An Assessment of Fecal Contamination in Rural Streams Impacted by Litter Applications

Yong Jin Lee[1] & Marirosa Molina[2]
NRC Postdoctoral Associate at USEPA[1], USEPA/NERL/ERD/EAB[2]

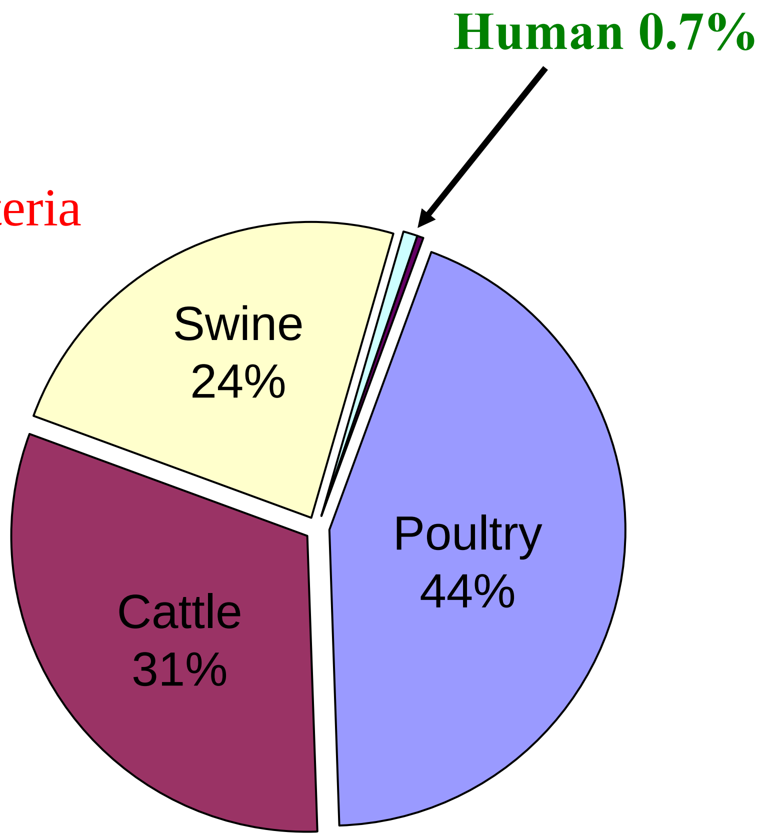
Microbial Source Tracking

Clean Water Act (1972)
Goal: Waters should be "fishable and swimmable"

However:
After 19% of US rivers/streams were sampled
    39% impaired
    35% of the impaired polluted by fecal bacteria

Types of sources

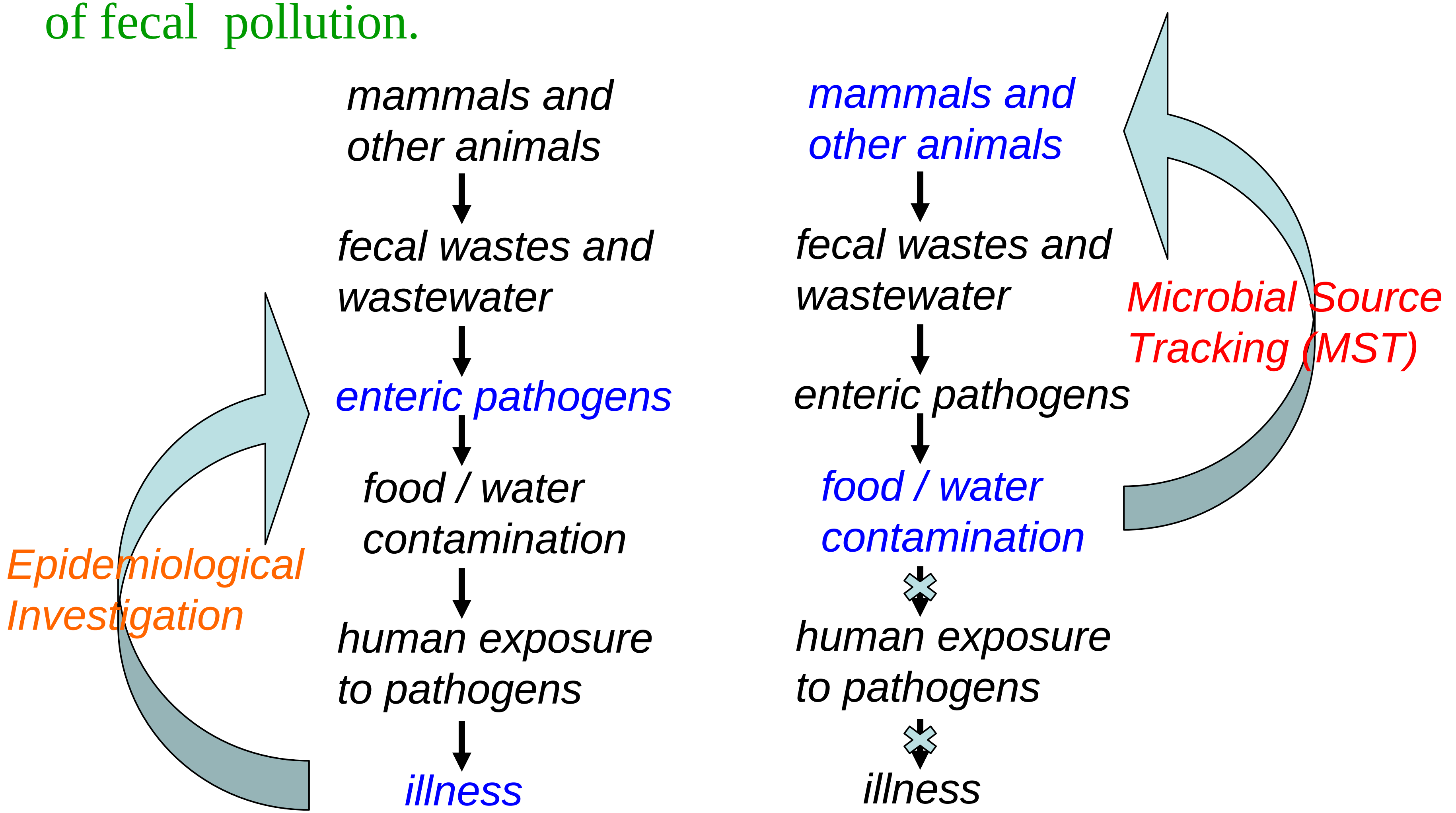
- Humans
- Domesticated animals
- Wildlife



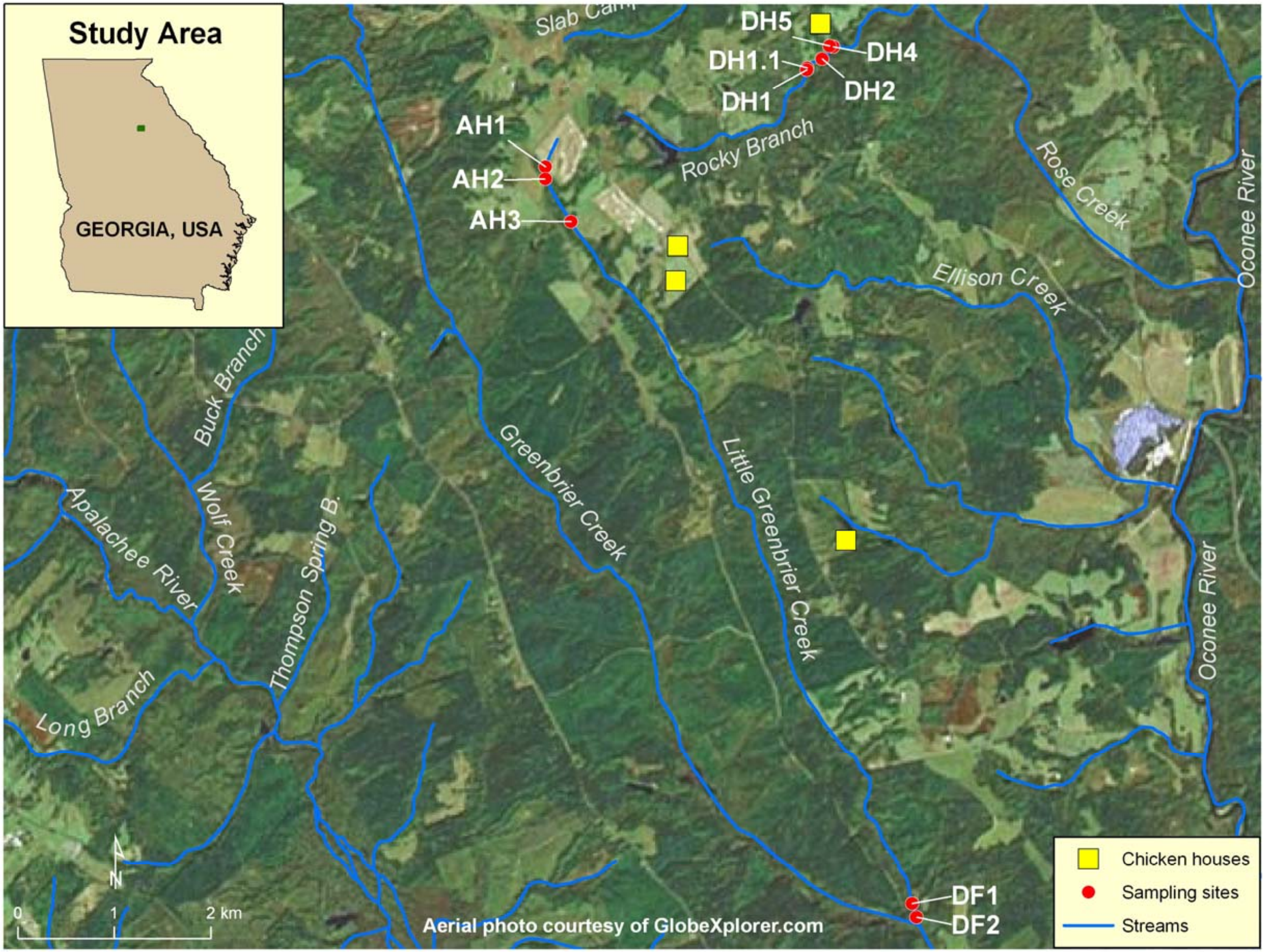
* Annual feces production in the U.S.
= **$1x10^{12}$ kg/year** + **wildlife**

Microbial Source Tracking

Matching microbial fingerprints from a polluted site with microbial fingerprints of animal fecal sources to define the origin of fecal pollution.



Study Sites



Study Area
GEORGIA, USA
Slab Camp
DH5
DH4
DH1.1
DH2
DH1
AH1
AH2
AH3
Rocky Branch
Rose Creek
Oconee River
Ellison Creek
Buck Branch
Greenbrier Creek
Little Greenbrier Creek
Apalachee River
Wolf Creek
Thompson Spring B.
Long Branch
Oconee River
N
0
1
2 km
Aerial photo courtesy of GlobeXplorer.com
DF1
DF2
Chicken houses
Sampling sites
Streams

Study Sites

Site	Description	Type of BMP	Projected sources of fecal impact
AH1	Head water of the creek near silage field	None	chicken, cattle, wildlife
AH2	Midstream near wetland area	"	
AH3	Stream next to pasture	"	
DH1	Upstream before farm	None	chicken, cattle, goat, wildlife
DH1.1	Intermittent stream adjacent to pasture	**Buffer strip**	
DH2	Stream near pasture	**Buffer strip**	
DH4	Stream after farm	None	
DH5	Pond water	**Reservoir**	
DF1	Stream near hay field	**Buffer strip**	chicken, cattle, horse, wildlife
DF2	Downstream from hay field	**Buffer strip**	

Experimental Scheme

Stream water potentially contaminated with chicken litters

↓

Filtration → *Enterococcal density* (CFU/100 ml)

↓

DNA extraction

↓ → *End-point PCR* → presence vs. absence

Q-PCR → quantitative assessment (*Brevibacterium spp.)*

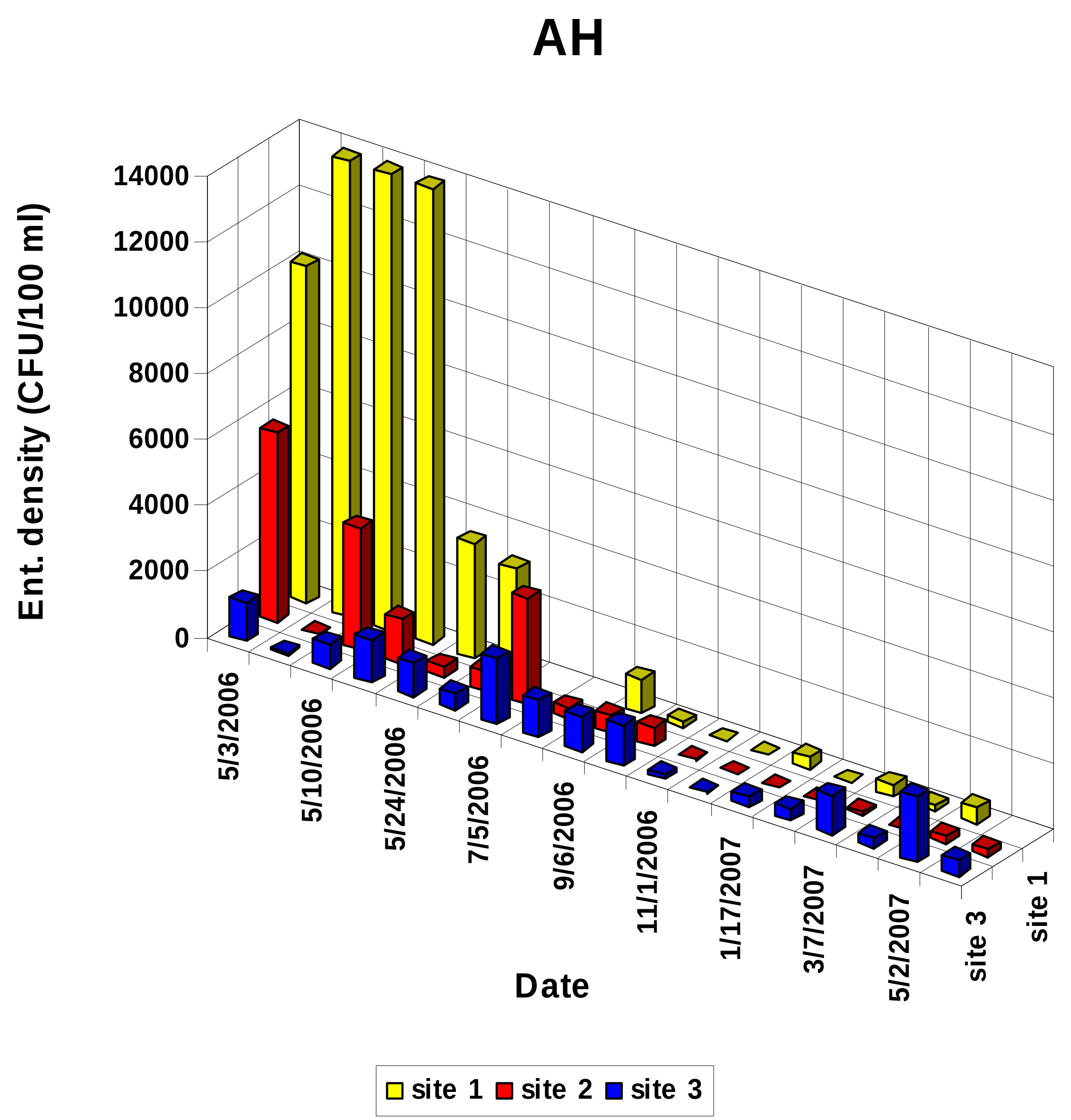
Environmental Parameters

Site	pH		Temp		Turbidity		
	Mean	95% CI	Mean	95% CI	Geometric mean	95% CI	
						lower bound	upper bound
AH1	6.58	±0.22	15.43	±2.60	38.10	5.76	252.00
AH2	6.48	±0.20	16.43	±2.56	46.29	30.93	69.27
AH3	6.73	±0.22	16.69	±2.59	19.57	16.29	23.53
DH1	7.16	±0.15	15.82	±2.65	4.95	3.39	7.21
DH1.1	7.01	±0.26	14.61	±2.96	13.21	8.84	19.74
DH2	7.06	±0.20	15.71	±2.70	3.85	2.74	5.40
DH4	7.07	±0.22	15.61	±2.76	4.07	2.74	6.05
DH5	7.22	±0.49	18.31	±4.02	29.29	20.14	42.60
DF1	6.98	±0.21	17.02	±3.69	9.16	5.33	15.73
DF2	7.02	±0.21	17.03	±3.79	18.36	10.14	33.17

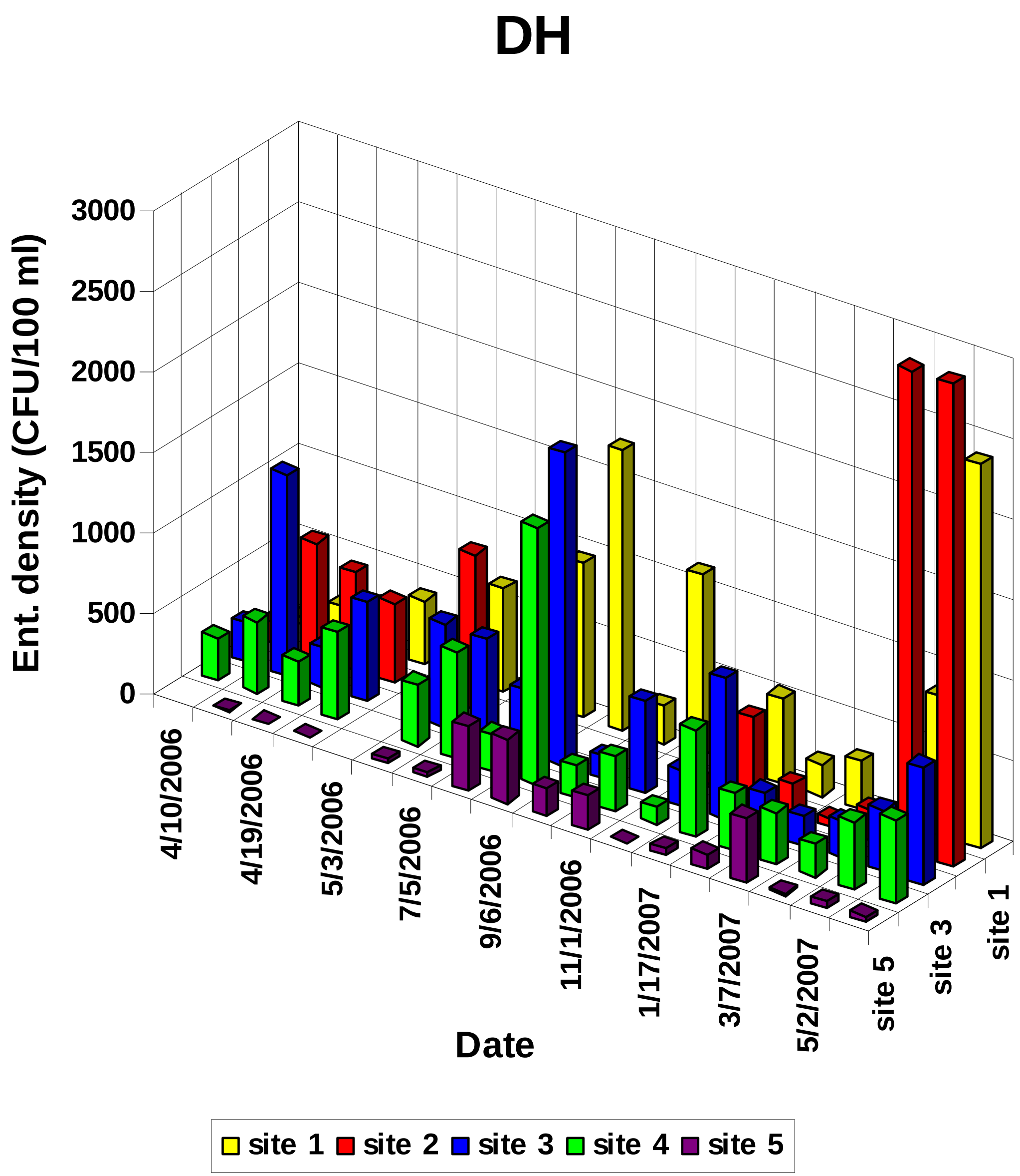
Enterococcal Density

Site	Enterococcal density (CFU/100 ml)		
	Geometric mean	95% CI	
		lower bound	upper bound
AH1	401	51	3161
AH2	136	29	633
AH3	561	323	975
DH1	389	200	756
DH1.1	358	137	934
DH2	435	300	631
DH4	369	271	503
DH5	29	7	117
DF1	391	256	597
DF2	438	280	685

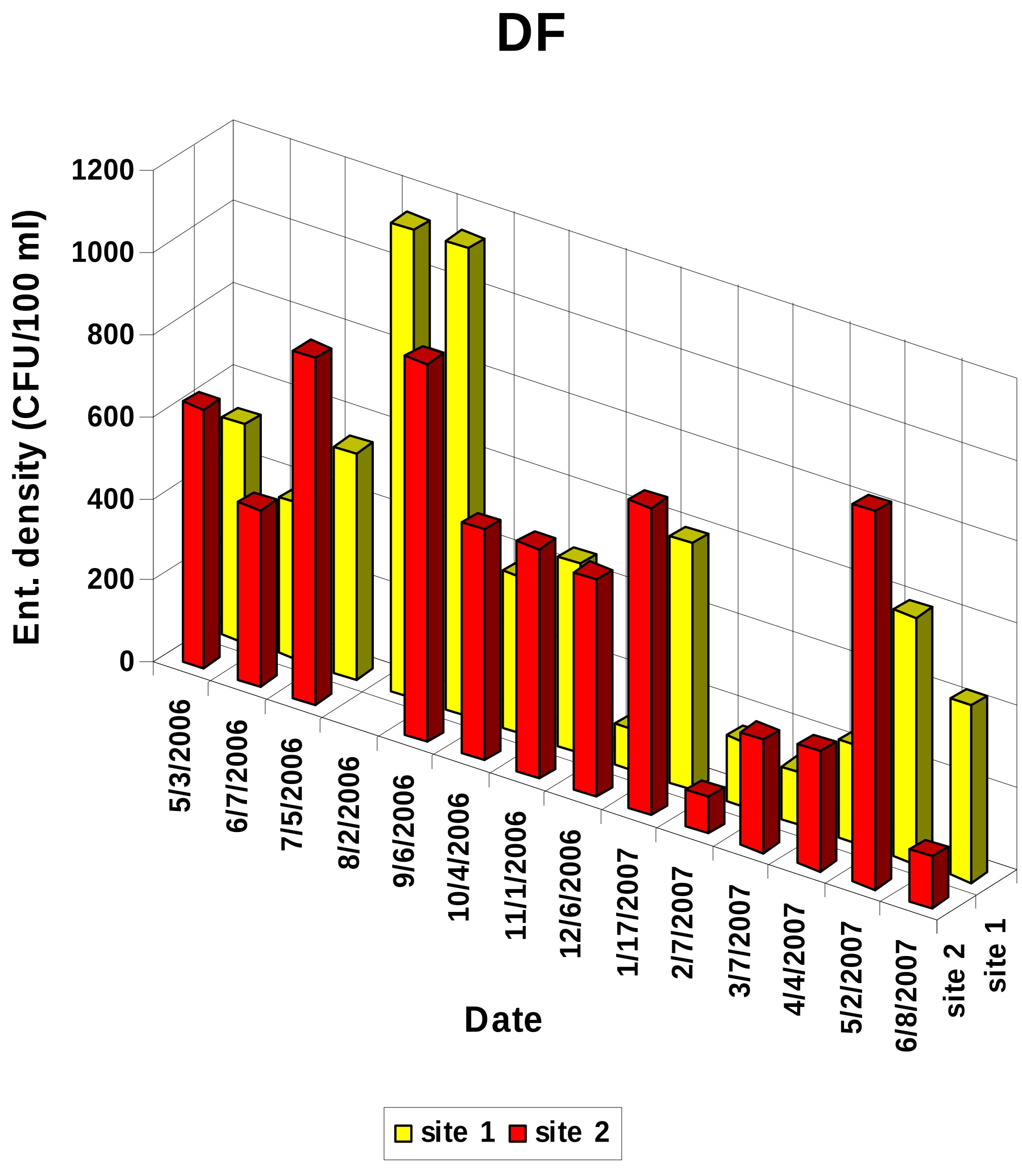
Enterococcal Density in AH



Enterococcal Density in DH



Enterococcal Density in DF



Molecular Markers

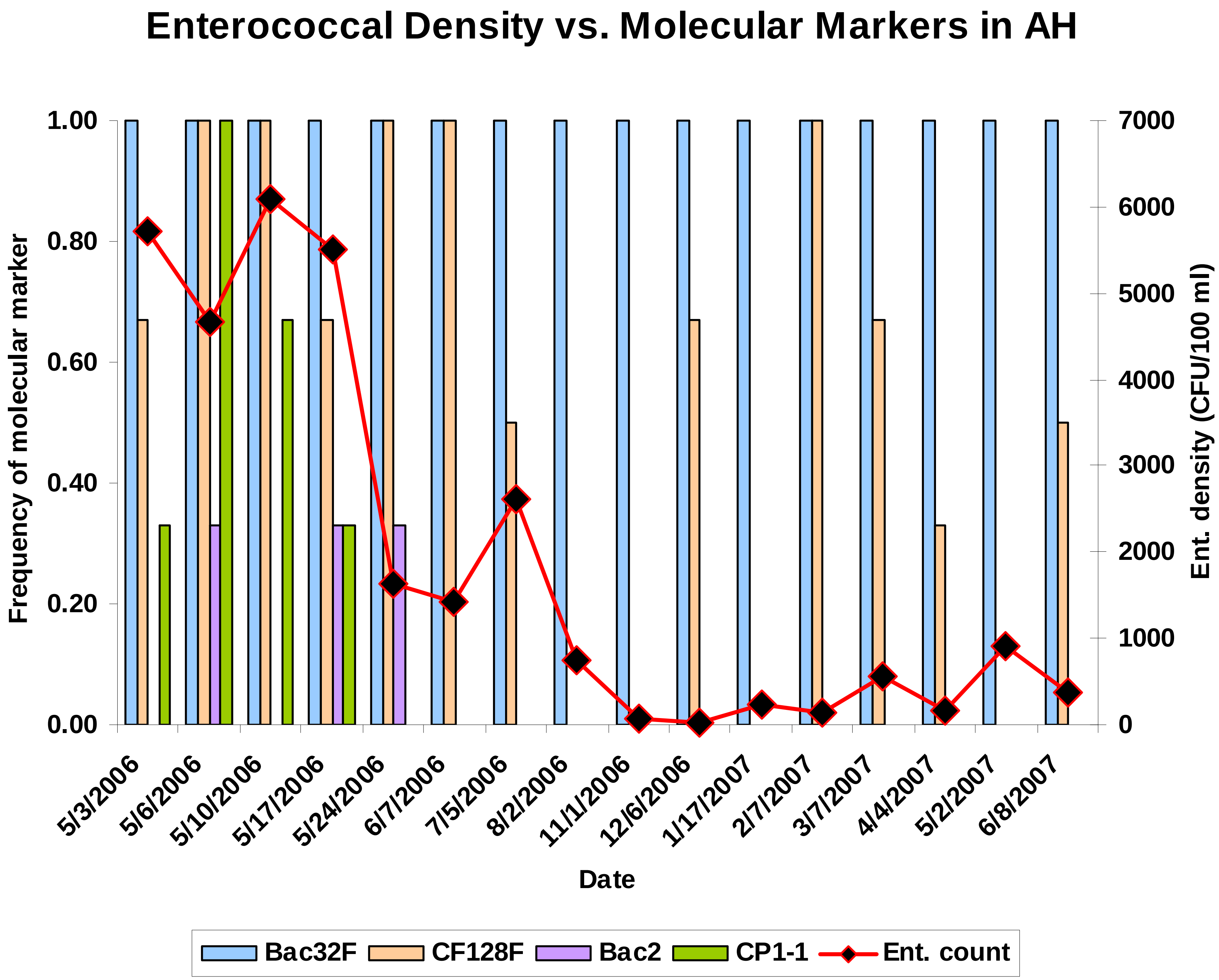
Primer	Sequence (5' to 3')	Expected size of PCR products (bp)	Target Animals
Bac32F	AACGCTAGCTACAGGCTT	676	Universal
Bac708R	CAATCGGAGTTCTTCGTG		
CF128F	CCAACYTTCCCGWTACTC	580	Ruminant
HF183F	ATCATGAGTTCACATGTCCG	525	Human
CP2-9	GTAAGACAGCAACCCCATGTA ACCTATGGTTCAACACGCTTTA	245	Chicken
CP1-74	AGACATCACCGGCAATAACTA CAAGGAGCTATGCCGCTTA	295	Chicken
CP3-49	GTCCAGCGCCTCATTGAT TGGTGATCGACTTTTCCAAT	329	Chicken
CB-R2-80	CGTGAATTTCCGCTACGA CCTCTTCCTTGCGTCCCA	287	Chicken
CB-R2-42	GACGAGATCTATATTTGCCTCA CGGAGCATATCCTACGATCA	265	Chicken
CP1-1	GGCAGGCATCAAGTCAACA TGGCAAAAGCAACTGTCATGGCA	281	Bird
CP2-10	CTTTGCTGCAAGCTCCTTGA TACGGAAGCGGAGGAAAG	276	Bird

Detection Limits

Primer	Detection limit (gDNA)
CP2-9	1×10^{-9}
CP1-74	5×10^{-10}
CP3-49	5×10^{-10}
CB-R2-80	1×10^{-9}
CB-R2-42	1×10^{-9}
CP1-1	5×10^{-11}
CP2-10	1×10^{-8}

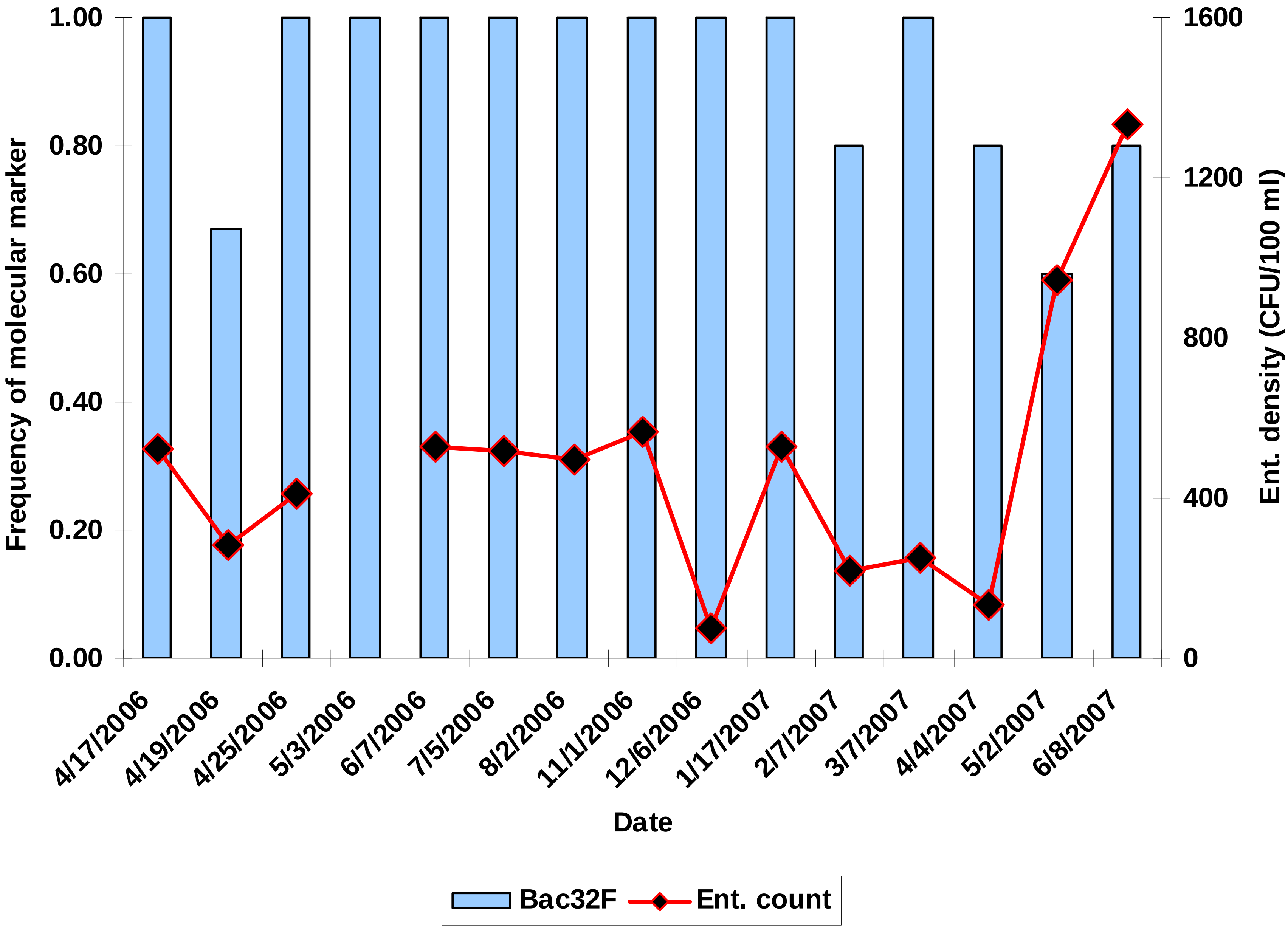
Presence of Molecular Marker

Site	Bac32 (universal)		CF128 (ruminant)		HF183 or HF134 (human)		Bac2 (cattle)		CP1-1 (bird)	
	Frequency	95% CI	Frequency	95% CI	Frequency	95% CI	Frequency	95% CI	Frequency	95% CI
AH1	1	0	0.54	0.14	0	0	0.23	0.10	0.15	0.07
AH2	1	0	0.50	0.14	0	0	0	0	0.19	0.09
AH3	1	0	0.69	0.12	0	0	0	0	0.13	0.06
DH1	1	0	0	0	0	0	0	0	0	0
DH1.1	0.91	0.05	0.09	0.05	0	0	0	0	0	0
DH2	0.93	0.04	0	0	0	0	0	0	0	0
DH4	1	0	0	0	0	0	0	0	0	0
DH5	0.73	0.11	0	0	0	0	0	0	0	0
DF1	1	0	0.75	0.11	0	0	0	0	0	0
DF2	1	0	0.83	0.08	0	0	0	0	0	0

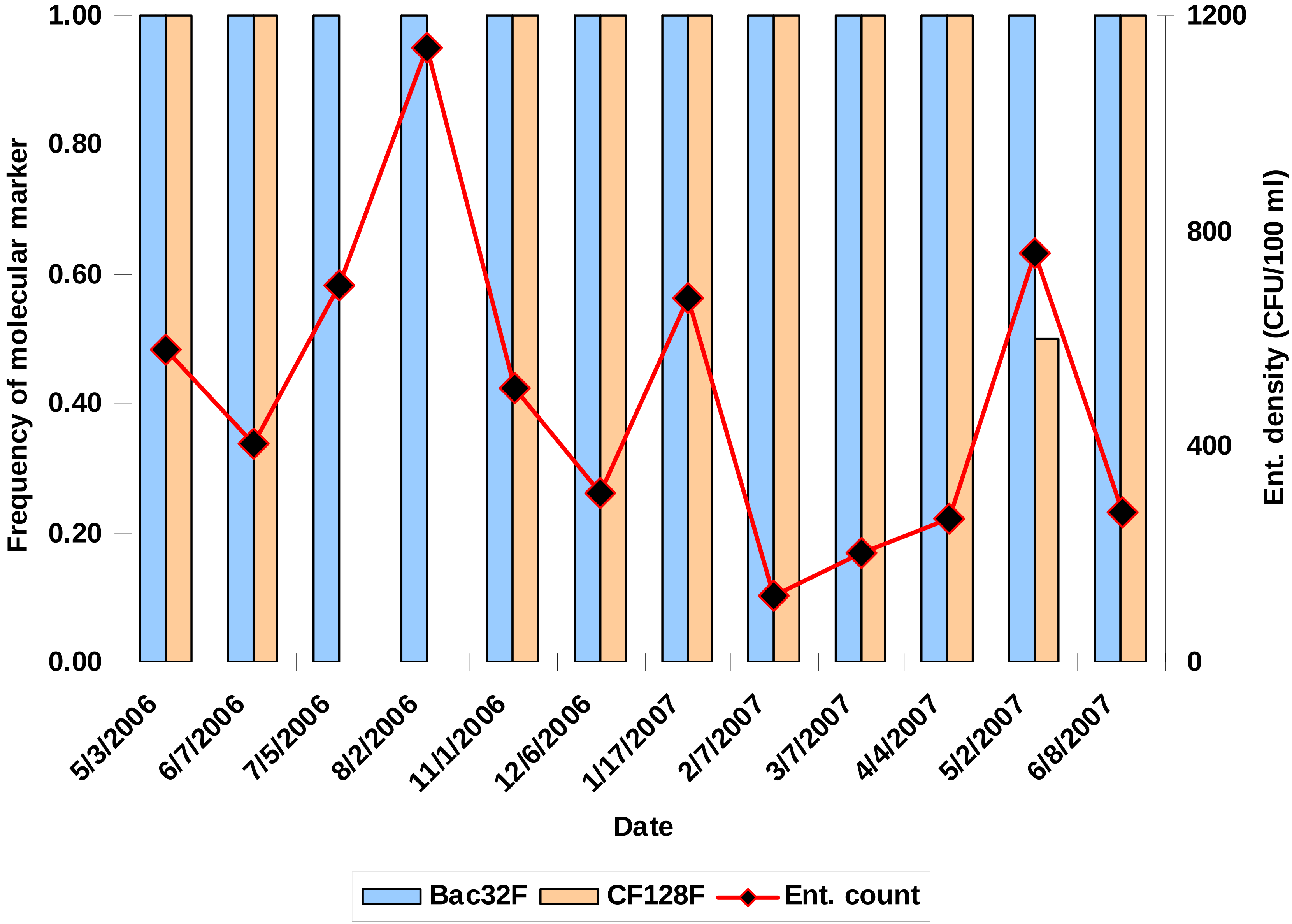


Enterococcal Density vs. Molecular Markers in AH
Frequency of molecular marker
Ent. density (CFU/100 ml)
1.00
0.80
0.60
0.40
0.20
0.00
7000
6000
5000
4000
3000
2000
1000
0
5/3/2006
5/6/2006
5/10/2006
5/17/2006
5/24/2006
6/7/2006
7/5/2006
8/2/2006
11/1/2006
12/6/2006
1/17/2007
2/7/2007
3/7/2007
4/4/2007
5/2/2007
6/8/2007
Date
Bac32F
CF128F
Bac2
CP1-1
Ent. count

Enterococcal Density vs. Molecular Markers in DH



Enterococcal Density vs. Molecular Markers in DF



Environmental Predictors for Markers

Binary Logistic Regression

Marker	Site	Significant predictor	*P*	Model fit (Deviance†)			Measures of association
				Chi square	*DF*	*P*	Somers' D‡
CF128F	AH DF	No significant predictors					
CP1-1	AH	three-day precipitation	0.009	7.757	6	0.256	0.65

† Deviance *P*-value > 0.05 indicates no significant lack of fit.

‡ Somers' D was calculated as the proportion of concordant pairs minus the proportion of discordant pairs.

Environmental Predictors for Ent. Density

Multiple Linear Regression

Site	Response	Explanatory Variables	Coefficient	*P*	Model Fit Adjusted R^2 (%)
AH	log (enterococcal density)	Temperature	0.22497	0.005	32.2
		Turbidity	0.00307	0.009	
DH		pH	-1.67550	0.000	26.3
		Turbidity	-0.03009	0.012	
DF		Turbidity	0.01977	0.029	20.5

Summary

- **AH** was found to be contaminated with multiple sources:
  - mostly cattle (56.9%).
  - chicken contamination was detected only after manure was spread on the hay field.
- **DH** had no source-specific contamination except once with CF128.
- **DF** showed 82.1% samples were positive for ruminant-specific marker but negative for cattle-specific marker (Bac2).
- **CP1-1** (bird-specific) showed the lowest detection limit among chicken-specific metagenomic markers and was the only marker detected from field samples.
- **Statistical analyses:**

  (a) **Binary logistic regression** analyses showed no significant predictors for source-specific markers except for CP1-1 that correlated with three-day precipitation.

  (b) **Multiple linear regression** showed that turbidity was the most significant predictor for enterococcal density, suggesting attachment of enterococci to particulate matters.
- **This study suggests that the establishment of an appropriate buffer zone or catchment is critical for reducing fecal pollution.**

Acknowledgement

EPA/NERL/ERD in Athens, GA
Jonathan Willis, Charles Budinoff, Ryan Jones

EPA/NRMRL in Cincinnati, OH
Dr. Jorge Santo-Domingo,
Dr. Jingrang Lu

National Research Council Research Associateship Programs

Owners of chicken farms