

Intestinal Parasitic Infections in Elementary Schools Children at Dilla Town and its Peripheral Villages

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Background and study aim: There is no information established about the diversity of human intestinal parasites at Dilla Town & its peripheral villages. The existence of various species of intestinal parasites in tropical and subtropical regions with moist and warm climate is expected to be high. Dilla Town and its peripheral villages is found in the tropics. The objective of this study was to establish a statistically verified status about the diversity of human pathogenic intestinal parasites at Dilla Town and its peripheral villages.

Patients and methods: Stools samples of 710 student children were collected in 0.85% saline solution from elementary schools and observed under microscope in the laboratory of parasitology. Microphoto-graphs of the diagnostic stages of the parasites found were taken with a digital camera from the fields of vision of the microscope.

Results: Out of 710 student children examined 257,225,200,170,107,61,40,9,8,

8 and 4 were positive, the corresponding infection rates being 36%, 32%, 28%, 24%, 15%, 9%, 6%, 1%, 1%, 1%, and 0.6% of *Ascaris lumbricoides*, *Entameba histolytica*, *Giardia lamblia*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Hookworms*, *Schistosoma mansoni*, *Taenia saginata*, *Enterobius vermicularis*, *Hymenolepis nana*, and *Fasciola hepatica* respectively. The infected children were given curative treatment.

Conclusion: Preventive measures against parasitic infections must be implemented by focusing on:

- Blocking transmission of infective stages,
- Delivering public health education,
- Providing the public with clean water supply for domestic use & drinking,
- Improving personal hygiene and environmental sanitation, and
- Identifying and treating infected individuals to prevent the spread of infections.

INTRODUCTION

Intestinal parasitic infections represent one of the most important infections that cause the global health problem of humans [1-4]. The sources of parasitic infections for man are poor personal hygiene, poor sanitation of the environment such as contamination of soil and water supplies with human excrement, wrong methods of sewage disposal, the parasites that adhere to edible vegetables and fruits, fingers, utensils, door handles, money, dust, flies that rest on food materials, reservoir hosts, and contaminated fingernails. It had been demonstrated that in the rural area of Cote d'Ivoire two children

were found infected with several intestinal parasites at the same time where one of them was infected with 8 and the other child had been harboring 10 different intestinal parasite species [5-9]. Gastrointestinal parasites of man persist to exist, causing morbidity and mortality in less developed tropical countries of the world. Parasitic infections are enhanced by poor personal hygiene, ignorance, poverty, poor environmental sanitation, and by warm and moist climatic conditions [10-13]. The infective filariform larvae and cercariae have the ability to pierce intact human skin and get into the blood stream [14].

PATIENTS AND METHODS

Student children of 8 different elementary schools at Dilla Town and its peripheral villages were the sites of sample taking. The specific study sites included: -Walame Don Bosco, Meskele Eyesus, Mekane Yesus, Dawit, Kofe, Dilla, Chichu, and Haroresa Elementary Schools. The total sample size of 710 student children was decided to be examined for the suspected intestinal parasites of man. The samples taken from student children of the schools cited above were fresh stools. The task of this survey with a high response rate was executed by way of randomized diagnostic tests. Collection of stools samples and documenting related information from the schools had been carried out repeatedly in 3 different durations from:

- 6/12/2006 to 10/6/2007,
- 10/9/2008 to 25/6/2009, and
- 4/11/2011 to 25/6/2012.

However, the deliberate follow up to be certain about the prevalence and persistence of the intestinal parasites of man at Dilla Town and its peripheral villages, was performed by taking fresh stools samples of ten students from each of the eight different elementary schools once every year from 2000 up to towards the beginning of 2014.

The statistics selected, being relevant to interpret and analyse the results of this study project were:- *histogram* and *correlation coefficient*. The fresh stools sample of each student child was examined with a compound light microscope at three stages:

- Direct wet mount,
- Concentration technique, and
- Permanent stained preparation.

Procedure

Direct wet mount:

- About 2.5 ml of fresh stools sample was taken in a small vial from each student of the schools selected. Immediately after that, 0.85% NaCl solution in distilled water and warmed to 37°C was added to each vial of fresh stools sample taken. Then, 1 drop of 0.85% warm (37°C) aqueous NaCl (saline solution) was placed on a clean slide.
- Following that, about 1 drop of the stools specimen (from that of any single student) was added to the slide and mixed with the drop of NaCl solution.
- The saline wet mount was covered with a cover slip and examined under a suitable

objective lens. This procedure of using warm saline solution was to allow determining the motility and gross morphology of trophozoites [11,15]. In the mean time, care was taken not to allow the sample on the slide to dry or cool; otherwise, the motility of trophozoites could have ceased. The stools specimen of any particular student child who was positive for the suspected parasites was preserved in 5% formalin (for protozoans) or in 10% formalin (for nonprotozoan parasites) to be used in the stages of Concentration Technique and Examination of Permanent Stained Preparation. The stools specimen of each student child was prepared, observed, and preserved exactly in this way.

Concentration technique:

- Involved concentrating the number of the diagnostic stages of the suspected parasites, primarily of cysts, eggs or larvae in the stool specimen that was collected and preserved in 5% or 10% formalin. These concentrated and preserved specimens were part of the complete examination and allowed the detection of small numbers of the parasites that could have been difficult. In order to concentrate the number of the diagnostic stages of the parasites, diethyl-ether had been mixed with the suspension of the stool sample. Then, the speed and concentration time were set at 1000 rpm for 2 minutes.
- Next, the parasites particularly the cysts, eggs, or larvae were expected to sediment at the bottom of the centrifuge tube and the floating stools debris was discarded.

Examination of permanent stained preparations:

- Detection and identification of intestinal protozoan and helminth parasites preserved in 5% or 10% formalin respectively would depend on the examination of a permanently stained smears under the oil immersion objective lens.
- These stained slides would provide a permanent record of the suspected intestinal parasites of man.
- The identifications in the stages (steps) of Direct Wet Mount and Concentration Technique would be tentative until confirmed by the permanent stained slide.
- The staining was with Safranin.
- About 3 drops of Safranin solution was added to the stools specimen suspension preserved in

formalin in a bottle of about 50 ml and waited for about 6 hours to get the diagnostic stages of the parasites stained [16,17].

- A drop of Yetwin Mounting Medium melted at 65⁰ C was placed on a clean slide, then on this drop of mounting medium, a drop of the stools specimen preserved in formalin and stained with Safranin was added and mixed well with the tip of a needle [18]. Next, the specimen was covered with a coverslip and left on a table for about 24 hours to let the mounting medium solidify and harden.
- Thereafter, the specimen in the hardened mounting medium was examined under the oil immersion objective lens to check the presence of the suspected intestinal parasites of man.

Additional methods:

- Baermann apparatus technique was employed for the diagnostic tests of *S. stercoralis* [19,20]. Here, the Low or Middle Power objective lens was used in observing under the compound light microscope.
- The suspensions of stools samples were made more diluted and thinner for the diagnostic examination of protozoan parasites than for those of helminths. In this case, High Power objective lens was used in observing under the compound light microscope.
- In all methods of this paper, from all fields of vision (i.e., low power-oil immersion) of the microscope, microphotographs of the parasites diagnosed were taken using a digital camera and transferred to a computer for further processing.

Treatment:

- Prescriptions and clinical supervision in the treatment of infected student children had been performed by an authorized medical doctor (i.e., Dr. Corazon B. JACA, FMA).
- Student children of 9 years and above in age were provided with and advised to take metronidazole 250 mg 1 capsule 3 times, i.e., after breakfast, lunch, and dinner daily for 7 consecutive days against *E. histolytica* or /and *G. lamblia* infections. On the other hand, student children of 8 years & below in age were provided with and advised to take metronidazole oral suspension 5 ml (equivalent to metronidazole 250 mg 1 capsule) 3 times, i.e., 5 ml after each of the three meals, daily for 7 consecutive days against *E. histolytica* or /and *G. lamblia* infections.

- Albendazole 400 mg 1 tablet single dose on empty stomach was prescribed against infections with intestinal parasitic helminths excluding *S. stercoralis*.
- Student children infected with *S. stercoralis* were given and advised to take albendazole 400 mg 1 tablet once immediately before going to bed for night sleep daily for 3 consecutive days.

RESULTS

The list of the most important diagnostic stages of intestinal parasites represented the actual parasitic protozoan and helminth species that were experimentally observed and recorded. This was done by way of randomized diagnostic tests. Out of 710 student children examined, the exact numbers of infected ones and the corresponding infection rates with each specific species of the pathogenic intestinal parasites was directly recorded. The analysis by the statistic of *correlation coefficient* had proved that the relation among the intestinal parasites investigated, due to various factors, in this study was very strong. The relation was nearly a perfect correlation, i. e., ± 1 . In this particular study the computed value of correlation coefficient was almost a perfect positive correlation (+1), because it was 0.99984, whereas the tabulated critical values are 0.754 & 0.874 at $\alpha = 0.05$, and $\alpha = 0.01$ levels of significance respectively for 5 degrees of freedom. The unprocessed raw data in the form of variables “X” and “Y” for Fig. 2 was that of Table 1.

Meaningfulness of applying the statistic of correlation can be imparted by citing several pairs or sets of paired variables that are appropriate to be explained using *correlation coefficient* as follows:

- educational qualification and salary, = stands for one pair of variables.
- money in the bank and interest,
- family size and weekly expenditures,
- property owned and taxes paid,
- hours of study and grades obtained,
- the time it takes a body to fall and the distance it falls,
- the price of steel and the quantity of it sold,
- married males and their incomes,
- carrot lengths and their maximum diameters,
- average industrial stocks and money supply, etc.

Example :

X	2	4	6	8	10
Y	1	2	3	4	5

Five sets of paired variables

The paired variables used in the statistic of **correlation coefficient** employed in this paper were:

- The number of students positive for *A. lumbricoides* and the infection rate of *A. lumbricoides*,
- The number of students positive for *E. histolytica* and the infection rate of *E. histolytica*,
- The number of students positive for *G. lamblia* and the infection rate of *G. lamblia*,
- The number of students positive for *S. stercoralis* and the infection rate of *S. stercoralis*,
- The number of students positive for *T. trichiura* and the infection rate of *T. trichiura*,
- The number of students positive for hookworms and the infection rate of hookworms,
- The number of students positive for *S. mansoni* and the infection rate of *S. mansoni*.

What were measured in the correlation coefficient were the relationship among the paired variables of the parasites and not the relationship of the number of infected students and the quantity of percentages. Each of the seven parasites produced only one pair. For example, the pair produced by *G. lamblia* consisted of the variables 200 and 28%.

X	257	225	200	170	107	61	40
Y	36%	32%	28%	24%	15%	9%	6%

Seven sets of paired variables
(of the seven parasites)

The percentile infection rate of each species of the intestinal parasites in the population of student children, were depicted in the statistic of histogram for impartive and practically meaningful impression.

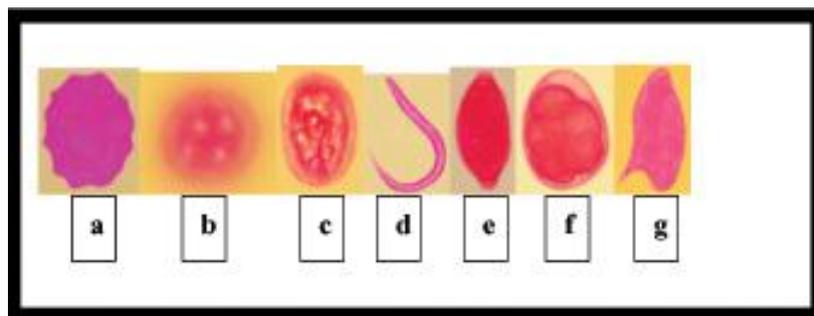


Figure (1): The list of of the most important diagnostic stages of intestinal parasites isolated from fresh stools samples of children at Dilla Town and its peripheral villages.

(a) *Ascaris lumbricoides* egg, magn†. X640; (b) *Entameba histolytica* cyst, magn. X640; (c) *Giardia lamblia* cyst, magn. X1600; (d) *Strongyloides stercoralis* rhabditiform larva, magn. X640; (e) *Trichuris trichiura* egg, magn. X640; (f) Hookworm egg, magn. X640; (g) *Schistosoma mansoni* egg, mag. X640.

Each of these seven pictures was colored using a computer Adobe Photoshop

•CS and transformed from its original magnified size to the resolution of 1200 pixels/inch with the quality of 12 (maximum) and large file compatible with A4 page format.

†magn. Stands for the term magnification that gives the size of an image and serves to explain how many times the actual size of the specimen was magnified to get that particular size of the image.

Table (1): The list of human intestinal parasites isolated from fresh stools samples of children at Dilla Town and its peripheral villages.

The parasites found, as a sample size n	No. of student children positive for the parasite out of 710 students examined, as a variable X	Infection rate in percentage, as a variable Y
1. <i>Ascaris lumbricoides</i>	257	36%
2. <i>Entameba histolytica</i>	225	32%
3. <i>Giardia lamblia</i>	200	28%
4. <i>Strongyloides stercoralis</i>	170	24%
5. <i>Trichuris trichiura</i>	107	15%
6. Hookworms	61	9%
7. <i>Schistosoma mansoni</i>	40	6%

$$\begin{aligned}
 r &= \frac{n\sum XY - \sum X \sum Y}{\sqrt{n\sum X^2 - (\sum X)^2} \sqrt{n\sum Y^2 - (\sum Y)^2}} \\
 &= \frac{7(28526) - 159000}{\sqrt{7(202344) - 1123600} \sqrt{7(4022) - 22500}} \\
 &= \frac{199682 - 159000}{\sqrt{1416408 - 1123600} \sqrt{28154 - 22500}} \\
 &= \frac{40682}{\sqrt{292808} \sqrt{5654}} \\
 &= \frac{40682}{541.12 \times 75.193} \\
 &= \frac{40682}{40688.44} \\
 &= \underline{0.99984}
 \end{aligned}$$

Figure (2): Correlation coefficient, r^{\neq} , depicting the same environment of Dilla Town and its peripheral villages being favorable for the variety of human intestinal parasites. \neq represents or stands as a symbol for the term “correlation coefficient”.

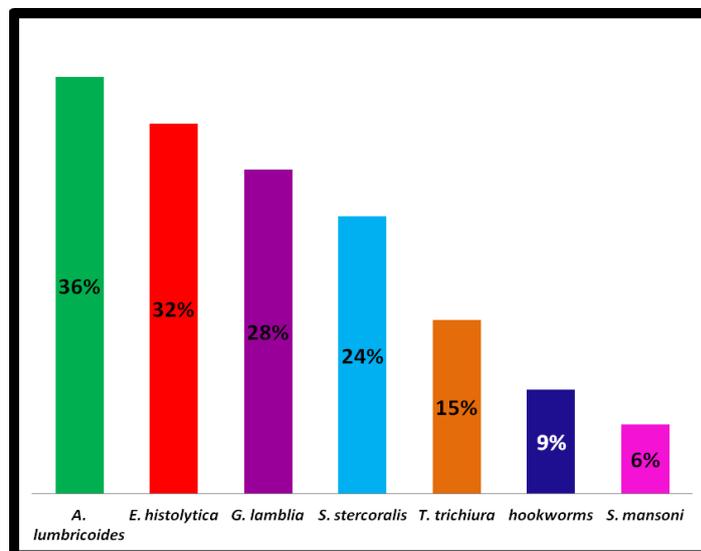


Figure (3): The **Statistic of Histogram**, demonstrating the list of different species of the intestinal parasites isolated from fresh stools samples of student children and the percentile infection rate in the children with each of these parasitic species.

DISCUSSION

The calculated *correlation coefficient*, or "r" value was greater than the critical values at both $\alpha = 0.05$ and $\alpha = 0.01$ levels of significance. Thus, the computed value was significant and demonstrated a very strong evidence for the fact that the same environment, i.e., Dilla Town & its peripheral villages was favorable for the variety of human intestinal parasites mentioned above as the climate of this very region is warm and moist. That was so because the computed value of r was 0.99984, being almost +1, or nearly a perfect positive correlation. The other major factors observed, by the researcher of this study project, to be responsible for the very strong correlation among the different species of intestinal parasites recorded include:- poor environmental sanitation, poor personal hygiene of the target children, lack of clean water for domestic use or drinking, and several poverty related problems. Multiple infections as many as 5 different species of pathogenic intestinal parasites were practically observed harbored in a single individual child.

The list of the most important diagnostic stages of intestinal parasites represents the first hand and pure identifying original data to be interpreted and analyzed. The set of data in Fig. 1 was a substantiated evidence for the existence of various species of intestinal parasites and multiple infections in the population of student children participated in this study project.

The data of Table 1 was an excellent set as an unprocessed raw data for statistical analysis and interpretation in Figs. 2 and 3. In brief, Table 1 was a complete statistical set to meet the need of a scholar reader about the planned study executed.

The infection rates in percentage with each specific species of intestinal parasites in the population of student children was imperatively devised and forwarded in the statistic of histogram.

In 710 student children examined the number of species of the intestinal parasites were 11; however, four of them were scanty in frequency and not in the rate that can be a threat for the health of human population of the area. The four species of intestinal parasites that had been observed to be rare in prevalence were *Taenia saginata*, *Enterobius vermicularis*, *Hymenolepis nana*, and *Fasciola hepatica* with the corresponding infection rates of 1%, 1%, 1%,

and 0.6% respectively. These four species of parasites were not included in the statistical analyses of Table 1 and Figs.1-3, because they were with negligible frequency in prevalence compared to the other 7 species of parasites.

CONCLUSION

In conclusion, the major preventive measures against parasitic infections in humans in the area where the research work has been executed must be by focusing on:

- Blocking transmission of infective stages,
- Delivering public health education,
- Providing the public with clean water supply for domestic use and drinking,
- Improving personal hygiene and environmental sanitation, and
- Identifying and treating infected individuals to prevent the spread of infections.

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Conflict of interest:

I confirm that I don't have any competitive conflict of interest with any body.

Ethical approval:

Ethical permission /clearance to perform the research work for the well-being of human subjects was obtained from:- Dilla University, the Office of Gedeo-Zone Administration, and the Directors of the schools involved in the study. The demands for the continuity of this study project and involvement by the participant student children & their parents was unusually high.

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