Helminth eggs in raw and treated wastewater in the Islamic Republic of Iran

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بيوض الديدان الطفيلية في الفضلات السائلة الخام والفضلات السائلة المعالجة في جمهورية إيران الإسلامية

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الخلاصـة: لتقييم نوع وحِمْل الديدان الطفيلية في الفضلات السائلة وجودة معالجتها، قام الباحثان في إطار هـذه الدراسة باختبار الفضلات السائلة الخام والمعالجة المأخوذة من ثماني محطات لمعالجة الفضلات السائلة في طهـران، ومحطتين في أصفهان، لتحري وجود بيوض الديدان الطفيلية، وذلك خلال العامَيْن 2002–2003. وتم فحص عينات الفضلات السائلة المأخوذة من مدخل ومخرج كل محطة من محطات المعالجة في عدة أحيان، باستخدام طريقة بيلينغر المعدلة. وأظهرت الدراسة أن الفضلات السائلة غير المعالجة الداخلة إلى محطات المعالجة في طهران، كانت تحتوي على أنواع مختلفة من بيوض الديدان وعلى أعداد من البيوض تزيد على مثيلها في محطات أصفهان. واشتملت بيوض الطفيليات التي تم التعرف عليها في محطات طهران: الصفر الخراطيني (الأسكاريس)، والدودة الشِّصِّية (الأنكلستوما)، والسُّرمية الدويدية، وأنواع الأسطونية الشعرية، وأنواع الدودة الشريطية، والمُحرشفة القَزَمة، ومتفرعة المِعَى المتغصِّنة؛ في حين تم التعرف في محطات أصفهان على الصفر الخراطيني، والاسطونية الشعرية، والمحرشفة القزمة. وبعد المعالجة انخفض عدد البيوض في كل لتر إلى بيضة واحدة أو أقل، وهو المعدل الذي ينسجم مع توصيات منظمة الصحة العالمية.

ABSTRACT To assess the type and load of helminths in wastewater and the quality of treatment, we examined the raw and treated wastewater of 8 wastewater treatment plants (WTP) in Tehran and 2 in Isfahan for the presence of helminth eggs during 2002–2003. Wastewater samples obtained from both inlet and effluent of each treatment plant were examined on several occasions using the modified Bailenger method. Untreated entry wastewater in Tehran WTPs contained a larger variety of helminth eggs than those of Isfahan, as well as higher total egg counts. The helminths identified in the influent of Tehran included *Ascaris lumbricoides*, hookworms, *Enterobius vermicularis*, *Trichostrongylus* spp., *Taenia* spp., *Hymenolepis nana* and *Dicrocoelium dendriticum*, while in Isfahan only *A. lumbricoides, Trichostriogylus* and *H. nana* were isolated. After treatment, the number of eggs/L fell to ≤ 1 egg/L.

Les œufs d'helminthes dans les eaux usées brutes et traitées en République islamique d'Iran

RÉSUMÉ Afin d'évaluer le type et la charge d'helminthes dans les eaux usées ainsi que la qualité du traitement, nous avons examiné les eaux usées brutes et traitées de 8 stations d'épuration à Téhéran et de 2 stations à Ispahan à la recherche d'œufs d'helminthes durant 2002-2003. Les échantillons d'eaux usées provenant des influents et des effluents de chaque station d'épuration ont été examinés à plusieurs reprises en utilisant la méthode de Bailenger modifiée. Les eaux usées entrantes non traitées des stations de Téhéran contenaient une plus grande variété d'œufs d'helminthes que celles d'Ispahan, ainsi qu'un plus grand nombre d'œufs total. Les helminthes identifiés dans les influents à Téhéran comprenaient les espèces suivantes : *Ascaris lumbricoides*, ankylostomes, *Enterobius vermicularis, Trichostrongylus* spp., *Taenia* spp., *Hymenolepis nana* et *Dicrocoelium dendriticum*, tandis qu'à Ispahan seuls *A. lumbricoides, Trichostriogylus* et *H. nana* ont été isolés. Après traitement, le nombre d'œufs par litre a diminué, passant à 1 œuf d'helminthe/L ou moins.

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Introduction

Wastewater reuse for irrigation is a common practice to overcome water shortage especially in countries with limited water resources [*1*]. However, there is the risk of transmission of diseases through excreted organisms in the case of irrigation with untreated wastewater. The World Health Organization (WHO) emphasizes that intestinal nematode infections constitute a major risk both to those working in the wastewater irrigated fields and to those consuming vegetables in the fields [*2*]. Therefore, in order to prevent the transmission of disease, it has been recommended that only treated wastewater be used for crop irrigation [*1*]. In this respect, quantitative guidelines are given by WHO for the helminth egg content of wastewater used in agriculture and aquaculture [*3*].

The Islamic Republic of Iran has in parts a semi-dry climate and there are insufficient water sources in some parts. In addition, the growing population of the country is leading to a worsening of the water shortage problem. Therefore, treated wastewater is now used as a water source for agriculture and irrigation of fields. Although health aspects of guidelines in such an application are necessary, there are few data on the diversity and concentration of helminth eggs in reused wastewater in the Islamic Republic of Iran.

We therefore aimed to evaluate the occurrence of parasite eggs in raw and treated wastewater of wastewater treatment plants (WTP) in two metropolitan cities of the Islamic Republic of Iran, the capital city Tehran, and Isfahan, in the central part of the country.

Methods

These 2 cities were chosen as they are among the biggest in the country and they both have complete WTP.

Samplings sites

Samples of raw and treated wastewater were obtained from 8 WTPs in Tehran [Sahebgharaniyeh, Gheytariyeh, Zargandeh, Shahrak-Gharb (Shahrak-Ghods), Shoosh, South, Ekbatan and Shahid Mahallati] and 2 in Isfahan (North and South) during 2002–2003. These WTPs are run by the Country Water and Wastewater Treatment Company which is a public sector company. In all of them, wastewater is treated by an activated sludge process except for Tehran South WTP where a stabilization pond is used [*4*].

Sampling collection

Sampling was carried out by WTP technicians and took place twice a week for a period of 8 weeks during the same time of the years at all the plants. On each occasion, samples of raw and treated wastewater, 20 litres of each, were taken from the inlet and outlet points. Therefore, for every WTP, 320 litres of untreated influent and 320 litres of final treated effluent were collected, making a total volume of 6400 litres for all 10 WTPs.

Identification and counting of helminth eggs

All samples from Tehran WTPs were examined in the Department of Medical Parasitology, School of Public Health and Institute of Health Research and those from Isfahan in the Isfahan Health Research

Centre, which is affiliated to the School of Public Health. Examinations of samples were carried out according to the modified Bailenger method [*1*]. Briefly, each sample was allowed to settle over 2 hours in the laboratory. Then 90% of the supernatant was removed using a siphon. The sediment was transferred to several tubes and centrifuged at 1000 g for 15 minutes. After that, the sediment of all tubes was recentrifuged, in one tube, at 1000 g for 15 minutes. The pellet was suspended in an equal volume of acetoacetic buffer, pH 4.5. Then 2 volumes of ether were added and mixed in a vortex mixer. The sample was then centrifuged at 1000 g for 15 minutes. After recording the volume of the pellet, it was resuspended in 5 volumes of zinc sulfate solution. The volume (X) of the final product (in mL) was recorded and then thoroughly mixed prior to transferring an aliquot to a new-style McMaster slide. Three such slides were prepared. The full slide was left to stand for 5 minutes and then examined under a microscope at 10× and 40× magnification. All the eggs seen were counted and the mean of the 3 slides was recorded. The number of eggs per litre was calculated from the equation:

$$N = AX/PV$$

Where N = number of eggs per litre of sample, A = the mean of counts from the 3 slides, X = volume of the final product (mL), V = original sample volume (L) and P = volume of the McMaster slide (0.15 mL).

The eggs were identified by their morphology and size, which was measured by a calibrated microscope.

Results

The quantification of helminth eggs in raw and treated wastewater samples from the 8 WTPs in Tehran is presented in Table 1. As this table shows, raw wastewater contained different groups of helminth including Nematoda [*Ascaris lumbricoides, Trichostrongylus* spp., *Enterobius vermicularis* and hookworms (*Ancylostoma duodenale* and *Necator americanus*)], Cestoda (*Taenia* spp., *Hymenolepis nana*) and Trematoda (*Dicrocoelium dendriticum*). There was a statistically significant difference between the total egg counts/L in the influent of all WTPs in Tehran (Poisson heterogeneity test $\chi^2_7 = 35.5$, $P < 0.001$); this difference was most significant between Shahrak-Gharb and Ekbatan (Poisson heterogeneity test, $\chi^2_1 = 16.22$, $P < 0.001$).

Table 2 shows the results of quantification of helminth eggs in raw and treated wastewater from the 2 WTPs of Isfahan. The influent of North WTP had a greater variety of helminth eggs than South WTP, as well as higher total egg counts/L. However, this difference was not significant (Poisson heterogeneity test, $\chi^2_1 = 0.082$, $P > 0.05$).

Of all parasitic eggs isolated in the raw wastewater in both Tehran and Isfahan WTPs, *A. lumbricoides* was the most frequently recovered (Tables 1 and 2). Regarding the effluent of treatment plants in both Tehran and Isfahan, the total number of eggs/L in all of them was ≤ 1 egg/L (Tables 1 and 2).

The mean total egg count/L in the influent of all WTPs in Tehran was much higher than that of Isfahan treatment plants (8.1 eggs/L versus 0.12 eggs/L); this difference was statistically significant (Poisson heterogeneity test $\chi^2_1 = 7.64$, $P < 0.01$). However, the difference between the mean total egg count/L in the effluent of Tehran treatment plants and those of Isfahan (0.025 eggs/L versus 0.025 eggs/L) was not statistically

Table 1 **Mean number of helminth eggs per litre in raw and treated wastewater in different treatment plants in Tehran**

Helminth group	Wastewater treatment plant															
	Saheb-gharaniyeh		Gheytariyeh		Zargandeh		Sharak–Gharb		Shoosh		South		Ekbatan		Shahid Mahalati	
	RW	TW	RW	TW	RW	TW	RW	TW	RW	TW	RW	TW	RW	TW	RW	TW
Nematoda																
Ascaris lumbricoides	2.5	0	7.5	0	2.0	0	12.15	0	10.9	0	6.2	0	0	0	2.2	0.5
Hookworm	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobius vermicularis	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1.1	0
Trichostrongylus spp.	0	0	0.5	0	0	0	7	0	0	0	1.1	0	0.9	0	1.1	0
Trichuris trichuria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cestoda																
Taenia spp.	0	0	3	0	0	0	1.25	0	0	0	0	0	0	0	0	0
Hymenolepis nana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trematoda																
Dicrocoelium dendriticum	0	0	0	0.5	0	0	0.65	0	0	0	1.1	0	0	0	0	0
Total	4.5	0	11	0.5	3	0	21.05	0	10.9	0	8.4	0	1.8	0	4.4	0.5

RW = raw wastewater; TW = treated wastewater.

Table 2 **Mean number of helminth eggs per litre in raw and treated wastewater in different treatment plants in Isfahan**

Helminth group	Wastewater treatment plant			
	South		North	
	RW	TW	RW	TW
Nematoda				
Ascaris lumbricoides	0.049	0	0.09	0.05
Hookworm	0	0	0	0
Enterobius vermicularis	0	0	0	0
Trichostrongylus spp.	0	0	0.05	0
Trichuris trichuria	0	0	0	0
Cestoda				
Taenia spp.	0	0	0	0
Hymenolepis nana	0	0	0.05	0
Trematoda				
Dicrocoelium dendriticum	0	0	0	0
Total	0.049	0	0.19	0.05

RW = raw wastewater; TW = treated wastewater.

significant (Poisson heterogeneity test χ^2_1 = 0.06, $P > 0.5$).

Discussion

The rapid population growth of the Islamic Republic of Iran in past 3 decades and the necessary requirement to increase food production on the one hand and the scarcity of water resources, especially in arid and semi-arid areas, on the other hand, have resulted in considerable attention being given to the use of wastewater for crop irrigation. However, a large variety of human pathogens are excreted in wastewater; of these, helminth infections pose the greatest risks and are a serious public health concern [*3*]. Therefore, when wastewater is intended to be reused in agriculture, the control of microbiological parameters at the level of international recommendation is important in order to obtain the benefits of wastewater reuse while at the same time eliminating the negative consequences. With this concern in mind, we evaluated the quality of final effluents and the effectiveness of wastewater treatment in removing helminth eggs in Tehran and Isfahan.

Raw wastewater samples at the WTPs contained different helminth eggs. The commonest species, however, was *A. lumbricoides*. This result concurs with other studies which have shown the frequent presence and high concentration of *Ascaris* eggs in raw sewage worldwide [*5,6*]. In addition, the resistance of *Ascaris* to external conditions [*7*] allows the eggs to remain viable for longer than other helminth eggs, e.g. hookworms [*8*].

There was a greater variety of parasite eggs in the wastewater influents in Tehran, as well as higher values of mean number of eggs/L, both for raw and treated wastewater. This was probably due to the migration of people from different rural areas to Tehran who were poorer and had poorer health. The results of recent studies on human intestinal parasites in Isfahan indicate a sharp drop in the rate of infection [*9,10*] compared with previous reports [*8*]. This may be linked with the low concentration of helminth eggs found in the influent of WTPs in Isfahan in our study. This suggests that enumeration of enteric pathogens in sewage may be used as an indicator of infection level within a community [*11*].

The decrease in the infection rates of human intestinal parasites applies to some extent for the whole country [*9,10*]. However, the decrease was most apparent in the case of ascariasis in Isfahan and hookworm in the north of the country [*12,13*]. In the past, Isfahan was known to have the highest infectivity rate for ascariasis (72%–98%) [*8*], when the use of domestic wastewater and night soil to fertilize land used for growing vegetables was very common. Since the presence of parasites in municipal sewage is associated with poverty and lack of adequate water and sanitation facilities [*14*], the significant drop in the *Ascaris* infection rate in recent reports (bringing levels to 1.3%–6.3%) [*9,10*] indicates an improvement in socioeconomic conditions and public health standards which could not be achieved without improvement of environmental health, sanitation, collection and treatment of wastewater.

The occurrence of *D. dendriticum* in municipal sewage do not usually indicate the infectivity of people with this helminth. This parasite, which uses 2 intermediate hosts in its indirect life cycle and is transmitted to its final host through ingestion of infected ants (the second intermediate host) is a common parasite of herbivorous animals in the Islamic Republic of Iran [*15*]. In most cases the presence of its eggs in human excreta is transitional and due to consumption of liver of infected animals [*8*].

The egg removal efficiency and quality of treated wastewater in all WTPs, both in Tehran and Isfahan, meet the current WHO's guideline, which states that only treated wastewater containing no more than 1 human intestinal nematode egg per litre should be used for irrigation [*3*]. The treated effluent of these WTPs can therefore be considered a safe source of water for agriculture, at least in terms of helminth eggs. Nonetheless, continued attention must be paid to wastewater parasitology for reused water especially where agricultural activity is common and intestinal parasites are a public health risk.

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