Title: Effects of Parasitism on Fecundity and Life History in Human Females

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Abstract

In animal studies, parasitism decreases overall reproductive effort, consistent with costs of both parasitism and reproduction. Yet when examined across the lifespan, parasitism can increase precocious reproduction, as effort is shifted earlier due to increased mortality or reproductive senescence, a response known as fecundity compensation. To date, studies have largely examined short-lived birds and rodents, and no studies have examined effects in humans. Here, we investigate whether intestinal parasites affect human fecundity with seven years of longitudinal data from the Tsimane, Bolivian forger-horticulturalists experiencing both natural fertility and a 70% helminth infection prevalence. We observed 184 nulliparous women, 45 of whom became pregnant during the study period, and 511 intervals following births for 432 women. Cox proportional hazard models were used to examine the effects of infection on pregnancy hazard, controlling for BMI. Hookworm was associated with both delayed first pregnancy (HR=0.38; p=0.003, median age 19.1 vs. 15.9) and extended interbirth intervals (HR=0.77, p=0.042; median IBI at age 20: 36.8 vs 33.9 months). In contrast, *A. lumbricoides* was associated with earlier first pregnancy (HR=2.24, p=0.002, median age 14.6) and shortened IBIs at younger, but not older, ages (at age 20: HR=2.33, p<0.001, median 27.1 months). Although parasitism affected pregnancy, odds of infection were not affected by reproductive state. While the effect of hookworm suggests overall costs, infection with *A. lumbricoides*, which often co-occurs with hookworm, suggests fecundity compensation. Our results suggest that helminths have consequences for human fertility, and provide an additional avenue for understanding demographic changes with modernization.

Significance

Parasites are known to affect fertility and reproduction in many animal species, but very little is known about the effects of parasites in humans. Here, we examine the effect of infection with the intestinal worms, hookworm and roundworm, on fecundity and birth spacing in indigenous women in the Bolivian lowlands, who on average have 9 children in their lives. We find that hookworm infection is associated with a later age of first birth and lengthened birth intervals, but roundworm is associated with earlier age of first birth and accelerated birth intervals. Our results suggest that parasites may have important effects on human reproduction that have been previously overlooked.

Introduction

In animal studies there are numerous examples of parasitism affecting host reproduction, including effects on sexual behavior, brood or litter size, offspring size, incubation periods, conception rates, and pregnancy loss (1–5). In many cases reproduction is negatively impacted, reflecting trade-offs between the interests of hosts and parasites. At its most extreme, some parasites will castrate their hosts to eliminate host investment into reproduction entirely, leaving parasites with long lived and resource rich hosts ripe for exploitation (6). Yet, in other cases, parasitism can increase host reproduction, particularly at younger ages (7–11).

To date there have been very few studies examining the effects of parasitism or infection on large bodied, long-lived mammals. One of the few exceptions found that infection with a transmissible cancer resulted in precocious reproduction in Tasmanian devils (8). In humans a few studies have examined amenorrhea secondary to infection (12), and sterility secondary to infections such as gonorrhea is certainly reported, but we know of no studies that have directly

examined the effects of parasitism on human fecundity, fertility[1], or birth spacing. Human life histories differ from those of species previously studies in terms of our longevity, delayed sexual maturity, and single-births, all features which might affect how our reproduction is affected by parasitic infection. The lack of studies on parasitism and human fertility is therefore surprising given the importance of understanding human-parasite interactions for human health and wellbeing, as well as for understanding demographic patterns in both the present and the past.

Here we examine the effect of parasitic helminth infections on human fertility using seven years of longitudinal data collected among the Tsimane. Tsimane are a group of about 15,000 forger-horticulturalists living in Amazonian lowlands of Bolivia. Tsimane are a natural fertility population, with an average total fertility rate of 9.1 children (13). Helminths are highly prevalent; the two most common infections being hookworm (*Ancylostoma duodenale* or *Necator americanus*), which infects 56% of the population, and the giant roundworm (*Ascaris lumbricoides*), which has a 15% prevalence (14, 15). They therefore represent an ideal natural population for examining the effects of helminth infection on human reproduction.

Although associated with anemia and other morbidities in other populations, helminth infections among the Tsimane are not associated with obvious morbidity and most patients do not know they are infected. In fact, helminths may provide some protection against other pathogens and auto-immune disorders (15, 16). Helminths also have significant effects on host immunity, typically characterized by Th2 biasing of immune responses (17, 18). Interestingly, pregnancy is also characterized by Th2 biasing, which is thought by some to be necessary for the mother to immunologically tolerate the fetus (19, 20).

[1] Note that we use the demographer's definitions of fertility and fecundity, in which capacity to reproduce is fecundity and reproductive output is fertility. Biologists typically reverse these two terms.

In general, the effect a parasite has on reproduction will depend on the interaction between the parasite's and the host's life histories. Parasites which infect and impose immediate costs, which are then reduced or cleared by host immunity, should result in short term reductions in current reproduction. In contrast, hosts should accelerate reproduction in response to parasites that impose long term, increasing costs because these parasites will reduce residual reproductive value more than current (21). The host life history is important in these interactions because it determines what might be considered long term versus short term infection. An infection lasting two weeks will have very different consequences for a short-lived insect versus a long-lived mammal. Additionally, the host's reproductive schedule may have consequences for the ability of an organism to shift reproduction. Organisms which produce large broods or litters may be better able to adjust reproductive output, whereas organisms such as humans which invest heavily in single offspring may have less flexibility.

Parasites that reduce residual reproductive value, either by gradually reducing fecundity (e.g. through castration) or by increasing mortality, can lead to precocious sexual maturity and increased reproductive effort early in life (8–11). The net effect is a shift in reproductive effort towards earlier ages, often with decreased net lifetime fertility. This is thought to represent adaptive phenotypic plasticity on the part of the host, and is therefore referred to as fecundity compensation, as the host compensates for loss of fertility in late age (11, 21). Typically helminths such as hookworm and *A. lumbricoides* live in the host for 1-10 years (22, 23). These helminths cannot reproduce in the host, but have external stages of the life cycle, which means that infected individuals do not have burdens that necessarily increase with time. At the same time, there are some indications that helminths may accelerate immunosenescence and increase risk of mortality from other infections (14, 24, 25).

Given these characteristics, we hypothesized that helminths might have either positive or negative effects on reproduction. Despite a lack of obvious morbidity, helminths do impose costs, most likely in part due to investment into immune function (26). At the same time, the immunological shifts caused by helminths might make pregnancy more likely, by biasing immune function toward greater fetal tolerance. Helminths might also be candidates for the investigation of fecundity compensation, given the potential for cumulative costs. We therefore investigate the effects of helminth infection on age of first reproduction and fecundity in a natural fertility human population.

Results

We observed 511 birth intervals for 432 women, 226 of which were complete intervals in which the next pregnancy was recorded (see Table S1 for full sample description). Controlling for age, age^2 and BMI, infection with hookworm during a birth interval (IBI) had a significant negative effect on the proportional hazard of becoming pregnant again (HR = 0.77, CI 0.54-0.82, p = 0.042) (Figure 1), extending the median IBI for a 35 year-old Tsimane woman from 42.1 to 52.2 months (Figure 2). In contrast, infection with *A. lumbricoides* increased the hazard of becoming pregnant again, particularly at younger ages (at age 20: HR = 2.33, CI 1.50-3.60, p < 0.001; at age 40 HR = 0.93, CI 0.62-1.42, p = 0.761) (Figure 1B), with a median IBI for an *A. lumbricoides* infected 35 year-old of 36.8 months.

In nulliparous young women, helminth infections were also associated with altered hazards of becoming pregnant for the first time. In 264 observations of 184 women age 10 to 30, 45 of whom later had observed pregnancies, hookworm was associated with a delayed age of first pregnancy (AFP) (HR = 0.38, CI 0.20 – 0.72, p = 0.003), with a median age of first

pregnancy of 19.1, compared to a median age of 15.9 for uninfected girls (Figure 1A). *A. lumbricoides* was associated with an accelerated age of first pregnancy (HR = 2.24, CI 1.33-3.78, p = 0.002) with a median age of 14.6.

To determine whether effects were due to helminth associated morbidity, both the AFP and IBI models were run including variables for either hemoglobin concentration or anemia status. Mean hemoglobin was 12.8 g/dL and anemia was relatively prevalent (18%). Hookworm was associated with slightly lower hemoglobin (mean 12.7 vs. 12.9 g/dL, t = 2.68, df=624, p = 0.008), but not with a higher prevalence of anemia. *A. lumbricoides* had no effect on either hemoglobin or anemia. Neither hemoglobin nor anemia was significant in any model for AFP or IBI, nor did inclusion of hemoglobin or anemia significantly alter the parameter estimates for hookworm and *A. lumbricoides*.

Although co-infection occurs frequently, these effects were largely independent of one another; including only one species in the model at a time did not substantially change parameter estimates (see SI), and we did not find significant interactions between species. Thus, coinfection was associated with accelerated reproductive schedules early in life and decelerated schedules at older ages (Figures 2), due to the combined independent effects of hookworm and *A. lumbricoides*.

Since a number of animal studies have shown that reproductive effort may affect susceptibility to parasites, and such effects could create the illusion of effects of infection on pregnancy, we also tested for effects of pregnancy on likelihood of becoming newly infected. Overall, pregnant women were marginally more likely to have hookworm (OR = 1.68, CI 0.96-2.94, p = 0.068), but no more likely to have *A. lumbricoides*. However, given a roughly two month period from infection to maturity for both species, and a typical lifespan of a few years

(22), infections during pregnancy would most likely be reflected in increased detection during the third trimester or in the year following birth. Broken out by trimester and lactational state, there were no significant differences in infection prevalence between reproductive states (Figure S2), and if anything higher prevalences were in the first two trimesters. Additionally, being pregnant did not increase the odds of being observed with an infection at the next visits. Using both binomial generalized mixed models and multistate Markov models to estimate the effects of pregnancy on transition rates between infection states, we again found no effect of pregnancy on likelihood of becoming infected with either helminth (see SI).

Discussion

Over the course of a woman's lifetime, helminth infections have substantial effects on women's fertility. Figure 2 shows the median IBIs, reproductive rates, and cumulative offspring-by-age with and without infections, as estimated by the combined effects on age of first pregnancy and inter-birth intervals discussed above. Although these are best thought of as hypothetical examples since most women will not be continuously infected over their entire lifetimes, they illustrate the range of possible effects. Whereas uninfected women will have a median of 8.54 children by age 45, hookworm infected woman will have only 6.59 and an *A. lumbricoides* infected woman will have 10.62.

How common are these effects likely to be? Among the Tsimane women included in this study, the prevalence of hookworm increases by age from 20% in the nulliparous sample, to 56% in women age 40-45, and the prevalence of *A. lumbricoides* declines from 38% in the nulliparous sample to 15% in women 40-45 (Table S1). However, virtually all women are likely infected with hookworm at various points in their lives. In a subsample from this study of 201 women

with four or more repeat observations, 96.6% were positive for hookworm at least once. Forty-five percent were observed with *A. lumbricoides* during one of the four observations.

At first glance, the finding that hookworm reduces fecundity, while *A. lumbricoides* increases it may seem surprising. If this were simply a case of costs associated with infection, then we would expect both parasites to have negative effects commiserate with their effects on energetic status and health. The fact that different parasites have different effects suggests a more complex mechanism. One possibility is that these two helminths produce different immune responses, which differentially affect reproduction. Pregnancy is characterized by Th2 biased peripheral immunity (19), including elevated IL-10, which has been implicated in immune tolerance of the fetus (20). Th2 biases also appear during the luteal phase of the menstrual cycle (27). These Th2 biases are similar to those produced by most helminths, including *A. lumbricoides* (17), suggesting that Th2 biases from helminth infections might increase tolerance of sperm or embryo, resulting in an increased chance of conception, such as we observe with *A. lumbricoides*. Why not for hookworm as well? In some reports, mono-infection with hookworm elicits a mixed Th1/Th2 response (28), while the Th1 portion of this response is suppressed in cases of coinfection (29). Th1 responses to leishmaniasis are associated with reduced implantation and increased fetal resorption (30), so mixed responses to hookworm might have results in either direction. These responses may be mediated when individuals are coinfected. It is worth noting that hookworm and *A. lumbricoides* have been found to have very different effects in other circumstances as well, for example with regard to malaria coinfection (31).

The age pattern of the effects of *A. lumbricoides* infection is suggestive of fecundity compensation, particularly when considered in tandem with hookworm coinfection, which is how it frequently occurs in this population (Figure 2). It is characterized by earlier reproduction

and shorter birth spacing early in reproduction. However, it is difficult to conclusively determine whether this represents fecundity compensation, rather than a byproduct of immune modulation. Fecundity compensation implies an adaptive reaction norm on the part of the host, in which infection serves as a cue to future conditions, causing the host to alter life history allocations. Overall, Tsimane women do reproduce earlier and more rapidly than women in populations with lower parasite burdens, and we might reasonably expect differences in comparison to other populations. However, here we are examining the effect of current infection status. If infection is serving as a cue then cumulative infection history might be more important than current status. However, it is also possible that current status captures some piece of a cumulative effect or running average of infection probability. In general, helminths are also not the type of parasite for which fecundity compensation is predicted, since they appear to have constant costs across the lifespan (21). However, there may be hidden costs associated with continuous infection that do accumulate with age, such as accelerated immunosenescence and depletion of naïve T-cells (24, 25). Finally, it is worth noting that if this is compensation, it appears to be doing more than simply compensating, but rather overshooting, in that *A. lumbricoides* infected individuals end up with higher fertility than uninfected individuals. However, this could be the product of an adaptive response that evolved under conditions of higher pathogen burden and mortality than the Tsimane currently experience. In fact, this and other results hint that *A. lumbricoides* infection may be associated with a suit of life history changes that would be expected if it were a cue to higher mortality, including not only the earlier and more rapid reproduction reported here, but also shorter adult stature (26, 32).

Our results have important implications for thinking about demographic patterns across populations, since these patterns are likely to be influenced not only by the presence or absence

of parasites, but by the specific species most prevalent. For example, there are a number of South American populations where *A. lumbricoides* is prevalent but hookworm is not (32). Demographic transitions are also likely influenced by changes in parasite loads. While increasing treatment of some helminths, like hookworm, might lead to increased fertility, treatment of others could lead to reduced fertility.

Although evidence is still somewhat sparse and sometimes contested, archaeological, genomic, and comparative data with other primates suggest that humans have likely been infected with helminths throughout our entire history (33). This presents the possibility that our physiology evolved with the expectation of some amount of helminth infection, e.g. that helminths are 'old friends' (34). Human life histories might have evolved to compensate for effects of infection, in terms of adjusting physiology for optimum fecundity and birth spacing. Of course, this begs the question of which helminths the system would expect. If helminth infections were not constant across time and space we might expect selection for plasticity. In this case, the only evolutionarily novel situation would likely be a complete absence of infections, the consequences of which might be increased Th1 biasing in immunity and potential suppression of fecundity. In part, this might relate to dramatic reductions in fertility in industrialized populations, even prior to birth control.

Materials and Methods

Subject Population

Tsimane are a rapidly expanding natural fertility population of about 15,000 forager-horticulturalists that live along the Maniqui River and surrounding areas in lowland Bolivia. Tsimane are largely self-sufficient and subsist primarily hunting, fishing, and cultivation of

plantains, rice, and manioc. 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 most choose to do so at least once. Approval for the study was granted by the Gran Consejo Tsimane and each study community. Individuals gave informed consent during medical visits and before each procedure. The study was approved by the IRBs at the University of California—Santa Barbara and the University of New Mexico. The Tsimane ethnographic context and project details, including methods of demographic collection, have been described in detail elsewhere (35–37)

Medical Surveillance

Study participants were seen by the mobile THLHP biomedical team who visited Tsimane villages annually from 2006-2013. 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 fecal samples, physicians administered vitamins and medications as warranted.

Fecal parasite identification

Fecal samples were analyzed using two methods. During all years, fecal samples were analyzed 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 100x and 400x (37, 38). Beginning in 2007, fecal samples were also preserved in 10% formalin solution following direct identification, and later analyzed using a modified Percoll (Amersham Pharmacia) technique (39). As we report

elsewhere, the Percoll method identified slightly more positive cases, but did not produce systematic biases in parasite identification (37). We therefore coded individuals as positive or negative for identified species in a single dataset, regardless of which method was used.

Identification of pregnancies

Pregnancy status was determined during medical visits through patient report, and in some cases with pregnancy tests. Doctors estimated gestation time using the date of last menstruation. Pregnancies were cross-validated against demographic interviews recording ages and birth dates of children. By cross-checking against the demographic interviews we were able to record pregnancies that occurred between medical visits, as well as a handful of pregnancies that were not recorded in medical charts, 89% of which were in the first trimester at the time of the medical visit. Cross-checking the two data sources also allowed us to verify estimates of conception dates using both dates of birth and estimated gestation time.

Data Analysis

Data on pregnancy hazard was analyzed using recurrent events cox-proportional hazard models with a counting process (40). The counting process formulation allows for not only recurrent events, but also time-varying covariates, which in this case included infection status and BMI. Analysis was done in R 3.0.3 (http://cran.us.r-project.org/) using *coxph* and *Surv* in the *survival* package. Since events (conceptions) occurred between observations of parasite status (in fact, no conceptions were directly observed) parasite status at conception was assumed to be the last observed infection status prior to the conception, with the constraint that the last observation had to have occurred after the last birth (i.e. during the interval). Additionally, for first

pregnancies we included eight pregnancies in which parasite status before the pregnancy was unknown, but status during the pregnancy was known. These were women who had not had medical visits prior to their pregnancy. For these women we used the infection status at the first medical visit as the status at conception. To determine whether these choices affected results, we ran several models with varying constraints. Overall the choice made little difference in results, so we report on more inclusive models that maximize sample size (see SI). Models were run controlling for BMI, age, and age^2.

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References

1. Møller A (1993) Ectoparasites increase the cost of reproduction in their hosts. *J Anim Ecol* 62:309–322.

2. Hurd H (2001) Host fecundity reduction: a strategy for damage limitation? *Trends Parasitol* 17:363–8.

3. Neuhaus P (2003) Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (Spermophilus columbianus). *Proc Biol Sci* 270 Suppl :S213–5.

4. Krishnan L, Guilbert LJ, Wegmann TG, Belosevic M, Mosmann TR (1996) T helper 1 response against Leishmania major in pregnant C57BL/6 mice increases implantation failure and fetal resorptions. Correlation with increased IFN-gamma and TNF and reduced IL-10 production by placental cells. *J Immunol* 156 :653–662.

5. Avitsur R, Yirmiya R (1999) The immunobiology of sexual behavior: gender differences in the suppression of sexual activity during illness. *Pharmacol Biochem Behav* 64:787–96.

6. Baudoin M (1975) Host castration as a parasitic strategy. *Evolution (N Y)* 29:335–352.

7. Adamo S (1999) Evidence for adaptive changes in egg laying in crickets exposed to bacteria and parasites. *Anim Behav* 57:117–124.

8. Jones ME et al. (2008) Life-history change in disease-ravaged Tasmanian devil populations. *Proc Natl Acad Sci U S A* 105:10023–7.

9. Thornhill JA, Jones JT, Kusel JR (2009) Increased oviposition and growth in immature Biomphalaria glabrata after exposure to Schistosoma mansoni. *Parasitology* 93:443.

10. Minchella D, Loverde P (1981) A cost of increased early reproductive effort in the snail Biomphalaria glabrata. *Am Nat* 118:876–881.

11. Schwanz LE (2008) Chronic parasitic infection alters reproductive output in deer mice. *Behav Ecol Sociobiol* 62:1351–1358.

12. Cejtin HE et al. (2006) Effects of human immunodeficiency virus on protracted amenorrhea and ovarian dysfunction. *Obstet Gynecol* 108:1423–31.

13. McAllister L, Gurven M, Kaplan H, Stieglitz J (2012) Why do women have more children than they want? Understanding differences in women's ideal and actual family size in a natural fertility population. *Am J Hum Biol* 24:786–99.

14. Martin M, Blackwell AD, Gurven M, Kaplan H (2013) in *Primates, Pathogens, and Evolution*, eds Brinkworth J, Pechenkina K (Springer, New York), pp 363–387.

15. Blackwell AD, Martin M, Kaplan H, Gurven M (2013) Antagonism between two intestinal parasites in humans: the importance of co-infection for infection risk and recovery dynamics. *Proc R Soc B* 280:20131671.

16. Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M (2014) Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. *Lancet Infect Dis* 3099:1–13.

17. Geiger SM et al. (2002) Cellular responses and cytokine profiles in Ascaris lumbricoides and Trichuris trichiura infected patients. *Parasite Immunol* 24:499–509.

18. Maizels RM, Yazdanbakhsh M (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol* 3:733–44.

19. Veenstra van Nieuwenhoven a. L (2003) The immunology of successful pregnancy. *Hum Reprod Update* 9:347–357.

20. Shurin M, Lu L, Kalinski P (1999) Th1/Th2 balance in cancer, transplantation and pregnancy. *Springer Semin Immunopathol* 21:339–359.

21. Forbes M (1993) Parasitism and host reproductive effort. *Oikos* 67:444–450.

22. Brooker S, Bethony J, Hotez PJ (2004) Human hookworm infection in the 21st century. *Adv Parasitol* 58:197–288.

23. O'Lorcain P, Holland C V (2000) The public health importance of Ascaris lumbricoides. *Parasitology* 121 Suppl:S51–71.

24. Kalinkovich A et al. (1998) Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. *Clin Exp Immunol* 114:414–21.

25. Van Baarle D, Tsegaye A, Miedema F, Akbar A (2005) Significance of senescence for virus-specific memory T cell responses: rapid ageing during chronic stimulation of the immune system. *Immunol Lett* 97:19–29.

26. Blackwell AD, Snodgrass JJ, Madimenos FC, Sugiyama LS (2010) Life history, immune function, and intestinal helminths: Trade-offs among immunoglobulin E, C-reactive protein, and growth in an Amazonian population. *Am J Hum Biol* 22:836–48.

27. Faas M et al. (2000) The immune response during the luteal phase of the ovarian cycle: a Th2-type response? *Fertil Steril* 74:1008–13.

28. Geiger SM et al. (2007) Stage-specific immune responses in human Necator americanus infection. *Parasite Immunol* 29:347–58.

29. Geiger SM et al. (2011) Necator americanus and Helminth Co-Infections: Further Down-Modulation of Hookworm-Specific Type 1 Immune Responses. *PLoS Negl Trop Dis* 5:e1280.

30. Krishnan L, Guilbert LJ, Wegmann TG, Belosevic M, Mosmann TR (1996) T helper 1 response against Leishmania major in pregnant C57BL/6 mice increases implantation failure and fetal resorptions. Correlation with increased IFN-gamma and TNF and reduced IL-10 production by placental cells. *J Immunol* 156 :653–662.

31. 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.

32. Cepon-Robins TJ et al. (2014) Soil-transmitted helminth prevalence and infection intensity among geographically and economically distinct Shuar communities in the Ecuadorian Amazon. *J Parasitol* preprint.

33. Hurtado AM, Frey M, Hill K, Hurtado I, Baker J (2008) in *Medicine and evolution: current applications, future prospects*, eds Elton S, O'Higgins P (Taylor and Francis Group, Boca Raton, FL), pp 153–180.

34. Rook GAW (2009) Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. *Immunology* 126:3–11.

35. Gurven M, Kaplan H, Supa AZ (2007) Mortality experience of Tsimane Amerindians of Bolivia: regional variation and temporal trends. *Am J Hum Biol* 19:376–98.

36. Gurven M et al. (2009) Inflammation and infection do not promote arterial aging and cardiovascular disease risk factors among lean horticulturalists. *PLoS One* 4:e6590.

37. Blackwell AD et al. (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 U.S. NHANES. *PLoS Negl Trop Dis* 5:e1218.

38. Vasunilashorn S et al. (2010) Blood lipids, infection, and inflammatory markers in the Tsimane of Bolivia. *Am J Hum Biol* 22:731–40.

39. Eberl M et al. (2002) A novel and sensitive method to monitor helminth infections by faecal sampling. *Acta Trop* 83:183–187.

40. Andersen P, Gill R (1982) Cox's regression model for counting processes: a large sample study. *Ann Stat* 10:1100–1120.

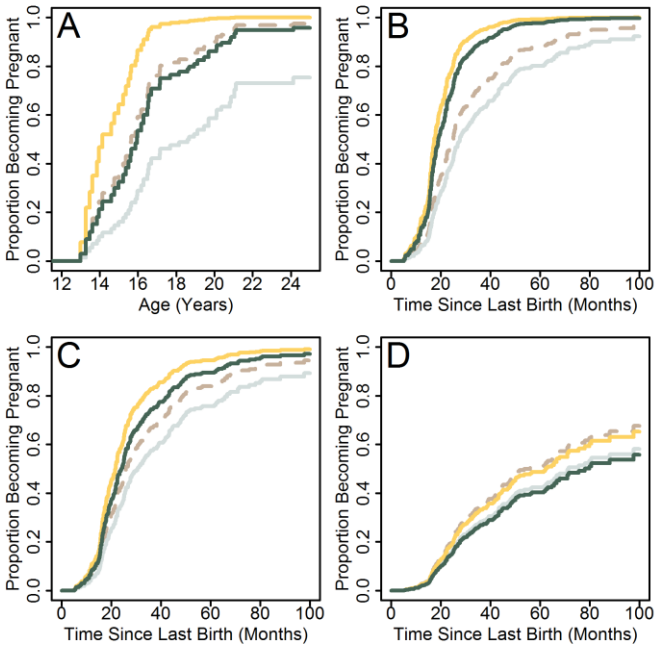

Figure 1. Effect of infection on likelihood of becoming pregnant. Effect of infection on age of first pregnancy (A) and on intervals between subsequent pregnancies at age 20 (B), age 30 (C), and age 40 (D). Survival curves are from cox-proportional hazard models controlling for BMI. Colors indicate uninfected (dashed grey), infected with hookworm (solid light blue), infected with A. lumbricoides (solid mustard), or coinfected with hookworm and A. lumbricoides (solid dark green).

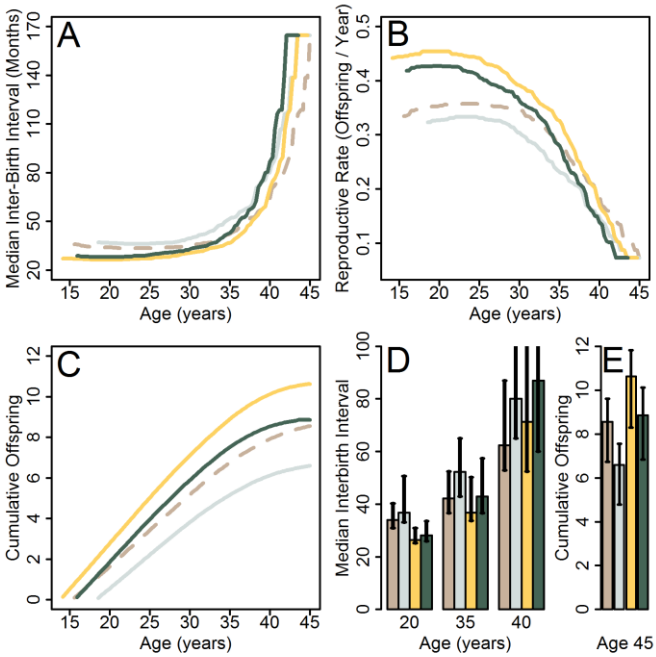

Figure 2. Predicted median interbirth intervals (A), reproductive rate (B), and cumulative offspring by age (C). Colors indicate uninfected (dashed grey), infected with hookworm (solid light blue), infected with *A. lumbricoides* (solid mustard), or coinfected with hookworm and *A. lumbricoides* (solid dark green). Also shown, median and 95% confidence intervals for three ages (D). Note that at age 40 the upper limits are essentially infinite since with the upper estimates less than half of infected women will have another child. Median and 95% confidence intervals for total fertility at age 45 are shown in (E). Predictions are derived from the models in Figure 1.

Supplemental Information

Descriptive Information

Table S1 gives descriptive information on the two samples used. Three individuals appear in both samples, but otherwise the nulliparous and multiparous samples are composed of different individuals. The multiparous sample has been broken out by age group. The sample is somewhat biased toward women over age 35, due to a deliberate oversampling of these women for other studies on aging. However, models all control for age, so this did not affect overall results.

Table S1. Descriptive statistics for the nulliparous and multiparous samples of Tsimane women

Study Group and Age Group	Obs[a]	N[b]	Birth Intervals Starting[c]	Pregnancies[d]	Age (SD)	Previous Pregnancies (SD)	Hookworm Prevalence	*Ascaris* Prevalence	Height in cm (SD)	Weight in kg (SD)	BMI (SD)
Nulliparous	264	184[e]		45	17.11 (2.68)	0	0.20	0.38	149.83 (4.74)	52.26 (8.16)	23.02 (2.85)
Multiparous											
15 – 19	40	26	28	14	18.03 (1.65)	1.54 (0.65)	0.27	0.23	149.70 (3.63)	50.40 (5.15)	22.48 (2.03)
20 – 24	88	58	55	37	22.27 (1.40)	2.92 (1.51)	0.49	0.16	151.08 (4.93)	52.80 (5.61)	23.13 (2.18)
25 – 29	92	57	52	34	27.58 (1.46)	4.53 (1.60)	0.40	0.21	150.33 (4.59)	55.22 (8.06)	24.39 (3.09)
30 – 34	105	65	64	41	32.47 (1.63)	6.53 (1.09)	0.39	0.23	151.92 (4.46)	57.72 (9.83)	24.95 (3.67)
35 – 39	193	121	123	53	37.86 (1.43)	7.76 (2.40)	0.56	0.22	151.53 (4.27)	57.85 (9.62)	25.17 (3.82)
40 - 45	324	193	189	47	42.85 (1.69)	8.93 (2.52)	0.56	0.15	151.65 (4.72)	56.96 (9.17)	24.74 (3.60)
All Ages	842	432[f]	511	226	35.37 (8.04)	6.95 (3.19)	0.48	0.19	151.28 (4.52)	56.10 (9.09)	24.48 (3.54)

[a] Observations of infection status, BMI, and pregnancy status. Means for age, previous pregnancies, height, weight, and BMI are means of all observations, and may include repeated data on the same individual. Prevalences reported here are similarly by observation, and not by individual.
[b] Unique individuals appearing in the age category
[c] Birth intervals beginning in this age group. Intervals begin on the birth date of the last offspring. Since some intervals overlap multiple age categories, this number may be lower that the number of unique individuals in that category.
[d] Intervals ending in recorded pregnancies. Intervals without recorded pregnancies are considered right censored.
[e] Three individuals appear in both the nulliparous and multiparous datasets, since we had data on both their first and later pregnancies. Otherwise, the two samples are composed of different individuals.
[f] Due to the longitudinal dataset, some individuals appear in more than one age group, so the total N is smaller than the sum of the Ns from each age group

Modelling the effects of infection on hazard of next pregnancy following a birth

We utilized a counting process approach for recurrent pregnancies in a cox-proportional hazards model, including only observations of infection status during the inter-pregnancy intervals, and excluding others (such as those during pregnancy). All observations of infection status therefore occurred during the inter-pregnancy interval. However, we wanted to verify that effects did not depend on the proximity of the infection observation to the following pregnancy, since some intervals were long and the parasite observation might fall long before the actually pregnancy. We therefore ran several models, restricting the time between the pregnancy and the observation to values ranging from 12 months to 48 months. Overall the choice had little impact on estimated hazard ratios (Table S2) and we therefore report the least restrictive model (with the largest sample) in the main body of the paper (Model 1.4). We also wanted to determine whether the effects of hookworm and *A. lumbricoides* were independent, and so ran models with only one parasite or the other. Hazard-ratio estimates were only slightly different when parasites were examined separately (Table S2, Models 1.4b and 1.4c), suggesting little mediation of one by the other.

Table S2. Recurrent events Cox proportional hazard models examining the effects of infection on pregnancy hazard.

	Model 1.1	Model 1.2	Model 1.3	Model 1.4	Model 1.4b	Model 1.4c
Restrictions						
Max Time Between Parasite Obs and Pregnancy	12	24	36	48	48	48
Mean Time Difference (Months)	5.39	7.86	8.94	9.62	9.62	9.62
SD Time Difference (Months)	3.59	5.83	7.31	8.51	8.51	8.51
Sample						
n	431	432	432	432	432	432
Observations	778	825	837	842	842	842
Pregnancies	162	209	221	226	226	226
Outcomes (HR (95%CI))						
Age (decades)*	7.85 (1.47-41.92)	7.43 (1.72-32.11)	7.08 (1.78-28.09)	6.60 (1.69-25.78)	7.24 (1.85-28.42)	6.39 (1.68-24.39)
Age^2 (decades)*	0.65 (0.50-0.83)	0.65 (0.52-0.82)	0.66 (0.53-0.82)	0.67 (0.54-0.82)	0.65 (0.53-0.8)	0.67 (0.54-0.82)
BMI	0.97 (0.92-1.03)	0.98 (0.93-1.03)	0.97 (0.93-1.02)	0.98 (0.93-1.02)	0.97 (0.93-1.02)	0.98 (0.94-1.03)
Hookworm	0.80 (0.59-1.08)	0.77 (0.6-1.00)	0.76 (0.59-0.98)	0.77 (0.60-0.99)	0.80 (0.62-1.03)	
Roundworm** (Age 20)	2.48 (1.42-4.3)	2.32 (1.47-3.67)	2.35 (1.52-3.64)	2.33 (1.50-3.60)		2.18 (1.41-3.36)
Roundworm** (Age 40)	0.79 (0.45-1.40)	0.90 (0.57-1.4)	0.89 (0.58-1.37)	0.94 (0.62-1.42)		0.97 (0.64-1.46)

*Age was continuous to the nearest tenth of a year, but is shown in decades to make the parameters more easily interpretable. **Roundworm is entered in the model as two terms, a main effect and age interaction term. However, for ease of interpretation we show the values age ages 20 and 40 rather than the parameter values.

Modelling the effect of infection on age of first pregnancy

We examined the robustness of our age of first pregnancy model by testing models with various restrictions on the timing of parasite observations relative to the timing of the first pregnancy (Figure S1, Table S3). Since there were some women were our first measure of parasite infection was during their first pregnancy, parasite observations ranged from four years before the pregnancy up to the date of their first birth, and we examined restrictions ranging from including only observations in year before the first pregnancy to including observations up to four years before as well as observations made during the pregnancy. Note that in all cases all observations before the pregnancy and up to the limit were included,

and observations during the pregnancy were included only for women without observations before the pregnancy.

In general the choice of restrictions affected the magnitude of hazard ratios, but did not affect the direction or general conclusions (Figure S1), although in the most restrictive models, the effect of hookworm did become non-significant. However, in these models, sample size was significantly reduced. In all other models hazard-ratios for hookworm and *A. lumbricoides* were significant and similar in magnitude (Table S3). This included models in which only one parasite was tested at a time (Models F1 and F2). The model we report in the main text is the least restrictive models, which maximizes the sample size.

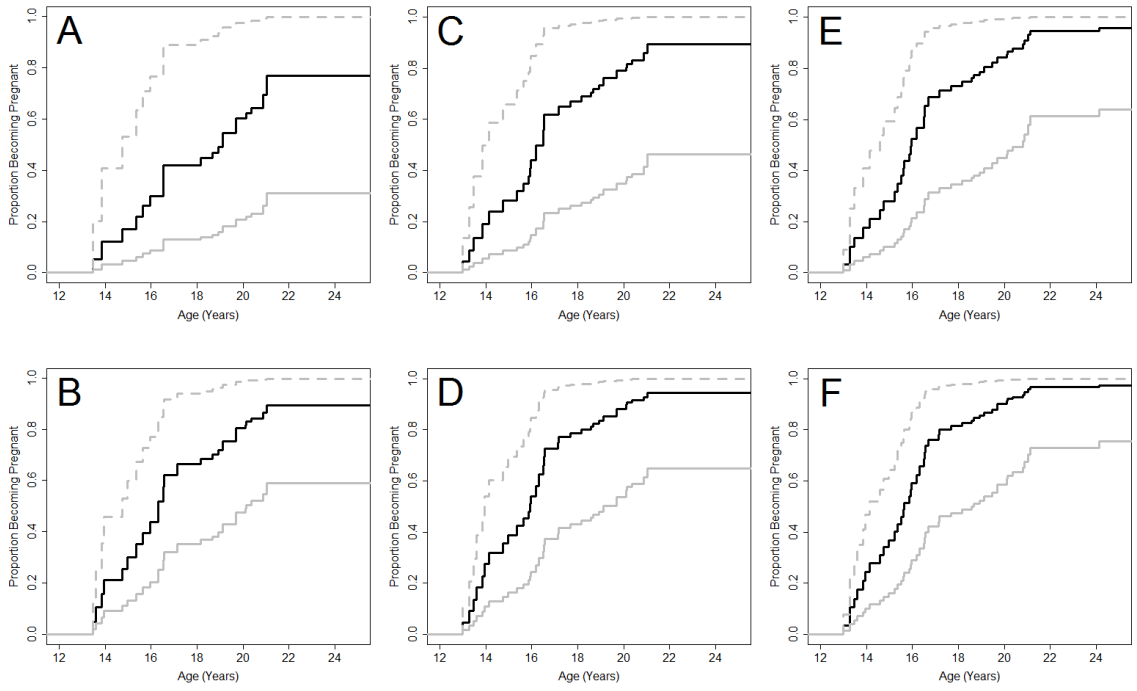

Figure S1. Cox-proportional hazard models with varying observation matching criteria. Survival curves from cox-proportional hazard models show the association between hookworm infection (solid grey) and *A. lumbricoides* infection (dashed grey), and coinfection (dotted black) on age of first pregnancy, relative to uninfected (solid black). Model details are shown in table S1. The model letter corresponds to the figure letter. Note that Model F is the same as the model shown in Figure 1A.

Table S3. Cox-proportional hazard models with varying inclusion criteria.

		Model A	Model B	Model C	Model D	Model E	Model F	Model F1	Model F2
Restrictions									
Maximum Time Difference (Months)		12	12	24	24	48	48	48	48
Include Observations During Pregnancy		No	Yes	No	Yes	No	Yes	Yes	Yes
Mean Time Difference (Months)		0.39	0.20	1.11	0.91	2.54	2.31	2.31	2.31
SD Time Difference (Months)		1.66	1.94	3.77	3.88	8.11	8.11	8.11	8.11
Sample									
n		176	184	176	184	176	184	184	184
Observations		235	243	246	254	256	264	264	264
Pregnancies		16	24	27	35	37	45	45	45
Outcomes									
Hazard Ratios (95% CI)	Hookworm	0.25 (0.06-1.11)	0.40 (0.13-1.20)	0.27 (0.12-0.64)	0.36 (0.17-0.79)	0.32 (0.17-0.63)	0.38 (0.20-0.72)	0.45 (0.24-0.85)	
	Roundworm	4.06 (1.36-12.12)	2.57 (1.12-5.87)	3.24 (1.61-6.52)	2.41 (1.36-4.26)	2.73 (1.5-4.94)	2.24 (1.33-3.78)		1.83 (1.08-3.11)
	BMI	1.16 (0.98-1.38)	1.14 (0.97-1.32)	1.10 (0.98-1.24)	1.10 (0.97-1.23)	1.06 (0.96-1.19)	1.07 (0.96-1.19)	1.10 (0.98-1.22)	1.09 (0.98-1.22)
Median AFB	Uninfected	19.70	16.53	16.49	16.17	15.96	15.87	15.36	15.94
	Hookworm	21.04	21.04	21.04	20.12	20.86	19.11	16.52	
	A. lumbricoides	15.36	14.98	14.14	13.95	14.60	14.60		14.98

Effects of pregnancy on infection

We used multistate Markov models (MSM) to examine the effect of pregnancy on likelihood of transitions from uninfected to infected states. Models controlled for BMI, age, and coinfection status. Pregnancy was not significantly associated with transitions from uninfected to infected states for either parasite using MSM models (Table S4). In binomial generalized linear mixed models (Table S5) being pregnant at the previous study visit was also not associated with altered odds of infection. Currently pregnant women were marginally more likely to have hookworm (OR = 1.68, p = 0.068). Broken out by trimester, there were no significant differences in likelihood of hookworm or *A. lumbricoides* infection, controlling for age, BMI, coinfection and repeat measures (Figure S2).

Table S4. Hazard ratios for covariates from multistate Markov models examining likelihood of transition between uninfected and infected states.

	Hookworm			***A. lumbricoides***		
		95% CI			**95% CI**	
Covariate	**HR**	**Lower**	**Upper**	**HR**	**Lower**	**Upper**
Age	1.03	1.00	1.07	0.97	0.92	1.02
BMI	0.95	0.89	1.01	1.05	0.96	1.15
Pregnancy	0.79	0.38	1.64	0.64	0.20	2.06
Coinfection	0.72	0.35	1.49	1.27	0.62	2.60

Table S5. Binomial generalized linear mixed models for hookworm and A. lumbricoides infection.

	Hookworm				**A. lumbricoides**			
		95% CI				**95% CI**		
Covariate	**OR**	**Lower**	**Upper**	**p**	**OR**	**Lower**	**Upper**	**p**
Age	1.04	1.01	1.07	0.016	0.96	0.92	1.01	0.136
BMI	0.93	0.88	0.98	0.007	1.01	0.92	1.11	0.821
Pregnant Now	1.68	0.96	2.94	0.068	0.95	0.41	2.16	0.897
Coinfected Now	1.70	0.99	2.89	0.053	1.93	1.02	3.64	0.043
Pregnant Last Year	1.10	0.50	2.46	0.811	0.77	0.21	2.89	0.700
A. lumbricoides Last Year	0.58	0.25	1.34	0.201	1.76	0.41	7.47	0.446
Hookworm Last Year	2.03	1.18	3.49	0.011	0.85	0.36	1.97	0.700

In addition to the parameters shown, models control for repeat measures with an individual level mixed effect term.

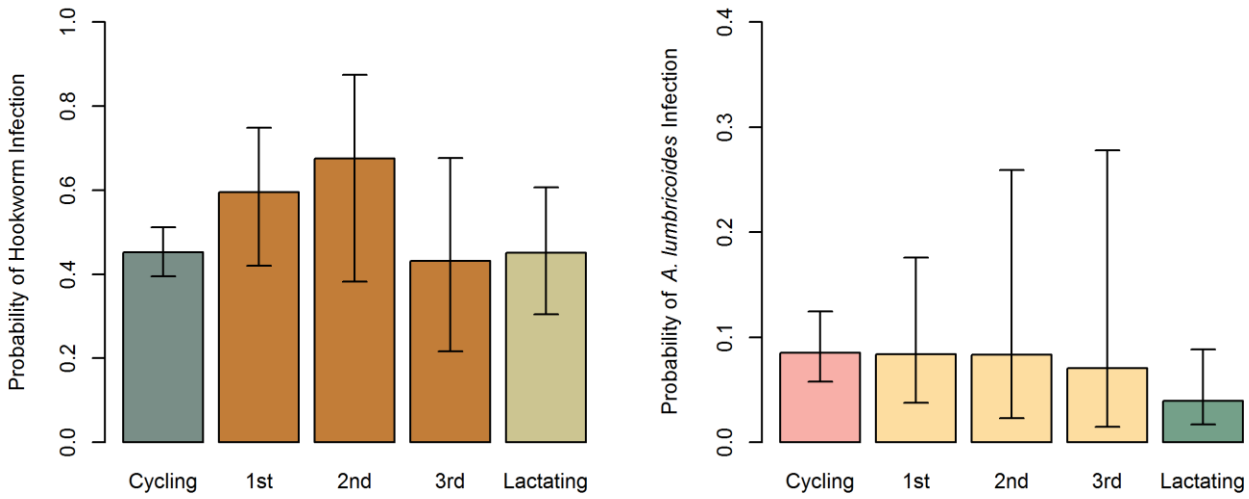

Figure S2. Predicted probability of helminths infection by reproductive status, controlling for age, BMI, and coinfection. Error bars show uncorrected 95% confidence intervals for the prediction. Correcting for multiple comparisons, there are no significant differences by reproductive state.