Most Probable Number Methods for Enumerating *Salmonella* and *Escherichia coli* O157:H7 in Environmental Waters

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Background

Agricultural animals such as beef and dairy cattle, swine, poultry, and wildlife are sources of *Salmonella* and *E. coli* O157:H7. Watersheds with animal agriculture and wildlife can impact surface waters and threaten public health as exemplified by the occurrence of spinach from Califonia contaminated with *E. coli* O157:H7. To understand better and manage the fate and transport of these pathogens in agricultural watersheds a method is needed to determine dilute concentrations of these pathogens in environmental waters.

Objective

Develop a culture-based most probable number (MPN) method to determine dilute concentrations of *Salmonella* and *E. coli* O157:H7 in surface waters of a watershed (Fig. 1) with animal agriculture (Fig. 2).

Materials and Methods

- Each MPN begins with a filtration step (Fig. 3). As much as 20 l of water from pond inflow (Fig. 4), pond (Fig. 5), and pond outflow (Fig. 6) is filtered through a 1-µm-pore-size 293 mm FALP filter.
- Filtered material is scrubbed off the filter with a brush in PBS, and consolidated by centrifugation.
- Pellets are resuspended in 5 ml PBS and used to inoculate tubes of Tetrathionate for *Salmonella* and Laural Tryptose Broth (LTB) for *E. coli*.
- Tubes are arranged in a 5-tube, four 10-fold dilution scheme.
- Positive Tetrathionate tubes are inoculated onto Brilliant Green agar.
- Presumptive *Salmonella* isolates are streaked on BHI agar plates.
- Colonies are stabbed into slants of Lysine Iron and Triple Iron agar.
- If both slants are positive confirmation is made with a TaqMan assay with primers and fluorescent probe for the junction between virulence genes *Sip*B and *Sip*C.
- Positive LTB tubes are spread on Sorbitol MacConkey plates.
- Suspected *E. coli* O157:H7 are inoculated on BHI plates and into tubes of LTB-MUG. Non-fluorescence and gas producing tubes are positive.
- Positive colonies are tested for glutamate decarboxylase (GAD) activity and latex agglutination.
- If both GAD and agglutination positive, confirmation is made with a TaqMan assay with primers and fluorescent probe for the virulence gene eaeA.
- MPN is calculated with FDA's preferred MPN method.

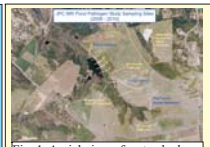

Fig. 1. Aerial view of watershed

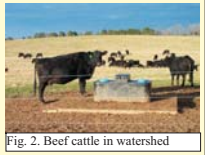

Fig. 2. Beef cattle in watershed

Fig. 3. Filter apparatus

Fig. 4. Pond inflow

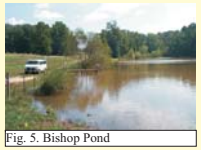

Fig. 5. Bishop Pond

Fig. 6. Pond outflow

Results

- Concentrations of *Salmonella* and *E. coli* 0157:H7 ranged from 0.1 to 3.4 and 0.1 to 18 MPN l^{-1}, respectively (Table 1).
- *Salmonella* was most frequently detected in pond inflow, and once each in pond and pond outflow.
- *E. coli* O157:H7 was detected equally in pond inflow and within pond, and once in pond outflow.

Results

- Pond inflow and outflow fluxes of *Salmonella* and *E. coli* O157:H7 ranged from <1.6 to 4.1 and <1.3 to 4.8 Log_{10} MPN h^{-1}, repectively (Table 2).
- The lower flux rates for 2007 may be attributable to a greater rainfall deficit compared to 2006 (Fig. 7).

Table 1. Detection and quantification of *Salmonella* and *E. coli* O157:H7 (EcO157:H7).

Year	Month	Pathogen	Inflow	Pond	Outflow
			MPN L^{-1}		
2006	May	EcO157:H7	ND[1]	ND	ND
	July	EcO157:H7	ND	**0.1**	**0.9**
	August	*Salmonella*	**0.1**	ND	**3.4**
	September	EcO157:H7	ND	ND	ND
	October	*Salmonella*	**0.2**	ND	ND
	November	EcO157:H7	**9.0**	ND	ND
	December	*Salmonella*	**0.55**	ND	ND
2007	January	*Salmonella*	ND	ND	ND
	February	EcO157:H7	**0.35**	**8.3**	ND
	March	*Salmonella*	ND	ND	ND
	April	*Salmonella*	ND	ND	ND
	May	EcO157:H7	**2.0**	ND	ND
	May	*Salmonella*	**0.1**	ND	ND
	June	EcO157:H7	ND	ND	ND
	July	*Salmonella*	ND	ND	ND
	August	EcO157:H7	ND	ND	ND
	September	EcO157:H7	ND	ND	ND
	October	*Salmonella*	ND	**0.9**	ND
	November	EcO157:H7	ND	**18**	ND

[1]ND = not detected.

Table 2. Flux of *Salmonella* and *E. coli* O157:H7 (EcO157:H7) in pond inflow and outflow.

Year	Month	Pathogen	Inflow	Outflow
			Log_{10} MPN h^{-1}	
2006	May	EcO157:H7	<2.3	2.5
	July	EcO157:H7	<2.0[1]	3.7
	August	*Salmonella*	2.8	4.1
	September	EcO157:H7	<2.1	<2.4
	October	*Salmonella*	3.0	<2.3
	November	EcO157:H7	4.8	<2.7
	December	*Salmonella*	3.9	<2.6
2007	January	*Salmonella*	<2.6	<3.0
	February	EcO157:H7	3.7	<2.6
	March	*Salmonella*	<2.3	<2.6
	April	*Salmonella*	<2.1	<2.5
	May	EcO157:H7	4.1	<2.4
	May	*Salmonella*	2.7	<2.1
	June	EcO157:H7	<1.9	<2.3
	July	*Salmonella*	<1.6	<1.7
	August	EcO157:H7	<1.6	<1.3
	September	EcO157:H7	<1.6	<1.8
	October	*Salmonella*	<1.8	<2.1
	November	EcO157:H7	<2.0	<2.1

[1]Flux values with a "less than" symbol is based on the low 95% confidence level of 0.015 MPN l^{-1}.

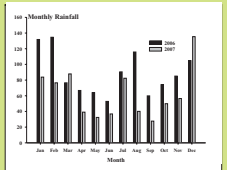

Fig. 7. Monthly rainfall (mm) for 2006 and 2007.

Conclusions and Impact

- These MPN methods are capable of quantifying dilute, viable concentrations of *Salmonella* and *E. coli* O1576:H7 in environmental surface waters.
- They are tools to be used to better understand fluxes of these pathogens in agricultural watersheds.
- As culture based methods they provide the means to develop a collection of these pathogens for source tracking and epidemiological purposes.

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