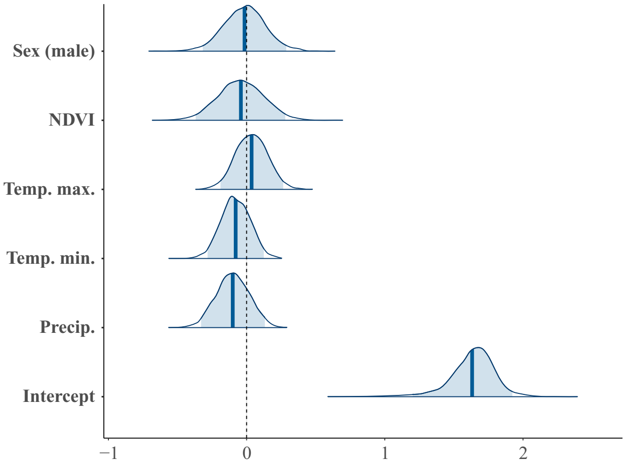

Figure 7 Posterior density plots from the Poisson hurdle GAMM showing the relationships between environmental variables (bi-weekly precipitation, minimum daily temperature, maximum daily temperature, NDVI), host sex and *Trichostrongylus* sp. intensity. Vertical lines represent the mean and area under the curves show 95% credible intervals. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

insect intermediate host, and that primates increase their insect intake under dry conditions when seasonally available or when food is more scarce (Garber, 1987; Chapman, 1988), it seems likely that the semi-arid conditions of the study site result in increased insect consumption, with a resultant increase in spirurid prevalence. Invertebrate foraging accounted for an average of 5.1% of all foraging events across the study period peaking at 10.34% which is higher than reported in some other vervets (Barrett, 2005).

Owing to the small sample size, statistical analysis could not be used to predict the presence or absence of *Oesophagastomum* sp., *Subulura* sp., and *Ternidens* sp. across the year and sample prevalence was presented as a general indication of prevalence in the population. We found only weak evidence of seasonal variation in parasite richness but did find variation in the occurrence of each genus across the year. We found that *Ternidens* sp. was only present from April to September, *Oesophagastomum* sp. was not recovered during spring while *Subulura* sp. was recovered throughout the year except for May and June, and prevalence was highest in summer. Further, sample prevalence for these genera in the current study was lower than reported in a short-term study the previous year (Blersch *et al.,* 2019). This suggests that, while there may be variation in nematode prevalence across the year, it may not be strictly seasonal or predicted by the environmental conditions at the time and that prevalence may oscillate biannually or even sporadically (Altizer *et al.,* 2006). This also highlights the importance of conducting long-term, seasonal studies when assessing parasite prevalence in a population as short-term studies may fail to accurately capture the full patterns of parasitism in the population.

The probability of *Trichostrongylus* sp. being present varied across the year but was only predicted by maximum daily temperature; that is, it was slightly more likely to occur when the maximum daily temperature was higher. Sample prevalence from May to July was lower (46%) than reported for the same period the previous year (63%). Experimentally, *Trichostrongylus* sp. infective larvae have been shown to be sensitive to both extremely high and low temperatures (Andersen, Wang & Levinem, 1966). Given that summer temperatures can exceed 40^{0}C, and that this would likely decrease infective stage larvae survival, our results suggest that there are other underlying drivers of *Trichostrongylus* sp. infection in the population and point to the need for additional host and environmental conditions to be considered. ?*Protospirura* sp. was present across the year and not predicted by environmental conditions. Given that ?*Protospirura* sp. transmission requires an intermediate host, this suggests that the monkeys either consume that host consistently across the year or that monkeys continue to be infected even if not actively consuming the intermediate host. This highlights the need to better understand intermediate host biology and has led to ongoing molecular analysis to identify the intermediate host of both ?*Protospirura* sp. and *Subulura* sp.

While commonly used in parasite studies, there is significant controversy regarding whether faecal egg counts can serve as a measure of parasite intensity or load (Gillespie, 2006). Host immunity, density-dependent factors and environmental cues affect worm ovulation (Christensen *et al.,* 1995; Stear *et al.,* 1995; Roepstorff *et al.,* 1996). Parasite age, fecundity and sex ratio also affect worm ovulation (Coadwell & Ward, 1982; Stear *et al.,* 1995; Roepstorff *et al.,* 1996). We have previously suggested that, in the short-term, the number of eggs shed in vervet monkey faeces points to an underlying infection rather than a stochastic event (Blersch *et al.,* 2019). Here, using hierarchical generalized additive models, we showed that, for *Trichostrongylus* sp. and, to a greater extent? *Protospirura* sp., this inference holds over the longer-term. Our models showed that there is global temporal dependence across an annual cycle and that this varies between individuals, with some individuals showing larger variation in egg shedding across the year than others. While it is possible that some individuals may vary more stochastically in egg shedding than others, inter-individual variation in estimate certainty is possibly a result of intervals between samples being larger in some individuals than others. These results suggest that there is underlying structure in faecal egg counts and highlights the importance of considering egg counts on an individual level as well as a population level. These patterns can be more carefully studied with more frequent sample collection.

Parasite intensity of ?*Protospirura* sp. varied across the year, peaking in austral spring whereas, *Trichostrongylus* sp. egg counts did not vary widely across the year. Rainfall was the primary predictor of variation in ?*Protospirura* sp. parasite intensity with egg counts being lower when precipitation was higher. Periodic low food and water availability at the field site have been linked to higher faecal glucocorticoid metabolite (fGCM) concentrations (Young *et al.,* 2019). Low parasite intensity during periods of high rainfall may be a result of improved host condition when environmental conditions are more favourable or may be a function of the effect of rainfall on the intermediate arthropod vector. This further points to the need to identify the insect intermediate host for this parasite.

We found no evidence of sex differences in overall parasite richness, prevalence or intensity. This is consistent with the findings of two previous studies on vervet monkeys (Wren *et al.,* 2015; Valenta *et al.,* 2017), as well as in an earlier short-term study at our field site (Blersch *et al.,* 2019). Given the mixed evidence in non-human primates, it is possible that sex bias in parasite infection is not prominent in vervet monkey populations. However, sex differences in parasite infections are thought to be the product of the physiological demands of pregnancy and lactation as well as the effects of sex hormones on immunosuppression (Moore & Wilson, 2002; Klein, 2004). As males and females have physiological demands that vary between the sexes across the year, there may be sex differences in seasonal variation of parasite intensity, richness and prevalence that are not captured by our models. This points to a need for more sex-specific seasonal measures, such as considering periods of lactation or pregnancy, to thoroughly assess the presence or absence of sex differences in parasite measures.

In summary, our results show that there is seasonality in both parasite prevalence and intensity in our study population predicted by precipitation and the maximum daily temperature. These vervets have a distinctively high overall parasite prevalence and intensity compared to other vervet populations living in tropical and sub-tropical zones. This study area is characterized by very high summer temperatures and very low winter temperatures with low annual rainfall (Pasternak *et al.,* 2013; McFarland *et al.,* 2015). With additional periodic, severe drought, this environment provides unique challenges to both host and parasites. This paves the way for more detailed research on how a primate living in an extreme environment copes with both environmental challenges and chronically high parasitic infection.

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Data accessibility

Data and scripts used for the analysis are available on GitHub https://github.com/blerschr/Vervet-monkey-parasites and archived in Zenodo (Blersch et al., 2020).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Figure S1. Results from the DHARMa non-parametric dispersion test showing underdispersion in the parasite richness dataset when modelling a Poisson distribution. ratioObsSim = 0.4945, *P*-value <- 2.2e-16.

Table S1. Summary statistics of Bayesian mixed-effects model for parasite richness (number of genera). CI, credible interval; SD, standard deviation.

Table S2. Summary statistics of Bayesian mixed-effects model for parasite richness (number of genera). CI, credible interval; SD, standard deviation.

Table S3. Summary statistics of Negative binomial Bayesian generalised additive mixed-effects model for parasite intensity (egg counts) of ?*Protospirura* sp. CI = credible interval; SD = standard deviation. Estimates where credible intervals do not cross zero are in bold.

Table S4. Summary statistics of Bayesian mixed-effects generalised additive hurdle model for parasite intensity (egg count) of *Trichostrongylus* sp. CI, credible interval; SD, standard deviation.