IV. RESULTS AND DISCUSSION

A. Classification of Known Isolates (Library Composition)

A total of 1,248 isolates were tested from the five sources and the isolates collected from these known fecal sources were analyzed using Logistic Regression (Table 5).

Table 5. Classification of 1,248 isolates of *E. coli* from known sources collected in the Coan River and Little Wicomico River watersheds. Correctly classified isolates (%) are shown in bold and the ARCC for the library was 71.9%. Each grid contains the number of isolates (top number) and the % classified (lower number).

Source	Bird	Human	Livestock	Pets	Wildlife	Totals
Bird	305	24	22	3	34	388
	84.72	7.38	9.21	2.78	15.74	
Human	24	243	29	31	19	346
	6.67	**74.77**	12.13	28.70	8.80	
Livestock	8	27	173	4	11	223
	2.22	8.31	**72.38**	3.70	5.09	
Pets	0	9	3	67	10	89
	0.00	2.77	1.26	**62.04**	4.63	
Wildlife	23	22	12	3	142	202
	6.39	6.77	5.02	2.78	**65.74**	
Totals	360	325	239	108	216	1248

One hundred and forty known source samples were collected during the course of the project: 42 bird scat samples (shore birds and waterfowl) yielded 360 isolates, 36 human samples (from septic tank pump-out trucks and single dwelling septic tanks) provided 325 isolates, 25 cattle and horse scat samples provided 239 isolates, 14 dog scat samples yielded 108 isolates, and 23 wildlife scat samples (deer, raccoon, muskrat) provided 216 isolates. The average rate of correct classification (ARCC) of the library was 71.9%, which was well above the background random classification level of 20% (based on 5 sources). The rates of correct classification (RCC) were 84.7% for birds, 74.8% for human, 72.4% for livestock, 62.1% for dogs, and 65.7% for wildlife (Table 5).

When the library was classified using a two-way split of human vs. non-human, the ARCC was 80.2% (68.3% RCC for human and 92% RCC for non-human). When the library was classified using a four-way split (combining birds with wildlife), the ARCC was 76.3% (71.0% RCC for human, 76.5% RCC for livestock, 76.8% for pets, and 81.2% RCC for wildlife + birds). The highest RCC for the human category (74.8%) was obtained with the five-way split (Table 5), and this was the reason that the five-way split was then used to classify *E. coli* isolates from water samples.

When the library was analyzed for artificial clustering, the ARCC was 24.8%, only 4.8% higher than random distribution of 20%, indicating that the library was of sufficient size to be used to classify unknown source isolates from water samples (Table 6). The random classifications (artificial clustering) were 22.2% for birds, 24.8% for human, 18.4% for livestock, 28.1% for dogs, and 29.6% for wildlife (Table 6). The largest artificial clusters were obtained with pets and wildlife, and indicated that these would be the sources where more isolates were needed if the library was to be expanded.

Table 6. Classification of 1,248 isolates of *E. coli* randomly assigned to source categories. Artificial clustering (%) are shown in bold and the ARCC for the library was 24.8%. Each grid contains the number of isolates (top number) and the % classified (lower number).

Source	Birds	Human	Livestock	Pets	Wildlife	Totals
Birds	57 **22.89**	52 20.80	41 16.40	42 16.87	38 15.20	230
Human	51 20.48	62 **24.80**	49 19.60	42 16.87	51 20.40	255
Livestock	30 12.05	35 14.00	46 **18.40**	46 18.47	40 16.00	197
Pets	48 19.28	51 20.40	59 23.60	70 **28.11**	47 18.80	275
Wildlife	63 25.30	50 20.00	55 22.00	49 19.68	74 **29.60**	291
Totals	249	250	250	249	250	1248

The library contained 565 duplicate isolates and 683 unique patterns. When the unique patterns were analyzed using jackknife analysis of individual isolates (performed by Dr. Bruce Wiggins, JMU), the ARCC was 72% (the ARCC of the library was 71.9%, Table 5). This equivalence in classification success indicates that this known source library is representative of the two watersheds. When the library was analyzed using jackknife analysis of individual samples instead of isolates, the ARCC was 64%, only 7.9% lower than the ARCC of 71.9% for the library (also demonstrating equivalence in classification success). Based on the jackknife analysis, the Minimum Detectable Percentage (MDP) for the Coan and Little Wicomico library was calculated. The mean expected frequency of misclassification (EFM) of this library is 6% ± 3% standard deviation (SD) (EFM and SD calculated for the library by Dr. Eric Smith, Statistics Department, VPI&SU). Multiplying the SD of 3 by four (equals 12) and adding this to the mean EFM (6%) results in a MDP of 18%. Multiplying the SD by three results in a level of confidence at the 99.9% level, so four is used as an additional measure to obtain a conservative estimate. The MDP, as proposed by Whitlock *et al.* (2002), reflects the amount of misclassification that occurs for a particular library, and is a conservative estimate of the lower limit for considering a source to be a significant contributor to a watershed. Whitlock *et al.* (2002) reported ARCCs of 27.9% and 28.9% for two randomly generated data sets and with four source categories, the probability that any one isolate would be assigned to one of the categories by chance was 25%. The low random ARCCs demonstrated that negligible random grouping occurred when analysis was performed on the relatively large library (2,398 isolates) used in their study.

B. Coan River Watershed Results

1. Division of Shellfish Sanitation Stations

The Division of Shellfish Sanitation, with the help of the VDH, conducted a shoreline survey to evaluate the shellfish stations along the Coan River (Killneck Creek, Mill Creek, Coan Mill Stream, Headley Creek, Boathouse Pond), The Glebe (Wright's Cove, Glebe Creek), Kingscote Creek, Garners Creek, Judith Sound, Balls Creek and all of their tributaries. The DSS and VDH discovered the types of potential pollutants, contributors of pollution, and whether the pollution was Direct or Indirect. As stated by the DSS and VDH, "Direct" indicates that the significant activity or potential pollutant has a direct impact on shellfish waters and directly enters into the water source. "Indirect" indicated that the significant activity or potential pollutant is a distance from the water source and is only impacted in the event of a heavy storm. Suspected sources of pollution include Human, Birds, Livestock, Pets and Wildlife. Not all these sources are suspected at each station. The suspected sources for each station (Table 7) are shown as suspected under the description.

Table 7. Coan River Watershed DSS Stations, Location, and Description

DSS STATION Latitude / Longitude	LOCATION	DESCRIPTION
C-7 38.00460954 / -76.47832973	North End of Kingscote Creek – Near Travis Point	Condemned - Seasonal Closure - Indirect Non-Sewage Waste Sites, Industrial Waste Sites, Boating Activity - Marinas Suspected Birds Human, Pets, and Wildlife
C-15 37.99208840 / -76.50150703	North East of Glebe Creek – Near Wright's Cove	Condemned - Seasonal Closure - Direct Onsite Sewage Deficiencies, Kitchen or Laundry Wastes - Contribute Pollution, Suspected Human, Wildlife, and Birds
C-16 37.99521938 / -76.50005378	North West end of Glebe Creek – Near Wright's Cove	Restricted - Indirect Onsite Sewage - Contributes Pollution, Suspected Wildlife and Birds
C-20 37.98123635 / -76.50173519	South East of Glebe Creek – Northeast of Lottsburg	Restricted - Indirect Onsite Sewage Deficiencies - Kitchen or Laundry Wastes - Contributes Pollution, Suspected Human
C-24 37.98416348 / -76.47421162	Part of Killneck Creek – South of Honest Point	Restricted - Indirect Onsite Sewage Deficiencies - Contributes Pollution - Suspected Birds, Human, and Pets
C-27 37.98159074 / -76.47017162	Southern Part of Coan River – Near Stevens Point	Restricted - Direct Onsite Sewage Deficiencies - Industrial Waste, Boating Activity, Suspected Birds Human, and Pets
C-33 37.97116223 / -76.47563654	South East Section of Coan River	Restricted - Indirect Onsite Sewage Deficiencies - Kitchen or Laundry Wastes -Contribute Pollution, Suspected Human and Wildlife
C-37.5Z 37.95872533 / -76.47813938	Southern Part of Coan River – Near Bundick	Restricted - Indirect Onsite Sewage Deficiencies - Contributes Pollution - Suspected Livestock and Wildlife
C-38 37.95757022 / -76.48159800	Southern Part of Coan River – near Bundick	Restricted - Boating Activity Under Surveillance – Suspected Birds and Wildlife

C. Analysis of Coan River Samples

1. *Eschericia coli* enumerations.

During the study period, 108 samples were collected from 9 sampling stations on the Coan River. The numbers of *E. coli* in these samples and the total amount of rainfall in

the 3 days previous to the sampling are shown in Table 8, listed by sample site and collection date. Forty-six of the samples (42.6%) had levels of *E. coli* that were above the Virginia standard of 14 *E. coli* /100 ml. Five of the nine stations (C-16, C-20, C-27, C-37.5Z, C-38) had consistently high fecal counts, with the geometric mean of the 12 monthly samples exceeding the Virginia standard. Over the nine stations, the months with the highest fecal counts were the April, August, September, and November samples. There appeared to be no correlation with rainfall in the fecal counts, as the April sample had only 0.01 inch or 0.0254 centimeters and the November sample had none in the preceding three days, while the August sample had 0.62 inch or 1.57 centimeters and the September sample had 1.0 inch or 2.54 centimeters in the preceding 3 days.

Table 8. Numbers of *E coli* isolates in the Coan River Watershed with rainfall 3 days prior to collection. Numbers in Bold exceed the State Standard

A. Samples collected at DSS station C-7

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C1	**18**	2.54
10/22/01	C10	4	0.00
11/19/01	C19	**16**	0.00
12/6/01	C28	5	0.05
1/17/02	C37	1	0.79
2/19/02	C46	0	0.00
3/20/02	C55	8	2.06
4/18/02	C64	9	0.03
5/1/02	C73	2	2.29
6/13/02	C82	1	0.00
7/15/02	C91	1	1.24
8/29/02	C100	10	1.57

B. Samples collected at DSS station C-15

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C1	13	2.54
10/22/01	C10	4	0.00
11/19/01	C19	4	0.00
12/6/01	C28	10	0.05
1/17/02	C37	2	0.79
2/19/02	C46	0	0.00
3/20/02	C55	**15**	2.06
4/18/02	C64	12	0.03
5/1/02	C73	1	2.29
6/13/02	C82	2	0.00
7/15/02	C91	3	1.24
8/29/02	C100	8	1.57

C. Sample collected at DSS station C-16

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C3	**26**	2.54
10/22/01	C12	12	0.00
11/19/01	C21	11	0.00
12/6/01	C30	**16**	0.05
1/17/02	C39	1	0.79
2/19/02	C48	1	0.00
3/20/02	C57	**14**	2.06
4/18/02	C66	**48**	0.03
5/1/02	C75	2	2.29
6/13/02	C84	**18**	0.00
7/15/02	C93	**18**	1.24
8/29/02	C102	**26**	1.57

D. Sample collected at DSS station C-20

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C4	**38**	2.54
10/22/01	C13	**18**	0.00
11/19/01	C22	**44**	0.00
12/6/01	C31	**17**	0.05
1/17/02	C40	3	0.79
2/19/02	C49	0	0.00
3/20/02	C58	**70**	2.06
4/18/02	C67	**17**	0.03
5/1/02	C76	3	2.29
6/13/02	C85	7	0.00
7/15/02	C94	4	1.24
8/29/02	C103	12	1.57

E. Sample collected at DSS station C-24

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C5	**25**	2.54
10/22/01	C14	3	0.00
11/19/01	C23	**18**	0.00
12/6/01	C32	5	0.05
1/17/02	C41	4	0.79
2/19/02	C50	0	0.00
3/20/02	C59	**20**	2.06
4/18/02	C68	4	0.03
5/1/02	C77	5	2.29
6/13/02	C86	8	0.00
7/15/02	C95	5	1.24
8/29/02	C104	**27**	1.57

F. Sample collected at DSS station C-27

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C6	23	2.54
10/22/01	C15	2	0.00
11/19/01	C24	30	0.00
12/6/01	C33	14	0.05
1/17/02	C42	1	0.79
2/19/02	C51	0	0.00
3/20/02	C60	9	2.06
4/18/02	C69	23	0.03
5/1/02	C78	11	2.29
6/13/02	C87	**113**	0.00
7/15/02	C96	**18**	1.24
8/29/02	C105	**48**	1.57

G. Sample collected at DSS station C-33

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C7	**43**	2.54
10/22/01	C16	**15**	0.00
11/19/01	C25	**26**	0.00
12/6/01	C34	12	0.05
1/17/02	C43	2	0.79
2/19/02	C52	0	0.00
3/20/02	C61	2	2.06
4/18/02	C70	10	0.03
5/1/02	C79	3	2.29
6/13/02	C88	4	0.00
7/15/02	C97	2	1.24
8/29/02	C106	**28**	1.57

H. Sample collected at DSS station C-37.5Z

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C8	**33**	2.54
10/22/01	C17	**21**	0.00
11/19/01	C26	**85**	0.00
12/6/01	C35	**18**	0.05
1/17/02	C44	**27**	0.79
2/19/02	C53	0	0.00
3/20/02	C62	12	2.06
4/18/02	C71	**59**	0.03
5/1/02	C80	6	2.29
6/13/02	C89	**21**	0.00
7/15/02	C98	**25**	1.24
8/29/02	C107	**58**	1.57

I. Sample collected at DSS station C-38

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	C9	**20**	2.54
10/22/01	C18	9	0.00
11/19/01	C27	**56**	0.00
12/6/01	C36	11	0.05
1/17/02	C45	2	0.79
2/19/02	C54*	0	0.00
3/20/02	C63	**23**	2.06
4/18/02	C72	**49**	0.03
5/1/02	C81	4	2.29
6/13/02	C90	3	0.00
7/15/02	C99	4	1.24
8/29/02	C108	26	1.57

2. Classification with ARA

ARA was used on a total of 2,233 unknown isolates from the Coan River Watershed.

Based on the Coan and Wicomico known source library, the 108 samples were classified by source. The results are shown in Table 9, listed by sample site and collection date. There was a strong human signature at all nine sampling stations and the percent of isolates classified as human averaged, over 12 months, above 50% for all stations except C-24 (46.5%). The 12-month averages for the percent human signature were 81.6% for C-7, 74.7% for C-15, 63.2% for C-20, and ranged from 45.8% to 58.0%

for the remaining five stations (Table 9). When comparing the five known source categories, human and bird sources were the most common with 95 and 33, respectively, of the 108 samples having percentages that exceeded the Minimum Detectable Percentage (MDP) of 18%. Livestock, pets, and wildlife signatures exceeded the MDP 15, 4, and 12 times, respectively, and are minor contributors to pollution in the Coan River compared to humans and birds.

There was very little seasonality in the results when comparing the wet and dry season averages for the human signature (Table 9). The human signature was higher in the wet season than the dry season for five of the stations, but the percent human isolates (averaged over all stations) for the wet season (67.0%) and the dry season (7.7%) were fairly close (and well above the MDP of 18%). For the 95 samples where the human signature exceeded the MDP, it was dominant in 70 of them, and was dominant in 12/12 samples for C-7 and 10/12 samples for C-15. On a per station basis, the 12-month average human signature exceeded the MDP at all 9 stations. Bird isolates were higher in the dry season than during the wet season for eight of the nine stations, but the seasonal averages over the year were 4.1% for the wet season and 16.7% for the dry season (both below the MDP of 18%). The seasonal average bird signature exceeded the MDP at four stations during the dry season (C-16, C-20, C-24, and C-27) and did not exceed the MDP at any stations during the wet season. On a per station basis, the 12-month average bird signature exceeded the MDP at 5 of the 9 stations (C-16, C-20, C-24, C-27, and C-37.5Z). The bird signature exceeded the MDP 33 times and in 15 of these samples the bird signature was dominant (for example, 91.7% for May at C-16), and the high bird

signatures were concentrated in the months of May, June, August, September, November, and December.

Table 9. Source Tracking percentages of *E. coli* in the Coan River watershed with seasonality results.

A. Samples collected at DSS station C-7

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C1	0.00	95.83	0.00	4.17	0.00
10/22/01	C10	0.00	95.83	0.00	0.00	4.17
11/19/01	C19	4.17	79.17	16.67	0.00	0.00
12/6/01	C28	16.67	70.83	12.50	0.00	0.00
1/17/02	C37	20.83	70.83	0.00	0.00	8.33
2/19/02	C46	4.17	95.83	0.00	0.00	0.00
3/20/02	C55	4.17	87.50	0.00	0.00	8.33
4/18/02	C64	4.17	91.67	0.00	0.00	4.17
5/1/02	C73	12.50	37.50	29.17	0.00	20.83
6/13/02	C82	0.00	91.67	0.00	0.00	8.33
7/15/02	C91	4.17	95.83	0.00	0.00	0.00
8/29/02	C100	4.17	66.67	16.67	4.17	8.33
	SUM	75.02	979.16	75.01	8.34	62.49
	AVERAGE	6.25	81.60	6.25	0.70	5.21
WET PERIOD						
JAN/FEB/MARCH		29.17	254.16	0.00	0.00	16.66
FOR SEASON		9.72	84.72	0.00	0.00	5.55
DRY PERIOD						
JULY/AUGUST/SEPT		8.34	258.33	16.67	8.34	8.33
FOR SEASON		2.78	86.11	5.56	2.78	2.78

B. Samples collected at DSS station C-15

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C2	0.00	95.83	0.00	4.17	0.00
10/22/01	C11	0.00	95.83	0.00	0.00	4.17
11/19/01	C20	25.00	45.83	29.17	0.00	0.00
12/6/01	C29	8.33	83.33	8.34	0.00	0.00
1/17/02	C38	0.00	95.83	0.00	0.00	4.17
2/19/02	C46	4.17	95.83	0.00	0.00	0.00
3/20/02	C56	4.17	91.67	0.00	0.00	4.17
4/18/02	C65	4.17	87.50	0.00	0.00	8.33
5/1/02	C74	58.33	25.00	4.17	0.00	12.50
6/13/02	C83	16.67	70.83	0.00	4.17	8.33
7/15/02	C92	0.00	83.33	4.17	0.00	12.50
8/29/02	C101	12.50	25.00	50.00	8.33	4.17
	SUM	133.34	895.81	95.85	16.67	58.34
	AVERAGE	11.11	74.65	7.99	1.39	4.86
WET PERIOD						
JAN/FEB/MARCH		8.34	283.33	0.00	0.00	8.34
FOR SEASON		2.78	94.44	0.00	0.00	2.78
DRY PERIOD						
JULY/AUGUST/SEPT		12.50	204.16	54.17	12.50	16.67
FOR SEASON		4.17	68.05	18.06	4.17	5.56

C. Samples Collected from DSS Station C-16

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C3	83.33	8.33	0.00	0.00	8.33
10/22/01	C12	8.33	75.00	4.17	0.00	12.50
11/19/01	C21	12.5	79.17	8.33	0.00	0.00
12/6/01	C30	16.67	70.83	12.50	0.00	0.00
1/17/02	C39	29.17	62.5	8.33	0.00	0.00
2/19/02	C48	4.17	95.83	0.00	0.00	0.00
3/20/02	C57	0.00	41.67	4.17	0.00	54.17
4/18/02	C66	0.00	83.33	0.00	0.00	16.67
5/1/02	C75	54.17	33.33	0.00	0.00	12.50
6/13/02	C84	4.17	8.33	0.00	62.50	25.00
7/15/02	C93	0.00	45.83	54.17	0.00	0.00
8/29/02	C102	41.67	50.00	8.33	0.00	0.00
	SUM	254.18	654.15	100.00	62.50	129.17
	AVERAGE	21.18	54.51	8.33	5.21	10.76
WET PERIOD						
JAN/FEB/MARCH		33.34	200.00	12.50	0.00	54.17
FOR SEASON		11.11	66.67	4.17	0.00	18.06
DRY PERIOD						
JULY/AUGUST/SEPT		125.00	104.16	62.50	0.00	8.33
FOR SEASON		41.67	34.72	20.83	0.00	2.78

D. Samples Collected from DSS Station C-20

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C4	83.33	12.50	0.00	0.00	4.17
10/22/01	C13	8.33	91.67	0.00	0.00	0.00
11/19/01	C22	12.50	75.00	12.50	0.00	0.00
12/6/01	C31	20.83	54.17	25.00	0.00	0.00
1/17/02	C40	20.83	58.33	4.17	0.00	16.67
2/19/02	C49	0.00	100.00	0.00	0.00	0.00
3/20/02	C58	0.00	62.50	0.00	0.00	37.50
4/18/02	C67	0.00	91.67	0.00	0.00	8.33
5/1/02	C76	29.17	58.33	4.17	4.17	4.17
6/13/02	C85	25.00	8.33	4.17	37.50	25.00
7/15/02	C94	12.50	75.00	8.33	0.00	4.17
8/29/02	C103	25.00	70.83	4.17	0.00	0.00
	SUM	237.49	758.33	62.51	41.67	100.01
	AVERAGE	19.79	63.19	5.21	3.47	8.33
WET PERIOD						
JAN/FEB/MARCH		20.83	220.83	4.17	0.00	54.17
FOR SEASON		6.94	73.61	1.39	0.00	18.06
DRY PERIOD						
JULY/AUGUST/SEPT		120.83	158.33	12.50	0.00	8.34
FOR SEASON		40.28	52.78	4.17	0.00	2.78

E. Samples Collected from DSS Station C-24

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C5	54.17	25.00	0.00	4.17	16.67
10/22/01	C14	0.00	95.83	0.00	0.00	4.17
11/19/01	C23	37.50	58.33	4.17	0.00	0.00
12/6/01	C32	16.67	62.50	16.67	4.17	0.00
1/17/02	C41	33.33	50.00	4.17	12.50	0.00
2/19/02	C50	0.00	0.00	0.00	0.00	0.00
3/20/02	C59	0.00	45.83	16.67	16.67	20.83
4/18/02	C68	4.17	66.67	0.00	0.00	29.17
5/1/02	C77	91.67	0.00	0.00	8.33	0.00
6/13/02	C86	66.67	4.17	20.83	4.17	4.17
7/15/02	C95	4.17	66.67	29.17	0.00	0.00
8/29/02	C104	4.17	83.33	12.50	0.00	0.00
	SUM	312.52	558.33	104.18	50.01	75.01
	AVERAGE	26.04	46.53	8.68	4.17	6.25
WET PERIOD						
JAN/FEB/MARCH		33.33	95.83	20.84	29.17	20.83
FOR SEASON		11.11	31.94	6.95	9.72	6.94
DRY PERIOD						
JULY/AUGUST/SEPT		62.51	175.00	41.67	4.17	16.67
FOR SEASON		20.84	58.33	13.89	1.39	5.56

F. Samples Collected from DSS Station C-27

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C6	25.00	25.00	8.33	25.00	16.67
10/22/01	C15	0.00	87.50	4.17	0.00	8.33
11/19/01	C24	54.17	33.33	8.33	4.17	0.00
12/6/01	C33	4.17	79.17	8.33	4.17	4.17
1/17/02	C42	12.50	62.50	16.67	4.17	4.17
2/19/02	C51	0.00	0.00	0.00	0.00	0.00
3/20/02	C60	8.33	75.00	8.33	0.00	8.33
4/18/02	C69	0.00	91.67	4.17	0.00	4.17
5/1/02	C78	95.83	4.17	0.00	0.00	0.00
6/13/02	C87	66.67	4.17	16.67	0.00	12.5
7/15/02	C96	41.67	20.83	25.00	4.17	8.33
8/29/02	C105	0.00	66.67	33.33	0.00	0.00
	SUM	308.30	550.00	133.30	41.70	66.70
	AVERAGE	25.70	45.80	11.10	3.50	5.60
WET PERIOD						
JAN/FEB/MARCH		20.83	137.50	25.00	4.17	12.50
FOR SEASON		6.94	45.83	8.33	1.39	4.17
DRY PERIOD						
JULY/AUGUST/SEPT		66.67	112.50	66.66	29.17	25.00
FOR SEASON		22.22	37.50	22.22	9.72	8.33

G. Samples Collected from DSS Station C-33

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C7	0.00	75.00	4.17	4.17	16.67
10/22/01	C16	4.17	75.00	4.17	0.00	16.67
11/19/01	C25	25.00	37.50	29.17	4.17	4.17
12/6/01	C34	12.50	62.50	20.83	4.17	0.00
1/17/02	C43	0.00	91.67	0.00	4.17	4.17
2/19/02	C52	0.00	0.00	0.00	0.00	0.00
3/20/02	C61	0.00	87.50	8.33	0.00	4.17
4/18/02	C70	8.33	75.00	4.17	0.00	12.50
5/1/02	C79	50.00	25.00	4.17	0.00	20.83
6/13/02	C88	4.17	41.67	16.67	16.67	20.83
7/15/02	C97	0.00	95.83	0.00	0.00	4.17
8/29/02	C106	8.33	29.17	54.17	0.00	8.33
	SUM	112.50	695.80	145.90	33.40	112.50
	AVERAGE	9.40	58.00	12.20	2.80	9.40
WET PERIOD						
JAN/FEB/MARCH		0.00	179.17	8.33	4.17	8.34
FOR SEASON		0.00	59.72	2.78	1.39	2.78
DRY PERIOD						
JULY/AUGUST/SEPT		8.33	200.00	58.34	4.17	29.17
FOR SEASON		2.78	66.67	19.45	1.39	9.72

H. Samples Collected from DSS Station C-37.5Z

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C8	4.17	62.50	12.50	4.17	16.67
10/22/01	C17	8.33	83.33	0.00	0.00	8.33
11/19/01	C26	50.00	41.67	8.33	0.00	0.00
12/6/01	C35	45.83	37.50	12.50	4.17	0.00
1/17/02	C44	0.00	100.00	0.00	0.00	0.00
2/19/02	C53	0.00	95.83	0.00	0.00	4.17
3/20/02	C62	0.00	91.67	0.00	0.00	8.33
4/18/02	C71	0.00	91.67	0.00	0.00	8.33
5/1/02	C80	41.67	4.17	41.67	0.00	12.50
6/13/02	C89	0.00	25.00	37.50	8.33	29.17
7/15/02	C98	0.00	37.50	20.83	0.00	41.67
8/29/02	C107	20.83	16.67	33.33	0.00	29.17
	SUM	170.80	687.50	166.70	16.70	158.30
	AVERAGE	14.20	57.30	13.90	1.40	13.20
WET PERIOD						
JAN/FEB/MARCH		0.00	287.50	0.00	0.00	12.50
FOR SEASON		0.00	95.83	0.00	0.00	4.17
DRY PERIOD						
JULY/AUGUST/SEPT		25.00	116.67	66.66	4.17	87.51
FOR SEASON		8.33	38.89	22.22	1.39	29.17

I. Samples Collected from DSS Station C-38

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C9	4.17	75.00	8.33	12.50	0.00
10/22/01	C18	4.17	66.67	0.00	8.33	20.83
11/19/01	C27	33.33	66.67	0.00	0.00	0.00
12/6/01	C36	0.00	25.00	0.00	29.17	45.83
1/17/02	C45	0.00	95.83	4.17	0.00	0.00
2/19/02	C54*	0.00	0.00	0.00	0.00	0.00
3/20/02	C63	0.00	100.00	0.00	0.00	0.00
4/18/02	C72	75.00	8.33	12.50	0.00	4.17
5/1/02	C81	54.17	25.00	16.67	0.00	4.17
6/13/02	C90	58.33	20.83	4.17	12.5	4.17
7/15/02	C99	4.17	62.50	12.50	0.00	20.83
8/29/02	C108	12.50	62.50	16.67	4.17	4.17
	* No isolates					
	SUM	245.80	608.30	75.00	66.70	104.20
	AVERAGE	20.50	50.70	6.30	5.60	8.70
WET PERIOD						
JAN/FEB/MARCH		0.00	195.83	4.17	0.00	0.00
FOR SEASON		0.00	65.28	1.39	0.00	0.00
DRY PERIOD						
JULY/AUGUST/SEPT		20.84	200.00	37.50	16.67	25.00
FOR SEASON		6.95	66.67	12.50	5.56	8.33

As with birds, the dry and wet season annual averages for livestock, pets, and wildlife were all below the MDP of 18% (Table 9). The pet signature did not exceed the MDP for any season at any site while the wildlife signature was barely above the MDP during the wet season at stations C-16 and C-20 (18.1% for both) and during the dry season at station C-37.5Z (29.2%). The pet signature exceeded the MDP just four times and was only dominant once (62.5% for June at C-16). There were twelve samples where wildlife exceeded the MDP, and only three samples where the wildlife signature was dominant (54.2% for March at C-16, 45.8% for December at C-38, and 41.7% for July at C-37.5Z). The livestock signature did not exceed the MDP at any station during the wet season, but exceeded the MDP during the dry season at six stations (C-15, C-16, C-24, C-27, C-33, and C-37.5Z), but was the dominant signature just five times.

In summary, the human signature was predominant and was slightly higher in the wet season than the dry season. Birds were second in importance to humans, and both birds and livestock were both more abundant in the dry season than the wet season, while the pets and wildlife signatures were essentially negligible. Averaging the percent classifications over all sources and samples provides an obvious ranking of the five sources: humans (59%), birds (17%), livestock (9%), wildlife (8%), and pets (3.0%).

3. Comparison of Source Tracking Results with Site Description

When comparing Table 7 and Table 11 the DSS stations that met the description were stations C-7, C-15, C-20, C-24 and C-27. Human and Birds dominated these stations. The stations that were inaccurate with meeting the descriptions found in Table 11 were stations C-16, C-33, C-37.5Z, and C-38 where human and birds dominated and a low percentage of wildlife were present

4. Comparison of 6-Hour and 24-Hour Samples

The July and August Coan River samples that were held overnight before processing were analyzed and compared to the 6-hour samples (Table 10). For the monitoring results, there was excellent agreement between the July (p =. 99) and August (p =. 90) 6 and 24 hr samples. For source tracking results, the source averages differed from each other by no more than 11% (well below the MDP). These results indicate that were no major differences between samples held for 6 versus 24 hours.

Table 10. Samples collected on July 15, 2002 (top table), and August 23, 2002 (bottom table). The 24-hour holding times are shown below the 6-hour samples. Paired test results were 0.99 for the July samples and 0.90 for the August samples.

Site #	Sample #	# of isolates	% Bird	% Human	%Livestock	%Pets	%Wildlife
C-7	C91	0.70	4.17	95.83	0.00	0.00	0.00
C-7	C91 (24)	0.30	8.33	91.67	0.00	0.00	0.00
C-15	C92	1.30	0.00	83.33	4.17	0.00	12.50
C-15	C92(24)	2.00	0.00	95.83	4.17	0.00	0.00
C-16	C93	4.70	0.00	45.83	54.17	0.00	0.00
C-16	C93(24)	6.00	4.17	20.83	70.83	0.00	4.17
C-20	C94	1.00	12.50	75.00	8.33	0.00	4.17
C-20	C94(24)	2.30	8.33	79.17	4.17	0.00	8.33
C-24	C95	2.70	4.17	66.67	29.17	0.00	0.00
C-24	C95(24)	2.30	25.00	50.00	16.67	0.00	8.33
C-27	C96	8.70	41.67	20.83	25.00	4.17	8.33
C-27	C96(24)	7.00	50.00	16.67	0.00	12.50	20.83
C-33	C97	2.70	0.00	95.83	0.00	0.00	4.17
C-33	C97(24)	1.00	4.17	87.50	4.17	0.00	4.17
C-37.5Z	C98	4.70	0.00	37.50	20.83	0.00	41.67
C-37.5Z	C98(24)	5.00	12.50	0.00	29.17	0.00	58.33
C-38	C99	2.00	4.17	62.50	12.50	0.00	20.83
C-38	C99(24)	2.70	4.17	62.50	25.00	0.00	8.33
Average	6hr	3.17	7.41	64.81	17.13	0.46	10.19
	24hr	3.18	12.96	56.02	17.13	1.39	12.50

Site #	Sample #	# of isolates	% Bird	% Human	%Livestock	%Pets	%Wildlife
C-7	C100	10.00	4.17	66.67	16.67	4.17	8.33
C-7	C100 (24)	11.70	12.50	25.00	45.83	0.00	16.67
C-15	C101	8.00	0.00	58.33	20.83	4.17	16.67
C-15	C101(24)	8.00	12.50	25.00	50.00	8.33	4.17
C-16	C102	25.30	4.17	33.33	41.67	4.17	16.67
C-16	C102(24)	16.70	41.67	50.00	8.33	0.00	0.00
C-20	C103	12.30	4.17	45.83	37.50	4.17	8.33
C-20	C103(24)	12.70	25.00	70.83	4.17	0.00	0.00
C-24	C104	27.30	4.17	37.50	4.17	0.00	54.17
C-24	C104(24)	25.30	4.17	83.33	12.50	0.00	0.00
C-27	C105	48.00	4.17	41.67	29.17	8.33	16.67
C-27	C105(24)	48.00	0.00	66.67	33.33	0.00	0.00
C-33	C106	28.00	0.00	41.67	25.00	16.67	16.67
C-33	C106(24)	27.00	8.33	29.17	54.17	0.00	8.33
C-37.5Z	C107	58.00	0.00	70.83	8.33	4.17	16.67
C-37.5Z	C107(24)	55.30	20.83	16.67	33.33	0.00	29.17
C-38	C108	26.30	12.50	62.50	16.67	4.17	4.17
C-38	C108(24)	17.00	8.33	50.00	33.33	0.00	8.33
Average	6 hr	27.02	3.71	50.93	22.22	5.56	17.59
	24 hr	24.63	14.81	46.30	30.55	0.93	7.41

5. Probabilities and Uncertainty

There is some level of uncertainty when applying unknown sources to actual stations. Molecular studies have taken the approach of evaluating the probability of each isolate and rejecting all isolates that match at less than 80%. The tables of Probabilities can be found in the Appendix for each source. In Table 11 the average corrected percentages are shown, and these percentages were isolates where their probabilities were greater than 80%. These percentages from Table 9 are actually the old percentages without the probability that sources may or may not be Bird, Human, Livestock, Pets, or Wildlife.

There have been many debates over molecular and phenotypic techniques in the bacterial source-tracking field when determining the validity of a study. One criticism that has been directed at ARA is that phenotypic methods are inaccurate and if individual isolates are evaluated at an 80% probability or greater, most if not all of the unknown isolates would be lost due to low probabilities. Simmons *et al.* (2002) performed a

molecular BST technique, Pulsed Field Gel Electrophoresis (PFGE), on the Four Mile Run Watershed in northern Virginia. Simmons *et al.* (2002) rejected 49% of the unknown isolates when using an 80% probability with PFGE. The study correctly classified 278 isolates out of a total 539 isolates. In Table 11 the percentages of unknown range from 44.7% for station C-17 to 79.2% for station C-33. Further study needs to be conducted to determine the results from station C-33. An average of 53% of all the unknown isolates were not correctly classified and were rejected using a 80% probability. Like Simmons *et al.* (2002) with PFGE about 50% of the unknown isolates were lost using an 80% probability that correlates with the results using the same probability with ARA. The phenotypic type and the molecular type of BST meet the same standard when using the 80% probability, which is a severe test for ARA.

Table 11. Coan River Corrected Percentage of Sources and Unknown Percentage

	AVERAGE CORRECT PERCENTAGES * OLD % in ( )					
DSS STATION	BIRD*	HUMAN*	LIVESTOCK*	PETS*	WILDLIFE*	UNKNOWN %
C-7	9.8 (6.3)	35.8 (81.6)	1.7 (6.3)	0.0 (0.7)	0.8 (5.2)	51.9
C-15	11.5 (11.1)	33.3 (74.7)	5.9 (8.0)	0.0 (1.4)	2.5 (4.9)	46.8
C-16	26.4 (21.2)	21.9 (54.5)	3.5 (8.3)	0.0 (5.2)	3.5 (10.8)	44.7
C-20	12.9 (19.8)	31.2 (63.2)	1.9 (5.2)	0.0 (3.5)	3.4 (8.3)	50.6
C-24	19.5 (26.0)	28.7 (46.5)	0.5 (8.7)	0.7 (4.2)	0.4 (6.3)	50.2
C-27	12.5 (21.2)	24.9 (45.8)	1.8 (11.1)	0.4 (3.5)	0.4 (5.6)	60.0
C-33	5.9 (9.4)	10.7 (58.0)	3.0 (12.2)	0.8 (2.8)	0.4 (9.4)	79.2
C-37.5Z	9.2 (14.2)	34.8 (57.3)	6.1 (13.9)	0.0 (1.4)	5.0 (13.2)	44.9
C-38	10.0 (25.7)	32.3 (50.7)	5.5 (6.3)	1.6 (5.6)	3.9 (8.7)	46.7

D. Little Wicomico River Watershed Results

1. Division of Shellfish Sanitation Stations

The DSS, with the help of the VDH, conducted a shoreline survey to evaluate the shellfish stations along the Little Wicomico River (Ellyson Creek, Bridgemans Back Creek, Spences Creek, Spring Cove, Sawmill Cove, Hansons Cove, Willis Creek, Sloop

Creek, Back Creek, Bridge Creek, Horse Pond, Sharps Creek, Slough Creek, Rock Hole), and all of their tributaries. The DSS and VDH discovered the types of potential pollutants, contributors of pollution, and whether the pollution was Direct or Indirect. As stated by the DSS and VDH, "Direct" indicates that the significant activity or potential pollutant has a direct impact on shellfish waters and directly enters into the water source. "Indirect" indicated that the significant activity or potential pollutant is a distance from the water source and is only impacted in the event of a heavy storm. Suspected sources of pollution include Human, Birds, Livestock, Pets and Wildlife. Not all these sources are suspected at each station. The suspected sources for each station (Table 12) are shown as suspected under the description.

Table 12. Little Wicomico River Watershed

DSS STATION Latitude / Longitude	LOCATION	DESCRIPTION
W-9W 37.87655569 / -76.28040642	South West Section of Little Wicomico – Near Bridge Creek	Condemned - Boating Activity and Marinas Contributes Pollution - Contributes Animal Pollution - Suspected Human, Birds, Livestock, Pets, and Wildlife
W-9X 37.87708291 / -76.27279482	South West Section of Little Wicomico – South of Sunny Bank	Restricted - Boating Activity and Marinas Contributes Pollution - Suspected Human and Birds
W-13.5Z 37.88823352 / -76.29356124	Western Section of Little Wicomico – Near Cod Creek	Condemned - Indirect Sewage Deficiencies - Suspected Human, and Wildlife
W-16 37.89868014 / -76.30398598	North West Section of Little Wicomico – South of Spring Cove	Restricted - Direct Non Sewage Sites Potential Pollution - Industrial Waste Sites - Suspected Human, Livestock and Wildlife
W-19 37.90947486 / -76.31633763	Western Tip of Little Wicomico – Northwest of Spring Cove	Restricted - Boating Activity and Marinas - Suspected Birds and Wildlife
W-20 37.91223493 / -76.31142869	Northwestern Tip of Little Wicomico – South of Hack Neck	Restricted - Animal Pollution Directly Contributes - Suspected Birds, Livestock, Pets and Wildlife

E. Analysis of Little Wicomico River Samples

1. *Eschericia coli* enumerations

During the study period, 72 samples were collected from 6 sampling stations on the Little Wicomico River. The numbers of *E. coli* in these samples, and the total amount of rainfall in the 3 days previous to the sampling are shown in Table 13, listed by sample site and collection date. Thirty of the samples (41.7%) had levels of *E. coli* that were above the Virginia standard of 14 *E. coli* /100 ml. Three of the six stations (W-16, W-19, and W-20) had consistently high fecal counts, with the geometric mean of the 12 monthly samples exceeding the Virginia standard. Over the six stations, the months with the highest fecal counts were the March, April, and September samples. There appeared to be no correlation with rainfall in the fecal counts, as the March sample had 0.70 inch or 1.78 centimeters of rain, the April sample had only 0.12 inch or 0.30 centimeters, and the September sample had 1.45 inch or 3.68 centimeters in the preceding three days.

Table 13. Numbers of *E coli* isolates in the Little Wicomico Watershed with rainfall 3 days prior to collection. Numbers in Bold exceed State Standard

A. Samples Collected from DSS Station W-9W

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	W1	9	3.68
10/22/01	W7	9	0.00
11/19/01	W13	6	0.00
12/6/01	W19	**20**	0.05
1/17/02	W25	1	0.79
2/19/02	W31	0	0.00
3/20/02	W37	**54**	1.78
4/18/02	W43	**16**	0.30
5/1/02	W49	5	2.29
6/13/02	W55	3	0.00
7/15/02	W61	3	1.25
8/29/02	W67	3	0.15

B. Samples Collected from DSS Station W-9X

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	W2	12	3.68
10/22/01	W8	3	0.00
11/19/01	W14	2	0.00
12/6/01	W20	6	0.05
1/17/02	W26	1	0.79
2/19/02	W32	0	0.00
3/20/02	W38	**18**	1.78
4/18/02	W44	13	0.30
5/1/02	W50	7	2.29
6/13/02	W56	2	0.00
7/15/02	W62	2	1.25
8/29/02	W68	2	0.15

C. Samples Collected from DSS Station W-13.5Z

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	W3	**30**	3.68
10/22/01	W9	3	0.00
11/19/01	W15	3	0.00
12/6/01	W21	11	0.05
1/17/02	W27	2	0.79
2/19/02	W33	1	0.00
3/20/02	W39	**58**	1.78
4/18/02	W45	**25**	0.30
5/1/02	W51	9	2.29
6/13/02	W57	2	0.00
7/15/02	W63	4	1.25
8/29/02	W69	3	0.15

D. Samples Collected from DSS Station W-16

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	W4	**32**	3.68
10/22/01	W10	9	0.00
11/19/01	W16	5	0.00
12/6/01	W22	**18**	0.05
1/17/02	W28	1	0.79
2/19/02	W34	1	0.00
3/20/02	W40	**71**	1.78
4/18/02	W46	**39**	0.30
5/1/02	W52	**46**	2.29
6/13/02	W58	6	0.00
7/15/02	W64	7	1.25
8/29/02	W70	**17**	0.15

E. Samples Collected from DSS Station W-19

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	W5	**77**	3.68
10/22/01	W11	**22**	0.00
11/19/01	W17	**23**	0.00
12/6/01	W23	**20**	0.05
1/17/02	W29	2	0.79
2/19/02	W35	13	0.00
3/20/02	W41	**53**	1.78
4/18/02	W47	**37**	0.30
5/1/02	W53	**17**	2.29
6/13/02	W59	**26**	0.00
7/15/02	W65	**33**	1.25
8/29/02	W71	13	0.15

F. Samples Collected from DSS Station W-20

DATE	Sample #	No. isolates/100 ml	Rain (centimeters)
9/25/01	W6	**55**	3.68
10/22/01	W12	13	0.00
11/19/01	W18	**36**	0.00
12/6/01	W24	**18**	0.05
1/17/02	W30	3	0.79
2/19/02	W36	2	0.00
3/20/02	W42	**60**	1.78
4/18/02	W48	**62**	0.30
5/1/02	W54	**37**	2.29
6/13/02	W60	**59**	0.00
7/15/02	W66	8	1.25
8/29/02	W72	**19**	0.15

2. Comparison of Source Tracking Results with Site Descriptions

When comparing Table 12 and Table 15 the DSS stations that met the description were stations W-9W and W-9X. Human and Birds dominated these stations. The stations that were inaccurate with meeting the descriptions found in Table 15 were stations W-13.5Z, W-16, W-19, and W-20 where human and birds dominated and a low percentage of livestock, pets, and wildlife were present

3. Classification with ARA

ARA was used on a total of 1,430 unknown isolates from the Little Wicomico River Watershed.

Based on the Coan and Wicomico known source library, the 72 samples were classified by source. The results are shown in Table 14, listed by sample site and collection date. There was a strong human signature at all six sampling stations and the average percent of isolates classified as human, over 12 months, ranged from 42.0% (W-20) to 64.2% (W-9X). When comparing the five known source categories, human and bird sources were the most common with 64 and 30, respectively, of the 72 samples having percentages that exceeded the Minimum Detectable Percentage (MDP) of 18%. Livestock, pets, and wildlife signatures exceeded the MDP 11, 5, and 18 times, respectively, and are minor contributors to pollution in the Little Wicomico River compared to humans and birds.

There was very little seasonality in the results when comparing the wet and dry season averages for the human signature (Table 14). The human signature was higher in the wet season than the dry season for five of the stations, but the percent human isolates (averaged over all stations) for the wet season (61.6%) and the dry season (53.9%) were fairly close (and well above the MDP of 18%). For the 64 samples where the human signature exceeded the MDP, it was dominant in 46 of them, and was dominant in 11/12 samples for W-9X and 10/12 samples for W-9W. On a per station basis, the 12-month average human signature exceeded the MDP at all 6 stations. Bird isolates were also very similar for both the wet and dry seasons and the seasonal averages over the year were 16.9% for the wet season and 15.5% for the dry season (both below the MDP of 18%). The seasonal average bird signature exceeded the MDP at two stations during both the wet and dry seasons (W-13.5Z and W-20). On a per station basis, the 12-month average bird signature exceeded the MDP at all 4 of 6 stations. The bird signature exceeded the

MDP for 30 samples and was dominant in 12 of those (for example, 100% for May at W-19) and the high bird signatures were concentrated in the months of April, May, June, August, November, December, and January.

As with birds, the dry and wet season annual averages for livestock, pets, and wildlife were all below the MDP of 18% (Table 14). The pet signature did not exceed the MDP for any season at any site while the wildlife signature was barely above the MDP during the wet season at stations W-9X and W-13.5Z (19.5% for both) and during the dry season at station W-20 (18.1%). The pet signature exceeded the MDP just five times and was only dominant twice (41.2% for December at W-9W and 58.3% for December at W-13.5Z). There were 18 samples where wildlife exceeded the MDP, and only three samples where the wildlife signature was dominant (62.5% for May at W-9W, and 37.5% for January and 50.0% for June at W-20). The livestock signature did not exceed the MDP at any station during the wet season, but exceeded the MDP during the dry season at two stations (W-13.5Z and W-16), but was the dominant signature just once (25.0% for December at W-20).

Table 14. Source Tracking percentages of *E. coli* in the Little Wicomico River watershed with seasonality results.

A. Samples Collected from DSS Station W-9W

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W1	4.17	75.00	4.17	16.67	0.00
10/22/01	W7	0.00	75.00	0.00	0.00	25.00
11/19/01	W13	29.17	54.17	12.50	4.17	0.00
12/6/01	W19	8.33	8.33	0.00	41.67	41.67
1/17/02	W25	25.00	70.83	0.00	0.00	4.17
2/19/02	W31	12.50	87.50	0.00	0.00	0.00
3/20/02	W37	12.50	67.50	0.00	0.00	0.00
4/18/02	W43	33.33	62.50	4.17	0.00	0.00
5/1/02	W49	33.33	0.00	4.17	0.00	62.50
6/13/02	W55	29.17	37.50	0.00	29.17	4.17
7/15/02	W61	0.00	58.33	29.17	0.00	12.50
8/29/02	W67	0.00	79.17	12.50	4.17	4.17
	SUM	175.00	608.30	66.70	95.90	154.20
	AVERAGE	14.60	50.70	5.60	8.00	12.80
WET PERIOD						
JAN/FEB/MARCH		50.00	225.83	0.00	0.00	4.17
FOR SEASON		16.67	75.28	0.00	0.00	1.39
DRY PERIOD						
JULY/AUGUST/SEPT		4.17	212.50	45.84	20.84	16.67
FOR SEASON		1.39	70.83	15.28	6.95	5.56

B. Samples Collected from DSS Station W-9X

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W2	4.17	70.83	12.50	8.33	4.17
10/22/01	W8	4.17	91.67	4.17	0.00	0.00
11/19/01	W14	8.33	83.33	8.33	0.00	0.00
12/6/01	W20	0.00	50.00	0.00	16.67	33.33
1/17/02	W26	20.83	62.50	4.17	0.00	12.50
2/19/02	W32	0.00	58.33	0.00	0.00	41.67
3/20/02	W38	16.67	66.67	8.33	4.17	4.17
4/18/02	W44	25.00	66.67	8.33	0.00	0.00
5/1/02	W50	62.50	20.83	0.00	8.33	8.33
6/13/02	W56	0.00	70.83	8.33	0.00	20.83
7/15/02	W62	4.17	62.50	29.17	0.00	4.17
8/29/02	W68	25.00	66.67	8.33	0.00	0.00
	SUM	170.80	770.80	91.70	37.50	129.20
	AVERAGE	14.20	64.20	7.60	3.10	10.80
WET PERIOD						
JAN/FEB/MARCH		37.50	187.50	12.50	4.17	58.34
FOR SEASON		12.50	62.50	4.17	1.39	19.45
DRY PERIOD						
JULY/AUGUST/SEPT		33.34	200.00	50.00	8.33	8.34
FOR SEASON		11.11	66.67	16.67	2.78	2.78

C. Samples Collected from DSS Station W-13.5Z

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W3	33.33	33.33	25.00	0.00	8.33
10/22/01	W9	12.50	70.83	4.17	12.50	0.00
11/19/01	W15	16.67	83.33	0.00	0.00	0.00
12/6/01	W21	0.00	37.50	0.00	58.33	4.17
1/17/02	W27	41.67	37.50	8.33	8.33	4.17
2/19/02	W33	0.00	70.83	0.00	0.00	29.17
3/20/02	W39	25.00	37.50	12.50	0.00	25.00
4/18/02	W45	45.83	25.00	16.67	0.00	12.50
5/1/02	W51	4.17	95.83	0.00	0.00	0.00
6/13/02	W57	4.17	70.83	12.5	4.17	8.33
7/15/02	W63	12.50	33.33	29.17	0.00	25.00
8/29/02	W69	45.83	50.00	4.17	0.00	0.00
	SUM	241.70	645.80	112.50	83.33	116.70
	AVERAGE	20.10	53.80	9.40	6.90	9.70
WET PERIOD						
JAN/FEB/MARCH		66.67	145.83	20.83	8.33	58.34
FOR SEASON		22.22	48.61	6.94	2.78	19.45
DRY PERIOD						
JULY/AUGUST/SEPT		91.66	116.66	58.34	0.00	33.33
FOR SEASON		30.55	38.89	19.45	0.00	11.11

D. Samples Collected from DSS Station W-16

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W4	8.33	50.00	12.50	16.67	12.50
10/22/01	W10	16.67	66.67	16.67	0.00	0.00
11/19/01	W16	50.00	45.83	4.17	0.00	0.00
12/6/01	W22	79.17	4.17	4.17	0.00	12.50
1/17/02	W28	16.67	83.33	0.00	0.00	0.00
2/19/02	W34	0.00	91.67	8.33	0.00	0.00
3/20/02	W40	16.67	50.00	0.00	20.83	12.50
4/18/02	W46	50.00	4.17	25.00	0.00	20.83
5/1/02	W52	0.00	95.83	0.00	0.00	4.17
6/13/02	W58	0.00	50.00	8.33	8.33	33.33
7/15/02	W64	8.33	66.67	12.50	4.17	8.33
8/29/02	W70	20.83	50.00	29.17	0.00	0.00
	SUM	266.70	658.30	120.80	50.00	104.20
	AVERAGE	22.20	54.90	10.10	4.20	8.70
WET PERIOD						
JAN/FEB/MARCH		33.34	225.00	8.33	20.83	12.50
FOR SEASON		11.11	75.00	2.78	6.94	4.17
DRY PERIOD						
JULY/AUGUST/SEPT		37.49	166.67	54.17	20.84	20.83
FOR SEASON		12.50	55.56	18.06	6.95	6.94

E. Samples Collected from DSS Station W-19

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W5	0.00	62.50	8.33	8.33	20.83
10/22/01	W11	12.50	66.67	4.17	0.00	16.67
11/19/01	W17	29.17	62.50	8.33	0.00	0.00
12/6/01	W23	45.83	12.50	0.00	0.00	41.67
1/17/02	W29	37.50	25.00	29.17	4.17	4.17
2/19/02	W35	0.00	62.50	8.33	0.00	29.17
3/20/02	W41	4.17	79.17	8.33	0.00	8.33
4/18/02	W47	4.17	45.83	8.33	0.00	41.67
5/1/02	W53	100.00	0.00	0.00	0.00	0.00
6/13/02	W59	0.00	29.17	8.33	4.17	58.33
7/15/02	W65	8.33	45.83	37.50	0.00	8.33
8/29/02	W71	25.00	58.33	4.17	12.50	0.00
	SUM	266.70	550.00	125.00	29.20	229.20
	AVERAGE	22.20	45.80	10.40	2.40	19.10
WET PERIOD						
JAN/FEB/MARCH		41.67	166.67	45.83	4.17	41.67
FOR SEASON		13.89	55.56	15.28	1.39	13.89
DRY PERIOD						
JULY/AUGUST/SEPT		33.33	166.66	50.00	20.83	29.16
FOR SEASON		11.11	55.55	16.67	6.94	9.72

F. Samples Collected from DSS Station W-20

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W6	4.17	50.00	4.17	29.17	12.50
10/22/01	W12	0.00	75.00	8.33	4.17	12.50
11/19/01	W18	41.57	58.33	0.00	0.00	0.00
12/6/01	W24	20.83	16.67	25.00	16.67	20.83
1/17/02	W30	16.67	20.83	20.83	4.17	37.50
2/19/02	W36	37.50	58.33	4.17	0.00	0.00
3/20/02	W42	20.83	79.17	0.00	0.00	0.00
4/18/02	W48	4.17	62.50	0.00	4.17	29.17
5/1/02	W54	87.50	4.17	0.00	4.17	4.17
6/13/02	W60	0.00	20.83	0.00	29.17	50.00
7/15/02	W66	62.50	8.33	4.17	0.00	25.00
8/29/02	W72	12.50	50.00	12.50	8.33	16.67
	SUM	308.20	504.20	79.20	100.00	208.30
	AVERAGE	25.70	42.00	6.60	8.30	17.40
WET PERIOD						
JAN/FEB/MARCH		75.00	158.30	25.00	4.20	37.50
FOR SEASON		25.00	52.80	8.30	1.40	12.50
DRY PERIOD						
JULY/AUGUST/SEPT		79.20	108.30	20.80	37.50	54.20
FOR SEASON		26.40	36.10	6.90	12.50	18.10

4. Probabilities and Uncertainty

There is some level of uncertainty when applying unknown sources to actual stations. Molecular studies have taken the approach of evaluating the probability of each isolate and rejecting all isolates that match at less than 80%. The tables of Probabilities can be found in the Appendix for each source. In Table 15 the average corrected percentages are shown, and these percentages were isolates where their probabilities were greater than 80%. These percentages from Table 14 are actually the old percentages without the probability that sources may or may not be Bird, Human, Livestock, Pets, or Wildlife.

There have been many debates over molecular and phenotypic techniques in the bacterial source-tracking field when determining the validity of a study. The belief is when using ARA the study is looking at individual isolates and evaluating isolates to an 80% probability would result is losing most if not all of the unknown isolates. Simmons *et al*. (2002) performed a molecular BST technique, Pulsed Field Gel Electrophoresis (PFGE), on the Four Mile Run Watershed in northern Virginia. Simmons *et al.* (2002) rejected 49% of the unknown isolates when using an 80% probability with PFGE. The study correctly classified 278 isolates out of a total 539 isolates. In Table 15 the percentages of unknown range from 43.5% for station W-9X to 55.8% for station W-20. An average of 52% of all the unknown isolates were not correctly classified and were rejected using an 80% probability. Like Simmons *et al.* (2002) with PFGE about 50% of the unknown isolates were lost using an 80% probability that correlates with the results using the same probability with ARA. The phenotypic type and the molecular type of

BST meet the same standard when using the 80% probability, which is a severe test for ARA.

Table 15. Little Wicomico Corrected Percentage of Sources and Unknown Percentage

DSS STATION	AVERAGE CORRECT PERCENTAGES * OLD % in ( )					
	BIRD*	HUMAN*	LIVESTOCK*	PETS*	WILDLIFE*	UNKNOWN %
W-9W	9.2 (13.2)	28.9 (49.0)	4.0 (5.6)	1.9 (8.0)	4.5 (12.8)	51.5
W-9X	17.4 (16.3)	24.6 (64.2)	4.1 (7.6)	1.6 (3.1)	8.8 (10.8)	43.5
W-13.5Z	13.5 (20.1)	18.6 (53.8)	4.5 (9.4)	4.4 (6.9)	10.5 (9.7)	48.5
W-16	20.3 (22.2)	21.4 (54.9)	2.6 (10.1)	0.0 (4.2)	0.4 (8.7)	55.3
W-19	14.5 (22.2)	23.9 (45.8)	2.5 (9.7)	0.8 (2.4)	3.4 (19.1)	54.9
W-20	17.1 (25.7)	19.8 (42.0)	1.8 (6.6)	1.4 (8.3)	4.1 (17.4)	55.8

F. Classifications and Number of Isolates

In source tracking the number of isolates evaluated is used as the basis for cost. A small number of isolates is not really representative as shown is Tables 16 and 17. It is important to understand the statistical limits of isolate numbers and to use adequate numbers to obtain a sure level of significance. Recent publications have used a small number of isolates, but in those cases statistical tests were not employed to examine the library and its suitability (Simmons *et al*., 2002).

In this project a total of 24 isolates was always attempted. Since a total of 24 isolates was used the library was much more representative than if 3 or 6 isolates was used (e.g. compare Tables 16 and 17). The objective of using 24 isolates is to determine whether or not the known source library was suitable for the samples that had been collected from the Coan River and the Little Wicomico River Watersheds. In this case, 24 isolates was much more representative and allowed for a more accurate classification over the known sources than using 3, 6, or even 12 isolates. Twenty-four or more isolates was needed in order to adequately measure bacterial variation. As shown in Table 16, the dominant source can be missed with 3 isolates, and several sources can be

missed if there are fewer isolates than possible sources. When classifying over three sources (Table 16) the dominant source is missed when using 3 isolates, and when classifying over five sources (Table 17), the dominant source is also missed with using 3 isolates. Using 24 isolates was much more representative when either classifying with 3 or even 5 sources.

Table 16: Antibiotic Resistance Analysis of *Escherichia coli* Classifications and Number of Isolates Over Three Sources

NUMBER	BIRD	HUMAN	LIVESTOCK	PETS	WILDLIFE
24	0.00	20.80	0.00	29.20	50.00
12	0.00	30.70	0.00	15.40	65.70
6	0.00	50.00	0.00	0.00	50.00
3	0.00	100.00	0.00	0.00	0.00

Table 17: Antibiotic Resistance Analysis of *Escherichia coli* Classifications and Number of Isolates Over Five Sources

NUMBER	BIRD	HUMAN	LIVESTOCK	PETS	WILDLIFE
24	16.67	20.83	20.83	4.17	37.50
12	8.33	25.00	33.33	0.00	33.33
6	16.67	50.00	0.00	0.00	33.33
3	0.00	66.67	0.00	0.00	33.33