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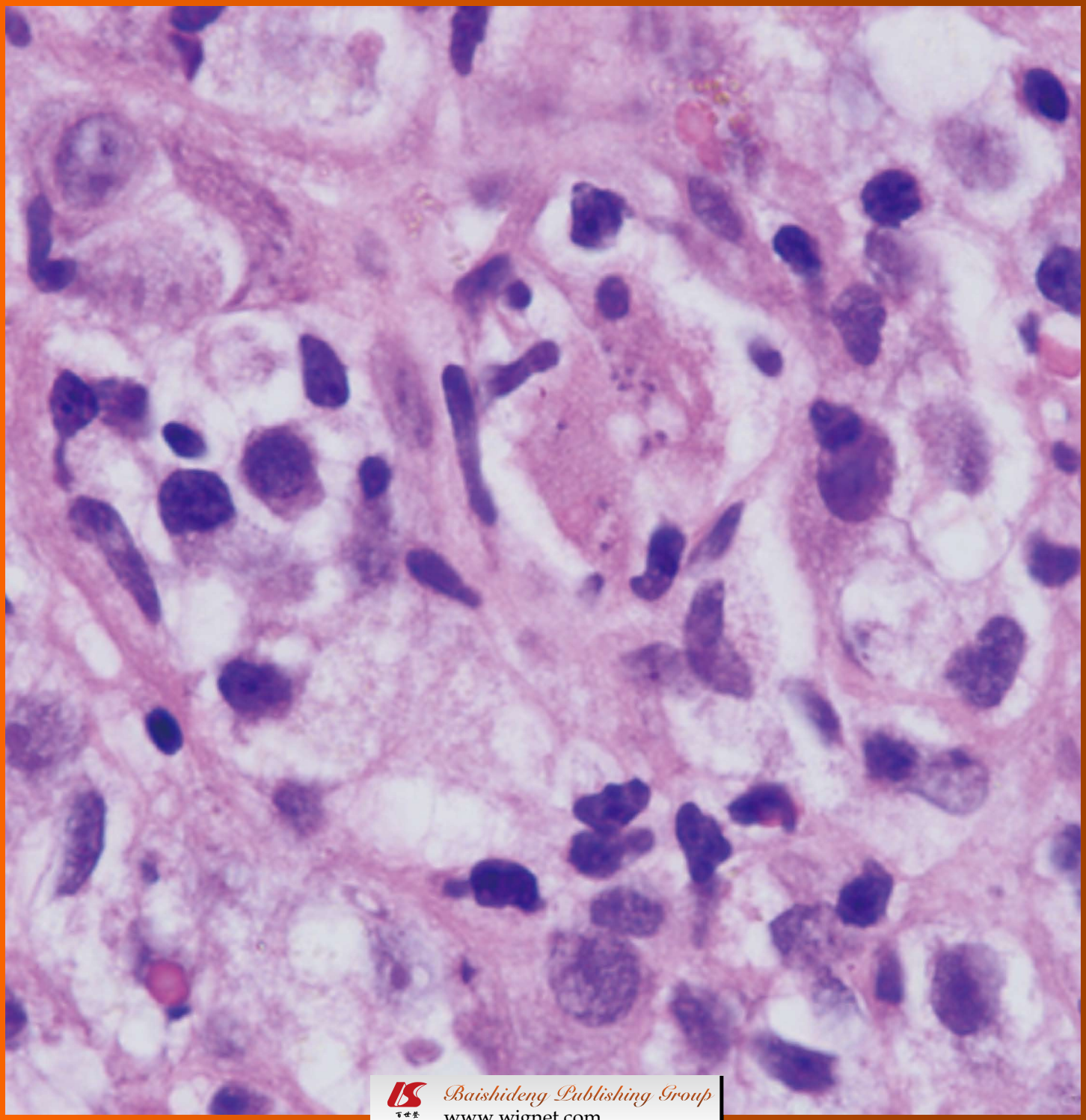

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BRIEF ARTICLE

Natural contamination of human hands with enteric parasites in Indian Subcontinent

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Abstract

AIM: To investigate the prevalence of enteric parasite contamination on hands and the potential role naturally contaminated hands may have in their transmission.

METHODS: Prior to initiating the survey, the protocol was reviewed and approved by respective Institutional Review Boards of each survey site (Dhaka, Bangladesh and Kolkata, India). Both stool and corresponding hand wash samples collected, were analyzed for the presence of enteric parasitic ova/(oo)cysts employing conventional microscopy coupled with permanent staining techniques. Additionally molecular approaches such as polymerase chain reaction (PCR) of enteric parasites recovered from both stool and corresponding hand wash samples, were also used to further confirm their identity.

RESULTS: A total of 972 stool samples were collected from both sites surveyed (300 volunteers from Kolkata, India and 672 from Dhaka, Bangladesh). Parasitic analysis revealed, 113 (38%) from Kolkata, India and 267 (40%) of stool samples from Dhaka, Bangladesh were positive for parasitic ova/(oo)cysts. When the corresponding hand wash samples were analyzed, 43 (14%) stool-positive volunteers in Kolkata, India and 47 (7%) in Dhaka, Bangladesh were positive for enteric parasitic ova/(oo)cysts. *Ascaris lumbricoides* (*A. lumbricoides*) ova and *Giardia lamblia* (*G. lamblia*) cysts predominated in hands wash samples from both sites surveyed (from India, *A. lumbricoides* ova, 53%; *G. lamblia* cysts 31% and from Bangladesh, *A. lumbricoides* ova, 47%; *G. lamblia* cysts 19%). Genotypic analysis of enteric parasitic ova/(oo)cysts obtained from both stool and corresponding hand wash samples taken from the same person were found to be identical.

CONCLUSION: These results suggest a possible role of hands contaminated with enteric parasites' ova/(oo)cysts in the transmission of these parasites highlighting another role of hand hygiene/proper hand washing in reducing the disease burden in low socio-economic communities.

Key words: Enteric parasites; *Ascaris lumbricoides*; *Giardia lamblia*; Natural contamination of hands

Core tip: The authors report contamination of human hands with enteric parasites in two independent sites surveyed in two developing countries of the Indian

Subcontinent. This study indicates that contamination of hands with parasite ova/(oo)cysts are common among those populations already infected and this may play a role in continued cycle of transmission and/or re-infection within the community.

Ijaz MK, Talukder KA, Aslam M, Haque R, Ganguly S, Azmi IJ, Hossain MS, Mukherjee AK, Raj D, Ahmed I, Kamal J, Rubino JR, Nur-E-Kamal A. Natural contamination of human hands with enteric parasites in Indian Subcontinent. *World J Clin Infect Dis* 2013; 3(2): 13-19 Available from: URL: http://www.wjgnet.com/2220-3176/full/v3/i2/13.htm DOI: http://dx.doi.org/10.5495/wjcid.v3.i2.13

INTRODUCTION

Parasitic infections are a global problem. Worldwide, more than a billion people are estimated to be infected with just one species of parasite [(*Ascaris lumbricoides* (*A. lumbricoides*)], mostly in derdeveloping countries[1,2]. Human association with enteric parasites extends into human history[3-5]. Some of these enteric parasitic agents, also called neglected intestinal parasites are responsible for causing not only chronic infection predisposing to malnutrition in children thereby lowering their resistance to infectious diseases, but also lead to malabsorption and further malnutrition by impairing intestinal absorption of nutrients critically required for child's growth and cognitive development[6,7]. This leads to the development of a vicious cycle of malnutrition - enteric pathogens - malnutrition synergy. For example, *A. lumbricoides*, a soil transmitted helminth (STH) sheds up to 200000 ova per day in the feces of infected person. With ineffective collection and treatment of human waste particularly in developing countries, *A. lumbricoides* ova widely contaminate the environment essentially maintaining a vicious cycle of malnutrition - enteric parasite - malnutrition synergy in human populations living under un-hygienic conditions as seen in most developing countries today[8,9]. Parasitic infections in humans are usually transmitted through fecal-oral route, using vehicles such as food, water environment, and hands contaminated with protozoa (oo)cysts and nematode ova, includes *Cryptosporidia*, *Giardia*, *Entamoeba histolytica*, *Enterobius* (pinworms), and *Ascaris* (roundworms)[10,11]. Such infections lead to loss of appetite, impaired digestion, malabsorption, and malnutrition leading to poor growth, cognitive development and predispose the vulnerable population to additional infectious agents including parasites[12].

Gastrointestinal parasitic infection has been significantly reduced in developed countries by improving sanitation and other hygienic measures including hand washing and adopting proper hand hygiene practices[13]. However, people living in low socioeconomic areas of the society in developing countries suffer from infections of various types of parasites. Although various vehicles (*e.g.*, food, water, feces, *etc.*) of transmission of enteric parasites from person to person have been reported, it remained to be demonstrated that hands of infected persons carry enteric parasites and are potential vehicle of their transmission. The scientific evidence describing intestinal parasites and bacterial contamination on paper currency from a developing country highlight the role of poor hand hygiene practices allowing dissemination of infectious diseases including enteric parasitic ova/(oo)cysts[11]. To our knowledge, this is the first report where enteric parasitic ova/(oo)cysts have been recovered from naturally contaminated hands of populations living in low socioeconomic communities of Indian Subcontinent (Bangladesh and India).

MATERIALS AND METHODS

Selection of human subjects

Human subjects were selected from the low socioeconomic communities of Dhaka, Bangladesh and Kolkata, India, living mainly in the slum area with poor sanitation facility and lack of good hygienic practices (*e.g.*, use of soap for hand washing after defecation; use of toilet tissue after defecation). A total of 300 volunteers from Kolkata, India and 672 volunteers from Dhaka, Bangladesh were selected in this study between April 2009 and June 2010. Volunteers for collection of stool and hand wash samples were selected independent of gender, age, religion, and race by a door to door visit procedure within the selected study region.

Sample preparation for microscopic analysis

Hands of the individual human subjects were washed with 100 mL of phosphate buffered saline (PBS) with rubbing. Total hand wash in PBS (100 mL) was centrifuged at 3000 rpm for 10 min to concentrate enteric parasitic ova/(oo)cysts. Hand wash samples collected from from both sites surveyed, were concentrated using the method of Ridley[14]. The concentrated ova/(oo)cysts thus obtained were suspended in 300 μL PBS. The concentrated ova/(oo)cysts were aliquoted into three parts: 100 μL was used for DNA isolation, 100 μL was used for microscopic analysis and 100 μL was stored at -80 ℃ for additional testing if required.

Microscopic analysis of stool and hand wash samples

Stool samples were tested for the presence of enteric parasitic ova/(oo)cysts using the methods published elsewhere[15]. In brief, a smear of feces in 0.9% saline was examined microscopically for the presence of enteric parasitic ova/(oo)cysts [*Entamoeba histolytica (E. histotylica)*, *Entamoeba dispar (E. dispar)*, *Giardia lamblia (G. lamblia)*, *Iodamoeba butschlii (I. butschlii)*, *Hymenolepis nana* (*H. nana*), *Trichuris trichiura* (*T. trichiura*), *hookworm*, *Cryptosporidium parvum (C. parvum)*, *Trichomonas hominis* (*T. hominis*), *Schistosoma*, *Blastocystis hominis (B. hominis)*, *Ascaris*, and *Taenia*]. Concentrated hand wash samples were directly observed under light microscope. Three separate techniques were used to identify the parasites in fecal samples: iodine wet mount

Table 1 Polymerase chain reaction primers used in this study

Target parasite	Target gene	Primer	Primer sequence (5'-3')	Annealing temperature	PCR Product size (bp)	Ref
Giardia lamblia	Beta-giardin	MAH433F MAH592R	CATAACGACGCCATCGCGGCTCTCAGGAA TTTGTGAGCGCTTCTGTCGTGGCAGCGCTAA	60	218	Rochelle *et al*[19]
Ascaris lumbricoides	rDNA	ITS-1F ITS-1R	TGCACATAAGTACTATTTGCGCGTAT TGATGTAATAGCAGTCGGCGG	60	82	Pecson *et al*[20]
Entamoeba histolytica	SSU rRNA	EH1 EH2	GTACAAAATGGCCAATTCATTCAATG ACTACCAACTGATTGATAGATCAG	51	128	Gonin *et al*[21]
Cryptosporidium sp.	SSU rRNA	18 SF 18 SR	TTCTAGAGCTAATACATGCG CCCTAATCCTTCGAAACAGGA	55	1325	Xiao *et al*[32]
Cryptosporidium sp.	Nested PCR for SSU rRNA		GAAGGGTTGTATTTATTAGATAAAG AAGGAGTAAGGAACAACCTCCA	55	825	Xiao *et al*[32]

PCR: Polymerase chain reaction.

staining for all parasites and parasitic ova/(oo)cysts[16]; modified Kinyoun's Acid fast staining for *Cryptosporidium sp.*[17] and Trichrome staining for *Giardia sp.* and *Entamoeba sp.*[16].

Polymerase chain reaction

Genomic DNA was isolated from stool samples according to the protocol described previously[18]. From handwash samples, total DNA was isolated by using DNA isolation kits (Invitrogen Life Technologies, Carlsbad, CA) according to the instructions provided by the manufacturer. All these DNA samples were used for the identification of enteric parasites present in the sample by polymerase chain reaction (PCR). Parasite-specific primers used in this study[19-21], their annealing temperature and respective PCR product sizes are listed in Table 1. PCR was performed according to the manufacturer's (Invitrogen Life Technologies, Carlsbad, CA) instruction. PCR product (DNA) was characterized by agarose gel electrophoresis. DNA was stained with ethidium bromide, visualized under UV light and images were recorded.

Ethical approval

Each survey site had their protocols reviewed and approved by their respective Institutional Review Boards (IRBs) prior to initiating the survey.

Genotyping of enteric parasites isolated from both stool and handwash samples

DNA was isolated from stool and hand wash samples of individuals, whose both hand wash and stool samples were positive for parasitic ova/(oo)cysts by microscopy. Every 15th Kolkata, India and every 10th Dhaka, Bangladesh handwash positive individual were analyzed this way. Segments of DNA known to be unique to each strain of enteric parasite were amplified by using specific primer sets (Table 1) for PCR. The PCR amplicons were purified with the GFXTM PCR DNA and gel band purification kit (Amersham Pharmacia, United States), and sequenced using the dideoxy-nucleotide chain termination method with the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction kit (Perkin-Elmer Applied Biosystems, Foster, CA) on an automated sequencer (ABI PRISM™ 310). The chromatogram sequencing files were inspected using Chromas 2.23 (Technelysium, Queensland, Australia). Sequence alignments were developed using CLUSTALX 1.81[22].

RESULTS

Recovery and identification of the types of enteric parasitic ova/(oo)cysts in stool samples

To better understand the effect of enteric parasitic burden on human health, we randomly surveyed a group of people living in low socioeconomic areas located in Kolkata, India and in Dhaka, Bangladesh. We found that 38% of the people in Kolkata and 40% in Dhaka were infected with enteric parasites. The different types of parasites detected by microscopy (morphology) are shown in Table 2. We also used PCR for DNA analysis to confirm the identity of these parasites (Figure 1). *A. lumbricoides* infection was the most prevalent (43% in Kolkata and 37% in Dhaka) followed by *Giardia* (26% in Kolkata and 10% in Dhaka) and 20% *Trichuris* in Dhaka. *Taenia* and *H. nana* infections were minimal (2%) in Kolkata and *T. hominis* and hookworm (0.5%) in Dhaka (Table 2). We did not find any pinworm in both study sites, most possibly due to the requirement of specific isolation protocol. These results indicate that a high percentage of people are infected with enteric parasites in both communities surveyed.

Recovery and identification of the types of enteric parasitic ova/(oo)cysts in hand wash samples

To determine if hands become contaminated in people infected with enteric parasites from their stool samples, handwash samples were examined for the presence of enteric parasitic ova/(oo)cysts. It was found that hands from 14% of the people in Kolkata, India and 7% in Dhaka, Bangladesh (Figure 2) that were contaminated with enteric parasites were also infected [stool-positive for enteric parasitic ova/oo(cysts)] with these enteric parasites in their gastrointestinal tract. Further analysis of these parasites by combined microscopy (Table 3),

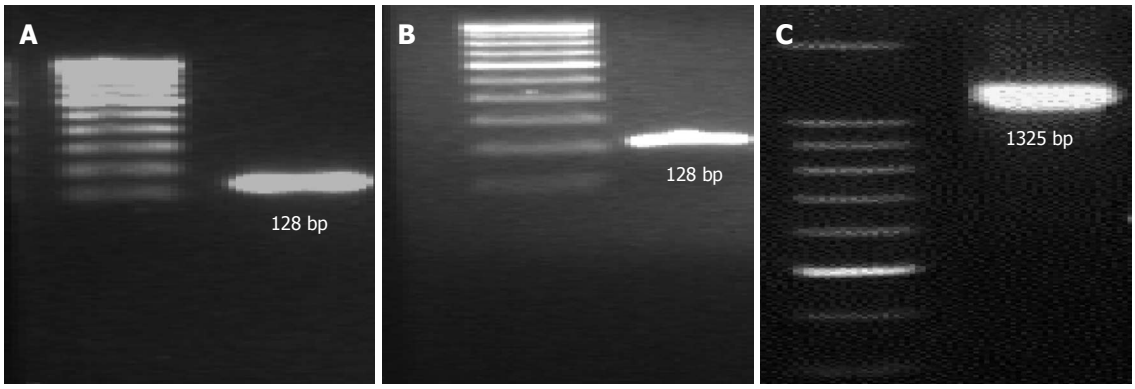

Figure 1 Identification of enteric parasites by polymerase chain reaction. Polymerase chain reaction (PCR) amplification of DNA sequences from stool and handwash samples containing. A: *Entamoeba histolytica*; B: *Giardia lamblia*; C: *Cryptosporidium sp.* Lane M: 1 kb DNA (Invitrogen), Lane E: 128 bp PCR product; Lane E: 128 bp PCR product; Lane C: 1325 bp PCR product ova/(oo)cysts.

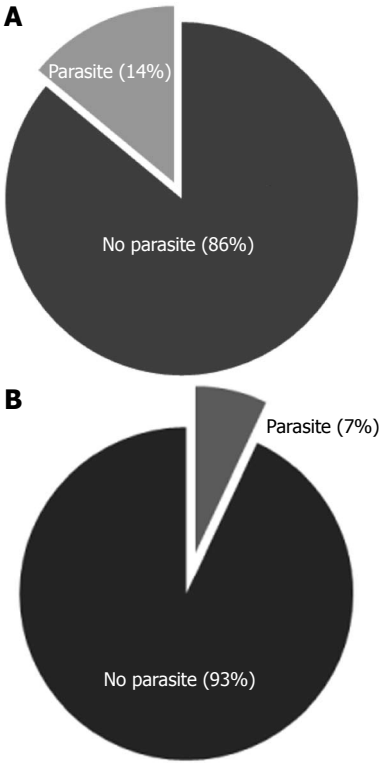

Figure 2 Identification of enteric parasites in hand wash. Hands of the individual human subject were washed with 100 mL of phosphate buffered saline (PBS) with rubbing several times. Total hand wash in PBS was centrifuged to concentrate enteric parasitic ova/(oo)cysts as described in the "Materials and Methods". Concentrated ova/(oo)cysts were studied by microscopic analysis and polymerase chain reaction (using genomic DNA). Presence of ova/(oo)cysts in hand wash samples from Kolkata, India (A) and Dhaka, Bangladesh (B) is shown in percentage.

PCR and permanent staining techniques revealed that *Ascaris* contamination was the most prevalent (53% in Kolkata and 47% in Dhaka) followed by *Giardia* (31% in Kolkata and 19% in Dhaka).

Genotyping of enteric parasitic ova/(oo)cysts isolated from stool and hand wash samples

In order to determine similarity between enteric parasitic ova/oo(cysts) isolated from stool samples and corresponding hands of volunteers, we genotyped these parasitic ova/(oo)cysts obtained from both stool and hand wash samples from the same person by nucleotide sequencing. It was found that PCR products obtained from stool and hand wash samples of each infected person were identical (Table 4). These results confirmed that hands of infected persons were contaminated with corresponding enteric parasite(s) present in their gastrointestinal tract.

Table 2 Characterization of enteric parasitic ova/(oo)cysts recovered from stool samples

Parasites	Prevalence in parasitic ova/(oo)cyst positive stool samples	
	Kolkata, India (n = 113)	Dhaka, Bangladesh (n = 269)
Intestinal protozoa		
Giardia lamblia	26%	10%
Cryptosporidium hominis.	10%	ND
Entamoeba histolytica	ND	7%
Blastocystis hominis	ND	7%
Iodamoeba butschlii	ND	6%
Trichomonas hominus	ND	0.50%
Soil-transmitted helminthes and schistosomes		
Ascaris lumbricoides	43%	37%
Trichuris trichiura	ND	20%
Hookworm	12%	0.50%
Hymenolepis nana	2%	1%
Taenia sp.	2%	ND
Schistosoma sp.	5%	ND

Percentage of each type of parasite found in stool samples from Kolkata, India and Dhaka, Bangladesh. ND: Not detected.

DISCUSSION

It is widely believed that hands are potential vehicle for transmission of infectious agents including enteric parasitic infection. To the best of our knowledge, no experimental evidence supporting this hypothesis regarding role of contaminated hands in dissemination of enteric parasites is available in scientific literature. In this multisite study, we surveyed intestinal parasitic infections in

Table 3 Identification of enteric parasites recovered from hand wash samples

Parasites	Prevalence of enteric parasitic ova /(oo)cysts in hand wash samples								
	Kolkata, India (*n* = 100)	Sex		Age (yr)		Dhaka, Bangladesh (*n* = 100)	Sex		Age (yr)	
		M	F	≤ 12	> 12		M	F	≤ 12	> 12
Intestinal protozoa										
Giardia lamblia	31%	64.5%	35.50%	35.50%	64.5%	19%	86%	14%	100%	0%
Cryptosporidium hominis.	5%	60%	40%	0%	100%	0%	0%	0%	0%	0%
Blastocystis hominis	ND	0%	0%	0%	0%	5%	0%	100%	0%	100%
Iodamoeba butschlii	ND	0%	0%	0%	0%	5%	0%	100%	0%	100%
Soil-transmitted helminthes and schistosomes										
Ascaris lumbricoides	53%	47.10%	52.90%	56.60%	43.40%	47%	68%	32%	100%	0%
Trichuris trichiura	ND	0%	0	0%	0%	24%	100%	0%	100%	0%
Hookworm	9%	44.40%	56.60%	0%	100%	0%	0%	0%	0%	0%
Schistosoma sp.	2%	0%	100%	100%	0%	0%	0%	0%	0%	0%

A portion of concentrated samples were smeared on microscopic slides and examined microscopically and/or by polymerase chain reaction. Percentages of different types of enteric parasites found in hand wash samples are shown. ND: Not detected. M: Male; F: Female.

Table 4 Genotyping of enteric parasites isolated from stool and hand wash samples by DNA sequencing

Enteric parasite studied	Number of samples studied	Number of samples genotyped with 100% similarity	
		Stool	Hand wash
Giardia lamblia	3	3	3
Entamoeba Histolytica	3	3	3
Trichuris trichiura	3	3	3
Giardia lamblia	5	5	5
Ascaris sp.	5	5	5
Trichuris trichiura	5	5	5

Similarities of enteric parasites (*Giardia lamblia*, *Cryptosporidium sp.*, *Ascaris lumbricoids*, and *Entamoeba histolytica*) found among the stool and hand wash samples collected from same individuals.

people from low socioeconomic communities in Bangladesh and India. We report presence of enteric parasitic ova/(oo)cysts on hands of people infected with parasite in their intestinal tracts and shedding their ova/(oo)cysts in stools. These results highlight the role of naturally contaminated hands with enteric parasitic ova/(oo)cysts in dissemination of enteric parasites in the communities with compromised hand hygiene and general hygiene practices.

In an analysis of randomly collected stool samples, we found that about 40% of human populations surveyed were infected with various enteric parasites. Our surveyed population in Dhaka, Bangladesh revealed that 7% of those volunteers infected with enteric parasites had their hands contaminated with parasitic ova/(oo)cysts. In a similar survey conducted in Kolkata, India we found about 14% of infected individuals had their hands contaminated with enteric parasitic ova/(oo)cysts. Difference in percentage of hand contamination of enteric parasites in two sites highlights the importance of analysis of increased sample size and number of sites to identify the cause of variation which could be one of the reasons accounting the for difference in two sites surveyed. Given, our study on contamination of hands with enteric parasitic ova/(oo)cysts was a snapshot in time, it is not known if the actual percentage of hand contamination with enteric parasites is more or less than what is being reported in this study. However, to the best of our knowledge, this is the first report demonstrating natural contamination of hands of human population (children and adults) infected with enteric parasitic ova/(oo)cysts which has also been confirmed by genotypic analysis to be the same parasites recovered from their stool samples.

Studies have shown that asymptomatic enteric infections (such as with *Cryptosporidium*, enteroaggregative *Escherichia coli*, and *Giardia*) are associated with retarded physical and cognitive development[23]. We have found a number of children stool-posive for enteric parasites, had their hands contaminated with these parasitic ova/(oo)cysts. This indicates a possible mechanism of transmission by self-inoculation of parasites in children maintaining the vicious cycle of enteric parasitic chain of infection that may lead to long term effect on their physical and cognitive development.

Hands contaminated with enteric parasitic ova/(oo)cysts can be a potential source of enteric parasitic infections in these communities and highlights the role proper hand hygiene practices could possibly have in reducing parasitic infection in these communities living in developing nations. People living in these communities are involved in working in food shops and other settings (*e.g.*, schools, hospitals and service industries). It appears

that natural contamination of hands of infected people could be a potential source of transmission of all enteric parasites in these hygienically-compromised communities. However, transmission of *Ascaris* through hand contamination remains unclear since *Ascaris* is known to require a contact of soil for hatching their eggs and they could have acquired *Ascaris* ova from the contaminated environment. Further studies are required to determine contribution of hand contamination in transmission of *Ascaris* in the community. In one study, school children from a slum in Visakhapatnam, south India were surveyed for intestinal parasitic load, found the prevalence rate for *A. lumbricoides* was 73%-75% followed by *T. trichiura* (66%) and hookworm (9%)[24,25]. Interestingly, re-infection prevalence post-treatment with albendazole reached pre-intervention level over a nine month period[26]. This highlights the potential role of hygiene to sustain the chemotherapeutic interventions programs designed for prevention and control these enteric parasites[3]. The problem is compounded by the fact that according to UNICEF and World Health Organization's (WHO) estimates, 1.1 billion people lacking safe water (1 in 6 people, or 18% of the world's 2005 population, projected to increase to 2.9 billion by 2025) and 2.4 billion lacking even pit latrines/adequate sanitation (4 in 10, or 42% of people, projected to be 4.2 billion by 2025)[27], consequently affecting adversely on the personal, domestic and community hygiene. Adopting holistic intervention approaches including improved hygiene, clean water supply along with nutrient supplement and chemotherapeutic intervention for combating infectious diseases including enteric parasites can potentially contribute not only to child's growth and cognitive development but also economic prosperity of the target population as experienced by developed nations. It is interesting to note that in emerging market such as India, the populations in general have more access to cell phones than toilets (http://www.inweh.unu.edu/News/2010-04_UNU-INWEH_News Release_Sanitation.pdf). Globally, roughly 1.5 billion individuals are infected with one of these parasites, *Ascaris*, primarily in Africa and Asia. In this regard, the developed nations are not fully immune to enteric parasites. Ascariasis is endemic in the United States as well. One study found that the prevalence of Ascariasis in the United States at about 4 million[28]. In a survey of a rural Nova Scotia (Canada) community, 28.1% of 431 individuals tested were positive for Ascaris, all of them being under age 20, while all 276 tested in metropolitan Halifax were negative[29] indicating disparity even within developed nations.

Therefore, according to UNICEF the role of water, sanitation, and hygiene (WASH) is critical for sustainable development contributing to the U.N.'s Millennium Development Goals which is to provide water and sanitation to fifty percent of population without access to safe water and basic sanitation, by 2015 (http://www.un.org/millenniumgoals/environ.shtml). Currently the UNICEF is promoting "WASH in schools to improve health" by lessening the spread of infectious diseases. Therefore, the potential mitigational role of hygiene in prevention of infectious agents including enteric parasites and thereby contributing to the child's physical and cognitive development cannot be under estimated[30]. According to WHO list of neglected tropical diseases (NTDs) (http://www.who.int/neglected_diseases/diseases/en/), the intestinal protozoa, STHs and schistosomes recovered from naturally contaminated hands of population surveyed, are amongst the main NTDs which can be prevented by adopting holistic approach including proper sanitation and hygiene measures[3,12].

In previous studies, bacteria/viruses have been reported to be present on hands and suggested to play an important role in their transmission in community[31]. In this study we report contamination of human hands with enteric parasites in two independent sites surveyed in two developing countries of the Indian Subcontinent. Our study indicates that contamination of hands with parasite ova/(oo)cysts are common among those populations already infected and this may play a role in continued cycle of transmission and/or re-infection within the community. Therefore, in addition to chemotherapeutic interventions, food and water sanitization, and regular hand hygiene practices would play a major role in reducing enteric parasitic infections. It will be useful to investigate the effectiveness of hand hygiene products (soap/hand wash agents) in removing enteric parasitic ova/(oo)cysts from naturally contaminated hands in these communities.

COMMENTS

Background

Gastrointestinal parasitic infection has been significantly reduced in developed countries by improving sanitation and other hygienic measures including hand washing and adopting proper hand hygiene practices. However, people living in low socioeconomic areas of the society in developing countries suffer from infections of various types of enteric parasites.

Research frontiers

Studies have shown that asymptomatic enteric infections (such as with *Cryptosporidium*, enteroaggregative *Escherichia coli*, and *Giardia*) are associated with retarded physical and cognitive development.

Innovations and breakthroughs

The results highlight the role of naturally contaminated hands with enteric parasitic ova/(oo)cysts in dissemination of enteric parasites in the communities with compromised hand hygiene and general hygiene practices.

Terminology

This study indicates that contamination of hands with parasite ova/(oo)cysts are common among those populations already infected and this may play a role in continued cycle of transmission and/or re-infection within the community.

Peer review

This is a well written manuscript that clearly demonstrates contamination of human hands with enteric parasites present in the gastrointestinal tract of the tested individuals themselves. The study is based on two independent sites surveyed in two developing countries, and the results found were very similar for both sites. The study demonstrates that the contamination of hands with parasite ova/(oo)cysts are common among low income populations infected with enteric parasites and indicate that this may play a role in continued cycle of transmission and/or re-infection within the community. This manuscript strengthens the importance of hand hygiene in reducing the spread of parasites in particular and infectious diseases in general.

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CASE REPORT

Liver biopsy for visceral leishmaniasis diagnosis in pregnancy: report of 2 cases

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Abstract

Visceral leishmaniasis (VL) or kala-azar is a zoonosis caused by intracellular protozoa of the *Leishmania* genus and is transmitted to humans by the bite of phlebotomine sandflies. It particularly affects cells in the phagocytic mononuclear system, accompanied by disturbances of cellular and humoral immunity. VL is potentially fatal and is characterized by fever, hepatosplenomegaly, diarrhea, epistaxis, jaundice, anemia, leucopenia, thrombocytopenia, hypoalbuminemia and hyperglobulinemia. Diagnostic suspicion is based on epidemiological, clinical and laboratory data and is confirmed by detecting the parasite in infected tissue. Splenic aspiration is the most sensitive method, followed by bone marrow aspiration (BMA) by sternal puncture, liver biopsy and lymph node aspiration; but, due to safety concerns, BMA is the most recommended method. VL is included as a target disease by players in drug research and development. Severe liver dysfunction associated with VL is uncommon. We report two VL cases in pregnant women from Bauru, Sao Paulo state, Brazil, considered an endemic area. The first of them developed hepatic failure due to fulminant hepatitis. In both cases, BMA was unable to find the protozoan; thus, liver biopsy was the only means of making the diagnosis.

Key words: Visceral Leishmaniasis; Infection in pregnancy; Liver biopsy; Bone marrow aspirate; Differential diagnosis

Core tip: Visceral leishmaniasis, which has many severe presentations, is an endemic disease found in many countries around the world, especially in South America, where Brazil is the most affected country. We herein present two cases of this disease affecting women during pregnancy, when the diagnosis and management can be very difficult. In both patients, the usual method of diagnosis failed so liver biopsy was the only option to make the correct diagnosis. Therefore, liver biopsy may be considered in special situations when severe visceral leishmaniasis is suspected, as in the cases herein presented.

Lima TB, Villar CR, Rodrigues MAM, Baima JP, Yamashiro FS, Franzoni LC, Caramori CA, Silva GF, Romeiro FG, Sassaki LY. Liver biopsy for visceral leishmaniasis diagnosis in

pregnancy: report of 2 cases. *World J Clin Infect Dis* 2013; 3(2): 20-24 Available from: URL: http://www.wjgnet.com/2220-3176/full/v3/i2/20.htm DOI: http://dx.doi.org/10.5495/wjcid.v3.i2.20

INTRODUCTION

Visceral leishmaniasis (VL) or kala-azar is a zoonosis caused by intracellular protozoa of the Leishmania genus. The disease is transmitted to humans by the bite of phlebotomine sandflies and Lutzomyia longipalis is one of the major transmitting agents. In Latin America, 90% of VL cases occur in Brazil, where the disease is found in 19 states and affects approximately 1600 cities[1,2]. The main reservoirs in wild and household environments are foxes and dogs, respectively[1,2]. The disease affects the phagocytic mononuclear system, leading to disturbances of cellular and humoral immunity[3,4]. The city of Bauru, 325 km from the state capital, is considered an endemic area for VL[5]. The disease affects individuals at any age and children under 10 years are commonly involved in endemic areas[6,7]. In contrast, VL is rare among pregnant women and there are few studies in these patients. The most common manifestations are fever, hepatosplenomegaly, diarrhea, epistaxis, jaundice, anemia, leucopenia, thrombocytopenia, hypoalbuminemia and hyperglobulinemia[1]. Diagnostic suspicion is based on epidemiological, clinical and laboratory data. In endemic regions, the diagnosis is confirmed by detecting the parasite in infected tissue and the first recommended option is bone marrow aspiration (BMA) by sternal puncture. Given that gestational VL is rare in South America, severe liver dysfunction associated with this diagnosis is uncommon[1]. Herein, we report two VL cases in pregnant women from Bauru, Sao Paulo state, Brazil, the first of which developed fulminant hepatic failure. In both cases, BMA was negative and liver biopsy was necessary to make the diagnosis.

CASE REPORT

Case 1

A 26 year old pregnant woman had a preterm vaginal delivery after 5 d of daily fever associated with hypogastric abdominal pain and foul lochia. She was initially treated with cephalothin and later with ampicillin, amikacin and metronidazole, prescribed due to suspected puerperal infection. She developed jaundice, choluria, hepatosplenomegaly and increased liver enzymes (Table 1). Imipenem and vancomycin were then introduced. Abdominal ultrasonography (US) and computed tomography showed no alterations in the liver or biliary tract. Transvaginal ultrasonography also showed no alterations. Serology for viral hepatitis, autoantibodies, ceruloplasmin, iron profiles, blood cultures and urocultures were negative. The patient had pancytopenia which was investigated by BMA but no specific alterations were found. Due to progressive liver failure, percutaneous liver biopsy was performed and the liver histology showed acute hepatitis, with intense mixed portal inflammation and structures compatible with amastigotes (Figure 1). The patient was treated immediately with liposomal amphotericin B, at a total dose of 20 mg/kg body weight, for 5 d. At that time, anemia, thrombocytopenia and abdominal pain worsened. Subsequent US identified a clot retained in the abdomen which was removed by exploratory laparotomy. Hemotherapeutic support was then initiated, including concentrations of red blood cells and platelets, as well as fresh plasma, cryoprecipitates and neutrophil colony-stimulating factors. The patient became unconscious, with increased serum ammonia and bilirubin levels concomitant with a progressive reduction in aminotransferases, thus characterizing hepatic failure due to fulminant hepatitis. Despite all efforts, she died on the 32nd puerperal day.

Case 2

A 31 year old woman in the 12th week of pregnancy arrived at the hospital with epigastric and right flank pain, progressive jaundice and choluria that had persisted for the previous 12 d. Physical examination showed jaundice without visceromegaly or fever. Laboratory tests suggested cholestasis and hepatocellular lesions. Serology for viral hepatitis was negative and no signs of biliary obstruction were found at abdominal US. BMA was performed but no specific findings were observed. Percutaneous liver biopsy was indicated and the histological analysis showed acute hepatitis and the presence of amastigotes, compatible with VL. Liposomal amphotericin B infusions were immediately initiated (at the 12nd gestational week) and this time the treatment was followed by clinical and laboratory improvement (Table 2).

DISCUSSION

There are no estimates of VL in pregnant women, particularly due to the small number of publications on such cases. In South America, VL in pregnant women is considered to be rare and the first Brazilian case was reported in 1993[8,9]. In case 1, clinical, laboratory and epidemiological data led to suspicion of VL because the patient had been residing in an endemic city (Bauru) and presented with associated symptoms of daily fever and acute hepatitis. Despite the possibility of trans-infectious hepatitis, the major causes of acute hepatitis were investigated, including viral diseases (hepatitis A, B and C, herpes simplex, cytomegalovirus, varicella, dengue fever, Epstein-Barr), which represent 40% of jaundice causes in pregnancy. These viral diseases do not usually affect the natural course of VL, except for hepatitis E and herpes simplex virus (HSV), which may lead to acute liver failure and fetal loss. Although HSV is considered to be a rare hepatitis agent, it can cause severe hepatitis in immunosuppressed individuals, neonates, pregnant women and transplant patients, with a mortality rate of up to 50% or even 60%[10,11]. The patient showed no

Table 1 Biochemical test profile, blood and coagulation tests of the 1st case

	Post-partum (d)				
	19	24	29	32	Normal range
Biochemical test profile					
GGT (U/L)	577	289	47	58	15-73
Alkaline Phosphatase (U/L)	1081	924	121	103	36-126
Aspartate transaminase (U/L)	338	3879	1212	429	30-110
Alanine transferase (U/L)	94	727	279	110	21-75
Albumin (g/dL)	2.3	2	1	1.5	3.5-5
INR	1.34	2.55	2.51	1.68	< 1.25
Total bilirubin (mg/dL)	13	15	10	16	0.2-1.3
Indirect bilirubin (mg/dL)	3	4	2	2	0-1.1
Direct bilirubin (mg/dL)	10	11	8	14	0-0.3
Blood and coagulation tests					
Platelets (× 10^3/mm^3)	155	132	55	22	140-440
Leukocytes (× 10^3/mm^3)	7.3	5	2.5	0.9	4-11
Hemoglobin (g/dL)	10	9.7	4.4	10.5	12-16
Factor Ⅴ (%)	-	70.2	40.7	64.8	50-150
Factor Ⅶ (%)	-	43.5	36	35.2	50-150
Fibrinogen (mg/dL)	-	164	90	193	146-380

GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio.

Table 2 Main laboratory tests of the 2nd case during LV treatment

	Gestational week				
	12	13	14	22	Normal range
GGT (U/L)	102	94	83	9	15-73
Alkaline Phosphatase (U/L)	116	106	109	45	36-126
Aspartate transaminase (U/L)	1711	2041	1905	16	30-110
Alanine transferase (U/L)	2198	2237	1809	15	21-75
Albumin (g/dL)	3.3	3.4	3.5	-	3.5-5
INR	1.29	1.51	1.69	1.2	< 1.25
Total bilirubin (mg/dL)	14.5	19.6	24.6	0.6	0.2-1.3
Indirect bilirubin (mg/dL)	1.6	2	2.4	0.4	0-1.1
Direct bilirubin (mg/dL)	10.7	15	16.3	0	0-0.3

GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio. LV treatment was initiated at the 12th gestational week.

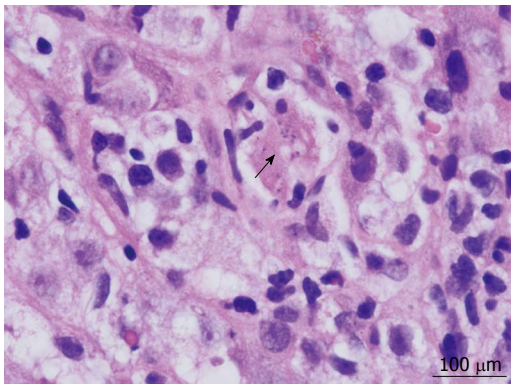

Figure 1 Pathological findings (hematoxylin/eosin staining × 1000 magnification-immersion oil). Liver biopsy. Structures compatible with amastigotes (arrow).

improvement after the use of broad spectrum antibiotics. Therefore, other possible hepatic diseases were also investigated: autoimmune hepatitis (AIH), Wilson's disease, as well as other lymphoproliferative, metastatic and metabolic diseases. With regards to the liver diseases associated with pregnancy, the most common are viral and drug-induced hepatitis, pre-eclampsia, acute hepatic steatosis in pregnancy (AHSP) and intrahepatic cholestasis in pregnancy (IHCP)[11-14]. The HELLP Syndrome (Hemolysis, Elevated Liver enzymes and Low Platelets) is a serious complication that most frequently occurs in the 3rd trimester but can also occur in the puerperium in 25% of cases[15]. However, the first case had no signs of hemolysis or the presence of schizocytes in the peripheral blood. AHSP occurs between the 30th and 40th pregnancy weeks and begins with the slow onset of anorexia, indisposition and cephalea, followed by vomiting, abdominal pain, fever and, at a later phase, jaundice[16-18]. In 2% of cases, AHSP causes acute hepatic failure, requiring urgent liver transplantation[19]. However, patients with AHSP tend to show clinical and laboratory

improvement after delivery, which did not happen in the case described. IHCP manifests in the 3rd gestational trimester with jaundice and pruritus in up to 70% of cases, but liver function deterioration is rare[12,18,20-22]. AIH affects young patients and due to the conditions of physiological immunosuppression induced by pregnancy, the activity of the disease may be exacerbated after delivery when immunological conditions are reversed. Again, case 1 did not meet the criteria established by the International AIH Study Group[17] so a diagnosis of AIH was rejected. When VL became the major diagnostic suspicion, other exams were considered. Splenic aspiration is the most sensitive method (90%-95%), followed by BMA (80%-90%), liver biopsy and lymph node aspiration, but BMA is the method recommended due to safety concerns[23]. When BMA is performed, the absence of leishmania in BMA does not preclude a diagnosis of the disease. For this reason, percutaneous liver biopsy was indicated and confirmed the diagnosis. Liver compromise resulting from VL has been reported in approximately 2% to 28% of cases[24]. Hepatomegaly may occur in up to 90% of cases, followed by slight increase in liver enzymes without severe disorders[25]. The presence of the parasites in Kupffer cells may be found in up to 40% of cases before treatment[26]. Severe cases with a bad prognosis have been associated with severe anemia, fever for longer than 60 d, diarrhea and jaundice[27]. Fulminant liver failure (FLF), which rarely occurs in VL, has been described more often in children[1]. According to a previous study by Singh *et al*[28] of 155 VL cases with liver compromise, moderate liver dysfunction was found in 16% and FLF in 1.6% of cases. Malatesha *et al*[29] reported an isolated case of an immunocompetent adult male with FLF by VL who recovered after therapy with amphotericin B. Unfortunately, our first case developed FLF without any response to liposomal amphotericin B. During the hospitalization, she developed multiple organ dysfunction and her 7 year old daughter was also hospitalized with fever and VL, which was successfully treated. If liver biopsy had not been performed to confirm the maternal disease, the child would not have been diagnosed so early. The rapid diagnosis was critical to the successful treatment of this child. Our second case was an oligosymptomatic (without fever or splenomegaly) pregnant woman. The lack of fever and visceromegaly could have delayed the diagnosis but the positive VL epidemiology was fundamental for the diagnostic suspicion. Once again, BMA was not confirmatory. The progressive increase in transaminases and liver dysfunction were the criteria used to indicate the liver biopsy. The American Association for the Study of Liver Diseases does not consider pregnancy to be a contraindication to this procedure[30]. Therefore, a diagnosis of VL was confirmed by the liver biopsy, allowing immediate treatment and resulting in the favorable outcome in this second case. Serological tests may be particularly useful for diagnosing VL given their high predictive value in the diagnosis of immunocompetent individuals. However, in severe cases from endemic areas, they are not sufficient to indicate the specific therapy, because the time required for cured individuals to return to a negative serology (anti-leishmania) is not known. Data suggest that a cellular immune response may still be present in subjects cured of the disease. This would explain the persistence of significant *Leishmania sp.* antibody titers in some subjects after treatment[31]. Thus, a positive test in the absence of clinical manifestations does not authorize the administration of therapy, which is not free of toxic effects.

VL has epidemiological importance in South America, especially in Brazil. Although the disease is rare among pregnant women and rarely causes severe liver dysfunction, both situations can be present in the same patient, requiring early and accurate diagnosis to reduce morbidity and mortality rates. The contribution of liver biopsy as an alternative to BMA in parasite detection was noteworthy in our cases. We conclude that other patients with VL in whom BMA is negative can obtain a correct diagnosis if liver biopsy is performed, as we showed in our last case.

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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