

Prevalence of Intestinal Parasitic Infections Among Rural Inhabitants of Hamadan City, Iran, 2012

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Background: Intestinal parasitic infections, particularly in the rural areas, are one of the most important indices of the hygiene status and sanitation level of the society.

Objectives: This study aimed to determine the prevalence of the intestinal parasitic infections among rural inhabitant of Hamadan City, Iran, 2012.

Patients and Methods: A total of 228 fecal samples were collected from 50 families in seven villages that were directly and indirectly involved in raising livestock and other domestic animals in spring of 2012. The demographic data were collected by interview and included age, sex, educational level, place of keeping animals, direct or indirect contact with animals, and occupation. Fecal samples were concentrated using formol-ether sedimentation technique and examined by iodine-stained wet mount method. Indistinguishable samples were assessed by trichrome staining method.

Results: Among 228 samples, 80 (35.1%) were diagnosed with parasitic infection, which separately included 43 cases of *Entamoeba coli* (18.9%), 32 *Blastocystis hominis* (14%), 16 *Endolimax nana* (7%), nine *Iodamoeba butschlii* (3.9%), five *Giardia lamblia* (2.2%), two *Taenia* species (0.9%), two *Hymenolepis nana* (0.9%), one *Chilomastix mesnili* (0.4%), one *Trichuris trichiura* (0.4%), and one *Entamoeba histolytica/dispar* (0.4%). No significant difference in infection rate was observed with regard to indirect or direct contact with livestock. Coinfection of *E. coli* and *B. hominis*, *E. coli* and *I. butschlii*, and *E. nana* and *G. lamblia* were statistically significant. Interestingly, no *Ascaris lumbricoides* ovum was seen in this population.

Conclusions: According to the results of the present study, the prevalence of some infections with intestinal parasites is high in the Hamadan City. Considering that most of the parasites are nonpathogenic, pathogenic ones have been reduced generally in comparison to the previous reports. Nevertheless, the existence of *Taenia* species and *H. nana* could not be ignored.

Keywords: Parasite; infection; Iran

1. Background

Intestinal parasitic infections are widespread and prevalent all around the world with higher rates in poor societies, tropical, and subtropical areas and affect fairly billions of peoples (1, 2). Hygiene of water for personal usage is a substantial factor in reducing prevalence of waterborne infections including dracunculiasis, schistosomiasis, giardiasis, amebiasis, and cryptosporidiosis. Morbidity and mortality of diarrhea and hookworm infection decrease by proper sanitation facilities (3-5). Poor sanitation, poverty, and inefficient health services are the major factors in high prevalence of parasitic infections (6, 7). It was estimated that by 1998, more than one billion people were chronically infected with soil-transmitted helminthes worldwide (7). Foods, especially soil-grown vegetables, are another source of infection; studies indicated the contamination of the market vegetables by par-

asitic cysts and ova in Iran (8-10). Hamadan City is located in west of Iran with mountainous climates, which is cold in winter with minimum temperature -28°C and average of 9°C. The occupation of the most of the rural inhabitants of Hamadan City is farming and animal husbandry. Before the performed mass chemotherapy in 1997, the prevalence of the parasitic infections, especially ascariasis, was high. Poor sanitation system and untreated waste water, which were widely used in vegetable gardens, were the cause of high prevalence of ascariasis in the past (11).

2. Objectives

This study aimed to investigate the prevalence of parasitic intestinal infections in the rural inhabitants of Hamadan City, Iran, in order to determine the possible changes in the prevalence of the infections and to evaluate the hygienic status of the rural residents.

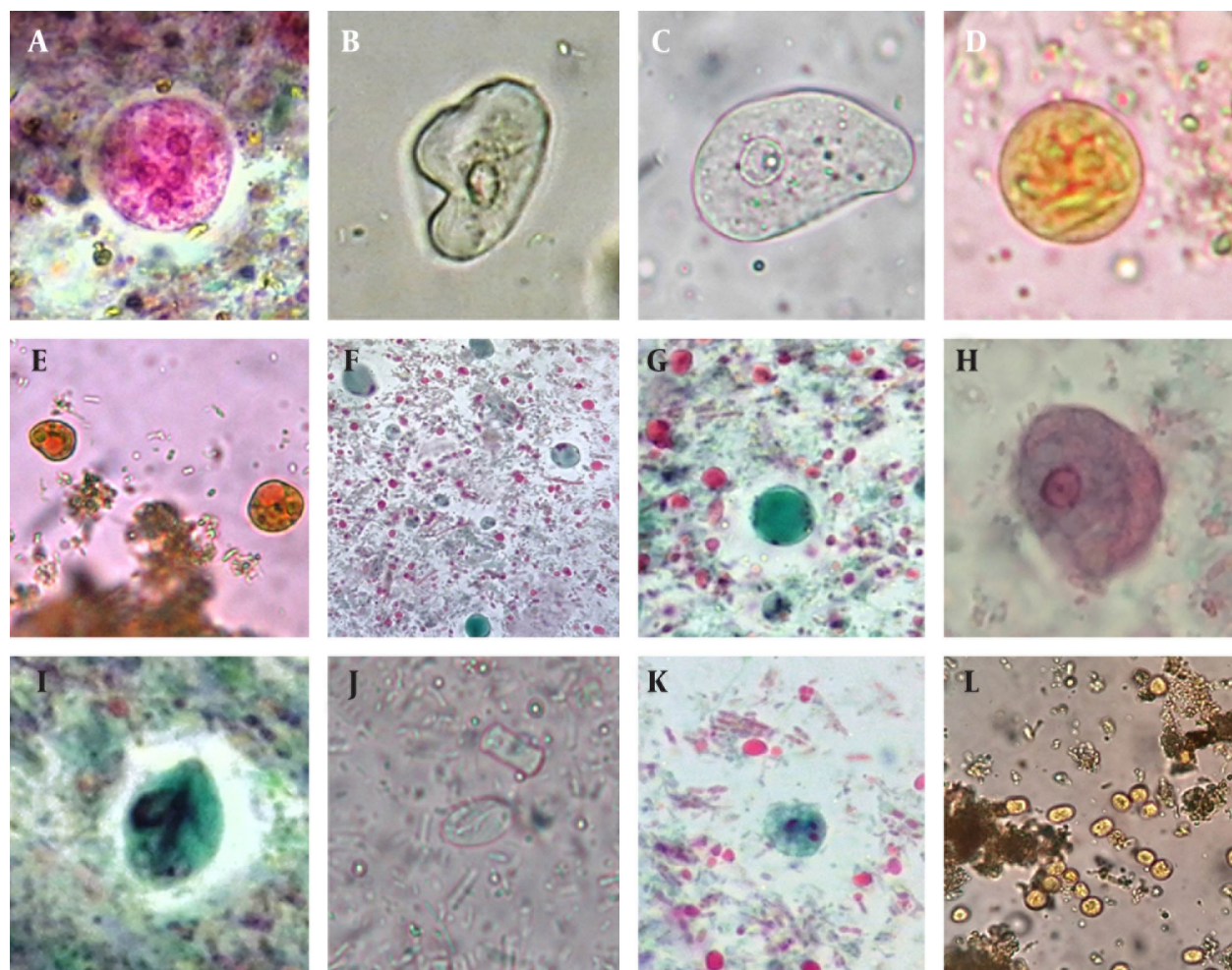
3. Patients and Methods

This cross-sectional study was conducted on rural inhabitants of Hamadan City, western Iran. A total of 228 fecal samples were collected from 50 families in seven villages near Hamadan City. Almost all of the families who were involved in farming activity or concentrating on animal husbandry including sheep, goats, and cattle were selected. Moreover, the studied society was in frequent contact with dogs, cats, and poultry. Sampling was begun with interviewing and recording the demographic data of the participant, which included age, sex, occupation, educational level, using animal fertilizer in their farm, place for keeping livestock, and being in direct or indirect contact with livestock. Collected fecal samples were transferred to the Parasitology Research Laboratory of Hamadan University of Medical Sciences as soon as possible. Samples were divided into two groups; one was preserved in polyvinyl alcohol for trichrome staining technique and the

other was concentrated using formol-ether sedimentation method (12) and sedimentary materials were used for wet mount slides with and without iodine stain. In brief, a pea size of fecal sample was emulsified in 7 mL of 10% formalin and was sieved by sterile gauze. Then 3 mL of ether was added to the fluid in centrifuge tube and was centrifuged at 3000 rpm for 60 seconds. The supernatants were disposed and the sedimentary materials were used for preparation of wet mount slides (12). The slides were examined under the light microscope using 100X and 400X magnification and were stained by Lugol's iodine when needed (Figure 1).

Indistinguishable fecal samples in wet mount slides underwent trichrome staining, in which cysts and trophozoites can be precisely identified (13). In this method, slides were immersed in iodine alcohol for ten minutes and then they were immersed two times in 70% alcohol for three to five minutes. Afterwards, they were stained with trichrome

Figure 1. Observed Trophozoites and Cysts of Protozoa in the Samples



a, *Entamoeba coli* cysts in trichrome staining method. b and c, *E. coli* trophozoite in wet mount slide. d, *E. coli* cyst containing splintered chromatoid bodies. e, *Iodamoeba butschlii* cysts iodine stained smear. f and g, *Blastocystis hominis* cysts in trichrome stained slides. h, *Entamoeba histolytica/dispar* trophozoite in trichrome stained slide. i, *Giardia lamblia* cyst in trichrome stained slide. j, *Giardia lamblia* cyst in wet mount preparation of feces. k, *Endolimax nana* cyst in trichrome stained smear. l, *Endolimax nana* cysts in iodine stained smear.

stain for ten minutes and were immersed in acid alcohol for a few seconds. Then the slides were immersed in 95% alcohol and absolute alcohol each for five minutes. The slides were kept in xylene for five minutes and after permanent mounting with Canada Balsam, they were examined under light microscope with magnification of 1000X (14). Data were analyzed by SPSS (version 16.2, SPSS Inc., Chicago, IL, USA) through Student t and Chi square tests.

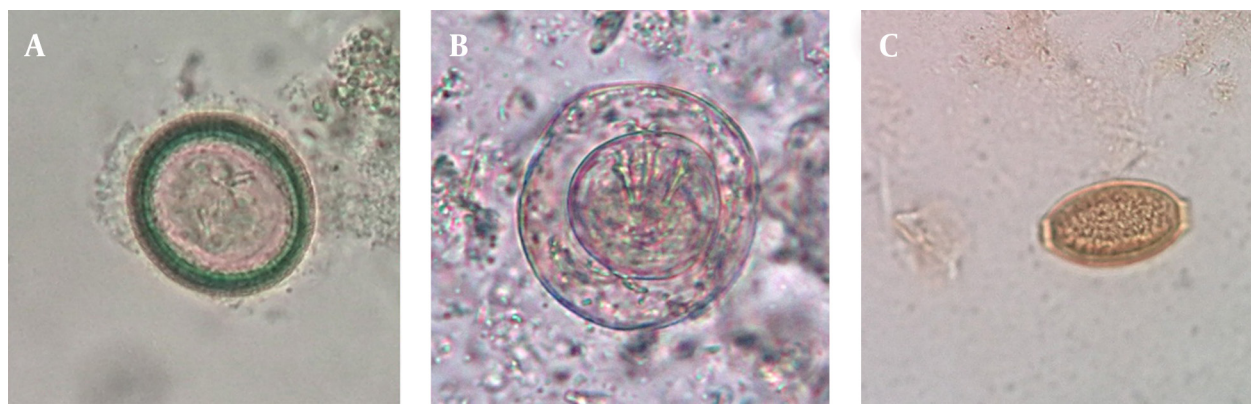
4. Results

A total of 228 samples from 135 males and 93 females with mean age of 31.66 ± 13.76 years (range, 1-58) were examined. Considering demographic characteristics, 30.7% and 69.3% had indirect and direct contact with livestock, respectively. The intestinal pathogenic or nonpathogenic parasites were present in 80 fecal samples (35.1%). The frequency of each parasite was as follows: 43 *Entamoeba coli* (18.9%), 32 *Blastocystis hominis* (14%), 16 *Endolimax nana* (7%), nine *Iodamoeba butschlii* (3.9%), five *Giardia lamblia* (2.2%), two *Taenia* species (0.9%), two *Hymenolepis nana* (0.9%), one *Chilomastix mesnili* (0.4%), one *Trichuris trichiura* (0.4%), and one *Entamoeba histolytica/dispar* (0.4%). Total helminthic and protozoal infections were seen in 5 (2.2%) and 75 (32.9%) individuals, respectively. Multi-infection with pro-

tozoa was diagnosed in 22 fecal samples (9.64%) including 19 samples (8.33%) with double infection and three samples (1.31%) with triple infection. The highest frequency of infection was observed with *E. coli* and the lowest with *E. histolytica/dispar*, *T. trichiura*, and *C. mesnili*. *Ascaris lumbricoides* ovum was seen in none of the examined fecal samples. Infection rate showed no difference between two groups who had indirect and direct contact with livestock ($P = 0.27$; OR = 1.07; and 95% CI, 0.90-1.28). Moreover, the infections separately showed no statistically significant difference between two groups. Some of the reported infections were zoonotic, which included *G. lamblia*, *I. butschlii*, *E. coli*, *C. mesnili*, *Taenia* species, *B. hominis*, *E. nana*, and *E. histolytica* (15, 16). Infection with *E. coli* was observed significantly lower in males than in females ($P = 0.01$; OR = 0.42; and 95% CI, 0.31-0.82). In addition, no significant difference in the mean age was detected between those with and without parasitic intestinal infection ($P = 0.92$, $t = -0.01$). Considering the infections separately, the mean age was lower in the individuals infected with *H. nana* ($P = 0.04$). Age distribution of infected individuals is shown in Table 1. Coinfection was observed with *E. coli* and *B. hominis* ($P = 0.004$), *E. coli* and *I. butschlii* ($P = 0.02$), and *G. lamblia* and *E. nana* ($P = 0.003$).

Table 1. Distribution of Intestinal Parasitic Infections Regarding Direct and Indirect Contact With Livestock and Comparison of Mean age of infected Individuals

Infection	Animal Contact		OR	95% CI	P Value	Mean Age	P Value
	Direct	Indirect					
<i>Entamoeba coli</i>							
Positive	31	12	1.180	0.566-2.461	0.659	35.77	0.179
Negative	127	58	1			32.8	
<i>Blastocystis hominis</i>							
Positive	24	8	1.388	0.590-3.264	0.451	34.72	0.466
Negative	134	62	1			32.46	
<i>Endolimax nana</i>							
Positive	13	3	2.002	0.552-7.262	0.282	28	0.086
Negative	145	67	1			33.14	
<i>Iodamoeba butschlii</i>							
Positive	7	2	1.576	0.319-7.786	0.442	34.67	0.722
Negative	151	68	1			32.7	
<i>Giardia lamblia</i>							
Positive	5	0	2.766	0.327-23.408	0.303	28.8	0.579
Negative	153	70	1			32.87	
<i>Taenia</i> species							
Positive	0	2	0.145	0.015-1.415	0.090	13	0.082
Negative	158	68	1			32.96	
<i>Hymenolepis nana</i>							
Positive	1	1	0.439	0.027-7.128	0.521	9.5	0.041
Negative	157	69	1			32.99	
Total infection							
Positive	58	22	1.265	0.695-2.304	0.441	32.64	0.922
Negative	100	48	1			32.86	

Figure 2. Observed Ova of Parasites in Studied Fecal Samplesa, *Taenia* species. egg; b, *Hymenolepis nana* egg; and c, *Trichuris trichiura* egg.

5. Discussion

In our study, *G. lamblia* (2.2%) and *E. histolytica/dispar* (0.4%) were respectively the most and the least prevalent pathogenic protozoa in Hamadan City; however, *E. coli* was the most prevalent nonpathogenic protozoa. With regard to the highest prevalence of pathogenic helminthes, *H. nana* and *Taenia* species both were observed with the same frequency (0.9%). Among pathogenic helminthes, the lowest infection rate was observed with *T. trichiura* (Figure 2).

Considering that in *taeniasis*, proglottids are mostly excreted from patients, finding the *Taenia* species ova in stool is not common. In our study, *Taenia* species ova were observed in the fecal sample of two individuals, which indicated that the proglottids of the cestode might had been degraded in the body or perhaps dog taeniid ova had contaminated the human foods or drinks. This fact might show that in reality, there would be more humans with *taeniasis* than those we observed in the Hamadan City. One of the interesting findings of this study was coinfection with *E. coli* and *B. hominis*, *E. coli* and *I. butschlii*, and *E. nana* and *G. lamblia*. In addition, *E. coli* infection was higher in women than in men and *H. nana* infection was seen in younger ages. Interestingly, we observed no *Ascaris lumbricoides* ovum in the studied population. Another aspect of the finding was a high rate of protozoal coinfection (9.64%).

Hamadan area had a high prevalence of ascariasis before mass chemotherapy in 1997; in fact, almost 53.3% of inhabitants of Hamadan province were infected at the time of mass chemotherapy. After two years of mass chemotherapy, the rate of ascariasis decreased to 6%; however, the same was not true for *G. lamblia* and *H. nana* (11). In our study, we observed no *A. lumbricoides* ovum in fecal samples, which showed a continuous reduction in ascariasis that could be the result of the mass chemotherapy in presence of a good hygienic and sanitation factors as well as promoted health standards in the society. Although the infection with *G. lamblia* has been slightly decreased over

the last decade in Hamadan area, hymenolepiasis remain almost unchanged in comparison to the report of Fallah et al. (11). In the present study, *H. nana* infection was observed almost in younger individuals with a mean age of 9.5 years. More prevalence of hymenolepiasis in younger individuals has been reported by many researchers (17-20). In addition, total infection rate showed no difference between those who were in indirect and direct contact with livestock. Therefore, people had the same risk of being infected by parasites irrespective of direct or indirect contact with livestock.

Nowadays reports from the area shows low prevalence of some intestinal parasites such as *Cryptosporidium* species, which is about 0.55% in renal transplant patients and 0.87% in farmers (21, 22). Another report from the Hamadan City shows relatively higher prevalence of the ascariasis (6.8%) and giardiasis (5.8%); however, that study was performed on patients with malignancies and eight years before our study (23). Recently, some reports from other parts of Iran are available. They reported the prevalence of some parasitic infections at the same or at lower rate than our report (24, 25).

In conclusion, the prevalence of pathogenic parasitic infections in rural inhabitants of Hamadan City was low and had been decreased during two last decades, but the existence of some helminthic infections such as *taeniasis* and hymenolepiasis was considerable.

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References

- Norhayati M, Fatmah MS, Yusof S, Edariah AB. Intestinal parasitic infections in man: a review. *Med J Malaysia*. 2003;**58**(2):296-305.
- Okay P, Ertug S, Gultekin B, Onen O, Beser E. Intestinal parasites prevalence and related factors in school children, a western city sample-Turkey. *BMC Public Health*. 2004;**4**:64.
- Esrey SA, Potash JB, Roberts L, Shiff C. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. *Bull World Health Organ*. 1991;**69**(5):609-21.
- Roach PD, Olson ME, Whitley G, Wallis PM. Waterborne Giardia cysts and Cryptosporidium oocysts in the Yukon, Canada. *Appl Environ Microbiol*. 1993;**59**(1):67-73.
- Zlobl TL. Amebiasis. *Prim Care Update Ob Gyns*. 2001;**8**(2):65-8.
- Albonico M, Crompton DW, Savioli L. Control strategies for human intestinal nematode infections. *Adv Parasitol*. 1999;**42**:277-341.
- Montresor A, Crompton D, Hall A, Bundy D, Savioli L. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. *World Health Organization*. 1998.
- Daryani A, Ettehad GH, Sharif M, Ghorbani L, Ziaei H. Prevalence of intestinal parasites in vegetables consumed in Ardabil, Iran. *Food Control*. 2008;**19**(8):790-4.
- Shahnazi M, Jafari-Sabet M. Prevalence of parasitic contamination of raw vegetables in villages of Qazvin Province, Iran. *Foodborne Pathog Dis*. 2010;**7**(9):1025-30.
- Fallah AA, Piralil Kheirabadi K, Shirvani F, Saei Dehkordi S. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Food Control*. 2012;**25**(2):617-20.
- Fallah M, Mirarab A, Jamalian F, Ghaderi A. Evaluation of two years of mass chemotherapy against ascariasis in Hamadan, Islamic Republic of Iran. *Bull World Health Organ*. 2002;**80**(5):399-402.
- Allen AV, Ridley DS. Further observations on the formol-ether concentration technique for faecal parasites. *J Clin Pathol*. 1970;**23**(6):545-6.
- Ustun S, Dagci H, Aksoy U, Guruz Y, Ersoz G. Prevalence of amebiasis in inflammatory bowel disease in Turkey. *World J Gastroenterol*. 2003;**9**(8):1834-5.
- Salleh FM, Anuar TS, Yasin AM, Moktar N. Wintergreen oil: a novel method in Wheatley's trichrome staining technique. *J Microbiol Methods*. 2012;**91**(1):174-8.
- Youn H. Review of zoonotic parasites in medical and veterinary fields in the Republic of Korea. *Korean J Parasitol*. 2009;**47** Suppl:S133-41.
- Abe N, Wu Z, Yoshikawa H. Zoonotic genotypes of *Blastocystis hominis* detected in cattle and pigs by PCR with diagnostic primers and restriction fragment length polymorphism analysis of the small subunit ribosomal RNA gene. *Parasitol Res*. 2003;**90**(2):124-8.
- Rim HJ, Chai JY, Min DY, Cho SY, Eom KS, Hong SJ, et al. Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. *Parasitol Res*. 2003;**91**(4):267-72.
- Park SK, Kim DH, Deung YK, Kim HJ, Yang EJ, Lim SJ, et al. Status of intestinal parasite infections among children in Bat Dambang, Cambodia. *Korean J Parasitol*. 2004;**42**(4):201-3.
- Mirdha BR, Samantray JC. *Hymenolepis nana*: a common cause of paediatric diarrhoea in urban slum dwellers in India. *J Trop Pediatr*. 2002;**48**(6):331-4.
- Sirivichayakul C, Radomyos P, Praevanit R, Pojjaroen-Anant C, Wisetsing P. *Hymenolepis nana* infection in Thai children. *J Med Assoc Thai*. 2000;**83**(9):1035-8.
- Jafari R, Gharibi Z, Fallah M. The Prevalence of Cryptosporidium Infection Among Renal Transplanted Patients in Hamadan City, West of Iran. *Avicenna J Clin Microb Infec*. 2014;**1**(1).
- Jafari R, Maghsood AH, Fallah M. Prevalence of Cryptosporidium Infection among Livestock and Humans in Contact with Livestock in Hamadan District, Iran, 2012. *J Res Health Sci*. 2012;**13**(1):86-9.
- Monsef AR, Hashemi SH, Abbasi M, Taherkhani H, Shalchi Z, Eliasi A. [Frequency of intestinal parasites in patients with malignancy, admitted in oncology ward of Sina Hospital, Hamadan, Iran]. *J Gorgan Univ Med Sci*. 2008;**9**(4):51-5.
- Tappeh Kh H, Mohammadzadeh H, Rahim RN, Barazesh A, Khashaveh S, Taherkhani H. Prevalence of Intestinal Parasitic Infections among Mentally Disabled Children and Adults of Urmia, Iran. *Iran J Parasitol*. 2010;**5**(2):60-4.
- Nasiri V, Esmailnia K, Karim G, Nasir M, Akhavan O. Intestinal parasitic infections among inhabitants of Karaj City, Tehran province, Iran in 2006-2008. *Korean J Parasitol*. 2009;**47**(3):265-8.