



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	Ms_IJTDH_29273
Title of the Manuscript:	Study of Neglected Tropical Diseases (NTDs): Gastrointestinal parasites in school children of Lolodorf neighborhood, south region, Cameroon.
Type of Article:	Original Research Article

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
Before going further in reviewing this Ms on intestinal parasites in south Cameroon, I must admit I am troubled by the methodology and somewhat by some descriptions or interpretations. Most respectfully, I like to invite the authors to better explain the following: It is stated that the survey was conducted in March. One stool sample was divided in two, which is perfectly accepted. However, one part was fixed and one part left unfixed to perform the Kato-Katz (KK) method. The laboratory was 150 k away: did they go daily to deliver the stools to a lab 150 k away?? When was the Kato-Katz method prepared: in the field? When was it read? At the end of the month, when they took all samples to the lab? The authors state that the KK was examined in less than 24 h (line 122) to avoid destruction of hookworm eggs. The technique as stated by WHO says that KK for hookworm eggs ought to be read between 30 and 60 minutes after preparation, otherwise hookworm eggs are lost. Therefore, I cannot understand, as explained in their methodology: 1) At what point in the study were stools taken to the lab? 2) How were the unfixed stools kept and handled before examination? 3) How long did it take to the stools (unfixed) to be examined? 4) Where and when was the KK performed? 5) If the KK was read 24 h later, there were no hookworm eggs to be seen, especially in a hot, humid climate as Cameroon. That is to say, that particular data is not fully correct and I don't accept it as is. Another doubtful finding is in regards to intestinal protozoa: Only <i>Entamoeba histolytica</i>/<i>E. dispar</i> and <i>Entamoeba coli</i> were recognized. No other intestinal commensal protozoa were diagnosed. If this is so, then these findings have to be firmly documented because to my knowledge they are unusual. Either the temporary Lugol stained smears require to be examined at 100X magnification (instead of Line 124, 40X) or iron hematoxylin smears have to be prepared and examined and those findings confirmed. Otherwise I accept those results with special reservation. As said, if the methodology is not explained more clearly, I cannot accept the results as such. There are a few other observations: In Table 3 there is a repeat of site results from Table 2, that is redundant. It would have been better to display the egg counts in a table rather than in the text. In Figure 1, when looking at the color legend, there is an extra colored cube that introduces confusion. Line 36: the word Among should be removed for clarity of the sentence. Lines 36 to 41: I believe Neglected Tropical Diseases deserve a better explanation. Lines 117 to 120: Kato-Katz technique, more than identification, it is a standardized method for estimating the intensity of the infection.	- It is stated in the methodology that the laboratory is situated at about 150 km from our study area. This distance is just an approximation which is normally less than the value indicated. However, to cover this distance takes a maximum of 3hours 30 minutes. - Concerning samples transportation to the laboratory, . samples were collected between 7h30 and 10 am by three groups of persons in the field (one group per village) and immediately after collection; the samples were transported by one person to the laboratory for subsequent analysis by technicians. Collection of samples continued the following day followed by transportation to the laboratory for immediate analysis. The authors did not find it necessary adding all these informations in the methodology. However, some corrections have been done as indicated in the text. - The unfixed samples were conserved in a cooler before transporting them. - For the analysis of Kato Katz slides (unfixed samples) within 24 hours, it is true that hookworm eggs may likely not be observed after a long period following collection of stool samples. Also, samples were read the same day within 24h following stool collection and not within 24h after preparation as previously written in the methodology (correction done in the text). It is also well documented in the Manual of basic technics for a health laboratory (WHO, 1980) that, three different forms of hookworm eggs can be observed depending on the duration after collection of stool sample: eggs observe in fresh stool (eggs with 4, 8 or 16 germ cells), egg observe in stool that have stayed for few hours (eggs having a unique mass of many small gray granulations) and eggs observed in those that have stayed between 12 to 48 hours (embryonated eggs) - Examination of slides for the identification of intestinal protozoan was done only at 40X magnification to observe amoeba cyst according to the formol-ether protocol. Other intestinal protozoan such as Giardia, are also normally observed at this same magnification like the works of Bechir et al., 2012 ; Akingbade et al., 2013 among others., but we did not observe them. It is true that other coloration techniques such as modified Ziel-Nelson permit observation at 100X magnification, but we did not use this technique. - The extra color on figure 1 has been deleted. - Correction made for line 36 to 41 (see text) - Correction on estimation of infection intensity using the kato technique is done in the text (methodology) - Concerning infection by age, some precisions have been made to give more clarity as can be seen in the text (line 148-151). - It is true that children between 6-10 years were more infected but a significant difference in eggload was observed only for <i>A. Lumbricoides</i> for children between 11-15 years . Following the reviewer's comment on eggload, a table has been included showing the summary of eggload variation in sex, age and village. - We agree with the reviewer that not all intestinal parasites have oral-fecal mode of



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In Table 3, I see some striking differences in the infection by ages: can the authors determine whether those were statistically significant or not? To my understanding, the age 6 to 10 years was more parasitized; then the authors proceed to state that the egg load was significantly high in the age group 11 to 15, but it is not shown in any Table. Line 208: OMS are the initials in French for the World Health Organization. Since this article is written in English, kindly correct them. The same in line 41 and line 42, SIDA should be AIDS. The authors insist in calling <i>Ascaris</i>, <i>Trichuris</i> and hookworm infections oral-fecal transmission. Another terminology for such infections is Soil Transmitted Nematodes or Soil Transmitted Helminths, therefore, oral-fecal is a misnomer. Eggs of <i>Ascaris</i> and <i>Trichuris</i> need a period in the soil before being infective and hookworm larvae infect the host percutaneously. May I suggest to remove oral-fecal from the text and look for a better term? There are more observations to the Ms, but before the methodology is not more clearly explained, I cannot accept the results of this Ms as presented.	contamination (eg hookworm). It has been précised in the text those that are concerned with this mode of contamination. Also, we consider oral-fecal contamination since for most intestinal parasites, the parasite is eliminated through the anus and after completing its developmental stages in the external environment, it is then ingested through the mouth (<i>A. Lumbricoides</i>, <i>T. Trichiura</i>, amoeba species etc). Even though the terminology Soil Transmitted Helminths is not used, the authors agree that the word stands the best to be used when talking of <i>A. Lumbricoides</i>, <i>T. trichiura</i> and hookworm. -
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