Epidemiological Studies on *Strongyloides stercoralis* at Dilla District, Ethiopia

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Background and study aim: Some authors have accepted parthenogenesis or asexual reproduction and hermaphroditism (protandrogony) to be the only mode of reproduction of parasitic female *Strongyloides stercoralis* in human hosts as parasitic males of it did not exist in human hosts. Therefore, the first objective was to work out the infection rate of *Strongyloides stercoralis* in the population of elementary schools children at Dilla district; secondly, to produce a visible evidence for the prese nce of many parasitic males of *Strongyloides stercoralis* as there are parasitic femal -es in fresh stools samples of human hosts; and thirdly, to replace the unfit term by a correct one.

Patients and methods: Stools samples were collected from student children of elementary schools, and observed under microscope in the laboratory of parasitology after employing Baermann apparatus technique.

Results: In the study a total of 710 student children were examined for *Strongyloides stercoralis* infection out of whom 142 (in 1st study) were positive, confirming the infection rate to be 20% or 198 positive (in 2nd study) the infection rate being 28% by the parasite. Then, the average infection rate was 24%. All developmental stages and sexes of the parasite were obtained in the study executed.

Conclusion: The presence of many parasitic males with everted spicules observed in fresh stools samples during this study had been a very strong evidence for the fact that male and female copulation & fertilization were naturally taking place among parasitic worms of *Strongyloides stercoralis* in human hosts. Parasitic and free living males of *S. stercoralis* have the same morphology including the curved or coiled posterior body part.

INTRODUCTION

Strongyloides stercoralis is known to have two life cycles: an internal sexual cycle, involving parasitic worms that constitute the parasitic generation, and the external sexual cycle, interacting among free-living worms that represent the free-living generation in soil [1-3]. It had been stated by authors in modern textbooks, journals, and on the internet that the type of reproduction in the parasitic generation of *S. stercoralis* in human hosts was only by parthenogenesis of parasitic females in the absence of parasitic males [4-7]. Due to this concept of parthenogenesis the parasitic male had been omitted in the figures that demonstrated the life cycle of the parasitic generation of *S. stercoralis* in all modern and relevant textbooks, journals, and on the internet. Not only that, there was an article on the internet which stated that the parasitic generation of *S. stercoralis* was known not to have parasitic males and the parasitic females used to reproduce only by the asexual method of reproduction [8]. In the parasitic generation, when the filariform larvae are in contact with skin, they penetrate the small cutaneous blood vessels and are carried through the right heart to the lungs [9]. Then, sexually mature parasitic females settle in the tissues of epithelial mucosa to lay eggs that hatch soon and are discharged in the stools each day [10,11]. When all or some larvae metamorphose into infective filari-form larvae autoinfection may be onset by invading the mucosa of the ileum or colon, travel to lungs a nd then return to the intestine to mature in the mucosa [12-15].

Disseminated strongyloidiasis had been reported in both of two recipients of kidney allografts from a single cadaver donor [16]. It was also reported that in a 53-year-old man who had lung cancer, fulminantly fatal strongyloidiasis had developed following postchemotherapy of immunosuppression, resulting in the death of the patient within 48 hrs [16]. The development of a florid strongyloidiasis was observed in a 45-year-old man, following anticancer chemotherapy when eggs of *S. stercoralis* were seen in the stools [17]. One scientific study has reported that almost all deaths due to helminths in the United States result from *S. stercoralis* hyperinfection mortality rates because the occurrence of hyperinfection can be as high as 87% [18].

Aim of the study

The aim of the study has three objectives to work out:

- First, to determine the infection rate of *S. stercoralis* in the population of elementary schools children at Dilla district;
- Second, to produce a visible evidence for the presence of many parasitic males of *S. stercoralis* as there are parasitic females in fresh stools samples of human hosts; and
- Thirdly, to replace the unfit morphologic term by a correct one. Is parthenogenesis or asexual reproduction true in the parasitic generation of *S. stercoralis* in human hosts?

Concerning some morphological features of this parasite, the part of the worm's body that is known as the tail is the posterior part of body beginning from cloaca in the parasitic males or beginning from anus in the parasitic females. Cloaca is the opening through which spicules are everted at times of copulation & fertilization and it is also the outlet of the digestive tract. The 3 stages of human strongyloidiasis are Intestinal Strongyloidiasis, Gastropulmonary Strongyloidiasis and Disseminated Strongyloidiasis [19-21]. Some of the clinical presentations of strongyloidiasis can be highlighted as:

- Cutaneous with larva currens (racing larvae), pruritic linear or serpiginous, creeping urticarial eruption, dermatologic lesions, and petechiae;
- Pulmonary with persistent wheezing, cough, and deteriorating respiratory status; and
- Intestinal with vomiting, abdominal pain, watery diarrhea and constipation.

PATIENTS AND METHODS

The suitable type of study selected to answer the question of this research work was the Cross Sectional Study. The statistical methods pre-planned to be employed in analyzing and interpreting the results were the expression by percentage and standard deviation.

Eight different elementary schools found at Dilla district were selected to be the sites of fresh stools sample collection from student children. It is important, from the ecological and geographical points of view, to notify that Dilla district is located in Gedio Zone that is found in:

- Southern Ethiopia,
- The continent of Africa, and
- The northern hemisphere between the tropic of cancer and the equator.

Collection of fresh stools samples and documenting related information from the schools had been carried out from 6/12/2006 to 10/06/2007 and again repeated in depth from 10/9/2008 to 25/6/2009. However, the deliberate follow up to be certain about the prevalence and persistence of the parasite at Dilla district, was performed by taking fresh stools samples of ten students from each of the eight different elementary schools every year from 2000 up to the beginning of 2014.

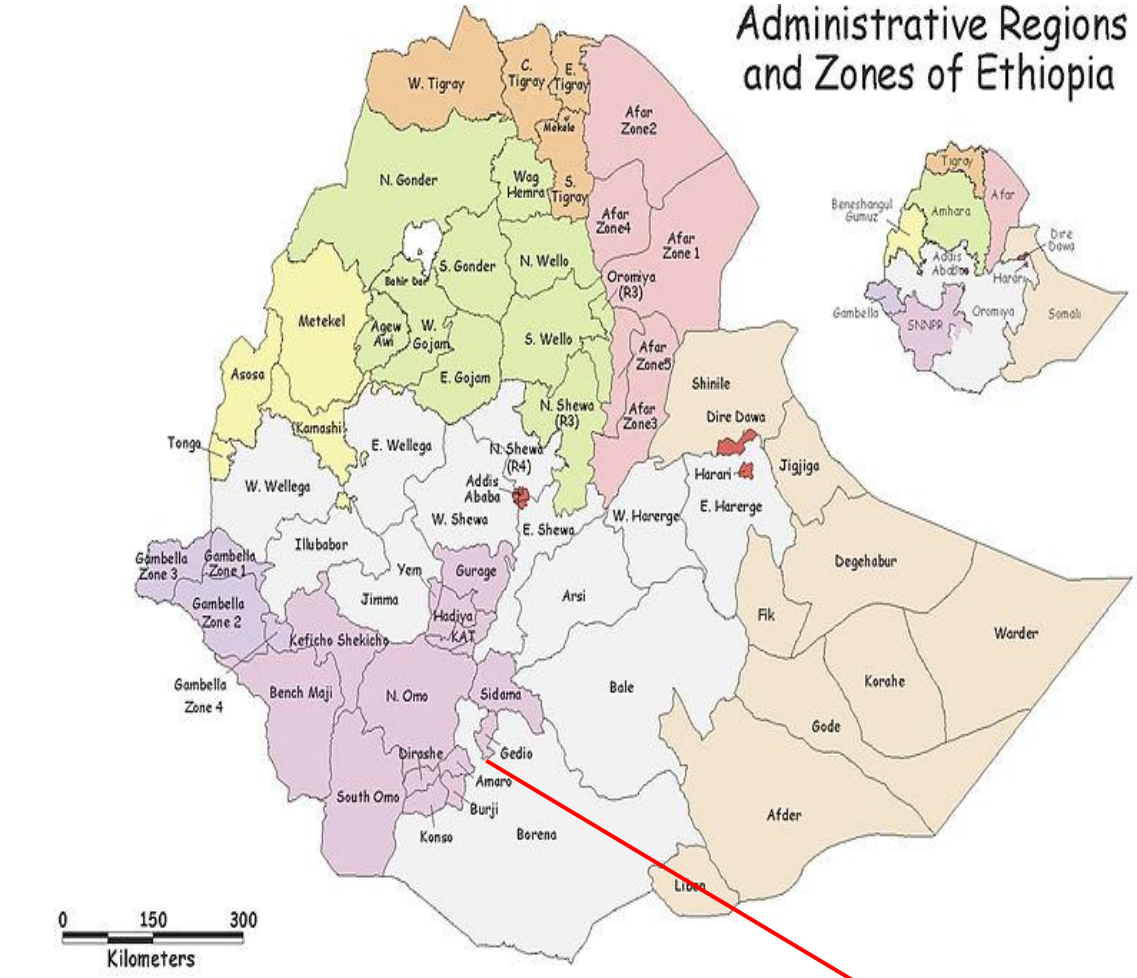

Figure (1): Map of administrative regions and zones of Ethiopia.

Specific sample size:

The sample size taken from the participant student children was 710. Each day, Monday through Friday (i.e. every week), fresh stools samples, of ten student children were taken to parasitology laboratory of Dilla University. When the sample was taken from the student child, he/she gave fresh stools sample in a bottle on which his/her I.D. No. was written. In addition to this, on that very day and moment a table that had columns with the headings of Date, Name of Child, I.D. No. of child, Class (grade), Age in Year, Sex, *S. stercoralis*, Education of parents, and Job of parents were filled by including the participation of the child for the necessary information, with the exception of the column under *S. stercoralis*, because it had to be filled either "–" or "+" for *S. stercoralis*, by the researcher after examining the fresh stools sample. Writing the name of the student child in the steps of raw data collection was important to identify the child for giving treatment if he/she had been found to be positive for the parasite, because many children could not remember their I.D. No. Of course, it was decided not to write the name of the student child in the report of the article.

Diagnostic Examination:

The diagnostic examination of fresh stools sample of each student child involved the following nine steps.

- Baermann funnel apparatus was constructed and the lower opening of the rubber tubing fitted to the stem of the funnel was closed.
- Water warmed to 40^0 C was poured into the funnel of the Baermann apparatus and the cheese cloth, that contained the fresh stools sample of the student child and tied with its peripheral edges to the rim of the funnel, was partially immersed in the water warmed to 40^0 C. This was done because if adults as well as juveniles of *S. stercoralis* were present in the stools, they would be attracted by the warm temperature of water (about 37.5^o C as there was dissipation of heat from the initial 40^o C of the added water to the surrounding materials and equipment) and escape into the warm water through the pores of the cheese cloth.
- After staying 1 hour and 30 minutes, the closed lower end of the rubber tubing was opened, releasing the water found in the funnel of Baermann apparatus into a 500 ml beaker. The stools left behind in the cheese cloth was thrown into the tube of toilet after being treated with a disinfectant (iodine solution) and washed away by a current of water.
- The water released and collected in the 500 ml beaker was centrifuged at a speed of 1000 rpm (revolutions of the rotor per minute) for 2 minutes using a manual centrifuge loaded with 4 centrifuge tubes and anchored to the edge of a table.
- From each centrifuge tube the supernatant was poured off into a waste collecting bucket to be thrown into the tube of toilet drainage line by treating with the disinfectant.
- Using a dropper, about 2 ml of the supernatant was added to the sediment of one of the 4 sediment containing centrifuge tubes and shaked well by closing its mouth with its own fittingly tight lid. The action of shaking was to change the sediment into a transferable suspension. The same suspension was
- Transferred to each of the remaining 3 centrifuge tubes one by one where in each case the centrifuge tube was shaked well and the sediment was changed into suspension.
- Next, the sediment collected in the form of suspension from 4 centrifuge tubes was poured into a test tube labeled with the I.D. No. of the student child from whom the fresh stools sample was taken to be examined. These seven steps were repeated for the fresh stools sample of each of the remaining 9 student children.
- Using a dropper, a drop of suspension was taken from the surface of bottom sediment of the labeled test tube suspension and placed on a clean glass slide and then covered with a cover slip. The preparation was examined under the low power objective of a research microscope to confirm the presence or absence of *S. stercoralis* in the fresh stools sample of the student child. The sample of each student child was examined in this way. The column under the heading of *S. stercoralis* for each student child was marked "–" indicating the absence or "+" confirming the presence of *S. stercoralis* in the fresh stools sample taken.
- The suspensions positive for *S. stercoralis* were fixed and preserved by adding 10% formaldehyde. Each container bottle of preservation in 10% formaldehyde was labeled *S. stercoralis* larvae/other stages including the date of collection and kept in a safe place in the laboratory of parasitology. Water emergence semi-concentration technique for detecting strongyloides larvae in feces was also used when there were needs to supplement the Baermann method **[22]**.

Water emergence semi-concentration technique for detecting S. stercoralis larvae in feces:

- A fresh (not more than 2 hours old) formed or semi-formed fecal specimen is required. The method is as follows:
- Using a piece of stick, make a central depression in the specimen contained in a vial or bottle. Fill the depression with warm water (about 37.50ºC).
- Incubate the specimen in a 35-37.50ºC incubator for 1.5 to 3 hours during which time the larvae will migrate out of the feces into the warm water.
- Using a plastic bulb pipette or Pasteur pipette, transfer some of the water to a slide and cover with a cover glass. Alternatively, transfer all the water to a conical tube, centrifuge, and transfer the sediment to a slide.
- Examine the preparation, under the low or middle power objective lens of a compound light microscope, for motile larvae of *S. stercoralis*.

Treatment :
The drug, that was available to treat the student children infected with *S. stercoralis* and ordered by the medical doctor assigned to assist the researcher of this study, was albendazole (Avion: Nabros, England) in the study project of 6/12/2006 to 10/06/2007. On the other hand, the drug of choice ordered by the medical doctor in the study of 10/09/2008 to 25/06/2009 was ivermectin (Ochoa: Ravenbhel, India).

The dose of albendazole:
Each infected child whose age was 9 years and above was advised to take two albendazole tablets at one time after dinner immediately before going to bed for night sleep daily for two consecutive days whereas those whose ages were 8 years and below were given 1 bottle (20 ml) albendazole oral suspension to take after dinner immediately before going to bed for night sleep daily for three consecutive days. It was notified that each tablet contained 200 mg albendazole USP whereas each bottle (20 ml) contained 400 mg albendazole USP.

The dose of ivermectin:
The prescription was stated as follows in proportion to individual student child's body weight. (Note: in this particular ivermectin 1 tablet is 6 mg in weight).

Body-weight	6 mg tablet of ivermectin
15-24 kg	0.5 tablet, single dose on empty stomach.
25-35 kg	1 tablet, single dose on empty stomach
36-50 kg	1.5 tablets, single dose on empty stomach
51-65 kg	2 tablets, single dose on empty stomach
66-79	2.5 tablets, single dose on empty stomach

Each student child was advised to take the tablet/s with a glass of water in the morning after waking up from bed and begin taking meal at noon.

Growth of free-living generation of *S. stercoralis* in the autoclaved topsoil in petridish incubated at 28^0C.

Topsoil that contained organic substance was taken and put into three different petridishes. Each of the petridishes was closed with its own lid and labeled 1, 2, and 3.

- Next, the petridishes with their contents of topsoil were autoclaved.
- The topsoil autoclaved in each of the Petri-dishes was inoculated with *S. stercoralis* from fresh stools sample obtained from a student child, infected with *S. stercoralis*, before he had been given treatment.
- Excess water was added to the topsoil of all the three petridishes and were incubated at 28^0 C on the same day.
- The topsoil of petridish No. 1, 2, and 3 were examined, using Baermann funnel apparatus technique to check the growth of free-living generation of *S. stercoralis*, after 11, 30, and 48 days of initial incubation respectively.
- The worms of the free-living generation of *S. stercoralis* collected from the three petridishes of topsoil using Baermann techniquewere fixed and preserved in 10% formaldehyde to be used for the preparation of permanent slides **[5]**.

Method of Safranin stain preparation :
I.1. Safranin O stock solution: Dissolve 2.5g safranin O Certistain in 100 ml of 96% ethanol. This is a stock solution.
2. For use: 10 ml of stock solution should be di luted with 90 ml of distilled water [23,24].
OR
II. 1. Safranin powder……0.1 g
2. Distilled water…….100 ml
The safranin powder is dissolved in the distilled water measured above.

Preparation of permanent slides and microphotographs :
In short, the preparation of *S. stercoralis* permanent slides was effectively done by applying the following Yetwin mounting medium **[25]**.

Yetwin Mounting Medium:
i 1. 10% bacto-gelatin, granular, aqueous 150.0 ml
2. Glycerin 50.0 ml
3. 1% chromium potassium sulfate aqueous (Chromium) 100.0 ml
4. Phenol (carbolic acid), melted 1.0 ml

ii. Gelatin was dissolved in boiling water (i.e., a 400 ml beaker, into which 10 g of gelatin & 90 ml of pure water were added, was immersed in a volume of boiling water in a larger heat-resistant dish) and glycerin was added to it. After mixing glycerin and 10% gelatin solution, 1% chromium potassium sulfate solution and phenol were added to the mixture of glycerin and 10% gelatin solution. The medium was liquefied in 15 minutes at 65ºC.

iii. Thereafter, the *S. stercoralis* worms were transferred from 10% formaldehyde directly into a drop of mounting medium, placed on a clean slide. The mounting medium with the worms was covered with a cover slip.

iv. Then, within overnight the gelatin hardened to form a permanent slide of *S. stercoralis* worms.

From the permanent slides prepared microphotographs of the larvae and other stages of S. stercoralis were taken using a digital camera from the fields of vision under suitable objective lenses of the compound light microscope.

RESULTS

The infection rate of *S. stercoralis* in the population of student children of elementary schools at Dilla district was 20% in the first study project (conducted during 6/12/2006 to 10/6/2007), but in the second one (done during 10/9/2008 to 25/6/2009), it went up to 28%. Why was that so? That was so, because a larger amount of sample size was taken & included, in the second study project than in the first one, from student children who were living in a remote village with poor environmental sanitation and covered with diversity of perennial plants, shrubs of densely planted coffee together with other giant trees where the soil was moist and warm, and the majority of student children were bare-footed as they used to come from poor parent families. As the result of those environmental conditions the worm-load of *S. stercoralis* in the population of student children was far higher in this particular remote village than in any other site school selected for sample taking. Due to those environmental and economic factors, the infection rate of *S. stercoralis* grew up to 28% in the second study project.With those practical results in mind, the infection rate of *S. stercoralis* at Dilla district was adjusted to 24%, taking the average infection rate of those two study projects

$$(\text{i.e.,}\ \frac{20\% + 28\%}{2} = \underline{\underline{24\%}}).$$

Several risk factors have been associated with human strongyloidiasis, including coinfection with HIV (Human Immunodeficiency Virus); HTLT-1 (Human T-cell Lymphotropic Virus type1) infection; diabetes mellitus; chronic alcoholism; asthma; tuberculosis; malnutrition; chronic pulmonary disease; leprosy; chronic renal failure; impaired bowel motility; immunosuppressive therapy for diseases such as rheumatic disease, malignancy or cancer, and organ transplants; and promiscuous defecation.

The difference in the infection rate of *S. stercoralis* in children due to the difference in the status of environmental sanitation & economic income in the families' residence areas of the children was analyzed by the statistic of standard deviation. In this case, the larger the standard deviation meant the greater the infection rate than the mean rate, manifesting at the epidemic level.

This was the statistical evidence for the fact that the poor status of environmental sanitation and poor economic income in the parent families' residence areas of elementary schools children had been one of the obvious causes for the increase of infection rate in the student children with *S. stercoralis*. This sanitation in the residence areas of the children was poor so that the pathogenic worm-load in the soil would be high and infect the bare-footed student children whose parents were poor and could not buy shoes for them.

Both parasitic male and female adults of *Strongyloides stercoralis* including the developmental stages had been isolated from fresh stools samples of the participant student children.

Growth of free-living generation of *S. stercoralis* in the autoclaved topsoil in petridishes incubated at 28ºC, showed the following result. In each of the three petridishes that were observed after 11, 30, and 48 days from the date of initial incubation, adults (males & females) and a large number of larvae were present. The purpose of growing free-living generation to compare the morphology of free-living males with that of parasitic males.

Safranin stain is not known at all to stain protozoa or any other parasite here before. When it was tried to stain the worms of *S. stercoralis*, for the first time, it gave a very good dyeing effect. It stained the worms red.

Tables 1 & 2, and Fig. 2 are given on following 3 consecutive pages.

Table (1): The infection rate with *Strougyloides stercoralis* and the cure rate of the drug albendazole against human stronglyoidiasis, 6/12/2206 to 10/06/2007

No. of students examined	No. of students positive for S. stercoralis	The drug used for treatment	No. of students cured by the treatment
710	142 (20%) [#]	Albendazole	138 (97%)[†]

[#]The percentile quantity in parenthesis adjacent to the value that meant "No. of Students positive for *S. stercoralis*," represented the infection rate of *S. stercoralis* in the population of student children whereas the one adjacent to the value that meant "No. of Students cured by the treatment," i.e.,

[†]represented the cure rate of the drug albendazole against human strongyloidiasis found at Dilla district.

Table (2): The increase of infection rate with *Strougyloides stercoralis* due to the poor status of sanitation and economic income

Infection rate of *S. stercoralis* in children from families of better (sanitation and economic) status-residence areas	Infection rate of *S. stercoralis* in children from families of poor (sanitation and economic) status-residence areas
38%	12%
11%	50%
10%	40%
15%	20%

$\overline{X}_1$=12%; S_1 =2.2% $\overline{X}_2$=37%; S_2 =12.5%

$\overline{X}_1$ or $\overline{X}_2$ stands for a sample mean and S_1 or S_2 represents the standard deviation of a sample. $\overline{X}_1$ and S_1 are variables for the children from families of better status in sanitation and in economic income whereas $\overline{X}_2$ and S_2 are for those from families of poor status in sanitation & in economic income.

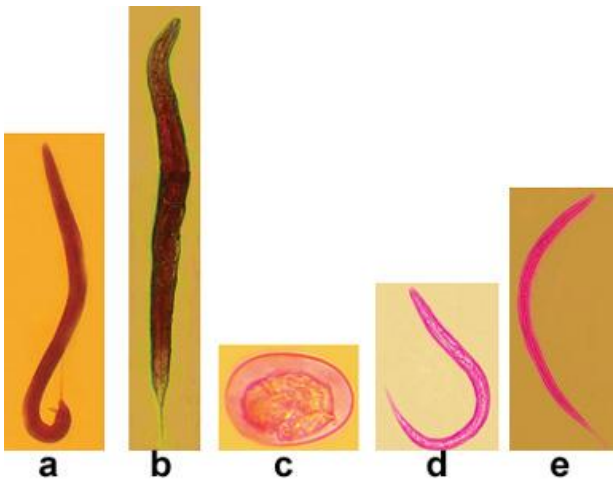

Figure (2): Microphotographs of different developmental stages and sexes of *Strongyloides stercoralis* isolated from fresh stools samples.

(a) parasitic adult male (stained with Safranin), magn[‡]. X64 ;
(b) parasitic adult female, magn. X64;
(c) egg, magn. X640; (d) rhabditiform larva, magn. X640; and
(e) filariform larva, magn. X320. Pictures (b), (c), (d) and (e) were colored by a Computer Adobe Phot oshop▪ CS. Each of these five pictures was 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 value of how many times the actual size of the specimen was magnified.

DISCUSSION

The results obtained in this study project can be defined as a set of achievement scored by way of cross-sectional type of study. Standard deviation of infection rate, in student children from families of better environmental sanitation and economic status, was far less (S_1 =2.2%) than in those from poor environmental sanitation and economic status (S_2 =12.5%). Larger S_2 indicated that the observed infection rate went up beyond the mean infection rate in the population of student children. Student children from families of better economic status did live a relatively more hygienic mode of life as they used to get water supply lines to wash their hands, clothes and bodies at their homes. Families might be in a better economic position by having enough capital to carry out their own private business work in the central part of the city with better sanitation that would be comfortable to be hygienic and buy shoes for their student children that could not be afforded and done by poor families. Parents who had educational skill and government job were economically self-sufficient so that they were able to buy shoes for their student children, resulting in reduction in the infection rate with *S. stercoralis*. The fact that poor environmental sanitation and poor economic income did form one of the obvious causes for the increase of infection rates in the student children was evidenced by the statistic of standard deviation and other angles.

When the safranin stain was tried to stain the worms of *S. stercoralis*, for the first time, it gave a very good dyeing effect. It stained the worms red. Actually, safranin is well known as the secondary stain (counter stain) applied to the fixed preparations of bacteria. If the bacteria are decolorized with alcohol, they will take up the safranin and appear red (gram-negative). If the bacterial cells are not decolorized , the safranin will have no effect on the already stained preparation, and the bacteria will remain blue or purple (gram-positive) [23, 24].

The student children who were positive for *S. stercoralis* infection were not revealing or not manifesting affectedness with the disease, being active in their daily lives like other student children who were without strongyloidiasis. On the other hand, the larvae of *S. stercoralis*, recovered from fresh stools samples of those infected student children, were practically observed moving actively in the fields of vision under the objectives of compound light microscopes. With this truth in mind, the student children who were positive for *S. stercoralis* infection and did not manifest affectedness with strongyloidiasis should be immunocompetent. In these infected children, adults and larvae of the parasite were confined to the digestive tract in which case the children were symptomless and the *S. stercoralis* infection they had was asymptomatic intestinal strongyloidiasis. In other words, it is neither at the "gastropulmonary strongyloidiasis" nor "disseminated strongyloidiasis" stage in these infected participant student children.

Here it could be understood that the parasite was silently hiding in the intestine of each of the infected student children to develop to the lethal conditions of strongyloidiasis whenever the immunity of the student child was broken down (weakened) by some risk factors. Such a hidden pathogenic parasite was found out from where it was hiding by carefully employing standard diagnostic procedures such as Baermann technique and displayed with all its developmental stages and sexes. Hence, the parasitic males of *S. stercoralis* are present together with their parasitic females in the bodies of human hosts and this verified evidence is a spectacularly targetful answer to the major question and objective of this study.

Some authors had generalized that parthenogenesis and protandrogony (i.e., hermaphroditism) were the methods of reproduction for *S. stercoralis* in human hosts as the parasitic males did not exist in human body [6]. *S. stercoralis* in humans are parthenogenetic and can produce offspring without being fertilized by the male. But the fact that parasitic males do exist can be demonstrated in experimentally infected dogs [5]. In other words, this group of thought stated that the adult female *S. stercoralis* is parthenogenetic & hermaphroditic in the mechanism of reproduction. No adult male *S. stercoralis* is known to exist, the adult female is considered as being parthenogenetic [26]. Another division of thought had concluded that asexual reproduction was the method for

the parasitic females of *S. stercoralis* to reproduce in human hosts for the very reason that parasitic males did not exist in the body of humans [8]. However, let us take that both parthenogenesis and asexual reproduction have the same meaning for the method of reproduction. Is there any evidence to generalize that adult female *S. stercoralis* is parthenogenetic and hermaphroditic? Each of these groups of thought did not have any trace of substantiated and persuasive scientific proof to be accepted in science. This was so because many parasitic males of *S. stercoralis* with spicules everted out of their spicule pouches were practically observed in fresh stools samples of participant student children. The presence of many parasitic males of *S. stercoralis* with everted spicules in fresh stools samples together with parasitic females was a very strong evidence for the fact that there was copulation & fertilization. Everted spicules of males are seen only at times of mating.

CONCLUSION

- The result of this study had identified the concepts of both parthenogenesis/asexual reproduction and protandrogony, in the parasitic generation of *S. stercoralis*, to be unscientific conclusions. The reports without adequate assessment and evidences, on the reproduction of *S. stercoralis* in the parasitic generation in human hosts that had been reacted to by this paper could not be denied because they were reported straight forward by authors in modern textbooks, journals, and on the internet. Due to those reports, in all modern human parasitology textbooks, journal, and on the internet, the males of *S. stercoralis* had been excluded (omitted) from the life cycle of its parasitic generation in human hosts. It was possible for copulation to take place between the parasitic males and females to result in fertilization in the lumen of the human host's gut and then the fertilized parasitic female could burrow into the intestinal mucosa to lay eggs that would hatch soon. It is just like a domestic cock and a hen where it is the hen which goes to a nest after mating to lay and incubate eggs and not the cock.
- Applying efficient preventive measures and devising effective treatment under clinical supervision against a pathogenic parasite depend on deep and detailed understanding about the biology and life cycle of the parasite.
- The term curved tail was used by authors for the posterior body part of males that belong to free living generation found in soil **[4]**. The term was not inclusive and unfit to define the actual taxonomic morphology of both free-living and parasitic males of *S. stercoralis*. The degree (extent) of being curved in the posterior body part of male *S. stercoralis* is greatly variable among the male worms of both free-living and parasitic ones in a similar way in extent. The morphology of both parasitic and free living males is the same. This was verified by growing free-living males in autoclaved topsoil that was inoculated with fresh stools sample obtained from an infected child before giving him treatment and incubated at 28ºC. When the morphology, including the variation in the degree of curvature or coiling of the posterior body part, of these free-living males was compared with that of parasitic males, it was found to be similar in both free living and parasitic ones. Therefore, the term practically ascertained to be correct to differentiate both the parasitic and free-living males from their respective females of *S. stercoralis* was a ventrally "curved or coiled posterior body part" in the males of this very parasite whereas that of the females was straight.
- In the life cycle of parasitic generation of *S. stercoralis* both parasitic male & female must be included just like the free-living male & female in their life cycle.
- This article is a realistic response to a chronic global problem that has remained unsolved for generations of man until now and needs world-wide attention of human parasitologists.

Funding:

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supervision were given by the Ethiopian Catholic Church (Dilla Don Bosco).

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 Gedio-Zone Administration, and the Directors of the schools involved in the study. The demand for the continuity of this study project and participation by the participant student children and their parents was unusually high.

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