Wat. Sci. Tech. Vol. 22, No. 9, pp. 51–57, 1990.

0273–1223/90 $0·00 + ·50

EXPERIMENTAL CONTAMINATION OF VEGETABLES WITH HELMINTH EGGS

J. L. Stien and J. Schwartzbrod

Department of Microbiology, Faculty of Pharmacy, Rue Albert Lebrun, 54000 Nancy, France

ABSTRACT

In countries with limited water resources, the agricultural use of treated wastewater represents an interesting alternative. A recently published international report (Engelberg report 1985) shows the importance of strict parasitological criteria for such reuse. In this study we have tested the survival of Ascaris eggs on different edible crops (lettuce, radishes, chives) and in different soils (clay, sand, silt) irrigated with water artificially contaminated. Leaves and roots from vegetables, surface and deep samples from soil were analyzed. In the soil the survival of Ascaris eggs is dependent on 2 factors : exposure to sunlight and soil type. Up to 20 days the Ascaris egg survival is significant,after which time it decreases quickly. In vegetable samples 10 days after the contamination by spraying no eggs are found on leaves of any crops. In roots samples after 10 days the Ascaris recovery is dependent on type of vegetables. These differences could be explained by different radicular system. After 45 days, the decrease is similar for all the crops. These results underline the importance of parasitological criteria for the agricultural wastewater reuse.

KEYWORDS

Wastewater reuse; Ascaris eggs; irrigation; vegetables contamination; parasitological criteria.

INTRODUCTION

The irrigation of crops with wastewater became popular in the late nineteenth and early twentieth century with the introduction of the water carriage system for domestic wastewater disposal. Sewage farms, as they were called, were introduced in Europe, in the Americas and in Australia but the practice became gradually less popular and almost completely disappeared soon after World War 1. However, in the past two decades, there has been a notable increase in the use of wastewater for crop irrigation, especially in the arid and seasonally arid areas of both industrialized and developing countries. This has occurred as a result of several factors :

- the increasing scarcity of alternative waters for irrigation
- the high cost of artificial fertilizers and the recognition of the value of the nutrients in wastewater, which significantly increase crop yield.
- the high cost of advanced wastewater treatment plants.
- the sociocultural acceptance of the practice.
- the recognition by water resource planners of the value of the practice.

However, there are possible effects on the health of farmers directly exposed to wastewater irrigation; on the public consuming edible crops contaminated by uncontrolled wastewater irrigation practices; on those consuming milk and meat derived from animals exposed to wastewater-irrigated pasture lands; and on population groups residing near wastewater-irrigated fields. These effects have to be carefully evaluated so that remedial measures can be taken to ensure that the public reaps the full benefits of a water recycling project.
Previous quality guidelines and standards for wastewater reuse have been based essentially on microbiological criteria in the absence of adequate epidemiological data. Recent epidemiological studies reviewed by Shuval et al. (1986) have indicated that, when untreated wastewater is used for crop irrigation, intestinal Nematodes present high actual risks, whereas there are lower bacterial risks than was previously suspected and little or no actual risks concerning virus (table 1).

TABLE 1 Relative Health Risks From Use Of Untreated Excreta And Wastewater In Agriculture And Aquaculture
(from Shuval et al. 1986)

Type of pathogen/infection	Excess frequency of infection or disease
Intestinal nematodes	High
Ascaris spp	
Trichuris spp	
Hookworms	
Bacteria	Lower
Bacterial diarrhoeas	
(e.g., cholera, typhoid)	
Viruses	
Viral diarrhoeas	Lowest
Hepatitis A	
Trematodes and Cestodes	From high to nil, depending upon
Schistosomiasis	the method of excreta use and local
Clonorchiasis	circumstances
Taeniasis	

On the basis of this new evidence, the World Health Organization recommended new Guidelines based on the Engelberg report (1985) which are shown in table 2.

These guidelines introduce a new, stricter approach concerning the need to reduce numbers of helminth eggs (Ascaris, Trichuris and hookworms) in effluents to a level of one or less per litre. With this new helminth standard, it is interesting to study the concentrations of helminth eggs in the environment (wastewater, sludge) and their behavior in soil or on vegetables.
There is much literature on helminth eggs concentrations in wastewater and sludge. There are wide variations in reported helminth eggs concentration in wastewater and in sludge which reflect both the effects of sociocultural and climatic factors, sanitary customs as well as differences in analytical techniques.
In soil, the survival time of helminth eggs varies considerably as shown in table 3.

TABLE 2 Recommended Microbiological Quality Guidelines For Wastewater Use In Agriculture[a]
(from W.H.O. 1989)

Category	Reuse conditions	Exposed group	Intestinal nematodes[b] (arithmetic mean no. of eggs per litre[c])	Faecal coliforms (geometric mean no. per 100 ml[c])	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks	Workers consumers public	$\leqslant 1$	$\leqslant 1000$[d]	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[e]	Workers	$\leqslant 1$	No standard recommended	Retention in stabilization ponds for 8-10 days or equivalent helminth and faecal coliform removal
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

a : In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account, and the guidelines modified accordingly.
b : Ascaris and Trichuris species and hookworms
c : During the irrigation period
d : A more stringent guideline ($\leqslant$200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.
e : In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

The maximum recorded survival time is 14 years for Ascaris eggs (Krasnonos 1978). Survival is usually promoted by cool, moist and shaded soil with the eggs situated under the surface of the soil. Exposure to sunlight and desiccation will reduce survival time very considerably. In soil, however, survival of helminth eggs may exceed the growth period of crops. Viable pathogens are therefore more likely found on root than on leaf crops. The information concerning the contamination of vegetables by helminth eggs following the use of sewage, nightsoil or sludge for fertilization has been a major concern of parasitologists in the USSR and in East Asia for many years as shown in table 4.

TABLE 3 HELMINTH SURVIVAL ON SOIL
(from Stien 1989)

	Survival time	Environmental conditions	Reference
Ascaris	5-6 months	Winter	Yoshida (1920)
	27-35 days	Dry Hot Summer	Rudolfs et al (1951)
	20 days	Sunny weather Sandy soil Temperature 30°C	Beaver (1952)
	5-7 years	Garden soil	Muller (1953)
	5 months		Forstner (1968)
	7 years		Parsons et al (1975)
	14 years		Krasnonos (1978)
	90 d - 7 years		Golveke (1983)
	many weeks	Bare soil	Wallis and Lehmann (1983)
	15 months	Moderate vegetation cover	"
	2 years	Heavy vegetation cover	"
	3 months	Clay soil and manure Temperature 25°C	O'Donnel et al (1984)
Taenia	15 months		Duthy and Van Someren (1947)
	58-159 days		Jepson and Roth (1952)
	49-90 days	Spring shaded soil	
	9-15 days	Summer shaded soil	
	53-64 days	Autumn shaded soil	Nadzafov and Chobanoy (1973)
	100-229 days	Winter shaded soil	quoted in Buerger (1984)
	27-68 days	Spring sunny soil	
	4-8 days	Summer sunny soil	
	20-38 days	Autumn sunny soil	
	73-207 days	Winter sunny soil	
	2 weeks	Summer	Coman (1975)
	Many months	Winter	
Trichuris	Many months		Levine (1968)
	3 months	25°C clay soil + manure	O'Donnel et al (1984)
Toxocara	8 months	25°C clay soil + manure	O'Donnel et al (1984)
	2 years	+4°C clay soil + manure	

TABLE 4 Helminth Survival And Development On Crops from Stien **(1989)**

COUNTRY	ENVIRONMENTAL CONDITIONS	VEGETABLES	RESULTS	REFERENCES
China	manure + human excreta + soil	Chinese cabbage	100 % positive samples	Ishikawa (1929)
	soil	lettuce spinach radish tomato	92 % 54 % 43 % 0 %	Yosesato and Sumi (1932)
	human excreta	celery, cabbage beans	0 %	Winfield and Yao (1937)
South Korea	nightsoil	greens	38 Ascaris eggs /100 g	Choi (1970)
		carrot	10.6 Ascaris eggs /100 g	
USSR	wastewater until harvest	cucumber tomato carrot	36 % eggs Ascaris viables	Vassilkova (1941)
	raw wastewater	tomato-cucumber	20 eggs /100 g vegetables	Vassilkova (1950)
	wastewater after irrigation	tomato-cucumber	3 eggs /100 g vegetables	
			98 % positives (Ascaris 94 %)	
	raw wastewater	vegetables	Trichuris 5 %	Biziulevicius (1954)
			(Enterobius 1 %)	
	no irrigation	vegetables	9-16 % positives	"
	nightsoil	fruits	71 % positives	Rosenberg (1960)
USA	wastewater	vegetables	6 % positives	Dunlop and Wang (1961)
		cabbage	0 %	
		lettuce	2 % positives	Rude et al (1984)
		carrot	2-4.1 % positives	(1984)
		radish	2.1-4.3% positives	
		spinach	9.8 % positives	

Pathogen survival periods on leaf and fruit crops tend to be shorter than the growth periods of most of these plants.

The present work includes 2 parts :

- study on artificially polluted soil with helminth eggs
- study on vegetables sprayed with helminth eggs suspensions.

MATERIAL and METHODS

- Collection of eggs. Eggs of Ascaris were used as a test organism because their great resistance to external conditions makes them most likely to be found viable in polluted soil or on vegetables. The human type is Ascaris lumbricoïdes. As no eggs of this organism were readily available it was decided to use those of the pig Ascaris suum. The eggs were obtained from the uteri of adult female worms collected from slaughterhouse. The eggs were squeezed from the uteri and suspended in distilled water.

- Soil samples. 3 types of soil were tested : sandy soil, clay soil, silty soil. 2 locations, "in sun and in shade", were studied. The soils were sprayed with a suspension of Ascaris eggs. The egg contamination was about 700 eggs per square centimetre, the soil depth 10 cm. The parasitological analyses of soil were made on surface samples and on samples 2 cm under the surface 20, 60, 95 days after the contamination.

. Vegetable samples 3 different edible crops were grown in silty soil free from helminth eggs (pH 6.2):
- lettuce (*Lactuca sativa*) because the leaves are eaten uncooked.
- radishes (*Raphanus sativus*) because the roots are eaten raw.
- chives (*Allium schoenoprasum*) because it is a vegetable crop with quick regrowth. Leaves and roots samples were studied 10, 45 and 60 days after irrigation of the vegetables with water artificially contaminated with *Ascaris* eggs (10^4 eggs/litre).

. Helminth eggs quantification. The evaluation of *Ascaris* in the samples was carried out in 3 steps. At first, vegetables or soil samples were mixed inner blender. Then, helminth eggs were concentrated by flotation method with potassium iodine mercurate solution (Janeckso-Urbanyi method). Finally, the *Ascaris* eggs were enumerated in a Mac Master cell under the microscope at a magnification of X 100.

RESULTS

The results of soil samples are summarized in table 5.

TABLE 5 Recovery Of Ascaris Eggs In Soil

	Exposure	Day 0		Day + 20		Day + 60		Day + 95	
		Surface	Depth	Surface	Depth	Surface	Depth	Surface	Depth
Sand	Shade	100 %	0 %	30.2 %	16.3 %	10.3 %	11.8 %	1.9 %	1.9 %
	Sunlight	100 %	0 %	22.6 %	5.4 %	12.4 %	2.5 %	0 %	0.2 %
Clay	Shade	100 %	0 %	37.3 %	23.4 %	17.7 %	4.6 %	9.7 %	1.7 %
	Sunlight	*ND	ND	ND	ND	ND	ND	ND	ND
Silt	Shade	100 %	0 %	55.8 %	33.7 %	20 %	15.9 %	5 %	1.3 %
	Sunlight	100 %	0 %	7.2 %	0.7 %	3.6 %	0.5 %	0.3 %	0.3 %

*ND : Not determined

They reveal that the survival in soil is important for 20 days. After 20 days, the survival decreases quickly according to the results of Beaver (1952) who observed the removal of *Ascaris* eggs from sandy and clay soil after 40 to 90 days. The survival on or in soil is dependent mainly on 2 factors :
. exposure to or protection from direct sunlight. Thus, in all soil samples, the highest percentage of eggs are found in shaded soil.
. soil type and moisture holding capacity. After 20 days in silty samples (depth and surface) 89.5 % of eggs are recovered, whereas in sandy samples (depth and surface) only 46.5 % of eggs are found.
Finally a difference is observed between surface samples and deep samples. After 20 days, 30.2 % of *Ascaris* eggs initially loaded are recovered in surface samples and only 16.3 % and 10.8 % respectively are found. This variation may be due to the percolation of rainfall. Moreover we have tested the viability of *Ascaris* eggs. In deep samples the viability of *Ascaris* eggs lasted twice as long as in surface samples.
The results of vegetable samples are collected in table 6.

TABLE 6 Recovery Of Ascaris Eggs On vegetables

Time after Spraying	Day 0	Days + 10	Days + 45	Days + 60
Lettuce				
Leaves	100 %	0 %	0 %	0 %
Roots		16.7 %	0.4 %	0.3
Radishes				
Leaves	100 %	0 %	0 %	0 %
Roots		76 %	3.4 %	3.3 %
Chives				
Leaves	100 %	0 %	0 %	0 %
Roots		23 %	10.2 %	1 %

10 days after the contamination, no eggs are found on leaves of vegetables. This rapid removal could be explained by the combined action of different factors : exposure to sunlight, irrigation. In roots samples, the recovery percentage depends on the type of vegetables. Indeed, after 10 days, low percentages (16.7 to 23) of Ascaris eggs survival are observed with lettuce and chives, whereas 76 % of Ascaris eggs are found on radish samples.

These observations could be explained by the difference in the arrangement of the roots. The radicular system of lettuce and chives is large and superficial whereas it is deep and restricted for the radishes. These arrangements were probably responsible for different moisture holding capacity. After 45 days, the decrease is similar for all the crops. These results are similar to those obtained by Rudolfs et al (1951) and Jackson et al (1978).

CONCLUSION

In this study, we have tested the survival of Ascaris eggs in soil (surface and deep samples) and on different edible crops (lettuce, radishes, chives) irrigated with artificially contaminated water. In soil, the results show that survival is important for 20 days then decreases quickly after. The recovery on or in soil is dependent mainly on 2 factors : exposure to sunlight and soil type. In vegetable samples 10 days after the contamination no eggs are found on leaves. In roots samples after 10 days the survival of Ascaris eggs depends on the type of vegetable. These observations could be explained by the difference in the arrangement of the roots. After 45 days, the decrease is similar for all the crops. These results confirm the importance of parasitological criteria for agricultural wastewater reuse.

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