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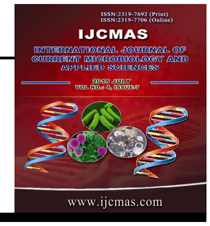

Case Study

A Case Report of Multiple Parasitic Infestations with Shigellosis

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A B S T R A C T

Enteroparasites are worldwide in distribution and enteroparasitic infections are usually seen in conditions with lowered immune response like malignancy, malnutrition, HIV infection, pregnancy, etc. In this report, we are discussing a case of 7 year old boy, who was admitted in Paediatric inpatient department of our hospital, with chief complaints of acute abdomen, loose stools and not accepting feeds. Routine stool examination showed rhabditiform larvae of *Strongyloides stercoralis* along with trophozoites of *Giardia lamblia*, cysts of *Entamoeba histolytica* and eggs of *Trichuris trichiura*. The soil transmitted threadworm, *Strongyloides stercoralis* is one of the most neglected among the so-called Neglected Tropical diseases. *Giardia lamblia* is considered to be the most common human protozoan enteropathogen worldwide. *E. histolytica* is a leading cause of mortality and morbidity in developing countries. While *Trichuris trichiura* is a common intestinal parasite of humans, primarily affecting caecum and appendix. On aerobic culture, non lactose fermenting colonies of *Shigella dysenteriae* type1 were isolated. Patient was started on Albendazole therapy, to which he responded well. This study reflects the importance of taking into consideration the possibility of such neglected parasitic infections, in order that their underreporting can be brought into check.

Keywords

Strongyloides stercoralis, Giardia lamblia, Entamoeba histolytica, Trichuris trichiura, Shigella dysenteriae

Introduction

Enteroparasites are widely distributed around the world, and infection usually varies according to region and age (Dinleyici *et al.,* 2003; Rajesh Karyakarte and AjitDamle, 2010). Conditions resulting into lowering of immune response like immunosuppressive therapy, HIV infection, malnutrition, pregnancy, etc., act as predisposing factors (Montes *et al.*, 2010; Siddiqui and Berk, 2001). *Strongyloides stercoralis*, is a nematode endemic in tropics and subtropics, mainly in hot and humid climates (Rajesh Karyakarte and AjitDamle, 2010; Montes *et al.,* 2010). It is more frequently seen in closed, low socio-economic communities with poor sanitation (Rajesh Karyakarte and AjitDamle, 2010; Montes *et al.,* 2010).

S. stercoralis is unique in its ability to replicate in the human host permitting ongoing cycles of autoinfection.

Strongyloidiasis can consequently persist for decades without further exposure to exogenous infection (Rajesh Karyakarte and AjitDamle, 2010). Chronic *S. stercoralis* infections can be asymptomatic or cause cutaneous, gastrointestinal and/or pulmonary symptoms (Montes *et al.,* 2010). Autoinfection can go unchecked and large numbers of invasive larvae may disseminate widely and cause hyperinfection, which can be fatal (Montes *et al.,* 2010).

Giardia lamblia is the only intestinal flagellate which causes endemic and epidemic diarrhoea in humans (Subhash Chandra Parija, 2013). It is well documented that in developing countries, infections are associated with poor sanitary conditions, poor water quality and overcrowding (Espelage *et al.,* 2010).

In developing countries, there is a very high prevalence and incidence of infection and data suggest that long-term growth retardation can result from chronic giardiasis (Adam, 2001).

Entamoeba histolytica is the third leading parasitic cause of death in the developing countries, infecting more than 10% of world's population (Subhash Chandra Parija, 2013). It is endemic in India and affects all age groups. Humans are affected through food and water contaminated with the cysts of *E. histolytica* due to feces, flies or unwashed hands of food handlers (Montes *et al.,* 2010).

T. trichiura causes worldwide parasitic infection most prevalent in tropical and subtropical areas. It is the third most common roundworm parasite in humans (Huang *et al.,* 2003). It is an infection of the large intestine and is called as whipworm (Nimir *et al.,* 2009).

Shigellosis is an important cause of bloody diarrhea in all age groups, especially in children (Dutta *et al.,* 2003). Poverty, poor sanitation, lack of personal hygiene and poor water supply are considered to be the major predisposing factors for *Shigella* infection (Srinivasa *et al.,* 2009). Amongst all the *Shigella* spp., *S. dysenteriae* and *S. flexneri* are most frequently isolated in developing countres (Huang *et al.,* 2009).

Here, we present a case report of a 7 year old boy who was admitted in our hospital with complaints suggesting gastrointestinal infection. On investigation, it revealed a rare combination of infection with multiple parasites which included *S. stercoralis, E. histolytica, G. lamblia* and *T. trichiura* along with *Shigella dysenteriae*.

Case report

A 7 year old boy, having quadriplegic spastic cerebral palsy with mental retardation, was brought to pediatric OPD with complaints of fever, abdominal pain, loose stools and not accepting feeds since 2–3 days. There was no history of vomiting.

Patient also had similar episodes of recurrent loose stools since 2 years, for which he was admitted in private hospital.

He was admitted to paediatric inpatient department.

Grossly the stools were yellowish green, semisolid, foul smelling and with a frequency of 10–12 per day.

On examination, he was irritable, pale, lethargic and cachexic with low grade fever not associated with chills or rigor. General condition was poor. His eyes were dry and platynychia was noted. Skin pinch retracted slowly which was suggestive of severe dehydration. Pulse was 126 beats/min, respiratory rate 20/min, blood pressure 90/70mmHg.

Anthropometrically, height was 16cm, weight 13kg, head circumference 45cm, chest circumference 49cm, mid-arm circumference 11cm, upper segment: lower segment 54:62. All this also suggested developmental delay.
Patient was a known case of cerebral palsy with history of convulsion episodes in the past, for which patient was on anti-convulsant therapy.

On cutaneous examination, there was marked urticaria along with pruritic, raised, erythematous lesions showing a typical course of larval migration.

The stool sample of the patient was send to microbiology department for further examination.

Microbiological workup

Grossly the stool was yellowish, semisolid and foul smelling.

On wet mount examination of stool, the following findings were noted:

Rhabditiform larvae of *S. stercoralis* (Fig.2):

- Size 200µmX15µm
- short buccal cavity
- double bulbed oesophagus
- a prominent genital primordium and
- anal pore from posterior end

Motile trophozoites of *Giardia intestinalis*:

- about 12µmX8µm size
- pear shaped and bilaterally symmetrical
- ventral disc, flagella, axostyles
- motile with a falling leaf appearance.
- Abundant in number

Cysts of *Entamoebahistolytica*:

- size 12µmx20 µm
- uninucleate to quadrinucleate

Eggs of *Trichuristrichiura*:

- bile stained barrel-shaped eggs
- protruding polar mucus plugs
- 50X25 µm in size
- Unsegmented ovum

On Gram stain of the stool sample, the larvae were stained Gram negative (Fig.3). Stool sample was further subjected to plate culture on blood agar. After incubation for 2days, visible trails of the larvae carrying the bacteria alongwith it, were seen (Fig.4). On aerobic culture, non-lactose fermenting colonies grew on MacConkey agar, which on further biochemical and motility testing gave following results:

- Non motile
- Indole not produced
- Methyl red positive
- Vogas-Proskauer reaction was negative
- Citrate not utilized
- Urea not hydrolysed
- On Triple sugar iron test, Alkali / Acid reaction
- Catalase negative
- Nitrate was reduced
- Mannitol not fermented
- Oxidase not produced
- Glucose fermented with no gas production
- Sucrose not fermented
- Ornithine not decarboxylated

On testing with antisera for serotyping, it was identified as *Shigella dysenteriae*. The strain was inferred to *be S. dysenteriae* type

1 based on biochemical reactions and serotyping.

Antibiotic susceptibility testing, using Kirby Bauer Disk diffusion method, was done against three drugs, i.e., Ciprofloxacin, Cotrimoxazole and Ampicillin, as mentioned in CLSI guidelines (CLSI, 2014). The isolate was found to be sensitive to Ciprofloxacin, while it was resistant to Ampicillin and Cotrimoxazole.

Repeat samples were collected on day 2 and day 3, after starting treatment, which showed marked decrease in larvae as well as other parasites. Also the health of the child improved after 3 days Albendazole therapy. He was further started on Metronidazole therapy for 14 days.

On complete blood count there was eosinophilic leucocytosis, hypocromia, microcytosis and anisocytosis. Ultrasonography of abdomen and pelvic scan had no significant finding.

Strongyloidiasis is caused by 2 species of the intestinal nematode Strongyloides. The most common and globally distributed human pathogen of clinical importance is *Strongyloides stercoralis*. The other species, *Strongyloides fuelleborni*, is found sporadically in Africa and Papua New Guinea (Siddiqui and Berk, 2001).

S. stercoralis was first reported in 1876 in the stools of French soldiers on duty in Vietnam who had severe diarrhea, and the disease the organism produces was known for many years as Cochin-China diarrhea (Siddiqui and Berk, 2001).

It has variable manifestations from asymptomatic to hyperinfection, or disseminated infection (Sharifdini *et al.*, 2014). Infections are mostly mild and often asymptomatic in otherwise healthy individuals (Salim *et al.,* 2014). Some patients have mild gastrointestinal, cutaneous, or pulmonary symptoms with or without fever (Sharifdini *et al.,* 2014).

Strongyloidiasis can occur in immunocompromised states such as hematologic malignancies, usage of corticosteroids or other immunosuppressive therapies, HIV infection and malnutrition (Dinleyici *et al.*, 2003; Rajesh Karyakarte and AjitDamle, 2010; Montes *et al.*, 2010). Risk factors for SS infection also include travelling to an endemic region and low socio-economic status with poor hygiene conditions (Dinleyici *et al.*, 2003).

In our case, patient was grossly malnourished with very poor hygiene status. This can be attributed to the mentally retarded state of the patient.

Chronic infections with *S. stercoralis* can be clinically inapparent or can lead to cutaneous, gastrointestinal, or pulmonary symptoms. Skin involvement is characterized by a migratory, serpiginous, urticarial rash, termed larva currens (Siddiqui and Berk, 2001).

The buttocks, groin, and trunk are more commonly affected by larva currens than the extremities and the head. Gastrointestinal symptoms of strongyloidiasis include diarrhoea, abdominal discomfort, nausea, and anorexia. Abdominal bloating is the most common complaint (Siddiqui and Berk, 2001).

However, in a majority of uncomplicated cases of strongyloidiasis, the intestinal worm load is often very low and the output of larvae is minimal. Eosinophilia is usually the only indication to the presence of *S. stercoralis* infection (Siddiqui and Berk, 2001).

In this case, patient had predominantly gastrointestinal and cutaneous symptoms. Patient had abdominal pain, diarrhoea, anorexia along with migratory urticarial rash over the trunk, buttocks and thighs, suggestive of larva currens. Also there was eosinophilia with anaemia.

Skin lesions, pulmonary and gastro-intestinal symptoms, and blood eosinophilia are reported as unspecific disease markers (Salim *et al.*, 2014).

The most commonly used conventional parasitological methods for detection of *S. stercoralis*include: Lugol iodine stain, Baermann concentration, formalin-ethyl acetate concentration, Harada-Mori filter paper culture, and agar plate cultures (Montes *et al.*, 2010; Siddiqui and Berk, 2001). However, agar plate culture and Baermann method are best suited (Sharifdini *et al.*, 2014).

The Lugol iodine mount of the patient's stool showed the presence of larvae with typical morphology of *S. stercoralis*, which eliminated the need of using concentration techniques for visualisation of larvae.

For agar plate culture, about 3g of fresh stool sample should be placed in the centre of the dish and incubated at 26–30°C for 48–72 hrs (Sharifdini *et al.*, 2014). Accordingly the stool was inoculated on blood agar plate and after incubation for 48 hrs., visible trails of the larvae carrying bacteria were noted, in the form of bacterial colonies.

Other than larvae of *S. stercoralis*, there were also cysts of *Entamoeba histolytica*, trophozoites of *Giardia lamblia* and eggs of *Trichuris trichura*; all of which suggests high grade parasitic infection. Similar kind of finding was obtained in one more study by Dinleyici EC *et al.*

Giardia lamblia infection is often acquired by drinking contaminated water or by person-to-person transmission among preschool children (Porter *et al.*, 1990). It is the only parasitic infection implicated in influencing the nutritive condition of the child with higher *G. lamblia* infection rates in undernourished children (Duran *et al.*, 2010). In humans, *Giardia lamblia*infections have a wide clinical spectrum ranging from asymptomatic carriage to long-lasting diarrhoea with malabsorption (Espelage *et al.*, 2010).

The child here was not only undernourished but also had a very poor sanitary living.

Trichuriasis is mostly observed in the age group of 2–7 years, where incidence of pica is highest (Nimir *et al.*, 2009). People living under poor hygienic conditions are at greater risk of developing trichuriasis-including institutionalized or mentally retarded persons and children of the primary school age. In more than half the cases of severe trichuriasis, there is a history of ingestion of non-food substances such as soil and wood.[8] Also hot and moist climate favor the worms' survival (Nimir *et al.*, 2009).

It is usually clinically asymptomatic. However, heavy infection, especially in small children, can cause gastrointestinal symptoms, such as abdominal pain, diarrhoea, nausea, vomiting, anorexia, constipation and chronic appendiceal syndrome (Huang *et al.*, 2003).

In this case, the child was noted to have the habit of eating soil, as indicated by attendant. This can be correlated to his age as well as the mentally retarded condition.

Of all serotypes of *Shigella*e, *Shigella* *dysenteriae* type 1 attracts special attention for its epidemic-causing potential and also for its association with most serious dysentery cases, with a high attack rate, high case-fatality rate, and various complications (Dutta *et al.*, 2003).

Though bloody diarrhea is a common presentation in *Shigella* infection, it was not present in our case. Such a finding was also noted in another study, where watery diarrhea has been noted as a more common presentation than bloody diarrhea in cases of *Shigella* infection (Huang *et al.*, 2009).

Oral rehydration is the principal means of management, because of the enteroinvasiveness antibacterial treatment may be necessary. While most of recent studies have reported multiple drug resistance in *Shigella* spp., it was not so in our case. One of the studies has noted highest resistance to Cotrimoxazole followed by Ampicillin (Huang *et al.*, 2009). Another study by Datta *et al.* (2003) shows multidrug resistance in *S. dysenteriae* type 1 isolates to at least 7 drugs, which included Ampicillin, Fluoroquinolones and Cotrimoxazole with hundred percent resistance.

According to Huang *et al.* (2009) *S. dysenteriae* is most frequently resistant to Ampicillin. This was also noted in our case; the isolate was resistant to Ampicillin and Cotrimoxazole while it was sensitive to Ciprofloxacin.

Because it is imperative to examine multiple stool samples to make a correct diagnosis according to Siddiqui and Berk (2001), stool sample was collected for 3 consecutive days and diagnosis was confirmed. But the parasitic load was reduced on each day since patient was started on therapy.

Many immunodiagnostic assays are available for detection of *S. stercoralis* infection. But they are not routinely used because of limited success. These include skin testing with larval extracts, indirect immunofluorescence analysis of fixed larvae, radio allegro sorbent testing for specific IgE, and gelatin particle agglutination. An ELISA test (Strongyloides antibody) for detecting the serum IgG against a crude extract of the filariform larvae of *S. stercoralis* is available only at specialized centers (Siddiqui and Berk, 2001). Also serological tests are available for diagnosis of Giardia like ELISA, indirect immunofluorescence and for *Entamoeba histolytica* such as PCR, indirect hemagglutination and counter immunoelectrophoresis. These were not done because of their non-availability.

Conclusion

Strongyloidiasis is becoming a major global health challenge that is underreported in many countries since it is not readily visible on routine procedures. All cases, especially children presenting with acute abdomen and diarrhoea associated with eosinophilia, should be investigated into depth, so as to avoid their misdiagnosis.

It is also essential to implement prevention efforts in endemic countries such as health education campaigns on the disease, proper sanitation through appropriate disposal of faecal material, regular de-worming and the use of protective footwear. Also habits like eating soil or pica should be discouraged.

Also it is important to make available a reliable data on neglected tropical diseases such as strongyloidiasis, so that indisputable causal link between infection and morbidity can be obtained.

Table.1 Other laboratory investigations were

Hemoglobin	6.9 g/dL	Neutrophils	65 %	Sr. Creatinine	1.4 mg%
WBC	$29120/mm^3$	lymphocytes	8 %	BUN	58 mg%
MCV	69.40 fL	Monocytes	3 %	Sr. Sodium	130 mEq/L
Platelet count	$285,000/mm^3$	Eosinophils	24 %	Sr. Potassium	3.3 mEq/L
HCT	22.90 %	Basophils	0 %	BSL	70 mg%
Sr. Bilirubin Indirect Direct	0.7 mg% 0.2 mg% 0.5 mg%	Total proteins Albumin Globulin	6.2 g/dL 3.2 g/dL 3.0 g/dL	SGOT SGPT	27 U/L 30 U/L

Fig.1 Showing urticarial rash and erythematous lesions along a typical course of larval migration

Fig.2 Showing rhabditiform larvae of *S. stercoralis* on wet mount of stool sample

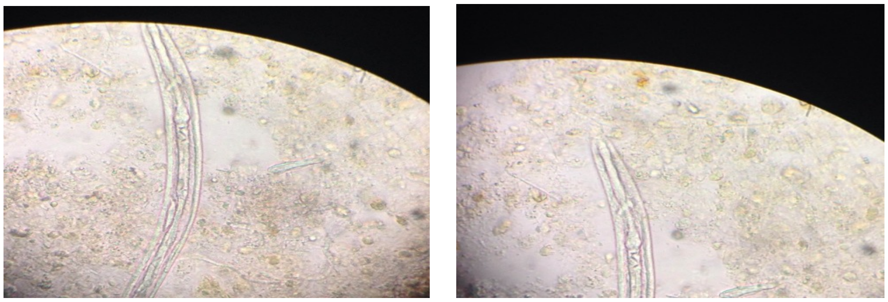

Fig.3 Showing Gram negatively stained larva of *S. stercoralis*

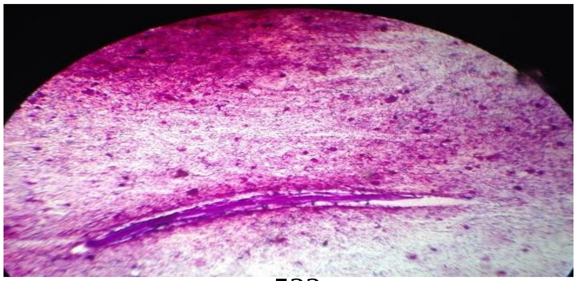

Fig.4 Showing trails of larvae carrying bacterial colonies along with them, on blood agar plate culture

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