Comparative community-level associations of helminth infections and microparasite shedding in wild long-tailed macaques in Bali, Indonesia

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SUMMARY

Helminthes have the capacity to modulate host immunity, leading to positive interactions with coinfecting microparasites. This phenomenon has been primarily studied during coinfections with a narrow range of geo-helminthes and intracellular microparasites in human populations or under laboratory conditions. Far less is known regarding differences in coinfection dynamics between helminth types, the range of microparasites that might be affected or the overall community-level effects of helminth infections on microparasites in wild systems. Here, we analysed the presence/absence and abundance patterns of enteric parasites in long-tailed macaques (*Macaca fascicularis*) on the island of Bali, Indonesia, to assess whether naturally occurring helminth infections were associated with increased shedding of the most common intracellular (*Cryptosporidium* spp., *Isospora* spp.) and extracellular (*Entamoeba* spp., *Giardia* spp.) microparasites. We also comparatively assessed the statistical correlations of different helminth taxa with microparasite shedding to determine if there were consistent relationships between the specific helminth taxa and microparasites. Helminth infections were associated with increased shedding of both intracellular and extracellular microparasites. Platyhelminthes repeatedly displayed strong positive correlations with several microparasites; while nematodes did not. Our results indicate that helminthes can influence microparasite community shedding dynamics under wild conditions, but that trends may be driven by a narrow range of helminthes.

Key words: Coinfection, helminth, microparasite, macaque, protozoa.

INTRODUCTION

Infection ecology is traditionally conceptualized within a single parasite–single disease paradigm (Anderson and May 1978). However, coinfections with multiple parasites are common in both human and wild populations (Pedersen and Fenton 2007; Steinmann *et al*. 2010), allowing for important potential interactions between different infectious agents (Petney and Andrews 1998; Fenton, 2008; Fenton *et al*. 2008; Graham 2008). Helminthes are ubiquitous, long-lived parasites, known to have strong immunomodulatory effects (Maizels *et al*. 2003; Hewitson *et al*. 2009), and as such, have been a major focus in the study of coinfection dynamics with other parasites (Jolles *et al*. 2008; Ezenwa and Jolles 2011). Several types of interactions are possible during coinfection between helminthes and other parasites, including combined stressor effects on shared hosts (Graham 2008; Marcogliese and Pietrock 2011) and competition between pathogens (Holmes 1961, Taraschewski 2006; Lagrue & Poulin 2008, Oros *et al*. 2009). However, helminth-mediated immune modulation is most often credited with driving synergistic interactions between microparasites and helminthes (Fenton *et al*. 2008; Graham 2008; Ezenwa & Jolles 2011; Geiger *et al*. 2011), and the ability of helminth infections to attenuate immunity to other infectious agents has been well documented during coinfections with several medically important intracellular parasites, including *Mycobacterium* spp. (Resende *et al*. 2007; Diniz *et al*. 2010; Ezenwa *et al*. 2010), Hepatitis C virus (Farid *et al*. 2005) and *Plasmodium* spp. (Nacher *et al*. 2002; Graham 2008; Hartgers *et al*. 2009; Knowles 2011; Brooker *et al*. 2012; Wang *et al*. 2013).

Helminthes have been proposed to synergistically interact with microparasites through two broad immunological mechanisms: (1) Th2 polarization and (2) generalized immune suppression. Th2 polarization is the most commonly invoked explanation for interactions between helminthes and microparasites (Fenton *et al*. 2008; Jolles *et al*. 2008; Ezenwa and Jolles 2011), due in part to the long recognized ability of helminthes to induce a strong humoral immune response (Thomas and Harn 2004). The Th2 polarization hypothesis states that the strong humoral, Th2-dependent, response to most helminth

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infections attenuates the generally antagonistic cell-mediated, Th1-dependent, immune response necessary to control most intracellular pathogens (Fenton *et al*. 2008; Fietta and Desante 2009). If this is the predominant mechanism by which helminthes interact with microparasites, such interactions should primarily occur between helminthes and intracellular parasites (Romagnani, 1997; Ezenwa and Jolles 2011). This contrasts to the second proposed mechanism by which helminthes may mediate interactions with microparasites, generalized immune suppression; several helminthes have been shown to attenuate both Th1 and Th2 immunity through the secretion of molecules with various broad-spectrum immunosuppressive effects (Maizels *et al*. 2003; Hewistson *et al*. 2009). These effects include the prevention of proper antigen presentation by dendritic cells (Carvalho *et al*. 2009; Terrazas *et al*. 2010), the promotion of a regulatory immune profile (Reyes *et al*. 2010; Klotz *et al*. 2011) and the inhibition of immune cell aggregation to the site of infection (Knox, 2007). Although these immune interactions are well documented physiologically, the overall effects of helminthes on microparasites in wild ecological systems are not, and the relative importance of helminth infections on overall microparasite community dynamics remains a topic of major interest (Sutherland *et al*. 2013). Several field studies have identified associations between particular helminthes and microparasites within wild systems (Jolles *et al*. 2008; Ezenwa *et al*. 2010; Ezenwa and Jolles 2011; Hamer *et al*. 2013; Moreno *et al*. 2013); however, these have tended to include only a relatively narrow range of helminthes and microparasites, preventing assessment of whole helminth community effects on microparasites communities, or comparisons of interactions across broad groups of helminthes and microparasites.

In this study, we analysed helminth and enteric protozoan shedding data to assess the potential impact of helminth infections on enteric microparasite community shedding, represented by the two most common intracellular (*Cryptosportidium* spp. and *Isospora* spp.) and extracellular (*Giardia* spp. and *Entamoeba* spp.) microparasites. We hypothesized that helminth infections would be positively associated with microparasite shedding. We also hypothesized that nematodes and Platyhelminthes may interact with microparasites differently due to their major evolutionary divergence (Poulin and Morand 2000; Philippe *et al*. 2005; Hewitson *et al*. 2009), and specifically compared the associations of each of these phyla with microparasite community shedding to test this hypothesis. As the different proposed mechanisms (i.e. Th2 polarization and general immune suppression) for helminth immune modulation predict different associations with microparasites (Ezenwa and Jolles 2011), we hypothesized that stronger associations would be seen between helminthes and intracellular microparasites than between helminthes and extracellular microparasites in accordance with the expectations of the Th2 polarization hypothesis. We tested all of these hypotheses using a single MANOVA, with protected *F*-tests as a *post-hoc* analysis (Spector 1977; Bray and Maxwell 1982; Haase and Ellis 1987; Warton and Hudson 2004). We also performed an analysis of genera-specific associations between helminthes and each microparasite using multifactor ANCOVAs to assess the consistency of interactions within each helminth phylum and the contribution of each helminth genus to our overall results.

METHODS

Sampling

Fecal samples ($n = 488$) were collected from wild long-tailed macaques (*Macaca fascicularis*) living in the vicinity of 15 temple sites on Bali as described previously (Lane *et al*. 2011). The habitat surrounding these sites is composed primarily of bamboo forest, rice agriculture, scrub lands, and wet and dry forest. Some sites also have considerable urban habitats in their near vicinity. Sites were well surveyed during the time period preceding and following sample collection, allowing for estimates of macaque population size and assessment of several habitat variables in the area surrounding each site (Fuentes *et al*. 2005; Loudon *et al*. 2006; Lane *et al*. 2011). Habitat information was not available for two smaller sites and these sites were excluded, resulting in a reduced sample size of $n = 474$ for analyses using habitat variables. Macaque populations are provided with varying degrees of provisioning by humans across sites (ranging from 0·5 to 100 kg/day of food) and data of provisioning and other human–macaque interactions was previously collected through a survey of local inhabitants and visiting tourists (Fuentes *et al*. 2005; Loudon *et al*. 2006; Lane *et al*. 2010; Lane-deGraaf *et al*. 2014). Considerable variation in interactions with humans, population size (ranging from 25 to 400 individuals), and landscape was noted and controlled for in our analysis (Lane *et al*. 2011). All sites had similar age-structures and sex ratios (Fuentes *et al*. 2005). Several helminthes are known to exist in this system and all helminthes that could be reliably identified were included in our analysis. A variety of enteric protozoans are also present but only the most common intracellular and extracellular protozoans were included. The only protozoan with greater than 10% prevalence excluded from this analysis was the commensal amoeba genus, *Endolimax*.

Fresh, non-dry, fecal samples were collected within a short time frame on the same day from each site to avoid pseudo-replication. On average, fecal collections represented approximately two-thirds

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of macaque population size at each site. Samples were collected in a single season, the summer of 2007; therefore, seasonal or temporal variation is not a confounding factor in this analysis. This corresponded to the Bali's dry season, and major variation in rainfall was not noted over our collection period. Furthermore, as each site was only collected from once on a single date, the effects of unobserved daily variation in rainfall should be largely controlled for by population blocking in our statistical analysis. Our sampling protocol is strongly biased towards sub-adults and adults; collection of infant feces from macaques is rare due to the small size and difficulty of detecting these specimens. One gram of each sample was used for fecal diagnosis of helminth infection on the day of collection; the remaining portion of each sample was stored for subsequent analyses, including the diagnosis of protozoan parasites.

Parasitological data collection

Parasitological data collection followed Lane *et al.* (2011). In brief, protozoan parasites were quantified as the number of infective stages identified across five trichrome-stained fecal smears examined over approximately 500 fields of view, at 1000× total magnification. *Crypotosporidium* spp. were quantified with the same methodology except with iodine used as a stain instead of trichrome, as this has been shown to be considerably more sensitive for detection of this microparasite (Garcia *et al.* 1983). Helminth infections were diagnosed using standing fecal flotation, with one gram of feces examined per sample. Helminthes and protozoans were identified to the lowest possible taxonomic level based on morphology. Eggs belonging to the order Strongylida were found and presumed to be hookworm (Family: Ancylostomatidae) on the basis of size general morphology, and presence in a primate host (Jones-Engel *et al.* 2004). All parasites except these hookworms could be identified to genus, and all of these hookworms are assumed to belong to the same genus (based on consistent egg morphology) for the purpose of our analysis. As our sampling protocol was non-invasive, we could not assess the intensity of worm infections or make diagnoses using adult worms. Microparasites were quantified as 'shedding abundance', i.e. the total number of infective stages counted for each sample across all fecal slides.

Taxa-specific associations between helminthes and microparasites

The associations of different helminth phyla with overall microparasite shedding patterns was assessed using a MANOVA with helminth phyla (four levels: infected with Platyhelminthes only, infected with Nematoda only, coinfected with Platyhelminthes and Nematoda and uninfected with helminth) as an independent variable, and shedding abundance of each of the four microparasites (*Cryptosporidium* spp., *Isospora* spp., *Giardia* spp. and *Entamoeba* spp.) as dependent variables (Warton and Hudson 2004). Population was included as a blocking effect in this MANOVA, as a control for differences between collection sites. Univariate ANOVAs (protected *F*-tests), with population as a blocking effect, were used as *post-hoc* tests to this MANOVA, as this has been demonstrated to be a superior method for interpretation of significant MANOVA results (Spector 1977; Bray and Maxwell 1982; Haase and Ellis 1987). Tukey–Kramer *post-hoc* tests on population adjusted least-squares means for helminth type were used with these univariate ANOVAs to determine specific differences in microparasite-shedding rates during infections with different helminth phyla. An additional MANCOVA, with accompanying *post-hoc* tests, was also constructed that controlled for the following site-specific landscape, macaque population and provisioning variables by including them as covariates with helminth type: population size (number of adults at site), forest cover (the m^2 of continuous forest surrounding site), elevation (the height above sea level in the centre of the temple associated with the macaque population as determined by GPS), weighted provisioning (total kg of food provided divided by the macaque population size), water days (the number of days in the year in which water was readily available as determined by survey of locals, surrounding geography and rainfall data), rice (the m^2 of rice cultivation surrounding each site) and urbanization (the m^2 of city surrounding each site) (for details on collection of landscape variables, see Southern (2002) and Lane *et al.* (2011)). As the results of both models were very similar and no significant associations found in the model blocking for population alone became non-significant when also controlling with the specific landscape and population effects, only the model blocking by population is reported.

In order to assess the specific helminth genera driving phyla level associations with microparasites, a series of ANCOVAs were used to assess associations between each specific helminth genus and each microparasite. Abundance shedding of each microparasite was modelled using presence–absence data for each genus of helminth as independent variables. The inclusion of all helminth genera in these models allowed us to control for the statistical effects of coinfection with different helminth genera through the use of type III Sums of Squares. This is a conservative approach as co-associations between some helminthes (Appendix: Table A1) may have increased type II error, thereby underestimating the number of genera with significant effects. The use of population assignment as a blocking effect was inappropriate for the genera-level analyses due to associations of collection sites with many helminth

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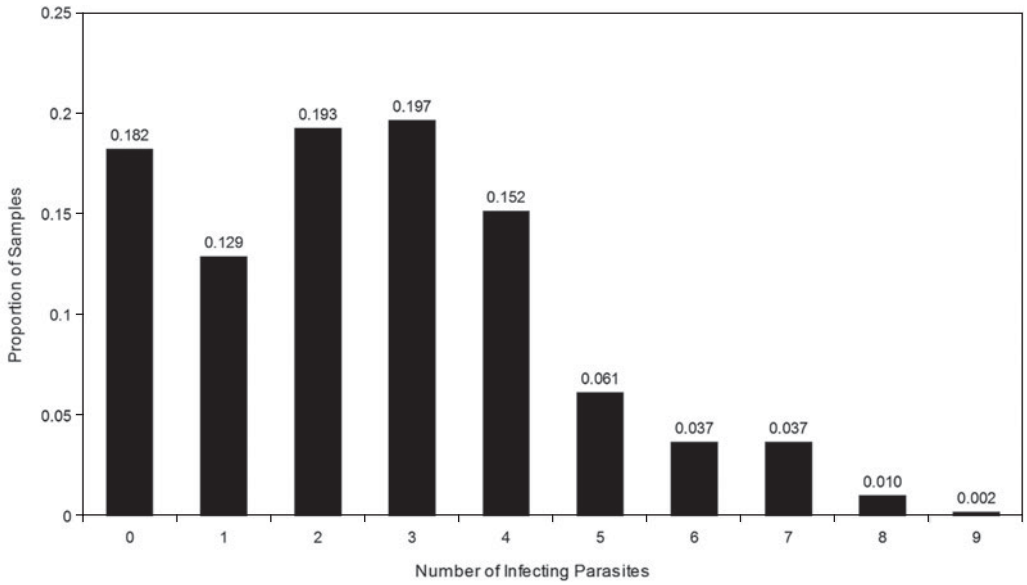

Fig. 1. Frequency of multiple parasitic infections in macaques living on Bali. The *X*-axis denotes the number of infections found in a sample and the *Y*-axis denote the proportion of samples with that number of infecting parasites ($N = 488$).

genera; instead, landscape- and population-level variables (population size, forest cover, elevation, weighted provisioning, water days, rice and urbanization) were included in these models as controlling covariates. All statistical tests were two-tailed and performed with SAS 9.3 software (SAS Institute, 2011).

RESULTS

Parasitological data and frequency of coinfections

Eighteen distinct monophyletic taxa of parasites were detected in these samples (Lane *et al*. 2011). Eight genera of helminthes were identified; hookworms could not be identified to genus but are assumed to represent a single genus for purposes of our analysis. Nine genera of enteric protozoans were found. Coinfections were common, with 69% of samples harbouring infections with more than one taxon of parasite (Fig. 1), and 49% of samples were infected with three or more parasite taxa. These estimates of coinfection rates are likely an underestimate of coinfections in these macaques, given that sporadic shedding should have resulted in reduced sensitivity in parasite diagnoses. As such, these coinfection rates should represent a lower bound.

Taxa-specific associations between helminthes and microparasites

Associations between microparasite shedding abundances and helminth infections differed significantly based on the phyla of infecting helminthes (Table 1). *Post-hoc* univariate ANOVAs showed that these differences were explained by differential shedding of *Cryptosporidium* spp., *Giardia* spp. and *Entamoeba* spp. in the presence of particular helminth phyla or combinations of helminth phyla (Fig. 2). No significant associations between helminth infections and *Isospora* spp. were found. Tukey–Kramer *post-hoc* tests, adjusted for collection site, were included in these ANOVAs to assess the specific associations between each helminth phylum and the shedding of each microparasite. Macaques infected with both Platyhelminthes and Nematoda shed significantly more *Cryptosporidium* spp. than either uninfected ($P = 0{\cdot}03$) or solely nematode infected ($P = 0{\cdot}03$) macaques; no differences in *Cryptosporidium* spp. shedding were found between solely Platyhelminthes-infected macaques and any other phyla-level groupings. Macaques infected solely with Platyhelminthes shed significantly more *Giardia* spp. than uninfected ($P = 0{\cdot}0124$) and nematode infected ($P = 0{\cdot}04$) macaques, but macaques infected with both nematodes and Platyhelminthes did not significantly differ from any of the other phyla level groupings (although they did if landscape variables are used as a control instead of population). Significantly more *Entamoeba* spp. were shed in the presence of Platyhelminthes, either on their own or with nematodes, than solely nematode-infected (Nematode-Platyhelminth: $P = 0{\cdot}0025$, Platyhelminth-only: $P = 0{\cdot}0007$) and uninfected macaques (Nematode-Platyhelminth: $P = 0{\cdot}0031$, Platyhelminth-only: $P = 0{\cdot}0002$).

In order to further explore differences in genera-level associations within each helminth phylum, multifactor ANCOVAs were used to examine associations between the presence–absence of each helminth genus and the shedding abundance of each microparasite using landscape and population variables (population size, forest cover, elevation, weighted provisioning, water days, rice and urbanization) as

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Table 1. Helminth phyla and site associations with microparasite shedding ($N = 488$)

Dependent variable(s)	Test	Effect	Λ_{Pillai}	F	r^2	$(\eta^2)^a$	P-value
Cryptosporidium, Isospora, Giardia, Entamoeba	MANOVA	Helminth phyla	0·08	3·11	–	–	0·0002
		Site	0·57	5·57	–	–	<0·0001
Cryptosporidium	ANOVA	Overall model	–	13·41	0·327	–	<0·0001
		Helminth phyla	–	2·75	–	0·012	0·0421
		Site	–	14·03	–	0·28	<0·0001
Isospora	ANOVA	Overall model	–	2·85	0·935	–	0·0001
		Helminth phyla	–	0·84	–	0·005	0·47
		Site	–	3·02	–	0·081	0·0002
Giardia	ANOVA	Overall model	–	4·99	0·15	–	<0·0001
		Helminth phyla	–	3·41	–	0·018	0·0176
		Site	–	3·52	–	0·089	<0·0001
Entamoeba	ANOVA	Overall model	–	6·73	0·196	–	<0·0001
		Helminth phyla	–	8·63	–	0·044	<0·0001
		Site	–	5·75	–	0·138	<0·0001

[a] (η^2) refers to the semi-partial eta-squared based on type III sums of squares controlling for other effects in the model and should be regarded as a lower bound.

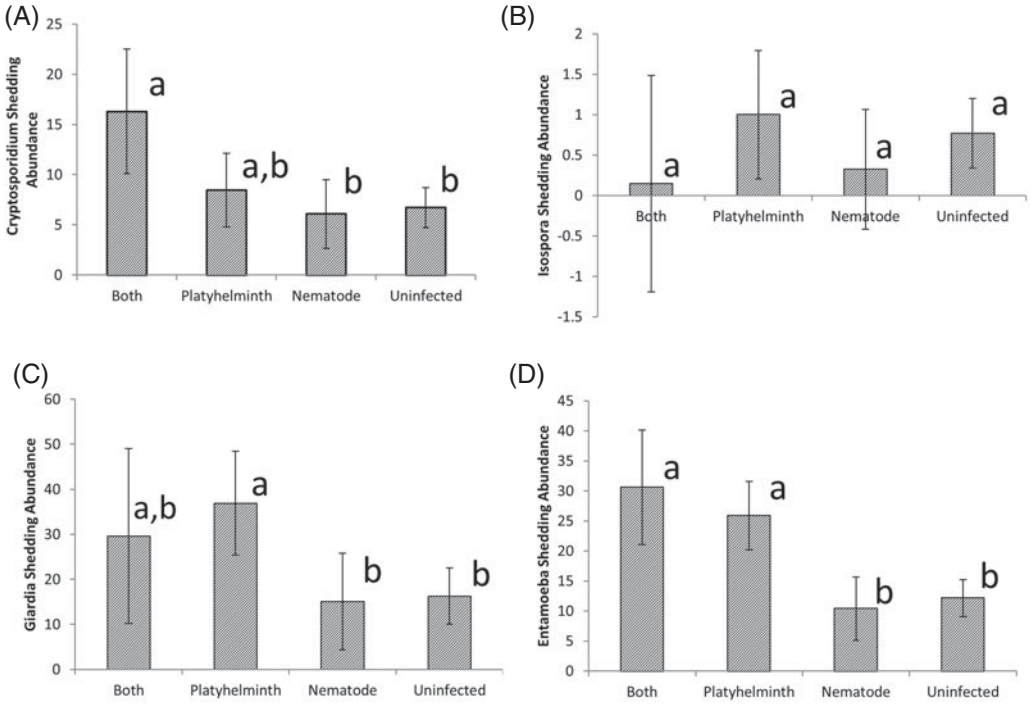

Fig. 2. Population-adjusted least-squares means ($N = 488$) for the shedding abundance, the number of infective stages per sample, of: (A) *Cryptosporidium*, (B) *Isospora*, (C) *Giardia* and (D) *Entamoeba*. Different letters denote significant differences. Error bars show 95% confidence intervals.

covariates. Although the overall models containing all genera were significant for all microparasites, only a few specific helminth genera (*Taenia, Alaria, Paragonimus* and hookworms) showed significant associations with the shedding abundances of any microparasites (Fig. 3). Additionally, several of these helminth genera showed significant associations with the shedding abundances of multiple microparasites (Table 2). *Taenia* spp. infections were positively associated with *Cryptosporidium* spp., *Giardia* spp. and *Entamoeba* spp. shedding abundances. *Paragonimus* spp. infections were positively associated with *Giardia* spp. and *Entamoeba* spp. shedding abundances.

DISCUSSION

Our study tested the hypothesis that helminthes would show associations with microparasites within our study system and that, based on the reported immunomodulatory abilities of helminthes, associations between helminthes and microparasite

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Table 2. Specific helminth genera and habitat associations with microparasite shedding ($N = 474$)

Microparasite	Significant model terms	F	r^2	$(\eta^2)^a$	P-value
Cryptosporidium	Overall model	11·16	0·268	–	<0·0001
	Taenia	6·17	–	0·01	0·0134
	Forest cover	50·86	–	0·081	<0·0001
	Elevation	60·66	–	0·097	<0·0001
	Water days	19·17	–	0·031	<0·0001
	Rice	32·58	–	0·052	<0·0001
Giardia	Overall model	6·94	0·185	–	<0·0001
	Taenia	17·69	–	0·032	<0·0001
	Paragonimus	30·00	–	0·0534	<0·0001
	Weighted provisioning	9·00	–	0·016	0·0028
	Rice	6·45	–	0·0115	0·0115
Entamoeba	Overall model	5·23	0·146		
	Taenia	13·93		0·026	0·0002
	Paragonimus	7·34		0·014	0·007
	Rice	8·32	–	0·016	0·0041

[a] (η^2) refers to the semi-partial eta-squared based on type III sums of squares controlling for other effects in the model and should be regarded as a lower bound.

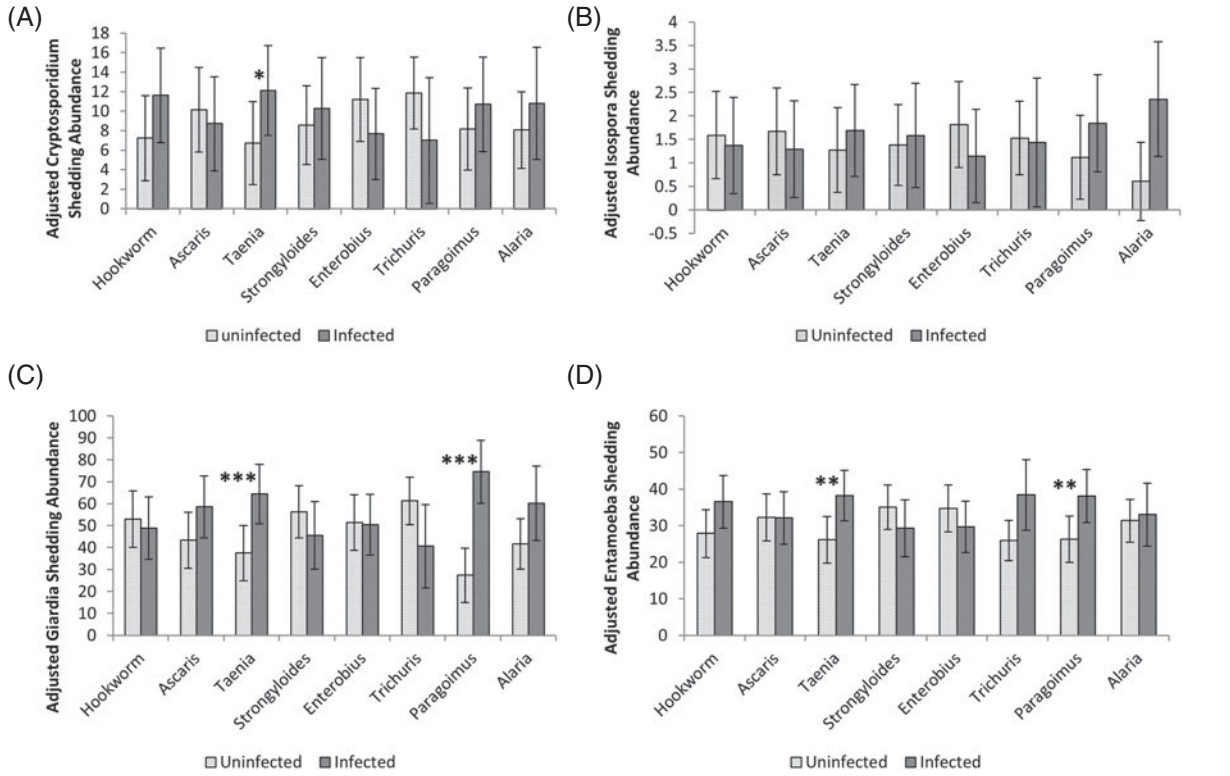

Fig. 3. Least-squares mean of shedding abundance, the average number of infective stages detected per sample, of microparasites by genera of infecting helminthes ($N = 474$) adjusted for population size, forest cover, elevation, weighted provisioning, water days, rice, urbanization and all other helminthes: (A) *Cryptosporidium*, (B) *Isospora*, (C) *Giardia*, (D) *Entamoeba*. Error bars denote standard error. * denotes significance at $P < 0·05$, ** significant at $P < 0·01$, *** significance at $P < 0·0001$.

shedding would be predominantly positive when they did occur. Although strictly correlative, our results support this hypothesis and demonstrate strong positive relationships between infections with certain parasitic worms and the shedding of both intracellular and extracellular enteric microparasites. In fact, observed associations were exclusively positive (no negative associations were found after controlling for population or landscape variables). We also hypothesized that helminthes and microparasites may show different patterns of association with one another on the basis of helminth phylogeny and intracellular *vs* extracellular status of the microparasite. We found that phylogenetically similar

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helminth taxa tended to consistently interact with a range of microparasites, but that these interactions occurred regardless of intracellular vs. extracellular status of the protozoans. Although overall, a greater number of associations with stronger effects (as indicated by F statistic values and P-values) were found with both of the extracellular protozoans than with either intracellular protozoan.

When conducting this analysis we considered that helminthes and microparasites may interact with one another differently depending on the specific taxa involved. Overall, our results indicate a potentially important role for Platyhelminthes in microparasite community dynamics: this phylum showed significant positive associations with three of the four microparasites (*Cryptosporidium, Giardia* and *Entamoeba*). In contrast, nematodes did not appear to be particularly influential on microparasite distributions in our study system, and no associations between any nematodes and mircroparasites were found after controlling for potentially confounding variables.

A causative role for helminthes in driving the interactions cannot be definitively established by a correlative study such as ours, as associations between parasites may occur as shared effects of hidden confounding variables on multiple parasites. We attempted to control against the effects of such hidden variables by alternately controlling for site of collection, as well as several population and landscape variables. As such, it is unlikely that hidden variables related to habitat confounded our results. There are some individual-level factors for which we did not control, most notably individual age and resistance to parasites. These factors may have influenced our results, but we expect these influences to be minimal as age-structure was highly similar across all groups and sampling was strongly biased towards adults. Moreover, it seems unlikely that factors such as these would have driven entirely positive interactions with only a few genera of platyhelminthes across multiple microparasites, and almost no interactions with nematodes, for many of which, immune status, diet and provisioning are known to be important determinants of distributions (Bradley and Jackson 2004; Weyher *et al*. 2006; MacIntosh *et al*. 2010; Nunn, 2012). In addition, the observed patterns would seem particularly unlikely to occur through common exposure as all three platyhelminth genera are characterized by different complex life-cycles, in contrast to the nematodes and microparasites which both possess much more similar direct life-cycles.

Although our current data preclude discerning a specific mechanism for the positive interactions we observed, our results are consistent with outcomes from other immunological studies involving helminth and microparasite interactions (Bednarska *et al*. 2008; Hamm *et al*. 2009; Hagel *et al*. 2011). Two hypotheses have been proposed to explain interactions between microparasites and helminthes, and these hypotheses make different predictions about the range of microparasites that helminthes may interact with:

(a) Th2 polarization by helminthes is expected to primarily drive interactions with intracellular microparasites; and
(b) helminth-mediated generalized immune suppression is expected to produce synergistic interactions with both intracellular and extracellular microparasites (Ezenwa and Jolles 2011).

We found relationships between helminth infections and both intracellular and extracellular microparasites, with extracellular interactions being more common with larger effects. Furthermore, there were no helminthes that interacted with only intracellular microparasites and not also with extracellular microparasites. These findings are not consistent with the predictions of the Th2 polarization hypothesis alone. Our results are more compatible with the predictions of generalized immune suppression by helminthes, although it is possible that both mechanisms may still be acting in this system.

Our results suggest that Platyhelminthes may play a particularly important and synergistic role in helminth–microparasite coinfection dynamics. All genera of microparasites, except *Isospora* spp., showed significant positive interactions with Playthelminthes at the phylum level, and two platyhelminth genera (*Taenia* and *Paragonimus*) showed significant interactions with multiple microparasites. This trend is particularly striking given the range of tissues and trophisms utilized by these platyhelminth genera. However, these trends are consistent with previous laboratory evidence indicating the ability of these taxa to affect generalized immune suppression in their hosts. *Taenia* have been demonstrated to suppress host immunity generally through a variety of mechanisms, including the promotion of alternatively activated macrophages (Reyes *et al*. 2010, 2011), interference in dendritic cell maturation (Terrazas *et al*. 2010) and prevention of neutrophil aggregation (Knox, 2007). We know of no specific work on the immunomodulatory capabilities of *Paragonimus*, but several immuno-suppressive secretory products identified from *Fasciola hepatica* have homologues in *Paragonimus* and other trematodes (Robinson *et al*. 2013). Many of these homologous secretory products have been shown to have generally immunosuppressive effects and to promote regulatory immune profiles. These genera, and the phylum Platyhelminthes in general, may represent particularly worthwhile candidates for future coinfection studies, especially given the overwhelming historical focus on nematodes (with the exception of *Schistosoma* spp.) in past coinfection studies (Nacher *et al*. 2002; Ezenwa *et al*. 2010; Hagel *et al*. 2011; Brooker *et al*. 2012).

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In complex ecological systems, it is possible that compensatory effects on the outcome of multiple parasitic infections may exist, and that chaotic influences may undermine whatever effects remain (Behnke *et al*. 2001). Using full models that include infections with all helminth taxa, our analysis was able to quantify the strength of interactions between helminth and microparasites providing new insight into the potential importance of helminth infections on wild microparasite community dynamics. Although substantial differences between mean microparasite shedding were observed during specific helminth infections, the overall amount of variation in microparasite shedding explained by helminth infections was relatively subtle, suggesting that a wide range of other ecological factors are exerting additional influences on microparasite distributions and perhaps swamping or attenuating the overall influence of helminthes on the microparasite distribution in our system. Another explanation for this relatively small amount of variation explained by overall helminth community could have been our inability to include helminth infection intensity in our model, as this has been suggested to be potentially important to coinfection dynamics (Fenton *et al*. 2008). Regardless, our results confirm that multiple parasitic infections, including infections with more than three parasites, are indeed extremely common in our system and that the effects of helminth communities were still large enough in many cases to be ecologically important. Moreover, we found that almost all helminth effects were driven by a small minority of taxa that may constitute particularly important 'keystone parasites' within the macaque parasite community.

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APPENDIX

Table A1. Helminth coinfection occurrences in abundance data set ($N = 488$)

	Hookworm	*Ascaris*	*Taenia*	*Strongyloides*	*Enterobius*	*Trichuris*	*Alaria*
Ascaris	**Wald = 41·67** **OR = 18·41** ***P* < 0·0001**	X	X	X	X	X	X
Taenia	**Wald = 21·79** **OR = 6·66** ***P* < 0·0001**	**Wald = 16·15** **OR = 4·99** ***P* < 0·0001**	X	X	X	X	X
Strongyloides	Wald = 1·12 OR = 2·283 *P* = 0·2897	**Wald = 12·36** **OR = 7·44** ***P* = 0·0004**	Wald = 0·25 OR = 1·39 *P* = 0·6139	X	X	X	X
Enterobius	Wald = 0·72 OR = 1·720 *P* = 0·3964	**Wald = 7·73** **OR = 3·99** ***P* = 0·0054**	Wald = 0·06 OR = 0·874 *P* = 0·8073	**Wald = 6·57** **OR = 4·69** ***P* = 0·0104**	X	X	X
Trichurisprev	**Wald** = 5·96 **OR** = 8·770 ***P* = 0·0146**	Undefined (complete overlap)	*P* = 0·9673 (no overlap at all)	No overlap *P* = 0·9836	Wald = 0·87 OR = 2·813 *P* = 0·3518	X	X
Alaria	Wald = 0·23 OR = 1·67 *P* = 0·6322	Wald = 2·49 OR = 3·564 *P* = 0·1147	Wald = 1·71 OR = 2·46 *P* = 0·1914	No overlap *P* = 0·9778	Wald = 2·09 OR = 3·20 *P* = 0·1480	**Wald = 3·87** **OR = 9·44** ***P* = 0·0491**	X
Paragonimus	Wald = 0·21 OR = 0·618 *P* = 0·6432	Wald = 0·079 OR = 1·237 *P* = 0·1146	Wald = 0·04 OR = 1·119 *P* = 0·8402	Wald = 0·004 OR = 1·070 *P* = 0·9488	Wald = 0·41 OR = 0·516 *P* = 0·5224	**Wald = 6·18** **OR = 9·141** ***P* = 0·0129**	**Wald = 7·623** **OR = 7·08** ***P* = 0·0058**