CONTAMINATION OF SOIL WITH HELMINTH PARASITE EGGS IN NEPAL

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Abstract. Contamination of soil with helminth eggs in Kathmandu Valley (n=122) and outside of Valley (n=34) in Nepal was investigated with the use of centrifugal floatation technique using sucrose solution (sp gr 1.200). The overall soil contamination rate was 36.5% (57/156). The prevalence was uniform in Kathmandu Valley (36.9%) and outside of the valley (35.3%). A mean of six helminth eggs per sample were detected out of which more than half were embryonated (potentially infective). In Kathmandu Valley, soil contamination rate was higher (48.3%) during wet season compared with that observed in dry season (33.3%) but without significant difference (p>0.05). Multiple species of helminth eggs were detected in 22.8% of total positive samples (n=57). Altogether five species of nematoda *(Ascaris lumbricoides, Toxocara* sp, *Trichuris trichiura, Capillaria* sp and *Trichostrongylus* sp) and two species of cestoda *(Hymenolepis nana* and *H. diminuta)* were recovered. *A. lumbricoides* was predominant in Kathmandu Valley while *Trichostrongylus* was the commonest one in outside of valley.

INTRODUCTION

Hundreds of millions of people in the world mainly in developing countries, even today, are infected with soil-transmitted helminths (STH) with a significant amount of morbidity and mortality (Chan *et al*, 1994; WHO, 1997). In some areas, nearly 100% of people are infected (van-Niekerk *et al*, 1979; Reily, 1980; Rai and Gurung, 1986). One estimate has put 120 million to 215 million cases of morbidity due to *Ascaris lumbricoides*, 90 million to 130 million due to hookworm and 60 million to 100 million due to *Trichuris trichiura* infections (Chan *et al*, 1994) with an annual death numbers of 60,000, 65,000 and 10,000, respectively (WHO, 1997). In addition, helminths of animal origin also pose a potential zoonotic health problem.

Elsewhere in the developing world, soil contamination rate reportedly range from 20% to 64% (Pezzani *et al*, 1996; Uga *et al*, 1997). A relatively stable soil contamination rate with *Ascaris* eggs throughout the year attributed to open and indiscriminate defecation has also been reported (Peng *et al*, 1996). Contamination of soil with parasite eggs, thus, constitute a most important risk factor for STH as well as for zoonotic helminth infections. In the industrialized countries, where STH prevalence is very low, investigators have focused their study on zoonotic helminth parasites such as *Toxocara* eggs contamination of sandpits in public parks (Uga *et al*, 1989; 1995a; Uga, 1993) including the measures to prevent their contamination and extermination of the eggs already present in the sandpits (Uga and Kataoka, 1995b).

Nepal, located in South Asia, is one of the least developed countries with nearly 22 million population living largely on agricultural subsistence. The varied geotopography with diverse climatic condition can be divided into three regions namely, the Mountains, the Hills, and the Terai (plains). The population densities and life style vary according to the region and ethnic groups, respectively. Approximately 70% of the health problems in Nepal are infections. Of them, STH infection alone is most important (Reily, 1980; Estevez *et al*, 1983; Rai and Gurung, 1986; Rai *et al*, 1994; 1995) and has been found to significantly affect on the nutritional status of Nepalese (Rai *et al*, 1998; 2000). However, the soil contamination with STH eggs of both human and animal origin has not been well investigated. Since the soil contamination with *A. lumbricoides* eggs

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alone indicate the status of environmental hygiene (Schulz and Kroeger, 1992), we investigated the status of soil contamination with parasitic helminth eggs in Kathmandu Valley and two rural areas in outside of the valley in Nepal.

MATERIALS AND METHODS

Soil sample collection site

Soil samples were collected in urban and suburban areas in Kathmandu Valley (n=122) and in two rural areas in outside of valley [Boya village development committee (VDC) in Bhojpur district in Eastern Region and Nandegada and Mastamandu VDCs in Achham district in Far-Western Region] (n=34) (Fig 1). In Kathmandu Valley, samples were collected from the roadside, playgrounds, bus parks, vegetable gardens, near community water tap and household surroundings during both the wet (June-September) and dry (November-April) seasons. Samples from outside of the valley were collected from vegetable gardens and household surroundings in dry season only.

Detection of helminth eggs

Approximately 200-250 g of soil samples from a depth of about 2 cm in an area not exposed to direct sun light was collected using a small shovel. The parasite eggs were detected by sucrose solution floatation method as described previously by Uga *et al* (1989). Briefly, soil samples were dried overnight at room temperature and sifted through a 150 mm mesh sieve. Approximately 2 g of powdery soil in a test tube was suspended in approximately 8.0 ml of Tween-20 solution (0.05%), centrifuged at 80*g* for 10 minutes and the supernatant was removed. The tube was filled up to 5/6 with sucrose solution (sp gr 1.200), vortexed and centrifuged at 190*g* for 10 minutes. The tube then was filled up to the top with sucrose solution so that only a small air bubble was formed when a coverslip was placed on the tube. After a final centrifugation at 25*g* for 5 minutes, the coverslip was lifted from the tube, placed on a clean glass slide and examined under the microscope for parasite eggs.

Statistical analysis

The findings were stratified against geographi-

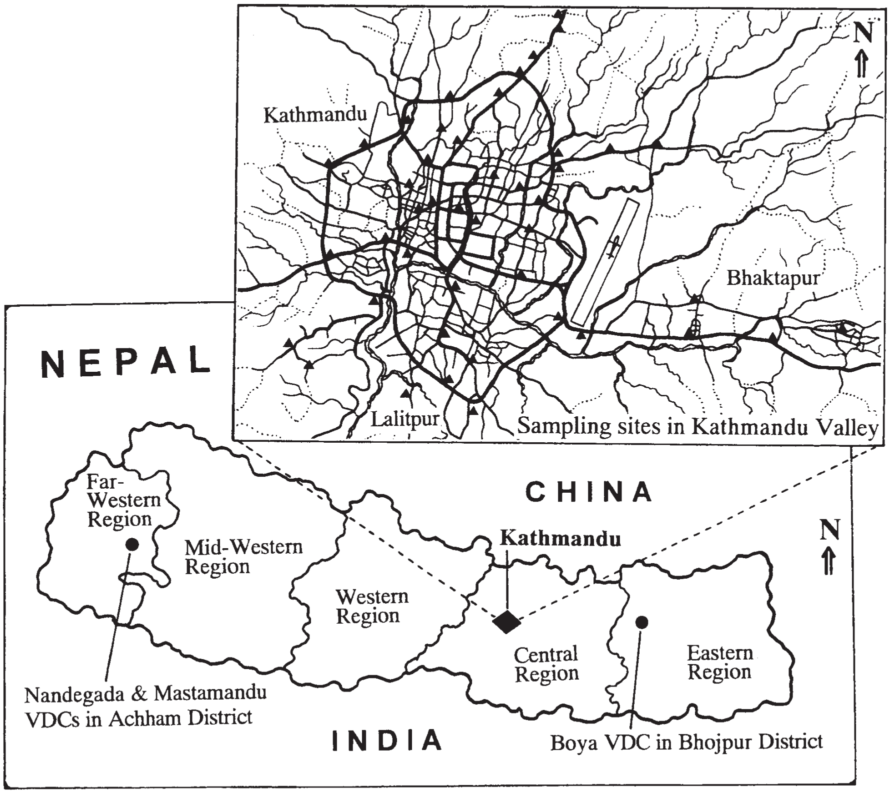


Fig 1–Map showing soil sampling sites in Kathmandu Valley and outside of the valley in Nepal.

Table 1
Type and frequency of helminth eggs recovered in two different seasons in Kathmandu Valley, Nepal.

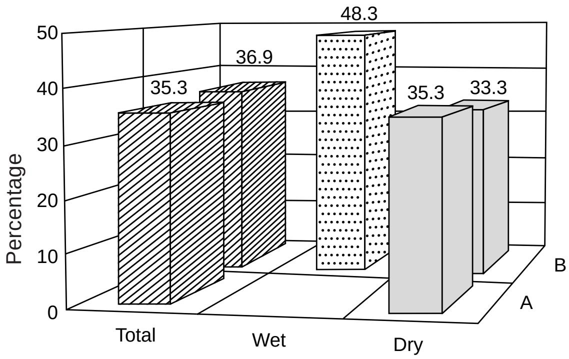
Parasites	Wet season (n = 29) Frequency[a] (%)	Dry season (n = 93) Frequency[a] (%)	Total (n = 122) Frequency[a] (%)
Ascaris lumbricpodes	11 (57.9)	22 (57.9)	33 (57.9)
Toxocara sp	6 (31.6)	7 (18.4)	13 (22.8)
Trichuris trichiura	2 (10.5)	6 (15.8)	8 (14.0)
Hymenolepis nana		1 (2.6)	1 (2.6)
H. diminuta		1 (2.6)	1 (2.6)
Capillaria sp		1 (2.6)	1 (2.6)
Total	19[b] (100.0)	38[b] (100.0)	57 (100.0)

[a]Frequency of parasites in, [b]14, [c]31 positive samples

Table 2
Type and frequency of helminth eggs recovered in outside of Kathmandu Valley, Nepal.

Parasites	Dry season only (n = 34) Frequency[a] (%)
Trichostrongylus sp	6 (46.1)
Trichuris trichiura	5 (38.5)
Ascaris lumbricoides	2 (15.4)
Total	13[b] (100.0)

[a]Frequency of parasites in [b]12 positive samples.



A=Outside of valley (Total n=34)
B=Kathmandu Valley (Total n=122)

Fig 2–Prevalence of soil contamination in Kathmandu Valley (during wet and dry seasons) and in outside of valley (during only dry season).

cal areas and seasons. Chi-square test was applied to assess the significant difference.

RESULTS

Of the total of 156 soil samples collected in urban and sub-urban areas in Kathmandu Valley (n=122) and rural areas in outside of valley (n=34) (Fig 1), 57 (36.5%) were found to be positive for helminth eggs (Fig 2). Helminth parasite eggs of both human (three species) and animal origin (four species) were recovered. All of the helminths species of animal origin were potential human pathogen (zoonosis). Soil contamination with helminth eggs was uniform in both Kathmandu Valley (36.9%) and outside of the valley (35.3%) (p>0.05). Soil contamination rate in the Kathmandu Valley during the wet season was higher (48.3%) than that found in dry season (33.3%). However, the difference was insignificant statistically (p>0.05) (Fig 2). Of the helminth eggs positive soil samples (n=57), 13 (22.8%) showed multiple helminth species.

A total of five species of nematoda eggs *(A. lumbricoides, Toxocara* sp, *T. trichiura, Capillaria* sp and *Trichostrongylus* sp*)* and two species of cestoda eggs *(Hymenolepis nana* and *H. diminuta)* were recovered (Tables 1 and 2). *A. lumbricoides* was the most predominant helminth in the Kathmandu Valley (57.9%) followed by *Toxocara* sp (22.8%) and others in both wet and dry seasons (Table 1). In contrast, *Trichostrongylus* sp topped the list of helminth species recovered in outside of the Valley (46.1%) followed by *T. trichiura* (38.5%) and *A. lumbricoides* (15.4%) (Table 2). The mean number of helminth eggs was six per sample and more than half of the eggs detected were fully embryonated (potentially infective).

DISCUSSION

Present study showed a considerably high soil contamination rate (36.5%) with helminth eggs of both human and animal origin as have been reported from elsewhere (Uga *et al*, 1995a; 1996; 1997; Peng *et al*, 1996). This finding was in agreement with the high prevalence of STH infection among Nepalese (Reily, 1980; Rai and Gurung, 1986; Rai *et al*, 1994; 1995) and the poor and/or non-existent sanitary systems. In some rural areas, no single pit latrine exists in the whole VDC area (having approximately 500-700 households). Local people defecate in the open on the farm land, the stream/river banks and in bushes (Rai *et al*, 1997b; Matsumura *et al*, 1998). People in the villages either have no footwear or usually wear thong-type sandals which afford poor protection against skin penetrating STH (hookworm and *Strongyloides stercoralis*). On the other hand, the influx of the rural population, many of them infected with STH, and unplanned urbanization appear to increase the parasitic infections in the cities (Crompton and Savioli, 1993). Recently, in the cities inside the Kathmandu Valley, a bed smelling street flood during and after rain has become a common phenomenon and this has been thought to be the cause of the re-emerging trend of hookworm infection during recent years (Rai *et al*, 1997a). In addition, indiscriminate defecation by the children particularly in peri-urban areas also exists. A half of the parasitic helminth eggs discharged in the environment reportedly come from children aged 2-15 years (Peng *et al*, 1996). Shrestha (1997), however, found a very low contamination rate of soil from kitchen gardens and around toilets with STH in a village 12 km away from the Kathmandu city. Shrestha's finding was in contrast not only to the results of present study but also to the indiscriminate open defecation habit of approximately half of the people (46.6%) and the high prevalence of STH infection (56.8%) in the area he surveyed. Such a discrepancy might have occurred due to the difference in the technique employed for the recovery of helminth eggs from soil samples.

The infective *Ascaris* eggs, in endemic areas, have reportedly been found adhering to cooking and eating utensils, fruit, vegetables, furniture, door handles, money and fingers (Kagei, 1983). Jonnalagadda and Bhat (1995) have reported the contamination of stored water used for drinking and/or cooking with parasitic helminth eggs in the neighboring country, India. Elsewhere, the soil contamination with parasite eggs reportedly range from 20 to 64% (Pezzani *et al*, 1996; Uga *et al*, 1997) and have primarily been associated with an open and indiscriminate defecation, poor or non-existent sanitation, the use of untreated night-soil as fertilizer and poverty. A soil survey in Bangladesh (Muttalib *et al*, 1983) has shown one hundred percent contamination of households (court yard, near latrine, kitchen area, garbage area, garden area, water source area). Hidayah *et al* (1997) in Malaysia found big family size, poor household hygienic score and poverty as potential risk factors for STH infections.

All of the helminths species of animal origin *(Toxocara* sp, *Trichostrongylus* sp, *Capillaria* sp and *H. diminuta)* were potential cause of parasitic zoonosis. In Kathmandu Valley, *Toxocara* sp ranked second to *A. lumbricoides* and appears to be associated with large numbers of stray dogs roaming around. The present finding was in agreement with the high *Toxocara*-seroprevalence (81%) reported earlier (Rai *et al,* 1996) indicating its public health importance in Nepal though cases of visceral larva migrans have not been reported yet due primarily to both the ignorance and lack of diagnostic facilities. *A. lumbricoides* topping the list of helminth recovered in Kathmandu Valley was in agreement with the findings in man (Rai *et al,* 1994; 1995). In contrast, *Trichostrongylus* sp was the commonest parasite detected in outside of the valley followed by *T. trichiura* and *A. lumbricoides* in spite of *A. lumbricoides* being the commonest parasite detected in fecal examination of locals (data not shown). This may be due to the fact that each household in the village keeps different kind animals for various purposes, including the use of animal dung as manure. In part, it may also be due to the difference in the resistance of parasite eggs to dryness as we collected the soil samples in outside of the valley only during dry season. However, in Kathmandu Valley, no difference was observed in two seasons and might be due to a relatively high humidity in the valley even in the dry season. Peng *et al* (1996) in China have also reported a relatively stable prevalence of soil contamination in and around the houses and vegetable gardens with *Ascaris* eggs throughout the year. Uga *et al* (1995a), however, found a significantly higher soil contamination rate during wet (rainy) season in Surabaya (Indonesia). Such a discrepancy might have occurred due to the geographical difference.

The present finding of soil contamination was in agreement with the high prevalence of STH infections among Nepalese (Reily, 1980; Estevez *et al*, 1983; Rai and Gurung, 1986; Rai *et al*, 1994; 1995). Considering the high soil contamination rate with parasitic helminth eggs of both human and animal origin in Nepal and the various kinds of public health problems including the economic burden associated with STH infections, an urgent measures in improving the basic environmental and sanitary conditions through a comprehensive community oriented health education program together with a periodic deworming is indicated.

ACKNOWLEDGEMENTS

We thank Mr Ram Nath Rai for his help in the soil sampling and their pre-processing (drying and sieving) in the field.

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