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Research Paper

DIVERSITY AND DIMENSIONS OF NEMATODE OVA: A TOOL IN WASTEWATER MANAGEMENT

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Abstract: Nematode ova were enumerated and dimensions were measured in raw and treated wastewater from two (2) Haya Water sewage treatment plants situated in Muscat, the capital city. A total of 100 wastewater samples of 5 liter volume per sample were collected from Darsait (DST) and Al Ansab (ANS) Sewage Treatment Plants (STP) from 30 March-08 April 2011 at 4 hour interval from 6:00 am -10:00 pm on a daily basis. The US EPA modified method, double flotation technique using Sodium Nitrate was used for the qualitative and quantitative determination of the parasitic ova, whereas, DP2-BSW software was applied for length/width measurement of the ova. Seven genera under Phylum Nematoda were found in the samples namely: *Ascaris, Trichuris, Capillaria, Enterobius, Ancyclostoma* (hookworm), *Toxocara,* and *Strongyloides.* Apparently, two types of cestode eggs under Phylum Platyhelminthes were also noted: *Taenia* and *Hymenolepis.* Size measurements were compared to publish dimensions. The study establishes the diversity, counts, and dimensions of the nematode ova content of the domestic wastewater in Muscat, Sultanate of Oman. As primary generated information, the profile will be useful in wastewater management and skill enhancement of the laboratory personnel.

Keywords: domestic wastewater, qualitative, quantitative, sewage treatment plant

INTRODUCTION

Global water scarcity is inevitable. Due to accelerated urbanization, rising population growth, and escalated standard of living, demand for more water is certain. In the Sultanate of

Oman, Sultan Qaboos University (SQU) had found that the daily average usage of water exceeds than in Europe and Japan. The world daily average is 120-140 liters per day, whereas, in Oman, the figure rises to 180 liters per day [1]. The reuse of sewage or wastewater is becoming increasingly widespread in order to address the water requirement for irrigation, recreation, industrial, and many other uses. Wastewater reuse is among the priority issues in the five-year development plan for Agriculture from 2006-2010 in Oman. The plan would give rise to construction of new treatment plants and development of the existing ones [2].

Haya Water vision is to provide desirable quality and adequate quantity of irrigation water as its commitment for greening the environment. However, associated to this practice is the health risk of nematode infection. Nematode eggs are parasitic worms that are more resistant to environmental conditions than bacteria and cannot be inactivated with chlorine, UV light, or ozone, at least in economical doses [3]. It is important that mitigating measures are considered with respect to health issues, which may arise. Public health is the prime concern in the reuse of wastewater. In order to safeguard public health, the World Health Organization (WHO) has established a microbiological guideline of <1 viable nematode ova per liter for restricted irrigation. Similarly, Ministerial Decision 145 /1993 issued by the Ministry of Regional Municipalities and Environment places the entire Sultanate under the same regulation. Water re-use for irrigation in terms of the number of viable nematode ova as well is < 1 ova /liter.

According to Jimenez [3], helminths ova are poorly known and understood in the environmental engineering field. There is a pushing need for more research and training of laboratory personnel from developing countries in order to gather more information about the helminth ova content and their removal in the currently used treatment processes. In Oman, there was no proper monitoring for helminths before 2004 [2]. The only available study about the nematode ova in Oman was done by Strauss in 1987 and the finding was, in raw sewage water, count of 100-200 eggs /liter was recorded. Most studies regarding the parasites deal with incidence of parasitism among children as an integral component of public health status.

At this point, Haya Water initiated a preliminary study on the nematode ova content of raw sewage and partially treated wastewater in order to establish the parasite diversity, count, and dimension. The profile is a realization of Haya Water support to scientific research alongside with technology application for water reuse. Vital information based on Science, along with personnel skills are essential tools for the success of any technology. This study will attempt to contribute significant primary data on nematode ova deliberately for use in the Sultanate. Likewise, this undertaking is aimed to increase awareness to protect the public and maintain ecosystem health through technology interventions that will be installed in the process.

MATERIALS AND METHODS

In the investigation conducted, a total of 100 wastewater samples corresponding to 500 liter of test material were collected. The selected sampling points are the inlet and clarifier at Darsait and Al Ansab Sewage Treatment Plants (STP) situated within Muscat governorate. Five liter of wastewater were collected by grab every four hours for five consecutive days per STP from 30 March to 08 April 2011. The time of sample collection was from 6 am - 10 pm daily.

The analytical technique applied is US EPA Modified Method, double flotation in Sodium Nitrate solution. Every sample was processed primarily by sedimentation, sieving, and decantation to reduce the original volume of 5 liter to about 200 milliliter. Turbid sample is

passed through a series of sieves whose mesh sizes ranged from 1000, 160, and 25 microns (µm) in order to get rid of unwanted particulates that can interfere in microscopy. Residues that remained in 25 µm sieve were collected. Centrifugation concentrates the sediments at the bottom of the tubes. The sediments are treated two times with the floating solution (Sodium Nitrate). Flotation works on the principle that most parasites eggs have specific gravity of 1.05-1.2 so they are less dense than the flotation solution [4]. As a result, in Sodium Nitrate solution (specific gravity =1.30), the ova are floated. Subsequent treatment with tap water (specific gravity = 1.00) prompted the eggs to sink to the bottom because the ova are denser than water. Concentration of the sample is by series of centrifugation.

Nematode egg examination for viability, count, and length-width measurements were performed by using Olympus CX-41 and BX-41 microscopes. Dimension of viable eggs was determined by using DP2-BSW software at 100x magnification. Evaluation of ova viability is based on the observation of the egg structures. For potentially viable ova, the unembryonated form has no visible defects or the embryonated form has larva detected inside. Whereas, non-viable ova have disorganized or damaged internal structures and outer shell.

RESULTS AND DISCUSSION

Diversity and Count

Diversity in this study refers to the various species of roundworm ova present in wastewater. The basis for diversity is the biological classification wherein organisms are categorized according to taxon and is part of the scientific Taxonomy (Table 1). This gives an idea of the nematode diversity in domestic wastewater.

Table 1 Classification of Nematode Ova Found in Haya Water Sewage Treatment Plants

Kingdom	Phylum	Order	Family	Genus
Animalia	Nematoda	Ascaridida	Ascarididae	1. *Ascaris*
		Enoplida	Trichuridae	2. *Trichuris*
		Enoplida	Trichuridae	3. *Capillaria*
		Oxyurida	Oxyuridae	4. *Enterobius*
		Ascaridida	Ascarididae	5. *Toxocara*
		Strongylida	Ancyclostomatidae	6. *Ancyclostoma*
		Strongylida	Strongyloidea	7. *Strongyloides*

Note: Cestode *Taenia* and *Hymenolepis* were also identified in the samples.

There were 7 genera of nematode ova found in the samples namely: *Ascaris, Trichuris, Ancyclostoma* (Hookworm)*, Toxocara, Strongyloides, Enterobius, and Capllaria* in their order of abundance. The type of nematode egg and their percentage in wastewater varies from each country. *Ascaris* is the most common and well-studied representing 71-85% in samples from developed countries [3]. The proportion of *Ascaris* in Muscat raw sewage was 52-71% and 80-88 % in partially treated wastewater. The egg is resistant, and with survival time usually of more than one year and the minimum infective dose is only one egg [5].

During the study, daily counts in raw sewage (SP_1) in DST and ANS ranged from 33-129 ova/liter and 21-408 ova/liter respectively. While, daily counts in wastewater before chlorination (SP_3), in DST and ANS ranged from 0-21 ova/liter and 0-12 ova/liter correspondingly. Cestode *Hymenolepis*, a rat tapeworm and *Taenia*, a beef tapeworm were also noted in significant numbers. *Taenia* was recorded only in ANS STP. This is most likely attributable to

slaughterhouse wastewaters. Comparatively, ANS STP registered lower counts of rat tapeworm.

Description of viable ova found in wastewater

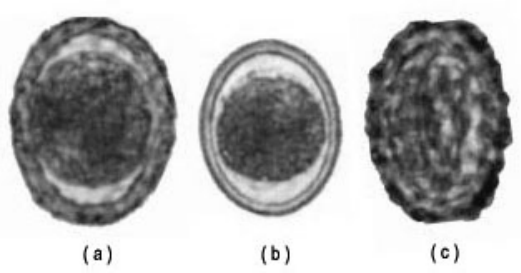

Fig. 1 *Ascaris* eggs at different stages. 1-cell stage (a) decorticated egg (b) and embryonated egg with mamillated layer (c)

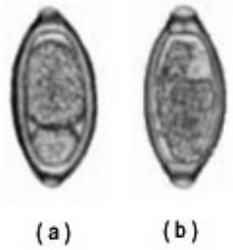

Fig. 2 *Trichuris* 2-cell stage (a) and embryonated egg (b)

Fig. 3 *Ancyclostoma* hookworm fertilized egg

Ascaris: Known as human roundworm. Distribution is worldwide. An estimated 807-1,221 million people in the world are infected with Ascaris [6]. Infection is caused by ingesting viable eggs. The eggs are golden brown color and contents are yellowish.
Counts: Enumeration in SP_1 samples from DST and ANS recovered 224 ova/liter (71%) and 517 ova/liter (52%) *Ascaris* eggs *respectively*. Whereas, in SP_3 samples, records were 45 ova/liter (88%) in DST and 24 ova/L (80%) in ANS.

Trichuris: Known as whipworm due to its distinctive whip-like shape. The species in human is *Trichuris trichura*. The whipworm has a worldwide distribution. An estimated 604-795 million people in the world are infected with whipworm [6]. Infection is caused by ingesting viable eggs. Lemon or football shaped with transparent polar plugs at both ends. Brownish shell color and contents are yellowish.
Counts: Enumeration in SP_1 of DST and ANS samples reflected 69 ova/liter (22%) and 405 ova/liter (41%) are *Trichuris*. There was no record of their presence in SP_3 samples.

Ancyclostoma: Known as hookworm. The species in human is *Ancyclostoma duodenale* and *Necator americanus.* The hookworm has a worldwide distribution. An estimated 576-740 million people in the world are infected with hookworm [6]. Infection is by penetration of immature form of the worm, mainly acquired by walking barefoot on contaminated soil. The shell is thin, smooth, and colorless. The contents are grayish in color.
Counts : Enumeration in DST-SP_1 and ANS-SP_1, revealed hookworm counts of 9 ova/liter (3%) and 33 ova/liter (4 %) respectively. SP_3 samples do not contained hookworm eggs.

Fig. 4 *Toxocara* embryonated egg

Toxocara: Known as dog and cat roundworm. Distribution is worldwide. Infection is by ingestion of embryonated egg. It is round with pitted thick shell and light tan in color.
Counts: No *Toxocara* was found in Darsait STP. ANS- SP_1 and SP_3 recorded 18 ova/liter (2%) and 6 ova/liter (20%) respectively. The eggs most likely come from the feces of domesticated dogs and cats.

Fig. 5 *Strongyloides* embryonated egg

Strongyloides: Known as threadworm. Distribution is worldwide. Infection is by invasion of infectious larvae or mature stage into the skin. It is oval shaped and thin shelled.
Counts: Enumeration at DST-Sp_1 and ANS –Sp_1, showed 6 ova//liter (1%) and 12 ova/liter are Strongyloides. While, in DST-SP_3, count of 6 ova/liter (6.12 %) was recorded. None of this parasite egg was found in ANS-SP_3.

Fig. 6 *Enterobius* embryonated egg

Enterobius: Known as pinworm. Distribution is worldwide. The species in human is *Enterobius vermicularis*. Infection is by ingestion of embryonated egg. Mostly infected are schoolchildren. The egg is thick shelled, colorless, and flattened on one side.
Counts : Enumeration at DST- SP_1 and ANS-SP_1 reflected 3 ova/liter (1%) and 3 ova/liter (0.3 %) are *Enterobius* The pinworm is absent in SP_3 water samples.

Fig. 7 *Capillaria* embryonated egg

Capillaria: First time record of this parasite was from the Philippines, although, occurrences were reported from Egypt, Iran, Indonesia, Korea, Thailand, Japan, UAE, and Yemen. Human may have acquired this parasite by eating raw small fresh water fishes in places where they have gone for work. The role of migratory fish eating birds influenced by changing weather condition may have contributed to the distribution of the parasite in other places [7]. Resemble *Trichuris,* peanut-shaped and with shallow polar plugs.
Count: Enumeration found 3 ova/liter at DST STP

Dimensions

Another tool for identification of ova is the length and width measurements. Body length and width serve as functional differentiating characteristic in Taxonomy. The unit of measurement for eggs is micrometer (µm). Specimen in water or fixative should always be measured.

Table 2 presents the dimensions of nematode egg per genera recorded at Haya Water Sewage Treatment Plants. Some genera were poorly represented in terms of density, nevertheless, as a primary data, the information is considered significantly valuable. Measurements were recorded using DP2-BSW software at 100x magnification.

Table 2: Dimension of nematode and cestode viable egg per genera found in domestic wastewater

Genera	Number of Viable Ova Measured	Haya Lab. Record Range of Values (µm)	Published Ranged of Values (µm)
Nematode			
Ascaris	276	42-48 / 67-79	35-50 / 45-75
Trichuris	156	23-30 / 48- 73	22-27 / 50-58
Capillaria	1	33 x 64	28-36 / 48-66
Enterobius	2	26-33 / 55-58	20-32 / 50-60
Toxocara	8	49-74 / 50-83	65-75 / 75-85
Ancyclostoma	13	39-57 / 64-74	36-40 / 64-76
Strongyloides	7	25-39 / 43-72	30-34 / 50-58
Cestode			
Taenia	28	29-37 / 31-41	30-35 / 35-40
Hymenolepis	108	49-65 / 59-88	60-80 / 70-85

Slight differences in egg size can be attributed to methodology used in measurement. Further, as cited, nematode egg size is shown to be highly variable, yet this variation is not associated with any other component of reproductive rate. This may be because of interspecific variation in eggshell thickness and complexity [8]. In the study undertaken, some genera were found in low numbers resulting in under or over estimation of size. Figures 8 and 9 present the relative sizes of nematode and cestode viable eggs encountered in the study.

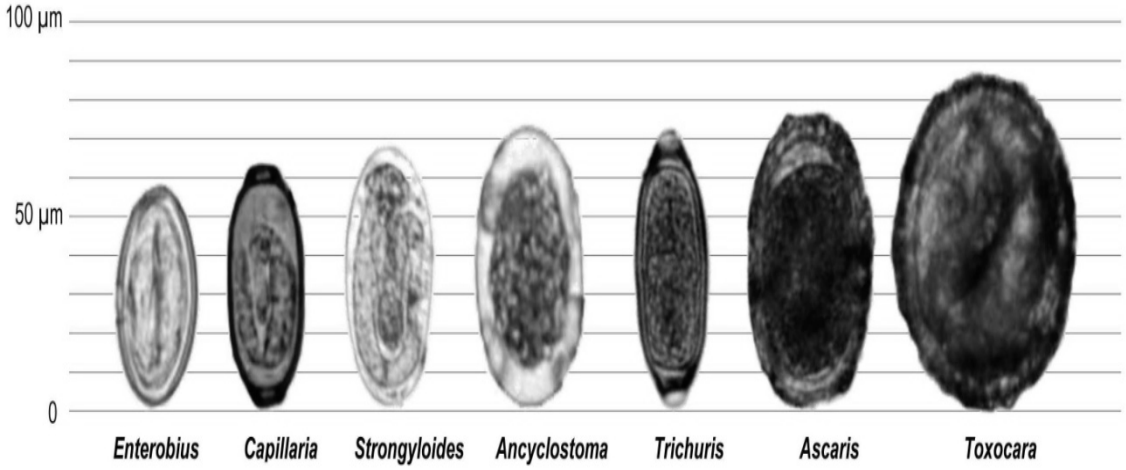

Fig. 8 Relative Size of Viable Nematode Ova Found in Muscat Domestic Wastewater

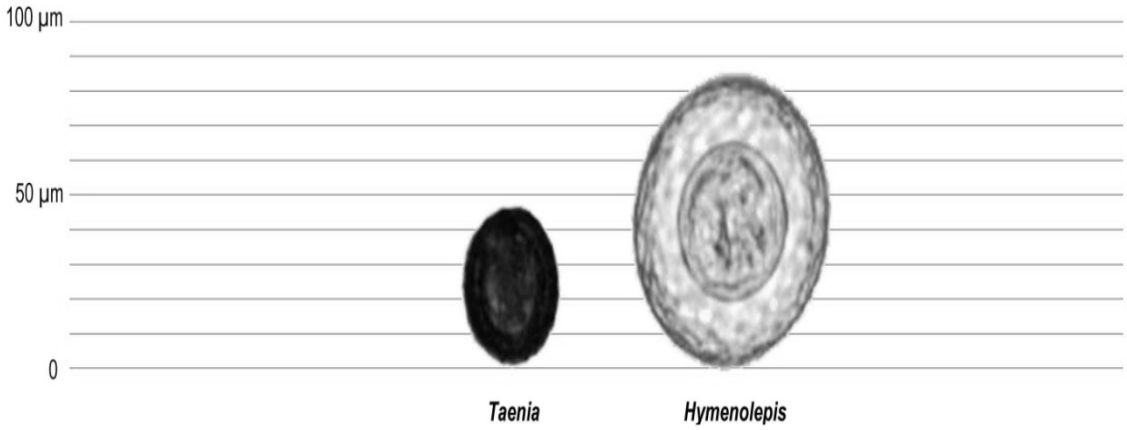

Fig. 9 Relative Size of Viable Cestode Ova Found in Muscat Domestic Wastewater

Total of (7) nematode and (2) cestode viable ova were identified in the wastewater samples analyzed. The different phases of wastewater treatment accounted for low counts in SP_3 samples. *Taenia,* a cestode, was found only in Al Ansab STP. Its prevalence indicates most likely contamination with slaughterhouse wastewater.

Ascaris eggs are the most common. Occurrences of other types determine the local pattern of parasitic infection. As cited, in the comparative study utilizing 5 different analytical techniques to determine the prevalence of intestinal parasitic infection among food handlers in Muscat, the findings identified *Ascaris lumbricoides, Trichuris trichura,* and *Ancyclostoma* (hookworm) as the common species found in 100 stool samples [9]. In Dhahira region, low parasitic helminth infection of 9.4% among undernourished children revealed the same community of nematodes [10].

CONCLUSION

Haya Central Laboratory concluded study has proved its capacity and expertise to provide evidence for a useful method intended for qualification and quantification of ova alongside with the Laboratory Analysts enhanced technical knowledge and skills. Align with wastewater management and public health issues, this study had generated valuable scientific information which are presently deficient in the country. Supplementary pictures of ova with their measurements are compiled at Haya Central Laboratory.

May we urge the readers and users to take critical appraisal of its usefulness and relevance.. It is our aim to review this work in the future with reference to analytical innovations yet to come.

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