

Natural contamination of human hands with enteric parasites in Indian Subcontinent

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Author contributions: All the authors contributed to this paper. Supported by President's Award, Medgar Evers College of the City University of New York and Reckitt Benckiser LLC, New Jersey, United States

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Received: February 4, 2013 Revised: April 7, 2013

Accepted: May 9, 2013

Published online: May 25, 2013

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.

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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 μ L PBS. The concentrated ova/(oo)cysts were aliquoted into three parts: 100 μ L was used for DNA isolation, 100 μ L was used for microscopic analysis and 100 μ L was stored at -80 °C 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. histolytica*), *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
<i>Giardia lamblia</i>	Beta-giardin	MAH433F	CATAACGACGCCATCGCGGCTCTCAGGAA	60	218	Rochelle <i>et al</i> ^[19]
		MAH592R	TTTGTGAGCGCTTCTGTCGTCGGCAGCGCTAA			
<i>Ascaris lumbricoides</i>	rDNA	ITS-1F	TGCACATAAGTACTATTGCGCGTAT	60	82	Pecson <i>et al</i> ^[20]
		ITS-1R	TGATGTAATAGCAGTCGGCGG			
<i>Entamoeba histolytica</i>	SSU rRNA	EH1	GTACAAAATGGCCAAATTCATTCAATG	51	128	Gonin <i>et al</i> ^[21]
		EH2	ACTACCAACTGATTGATAGATCAG			
<i>Cryptosporidium sp.</i>	SSU rRNA	18 SF	TTCTAGAGCTAATACATGCG	55	1325	Xiao <i>et al</i> ^[32]
		18 SR	CCCTAATCCTTCGAAAACAGGA			
<i>Cryptosporidium sp.</i>	Nested PCR for SSU rRNA		GAAGGGTGTATTATTAGATAAAAG AAGGAGTAAGGAACAACCTCCA	55	825	Xiao <i>et al</i> ^[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 hand-wash 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 GFX™ 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),

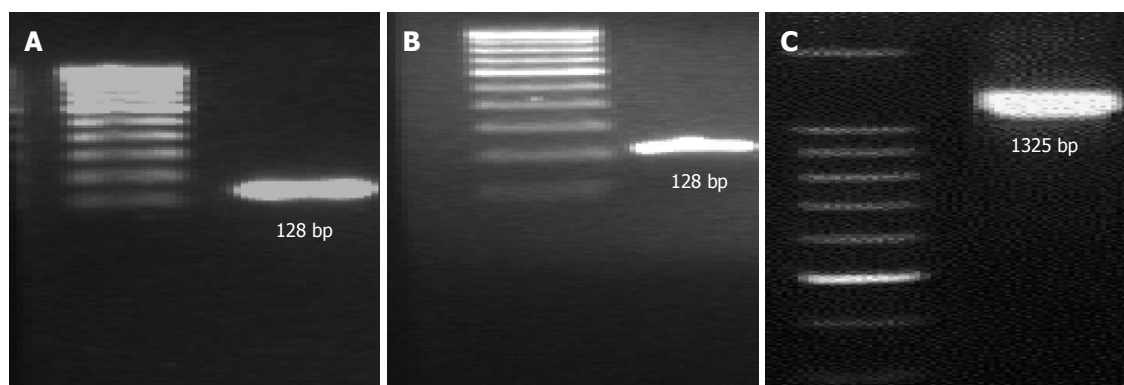


Figure 1 Identification of enteric parasites by polymerase chain reaction. Polymerase chain reaction (PCR) amplification of DNA sequences from stool and handwash samples containing: A: *Entamoeba histolytica*; B: *Giardia lamblia*; C: *Cryptosporidium* sp. Lane M: 1 kb DNA (Invitrogen), Lane E: 128 bp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

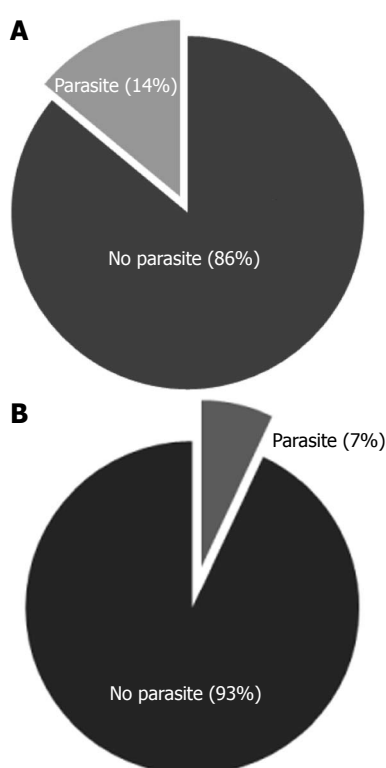


Figure 2 Identification of enteric parasites in hand wash. Hands of the individual human subject were washed with 100 mL of phosphate buffered saline (PBS) with rubbing several times. Total hand wash in PBS was centrifuged to concentrate enteric parasitic ova/(oo)cysts as described in the “Materials 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 cor-

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		
<i>Giardia lamblia</i>	26%	10%
<i>Cryptosporidium hominis</i> .	10%	ND
<i>Entamoeba histolytica</i>	ND	7%
<i>Blastocystis hominis</i>	ND	7%
<i>Iodamoeba butschlii</i>	ND	6%
<i>Trichomonas hominus</i>	ND	0.50%
Soil-transmitted helminthes and schistosomes		
<i>Ascaris lumbricoides</i>	43%	37%
<i>Trichuris trichiura</i>	ND	20%
Hookworm	12%	0.50%
<i>Hymenolepis nana</i>	2%	1%
<i>Taenia</i> sp.	2%	ND
<i>Schistosoma</i> sp.	5%	ND

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

responding 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.

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 multi-site 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										
<i>Giardia lamblia</i>	31%	64.5%	35.50%	35.50%	64.5%	19%	86%	14%	100%	0%
<i>Cryptosporidium hominis</i>	5%	60%	40%	0%	100%	0%	0%	0%	0%	0%
<i>Blastocystis hominis</i>	ND	0%	0%	0%	0%	5%	0%	100%	0%	100%
<i>Iodamoeba butschlii</i>	ND	0%	0%	0%	0%	5%	0%	100%	0%	100%
Soil-transmitted helminthes and schistosomes										
<i>Ascaris lumbricoides</i>	53%	47.10%	52.90%	56.60%	43.40%	47%	68%	32%	100%	0%
<i>Trichuris trichiura</i>	ND	0%	0	0%	0%	24%	100%	0%	100%	0%
Hookworm	9%	44.40%	56.60%	0%	100%	0%	0%	0%	0%	0%
<i>Schistosoma sp.</i>	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
<i>Giardia lamblia</i>	3	3	3
<i>Entamoeba Histolytica</i>	3	3	3
<i>Trichuris trichiura</i>	3	3	3
<i>Giardia lamblia</i>	5	5	5
<i>Ascaris sp.</i>	5	5	5
<i>Trichuris trichiura</i>	5	5	5

Similarities of enteric parasites (*Giardia lamblia*, *Cryptosporidium sp.*, *Ascaris lumbricoides*, 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 contami-

nation 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-positive 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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