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Strongyloidiasis in HIV- HCV co-infected patients

Haodijam Ranjana Devi[1] and Laitonjam Anand Singh[2]
1 *Post Doctoral Researcher (Parasitology)*
Department of Zoology, Centre for Advanced Study in Life Sciences
Manipur University, Canchipur - 795 003, Imphal, Manipur (India)
2 *Research Scientist – II*
Viral Research & Diagnostic Laboratory (VRDL)
Regional Institute of Medical Sciences,
Lamphelpat – 795004, Imphal, Manipur (India)

Abstract
Background: *Quite a good number of HIV- HCV co-infected patients are there in Manipur, a tiny state situated in the north eastern region of India. These patients are reported to have experienced a number of gastrointestinal disorders including diarrhoea that might have been caused by a number of pathogens. Thus, visualizing the importance of identifying the aetiologic agent(s) especially of parasitic origin associated with this group of patients and also as a part of continuing research programme towards assessing the occurrence of intestinal parasitic infection among the HIV-HCV co-infected patients, a coproparasitological study was carried out at Chandel, a tribal dominated district of Manipur (India).*
Materials and Methods: *Three (03) stool samples, which consisted of two consecutive fresh and one preserved in 10% formol saline were collected from 36 HIV-HCV co-infected diarrhoeal tribal patients who had been admitted in the different drug de-addiction and rehabilitation centres located in the different areas of Chandel district of Manipur (India) during the period from June to September 2019. There were 29 males and 7 females. The age of the patients ranged from 30 to 48 years. Of the 29 male patients, 7 were injecting drug users while 6 were habitual drinkers (alcoholics). Of the 7 females, 3 were commercial sex workers (CSW). The samples were then brought to the Parasitology Laboratory of the erstwhile Department of Life Sciences, Manipur University for laboratory examination. Detection, recovery and identification of the parasites were done by employing the techniques of normal saline method; iodine wet preparation method; formol ethyl acetate/diethyl ether concentration technique; modified Baermann funnel technique and agar plate method. A stereoscopic dissecting binocular microscope (Olympus) and a high resolution compound microscope (Nikon, Eclipse - 200) were used for studying the morpho-anatomical structures of both the larvae and adult parasite(s) and a calibrated binocular compound microscope (Olympus) was also employed for ocular micrometry.*
Results: *During the study, of the 36 HIV-HCV co-infected diarrhoeal patients screened for the presence of intestinal parasites, 15(41.6%) were found to be infected with Strongyloides stercoralis. None of these patients were found positive for other parasites. There were 11 males and 4 females. Of the 11 males, 5 (45.4%) were intravenous drug users and of the 4 females, 2 (50%) were commercial sex workers. The age of the patients ranged from 30 to 48 years. Their CD4 T lymphocyte counts ranged from 116 to 220 cells/µl of blood. All the 15 patients complained of mild fever and cough for the last 3-4 months. They also complained of experiencing diarrhoea on and off in the preceding 2-3 months. During laboratory investigation, while rhabditiform larva was observed in almost all the positive patients, only 7 patients were found to have harboured adult male and female parasites besides the rhabditiform larva.*
Conclusion: *The study reveals that Strongyloides stercoralis is one of the important parasitic agents associated with HIV-HCV co-infected diarrhoeal tribal patients of Chandel district. The present study highlights the importance of carrying out such study in the other parts of the state with a view towards assessing the occurrence/prevalence and endemicity of the disease so that appropriate precautionary and preventive measures can be taken up in the best interest of the people of the state in general and HIV-HCV co-infected patients in particular.*
Key Words: *HIV- HCV co-infection, Strongyloides stercoralis, rhabditiform larva.*

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I. Introduction

Strongyloidiasis is an intestinal parasitic disease caused by the extracellular luminal parasitic nematode – *Strongyloides stercoralis*. This nematode also known as threadworm (in Great Britain) / pinworm (in USA) is reported to have infected 50-100 million people at a given point of time with 1000 deaths per year [1,2]. In non-HIV individuals, strongyloidiasis usually produces asymptomatic or mild infection and rarely produces major health issue. In HIV/AIDS patients, strongyloidiasis is considered as one of the most important opportunistic infections because HIV/AIDS patients infected with *S. stercoralis* are prone to develop massive strongyloidiasis called hyper infection syndrome. In HIV/AIDS patients, opportunistic gut infections caused by the colonization of the GI tract by opportunist parasites including *S. stercoralis* may cause intestinal disorders by disrupting the normal absorptive functions of the small intestine [3].

In Manipur, a number of earlier workers have reported the association of this nematode with HIV patients [4-10]. However, literature review reveals no case of HIV-HCV co-infected patient to have been afflicted with strongyloidiasis. The present paper highlights the occurrence of this opportunist nematode in HIV- HCV co-infected patients of Chandel district of Manipur, India.

II. Materials And Methods

Three (03) stool samples, which consisted of two consecutive fresh and one preserved in 10% formol saline were collected from 36 HIV-HCV co-infected diarrhoeal patients who had been admitted in the different drug de-addiction and rehabilitation centres located in the different areas of Chandel district of Manipur (India) during the period from June to September 2019.

There were 29 males and 7 females. The age of the patients ranged from 30 to 48 years. Of the 29 male patients, 7 were injecting drug users while 6 were habitual drinkers (alcoholics). Of the 7 females, 3 were commercial sex workers (CSW). 20 apparently healthy neither HIV nor HCV infected individuals were taken as control group. They were counsellors and helpers working in the different drug de addiction and rehabilitation centres from where the samples were obtained.

All the patients were enjoying antiretroviral treatment (*ART*) and were also tribal. Moreover, with the exception of six (06) patients, all the other patients had been receiving anti HCV drugs. These six clients were HCV treatment naive at the time of sample collection as they had been recently/newly diagnosed (only 05 days back) as afflicted with HCV infection.

The samples were brought to the Parasitology Laboratory of the erstwhile Department of Life Sciences, Manipur University for laboratory examination. Detection, recovery and identification of the parasites were done by employing the techniques of normal saline method; iodine wet preparation method; formol ethyl acetate/diethyl ether concentration technique; modified Baermann funnel technique and agar plate method [11-14]. A stereoscopic dissecting binocular microscope (Olympus) and a high resolution compound microscope (Nikon, Eclipse - 200) were used for studying the morpho-anatomical structures of both the larvae and adult parasite(s) and a calibrated binocular compound microscope (Olympus) was also employed for ocular micrometry.

The adult parasites were first washed with normal saline (0.9%), followed by physiological saline (0.9%) and PBS (P^H = 7-8) so that all the faecal debris and other dirt that remain sticked with the cuticle get detached from the worm. It was then observed using a stereoscopic dissecting microscope for the presence of ♂ and ♀ parasites. These adult worms were then treated with 70% Glycerine Alcohol (GA) in a cavity block and were then kept for about three months in a desiccator packed with calcium carbonate as drying agent with periodic changing of GA and drying agent for every alternate week. The parasite(s) were then checked for every 15 days by removing them from the desiccator and by observing under a stereoscopic dissecting microscope to monitor the appearance of any undesirable change or alterations in the gross structure of the parasite and also to ascertain whether the parasite is free from all faecal contaminants and debris. The parasites were also gently washed once every 15 days by slowly rotating the desiccators in a circular motion to get rid of faecal debris that remained attached with the cuticle. Only when the adult parasites are free of all faecal debris, confirmed by the presence of a highly transparent cuticle with internal structures clearly visible, semi-permanent slides were prepared by using GA as the embedding medium (also by employing glass wool, cover slip having 0.8 mm - 1 mm thickness and circular glass slide cover bearing No.0) and the edges of the slides are sealed with nail polish or any other suitable sealing medium. The specimen in the slide was used for microphotography and ocular micrometry.

A brief clinical, health & hygienic practices including socioeconomic history/ background were also evaluated at the time of sample collection.

III. Results

Of the 36 patients examined, only 15 (41.6%) patients were found positive for strongyloidiasis. Of the 15 strongyloidiasis positive patients, 11 were males and 4 were females. Of the 11 males, 5 (45.4%) were intravenous drug users and of the 4 females, 2 (50%) were commercial sex workers. The age of the patients

ranged from 30 to 48 years. Their absolute CD4 T – lymphocyte counts ranged from 116 cells/µl to 220 cells/µl of blood. All the 15 patients complained of mild fever and cough for the last 3 - 4 months. They also complained of experiencing diarrhoea on and off in the preceding 2 to 3 months.

When the stool samples were examined using a compound microscope, a large number of motile larvae along with many matured worms were observed. Using a high powered stereoscopic binocular dissecting microscope, the larvae were first examined followed by the adult worms for the presence of systematically important and taxonomically valid morpho-anatomical features and other important specific features and characters pertinent to the rhabditiform larvae (L_1)/adults of *Strongyloides stercoralis.*

Then the larvae/ adults were critically examined under a compound microscope having a high resolving power (Nikon, Eclipse - 200) and finally measurements were recorded using a calibrated compound microscope (Olympus).

While rhabditiform larvae were observed in almost all the positive patients, adult male, female and larvae were observed in only seven (07) patients.

The diagnostic feature(s) as seen and recorded when observed under the microscope having high resolving power is detailed below:

A) **Larva of *Strongyloides stercoralis***:

Diagnosis: The larva has the following features -

i) a shallow buccal cavity having a length of 4µm
ii) a vermiform body having a length of 243.6µm [range: 200-250µm] and a breadth of 12.5µm [range: 11-15µm]
iii) a short oesophagus which consisted of a corpus and an end bulb with an isthmus [collectively referred to as doubled bulb oesophagus]
iv) a germinal primordium having a dimension of 22µm
v) a distinct attenuated tail
vi) shows whip like movement on agar plate

Based on the presence of the above mentioned diagnostic characters and especially the presence of a 4µm long buccal cavity which is the most important and taxonomically valid identifying criterion [12] for the identification of the rhabditiform larva (e) (L_1 stage) of *Strongyloides stercoralis*, the present specimen has been identified and confirmed as the L_1 stage larvae (rhabditiform) of *Strongyloides stercoralis*.

B) ♀ ***Strongyloides stercoralis*:**

Diagnosis:

Body - worm like, vermiform, transparent with pointed posterior end; Dimension: Length: 2126 µm [range: 2000 – 2500 µm, i.e. 2- 2.5 mm], Breadth: 43 µm [range: 40-50 µm]; Buccal cavity: indistinct, with four small lips; Oesophagus : cylindrical, muscular, extends up to the anterior 1/3rd of the body; Intestine: distinct, extends up to 2/3rd of the posterior end; Oesophago-intestinal valve : distinct; Anus: distinct, mid ventral at a short distance from the caudal end; Ovary: didelphic; Vulva: distinct, equatorial; Vulval flap: distinct with thickened wall; Vulval opening: slit like; Amphid: present, indistinct; Caudal gland: absent; Phasmid (caudal): present.

Based on the presence of the above mentioned taxonomically important characters, the present specimen has been identified and confirmed as the adult ♀ *Strongyloides stercoralis*. However, the length of the present specimen is much shorter compared to the parthenogenetic parasitic female – the usual form of female generally found in the human host, which is approximately three times (3x) longer than the present specimen. The position of the vulva in the present specimen is equatorial, i.e. it is situated in the mid portion/middle (i.e. equator) of the body.

In parasitic female, vulva is located slightly posterior to the equator (post equatorial, i.e. situated at a little distance from the middle of the body towards the posterior/caudal end). In the present study, though the specimen examined was from human stool, the presence of vulval opening at the equator (i.e. middle) of the body shows that these are free living females.

The possible reason for the observation of free living adult worm, especially the female in the present samples might be due to the transformation of L_3 stage (infective filariform larvae) into that of the adult (as a result of 4th moult) while they were still passing through the intestinal lumen.

C) ♂ ***Strongyloides stercoralis:***

Diagnosis:

Body – worm like, vermiform, transparent, slender, Stouter compared to female; Dimension: Length: 1223µm [range: 900 – 1300 µm], Breadth: 40 µm [range: 40-50 µm]; Buccal Cavity – short; Post caudal end: spirally

coiled; Reproductive accessories: i) Copulatory Spicule: 01 pair, distinct; ii) Gubernaculum: 01 unpaired, distinct.

Fig. I Rhabditiform larva (L_1 stage) of *Strongyloides stercoralis* showing a short buccal cavity [Ethyl acetate concentration technique, stained with Dobell's Iodine, 10x]

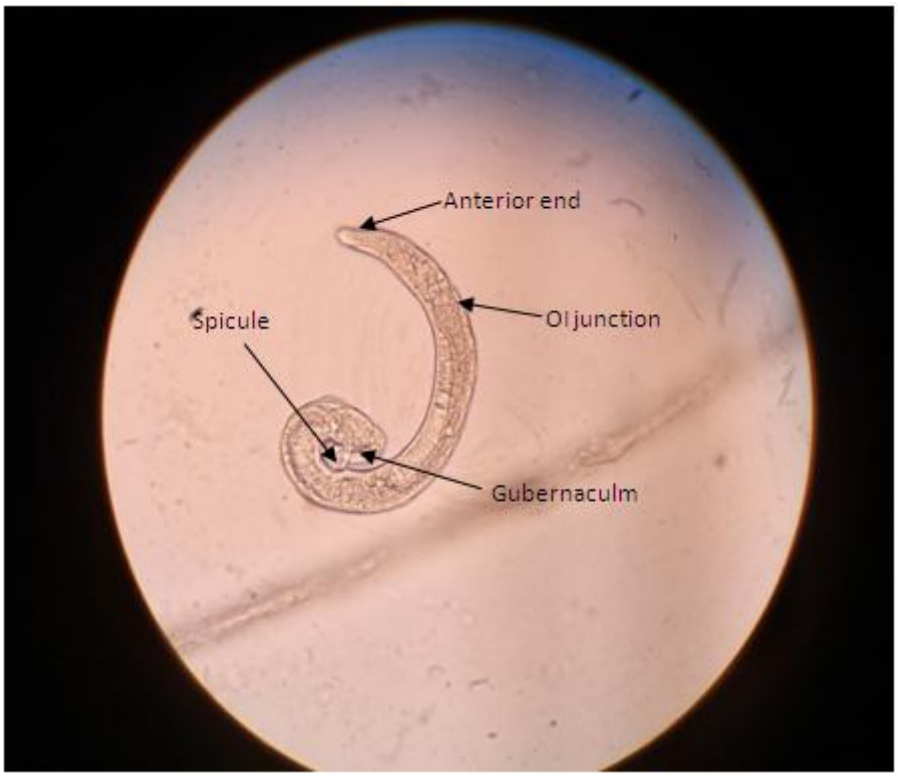

Fig. II ♂ *Strongyloides stercoralis* showing spicules and gubernaculum [GA mounted, 20x]

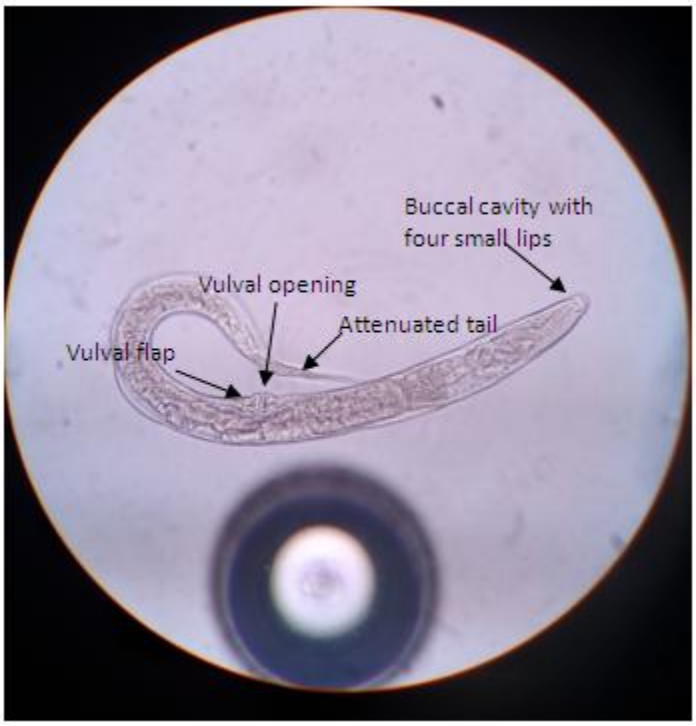

Fig. II ♀ *Strongyloides stercoralis* showing vulva and OI junction [GA mounted, 20x]

IV. Discussion

Strongyloides stercoralis is a facultative parasite capable of thriving both in the human host and environment. It is a soil transmitted disease that mainly affects the people living in the tropical and subtropical regions. Infection occurs percutaneously through the penetration of the skin mediated by the infective filarifrom larvae (L_3 stage). Acute infection may produce urticarial rash at the filariform larvae penetration site of the skin and symptoms such as dyspnea, cough and wheezing may occur when the larvae invade the lungs. In immuno-competent individuals, the parasite may elicit some minor health problems like transient diarrhoea, abdominal pain and occasional cramps, however, up to 30% of the infected patients may show no signs and symptoms and usually remain asymptomatic or with mild health problems.

In Manipur, this parasite has been reported from both non-HIV population and HIV/AIDS patients, although occurrence of the disease seems to be higher in HIV patients because of the opportunistic nature of the parasite.

Previous workers have documented occurrence of this nematode amongst the HIV patients of Imphal, Churachandpur and Ukhrul districts of the state, with a prevalence rate ranging from 3.2% to 27.2% depending upon various factors [4, 5]. *S. stercoralis* is considered to be strongly associated with a Th 2 cytokine shift. Therefore, patients with strongyloidiasis could be associated with rapid immuno-competence deterioration. In another aspect, patients diagnosed with either HIV or HIV-HCV co-infection, have greater chances or likelihood of developing disseminated strongyloidiasis.

Although early diagnosis/detection is the best option for the in time treatment of the patient, strongyloidiasis is difficult to diagnose because the parasite load is low and the larval output is irregular and chances of detecting the egg or adult (in the stool) is extremely low. Moreover, many a times, detection can't be done by the routine diagnostic procedures, but frequently requires special parasitological techniques like modified Baermann funnel technique and ethyl acetate or diethyl ether concentration technique to detect the presence of either the larva (L_1 stage) or adult.

The L_3 stage (filariform larva) is the infective stage of the parasite. Female parasite may be either free living or parasitic and parasitic females are approximately three times longer than the free living counterpart. In parthenogenetic parasitic females, vulva is located slightly posterior to the equator (post equatorial), while in free living forms, it is situated at the equator, i.e. at the middle of the body.

Generally, parasitic males do not exist in infected humans as they have no penetrating power. In case, these males survive in the human host, they are generally found as free living form in the lumen of the large intestine feeding on host tissue exudates and other commensals and bacteria present in the large intestine.

The present study throws light on the importance of conducting such types of study in other tribal inhabited rural areas of the state so that endemicity of the disease can be established. Moreover, with a view

towards minimizing the occurrence of the disease in an area, the local population can be sensitized by educating them the importance of maintaining health and hygienic practices besides imparting the basics of health education.

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