Preliminary Assessment of Microbial Risk to Lettuce from Canine Waste on Canal Banks

Dr. Charles. A. Sanchez, Professor of Soil, Water, and Environmental Sciences, Yuma Agricultural Center

Dr. Jorge Fonseca, Associate Professor, Department of Plant Sciences, Yuma Agricultural Center

Dr. Kurt Nolte, County Director, Yuma County

Introduction

A number of studies have shown that irrigation water is a potential source of microbial contamination to vegetables (Islam et al., 2004; Steele and Odumeru, 2004; Stine et al., 2005; Fonseca 2006a). Surface waters are much more susceptible than ground water to microbial contamination (Steele and Odumeru, 2004). Irrigation water can be contaminated by manures used in agricultural operations, runoff from areas used for animal husbandry operations (Ackers et al., 1998; McGechan and Vinten, 2004) and wildlife (Wallace et al., 1997; Alderisio and DeLuca 1999). Where irrigation conveyance systems traverse urban areas there is also a potential elevated risk of contamination from domestic pet waste (Tauxe, 1997).

Several miles of canals used for irrigation of produce in the Yuma Valley traverse the city. These include the East and West Main Canals as well as secondary channels such as the Thacker and Central Canals. Most of these canal banks are used for recreation and exercise by city residents and their domestic pets. Further, all of these canal banks within and near the city limit are littered with canine excrement. It has been shown that a single gram of dog feces can contain 23 million fecal coliform bacteria, an amount 2 log units higher than that produced by the average cow. Dogs can also be significant hosts of both *Giardia* and *Salmonella* (van der Wel, 1995). In the United States, the 50 million registered dogs produce an average 0.32 lbs of fecal matter/day, and the potential risk of this may be affected by hygiene and type of food used (Godfrey, 1992).

Escherichia coli is part of the normal intestinal flora in dogs, in fact, it has been diagnosed as the causative agent of death shortly after birth (Olson and Mather, 1978; Munnich and Lubke-Becker, 2003) or in adult dogs due to kidney failure. *Escherichia coli* O157:H7 infection has often been documented in dogs but never in cats, however, most of these reports have been associated with the farm environment (Trevena et al., 1996) suggesting that dogs may play a role as vector of *E. coli* from cattle or even humans.

It is possible that some dogs pose potential risk for contaminated water in canal water, however, the decay rate of fecal coliforms in water, such as the Colorado river, is unknown and this needs to be examined. Persistence of fecal indicators vary depending on the water quality (Lim and Olivieri, 1982; Anderson et al., 2005).

We have no information on the potential risk of microbial contamination of irrigation water adjacent to this pet waste nor the potential for contamination of lettuce irrigated with this water downstream. The objective of this study is to characterize the microbial risk of this waste to lettuce. We envision these data will be used as an educational tool to effect behavioral change in the management of pet waste by the irrigation districts, city employees, and city residents who use the canal to exercise their pets.

Materials and Methods

This study was conducted on the East Main Canal because we made the visual observation that the mass of waste appears larger than other canals and this conveyance channel is sloped off the Yuma Mesa allowing for more opportunities for water contamination by gravity and erosion. We selected a 0.75 mile stretch of one bank of the East Main canal to collect and determine microbial activity of pet waste. This stretch is from 16th street to 22th street. Essentially all the waste in this 0.75 mile section was gathered and weighed. Sub samples collected from this waste was processed and stored for moisture and microbial analysis. Waste samples were collected on Dec. 7, 2007, Jan. 7, 2008, Feb. 3, 2008, March 2, 2008, and April 4, 2008.

We collected water samples from the canal 2 miles upstream from our first sampling point (near the Yuma siphon on 1st street), near 16th street where the waste survey began, on 22nd street where waste collection was terminated, on 24th street, on 32nd (County 11th) street and on County 11&1/2. Water samples were collected on the same date as the canine waste samples, except for Dec. 7. We collected lettuce from a furrow irrigated field on County 11&1/2 on February 24 and from a sprinkler irrigated field from County 11 on February 27.

The microbial analysis was performed at the Agricultural Center, where space and instrumentation is in place for food and water microbiology studies. We analyzed the canine feces, water and lettuce samples for total coliforms, fecal coliforms and generic *Escherichia coli*.

Determination of E. coli, total coliforms and fecal coliforms in dog feces.
Fecal samples were collected and stored at 6^{o}C (this is a guess at what the outside coolers are, they changed) prior to testing. From each sample 10 g of sample was aseptically added to 90 ml of water and blended for 1 minute at 230 rpm, or course samples were blended by hand by continuous manipulation (ie hand beat 230 times). Samples were plated by serial dilutions from the stomached sample onto Petrifilm EC plates (3M) and and coliform plates and incubated at 35oC and 44.5oC respectively. Colonies were counted for total coliforms and E. coli after 24 hrs and again after 48 hrs for E. coli.

Lettuce Samples
From each of the lettuce samples 10 g from the outside leaves were removed and blended with 0.1 % peptone water for 1 min in a blender at 230 rpm. A 1 ml sample from this was than plated onto Petrifilm EC plates (3M) and Petrifilm coliform plates. These were incubated at 35oC and 44.5oC respectively for 24 h for total coliforms and thermotolerant coliforms, and for 48 hrs for E. coli checking at 24 hrs for blue formation.

Water samples

Initial water samples were conducted using Colilert (IDEXX, AOAC 991.15) by adding 5 ml of water sample to each supplied vial as outlined in manufactures guidelines. The remaining water samples were tested for generic E. coli, coliforms and thermotolerant coliforms conducted by membrane filtration of a 100 ml sample onto either mColiblue incubated at 35oC for 24 hrs for E. coli and total coliform enumeration or mFC at 44.5^{o}C for 24 hrs for thermotolerant bacteria. Quantification of *E. coli* O157:H7 was done using multiplex real-time PCR by submitting samples to a local IEH laboratory.

Results and Discussion

The locations, dates, and microbial assessments of canine waste are shown in Figure 1. There is wide variation in the relative amounts of total coliform, fecal coliform, and generic *E. coli* found in canine waste. Although we found no pathogenic *E. coli* (O157:H7) in any subsample of canine waste tested, the other parameters show the potential of this waste to complicate microbial water quality assessments if it contaminates nearby irrigation water.

The microbial quality of irrigation water found in the adjacent canal is shown in Table 1. Of all the samples collected, only the sample collected at 24th street on Feb. 3 would potentially prompt the follow up testing by the Arizona Leafy Green Marketing Agreement (ALGMA). This sample showed *E. coli* levels of 140 cfu/100 ml. If we assumed this was a geometric mean of several samples the levels would be above the 126cfu/100 ml metric mandated in the marketing agreement. For a single sample, the levels should be <235 cf/100, which then make all samples within the permitted limits. It is interesting to note that on the 5th evaluation, taken at the end of the season, all samples where high in total coliforms. In the past total coliforms were considered as indicators, and it still is a way to observe fluctuation of the bacteria population, with a recommended threshold of <1000 cfu/100ml. If this was still the case essentially all of the water samples on the 5th sample date would have been outside compliance. It is one reason why we have recommended due diligence with water samples on the harvest months that are hotter (November, April). It is important to note that no water sample was positive for *E. coli* O157:H7. We have reported (California Leafy Green Research Board) that surrogate bacteria inoculated onto dog feces decline very rapidly when feces are subjected to field conditions in Yuma,AZ. This situation does not occur with cow or bird feces, in which E. coli survive for a substantially longer time period.

Evaluations of lettuce grown within 2 miles downstream of this waste, shows no problematic levels of microbes. These data show aerobic bacteria population in the range of Log 4 which is common for lettuce according to results obtained in other years in the Yuma area. No *E. coli* was detected in the lettuce sampled. In previous studies we have shown risks of contamination are greater for sprinkler irrigated lettuce compared to furrow irrigated lettuce. In this study we found no microbial issues associated with lettuce irrigated by sprinkler through maturity.

At this time we do not know to what extent limited transport of the waste into the canal or simple dilution with the large volume of water in the canal limited contamination of the water, and subsequent contamination of crops below thresholds. Transport into the canal by humans (i.e. kicking waste into the canal), wind, or water is likely limited, and the microbial populations associated with the waste that does make it into the canal is likely diluted below threshold

levels. It is possible for risks to be elevated during extreme rainfall events where more waste is washed into the canal. The only significant rainfall during the study period was 0.31 inches that fell on Jan. 26 and 27. Interestingly, it was the Feb. 3 sampling where we collected a water sample potentially above the above the 126cfu/100 ml ALGMA metric. This agrees with other observations that have been made in the past by other researchers (Gerba, 2007, personal communication), indicating that the greatest risk normally follows the week after a rainfall event.

Overall, results from this study indicate the microbial risk associated with canine waste on canal banks is low. However, we need to take into consideration that this study limited the analyses to bacteria indicators that are normally evaluated to comply with current industry regulations. Dog feces pose additional potential risks, particularly with certain parasites that may harm humans. Furthermore, in contrast to many other risks, this is a risk that can be entirely avoided. We have made the observation that on canals in other regions, such as Phoenix, cleaning up after pets is enforced by ordinance and potential fines. In these areas we observed no waste on the canal walking paths. A program of education, and if necessary enforcement, could eliminate this risk in the Yuma area.

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Table 1. Water quality at various locations within canal as affected by distance relative to canine waste samples.

	Location	Total Coliforms	Fecal Coliforms	E. Coli
		cfu/100ml		
Jan. 7	1st	44	1	1
	16th	41	0	9
	22nd	8	0	0
	24th	5	0	0
	32nd	14	0	0
	Co 11&1/2	11	2	1
Feb. 3	1st	332	0	0
	16th	35	1	0
	22nd	448	0	0
	24th	146	2	140
	32nd	56	1	0
	Co 11&1/2	30	1	0
March 2	1st	142	3	6
	16th	144	17	4
	22nd	51	0	3
	24th	64	0	1
	32nd	113	1	5
	Co 11&1/2	nd	nd	nd
April 4	1st	740	1	2
	16th	1320	40	4
	22nd	1260	74	0
	24th	2520	37	0
	32nd	1440	103	1
	Co 11&1/2	11000	10	0

Table 2. Microbial quality of lettuce downstream of canal sample for canine waste.

		Lettuce Type	Aerobic bacteria	Total Coliforms
			cfu/g	
Feb. 24	Furrow	Romaine	14400	0
		Green Leaf	23500	0
		Red Leaf	35400	10
		Boston	7100	0
Feb. 27	Sprinkler	Green Leaf	13200	120
		Green Leaf	14400	0
		Green Leaf	12800	0
		Red Leaf	70600	30
		Red Leaf	13500	0
		Red Leaf	11200	0
		Boston	86100	50
		Boston	29600	0

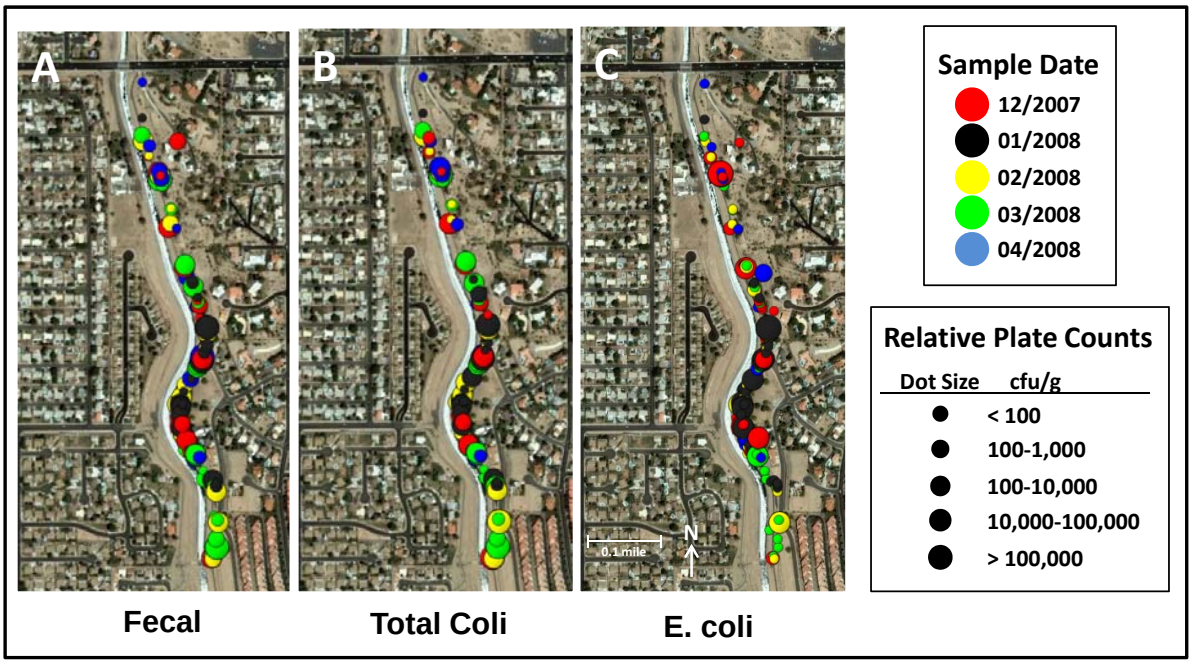

Figure 1. Locations and microbial contents of canine waste on East Main Canal.