Microbial Pathogen and Indicator Survival in Fescue Soils with Livestock Amendments

Kimberly Cook[1], Annesly Netthisinghe[2], Rebecca Gilfillen[2] and Paul Woosley[2]
(1)USDA-ARS, Bowling Green, KY
(2)Department of Agriculture, Western Kentucky University, Bowling Green, KY

Kim Cook
USDA-ARS- AWMRU
230 Bennett Lane,
Bowling Green, KY 42104
E-mail:
kim.cook@ars.usda.gov

BACKGROUND

- Studies show that manure-borne pathogens may persist in livestock manures for weeks or even months with their survival depending on the organism, and soil biological, chemical and physical conditions.
- Although the majority of bacteria associated with manures are beneficial and/or innocuous, the potential for contamination of agricultural environments, livestock and crops with manure-borne pathogens necessitates greater knowledge of their persistence in the field.

Manure-Borne Gastrointestinal Pathogens

- Two of the most common causes of diarrheal illness in the U.S. and around the world are usually transmitted to humans by eating foods contaminated with animal feces.

***Campylobacter* sp.**

- Estimated to affect over 2.4 million persons every year
- Despite its relative sensitivity to environmental conditions it is a leading cause of human gastroenteritis worldwide

***Salmonella* sp.**

- Unlike other foodborne illnesses, *Salmonella* infections have not decreased during the past 15 years and have instead increased by 10 percent
- More than 1 million people in this country become ill from *Salmonella* each year

Indicators of Pathogen Contamination

- Fecal indicator bacteria are used to detect fecal contamination and potential for pathogen presence
- Present in contaminated materials in higher concentrations than pathogens and are easier to culture
- Questions remain regarding accuracy for predicating pathogen presence

OBJECTIVE

- The objective of this study was to evaluate the persistence of bacterial pathogens and indicators associated with poultry and dairy manures following addition to field plot soils with tall fescue

MATERIALS AND METHODS

- The field experiment was established with four replicates of tall fescue with soils which were either un-amended **(C)** or amended with dairy manure **(DM)**, poultry litter **(PL)** under conventional till **(CT)** or no till **(NT)** management.
- Soil samples were collected from 15 cm cores on days 1, 2, 4, 7, 15, 21, 35, 42, 57 and 84. Three cores were taken from each 3 m x 6 m field plot. The cores were combined and mixed thoroughly before sub-samples were taken for microbiological and chemical analyses. The soil corer was sterilized with 70% ethanol between treatments and untreated controls were always sampled first.
- Soils from each treatment were plated onto selective media and/or placed into enrichment broths. DNA from soil samples (300mg) was extracted & quantitative, real-time PCR (qPCR) was used to determine the cell concentration using primers, probes and qPCR protocols as shown in Table 1

Table 1. Sequences, target size and Tm of primers used in this study

Organism	Target	Oligo	Sequence (5'-3')	Tm (°C)	Insert size (bp)	Copy Number
All Bacteria	16S rRNA gene	1055-F	ATG GCT GTC GTC AGC T	54.0	337	4
		1392-R	ACG GGC GGT GTG TAC	59.0		
		B16s-Taq115-F	CAA CGA GCG CAA CCC			
Escherichia coli	*uidA*	UidA784 F	GTG TCA TAT CTA CCC GCT TCG C	66.5	82	1
		UidA866 R	AGA ACG CTT TGT GGT TAA TCA GGA	66.5		
		UidA807FAM	TCG GCA TCC GGT CAG TGG CAG T			
***Enterococci* sp**	23S rRNA gene	ECF-748F	AGA AAT TCC AAA CGA ACT TG	53.1	106	4
		ENR-854R	CAG TGC TCT ACC TCC ATC ATT	55.4		
		Enterococci-Gl813tQ	TGG TTC TCT CCG AAA TAG CTT TAG GGC TA			
***Bacteroides* sp.**	16S rRNA gene	AllBac296-F	GAG AGG AAG GTC CCC CAC	63.6	106	6
		AllBac412-R	CGC TAC TTG GCT GGT TCA G	63.5		
		AllBac375-Bhq-R	CCA TTG ACC AAT ATT CCT CAC TGC TGC CT			
***Salmonella* sp.**	*ttrBCA*	ttr-4-sal-F	AGC TCA GAC CAA AAG TGA CCA TC	65.5	94	1
		ttr-6-sal-R	CTC ACC AGG AGA TTA CAA CAT GG	64.4		
		ttr-5-sal-P	CAC CGA CGG CGA GAC CGA CTT T			
Campylobacter jejuni	Genome fragment	Nogova F	CTG AAT TTG ATA CCT TAA GTG CAG C	63.6	86	1
		Nogova R	AGG CAC GCC TAA ACC TAT AGC T	63.9		
		NogCampBHQ	TCT CCT TGC TCA TCT TTA GGA TAA ATT CTT TCA CA			

EXPERIMENTAL SITE

Western Kentucky University
Agricultural Research Complex,
Bowling Green, KY

Tall fescue forage grass established with Conventional till (CT) or No Till (NT)

Manure surface applied to 3 m x 6 m field plots

Soil Samples were taken to a depth of 15 cm

Manures on surface of forage grass

ACKNOWLEDGEMENTS

We are grateful for the technical assistance of Rohan Parekh and Tinesha Simmons. This research is supported under the USDA-CRIS project Efficient Management and Use of Animal Manure to Protect Human Health and Environmental Quality (Project No. 6445-12630-003-00D).

RESULTS & CONCLUSIONS

Fig 1. Concentration of enterococci in soils amended with poultry litter

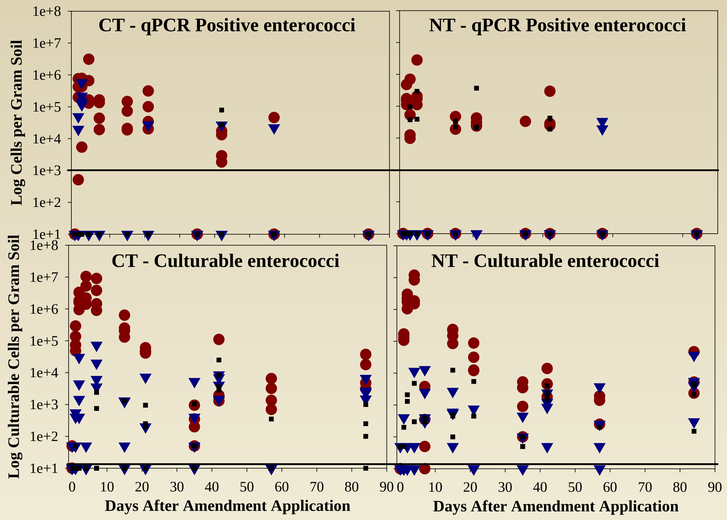


Fig. 2. Concentration of Bacteroides in Soils

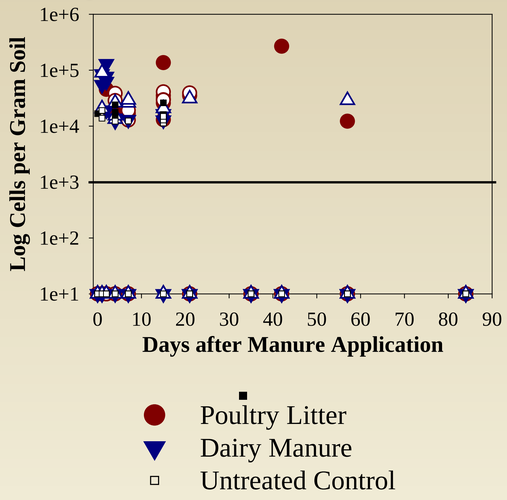
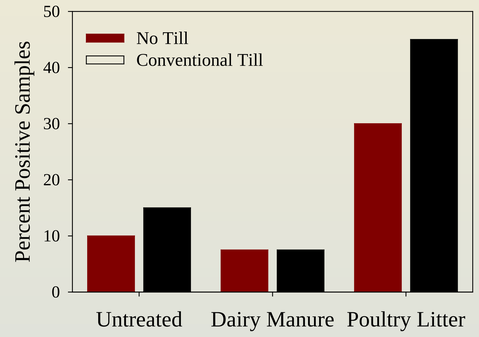


Table 2. Percent positive *Salmonella enterica* enrichment cultures (RV10)

Day	No Amendment NT	No Amendment CT	Dairy Manure NT	Dairy Manure CT	Poultry Litter NT	Poultry Litter CT
1	0	0	25	0	0	0
2	0	0	0	25	0	0
4	25	50	50	0	25	0
7	50	25	0	0	25	50
15	0	25	0	0	25	75
21	0	25	0	0	100	75
35	0	0	0	50	50	50
42	25	25	0	0	25	75
57	0	0	0	0	0	75
84	0	0	0	0	25	50
Total Positive	**10.00**	**15.00**	**7.50**	**7.50**	**30.00**	**45.00**

NT = No Tillage
CT = Conventional Tillage

Fig. 3. Soil Enrichment for *Salmonella enterica*



RESULTS

Indicator Detection: Enterococci (Fig. 1)

- Concentrations of enterococci were similar in conventional and no till soils
- Around 3 x 10^5 cells g^{-1} soil qPCR positive and around 1 x 10^6 cells g^{-1} soil culturable for the first 21 days after application with poultry litter
- Time for a 90% decrease in culturable populations in poultry litter amended soils was 7.4 and 9.2 days for conventional till and no till, respectively
- Time for a 90% decrease in PCR positive poultry litter amended soils was 24.1 and 25.2 days for conventional till and no till, respectively
- Enterococci were only detected above background levels in dairy manure amended soils on day 2 (2.6 x 10^5 cells g^{-1} soil) by qPCR and by culture methods only on days 2 and 4 (around 1.0 x 10^4 cells g^{-1} soil) and only in conventional till soils

Indicator Detection: *Bacteroides* (Fig. 2)

- Generally exhibited less background (qPCR only) but limits of detection may be reduced due to lower concentrations in manures
- In poultry litter amended soils *Bacteroides* were detected in day 15 samples (5.0 x 10^4 cells g^{-1} soil) but were generally only intermittently detected
- In dairy manure *Bacteroides* concentrations averaged around 1.0 x 10^4 cells g^{-1} soil for the first 14 days – with little background

Pathogen Detection: *Salmonella* (Fig. 3 and Table 2)

- **Detected above background levels only in enrichment broths (RV10) from soils amended with poultry litter**
- **Detected 7 days after application – which correlated with the first precipitation totaling 1.3 cm**
- **Conventionally tilled soils had higher numbers of positive enrichment samples than did no till soils**
- **Occurrence of *Salmonella* was not well correlated with that of the indicators enterococci or *Bacteroides***

Pathogen Detection: *Campylobacter*

- Although present at high concentrations in the initial poultry litter used for application (2 x 10^6 cells g^{-1} litter), *Campylobacter* was not detected in enrichment culture and was only intermittently detected in soil samples amended with poultry litter
- Efforts continue to detect and monitor die-off of this organism in poultry litter amended soils and in collected grass samples

CONCLUSIONS

While occurrence of indicators decreased after the first 4 days of the experiment, *Salmonella* were found in soils after 7 days and were dominant between 7 and 42 days; likely due to manures being retained on vegetation

Lack of detection of *Campylobacter* in poultry litter amended soils is a concern and suggests that inability to enrich for this pathogen adequately (due to fastidious nature and viable but non-culturable state) may lead to under-estimation of its prevalence and/or survival

Sampling of grass taken at each time point may prove to more accurately reflect pathogen/indicator presence than soil samples