Metronidazole Therapy for Treating Dientamoebiasis in Children Is Not Associated With Better Clinical Outcomes: A Randomized, Double-Blinded and Placebo-Controlled Clinical Trial

Dennis Röser,1 Jacob Simonsen,2 Christen Rune Stensvold,1 Katharina E. P. Olsen,1 Peter Bytzer,3 Henrik V. Nielsen,1 and Kåre Mølbak2

1Department of Microbiology and Infection Control, and 2Division of Health Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark, and 3Faculty of Health Sciences, Department of Medicine, Copenhagen University, Køge University Hospital, Denmark

**Background.** There is a paucity of evidence documenting the pathogenicity of *Dientamoeba fragilis*, an intestinal protozoan common in children. As case reports on successful treatment are numerous, many authors advocate treatment, despite no placebo-controlled trials being available. Metronidazole is often used for treatment, though eradication rates are relatively low (60%–80%). In the present study we determined the clinical and microbiological efficacy of metronidazole in Danish children.

**Methods.** In this parallel placebo-controlled double-blinded trial, children aged 3–12 years with >4 weeks of gastrointestinal symptoms were allocated using block randomization in a 1:1 ratio to a 10-day course of oral metronidazole or placebo. Primary outcome was change in level of gastrointestinal symptoms, measured on a visual-analog-scale (VAS), and secondary outcome was eradication of *D. fragilis* infection. Participants, caregivers, investigators, and sponsor were all blinded to group assignment. The trial was registered with clinicaltrials.gov (NCT01314976) prior to start.

**Results.** Of 96 participants, 48 were allocated to the metronidazole and placebo group each. Mean VAS change from pre- to post-treatment did not differ significantly (*P* = .8) between the metronidazole (−1.8 CI, [−2.5, −1.1]) and the placebo group (−1.6 CI, [−2.3, −0.9]). Eradication of *D. fragilis* was significantly greater in the metronidazole group, although it declined rapidly from 62.5% 2 weeks after end of treatment to 24.9% 8 weeks after end of treatment.

**Conclusions.** These findings do not provide evidence to support routine metronidazole treatment of *D. fragilis* positive children with chronic gastrointestinal symptoms. Study funded by Statens Serum Institut.

**Clinical Trials Registration.** Trial was registered with clinicaltrials.gov (NCT01314976).

**Keywords.** antimicrobial; *Dientamoeba fragilis*; pediatric; pathogenicity; RCT.

The management of children with chronic gastrointestinal (GI) symptoms of presumed infectious etiology is a challenge to both primary and secondary healthcare. The intestinal protozoon *Dientamoeba fragilis* is common in both healthy and sick children [1, 2] and is present in as many as 70% of 7-year-old children suspected of enteroparasitic disease in Denmark [3]. Numerous case reports have described alleviation of symptoms upon treatment and/or eradication of *D. fragilis* [4–6], and also cohort and case-control studies have linked *D. fragilis* infection to GI symptoms [7–9], in some cases particularly so in children [10, 11]. As such, many health professionals consider *D. fragilis* a potential pathogen and propose treatment when diagnosed in
the absence of other likely explanations of the patients’ symptoms. However, the demonstration of association between *D. fragilis* infection and GI symptoms is not evidence for causality, and many studies do not report any such association [1,12,13].

Various antimicrobial agents have historically been attempted for the treatment of dientamoebiasis, and although many presently used drugs have shown in-vitro effectiveness against *D. fragilis* [14–16], present recommendations are largely based on small numbers of nonrandomized studies [17]. Only 2 randomized controlled trials have been published [18,19]. One trial showed diphetarsone, an arsenical compound no longer in use, to be superior to placebo in self-assessed clinical outcome, but was only published in abstract form [18]. The other trial compared 2 nitroimidazoles, metronidazole and ornidazole, showing ornidazole to be both clinically and microbiologically superior to metronidazole; however, the trial did not include a placebo group [19], and symptom alleviation after treatment was not significantly associated with eradication of *D. fragilis* (Özgür Kurt, personal communication).

In Denmark, the only registered drug for treating dientamoebiasis is metronidazole [20]. Although the clinical efficacy of metronidazole can be difficult to ascertain, the eradication rate has been reported to range from 60% to 80% [4,21]. As such, there is a need for controlled studies critically examining the clinical and microbiological effect of antimicrobials presently used to treat *D. fragilis*. With the apparent predilection of *D. fragilis* in children, both in terms of prevalence [2] and possibly GI symptoms [11], studies targeting a symptomatic pediatric cohort appear relevant. The aim of the present study was therefore to determine the clinical efficacy of metronidazole treatment of *D. fragilis*-carrying children with chronic GI complaints.

**METHODS**

**Study Design**

The study was a parallel double-blind, randomized, placebo-controlled trial designed to determine the clinical and microbiological efficacy of metronidazole in *D. fragilis*-infected Danish children with chronic GI complaints; it was conducted in the Copenhagen metropolitan area, Denmark.

Primary outcome was change in the level of GI symptoms, as assessed by parents on a visual analog scale (VAS), from pretreatment to 14 days after end of treatment; secondary outcome was eradication of *D. fragilis*. Both outcomes were specified prior to trial initiation.

Written informed consent was signed by parents/legal guardians, and when appropriate, consent from the child was also retrieved.

The study was approved by the Regional Committee on Health Research Ethics (H-1-2011-035) and was initiated on 1 July 2011, with an expected 2-year enrollment (predefined cutoff defined by the trial steering group). However, inclusion was halted prematurely on 1 March 2013 as reorganization of regional diagnostic services outside of study resulted in fewer samples sent to Statens Serum Institut for *D. fragilis* testing, which precluded screening for trial candidates. This had little consequence to the trial, because at this point 96 of the targeted 100 patients had already completed the trial. No change in process was necessary throughout the study period.

**Participants**

From July 2011 to March 2013, patients from both primary and secondary care settings submitting fecal samples to Statens Serum Institut for investigation of *D. fragilis* were screened. A trial invitation letter was sent if patients were (i) positive for *D. fragilis* in real-time polymerase chain reaction, (ii) 3–12 years of age, (iii) not recently (0–3 months) diagnosed with *D. fragilis*, and (iv) living in the island of Zealand (which includes the capital region). Responders were further screened by phone interview, identifying patients (i) not treated with metronidazole within past 3 months and (ii) with ≥2 weeks of GI symptoms (up to date of interview), including either ≥3 episodes of diarrhea per week, ≥3 episodes of stomach pain per week, or ≥2 other GI related complaints (loss of appetite, failure to thrive, anal itching, flatulence, or otherwise abnormal stool), occurring weekly. Exclusion criteria were (i) concurrent laboratory confirmed GI infection (not including *Enteroabius vermicularis* [pinworm]), (ii) noninfectious GI or liver disease, or (iii) intolerance or allergy toward metronidazole, (iv) weight >50 kg, or (v) inability of parents to provide informed consent or complete trial diary.

Trial enrollment was conducted consecutively at the Departments of Pediatrics at Roskilde and Herlev Hospitals and included a standard pediatric examination. Parents completed a structured pretreatment “self-assessed” questionnaire, which used an unnumbered VAS scale with ends denoting “no symptoms” and “worst experienced level of symptoms,” addressing the 14 days prior to trial enrollment (protocol and questionnaires available upon request). Fecal sampling containers were issued for immediate return to Statens Serum Institut, and test results were given by phone. Drug formulations were sent by postal mail along with questionnaires, diary, and sampling kits, which were collected on days 14, 28, 42, and 56 after end of treatment (Figure 1).

**Randomization**

Participants were randomized using block randomization with randomly selected block sizes in a 1:1 ratio. Randomization was performed by pharmacist Alice Rosendahl from the Capital Region Pharmacy, who also prepared the sequentially numbered drug containers. The randomization code was kept at the Capital Region Pharmacy until end of trial and was masked to sponsor, investigator, and patients until end of trial.

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**REFERENCES**

[1] Advertisement

[2] Advertisement


[7] Advertisement

[8] Advertisement

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Interventions
Participants were allocated to either metronidazole (40 mg/kg/day, 3 daily dosages, 10 days) or placebo. Oral metronidazole solutions were produced by Sanoﬁ-Aventis (Flagyl 40 mg/mL), and placebo formulations were manufactured by the Capital Region Pharmacy to be identical in appearance, taste, and texture.

Statistical Analyses
The null hypothesis was that there is no clinical or microbiological measurable effect of metronidazole compared to placebo. The sample size was calculated using 80% power and an error of 5%. The expected clinical efficacy of metronidazole was set to 60%, in line with reported eradication rates, and the effect of placebo, in terms of spontaneous clinical improvement, was set to 20%. Based on this, we estimated a sample size of 100 children, allowing up to 20% loss to follow-up.

Analysis of the primary outcome measure was performed using a 2-sided t-test (5% α level, assuming normal distribution and homogeneity of variance). The analysis of primary outcome measure was tested for robustness in order to optimize the fit of the model, by applying the Box-Cox method for best possible power transformation to obtain normality of data [30]. Intention-to-treat analyses included all patients who submitted a fecal sample and questionnaire prior to treatment start, whereas per-protocol analyses included only those patients who submitted fecal samples and questionnaires both prior to treatment start and 14 days after end of treatment. The analysis was designed and tables prepared prior to unblinding of data. Interim analysis was not performed. Analysis of secondary outcome
measure was performed using binary regression with log link and was also designed prior to unblinding of data. Tertiary analyses were performed by analysis of variance, χ²-tests, Wald and Mantel-Haenszel methods [31], and were designed after unblinding of the data. SAS statistical software 9.4 (SAS Institute Inc., Cary, NC) was used for the data analysis.

Role of the Funding Source
The trial was funded by Statens Serum Institut (sponsor), with no external funding. Collection, analysis, and interpretation of data, as well as decision to submit the article for publication, were done solely by the authors, who all had full access to trial data.

RESULTS
A total of 1024 patients were invited to participate in the trial during the trial period. Of these, 154 responded by phone after a mean period of 13 days. Subsequent to phone interview, consultation and confirmatory fecal analyses, 48 failed to meet inclusion criteria, whereas 106 proceeded to receive intervention (Figure 1). Median time from parent response to trial initiation consultation was 5 days, and median time from sample collection (after consultation) to treatment start was 15 days. Most parents reported abdominal pain as the primary (most adversely affecting) GI symptom. Median duration of GI symptoms prior to inclusion was 11 months. Almost no parents reported patients’ stools as hard; equally many reported either solid or mushy/fluid stools. Loss of appetite and anal itching were the 2 most common GI symptoms otherwise reported (Table 1).

Per-protocol analysis of the primary outcome measure showed that both the metronidazole and the placebo group reported a decrease in self-reported GI illness; however, there was no significant difference between the 2 types of intervention (Table 2).

Analysis of the secondary outcome measure showed a significant association between intervention and post-treatment infection with D. fragilis. The 2-week D. fragilis eradication rate of metronidazole (adjusted for effect of placebo) was 62.5%, rapidly declining to only 24.9% 8 weeks after end of treatment (Table 3, Figure 2).

Tertiary analyses showed no significant change in primary and secondary outcome measures when stratifying analyses by any of the following parameters: age, gender, duration of GI symptoms, primary GI symptom, intervention, primary and secondary outcome, and E. vermicularis coinfection (Supplementary Table 1). Recalculating primary outcome against later follow-up points did not change findings nor disclose any trends for possible treatment effect (Supplementary Table 2). A pinworm prevalence of 24% was observed, and analysis showed a relative risk of 1.20 (95% CI, 1.01, 1.44) for post-treatment D. fragilis infection when being pinworm positive.

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of Patients by Intervention Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>METRONIDAZOLE (N, %)</td>
</tr>
<tr>
<td>BOYS</td>
</tr>
<tr>
<td>25 (46.3%)</td>
</tr>
<tr>
<td>PRIMARY GASTROINTESTINAL SYMPTOMS</td>
</tr>
<tr>
<td>ABDOMINAL PAIN</td>
</tr>
<tr>
<td>METRONIDAZOLE (N, %)</td>
</tr>
<tr>
<td>35 (64.8%)</td>
</tr>
<tr>
<td>DIARRHEA</td>
</tr>
<tr>
<td>METRONIDAZOLE (N, %)</td>
</tr>
<tr>
<td>16 (29.6%)</td>
</tr>
<tr>
<td>OTHER</td>
</tr>
<tr>
<td>METRONIDAZOLE (N, %)</td>
</tr>
<tr>
<td>3 (5.6%)</td>
</tr>
</tbody>
</table>

Average stool consistency
Normal stool: 28 (51.9%), 23 (44.2%)
Mushy, fluid stool: 22 (40.7%), 27 (51.9%)
Not known stool: 2 (3.7%), 0 (0%)

Abdominal pain episodes
Diarrhea episodes
Other symptoms

* Median age was 7.5 (3–12) and 6 (3–11) years in the metronidazole and placebo group, respectively.
* Within present period of gastrointestinal illness, the symptom/complaint which most adversely affected the patient’s well-being. Median duration was 12 (1–72) and 8.5 (1–72) months in the metronidazole and placebo group, respectively.
* Other symptoms included constipation, encopresis, and excessive flatulence in the metronidazole group, and excessive flatulence, nausea (×3), gastric reflux, and vomiting in the placebo group.
* As noted within the 2 months prior to patient enrollment in trial.
real-time PCR Ct-values and level of GI symptoms, neither at trial entry nor post-treatment, and no differences between intervention groups were detected (data not shown).

Overall, the trial participants were compliant, and there was little missing data; all patients (excluding dropouts) supplied data for primary and secondary outcome measures. Almost all intervention dosages (>99%) were taken as scheduled. No serious adverse events were reported, and emergency unmasking of data was not necessary during trial period. Adverse effects were as expected; the most commonly reported in both metronidazole and placebo group was nausea (65% and 42% of patients) and vomiting (33% and 10%).

**DISCUSSION**

This is the first randomized, placebo-controlled trial of metronidazole for the treatment of dientamoebiasis in children. We found that metronidazole did not significantly reduce GI symptoms compared with placebo. In addition, we showed that the ability of metronidazole to eradicate *D. fragilis* was initially moderate (62.5%), but that this effect declined rapidly over a period of 6 weeks to only 24.9%.

Furthermore, tertiary analyses did not show greater clinical effect in eradicated vs noneradicated patients. It could be argued that lingering adverse effects of metronidazole could mask a greater "true" clinical effect of metronidazole, and that the time period chosen to observe the primary measure of effect, that is, the 2 weeks immediately following treatment, was too close to the treatment period. However, bearing in mind that testing against later follow-up points did not show any greater effect, this is likely not the case. Our choice of antimicrobial intervention might have restricted efficacy, but the chosen drug (metronidazole) was the only registered drug for intestinal amoebic infections in Denmark [20] and thus relevantly reflected daily clinical practice. And again, as our results did not disclose greater clinical effect in eradicated vs noneradicated patients, it remains uncertain if another drug would have produced different results. Poor adherence could have diminished

### Table 2. Primary Outcome Measure: Change in Level of Gastrointestinal Symptoms by Intervention Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention</th>
<th>No. of Patients</th>
<th>Pretreatment VAS Mean (95% CI)</th>
<th>Post-treatment VAS Mean (95% CI)</th>
<th>Difference Mean (95% CI)</th>
<th>Statistical Significance&lt;sup&gt;+&lt;/sup&gt;</th>
<th>P Value</th>
<th>P Value (Adjusted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention to treat&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Placebo</td>
<td>52</td>
<td>5.24 (4.71, 5.78)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>54</td>
<td>5.35 (4.83, 5.87)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Per protocol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Placebo</td>
<td>48</td>
<td>5.32 (4.75, 5.88)</td>
<td>3.75 (3.08, 4.42)</td>
<td>−1.57 (−2.28, −0.85)</td>
<td>P = .704</td>
<td>P = .818</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>48</td>
<td>5.28 (4.71, 5.84)</td>
<td>3.52 (2.85, 4.18)</td>
<td>−1.76 (−2.47, −1.05)</td>
<td>P = .001</td>
<td>P = .45</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Total level of gastrointestinal symptoms as registered by parent-assessed visual analog scale (VAS-scale), and recorded at pretreatment consultation and at follow-up day 14 after end of treatment.

<sup>b</sup> Test for no difference between metronidazole and placebo intervention, calculated using a two-sided t-test (5% alpha level, assuming normal distribution and homogeneity of variance); adjusted denotes adjustment for age and gender. Box-Cox analysis showed that, within the family of power transformations ($y_λ$), the raw value ($y_1$) was very close to the best possible transformation ($λ = 0.92$, 95% CI [0.44–1.45]) in order to obtain normality of data (VAS difference).

<sup>c</sup> Intention-to-treat group includes all patients submitting sample and questionnaire at pretreatment consultation.

<sup>d</sup> Per-protocol group includes all patients submitting sample and questionnaire at both pretreatment consultation and at follow-up day 14 after end of treatment.

### Table 3. Secondary Outcome Measure: Post-treatment Infection of *Dientamoeba fragilis* by Intervention Group

<table>
<thead>
<tr>
<th>Intervention Effect on <em>D. fragilis</em> Infection&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Follow-up Day 14</th>
<th>Follow-up Day 28</th>
<th>Follow-up Day 42</th>
<th>Follow-up Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole, n/N (%)</td>
<td>14/48 (29.2)</td>
<td>14/44 (31.8)</td>
<td>18/41 (43.9)</td>
<td>23/41 (56.1)</td>
</tr>
<tr>
<td>Placebo, n/N (%)</td>
<td>44/48 (91.7)</td>
<td>39/44 (88.6)</td>
<td>37/43 (83.7)</td>
<td>34/42 (81)</td>
</tr>
<tr>
<td>P Value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Metronidazole eradication rate (%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.5</td>
<td>56.8</td>
<td>39.8</td>
<td>24.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Infection in terms of either positive or negative *D. fragilis* real-time polymerase chain reaction at days 14, 28, 42, and 56 after end of treatment.

<sup>b</sup> Test for no difference between interventions calculated using binary regression with log link.

<sup>c</sup> Calculated as rate of infection for the placebo group minus the metronidazole group, that is, 91.7–29.2 for follow-up day 14.
efficacy, but with patients reporting >99% of intervention dosages taken as per schedule, this is not likely. Reduced power might have hampered our ability to show an effect of treatment; however, since 96 of the targeted 100 trial participants were included, and considering that analyses of scenarios of maximum treatment effect in dropouts did not allow for significant findings, this is not likely. The applicability of our findings is strengthened by the controlled design of the trial.

As the exact clinical profile of a *D. fragilis* infection is not known, our inclusion criteria were designed to be pragmatic and broad, focusing on the 2 most commonly described complaints in *D. fragilis* infections, that of abdominal pain and diarrhea. This is important because there is no consensus concerning the clinical diagnosis of dientamoebiasis. This notion infers a risk of bias, as physicians usually employ specific investigations only on patients who harbor “expectable” symptoms, which, in the case of a possible GI infection, often includes diarrhea and abdominal pain. However, when the target microorganism occurs commonly in asymptomatic individuals as well, such expectations may be falsely reflected on the reported clinical profile; in terms of the present trial, the risk is that our inclusion criteria could be structured inaccurately. Such a risk is difficult to assess; however, our cohort did have similar clinical characteristics to previous observational studies. We included children, who, in some studies, have been suggested to harbor symptoms more often than adults when infected with *D. fragilis*, and also restricted inclusion to patients with a history of chronic GI symptoms (no less than 4 weeks), decreasing our likelihood of including patients with acute disease only. Also, stratifying the analyses as per particular subgroups (Supplementary Table 1) did not disclose any trends for primary or secondary outcome.

The participation rate was low (10%), possibly due to trial invitation being conducted by mail, as opposed to during consultation; the consequence is that our trial participant cohort could differ from our trial candidate cohort in terms of clinical profile. We used a VAS-scale to assess the clinical efficacy of intervention. Parents were instructed to record a single value on the VAS-scale to reflect the total level of GI symptoms that the patient experienced. However, we have no way of knowing how meticulously parents approached this assignment, nor how great the stochastic variability was in terms of reproducibility. As such, the use of a VAS-scale, as opposed to a multiple-item questionnaire or similar record, might have restricted our ability to correctly assess the primary outcome. However, a VAS-scale is a validated clinical tool that has previously been used to address severity of abdominal pain in pediatric intestinal protozoal infections [32]. A multiple-item questionnaire would have had to be weighed against the ill-defined clinical profile of *D. fragilis*, which would carry a significant risk of bias. Additionally, a VAS-scale produces a single unequivocal value for statistical significance testing, greatly reducing risk of investigator bias. Finally, the presently observed (and expected) post-treatment decrease in mean VAS in the placebo group supports our use of a VAS-scale as a viable outcome measure, especially considering that scoring was performed by parents.

Our finding of a metronidazole eradication rate of 62.5% 2 weeks after end of treatment is in line with previously reported rates of eradication. As molecular typing tools are not yet available for *D. fragilis* [33], it was not possible to distinguish recrudescence from reinfection, in terms of explaining the rapidly increasing post-treatment *D. fragilis* infection rate in the metronidazole group. However, considering the rate of spontaneous clearance in the placebo group, and the generally high prevalence of *D. fragilis* in children [2], re-infection, reflecting a high infection pressure, may be more likely. Although the mode of transmission of *D. fragilis* remains a topic of debate [34], the eggs of pinworm have repeatedly been suggested as a potential vector [35]. The present finding of a positive association between post-treatment pinworm and *D. fragilis* infection supports this hypothesis.

The median age of trial subjects was 7 years, which is the age when the prevalence of *D. fragilis* in Danish patients peaks [2]. It is likely that spontaneous clearance is common in this age group and that this explains the fast decreasing post-treatment infection rate in the placebo group. This is consistent with the observed trend for lower levels of post-treatment infection in older vs younger children (Supplementary Table 1).

This trial does not demonstrate clinical improvement in *D. fragilis* positive patients following metronidazole treatment.
beyond that of placebo nor that eradication of *D. fragilis* has any particular clinical effect. This does not preclude the possibility that *D. fragilis* in other settings, by distinct strains or different burden of parasites, may be a cause of illness. But the disease-to-infection rate must be very low and the number-needed-to-treat proportionally high.

From an ethical perspective, treatment should always be balanced in terms of likely benefit vs potential harm. In terms of severity, dientamoebiasis has not been associated with deaths or serious illness, and the clinical severity might be viewed as moderate or even low. On the other hand, antimicrobial treatment may have serious or even long-lasting side-effects on top of its potential to promote microbial resistance. The failure of the present trial to show a clinical benefit of metronidazole in children with *D. fragilis* and long-term GI complaints should seriously question the routine use of this treatment approach.

Furthermore, we recommend that all future studies examining the clinical effect of treating *D. fragilis* adopt a randomized, placebo-controlled, and blinded approach and take serious considerations as to the specific effect of eradication, if any. And if *D. fragilis* is unequivocally linked to disease at some point, the present trial serves to underline the importance of identifying individuals with disease, as opposed to infection. In this respect, a well-documented clinical profile in dientamoebiasis is essential.

In conclusion, metronidazole provides no clinical benefit for children with chronic GI complaints who have *D. fragilis* in their stools, and the microbiological effect of metronidazole is only moderate and transient.

**Supplementary Data**

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Contributors.** D. R., P. M. B., H. V. N., and K. M. proposed the initial idea for the trial. All authors contributed to the development of the protocol and trial management. D. R. led the day-to-day management of the trial (supervised by H. V. N. and K. M.), with D. R., K. E. P. O. and H. V. N. managing the analysis of the samples. D. R., J. S., and K. M. analyzed the data; all authors interpreted the data and contributed to the write-up.

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