PARASITOLOGY

Helminth infection, fecundity, and age of first pregnancy in women

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Infection with intestinal helminths results in immunological changes that influence co-infections, and might influence fecundity by inducing immunological states affecting conception and pregnancy. We investigated associations between intestinal helminths and fertility in women, using 9 years of longitudinal data from 986 Bolivian forager-horticulturalists, experiencing natural fertility and 70% helminth prevalence. We found that different species of helminth are associated with contrasting effects on fecundity. Infection with roundworm (*Ascaris lumbricoides*) is associated with earlier first births and shortened interbirth intervals, whereas infection with hookworm is associated with delayed first pregnancy and extended interbirth intervals. Thus, helminths may have important effects on human fertility that reflect physiological and immunological consequences of infection.

Dysregulated immune function, and in particular autoimmune disease, has negative impacts on virtually every aspect of fecundity, including ovarian function, implantation, and pregnancy loss (*1*, *2*). Healthy pregnancy is also associated with shifts in immune responses. During the luteal phase of the menstrual cycle, regulatory and type 2 (T_H2) T cell responses increase (*3*). If conception occurs, these shifts continue through pregnancy (*4*) and help to suppress type 1 (T_H1) T cell responses, increasing maternal tolerance of an immunologically distinct fetus (*3*). Because pregnancy is both affected by and alters immunity, parasites that result in systemic immunological changes might be expected to affect fecundity by altering the host's immune responses. Helminths, such as hookworm (*Ancylostoma duodenale* or *Necator americanus*) and giant roundworm (*Ascaris lumbricoides*), each infect 500 million to 800 million people (*5*) and are associated with immunological changes, such that host helper T cell populations generally shift from T_H1 toward T_H2 responses (*6*, *7*) and the suppressive activity of regulatory T cells increases to modulate both T_H1 and T_H2 responses (*8*, *9*). These shifts can alter susceptibility to other infectious diseases, such as malaria (*10*), giardiasis (*11*), and tuberculosis (*12*); are associated with reductions in many diseases that have inflammatory or autoimmune etiology (*13*); and also resemble the T cell patterns that occur during pregnancy.

In humans, some helminth parasites can directly infect the reproductive organs or the fetus; for example, the filarial roundworm, *Wuchereria bancrofti*, can cause elephantiasis of the genitals (*14*). Animal studies have also examined life history changes associated with parasitism (*15*). Yet there are few data on the effects of intestinal helminth infections on human fecundity, fertility, or birth spacing. We prospectively examined the effect of helminth infection on the fecundity of women. We used 9 years of longitudinal data collected on 986 Tsimane forager-horticulturalist women living in the Amazonian lowlands of Bolivia (table S1). Tsimane are predominantly a natural fertility population, with infrequent (<5% prevalence) use of pharmaceutical contraceptives and a total fertility rate of nine births per woman (*16*). Helminths infect 70% of the population, the most common being hookworm (56%) and *A. lumbricoides* (15 to 20%) (*11*, *17*).

In both animal and human studies, parasites have been shown to influence host reproduction via sexual behavior, brood or litter size, offspring size, incubation periods, conception rates, and pregnancy loss (*18*–*22*). In most cases, parasitism reduces host reproduction by compromising reproductive organs or reducing energy budgets (*14*, *23*). However, among Tsimane adults, morbidity from intestinal helminth infections is low, particularly for *A. lumbricoides*. Controlling for age and co-infection in our sample, hookworm infection is associated with slightly lower body mass index (BMI) (generalized linear model, $\beta = -0.77$ kg/m^2, $P < 0.001$) and hemoglobin ($\beta = -0.19$ g/dl, $P = 0.005$), whereas *A. lumbricoides* is not ($\beta = -0.34$ kg/m^2, $P = 0.180$; $\beta = -0.07$ g/dl, $P = 0.413$). However, helminth infection is also associated with reductions in other infections, such as the intestinal protist *Giardia lamblia* (*11*). We hypothesized that intestinal helminths might increase fecundity because the associated immunological changes, resembling those occurring during pregnancy, modulate inflammatory responses that might otherwise impair fertility.

By using Cox proportional hazards models, we tested whether helminth infection was associated with changes in birth spacing for 561 multiparous women and the age of first pregnancy (AFP) for 425 nulliparous women (*24*). Consistent with our hypothesis, compared to being uninfected, *A. lumbricoides* infection was associated with an earlier AFP hazard ratio [(HR) = 3.06, confidence interval (CI) 1.91 to 4.91, $P < 0.001$ (Fig. 1 and Table 1)] and an increased hazard of pregnancy under age 32 years (at age 20: HR = 1.64, CI 1.16 to 2.33, $P = 0.005$). This association declines with age (interaction between *A. lumbricoides* and age: HR = 0.68 per decade, CI 0.51 to 0.89, $P = 0.006$) and becomes significantly negative by the age of 46 years (HR = 0.62, CI 0.38 to 1.00, $P = 0.05$). However, these late-life

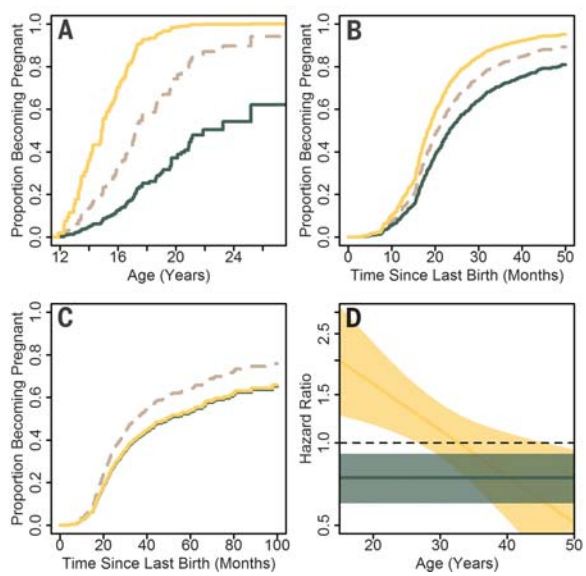

Fig. 1. Associations between infection and likelihood of becoming pregnant. (**A** to **C**) Kaplan-Meier curves from Cox proportional hazard models (table S2), representing the time to first pregnancy (A), and time to subsequent pregnancies at age 25 years (B) and age 40 years (C). Hazard ratios for conception associated with infection across ages are shown in (**D**). Colors indicate uninfected (dashed brown), infected with hookworm (solid dark green), or infected with *A. lumbricoides* (solid yellow).

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negative associations are outweighed by positive associations during early life, such that a woman with *A. lumbricoides*, projected across her life span, would expect to have two more children than a woman who was never infected (Fig. 2).

In contrast, infection with hookworm was associated with a delayed age of first pregnancy (HR = 0.33, CI 0.20 to 0.54, $P < 0.001$) and a reduced hazard of subsequent pregnancies at all ages (HR = 0.71, CI 0.58 to 0.86, $P < 0.001$). A woman chronically infected with hookworm would be predicted to have three fewer children than an uninfected woman (Fig. 2). We found no interaction between infections, such that co-infection is associated with the additive effects of hookworm and *A. lumbricoides*.

These results are not altered by controlling for other likely confounds affecting fecundity or fecundity-altering behaviors, including physical condition, education (a proxy of acculturation), village location, season, and secular changes, even though these variables do affect fertility [tables S2 and S3, also see (*25*)]. The results are also not mediated by other comorbid infections or illnesses (table S4). Twenty percent of infected women were given antihelminthic drugs during medical visits. Receipt of antihelminthics was itself associated with a lower hazard of conceiving (HR = 0.75, CI 0.58 to 0.97, $P = 0.03$); however, neither controlling for treatment in models nor excluding these women appreciably altered hazard ratios from infection with either hookworm or *A. lumbricoides*. The results are also not driven by changing infection hazard during pregnancy. Although pregnancy is associated with an increased likelihood of hookworm infection, particularly in late pregnancy (table S6 and fig. S8), this relationship does not mediate the association between infection and conception hazards (*24*). Instead, it appears that hookworm-infected women occasionally clear their infections, during which time they become pregnant, followed quickly by subsequent reinfection with hookworm. Lastly, these associations are unlikely to be caused by consistent differences between individual women (e.g., genetic pleiotropies) that affect both fertility and risk of infection, because past parity is unrelated to likelihood of current infection [hookworm: odds ratio (OR) = 0.98 per birth, CI 0.90 to 1.08, $P = 0.65$; *A. lumbricoides*: OR 1.05 per birth, CI 0.93 to 1.18, $P = 0.46$].

Finding that hookworm and *A. lumbricoides* have contrasting associations with fecundity may seem unexpected. However, we suggest two reasons why we might observe such a pattern. First, although helminths are often discussed as if interchangeable, hookworm and *A. lumbricoides* do not have identical effects on the immune system. Whereas *A. lumbricoides* is

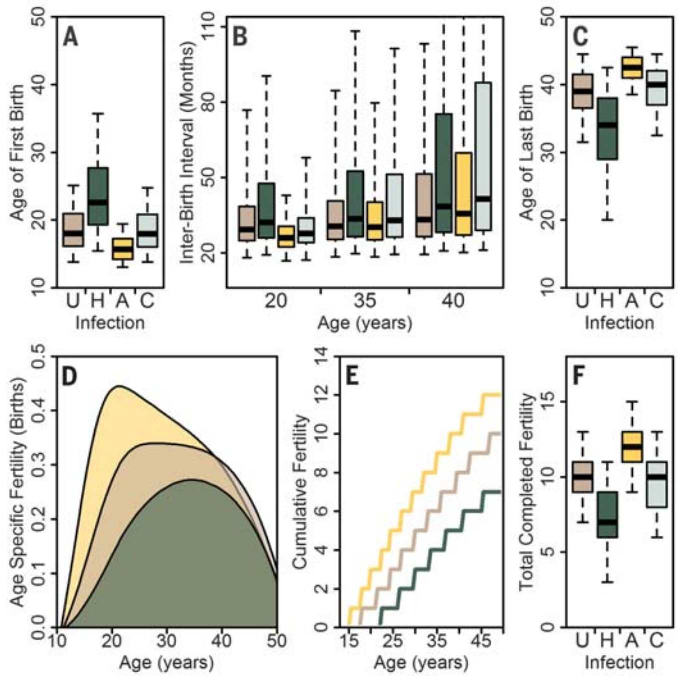

Fig. 2. Reproductive careers predicted from Cox proportional hazard models, showing the expected distributions of reproductive values for hypothetical women with persistent parasite status throughout life. Outcomes include age at first birth (**A**), interbirth intervals (**B**), age at last birth (**C**), age-specific fertility (births/woman per year) (**D**), median cumulative fertility over time (**E**), and total completed fertility by age 50 (**F**). Colors indicate uninfected (U, brown), infected with hookworm (H, dark green), infected with *A. lumbricoides* (A, yellow), or co-infected with hookworm and *A. lumbricoides* (C, light blue). Box plot whiskers display the 5th and 95th percentiles; bodies, the 25th, 50th, and 75th. Predictions are derived from the models in Fig. 1.

Table 1. Cox proportional hazard models. Models also include generalized estimating equation cluster terms for individual and village. For each model, the number of individuals (*n*), number of medical observations (obs), and number of observed pregnancies (preg) are given. Dashes indicate variables not applicable for a given model or excluded by AIC. Details and additional excluded variables are given in tables S2 and S3.

	Age of first pregnancy (*n* = 425, obs = 639, preg = 87)			**Time to next pregnancy (*n* = 561, obs = 1623, preg = 405)**		
Variable	**Exp(β)**	**95% CI**	***P***	**Exp(β)**	**95% CI**	***P***
Age (decades)*	–	–	–	1.00	(0.80–1.25)	0.992
Age^4 (decades)*	–	–	–	0.95	(0.93–0.96)	<0.001
Hookworm	0.34	(0.20–0.58)	<0.001	0.74	(0.60–0.91)	0.004
A. lumbricoides†	3.06	(1.91–4.91)	<0.001	1.64	(1.16–2.33)	0.005
A. lumbricoides × age*	–	–	–	0.68	(0.51–0.89)	0.006
Treatment with antihelminthic	0.43	(0.19-0.97)	0.042	0.75	(0.58–0.97)	0.027
Education (years)	–	–	–	0.92	(0.86–0.99)	0.017
Speaks Spanish	–	–	–	0.74	(0.57–0.95)	0.018
Distance to town (10 km)	–	–	–	0.96	(0.91–1.00)	0.075
Season (P-spline)	–	–	<0.001	–	–	<0.001

*Age is centered at 20 years. Age was continuous to the nearest tenth of a year but is shown in decades to make the parameters more easily interpretable. Because age-related changes in fecundity are nonlinear, transformations ranging from age^2 to age^5 were compared by AIC to select the age transformation (age^4) that best fit the data (fig. S3). †For the time to next pregnancy model, the roundworm parameter represents the hazard ratio at age 20.

associated with a polarized T_H2 response (*6*), the response to hookworm has been reported as a mixed T_H1/T_H2 response (*26*, *27*). Hookworm and *A. lumbricoides* also have differing effects on other diseases, such as malaria (*10*). Thus, the response to *A. lumbricoides* may be more favorable to conception and implantation, because it more closely resembles the immunological state in pregnancy and less closely resembles proinflammatory states that suppress fecundity. Second, hookworm infection may be more costly than *A. lumbricoides* infection, such that the costs imposed by infection, such as anemia and nutritional loss, outweigh any effect of immune modulation. Although we do not have direct measures of parasite load, hookworm is associated with both lower BMI and lower hemoglobin for women in our sample, whereas *A. lumbricoides* is not. Future studies will need to investigate the importance of parasite burden in these associations.

Although consistent with our hypothesis, it is still unexpected to see positive associations between fecundity and *A. lumbricoides* infection, given that most parasites decrease reproduction. However, this association might instead be understood not as de novo increases in fecundity, but as the suppression of responses that would otherwise decrease fecundity. For example, most organisms down-regulate reproductive effort during acute illness because inflammation suppresses reproductive function (*28*). If *A. lumbricoides* infection modulates inflammatory responses, then it might also limit inflammation-induced reproductive suppression, as well as sickness behavior and associated reductions in sexual activity (*29*, *30*). If so, then the effects of *A. lumbricoides* might only be observed in the presence of other illnesses or conditions resulting in excess inflammation. An additional possibility is that the increase in fertility represents fecundity compensation, a host response in which reproductive effort is shifted toward earlier ages to compensate for increasing morbidity or mortality (*15*). However, our analysis cannot fully evaluate these kinds of lifetime or cumulative effects, because our longitudinal sample remains short relative to the human life span.

Regardless of mechanism, these results indicate that across populations, helminths may have unappreciated effects on demographic patterns, particularly given their high global prevalences (*5*).

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SUPPLEMENTARY MATERIALS

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CANCER IMMUNOLOGY

Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1

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Antitumor immunity driven by intratumoral dendritic cells contributes to the efficacy of anthracycline-based chemotherapy in cancer. We identified a loss-of-function allele of the gene coding for formyl peptide receptor 1 (FPR1) that was associated with poor metastasis-free and overall survival in breast and colorectal cancer patients receiving adjuvant chemotherapy. The therapeutic effects of anthracyclines were abrogated in tumor-bearing $Fpr1^{-/-}$ mice due to impaired antitumor immunity. Fpr1-deficient dendritic cells failed to approach dying cancer cells and, as a result, could not elicit antitumor T cell immunity. Experiments performed in a microfluidic device confirmed that FPR1 and its ligand, annexin-1, promoted stable interactions between dying cancer cells and human or murine leukocytes. Altogether, these results highlight the importance of FPR1 in chemotherapy-induced anticancer immune responses.

The success of anticancer chemotherapy is linked to a durable tumor-targeting immune response (*1*). Accordingly, the presence of tumor-infiltrating dendritic cells (DCs) and $CD8^+$ T lymphocytes at diagnosis increases the likelihood of breast cancer patients responding to anthracyclines (*2*–*6*). One mechanism through which anthracyclines can stimulate an antitumor immunity is by inducing immunogenic cell death (ICD), and this mechanism implies