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Isolation, Identification of the Waterborne Protozoan Parasites *Cryptosporidium* Spp. and *Giardia* Spp. and their Presence on Restricted and Unrestricted Irrigated Vegetables in Israel

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ABSTRACT

Cryptosporidium parvum *and* Giardia lamblia *are protozoan parasites that cause major human infections through waterborne transmission. The presence of these two parasites is closely linked with effluent reuse. Since most of the effluent, primarily in arid and semi-arid regions is reused for agricultural irrigation, extra work was conducted in order to detect these parasites in wastewater, the applied effluent, soil and crops. Unrestricted irrigation of a variety of vegetables with low quality effluents could lead to crop contamination with large amounts of* Cryptosporidium *oocysts and* Giardia *cysts. A special case was observed with zucchini that will be discussed further. Sprinkle, surface and subsurface dripping irrigation revealed that the last two methods are much safer for unrestricted irrigation, using the soil as an efficient filter matrix. However, from the obtained results (using a new method for parasites recovery from soil) both protozoan parasites were isolated from soil revealing migration to a depth of 60 and 90 cm bellow the surface when subsurface dripping irrigation was used. All these results will be further discussed as linked to optimization of secondary wastewater reuse in order to minimize environmental risk.*

1. Introduction

Since the first environmental study, human cryptosporidiosis has been described in various countries and continents and reported mean prevalence of

the disease is between 1 and 3% in Europe and North America, and ranging from 5% in Asia to approximately 10% in Africa (Current, 1994). In Israel cryptosporidiosis has been described among Bedouin communities in the southern communities (El-On *et al.*, 1994; Frazer, 1994). Contamination of surface and ground waters by *Cryptosporidium* oocysts and *Giardia* cysts occurs due to contact with sewage effluent, feces, slurry discharges or runoff from land.

Many surveys, which were conducted mainly in the USA and the UK, showed widespread prevalence of both parasites in many raw and some treated waters. The methods which were and still are used for the concentration and detection of both parasites are inefficient and the numbers which have been detected are probably an under estimation. Studies of surface waters conducted in North America have showed that *Cryptosporidium* and *Giardia* presence is ubiquitous. Data from seven studies showed presence of *Cryptosporidium* oocysts of 9.1% to 100% of the samples with oocyst concentrations ranging from 0 to 240 per liter (Rose, 1988; Ongerth and Stibbs, 1987; Rose et al., 1988; Madore et al., 1987; Roach et al., 1993). Data acquired between 1979 and 1986 showed that *Giardia* cysts are common contaminants of surface water sources in North America, 18% out of 4423 samples were positive for cysts (Hibler, 1988). A survey in 66 surface water treatment plants in the USA and one Canadian provincial plant showed that *Giardia* cysts were present in 17% out of 83 filtered water effluents and *Cryptosporidium* oocysts were present in 27% of the drinking water samples. Intensive monitoring was conducted from 1998 to 1993 in the USA showed that out of 347 surface water samples, 53.9% were positive for *Giardia* and 60.2% were positive for *Cryptosporidium* (LeChevallier and Norton, 1995). A recent survey in 199 groundwater sites, showed that 12% of the 463 samples were positive for *Cryptosporidium* and/or *Giardia* (Hancock *et al.*, 1997). Reported levels for *Cryptosporidium* were higher for those of *Giardia* and according to the author views the reason for this is probably due to more efficient methods for collecting *Cryptosporidium*. In the UK, numerous reports indicated that all types of surface waters surveyed were positive for both *Cryptosporidium* and *Giardia* (Smith et al., 1990; Humphreys et al., 1995; Anon, 1992; Anon, 1990; Smith et al., 1993).

In Israel, a preliminary survey that was conducted in 1992 in north of the country surface waters showed that maximal levels of *Cryptosporidium* cysts were 0.5/L and *Giardia* cysts 0.09/L (Armon et al., 1994). Data on reused water is scarce and the present study reveals information base on irrigation sources and their impact on soil and crops.

2. Methodology

2.1 *C. parvum* oocysts and *G. lamblia* cysts used in concentration methods

Cryptosporidium parvum oocysts from two sources were evaluated. GCH1 isolate was kindly donated by Prof. S. Tzipori, Division of Infectious Diseases, School of Veterinary Medicine, Tufts University, MA, U.S.A. A second isolate was purchased from Moredun Research Institute, Edinburgh, U.K. *Giardia lamblia* cysts were isolated from faecal material of infected patients (kindly supplied by the parasitology Laboratory, Hillel Jaffe Hospital, Hadera, Israel) concentrated and purified by sucrose gradient. Both oocysts and cysts were

stored in 2.5% potassium di-chromate solution at 4°C. Some aliquots were fixed in 10% buffered formalin.

2.2 Monoclonal Antibodies

Fluorescent monoclonal antibodies (Mab) AquaGlo G/C (Waterborne Inc., New Orleans, LA, USA) against both *Giardia* and *Cryptosporidium* were used according to the manufacturer's instructions for simultaneous detection in water samples, or Crypt-A-Glo (Waterborne Inc.) against *Cryptosporidium* only.

2.3 Concentration from small and large volumes of surface waters

Concentration of samples in 10 to 800 L of surface water was performed by two concentration methods: 1) large volume filtration (polypropylene wound 10" long cartridge filter, 1μm porosity rating, Filterite, MEMTEC America Corp., U.S.A.) (Rose *et al.*, 1991; APHA, 1992) and 2) inorganic flocculation as already described (Vesey *et al.*, 1993). Filtration was used for concentration of *G. lamblia* and *C. parvum* from large volumes of water and inorganic flocculation for *C. parvum* concentration from 10-L volume.

2.4 Microscopical oocysts identification and viability test

Ten-microliter aliquots of oocysts suspension were stained with monoclonal antibodies (Waterborne Inc., USA) and placed on a twelve microwells slide for microscopic examination. Using Zeiss epifluorescence microscope, the oocysts were identified and enumerated by blue filter block (excitation 490 nm, emission 510 nm). When necessary, viability was assessed by inclusion or exclusion of fluorogenic vital dyes (DAPI and PI); UV filter block for DAPI (excitation 335 nm, emission 450 nm) and green filter block for PI (excitation 488 nm, emission 610 nm) (Campbell et al., 1992). A minimum of 100 oocysts was counted in each well, and all experiments were performed in duplicates.

2.5 Concentration method of oocysts and cysts from soil samples

A low cost method for recovery of *Cryptosporidium* oocysts from soil samples irrigated with treated effluents was developed (Zilberman & Armon, 2000). Briefly the method is a modification of an other procedure previously used for extraction *C. perfringens* spores from bottom sediment samples (Emerson & Cabelli, 1982) that comprise phase separation of PEG (60%) and sucrose (25%).

3. Results

3.1 Isolation and enumeration of Cryptosporidium oocysts and Giardia cysts from surface water, wastewater and effluent in Israel

Wastewater and effluents from north and south sources in Israel were tested for presence of *Cryptosporidium* oocysts and *Giardia* cysts. Table 1 shows the percentage and the range of oocysts and cysts in wastewater and effluent in northern part of Israel (activated sludge plants). Due to low recoverability of the cartridge filtration it can be assumed that the absolute numbers are higher. *Cryptosporidium* was isolated in 100% and *Giardia* in 75% of the samples. It should be mentioned that the sewage treatment plant (Haifa STP) that was

sampled, collects sewage from a larger population compared with sewage treatment of the Southern area which is less populated.

Tables 2 and 3 shows the isolation and enumeration of oocysts and cysts in wastewater and effluent of Southern part of Israel (Arad and Chafetz-Haim)(mainly oxidation and stabilization ponds).

Table 1. *Enumeration of Giardia and Cryptosporidium in raw sewage and effluent in northern part of Israel (1997)*

Protozoan Parasite	No. of samples tested[a]	% positive	Range/L
Cryptosporidium spp.	4	100	8.2 - 270
Giardia spp.	4	75	0 -300

[a] Result of a cartridge filtration of 200-L raw sewage and effluents.

In these cases *Giardia* cysts outnumbered *Cryptosporidium* oocysts. Assuming that wastewater from this area is mixed with animals waste (sheep, goat and cattle) the higher number of *Giardia* cysts is appropriate. Following oxidation and stabilization ponds the number of cysts and oocysts dropped to 0/L.

Table 2. Giardia *cysts and* Cryptosporidium *oocysts enumeration in Arad wastewater samples (Southern Israel, 26 March 1998)*

Wastewater Sample	*Giardia* /L	*Cryptosporidium* /L
Settling pond outlet	548	48
Facultative pond outlet	167	24
Southern stabilization reservoir	0	0
Central stabilization reservoir	0	0
Reservoir outlet	0	0

Table 3. Giardia *cysts and* Cryptosporidium *oocysts enumeration in Arad and Chafets-Chaim (Southern part of Israel) wastewater samples (26 March, 1998)*

Wastewater Sample	*Giardia* /L	*Cryptosporidium* /L
Arad		
Arad (raw wastewater)	340	0
Arad (after oxidation pond)	0	0
Arad (after 1st reservoir)	0	0
Arad (irrigation) following reservoir	0	0
Chafets-Chaim		
Inlet to reservoir	170	0
Reservoir outlet	0	0

Table 4 shows the isolation and enumeration of oocysts and cysts in surface waters in the northern part of Israel. The Southern part of the country does not have continuous surface waters (rivers, lakes, streams or creeks) and the main drinking water source is groundwater and the national water carrier. The results presented in Table 4 reveals that surface water of different streams are contaminated with both protozoan parasites (ranging for Giardia from 0/100 L to

78.3/100L and for Cryptosporidium from 0/100L to 185/100L). The higher and more frequent isolation of Cryptosporidium oocysts in these surface waters could be attributed to agricultural activity (cattle growing) in the Lake Kinneret basin.

Table 4. Enumeration of *Giardia* cysts and *Cryptosporidium* oocysts concentrated from surface water in the northern part of Israel (by high volume cartridge filtration method)

Sampling Site	Date sampled	Volume Filtered (L)	*Giardia* cysts/100 L*	*Cryptosporidium* oocysts/100 L**
Dan stream	May-95	1000	38	190
Jordan river	May-95	700	10.7	139.3
Banias stream	June-95	500	13.3	80
Dan stream	June-95	800	7.4	68
Parshal	June-95	500	23.2	0
Jordan river	July-95	600	0	35.3
Hazbani stream	July-95	800	0	4
Parshal	July-95	200	78.3	0
Dan stream	July-95	1100	0	56.2
Jordan river	July-95	700	0	24.8
Jordan river	July-95	600	22	211
Banias	July-95	550	5	35.4
Parshal	July-95	350	0	0
Jordan river	November-95	500	0	185
Jordan river	January-96	400	0	95
Gonen spring	October-95	800	ND	0.54
Ein-Zahav spring	October-95	800	ND	0.54

ND - not done

* *Giardia* cysts recovery (approx. 26%)

** *Cryptosporidium* oocysts recovery (approx. 1.8%)

*** High turbidity (due to algal bloom)

3.2 Isolation and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts from soil samples sub-surface irrigated with effluents

The next stage of the present study was to look for the presence of oocysts and cysts in soil samples irrigated with different irrigation techniques (sprinkler, surface and subsurface dripping). Table 5 reveals the presence of cysts and oocysts at different soil depths following sprinkler irrigation at two sites. At the first site (Arad) *Giardia* cysts were not detected at 0 and 30 cm depths, however Cryptosporidium oocysts were detected at 30cm depth. At a second site (Chafets-Chaim) both parasites were isolated at 0 and 30 cm depths in large numbers.

Other two irrigation methods: surface and subsurface dripping were tested for their potential to disseminate *Giardia* cysts and *Cryptosporidium* oocysts in soil matrix (Tables 6, 7 and 8).

The obtained results are part of a major project on dripping irrigation (surface and subsurface) with oxidation pond effluent of different crops (vineyard,

almond trees and cabbage). There are two major observation from the obtained results: a) both protozoan parasites can migrate up to a depth of 60 cm; b) there is no special pattern of migration as can be seen from the variety of depth that both parasites were isolated.

Table 5. *Cryptosporidium* oocysts and *Giardia* cysts enumeration in soil samples (23 November, 1999)

Soil Sample	Soil moisture% by weight	Giardia/g	Cryptosporidium/g
Arad - Wheat-preparations for sowing			
Sprinkler effluent (0 cm deep)	23.5	0	0
Sprinkler effluent (30 cm deep)	25.1	0	40
Chafets-Chaim-Wheat-preparations for sowing			
Sprinkler effluent (0 cm deep)	28.6	120	160
Sprinkler effluent (30 cm deep)	23.7	80	180

Table 6. *Cryptosporidium* oocysts and *Giardia* cysts enumeration in soil samples in Arad (27 October, 1998)

Soil Sample	Soil moisture% by weight	Giardia/g	Cryptosporidium/g
Arad-Vineyard			
Drip surface effluent (0 cm deep)	20.4	0	0
Drip surface effluent (30 cm deep)	21.8	0	47
Drip surface effluent (60 cm deep)	20.6	43	0
Drip subsurface effluent (0 cm deep)	8.5	39	118
Drip subsurface effluent (30 cm deep)	16.1	ND*	0
Drip subsurface effluent (60 cm deep)	27.4	52	52

ND - Not Determined

Table 7. *Cryptosporidium* oocysts and *Giardia* cysts enumeration in soil samples (8 March, 1999)

Soil Sample	Soil moisture% by weight	Giardia/g	Cryptosporidium/g
Arad-Almonds			
Drip surface effluent (0 cm deep)	15.6	0	0
Drip surface effluent (30 cm deep)	16.6	37	73
Drip surface effluent (60 cm deep)	16.8	0	0
Drip surface effluent (90 cm deep)	17.6	0	37
Drip subsurface effluent (0 cm deep)	12.8	0	0
Drip subsurface effluent (30 cm deep)	18.8	0	0
Drip subsurface effluent (60 cm deep)	18.5	0	0
Drip subsurface effluent (90 cm deep)	18.6	0	0

The last part of the present study was to detect *Giardia* cysts and *Cryptosporidium* oocysts on vegetable crops that reach Haifa' Health laboratory (Ministry of Health). Part of the crops reach the laboratory from the Palestinian Authority areas (Fig. 1) and part from the Galilee agricultural areas (Israel) (Fig. 2) for routine microbiological testing. According to our information some of the Palestinian farmers irrigate their crops with raw sewage while the Israeli farmers use different qualities of effluents and also drinking water. As can be seen from Figure 1 the poor quality water used for irrigation strongly contaminate crops with protozoan parasites. A special case was found with zucchini that was the most predominant crop harboring *Cryptosporidium* oocyst. This vegetable has a hairy and sticky outer husk that enhances oocyst sticking. In case of better irrigation water quality the contamination with protozoan parasites is much lower, yet it did not reach zero contamination (Fig. 2).

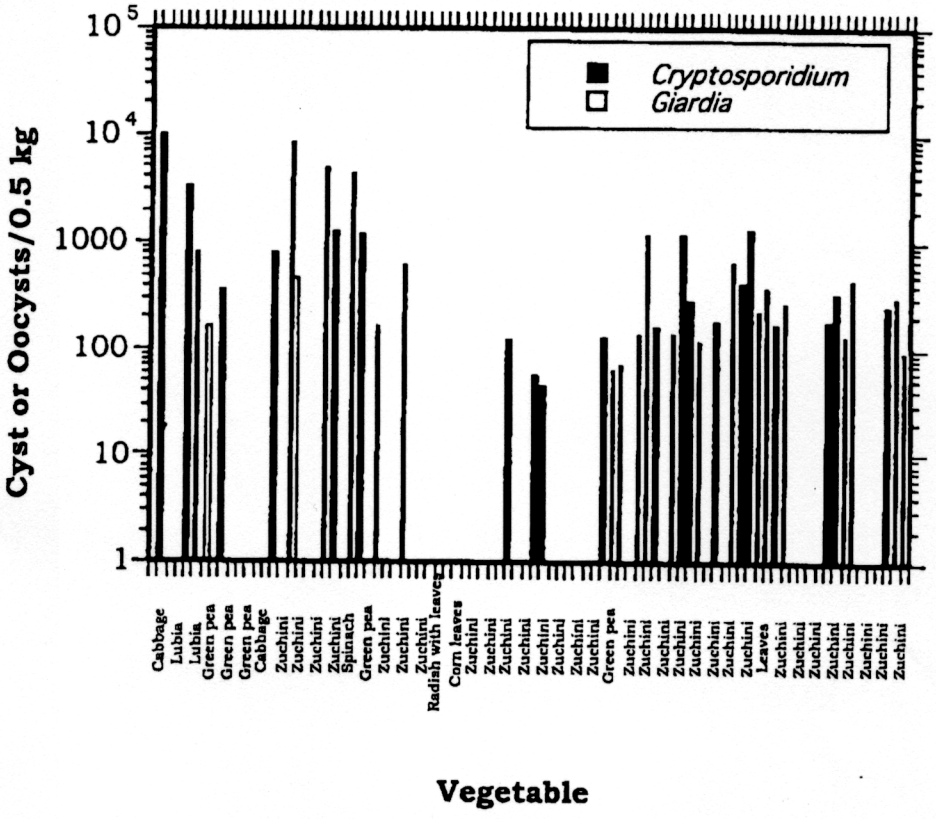

Fig. 1 Presence of *Cryptosporidium* oocysts and *Giardia* cysts on vegetables irrigated with poor quality effluents (West Bank).

4. Conclusions

1. *Cryptosporidium* and *Giardia* were isolated and enumerated in Israel sewage, effluent and surface waters supporting previous results (Zuckerman *et al.*, 1997).
2. Agricultural areas have an important impact on the quality of water sources. Lake Kinneret basin is a good example. Intense cattle activity increases the input of *Cryptosporidium* oocysts into surface waters.
3. Sprinkle, surface and subsurface dripping irrigation does not avoid soil and crops contamination with protozoan parasites.

4. Soil irrigated with effluents containing protozoan parasites was found to contain both parasites at different depths (up to 60cm).
5. Crops irrigated with poor quality reused water were found to be heavily contaminated with mainly Cryptosporidium but also with Giardia.
6. In summary, efficient wastewater treatment is a major prerequisite to reduce soil and crops contamination with *Giardia* and *Cryptosporidium*.

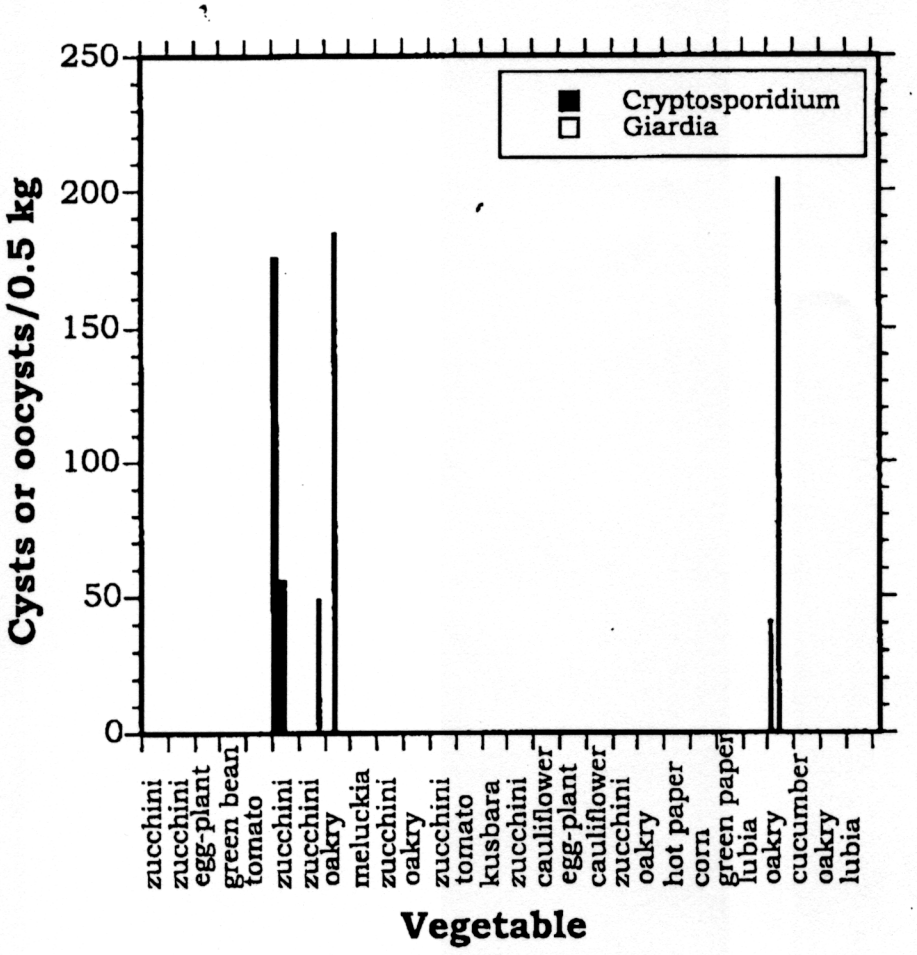

Fig. 2 Presence of Cryptosporidium *oocysts and* Giardia *cysts on vegetables irrigated with good quality effluents (Northern part of Israel).*

Table 8. Cryptosporidium *oocysts and* Giardia *cysts enumeration in soil samples in Chafets-Chaim (14 September, 1998)*

Soil Sample	Soil moisture% by weight	Giardia/g	Cryptosporidium/g
Chafets-Chaim-Cabbage			
Drip surface effluent (0 cm deep)	11.1	39	116
Drip surface effluent (30 cm deep)	13.5	0	28
Drip subsurface effluent (0 cm deep)	13.0	0	60
Drip subsurface effluent (30 cm deep)	14.6	44	44

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