Assessment of fecal calprotectin and fecal occult blood as point-of-care markers for soil-transmitted helminth attributable intestinal morbidity in a case-control substudy conducted in Côte d’Ivoire, Lao PDR and Pemba Island, Tanzania

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ABSTRACT

Background: Infections with soil-transmitted helminths (STHs) may result in chronic inflammatory disorders affecting the human host. The objective of this study was to evaluate Fecal Calprotectin (FC) and Fecal Occult Blood (FOB) in individuals infected and non-infected with STHs to identify potential intestinal morbidity markers.

Methods: Stool from participants diagnosed positive for Trichuris trichiura and concomitant STH infections from three countries was used to perform FC and FOB point-of-care assays. Simultaneously, identified STH negative participants underwent FC and FOB testing as controls. Potential associations between test results and determinants were analyzed using multivariable logistic regression.

Findings: In total, 1034 T. trichiura infected cases (mostly light infections) and 157 STH negative controls were tested for FC and FOB. Among all participants tested, 18.5% had > 50 μg/g FC concentration, while 14 (1.2%) were positive for FOB. No statistically significant association was found between T. trichiura infection or Ascaris lumbricoides co-infection and FC concentration, while an inverse association (odds ratio (OR): 0.45, 95% credible intervals (CrI): 0.26, 0.75) was found between hookworm co-infection and FC concentration. In Lao PDR, the proportion of participants in the > 50 μg/g FC category was significantly higher in the oldest age category compared to the 5–11 years group (OR: 3.11, 95% CrI: 1.62, 7.24). Too few participants were found positive for FOB to derive any conclusions.

Interpretation: Studies are needed to better understand the relationship between intestinal morbidity and STH infections. Suitable, standardized, low-cost markers of STH attributable morbidity to better monitor the impact of STH control interventions are necessary.

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1. Introduction

More than 1-5 billion people worldwide are infected with soil-transmitted helminths (STHs), namely Ascaris lumbricoides, hookworm (Ancylostoma duodenale and Necator americanus), and Trichuris trichiura [1]. Helminthiases typically affect the poorest and the most marginalized populations, particularly in tropical and subtropical regions, where access to water, sanitation, and hygiene is inadequate [2,3]. STH infections manifest, if left untreated, as generally asymptomatic chronic infections causing both concurrent and delayed-onset pathologies affecting the human host [4]. Chronic infections and/or infections of heavy intensities can result in malnutrition, malabsorption, reduced growth rate, intestinal obstruction, poor iron status, and iron deficiency.

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Research in context

Evidence before this study

Long-term infections with soil-transmitted helminths (STHs) contribute to substantial morbidity; however, evidence on appropriate point-of-care indicators of STH attributable morbidity is sparse. We searched in PubMed for all articles published before Nov 2, 2020 which mentioned “helminth”, “Trichuris trichiura”, “Ascaris lumbricoides”, or “hookworm” and “fecal calprotectin” or “fecal occult blood” in the abstract, without language restrictions. Contradictory findings result from a small body of evidence (nine studies) on the association between STH infection status, infection intensity and intestinal morbidity, using Fecal Calprotectin (FC) or Fecal Occult Blood (FOB) as indicators. Only one study used novel rapid diagnostic immunoassay tests for FOB detection, while none had a sufficient sample size to derive meaningful conclusions.

Added value of this study

This is the first large scale study testing FC and FOB as potential proxy markers for STH attributable intestinal morbidity in three different countries. No association between the presence of intestinal inflammation or mucosal bleeding, assessed with FC and FOB, and T. trichiura and A. lumbricoides infection status was found, while a negative association between FC concentration and hookworm infection was found.

Implications of all the available evidence

Further research should focus on the development and evaluation of potential morbidity markers, as the appropriate monitoring of STH attributable morbidity might become as important as diagnosing the infection itself. Thus, appropriate indicators of helminth attributable morbidity are still lacking.

anemia [4–9], particularly in those on marginal diets [10]. Long-term consequences include subtle effects on cognition [11], educational performance [12], and school absenteeism [13], impacting individuals’ workforce potential, and economic progress among affected groups [14].

Embryonated T. trichiura eggs hatch in the small intestine and potentially attach to the mucosa in the large intestine, whereas larvae penetrate the epithelial cells for subsequent growing and molting into adult stages [15]. Adult worms embed their head part into a intracellular niche in the large intestine [16] causing petechial lesions, blotchy mucosal hemorrhage, and oozing leading to both mucosal and systemic immune responses [17–19]. Similarly, hookworm larvae use their cutting plates to attach to the mucosa to begin feeding and molting into adult worms, attributing to intestinal morbidity [20]. In contrast, A. lumbricoides feeds passively and never attaches directly to the mucosa [4,21].

The present goal of global control programs recommended by the World Health Organization (WHO), is to reduce morbidity with preventive chemotherapy (i.e. administration of albendazole or mebendazole without prior diagnosis) to at-risk populations (i.e. school-aged children and women of reproductive age) with accompanying improvements in access to clean water and sanitation to reduce worm burden associated morbidity [22]. Inherent with such control interventions is the necessity to define and validate indicators of helminth attributable morbidity. Even though in most cases morbidity is associated with infection intensity [23], assessed by classic microscopic diagnosis detecting eggs in the feces, appropriate morbidity parameters are of pivotal importance as they provide additional information on the degree to which the STH infection affects the patient. This is particularly important after anthelmintic treatment, when intensity of infection has reduced markedly, but morbidity still is present [24]. A better clinical understanding of measurable reductions in STH attributable morbidity in response to anthelmintics is needed to appropriately shape and evaluate ongoing or future STH control programs [25].

Fecal biomarkers are promising non-invasive indicators possibly reflecting mucosal inflammation or damage, as molecules from the intestinal mucosa are transported in passing with the feces [26]. It is a well-known phenomenon that the occurrence of blood in feces can be indicative of pathologic changes, especially in the context of malignancies or inflammation [27].

Fecal Calprotectin (FC), a neutrophil cytoplasmatic multimeric complex of the calcium-binding proteins, is abundant in neutrophil granulocytes, monocytes, and early stage macrophages [28,29]. Translocation of these cells into the intestinal mucosa and degranulation inside the intestinal lumen leads to increased secretion of FC as a response to local inflammation [30,31]. Given the fact that FC levels are stable in feces and not influenced by systemic infections, FC is an interesting biomarker for understanding the association between intestinal infection and inflammation by measuring the localized intestinal inflammation [19,32–34]. Therefore, FC is widely used across gastroenterology practices as a non-invasive surrogate marker for disease activity and response to treatment [35,36]. In the past years, its use in enteric infections is increasing, particularly as a correlative marker for clinical severity and in evaluating bacterial and viral pathogens [37].

Fecal blood is a late symptom of inflammatory tissue damage [30]. For example, blood loss due to T. trichiura infections has been estimated to be 5 μL per adult worm per day [38]. Thus, fecal occult blood (FOB) might be another candidate for a morbidity marker of helminthiasis. FOB tests have been previously used for identifying blood loss in hookworm infection, trichuriasis, and intestinal schistosomiasis [39–42]. However, most studies used guaiac-based FOB tests that are known to be less sensitive than immunochemical assays [43].

Rapid immunological FC and FOB dipstick tests are non-invasive, easily preserved, and reliable diagnostic tools allowing immediate detection of FC for diagnosing intestinal inflammation and FOB for occult blood in the feces indicating intestinal morbidity [44]. Characteristics and applicability of field-appropriate diagnostic tools using proxy markers for STH morbidity are not well investigated to date [42]. Moreover, previous work on morbidity indicators is mostly limited to research on schistosomiasis causing gastrointestinal morbidity [41–43,45–47]. Although, some case reports suggest mucosal damage as a consequence of STH infections [48,49], evidence on the association between infection and intestinal inflammation is lacking [8].

The objective of the current study was to investigate FC and FOB as potential STH gut morbidity markers as a tool to monitor the impact of community-level deworming. Using FC and FOB as biomarkers, we aimed at assessing the potential association between the presence of intestinal inflammation and STH infection status.

2. Methods

2.1. Study design

The presented data derive from a randomized controlled trial (RCT) assessing efficacies of ivermectin-albendazole and albendazole alone against T. trichiura and concomitant STH infections in participants aged 6–60 years. The study was conducted in communities in the Lagunes region in Côte d’Ivoire, in the Luang Prabang Province in Lao PDR, and in South Pemba on Pemba Island, Tanzania during the screening period (Nov 2018 to Dec 2019) of the trial. Prior to study initiation, ethical clearance was granted by the Ethics Committee of Northwestern and Central Switzerland (EKNZ; reference no.: BASEC Nr Req-2018–00494), the Zanzibar Medical Research and Ethics Committee (ZAMREC, reference no.: ZAMREC/0003/Febr/2018), the Comité National d’Éthique et de la Recherche, Ministère de la Santé et de Lutte contre le SIDA (reference no.: 088–18/MSHP/CNESVS-
thorchis viverrini infection and infections of A. lumbricoides, and hookworm) within 60 min after preparation to avoid over-clearing of hookworm eggs [51] following the World Health Organization (WHO) standard procedures [52]. Additionally, in Lao PDR, Kato-Katz thick smear slides were examined for Opisthorchis viverrini infection and infections of Strongyloides stercoralis were classified as larvae-positive or negative using the Baermann technique [53]. To assure high quality of the microscopic evaluation, 10% of all Kato-Katz slides were randomly chosen, re-labeled, and re-examined for A. lumbricoideaes and T. trichiura. In brief, microscopic results were considered inconsistent if there was a difference in presence/absence of a specific helminth species, or if differences in egg counts exceeded (i) 10 eggs for Kato-Katz thick smears with ≤100 eggs, or (ii) exceeded 20% for Kato-Katz thick smears with >100 eggs. In case of discrepancies between the original and the quality control read, slides were read by a third independent microscopist and results were discussed until consensus was reached [54].

Of those found positive for T. trichiura, a random subsample of unique ID numbers (generated by a co-investigator not involved in the laboratory work) were chosen to undergo FC and FOB testing the same day. At the same time, of those participants who were found negative for T. trichiura, A. lumbricoidees, and hookworm infections were not included in this study. All included participants donated two fecal samples in order to increase the precision of the STH infection result. On Pemba Island, the first of the two fecal sample was used for subsequent rapid diagnostic testing (RDT). Due to the low prevalence of T. trichiura infections in the communities chosen in Lao PDR and Côte d’Ivoire, it was decided to use the second donated stool sample to ensure participants in this study would also be included in the larger trial, which had a minimum infection intensity cut-off of 100 T. trichiura eggs per gram of stool.

Cases that were subsequently enrolled in the clinical trial, were clinically examined by a physician and their height, weight, and hemoglobin levels recorded. However, negative controls, whom were not eligible for trial inclusion, did not undergo clinical examination.
2.3. Point-of-care tests: fecal calprotectin and fecal occult blood

A semi-quantitative chromatographic immunoassay (Actim® Fecal Calprotectin test, Medix Biochemica, Finland) was applied for FC detection according to the manufacturer’s instructions. In brief, the participant number was first written on the Specimen Dilution Buffer tube (3 mL) before unscrewing. The sampling stick, attached to the cap, was first twisted into different places of the fecal sample in order to make sure that both slits at the head of the stick contained stool. The sampling stick was put back in the tube and shaken to suspend the feces in the buffer. A dipstick was then inserted into the perforation area of the buffer tube before inverting the tube by 90° for two seconds. The tube was then placed on a flat surface in an upright position. Results were read after ten minutes with one test line indicating a valid test result with a FC level < 50 μg/g, two lines indicating a level of 50–200 μg/g, and three lines indicating a level > 200 μg/g of FC concentration in the test specimen based on reference values provided by the manufacturer. FC levels < 50 μg/g were interpreted as no inflammation, 50–200 μg/g as possible inflammation and > 200 μg/g of FC as likely active inflammation, similar to suggestions by Bressler et al [55].

Fig. 2. Prevalence of ≥ 50 μg/g FC concentration by EPG for Trichiura trichiura infections, Ascaris lumbricoides, and hookworm co-infections, and, in addition, by country (middle row), and age group (bottom row).

Abbreviations: A. lumbricoides, Ascaris lumbricoides; EPG, eggs per gram; FC, fecal calprotectin; T. trichiura, Trichuris trichiura.
A simple immunochemical test was applied for FOB (Actim® Fecal Blood test, Medix Biochemica, Finland) detection, following the manufacturer’s instructions. Unlike traditional guaiac tests, this test is based on highly specific monoclonal antibodies that only detect human hemoglobin, thus, food substances containing hemoglobin or peroxidase activity do not influence test results. Test procedure was similar to the aforementioned FC test, however, positive results were readable immediately as soon as two blue lines appeared, negative samples remained with only one blue line after ten minutes. Detailed test characteristics (e.g., sensitivity and specificity) are summarized by the manufacturer and published online [56].

Fecal rapid tests were conducted by laboratory personnel not involved in assessing parasitological data and masked to the identity of participants providing samples. The results from each test were then recorded on a personal log form by the technician involved in the fecal rapid diagnostic testing. If no control line appeared, the result was recorded as invalid and was repeated with a new dipstick.

### 2.4. Outcomes

The objective of this study was to evaluate FC and FOB as potential STH gut morbidity markers. Adjusted odds ratios of FC and FOB values were calculated for STH infected participants compared to STH negative controls.

### 2.5. Sample size

We aimed for a total sample size of 1050: 300 T. trichiura positive individuals and 50 individuals negative for all STH infections per country. This study was conducted within the framework of a RCT; therefore, no separate sample size calculation has been conducted. Since prevalence of FC and FOB is unlikely very rare and varies drastically by setting, a sample size of above 1000 was deemed to be sufficient. Moreover, experience has shown that the study would be sufficiently powered unless the outcome or the exposure is rare. We estimated we would be able to detect a halving of risk with at least 80% power if the prevalence in the non-exposed group is at least 10% or higher.

### 2.6. Statistical analysis

Data were double entered into Epilinfo 3.5.4 by two independent data clerks and crosschecked using the data compare tool of Epilinfo 3.5.4 Any discrepancies between the two datasets were resolved by consulting the original hardcopy. Descriptive analysis was done in Stata IC 15 (StataCorp.; College Station, TX), whereas all statistical estimations were performed in R 3.5.1 (RStudio, PBC, Boston, MA). Proportions of infection with T. trichiura and co-infections with A. lumbricoides and/or hookworm were assessed for all participants in all three settings, while for Lao PDR O. viverrini and S. stercoralis co-infections were additionally assessed.

Results from the duplicate Kato-Katz smears from each of the two stool samples were summed and multiplied by a factor of six to be expressed as mean egg count per gram (EPG) of feces. Associations between FC with participant’s age, sex, country, and parasitic infections was assessed using Bayesian logistic regression with logit link to estimate odds ratios (ORs) with corresponding 95% credible intervals (CrI). The Bayesian framework - as implemented in the R package ‘rstanarm’ (v 2.19.3) - was preferred to avoid potential quasi separation problems. We used the default weakly informative prior distributions for all parameters, i.e. normal priors with mean 0 and standard deviation 2.5. The Markov chain Monte Carlo algorithm drew 1000 samples from 4 chains after a warm-up of additional 1000 samples per chain. Convergence was assessed by the R-hat statistic. Smoothing lines in figures were predicted via generalized additive models for binomial data. Due to the low number of individuals (n = 141189) testing positive for FOB, it was decided to forego formal statistical testing and present the results descriptively.

### 2.7. Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

### 3. Results

#### 3.1. Participants characteristics

![Fig. 1](image-url) shows the study design by setting. Out of the 6567 participants screened, 2983 (454%) were positive for T. trichiura infection and 3584 (54.6%) were negative for all STH infections. From these, 1034 of the 2983 T. trichiura positive cases and 157 of the STH negative cases were randomly selected for FC and FOB testing.

Baseline parasitological and demographic characteristics of participants surveyed for FC and FOB are summarized in Table 1. Of those participants infected with T. trichiura (n = 1034), 51.5% were female, while the mean age of those was 18.7 (±15.0) years. The identified STH negative participants (n = 157) were slightly older with a mean age of 30.1 (±16.2), while 66.2% of those participants were female.

### Table 1: Baseline characteristics of T. trichiura positive cases and STH negative controls.

| Characteristic                        | T. trichiura positive, n (%) | STH negative, n (%) |
|---------------------------------------|------------------------------|---------------------|
| Total of participants included, N. (%) | 1034 (86.8)                  | 157 (13.2)          |
| Females, n (%)                        | 533 (51.5)                   | 104 (66.2)          |
| Mean age, years (SD)                  | 18.7 (15.0)                  | 30.1 (16.2)         |
| Mean BMI, kg/m² (SD)                  | 18.1 (4.6)                   | NA                  |
| Mean hemoglobin, g/L (SD)             | 124.6 (14.5)                 | NA                  |
| T. trichiura infection                |                              |                     |
| Geometric mean EPG                    | 492.0                        |                     |
| Light                                 | 784 (75.8)                   |                     |
| Moderate                              | 240 (23.2)                   |                     |
| Heavy                                 | 10 (1.0)                     |                     |
| A. lumbricoides co-infection          |                              |                     |
| Infected, n (%)                       | 345 (33.3)                   | NA                  |
| Geometric mean EPG                    | 4317.9                       |                     |
| Light                                 | 167 (48.4)                   |                     |
| Moderate                              | 150 (43.5)                   |                     |
| Heavy                                 | 28 (8.1)                     |                     |
| Hookworm co-infection                 |                              |                     |
| Infected, n (%)                       | 338 (32.7)                   | NA                  |
| Geometric mean EPG                    | 559.4                        |                     |
| Light                                 | 253 (74.9)                   |                     |
| Moderate                              | 49 (14.5)                    |                     |
| Heavy                                 | 36 (10.7)                    |                     |
| S. stercoralis co-infection           |                              |                     |
| Infected/Surveyed, n (%)              | 49/277 (17.7)                | NA                  |
| O. viverrini co-infection             |                              |                     |
| Infected, n (%)                       | 54/300 (18.0)                | NA                  |

Abbreviations: A. lumbricoides, Ascaris lumbricoides; BMI, body mass index; EPG, eggs per gram; NA, not applicable; O. viverrini, Opisthorchis viverrini; SD, standard deviation; S. stercoralis, Strongyloides stercoralis; T. trichiura, Trichuris trichiura.

a Baseline characteristics were assessed for 1191 participants surveyed for FC. Additionally, 2 participants missed FOB testing, however, baseline characteristics between FC and FOB are thus very similar.

b BMI and hemoglobin was only assessed in participants attending subsequent clinical examination.

c Infection intensities were classified according to WHO recommendations, based on guidelines established by Montresor et al. (1998).

d O. viverrini and S. stercoralis co-infections were only assessed in Lao PDR.
In addition to *T. trichiura* infection, 345 (33.3%) participants were co-infected with *A. lumbricoides*, while 338 (32.7%) participants harbored a hookworm co-infection. Among those participants in Lao PDR, 18.0% were found to be co-infected with *O. viverrini* and 17.7% with *S. stercoralis*. Mean body mass index (BMI) and mean hemoglobin concentration were 18.1 (±4.6) kg/m² and 124.6 (±14.5) g/L, respectively.

Medium EPGs were 402, 5508, and 759 for *T. trichiura* infections and *A. lumbricoides* and hookworm co-infections, respectively (Table 2). Table 3 shows that the EPG distribution differs among settings with Pemba Island, having a higher median EPG (558, IQR 330–2000) than Côte d’Ivoire (median EPG: 408, IQR 198–1068), and Lao PDR (median EPG: 252, IQR 150–543) for those with *T. trichiura* infection. *A. lumbricoides* co-infection was found in 35.9%, 36.0%, and 27.8% of study participants in Côte d’Ivoire, Lao PDR, and Pemba Island, respectively; though the proportion of *T. trichiura* infected participants with hookworm co-infection was higher in Lao PDR (91.7%) compared to Côte d’Ivoire (7.6%) and Pemba Island (9.9%).

### 3.2. Fecal rapid diagnostic test results

As shown in Table 2, of those diagnosed with *T. trichiura* (n = 1034), most participants (842 (86.7%)) were found to have a normal FC concentration of < 50 μg/g, while 149 (12.5%) were found to have elevated FC concentrations of 50–200 μg/g. Only a few participants (43 (3.6%)) were identified with high (>200 μg/g) FC levels. Most participants harboring a co-infection with *A. lumbricoides* (78.9%) or hookworm (79.0%) were found to have a normal FC concentration of < 50 μg/g.

Negative controls showed a similar distribution of FC levels; the majority 129 (82.2%) had a normal FC concentration of < 50 μg/g, while 18 (11.4%) were identified with FC concentrations of 50–200 μg/g and 10 (6.4%) with high FC concentrations of > 200 μg/g. Age, Sex, BMI, and hemoglobin level were similar among the different FC levels.

During the study, 1189 participants were surveyed for FOB, while only a minority of 14 (1.2%) participants were found to be positive. Most of the participants (78.6%) tested positive for FOB were found in the Asian setting. Country specific study results are summarized in Table 3.

**Table 2**

Test results of fecal calprotectin and fecal occult blood in *T. trichiura* positive and STH negative participants.

| Number of test results, n (%) | < 50 μg/g | Fecal calprotectin | 50–200 μg/g | > 200 μg/g | Negative | Fecal occult blood | Total N(%) |
|-------------------------------|----------|--------------------|-------------|------------|----------|-------------------|-------------|
| **T. trichiura positive, n (%)** | 971 (81.5) | 167 (14.9) | 53 (4.5) | 1175 (98.8) | 14 (1.2) | 1191 (100) |
| Females, n (%) | 436 (81.8) | 77 (14.4) | 20 (3.8) | 520 (97.9) | 11 (2.1) | 533 (100) |
| Mean age, years (SD) | 18.0 (14.3) | 22.1 (17.5) | 21.1 (17.4 | 18.6 (14.9 | 29.6 (18.2) | 18.5 (15.0) |
| Mean BMI, kg/m² (SD) | 18.0 (4.5) | 18.9 (5.1) | 18.0 (4.5) | 18.1 (4.6) | 19.2 (3.7) | 18.1 (4.6) |
| Mean hemoglobin, g/L (SD) | 124.6 (14.5) | 124.6 (14.3) | 126.0 (14.9) | 124.6 (14.4) | 123.3 (20.6) | 124.6 (14.5) |
| Median EPG (IQR) | 408 (216–948) | 360 (210–966) | 390 (186–720) | 405 (210–960) | 279 (138–432) | 402 (210–948) |
| **Co-infections with A. lumbricoides** | | | | | | |
| Positive (%) | 273 (78.9) | 15 (4.6) | 19 (5.5) | 343 (99.1) | 2 (0.9) | 346 (100) |
| Median EPG (IQR) | 4716 | 9864 | 10,716 | 5508 | 12,861 | 5508 |
| **Co-infections with hookworm** | | | | | | |
| Positive (%) | 271 (77.9) | 49 (14.5) | 19 (5.6) | 330 (97.3) | 9 (2.7) | 339 (100) |
| Median EPG (IQR) | 714 | 954 | 522 | 729 | 1158 | 759 |
| **Co-infections of T. trichiura** | | | | | | |
| with 1 other STHs | 442 (79.0) | 87 (15.5) | 31 (5.5) | 548 (98.2) | 10 (1.8) | 560 (100) |
| with 2 other STHs | 101 (82.1) | 15 (12.2) | 7 (5.7) | 122 (12.0) | 1 (8.3) | 123 (100) |
| with *O. viverrini* | 43 (79.6) | 8 (14.8) | 3 (5.6) | 53 (98.1) | 1 (1.9) | 54 (100) |
| with *S. stercoralis* | 39 (79.6) | 7 (14.3) | 3 (6.1) | 48 (98.0) | 1 (2.0) | 49 (100) |
| **STH negative, n (%)** | 129 (82.2) | 18 (11.4) | 10 (6.4) | 155 (98.7) | 2 (1.3) | 157 (100) |
| Females, n (%) | 89 (69.0) | 9 (50.0) | 6 (60.0) | 104 (67.1) | 0 (0.0) | 104 (100) |
| Mean age, years (SD) | 31.3 (15.4) | 32.4 (20.5) | 20.9 (16.1) | 30.1 (16.1) | 30.5 (29.0) | 21.1 (17.4) |

**Abbreviations:** A. lumbricoides, Ascaris lumbricoides; BMI, body mass index; EPG, eggs per gram; IQR, interquartile range; O. viverrini, Opisthorchis viverrini; SD, standard deviation; S. stercoralis, Strongyloides stercoralis; T. trichiura, Trichuris trichiura.

* a 1191 FC test results and 1189 FOB test results were obtained, as not enough stool was collected from two individuals to conduct FOB tests.

* b Data was only collected in 954 individuals for FC and 953 individuals for FOB, as STH negative and other possibly eligible participants did not attend subsequent clinical examination.

* c Data was only collected in 957 individuals for FC and 956 individuals for FOB, as STH negative and other possibly eligible participants did not attend subsequent clinical examination.

* d O. viverrini and S. stercoralis infections were only assessed in Lao PDR.
observed in a small sample size among the Shuar of Amazonian subcontinent on a larger scale the previous findings. The results we found suggest that low FC levels, regardless their infection intensity, while no significant relationships were found among adults [57].

Surprisingly, we only found 12 FOB positive results among all STH infected participants. Though we did not formally apply a statistical test for FOB, due to the low number of positive test results, our findings correlate fairly well with Raj et al. as they also did not find a significant difference in the rate of FOB between *T. trichiura* or *A. lumbricoides* positive and negative children [70]. These findings are supported by Wakid who did not detect significant evidence on intestinal parasitic infections, including STHs, and positive FOB tests [71]. In contrast, the findings of Kanzaria et al. and Wanachiwanawin et al. support a relationship between FOB and moderate and heavy *T. trichiura* infections only [40,43].

Different findings were documented for schistosomiasis, a strong association between prevalence and intensity of *Schistosoma mansoni* infection and FOB was observed after repeated treatment over a period of one year in a cohort of young children [41]. These findings seem to be supported by Bustinduy et al. who found a significant correlation between FOB and moderate and heavy egg intensities of *S. mansoni* infection [46]. Moreover, Kanzaria et al. found a positive correlation between FOB and *Schistosoma japonicum* [43]. However, comparison to these studies on schistosomiasis needs to be interpreted with caution, as the host-parasite interaction differs compared to STHs. In the case of intestinal schistosomiasis, the disease is progressed by the chronic and downregulated granulomatous response to entrapped eggs causing polyposis and pseudopolyps leading to rectal bleeding [72].

According to our findings, we suggest that intestinal inflammation and mucosal bleeding caused by STH infections potentially are very low-grade. There might be an age attributable reduction in the rate of parasite establishment, survival, and fecundity due to acquired immunity [73]. Moreover, immune system response to presence of STH ova might differ between the chosen settings. Additionally, blood loss in STH infected individuals might particularly result from the

**Table 3**

| Age, sex, and co-infection for participants surveyed for fecal calprotectin and fecal occult blood by country. |
|---------------------------------------------------------------|
| Participants, n | Côte d'Ivoire | Lao PDR | Pemb Island |
|-----------------|---------------|--------|------------|
|                  | T. trichiura positive | all STH negative | T. trichiura positive | all STH negative | T. trichiura positive | all STH negative |
| Mean age in years (SD) | 163 (13 6) | 35 (14 8) | 283 (17 5) | 246 (17 5) | 130 (8 8) | 249 (14 3) |
| Age categories | 5–11 years (%) | 251 (61 2) | 8 (16 0) | 92 (30 7) | 20 (40 0) | 182 (56 2) | 2 (3 6) |
| 12–34 years (%) | 99 (24 2) | 18 (36 0) | 91 (30 3) | 11 (22 0) | 126 (38 9) | 22 (40 0) |
| 35–64 years (%) | 60 (14 6) | 24 (48 0) | 117 (39 0) | 19 (38 0) | 16 (4 9) | 31 (56 4) |
| Females (%) | 209 (51 0) | 29 (58 0) | 157 (52 3) | 31 (62 0) | 167 (51 5) | 43 (78 2) |
| Median EPG (IQR) | 408 (198–1068) | 0 (0) | 252 (150–543) | 0 (0) | 558 (330–1224) | 0 (0) |
| A. lumbricoides infection (%) | 147 (35 9) | 0 (0) | 108 (36 0) | 1 (2 0) | 90 (27 8) | 0 (0) |
| Hookworm infection (%) | 31 (7 6) | 1 (2 0) | 275 (91 7) | 0 (0) | 32 (9 9) | 0 (0) |
| O. viverrini infection (%) | ND | ND | 54 (18 0) | 1 (2 0) | ND | ND |
| S. stercoaris infection (%) | ND | ND | 49 (17 7) | 0 (0 0) | ND | ND |
| Fecal calprotectin | | | | | | |
| < 50 μg/g (%) | 311 (75 9) | 40 (80 0) | 231 (77 0) | 33 (66 0) | 300 (96 0) | 55 (100 0) |
| ≥ 50 μg/g (%) | 99 (24 1) | 10 (20 0) | 69 (23 0) | 17 (34 0) | 24 (7 4) | 0 (0) |
| Fecal occult blood | | | | | | |
| Positive (%) | 2 (0 5) | 0 (0) | 9 (3 0) | 2 (4 0) | 1 (0 3) | 0 (0) |
| Negative (%) | 407 (99 5) | 50 (100 0) | 291 (97 0) | 48 (96 0) | 322 (99 7) | 55 (100 0) |

Abbreviations: A. lumbricoides, Aisacris lumbricoides; ND, not determined; O. viverrini, Opisthorchis viverrini; SD, standard deviation; STH, soil-transmitted helminths; S. stercoaris, Strongylus stercoaris; T. trichiura, Trichuris trichiura.

4. Discussion

We applied FC and FOB tests to assess the relationship between STH infections and intestinal inflammation or mucosal bleeding in three different countries. It is of pivotal importance to find an affordable, standardized and simple point-of-care test to assess STH attributable morbidity to better survey control interventions. Evidence on the association between local inflammation and STH infections is scarce; however, key problems with much of the literature are the generally small sample sizes [40,57,58] and the use of different biomarkers [19] for intestinal inflammation leading to contradictory results.

We found no associations of FC and FOB with *T. trichiura* infection and *A. lumbricoides* co-infection status and intensity in each of the three settings tested, demonstrating that FC and FOB are not good proxy markers for STH attributable gut morbidity. Known immunoregulatory properties of STHs have shown a down-regulation of host response to limit inflammation and tissue damage, which may explain our findings [59,60]. Albeit our results show a slight protective effect of hookworm co-infection, this might be attributed to the hypothesis that hookworm infection causes a dampening effect on FC levels by inhibiting neutrophils, the main calprotectin-producing cell type [61]. Of note, *N. americanus* is expected to be the predominant hookworm species in our settings, ingesting around 0.001 ml blood per day [62–65]. Our results suggest age and setting are greater predictors of FC concentration than the presence of helmith infection. Though age has been thoroughly established as a predictor for inflammation in the body [66,67], the root cause of why setting is predictive of high calprotectin is yet to be determined although different diets and exercise might play a role [68].

Our findings add to the small body of evidence currently available on FC concentration and helmith infections. The results we found substantiate on a larger scale the previous findings of de Gier and colleagues, who reported no association between FC concentration and STH infection in 2018 [19]. Additionally, de Gier et al. reported no association between hookworm and FC concentration at baseline or at seven months follow-up [69]. Copon-Robins et al. similarly observed in a small sample size among the Shuar of Amazonian Ecuador that the relationship between infection and intestinal inflammation were age- and species-specific. These researchers found children singly infected with *T. trichiura* to have significantly lower FC levels, regardless their infection intensity, while no significant relationships were found among adults [57].

The findings of de Gier and colleagues were documented for schistosomiasis, a strong association between prevalence and intensity of *Schistosoma mansoni* infection and FOB was observed after repeated treatment over a period of one year in a cohort of young children [41]. These findings seem to be supported by Bustinduy et al. who found a significant correlation between FOB and moderate and heavy egg intensities of *S. mansoni* infection [46]. Moreover, Kanzaria et al. found a positive correlation between FOB and *Schistosoma japonicum* [43]. However, comparison to these studies on schistosomiasis needs to be interpreted with caution, as the host-parasite interaction differs compared to STHs. In the case of intestinal schistosomiasis, the disease is progressed by the chronic and downregulated granulomatous response to entrapped eggs causing polyposis and pseudopolyps leading to rectal bleeding [72].

According to our findings, we suggest that intestinal inflammation and mucosal bleeding caused by STH infections potentially are very low-grade. There might be an age attributable reduction in the rate of parasite establishment, survival, and fecundity due to acquired immunity [73]. Moreover, immune system response to presence of STH ova might differ between the chosen settings. Additionally, blood loss in STH infected individuals might particularly result from the
whenever possible, by partially re-labeling and re-examining results. Moreover, as in many multicountry research, various challenges arose across multiple settings. Differences in diet, culture, and social norms were unable to be accounted for and could potentially bias results. In addition, varying seasonality, host susceptibility, and parasite strains would have an uncontrollable impact on the findings of this study. In spite of these challenges, the results of this study add to the expanding body of literature of STH attributable morbidity. Lastly, the generalizability of our findings are limited to populations in resource-limited settings with recurring and chronic STH infections.

In conclusion, we were not able to detect intestinal morbidity in our study population using FC and FOB as proxy markers. Other potential markers of intestinal inflammation including antityrpsin, eosinophilic protein X, TNFα, or lysozyme have been suggested for diagnosing inflammatory bowel disease, which may be expanded to test for STH attributable intestinal morbidity. However, these test suffer from low diagnostic performance or have not been investigated well enough [78]. Furthermore, markers assessing other facets of STH attributable intestinal morbidity, such as mucosal changes, are needed. Thus, field applicable morbidity markers for STH infections need to be further explored to appropriately monitor and evaluate current global control strategies as monitoring levels of morbidity might become as important as diagnosing the infection itself.

5. Author contributions

EH and JK planned and designed the study; CP, LK, SW, SS, SMA, ShMA, JTC, EH, and JK conducted the study; JH and CP did the formal analyses; LK, CP, and JK interpreted the study data and drafted the manuscript. All authors read and approved the final version of the manuscript and took responsibility to submit this manuscript for publication.

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Data sharing

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request after publishing the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.100724.

References

[1] Pullan RL, Smith JL, Jasrasaria R, Brooker S. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasites Vector 2014;7:37.
[2] World Health Organization. Closing the gap in a generation: health equity through action on the social determinants of health. Final report of the commission on social determinants of health. Geneva: World Health Organization; 2008.
[3] Jourdan PM, Lambertorn PHL, Fenwick A, Addiss DG. Soil-transmitted helminth infections. Lancet 2018;391:252–65.
[71] Wakid MH. Fecal occult blood test and gastrointestinal parasitic infection. J Parasitol Res 2010;2010:434801.

[72] Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet 2014;383:2253–64.

[73] Wakelin D. The role of the immune response in helminth population regulation. Int J Parasitol 1987;17:549–57.

[74] Osazuwa F, Ayo O, Imade P. A significant association between intestinal helminth infection and anaemia burden in children in rural communities of Edo state, Nigeria. N A J Med Sci 2011;3:30–4.

[75] Bogoch II, Speich B, Lo NC, et al. Clinical evaluation for morbidity associated with soil-transmitted helminth infection in school-age children on Pemba Island, Tanzania. PLoS Negl Trop Dis 2019;13:e0007581.

[76] Bjarnason I. The use of fecal calprotectin in inflammatory bowel disease. Gastroenterol Hepatol 2017;13:53–6.

[77] Lin JF, Chen JM, Zuo JH, et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. Inflamm Bowel Dis 2014;20:1407–15.

[78] Lehmann FS, Burri E, Beglinger C. The role and utility of faecal markers in inflammatory bowel disease. Therap Adv Gastroenterol 2015;8:23–36.