Effect of primary schools' sanitation and hygiene on soil-transmitted helminth infection: An approach for research in a rural district of KwaZulu-Natal, South Africa

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Abstract

Soil-transmitted helminths (STHs) cause some of the most prevalent infections worldwide, and are fundamentally associated with poor sanitation and hygiene conditions. The worm eggs require soil to develop into infectious stages, and soil contamination is thus the link between sanitation and infection. Children are particularly susceptible to STHs. In theory, it is likely that the sanitation, hygiene and soil contamination of STH in schools would be associated with the level of infection among the learners. This is however a severely neglected area of research, where the few studies conducted only to a limited extent have succeeded in providing evidence for an association. The Ugu District of KwaZulu-Natal (KZN), South Africa, is highly burdened with helminthiasis. It is a suggested study area as it already has data of the learners' level of infection available. This project sought to develop an approach for research of the association between toilet and hand washing facilities in primary schools and infection with *A. lumbricoides* and *T. trichiura* among the learners.

A method for evaluating the toilet and washing facilities is presented along with a method for the quantification of STH eggs in soil. The paper presents a relatively quick and inexpensive way to investigate the association between sanitation, hygiene, soil contamination and infection, in the setting of primary schools. The overwhelming extent of disease burden, the theoretical likelihood of an existing association and the considerable lack of research, is likely to make the study worthwhile, despite its several limitations.

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Introduction

Globally, over one billion individuals have been estimated to be infected with soil-transmitted helminths (STHs) (1), making these parasites responsible for some of the most prevalent infections of humankind (2). The most frequent infections are caused by roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura)* and hookworm (*Necator americanus* and *Ancylostoma duodenale*), where co-infestation with more than one of the species is common (3). STH infection is a disease of deprived communities in tropical and subtropical countries (3). It is fundamentally linked to poor sanitation and hygiene conditions as the parasite eggs must spread from feces to soil in order to become infectious. Kwa-Zulu Natal (KZN) is the third poorest province in South Africa (4). Results from a study on adult patients indicate that it has a high prevalence of *A. lumbricoides* and *T. trichiura*, and lower prevalence of the hookworm species (4). This project will thus only treat the former two species.

The burden of disease

Listed among the neglected tropical diseases as specified by the World Health Organization (WHO) (5), the STHs are "increasingly recognized as a significant public health problem, particularly in developing countries" (6). The general level of infection has however remained unacceptably high over the past twenty years, despite a substantial decline in some regions (1). The southern part of Sub-Saharan Africa has had a constant STH infection prevalence in this period, that combined with a growing population results in an increased infection count (1, 2). Hence, this population now has the greatest prevalence rate of trichuriasis (33,1%) in the world, while ascariasis remains less common (12,2%) (1).

Infestation with STHs result in a chronic infection as the parasites can live for several years in the human intestines (7). Although the worms cause thousands of deaths each year, the true burden of the disease is its morbidity rather than the mortality (8). It is estimated that more than 1,7 million years lived with disability (YLDs) are attributable to ascariasis and trichuriasis (1). The intensity of infection is a major predictor for morbidity (8). Heavy infections can cause upper (*A. lumbricoides*) or lower (*T. trichiura*) abdominal pain (3, 7, 8). In ascariasis, a high intensity infection can give partial bowel obstruction. Larvae may cause an eosinophilic inflammatory response, manifesting as asthma, or migrate to surrounding digestive organs or through the intestinal wall. Heavy trichuriasis can cause haemorrhages, mucopurolent bloody stools and symptoms of dysentery with rectal prolapse. Most infected individuals harbor just a few worms (8). Although the worm burden most frequently is insufficient to manifest as any of the above-mentioned severe symptoms, even light infections can have insidious effects on absorption and nutritional status (3, 7, 8).

A primary school problem

School-age children have the peak prevalence and intensity of both ascariasis and trichuriasis (6, 9), and "chronic infection negatively affects all aspects of a child's health, nutrition, cognitive development, learning, and educational access and achievement" (9). This makes STHs "one of the world's most important causes of physical and intellectual growth retardation" (3).

In their school, the learners come together in a large number on a daily basis, play in the soil, and are all exposed to the school's hygiene and sanitary conditions (10). The school environment thus correlates the exposure of the learners to one another. When there additionally is evidence to suggest a stronger association of sanitation with helminth infection in children than in the whole population (11), it seems credible that the sanitation in schools will have an effect on the learners' overall infection status.

Disease control

The means for reducing the extent of STH infections are a triad consisting of deworming with drugs, improved sanitation to reduce soil contamination and health education (12). STH infections can be treated safely and inexpensively with different antihelmintic agents (6). A systematic review and meta-analysis evaluating post-treatment follow-ups did however show that the prevalence of both *A. lumbricoides* and *T. trichiura* usually returns to levels close to the initial pre-treatment within a year (13). Hence, in the absence of sanitation, treatment must be repeated periodically (14). Health education is important as the availability of sanitation and hygiene facilities does not ensure that they are used competently or even at all. Hygiene behavior thus plays a modifying role of the facilities effectiveness in disease control.

Sanitation and hygiene: transmission barriers

STH infections are spread through fecal-oral transmission. Adult parasites inhabit the lumen of the gastrointestinal tract where they reproduce sexually (7). The transmission to a new individual (Figure 1) begins with the deposition of STH eggs from feces into warm and moist soil (9).

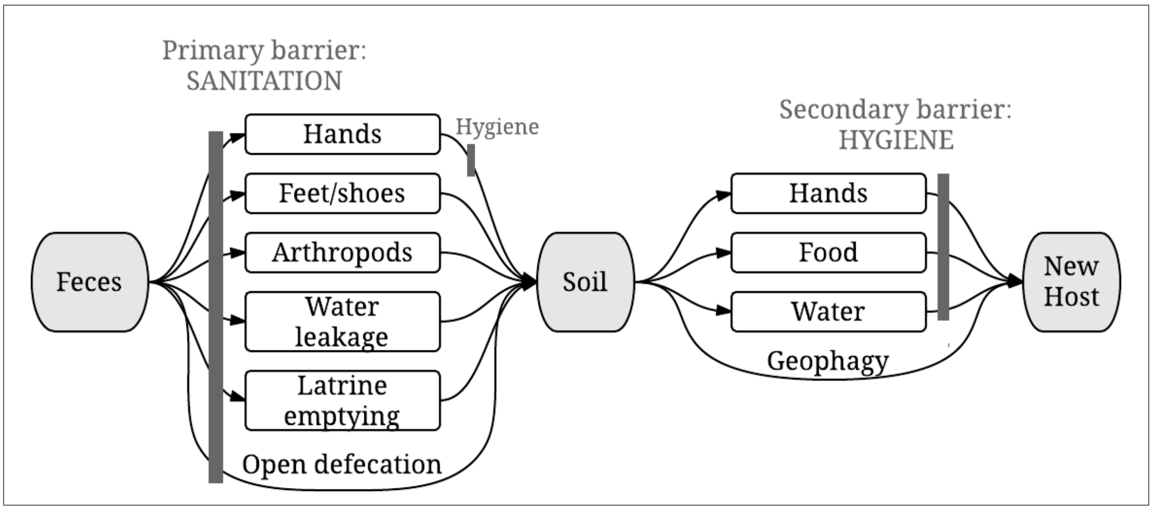

Figure 1 The transmission route for *A. lumbricoides* and *T. trichiura* (12, 14, 15).

If open defecation occurs, the eggs will be transferred to soil directly. Other possible transmission routes from feces to soil may be the individual's hands, feet or shoes (12, 14, 15). Depending on the type of latrine available, different arthropods may access the feces and act as a carrier for the eggs. Moreover, if the excreta is not contained adequately, it can leak into the surrounding soil. Finally, contamination will occur if the latrines are emptied while the eggs are still viable. The last step includes the use of latrine content as an agricultural fertilizer.

Adequate sanitation, being the safe disposal of human excreta, will act as a primary barrier to stop the transmission of eggs from feces to soil. An optimal toilet will not contaminate feet or shoes, limit the arthropods' access to feces, not cause any water leakage to surrounding soil and finally limit the need of latrine emptying. Toilets can thus be considered the most important component of the primary transmission barrier, even though other sanitation facilities and services can contribute. Types of toilets can roughly be divided into two groups: unimproved facilities that do not ensure safe disposal of human excreta and improved facilities that do (16). Inadequate sanitation facilities include the ordinary pit latrine, buckets and no toilets. Flush toilets, ventilated improved pit (VIP) latrines and composting toilets are considered as adequate (10, 16). There is "no credible evidence that the health of sanitation cannot be achieved by dry latrines, if they are properly built and maintained."(17), and the first step on the sanitation ladder from open defecation to fixed-location defecation makes the biggest change both socially and in terms of health (14).

The second part of the transmission route (Figure 1) is the ingestion of a matured egg by a host. As the STHs do not complete the entire reproduction cycle in the human host, each worm in a human body is a result of an infection event (11). The transfer of eggs from soil to humans can occur directly through geophagy, i.e. the practice of eating soil. Alternatively, the eggs can be transported by hands that have been in contact with contaminated soil, by vegetables grown in this soil or by drinking water from contaminated sources (12, 14, 15).

WHO defines hygiene as “conditions and practices that help to maintain health and prevent the spread of diseases”, and includes sanitation in this term (18). Hygiene measures, specifically hand washing before meals, adequate food preparation and safe drinking water, limits the transfer of infectious eggs from soil to new hosts. Hygiene will thus be a secondary barrier against STHs. Hand washing can in theory additionally limit the contamination of soil from hands, although this is likely to be a less important contributor to disease control.

Soil contamination

Finally, the discussed model for STH transmission shows that soil is the intermediate medium which links sanitation to infection. Investigations into the relationship between sanitation facilities and level of infection could thus benefit from a parallel assessment of soil contamination.

Objectives

This paper seeks to develop an approach for research of the association between toilet and hand washing facilities in primary schools and the infection with *A. lumbricoides* and *T. trichiura* among the learners, in the setting of a rural district of South Africa. The proposed study comprise three separate investigations which are [1] the correlation between sanitation/hygiene and infection, [2] the correlation between sanitation/hygiene and soil contamination and [3] the correlation between soil contamination and infection.

The needs for research: A review

It is a well-established fact that sanitation and hygiene facilities has fundamental health implications, including impact on the transmission of STH infections (17). It has however been noted that the opportunity to prevent the devastating extent of STH infections by improvement of these facilities, seems to lack the attention of the international public health community (14, 17). A systematic review and meta-analysis confirmed that the "availability of sanitation facilities was associated with significant protection against infection with soil-transmitted helminths"(11). However, the authors noted several aspects where research should be improved. Firstly, only two of the identified studies assessed the effect of sanitation on the intensity of infection, although this variable is directly related to morbidity and transmission rate. Secondly, most of the studies did not mention the type of facilities available or the coverage level of the facilities. Thirdly, it was noted that the toilet facilities were primarily assessed by questionnaires, but that this method is prone to bias and should be replaced by direct observations. The effects of hand washing on the prevalence of STH infections has been inconclusive, although use of soap showed to be protective against ascariasis with respect to prevalence (19).

The role of toilet and hand washing facilities in primary schools

Although there are several studies investigating the prevalence of STH infections in primary schools, research that evaluates the role of the schools' sanitation and hygiene upon this prevalence seems to be neglected.

Of the studies that do investigate this topic, a recent cluster-randomized trial in Kenya conducted a school-based water treatment, hygiene and sanitation intervention after deworming (20). The schools in the intervention group received among other improvements latrines to meet a set learner-latrine ratio. The overall intervention significantly reduced reinfection prevalence and intensity of *A. lumbricoides*, but this was only true for girls if the effect was considered for boys and girls separately. There was no significant evidence of a reduction in *T. trichiura* infections, although it was noted that the role of behaviors may have moderated the effect of the intervention. It is not possible to isolate the role of toilet and hand washing facilities from the data.

A cross-sectional study conducted among Pacific schoolchildren found that an inadequate water supply and absence of regular maintenance services were associated with helminthiasis

(21). Availability of excreta disposal, toilet paper, handbasins and soap did not show any significant association. Yet, the authors concluded that adequate toilet and hand washing facilities had to be made available to ensure basic public health.

Other descriptive studies have suggested poor sanitation in schools as a risk factor of STH infections among learners, but their objective has not been to provide evidence for this association (22-24). None of the studies found specifically investigates the type of toilet available or the overall standard of the facilities.

The role of soil contamination in primary schools

A study in Vietnam found that the soil surrounding the toilets was the soil in schools most contaminated with *A. lumbricoides* eggs (25). However, there is to the author's knowledge no study that explicitly investigates the association of sanitary conditions in schools with the concentration of helminth eggs in soil. Such an investigation could quantify different toilet facilities ability to act as a barrier against transmission.

The study in Vietnam also found a positive correlation between the contamination rate of soil surrounding the latrines and the prevalence of ascariasis among the learners (25). This suggests that assessment of soil contamination in schools can be used to estimate helminthiasis among learners in the absence of stool samples. An earlier study conducted in a village in Thailand equally found a set correlation between soil contamination by *A. lumbricoides* and *T. trichiura* around the domicile and infection of these parasites in household members (26). A Jamaican study from two children homes showed that the number of STH eggs in soil will quickly diminish when people living in the area is treated for the infections (27), all this indicating a strong relationship between soil contamination and helminthiasis.

Materials and methods

Study area and population

KwaZulu-Natal (KZN) is an eastern province of South Africa. When the South African Department of Basic Education in 2011 provided an overview of the education infrastructure in the country, they found that 51% of the ablution sites in the schools of KZN were inadequate, mainly constituing of ordinary pit larines (28). Furthermore 11% of the school sites did not have a water supply, while an additional 20% had a water supply that was unrealiable.

The study will be conducted in primary schools in the Ugu District, located in the coastal southern tip of KZN. This area has a 92% black population and approximately 48% of the population is younger than 20 years (29). In 2008 58% of households had an income levels below the Minimum Living Level (MLL), which is set where the lack of resources fail to meet the basic needs (29). This puts the level of poverty in the district above the average level of level of KZN, as well as the country. Sanitation backlogs in schools remain a major challenge (29). The former Port Shepstone health region, which comprised Ugu, was found to be the health region in KZN with the highest prevalence rate of ascariasis and second highest rate of trichuriasis (4).

Sample size

The study is nested in a larger study that has been conducted in 18 primary schools. This study will collect data from the same schools.

Investigations

Stool collection and analysis

Stool samples from learners were collected and analyzed in 2009-10. The data for the prevalence and intensity of infection with *A. lumbricoides* and *T. trichiura* will be used in this study.

Toilet and washing facility observation

The standards will be recorded as specified in the Appendix. The variables are modified from WHO's WASH (Water, Sanitation and Hygiene) guidelines for schools in low-cost settings (30) and an overview of sanitation technology for the control of intestinal helminths (31). The facilities for boys and girls will be assessed separately.

Soil sample collection

The toilets will be in the center of the soil sampling area. Two soil samples will be taken from each school: Sample A close to the toilet and Sample B five meters away. The total weight of either sample A or B should be 200-300 g. The sampling will be repeated if the toilets are separated for boys and girls. In situations where it is impossible to take a sample (toilets are inside the school building, they are surrounded by concrete instead of soil etc.), samples of sand or dust will be collected if possible. Soil from the school yard will additionally be sampled in such cases, as well as in schools where there is no toilet available. Due to possible physical obstacles and the need for an adapted sampling method for some schools, it is advisable to draw a map of each school and take pictures to document where the samples were actually taken.

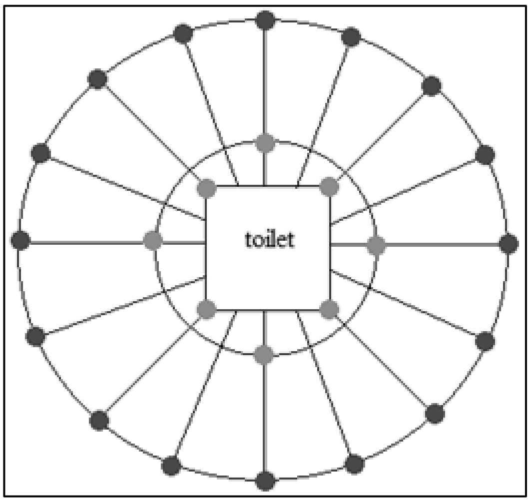

Figure 2 Spots for collecting the sub-samples of Sample A (grey) and Sample B (black)

For a soil sample to be representative for the distance away from the toilet, several sub-samples must be taken (Figure 2). Sample A should be collected from each corner of the toilet and 50 cm perpendicularly out from each wall. Sample B is constituted of 16 evenly distributed sub-samples taken with the aid of a five-meter rope. A sampling device made up of half a steel pipe and a garden hose will be used to collect soil from the surface to 5 cm down. If there is a small obstacle (a stone, a tree etc.) in the spot where a sub-sample should be collected, the sample will be taken directly to the left of the obstacle. The samples can be stored in a refrigerator in the laboratory until the conduction of the soil analysis.

Soil analysis

The soil analysis must be performed for Sample A and Sample B separately (Figure 3). Each round of analysis includes two steps: The quantification of eggs in soil (Sample 1) and the determination of the soil's dry weight (Sample 2). Sample 1 and 2 should be weighed out subsequently with no major delay to avoid evaporation of water from either sample.

Plant material, gravel etc. that might be in the sample, should be removed before the analysis. It is important that a sample is mixed thoroughly, as only a portion of it will be analyzed.

Approximately 5 grams of soil must be weighed out three times (total of 15 grams) and transferred to centrifuge tubes (Figure 4). The exact total weight of the soil is recorded as *g soil Sample 1*.

A. lumbricoides eggs will normally stick together when in contact. The eggs will be separated when the outer layer is altered by leaving the soil in a 0,5 M NaOH solution in the fridge (5˚) for 16-18 hours (Figure 5). It is important that the treatment with NaOH does not exceed this amount of time, as the eggs will then dissolve. The tubes will be centrifuged for 7 min at 1200 RPM, and the supernatant removed (Figure 6).

The pellet will be subsequently mixed with flotation fluid (400 g of Epsom salt ($MgSO_4$) in 1 liter of water) and centrifuged for 7 min at 1200 RPM. The supernatant, now containing the STH eggs, should be collected in a beaker. This procedure is repeated once. The lids of the centrifuge tubes may contain eggs, and it is important to rinse these using a wash bottle (Figure 7).

The content of the beaker will be sieved through a 100 µm sieve (catching larger particles) and then directly through a 20 µm sieve (catching the STH eggs) (Figure 8). The content of the 20 µm sieve will have to be rinsed thoroughly with (ideally sterilized) water as the eggs cannot survive in the flotation fluid for more then 30-45 min (Figure 9). The content of the 20 µm sieve will be transferred to centrifuge tubes and centrifuged for 7 min at 1200 RPM. The supernatant can be discarded.

Finally, the pellet should be resuspended in a few drops of water, and the liquid transferred to as many microscope slides as necessary. The slides will be examined microscopically with the 10x lens for the presence of eggs, and the 40x lens to determine the species found. The number of *A. lumbricoides* and *T. trichiura* eggs will be recorded separately as *number of eggs Sample 1*. (Figure 10)

The dry weight of the soil is determined as the water content of the different soil samples may vary. After the distribution of the soil into centrifuge tubes the remaining soil will be weighed and recorded as *g wet soil Sample 2*. The sample is then left to dry in an oven at 105 °C until constant weight is achieved, normally within 24 hours. The *g dry soil Sample 2* is finally recorded.

The number of eggs per gram of soil can then be calculated:

$$\frac{\text{Eggs}}{\text{g soil}} = \frac{\text{Number of eggs Sample 1 x g wet soil Sample 2}}{\text{g soil Sample 1 x g dry soil Sample 2}}$$

Ethical issues

Ethical clearance is obtained from the Biomedical Research Ethics Administration (BREC) of the University of KZN. Each school will receive a letter that gives information about the objectives and nature of the study, as well as contact details to the research group involved. The school's principal will have to give written permission before the collection of data can begin. The schools will be assigned identity numbers and all information about the schools will be confidential. Soil samples will be limited to the amount of soil required. The researcher will use protective gloves throughout the collection and analysis of the soil, and the work bench will be covered with disposable sheets. The soil will be disposed as biological waste after the completion of the analysis.

Statistical plan

The analyses will be performed using SPSS. 95% confidence intervals will be calculated and p-values $\leq 0{,}05$ will signify an association.

Analysis plan

This is an observational study with an ecological nature as both exposures and outcomes are measured on a group level, and the schools will be compared against each other.

Completing the data collection, the researcher will be left with three types of variables measured in each school (Table 1). The variables for assessing the quality of the toilet and hand washing facilities (variable A-O) are qualitative with a rank order as specified in the Appendix. Some of them can be further divided to represent boys and girls separately. The variables for the concentration and distribution of eggs in the soil (variable P-S) and for the prevalence and intensity of infection in learners (variable T-W) are all quantitative.

Variables for assessing the quality of the toilet and hand washing facilities	
A	Distance between school building and toilet facilities
B	Numbers of cubicles and urinals / number of learners
C	Type of toilet facilities
D	Toilet paper availability
E	Privacy
F	Toilet lid availability
G	Cleanness
H	Odor
I	Insects
J	Distance between toilet facilities and washing facilities
K	Number of hand washing units / number of cubicles
L	Number of hand washing units / number of learners
M	Water availability
N	Soap availability
O	Drying facility availability
Variables for the concentration and distribution of eggs in the soil	
P	Concentration of *A. lumbricoides* eggs in the soil close to the toilets
Q	Concentration of *A. lumbricoides* eggs in the soil far from the toilets
R	Concentration of *T. trichiura* eggs in the soil close to the toilets
S	Concentration of *T. trichiura* eggs in the soil far from the toilets
Variables for the prevalence and intensity of infection in learners*	
T	Prevalence of *A. lumbricoides* in learners
U	Intensity of infection of *A. lumbricoides* in learners
V	Prevalence of *T. trichiura* in learners
W	Intensity of infection of *T. trichiura* in learners

Table 1 Variables that will be measured in the data collection of each school. *Data were collected in 2009-10.

The proposed study does in reality comprise three separate investigations which are [1] the correlation between sanitation/hygiene and infection, [2] the correlation between sanitation/hygiene and soil contamination and [3] the correlation between soil contamination and infection. Comparing any variable from one of the three groups to a variable from another group will address one of these correlations. The assessment of the association between a quantitative and a qualitative variable could be done by a 2x2 contingency table where each variable is divided in two. A chi-squared test should then be applied. Regression analysis could be used when comparing two variables that are both quantitative. The relationship between toilet facilities and soil contamination can be further explored by testing for a significant difference in concentrations of eggs in the soil close and further away from the toilet.

Discussion

Primary schools' sanitation and hygiene in relation to soil-transmitted helminths were in the presented review found to be a much neglected area of research. Moreover, the studies conducted have to a limited extent been able to document an association. A few studies were found to investigate the extent to which school soil acts as a link between sanitation and infection, but this topic is also in need of further exploration.

The study described in this paper approaches the indicated gaps of research. As ecological studies in general, the methods propose a relatively quick and inexpensive way to investigate the topic in question. Furthermore, the study does to a very little extent interfere with the schools and the learners. It treats both prevalence and intensity of infection. It will provide data of the overall standard of the facilities, not simply its availability, and this will be assessed by direct observation.

Limitations

The outlined study uses data of learners' infection status from 2009-10. This means that the exposure (standard of facilities and soil contamination) is measured years after the outcome (infection level) has been established. Depending on how far into the future the data collection will be conducted, the researcher should consider to simultaneously recollect data for the prevalence and intensity of infection among learners. This would however make the study considerably more substantial and involve direct contact with the learners. If a new data collection is not conducted, it is advisable to gather information on possible changes made since 2009-10, and take these changes into account in the analyses. This can be done by developing a questionnaire to be answered by the schools' administrative staff. Changes in the composition of students (number, age, sex), advancements in sanitation and hygiene services and practices, as well as any major development in the surrounding community should be noted. Schools where substantial changes have taken place must be excluded from the analyses.

The STH eggs in soil are known to aggregate in hotspots such as paths. The method for soil sampling tries to take this into account by systematically taking several evenly distributed sub-samples, in order to find the average concentration of the eggs around the toilet. However, the number of sub-samples may show to be inadequate, where unintentionally sampling from certain hotspots will have a great influence on the calculated concentration.

Moreover, the presented method suggests analyzing only a portion of the soil collected in order to save time. This will again increase the chance of analyzing soil that in fact is not representable for the soil at a certain distance from the toilet.

There are several characteristics of the soil that may have an influence on the STH eggs. The soils physical and chemical characteristics are not measured in this study, and can thus not be adjusted for in the analyses. The researcher should aim at sampling soil during the same season as the learners' stools were collected, as this will reduce the differences in these characteristics between the two data collections. However, the STH eggs are known to be extremely resistant, and may not be greatly affected by the existing differences in soil properties. In addition, the study does not record the developmental stages of the eggs found, so that the survival of the eggs is the only factor that would influence the results.

The number of schools planned to be investigated in this study, may be too few to draw any conclusions. If only 18 schools are investigated, cells in the contingency tables of the analysis will easily be sparsely populated, thus violating the contingency table analysis assumptions (32). Then alternative statistical methods will have to be applied. The data already collected showed no statistical significant difference in level of infection among the schools. The greater part of the primary schools in Ugu empirically has quite similar standards of their toilet and hand washing facilities. When there is neither any major contrast in the level of exposure nor in the measured outcome, the number of schools included in the study needs to be accordingly high to statistically document a correlation.

The methods for collecting data presented in this paper will result in a great number of different variables. Preforming all the possible statistical analyses will give a high risk of incorrectly establishing a correlation between some of the variables, i.e. committing a type I error. It is therefore advised to limit the number of comparisons being made. One way to do this could be to score the variables assessing the toilet and hygiene facilities according to their rank, giving different number of points for different criteria. Then the derived variable representing the overall standard of the facilities could be compared to the concentration of eggs in soil and the infection level among learners. However, this method adds the challenge of denoting maximum number of points and hence importance to each variable. After all this is to some extent what the outlined study seeks to establish. Perhaps it will be possible to develop such a system after the collection of data, but this will put the researcher at risk of manipulating the scoring system as to fit the hypothesis being tested. Alternatively, the number of variables representing the standard of the toilet and hand washing facilities could be reduced. For instance, the focus could be solely on investigating the type of toilets at the school or the toilet per learner ratio.

The introduction showed that toilet and hand washing facilities constitute a great part of the barriers against *A. lumbricoides* and *T. trichiura*. The rest of the barrier in the school, including safe drinking water and food preparation, will be confounding factors in the study. The importance of hygiene behavior must additionally be acknowledged, although the proposed study does not provide means to control for its influence. Also, even though the study will provide data for the standard of the hand washing facilities, it does not investigate the infestation of eggs on the learners' hands. The effect of the hand washing facilities will therefore only be measured indirectly.

All exposures to infectious STH eggs outside the school will be other important confounding variables. This includes the level of sanitation and hygiene in the learners' homes. The extent of poverty in the school and in the catchment community is however correlated, and a study investigating both sanitation in schools and in the surroundings noted that the environmental influences of the two arenas in most cases were similar (21). The study outlined in this paper would nevertheless benefit from an overview of the sanitation and hygiene situation in the learners' households.

The study proposes a design where the exposure to a group of learners is compared to the general level of infection among them. In the interpretation of the results, it is important to avoid committing an ecological fallacy where the documented relationship between sanitation, hygiene and infection on group level is uncritically applied to a learner as an individual. It is noted that such a study design in general has a low level of evidence as we do not know if the individuals that constitute the group in fact were exposed to the measured variable (33). However, in this particular case it must be safe to assume that the learners are in contact with the toilet and hand washing facilities at their school, and they are certainly to some extent in contact with the school's soil. Furthermore, ecological variables play an essential role in the transmission of infectious diseases, and it may be productive to investigate the causes of infection on a group level. The real problem with conducting the study exclusively on a group level is however the already mentioned difficulty in adjusting for individual factors, such as exposure at home.

Finally, the direction of the casual relation between soil contamination and level of infection will be ambiguous (26). A high level of infection among learners will generally to a greater extent contaminate the soil than if the infection level is low. The other way around, will extensive soil contamination by helminth eggs unquestionably yield a higher level of infection than what a lower contamination level would cause.

Impact and further research

There are several limitations to the proposed study that might make it impossible to draw any absolute conclusion from its results. However, the association between sanitation, hygiene, soil and infection in schools is shown to be a neglected research area of what is already a neglected tropical disease. This combined with the theoretical likelihood of an association and the deleterious effects the disease has on children, a mere indication of an association might also be considered valuable.

South Africa did in November 2013 publish nationwide legally binding standards for school infrastructure, including the access to water and the safe disposal of human excreta (34). While this is a crucial step in the right direction, the rural parts of the country have a long way to go to implement these new standards. They could therefore still benefit from more evidence for their advatages.

The outlined study does in reality consist of three separate investigations, where any one of them could be conducted exclusively to limit the scope of the investigation. On the other hand, the study design could be expanded in order to better control confounding variables. Soil samples from the school yard, observations of hygiene behavior or evaluation of the sanitation in the catchment area of the schools would for instance benefit the proposed investigations. The study design might be used in other endemic sites, and is not limited to the Ugu District. The possibilities for research in this field are numerous, and the hope is that the proposed study could draw attention to the issue and encourage further research.

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Figures

Figure 3 Sample A and B

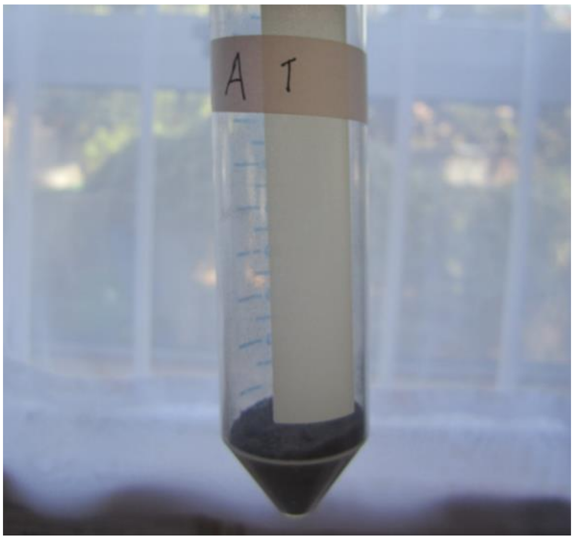

Figure 4 A 50 ml centrifuge tube with 5 grams of soil

Figure 5 Soil mixed with 0,5 M NaOH

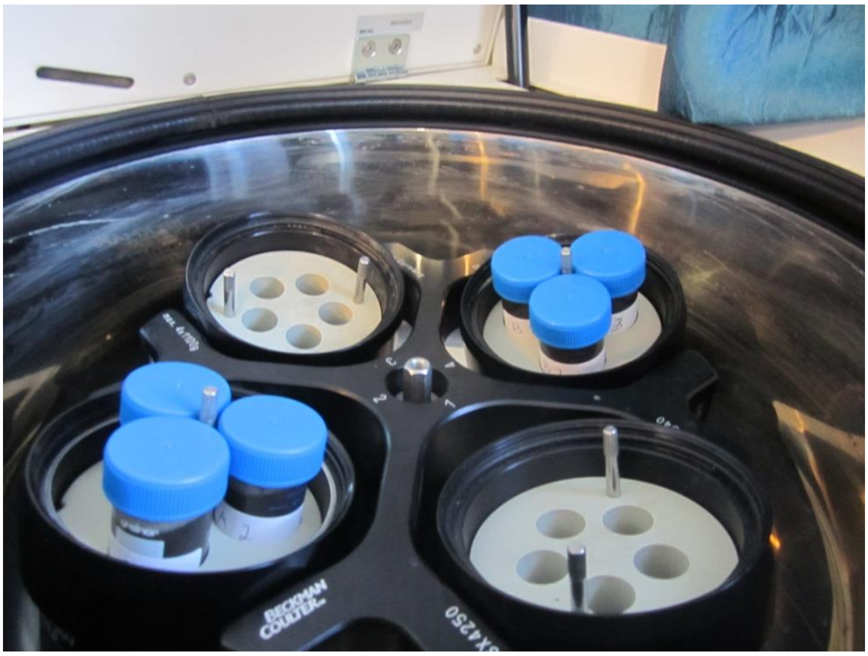

Figure 6 A centrifuge with a maximum capacity of six 50 ml tubes

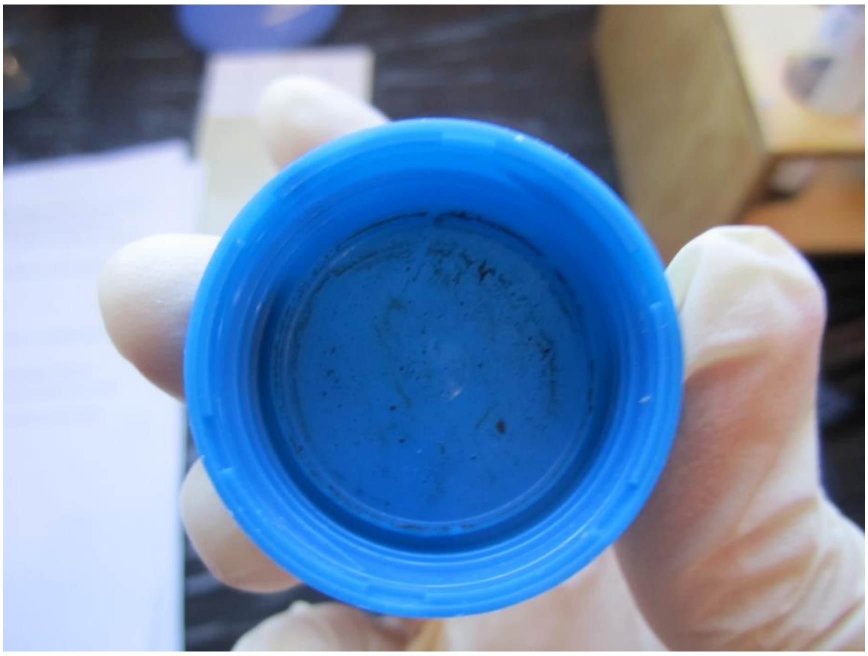

Figure 7 A centrifuge tube lid after centrifugation with flotation fluid. The eggs will flotate and can stick to the lid.

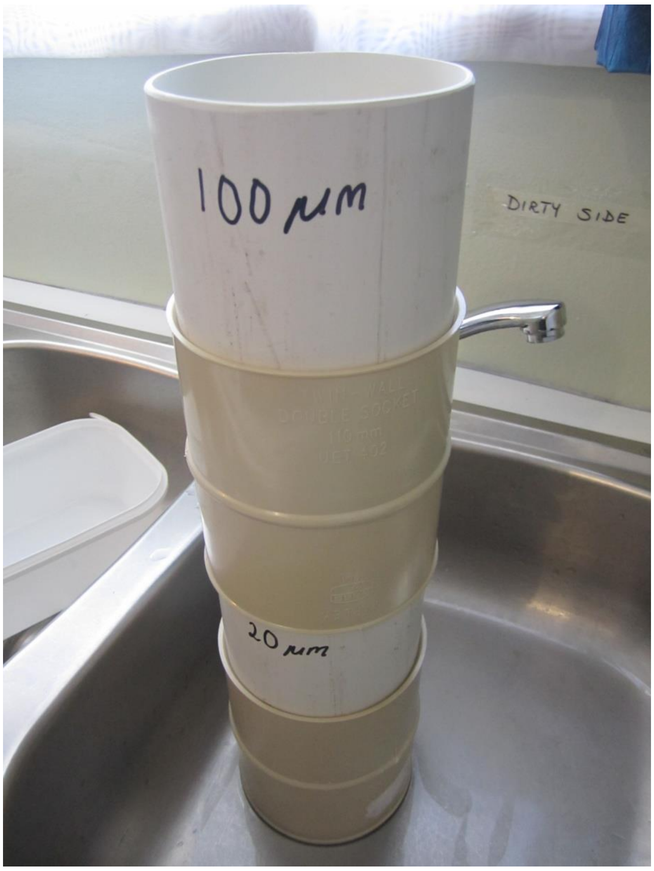

Figure 8 The 100 µm sieve on top of the 20 µm sieve allowing the researcher to use both sieves simultaneously.


Figure 9 Washing of the sieves

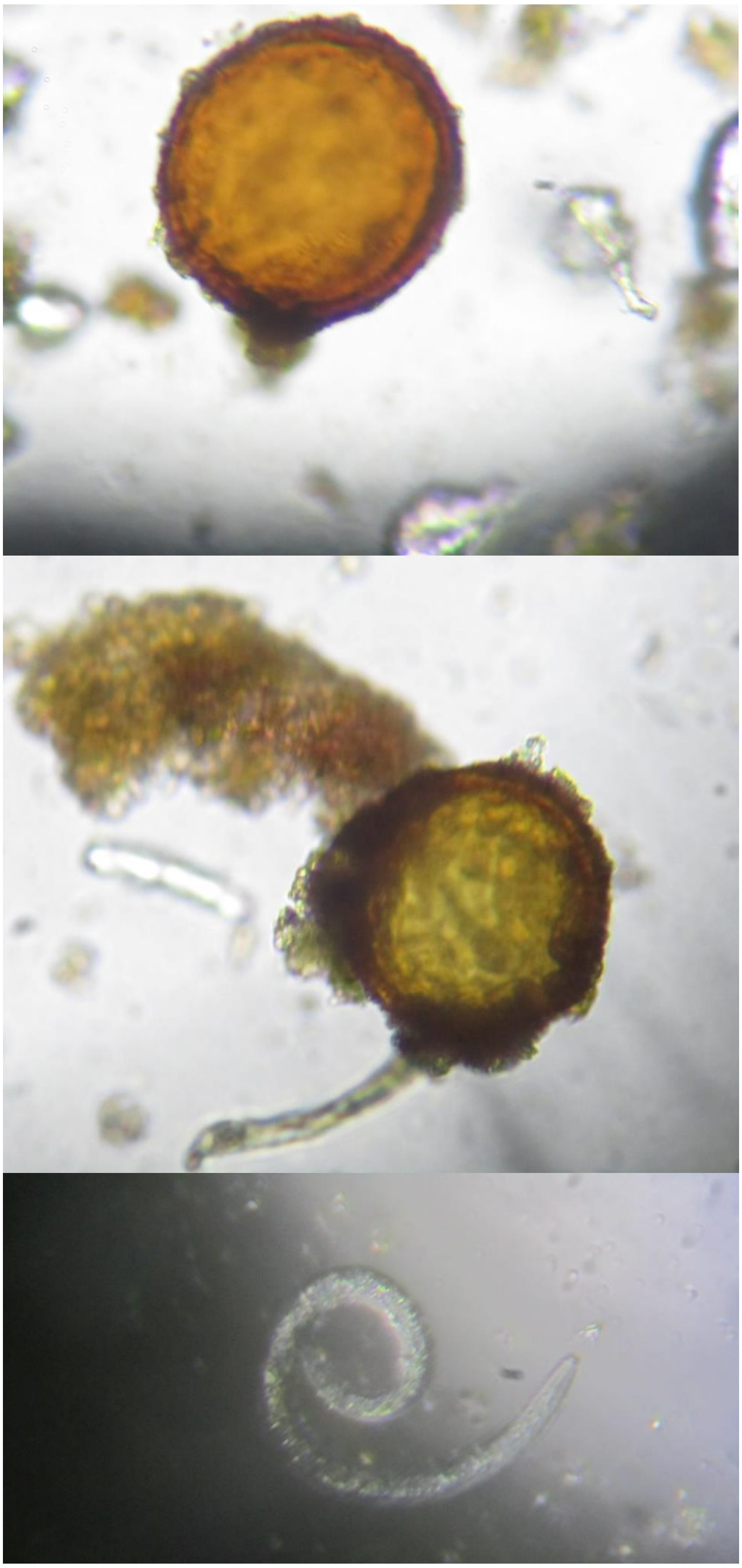

Figure 10 Pictures taken through the microscope at 40x magnification during a run-through of the outlined soil analysis. The species are not determined.

Appendix

Name of school ______________________________

	Boys	Girls	Total
Enrolment			
Number of cubicles and urinals			
Number of hand washing units			

	Variable		Comments
A	Distance between school building and toilet facilities		
B	Number of cubicles and urinals / number of learners (Fill in later)		
C	Type of toilet system		
1	Off-site wet flush toilet system		
2	Ventilated improved pit (VIP) latrine		
3	Compost toilet, Enviro loo		
4	Pour-flush toilet, Septic tank and soakaway, Aqua privy		
5	Pit latrine		
6	Bucket		
7	None or none working		

D	Toilet paper available		
1	Yes		
2	No		
E	Privacy		
1	Door with lock		
2	Door without lock		
3	No door, turned towards private area		
4	No door, turned towards common area		
5	No walls		
F	Toilet lid available		
1	Yes		
2	No		
G	Cleanness		
1	Clean		
2	Mediocre		
3	Dirty		
H	Odor		
1	None		
2	Light		
3	Heavy		
I	Insects		
J	Distance between toilet facilities and washing facilities		
K	Number of hand washing units/ number of cubicles (Fill in later)		
L	Number of hand washing units/ number (Fill in later)		

M	Water availability		
1	Hot water		
2	Cold water		
3	None		
N	Soap availability		
1	Dispenser		
2	Bar		
3	None		
O	Drying facility		
1	Disposable paper or hand dryer		
2	Cloth towel		
3	None		