

Wildlife identified as major source of *Escherichia coli* in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints

J.A. Somarelli^a, J.C. Makarewicz^a, R. Sia^b and R. Simon^c

^aDepartment of Environmental Science and Biology, State University of New York at Brockport, Brockport, NY 14420, USA

^bDepartment of Biological Sciences, State University of New York at Brockport, Brockport, NY 14420, USA

^cDepartment of Biology, State University of New York at Geneseo, Geneseo, NY 14454, USA

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Abstract

The presence of *Escherichia coli* in recreational and potable waters is a major concern to the general public as elevated levels of *E. coli* suggest the presence of pathogenic bacteria and viruses. Unfortunately, traditional microbial techniques do not allow specific identification of the source of *E. coli*. This reduces the ability to target management practices that reduce bacterial contamination. In the Finger Lakes region of western New York, USA, wildlife resides in relatively high densities on watersheds dominated by people and dairy farms, and as a result, the sources of fecal degradation of potable and recreational waters are often unknown. In the Conesus Lake watershed, the sources of microbial contamination were assessed using Rep-PCR molecular tools, a method of amplifying repetitive DNA sequences found throughout the *E. coli* genome to produce distinct fingerprints for a given ecotype. Molecular fingerprints of *E. coli* isolated from regional populations of cattle, humans, geese and deer were compared to *E. coli* isolated from stream water samples. Canonical discriminant function analysis indicated that the DNA fingerprints of the original source group isolates were correctly predicted 90.2% of the time. Since land use in the sub-watersheds was dominated by dairy and cash crop farms, it was expected that the majority of *E. coli* isolated would be identified as cows; however, an unexpectedly high percentage of isolates were identified as wildlife (geese and deer). Geese were the dominant source of *E. coli* (44.7–73.7% of the total sources) in four sub-watersheds followed by cows (10.5–21.1%), deer (10.5–18.4%), humans (5.3–12.9%) and unidentifiable sources (0.0–11.8%). Management practices intended to decrease the number of cattle or the amount of manure spread in a sub-watershed were reflected in a decrease of *E. coli* ecotypes associated with dairy cows.

Comments

The Finger Lakes area is similar to many parts of Ontario. 78.9% to 89.5% of the *E. coli* is from sources other than agriculture in a watershed dominated by people, dairy farms and wildlife. This study use Rep-PCR methodology and was published in 2006.

Repetitive element (REP)-polymerase chain reaction (PCR) analysis of *Escherichia coli* isolates from recreational waters of southeastern Lake Huron

Can. J. Microbiol. 55(3): 269–276 (2009)

Tanya Kon, Susan C. Weir, E. Todd Howell, Hung Lee, and Jack T. Trevors

Abstract: Repetitive element-polymerase chain reaction (REP-PCR) DNA fingerprinting and library-based microbial source tracking (MST) methods were utilized to investigate the potential sources of *Escherichia coli* pollution in recreational waters of southeastern Lake Huron. In addition to traditional sources such as humans, agriculture, and wildlife, environmentally persistent *E. coli* isolates were included in the identification library as a separate library unit consisting of the *E. coli* strains isolated from interstitial water on the beach itself. Our results demonstrated that the dominant source of *E. coli* pollution of the lake was agriculture, followed by environmentally adapted *E. coli* strains, wildlife, and then humans. A similar ratio of contributing sources was observed in all samples collected from various locations including the river discharging to the beach in both 2005 and 2006. The high similarity between the compositions of *E. coli* communities collected simultaneously in the river and in the lake suggests that tributaries were the major overall sources of *E. coli* to the lake. Our findings also suggest that environmentally adapted strains (EAS) of *E. coli* should be included as one of the potential sources in future microbial source tracking efforts.

Comments

In the agriculturally dominated 18-Mile Creek watershed 99% of the fecal material is from livestock sources (Fleming and Ford, 2001). Even under these circumstances, more than 40% of the *E. coli* is from sources other than agriculture. The authors report that the average rate of “correction assignment” was 66.9% and acknowledge that the assignment of unknown samples to an agricultural source is likely biased high.

Lake Huron At Ashfield Township Park and from the Eighteen Mile River							
Source Category							
Location	Year	CFU/100 ml	Agricultural	Wildlife	Human	EAS	Unidentified
5-lake	2005	2 - 1500	60.0	13.6	2.9	15.9	7.9
Stations	2006	1 - 2800	60.0	12.9	2.6	15.5	9.0
18-Mile	2005	11 - 4900	60.4	12.6	3.2	16.1	7.6
River	2006	22 -6500	59.2	7.7	1.4	22.5	9.2

*EAS = Environmentally adapted strains

Lake Huron At Ashfield Township Park and from the Eighteen Mile River						
Location	Year	CFU/100 ml	Max CFU	Agricultural	Max CFU (Ag-Sources Eliminated)	
5-lake	2005	2 - 1500	1500	60.0		600
Stations	2006	1 - 2800	2800	60.0		1120
18-Mile	2005	11 - 4900	4900	60.4		1940
River	2006	22 -6500	6500	59.2		2652

Faune et contamination des sources d'eau par *Cryptosporidium* et *Giardia*

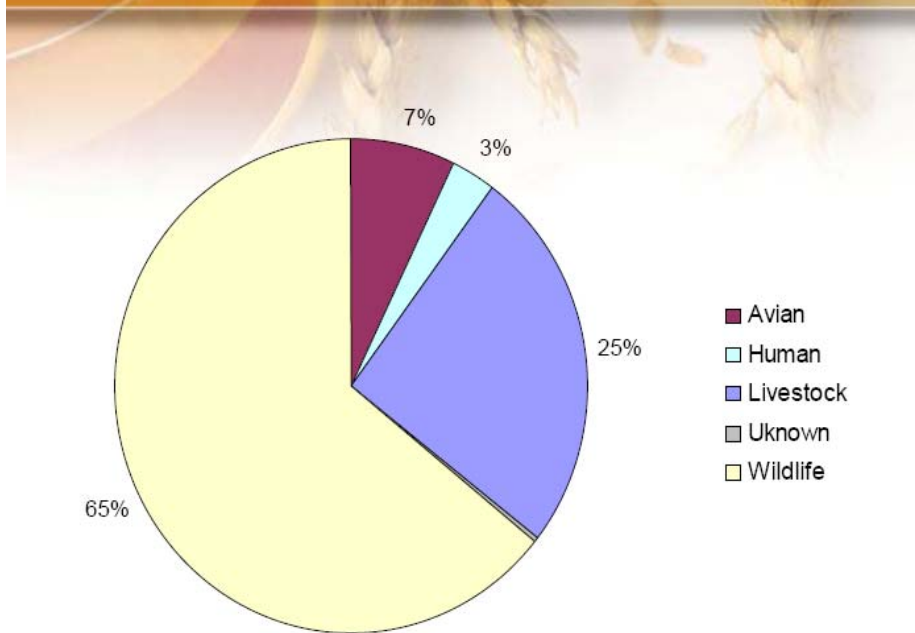
Edward Topp, Norma Ruecker, Graham Wilkes, David R. Lapen, Norman F. Neumann

South Nation River study area (Power Point Presentation)

- 24 discrete sampling sites ~200 km²
- Widely varying stream order Wide range of land use, with varying density of potential fecal sources:
 - a. urban and rural residences
 - b. livestock/dairy production
 - c. pasture
 - d. wildlife habitat
- Sample every 2 weeks unless frozen 2004-2008 [n~ 3500]
- Climate constant across sites
- Widely varying climate and seasonal trends
- *Cryptosporidium* spp. and *Giardia* spp. are ubiquitous enteric pathogens that infect humans, domesticated animals and wildlife [Savioli et al. 2006]
- Their taxonomy, host specificity and epidemiology is complex.
- The majority of the *Cryptosporidium* species detected are
 - not types that are pathogenic for humans
 - are of wildlife origin
- However species distribution varies enormously in watershed
 - due to the distribution of wildlife habitat and farms?

Diversity of <i>Cryptosporidium</i> species/genotypes	Host source	# of occurrences	Prevalence	Pathogenicity
<i>C. andersoni</i>	Livestock	63	34.1%	Non-pathogenic
<i>Cryptosporidium</i> muskrat genotype II	Wildlife	58	31.4%	Non-pathogenic
<i>Cryptosporidium</i> muskrat genotype I	Wildlife	28	15.1%	Non-pathogenic
<i>Cryptosporidium</i> genotype W12	Wildlife	25	13.5%	Non-pathogenic
<i>Cryptosporidium</i> genotype W25	Wildlife	22	11.9%	Non-pathogenic
<i>C. baileyi</i>	Avian	12	6.5%	Non-pathogenic
<i>Cryptosporidium</i> cervine genotype	Wildlife	10	5.4%	Potential zoonotic
<i>C. meleagridis</i>	Avian	6	3.2%	Potential zoonotic
<i>Cryptosporidium</i> vole genotype	Wildlife	5	2.7%	Non-pathogenic
<i>C. hominis</i>	Human	5	2.7%	Pathogenic
<i>Cryptosporidium</i> fox genotype	Wildlife	4	2.2%	Non-pathogenic
<i>Cryptosporidium</i> deer mouse genotype III	Wildlife	4	2.2%	Non-pathogenic
<i>C. parvum</i>	Human	3	1.6%	Pathogenic
<i>Cryptosporidium</i> skunk genotype	Wildlife	2	1.1%	Potential zoonotic
<i>Cryptosporidium</i> genotype W19	Wildlife	2	1.1%	Non-pathogenic
Unknowns	unknown	1	0.5%	Unidentified
<i>Cryptosporidium</i> mouse II genotype	Wildlife	1	0.5%	Non-pathogenic

Percent host sources of *Cryptosporidium* in SNW 2004-2009



Comments:

More than 75% of the *Cryptosporidium* is linked with sources other than livestock in the South Nation River watershed.

Other comments based on follow up discussion with Dr. Ed Topp.

- A non-technical summary is not yet available for the Eastern Ontario study. The available papers 'appear alarmingly technical'. The focus is on the relationship between *E. coli* and pathogens, not *E. coli* source diagnosis.

Overall, general comments

- The current estimate of fecal source using *E. coli* in the Eastern Ontario watersheds is that about 2/3 isolates are of livestock origin [dairy and beef in this area].
- Some pathogens [eg. *Listeria*] are likely [but perhaps not solely] of bovine origin, based primarily on land use analysis
- **Associations between *E. coli* and pathogens are often weak.**
- *Cryptosporidium* is interesting, likely large wildlife input, but of species that are not pathogenic for humans.
- There are a number of source tracking tools that are being developed that don't require a "reference library". The field is evolving rapidly, there are new tools being developed constantly, so the state of the science is still largely in the research realm rather than the commercial off-the-shelf. The idea is you could take a water sample, extract DNA from it, and use some lab tricks [a method called PCR] to detect DNA sequences that are implicit evidence for [for example] cattle, goose, gull, pig contamination. Compared to the library-dependent methods, this approach has the potential to reduce the cost of MST studies, and hopefully eliminate the geographic specificity problems associated with libraries built outside the reference area. But a big caveat, how complicated is the scenario, how likely is one to detect mixed contaminants at the inlet?

Sources of Fecal Pollution in Virginia's Blackwater River

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ABSTRACT

Amy M. Booth,¹ Charles Hagedorn,² Alexandria K. Graves,³ Sarah C. Hagedorn,⁴ and Karen H. Mentz⁵

¹Research Associate, Dept. of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State Univ., 330 Smyth Hall, Blacksburg, VA 24061.

²Professor, Dept. of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State Univ., 330 Smyth Hall, Blacksburg, VA 24061.

³Doctoral Candidate, Dept. of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State Univ., 330 Smyth Hall, Blacksburg, VA 24061.

⁴Marine Sciences Laboratory, Duke Univ., Beaufort, NC 28516.

⁵Masters Candidate, Dept. of Environmental Engineering, Univ. of Florida, Gainesville, FL 32611

Sources of fecal pollution in the Blackwater River in south-central Virginia were identified. The study area encompassed intensive dairy and beef farming, abundant wildlife populations, homes with on-site septic systems, and four stream segments listed as impaired due to high-fecal coliform concentrations. A library of antibiotic resistance profiles was developed for 1,451 *Enterococcus* isolates from human, wildlife, and livestock. A discriminant analysis model was used to classify the isolates by source and calculate rates of correct classification (RCC) for each source. RCCs for the known source library were 82.3% for human, 86.2% for livestock, and 87.4% for wildlife. Profiles were determined for *Enterococcus* isolates from stream samples collected periodically from August 1999 to August 2001 (a total of 8,542 isolates) and compared against the known source library. Livestock contributed the highest percentage of isolates (47.6%) in the four segments studied, followed by wildlife (29.1%), and human (24.9%). The results indicate that reducing fecal pollution will require consideration of all three source categories. The results from this research are being used to develop total maximum daily load project allocations for fecal coliforms in the Blackwater River.

Comments

In this mixed use watershed more than 53% of the E.coli is from sources other than agriculture. This study used a library of antibiotic resistance profiles rather than the DNA-based Rep-PCR method.

Multiple lines of evidence to identify the sources of fecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario

Thomas A. Edge, Stephen Hill
National Water Research Institute, Water Science & Technology Directorate,
Environment Canada, 867 Lakeshore Road, Burlington, Ont.,
Canada L7R 4A6

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ABSTRACT

Multiple microbial source-tracking methods were investigated to determine the source of elevated *Escherichia coli* levels at Bayfront Park Beach in Hamilton Harbour, Lake Ontario.

E. coli concentrations were highest in wet foreshore sand (114,000 CFU/g dry sand) and ankle-depth water (177,000 CFU/100 mL), declining rapidly in deeper waters. Many gull and geese droppings were enumerated each week on the foreshore sand within 2m of the waterline. Both antimicrobial resistance analysis and rep-PCR DNA fingerprinting of *E. coli* collected at the beach and nearby fecal pollution sources indicated that *E. coli* in sand and water samples were predominantly from bird droppings rather than from pet droppings or municipal wastewater. Both methods indicated a trend of decreasing bird contamination, and increasing wastewater contamination, moving offshore from the beach. When foreshore sand was treated as a reservoir and secondary source of *E. coli*, waterborne *E. coli* were found to be more similar to sand isolates than bird or wastewater isolates out to 150m offshore.

Multiple lines of evidence indicated the importance of bird droppings and foreshore sand as primary and secondary sources of *E. coli* contamination in beach water at Bayfront Park.

Comments

This study demonstrates the very high levels of *E. coli* (177,000 CFU/100 ml) that can be associated with gull and geese droppings. It also shows that carefully designed MST studies and closer scrutiny indicates that widely perceived notions of prominent fecal pollution sources may not be as significant as first thought (e.g. gull and geese vs sewage plant effluents in Hamilton Harbour).

Spatial and Annual Variability in Concentrations and Sources of *Escherichia coli* in Multiple Watersheds

CINDYL MEAYS, *, †, ‡ KLAAS BROERSMA, † RICK NORDIN, ‡ ASIT MAZUMDER, ‡ AND MANSOUR SAMADPOUR §

Water and Watershed Research Program Department of Biology University of Victoria, P.O. Box 3020 Stn CSC, Victoria, British Columbia V8N 3N Canada
Agriculture and Agri-Food Canada, 3015 Ord Road, Kamloops, British Columbia V2B 8A9 Canada
Institute for Environmental Health Inc., 15300 Bothell Way N. E., Lake Forest Park, Seattle, Washington 98155

Environ. Sci. Technol. **2006**, 40, 5289-5296

ABSTRACT

Nonpoint source fecal contamination is a concern for drinking water supplies worldwide. In this study, 4812 *E. coli* isolates were classified to source. Results of this experiment show that the fecal coliform (FC) counts varied by year, month, and site, for each of the watersheds sampled. For both years, the lowest FC counts tended to be at the highest elevation sites followed by the drinking water intake sites at the lowest elevation. The highest FC counts tended to be at the mid-elevation sites on BX, Deer, and Duteau Creeks. The sources of *E. coli* varied significantly with stream for 2003 and 2004 ($P < 0.001$, $df = 39$), although the main sources of *E. coli* (avian, deer/elk, canine, rodent, bovine, and bear) tended to be similar between watersheds. The dominant sources of *E. coli* changed from 2003 (avian, deer/elk, and canine) to 2004 (avian, bovine, and rodent). It is important to look at the results of more than 1 year of source tracking data to get a better picture of the dominant sources within a watershed. Overall, wildlife was the largest contributor of *E. coli* to the watersheds in both 2003 (> 84%) and 2004 (>73%).

Comments

This study collected data from 4 watershed in the Vernon, BC area. Wildlife contributed 73% to 84% of the total *E. coli* to the watershed. Background *E. coli* levels ranged from 18 to 2922 FC/100 ml. Bovine source *E. coli* varied according to sampling year and watershed, and ranged from 1.3% - 20.0% of the total *E. coli* load. Avian sources ranges from 10.1 - 40.7% of the total *E. coli* load.

The authors concluded that managing wildlife is a necessary step in reducing fecal bacteria and pathogen loading in drinking water watersheds.

This study demonstrates that fecal pollution arises from multiple sources in watersheds. By positively identifying the sources of fecal pollution, land managers and stakeholders can develop watershed management plans to reduce fecal pollution by livestock, humans, and wildlife. Managing wildlife is more difficult than livestock; however, it is a necessary step in reducing fecal bacteria and pathogen loading in drinking water watersheds.

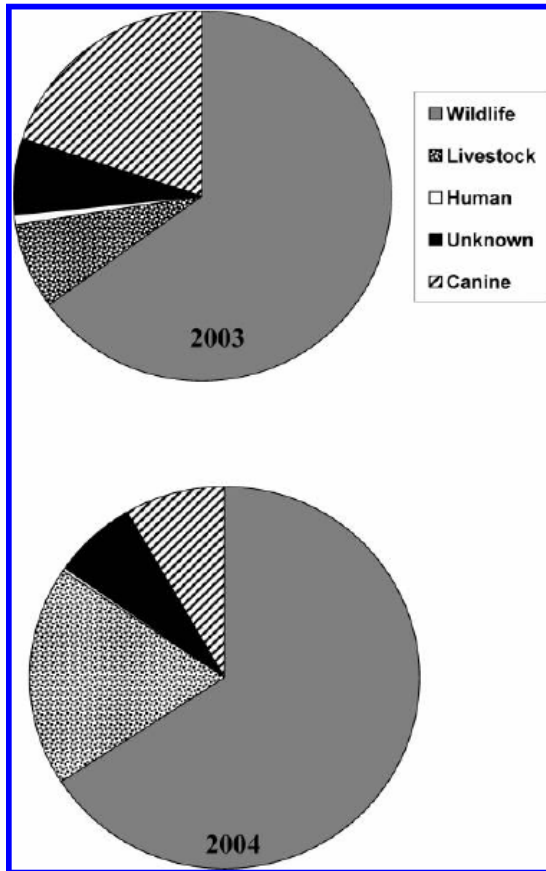


FIGURE 3. Pie charts of main sources of *E. coli* for 2003 and 2004. Note: Canine was separated from wildlife because although a large portion would be from coyote and some wolf, it could also include domestic dog.

TABLE 12. Total^a and Percentage (in parentheses) of *E. coli* for Each Source by Stream for 2003 and 2004

	BX	Deer	Duteau	S.Fortune	sum
2003					
avian	220 (26.5)	81 (10.1)	201 (21.4)	107 (40.7)	609 (21.5)
bear	65 (7.8)	136 (17.0)	56 (6.0)	15 (5.7)	272 (9.6)
bovine	11 (1.3)	72 (9.0)	87 (9.2)	13 (4.9)	183 (6.5)
canine	202 (24.3)	134 (16.7)	191 (20.3)	37 (14.1)	564 (19.9)
deer/elk	159 (19.1)	169 (21.1)	266 (28.3)	33 (12.5)	627 (22.1)
feline	31 (3.7)	5 (<1)	16 (1.7)	3 (1.1)	55 (1.9)
horse	26 (3.1)	2 (<1)	2 (<1)	0 (0)	30 (1.1)
human	4 (<1)	14 (1.7)	8 (<1)	0 (0)	26 (<1)
moose	0 (0)	0 (0)	0 (0)	1 (<1)	1 (<1)
rabbit	2 (<1)	2 (<1)	0 (0)	2 (<1)	6 (<1)
raccoon	21 (2.5)	11 (1.4)	29 (3.1)	6 (2.3)	67 (2.4)
rodent ^b	65 (7.8)	47 (5.9)	55 (5.8)	35 (13.3)	202 (7.1)
squirrel	1 (<1)	0 (0)	0 (0)	1 (<1)	2 (<1)
unknown	24 (2.9)	129 (16.1)	30 (3.2)	10 (3.8)	193 (6.8)
sum	831 (100)	802 (100)	941 (100)	263 (100)	2837 (100)
2004					
avian	131 (24.3)	145 (28.1)	161 (24.7)	85 (32.0)	522 (26.4)
bear	28 (5.2)	22 (4.3)	57 (8.7)	21 (7.9)	128 (6.5)
bovine	103 (19.1)	103 (20.0)	130 (19.9)	25 (9.4)	361 (18.3)
canine	33 (6.1)	58 (11.2)	55 (8.4)	18 (6.8)	164 (8.3)
deer/elk	37 (6.9)	33 (6.4)	61 (9.3)	30 (11.3)	161 (8.2)
feline	0 (0)	0 (0)	3 (<1)	0 (0)	3 (<1)
horse	6 (1.1)	0 (0)	0 (0)	0 (0)	6 (<1)
human	1 (<1)	2 (<1)	2 (<1)	0 (0)	5 (<1)
moose	10 (1.9)	22 (4.3)	5 (<1)	4 (1.5)	41 (2.1)
rabbit	1 (<1)	1 (<1)	1 (<1)	0 (0)	3 (<1)
raccoon	29 (5.4)	36 (7.0)	22 (3.4)	10 (3.8)	97 (4.9)
rodent ^b	97 (18.0)	59 (11.4)	118 (18.1)	60 (22.6)	334 (16.9)
squirrel	2 (<1)	5 (1.0)	5 (<1)	0 (0)	12 (<1)
unknown	62 (11.5)	30 (5.8)	33 (5.1)	13 (4.9)	138 (7.0)
sum	540 (100)	516 (100)	653 (100)	266 (100)	1975 (100)

^a Total is the total number of *E. coli* isolates ribotyped of each source.

^b Rodent includes beavers and all other rodents.