FINAL REPORT

AUG 0 8 2001

1999 Project Abstract
For the Period Ending June 30, 2001

TITLE:	W13 Tracking Sources of Fecal Pollution Using DNA Techniques
PROJECT MANAGER:	DR. MICHAEL J. SADOWSKY
ORGANIZATION:	University of Minnesota
ADDRESS:	Department of Soil, Water, and Climate; 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN 55108
WEB SITE ADDRESS:	http://www.ecolirep.umn.edu
LEGAL CITATION:	ML 1999, Chap. 231, Sec. 16, Subd. 6(d).

APPROPRIATION AMOUNT: $300,000

Overall Project Outcomes and Results: The rep-PCR DNA fingerprint technique, using repetitive DNA sequences, was investigated as a means to differentiate human from animal sources of fecal pollution. BOX PCR primers were used to generate 2466 DNA fingerprints from *Escherichia coli* strains from human and animal sources in Minnesota (humans, dogs, cats, horses, deer, geese, ducks, chickens, turkeys, cows, pigs, goats, and sheep). This constituted a known source DNA fingerprint library. Fingerprints were analyzed using curve-matching algorithms. Jackknife analyses indicated that 70.2 – 96.2% of animal and human isolates were assigned into the correct source groups. However, when only unique isolates were examined (isolates from a single animal having distinct DNA fingerprints), Jackknife analyses indicated that 52.8 – 78.5% of the isolates were assigned to the correct source group. BOX DNA fingerprints were generated from 300-400 *E. coli* isolated from each of four Minnesota watershed areas (Mississippi River, Prairie Creek, Rush River, and Grindstone River) and compared to those in the known source fingerprint library. Based on similarity cut-off values of 80% or greater, about 83% of the environmental isolates could be assigned to a source group. In general, the rural sites were dominated by *E. coli* bacteria originating from livestock species, while the urban site was impacted by a mixture of domestic animals and wildlife. Database size was found to be important in accurately determining sources of fecal pollutants. Taken together, our results indicate that rep-PCR using the BOX A1R primer may be a useful and effective tool to rapidly determine sources of fecal pollution.

Project Results Use and Dissemination: Results from this project can be found at http://www.ecolirep.umn.edu. The DNA fingerprint library is currently being used to determine potential sources of fecal pollution in three watershed areas in Minnesota (part of our 2001 LCMR appropriation). Results from this current project have been presented at many local and national conferences.

Date of Report: July 1, 2001
LCMR Final Work Program Report

Date of Work Program Approval: June 10, 1999

Project Completion Date: June 30, 2001

Modified LCMR Work Program 1999

I. PROJECT TITLE: W13 Tracking Sources of Fecal Pollution Using DNA Techniques

Project Manager:	Dr. Michael J. Sadowsky
Affiliation:	University of Minnesota
Mailing Address:	Department of Soil, Water, and Climate; 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN 55108
Telephone Number:	(612) 624-2706 **Email:** Sadowsky@soils.umn.edu **FAX:** (612) 625-6725
Web Page Address:	http://www.ecolirep.umn.edu

Total Biennial Project Budget:

$ LCMR	$300,000	$ Match	0
- $ LCMR Amount Spent	$297,099	- $ Match Amount Spent	0
= $ LCMR Balance	$ 2,901	= $ Match Balance	0

A. Legal Citation: ML 1999, Chap. 231, Sec. 16, Subd. 6(d).

Tracking Sources of Fecal Pollution Using DNA Techniques
$150,000 the first year and $150,000 the second year are from the trust fund to the University of Minnesota to define sources of fecal pollution in waters.

B. Status of Match Requirement: Not Applicable

II. and III. FINAL PROJECT SUMMARY:

The rep-PCR DNA fingerprint technique, using repetitive DNA sequences, was investigated as a means to differentiate human from animal sources of fecal pollution. BOX PCR primers were used to generate 2466 DNA fingerprints from *Escherichia coli* strains from human and animal sources in Minnesota (humans, dogs, cats, horses, deer, geese, ducks, chickens, turkeys, cows, pigs, goats, and sheep). This constituted a known source DNA fingerprint library. Fingerprints were analyzed using curve-matching algorithms. Jackknife analyses indicated that 70.2 – 96.2% of animal and human isolates were assigned into the correct source groups. However, when only unique isolates were examined (isolates from a single animal having distinct DNA fingerprints), Jackknife analyses indicated that 52.8 – 78.5% of the isolates were assigned to the correct source group. BOX DNA fingerprints were generated from 300-400 *E. coli* isolated from each of four Minnesota watershed areas (Mississippi River, Prairie Creek, Rush River, and

Grindstone River) and compared to those in the known source fingerprint library. Based on similarity cut-off values of 80% or greater, about 83% of the environmental isolates could be assigned to a source group. In general, the rural sites were dominated by *E. coli* bacteria originating from livestock species, while the urban site was impacted by a mixture of domestic animals and wildlife. Database size was found to be important in accurately determining sources of fecal pollutants. Taken together, our results indicate that rep-PCR using the BOX A1R primer may be a useful and effective tool to rapidly determine sources of fecal pollution.

Results from this project can be found at http://www.ecolirep.umn.edu. The DNA fingerprint library is currently being used to determine potential sources of fecal pollution in three watershed areas in Minnesota (part of our 2001 LCMR appropriation). Results from this current project have been presented at many local and national conferences.

IV. OUTLINE OF PROJECT RESULTS:

Result 1 – Acquisition of fecal coliform bacteria from known sources

The initial project work plan called for isolation of approximately 1600 *E. coli* bacteria from several known sources including cattle, swine, chickens, turkeys, waterfowl (ducks and geese), domestic pets (cats and dogs), humans. The goal was to obtain approximately 200 fecal coliform isolates, from at least 10 different locations, from each animal or human source. We proposed to isolate four *E. coli* from each of 50 individuals for each source. The number of *E. coli* bacteria isolated and characterized was subsequently increased (see below). Goals were set based on the perceived importance of the contribution of the animal group to environmental fecal pollution. Therefore, more isolates were obtained for cows, pigs, and humans than for goats and cats. The identity of fecal coliform bacteria was tested by using selective and differential laboratory media and biochemical tests. Only *E. coli* isolates were used for our studies. To reduce possible feed bias effects on coliform population structure, we used individuals from locations throughout the state. The confirmed *E. coli* isolates were cataloged, preserved in glycerol and stored at -70°C until used for DNA fingerprinting.

This portion of the project has required cooperation with many state and local government staff, University of Minnesota staff and faculty, private citizens, in addition to the project cooperators. These have included:

- Minnesota State Fair staff and participants
- Minnesota DNR staff at Roseau River, Lac Qui Parle, and Carlos Avery Wildlife Management Areas
- Hennepin and Washington County Parks staff and deer hunters
- Private citizen trappers
- Staff of Ramsey County Humane Society and Humane Society of Pierce-St. Croix, Inc.
- VA Hospital staff
- U of M Agricultural Experiment Station staff and faculty
- U of M Microbiology students
- U of M staff, friends and family members

Table 1. Human and animal sources of *E. coli*

Animal Source	Animals Sampled	*E. coli* Isolates Obtained	Goal	Percent Complete
Cat	38	111	100	111
Chicken	87	239	200	120
Cow	125	345	300	115
Deer	65	189	200	94
Dog	74	218	200	109
Duck	42	125	100	125
Goat	40	120	100	120
Goose	73	206	200	103
Horse	49	144	100	144
Human	197	313	300	104
Pig	112	335	300	112
Sheep	38	118	100	118
Turkey	70	209	200	104
Total	1010	2672	2400	111

Result 1of the project was essentially conducted as originally proposed. However, a few LCMR-approved changes were made. The changes were to: increase the total number of isolates acquired from known animal and human sources from 1600 to approximately 2400; sample a wider variety of animal types (deer, goats, sheep, and horses were added); and decrease the number of *E. coli* isolated from each individual animal from four to three. This latter change was made after initial DNA fingerprinting results showed that, in many cases, two or more isolates obtained from the same animal yielded identical DNA fingerprints. We felt that by decreasing the number of *E. coli* isolated from each individual and by increasing the number of individual animals sampled, we would increase the representation of *E. coli* population in our database. We also believed that these changes to the project created a more comprehensive collection of *E. coli* isolates from which to obtain DNA fingerprints and increased the overall robustness of our database.

Out of the 2672 *E. coli* strains obtained, 219 isolates gave at least one atypical result when examined in our routine biochemical screening tests. The biochemical characteristics of these isolates were examined further by using the API 20E system. Results of this analysis indicated the majority of these isolates (167) were bona fide *E. coli,* while the remainder (52) could not be confirmed as this bacterium. The latter group was not used in rep-PCR analysis or included in the DNA fingerprint database.

We recently entered into an agreement with Bacterial Bar Codes, Inc. (Houston, Texas) to sell them 1010 *E. coli* bacteria that were isolated during Result 1 of this 1999 W13 LCMR-sponsored project. The sale generated $10,100 in program income. These funds have been deposited in an auditable account managed by the Sponsored Projects Administration at the University of Minnesota. As per our discussions with LCMR staff, these funds will be used to offset increases in fringe benefit rates for project personnel and to conduct additional biochemical testing of atypical *E. coli* bacteria in our 2001LCMR-funded project.

Budget:

LCMR Budget:	$101,800	Match:	$0
Balance:	$ 304	Match Balance:	$0

Result 2 - Generate DNA fingerprints from known isolates using PCR techniques and gel electrophoresis.

In our initial studies we examined approximately 160 *E. coli* isolates from numerous individuals from seven different animal species. DNA fingerprints for 125 of the *E. coli* isolates were generated using both BOX and REP primers. The resulting DNA fingerprints were captured into TIFF files, normalized to molecular weight and internal standards, and compared and analyzed using BioNumerics software. The relatedness of isolates to each was determined by a number of statistical methods including: cluster analysis; principal components analysis; and discriminant analysis. BOX DNA fingerprints from 154 *E. coli* isolates were analyzed using the Jaccard band-matching algorithm. Jackknife analysis of the resultant similarity coefficients indicated that 100% of the chicken and cow isolates, and between 78-90% of the human, goose, duck, pig, and sheep isolates were assigned into the correct source group. A dendrogram constructed using Jaccard similarity coefficients almost completely separated human from non-human isolates. Multivariate Analysis of Variance (MANOVA), a form of discriminant analysis, successfully differentiated the isolates and separated them into their respective source groups. Taken together, our results using a small database indicated that rep-PCR using the BOX A1R primer is useful and effective tool to rapidly determine sources of fecal pollution.

In our original submission we proposed to DNA fingerprint each of the *E. coli* isolates using two different rep-PCR primers, REP I and BOX A1R. We hypothesized that the combined BOX+REP fingerprints generated from both primers would provide a more robust and discriminatory DNA fingerprint database than a database of DNA fingerprints generated by either of the two primers alone. Statistical analysis showed that DNA fingerprints generated by using the REP primers were not as effective as the BOX-derived fingerprints for correctly classifying many animal isolates. Furthermore, there was no improvement in the in the grouping of strains when BOX-plus-REP DNA fingerprint data were used compared to BOX-derived fingerprints alone.

From this we have concluded that the use of the REP primer in addition to the BOX primer does not provide additional useful information, and that channeling resources away from use of REP primers into other activities would contribute more to the success of the project. Given these results, we received permission from the LCMR to revise our workplan to discontinue the use of REP DNA fingerprinting, and to isolate more *E. coli* from a wider variety of animal sources.

Results from these studies were published in Applied and Environmental Microbiology. The citation is: Dombek, P.E., L.K. Johnson, S.T. Zimmerley, and M.J. Sadowsky. 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. **66:**2572-2577.

The final DNA fingerprint database now contains 2466 entries from known human and animal sources (Table 2). The library database has been stored on floppy and hard disks and backed-up on Zip disks for safety purposes. A rep-PCR web site has been constructed as can be found at www.ecolirep.umn.edu. The web site contains background information, detailed descriptions of our methods, links to related water quality and microbiology sites, links to pertinent references, a glossary of terms, and links to news items describing outbreaks of water-borne diseases caused by fecal bacteria. The web site is housed and

maintained at the University of Minnesota Department of Soil, Water, and Climate. While we originally proposed to make the fingerprint library available to the scientific community as TIFF file downloads, and as a bundled database for use by individuals having BioNumerics software, we received notice from Bacterial Barcodes, Inc. (Houston, TX) that we would be infringing on their patent if we posted the actual DNA fingerprints on our web site. However we were informed by their counsel that we were free to use the database for our own research, share the database with individuals doing collaborative research with us, publish results from our research , disseminate our results to others at meetings and in publications, and continue research using their patented technology.

Table 2. Human and animal *E. coli* DNA fingerprints in database

Animal Source	Fingerprints in Database	Goal	Percent Complete
Cat	108	100	108
Chicken	231	200	116
Cow	299	300	100
Deer	180	200	90
Dog	197	200	98
Duck	122	100	122
Goat	104	100	104
Goose	200	200	100
Horse	114	100	114
Human	307	300	102
Pig	302	300	101
Sheep	100	100	100
Turkey	202	200	101
Total	2466	2400	103

The resulting 2466 DNA fingerprints have been scanned into digital images, converted to TIFF files, normalized to molecular weight and internal standards, and compared and analyzed using BioNumerics software. The relatedness of isolates to each other was determined by cluster and principal component statistical analyses. Dr. Linda Kinkel, Dept. of Plant Pathology, University of Minnesota, who is an expert in statistical and epidemiological analyses, has provided her expertise to us in analyzing our results. As discussed above, the resulting DNA fingerprints now comprise the DNA fingerprint library database. The complete database of *E. coli* DNA fingerprints have been used to analyze *E. coli* obtained from environmental sources (see Result 3). The fingerprints have been analyzed in detail using both band- and curve-matching algorithms. Jackknife analyses indicated that 70.2 – 96.2 % of animal and human isolates were assigned into the correct source groups (Table 3). However, when only unique isolates, 1616, were examined (those isolates from a single animal having distinct DNA fingerprints), Jackknife analyses indicated that 52.8 – 78.5 % of the isolates were assigned to the correct source group (Table 4). This indicates that: 1) inclusion of more than one identical isolate from the same individual animal skews or biases accurate determination of potential source groups, and 2) the current library size does not capture all the genetic diversity present in *E. coli*.

Table 3. Jackknife analysis of all 2466 *E. coli* in DNA fingerprint library

Assigned to:	*E. coli* isolated from:											
	Deer	Chicken	Pig	Human	W/fowl	Cow	Turkey	Dog	Sheep	Horse	Cat	Goat
Deer	**87.7**	1.7	1.3	1.0	3.4	1.7	1.5	0.5	2.0	0.0	0.9	1.0
Chicken	1.1	**81.8**	4.0	2.9	2.2	4.4	1.0	1.0	2.0	3.5	0.9	0.0
Pig	1.7	3.0	**79.2**	3.6	3.7	4.0	1.5	0.0	3.0	8.8	0.0	1.0
Human	1.1	0.4	0.7	**77.9**	2.8	3.3	1.5	0.0	2.0	1.8	0.9	0.0
W/fowl	1.7	1.7	4.0	2.9	**79.8**	2.3	1.0	0.5	2.0	3.5	0.0	0.0
Cow	1.1	1.7	3.0	1.0	3.4	**81.3**	1.0	2.6	4.0	3.5	0.0	0.0
Turkey	2.8	3.5	2.3	2.6	0.3	0.0	**89.6**	0.5	0.0	2.6	0.0	0.0
Dog	1.7	0.9	0.7	2.0	0.3	1.0	1.0	**90.3**	0.0	0.9	1.9	0.0
Sheep	0.6	1.7	2.6	2.6	1.9	0.7	2.0	0.5	**80.2**	4.4	0.9	0.0
Horse	0.0	2.2	1.7	1.0	1.2	0.7	0.0	1.0	3.0	**70.2**	0.0	1.9
Cat	0.6	1.3	0.7	2.6	0.9	0.7	0.0	1.5	1.0	0.9	**94.4**	0.0
Goat	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.0	0.0	0.0	**96.2**

Done using Pearson's correlation, maximum similarity, and 1% optimization

Table 4. Jackknife analysis of 1616 unique *E. coli* in DNA fingerprint library

Assigned to:	*E. coli* isolated from:											
	Goat	Sheep	Horse	W/fowl	Deer	Pig	Chicken	Cow	Turkey	Cat	Human	Dog
Goat	**72.9**	1.5	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Sheep	2.1	**58.2**	7.0	3.2	1.0	4.0	3.2	3.0	3.1	0.0	3.5	3.0
Horse	6.3	3.0	**55.8**	2.8	0.0	3.1	4.4	2.0	0.0	1.9	2.2	5.0
W/fowl	0.0	6.0	4.7	**59.7**	5.0	6.1	2.5	4.4	2.3	7.6	4.9	2.0
Deer	2.1	1.5	0.0	4.2	**55.0**	1.8	3.2	1.5	3.1	1.9	1.3	3.0
Pig	2.1	6.0	12.8	6.9	3.0	**66.7**	6.3	5.4	3.1	3.8	6.2	5.0
Chicken	4.2	4.5	5.8	5.1	6.0	5.7	**63.3**	12.8	3.1	5.7	4.9	2.0
Cow	8.3	10.5	5.8	7.4	10.0	4.4	7.0	**63.1**	2.3	7.6	2.7	6.9
Turkey	0.0	3.0	3.5	2.8	11.0	4.4	5.7	1.5	**78.5**	1.9	4.4	3.0
Cat	0.0	1.5	1.2	1.4	1.0	0.4	1.9	0.5	0.0	**52.8**	3.1	4.0
Human	0.0	3.0	2.3	6.5	3.0	2.2	1.9	3.9	3.1	7.6	**63.3**	4.0
Dog	2.1	1.5	1.2	0.0	4.0	1.3	0.6	2.0	1.5	9.4	3.5	**60.4**

Done using Pearson's correlation, maximum similarity, and 1% optimization

However, the DNA library database in its current size is capable of clearly differentiating *E. coli* originating from animal or human sources (Table 5), 96% and 64% of non-human and human isolates, respectively, were assigned to the correct source group. The difference in these two numbers reflects the fact that the library consists of many more animal than human isolates, and captures more of the animal diversity that is present.

Table 5. Jackknife analysis of human animal DNA fingerprints.

Assigned to:	*E. coli* isolated from:	
	Animal	Human
Animal	**96**	36
Human	4	**64**

Done using Pearson's correlation, maximum similarity, and 1% optimization

Moreover, when a limited subset of the fingerprint database consisting of humans, cows, pigs, and

turkeys, was examined by Jackknife analysis, 77 – 79% of *E. coli* isolates were assigned to the correct source group (Table 6). Accordingly, these results indicate that a targeted subset of the DNA fingerprint database should be used to more precisely determine sources of fecal pollutants in watersheds. For example, in watershed where impacts are expected to be due to farm animals and waterfowl, a subset of the DNA fingerprint database library consisting of these animals should be initially used.

Table 6. Jackknife analysis of human, pig, cow and turkey DNA fingerprints.

	E. coli isolated from:			
Assigned to:	Human	Pig	Cow	Turkey
Human	**77**	8	8	8
Pig	10	**79**	11	9
Cow	7	9	**77**	5
Turkey	6	4	4	**79**

Done using Pearson's correlation, maximum similarity, and 1% optimization

Budget:

LCMR Budget:	$105,051	Match:	$0
Balance:	$ 385	Match Balance:	$0

Result 3 - Isolation of fecal coliform bacteria from watershed areas, rep-PCR DNA fingerprint analysis, and comparison of fingerprints to established database.

We have isolated coliform (*E. coli*) bacteria from four watershed areas with a history of elevated fecal coliform counts. These areas included: 1) Rush River (Sibley County), 2) Grindstone River (Pine County), 3) Mississippi River (Hennepin County) and 4) Prairie Creek (Rice County).
This result has required cooperation with local government staff to acquire water samples and/or fecal coliform isolates. These cooperators have included:

- Metropolitan Council Environmental Services staff
- Pine County Soil and Water Conservation District staff
- Sibley County Soil and Water Conservation District staff
- Carleton College faculty and students

All environmental water samples were analyzed for fecal coliform by MCES staff. The microbiological counting plates generated by these analyses were obtained by our staff to isolate and identify *E. coli.* Coliform bacteria were authenticated as described in Result 1. Of the 1483 environmental *E. coli* strains obtained, 76 isolates gave at least one atypical result when examined in our routine biochemical screening tests, and were examined further by using the API 20E system. Results of this analysis indicated the majority of these isolates (66) were bona fide *E. coli,* while the remainder (10) could not be confirmed as this organism. The latter group was not used in rep-PCR analysis or for inclusion in the DNA fingerprint database. A summary of isolates obtained is included in the Table 7 below.

Table 7. Environmental sources of *E. coli* obtained

Stream	Sites Sampled	Times Sampled	Isolates Obtained	Goal	Percent complete
Mississippi River	1	19	357	300	119
Grindstone River	6	3	358	300	119
Prairie Creek	varied	varied	439	300	146
Rush River	6	3	329	300	110
Total			1483	1200	124

DNA from the confirmed environmental coliform isolates were subjected to rep-PCR DNA fingerprinting using BOX A1R primers (Table 8) as is described in Result 2 above. The resulting DNA fingerprints were compared to those in the constructed DNA fingerprint database created in Result 2.

Table 8. Environmental *E. coli* in DNA fingerprint database

Stream	Fingerprints in database	Goal	Percent Complete
Mississippi River	338	300	113
Grindstone River	348	300	116
Prairie Creek	428	300	143
Rush River	322	300	107
Total	1436	1200	120

Source group identification was achieved by comparison of DNA fingerprints from the river isolates to the 2466 *E. coli* DNA fingerprints in our database. For our analyses, an environmental organism was assigned to an animal source group if it had >80% match to a DNA fingerprint pattern in the database library based on Pearson maximum similarity (Table 9).

Table 9. Environmental *E. coli* assigned to animal source groups.

Stream	Fingerprints in Database	>80% Similarity	Percent assigned
Mississippi River	338	256	76
Grindstone River	348	297	85
Prairie Creek	428	364	85
Rush River	322	276	86

Results of these studies indicated that a majority of the environmental *E. coli* isolates (mean of 83%) were found to match those in our DNA fingerprint library, at a >80% similarity cutoff. About 25% of the Prairie Creek isolates were determined to originate from cows, while chickens and pigs each contributed about 15% and 12%, respectively, of the isolates (Figure 1). In this watershed, wildlife and domestic pets were minor contributors to the total *E. coli* load. At the Mississippi River site, waterfowl, dogs, and cows each contributed 12 - 16% of the total *E. coli* load, while isolates from the other animals contributed

lesser amounts (Figure 2). Results in Figure 3 show that the *E. coli* from the Grindstone River sites were mainly dominated by cows (about 25%), with human, sheep, and waterfowl each contributing about 10 - 12% of the total *E. coli*. Lastly, the Rush River sites (Figure 4) were dominated by *E. coli* bacteria originating from waterfowl (about 30%), although chickens and cows contributed about 15% of the isolates. Taken together, these results show that, in general, the rural sites were more heavily dominated by *E. coli* from livestock species, especially cows, while river water from the urban site contained *E. coli* bacteria from more diverse sources. Moreover, our studies demonstrate that rep-PCR DNA fingerprint technique is a promising method to determine sources of fecal pollution in waterways.

However, when the environmental *E. coli* isolates are compared to those in our DNA fingerprint library in a more stringent manner (>90% similarity cutoff), a much smaller number of the isolates (mean of 28%) could be grouped with those in the library. This indicates that: 1) the diversity of environmental *E. coli* is much greater than originally anticipated; 2) the database library needs to be expanded to include more isolates from each of the animal sources; and 3) that additional potential animal sources need to be examined. We are currently expanding the database library to include more beaver, deer, waterfowl, and human *E. coli* isolates (with funding from Sea Grant and U.S. EPA) and this should aid us in further increasing the accuracy of the database and its ability to discriminate among sources of *E. coli* in water. Moreover, we are currently examining alternate methods for detection of rep-PCR fragments, and to standardize fingerprint patterns to reduce gel-gel variability and increase the resolving power of rep-PCR. One method that holds promise is: FERP (Flurophore Enhanced Rep-PCR), where DNA fragments are separated on a DNA sequencing gel and detected with a laser. Nevertheless, results from the current study indicate that the large scale *E. coli* database is a useful tool in distinguishing between human and animal source of fecal pollution. As such, this tool will be very useful for water quality managers and regulators in remediating waterways contaminated with fecal bacteria. We are currently consulting with a statistician to help us better differentiate among animal sources and to determine the optimum number of *E. coli* isolates in the known source database that are needed to capture the greatest amount of genetic diversity.

Results from the above studies will be submitted for publication in *Applied and Environmental Microbiology* (a peer-reviewed scientific journal) in the Fall.

Budget:

LCMR Budget:	$93,149	Match:	$0
Balance:	$ 2,212	Match Balance:	$0

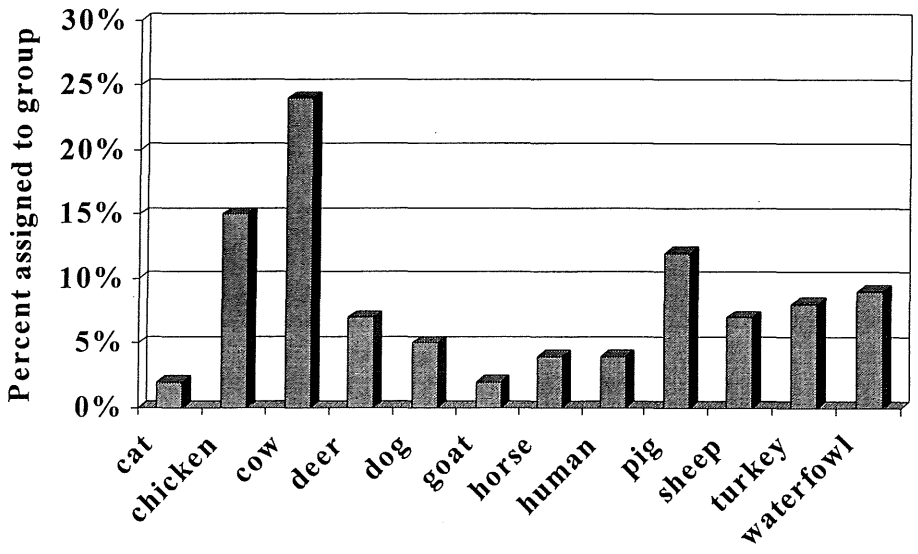

Figure 1. Prairie Creek isolates assigned to source group. 364 of 428 isolates (85%) yielded Pearson Maximum Similarity > 80%

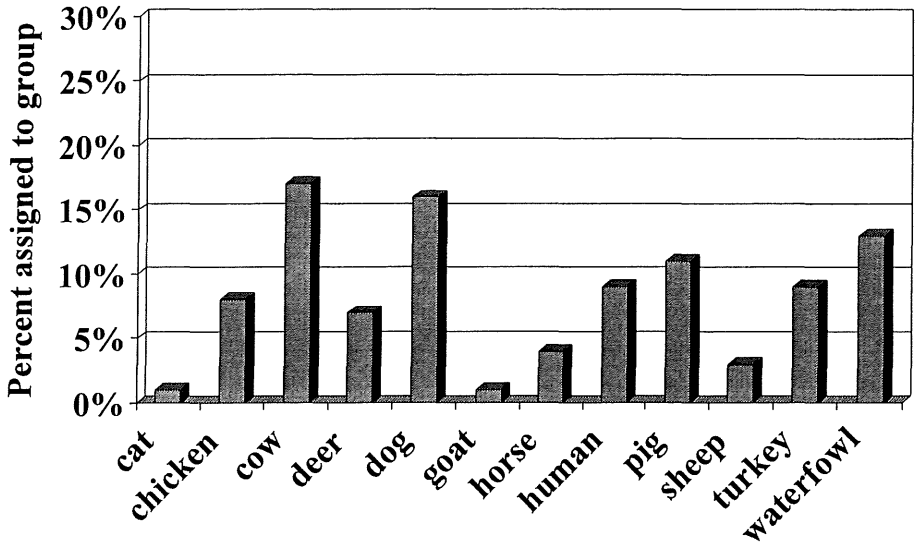

Figure 2. Mississippi River isolates assigned to source group. 256 of 338 isolates (76%) yielded Pearson Maximum Similarity > 80%

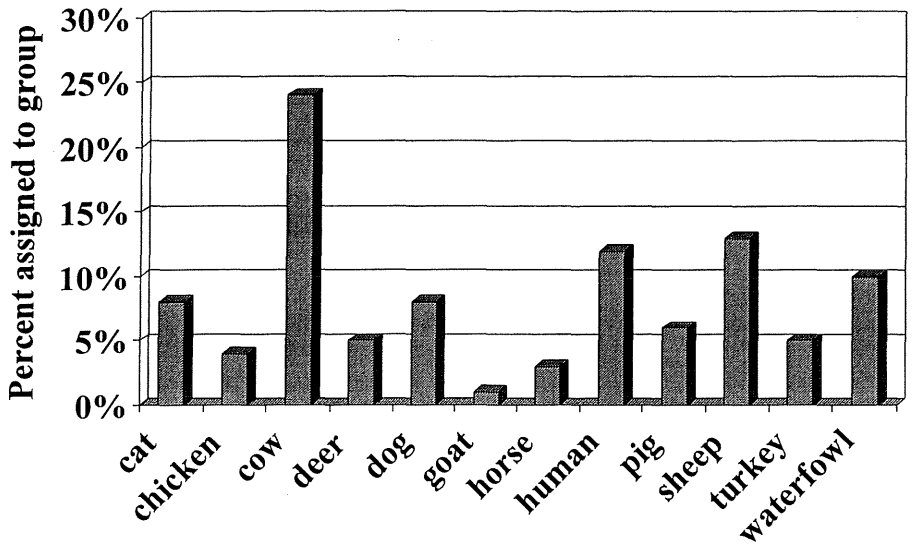

Figure 3. Grindstone River isolates assigned to source group. 297 of 348 isolates (85%) yielded Pearson Maximum Similarity > 80%

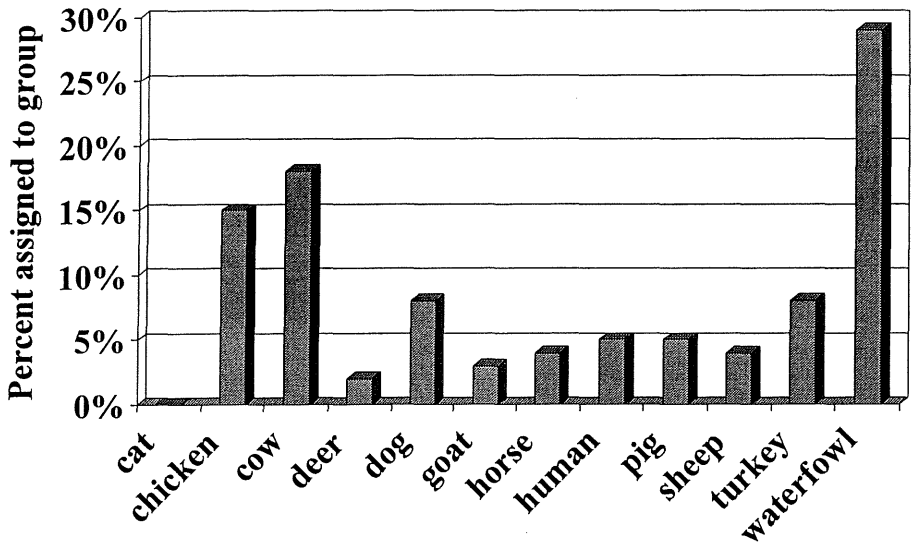

Figure 4. Rush River isolates assigned to source group. 276 of 322 isolates (86%) yielded Pearson Maximum Similarity > 80%

V. DISSEMINATION ACTIVITIES

There has been a great deal of interest in our project by State and county staff working in the areas of pollution control and water resources management. These individuals view the development of a method to track sources of fecal pollution problems as an important decision-making tool for prioritizing pollution abatement efforts. An *E. coli* DNA fingerprint Website has been created to describe our work and can be viewed at: http://www.ecolirep.umn.edu. Results from this project have been also disseminated in reports made to the LCMR on the indicated dates, in periodic updates made to cooperators at MCES and MPCA, in a scientific publication in peer-reviewed journal, and at local, state, and national conferences.

The following additional dissemination activities have been completed during the course of this project:

- Publication of a peer-reviewed journal article describing our initial success in discriminating between *E. coli* DNA fingerprints. A copy of the article is included with this report.
  Dombek, P.E., L.K. Johnson, S.T. Zimmerley, and M.J. Sadowsky. 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. **66:** 2572-2577.
- Presentation of data to the Minnesota River Basin Joint Powers Board Technical Advisory Committee meeting, January 2000
- Presentation of data at the Cannon River Watershed Partnership Summit 2000 conference, February 2000
- Poster presentation at Minnesota Water 2000 Conference, sponsored by University of Minnesota Water Resources Center, April 2000
- Presentation to MPCA Central Office staff, April 2000
- Presentation to MPCA Rochester Office staff (also attended by LCMR staff), May 2000
- Presentation at the Minnesota River Basin Joint Powers Board river trip, July 2000
- Presentation at Texas A&M University, Dept. of Poultry Science, September 2000
- Presentation at the ASA-CSSA-SSSA Annual Meeting, November 2000
- Poster presentation at EPA Great Lakes Beach Conference, February 2001
- Presentation at the MPCA County Feedlot Officer Conference, March 2001
- Presentation to PICKM (Pine, Isanti, Chisago, Kanabec & Mille Lacs counties) citizen volunteer water monitoring group, March 2001
- Presentation to Straight River TMDL Steering Committee, April 2001
- Poster presentation at the American Society for Microbiology Annual Meeting in May, 2001
- Symposium presentation at the American Society for Microbiology Annual Meeting in May, 2001
- Presentation at American Farm Bureau Federation Watershed Heroes Conference, June 2001

VI. CONTEXT

High levels of fecal bacteria in Minnesota's rivers, lakes and streams threaten the use of these water resources for swimming and other forms of water recreation. No one questions the importance of Minnesota's water resources, both for the contribution they make to the quality of life for Minnesota residents, and for sustaining the State's important tourism industry. A better understanding of the source of fecal contamination will be a valuable tool in efforts to minimize the deleterious environmental consequences of fecal pollution. The human health risks of ingesting water contaminated with human fecal materials is well documented. Also, there is increasing concern about possible pathogens associated with fecal material from animal sources (e.g. the *Cryptosporidium* outbreak in Milwaukee).

Many of Minnesota's rivers and streams do not achieve the Clean Water Act "swimmable" goal due to elevated numbers of fecal coliform bacteria. Sources of fecal coliform bacteria include runoff from feedlots and manure-amended agricultural land, wildlife, inadequate septic systems, urban runoff, and sewage discharges. The State's water quality standard for fecal coliform bacteria is 200 microorganisms per 100 milliliters of water (as a monthly average). This number is used as an indicator of the possible presence of human pathogenic microorganisms. According to the last (1996) report to Congress on the condition of Minnesota's rivers, lakes and streams (as reported by the Minnesota Pollution Control Agency), 47% of the river miles assessed could not support swimming due to high levels of fecal bacteria.

The ability to distinguish between human and animal sources of fecal contamination is an important assessment tool. From a public health perspective, fecal contamination originating from human sources poses a greater human health risk than that originating from animal sources. Armed with knowledge about contamination sources, agencies could respond more quickly and more directly to inform that segment of the population at the greatest risk, without unnecessarily alarming people at low or insignificant risk. From a water quality perspective the ability to narrow the source of fecal contamination among the many potential sources could facilitate a more tailored and cost effective pollution abatement effort.

Conventional analytical methods cannot differentiate between sources of fecal pollution giving rise to elevated fecal coliform counts. While various methods have been proposed to determine the source of water-borne fecal contamination, many problems with these procedures are yet to be satisfactorily resolved. Recent developments in molecular biology are expected to provide the answer. Modern molecular biological approaches have been used to detect and track coliform bacteria and specific microbial pathogens in water. The polymerase chain reaction (PCR) technique, coupled with the use of specific nucleic acid primers and gene probes, has been used successfully to detect *E. coli* and the enteric pathogens, *Salmonella* and *Shigella,* in water. One PCR technique, called rep-PCR DNA fingerprinting, can be used to identify coliform bacteria, much as DNA fingerprinting techniques have been used in paternity and forensic cases. Organisms yielding indistinguishable DNA banding patterns can be regarded as being identical or near-identical, and as such, define the source of the fecal contamination. The rep-PCR technique provides the necessary sensitivity to differentiate between strains of fecal coliform bacteria originating from different human and animal sources. Of the various genetic fingerprinting strategies, rep-PCR is a relatively simple and cost effective technique which can be adapted for high throughput applications.

The project examined four river watershed areas determined by the cooperating agencies (MPCA and MCES) to represent a range of water quality challenges. The selected locations provided case studies to evaluate the utility of the DNA fingerprinting method for tracking sources of fecal contamination. The project provides a scientifically sound basis for prioritizing pollution control efforts so that resources can be efficiently and effectively allocated to lower fecal coliform counts and achieve water quality goals in these rivers. Beyond the specific applications described here, the project has potential national importance as it adds to the database of DNA fingerprints that can be applied to future pollution tracking efforts.

VII. COOPERATION:

Cooperation among agencies has allowed for the most efficient use of resources. Fecal coliform isolates generated from ongoing water quality monitoring projects at MPCA and MCES have been utilized in the current project and have enhanced all activities. There is widespread interest in the application of genetic

fingerprinting techniques to tracking pollution sources, and a nascent movement toward creating a national database of DNA fingerprints. Dr. Gary Wagenbach (Carleton College), as part of their ongoing fecal coliform monitoring programs, was helpful in providing samples from Prairie Creek (Rice County). Fecal coliform isolates from the Mississippi River (Hennepin County) site, above Lock and Dam #1, were obtained by MCES. Additional isolates were obtained by us from river water as part of Result 3 action plan

The following individuals were cooperators on the research project. They received no LCMR funds.

Dr. Robert Polta - Metropolitan Council Environmental Services
David Maschwitz - Minnesota Pollution Control Agency
Dr. Gary Wagenbach - Department of Biology, Carleton College

In our original LCMR proposal (dated 5/28/98) and subsequently submitted Research Addendum (dated 12/21/98) we listed a non-required, in-kind match of $60,000 to be provided by MCES to aid in the completion of our project objectives. This commitment was to be in the form of MCES staff time. Due to downsizing of MCES technical staff and research department, the organization was not able to provide personnel to the project. Consequently, MCES agreed to supply us with an equivalent in-kind match consisting of approximately $39,000 in cash and $21,000 in supplies and equipment donation. The $39,000 contribution allowed us to hire additional staff to aid us in the acquisition of fecal coliform bacteria from known and environmental samples. The additional staff offset the loss of MCES staff time on the project.

VIII. LOCATION:

The project examined four river watershed areas determined by the cooperating agencies (MPCA and MCES) to represent a range of water quality challenges. The selected locations provided case studies to evaluate the utility of the DNA fingerprinting method for tracking sources of fecal contamination. The project provides a scientifically sound basis for prioritizing pollution control efforts so that resources can be efficiently and effectively allocated to lower fecal coliform counts and achieve water quality goals in these rivers. The laboratory work was done at The Department of Soil, Water, and Climate, University of Minnesota, St. Paul and at Metropolitan Council Environmental Services (MCES) in St. Paul. As discussed above, field sites for obtaining environmental coliform isolates were: Rush River (Sibley County), Grindstone River (Pine County), Prairie Creek (Rice County), and Mississippi River above Lock and Dam #1 (Hennepin County). The sites were chosen by the cooperators due to the continual presence of high fecal coliform counts in these river waters.

Attachment A – Deliverable Products and Related Budget[1] LCMR Project Biennial Budget

Budget Item	Result 1 Acquire E. coli from known sources		Result 2 Generate DNA fingerprints for known source E. coli		Result 3 Isolate and DNA fingerprint environmental E. coli		ROW TOTAL	
	Expenses	Budgeted	Expenses	Budgeted	Expenses	Budgeted	Expenses	Budgeted
Wages, salaries & benefits								
Senior Scientist	61,773	60,579			41,182	40,386	102,955[5]	100,965
Post-Doc			50,328	48,883	24,788	24,077	75,116[6]	72,960
Student Workers/ Jr. Scientist	7,695	7,721	7,886	8,264	3,895	4,167	19,476	20,152
Printing & advertising			9	50	0	950	9	1,000
Local automobile mileage	1,470	1,800			668	1,800	2,138	3,600
Other travel expenses in Minnesota	333	500			5	0	338	500
Travel outside Minnesota	210[2]	0			500	500[3]	710	500
Office supplies (general supplies)	2,905	2,919	1,847	2,206	2,080	2,485	6,832	7,610
Other supplies (laboratory supplies)	21,629	22,800	16,996	17,917	17,819	18,784	56,444	59,501
Office equip & computers (includes software)			12,229	12,360			12,229	12,360
Other capital equipment[4]	5,481	5,481	15,371	15,371			20,852	20,852
COLUMN TOTAL	$ 101,496	$ 101,800	$ 104,666	$ 105,051	$ 90,937	$ 93,149	$ 297,099	$ 300,000

[1] Budgeted amounts based on modified workprogram approved on Oct. 11, 2000 by letter from John Velin. Due to the nature of our research project, activities for the various result categories are carried out simultaneously, and staff time and supplies are shared between result sections. Consequently, expenses in wages, salaries & benefits, and supplies for each result are estimates based on percentage allocations. All expenses are based on information obtained from the University of Minnesota Financial Reporting System on August 2, 2001.

[2] Travel to Grantsburg, WI to collect duck samples. Approval for this out-of-state travel was not requested due to close proximity to Twin Cities.

[3] Approval for travel to EPA conference in Chicago granted in e-mail dated Jan. 3, 2001.

[4] Equipment is defined by University of Minnesota as that costing greater than $2,500.

[5]Overage in salary and fringe benefit category in the Sr. Scientist position was due to an unbudgeted automatic, across-the-board, salary increase and an increase in the U of M fringe benefits rate that occurred during the project period.

[6] Overage in salary and fringe benefit category in the Post-Doc position was due to a one time vacation balance payout on termination of the position, an unbudgeted automatic, across-the-board, salary increase, and an increase in the U of M fringe benefits rate that occurred during the project period.