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ORIGINAL RESEARCH

A Study on Hookworm infections- *Ancylostoma duodenale* and *Necator americanus* in patients reporting at a tertiary care hospital in Raipur

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ABSTRACT

Objective: Hookworm infestation contribute significantly to the disease burden in developing nations. The purpose of this study is to know about hookworm infections- *Ancylostoma duodenale* and *Necator americanus*, in patients reporting at tertiary care hospital in Raipur. **Methods**: This was a retrospective observational study. A total of 659 stool samples received from patients with various gastro-intestinal disturbances were microscopically screened for the presence of hookworm eggs. Hookworm eggs in freshly passed unfixed stool samples were cultured to the infective third stage filariform larvae at room temperature, by the Harada Mori filter paper strip method. The cultured filariform larvae were examined under the microscope; both iodine stained and unstained larvae were examined and identified according to the criteria of WHO. **Results:** Out of 659 stool samples that were microscopically screened for the presence of hookworm eggs, 50 samples were found positive for different parasitic infections of which 20 cases of hookworm infection were recorded giving us an overall infection rate of 40%. Of the 10 hookworm positive coproculture *N.americanus* filariform larvae were recovered from a total of 9 [90%] while *A.duodenale* larvae were recovered from a total of 3[30%] patients. Only 2[20%] of the coprocultures had double species infections as evidenced by the recovery of the filariform larvae of both species. However in all mixed infection higher number of *N.americanus* larvae were recovered compared to *A.duodenale*, the former ranging from 62-75% and the latter ranging from 25-60%. **Conclusions**: Hookworm infection ranks quite high among the patients with different gastro intestinal disturbances. This is alarming as because hookworm has been associated with impaired cognitive functions and decreased future economic productivity.

Key words: Hookworms, Harada Mori filter paper strip method, Harada Mori filter paper strip method, coproculture

INTRODUCTION

Hookworm infection is caused by blood feeding nematode parasites It is soil transmitted and ranks amongst the most wide spread intestinal helminthic infection Approximately 740 million people of the world are infected with hookworms [1]. The annual number of deaths was approximately 65,000 as a result of hookworm anemia [2] The highest prevalence of hookworm occurs in Sub-Saharan Africa and Eastern Asia High transmission also occurs in other areas of rural poverty in the tropics including Southern China [3], the Indian Sub-Continent and the Americas [3] In all regions there is a striking relationship between hookworm prevalence and low socioeconomic status [1]

There are two major species of hookworms that affect

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humans namely *Necator americanus* and *Ancylostoma duodenale*. In the tropics, many people are infected simultaneously with both the species Hookworm infection is widely prevalent in India [4] *N.americanus* is predominant in South India while *A.duodenale* in North India .Recently another species *A.celanicum* has been reported from a village near Kolkata[5] The heavily infected areas are found in Assam, W Bengal Bihar, Orrisa, Tamil Nadu, Kerela and Maharashtra It is believed that 60-80% of the population of certain areas of W.Bengal, UP Bihar and eastern coast of Tamil Nadu and Andhra are infected with hookworms Infection rate contently stands at 200 million [6]

The adult worms attach to the mucosa of the small intestine and suck blood of the host Heavily infected individuals suffer a huge blood loss leading to chronic hookworm anemia In children hookworm anemia leads to profound alterations in intellectual, cognitive and physical growth [7] There is significant differences in the life history of the two species of human hookworms Humans acquire infection either by exposing skin to soil contaminated with *A. duodenale* or *N. americanus* larvae or even by ingesting products contaminated with A. duodenale larvae [7] Moreover some *A.duodenale* larvae may undergo a period of extra intestinal dormancy after penetrating the skin before resuming migration to the gut.Dormancy can last weeks or months. The repositories of these dormant larvae may be muscle tissues or the dormant larvae may enter mammary glands and breast milk which may account for cases of infantile ancylostomiasis in Africa, China and India[2]. Larval latency complicates treatment and control of ancylostomiasis since dormant nematode larvae resist most anti helminthics and resume development at a later date[2] Rossignol et al in 1998 reported that administration of only one anti helminthics may not be sufficient to kill both the species, this means that distinguishing the two species may be of importance to prevent treatment failure in a particular population, therefore it is emphasized that knowledge of the species infecting a particular human population is necessary in order to direct treatment to be successful in the long term [11]

Adults are parasitic in the upper part of the jejunum, sucking in the mucosa and biting it with cutting plates or ventral teeth Mainly due to the differences in the sucking power of the oesophagus, the amount of blood loss is five times or more large in *A.duodenale* infection than in *N.americanus* infection Besides the strength of sucking power, the shape of biting organs, the size of worms, etc may be responsible for the difference. The eggs of the two species are similar and not readily distinguishable from one another by classical parasitological methods. Therefore, individual isolates are easily not speciated and treatment relies on past epidemiological data to estimate which species is predominant in a particular area [1].

We can differentiate the two species from the morphology of the adult worms [8]. However, the adult stages are rarely available from patients and usually stool is examined for ova and reported without speciation. Because hookworms do not multiply in humans, the morbidity of hookworms is highest in patients that harbor large number of adult parasites. Estimates of intensity of hookworm infections are typically obtained by using quantitative fecal egg counts as a surrogate marker for worm burden. The WHO defines moderate intensity infections as those with 2000-3999 eggs per gram of faeces, and heavy intensity infections as those with 4000 or more eggs per gram [9].

WHO in 1981, recommended identification of the third stage filariform larvae for identification of the species after being reared from eggs in coprocultures. However morphological characters in filariform larvae are subtle and requires expertise [10]. Although some studies have shown that *N.americanus* is ubiquitous and dominant hookworm in some areas of a particular country in a particular time the contrary can be ruled out by a larger study of different areas and only selected in which epidemiological situations may differ. Since the relative distribution of the two species may vary from one endemic locality to the other similar studies need to be carried out in all endemic parts of the country [11].

As no epidemiological study on human hookworms was done earlier in Nava Raipur area, Chhattisgarh we report on a study to estimate the proportion of hookworm infections represented by *N.americanus* and *A.duodenale* in patients reporting at Shri Rawatpura Institute of Medical Sciences and Research (SRIMSR), Nava Raipur. The study shall be able to determine the prevalent species of hookworm in and around Raipur. The information so obtained from the study shall be useful to the clinicians for clinical assessment of the infection and prescribe the appropriate drug.

MATERIALS AND METHODS

The study was conducted from July 2023 to December 2024, in the department of Microbiology, SRIMSR, Nava Raipur. The study population included patients reporting at SRIMSR,Nava Raipur with gastrointestinal disturbances.

SPECIMEN COLLECTION

1. Patients were advised to pass stool directly on to a clean, leak proof container devoid of any antiseptics or disinfectants.
2. A tea spoon amount faces or about 10 ml of fluid specimen was collected for routine examination and culture [16].

TRANSPORTATION

Stool samples were examined immediately upon reaching the laboratory.

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MACROSCOPIC EXAMINATION

It was done to observe colour, consistency, presence of blood and mucus.

MICROSCOPIC EXAMINATION

Direct faecal smear or wet films were made mixing a small amount of stool with a drop of normal saline making smooth thin preparations which were then examined first under low power and then under the high power lens of the light microscope. [cheesebrough M]

Eggs were recognized by their

- Size -65x40 µm
- Colour –colourless
- Morphological features -oval in shape containing an ovum which appears segmented. [4-8 cell stage] [2].
- Negative samples were re examined by floatation with saturated sodium chloride solution. Positive samples were also re examined by floatation in order to compare egg counts.

CULTURE

Hookworm eggs in freshly passed unfixed stool samples were cultured to the infective third stage filariform larvae at room temperature, by the Harada Mori filter paper strip method[2].

- In this method 0.5-1 gm of fresh fecal material was smeared on to a narrow strip of filter paper[3/8 by 5 in] slightly tapered at one end.
- 3-4 ml of distilled water was added to a 15 ml conical tube[identify the specimen]
- The filter paper strip was inserted into the tube so that the tapered end was near the bottom of the tube, water level being approximately ½ inch below fecal matter.
- The tubes were maintained upright on the rack at 25-28 degree Celsius; distilled water was added to maintain original level. The tubes were incubated for-10 days and examined every alternate day by withdrawing a small amount of fluid from the bottom of the tube.

LARVAL EXAMINATION

The cultured filariform larvae were examined under the microscope; both iodine stained and unstained larvae were examined and identified according to the criteria of WHO[1981].

The following key points were taken into consideration for the identification of the filariform larvae of the two hookworm species:

Necator americanus	***Ancylostoma duodenale***
1. Body not including sheath 500-600 µm long.	1. Body not including sheath 600-700 micromtre long.
2. Anus to tail tip <73µm long.	2. Anus to tail tip >73 µm long.
3. Intestine at oesophagointestinal junction as wide as oesophageal bulb.	3. Intestine at oesophagointestinal junction narrower than the oesophageal bulb.
4. Buccal spears through out length.	4. Buccal spears inconspicuous.
5. Conspicuous transverse striations present on sheath especially in the tail region.	5.Transverse striations on sheath in tail region inconspicuous.
6. Oral cavity spear shaped.	6. Oral cavity closed,not clearly seen.
7. Genital primordium located at the centre of the intestine.	7. Genital primordium located posterior to the centre of the intestine.

RESULTS

A total of 659 stool samples from patients reporting at SRIMSR,Nava Raipur with gastro intestinal problems such as diarrhoea, vomiting ,abdominal pain were studied for hookworm infection during the period from July '23 to December '24. 50 samples were positive for total parasitic infections of which 20[40%] fecal samples were positive only for hookworms. Thus, the overall prevalence rate of hookworm infestation was found out to be 40%. Infection rates were higher in males 13 cases [65%] than in females. 7 cases [35%], with a p-value of less than 0.876.

TABLE 1: Age and sex distribution of the patients with hookworm infection.

AGE IN YEARS	MALES	FEMALES
< 10	-	-
11-20	1	1
21-30	2	1
31-40	4	1
41-50	4	1
51-60	1	-
61-70	1	2
> 71	-	-

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Hookworm infection increased with age, peaked in age group 31-50 in males and in the age group of 61-70 years in females [table- 1]. In addition, 100 Hookworm egg positive and negative fecal samples were cultured from the period of July '23 to December '24 to recover the infective filariform larvae using the Harada Mori coproculture method. Only 10 Hookworm egg positive fecal samples yielded the larvae. The results of the study for the first time showed that both *N.americanus* and *A.duodenale* to be present in Nava Raipur. The former species was relatively more common than the latter. Mixed infection was detected in 2 cases.

TABLE 2: Relative prevalence of N.americanus and A.duodenale.

HOOKWORM SPECIES	NUMBER OF CASES OBSERVED	PREVELANCE[% OF TOTAL HOOKWORM POSITIVE PATIENTS]
N.americanus	9	90%
A.duodenale	3	30%

Of the 10 Hookworm positive co-procultures, *N.americanus* filariform larvae were recovered from a total of 9 [90%], while *A.duodenale* filariform larvae were recovered from 3 [30%] of the patients [table- 2]. Only 2 [20%] subjects were simultaneously infected with both species of Hookworms as evidenced by the recovery of filariform larvae of both species [*N.americanus* and *A.duodenale*]. However in the mixed species infections, higher numbers of *N.americanus* larvae were recovered compared to *A.duodenale*, with the former ranging from 62.5-75% and the latter ranging from 25-60%.

In the present study, filariform larvae of *N. americanus* varied in length from 400-555 um [with majority measuring between 475-550 um], while *A.duodenale* larvae varied in length between 500-610um [majority measuring about 560-600um]. In the present study, most of the hookworm eggs were ranging in size from 71 x 46µm.(Fig. 1 and 2)

Fig. 1 - A – A.duodenale filaraiform larvae B - N.americanus filariform larvae

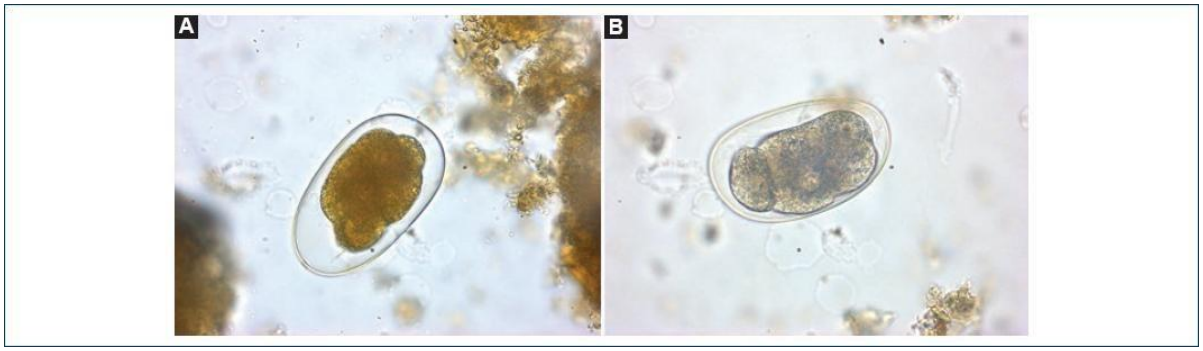

Fig. 2 - A/B- Embryonated Hookworm Ova

DISCUSSION

The study revealed that the hookworm infection rate in patients attending SRIMSR hospital from the period of July 2023 to December 2024 was 40% thereby highlighting the fact that hookworm infestation is high in and around parts of Nava Raipur. Although the results were insignificant [p<0.876], hookworm infestation in males [65%] was still higher than that in females [35%]. This result is in agreement with similar studies done in Kanpur, India and another one at Itagua, Paraguay [14,12].

This study also found that hookworm infestation rates

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increased with age, peaking in the age-group of 31-50 years in the case of males and in 61-70 age groups in the case of female patients. This is in accordance to a similar study carried out in Paraguay, Brazil were they found out that hookworm prevalence increased with age ,reached a maximum in the 29-30 age group and then declined. Such age intensity pattern is more commonly seen in other nematode infections such as *Trichuris trichura* and *Ascaris lumbricoides* but is rare in the case of hookworms. Differences in the age intensity patterns of hookworms may be due to the differences in the relative abundance of *N.americanus* and *A.duodenale*, perhaps because of differences in transmission dynamics of mixed infection [17].

This study further showed that *N.americanus* was the dominant hookworm species in patients with hookworm egg positive stool samples, attending SRIMSR hospital from the period of July 2023 to December 2024,as it solely accounted for 70% of all hookworms infections. This result is in agreement with previous studies carried out in other parts of the world, Paraguay and Nigeria [11,12]. However recent studies carried out in Pondicherry and in Kanpur, India [13,14] found out that *A.duodenale* was the more dominant species. Such differences in the regional distribution of the two species are not clearly understood and further studies need to be carried out. In a study conducted in Lagos, Nigeria [18] showed that apparently all infections with A.duodenale occurred always in association with *N.americanus* [mixed hookworm infections], with the latter species occurring independent of the former, and found that *A.duodenale* accounted for less than 4.5% of hookworm infection from Lagos population.

In the present study although a few samples yielded larvae *A.duodenale* was solely responsible for only 10% of all Hookworm infections, nevertheless occurred concurrently with *N.americanus* [mixed hookworm infection]in 20% of the subjects. These patients were probably more heavily exposed as to have been infected with both species. Inspite of the fact that a female *A.duodenale* lays an average of 30,000 eggs/day as compared to about 9000 by an adult female *N.americanus* [19]. Much higher numbers of *N. americanus* infective larvae were recovered in coprocultures compared to *A. duodenale* in all mixed infections suggesting that much higher number of adult female egg laying *N.americanus* worms were present compared to *A.duodenale* in all mixed infections, as all the eggs were subjected to the same culture conditions.

Assessment of all public health significance hookworm infection should not, as has been the case over the years, be focused only on estimation of the number of hookworm infections, rather it should also include identification of the infecting hookworm species. This is vital to the evaluation of hookworm infection as a public health problem were therapy and control of the disease should be specific and targeted at the infecting hookworm species. Well known anti Helmintics such as Alcopar and Pyrantel are known to be comparatively less effective against *N.americanus* than *A.duodenale* [20]. Moreover the ability of *A.duodenale* larvae to undergo latency in the host is surely going to complicate treatment [2]. Also in hookworm infection, the degree of severity varies with the infecting hookworm species. In a study carried in Zanzibar school children showed that infection with *A.duodenale* has a much greater impact on anemia then infection with *N.americanus.* Therefore were A.duodenale is more prevalent the effect on anemia might be greater. This is of a great public health significance [21].

SUMMARY AND CONCLUSION

A total of 659 stool samples received from patients with various gastro-intestinal disturbances ranging from anemia to vomiting and abdominal pain attending SRIMSR, were studied for hookworm infestation between July '23 to December'24. In addition, 100 stool samples were cultured, during the said period , using the Harada –mori coproculture method for recovery and identification of the infective filariform larvae. Out of 659 stool samples that were microscopically screened for the presence of hookworm eggs, 50 samples were found positive for different parasitic infections of which 20 cases of hookworm infection were recorded giving us an overall infection rate of 40%. While of the 100 stool samples cultured, only 10 hookworm egg positive samples yielded larvae, while no larvae could be isolated from the hookworm egg negative samples. The result of the study showed that both hookworm species to be present. Of the 10 hookworm positive coproculture *N.americanus* filariform larvae were recovered from a total of 9 [90%] while *A.duodenale* larvae were recovered from a total of 3[30%] patients. Only 2[20%] of the coprocultures had double species infections as evidenced by the recovery of the filariform larvae of both species. However in all mixed infection higher number of *N.americanus* larvae were recovered compared to *A.duodenale*, the former ranging from 62-75% and the latter ranging from 25-60%.

From the study it is clear that hookworm infection ranks quite high among the patients visiting SRIMSR with different gastro intestinal disturbances. This is alarming as because hookworm has been associated with impaired cognitive functions and decreased future economic productivity. Although very few stool samples yielded the larvae, *N.americanus* was the predominant hookworm species in the present study. *A.duodenale* also occurred. The results of the present study are reliably guaranteed by the Harada Mori culture method which is commonly used for the culture of hookworm eggs and in WHO 1981 identification scheme.

Identification of the infective hookworm species are based on the above is tedious and time consuming as

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it requires cultures of eggs and microscopic examination of numerous larvae before coming to a conclusion. Like other neglected diseases in the Third World, chronic infections with hookworms promotes long term morbidity and increases the likelihood that an afflicted population will remain mired in poverty. A rapid and simple method thus represents not only considerable savings in time over current methods, but also a more economical and efficient approach for the utilization of the already overburdened present healthcare delivery systems.

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