Pollution Source Identification through *Pseudomonas aeruginosa* Typing Procedures

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Prepared by:

Cathy Quinlan, M.A.
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Sponsored by:

Ontario Ministry of the Environment and
St. Clair Region Conservation Authority

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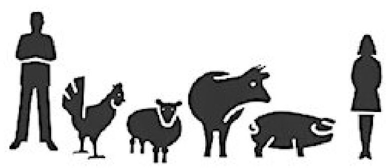

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EXECUTIVE SUMMARY

High bacteria levels in many southern Ontario swimming areas has compelled researchers to search for a means of identifying the sources of the bacteria. The aim of this study was to develop and test a method of categorizing strains of *Pseudomonas aeruginosa* bacteria according to their host species (eg. cow, pig, human). This bacterium is found in the intestines of warm blooded animals and in natural waters polluted with human and animal feces.

The Bear Creek watershed in Warwick Township, Lambton County, was chosen for this study. Water samples were collected from creeks, drains, tile outlets near various livestock farms and at the beach in the downstream Warwick Conservation Area Reservoir. Sediment and manure samples were also collected. The P. *aeruginosa* found in these samples were serotyped and then pyocin typed in the laboratory of the Ministry of the Environment in London.

Out of 245 water samples that were analyzed, 99 (40%) did not contain *Pseudomonas* bacteria and so could not be typed. The frequency of occurrence of this bacterium was highest in the tile outlets and lowest at the beach.

Serotype #6 occurred 34% of the time followed by serotypes #1 and #3 which were detected 19 and 10% of the time respectively. These results parallel the findings of other studies. The serotyping results did not prove to be useful since no link could be made between one serotype and one pollution source.

Pyocin typing was then carried out on the isolates. Out of the 342 isolates which were analyzed, 211 different sero-pyocin type combinations were recorded. This included 154 unique pyocin types. Pyocin type 77773, the maximum number possible, occurred most frequently and ubiquitously. Pyocin types beginning with the number 7 (7-series) were the most common (38%) followed by those beginning with the number 5 (26%). Other studies have found the 7-series to be very common as well.

The results were separated and grouped according to the source of the pollution. A large range of sero-pyocin types were recorded for each source. Most of the types occurred only once, although the data base was small in some cases. The sero-pyocin types found in the sediments taken near the water sampled rarely matched those found in the water. In addition, the limited data from manure did not provide any good links either. Only one sero-pyocin type found at the beach could be matched to the same type found upstream.

Detecting *Pseudomonas* in manure samples was difficult due to the low frequency of occurrence of this bacterium in individual animals and the competition with the more numerous fecal coliforms. The use of Nalidixic Acid in the lab was useful in killing the fecal coliforms. Also, composite samples from liquid manure pits increased the likelihood of picking up *Pseudomonas* compared to individual animal droppings.

Some sero-pyocin types were found frequently at specific sites and also at numerous other sites. These types appear to be ubiquitous and may be present everywhere and thus not indicative of any one particular host species.

The sero-pyocin types which are unique to one host species may be very numerous. A very large data base of samples will be needed in order to discover all of the potential types which exist from one type of animal. The list will undoubtedly be very large considering 211 different sero-pyocin types have already been found from a limited sample size of 342 isolates in this study.

ACKNOWLEDGEMENTS

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CHAPTER 1

BACKGROUND

1.1 INTRODUCTION

Beach closures due to high bacteria levels have become a common occurrence in Southern Ontario. Over the last five years, several studies have been carried out by the Ministry of the Environment (MOE) and Conservation Authorities in an attempt to identify the sources and solutions to this widespread problem.

To date, laboratory tests have focussed on counting the numbers of indicator bacteria in a water sample, not identifying their source of origin. It is very difficult to determine the specific source of bacteria (eg. hog, cow, human) in a watercourse or lake since numerous sources of bacterial contamination exist in the upstream watersheds.

Recently, however, staff at the Microbiology Department of the Ministry of the Environment in London, have experimented with bacteria typing techniques to determine the sources of *Pseudomonas aeruginosa* in environmental samples. *P. aeruginosa* is a bacterium found in the feces of animals and humans. The technique is based on the assumption that different species of animals would produce different strains of *Pseudomonas aeruginosa*.

Time and money could be saved if the sources of the bacteria found at a beach or in a stream could be identified and linked to a specific host species (ie. human, hog, etc.). Remedial measures could then be put into place which focus on the most significant sources of pollution. Thus, money would not be wasted in correcting operations which were not contributing significantly to the pollution problem.

Goal:

The goal of this study was to develop and test a method of identifying and categorizing *Pseudomonas aeruginosa* bacteria according to their host species. The secondary goal was to use the results of this study to determine which activities or types of farms are the largest contributors of bacterial pollution in Bear Creek and at the Warwick Conservation Area reservoir.

1.2 STUDY FUNDING

Funding for this project was received from the Ministry of the Environment out of the London Region Agricultural Fund. The study began in the spring of 1989 with a 12 month contract. Towards the end of this contract, it was apparent that more data was required and so an additional 6 month contract was funded. This second contract began in the summer of 1990 and continued until the winter of 1990/1991.

1.3 THE BEAR CREEK WATERSHED

The Bear Creek watershed was chosen for study due to the availability of background data from an earlier water quality study in 1988-1989. The study revealed high bacteria levels were present throughout the watershed and in several specific tile outlets. The area is also intensively farmed.

The watershed is located in the northeast corner of Lambton County in the Township of Warwick approximately 40 km east of the City of Sarnia. The situation of the Bear Creek watershed within the St. Clair Region Conservation Authority (SCRCA) and Southern Ontario is illustrated in Figure 1. The Bear Creek watershed forms the upper basin of the North Branch of the Sydenham River.

The study area is characterized by a flat glacial lake-bed topography of clay and sand plains. Perth and Huron clay soils of the Grey-Brown Podzolic Soil Group predominate.

Land use in this watershed is predominantly agricultural. Hog operations are the most common type of livestock farm. Dairy and beef cattle farms are also abundant.

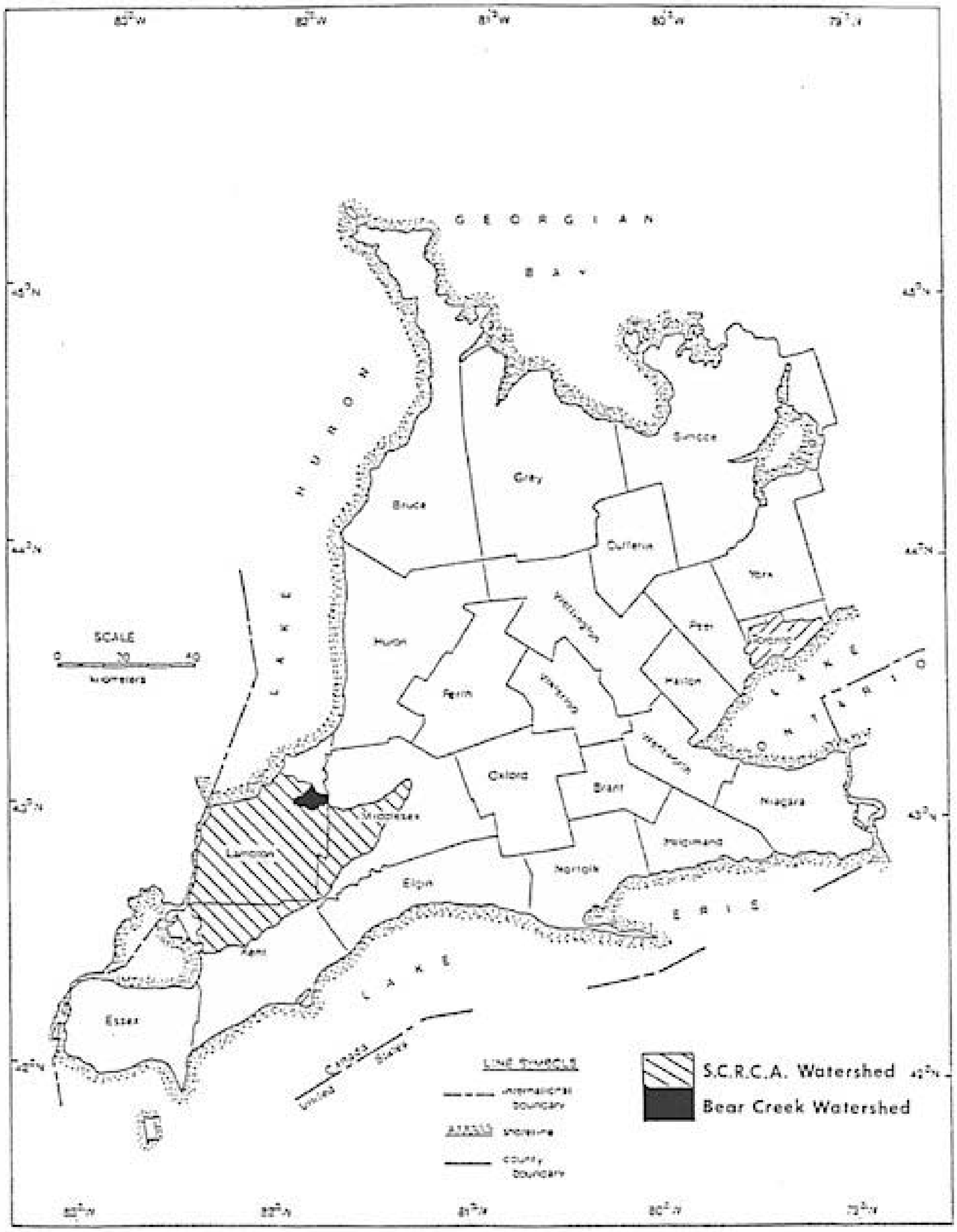


Figure 1: The situation of the St. Clair Region Conservation Authority and Bear Creek within Southern Ontario.

Corn, wheat and beans are the dominant crops. There are some non-farming residences scattered throughout the watershed, but most of these are concentrated in the Police Village of Warwick.

Figure 2 illustrates the drainage pattern of Bear Creek and its associated tributaries. The Warwick Conservation Area and Reservoir is situated at the downstream end of the watershed in the Police Village of Warwick. This man-made reservoir is approximately 6 hectares (15 acres) in size. The beach at this reservoir has been closed to the public for swimming for various periods of time over the past 5 years due to high fecal coliform levels.

Warwick Township is located in a warm and humid region of Southwestern Ontario. The area is denoted by warm summers, mild winters and a long growing season. On average, there are 211 days in the growing season (days over 6°C or 42°F) and 150 frost-free days.

Due to the lake-effect, Lambton County experiences significantly more cloud and precipitation and moderate temperatures than areas farther inland (Dept. of Transport, 1962 and 1967). Mean annual rainfall in the Warwick area is 76-86 centimeters or 30-34 inches. The summers of 1988 and 1989 were particularly dry and warm. The year 1990 experienced more normal conditions.

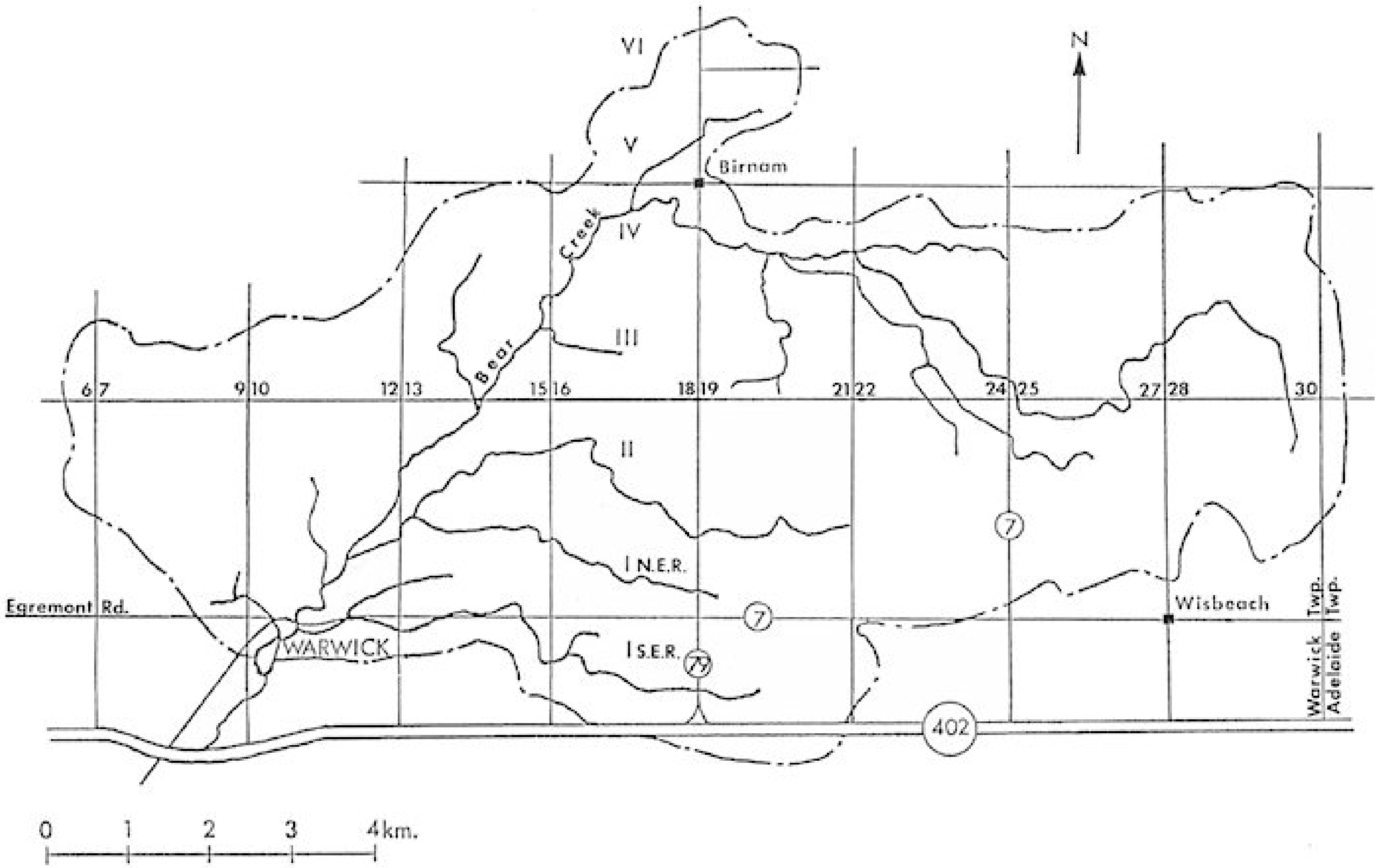


Figure 2: The Bear Creek Watershed showing the watercourse and major roads.

CHAPTER 2

METHODS

2.1 SELECTION OF ZONES

Three zones or study areas along Bear Creek and its tributaries were selected for sampling. These are illustrated in Figure 3. Relatively small areas were needed to locate and track the bacteria both in tile outlets and the receiving stream. The specific sampling sites within Zones I, II and III are illustrated in Figures 4, 5 and 6 respectively.

These zones were chosen for a number of reasons. Firstly *Pseudomonas aeruginosa* was detected routinely in these tile outlets and in the receiving stream in earlier sampling inventories (Quinlan, 1989). This is important since *P. aeruginosa* is not as common as other bacteria in environmental samples. In fact, it took some searching to find tiles which routinely contained *P. aeruginosa*.

Efforts were made to select tile outlets where the source of the water was fairly obvious and singular. For example, tile outlets were chosen where only one source of pollution seemed possible such as in an area where only hog farms were present. Tiles were avoided where a number of different potential sources were possible such as near a number of different farms types. An attempt was made to find tiles which could be used as samples of human, hog, dairy and cattle waste for comparison purposes.

Lastly, areas were chosen where there was relatively easy to access from the road or field. This was important as samples had to be taken repeatedly.

2.2 SAMPLING

Water sampling was first attempted during the summer of 1989. However, this was not successful as most of the tiles were not flowing due to the unusually dry summer conditions. Sampling was initiated again in October and November of 1989

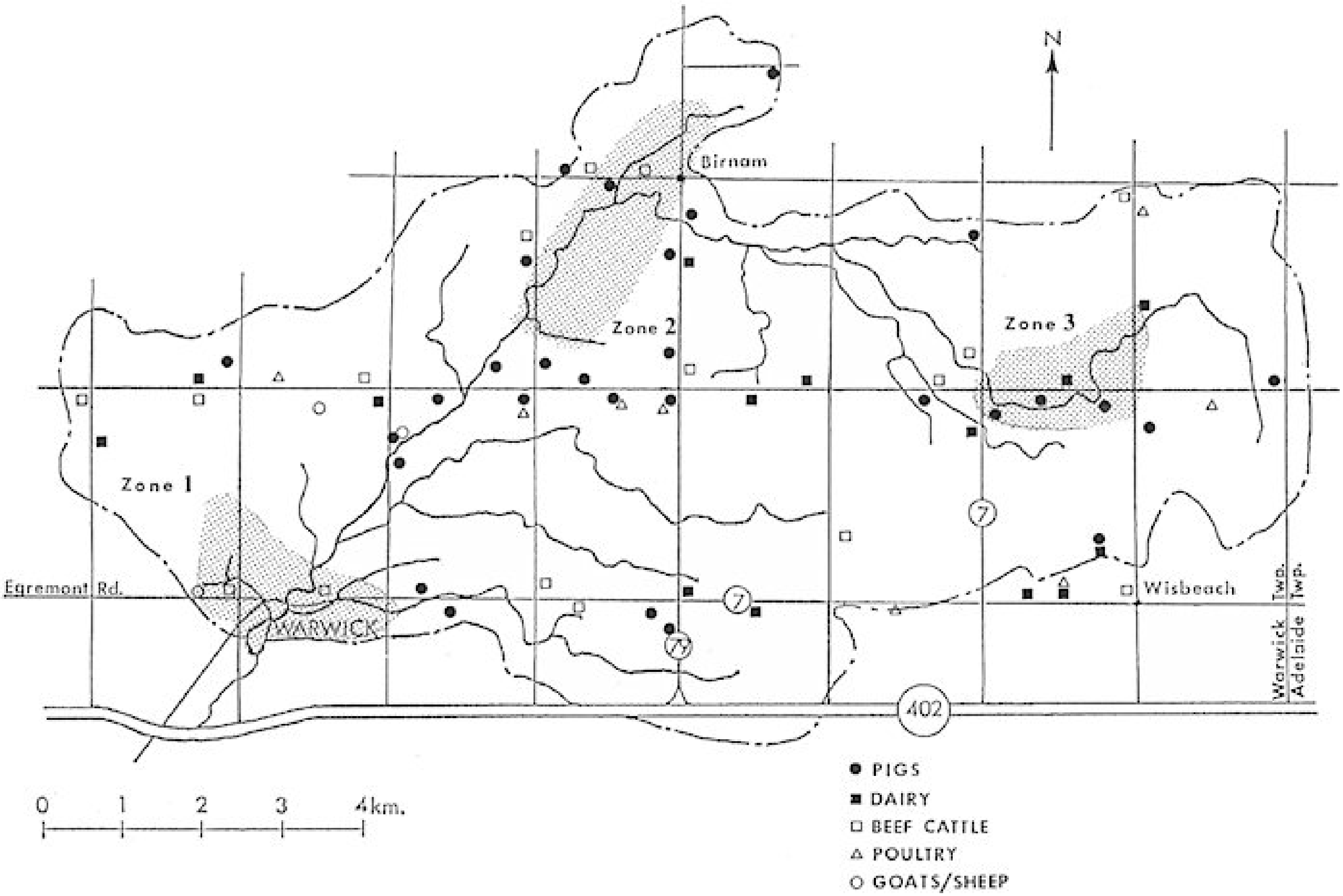


Figure 3: Location of Study Zones within the Bear Creek Watershed.

when the wetter conditions caused the tiles to flow and ceased with the onset of winter. Sampling resumed in the summer of 1990 and into the following fall and winter.

During the summer of 1990, sediment samples were collected at or near the locations where the water samples were taken. Sterile scoops were used to scrape off the top few centimeters of sediment from the creek bed or beach. The samples were dropped into a sterile plastic bottle and delivered to the laboratory for analysis along with the water samples.

In the winter of 1990/1991 several manure samples were collected from a variety of farms throughout the watershed. A 500 ml sterilized plastic jar was filled with fresh solid or liquid manure from the barn or manure pit.

Water samples were collected in a downstream to upstream direction. A 250 ml sterilized glass bottle was filled at each location. The samples were then delivered the same day to the MOE lab in London for routine and special analysis. Under routine analysis, the samples were tested for the presence and concentration of the following groups of bacteria:

fecal coliform
Escherichia coli
fecal *Streptococci*
Pseudomonas aeruginosa

The *Pseudomonas aeruginosa* colonies were then picked and saved for serotyping and pyocin typing. These procedures are discussed in the next chapter.

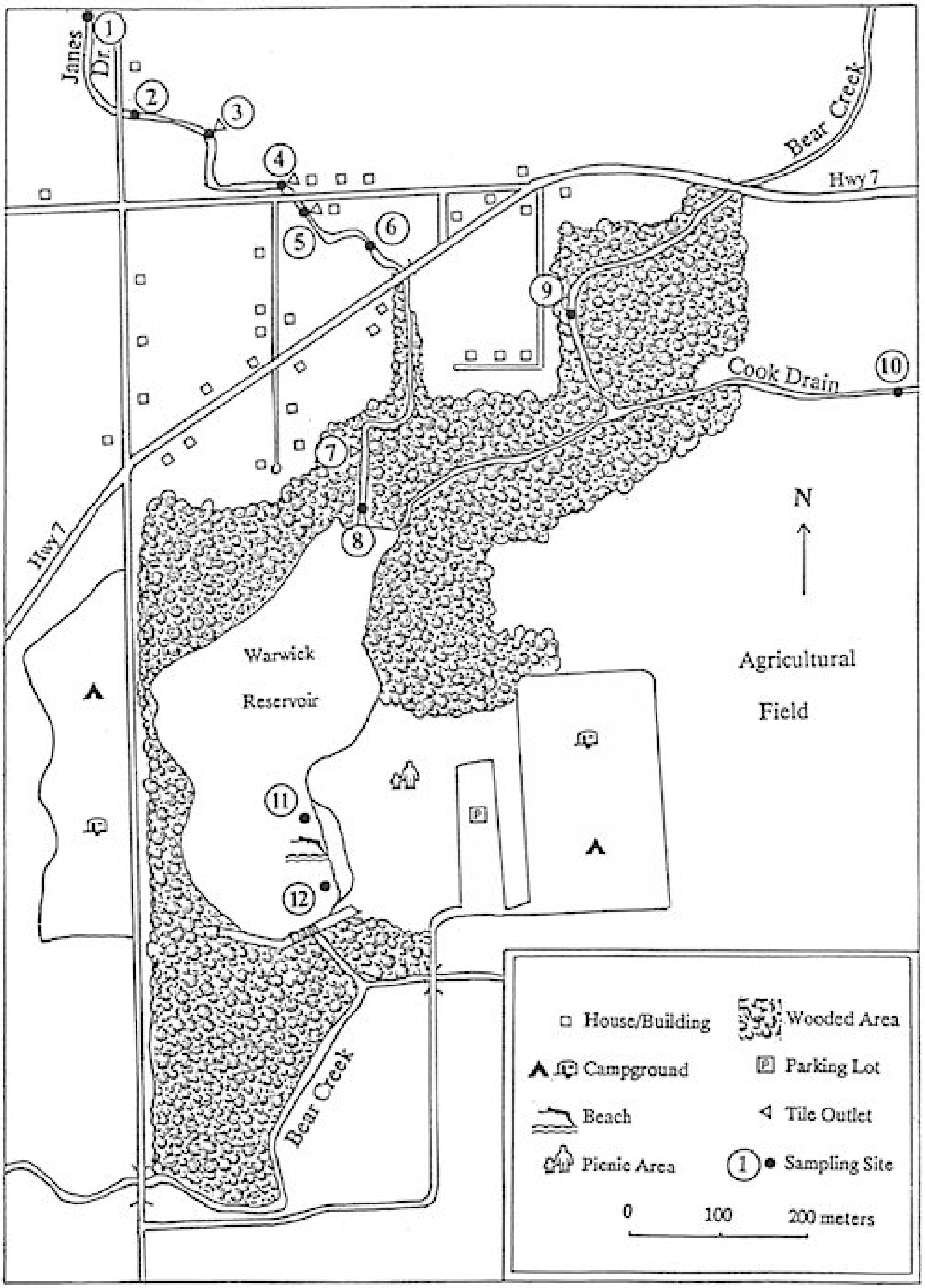


Figure 4: Location of sampling sites in Zone I.

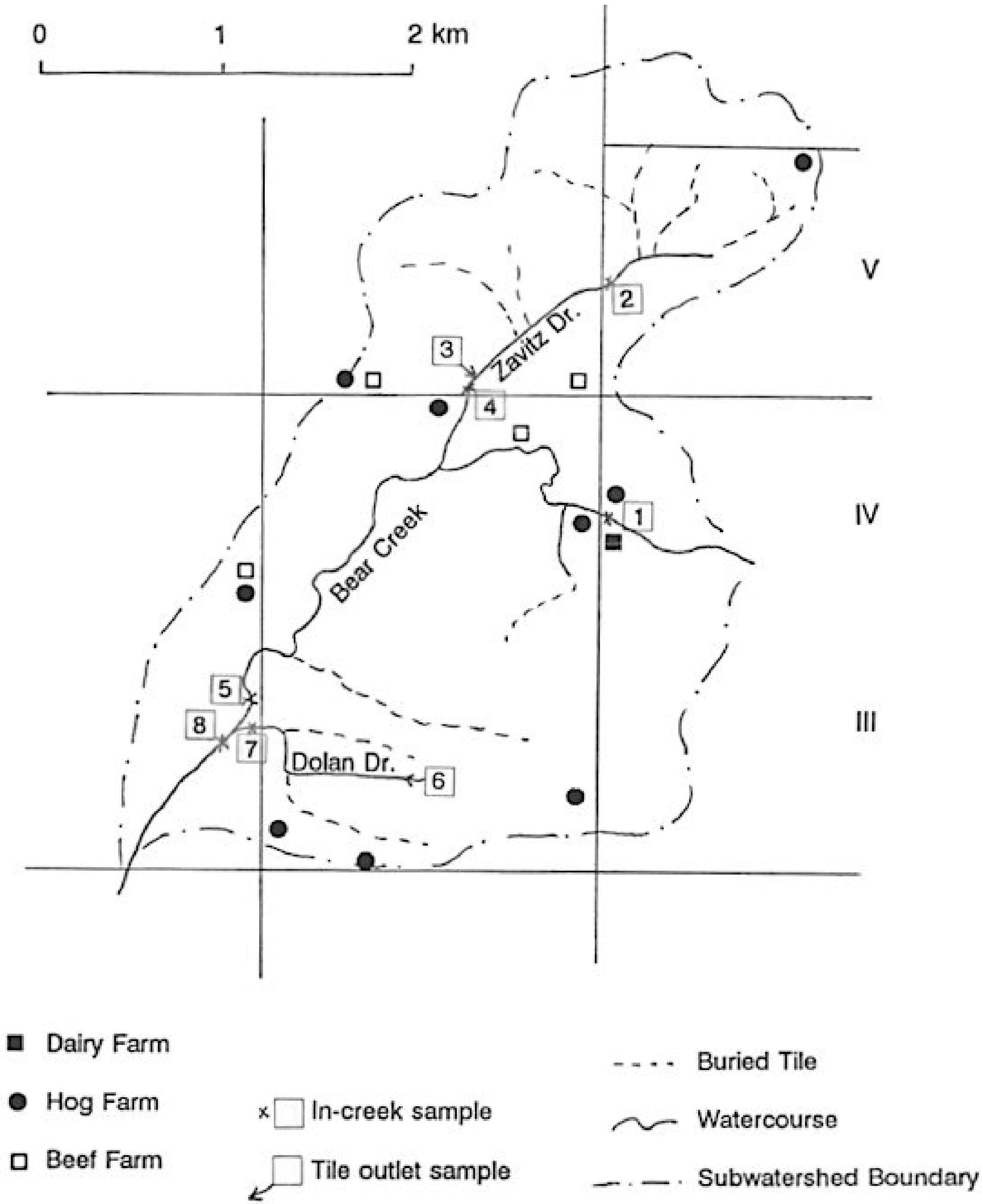


Figure 5: Location of the sampling sites in Zone 2.

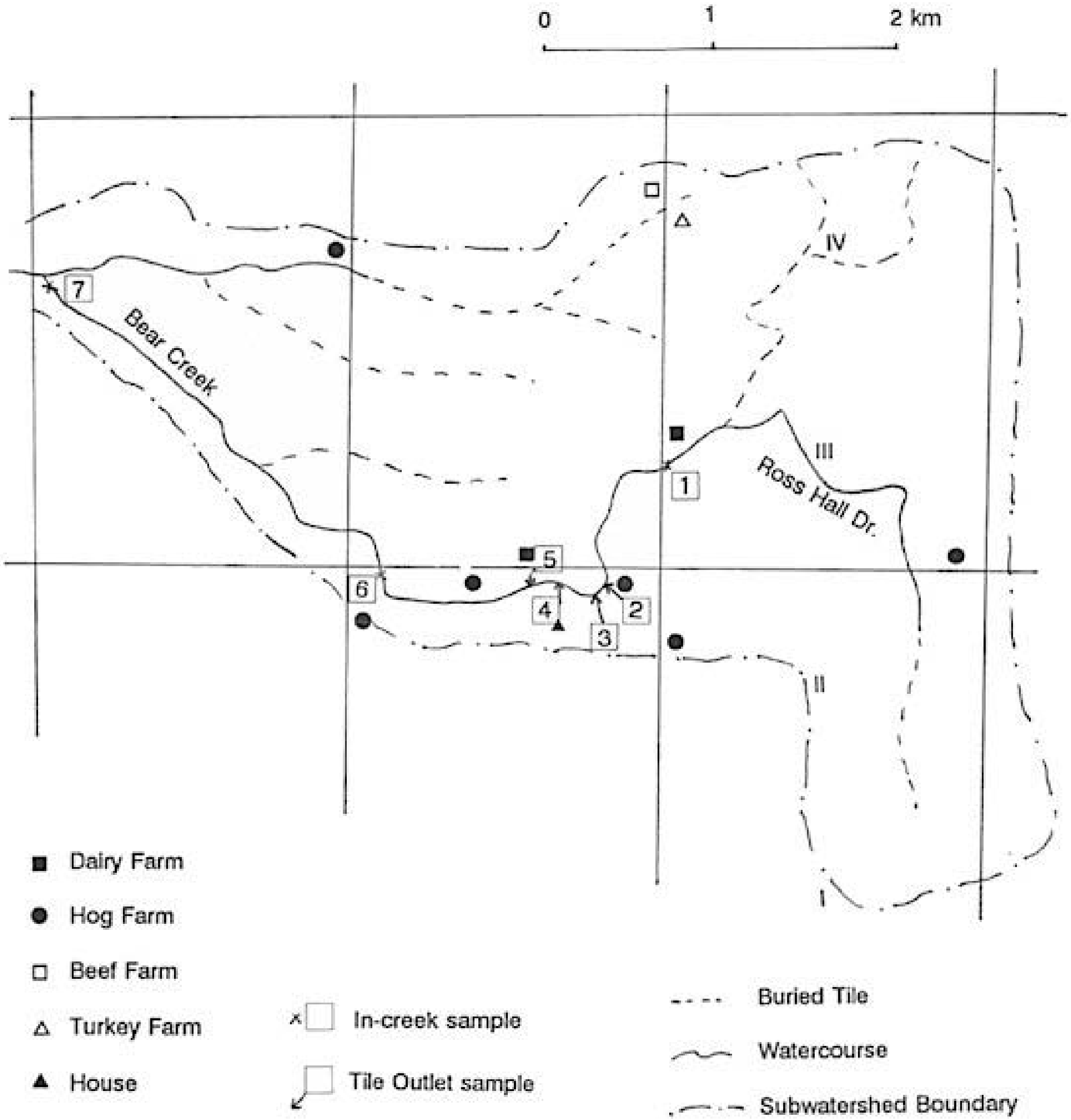


Figure 6: Location of the sampling sites in Zone 3.

CHAPTER 3

BACTERIA TYPING

3.1 CHARACTERISTICS OF *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an intestinal, pathogenic bacterium found primarily in the feces of warm blooded animals and humans. It can also be found in natural waters which are polluted with human and animal waste. When present in bathing water, it can cause eye and ear infections (Jones and Barlett, 1985). It can also cause other infections when ingested in contaminated food or water. This is especially true in institutions where it can spread readily and from a number of sources. *P. aeruginosa* is very resistant to antibiotics and so infections of this bacteria can be difficult to eradicate and have particularly serious consequences.

Cultures of *P. aeruginosa* smell of grapes and this is a diagnostic indicator of its presence. *Pseudomonas aeruginosa* is a strictly aerobic bacterium which means it needs oxygen to survive. In the intestines of mammals, conditions are anaerobic. However, when the organism is excreted from the animal it is exposed to air and its survival is enhanced. In contrast, Fecal coliforms and E. coli bacteria tend to die off when exposed to air.

In open watercourses, *P. aeruginosa* does not multiply readily. However, when temperatures are warm and there is an ample supply of nutrients such as in milkhouse wash water, multiplication can occur. *P. aeruginosa* does not tolerate very cold temperatures (below 2 or 3°C) but survives for long periods of time in the summer. It is stable from 5-10°C but is killed if frozen or dried out. It is also unaffected by pH, metals, antibiotics, etc. In other words, it is a very hardy species.

P. aeruginosa is useful for analysis because it can be found in nature, is physiologically adaptable, and is resistant to many microbial agents.

Presence in the Environment:

P. aeruginosa is not found in the gut of every human or animal. For example, less than 80% of cattle contain it. In contrast, human sewage samples and samples from liquid manure pits often contain *P. aeruginosa* since they are composites, combining the feces of several individuals. Concentrations can be as high as 75,000/100 ml in sewage samples.

3.2. BACTERIA TYPING METHODS

There are basically three laboratory techniques used to identify *Pseudomonas aeruginosa*. These are: serotyping, pyocin typing and phage typing. A description of each of these procedures as conducted by the MOE lab is included in Appendix A. Descriptions can also be found in Govan (1978).

Serotypes are expressed as a number from 1 to 13. Occasionally, when the laboratory procedure is repeated, a certain strain will record a different serotype from the type it recorded initially. These multiple types are separated by a comma (ie. 2,3 or 7,8).

There is limited data on animal sources of *P. aeruginosa* and consequently there are no particular serogroups of *P. aeruginosa* associated with animals documented to date. The incidence of each serogroup or strain apparently varies with the herd examined.

Serogroups in surface and sewage water are similar to the serogroups found in healthy people living in the same area (Lanyi *et al*, 1966-67). Cross contamination can also occur between humans and their livestock.

Pyocin types are expressed as a five-digit number. The numbers and their order identify a particular unknown strain or isolate of *P. aeruginosa* quite specifically. The maximum number that can be recorded is 77773. A pyocin number of 00000 is deemed untypeable (see Appendix A for further details). There are 105 different pyocin types which have been determined to date.

As in the serotypes, occasionally when the pyocin test is repeated, a different pyocin type is produced. These multiple pyocin types are expressed as a two or three number combination separated by a comma (,) such as 77773,76773.

Manure testing:

In total, sixteen separate manure samples, both liquid and solid, were analyzed. In the first set of samples which were collected, *Pseudomonas aeruginosa* was not detected. Initially it was thought that the great number of fecal coliform and *E. coli* bacteria in the manure made it difficult to find the *P. aeruginosa* which is present in lower numbers.

To reduce the number of fecal coliforms in the manure samples, Nalidixic Acid was injected into the sample in the lab. Nalidixic Acid kills fecal coliform bacteria but has no effect on *Pseudomonas* bacteria. Unfortunately, the next set of manure samples analyzed did not reveal the presence of *P. aeruginosa* even with the acid added except for one sample of liquid swine manure.

While the fecal coliforms may have been hindering the detection of *Pseudomonas*, it was obvious there was another factor acting here as well. It was postulated that due to the fact that only 10-20% of livestock actually contain *P. aeruginosa* in their gut, fecal samples from one animal would have a similar chance of containing this bacteria. Samples of liquid swine manure have a greater chance of containing *P. aeruginosa* since the feces of several hundred pigs are mixed together in the pit. In fact, one of the liquid swine samples did contain *P. aeruginosa* and was subsequently serotyped and pyocin typed.

3.3 LITERATURE REVIEW

Very few studies have been carried out on bacteria typing techniques using environmental samples.

A recent study conducted by the Toronto office of the Ministry of the Environment utilized the technique of Endonucleal Restriction (MOE, 1989). This is a fingerprinting technique used to identify bacteria and their sources. The technique involves extracting the DNA from the bacteria cell and subsequently digesting it to break up the DNA into nucleotides. This system has been successfully used in hospitals to track sources of infection. The researchers from MOE Toronto used this technique on fecal samples of ducks and geese collected at Metropolitan Toronto beaches. Dozens of patterns emerged from the goose manure and a computer was needed to store and analyze all of the information. They concluded that the technique was too time consuming, costly, and the results too variable to draw conclusions.

A later study conducted in Spain (de Vicente et al, 1989) examined the serological and pyocinogenic characteristics of *Pseudomonas aeruginosa* strains isolated from polluted natural waters. Their main focus was to document the distribution of various strains in fresh water versus sea water. No attempt was made to link the strains to a particular source of pollution. The study did find that serotypes 1 and 6 were the most prevalent especially under high pollution conditions.

CHAPTER 4

RESULTS AND DISCUSSION

The results of the laboratory analysis for all of the water samples collected are included in Appendix B. Listed are the concentrations of fecal coliforms, fecal *streptococci, E. coli* and *Pseudomonas* as well as the serotypes and pyocin types for each isolate. In many cases, several isolates were tested per sample. These are separated by a semicolon (;). Where multiple pyocin types or serotypes were recorded for a particular isolate these are separated with a dash (-) in the Appendix tables only due to the small print and the density of the data. Normally a comma (,) is used.

4.1 OCCURRENCE OF *PSEUDOMONAS* IN THE SAMPLES

A total of 245 water samples were submitted to the laboratory for analysis. Of these 99 (40%) did not contain *P. aeruginosa* bacteria and so could not be serotyped or pyocin typed. The remaining 146 (60%) samples did contain *Pseudomonas aeruginosa*.

A breakdown of the percentage of samples and the concentration of *P. aeruginosa* is listed in Table 1. Most samples contained *P. aeruginosa* in low concentrations below 20/100 ml water. However, 20% of the samples did contain this bacterium in concentrations above 100/100 ml water.

Further breakdown of the data was carried out to examine the concentration of *Pseudomonas aeruginosa* at various sources (ie. tile outlets, drains, creek, beach). This data is listed below.

As expected, *P. aeruginosa* was absent in over half of the samples taken from the beach and Bear Creek. This is likely due to the their distance from direct pollution sources and the dilution factor. Samples from the beach contained *Pseudomonas* less than 20% of the time.

Table 1. Frequency of occurrence and concentration of *Pseudomonas* in samples.

Conc. of *P. aeruginosa* (#/100 ml water)	Number of samples	Percentage of samples
0	99	40
4 - 19	63	26
20 - 99	36	15
100 - 999	38	16
> 1000	9	5
Total	245	100

Table 2. Frequency of occurrence of *Pseudomonas* in different sampling sources.

Conc. of *P. aeruginosa* (#/100 ml water)	% of Samples Containing *Pseudomonas*			
	Tiles	Drains	Creek	Beach
0	16	48	58	81
4 - 19	26	30	22	13
20 - 99	23	12	6	6
> 100	34	10	14	0
Total	100	100	100	100

The tile outlets and their receiving drains, on the other hand, contained *P. aeruginosa* more than half of the time. Over 50% of the samples taken from tile outlets contained *P. aeruginosa* in levels above 20/100 ml.

The low frequency of *Pseudomonas aeruginosa* in the samples illustrates the difficulty of this type of study. A great number of samples need to be collected to make up an adequate data base.

Pseudomonas **versus Fecal coliform concentrations**

In general, low *P. aeruginosa* levels were associated with low fecal coliform levels and high *P. aeruginosa* concentrations were present when fecal coliform levels were high. Table 3 summarizes the relationship between these two groups of bacteria. This is an important relationship to note since this study examined *Pseudomonas aeruginosa* bacteria and health officials use fecal coliforms to determine swimming water safety. These figures illustrate that indeed we can look at *P. aeruginosa* as an indicator of general bacterial pollution which influence our swimming areas.

Table 3. *Pseudomonas* versus Fecal coliform concentrations**.**

Pseudomonas aeruginosa (# colonies/100 ml) (range of levels)	Fecal coliform (# colonies/100 ml) (geometric mean conc.)
absent	3,877
4 - 19	128,882
20 - 99	157,529
100 - 999	138,511
> 1000	3,051,144

4.2 SEROTYPING RESULTS

A few common serotypes were found repeatedly in this study. The most common was serotype #6 which was found 34% of the time followed by serotypes #1 and #3 which were detected 19 and 10% of the time respectively. Serotypes #1 through 13 (excluding 12) were all detected at least once. In addition, 11 different multiple serotypes were also recorded (ie. 2,5 and 7,8).

Table 4 lists the percentage occurrence of each serotype found in this study along with the results from other studies in Southwestern Ontario. In all cases serotypes 6, 1 and 3 were the most common. The most uncommon serotypes in this study were #5, 7, 12 and 13 which were found less than 1% of the time.

The results indicate that serotyping is not very useful on its own because of the lack of differentiation. The common serotypes were found virtually throughout the study area in tiles, drains, creeks and at the beach. No link could be made between one serotype and one pollution source.

Table 4. Serotypes of *Pseudomonas aeruginosa* from Various Sources.

Percent Occurrence For Serotypes Of *Pseudomonas Aeruginosa*

Serotypes –>	1	2	3	4	5	6	7-8	9	10	11	13-14	16	Other
BEAR CR *	17	2	9	5	1	31	6	3	3	2	0	0	21
BEACH	34	0	8	7	7	26	7	3	2	9	1	0	--
RIVER	10	2	14	4	4	53	8	3	0	2	2	0	--
TRIG.	12	3	15	6	6	26	6	12	1	7	3	0	--
AUSABLE	15	2	6	6	5	50	4	6	1	3	0	1	--
STP'S	11	17	7	0	4	35	0	5	5	6	0	0	8

* BEAR CREEK = THIS STUDY STP's = Sewage Treatment Plants

DATA OBTAINED FROM MOE LONDON

4.3 PYOCIN TYPING RESULTS

Due to the lack of differentiation in the serotype analysis, pyocin typing was carried out on the isolates. Of the 342 isolates which were analyzed, 211 different serotype-pyocin type combinations were found. Thus 62% of the isolates analyzed in this study produced unique, one-of-a-kind, sero-pyocin types. Previous studies found only 105 pyocin types.

Although 211 sero-pyocin types were found, this included only 154 unique pyocin types. This is because some pyocin types were associated with a number of different serotypes. For example, pyocin type 77773 occurred 60 times but it was found in combination with 12 different serotypes. Therefore the serotype-pyocin type combination 6-77773 may not be the same as 10-77773 or 1-77773. A list of all of the pyocin types recorded in ascending order is listed in Appendix C.

Below is a list of the frequency of occurrence of the pyocin types by their series number. For example the 5-series includes pyocin types which begin with the number 5 such as 57773 or 56373. The results indicate that the 7-series is the most common overall accounting for 38% of the isolates followed by the 5-series with 26% and the 4-series with 12%.

0-Series	=	7%
1-Series	=	8%
2-Series	=	3%
3-Series	=	3%
4-Series	=	12%
5-Series	=	26%
6-Series	=	4%
7-Series	=	38%

Tables 5a-5c summarize the results of the serotyping and pyocin typing for each sampling location by Zone. The number of isolates analyzed per site varied due to the fact that *Pseudomonas aeruginosa* was not present consistently at all sites.

Table 5a. Results of analysis for Zone 1.

ZONE1. SEROTYPES AND PYOCIN TYPES BY L0CATION

Janes Dr. Upstm (Cattle, Dairy)		Munic Tile (Cattle)		Small Tile (Septic)		Apartment Tile (Septic)		Janes Drain Downstream		Janes Drain Beach Inlet		Bear Creek Beach Inlet		Cook Drain		Left + Right Beach	
	1		2		3		4		5		6		7		8		9+ 10
1	10002 (3)	1,2	00203	1	00031 (2)	1	30472	1	00032	1	10001 (2)	6	11003	3	76340	1	10002
1	14022 (2)	6	16373	1	00032	2,5	40220	6	01223	N	56373	6	31002 (2)	3	76740	2,3	51202
1	50023	N	40002	1	04072	2,5	40222	1	10002 (4)	6	56373	3	40620	7,8	74763	2,3	61220
8	74373	N	40223 (2)	1	10130	3	40431	1	10020	3	71602	6	61002 (2)	4	7777 (3)	2,5	74771
5	75773	1,2	40223	1	10220	3	40631	1	10022	6	76776	6	61013	----	--------	9	74771
10	77773	N	50201			N	41433	1	10030					6	73573	2,3	76740
		6	56373			3	47633	1	10072					2	75771	4	77672
6,13	41623	6	76373			3	50623	1	10420					2	75773	----	-------
6,13	57723	11	77773			3	50631 (2)	1	30232					8	76373	6	56773
		6	77773			3	53673	3	40022								
		N	77772 (3)			6	56773 (3)	N	40203								
						1	57373	3	40621								
						1	57773	3	40631								
						6	57773 (2)	6	41223								
						N	57773	1	50000								
						6,10	57773	1	50002								
						2,5	70473	1,2	50223								
						N	70633	2	51213								
						3	70673	6	56373								
						3	73772	1,2	57773								
						2,5	74773	4	67651								
						N	76373	N	70032								
						6	76373	1	70252								
						10	76753, 72753	3									
						10	76763	8	74373								
						6	76773	4	77773 (5)								
						4	77772	6	77773								
						4	77773 (2)	------	---------								
						1	77773	6	56773								
						6	77773										

---- = water samples above, sediments below line

1 10002 (3)

serotype — pyocin type — # times found

Table 5b. Results of analysis for Zone 2.

ZONE 2. SEROTYPES AND PYOCIN TYPES BY LOCATION

Bear Creek Upstream		Zavitz Drain Tile Outlet (Hog + Cattle)		Zavitz Drain Downstream		Bear Creek Downstream		Dolan Drain Tile Outlet (Hog)		Dolan Drain Downstream		Bear Creek Downstream	
1		3		4		5		6		7		8	
6	56773	9	70663	3	40020	6	05323	6	00000	6	00433 (3)	N	02603
6	62453	6	76173, 76373	3	72073	6	05725	1	00433	1	02461	N	04723
12,13	74771,74773	7,8	76373			3	30461	6	00433	1	70023	3	40022
13	74773	6	77773 (2)			3	40000	6,13	01413	6	20013 (2)	3	50022
7	77173	10	77773			1	40020	6,13	01473	6	20413	4	54363
11	77773					1	40202	6	01473	6	20453	6	57773
						N	74776, 74773	N	01473	6	20473	6,13	57773
						11	77773	6	01621	6,73	30033	6	57773
						6	77773 (2)	6	11473	6	31433	6,13	57773
								6	20033	6,13	35473	6	57773
								6	20433 (2)	1	40003	6	57773
								6	21563	2,13	40013	6	74771
								1	40002	1	40413 (2)	N	74773
								2	40003	6	40413	4	74773
								7	40033	2,11	40433	6	76753
								6	40220	2,17	44403	4	77773
								N	40413	1	50630	6	77773
								1	41013	7	52210		
								6,13	41413	17	54363 (2)		
								1	42571	6	56373 (2)		
								6	51433	6	56773		
								6	57473	N	57773		
								2,5	56353	6,13	57773 (3)		
								6	56373	6	57773 (2)		
								6,13	57773 (2)	2	60013		
								6	57773 (3)	9	70443		
								6	60413	6	75433 (2)		
								9	61413	1	76773		
								1,2,3	61433	6	77573		
								1	62362	7,8	77773		
									62363	6,13	77773		
								9	70443 (3)	6	77773		
								1	71413	11	77773		
								6	71433 (2)	----	-------		
								5	74771	5,16	76760		
								6	75433	4,71	77772		
								7	76223	77	77772		
								6	77033	2,11	77773		
								2,6	77773				
								11	77773				
								----	----------				
								6	20432				
								6	57773				
								6	60012				
								1,2	60012				
								1,10	60012				
								N	60032				
								1,10	62012				
								6	70132				

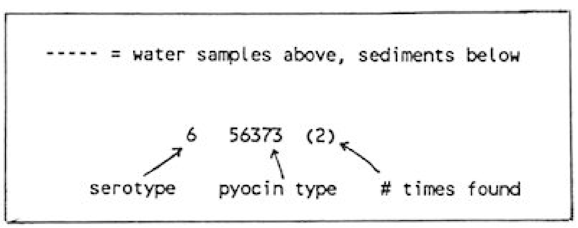
---- = water samples above, sediments below line

6 56773 (2)

serotype — pyocin type — # times found

Table 5c. Results of analysis for Zone 3.

Ross-Hall Drain Upstream		Municip Tile #2 (Hog)		Municip Tile #1 (Hog)		Tile Outlet (Septic)		Tile Outlet (Dairy)		Ross-Hall Drain Downstream		Rear Creek Downstream	
1		2		3		4		5		6		7	
8	50463	1	00022	1	10223	3	43473	1	10413,12413	1	10223	1	10032
8	74763	1	10235	6	56372 (2)	3	52673	6	32051	6	35163	1	40020,40022
7,8	77772	6	16560	2,4	77563	6	56373 (5)	2	40600	2,3	40620	1	50022
7,8	77772	6	22371	7,8	77772	6	56373,76373	6	56373 (2)	3	52673 (4)	13	54373
----	--------	3	40200	7,8	77773 (7)	7,8	56542	10	56773 (2)	N	54373	9	74763
1	10420	6	57773	7	77773	7,8	57562	6	56773 (3)	3	56373	6	76371
		4	73573,77573	8	77773(2)	2	57573	9	71601	6	56373	----	----------
		5	74373	2	77773	6	57773 (2)	2	77773	6	56773	6	55373
		10	74763	10	77773	6	76773			5	74763	6	56372
		9	75761	6	77773	6	77773 (2)			3	77753	6	56373
		6	76773			10	77773			6	77773	6	56773
		8	77772 (3)									7,8	74760
		8	77773 (2)							2,3	50063	7,8	76760
		1	77773							2,3	50220	7,8	77772
		7,8	77773(2)							2,3	50222	7,8	77773
										2,3	52473		
										2,3	52663		
										2,3	53473		
										7,8	77773		



4.4 TRACING TYPES FROM UPSTREAM TO DOWNSTREAM SITES

All of the sero-pyocin types recorded in the water and sediment samples were pooled and sorted to see if certain types occurred in more than one sampling site. Table 6a lists the results for the sero-pyocin types and table 6b lists the results for the pyocin types alone.

In total only 26 of the 211 sero-pyocin types found in this study were recorded in more than one sampling location. Of these, 13 (50%) were found in different sites within the same zone but not in the other two zones. Another eight types (31%) were found in two zones and the remaining five types (19%) were recorded in all three zones. The five types which occurred in all three zones may be considered ubiquitous and are not likely to be indicative of any one source. They are:

6-56373
6-56773
6,13-57773
6-77773
10-77773

Sixteen sero-pyocin types were found in a specific tile outlet and again downstream in the same zone. An additional 8% (2/26) were found upstream and downstream in the water of a particular drain or creek. Thus, 70% of these sero-pyocin types could be traced from an upstream site (usually a tile outlet) to a downstream site in the creek. However, many of these are the ubiquitous types listed above and may not necessarily indicate that we traced the type from a location upstream. They may, in fact, simply be present everywhere.

Sediment samples collected near tile outlets did not share similar sero-pyocin types as the water sample from that tile outlet, with one exception. Type 6,13-57773 was found in the tile outlet on Dolan Drain and in the sediment samples taken close by. However, this is a ubiquitous type as well.

Table 6a. Sero-pyocin types found in more than one site or zone.

SERO-PYOCIN	SITE/ZONE										# SITES	# ZONES	# ISOLATES
1- 00032	3/1	5/1									2	1	2
6 - 00433	6/2	7/2									2	1	5
1- 10002	1/1	5/1	9/1								3	1	8
1- 10223	3/3	6/3									2	1	2
1- 10420	5/1	1/3s									2	2	2
3 - 40022	5/1	8/2									2	2	2
3 - 40631	4/1	5/1									2	1	2
3 - 52673	4/3	6/3									2	1	5
6 - 56372	3/3	7/3s									2	1	4
6 - 56373	2/1	5/1	6/1	6/2	7/2	4/3	5/3	6/3	7/3s		9	3	15
6 - 56773	4/1	5/1s	9/1s	½	7/2	5/3	6/3	7/3s			8	3	12
6 - 57773	4/1	6/2	6/2s	7/2	8/2	2/3	4/3				7	3	15
6,13-57773	4/1	6/2	6/2s	7/2	8/2	2/3	4/3				7	3	7
9 - 70443	6/2	7/2									2	1	4
8 - 74373	1/1	5/1	2/3								3	2	3
6 - 75433	6/2	7/2									2	1	3
6 - 76373	2/1	4/1									2	1	5
6 - 76773	4/1	2/3	4/3								3	2	4
7,8- 77772	1/3	3/3	7/3s								3	1	4
7,8- 77773	7/2	2/3	3/3	6/3s	7/3s						5	2	12
1- 77773	4/1	2/3									2	2	2
2 - 77773	3/3	5/3									2	1	2
4 - 77773	4/1	5/1	8/1	8/2							4	2	11
6 - 77773	2/1	4/1	5/1	3/2	5/2	7/2	8/2	3/3	4/3	6/3	10	3	13
8 - 77773	2/3	3/3									2	1	4
10- 77773	1/1	3/2	3/3	4/72							4	3	4
11- 77773	2/1	1/2	5/2	6/2	7/2						5	2	5

2/1 = site 2 zone 1 2/1s = site 2 zone 1 sediment (See Table 2 for key to sites and zones)

SERO-PYOCIN TYPES FOUND IN SEVERAL SITES AND ZONES

6 - 56373
6 - 56773
6 - 57773
6,13 - 57773
7,8 - 77773
4 - 77773
6 - 77773

Table 6b. Pyocin types found in more than one site or zone.

PYOCIN	SITE/ZONE									# SITES	# ZONES	# ISOLATES
00032	3/1	5/1								2	1	2
00433	6/2	7/2								2	1	5
10002	1/1	5/1	9/1							3	1	3
10223	3/3	6/3								2	1	2
10420	5/1	1/3s								2	2	2
40002	2/1	6/2								2	2	2
40003	6/2	7/2								2	1	2
40020	4/2	5/2								2	1	2
40022	5/1	8/2								2	2	2
40220	4/1	6/2								2	2	2
40413	6/2	7/2								2	1	4
40620	7/1	6/2								2	2	2
40631	4/1	5/1								2	1	2
50022	8/2	7/3								2	2	2
52673	6/3	4/3								2	1	5
54363	7/2	8/2								2	1	3
54373	6/3	7/3								2	1	2
56372	3/3	7/3s								2	1	4
56373	2/1	5/1	6/1	6/2	7/2	4/3	5/3	6/3	7/3	9	3	17
56773	4/1	5/1s	9/1s	1/2	7/2	5/3	6/3	7/3s		8	3	14
57773	4/1	5/1	6/2	6/2s	7/2	8/2	4/3	2/3		8	3	26
70443	6/2	7/2								2	1	4
74373	1/1	5/1	2/3							3	2	3
74763	8/1	1/2	2/3	6/3	7/3					5	2	4
74771	9/1	6/2	8/2							3	2	4
74773	4/1	1/2	8/2							3	2	4
75433	6/2	7/2								2	1	3
75773	1/1	8/1s								2	1	2
76373	2/1	4/1	8/1s	3/2						4	2	5
76740	8/1	9/1								2	1	2
76760	7/2s	7/3s								2	2	2
76773	4/1	7/2	2/3	4/3						4	3	4
77772	4/1	7/2s	1/3	2/3	3/3	7/3s				6	3	10
77773	2/1	4/1	5/1	8/1	3/2	5/2	6/2	7/2	7/2s	17	3	60
cont'd	8/2	2/3	3/3	4/3	5/3	6/3s	6/3	7/3s				

6/2 = SITE 6, ZONE 2
6/2s = SITE 6, ZONE 2, SEDIMENTS

Seven sero-pyocin types found in sediments matched types found further upstream in the water and in sediments. For example 6-56773 was found in the sediments of Janes Drain as well as the beach sediments and upstream in the tile outlet (septic). As well, 7,8-77773 was found in two municipal tile outlets in Zone **3** as well as in the sediments collected at two downstream locations.

Beach Samples:

Table 7 lists the small number of isolates found in the water at the Warwick Beach and any matches in serology upstream. Only sero-pyocin type 1-10002 was found at both the upstream and downstream sections of Janes Drain in the Village of Warwick and at the beach. Pyocin type 74771 was also found at the tile outlet in Dolan Drain of zone 2 near hog farms, although the serotypes did not match. No other matches were found. Additional sampling at the beach may reveal further matches.

The discovery of 1-10002 at these three locations may indicate the study was successful in tracing a particular sero-pyocin type from an upstream to downstream location. A much larger data base is required before conclusions can be drawn, however.

Table 7. Matching Sero-Pyocin and Pyocin Types at Warwick Beach.

Sero -	Pyocin Type	Location
1-	10002	Janes Dr., upstream + downstream
9-	74771	Dolan Tile Outlet (pyocin type only)
2,5-	74771	Dolan Tile Outlet (pyocin type only)
2,3-	51202	No match
2,3-	61220	No match
2,3-	77672	No match
4-	77672	No match

4.5 TILE OUTLETS NEAR HOG FARMS

The results of the sample analysis from three tile outlets which were thought to be polluted with bacteria from nearby hog farms were pooled to see if there were any trends. The serotypes and pyocin types are listed in Table 8. The results of the analysis carried out on the liquid hog manure are also given as well as results from the Ausable Bayfield Conservation Authority (ABCA, 1988).

Of the 90 isolates analyzed, 65 unique sero-pyocin types were recorded, including 51 different pyocin types. Thus 72% of the isolates were one-of-a-kind sero-pyocin types. This is a large degree of variability. It is interesting to note that serotype #3, which is usually quite common (see Table 1), was only found once.

The sero-pyocin types which occurred most frequently in tiles near hog farms in descending order were:

7,8-77773
6-57773
9-70443
8-77772
6-77773

Fifty-one percent of the pyocin types found in the tile outlets were of the 7-pyocin series. The 0-series, 4-series and 5-series were also present 10% or more of the time.

Of all of the sero-pyocin types found in the samples collected in the ABCA sites, only 6-56373 was also found the tile outlets of this study. The other types found in their sites were unique and not found in this study.

Sero-pyocin type 5-74171 which was found in the hog manure did not match any of the types found in the tile outlets. The sediment samples which were taken near these tile outlets did not match any of the sero-pyocin types of the water samples either. However, the data base was small for both the manure and sediments and so the full range of possible types are yet to be recorded.

Table 8. Serotypes and Pyocin types found near Hog Farms.

SERO-PYOCIN TYPES OF TILE OUTLETS IN BEAR CREEK	(cont'd)	(cont'd)	SEDIMENTS	HOG MANURE	ABCA SAMPLES
6 - 00000	1 - 40002	9 - 70443 (3)	6 -20432	5 - 74171 (2)	6 - 01211 (2)
1 - 00022	2 - 40003	9 - 70663	6 -57773		6 - 36753
1 - 00433	1 - 40033	1 - 71413	6 -60012		3 - 40000
6 - 00433	3 - 40200	6 - 71433 (2)	1,2 -60012		1 - 40062 (2)
6,13 - 01413	6 - 40220	5 - 74373	1,10 -60012		6 - 46373 (3)
N - 01473	N - 40413	10 -74763	N -60032		6 - 46773 (2)
6,13-01473	1- 41013	5- 74771	1,10 -62012		6 - 56373 (14)
6 - 01473	6,13-41413	6 - 75433	6 -70132		
6- 01621	1 - 42571	9 - 75761			
1- 10223	6 - 51433	1 - 76223			
1- 10235	6 - 51473	7,8-76373			
6 - 11473	2,- 56353	6 - 76773			
6 - 16560	6 - 56372 (2)	6 - 77033			
6 - 20033	6 - 56373	2,4-77563			
6 - 20433 (2)	6,1 57773 (2)	7,8-77772			
6 - 21563	6 - 57773 (4)	8 - 77772 (3)			
6 - 22371	60413	2,6-77773			
	61413	7,8-77773 (9)			
	1,2,- 61433	1 - 77773			
	- 62362	2 - 77773			
	62363	6 - 77773 (3)			
		7 - 77773			
		8 - 77773 (4)			
		10 - 77773 (2)			
		11 - 77773			
		4- 73573,77573			
		6- 76173,76373			

	MOST COMMON TYPES			FREQUENCY OCCURRENCE OF PYOCIN SERIES	
%	sero-pyocin	%	pyocin	0-SERIES	= 10%
				1-SERIES	=4%
10	7,8 - 77773	26	77773	2-SERIES	=6%
4	6 - 57773	4	57773	3-SERIES	=0%
3	9 - 70443	4	70443	4-SERIES	= 10%
3	8 - 77772	3	01473	5-SERIES	=13%
3	6 - 77773			6-SERIES	=6%
				7-SERIES	= 51%

4.6 WATER SAMPLES NEAR CATTLE AND DAIRY FARMS

The data from the two sites (Site 1, Zone 1 and Site 2, Zone 3) which drained areas near beef and dairy farms were pooled. The results are listed in Table 9.

Although the data base is small, of the 17 isolates analyzed, the most common sero-pyocin types recorded were 1-10002 and 6-56773. Pyocin type 56773 occurred 25% of the time but may be a ubiquitous type. Although no results from cow manure were obtained from this study, results from cow manure and water samples near cattle farms in the Ausable Bayfield Conservation Authority watershed are given for comparison.

Only pyocin type 77773 and 56373 was common to both the manure and the water samples. However, these pyocin type are very common throughout the watershed and is unlikely to be indicative of just cow manure. No matches between sero-pyocin types were found in the water samples and the sediments. The data base for the sediments was very small (2 isolates).

Overall, the 5-Series of pyocin types was the most common in the samples near beef and dairy operations.

4.7 SEPTIC SAMPLES AND SEWAGE TREATMENT PLANT STUDY

Septic systems work on an anaerobic principle to decompose waste. *P. aeruginosa,* on the other hand, is a strictly aerobic bacterium but interestingly enough, it was found in tile outlets which drained malfunctioning septic systems. This suggests that the anaerobic breakdown is bypassed in these illegal systems.

The MOE lab in London has completed pyocin typing on samples of sewage waste taken from the Sewage Treatment Plants (STP's) of 22 small towns and villages in southwestern Ontario (Appendix D). Table 10 compares these results those from the Bear Creek watershed and the ABCA water samples.

Table 9. Serotypes and pyocin types found near dairy and beef farms.

Water Samples Sero - Pyocin Type			Sediments Sero - Pyocin Type	
(3)	1	-10002	6,13	-41623
(2)	1	-14022	6,13	-57723
	1	-10413,12413		
	6	-32051		
	2	-40600		
	1	-50023		
(2)	10	-56773		
(3)	6	-56773		
	9	-71601		
	2	-77773		
	10	-77773		

Ausable Bayfield Conservation Authority Samples

Water Samples: Cattle Farm	Water Samples: Dairy Farms		Manure Dairy
46373	76373		00762
56373	77772		23763
77773	77773		24372, 25372
			21272, 20272
		(5)	77773
		(2)	56373

(3) = found 3 times

Table 10. Serological results of STP and septic tile samples.

SEWAGE TREATMENT PLANT						SEPTIC TILE SAMPLES			
SEROTYPE	PYOCIN	SEROTYPE (cont'd)	PYOCIN	SEROTYPE (cont'd)	PYOCIN	SEROTYPE	PYOCIN	SEROTYPE (cont'd)	PYOCIN
1	N	2,3	50001	9	70641	1	30472	2,5	70473
3	N	3	52222	9	72401	2,5	40220	N	70633
N	00000	9	53141	N	72771	2,5	40222	3	70673
N	00251	9	53540	2,3	73020	3	40431	3	73772
1,6	00440	6	54773	9	73140	3	40631	2,5	74773
N	00632	1	56160	5	73161	N	41433	N	76373
3	02441	6	56373 (4)	N	73473	3	43473	6	76373
1	10020	1,2,3,4	56773	10	73571	3	47633	10	76763
1	10040	6	56773 (3)	2	76373	3	50623	6	76773 (2)
1	10203	6	57373 (2)	6	76373	3	50631 (2)	4	77772
1	10213	6	57771	10	76731	3	52673	1	77773
1	10440	1,2	57773	6	76751	3	53673	4	77773 (2)
1	10532	N	57773	6	76771 (3)	6	56373 (5)	6	77773 (3)
1	10632	6	57773 (7)	6, 11	76773	7,8	56542	10	77773
1	12672	10	57773	2	76773	6	56773 (3)	10	76753,76773,72753
6	14542	3	64423	5	76773	6	56373, 76373		
1	20040	6	66751	6	76773	1	57373		
N	30432			11	77371 (4)	7,8	57562		
1	30472			6	77771	2	57573		
1	31533			1,2,10	77773	6,10	57773		
1	40022			2,7,8	77773	N	57773		
N	47633			N	77773 (2)	1	57773		
3	41231			1	77773	6	57773		
3	41623			5	77773 (2)				
3	41661			6	77773 (7)				
1	42670			10	77773 (2)				
6	46551			11	77773 (2)				
1	46770								

Four most commonly occurring sero-pyocin types in descending order:

S.T.P.'s		Bear Creek		ABCA
6 -	77773	6 -	56373	57773
6 -	57773	6 -	57773	77773
11-	77371	6 -	56773	56773
6 -	56373	6 -	77773	

Of the 100 isolates analyzed from the STP's, 66 different sero-pyocin types were found including 56 different pyocin types and 15 different serotypes. The large variability in pyocin types leaves little room for trend development since 66% of the isolates produced sero-pyocin types which were recorded only once.

The most common pyocin types in the STP's and septic tile outlets were the 5-series and 7-series. Common pyocin types included 77773 and 57773. The most common serotype was #6 which occurred over a third of the time. Serotypes #1 and #3 were also common.

4.8 SERO-PYOCIN TYPES FOUND IN DIFFERENT TILES

Table 11 compares the sero-pyocin types and pyocin types found in different types of tile outlets and manure samples. Only those found in more than one different source are listed. Many of these types are similar to those termed ubiquitous in section 4.4. This comparison further supports the theory that these sero-pyocin types and pyocin types may not be indicative of any particular source.

Only samples near hog farms had pyocin types of the 0, 2 and 6-series. There were no types of the 3-series for the samples near hog farms. This may be the beginning on an indication towards trend development in isolating hog pollution from other types of pollution sources.

Table 11. Sero-pyocin and pyocin types with matches from various sources.

	Hog (Tiles)	Cow (Tiles)	Septic (Tiles)	STP (Sewage)	Dairy (Manure)	Hog (Manure)
10 - 77773	x	x	x	x		
6 - 77773	x		x			
2 - 77773	x	x				
1 - 77773	x		x	x		
6 - 76773	x		x	x		
6 - 57773	x		x	X		
6 - 56773		x	x	x		
6 - 56373	x		x	x		
77772	x		x			
76373	x		x	x		
40220	x		x			
77773	x	x	x	x	x	x

4.9 DISCUSSION

Many studies have suggested that the 7-series (pyocin types which begin with the number 7 such as 76373) are very common. The most common types may be ubiquitous or common to several sources Oanzen, 1989). In this study, the pyocin 5-series and 7-series were very common throughout. Many of these types appeared to be ubiquitous.

The rare or unusual types may prove to be more useful as they could be correlated with site specific sources. However, a large enough data base would be needed to discover all of the potential types which are likely to occur. This will be quite an extensive list considering 211 different sero-pyocin types have already been found from the 342 isolates analyzed in this study.

Although different serotypes can produce the same pyocin type, pyocin types may not rely on serotypes in environmental samples (Palmateer, pers. comm.). As a result, pyocin types may be examined in isolation of the serotypes. This is in contradiction with the literature which suggests there is a correlation between the results of these two techniques. However, the literature is largely based on clinical samples. Regardless, the large variability in the pyocin types does not help to discern animal sources either.

The fact that pyocin type 77773 is the maximum number possible under this system combined with the fact that it was the most common type found in this study may indicate some limitation in the laboratory technique. Additional tester strains may subdivide these types further. However, it is difficult to find good reliable and reproducible tester strains on the market.

Cross contamination between animals and humans can further cloud the issue. Other studies have found that humans can exhibit the same pyocin types as the livestock they work with. The cleanliness level of the operation probably affects the closeness of this relationship. Thus, it may not always be possible to separate the septic from the manure waste in a water sample taken at a tile outlet or downstream of a particular farm since the pyocin types may be the same.

In summary, the extremely large number of sero-pyocin types found in this limited study, makes it impossible to sort the types by animal source.

Since the samples taken near hog farms, cattle farms and septic systems recorded a number of types not found elsewhere, indicative types may be numerous. Initially it was thought that if a particular tile outlet or manure sample recorded a specific pyocin type over and over, that this would be its indicative or tell-tale type. However, the types which were found repeatedly at one site also tended to be common throughout the study area watershed. Thus, simply because a particular tile outlet or manure sample recorded a sero-pyocin type frequently, did not necessarily link that sero-pyocin type uniquely to that source.

Once all of the ubiquitous or common types are discovered, they should be set aside to focus more closely on the unique pyocin types. Since over 200 sero-pyocin types have already been found from this limited sampling study, enormous amounts of sampling will be needed to discover all of the potential types which exist. Manure samples from a variety of livestock animals are necessary as well. However, the difficulty of isolating Pseudomonas in these samples remains a challenge.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

This study proved to be a valuable trial for testing the practicality of using pyocin typing and serotyping methods to trace the sources of bacteria in environmental samples. Although it is premature to draw conclusive links between specific pyocin types and specific animal sources, a number of valuable facts were revealed especially in regards to methodology.

1. The study revealed that a large number of samples are required to provide a suitable data base. This is due to the fluctuating occurrence of *P. aeruginosa* in the environment. This bacterium is not as common or as numerous as other intestinal species of bacteria in fecal material and in the environment. A large data base is also needed due to the incredibly large variability in sero-pyocin combination types found. Many more are likely still to be found.

2. One can increase the chances of detecting *P. aeruginosa* in water samples by selecting sites very close to pollution sources and in areas where it has been routinely detected in the past.

3. Manure sample analysis proved to be difficult due the low occurrence of *Pseudomonas* in individual droppings. Composite manure samples such as from a liquid manure pit increase the chances of finding *Pseudomonas* due to the mixing of large numbers of individual fecal droppings in one tank. Nalidixic Acid can be added to the samples in the laboratory to kill the fecal coliforms which may overcrowd the *Pseudomonas*.

4. A large data base is also required from one specific pollution source to separate the trends in pyocin types from the anomalies. This is due to the large variability in pyocin types which exist from a single pollution source such as a tile outlet or

a manure sample. For example, it would be dangerous to predict a trend in pyocin types from human waste based on only five samples since the number of seropyocin types is well over 60.

5. There are several sero-pyocin types which occur quite widely and frequently and may be considered ubiquitous in environmental samples. These should not be used as indicator types of a particular pollution source. These ubiquitous types tend to be of the 5 and 7-pyocin series.

5.2 RECOMMENDATIONS

1. The MOE lab in London should continue to separate out the *Pseudomonas aeruginosa* bacteria from a variety of incoming laboratory samples. They can request the samples be labelled according to suspected source (ie. poultry manure, human waste, etc.). The bacteria can be stored and later serotyped and pyocin typed at a convenient time. The results can be added to the data base already established from this and other studies.

2. The SCRCA should assist the Ministry by recording any submitted samples according to suspected animal or human source. The Authority should also assist the Ministry with any discussion or interpretation of methods and results.

3. Other approaches and techniques which seek to identify bacterial pollution by source should be explored.

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PERSONAL COMMUNICATIONS

Garry Palmateer, February 1990. Ministry of the Environment, London. Head of Microbiology.

Edna Jansen, February, 1990. Ministry of the Environment, London. Microbiologist.

GLOSSARY OF TERMS

Aerobic -	requiring the presence of oxygen
Anaerobic -	functioning without the presence of oxygen
Bacteria -	plural of bacterium
Bacterium -	microscopic unicellular organisms, some causing disease
Feces -	(faeces) - waste matter discharged from bowels
Microbe -	minute living being, micro-organism, especially of disease causing bacteria Microbial Agent

APPENDICES

APPENDIX A (i)
SEROTYPING, PYOCINT TYPING AND PHAGE TYPING

SEROTYPING

Serotyping distinguishes *Pseudomonas aeruginosa* bacteria on the basis of an antigen-antibody reaction. Antibodies are produced in human and animal bodies to fight off antigens. Antigens are disease-causing viruses and bacteria such as *Pseudomonas aeruginosa*. The cell structure of each antibody is specific so that it will only attack and kill its targeted antigen.

In this seroryping procedure, a known antibody is added to an unknown strain of P. aeruginosa in a petri dish and the reaction is observed. If the cells join together like a lock-and-key the cells appear clumped and it is deduced that the antibody has attached to its targeted antigen. If the cells do not attach, other antibodies must be added, one at a time, until a match or serotype is found.

There are only 17 known serotypes and often a few common types appear over and over again in animal and human waste. This is not very useful for our purposes. Therefore, to further differentiate the cells, pyocin typing can be used. For example, a group of samples which were all serotyped as #1 can be further differentiated into a number of different pyocin types.

PYOCIN TYPING

Pyocin typing uses the ability of *Pseudomonas* to produce a protein substance known as pyocin. Pyocins are antibiotic substances which are lethal to other strains of *Pseudomonas* or closely related species. Strains of *P. aeruginosa* are immune to their own pyocin (Govan, 1978).

Procedure:

The following is a summary of the procedure used by Govan (1978). The detailed procedure is in Appendix A.

Exactly 0.3 ml of the unknown isolate is inoculated in a particular pattern (or placed) onto an agar base in a petri dish. The dish is then incubated for 6 hours at 30 C so that the *Pseudomonas aeruginosa* can grow, replicate and produce its pyocin. The petri dish is then removed from incubation and all living bacterial cells are killed using chloroform. The pyocin is then residual on the agar base.

Small amounts of 14 known tester strains of *Pseudomonas* are dropped or overlaid onto the petri dish. The dish is then incubated for another 18 hours at 35 C to allow the tester Pseudomonas to grow and multiply. When the cells cover the agar base, they appear green in colour and this is termed a lawn.

At each location where a tester strain was added, the reaction is observed. If the pyocin inhibited the growth of the tester *Pseudomonas* lawn, there would be a clearing in the lawn (zone of inhibition), or a place where no green colour is visible. This is termed a positive reaction. Alternatively, if the tester *Pseudomonas* continued to grow unaffected by the pyocin, the lawn would appear unaffected and this is termed a negative reaction.

All of this information is recorded on a spread sheet using the octocoding system. A sample sheet is illustrated in Table 1. Under this system, the 14 known tester samples are grouped into three's resulting in 5 major columns. Number values are assigned to each tester. Each grouping of three is assigned number values of 1, 2 and 4 respectively. For example, the first isolate (#15826-1) recorded three pluses in the first column. Thus the values assigned would be the sum of 1+2+4=7. If there is a negative (-) recorded, a zero value is assigned. For example, isolate #15826-3 recorded two negatives and one positive in the first column and this works out to 0+0+4=4.

Occasionally one of the fourteen known reacts positively with the sample, but when the process is repeated, it reacts negatively. These isolates are then recorded with two 5-digit numbers to reflect this. For example, isolate number 15936-2 is denoted by pyocin type 12413 and 10413. The second digit recorded a '2' the first time the experiment was run, yet a '0' the second time.

PHAGE TYPING

Phage typing can be carried out to further differentiate the pyocin types. Of the 3 methods, this is probably the most laborious to undertake. it is also less reproducible than pyocin and serotyping.

Phage are viruses which need a bacterial host such as *Pseudomonas* to survive. Phage can be present at any time and may interfere with the serotyping and pyocin typing techniques. There are 7 different phages which can be identified under an electron microscope. To date, the MOE lab has only an enumeration technique for *Salmonella* and *E. coli*. The technique is therefore not available for *Pseudomonas*.

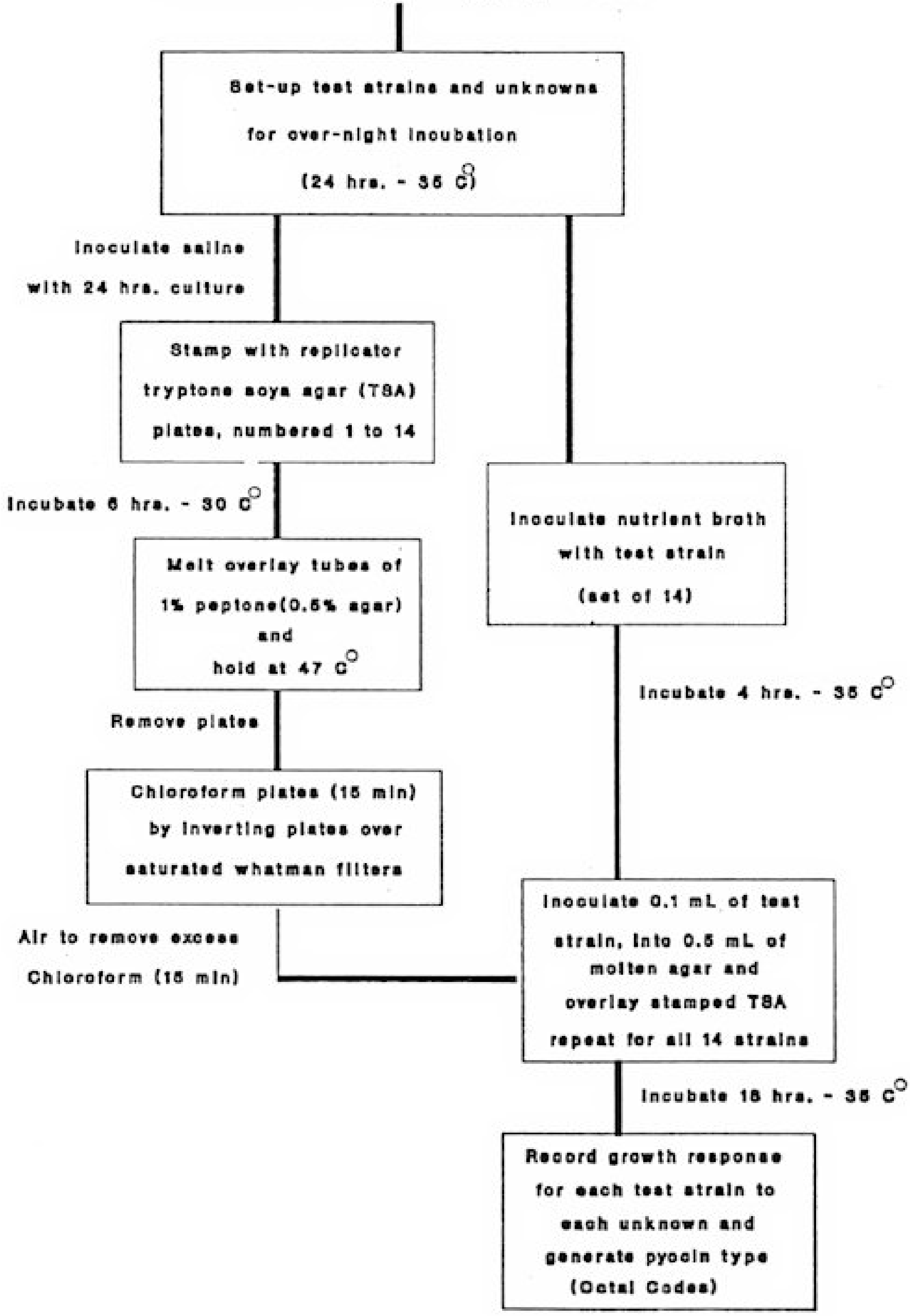
APPENDIX A (ii)

Octocoding chart for pyocin types in Warwick Township.

ISOLATE #	PYOCIN STRAINS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	PYOCIN TYPE
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
15826-1	7			7			7			7			3		77773
2	−	−	+	−	−	−	−	−	−	−	+	−	−	−	
15826-3	4			0			0			2			0		40020
3	−	−	+	−	−	−	−	−	−	−	+	−	−	−	
15827-1	4			0			0			2			0		40020
4	−	−	+	−	−	−	−	−	−	−	+	−	−	+/−	40020
15934-1	4			0			0			2			2/0		40022
5	+	−	−	−	−	−	−	−	−	(+)	+	−	−	+	
15934-2	1			0			0			3			2		10032
6	+	+	−	+	−	+	+	−	−	−	+	+	+	+	
15935-1	3			5			1			6			3		35163
7	+	−	−	−	−	−	−	+	−	−	+	−	+	+	
15935-2	1			0			2			2			3		10223
8	(+)	+	−	−	+	−	−	+	+	+	−	+	+	−	
15936-1	3			2			6			5			1		32651
9	(+)	−	−	−	+/−	−	−	−	(+)	(+)	−	−	+	+	12413
15936-2	1			2/0			4			1			3		10413
10	+	−	+	−	+	−	+	+	−	+	+	+	+	+	
15937-1	5			2			3			7			3		52373
11	+	−	+	−	+	+	+	+	−	+	+	+	+	+	
15932-2	5			6			3			7			3		56373
12	+	−	−	−	−	−	−	(+)	−	(+)	+	−	++	+	
15938-1	1			0			2			3			3		10233
13	+	+	+	−	−	+	+	+	−	+	+	+	+	+	
15938-2	7			4			3			7			3		74373
14	+	−	(+)	−	−	−	−	−	+	−	(+)	(+)	+	(+)	
15939-1	5			0			4			6			3		50463
15	+	+	+	−	−	+	+	+	+	−	+	+	+	+	
15939-2	7			4			7			6			3		74763
16	+	+	+	−	−	+	+	+	+	−	+	+	+	+	
15939-2 NC 11 11-??	7			4			7			6			3		74763
17	(+)	−	−	−	−	−	−	−	−	+	+	−	−	−	
16492-1	1			0			0			3			0		10030

APPENDIX A (iii)

Flow Chart for Pyocin Typing Method



APPENDIX B

BACTERIA AND SEROLOGY RESULTS

BACTERIA AND SEROLOGY RESULTS

ZONE 1 - VILLAGE OF WARWICK AND JANES DRAIN

LOCATION	LAB #	DATE	F. Colif	E. Coli	F. Strep	P. Aeru	Serotype	Pyocin Type	Suspected Source
1 JANES	11286	30 AUG 89	1300	1300	460	0	--------	--------	- summer cattle
DRAIN	16495	20 NOV 89	3300	3200	2400	0	--------	--------	pasture upstream
UPSTM	16824	22 NOV 89	1100	900	680	0	--------	--------	- otherwise just field
	17166	28 NOV 89	1500	1300	5500	0	--------	--------	- dairy + beef farm
	3466	28 MAY 90	80	10	100	0	--------	--------	about 3 km upstm
	3630	29 MAY 90	100	100	110	0	--------	--------	
	3862	31 MAY 90	116	88	252	0	--------	--------	
	5970	3 JUL 90	1600	1400	620	0	--------	--------	
	6425	9 JUL 90	10000	5800	3300	104	1;10;1;1;1;8	14022;77773;10002;10002;50023;74373	
	6641	10 JUL 90	5500	5500	1600	4	1;1	14022;10002	
	6777	11 JUL 90	3700	3600	1050	0	--------	--------	
	18215	6 DEC 90	1000	1000	310	4	5	75773	
2 MUNIC	3467	28 MAY 90	11300	7500	120	12	6;11	76373;77773	-16-24°tile outlet
TILE	5971	3 JUL 90	240000	120000	1500	0	--------	--------	- drains field
OUTLET	6424	9 JUL 90	29000	29000	1600	680	6;n;6;1-2;n;n	16373;50201;77773;00203;40002;77773	- house nearby
	6640	10 JUL 90	13600	4300	250	152	1-2;n;n;1-2;6;n	40223;77773;77773;40223;56373;40223	- no farms nearby
	6776	11 JUL 90	26000	20000	200	252	--------		
	18214	6 DEC 90	820	510	60	16	N/A	N/A	
3 TILE	14688	23 OCT 89	1600	1000	100	140	1	04072	-4 " plastic tile
OUTLET	16494	20 NOV 89	130	70	10	4	1;1	10220;10130	- eavestrough/grey water
EAVES	17759	28 NOV 89	100	100	140	600	1;1	00032-00031;00032	
4 TILE	14687	23 OCT 89	17200000	17200000	1000	1500	2-5;2-5;3	70473;74773;73772	- 12"tile, grey +slimy
OUTLET	15400	1 NOV 89	690000	690000	39000	20000	10	76753-76773-72753	- from apartment septic
APTS	16493	20 NOV 89	105000	105000	51000	600	3;4	50631;77773	- disconnected Dec 90
	16823	22 NOV 89	41000	41000	10200	600	2-5;2-5	40220;40222	- probably septic from
	17158	28 NOV 89	31000	29000	2100	260	3	40431	other town homes too
	3464	28 MAY 90	37000	37000	40	600	6;6	76773;76373	
	3629	29 MAY 90	600	600	600	600	1;3	77773;47633	
	3861	31 MAY 90	34400	28600	10	1500	n;n;4;3;6	70633;77773;70673;77773	
	5969	3 JUL 90	210000	70000	2100	4	N/A	N/A	
	6423	9 JUL 90	5300000	300000	6000	1500	6;n;6;1;n;3	56773;57773;56773;57773;76373;40631	
	6639	10 JUL 90	81000	61000	12000	390	6;6;10;3;1;4	57773;57773;76763;50623;57373;77772	
	6775	11 JUL 90	160000	110000	10000	1500	6-10;n;3;6;3	57773;41433;53673;56773;50631	
	18213	6 DEC 90	1000	1000	10	4	1	30472	

5 JANES DRAIN DOWNST	11284	30 AUG 89	1100	600	210	48	1	70252	- in-drain sample about 20 m downstream of apartment
	16492	20 NOV 89	1500	1500	1500	600	1;1	10030;10020	
	16822	22 NOV 89	2000	1700	1200	12	1	00032	
	17157	28 NOV 89	1000	1000	3100	10	3	40022	
	3465	28 MAY 90	1460	760	310	16	1;1	10072;30232	
	3628	29 MAY 90	790	660	390	8	N/A	N/A	
	3860	31 MAY 90	580	580	350	0	--------	--------	
	5968	3 JUL 90	5300	3600	840	88	4;4;n;4;4;3;4;3;4;4	77773;77773;70032;77773;77773;40631;77773;70671;67651	
	6422	9 JUL 90	1500	1500	1360	104	n;6;6;6;1;1	40203;41223;56373;01223;10002;50002	
	6638	10 JUL 90	2900	2500	1500	20	1;1-2;1;1;8;1	10022;57773;10002;10002;74373;10002	
	6774	11 JUL 90	4900	3600	790	48	1;6;2;1;1,2;3	10420;77773;51213;50000;50223;40621	
	18312	6 DEC 90	850	410	390	0	--------	--------	
6 JANES DR. INLET	3460	28 MAY 90	800	480	230	0	--------	--------	- in-drain sample about 10 m upstm of lake
	3623	29 MAY 90	240	70	180	0	--------	--------	
	3856	31 MAY 90	10	10	50	0	--------	--------	- about 1km downstm of apartment
	5966	3 JUL 90	90	20	170	0	--------	--------	
	6420	9 JUL 90	330	260	230	12	1;n;6;1	10001;56373;56373;10001	
	6636	10 JUL 90	150	40	120	0	--------	--------	
	6772	11JUL90	70	60	40	0	--------	--------	
	18210	6 DEC 90	540	390	1500	8	6;3	76776;71602	
7 BEAR CREEK INLET	3461	28 MAY 90	250	160	110	0	--------	--------	- Bear Creek, Main branch
	3624	29 MAY 90	210	200	80	0	--------	--------	- about 40 m upstm of lake
	3857	31 MAY 90	52	64	64	0	--------	--------	
	5967	3 JUL 90	1090	980	550	0	--------	--------	
	6421	9 JUL 90	4300	3300	3600	156	6;6;6;6;6;3	11003;31002;31002;61002;61002;40620	
	6637	10 JUL 90	1000	100	390	0	--------	--------	
	6773	11JUL 90	500	440	450	0	--------	--------	
	18211	6 DEC 90	120	50	450	4	6	61013	
8 COOK DRAIN	3468	28 MAY 90	20	10	100	0	--------	--------	- in-drain sample near school
	3631	29 MAY 90	80	10	10	0	--------	--------	- drains area of mostly hog farms
	3863	31 MAY 90	160	124	84	0	--------	--------	
	5972	3 JUL 90	120	60	180	8	3;3	76740;76340	
	6426	9 JUL 90	2900	1700	3900	10	N/A	N/A	
	6642	10 JUL 90	840	700	1600	0	--------	--------	
	6778	11JUL90	250	180	850	0	--------	--------	
	18216	6 DEC 90	1250	740	290	16	4;4;7-8;4	77773;77773;74763;77773	

9 LEFT	3463	28 MAY 90	70	50	20	0	--------	--------	- Warwick Conser. Area beach
BEACH	3626	29 MAY 90	68	44	20	0	--------	--------	
WARWIC	3859	31 MAY 90	10	10	10	0	--------	--------	
	5964	3 JUL 90	20	10	20	0	--------	--------	
	6418	9 JUL 90	60	20	260	0	--------	--------	
	6634	10 JUL 90	110	110	150	0	--------	--------	
	6770	11JUL 90	10	10	30	8	1	10002	
	18208	6 DEC 90	1000	1000	1500	4	9	74771	
10 RIGHT	3462	28 MAY 90	30	30	10	0	--------	--------	- Warwick Conser. Area beach
BEACH	3625	29 MAY 90	100	30	4	0	--------	--------	
WARWIC	3858	31 MAY 90	10	10	10	0	--------	--------	
	5965	3 JUL 90	30	30	10	0	--------	--------	
	6419	9 JUL 90	140	80	150	0	--------	--------	
	6635	10 JUL 90	110	40	100	0	--------	--------	
	6771	11JUL 90	30	20	340	0	--------	--------	
	18209	6 DEC 90	100	100	2100	20	2-3;2-3;2-3;4;2-5	61220;76740;51202;77672;74771	

ZONE 2 - BEAR CREEK, DOLAN MCKENZIE DR., ZAVITZ DR.

LOCATION	LAB #	DATE	F. COLIF	E. COLI	F. STREP	P. AER	SEROTYPE	PYOCIN TYPE	SUSPECTED SOURCE
1 BEAR	16503	20 NOV 89	3800	3700	3300	8	1;11	77173;77773	- in-creek sample
CREEK	16832	22 NOV 89	300	300	410	4	6	56773	- drains about eastern
UPSTM	17168	28 NOV 89	4400	3400	72000	120	12-13;6	74771-74773;62453	half of watershed
2 ZAVITZ	15404	1 NOV 89	20	10	90	0	-----	-----	- in-drain sample
DRAIN	16502	20 NOV 89	100	50	370	0	-----	-----	- quarry and one hog
UPSTM	16831	22 NOV 89	100	10	250	0	-----	-----	farm upstream
	17167	28 NOV 89	60	10	1200	0	-----	-----	
3 TILE	14931	25 OCT 89	4800	3800	20	8	-----	-----	- 12" tile outlet
OUTLET	15403	1NOV 89	54000	54000	1000	16	6	77773	- drains small area of field
ZAVITZ	15828	8 NOV 89	21500	12000	690	0	-----	-----	- one hog and beef farm
DRAIN	16151	14 NOV 89	7100	3300	1100	0	-----	-----	½ km away
	16501	20 NOV 89	1500	1500	1500	16	6;9	77773;70663	
	16830	22 NOV 89	1600	5400	50	8	6	76173-76373	
	17166	28 NOV 89	770	660	1000	8	7-B;10	76373;77773	

4 ZAVITZ	11279	30 AUG 89	1500	2100	5300	16	3	72073	- in-drain sample
DRAIN	15404	1 NOV 89	20	10	90	0	--------	--------	- 10 m downstream of
DOWNST	15827	8 NOV 89	2800	500	3800	4	3	40020	tile above
	16750	14 NOV 89	1000	400	400	0	--------	--------	
	16500	20 NOV 89	7000	300	600	0	--------	--------	
	16829	22 NOV 90	230	50	210	0	--------	--------	
	17165	28 NOV 89	50	40	1200	0	--------	--------	
5 BEAR	11278	30 AUG 89	530	400	510	0	--------	--------	- in-creek sample
CREEK	15826	8 NOV 89	27000	3000	27000	116	1;11	40020;77773	- Bear Creek
DOWNST	16149	14 NOV 89	130	20	100	0	--------	--------	
OF 3 +	16499	20 NOV 89	1300	1300	1800	0	--------	--------	
UPSTM	16828	22 NOV 89	860	760	1200	8	1;3	40202;30461	
OF 6	17164	28 NOV 89	14000	14000	51000	1300	3;n	40000;74776- 74773	
	4126	5 JUN 90	52	28	44	0	--------	--------	
	4314	7 JUN 90	64	64	120	0	--------	--------	
	4488	11JUN 90	100	80	150	0	--------	--------	
	4722	12 JUN 90	150	150	290	0	--------	--------	
	4841	14 JUN 90	180	180	320	0	--------	--------	
	6993	16 JUL 90	130	130	1200	0	--------	--------	
	7173	17 JUL 90	150	130	3300	0	--------	--------	
	7567	23 JUL 90	364	244	348	0	--------	--------	
	7836	24 JUL 90	244	244	510	0	--------	--------	
	8002	26 JUL 90	170	50	100	4	N/A	N/A	
	15633	7 NOV 90	5100	3800	740	128	6;6	77773;77773	
	16205	14 NOV 90	70	30	60	0	0	--------	
	16338	15 NOV 90	50	40	80	12	6;6	05323;05723	
6 TILE	15402	1 NOV 89	1500	1500	210	600	N/A	N/A	- 16-24"tile outlet
OUTLET	15825	8 NOV 89	33000	2700	32000	392	1;9;9	76223;70443;70443	- drains field of cabbage + corn
DOLAN	16147	14 NOV 89	57000	38000	31000	0	0	--------	- several hog farms in area
DRAIN	16498	20 NOV 89	3100	3100	43000	168	1	62363-62362	
START	16827	22 NOV 89	132000	67000	40	28	6;6	40220;60413	
	17163	28 NOV 89	1100	1000	6000	40	6;9	56373;70443	
	4123	5 JUN 90	600	600	600	96	9;6	61413;56373	
	4311	7 JUN 90	2700	2400	50	84	6;2	77033;40003	
	4485	11JUN 90	3600	2600	390	368	1;1-2-3-4;6;6	71413;61433;20433;20033	
	4719	12 JUN 90	1100	200	30	252	1;1;1;n	40033;41013;40002;40413	
	4838	14 JUN 90	130000	240000	130000	4	6-13;2-6	57773;77773	
	6990	16 JUL 90	10700	10700	1000	4	1	00433	
	7170	17 JUL 90	17000	17000	630	4	6	57773	
	7564	23 JUL 90	66000	60000	2400	4	2-5;6;11	56353;51473;77773	
	7833	24 JUL 90	52000	52000	5600	1500	6;6;6;6;6-13;6-13	51433;01621;57773;57773;41413;01413	
	7999	26 JUL 90	65000	51000	4300	4	6;6;6;6	71433;71433;75433;20433	
	15631	7 NOV 90	330	350	630	36	1;5;6,13	42571;74771;01473;NA	
	16203	14 NOV 90	5400	4800	180	250	6;6	00000;00433	
	16336	15 NOV 90	240000	240000	1500	600	6;6;n;6	11473;01473;01473;21563	

7 DOLAN	15824	8 NOV 89	110000	60000	24400	368	1	10023	- in-drain sample
DRAIN	16148	14 NOV 89	100	100	400	8	N/A	N/A	- Dolan McKenzie Drain
DOWNST	16497	20 NOV 89	3800	2400	2100	124	1;11;11	54363;54363;77773	- 1 km downstream of
OF 6	16826	22 NOV 89	10000	6800	3100	60	6;6	77573-77773;56373	above tile outlet
	17162	28 NOV 89	700	600	12200	50	1;9	76773;70443	
	4124	5 JUN 90	1010	1000	376	16	1;1	52210;50630	
	4312	7 JUN 90	270	270	110	4	6	20013	
	4486	11JUN 90	290	150	100	0	--------	--------	
	4720	12 JUN 90	150	80	390	244	1;1;6;1	40413;40413;40413;40003	
	4839	14 JUN 90	45000	45000	12000	4	2-11;2-11;7-8	44403;40433;77773	
	6991	16 JUL 90	1440	480	5100	4	6-13;n;6-13	57773;57773;57773	
	7171	17 JUL 90	1220	1260	3800	76	6;6;6;6;2;6	57773;20013;20413;57773;60013;56373	
	7565	23 JUL 90	830	830	1020	4	6;6-13	20453;57773	
	7834	24 JUL 90	2600	2600	3200	568	6-13;6;6;6;2-13;6-13	30033;75433;75433;31433;40013;35473	
	8000	26 JUL 90			1300	0	--------	--------	
	15632	7 NOV 90	650	260	160	128	1;6	02461;56773	
	16204	14 NOV 90	350	250	40	16	6;6;6;6	00433;00433;00433;20473	
	16337	15 NOV 90	40	40	80	8	6-13	77773	
8 BEAR	15823	8 NOV 89	10300	10200	11400	20	3;3	40022;50022	- in-creek sample
CREEK	16146	14 NOV 89	200	100	100	0	--------	--------	- Bear Creek downstream
DOWNST	16496	20 NOV 89	1300	1000	1100	20	4;4	747773;77773;54363	of above tile
	16825	22 NOV 89	1000	700	1700	0	--------	--------	- downstream of Zones 2 + 3
	17161	28 NOV 89	9900	8600	65000	1330	6;6	76753;74771;74773	
	4125	5 JUN 90	140	92	68	0	--------	--------	
	4313	7 JUN 90	140	70	92	0	--------	--------	
	4487	11JUN 90	60	40	40	0	--------	--------	
	4721	12 JUN 90	150	130	220	0	--------	--------	
	4840	14 JUN 90	4600	4600	3100	0	--------	--------	
	6992	16 JUL 90	460	330	2000	0	--------	--------	
	7172	17 JUL 90	330	350	1000	0	--------	--------	
	7566	23 JUL 90	308	260	420	0	--------	--------	
	7835	24 JUL 90	480	480	900	16	6;6-13;6;6-13	57773;57773;57773;57773	
	8001	26 JUL 90	310	270	1000	8	6;6	57773;57773	
	15634	7 NOV 90	3900	2100	6800	156	n;6	02603;77773	
	16206	14 NOV 90	50	10	10	0	--------	--------	
	16339	15 NOV 90	40	40	80	8	n	04723	

ZONE 3 - BEAR CREEK, ROSS-HALL DRAIN

LOCATION	LAB #	DATE	F. COLIF	E. COLI	F. STREP	P. AER	SEROTYPE	PYOCIN TYPE	
1 ROSS	11281	30 AUG 89	460	460	1500	4	N/A	N/A	ZONE 3
DRAIN	15939	9 NOV 89	590	300	960	20	8;8	74763;50463	SUSPECTED SOURCE
UPSTM	16670	21 NOV 89	10	10	100	0	------	-----	
	16998	27 NOV 89	380	240	1500	0	-----	-----	- in-drain sample
	5002	18 JUN 90	5200	3700	1000	0	-----	-----	- drains area of mixed farms:
	5364	21JUN 90	Z80	230	780	0	-----	-----	dairy, beef, hog + poultry
	5465	25 JUN 90	280	240	580	0	-----	-----	
	5727	27 JUN 90	690	120	220	12	7-8;7-8	77772;77772	
	17608	28 NOV 90	300	300	1000	0	-----	-----	
	17708	29 NOV 90	30	30	480	0	-----	-----	
2 MUNIC	14694	23 OCT 89	12000	7900	230	0	-----	-----	- 16" tile, fast flowing
OUTLET	15938	9 NOV 89	?	8300	8800	10	1;5	10235;74373	- drains area of hog farms
HOG?	16669	21 NOV 89	34000	34000	1900	136	1;6;6	00022;22371;76773	and one poultry farm
	16997	27 NOV 89	1400	900	6600	52	3;4	40200;73573-77573	
	5001	18 JUN 90	4500	4000	490	12	N/A	N/A	
	5363	21JUN 90	27000	10600	890	76	8;1;10;7-8	77773;777T3;74763;77773	
	5464	25 JUN 90	7800	3000	1180	84	6;8;8;8	16560;77772;77772;77772	
	5726	27 JUN 90	200	100	340	0	-----	-----	
	17707	29 NOV 90	4900	4900	2600	32	6;8;9;7-8	57773;77773;75761;77773	
3 MUNIC	14693	23 OCT 89	2800	300	340	0	-----	-----	- 16" tile, new
OUTLET	16668	21 NOV 89	1500	1500	470	8	7-8;7	77773;77773	- drains field near
HOG?	169996	27 NOV 89	160	160	3300	0	-----	-----	hog farms
	5000	18 JUN 90	270	130	310	0	-----	-----	
	5362	21JUN 90	1500	1500	430	36	8;8;7-8;7-8	77773;77773;77773;77773	
	5463	25 JUN 90	600	520	568	64	7-8;7-8;2;7-8	77773;77773;77773;77773	
	5725	27 JUN 90	120	40	170	12	6;6;7-8	56372;56372;77772	
	17607	28 NOV 90	2000	2000	47000	30	1;10	10223;77773	
	17706	29 NOV 90	12000	7000	40000	28	2-4;7-8;7-8;7-8	77563;77773;77763;77763	
4 TILE	14692	23 OCT 89	4000000	4000000	100	1500	6	56373	- 12"tile, grey, slimy, t.p.
OUTLET	15406	1 NOV 89	1650000	1650000	100	600	6	76373-56373	- home nearby and hog farms
SEPTIC	75937	9 NOV 89	840000	430000	100	488	6;6	56373;56373	
	16667	21 NOV 89	520000	31000	1000	28	6;6	56373;56373	
	16995	27 NOV 89	1100	1100	1600	0	-----	-----	
	4999	18 JUN 90	680000	330000	100	280	N/A	N/A	
	5361	21JUN 90	4800000	380000	2700	80	3;2;3;6	52673;57573;43473;57773	
	5462	25 JUN 90	2400000	1080000	270	10	6	76773	
	5724	27 JUN 90	3500000	800000	100	10	6	57773	
	17606	28 NOV 90	850000	500000	1000	210	6	77773	
	17705	29 NOV 90	380000	220000	730	192	10;7-8;6;7-8	77773;56542;77773;57562	

5 TILE	14691	23 OCT 89	15200	100000	10	20	10;10	76773;76773	- 12" tile outlet
OUTLET	15936	9 NOV 89	11200	530	6100	32	1;6	12413-10413;32051	- dairy barn nearby
DAIRY?	15405	1 NOV 89	690	590	1400	0	--------	--------	
	16666	21 NOV 89	550	440	870	0	--------	--------	
	169944	27 NOV 89	84000	54000	100	72	6;6	56373;56773;56773	
	4998	18 JUN 90	230	130	590	28	N/A	N/A	
	5360	21 JUN 90	250	60	90	0	--------	--------	
	5461	25 JUN 90	600	440	600	36	6;6;6	56773;56773;56773	
	5723	27 JUN 90	36	12	380	0	--------	--------	
	17605	28 NOV 90	500	500	2700	28	2;2	40600;77773	
	17704	29 NOV 90	10	10	140	0	--------	--------	
	17709	29 NOV 90	3000	1700	180	4	9	71601	
6 ROSS	15935	9 NOV 89	920	630	860	124	1;6	10223;35163	- in-drain sample
DRAIN	16665	21 NOV 89	1300	700	1600	4	5	74763	- downstream of above tiles
DOWNST	16993	27 NOV 89	1200	900	8800	32	3;6	56373;56373	
	4997	18 JUN 90	5100	1300	610	40	N/A	N/A	
	5359	21JUN 90	760	760	910	56	3;3;3;3	52673;52673;52673;52673	
	5460	25 JUN 90	270	200	540	12	n;6;3	54373;56773;77753	
	5722	27 JUN 90	230	130	610	0	--------	--------	
	17604	28 NOV 90	500	400	2000	72	6;2-3	7/113;40620	
	17703	29 NOV 90	1000	50	1100	0	--------	--------	
7 BEAR	11280	30 AUG 89	100	90	2700	0	--------	--------	- in-creek sample
CREEK	15934	9 NOV 89	640	410	880	88	1;1	10032;40020-40022	- downstream of zone 3
DOWNST	16664	21 NOV 89	1500	100	1800	8	1;9	50022;74763	- upstream of zone 2
	16992	27 NOV 89	1100	500	9100	8	6;13	76371;54373	

SEDIMENTS ZONE ONE

#LOCATION	LAB #	DATE	F. COLIF	E.COLI	F. STREP	P. AER	SEROTYPE	PYOCIN TYPE	SUSPECTED SOURCE
1 UPSTM	3475	28 MAY 90	4400	4400	--------	0	--------	--------	
OF APT	5962	3 JUL 90	100000	700000	--------	80	6-13;6-13	57723;41623	
5 DOWNST	3474	28 MAY 90	320000	320000	--------	0	--------	--------	
OF APT	5961	3 JUL 90	340000	34000	--------	40	6	56773	
6 JANES	3469	28 MAY 90	320000	180000	--------	0	--------	--------	
DRAIN INLET	5959	3 JUL 90	4000	4000	--------	0	--------	--------	
7 MAIN	3470	28 MAY 90	180000	100000	--------	0	--------	--------	
BRANCH INLET	5960	JUL 90	16600	4000	--------	0	--------	--------	
8 COOK	3473	28 MAY 90	34000	34000	--------	0	--------	--------	
DRAIN	5963	3 JUL 90	28000	4000	--------	148	8;2;2;6	76373;75773;75771;73573	
9 LEFT	3472	28 MAY 90	10000	10000	--------	0	0	--------	
BEACH	5957	3 JUL 90	4000	4000	--------	40	6	56773	
10 RIGHT	3471	28 MAY 90	16000	4400	--------	0	0	--------	
BEACH	5958	3 JUL 90	4000	4000	--------	40	N/A	N/A	

ZONE TWO

#LOCATION	LAB #	DATE	F. COLIF	E.COLI	F. STREP	P. AER	SEROTYPE	PYOCIN TYPE	SUSPECTED SOURCE
5 BEAR C UPSTM	4443	11JUL 90	5000	6000	------	------	0	------	
6 TILE OUTLET	4440	11JUL 90	100000	100000	------	24	6;6;1-2;1-10;6;6;n;1-10	57773;60072;60012;62012;70132;20432;60032;60012	
7 DOWNST OF TILE	4441	11JUL 90	320000	320000	------	9	5-16;2-11;4-11;11	76760;77773;77772;77772	
8 DOWNST BEAR CR	4442	11JUL 90	100000	100000	------	------	0	------	

ZONE THREE

1 UPSTM ROSS-HALL	5468	25 JUN 90	16000	2000	36	1	10420
3 DOWNST OF 1,2	5467	25 JUN 90	16000	8000	398	2-3;2-3;2-3;2-3;2-3;2-3;7-8	53473;52473;50063;50220;40220;52663;50222;77773
6 DOWNST OF ALL	5466	25 JUN 90	600000	340000	381	6;7-8;7-8;6;7-8;6;6;6;7-8	55373;74760;77772;56372;76760;56372;56773;56373;77773

MANURE SAMPLES

LAB #	DATE	MANURE	ANIMAL	P. AERUGIN	SEROTYPE	PYOCIN TYPE
16616	14 NOV 90	SOLID	BEEF CATTLE	0	------	------
16717	14 NOV 90	LIQUID	PIGS	0	------	------
16618	14 NOV 90	SOLID	DAIRY COW	0	------	------
16619	14 NOV 90	LIQUID	POULTRY + PIG	0	------	------
16620	14 NOV 90	LIQUID	PIG	0	------	------
16621	14 NOV 90	SOLID	PULLETS	0	------	------
18206	6 DEC 90	SOLID	HORSE	0	------	------
18207	6 DEC 90	SOLID	GOAT	0	------	------
20453	24 JAN 91	SOLID	HORSE	0	------	------
20454	24 JAN 91	SOLID	GOAT	0	------	------
20455	24 JAN 91	LIQUID	PIG	present	5;5	74171;74171
20456	24 JAN 91	LIQUID	PIG	0	------	------
20457	24 JAN 91	SOLID	CATTLE	0	------	------
21044	5 FEB 91	SOLID	GEESE, CAN	0	------	------
21045	5 FEB 91	LIQUID	PIG	0	------	------
21046	5 FEB 91	SOLID	CATTLE	0	------	------

ABBREVIATIONS

n = NO SEROTYPE PRODUCED
N/A = DATA NOT AVAILABLE DUE TO LAB ACCIDENT
DASH (-) 77773-77771 = COMBINATION PYOCIN TYPE FOR SAME ISOLATE
SEMI-COLON (;) 77773;77771 = TWO SEPARATE PYOCIN TYPES FOR TWO DIFFERENT ISOLATES

MUNIC = MUNICIPAL
F. COLIF = FECAL COLIFORM
E. COLI = ESCHERICHIA COLI
F.STREP = FECAL STREPTOCOCCI
P.AERUG = PSEUDOMONAS AERUGINOSA

APPENDIX C

LIST OF ALL PYOCIN TYPES RECORDED IN THIS STUDY

C (i) PYOCIN TYPES SORTED UNDER THEIR SEROTYPES: SEROTYPES 1 - 11.

C(ii) PYOCIN TYPES SORTED UNDER THEIR SEROTYPES: UNSPECIFIED, NON-TYPEABLE AND COMBINATION SEROTYPES.

APPENDIX C(i)

PYOCIN TYPES SORTED UNDER THEIR SEROTYPES: SEROTYPES 1- 11

1	2	3	4	5	6	7	8	9	10	11
10413,12413	40003	74773,74776	73573,77573	74373	74771,74773	77773	50463	61413	72753,76753,76773	(2) 54363
62362,62363	40600	30461	54363	74763	77573,77773		74373	70443	(2) 56773	74771
22	51213	40000	67651	74771	0		74763	70443	74763	74773
31	57573	40020	74773		(4) 433		75773	70443	76763	(5) 77773
(3) 32	60013	(2) 40022	77772		1223		77772	70443	(3) 77773	
433	77773	40200	(8) 77773		1473		77772	70663		
2461		40431			1621		77777			
4072		40620			5323		77773			
(2) 10001		40621			5725		77773			
(7) 10002		(2) 40631			11003		77773			
10020		43473			11473					
10022		47633			16373					
10023		50022			16560					
10030		50623			(2) 20013					
10072		(2) 50631			20033					
10130		(5) 52673			20413					
10220		53673			(2) 20433					
(2) 10223		56373			20453					
10235		70671			20473					
(2) 10420		70673			21563					
12413		72073			22371					
(2) 14022		73772			(2) 31002					
30232		76340			31433					
40002		76740			32051					
40003		77753			35163					
40020					40220					
40033					40413					
40202					41223					
(2) 40413					51433					
41012					51473					
42571					55373					
50000					(4) 56372					
50002					(17) 56373					
50023					(8) 56773					
57373					(13) 57773					
57773					(2) 61002					
70232					62453					
71413					71433					
74373					75433					
76223					76173					
76773					76373					
77173					76753					
(2) 77773					(3) 76773					
					77033					
					(10) 77773					

APPENDIX C(ii)
PYOCIN TYPES SORTED UNDER THEIR SEROTYPES: UNSPECIFIED, NON-TYPABLE AND COMBINATION SEROTYPES

UNSPECIFIED		NON-TYPEABLE		SEROTYPE COMBINATIONS			COMBINATIONS		
	10002		1473		1,2	203		1,2,3,4	61433
	10032		2603		1,2	40223		2,11	40433
	20432		4723		1,2	50223		2,11	44403
	40020		40002		1,2	57773		2,13	40013
	40022		40203		2,3	50063		6,10	57773
	40620	(2)	40223		2,3	50220		6,13	1413
	50022		40413		2,3	50222		6,13	1473
	51202		41433		2,3	52473		6,13	30033
(2)	54373		50201		2,3	52663		6,13	35473
	56542		56373		2,3	53473		6,13	41413
(4)	56773	(2)	57773		2,5	40220		6,13	41623
	57562		70032		2,5	40222		6,13	57723
	57773		70633		2,5	56353	(7)	6,13	57773
	60012		76373		2,5	70473			
	60012	(3)	77773		2,5	74773			
	60012				2,6	77773			
	60032				7,8	74760			
	60413				7,8	76373			
	61220				7,2	76760			
	62012			(4)	7,8	77772			
	70132			(10)	7,8	77773			
	71601								
	71602								
	73573								
(2)	74763								
(2)	74771								
	75761								
	75771								
	75773								
	76371								
	76373								
	76740								
	76760								
	76776								
	77563								
	77672								
(2)	77772								
(10)	77773								

APPENDIX D
SEROLOGY OF SAMPLES TAKEN FROM SEWAGE TREATMENT PLANTS IN ONTARIO

SOURCE	SEROTYPE	PYOCIN	ISOLATE	SOURCE	SEROTYPE	PYOCIN	ISOLATE
BRIGDEN	6	56373	21483	DURHAM	3	64423	22292
BRIGDEN	6	56373	21483	DURHAM	6	46551	22292
BRIGDEN	6	56373	21483	DURHAM	6	66751	22292
COMBER	2,7,8	77773	21507	DURHAM	6	76751	22292
COMBER	6	57773	21507	OWEN SOUND	9	70641	22293
COMBER	1	12672	21507	OWEN SOUND	6	57771	22293
COMBER	1	42670	21507	OWEN SOUND	N	30432	22293
HURON PARK	6	77773	21508	OWEN SOUND	6	14542	22293
HURON PARK	3	52222	21508	PT EDWARDS	N	73473	22294
HURON PARK	N	40633	21508	PT EDWARDS	6,11	76773	22294
HURON PARK	1	10203	21508	PT EDWARDS	9	72401	22294
MILVERTON	1,2	57775	21509	PT EDWARDS	6	77773	22294
MILVERTON	6	57773	21509	SOUTHAMPTON	3	2441	22295
MILVERTON	N	51113	21509	SOUTHAMPTON	6	76771	22295
MILVERTON	N	77773	21509	SOUTHAMPTON	11	77371	22295
PETROLIA	N	632	21728	SOUTHAMPTON	11	77371	22295
PETROLIA	1	10632	21728	STRATFORD	9	53540	22296
PETROLIA	10	76731	21728	STRATFORD	11	77371	22296
PETROLIA	6	54773	21728	STRATFORD	9	53141	22296
OIL SPRINGS	2,3	50001	21730	STRATFORD	11	77371	22296
OIL SPRINGS	2,3	73020	21730	VAVASTRA	9	73140	22297
OIL SPRINGS	1	10213	21730	VAVASTRA	1	10440	22297
OIL SPRINGS	6	56373	21730	VAVASTRA	3	N	22297
WYOMING	6	77773	21731	VAVASTRA	1	10040	22297
WYOMING	6	77773	21731	THAMESVILLE	6	76771	22316
WYOMING	6	77773	21731	THAMESVILLE	5	73161	22316
WYOMING	N	251	21731	THAMESVILLE	6	77771	22316
NEWSTADT	1,2,3,4	56773	21732	THAMESVILLE	1,6	440	22316
NEWSTADT	6	56773	21732	ALVINSTON	N	72771	22317
NEWSTADT	1	N	21732	ALVINSTON	10	73571	22317
NEWSTADT	1	7777S	21732	ALVINSTON	1	20040	22317
BRUCE ENERGY	6	76373	21733	ALVINSTON	3	41231	22317
BRUCE ENERGY	1	56160	21733	AND TWP	6	57773	22602
BRUCE ENERGY	6	76773	21733	AND TWP	2	76373	22602
BRUCE ENERGY	1	46770	21733	AND TWP	11	77773	22602
BRUCE ENERGY	7	10532	21879	AND TWP	10	77773	22602
BRUCE ENERGY	1	40022	21879	COLC. TWP	5	76773	22603
BRUCE ENERGY	1	31533	21879	COLC. TWP	6	57773	22603
BRUCE ENERGY	6	57373	21880	COLC. TWP	1	10020	22603
BRUCE ENERGY	6	57373	21880	COLC. TWP	10	57773	22603
BRUCE ENERGY	6	56773	21880	HARROW	11	77773	22604
BRUCE ENERGY	6	57773	21880	HARROW	2	76773	22604
BRUCE ENERGY	6	56773	21882	HARROW	10	77773	22604
BRUCE ENERGY	6	57773	21882	HARROW	5	77773	22604
BRUCE ENERGY	6	77773	21882	MCGREGOR	6	77773	22605
BRUCE ENERGY	6	57773	21882	MCGREGOR	1,2,10	77773	22605
WATFORD	N	77773	21729	MCGREGOR	3	41661	22605
WATFORD	1	30472	21729	MCGREGOR	5	77773	22605
WATFORD	3	41623	21729				
WATFORD	6	76771	21729				