



SDI Review Form 1.6

Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	Ms_IJTDH_26048
Title of the Manuscript:	Soil-Transmitted Helminth Infections and Associated Risk Factors in a Neglected Region in the Upper Nkongho-mbo Area, South-west Region, Cameroon
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Ms_IJTDH_26048 studied soil transmitted helminth infections and risk factors in a neglected rural area of Cameroon. The purpose was to determine prevalence and intensity of STH and risk factors in transmission. 1) Methodology – the sample collection was selectively purposive (line 72) meaning? were the participants selected at random? How were they identified in the community itself? Where did the participants gather? Were they chosen going from house to house? This requires better explanation. 2) Stool samples were analyzed by the formol-ether concentration method (line 87). This method is not well suited to determine intensity of infection, unless a known amount of stool is placed in a known volume of diluent (formalin). Was this the case? Explain. Later in the text one reads: (lines 141-142) “mean worm burden”. Were the participants given anthelmintic drugs and the actual worms recovered from stools counted? Yet, line 142 states epg (eggs per gram). Egg counts are merely estimates of egg output; they cannot be used to determine the number of worms actually present in the individual cases. The World Health Organization and parasitologists in general are aware that to estimate intensity (eggs per gram of stool) the Kato Katz method is the standard method recommended and accepted.	Parents preferred sending their children ahead for the sample collection because they were convinced that mostly children harboured the worms since deworming has always been for children only.. Sampling was from house to house. The participants were selected at random but we made sure that all the age groups were represented and in the case where more children Had already been sampled in a community, we now collected samples only from parents, To us the formol ether is the best method Kato Katz had the main disadvantage of hookworm eggs melting out with the clearing of the sample. We had a scoop spoon that was 0.2 grams and the method is standard with 7mls of 10% fomaline and 3mls of diethyl ether as the diluents. Please the full method is in the Danish Bilharziasis laboratory manual that was written by Christensen <i>et al</i> in 1984. A small stool sample (0.2 grams) was placed in a mortar and mixed with 7 ml of 10% formaline. A sieve was used to filter the sample into a centrifuge tube and 3 ml of diethyl ether added and shaken vigorously. The mixture was then centrifuged at 2000rpm for 2 minutes. A drop of Lugol iodine was then added to the deposit and



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	3) The authors please explain how did they estimate intensity of infection in stools? 4) Soil samples (200 g) were also analyzed from 112 sites. More than 48 pounds of soil were processed in the lab. What was the exact method of soil examination? Also, hookworm eggs develop very fast under known conditions of humidity and temperature and larvae hatch in one or two days in the soil. Please explain what kind of stage was recovered in the case of hookworms? 5) Authors did not explain what kind of region is it (jungle, savannah, semi arid), rainy season from when to when, etc. (lines 194-195)?? What ecological determinants? Again line 201 whipworm is less common at high altitude. Please offer information of altitude. 6) were the eggs recovered from the soil viable? Were there only empty egg shells? Did they have a larva inside? 7) the eggs depicted in Plate 1 were they recovered from the stool or from the soil? 8) a reference is given for the sucrose flotation method (19), but authors forgot to properly cite the journal. 9) line 90: what do the authors mean by health seeking behaviour among participants? What was their level of education? Nothing of this is discussed in the MS. 10) lines 95-97 egg counts were found not normally distributed. This is not explained in the Results. 11) Unless authors provide a proven acceptable method for egg counts, the intensity of infection in this MS is not acceptable. 12) Table 2 needs better distribution of spaces. Example: Njungo does not have a line for itself, 18 is on top and 7 by itself, very confusing; same for totals. 13) Could the Table be better presented, as example: 34 (37.3); 22 (44.9)? 14) Table 3 is confusing: (%) and (number); kindly make it clearer.	observed under the microscope. Within 45 minutes of preparation the faecal smears were examined using the x10 objective of the light microscope for identification and enumeration of helminthe eggs. Infection intensities were expressed as eggs per gram (mean count/x40 field). The different heminthe species were identified using standard identification tables. The Participants were given anthelmintic drugs but what you see in lines 141-142 is an error. We are very aware of the Kato Katz method but it is a fact that formol ether concentration method is the best all round method for diagnosing intestinal helminths. 4. Approximately 200g of soil samples from a depth of about 2 cm in an area not exposed to direct sun light was collected using a small shovel. The parasite eggs were detected by sucrose solution floatation method as described previously by Uga et al (1989) and Rai <i>et al.</i> (2000).. Briefly, soil samples were dried overnight at room temperature and sifted through a 150mm mesh sieve. Approximately 2 g of powdery soil in a test tube was suspended in approximately 8.0 ml of Tween-20 solution (0.05%), centrifuged at 2000rpm for 10 minutes and the supernatant was discarded. The tube was again filled up, this time with sucrose solution (specific gravity 1.200), up to 5/6, vortexed, and centrifuged at 3000rpm for 10 minutes. The tube was then filled up to the top with sucrose solution so that only a small air bubble was formed when a coverslip was placed on the tube. The tube was then topped up with
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	15) line 140 thereon: as said before, what is meant by worm burden and epg?? The methods employed do not allow for standardized egg counts. 16) Table 6 (Line 164) reads in the title: number infected (%) in each age group; however, in the Table the number is in parenthesis and not the percentage. 17) line 166: in the methodology, soil was collected from 3 different sites: toilet area, community playground and schoolyard. Was kind of soil was it? Sandy? Humus? Silt? Which sites were more polluted? Were soils analyzed separately? How did they process more than 48 pounds of soil? Again, what was recovered? empty egg shells or were the eggs viable, or with a dead larva? 18) although not mentioned in text, Table 7 shows Hookworm (eggs?) collected from soil. Kindly explain. 19) for unfamiliar readers, please explain jetty toilet. 20) lines 156-162. The authors do not mention age group 0-5 and 6-12 y old in regards to infection. None of the members in that age groups were infected? 21-50 is a large age span, compared to 13-20 or 6-12. Any comments? How did the age groups differ among each other? (table 6) $p = 0.001$ in regards to what compared to what? 21) What about other parameters asked? Education? Healthy practices? 22) for the Discussion, how many times had the participating population been dewormed? ...despite more than a decade....(lines 59-61), what about exploring reinfection conditions more extensively? 23) line 195: Ascaris app? 24) line 198: what is the observed epidemiology of hkw? is that area endemic for hkw? Or sporadic only when it rains? Or increases during agricultural soil preparation? Promiscuous defecation around agricultural sites? Use of night soil?	more sucrose solution until it formed a meniscus. A cover slip was placed over the meniscus carefully without trapping air bubbles. The setup was allowed to stand for about 15 minutes after which the cover slip was lifted with the adherent drop of solution. It was placed on a clean glass slide and examined for parasite eggs under the 10X objective of the OLYMPUS CH light microscope. Samples for examination were prepared in duplicates. We collected 200 grams of soil from each site but we were processing 2 grams for each sample. . 5). Details have been included in the work. 6. Most of the eggs that were recovered from the study were viable eggs. 7. Most of the eggs on plate 1 were from stool samples but some were from the soil. 8. Corrections effected' 9. This is to indicate the interest of the participants ingoing to a medical centre when they notice any disease symptoms. 10. We discussed the fact that the data of intensity showed extream variability indicating the fact that STHs are typically over-dispersed in the population with a few individuals harbouring infections of high intensity. 11. who accepts a method for egg counts? You are just being unnecessarily bias and you do not want to learn. So if the kato Katz is no available there will be no egg counts? I have given you the method. Take decision because that is what we used. Instead of correcting the work, you spend your time criticizing. 12. Done. 13 Done
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	25) lines 203 to 209: not acceptable for grounds already explained. 26) lines 212-215: it was said before that deworming efforts have taken place during 10 years. So, children had been dewormed previously. Infections are endemic by the presence of infected individuals, adequate soil, rain and temperature conditions and frequent soil pollution. What is the real picture in the region examined? 27) again, line 232: to examine the soil for hkw larvae, one applies wet gauze pads to the soil and then recover larvae by a modified Baermann method. There should be no hkw eggs in soil, therefore these results are questionable. 28) the study has no conclusions. 29) References need a title. 30) References have to be carefully reviewed and corrected to the journal format. 31) Authors have to carefully review punctuation in the reference citing. There are extra period everywhere. In summary, I could gather that deworming has been taking place during more than 10 years without a previous baseline study to determine prevalence and intensity of STH; that most likely drugs have been administered to school children (not to school-age children) at undetermined periods and no follow-up evaluations or monitoring has taken place all that time. Therefore, it is likely that results of deworming efforts cannot be properly measured. Also, because deworming has been concentrated on children, neglecting an adult population that probably was infected in unknown percentage and age distribution, STH infections continue unchanged in the studied area of Cameroon. The authors discuss concentration of STH infection in adult population at the end of the MS, but conclusions are	14. done 15. An error. Corrected 16. Done 17. I am sure that the question has been answered before. It was top soil. We analysed the soils only following the villages. Accessibility to the area is very difficult. We are presently carrying out another work on soil contamination in the region specifically. 18. Eggs of hookworm are passed out in faeces and under favourable conditions they hatch in about 2 days. Jetty toilets are actually toilets with star cases (steps made of wood) that climb on top of pig fences. And so we could easily recover eggs of hookworms around the area that were freshly deposited. 19. done 20. It is said that details are on the table. <i>You</i> cannot say everything and then give a table. It is repetition. We tried to group the ages following pre primary school, Primary school, Secondary school. After that age, we have most of the inhabitants becoming farmers which is the 21-60 years age group and after that the strength reduces to do farming since most of the infections are from their activities. 21. Most Journal are not interested in results that are not significant and so when the results are not significant that factor is not important. 22. The school children are dewormed every 3 months even though some of the teachers may collect the drugs and sell instead of giving the children since the place is inaccessible to the government.. 23. Done.
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	missing. Although the purpose of the study was different, the authors could point out the futility of drug distribution without proper gathering of baseline data followed by monitoring and evaluations. Methodology is weak, both in stool and soil examinations description. Intensity of infection is unacceptable in base of the methods employed.	24. most of the hookworm infections are gotten at the beginning of the rains in early February which also marks the beginning of intensive farming activities. 25. That is your opinion because we have been using this method long for intensivity of infection and the results are quite good.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		