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The Risk of Infection from *Giardia lamblia* due to Drinking Water Supply, Use of Water, and Latrines among Preschool Children in Rural Lesotho

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Esrey S A (Department of International Health, Johns Hopkins University, School of Hygiene and Public Health, 615 N Wolfe St, Baltimore MD 21205, USA), Collett J, Miliotis M D, Koornhof H J and Makhale P. The risk of infection from *Giardia lamblia* due to drinking water supply, use of water and latrines among preschool children in rural Lesotho. *International Journal of Epidemiology* 1989, **18**: 248–253.
Stool samples were collected from 267 rural, preschool children in four districts in Lesotho during October–November, 1984. Sixty-three children (23.6%) were tested positive for *Giardia lamblia*, the most commonly recovered parasite from stool samples. The use of low amounts of water for personal hygiene was associated significantly with having *G. lamblia* (OR = 2.42), but the use of traditional, non-improved drinking water sources (OR = 1.38) or lack of latrines (OR = 0.94) was not. Although *G. lamblia* may be primarily waterborne in developed countries, the amount of water that is used for personal and domestic hygiene may be more important than the quality of drinking water in developing countries. Other risk factors that were identified to be associated significantly with having or not having *Giardia* were child older than 24 months (OR = 6.79), mother less than 20 years of age (OR = 5.18), residing in Mohales Hoek district (OR = 2.33), and possessing several agricultural tools (OR = 0.70).

Giardia lamblia is a common protozoan found throughout the world. The percentage of carriers of *G lamblia* varies considerably by geography and age, but asymptomatic excretion globally may be as high as 10%.[1] Prevalence estimates for developing countries may be higher, particularly among young children, since prevalence among children is about three times higher than among adults.[2–4] Infection rates for children are variable throughout the world and may exceed 50% in some areas.[5,6]

Although *G lamblia* was once thought to be non-pathogenic,[7] it is now known to be a leading cause of acute diarrhoea. Clinical manifestations vary from asymptomatic to chronic diarrhoea. Intestinal damage due to *G lamblia* may result in disaccharidase deficiency, steatorrhoea, vitamin A[8] and vitamin B_{12}[9,10] malabsorption, and weight loss.[11,12] Available treatments are effective,[13] but the vast majority of infected people in developing countries remain undiagnosed and never receive treatment. Efforts at prevention should therefore prove more effective in reducing morbidity due to *G lamblia* since treatment is unlikely.

G lamblia is generally thought to be transmitted through drinking water[14–23] that has become contaminated with human or animal faeces. It has been recovered from untreated and municipal water supplies.[21] *G lamblia* is also thought to be transmitted by food[24,25] and person-to-person contact.[26,27] Two observations suggest that personal hygiene may play a role in the transmission of *G lamblia* that is possibly more important than that of drinking water. First, infectious doses of *G lamblia* may be low so that a few viable cysts (40–100 cysts) from food[24] or hands may be sufficient to lead to infection. Second, although many endemic areas may have occasional

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waterborne outbreaks of *G lamblia*, these outbreaks are too seldom and infrequent to sustain high rates of endemic infection. Furthermore, in many other endemic areas, waterborne outbreaks have not been reported.

Most studies of *G lamblia* transmission have been confined to Western countries, where waterborne outbreaks have been well documented. Less is known about other sources of transmission, particularly in developing countries, although *G lamblia* is known to be a leading cause of diarrhoea. Since transmission is by the fecal-oral route, the elimination of faeces from the environment, by improved disposal of human and possibly animal faeces, should reduce infection rates. Similarly, the use of larger amounts of water for good personal hygiene should also be effective in reducing infection due to *G lamblia*. It is the purpose of this paper to examine these factors, while controlling for the effect that other risk factors may have on the transmission of *G lamblia*.

METHODS

Stool samples and socioeconomic data were collected from 267 children, regardless of their health status, in 21 villages in rural Lesotho. The children were a subset of a larger group of children (n = 600) that were part of a health impact evaluation of a rural water supply programme, which was conducted from July 1984 to February 1985.[28] The 21 villages were located in the lowland and foothill areas of four districts, Butha Buthe, Leribe, Mafeteng, and Mohales Hoek.

Socioeconomic information on 267 children was collected during July and August 1984. On the basis of a questionnaire administered to mothers, variables were categorized to reflect environmental, child, maternal, and household characteristics.

Three environmental dummy variables were presence or absence of latrines for the household, water use of less than versus more than or equal to eight litres per capita per day, and exclusive versus non-exclusive reliance on an improved water supply. Improved water supplies were either taps or hand pumps.

Most improved villages were serviced by taps, in which spring water passed down a gradient through a silt box to a storage tank that was located as close as possible to consumers, but at least five metres above the highest tap. When springs were unavailable in the lowlands, hand pumps were situated on boreholes, which averaged 50 metres in depth. Each tap or hand pump was installed within 150 metres the household, but often much closer, and each serviced no more than 100 people.

Child characteristic dummy variables were sex, age (over versus under or equal to 24 months), and breastfeeding. Six maternal dummy factors were controlled in the analyses: mother's age (under versus over or equal to 20 years of age), whether or not the mother was literate, pregnant, or married, and the mother's occupation (knits/sews or brews beer).

Household characteristics included the following continuous (c) and dummy (d) variables: household size (c), whether or not remittances were sent to the household from South Africa (d), household possessions (d) of more versus less than seven items from a list of 12 possessions, ownership of agricultural equipment (c), district of residence (d) (Mohales Hoek versus the other three districts), and whether or not a child came from a village with or without an improved water supply (d).

Stool samples were collected during October and November 1984, three months after the collection of socioeconomic data. Stool containers and paper plates were dispensed to mothers the afternoon prior to stool collection, and the mothers were asked to deposit the children's stools in the containers. The containers were collected the following morning, usually shortly after the child defaecated, and immediately packed in ice in a styrofoam box and transported to Maseru, the capital. Samples were flown to Johannesburg and transported to the South African Institute for Medical Research on the same afternoon that stools were collected. Samples were then divided into three containers for bacteriological, parasitological, and virological examination.

The stools partitioned for parasitological analysis were stored at 4°C before they could be processed by the merthiolate-iodine-formaldehyde (MIF) method.[29] A small sample of faeces (about 150 mg) was mixed with three ml of MIF. The fecal-MIF mixture was strained through three layers of surgical gauze into a centrifuge tube. Two ml of ether were added and mixed thoroughly for 30 seconds using a pasteur-pipette. The top three layers (ether, a plug of faecal debris, and MIF) were poured off leaving a fourth layer, the sediment, which was resuspended in the small amount of remaining fluid and examined microscopically. All stool samples were analysed without any knowledge of the socioeconomic status of the children or their village of residence.

Statistical analyses were done in SYSTAT, version 3.0,[30] on an AT&T 6300 Personal Computer. A supplementary logistic regression program[31] was used for the multivariate logit analysis when estimating the odds ratios. Two-tailed 95% confidence intervals were calculated on the environmental variables as well as on the controlling variables.

RESULTS

Several parasites were recovered from the stool samples (Table 1). Helminths were rare, but protozoa were detected with greater frequency. Only *G lamblia* was detected with enough frequency to examine the epidemiology of the protozoan. Among those children testing positive for *G lamblia*, only 11.3% had had diarrhoea in the last 24 hours according to the mother's definition. These protozoa were seldom found among infants and the highest prevalence was among two-year-old children (Table 2). *G lamblia* was recovered from 20.7% and 26.2% of males and females, respectively.

Forty-three per cent of the children came from families with an average water use of eight or more litres per capita per day (lcd); 57% of the families used less than eight lcd (Table 3). Greater per capita per day water use was protective since those children whose families used less than eight litres per capita per day were more than two times (unadjusted odds ratio (OR) = 2.31, 95% confidence interval (CI) = 1.25 − 4.26) as likely to have *G lamblia* than those whose families used eight or more litres per capita per day (Table 4).

Twenty-eight per cent of the children came from families that relied exclusively on improved water supplies for their drinking and cooking needs (Table 3). Children of non-exclusive users of the improved water supplies, those families with or without access to improved water supplies, were more than twice as likely (unadjusted OR = 2.16; 95% CI = 1.05 − 4.35) to have *G lamblia* as those whose

TABLE 1 *Prevalence of parasites in 267 stool samples among preschool children in rural Lesotho*

Parasite	Positive	
	Per cent	Number
Protozoa		
Chilomastix mesnili	17.6	(47)
Endolimax nana	3.0	(8)
Entamoeba histolytica	2.6	(7)
Entamoeba coli	2.6	(7)
Giardia lamblia	23.6	(63)
Helminths		
Ascaris ova	0.0	(0)
Enterobius vermicularis	0.4	(1)
Hookworm	0.0	(0)
Hymenolepis nana	1.9	(5)
Taenia sp	0.7	(2)
Total number of parasites in stool		
None	62.2	(166)
One	25.1	(67)
Two	10.9	(29)
Three	1.5	(4)
Four	0.4	(1)

TABLE 2 *Percentage of children excreting Giardia lamblia by age*

Age in months	Sample size	Per cent positive
<13	48	2.1
13−24	69	17.4
25−36	63	41.3
37−48	45	28.9
>48	42	26.2
Total	267	23.6

TABLE 3 *Distribution of Giardia lamblia among preschool children in rural Lesotho by presence or absence of environmental risk factor*

Environmental risk factor	*Giardia lamblia*	
	Positive	Negative
Water use in litres per capita per day		
< 8 lcd	45	106
≥ 8 lcd	18	98
Drinking water source		
Non-exclusive or non-use	52	140
Exclusive use of improved supply	11	64
Sanitation		
No latrine	50	143
Latrine	13	61

TABLE 4 *Environmental and other determinants of Giardia lamblia among preschool children in rural Lesotho*

Risk factor	Odds ratio (95% confidence interval)	
	Unadjusted	Adjusted
Environmental characteristics		
Low water use	2.31 (1.25−4.26)	2.42 (1.12− 5.23)
Non-exclusive water use	2.16 (1.05−4.35)	1.38 (0.53− 3.57)
No latrine	1.64 (0.83−3.23)	0.94 (0.39− 2.27)
Child characteristics		
Greater than 24 months	4.00 (2.05−7.81)	6.79 (2.41−19.09)
Male	0.74 (0.42−1.32)	0.85 (0.42− 1.69)
Breastfeeding	0.42 (0.23−0.78)	1.50 (0.54− 4.15)
Maternal characteristics		
Less than 20 years	2.82 (1.06−7.49)	5.18 (1.48−18.09)
Literate	0.60 (0.20−1.82)	0.56 (0.13− 2.41)
Pregnant	2.68 (1.31−5.47)	1.75 (0.72− 4.27)
Married	1.16 (0.52−2.57)	1.77 (0.64− 4.87)
Knits/sews	2.28 (0.78−6.68)	2.85 (0.76−10.75)
Brews beer	1.71 (0.96−3.05)	1.70 (0.83− 3.47)
Household characteristics		
Household size	0.95 (0.82−1.09)	1.00 (0.84− 1.21)
Remittances	0.91 (0.44−1.88)	1.00 (0.38− 2.62)
Household possessions (few)	1.83 (1.02−3.28)	1.99 (0.93− 4.29)
Agricultural possessions	0.73 (0.57−0.94)	0.70 (0.51− 0.96)
District (Mohales Hoek)	2.69 (1.41−5.13)	2.33 (1.04− 5.21)
Improved group	0.59 (0.33−1.04)	0.89 (0.40− 1.98)
Constant		0.02 (0.00− 0.29)

families relied exclusively on improved water supplies (Table 4).

Latrines were available for families of 28.0% of the children under investigation (Table 3). Unadjusted effects indicated that the presence of a latrine was protective since children in families without a latrine were 1.64 times (95% CI = 0.83 – 3.23) more likely to have *G lamblia* than children with an available latrine.

These environmental effects were re-examined while other factors (see Methods and Table 4) were controlled. Low water use was still associated with an increased risk of having *G lamblia* (adjusted OR = 2.42). These effects were similar to the unadjusted effects, and the 95% interval did not include 1.00. The effect of exclusive water use was somewhat diminished although still protective since non-exclusive users were still more likely to have *G lamblia* (adjusted OR = 1.38). The 95% confidence interval, however, included 1.00. The protective effect of latrines disappeared when the other factors were entered into the regression (adjusted OR = 0.94).

Several of the controlling variables were associated particularly with having *G lamblia* (Table 4). Children two years of age or older (56% of the sample) were more than six times (adjusted OR = 6.79) as likely to have *G lamblia* as children under two. A child with a mother under 20 years of age (7% of the sample) was more likely to have *G lamblia* than a child whose mother was older than 20 (adjusted OR =5.18). Children from Mohales Hoek district (20% of the sample) were more than twice as likely to have *G lamblia* than children from any of the other three districts (adjusted OR = 2.33).

DISCUSSION

The amount of water used was more important than the source of drinking water or the presence of latrines in lowering infection due to *G lamblia*. Water use, as measured by litres per capita per day, was associated with infection due to *G lamblia* more than was the source of drinking water, as measured by exclusive use of the improved water supplies for drinking and cooking needs. The results related to water quantity were similar in the univariate and multivariate models, but the results related to drinking water source were diminished when the effects due to water quantity, as well as to other factors, were included in the model. Similarly, the protective effects of latrines disappeared when other factors were included in the model.

Examination for *G lamblia* cysts in stools is less reliable than is examination by duodenal aspirates[7,32] or biopsy,[13,33,34] particularly when only one stool is examined. Thus, many children may have been classified incorrectly as negative for *G lamblia*. However, the associations found above are likely to be true because no differential bias in isolation rates is likely to occur among the exposed and unexposed.

Stool detection rates for *G lamblia* found in this sample of rural children were similar to those in previous reports about urban children living in Lesotho. Stool isolation rates of 19.4% among school children 60 – 180 months of age[35] and 27.4% among children 2 – 80 months of age living in Maseru[36] have been reported from studies using the same technique as that used in this study.

Although improved water supplies were more widely available in urban than in rural areas, infection rates in Maseru, the capital, were similar to those found in rural areas, suggesting that drinking water source was not as important as the amount of water used or other factors. If source of water was important urban detection rates would be expected to be lower than rural detection rates since a higher percentage of the urban population has access to improved water supplies.

The weak association between drinking water source and risk of *G lamblia* may be explained by one of two possibilities. *G lamblia*, in general, may not be found in traditional water sources, and efforts to improve drinking water may not effect the protozoan to any measurable degree. A more likely explanation is that *G lamblia* may frequently be found in traditional water sources, and not in the improved water at its collection point. *G lamblia*, however, may be introduced into the drinking water by poor water storage practices in the home thereby negating the benefits of improved water. Although the water source was not examined for *G lamblia*, recontamination of water stored in the home did occur in this sample.[28] Therefore, efforts to provide uncontaminated drinking water may result in less *G lamblia* if home water storage practices insure that recontamination does not occur.

The behavioural mechanisms whereby the quantity of water used is related to a lower risk of *G lamblia* is not known. More detailed information on how the increased water is used would be necessary before specific recommendations on hygiene behaviour could be made. Mechanisms of better water use could be associated with the handling and preparation of food, the ability to keep food preparation and storage areas cleaner, or more frequent hand washing and personal hygiene measures unrelated to food preparation.

An important risk factor for *G lamblia* was young mothers, who provided a stronger determinant of

G lamblia than quantity of water used. Younger mothers may have less experience in child care than older mothers, and they may be less hygienic, which may not be related to daily quantity of water used. This is a high risk group that should be targeted for health education either at clinics or in the community.

Amount of water used was also related to diarrhoea and growth of children in this sample.[28] One of the mechanisms whereby water use may reduce diarrhoea and improve child growth is by reducing infection with *G lamblia*. In this sample *G lamblia* was not associated with diarrhoea, on the basis of 24-hour recall; it was, however, associated with child growth.[28]

In this study water quantity was more important than water quality in preventing infection with *G lamblia*. Recent reviews of water and sanitation health impact studies have also concluded that increasing water quantity in areas of low or moderate water use is more effective for improving health than is water quality.[37,38] Although waterborne *G lamblia* may be a problem in developed countries, where other routes of transmission are less important, in developing countries where other routes of transmission continue to exist waterborne *G lamblia* may not be an issue. The specific mechanisms whereby the risk of *G lamblia* could be reduced are poorly understood. This includes behaviour related to the recontamination of water used for drinking as well as the behaviour associated with the transmission of *G lamblia* independently of drinking water.

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