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ORIGINAL PAPER

# Seroprevalence of five parasitic pathogens in pregnant women in ten Caribbean countries

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**Abstract** To date, published epidemiological studies of parasitic infections in humans in the Caribbean region are very limited. Here, we report the seroprevalence of five parasitic pathogens, including *Ascaris lumbricoides*, *Entamoeba histolytica*, *Giardia lamblia*, *Schistosoma mansoni*, and *Toxocara canis* in 435 serum samples collected between 2008 and 2011 from pregnant women in ten Caribbean islands. We tested the serum samples for IgG antibodies against the five parasites by enzyme-linked immunosorbent assay (ELISA). Among them, 66.2 % were serologically positive for at least one parasite. The most prevalent parasite was *G. lamblia* (40.5 %), followed by *A. lumbricoides* (37.9 %), *T. canis* (14.5 %), *E. histolytica* (6.7 %), and *S. mansoni* (3.0 %). Evidence of infections of *G. lamblia* and *A. lumbricoides* were detected in all ten Caribbean countries. Seroprevalence estimates significantly differed between countries for *A. lumbricoides*, *E. histolytica*, and *T. canis* (*p*-values <0.001). For *S. mansoni*, significance was observed by Fisher's exact test ($p = 0.013$) but not by multiple comparisons. The prevalence of *G. lamblia* was not significantly different between countries ($p = 0.089$). A significant negative correlation between the gross domestic product (GDP) per capita and overall seroprevalence by country was also observed (Pearson's $r = -0.9202$, $p = 0.0002$). The data strongly indicates that neglected parasitic infections remain a significant health burden on people in these countries. Thus, justification has been provided to regional health planners to enhance existing public health surveillance programs on parasitic diseases and to heighten the public's awareness through education and outreach programs on how they can minimize the occurrence of parasitic infections.

**Keywords** Seroprevalence · Pregnant women · Caribbean · *Ascaris lumbricoides* · *Entamoeba histolytica* · *Giardia lamblia* · *Schistosoma mansoni* · *Toxocara canis*

Rosina C. Krecek and Guan Zhu contributed equally to this work.

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## Introduction

Intestinal parasites are a significant cause of morbidity and mortality around the world, in which an estimated two billion people are infected by at least one intestinal parasite and more than half of the world population is at risk for infection (Al-Delaimy et al. 2014). Intestinal parasites comprise a large, diverse group of pathogens, such as water-borne protozoa (e.g., *Entamoeba histolytica* and *Giardia lamblia*, syn. *G. intestinalis*) and soil-transmitted helminth (STH) nematodes (e.g., *Ascaris lumbricoides* and *Toxocara* spp.). *G. lamblia* causes giardiasis and is the most commonly diagnosed gut parasite worldwide, with an estimated 280 million people suffering from symptomatic infections annually (Ankarklev et al. 2010; Mastronicola et al. 2014). *E. histolytica* causes amebiasis, for which the symptoms can range from mild diarrhea to dysentery. In some cases, *E. histolytica* can breach the intestinal mucosal barrier and cause liver abscesses (Stanley 2003). Amebiasis is the fourth leading cause of death due to protozoan infections with approximately 48 million new cases and 55,000 deaths around the world annually (Lozano et al. 2012; WHO 1998).

In the STH group, *A. lumbricoides* infects more than one billion people globally (Bethony et al. 2006). It is one of the major causes of morbidity and has been linked with impaired growth and development in a pre-school age and school age children in developing countries (Hotez 2008). *Toxocara* parasites are the causative agents of toxocariasis in dogs and cats, in which *T. canis* and *T. cati* are also zoonotic, infecting both humans and animals, most frequently occurring in humans as visceral larva migrans. Although the global epidemiological estimate of human toxocariasis is limited (Smith et al. 2009), several regional studies indicate that this parasite is common in both developing and developed countries and may infect up to 2.8 million individuals annually around the world (Hotez and Wilkins 2009; Lee et al. 2014). STH parasite species are globally distributed, including the Caribbean region.

The flatworm trematode *Schistosoma* causes schistosomiasis in humans and animals. A recent estimate suggests that at least 230 million persons globally are infected with *Schistosoma* (Colley et al. 2014) and 700 million people in >70 countries are at risk of *Schistosoma* infection (Steinmann et al. 2006). Schistosomiasis infections can lead to death if no treatment is given. In 2003, over 280,000 persons died of schistosomiasis in sub-Saharan Africa alone (van der Werf et al. 2003). There are mainly six *Schistosoma* species infecting humans in various regions defined by their respective intermediate host snail's habitat ranges, in which *S. mansoni* is the only species present in the Americas (Colley et al. 2014).

The above five parasitic diseases are listed among the neglected tropical diseases (NTDs) that mainly affect populations living in tropical and sub-tropical conditions (Hotez et al. 2014; Torgerson et al. 2014). Although the burden of NTDs in the Caribbean has already been recognized (Ault et al. 2012; Hotez et al. 2008), only a few national surveys on the prevalence and intensity of these neglected parasitic diseases have been performed in the region (Saboya et al. 2013). In fact, a recent review by the Pan American Health Organization/World Health Organization (PAHO/WHO) indicated that "there is still an important lack of data on prevalence and intensity of infection to determine the burden of disease based on epidemiological surveys, particularly among preschool age children. This situation is a challenge for the Latin America and Caribbean (LAC) given that adequate planning of interventions such as deworming requires information on prevalence to determine the frequency of needed anthelmintic drug administration and to conduct monitoring and evaluation of progress in drug coverage" (Saboya et al. 2013).

The Caribbean EcoHealth Programme (CEHP) provided an opportunity to collect more than 435 serum samples from pregnant women in ten English-speaking Caribbean countries (Forde et al. 2011). These samples were previously evaluated for the presence of chemical toxicants such as persistent organic pollutants, pesticides, heavy metals, and zoonotic infections (Dewailly et al. 2014; Forde et al. 2014a; Forde et al. 2014b; Forde et al. 2015; Wood et al. 2014). From these samples, the sera for five parasites were also evaluated, namely, *A. lumbricoides*, *E. histolytica*, *G. lamblia*, *S. mansoni*, and *T. canis*. This serological survey represents part of ongoing efforts to fill in the knowledge gap on the epidemiology of common parasitic infections in the Caribbean.

## Materials and methods

### Ethics, consent, and permissions

This study used archived serum samples collected between August 2008 and April 2011 from healthy delivering women (≥18 years old) from ten English-speaking Caribbean countries, for which the protocols were individually approved by the corresponding institutional review boards or ethics committees in the participating countries and the institutions of the principal investigators (Dewailly et al. 2014; Forde et al. 2014b; Wood et al. 2014). For this study, ethics approval was obtained from the Texas A&M University's Institutional Review Board (approval no. IRB2015-0554D). All participants received and signed a written consent form.

### Collection of serum samples

A total of 442 specimens were originally collected from pregnant women in Antigua-Barbuda, Belize, Bermuda, Dominica, Grenada, Jamaica, Montserrat, St. Kitts-Nevis, St. Lucia, and St. Vincent-Grenadines using recruitment and sampling protocols based on the Arctic Monitoring and

Assessment Programme (AMAP) (http://www.amap.no), which is described in greater details elsewhere (Forde et al. 2011; Van Oostdam et al. 2004). Specimens (up to 50 from each country) were initially processed and stored at Ross University School of Veterinary Medicine in St. Kitts. During the sample collection, participants were asked to voluntarily answer a questionnaire consisting of 27 potential risk factors such age, occupation, involvement of animal care/contact (yes/no), and rat infestation at home or work (yes/no). For this study, aliquots of 435 specimens were sent to Texas A&M University for serological analysis of parasitic infections. In the associated data sheets, protected health information was removed (i.e., de-identified) in accordance with the US Health Insurance Portability and Accountability Act Privacy Rule.

### Antibody detection and optical density data analysis

Commercial Enzyme-Linked Immunosorbent Assay (ELISA) Kits were used to detect IgG antibodies against *A. lumbricoides* (catalog no. 40-521-475052), *E. histolytica* (no. 40-521-475086), *S. mansoni* (no. 40-521-475124), *T. canis* (no. 40-521-475130), and *G. lamblia* (no. KTR-846). The ELISA Kit for *G. lamblia* was purchased from Epitope Diagnostics (San Diego, CA), while the other four kits were purchased from GenWay Biotech (San Diego, CA). All kits were in 96-well format. Each detection used 1.0-μL serum sample diluted by ×100 with diluent provided in the ELISA Kits and following the procedures recommended by the manufacturers. The optical density values at 450 nm ($OD_{450}$) were read in a Multiskan spectrophotometer (Thermo Scientific Inc., MA). In all experiments, each ELISA plate included one substrate blank, one negative control, two cutoff controls, and one positive control. For *G. lamblia*, IgG concentrations were calculated according to the intra-assay standard curves (positive >10 U/mL, uncertain (gray zone) 5–10 U/mL, negative <5 U/mL). For the other four parasites, a sample was considered as serologically positive, uncertain (gray zone), or negative if the mean absorbance value was >110, between 90 and 110, or <90 %, respectively, of the cutoff controls.

### Statistical analysis

For each parasite, prevalence estimates were compared between countries using Fisher's exact test as an overall test and for all the two-way comparisons. The *p* values for the two-way comparisons were adjusted for multiple comparisons using the Benjamini-Horcheburg false discovery rate method. To evaluate serological co-positivity, data from one parasite were arbitrarily designated as the outcome and those from another one were arbitrarily designated as the predictor. The association between each outcome-predictor pair was modeled using the SAS procedure "proc surveylogistic." In addition to the outcome and predictor, the model specified country as a cluster effect. All possible pairs between the five parasites were examined. Potential risk factors included participant's age, occupation, cared for animals (yes vs. no), and the presence of rats at home (yes vs. no). A normal probability plot showed that age followed an approximately normal distribution. To test the association between age and serology results for each of the parasites, groups of serology findings (positive, negative, and unclear) were compared using mixed-model analysis of variance. The linear model specified age as the outcome, parasite as a fixed effect, and country identification as a random effect to adjust for clusters of observations within a country. For the categorical risk factors, associations between each factor and each parasite were tested using Mantel-Haenszel chi-square with country as a blocking factor.

Correlation between the seroprevalence and gross domestic product (GDP) per capita by country was also evaluated by Pearson's correlation coefficient test. The GDP on purchasing power parity (PPP) data for nine countries were obtained from the World Bank database (http://data.worldbank.org/indicator/NY.GDP.PCAP.PP.CD). The GDP-PPP data are more useful than regular GDP per capita in comparing living standards between nations (Goossens et al. 2007). We averaged the GDP-PPP values from 2008 to 2011 when samples were collected. The GDP data for Montserrat were unavailable at the World Bank and separately obtained from the Central Intelligence Agency's World Factbook that was estimated in 2006 (https://www.cia.gov/library/publications/the-world-factbook). All GDP-PPP data were expressed in international dollars (Int$). In all tests, statistical significance was set to $p < 0.05$. Analyses were performed using SAS v9.4 (Cary, NC) or GraphPad Prism v5.0f (La Jolla, CA).

## Results

### Overall seroprevalence profiles for the five parasites

A total of 435 serum samples were acquired from pregnant women residing in the following ten English speaking Caribbean countries: Antigua-Barbuda, Belize, Bermuda, Dominica, Grenada, Jamaica, Montserrat, St. Kitts-Nevis, St. Lucia, and St. Vincent-Grenadines (Table 1, Fig. 1, and Supplemental Tables S1 and S2). IgG levels were detected against five parasites by indirect ELISA. Approximately two thirds of all tested samples were seropositive to at least one parasite (i.e., 66.2 ± 4.5 % overall seroprevalence) (Table 1 and Fig. 2). The seroprevalence was the lowest in Bermuda (34.0 ± 13.1 %) but ranged much higher in the other nine countries (61.4 ± 12.8 to 84.0 ± 10.2 %) (Table 1). Of the five parasites, *G. lamblia* (40.5 ± 4.6 %) and *A. lumbricoides* (37.9 ± 4.6 %) had the highest overall seropositive rates and were detected in all ten countries. The next most prevalent parasite infection was *T. canis* (14.5 ± 3.3 %

**Table 1** Seroprevalence of five parasitic infections in pregnant women from ten Caribbean countries as determined by IgG ELISA

| Country | Overall | | Seroprevalence by parasite, *N* (% ± 95 % CI) | | | | |
|---|---|---|---|---|---|---|---|
| | Sample *N* | Positive *N* (% ± 95 % CI) | *Ascaris lumbricoides* | *Entamoeba histolytica* | *Giardia lamblia* | *Schistosoma mansoni* | *Toxocara canis* |
| Antigua-Barbuda | 39 | 25 (64.1 ± 15.1) | 7 (17.9 ± 12.0) | 0 | *17 (43.6 ± 15.6)* | 2 (5.1 ± 6.9) | 5 (12.8 ± 10.5) |
| Belize | 49 | 37 (75.5 ± 12.0) | *25 (51.0 ± 14.0)* | 0 | 23 (46.9 ± 14.0) | 4 (8.2 ± 7.7) | 14 (28.6 ± 12.6) |
| Bermuda | 50 | 17 (34.0 ± 13.1) | 3 (6.0 ± 6.6) | 0 | *14 (28.0 ± 12.4)* | 0 | 0 |
| Dominica | 50 | 35 (70.0 ± 12.7) | *23 (46.0 ± 13.8)* | 4 (8.0 ± 7.5) | *23 (46.0 ± 13.8)* | 1 (2.0 ± 3.9) | 7 (14.0 ± 9.6) |
| Grenada | 46 | 31 (67.4 ± 13.5) | *26 (56.5 ± 14.3)* | 15 (32.6 ± 13.5) | 12 (26.1 ± 12.7) | 0 | 7 (15.2 ± 10.4) |
| Jamaica | 47 | 34 (72.3 ± 12.8) | 17 (36.2 ± 13.7) | 1 (2.1 ± 4.1) | *20 (42.6 ± 14.1)* | 1 (2.1 ± 4.1) | 3 (6.4 ± 7.0) |
| Montserrat | 15 | 10 (66.7 ± 23.9) | 5 (33.3 ± 23.9) | 0 | *6 (40.0 ± 24.8)* | 0 | 2 (13.3 ± 17.2) |
| St. Kitts-Nevis | 44 | 27 (61.4 ± 12.8) | 11 (25.0 ± 12.8) | 3 (6.8 ± 7.4) | *25 (56.8 ± 14.6)* | 0 | 5 (11.4 ± 9.4) |
| St. Lucia | 45 | 30 (66.7 ± 13.8) | *16 (35.6 ± 14.0)* | 5 (11.1 ± 9.2) | *16 (35.6 ± 14.0)* | 5 (11.1 ± 9.2) | 4 (8.9 ± 8.3) |
| St. Vincent-Grenadines | 50 | 42 (84.0 ± 10.2) | *32 (64.0 ± 13.3)* | 1 (2.0 ± 3.9) | 20 (40.0 ± 13.6) | 0 | 16 (32.0 ± 12.9) |
| Total | 435 | 288 (66.2 ± 4.5 ) | 165 (37.9 ± 4.6) | 29 (6.7 ± 2.3) | *176 (40.5 ± 4.6)* | 13 (3.0 ± 1.6) | 63 (14.5 ± 3.3) |
| *p*-value (by country)[a] | | <0.001 | <0.001 | <0.001 | 0.089 | 0.013 | <0.001 |

Values in italic indicate the most prevalent parasite infections in individual countries

[a] *p* values by country were calculated by Fisher's exact test

overall rate, which was detected in nine of the ten sampled countries). *E. histolytica* (6.7 ± 42.3 %) was detected in six countries and *S. mansoni* (3.0 ± 1.6 %) in five countries (Table 1 and Fig. 2). *G. lamblia* (ranging from 26.1 ± 12.7 to 56.8 ± 14.6 %) and *A. lumbricoides* (ranging from 6.0 ± 6.6 to 64.0 ± 13.3 %) were detected in all ten Caribbean countries, and dominated five and three, and tied in two countries, respectively (Table 1, highlighted in italic).

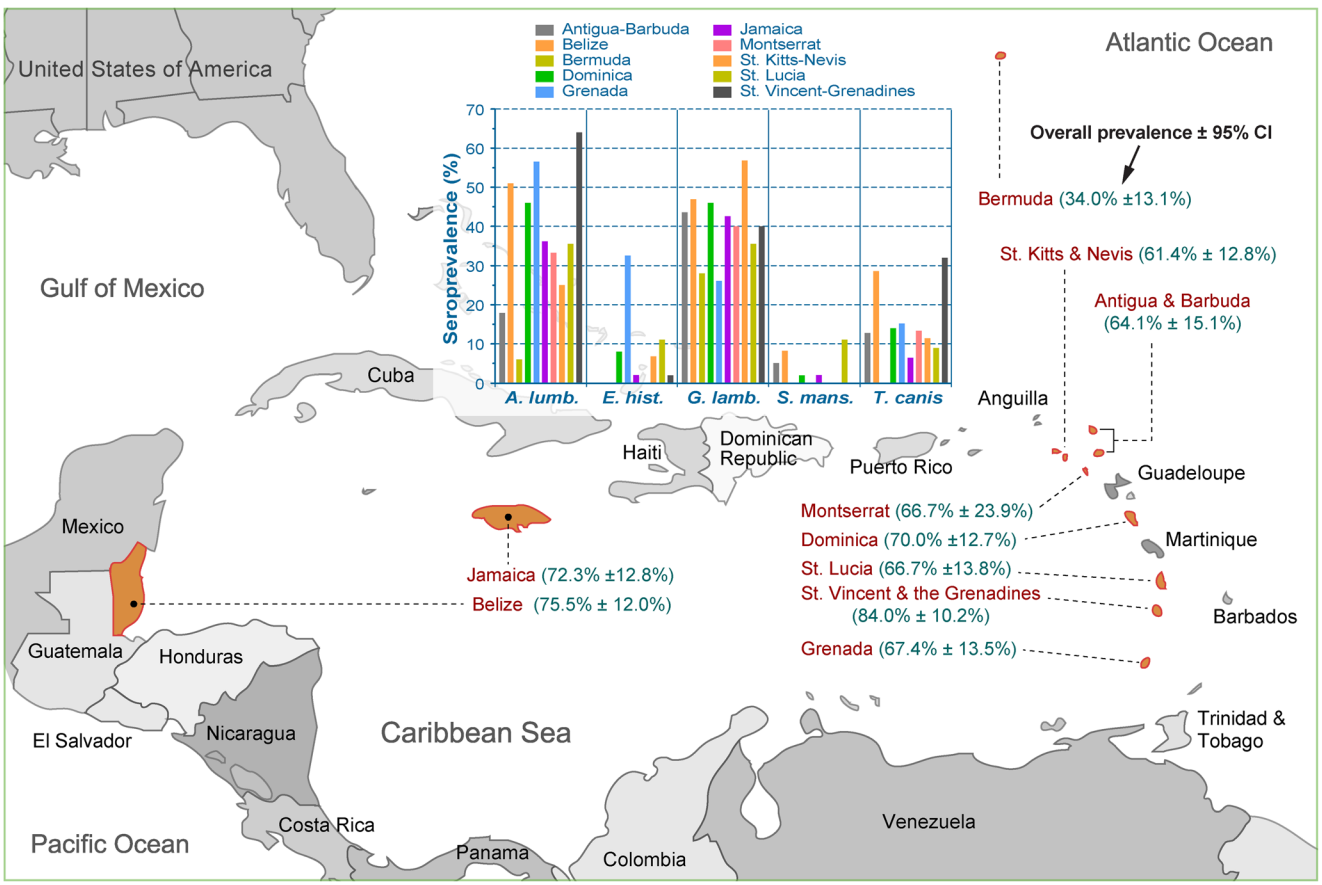


**Fig. 1** Regional map shows the overall seroprevalence of five parasite infections in the pregnant women from ten Caribbean countries. *Inset chart* shows the seroprevalence of individual parasites by countries

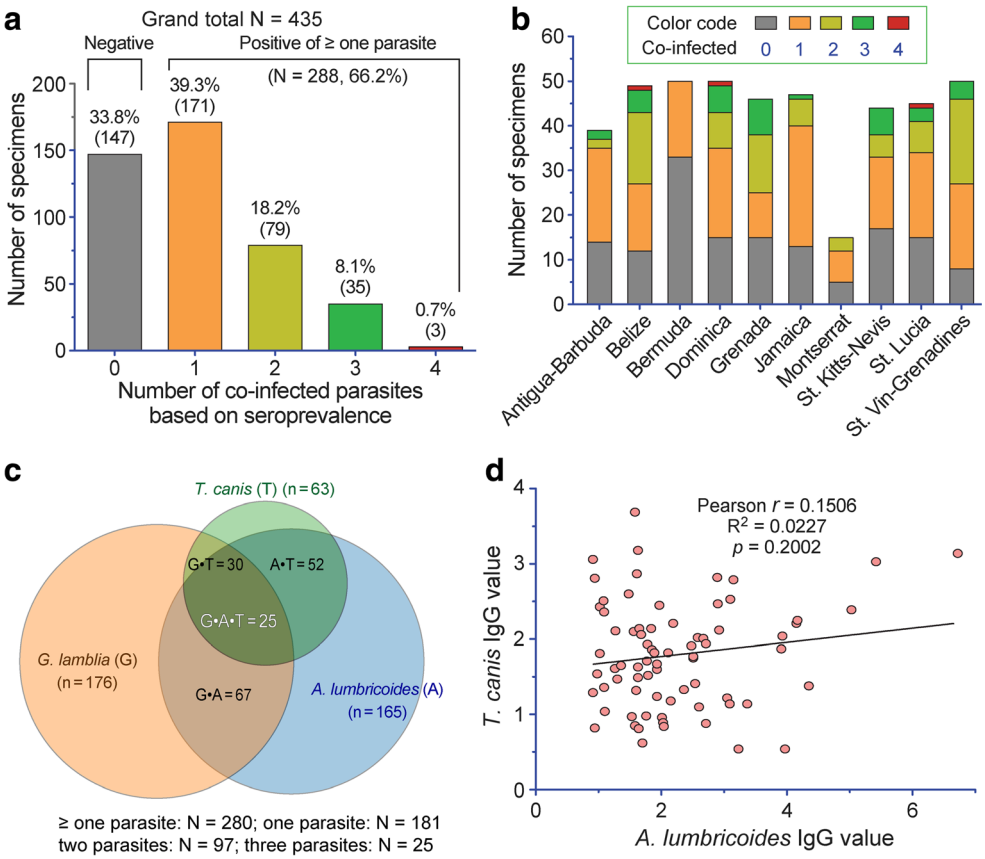


**Fig. 2** Seroprevalence and co-positivity of parasites in the pregnant women from ten Caribbean countries as determined by IgG ELISA. **a** Numbers of specimens that were negative to any of the five parasites or seropositive with one to four parasites in all 435 samples. **b** Numbers of specimens that were negative to any of the five parasites or seropositive with one to four parasites by country. **c** Proportional Venn diagram illustrating the co-positivity between *A. lumbricoides*, *G. lamblia*, and *T. canis*. **d** Correlation of the IgG levels between *A. lumbricoides* and *T. canis* in samples that were seropositive or uncertain (*gray zone*) to at least one parasite. The IgG levels were expressed as folds of corresponding cutoff controls. *p* values were calculated by Pearson's coefficient test

The overall seroprevalence profiles differed significantly between countries ($p < 0.001$ by Fisher's exact test) (Table 1). For individual parasites, significant difference was observed for *A. lumbricoides*, *E. histolytica*, and *T. canis* ($p < 0.001$) and for *S. mansoni* ($p = 0.013$) (Table 1). For *A. lumbricoides*, prevalence was highest in St. Vincent-Grenadines (64.0 %). This prevalence was significantly greater than that in Antigua-Barbuda, Bermuda, Jamaica, St. Kitts-Nevis, and St. Lucia but similar to those in Belize, Dominica, Grenada, and Montserrat (Table 2). The country with the lowest prevalence for *A. lumbricoides* was Bermuda (6.0 %). This prevalence was not significantly different from that in Antigua-Barbuda, Montserrat, and St. Kitts-Nevis (Table 2). For *E. histolytica*, prevalence was highest in Grenada (32.6 %). This prevalence was significantly greater than that for the other nine countries (Table 2). The other nine countries were not significantly different from each other. For *T. canis*, the country with highest prevalence was St. Vincent-Grenadines (32.0 %). This prevalence was significantly greater than that for Bermuda, Jamaica, and St. Lucia (Table 2). The country with the lowest prevalence was Bermuda (0 %). This prevalence was not significantly different from the prevalence for Jamaica, Montserrat, St. Kitts-Nevis, and St. Lucia. Significance associated with *S. mansoni* ($p = 0.013$; Table 1) disappeared after multiple comparisons (see Table 2). Prevalence of *G. lamblia* was not significantly different between countries ($p = 0.089$).

## Association analyses of specimens that were seropositive for multiple parasites

Among the 288 (66.2 %) seropositive samples, 171 (39.3 %), 79 (18.2 %), 35 (8.1 %), and 3 (0.7 %) specimens were seropositive of IgG antibodies for one to four parasites, respectively (Fig. 2a, b), indicating that either co-infections or sequential infections were common in the pregnant women populations. Serological co-positivity with two parasites were more common than those co-positive with three or four parasites and present in all ten countries (Fig. 2a). *G. lamblia* ($n = 176$) and *A. lumbricoides* ($n = 165$) were the top two parasites in this survey. However, co-positivity for these two parasites appeared to be relatively low (i.e., $n = 67$, or 24.5 %, out of the total 274 samples that were seropositive for at least one of the two parasites) (Fig. 2c). The lack of significant association of co-positivity by these two parasites was also supported by statistical analysis ($p = 0.1483$) (Table 3).

A statistically significant positive association was observed between the two roundworms *A. lumbricoides* and *T. canis* ($p < 0.0001$, odds ratio (OR) = 13.7) (Table 3). It is noticeable that the majority of the *T. canis*-seropositive samples ($n = 63$) were also positive for *A. lumbricoides* ($n = 52$, 82.5 %) (Table 3 and Fig. 2c). However, there was a much lower rate of *T. canis* in the *A. lumbricoides*-positive samples (i.e., 31.5 % co-positive with *T. canis*). A significant positive association was also

**Table 2** Two-way prevalence comparisons between countries within each parasite

| Parasite | Country | Prevalence (%) | Antigua-Barbuda | Belize | Bermuda | Dominica | Grenada | Jamaica | Montserrat | St. Kitts-Nevis | St. Lucia |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ascaris lumbricoides* | Antigua-Barbuda | 17.9 | | | | | | | | | |
| | Belize | 51.0 | *0.0104* | | | | | | | | |
| | Bermuda | 6.0 | 0.1233 | *0.0000* | | | | | | | |
| | Dominica | 46.0 | 0.0591 | 0.5875 | *0.0000* | | | | | | |
| | Grenada | 56.5 | *0.0045* | 0.5875 | *0.0000* | 0.6721 | | | | | |
| | Jamaica | 36.2 | 0.1971 | 0.4601 | *0.0032* | 0.4601 | 0.1556 | | | | |
| | Montserrat | 33.3 | 0.5400 | 0.5824 | 0.0638 | 0.5824 | 0.2582 | 1.0000 | | | |
| | St. Kitts-Nevis | 25.0 | 0.2264 | 0.0610 | 0.0833 | *0.0379* | *0.0045* | 0.5125 | 0.6458 | | |
| | St. Lucia | 35.6 | 0.1001 | 0.3498 | *0.0045* | 0.1854 | 0.0772 | 0.8799 | 1.0000 | 0.6014 | |
| | St. Vincent-Grenadines | 64.0 | *0.0000* | 0.1233 | *0.0000* | 0.2264 | 0.5875 | *0.0104* | 0.0653 | *0.0000* | *0.0034* |
| *Entamoeba histolytica* | Antigua-Barbuda | 0.0 | | | | | | | | | |
| | Belize | 0.0 | 1.0000 | | | | | | | | |
| | Bermuda | 0.0 | 1.0000 | 1.0000 | | | | | | | |
| | Dominica | 8.0 | 0.3140 | 0.1133 | 0.1008 | | | | | | |
| | Grenada | 32.6 | *0.0000* | *0.0000* | *0.0000* | *0.0208* | | | | | |
| | Jamaica | 2.1 | 1.0000 | 1.0000 | 1.0000 | 0.3766 | *0.0000* | | | | |
| | Montserrat | 0.0 | 1.0000 | 1.0000 | 1.0000 | 0.5744 | *0.0064* | 1.0000 | | | |
| | St. Kitts-Nevis | 6.8 | 0.5744 | 0.4569 | 0.4569 | 0.4774 | *0.0008* | 0.8156 | 1.0000 | | |
| | St. Lucia | 11.1 | 0.3759 | 0.1762 | 0.1762 | 0.9929 | *0.0235* | 0.3766 | 0.5744 | 0.7920 | |
| | St. Vincent-Grenadines | 2.0 | 1.0000 | 1.0000 | 1.0000 | 0.3766 | *0.0000* | 1.0000 | 1.0000 | 0.8156 | 0.3766 |
| *Giardia lamblia* | Antigua-Barbuda | 43.6 | | | | | | | | | |
| | Belize | 46.9 | 0.9676 | | | | | | | | |
| | Bermuda | 28.0 | 0.5757 | 0.4733 | | | | | | | |
| | Dominica | 46.0 | 0.9676 | 1.0000 | 0.5687 | | | | | | |
| | Grenada | 26.1 | 0.5757 | 0.4620 | 0.8434 | 0.4733 | | | | | |
| | Jamaica | 42.6 | 1.0000 | 0.9657 | 0.5757 | 0.9676 | 0.5687 | | | | |
| | Montserrat | 40.0 | 1.0000 | 0.9676 | 0.8738 | 0.9676 | 0.9443 | 1.0000 | | | |
| | St. Kitts-Nevis | 56.8 | 0.6865 | 0.7646 | 0.1440 | 0.6982 | 0.1440 | 0.5757 | 0.7580 | | |
| | St. Lucia | 35.6 | 0.8434 | 0.5757 | 0.5757 | 0.5757 | 0.9011 | 0.7646 | 1.0000 | 0.4733 | |
| | St. Vincent-Grenadines | 40.0 | 0.9676 | 0.8773 | 0.6897 | 0.9657 | 0.5757 | 0.9676 | 1.0000 | 0.5757 | 0.7580 |
| *Schistosoma mansoni* | Antigua-Barbuda | 5.1 | | | | | | | | | |
| | Belize | 8.2 | 1.0000 | | | | | | | | |
| | Bermuda | 0.0 | 0.5676 | 0.2403 | | | | | | | |
| | Dominica | 2.0 | 0.8820 | 0.4403 | 1.0000 | | | | | | |
| | Grenada | 0.0 | 0.5763 | 0.4403 | 0.7787 | 1.0000 | | | | | |
| | Jamaica | 2.1 | 0.8820 | 0.6995 | 0.7787 | 1.0000 | 1.0000 | | | | |
| | Montserrat | 0.0 | 1.0000 | 0.9675 | 0.7787 | 1.0000 | 1.0000 | 1.0000 | | | |
| | St. Kitts-Nevis | 0.0 | 0.5763 | 0.4403 | 0.7787 | 1.0000 | 1.0000 | 1.0000 | 0.7787 | | |
| | St. Lucia | 11.1 | 0.7787 | 1.0000 | 0.2403 | 0.4403 | 0.3023 | 0.4403 | 0.7155 | 0.3128 | |
| | St. Vincent-Grenadines | 0.0 | 0.5676 | 0.2403 | 0.2403 | 1.0000 | 0.7787 | 0.7787 | 0.7155 | 0.3128 | 0.2403 |

**Table 2** (continued)

| Parasite | Country | Prevalence (%) | Antigua-Barbuda | Belize | Bermuda | Dominica | Grenada | Jamaica | Montserrat | St. Kitts-Nevis | St. Lucia |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Toxocara canis* | Antigua-Barbuda | 12.8 | | | | | | | | | |
| | Belize | 28.6 | 0.2847 | | | | | | | | |
| | Bermuda | 0.0 | *0.0360* | *0.0000* | | | | | | | |
| | Dominica | 14.0 | 1.0000 | 0.4228 | *0.0135* | | | | | | |
| | Grenada | 15.2 | 1.0000 | 0.4584 | *0.0120* | 1.0000 | | | | | |
| | Jamaica | 6.4 | 0.9494 | *0.0315* | 0.1288 | 0.5478 | 0.5807 | | | | |
| | Montserrat | 13.3 | 1.0000 | 0.5807 | 0.1288 | 1.0000 | 1.0000 | 0.9494 | | | |
| | St. Kitts-Nevis | 11.4 | 0.9494 | 0.0756 | 0.0791 | 0.5807 | 0.7001 | 0.7388 | 1.0000 | | |
| | St. Lucia | 8.9 | 1.0000 | 0.0756 | 0.0791 | 0.7644 | 0.8299 | 1.0000 | 0.9494 | 1.0000 | |
| | St. Vincent-Grenadines | 32.0 | 0.1288 | 0.5516 | *0.0000* | 0.0842 | 0.1288 | *0.0162* | 0.4318 | 0.0842 | *0.0444* |

*p* values were generated using Fisher's exact test and adjusted for multiple comparisons using the Benjamini-Horcheburg false discovery rate method. Values in italic indicate *p* values ≤0.05

**Table 3** Association analysis of serological co-positivity by any two parasites in the pregnant women from ten Caribbean countries

| Outcome parasite | Predictor parasite | | Outcome serology | | Odds ratio (95 % CI) | *p* value[a] |
|---|---|---|---|---|---|---|
| | | +/− | Positive *n* (%) | Negative *n* (%) | | |
| *Ascaris lumbricoides* | *Entamoeba histolytica* | + | 19 (70.4) | 8 (29.6) | 3.8 (1.6–8.9) | 0.0018** |
| | | − | 131 (38.3) | 211 (61.7) | | |
| | *Giardia lamblia* | + | 67 (43.2) | 88 (56.8) | 0.4 (0.1–1.4) | 0.1483 |
| | | − | 2 (66.7) | 1 (33.3) | | |
| | *Schistosoma mansoni* | + | 7 (63.6) | 4 (36.4) | 2.5 (0.6–9.6) | 0.1863 |
| | | − | 157 (41.3) | 223 (58.7) | | |
| | *Toxocara canis* | + | 52 (86.7) | 8 (13.3) | 13.7 (5.4–34.4) | <0.0001*** |
| | | − | 104 (32.2) | 219 (67.8) | | |
| *Entamoeba histolytica* | *Giardia lamblia* | + | 14 (8.3) | 154 (91.7) | N/A | N/A |
| | | − | 0 (0.0) | 3 (100.0) | | |
| | *Schistosoma mansoni* | + | 3 (25.0) | 9 (75.0) | 4.9 (1.1–22.3) | 0.0394* |
| | | − | 25 (6.4) | 368 (93.6) | | |
| | *Toxocara canis* | + | 1 (1.7) | 58 (98.3) | 0.2 (0.1–0.7) | 0.0093** |
| | | − | 26 (7.7) | 313 (92.3) | | |
| *Toxocara canis* | *Giardia lamblia* | + | 30 (17.6) | 140 (82.4) | 0.6 (0.1–4.1) | 0.6381 |
| | | − | 1 (25.0) | 3 (75.0) | | |
| | *Schistosoma mansoni* | + | 3 (27.3) | 8 (72.7) | 2.2 (0.7–7.3) | 0.1811 |
| | | − | 59 (14.4) | 352 (85.6) | | |
| *Schistosoma mansoni* | *Giardia lamblia* | + | 6 (3.4) | 168 (96.6) | N/A | N/A |
| | | − | 0 (0.0) | 4 (100.0) | | |

Serologically uncertain (gray zone) samples were excluded from the analysis

*N/A* not available

[a] *p* values were determined by logistic regression (SAS proc surveylogistic)

*$p < 0.05$

**$p < 0.01$

***$p < 0.001$

observed between *A. lumbricoides* and *E. histolytica* ($p = 0.0018$, OR = 3.8). Similarly, there were significant positive associations between *E. histolytica* and *S. mansoni* ($p < 0.05$, OR = 4.9) and negative association between *E. histolytica* and *T. canis* ($p < 0.01$, OR = 0.2) (Table 3).

Several studies have shown that some commercial ELISA Kits for the two roundworms might cross-react with each other (Kennedy et al. 1989; Nunes et al. 1997); however, the likelihood that this happened in this study is very low. Firstly, we can rule out that the *T. canis* kit cross-reacted with IgG against *A. lumbricoides* based on the much lower seroprevalence of *T. canis* (i.e., much higher seropositive rates for *T. canis* was expected if *T. canis* kit could falsely react with specimens containing IgG to *A. lumbricoides* only). The same criterion was unable to exclude the possibility that the kit for *A. lumbricoides* cross-reacted with specimens containing only *T. canis* IgG antibodies, because the low *T. canis* infection rates could be masked by the much higher rates of *A. lumbricoides*. On the other hand, if it was true, a significant correlation on the ELISA $OD_{450}$ levels between the two parasites would be expected (i.e., a sample with higher level of *T. canis* IgG antibodies would give a higher $OD_{450}$ value by *A. lumbricoides* IgG kit). To evaluate this possibility, the Pearson's coefficient test was conducted on *T. canis*-positive specimens plus those in the gray zone ($n = 74$), in which we observed an apparent lack of correction (Pearson's $r = 0.1506$, $R^2 = 0.0227$, $p = 0.2002$) (Fig. 2d). Although one could not fully exclude the possibility of a certain level of cross-reaction between the two parasites (and among the other parasites as well), our analysis concluded that the detections were specific and the observed co-positivity were mostly true.

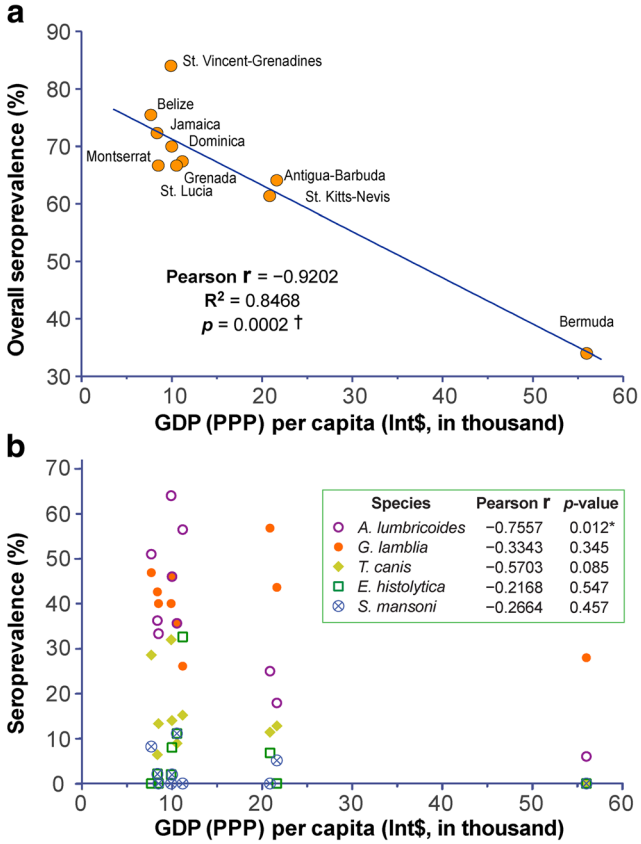


**Fig. 3** Negative correlation between gross domestic product on purchasing power parity (GDP-PPP) in international dollars (Int$) and seroprevalence of parasitic infections in the pregnant women from ten Caribbean countries. **a** Correlation between GDP-PPP and the overall seroprevalence by country. **b** Correlation between GDP-PPP and individual parasitic infections by country. *p* values were calculated by Pearson's coefficient test. $^{*}p < 0.05$, $^{\dagger}p < 0.001$

### Potential risk factor analysis

Individual risk factors were collected through filling out questionnaires by participants, and only partial data were available for different risk factors including age ($n = 280$), occupation ($n = 262$), caring for animals ($n = 293$), and rats at home or work ($n = 294$). No significant associations were observed between any of these potential risk factors and the seroprevalence of each parasite (Supplemental Table S3). An association study was also performed between age and seroprevalence of individual parasites and resulted in no statistical significance between age and any of the five parasites (Supplemental Table S4).

On the other hand, we observed significant negative correlation between the levels of GDP-PPP per capita of the Caribbean countries and the overall seroprevalence of parasitic infections ($p = 0.0002$ and Pearson's coefficient $r = -0.9202$) (Fig. 3a). When individual parasites were analyzed, significant negative correlation was only present between GDP levels and seroprevalence of *Ascaris* ($p = 0.012$, $r = -0.7557$) (Fig. 3b). In this analysis, the average GDP-PPP per capita data between 2008 and 2011 were used because samples were collected in these years. The GDP-PPP per capita levels varied substantially among the ten Caribbean countries and could be roughly classified into three groups. The top group consisted of one country (Bermuda, Int$55,692). The middle group consisted of two countries (Antigua-Barbuda, Int$21,633 and St. Kitts-Nevis, Int$20,895), while the third group was comprised of the remaining seven countries with GDP-PPP per capita ranging from Int$11,182 (Grenada) to Int$7693 (Belize). The top group had the lowest overall infection rate and least number of detected parasites (34.0 ± 13.1 %, two parasites) that were much higher in the second (61.4 ± 12.8 to 64.1 ± 15.1 %, four parasites) and third (66.7 ± 23.9 to 84.0 ± 10.2 %, three to five parasites) groups (Fig. 3a and Table 1).

## Discussion

Parasites are the causative agents of many NTDs that are important to public health. However, there have been only

limited epidemiological surveys on the human parasites in the Caribbean region, particularly the lack of multi-national surveys on gut parasites. In fact, literature searches suggest that there is a lack of epidemiological data on gastrointestinal parasites in most Caribbean countries. Among the few published studies, a national retrospective survey in St. Lucia analyzed 10,508 stool sample records between 2002 and 2005 that were examined by direct smear method by hospitals and private testing laboratories and yielded an overall parasite prevalence of 26.1 % (Rajini and Hunjan 2010). The prevalence for individual parasites were *Strongyloides* (2.9 %), *A. lumbricoides* (2.5 %), *Trichuris trichiura* (2.5 %), *S. mansoni* (0.3 %), *Taenia* spp. (0.1 %), *Entamoeba coli* (5.6 %), *Endolimax nana* (4.1 %), *Iodamoeba butschli* (1.1 %), *E. histolytica/dispar/moshkovski* (1.1 %), *G. lamblia* (0.6 %), and *Entamoeba hartmanni* (0.2 %) (Rajini and Hunjan 2010). In another survey on school children in south St. Lucia, 61.6 % of the 554 participants were infected by at least one of the parasites, including *A. lumbricoides* (15.7 %), hookworm (11.9 %), *Strongyloides* (9.7 %), *T. trichiura* (4.7 %), *S. mansoni* (0.6 %), *Taenia solium* (0.8 %), *Enterobius vermicularis* (2.1 %), *E. coli* (9.7 %), *I. butschlii* (5 %), *E. histolytica* (1.1 %), *G. lamblia* (1.8 %), and *E. nana* (2.1 %) (Kurup and Hunjan 2010). A third survey identified in the literature studied the intestinal parasites in 672 participants from five villages in southern Belize, in which 66 % of the population were infected by intestinal parasites, including hookworm (55 %), *A. lumbricoides* (30 %), *E. coli* (21 %), *T. trichiura* (19 %), *G. lamblia* (12 %), *I. beutschlii* (9 %), and *E. histolytica/dispar* (6 %) (Aimpun and Hshieh 2004).

The present study appears to represent the first published multi-national survey on the seroprevalence of five neglected parasites in pregnant women residing in ten Caribbean countries. The overall prevalence of any parasitic infections was high (66.2 ± 4.5 %) but similar to those revealed in children in St. Lucia (61.6 %) and in the five villages of Belize (66 %) (Aimpun and Hshieh 2004; Kurup and Hunjan 2010). The relatively higher prevalence for the gut parasites in this study was likely due to the facts that (1) ELISA detection is more sensitive than direct microscopic examination of stool smears (Hawash 2014; Savioli et al. 2006) and (2) this study evaluated the IgG levels in sera, in which a positive detection might indicate either current or past infection. Nonetheless, these data clearly indicate that parasitic infections are a significant public health concern in pregnant women in the Caribbean region.

NTDs mainly affect socio-economically disadvantaged populations and nations (Choy et al. 2014; Hotez 2008; King 2010; Mendonca et al. 2012; Saboya et al. 2013). This study provides supporting evidence that living standards play an important role in the burdens of parasitic diseases given the strong negative correlation between the GDP-PPP data and overall seroprevalence values in the ten Caribbean countries (Fig. 3a). Ascariasis and giardiasis were the two most common infections; however, only the seroprevalence of ascariasis was significantly negatively correlated with living conditions ($p = 0.012$) (Fig. 3b). This is likely due to the fact that *A. lumbricoides* is mainly soil transmitted and subjected more to hygiene and living conditions, whereas the zoonotic *G. lamblia* is mainly a water- or food-borne parasite and can be present in drinking and/or recreational waters. While this geographical region or the socio-economic status may affect overall prevalence of *G. lamblia*, the presence of *Giardia* cysts in raw/untreated waters (one of the main transmission sources) had no substantial difference when compared between developed and developing countries (Nasser et al. 2012). It is true that *Giardia* infection is also common in developed countries, although generally at lower prevalence (Esch and Petersen 2013; Ortega and Adam 1997). For example, in the USA and territories, there were estimated 1.2–2 million (but up to 8 million) cases of giardiasis occurring annually (Yoder et al. 2007; Yoder et al. 2012; Yoder et al. 2010).

We also observed a significant association of infections between *T. canis* and *A. lumbricoides*. A similar association between these two roundworms was also reported in a study conducted in India (Ahmad et al. 2002). It is likely that these two parasites share a common mode of transmission that is influenced by human living conditions. Both *T. canis* and *A. lumbricoides* eggs need one to several weeks of development in soil to become infective, and this embryonation requires similar temperature and humidity conditions (Azam et al. 2012; Gamboa 2005; Kim et al. 2012). Because fresh eggs are not infective, direct contact with dogs (definitive host for *T. canis*) might play a less significant role in the acquisition of *T. canis* infection as described by others and revealed by association analysis here (Supplemental Table S3) (Deplazes et al. 2011; Keegan and Holland 2010).

The seroprevalence for *E. histolytica* (6.7 ± 2.3 %) and *S. mansoni* (3.0 ± 1.6 %) was generally low, except for those of *E. histolytica* in Grenada (32.6 ± 13.5 %) and *S. mansoni* in St. Lucia (11.1 ± 9.2 %) and Belize (8.2 ± 7.7 %). Schistosomiasis was historically endemic in the Caribbean islands but largely eliminated after the massive schistosomiasis control and elimination programs (e.g., in the mid-2003, the estimated prevalence in St. Lucia was <0.1 % and fully eliminated in Montserrat) (Gryseels et al. 2006; Rollinson et al. 2013). However, schistosomiasis is still endemic in the Caribbean (e.g., prevalence in St. Lucia in 2010 was <10 % as estimated by WHO) (Colley et al. 2014; Rollinson et al. 2013). An increased prevalence of schistosomiasis between 1998 and 2007 was also reported in St. Lucia (Schneider et al. 2011). Taken together, our results show that schistosomiasis remains a significant public health concern in at least some Caribbean countries, particularly in St. Lucia and Belize.

Finally, we recognized several limitations of this study. Among them, the first concerns the sample sizes. Although the original goal to collect 50 specimens from each country exceeded

that recommended by the AMAP protocol (i.e., 30 specimens per country) (Forde et al. 2011; Van Oostdam et al. 2004), the sample sizes were still relatively small, and the 50-specimen goal was not reached for all countries. In Montserrat where the population was slightly over 5000 (or more than ten times less than those of other countries in the study), only 15 samples were collected. On the other hand, the populations on most countries/islands were smaller than 100,000. Therefore, the sample sizes were representative to certain degree for providing a good snapshot on the epidemiology in pregnant women.

Another limitation was the method used in determining the IgG. These data only represent historical infections. Methods to detect IgM for each parasite need to be developed in the future to study acute infections. Finally, the questionnaires used in this study were not comprehensive enough to differentiate risks according to the different parasite exposures (e.g., proximity to freshwater and *S. mansoni* infection). Additionally, although we observed statistically significant negative correlation between income and seroprevalence, other factors such as parasite heterogeneity might also contribute to the different infections. Thus, future studies employing a more comprehensive questionnaire design, a larger sample size, information on medical assessments and clinical symptoms, and additional analytical techniques (e.g., detection of IgM antibodies and pathogen antigens) are needed in order to gather better epidemiologic data on the incidences and prevalence of these parasitic infections in the Caribbean.

## Conclusions

Parasites cause a significant proportion of neglected tropical diseases, but published data on the epidemiology of parasitic infections in the Caribbean countries is conspicuously limited. In this study, the seroprevalence of *A. lumbricoides*, *E. histolytica*, *G. lamblia*, *S. mansoni*, and *T. canis* in 435 pregnant women in ten English-speaking Caribbean countries between 2008 and 2011 was assessed. A high overall seroprevalence of 66.2 % was found for these Caribbean countries. While *G. lamblia* and *A. lumbricoides* were found to be the most prevalent parasites and detected in all ten countries, the profiles of parasitic diseases varied from one country to another. Further, living standards as assessed using the proxy measure of GDP-PPP per capita in countries showed a significant negative correlation. This study provides the necessary justification for additional comprehensive surveys on the burdens of parasitic diseases to be conducted in the Caribbean.

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