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Antagonism between two intestinal parasites in humans: the importance of co-infection for infection risk and recovery dynamics

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Co-infection may affect transmission and recovery from infection, but remains an understudied element of disease ecology, particularly with regard to antagonism between parasites sharing a host. Helminth and giardia infections are often endemic in the same populations and both occupy the small intestine; yet few studies have examined interactions between these parasites. We report on helminth–giardia co-infections in a panel study of forager–horticulturalists in the Bolivian lowlands. Parasites were identified in faecal samples from 3275 participants, collected during 5235 medical exams over 6 years. Longitudinal co-infection patterns were examined using logistic mixed and multi-state Markov models. The most prevalent infections were hookworm (56%), *Giardia lamblia* (30%) and *Ascaris lumbricoides* (15%). Cross-sectionally, hookworm and *A. lumbricoides* were negatively associated with *G. lamblia* (OR = 0.60; OR = 0.65, respectively). Longitudinally, giardia infection was less likely in helminth-infected individuals (HR: 0.46). Infection with helminths was also less likely for individuals infected with giardia (HR: 0.71). Finally, treatment with mebendazole reduced subsequent hookworm infections, but resulted in a marginal increase in the odds of *G. lamblia* infection. Our results provide evidence for an antagonistic relationship between helminths and giardia, and suggest that co-infection should be considered in disease transmission models and treatment decisions.

1. Introduction

A complete understanding of pathogen and parasite ecology requires knowledge about how pathogens and parasites interact with one another as they compete for limited hosts and other resources. Co-infection with multiple species of parasite or pathogen is likely to be the rule rather than the exception in most biological systems, including human populations with limited medical access. Although co-infection studies are common for diseases in which the effects of co-infection are dramatic (e.g. HIV [1]) or where infections are similar (e.g. multiple species of helminth [2]), relatively little is known about the interactions between many common parasites and pathogens. Investigation of co-infection is important, since co-infection may alter both treatment and susceptibility to infection [3,4].

Helminths are common parasites with significant potential for interactions with other pathogens and parasites. Several recent studies have demonstrated that helminth infection modifies risks for many other infections. In part, these interactions may arise because helminths have significant effects on host immune function, for example affecting the balance of T_H1, T_H2 and regulatory T cells [5,6]. In some cases, these changes may protect against other conditions, such as severe malaria [7]. However, the immunomodulatory effects of helminths may also limit the effectiveness of vaccination and increase susceptibility to other infectious diseases [4,8–11].

Giardia lamblia (a.k.a. *Giardia intestinalis, Giardia duodenalis*) is a water-borne, intestinal protozoa that is estimated to infect around a billion people worldwide, many of which are in developing and subsistence populations [12]. Although *G. lamblia* infection may appear asymptomatic, particularly in individuals living with endemic infection, for many others *G. lamblia* is associated with significant morbidity, including diarrhoea, cramping, bloating and weight loss. In particular, these symptoms can adversely affect young children, affecting growth, school performance and quality of life [12].

Both giardia and helminths disproportionately affect individuals in underdeveloped areas, and often co-infect the same individuals. However, very few studies have examined interactions between giardia and helminths, despite the potential for interactions due to occupation of similar intestinal substrates and the involvement of shared immune defences in fighting both types of parasites. The few reports that exist suggest that infection with helminths may alter immune responses to giardia [13], that giardia and helminth co-infections may occur less frequently than would be expected (suggesting antagonism) [14], and that treatment for helminths may increase susceptibility to *G. lamblia* [15]. This last possibility has strong implications for treatment plans in areas where both are endemic. There is currently no consensus as to the value of treating helminth infections in endemically infected populations. On the one hand, helminth infections are associated with significant morbidity, including growth deficits and anaemia [16]. On the other hand, individuals treated for helminths in highly endemic populations may become reinfected, limiting the effectiveness of one-time or even periodic treatment [17,18]. If helminth infections are antagonistic to *G. lamblia* infections, then this may further alter the cost–benefit calculus for treatment, and initiatives such as frequent deworming of an entire population may need to be reconsidered. Given that giardia and helminths each infect around 15% of the world's population, understanding the relationship between these two pathogens has significant consequences for world health.

Given the paucity of reports examining the effect of helminth infection on giardia infection and vice versa, we report on infections in a population of lowland Bolivian forager–horticulturalists, where both infections are chronic and endemic. We use data from an on-going, multi-year, longitudinal panel study to address the following questions. What is the relationship between helminth and giardia infections? Does infection with one alter the odds of infection with the other? Does treatment for one alter the odds of infection with the other?

2. Material and methods

(a) Subject population

Tsimane are forager–horticulturalists who live along the Maniqui River and surrounding areas in lowland Bolivia; they subsist primarily on cultivation of plantains, rice, manioc and corn, as well as hunting, fishing and gathering. The data for this study were collected in 83 Tsimane villages representing a modest range of environmental (forest versus riverine) and economic (market integrated versus isolated) situations. Since 2002, the Tsimane have been participants in the on-going Tsimane Health and Life History Project (THLHP; http://www.unm.edu/~tsimane). All Tsimane residing in study villages are eligible to participate in the study, and the vast majority

of the Tsimane population chooses to do so at least once. THLHP researchers work extensively in Tsimane villages, collecting demographic, anthropological and biomedical data, while also providing primary medical care. Helminth infections are highly prevalent, infecting 57% of the population [19].

(b) Data collection and medical care

Study participants were seen by the mobile THLHP biomedical team who visited Tsimane villages annually from 2006 to 2010. Patients seen by THLHP physicians were given routine physical exams (patient history, symptom investigation, blood pressure and temperature, height and weight). Following on-site analysis of participant blood and faecal samples, physicians administered vitamins, antibiotics, anti-protozans and anti-helminthics as warranted. Ethnographic and epidemiological information on the Tsimane, methods for age estimation, subject sampling, biomarker collection and physician diagnostics have been described elsewhere [19,20].

For this study, we considered all medications prescribed by project doctors known to be effective as anti-protozoal and anti-helminthic agents. Information on treatments given and infection status during the previous examination was available for 1082 individuals at 1731 medical visits. Patients were treated with four relevant medications: albendazole, mebendazole, metronidazole and tinidazole. Albendazole and mebendazole were prescribed as anti-helminthics but also have the potential for anti-protozoal effects [12,21]. Metronidazole and tinidazole were prescribed as anti-protozoal agents; neither is known to be effective against helminths. Patients were treated if they had either a positive faecal analysis or symptoms indicative of infection. In most cases, albendazole was prescribed as a single 400 mg dose. Mebendazole was prescribed either as a single dose of 500 mg or as 100 mg twice daily for 3 days. Metronidazole was prescribed at 250 or 500 mg three times a day for 7 days. Tinidazole was given as a single 2000 mg dose.

Owing to the difficulty of working in an isolated rural Bolivian context, the choice of medication was made by study doctors based on availability. Albendazole in particular was either unavailable or prohibitively expensive during most of the study, and was used only in the later part of 2008 and 2009. At all other times mebendazole and metronidazole were prescribed. Not all patients were prescribed medications (see below). In many cases, patients either would leave the site of the mobile medical care before faecal analysis was completed, or would decline treatment. In other cases, neither medication was available.

(c) Faecal analysis

Faecal samples were analysed using two methods. Starting in 2004, faecal samples were analysed for the presence of helminth eggs, larvae, protozoa and other parasites by direct identification on wet mounts. Duplicate mounts were prepared with 0.9% saline solution and iodine solution, respectively, and examined at $100\times$ and $400\times$ [19,22]. Beginning in 2007, faecal samples were also preserved in 10% formalin solution following direct identification, and later analysed using a modified Percoll (Amersham Pharmacia) technique [23]. As we report elsewhere, the Percoll method identified slightly more positive cases, but did not produce systematic biases in parasite identification [19]. We therefore coded individuals as positive or negative for identified species in a single dataset, regardless of which method was used.

(d) Statistical analysis

Logistic mixed models were used to examine odds ratios for infection status given co-infection and treatment variables. Random effects were included to control for repeated observations on the same individual and within village correlations. Since helminth

Table 1. Sample characteristics. Twenty-three participants were age 18 or under during some examinations and over 18 during others. Observations from these individuals contribute to both means. Juveniles are 18 and under, whereas adults are older than 18.

	juveniles		adults	
	female (n = 804, obs = 952)	male (n = 766, obs = 886)	female (n = 868, obs = 1692)	male (n = 860, obs = 1670)
	mean $\pm$ s.d.	mean $\pm$ s.d.	mean $\pm$ s.d.	mean $\pm$ s.d.
age (years)	6.3 $\pm$ 4.4	6.2 $\pm$ 4.3	48.5 $\pm$ 14.2	50.4 $\pm$ 14.1
height (cm)	105.6 $\pm$ 25.5	105.2 $\pm$ 25.3	150.8 $\pm$ 4.9	162.1 $\pm$ 5.7
weight (kg)	20.8 $\pm$ 12.8	20.1 $\pm$ 11.7	55.3 $\pm$ 9.6	63 $\pm$ 8.6
body mass index (kg m^{-2})	17 $\pm$ 2.7	16.8 $\pm$ 2	24.3 $\pm$ 3.8	24 $\pm$ 2.8

infections often show nonlinear age patterns, age was modelled with thin-plate regression smoothing splines in generalized additive mixed models [24]. For models in which the spline reduced to a single degree of freedom, splines were refitted with natural log age, which forces the spline to fit early life patterns. To test for interactions between predictors and sex, we included sex-by-parameter interaction terms in models one term at a time, retaining only those interactions that were significant. For all regression analyses, we controlled for age, sex and study year. In treatment analysis (§3d) we also controlled for infection status with the parasite in question at the previous visit. In treatment models, all age terms reduced to linear parameters. We therefore report age as a linear parameter for these models.

Since cross-sectional analyses are of limited usefulness in establishing causal direction, we used a Markov multi-state model (MMSM) to examine longitudinal data in sequence. The model allows repeated observations at arbitrary times to be used to estimate instantaneous rates of transition from one state to another; in this case, transitions between infection states over time [25]. More specifically, these represent the probabilities of recovery or infection at any given moment in time, which can be used to calculate the likelihood of transition over a defined period of time. Hazard ratios can also be calculated for comparable transition intensities. Our model includes four disease states: uninfected (U), hookworm-infected (H), giardia-infected (G) and co-infected (C). Transition intensities are estimated from continuous time observations, which are linked together by subject to model transitions for each individual. Models included eight intensity transitions (figure 2a). Direct transitions between C and U were not allowed in the model; instead recovery and co-infection require two transitions—first to a single infection state (H or G) and then to U or C. Because the model includes eight basic parameters, all of which can interact with covariates, degrees of freedom are rapidly lost when including covariates. We therefore used a modelling strategy in which covariates were checked for significance and removed in a backward stepwise manner, and in which covariates were constrained to affect only certain transition intensities, as we describe later.

First, a basic model was first fitted with no covariates. Starting transition intensities between infection states were arbitrarily set at 1.0 (equivalent to a mean infection duration of approx. 1 year). However, models were relatively insensitive to starting values; values between 0.1 and 2.0 produced similar final models. Owing to a very low number of individuals who transitioned from a co-infected to a giardia-only state, initial models had difficulty converging on this intensity. We therefore constrained this transition intensity to be equal to the transition from a helminth-only to an uninfected state, since both transitions represented recovery from a helminth infection.

The transition intensity matrix from the basic model was used as the initial values matrix for subsequent models. In these models, all eight transition intensities were unconstrained. Study year was constrained to affect only infection rates, but not recovery rates, while medication was modelled as affecting recovery rates, but not infection rates. We began with all four treatment variables and study year in the model, and then removed non-significant covariates one at a time. After each parameter change, model AIC was assessed. We proceeded until model AIC was minimized. Once this model was settled on, sex-specific effects and interactions were screened for by running separate male and female models, and comparing transition intensities and hazard ratios between these models. Significant interactions were included in the final model. Finally, to verify that effects were not due to nonlinear age patterns, we ran separate models on younger (less than or equal to 16) and older (greater than or equal to 40) participants. All models and analyses were run in R v. 3.0.11 (http://cran. r-project.org/) using *lmer* in the *lme4* package, *msm* in the *msm* package, and *gamm* in the *mgcv* package. See the electronic supplementary material for the data used in these analyses.

3. Results

(a) Physician examinations

Between July 2006 and May 2012, 3275 participants age 0–90 (mean 34.2 $\pm$ 23.7, 51% female) were seen over the course of 5235 physician examinations (table 1). Individuals were seen between one and five times each (mean 1.6 $\pm$ 0.9). On average, subsequent visits were just over 1 year apart (1.20 $\pm$ 0.32 years). Sixty-five per cent of physician examinations were with adults (over age 18), although only 53% of individual participants were over age 18.

(b) Helminth and protozoan infection prevalences

Table 2 shows the prevalences for the principal species of helminths and giardia among juveniles and adults, respectively. The most common parasites found were hookworm, *G. lamblia* and *Ascaris lumbricoides*, found in 56%, 30% and 15% of faecal samples, respectively. Over the course of the study, prevalences changed significantly (figure 1), with helminths becoming more prevalent and giardia infections declining. We therefore controlled for study year in all additional analyses. Controlling for study year, age and repeat observations, males were less likely to be infected with *A. lumbricoides* (OR = 0.75, $t_{5,171} = -2.46$, $p = 0.01$), and more likely to be infected with *S. stercoralis* (OR = 1.55, $t_{5,171} = 2.60$, $p = 0.01$). Age patterns were significantly nonlinear, particularly during childhood, when infection prevalences are increasing
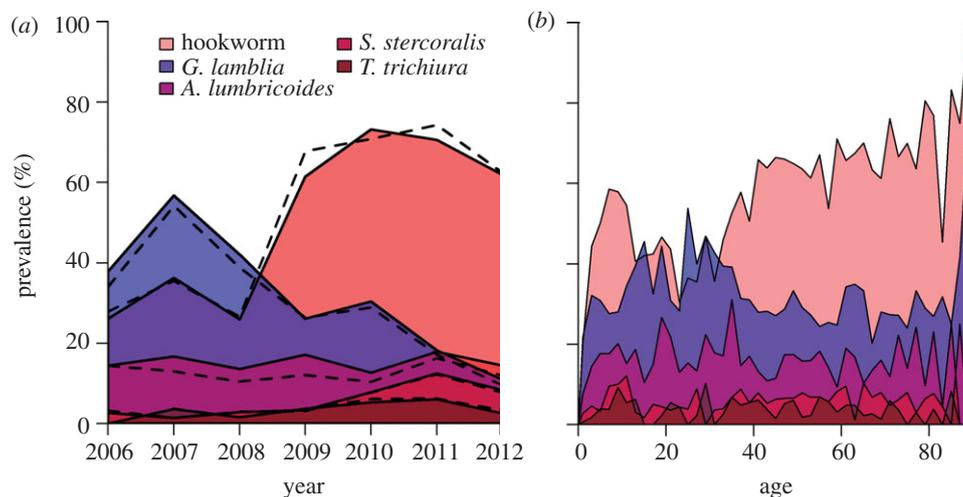
Figure 1. Prevalence of helminths and giardia. (*a*) Prevalences of giardia and helminths by study year. Both uncorrected prevalences (filled polygons) and prevalences corrected for age and village membership (dashed lines) are shown. (*b*) Total uncorrected infection prevalences for each species by 2-year age group. In both panels, prevalences are overlapping, not stacked. (Online version in colour.)

Table 2. Prevalence of tsimane helminth and giardia infections. Twenty-three participants were age 18 or under during some examinations and over 18 during others. Observations from these individuals contribute to both prevalences. Juveniles are 18 and under, whereas adults are older than 18.

	juveniles		adults	
	female (%)	male (%)	female (%)	male (%)
any helminth	56.1	52.9	68.0	70.2
hookworm	46.5	44.2	60.0	63.1
A. lumbricoides	16.9	13.8	16.7	14.0
T. trichiura	3.3	4.1	3.7	4.3
S. stercoralis	5.8	6.0	4.9	8.0
G. lamblia	29.6	31.3	30.6	29.8
G. lamblia + any helminth	12.7	13.8	16.4	16.0
participants/observations	804/952	766/886	868/1692	860/1670

(figure 1*b*). More detailed age patterns for this population have been described elsewhere [19].

(c) Coinfection risk

Table 3 presents the results of logistic regression analyses in which the presence/absence of each individual parasite is regressed on the presence/absence of the others. In general, having one helminth infection was predictive of having another helminth infection. Hookworm infection was associated with greater odds of *Strongyloides stercoralis* infection (OR = 37.64, $z = 6.48$, $p < 0.001$); *S. stercoralis* was predictive of hookworm infection (OR = 3.63, $z = 7.60$, $p < 0.001$); *A. lumbricoides* infection was associated with greater odds of *Trichuris trichiura* (OR = 3.99, $z = 3.06$, $p = 0.002$) and *T. trichiura* was associated with *A. lumbricoides* (OR = 2.55, $z = 5.26$, $p < 0.001$). By contrast, helminths and giardia were negatively associated. *Giardia lamblia* infection was associated with lower odds of infection with hookworm (OR = 0.60, $z = -7.42$, $p < 0.001$), *A. lumbricoides* (OR = 0.63, $z = -4.58$, $p < 0.001$) and *S. stercoralis* (OR = 0.50, $z = -1.69$, $p = 0.09$). Conversely, hookworm, *A lumbricoides* and *S. stercoralis* were

associated with lower odds of *G. lamblia* infection (OR = 0.60, $z = -7.44$, $p < 0.001$; OR = 0.65, $z = -4.47$, $p < 0.001$; OR = 0.75, $z = -1.80$, $p = 0.07$, respectively). *T. trichiura* was positively associated with *G. lamblia* (OR = 2.62, $z = 2.25$, $p = 0.02$), although the converse association was not found (OR = 0.94, $z = -0.33$, $p = 0.74$). These associations were not dependent upon whether a linear or nonlinear age term was used. Additionally, we ran a series of models to test for sex-specific associations. No significant interactions with sex were found.

(d) Effect of treatment during the subsequent visit on risk of infection

Over the course of the study, 54% of patients infected with a helminth were given an anti-helminthic, while 57% of patients infected with giardia were given an anti-protozoan. Of those who received an anti-helminithic, 71% received mebendazole. Owing to co-infection, 25% of the helminth-infected patients also received an anti-protozoan and 26% of giardia-infected individuals received an anti-helminthic.

5

Table 3. Odds ratios for infection with one parasite given infection with another. Odds ratios were calculated in generalized additive logistic mixed models with all independent variables in one model, controlling for nonlinear age effects with a spline term, and controlling for repeat observations and within community correlation with random effects terms (obs = 5171; n = 3275). Parameter significance is indicated by asterisks.

	dependent				
independent	*G. lamblia*	hookworm	*A. lumbricoides*	*T. trichiura*	*S. stercoralis*
G. lamblia		0.60***	0.63***	2.62*	0.50#
hookworm	0.60***		1.05	2.27#	37.64***
A. lumbricoides	0.65***	1.02		3.99**	0.61
T. trichiura	0.94	1.20	2.55***		2.36
S. stercoralis	0.75#	3.63***	0.82	2.10	
sex (male)	0.71***	1.33***	0.98	1.23#	2.00***
study year	0.99	1.01	0.77**	1.25	1.50
age (EDF)[a]	(3.99[b])***	(7.89)***	(2.96[b])**	(1.02)	(1.00[b])

[a]The parameter given is the estimated degrees of freedom for the spline.
[b]These age terms were fitted to log age.
#$p \leq 0.10$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

Table 4. Odds ratios for infection based on receipt of anti-helminthic or anti-protozoal agents and infection status at the previous medical visit. Models were run using only observations with complete data (participants = 1082, obs = 1731). Parameter values are odds ratios estimated in separate generalized logistic mixed model for each parasite or pathogen, controlling for repeat observations and within community correlation with random effects.

	dependent					
independent	*G. lamblia*	hookworm	*A. lumbricoides*	*T. trichiura*	*S. strongyloides*	any helminth
albendazole	2.14***	1.43	0.74	4.10**	0.91	1.56#
mebendazole	1.27#	0.72*	1.06	1.18	1.04	0.71*
metronidazole	1.02	1.01	1.17	0.51	0.80	0.91
tinidazole	1.12	1.23	0.75	0.36	1.06	0.87
sex (male)	0.90	1.12	0.78#	1.47	1.99**	0.98
study year	0.65***	1.46***	1.10#	1.13	1.37***	1.61***
infected at previous visit[a]	0.97	0.99	2.57***	7.69***	1.61	1.2
age (decades)	0.97	1.08**	0.97	0.86#	1.03	1.07*

[a]Infected at the previous visit with the parasite listed as the dependent variable for a given column.
#$p \leq 0.10$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

We examined the effect of treatment on infection at the subsequent visit (table 4). The most common helminth treatment, mebendazole, was associated with a decreased likelihood of hookworm infection at the subsequent visit (OR = 0.72, $z = -2.36$, $p = 0.02$). The 1-year impact of mebendazole treatment was modest: 69% of individuals infected with hookworm at one visit were infected a year later without treatment, with mebendazole reducing this only to 63%. Individuals who received mebendazole were also marginally more likely to have *G. lamblia* infections 1 year later (OR = 1.27, $z = 1.66$, $p = 0.10$). By contrast, albendazole was associated with increased odds of subsequent *T. trichiura* (OR = 4.10, $z = 3.38$, $p = 0.001$) and giardia infection (OR = 2.14, $z = 3.83$, $p < 0.001$). Metronidazole and tinidazole treatments were not significantly associated with changes in the odds of infection in the subsequent year for any helminth or giardia. This did not change when these two anti-protozoans were pooled together into a single variable.

When we tested for interactions by sex, we found a significant interaction between mebendazole treatment and sex on *Ascaris* infection in which treatment was associated for greater odds of infection in males, and lower odds in females (OR$_{males}$ = 1.53, OR$_{females}$ = 0.74, interaction: $z = 2.21$, $p = 0.03$). However, when males and females were analysed in separate models, neither of these odds ratios was significant (OR$_{males}$ = 1.45, $z = 1.56$, $p = 0.12$; OR$_{females}$ = 0.72, $z = -1.28$, $p = 0.20$).

(e) Changes in infection status over time

An MMSM was used to examine transitions between four disease states over time: uninfected, hookworm-infected, giardia-infected and co-infected (figure 2). After examining covariates, the final model included mebendazole treatment, albendazole treatment, study year and a sex-specific hazard for hookworm infection. Males were more likely to become
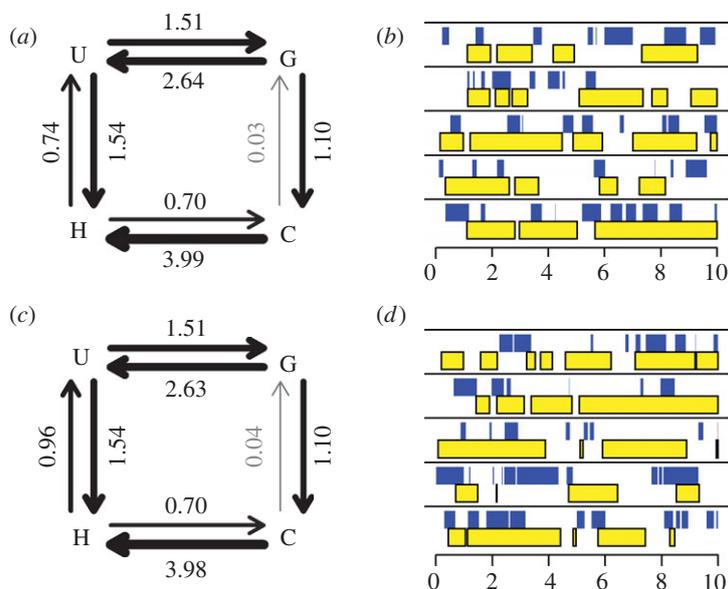
Figure 2. Multi-state Markov models. (*a,c*) Arrows and values indicate the estimated instantaneous transition intensities between the possible states of uninfected (U), hookworm-infected (H), giardia-infected (G) and co-infected with both (C). (*b,d*) Examples of simulated 10-year infection histories for hookworm (lower, light bars) and giardia (upper, dark bars). (*a,b*) With no treatment, (*c,d*) with annual mebendazole treatment. (Online version in colour.)

infected with hookworm (HR: 1.27, 95% CI (1.07, 1.52)). Apart from a sex difference in hookworm infection, no other interactions by sex were found. Anti-protozoans were not significant in any model and did not improve model fit (by AIC), so were not included in final models. Models run separately by age cohort also did not differ significantly in model parameters.

The model confirms that individuals infected with hookworm are significantly less likely to become infected with giardia (H → C/U → G; HR: 0.46, 95% CI (0.36, 0.60)). Similarly, infection with hookworm is less likely for individuals with giardia (G → C/U → H; HR: 0.71, 95% CI (0.57, 0.90)). Additionally, recovery from giardia is more likely when co-infected with helminths (C → H/G → U; HR: 1.51, 95% CI (1.31, 1.74)). However, recovery from helminth was much less likely when co-infected with giardia (B → G/H → U; HR: 0.04, 95% CI (0.02, 0.07)). Overall, the model predicts that over a 10-year period the average Tsimane will be infected with helminths for 6.63 years (95% CI (6.18, 7.08)) and with giardia for 2.14 years (95% CI (1.86, 2.47)).

Treatment with mebendazole (figure 2*c,d*) significantly increased the recovery rate from hookworm-infected to uninfected (H → U and B → G; HR: 1.30, 95% CI (1.02, 1.65)), but did not affect the giardia recovery rate (HR: 1.00, 95% CI (0.79, 1.26)). In a model assuming annual mebendazole treatment, time infected with helminths was 6.12 years out of 10 years (95% CI: (5.57, 6.76)), not significantly less time than without treatment. Time infected with giardia was also not significantly changed (2.24 years; 95% CI: (1.88, 2.67)). Although the inclusion of albendazole improved model fit, albendazole was not significantly associated with likelihood of giardia recovery (HR: 0.81, 95% CI (0.57, 1.15)) or hookworm recovery (HR: 0.88, 95% CI (0.60, 1.28)).

(f) Individual differences or parasite interaction

Negative associations between helminths and giardia might be due to antagonism between parasites, or to host differences in genetic or phenotypic susceptibility. To parse this

out, we examined correlations between individual frequency of hookworm infection and frequency of giardia infection. If negative associations are due to host differences, we would expect giardia infections to cluster in some hosts and helminth infections in others, and for these differences to persist over time. By contrast, if negative associations are due to parasite interactions, individuals should cycle between infections, but end up with similar infection frequencies given sufficient time. Indeed, for individuals sampled two ($r = -0.22$, d.f. = 789, $p < 0.001$) or three ($r = -0.19$, d.f. = 245, $p = 0.002$) times, frequencies of hookworm and giardia infections were negatively correlated. However, for individuals sampled four or five times there was no correlation between frequencies ($r = -0.06$, d.f. = 189, $p = 0.43$), suggesting that differences are due not to persistent host characteristics but to short-term dynamics.

4. Discussion

We report on helminth and giardia infections in an on-going longitudinal study of a lowland Bolivian population. We find significant evidence for an interaction between helminth and giardia infections. To put the odds ratios we report into perspective, although 42% of those without helminth infection were infected with *G. lamblia*, we find *G. lamblia* infections in only 24% of those infected with helminths. Similarly, of those with *G. lamblia* infection, 50% were infected with at least one helminth, compared with 70% of those without *G. lamblia*.

Taken alone, the estimation of odds ratios from cross-sectional data is of somewhat limited value. Odds ratios may uncover associations between parasites, but generally cannot illuminate causality. Additionally, some associations may be mediated by third variables. We therefore analysed the time course of infections using longitudinal data in a state-based model. Although both types of analysis suggest mutual antagonism, in longitudinal analysis, we find that co-infected individuals are much more likely to clear giardia rather than

hookworm, and the inhibition of giardia infection by hookworm is more pronounced than the converse. Additionally, although males in cross-sectional analysis were more likely to have hookworm and less likely to have giardia, in the state-based model the only significant sex difference was an increased hazard of hookworm infection for males, suggesting that the increased likelihood of male hookworm infection may drive males' lower odds of giardia infection.

The negative association between hookworm and giardia does not appear to be driven by differential host sensitivity to these two parasites. It is not the case that some individuals suffer from giardia and others from hookworm, since we would expect this to result in persistent negative correlations between individual infection frequencies. Rather, over a longer time period, frequency of hookworm infection is uncorrelated with frequency of giardia infection. Instead, individuals alternate between infections. This can be seen in simulated infection histories (figure 2b,d). Individuals may be co-infected, but more frequently they become infected with one parasite when not infected with the other. This is particularly apparent in the mebendazole treatment condition, in which hookworm infections are disrupted (figure 2d).

The apparent antagonism may reflect competitive inhibition or cross-immunity. In a murine model, G. lamblia, which reside on microvilli in the small intestine, were inhibited by Trichinella spiralis when these helminths inhabited the small intestine but not at later stages when they moved to muscular tissue, suggesting a physical or localized, rather than systemic immune interaction between the two species [14]. In our study, A. lumbricoides, S. stercoralis and hookworm, all of which inhabit the small intestine, were negatively associated with G. lamblia, whereas T. trichiura (with adults located further down in the large intestine) was not, consistent with either a physical interaction or the effect of a localized immune response. Studies have also shown that G. lamblia clearance and protective immunity are mediated by mixed T_H1 and T_H2 cytokine production (characterized by both INF-γ and IL-4), as well as T_H2 antibody responses (IgA, IgG, IgE) [26–28]. Therefore, it is possible that helminth-induced T_H2 activity may provide some cross-immunity against G. lamblia. The details of this interaction will need to be investigated in future studies.

Owing to its large size and longitudinal analysis, our study provides strong support for an interaction between helminths and giardia. However, there are several limitations that should be noted. First, infectious status among the Tsimane was diagnosed only on the basis of a single faecal sample, which may underestimate the true prevalence of both single and multiple species infections in this population. Second, study visits were on average 1.2 years apart, limiting our ability to examine short-term dynamics of reinfection or changing infection status. Finally, treatment in our study was not truly randomized, but depended on logistical and clinical factors. Additionally, although most Tsimane have limited access to medications other than those provided by our medical team, we cannot rule out the possibility that some medications may have been obtained from other sources between study visits.

Not only do we see fewer G. lamblia/helminth co-infections than would be expected by random assortment, but our analyses suggest that treatment with albendazole or mebendazole may increase the odds of giardia infection, consistent with the only other similar study we are aware of [15]. Our analyses suggest that this is not a direct effect of the drug administered, but that the removal of helminths increases giardia susceptibility. The apparent interaction between hookworm, A. lumbricoides and G. lamblia presents a conundrum for treatment decisions in regions in which infection with these species is common. If occasional treatment for helminths increases susceptibility to G. lamblia, should treatment be provided? Only a careful analysis of the impact of each species on quality of life can answer this question. In our study, treatment did not significantly affect time spent with either infection. However, an open question is whether treatment for helminths increases susceptibility to giardia infection in the weeks or months following treatment, since this is the time period in which we would most expect to see an effect. Given the highly significant negative associations between G. lamblia and hookworm, the lack of significant changes in time spent infected in the longitudinal model probably reflects the time between samples and the overall rapid reinfection rates for both giardia and hookworm. We suspect a shorter sampling period might reveal stronger effects.

Our results nonetheless show that treatment for either parasite is unlikely to be effective without changes in sanitation and access to clean water. Although mebendazole was associated with reduced odds of being infected with hookworm 1 year later, the reduction was minimal. Our results also suggest that in the neotropics, mebendazole may be more effective at treating the predominant helminth, hookworm, than is albendazole. Albendazole was associated with an increase in T. trichiura and G. lamblia. Typical albendazole treatment recommendations for these species require multiple days of treatment, which may present a problem due to patient compliance. While this may explain the lack of efficacy, we do not have a clear explanation for the apparent increases. However, we caution against drawing strong conclusions since albendazole was used primarily in only one study year. Additionally, the effect of albendazole was not significant in the state-based model. Finally, we found no lasting effect of anti-protozoan treatment. Thus, anti-protozoans may provide short-term benefits, but do not appear to have lasting effects due to constant reinfection.

Overall, these results illustrate the importance of considering co-infection in epidemiological models and treatment strategies. Co-infection affects both recovery and reinfection, particularly for infectious agents that directly compete for substrate or resources, or which induce similar immune responses.

References

1. Huckans MS, Blackwell AD, Harms TA, Indest DW, Hauser P. 2005 Integrated hepatitis C virus treatment: addressing comorbid substance use disorders and HIV infection. *AIDS* **19**, S106–S115. (doi:10.1097/01.aids.0000192078.49185.b0)

2. Lello J, Knopp S, Mohammed KA, Khamis IS, Utzinger J, Viney ME. 2013 The relative contribution of co-infection to focal infection risk in children. *Proc. R. Soc. B* **280**, 20122813. (doi:10.1098/rspb.2012.2813)

3. Telfer S, Lambin X, Birtles R, Beldomenico P, Burthe S, Paterson S, Begon M. 2010 Species interactions in a parasite community drive infection risk in a wildlife population. *Science* **330**, 243–246. (doi:10.1126/science.1190333)

4. Labeaud AD, Malhotra I, King MJ, King CL, King CH. 2009 Do antenatal parasite infections devalue childhood vaccination? *PLoS Negl. Trop. Dis.* **3**, e442. (doi:10.1371/journal.pntd.0000442)

5. Yazdanbakhsh M, Kremsner PG, Van Ree R. 2002 Allergy, parasites, and the hygiene hypothesis. *Science* **296**, 490–494. (doi:10.1126/science.296.5567.490)

6. Maizels RM, Yazdanbakhsh M. 2003 Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat. Rev. Immunol.* **3**, 733–744. (doi:10.1038/nri1183)

7. Fernández-Niño JA *et al*. 2012 Paradoxical associations between soil-transmitted helminths and *Plasmodium falciparum* infection. *Trans. R. Soc. Trop. Med. Hyg.* **106**, 701–708. (doi:10.1016/j.trstmh.2012.07.012)

8. Elias D, Wolday D, Akuffo H, Petros B, Bronner U, Britton S. 2001 Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guérin (BCG) vaccination. *Clin. Exp. Immunol.* **123**, 219–225. (doi:10.1046/j.1365-2249.2001.01446.x)

9. Wammes LJ *et al*. 2010 Regulatory T cells in human geohelminth infection suppress immune responses to BCG and *Plasmodium falciparum*. *Eur. J. Immunol.* **40**, 437–442. (doi:10.1002/eji.200939699)

10. Elias D, Britton S, Aseffa A, Engers H, Akuffo H. 2008 Poor immunogenicity of BCG in helminth

11. Walson J *et al*. 2008 Albendazole treatment of HIV-1 and helminth co-infection: a randomized, double blind, placebo-controlled trial. *AIDS* **22**, 1601–1609. (doi:10.1097/QAD.0b013e32830a502e)

12. Escobedo AA, Almirall P, Robertson LJ, Franco RMB, Hanevik K, Mørch K, Cimerman S. 2010 Giardiasis: the ever-present threat of a neglected disease. *Infect. Disord. Drug Targets* **10**, 329–348. (doi:10.2174/187152610793180821)

13. Hagel I, Cabrera M, Puccio F, Santaella C, Buvat E, Infante B, Zabala M, Cordero R, Di Prisco MC. 2011 Co-infection with *Ascaris lumbricoides* modulates protective immune responses against *Giardia duodenalis* in school Venezuelan rural children. *Acta Trop.* **117**, 189–195. (doi:10.1016/j.actatropica.2010.12.001)

14. Chunge RN *et al*. 1991 Longitudinal study of young children in Kenya: intestinal parasitic infection with special reference to *Giardia lamblia*, its prevalence, incidence and duration, and its association with diarrhoea and with other parasites. *Acta Trop.* **50**, 39–49. (doi:10.1016/0001-706X(91)90071-Q)

15. Rousham E. 1994 An increase in *Giardia duodenalis* infection among children receiving periodic Anthelmintic treatment in Bangladesh. *J. Trop. Pediatr.* **40**, 329. (doi:10.1093/tropej/40.6.329)

16. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. 2008 Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* **118**, 1311–1321. (doi:10.1172/JCI34261)

17. Anderson R, Medley G. 1985 Community control of helminth infections of man by mass and selective chemotherapy. *Parasitology* **90**, 629–660. (doi:10.1017/S0031182000052288)

18. Jia T-W, Melville S, Utzinger J, King CH, Zhou X-N. 2012 Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Negl. Trop. D* **6**, e1621. (doi:10.1371/journal.pntd.0001621)

19. Blackwell AD, Gurven M, Sugiyama LS, Madimenos FC, Liebert MA, Martin MA, Kaplan HS, Snodgrass JJ. 2011 Evidence for a peak shift in a humoral response to helminths: age profiles of IgE in the Shuar of

Ecuador, the Tsimane of Bolivia, and the US *NHANES*. *PLoS Negl. Trop. D* **5**, e1218. (doi:10.1371/journal.pntd.0001218)

20. Gurven M, Kaplan H, Winking J, Eid Rodriguez D, Vasunilashorn S, Kim JK, Finch C, Crimmins E. 2009 Inflammation and infection do not promote arterial aging and cardiovascular disease risk factors among lean horticulturalists. *PLoS ONE* **4**, e6590. (doi:10.1371/journal.pone.0006590)

21. Sadjjadi SM, Alborzi AW, Mostovfi H. 2001 Comparative clinical trial of mebendazole and metronidazole in giardiasis of children. *J. Trop. Pediatr.* **47**, 176–178. (doi:10.1093/tropej/47.3.176)

22. Vasunilashorn S, Crimmins EM, Kim JK, Winking J, Gurven M, Kaplan H, Finch CE. 2010 Blood lipids, infection, and inflammatory markers in the Tsimane of Bolivia. *Am. J. Hum. Biol.* **22**, 731–740. (doi:10.1002/ajhb.21074)

23. Eberl M, Al-Sherbiny M, Hagan P, Ljubojevic S, Thomas AW, Wilson RA, Institut B. 2002 A novel and sensitive method to monitor helminth infections by faecal sampling. *Acta Trop.* **83**, 183–187. (doi:10.1016/S0001-706X(02)00089-X)

24. Fenton A, Viney ME, Lello J. 2010 Detecting interspecific macroparasite interactions from ecological data: patterns and process. *Ecol. Lett.* **13**, 606–615. (doi:10.1111/j.1461-0248.2010.01458.x)

25. Jackson C. 2011 Multi-state models for panel data: the msm package for R. *J. Stat. Softw.* **38**, 1–28.

26. Matowicka-Karna J, Dymicka-Piekarska V, Kemona H. 2009 IFN-gamma, IL-5, IL-6 and IgE in patients infected with *Giardia intestinalis*. *Folia Histochem. Cytobiol.* **47**, 93–97. (doi:10.2478/v10042-009-0013-3)

27. Jiménez JC, Pinon A, Dive D, Capron M, Dei-Cas E, Convit J. 2009 Antibody response in children infected with *Giardia intestinalis* before and after treatment with Secnidazole. *Am. J. Trop. Med. Hyg.* **80**, 11–15.

28. Abdul-Wahid A, Faubert G. 2008 Characterization of the local immune response to cyst antigens during the acute and elimination phases of primary murine giardiasis. *Int. J. Parasitol.* **38**, 691–703. (doi:10.1016/j.ijpara.2007.10.004)