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Short Report: Parasite Infection and Tuberculosis Disease among Children: A Case–Control Study

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Abstract. We conducted a case–control study to examine associations between parasite infection, including protozoa infection, and tuberculosis (TB) in children in Lima, Peru. We enrolled 189 matched-pairs. In multivariable conditional logistic regression analyses, *Blastocystis hominis* infection (rate ratio = 0.30, 95% confidence interval = 0.14–0.64, $P = 0.002$) was strongly associated with a lower risk of TB. We observed a statistically significant inverse linear dose-response relationship between *Blastocystis hominis* infection and TB. These findings should be confirmed in future prospective studies.

Globally, infectious diseases account for more than half of deaths among children less than five years of age.[1] Studies examining the ways in which some infectious diseases interact identify worrisome synergistic relationships in which infection with one disease increases susceptibility to or worsens the prognosis of another.[2–4] The relationship between parasite infection and tuberculosis (TB) has gained increasing attention over the past decade: both animal studies and epidemiologic studies in humans have found evidence indicating that chronic helminth infection may increase the risk of TB and reduce the effectiveness of the Bacillus Calmette–Guérin vaccine.[5,6]

Protozoa infection, another type of parasitic infection, is common in urban areas lacking clean water access and may result in severe malnutrition as a consequence of chronic diarrhea or anorexia. Protozoa infection can be asymptomatic and may result in elevated levels of cytokines such as interferon-γ (IFN-γ),[7,8] a critical mediator in the host immune response to *Mycobacterium tuberculosis*. We report on the relationship between various parasite infections, including protozoa infection, and TB among children in Lima, Peru.

We conducted this case–control study in two of five health regions in the Lima metropolitan area as described.[9] Parasite infestation, with protozoa pathogens in particular, is common in Lima, with more than 40% of adults and children infected.[10,11] Eligible cases were children < 15 years of age who received an initial TB diagnosis at the Instituto Nacional de Salud del Niño, the main pediatric tuberculosis referral center in Peru, or a participating health clinic during the study period of February 2010–September 2011. Healthy controls (i.e., no chronic cough or fever) without a history of TB were identified by using a random walk or friend referral and matched to cases by neighborhood, age, and enrollment date. Ethical approval for this study protocol was granted by the Office of Human Research Administration at the Harvard School of Public Health in Boston, Massachusetts and the Instituto Nacional de Salud del Niño in Lima, Peru. Guardians provided informed consent, and children ≥ 8 years of age provided informed assent.

To assess parasite infection, we requested that children provide two scotch-tape specimens applied to the anal area for identification of *Enterobius vermicularis*[12] and three stool samples: the first two samples were preserved with 10% formalin and the third was a fresh sample. An accredited laboratory (Blufstein Laboratorio Clínico, S.A.) in Lima examined stool samples by using direct smear microscopy and spontaneous sedimentation methods and scotch tape specimens by using the Graham method.[12] Laboratory personnel were blinded to the case status of participants. We excluded pairs in which either the case or control did not contribute ≥ 1 stool and scotch tape specimen. Children and their guardians were asked to respond to an interview related to sociodemographic, clinical, and lifestyle factors.

We conducted conditional logistic regression analyses, stratified by each matched pair. To create the final multivariable model, we included binary variables for the presence of infection with any parasite species that was found in at least 5% of controls; we also included variables that were identified *a priori* as potential confounders (Table 1). To examine a potential dose response relationship between protozoa infections and TB, we categorized each person's disease burden with a particular pathogen as none, scarce (1+, defined as 1–2 parasites per field), light (2+, defined as 3–4 parasites per field), moderate (3+, defined as 5–6 parasites per field), and heavy (4+, defined as > 6 parasites per field). We conducted sensitivity analyses among only those participants with all three stool samples.To address missing data in multivariable analyses, we imputed data sets using Markov Chain Monte Carlo methods (SAS MI procedure; SAS Institute, Chicago, IL) and estimated adjusted rate ratios by pooling across data sets.

A total of 189 of 194 matched pairs (97.4%) were included for analysis. Ninety-two percent of controls were identified by using a random neighborhood walk technique. In 29 cases (15.3%), TB was confirmed by positive smear microscopy or positive culture. Controls were enrolled a median of 8 days after case enrollment (interquartile range [IQR] = 5–12.5 days). Characteristics of cases and controls are shown in Table 1. Household ownership of animals included cats, dogs, canaries, parrots, hamsters, chickens, turkeys, ducks, rabbits, guinea pigs, and cows. All three stool specimens were collected in 64% of cases and 70% of controls, and 91% and 96%, respectively, provided at least two stool specimens.

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TABLE 1
Tuberculosis-related characteristics of cases and controls, Lima, Peru

Variable	No.	Controls, n = 189, no. (%)	Cases, n = 189, no. (%)	P*
Age, years†‡	378	6.70 (4.19)	6.73 (4.55)	–
Male sex	378	87 (46.03)	100 (52.91)	0.19
Hospitalized ≥ 8 hours in past 2 years	368	20 (10.75)	29 (15.93)	0.14
Household member treated for parasite infection in past year	366	27 (14.67)	19 (10.44)	0.23
Treated for parasite infection in past year	370	35 (18.92)	30 (16.22)	0.42
Immigrant from outside Lima	370	19 (10.22)	29 (15.76)	0.13
Previous close contact with someone with tuberculosis	347	37 (20.79)	122 (72.19)	< 0.0001
House lacks any exterior windows	367	24 (12.97)	33 (18.13)	0.18
Toilet or latrine used exclusively by household	370	143 (77.30)	142 (76.76)	0.90
House has a kitchen	371	128 (68.82)	129 (69.73)	0.91
Household owns motorized form of transport	372	36 (19.35)	29 (15.59)	0.38
House has a dirt floor	372	23 (12.37)	12 (6.45)	0.06

*From univariable conditional logistic regression analysis.
†Continuous variable, mean and standard deviation presented
‡Cases and controls were matched by age; therefore, no *P* value is provided.

Among cases, the median time from TB treatment initiation to first stool sample collection was 1 day (IQR = -1–3 days). Two tape specimens were obtained from 89% cases and 93% of controls. Similar prevalences of diarrhea in the month before enrollment were reported for cases and controls (26.1% versus 29.6%, respectively; $P = 0.55$).

Protozoa infections constituted most parasite infections: *Blastocystis hominis*, *Endolimax nana*, *Giardia lamblia, Entamoeba coli* were present in 43%, 19%, 16%, and 13% of controls, respectively (Table 2). Infection with *B. hominis* was inversely associated with TB by univariable analysis (rate ratio [RR] = 0.45, 95% confidence interval [CI] = 0.28–0.74, $P = 0.002$) (Table 2) and remained independently associated with TB (RR = 0.30, 95% CI = 0.14–0.64, $P = 0.002$) by multivariable analyses. In the same multivariable analysis, animal ownership also demonstrated a protective relationship with TB of statistical significance (RR = 0.46, 95% CI = 0.22–0.99, $P = 0.05$). The association between *B. hominis* infection and TB was consistent in a multivariable sensitivity analysis that included the subset of 254 children (67.2%) with all three stool specimens (RR = 0.16, 95% CI = 0.02–1.31, $P = 0.09$).

Among persons infected with *B. hominis*, most (63.7%) had a light parasite burden and 13.3% and 22.2% had scarce and moderate parasite burdens, respectively. Only one child had a heavy (4+) *B. hominis* burden. Therefore, moderate and heavy burdens were grouped for the dose-response analysis. We found that greater *B. hominis* burdens were associated with lower rates of TB. Each increase in *B. hominis* burden category was associated with a 42% decrease in the rate of TB (RR = 0.58, 95% CI = 0.40–0.82, $P = 0.002$).

We found that infection with *B. hominis* was associated with a lower risk of TB. Furthermore, *B. hominis* burden demonstrated a linear dose response with TB risk. These findings persisted after adjustment for other parasite infections and numerous demographic and socioeconomic risk factors. The observation that household animal ownership, a potential risk factor for *B. hominis*,[13,14] was also associated with reduced TB risk further supports our findings. Although the pathogenicity of *B. hominis* infection is debated and may be subtype dependent, asymptomatic infection is common.[13–15] Although the treatment of symptomatic diarrheal illness is unequivocally crucial to the nutritional status and overall health and well-being of children, these findings raise the question of whether chronic asymptomatic infection with *B. hominis* may provide protection against TB.

Recent work introduces the possibility of a protective relationship between *Helicobacter pylori*, an asymptomatic gastrointestinal infection, and TB. Perry and others observed that household contacts without TB were significantly more likely to be infected with *H. pylori* than the prevalent TB cases in their households, and that cynomolgus macaques with *H. pylori* were less likely to progress to TB within 6–8 months of challenge with *M. tuberculosis*.[16] The authors also reported higher TB antigen–induced IFN-γ levels and an enhanced Th-1 response among persons with latent tuberculosis infection and *H. pylori* infection compared with those with latent

TABLE 2
Prevalence of parasite infection and associations with tuberculosis, Lima. Peru*

Variable	No.	Controls, n = 189, no. (%)	Cases, n = 189, no. (%)	Univariable RR (95% CI)	P	Multivariable RR,† (95% CI)	P
Chilomastix mesnili	378	1 (0.53)	2 (1.06)	–	–	–	–
Entamoeba histolytica	378	1 (0.53)	3 (1.59)	–	–	–	–
Iodamoeba butschlii	378	4 (2.12)	2 (1.06)	–	–	–	–
Hymenolepis nana	378	0 (0)	2 (1.06)	–	–	–	–
Trichuris trichiura	378	0 (0)	1 (0.53)	–	–	–	–
Strongyloides stercoralis	378	0 (0)	1 (0.53)	–	–	–	–
Blastocystis hominis	378	82 (43.39)	54 (28.57)	0.45 (0.28–0.74)	0.002	0.30 (0.14–0.64)	0.002
Giardia lamblia	378	30 (15.87)	19 (10.05)	0.59 (0.32–1.10)	0.10	0.52 (0.20–1.33)	0.17
Entamoeba coli	378	24 (12.70)	29 (15.34)	1.29 (0.69–2.44)	0.42	0.83 (0.32–2.16)	0.71
Endolimax nana	378	35 (18.52)	40 (21.16)	1.17 (0.72–1.90)	0.54	1.72 (0.78–3.77)	0.18
Enterobius vermicularis	378	23 (12.17)	24 (12.70)	1.05 (0.58–1.88)	0.88	0.77 (0.31–1.92)	0.58
Has animal(s)	371	131 (70.43)	107 (57.84)	0.52 (0.32–0.86)	0.01	0.46 (0.22–0.99)	0.05

*RR = rate ratio; CI = confidence interval.
†Multivariable estimates adjusted for infection with other parasites and animal ownership (as shown), matching factors (age, neighborhood, enrollment date), as well as sex, hospitalization in prior two years, immigrant status, individual and household parasite treatment history, prior close tuberculosis contact, and the following household characteristics: dwelling lacks windows to exterior, toilet or latrine used exclusively by household, dwelling has a kitchen, dwelling has a dirt floor, and motorized vehicle ownership.

TB infection but no *H. pylori* infection. This latter observation represents a potential mechanism through which infection with *B. hominis* may lead to a reduced TB risk.

Protozoa infection may be accompanied by pro-inflammatory responses: studies have found increased serum levels of IFN-γ among those infected with *Giardia*.[7,8] Although a recent study from China showed similar cytokine profiles in adults with and without *B. hominis*,[17] children with repeated exposure to or chronic infection with certain subtypes of *B. hominis* might manifest a pro-inflammatory response that provides protection against *M. tuberculosis* infection or TB. Interestingly, one large cross-sectional study conducted in Lima found that *B. hominis* was significantly less prevalent in persons infected with human immunodeficiency virus than in persons not infected with this virus,[18] and a study of children in Colombia found higher vitamin A levels, reduced gastrointestinal morbidity, and better school attendance among children with *B. hominis* infection.[19]

This case–control study design assessed TB and parasite burden simultaneously and therefore, we cannot determine the directionality of the observed relationship. It is possible, for example, that a pro-inflammatory immune response to TB provided protection against *B. hominis* infection or reduced the duration of *B. hominis* infection when it occurred. Similarly, if even short exposure to TB treatment affected the presence or shedding of *B. hominis,* this could also explain our findings. Furthermore, we cannot rule out the possibility that an unknown factor increased the risk of TB while conferring protection against *B. hominis*; or conversely, that an unknown factor increased the risk of *B. hominis* infection but protected against TB. Finally, although we knowledge that parasite quantification is subject to misclassification, studies of *B. hominis* have reported associations between intensity of infection and the presence of symptoms,[20] suggesting that quantification may be clinically relevant in spite of misclassification. Prospective studies that include consideration of *B. hominis* subtype, as well as cytokine profiles in children with and without infection, may help to further elucidate the relationship.

The study of TB in the context of other co-occurring infections coincides with the relatively new microbiomic approach to research and may lead to a greater understanding of the ways in which microbes interact and co-evolve in the human host.[21] A factor conferring a level of protection as strong as that reported here is worthy of further exploration. A causal relationship between *B. hominis* infection and TB could have critical implications for vaccine development as well as policies related to the treatment of asymptomatic infection.

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