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## Comparison of Parasitic Contamination in a Society Based on Measurement of the Domestic Raw Wastewater Pollution and Clinical Referrals

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### ABSTRACT

Nowadays, the rate of parasitic contamination can be estimated in at least two ways. One is based on clinical referrals and the other is via measurement of the contamination in domestic raw wastewater. This study aimed to investigate the difference of results between these types. This study was carried out during five months during spring and summer seasons. In total, 90 samples were collected every 5 days from the input raw wastewater and to determine the concentration of ova, samples were analyzed by Mc Master slide according to Bailenger method. The data obtained from the laboratories of the city of Kermanshah in published papers and thesis done as results of clinically parasitic infection was considered. The results indicated that greatest number of parasite eggs in raw wastewater of Kermanshah belongs to *Ascaris lumbricoides* and *Hymenolepis nana* eggs. While, the results of parasitic contamination considering clinical referrals were lowest number. *Trichuris trichiura* reported relatively low rate in both the two types. The statement of parasitic contamination based on clinical referrals and raw sewage was dissimilar and it is more reliable to measure parasitic contamination of sewage than to examine the clinical referrals since the former results sound more real rather latter. The identified parasitic agents in this condition belong to the community as a whole.

**Key words:** Parasitic contamination, raw wastewater, clinical referrals, Kermanshah

### INTRODUCTION

Parasitic diseases are one of the most common illnesses on world (Sharafi *et al.*, 2012a). More than 40 million people are infected with these diseases worldwide and more than 10% of people on planet Earth are at risk of the parasitic infections (Jimenez, 2007). Monitoring and measuring the exact amount and type of parasitic infections in specific geographic area is very important. Since, identification of the parasite has the highest prevalence in a specific region, the exclusive ways to control it will be more followed. However, the amount and type of parasitic infection needs to be determined in a community using the true facts. Studies have shown that maximum and minimum of the parasitic pollution of *Trichuris trichiura* is in the Caspian Sea sides and Sistan-Baluchistan and West regions of the country. Additionally, in cities Tehran, Kermanshah, Shahrekord and Isfahan the maximum rate of parasitic infection has been recorded in relation to

*Ascaris lumbricoides* (Chapman *et al.*, 2005). As parasitic diseases may be transmitted through various ways such as contaminated meat of animal that fed on infected pasture, agricultural crops are irrigated with raw wastewater as well as contaminated water to urban wastewater. Therefore, the most important sources of distribution of infectious agents are principally wastewater (Zamo *et al.*, 2002; Sharafi *et al.*, 2012b). Per each liter of urban raw sewage possibly are found 600 intestinal nematode eggs, 32 hookworms, 1 schistosoma, 10 measles and 120 *Trichuris trichiura* (Tchobanoglus and Burton, 2003; Molleda *et al.*, 2008). Taking altogether, nowadays, the rate of parasitic contamination can be examined in at least two ways. One is based on clinical referrals (Vejdani *et al.*, 2002; Hamzavi, 2003; Ranjbar *et al.*, 2005; Nazari, 1996; Alvani, 1996; Rajabi, 2002) and the other is via measurement of the pollution in domestic raw wastewater (Miranzadeh and Mahmodi, 2002; Mahvi and Kia, 2006; Chapman *et al.*, 2005; Zamo *et al.*, 2002). Though, the infected patients in most of times refer to medical clinics when needed, thus in most of communities the clinical referrals pertain to accessible information in reporting rate of community parasitic infection.

Here a question may rise. Could the reported parasitic infection driven from measurement of clinical referrals and domestic raw sewage parasitic infection (both rate and type) differ for a single community? What is the source of this difference, then? And which method states factual results? The present study intends to answer the research questions by measuring parasitic infection of urban raw sewage in Kermanshah and comparing it with existing results based on the clinical referrals (for the city Kermanshah).

## **MATERIAL AND METHOD**

This cross-sectional study was performed within 5 months during spring and summer seasons. For measuring the parasitic pollution of raw wastewater, Samples were collected every 5 days from the input raw wastewater (screening unit) with 2 L volume. The frequency of raw wastewater samples in each month was 6 times. After mixing together, they were divided into two identical shares, then each one analyzed separately. Therefore, in total 60 samples were collected and analyzed (each sample with 1 L volume). Sampling days were randomly selected and the samples were delivered to a microbiological laboratory in faculty of health, Kermanshah University of medical sciences in order to parasitological experiments.

When the parasitic pollution of Kermanshah province cities raw wastewater was measured, the related documents on evaluation of parasitic infection rate considering the clinical referrals in the province were reviewed. These included all dissertations, paper and conference articles as well as final research findings of Kermanshah University of Medical Sciences research projects within previous decades.

Parasitological analysis was conducted based on modified Bailenger method with McMaster counting slides (with volume held under the grid equal to 0.3 mL) (Ayres and Mara, 1996). First, the samples were allowed to sediment over 2 h. Then 90% of supernatant was removed using a siphon. The sediment was carefully transferred to several centrifuge tubes and centrifuged at 1000×g for 15 min and supernatant was removed. All the sediments were transferred to one tube and recentrifuged at 1000×g for 15 min. Pellet created after recentrifuging was suspended in an equal volume of acetoacetic buffer, pH 4.5. Then two volumes of ethyl acetate were added and the solution was mixed thoroughly in a vortex mixer. The sample was centrifuged at 1000×g for 15 min. The sample in the test tubes was separated into three distinct phases. All the non-fatty heavier debris, including parasite eggs, larvae and protozoa were in the bottom layer. Above of it was the

buffer and the fatty and other material moved into the ethyl acetate and forms a thick dark plug at the top of the sample. Upper dark layer and middle clear layer were drained. Afterwards the pellet was resuspended in five volumes of zinc sulfate solution 33% (density =1.18 kg m<sup>-3</sup>) and mixed thoroughly. The volume of the final product (mL) was recorded.

An aliquot of sample was quickly transferred to a McMaster slide using a Pasteur pipette (with volume 0.3 mL) for final examination. Before examination the full McMaster slide was leaved to stand on a flat surface for 5 min. This allowed all the eggs to float to the surface. The McMaster slide was placed on the microscope stage and examined under 40 and 100X magnifications. All the eggs seen within the grid in both chambers of the McMaster slide were counted. The number of eggs per liter was determined from the Eq. 1:

$$N = AX/PV \tag{1}$$

Where:

- N = No. of eggs per liter of sample
- A = No. of eggs counted in the McMaster slide
- X = Volume of the final product (mL)
- P = Volume of the McMaster slide (0.3 mL)
- V = Original sample volume (L)

Finally, because all findings had distribution that were not statistically normal (p-value<0.05), the data related to parasite eggs and protozoan cysts contents of raw wastewater produced in spring and summer seasons were compared by Mann-Whitney test Table 1. All statistical tests were carried out using SPSS-Version.16 with a level of significance of 0.05.

## RESULTS AND DISCUSSION

The mean, minimum and maximum counts of parasite eggs and protozoan cysts found in raw wastewater samples are shown in Table 2. Necessary to say, enterobius vermicularis and fasciola hepatica parasites eggs were only observed in one sample of raw wastewater of Kermanshah.

Regarding the parasitic infection recorded by clinical referrals in Kermanshah province, the latest available and valid data are for 2008 that show 10.7% prevalence rate of parasitic infection in 2310 samples. The highest rate of recorded parasitic infection belongs to *Giardia lamblia* (5.8%). The patients' information collected from reference laboratory and special clinic of Medical Sciences University during 2001-2007 no infection to *Ascaris lumbricoides* and *Trichuris trichiura* was observed. The rate and type of parasitic infection from years 1991-2008 according to clinical referrals in city Kermanshah for *Ascaris lumbricoides*, *Trichuris trichiura*, *Hymenolepis nana* and *Giardia lamblia* parasites are presented in Table 3.

Table 1: Statistic results from Mann-Whitney test on achieved data

Interpretation	p-value	Nonparametric statistic tests	Application (purpose)
Mann-Whitney	<0.0001	There is a statistically significant difference between parasite eggs contents of raw wastewater produced in spring and summer seasons	Comparison of parasite egg counts of raw wastewater produced in spring and summer seasons
	0.004	There is a statistically significant difference between protozoan cysts contents of raw wastewater produced in spring and summer seasons	Comparison of protozoan cyst counts of raw wastewater produced in spring and summer seasons

Table 2: Mean, minimum and maximum counts of parasite eggs and protozoan cysts in raw wastewater of Kermanshah province (count per liter of wastewater)

Parameters	Total protozoan cysts	Nematoda egg	Total parasite eggs	Amoeba cyst	<i>Giardia</i> cyst	<i>Trichuris trichiura</i> egg	<i>Hymenolepis nana</i> egg	<i>Ascaris lumbricoides</i> egg
Mean	30.1	45.75	50.27	19.33	10.77	0	4.52	45.75
Minimum	0	0	0	0	20	0	0	0
Maximum	10.57	175	225	93.3	40	0	50	175

Table 3: Frequency of prevalence of parasitic contamination reported from referrals to reference laboratory and special clinic of Kermanshah University of Medical Sciences (%)

<i>Giardia lamblia</i>	<i>Trichuris trichiura</i>	<i>Hymenolepis nana</i>	<i>Ascaris lumbricoides</i>	References
18.2	0.9	2.30	3.40	Nazari (1996)
15.9	0.16	1.47	1.24	Vejdani <i>et al.</i> (2002)
05.8	0.0	0.30	0.00	Hamzavi (2010)
13.3	0.0	0.20	0.00	Hamzavi (2003)

The findings indicated that greatest number of parasite eggs in raw wastewater of Kermanshah belongs to *Ascaris lumbricoides* and *Hymenolepis nana* eggs; however, no *Trichuris trichiura* egg found in the raw wastewater. It may be due to high resistance of *Ascaris* eggs than other parasites such as hooking worms and *Trichuris trichiura* against unfavorable environmental conditions. These findings along with the results of similar studies conducted in Tehran, Isfahan and Shahrekord (Miranzadeh and Mahmoudi, 2002; Mahvi and Kia, 2006; Arbabi and Zahedi, 1998; Bitton, 2005; Sharafi *et al.*, 2012c) indicate that the *Ascaris* worm infection in Iranian society is currently higher than other parasites.

According to Miranzadeh and Mahmoudi results, the maximum and minimum numbers of nematode egg of input wastewater to treatment plant of Shoush town, Tehran were *Ascaris lumbricoides* and *Trichuris trichiura*, respectively (Miranzadeh and Mahmoudi, 2002). Also, Mahvi and Kia in their study founded that the *Ascaris lumbricoides* egg in input wastewater of 8 Tehran treatment plants and 2 Isfahan treatment plants was significant (Mahvi and Kia, 2006). Additionally, Arbabi and Zahedi reached the same fact about raw wastewater of Shahrekord (Arbabi and Zahedi, 1998). This is in line with Jimenez study where information about parasitic contamination of raw wastewater of different countries including USA, Germany, Pakistan, Egypt, Brazil and some other countries has been discussed (Jimenez, 2007). The reason for relatively high pollution of *Ascaris lumbricoides* compared to other parasites eggs in most of regions could be the ability of female *Ascaris* to produce 200,000 eggs per day, while eating a few infectious eggs may cause pneumonia disease (Loeffler's syndrome). Though *Ascaris* smooth transition increases infection in a community, Therefore, some other conditions including climate, geographical conditions, culture and health habits of people can contribute in dominance of a parasite in a community (Zamo *et al.*, 2002). The Zamo's *et al.* (2002) study showed that the most dominant parasite egg found in tested raw wastewater belongs to toxocara in Kenitra, Morocco. The frequency of *Ascaris lumbricoides* was even much lesser than *Trichuris trichiura* egg (Zamo *et al.*, 2002).

Since, the *Trichuris trichiura* survive well in warm and humid regions at 22-26°C in moist soils with enough shadow, existence of the *Trichuris trichiura* egg in two cities of Gilan-e-Gharb and Sarpolezahab could be an ideal condition for the parasite egg survival. The Iranian studies indicated that the maximum *Trichuris trichiura* contamination will be along the Caspian Sea and the minimum rate in Sistan- Baluchistan and western regions (Sharafi *et al.*, 2012a).

According to Mann-Whitney test results, raw wastewater parasitic contamination levels in spring were higher than summer in all studied cities. Considering that all the raw sewage samples in this study were collected in non-rainy days as well as the fact that water consumption in summer is higher than spring (leading to less wastewater production in spring), may explain the higher parasitic contamination in the raw sewage produced in spring.

Measuring parasitic infection considering clinical referrals, the recorded prevalence of *Ascaris lumbricoides* and *Hymenolepis nana* had the lower percentage (Vejdani *et al.*, 2002; Nazari, 1996; Hamzavi, 2003; Hamzavi, 2010). While, the results of parasitic contamination of domestic raw sewage measurement indicated that these two parasites were significantly prevalent in the domestic sewage.

How many cyst and parasite egg every parasite disposes differ based on the parasite nature. Additionally, the resistance and survival time in the environment such as system of sewer collection and pretreatment systems impact significantly differ (Jimenez, 2007). For instance, the *Ascaris* egg proved higher resistance than other nematodes egg against unfavorable environmental conditions. Thus, to rely solely on the clinical referrals or epidemiological evidences to predict protozoa prevalence in a community would make no sense. It does not mean that the number of protozoa cyst in entered sewage into treatment plant should precede since these two issues (measurement of infection based on clinical referrals and this measurement about sewage) totally differ from each other. The matter of parasitic contamination measurement in sewage primarily relates to the environmental microbiology. It is a matter of concern in reuse of sewage in agricultural irrigation or discharge into acceptor water.

Furthermore, regarding parasitic contamination difference based on clinical referrals and raw sewage, this question raises that whether all of infected patients to parasitic agents will refer to medical clinics to get examined and then some precise comment can be made on rate of parasitic infection (the rate of infection to *Ascaris* in Kermanshah is slight) or it won't. If it is possible to state that the patients referred to these clinics were similar to those who did not refer both from rate and type of infection view. or it could be possible to generalize the obtained results to the whole with higher level of confidence. Therefore, as the findings suggest the statement of parasitic infection according to clinical referrals and raw sewage contamination was dissimilar and it is more reliable to measure parasitic contamination of sewage than to examine the clinical referrals since, the former results sound more real rather latter. The identified parasitic agents in this condition belong to the community as a whole.

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