Intestinal Parasites and Haemoglobin Concentration in the People of Two Different Areas of Nepal.

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ABSTRACT

Introduction The intestinal protozoa and helminths have primarily been attributed to the poor socio-economic concern and over dispersion of parasites in Nepal. Low birth-weight, low productivity in adulthood, low haemoglobin concentration, chronic loss of blood and iron are related to parasite infection.

Objectives This study was conducted to determine the prevalence of the intestinal parasites and to evaluate the types of intestinal parasites and haemoglobin concentrations in the people of two areas of Nepal.

Methods The cross-sectional descriptive type of study was conducted from April to October 2005 in Kirtipur, Kathmandu and Gunjanagar VDC, Chitwan, Nepal. A total of 400 stools were processed by using a standard formalin-ethyl acetate concentration method, direct light microscopy, modified acid fast stain, oculo-micrometer and bisporulation assay. The blood was collected from the 59 solitary parasite positive persons, one concomitantly infected person and 17 parasite non-infected persons and examined by colorimeter.

Results The total prevalence of intestinal parasites was 42.0 percent in which the prevalence of males and females was 35.2 percent (58/165) and 46.8 percent (110/235) respectively with statistically significant (P<0.05, 95% CI). There was statistically significant of low concentration of haemoglobin in the helminths and protozoa infected males and females with different age groups (P<0.05, 95% CI).

Conclusion The reported prevalent parasites might be the auxiliary means of causing anaemia in the people of Nepal. The treatment of intestinal helminths, protozoans including coccidia and of anaemia must be followed by haematological examinations to evaluate other inducing cause of anaemia.

Key words Intestinal parasites, Haemoglobin, Nepal.

Introduction

The involvement of protozoan agents *Giardia lamblia, Entamoeba histolytica, Balantidium coli,* coccidia agents like *Isospora, Cryptosporidium, Microsporidia and Cyclospora* and intestinal nematodes constitute the highest groups of parasites known to infect the human health[1, 2]. This has primarily been attributed to the absence of potable drinking water, proper sanitary habits, good faecal disposal system, and poor socio-economic concern and over dispersion of parasites within the human communities[3]. Intestinal parasitosis is highly prevalent in rural communities of Nepal and constitutes an important cause of morbidity and mortality among Nepalese people[4]. Low birth-weight, low productivity in adulthood, stunted growth, low haemoglobin concentration, chronic loss of blood and iron are related to parasite infection[5, 6]. Besides disrupting growth, iron deficiency increases susceptibility to infections, interferes in mental activity leading to apathy, irritability and lowers powers of concentration as well as a reduced learning capacity[7]. This study was conducted to determine the prevalence of the intestinal parasites and to evaluate the types of intestinal parasites and haemoglobin concentrations among the studied people of different geographical areas of Nepal.

Methods

Study Area

The study was conducted from April 2005 to October 2005 in Kirtipur Municipality, Kathmandu and Gunjanagar

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Village Development Committee (VDC), Chitwan, Nepal. Kirtipur, the city of glory, is one of the oldest settlements in the Kathmandu valley. Gunjanagar is an agricultural area located in the western part of Chitwan District and is about 161 km southwest of Kathmandu, the capital of Nepal. This village is situated in about 15 km west of Bharatpur Municipality. It is surrounded by the Sharadanagar VDC in the east, Shukranagar VDC in the south and Dibyanagar VDC in the west and Narayani River in the north.

Study design
The study was cross-sectional descriptive type.

Stool Sampling
A total of 400 stools were randomly collected from the 400 people of these study areas with 200 samples from each locality. The door-to-door household survey was conducted for the stool collection. In both locality, we screened stool samples and recorded about age, sex and clinical symptoms.

Laboratory
The experiments were conducted in the Central Department of Zoology, Tribhuvan University, Kirtipur. Stool specimens were processed using a standard formalin-ethyl acetate concentration method and examined by two methods: direct light microscopy at normal saline (0.9%) and stool smear stained with modified acid fast stain for the coccidian parasites. Measurements of oocysts were done with ocular micrometer to distinguish the oocysts of *Cyclospora* from the oocysts of *Cryptosporidium* and *Isospora*. All stool samples were preserved in 2.5 percent potassium dichromate solution. All the *Cyclospora* positive specimens were stored at an ambient temperature (approximately 23 degree centigrade) and were examined at regular intervals over a period of 2 weeks starting from the time of excretion[8].

Haematological study
The blood was collected from the 59 solitary parasite positive persons, 1 concomitantly infected person and 17 parasite non-infected persons. The blood samples were taken from the *Cyclospora* (1 male) and *Cryptosporidium* infected persons (1 male) and concomitantly infected person (1 male) at twice at the gap of 9 days. As soon as the blood was collected in the vile, Ethylene Diamine Tetra-Acetate (EDTA) was added to prevent from clotting. They were brought in a private laboratory in Kathmandu and haemoglobin was tested with colorimeter.

Statistical analysis
The results were analyzed by using the chi-square test (Chi^2-test). The data were determined to have statistically significant at 95 percent Confidence Interval (CI) if the P value was less than 0.05.

Limitation
A person was considered low haemoglobin count when the haemoglobin was less than 13.5g/dl in males and 11.5g/dl in females[9]. It may not follow the rules of classification of low haemoglobin concentration for all ages given by other health agencies.

Results

The table 1 shows total prevalence of 42.0 percent (168 out of 400 persons) intestinal parasites. In Kirtipur, out of 200 patients, 107 were found to be infected with parasites with the prevalence of males and females 48.3 percent (42 out of 87) and 57.5 percent (65 out of 113) respectively with statistically significant ($P<0.05$, 95% CI). Similarly, in Gunjanagar, out of 200 persons, 61 were found to be infected with intestinal parasites in which prevalence of males and females was 20.5 percent (16 out of 78) and 36.9 percent (45 out of 122) with statistically insignificant ($P>0.05$, 95% CI). The prevalence of parasites in d”10 years age children and >10 years persons was 52.5 percent (43.0% males and 60.8% females) and 33.2 percent (26.6% males and 37.0%) respectively with statistically insignificant ($P>0.05$, 95% CI). The total prevalence of males and females was 35.2 percent (58 out of 165) and 46.8 percent (110 out of 235) respectively with statistically significant ($P<0.05$, 95% CI).

The prevalence of individual parasites in 400 persons was recorded as: *Ascaris* 10.3 percent (41 persons), *Giardia* 8.3 percent (33 persons), *Entamoeba* 5.3 percent (21 persons), *Trichuris* 5.0 percent (20 persons), *Hymenolepis* 4.0 percent (16 persons), hookworm 3.8 percent (15 persons), *Strongyloides* 2.5 percent (10 persons), *Cyclospora* 1.8 percent (7 persons), *Cryptosporidium* 1.0 percent (4 persons) and *Enterobius* 0.25 percent (1 person). Out of 168 positive samples, only 12 persons (7.1%) showed co-infection. In the present study, the prevalence of helminths and protozoan was 61.3 percent (103 out of 168) and 38.7 percent (65 out of 168) respectively. There was significant difference of protozoa and helminths infection in males and females of different age-groups ($P<0.05$, 95% CI).

The table 2 reflects the haemoglobin concentration of intestinal parasite positive and negative persons. A 35-year male person, who worked in the Metropolitan Solid Waste Management in Bishnumati River in Balkhu, was infected with all above nine parasites, except *Cryptosporidium*, with haemoglobin count 7.0. He had the same haemoglobin concentration and parasitic species after collection of blood and stool at two times at the gap of 9 days.

Out of 77 blood samples examined, 31 samples (40.3%) were found to have low Hb concentration. The total prevalence of males and females with low Hb was 45.2 percent (14 out of 31) and 37.0 percent (17 out of 47) respectively with statistically insignificant ($P>0.05$, 95% CI). The prevalence of low Hb in parasite positive males and females was 53.8 percent (14 out of 26) and 47.1 percent (16 out of 34) respectively with statistically insignificant ($P>0.05$, 95% CI).

The prevalence rate of low Hb persons in parasite positive and parasite negative was 50.0 percent (30 out of 60) and 5.9 percent (1 out of 17) respectively.

Out of 59 solitary infected low Hb persons, 33.3 percent (7 out of 21) were protozoan infected and 57.9 percent (22 out of 38) were helminths infected persons with statistically insignificant (P>0.05, 95% CI). However, there was statistically significant of low concentration of Hb in the helminths and protozoa infected males and females with different age groups (P<0.05, 95% CI). The relationship of different parasites with low concentration of Hb was significant (P<0.05, 95% CI).

The prevalence of low Hb count in different parasite positive persons was as follows: *Strongyloides* (80.0%) with the lowest 7.0 Hb count, hookworm (75.0%) with the lowest 7.0 Hb count, *Hymenolepis* (57.1%) with the lowest 12.0 Hb count, *Trichuris* (50.0%) with the lowest 9.0 Hb count, *Ascaris* (40.0%) with the lowest 8.0 Hb count, *Entamoeba* (40.0%) with the lowest 9.0 Hb count, *Cryptosporidium* (33.3%) with the lowest 7.0 Hb count, *Cyclospora* (25.0%) with the lowest 7.5 Hb count, and *Giardia* (25.0%) with the lowest 10.0 Hb count. The blood and stool of the *Cyclospora* infected male children (8 years) and *Cryptosporidium* infected male children (6 years) were tested at twice at the gap of 9 days. Both of them showed constant Hb count 7.5 and 7.0 respectively.

Table 1: Prevalence of Intestinal Parasite with Age-groups and Sex of the Persons in Kirtipur and Gunjanagar, from April 2005 to October 2005.

Age Groups of the Persons	Prevalence in Different study Areas						Total Prevalence in Different Sexes		Total Prevalence n/N
	Kirtipur			Gunjanagar					
	Males	Females	Total	Males	Females	Total	Males	Females	
≤ 10 yrs.	55.80% (29/52)	77.5% (31/40)	65.2% (60/92)	23.5% (8/34)	49.1% (28/57)	39.6% (36/91)	43.0% (37/86)	60.8% (59/97)	52.5% (96/183)
>10 yrs.	37.1% (13/35)	46.6% (34/73)	43.5% (47/108)	18.2% (8/44)	26.2% (17/65)	22.9% (25/109)	26.6% (21/79)	37.0% (51/138)	33.2% (72/217)
Total	48.3% (42/87)	57.5% (65/113)	53.5% (107/200)	20.5% (16/78)	36.9% (45/122)	30.5% (61/200)	35.2% (58/165)	46.8% (110/235)	42.0% (168/400)

Table 2: Status of Hb in Gram/Deciliter (g/dl) in Different Types of Parasites Infected and Non-infected Males and Females, in Kirtipur and Gunjanagar from April 2005 to October 2005.

Types of Parasites in Stool Tests	No. of Males with Different Hb Counts (g/dl)		No. of Females with Different Hb Counts (g/dl)		Total Prevalence of low Hb Patients
	Low Hb (<13.5)	Normal Hb(>13.5)	Low Hb (<11.5)	Normal Hb(>11.5)	
Mixed infection	1	0	0	0	1/1 (100.0%)
Strongyloides	0	1	4	0	4/5 (80.0%)
Hookworm	1	2	5	0	6/8 (75.0%)
Hymenolepis	3	1	1	2	4/7 (57.1%)
Trichuris	1	1	3	3	4/8 (50.0%)
Ascaris	2	2	2	4	4/10 (40.0%)
Entamoeba	3	4	1	2	4/10 (40.0%)
Cryptosporidium	1	0	0	2	1/3 (33.3%)
Cyclospora	1	1	0	2	1/4 (25.0%)
Giardia	1	0	0	3	1/4 (25.0%)
No parasite	0	5	1	11	1/17 (5.9%)
Total	14	17	17	29	31/77 (40.3%)

Discussion and Conclusion

In the present study, the prevalence of *A. lumbricoides* was the highest and showed that it was the most common helminth in Nepal similar to other studies[2,10,11]. The high rate of hookworm and *Strongyloides* in Kirtipur might be due to the fewer sandals bearing habit and low rate in Gunjanagar might be due to sandal bearing habit of the persons of the related area. Besides, open defecation is not found in Gunjanagar and water doesn't become medium of larval transportation which is present in Kirtipur area. The high prevalence of helminths than protozoa shows that soil transmitted helminth has been increasingly recognized as an important public health problem, particularly in developing countries[1,12]. The only one pinworm infection might be explained on the basis of rare appearance of eggs because of the direct wet mount technique and not applying the Scotch Tape method[13,14].

Regarding the protozoan parasite, the prevalence of *G. lamblia* was the highest (8.3%), followed by *E. histolytica* (5.3%) and *Cyclospora* (1.8%) which resembles with other researches[15,16,17].

Giardia infections are very common in developing countries ranging from about 5–43 present[18,19,20] similar to present results**.** Consumption of contaminated food, water or untreated water contaminated by human sewage or by wild rodents, cross-connections or damage in water-distribution systems, ineffective filtration are the transmission of *Giardia* cysts[21,22] which explains such a high rate of prevalence.

In the developing world, amoebiasis causes some 450 million infections *per annum*, about 50 million incidents and about 1,00,000 deaths[23]. Invasive amoebiasis is prevalent in the whole of South East Asia and the Indian subcontinent[14]. The present prevalence of *Entamoeba histolytica* is similar to other studies[11,15] with similar modes of transmission of *Giardia*.

The prevalence of *Cyclospora* is lower than previous studies in Nepal[24,25,26,27]. It is transmitted from the contact of waste disposal deposits, contaminated soil and water[24,25,26,28,29,30,31] which might be absent in Gunjanagar because of the water drinking practices from the deep ringed-wells or tap water and well-toilet facilities. *Cyclospora* is found in surface water when the organic content is high, and should not be found in subsurface water[32]. This explains the presence of all *Cyclospora* samples in Kirtipur only and absence from Gunjanagar.

The entire Cryptosporidial sample was present in Gunjanagar and none in Kirtipur. The people of Gunjanagar might have a high contact with carriers of *C. parvum* such as cattle, which is less common in Kirtipur community[33,34,35]. Cryptosporidiosis is seen during rainy season, reflecting the increased oocysts contamination of surface due to farming practices as a sludge spreading and heavy seasonal rains[36,37]. The prevalence rate one percent of *Cryptosporidium* in this study was exactly similar to that from Guatemala[38] and this low rate might be due to the absence of molecular study in the present research.

The table 2 shows high percentage of persons with low haemoglobin concentration due to helminths infection. This proves these parasites might be the auxiliary organisms of anaemia in Nepal. The most important cause of pathological chronic loss of blood and iron in the tropics is hookworm, and other soil-transmitted helminths reported in the present study[39,40,41].

The irregular patterns of low Hb count in the protozoan infected persons show that others factors may assist them to cause anaemia[14,42]. However, presence of low Hb both in males and females infected with *Entamoeba histolytica* proves that this is the causative agent of low Hb concentration. It secretes proteolytic enzymes that dissolve host tissues and host cells and engulfs RBCs[14]. The respective Hb counts 7.5 and 7.0 tested at twice in the *Cyclospora* (8 years age) and *Cryptosporidium* (6 years age) infected males prove that these coccidia induce low Hb in the absence of good immune system.

The table 2 reflects the predominance of low Hb in the helminths infected than protozoan infected males and females with different age groups with statistically significant ($P<0.05$, 95% CI)[43]. This may be due to different life cycle pattern and habit and habitat of protozoa and helminths parasites.

The presence of low Hb below the 10 year-children might be explained on the basis of multiple factors such as nutrition, immune system, socioeconomic status or other unknown factors[41,43,44,45]. The highest percentage of the low Hb persons above 10 years age show that anaemia due to parasitism might become a public health problem having an impact on the economy of Nepal[46].

The total prevalence of anaemia was 40.3 percent in which males were 45.2 percent and females were 54.8 percent with statistically insignificant ($P>0.05$, 95% CI). This might be due to the poor socio-economic status, low nutrition, low immune system and loss of blood through child delivery, menstruation of the females[9,47]. Anaemia in pregnancy is aggravated by low nutritional status of subjects whose staple foods, such as rice, cassava and maize are poor sources of folate, and iron[48,49] which might be explained in Nepalese context.

The detection of 7.0 haemoglobin counts at the 35 year-male person in the study might be explained on the basis of blood loss due to concomitant parasitic infection or increased parasitic burden[50,51,52].

The presence of 50 percent low Hb patients (30 out of 60) with different parasitic infection in the table 2 shows that the reported parasites are the auxiliary means of causing anaemia in the people of Nepal. Our results may probably be related to the low burden of parasites in the sample. The molecular diagnosis method should confirm the frequency of cysts, oocysts and ova to evaluate the haemoglobin. The results show that the treatment of intestinal helminths, protozoans including coccidia and of anaemia must be followed by haematological examinations, so that any other inducing causes of anaemia can be evaluated and properly treated.

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