To the Graduate Council:

I am submitting herewith a thesis written by Theresa Louise Ervin entitled "Incidence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* species on a Tennessee dairy farm". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biosystems Engineering.

Dr. Ronald E. Yoder, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Interim Vice Provost and
Dean of the Graduate School

Incidence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Species on a Tennessee Dairy Farm

A Thesis

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Degree

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Dedication

This thesis is dedicated to my parents, Gay and Bo Ervin, for always supporting me and reminding me that I could do it even when I thought I couldn't.

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Abstract

In response to public awareness of foodborne pathogens due to recent outbreaks of illness, and to the call for control of pathogens to begin on the dairy farm, a study was conducted on the survival and movement of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* species on a dairy farm in middle Tennessee. Samples were taken from various locations around the farm, including water samples from up- and down-stream in a creek running through the farm. Samples were also collected from free stall bedding, silage, separator liquids, separator solids, pumped liquid from a holding pond, feed, and bulk tank milk. Three samples were taken monthly at each location for 12 months. These samples were evaluated to determine possible sources and reservoirs of the pathogens on the dairy farm and to identify seasonal trends in pathogen presence.

No *Escherichia coli* O157:H7 was found in any of the samples. *Listeria monocytogenes* was present most often in the pumped holding pond liquid and the separator liquid, with each producing positives in 31% of the samples. The bedding and silage samples were negative for the pathogen for the entire testing period. The up- and downstream samples each produced one positive sample, in December and September respectively. *Listeria monocytogenes* was found in the November sample of irrigated pasture soil; irrigated soil samples were also collected during September. A possible seasonal trend was identified in *Listeria monocytogenes*, with more positive samples collected during cooler months.

In contrast to *Listeria monocytogenes*, *Salmonella* were found widely across the farm. Pumped liquid exhibited the highest incidence of *Salmonella* occurrence with 34

(94%) of the 36 samples testing positive. The manure solids separator was also identified as a potential control point with separator liquid and separator solid samples returning *Salmonella* positives on 72% and 81%, respectively. The downstream samples yielded 27% positives, whereas the upstream samples identified isolates in 42% of the samples collected. All of the sampling locations yielded at least two positive *Salmonella* samples.

Three times within the testing period comparative samples were collected from within a nearby city to supply a reference for pathogen presence. The comparative stream samples from the city yielded one *Listeria monocytogenes* positive sample for each location in September. Comparative samples collected below a waste treatment discharge provided 44% positive *Salmonella* isolates.

After testing was completed, locations associated with waste, such as the manure solids separator and the holding pond, were identified as potential control points for *Listeria monocytogenes* and *Salmonella*. Based on these findings, possible methods for reducing pathogen presence on the farm include composting the separator solids prior to being used for bedding and possibly irrigating silage corn with holding pond water. The farm will never be a sterile environment, but pathogen concentrations can possibly be reduced by setting up critical points throughout the farm and controlling the use of the products at these points.

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Chapter I

Introduction

In 1959, the National Aeronautics and Space Administration (NASA) developed Hazard Analysis Critical Control Points (HACCP) to assure the safety of food to be used in space. This system was based on identifying points in a process that can be controlled to alter the quality and safety of the product. The HACCP system was introduced publicly in the United States in 1971 as a solution to microbiological problems with low-acid canned foods and accepted worldwide in 1985 by the Food and Agriculture Organization/World Health Organization. In 1996, the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) mandated the use of the HACCP system on meat and poultry products to reduce the occurrence and numbers of pathogens, reduce the incidence of foodborne illness associated with consuming these products, and provide a framework for modernizing the inspection system of meat and poultry. According to data from the Centers for Disease Control (CDC), from 1996 to 1999 the rates of *Escherichia coli* O157 infection decreased 22%. From 1996 to 1998, the overall incidence of salmonellosis decreased 15%, but the incidence increased 20% from 1998 to 1999. During the 4-year period, the incidence of listeriosis remained unchanged.

The goal of this study was to identify the sources and reservoirs of pathogens on a Tennessee dairy farm by testing a variety of locations on the farm. The sources are identified as possible entry points for pathogen growth on the farm, and the reservoirs are

identified as locations where pathogens often grow and multiply. The specific objective of the study was to monitor the presence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* species for one year. Samples were taken from nine locations on the farm, including water samples from up- and down-stream in a stream running through the farm. Samples were also taken of free stall bedding, silage, separator liquids, separator solids, pumped liquid from a holding pond, feed, and bulk tank milk. Twice during the study period, soil samples were collected from the pasture irrigated with the holding pond water on the dairy. These data can be used to help dairy farmers determine appropriate locations to set up a generic system, similar to the HACCP system, to possibly reduce the concentration of pathogens throughout the farm.

To determine the incidence of these pathogens in public areas off of the farm, soil samples were collected three times within the testing period from a little league baseball park. Additionally, off-farm water samples were collected from a stream above and below a municipal water treatment outfall, and water samples were also collected from another stream below an industrial park.

The abundance of pathogens in the dairy environment and the movement of birds and animals in and out of the dairy make it impossible to eliminate them completely. By testing sources around the dairy and identifying the critical control points, it may be possible to reduce the pathogens on the farm through improved farm management practices. This 12-month study identified sources and reservoirs of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* species for possible future use in developing control points on the farm.

Chapter II

Review of the Literature

In the past few years, the public's awareness of foodborne pathogens has increased due to outbreaks like the one at Jack in the Box fast-food restaurants in 1993 that killed three children and sickened several hundred. Publicity about outbreaks across the country has increased consumer calls for stricter regulations on the food production and service industry. Hazard Analysis Critical Control Points (HACCP) were first introduced in 1959 to ensure that the space program would have safe food. In 1971, the HACCP system was first introduced to the public as a way for industry to control and prevent problems that occur in processing plants, and to ensure safe food by detecting problems before the final product. To implement HACCP principles, the production process must be monitored and the results of the monitoring be used to identify and correct risk factors.

In 1996, the USDA FSIS mandated that meat and poultry producers implement a HACCP system, and by January 2000 all slaughter plants were mandated to have implemented a HACCP system. These regulations affect meat processors and dairy farmers, because approximately 8.6% of the 2.8 million cattle slaughtered in the United States for ground beef each month are dairy cattle (Faith et al., 1996). The dairy industry is feeling public pressure to provide the purest product possible. Some public advocates call for stopping food contamination before it reaches the processing plant. In order to

implement on-farm HACCP programs, areas of risk must be correctly identified, and tests must be developed and applied that are accurate and fast.

A 1991 report from the Centers for Disease Control and Prevention (CDC) identified *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* species, *Campylobacter jejuni,* and *Cryptosporidium* species as targets for control on dairy farms (Cullor, 1995). *Campylobacter jejuni* and *Cryptosporidium* species are more commonly found in poultry, swine, and small animal waste than in dairy waste (Cullor, 1995).

Various approaches have been used to monitor pathogens in the farm environment. Typically, studies have been done on a group of pathogens in a certain place on the farm, or on one particular pathogen in the farm environment. Due to the abundance of pathogens on dairy farms, it is more common to see studies on a specific pathogen. One pathogen is usually monitored in several different locations throughout the farm. By studying a pathogen at different locations on a farm, magnitude can be added, allowing for a better understanding of the actual path of the bacteria.

Studying pathogens in raw milk has been used to determine the pathogens that may be leaving the farm (Rohrbach et al., 1992). While this method is adequate for identifying pathogens that enter the milk processing plant, it is inadequate for the meat processing industry and for determining the quality of the farm environment.

Escherichia coli O157:H7

Characteristics of *Escherichia coli* O157:H7

Escherichia coli is commonly found in the intestines of all animals and humans and is commonly found in feces. Normally, the dominance of *E. coli* in the intestines is

useful, because it suppresses harmful bacteria. However, *Escherichia coli* O157:H7 is a verotoxin-producing strain of *E. coli*, capable of producing large quantities of one or more related potent toxins that cause severe damage to the lining of the intestines. This strain produces serious symptoms, such as bloody diarrhea and kidney failure, and can cause death in animals and humans. In infants, death can occur in about 24 hours, and the use of antibiotics makes the condition worse.

The *E. coli* O157:H7 bacteria can survive a wide range of conditions (Pell, 1997). The optimum temperature for growth of *Escherichia coli* O157:H7 is 37 °C, but cooking to 60 °C will significantly reduce pathogen presence. Death of most of the pathogens occur when food is cooked to 60 °C and remains at that temperature for a few seconds, which is enough time to kill the pathogen in most cases. *Escherichia coli* O157:H7 was implicated in 75 outbreaks of illness associated with pathogens between 1982 and mid-1995 (Faith et al., 1996). Possible sources of *Escherichia coli* O157:H7 on the dairy farm, besides fecal matter, are in cattle feeds and silage. The feeds often contain naturally occurring *Escherichia coli* O157:H7, but are more likely contaminated by the pathogen during delivery or while stored on the farm (Lynn et al., 1998). The bacterium has been known to sicken swimmers in contaminated lakes; however, it is not a significant problem in potable water, because heat and chlorine kill it (Cliver and Atwill, 1997). According to the Center for Disease Control (CDC), in 1999 infections per 100,000 people from *Escherichia coli* O157 ranged from 0.6 in Georgia to 5.8 in New York.

Escherichia coli O157:H7 in the farm environment

Due to recent outbreaks and the nature of the pathogen, *Escherichia coli* O157:H7 has become the focus of many studies. In a study conducted by Hancock et al. (1998) on non-bovine sources of *E. coli* O157 on cattle farms, samples were collected from four cattle farms on three occasions separated by approximately one month starting in July and ending in November. Samples were collected from cattle, feed, birds, rodents, flytraps baited with autoclaved ground beef, water troughs, wildlife, cats, dogs, and horses. No *E. coli* O157 isolates were found in the rodents, cats, wildlife, or cattle feed samples. The other samples ranged from positives of 3.3% for the fly samples to 0.5% for bird samples pooled during collection. Both water trough samples and canine fecal samples yielded 3.1% positive samples. Feedlot samples and dairy cattle samples identified 2.9% total occurrence. It was concluded that the means by which *E. coli* O157 is sustained and transmitted on farms is unknown, but it raises the possibility that cattle are not the only reservoir for the pathogen.

A study conducted by Rahn et al. (1997) contrasted with these findings by concluding that dairy cattle are a more frequent reservoir for *E. coli* O157:H7 than is the dairy farm environment. The Rahn et al. study was over a one-year period on eight Ontario dairy farms, and cats, rodents, birds, cows, calves, manure piles, waterbowls, floors, walls, calf hutches, and milk filters were studied. Only the samples associated with the cattle on the farm tested positive for *E. coli* O157:H7 isolates. Different methods of isolation were used in the contrasting studies.

Four Wisconsin dairy farms were monitored for *Escherichia coli* O157:H7 for fourteen months by Shere et al. (1998) by collecting samples from each farm from 15

cohort heifers, bedding, cattle in contact with the cohort cattle, feed, water, and other animals in contact with the cohort cattle. The study found that two of the herds studied remained negative, and one herd tested negative for a three-year period. The remaining herd tested positive, and the pathogen was believed to originate at a point source, probably contaminated drinking water.

Herriott et al. (1998) studied the relationship between herd management practices and the prevalence of *Escherichia coli* O157:H7 on 36 dairy farms. This study focused on the differences between the herd management practices and the levels of the pathogen in the environment. They found that feeding corn silage, grain screens, and ionophores to heifers, and feeding animal by-products to adults was in direct correlation with higher occurrences of *Escherichia coli* O157:H7 in dairy cattle. Lynn et al. (1998) evaluated cattle feeds from commercial and farm sources for *Escherichia coli* presence. The study showed 63 of 209 samples (30.1%) contained *Escherichia coli*, but none of the samples tested were positive for *E. coli* O157. While all of the samples may not have been positive, it was concluded that all *E. coli* could replicate in feeds under conditions that may be present on cattle farms in warm weather. Conner and Kotrola (1995) demonstrated that *E. coli* O157 would survive and replicate at temperatures between 25 and 30 °C.

To get a better understanding of pathogens consumed by cattle, Grant et al. (1995) monitored bacterial groups in corn and grass silage from the Washington State University Dairy Forage Research Facility. They found that high temperatures and low pH in the silage are critical to the number of pathogens that are able to survive. Enterohemorrhagic *E. coli* was absent from all 46 samples collected.

Hancock et al. (1997b) studied the effects of manure-handling practices on the prevalence of *E. coli* O157 in cattle. For six months, the study was conducted using samples from 36 dairy herds in the Pacific Northwest. Only 179 (1.41%) of the 12,664 samples collected tested positive for *E. coli* O157. The resulting data showed no important risk to heifers confined in pasture lots that had or had not had manure applied, and that the destination and handling practices of stored manure appeared to have little or no impact on the ecology of *E. coli* O157. A trend observed within herds, however, revealed that herds maintained a relatively low or high presence of *E. coli* O157, ranging between 0% and 5.5%. An earlier study done by Hancock et al. (1994) suggested a slight association between slurry application to pasture and the isolation of E. *coli* O157:H7, however, additional studies were suggested.

Porter et al. (1997) evaluated detection methods and the distribution of *E. coli* O157 from asymptomatic dairy cattle by testing a variety of environmental samples. These samples included water, manure, slurry, grass, and soil. The study found *E. coli* O157 in detectable numbers from the milking area and the slurry pit area. After the slurry pit entry, however, the cells appeared to die. Based on this idea, Porter et al. proposed a method for controlling *E. coli* O157 on the farm by using a slurry pond to contain the waste.

Cliver and Atwill (1997) suggested water as a vehicle for *E. coli* O157:H7, and a study done by Flint (1987) observed untreated river water at various temperatures to determine survival times of *E. coli*. The pathogen appeared to disappear between four and 11 days in water collected above or below a sewage outfall at temperatures that would compare to those found in nature. At 4 °C, there was little difference between the

survivals in the two samples; however, as the temperature increased to 15 °C, 25 °C, and 37 °C the survival rate was longer for the samples collected below the outfall.

A fifteen-month study conducted by Mechie et al. (1997) on *Escherichia coli* O157:H7 in a dairy herd found that 153 (4.3%) of 3593 bovine rectal swabs tested were positive. It was also found that 74% of the animals that excreted the pathogen only did so on one occasion, and no *E. coli* O157 was isolated from the bulk tank milk. The excretion rate of the pathogen during a 12-month period was found to be lowest in lactating cows (0.9%) and highest in heifers (14.2%). Other studies done by Hancock et al. (1997a) and Zhao et al. (1995) agree with these findings showing a lower occurrence of isolates in lactating cows than in heifers and calves. One explanation for this phenomenon is the younger cattle may be more vulnerable to exposure to bacterial and viral organisms than older, larger cattle (Dargatz et al., 1997).

Regional distribution of *E. coli* O157 was tested with fecal samples collected from 100 feedlots in 13 states by Hancock et al. (1997c). Out of the 100 feedlots, 61 provided isolates of *E. coli* O157:H7; however, based on the distribution of the isolates there was no evidence of regional distribution. Based on this study and others (Rahn et al, 1997; Hancock et al., 1994; Hancock et al., 1997a), seasonal shedding of the pathogen by the animal appears to be more of a concern than shedding due to regional distribution based on geographic differences.

Characteristics of *Listeria monocytogenes*

Listeria monocytogenes is a bacterium that has been isolated from 42 species of mammals, 22 species of birds, and from fish, crustaceans, and insects. It is also found naturally in plant and soil environments (Pell, 1997). Its optimum growth temperature is 30 °C, and it is one of the most heat resistant pathogens; however, *Listeria monocytogenes* can be significantly reduced or killed, depending on environment, by elevating the temperature to 60 °C. *Listeria monocytogenes* is known to bring about abortion, stillbirth, or neonatal sepsis in humans and is usually fatal in sheep and pigs (Cullor, 1995). In immunocompromised patients, *Listeria monocytogenes* may attack the nervous system causing meningitis. Because of its abundance in the environment, consumers have called for the areas of its known origin to be monitored and controlled. In 1995, *Listeria monocytogenes* caused over half of all dairy-related Class I recalls (Arimi et al., 1997). A Class I recall, as defined by the FSIS, involves a health hazard situation where there is a reasonable probability that eating the food will cause health problems or death. *Listeria monocytogenes* is found in healthy cows and in the environment (Ueno et al., 1996), but it is most commonly isolated from improperly fermented silage (Ryser et al., 1997). Consumption of the tainted silage will eventually lead to the shedding of the pathogen by the animal into the raw milk (Husu, 1990). The CDC determined that 113 cases of listeriosis were laboratory confirmed during 1999.

Listeria monocytogenes in the farm environment

To get a better idea of the paths of the pathogen in the environment, studies are often done focusing on one pathogen at several locations. By identifying the behavior of

the pathogen, a better understanding of how to control it can be obtained. A typical location to search for *Listeria monocytogenes* is in silage. In a study by Ryser et al. (1997), *Listeria monocytogenes* was isolated from 1 of the 13 hay silage samples with a pH between five and six, and 1 of 17 corn silage samples with a pH between four and five. Out of 129 total corn silage samples, three samples tested positive for *Listeria monocytogenes.* Two of the 76 hay silage samples were positive, and none of the five grass silage samples tested positive. Perry and Donnelly (1990) studied how the methods of storage affect the pH of silage and the incidence of *Listeria.* They isolated *Listeria* in 33.3% of the 12 Harvestore silos sampled, 23.4% of the 77 upright silos sampled, 16.2% of 74 bunker silos sampled, and 12.5 % of the 16 Ag Bags tested. They concluded that a larger number of samples should be collected from Harvestore silos and Ag Bags for added accuracy. Fenlon et al. (1989) compared the difference in *Listeria monocytogenes* contamination between bagged and wrapped big bale silage and found that there was no significant difference. The study did show that big bale silage was prone to contamination, but that *Listeria* could be greatly reduced by the removal of obviously spoiled material before feeding.

Arimi et al. (1997) tried to track *Listeria monocytogenes* from the processing plant back to the farm using 475 *Listeria* strains collected over the past 10 years from 388 dairy processing plant environments, 14 bulk tank, 44 silage, 26 udder, and three clinical samples from listeriosis infected dairy cattle. By using an automated Riboprinter microbial characterization system, the samples were ribotyped. A total of 388 different ribotypes of *Listeria* were found; however, eight *L. monocytogenes* and 12 non- *L. monocytogenes* ribotypes were common to the farm and processing environments

implicating a path for pathogens. Husu et al. (1990) suggested that poor milking and barn hygiene increased the risks of contamination of milk by *Listeria monocytogenes* in barns, but that effective cleaning of the udders and careful washing of the milking equipment prevented contamination of the milk.

For six months, Ueno et al. (1996) studied the prevalence of *Listeria monocytogenes* in the environment of five dairy farms in Japan. The rectal contents of a healthy cow, straw on the floor, straw in the barn, and silage were tested, and three of five farms tested positive for the pathogen. *Listeria monocytogenes* was isolated from the brain stem and the straw around a cow on one farm, from the straw on the floor on the second farm, and from the straw in the barn and silage in the last farm.

Husu (1990) also studied the occurrence of *Listeria monocytogenes* in dairy cattle feces. The data for the paper were compiled from two studies. The first study was conducted over a two-year period and involved 249 farms. The second study involved 80 different farms and the collection of raw milk, grass silage, pasture grass, and fecal material on a four-season collection schedule. *Listeria* species were isolated from 373 (9.6%) of the 3,878 fecal samples examined in the first study. *Listeria monocytogenes* existed in 6.7% of the samples. Husu determined that *Listeria* species were excreted by at least one dairy cow on 114 (45.8%) of the 249 herds tested. On the 80 tested farms, listeriae was detected in silage on 39 farms (48.8%), and on 24 of these 39 farms (61.5%) *Listeria* species were also isolated from the feces. There also appeared to be no significant difference between feed contamination and fecal positives. More fecal isolations were made on farms where *Listeria* was detected in feed than on those in which silage or pasture grass was found to be *Listeria* negative. The prevalence of *Listeria*

species was also found to be higher in pasture grass (64.7%) than in grass silage (22.7%), but in August, after cattle had been grazing all summer, there were no *Listeria* found in the 74 milk samples examined from the farms.

Slade et al. (1988) studied the incidence of *Listeria* species in raw milk in Ontario. The results showed 36 (11.4%) of 315 milk samples contained *Listeria* species, and 17 (5.4%) showed the presence of *Listeria monocytogenes*. These findings agree favorably with those of Lovett et al. (1987) for a study done in the United States. The study in the United States showed that there was an overall *Listeria* species incidence of 12.6% and the incidence of *Listeria monocytogenes* was 4.2%; however, the results of another study done in Ontario by Farber et al. (1988) disagreed with the previously mentioned studies by producing only six (1.3%) of 445 raw milk samples *Listeria monocytogenes* positive. The Farber et al. study does agree with the overall incidence showing 12.4% *Listeria* species positive samples. In a later study done by Steele et al. (1997) in Ontario, *Listeria monocytogenes* was found in 2.73% of the raw milk samples.

Salmonella

Characteristics of *Salmonella*

Salmonella were responsible for 28% of foodborne disease outbreaks and 45% of foodborne disease cases that had the cause identified between 1983 and 1987 (Losinger et al., 1995). *Salmonella* are present in the feces of both warm and cold-blooded animals. It grows best at 37 °C, but *Salmonella* are significantly reduced or killed at 60 °C depending on the environment in which the pathogen is found. Infections caused by *Salmonella* occur when contaminated food or water is ingested. While most people

associate *Salmonella* with poultry, it has been traced to raw milk, ice cream, and undercooked beef (Cullor, 1995). *Salmonella* can cause nausea, vomiting, cramps, diarrhea, and, in some cases, arthritis in humans (Pell, 1997); the pathogen has the ability to invade the blood stream through the intestinal wall and attack the joints to cause arthritis. According to the CDC, in 1999, the incidence per 100,000 people was higher for salmonellosis with 17.7 than any of the other nine laboratory-confirmed diseases induced by pathogens

Salmonella in the farm environment

While mainly thought of as a poultry pathogen, *Salmonella* have been studied on dairy farms and in the products that are found there. The FDA conducted a study on the *Salmonella* contamination of feed that is consumed by dairy cattle (McChesney et al., 1995). This study tested products from processors that used animal and plant protein in their meals. Within the samples collected, 56.4% of the animal protein samples and 36.0% of the plant protein samples tested positive. The higher occurrence of *Salmonella* in animal protein samples can be traced to meat and bone meal (MBM). Bender et al. (1997) report that 27% of the MBM produced in the US is used as a source of protein for dairy and beef cattle, and in Minnesota dairy production it is estimated that 20-30% of the rations contain MBM. *Salmonella* attack calves and pregnant cows, often causing death (Clegg et al., 1983).

Durand et al. (1987) studied the resistance to various antibiotics of *Salmonella* and *Escherichia coli* isolated from farm feeds. It is a common practice to add certain antibiotics to feeds for young animals to promote their growth, however, over time pathogens build resistance to antibiotics that are found in feeds. This can be hazardous

for animals and humans. The study found the 128 *Salmonella* isolates investigated to be totally resistant to penicillin, methicillin, and bacitracin. This total resistance increases the probability of the animals being a carrier of the pathogen.

Studies conducted by both Harbourne et al. (1978) and Smith et al. (1978) evaluated surface waters in England for *Salmonella* contamination. Harbourne et al. collected samples at the outfalls of three sewage works and at various points in the rivers above and below these outfalls for a 12-month period. During this time, *Salmonella* were isolated 316 times, and 29 different salmonella serotypes were recovered from the rivers. Smith et al. examined an impounding reservoir, three rivers, and 10 streams that were chosen due to their accessibility to farm livestock. Samples were collected every two-weeks from January to March 1976. *Salmonella* species were isolated from 22 (39%) of the 57 samples examined. Positive samples were collected from 10 (71%) of the 14 locations. Possible causes of the contamination ranged from bird feces to sewage effluent to farm effluent.

Milk has been a popular location to test for *Salmonella.* In studies done by McManus and Lanier (1987), McEwen et al. (1988a), and McEwen et al. (1988b) the presence of *Salmonella* in milk ranged from 4.7% to 2.9% to 1.23% respectively. McManus and Lanier conducted their study by collecting raw milk samples from bulk tank trucks from Wisconsin, Michigan, and Illinois, and isolated *Salmonella* from 32 of 678 samples. These samples were obtained from tankers that contained milk from several producers giving a more accurate representation of the incidence of the pathogen in the raw milk supply because of the large number of farms represented. McEwen et al. (1988a) attempted to determine the prevalence and geographic distribution of *Salmonella*

on dairy farms in southwestern Ontario. Milk filters from 511 randomly selected dairy farms were tested and 22 (2.9%) were *Salmonella*-positive. Possible outside contamination could have occurred in the collection of the filters, because many of the milk filters were visibly contaminated with plant material and other debris that had been collected by the vacuum pump of the milking system. One year later, McEwen et al. (1988b) tested farms in four study periods; many of the farms that participated in the previous study participated again. The resulting positives were fewer than in the previous year, with the average of the four periods equaling 0.455%.

Giles et al. (1989) discovered the impact of one *Salmonella*-positive cow in a study while isolating *Salmonella typhimurium*. The study was conducted on a dairy farm that had a persistent history of *Salmonella typhimurium*. After testing, it was determined that 26 (35.6%) of 73 milk filter samplings were positive. Further testing of individual milk samples identified one cow as the excreter and at slaughter the pathogen was recovered from the animal's mammary gland.

In 1989, Pacer et al. conducted a prevalence study on 75 randomly selected California dairies. Cotton swabs covered in manure were collected on the first 10 milking and first 10 non-lactating cows observed defecating. By this method, 12 (16%) of the 75 farms tested positive. A later study conducted by Smith et al. (1994) suggested that the Pacer et al. study underestimated the prevalence of the pathogen. The later study based the presence of *Salmonella* species on the presence of serum IgG, which characterizes organism carriers. Overall, the study found that 75% of the 60 dairies had cows with serologic evidence of recent exposure to salmonellae. The high percentage was explained by better means of identification of positives, and by poor management

practices on the farms. Some examples of poor management practices include sick animals not being quarantined from healthy animals and the same front-end loader used to transport dead animals also being used to move feed.

Often families that live on dairies drink milk directly from the bulk milk tank before it has been pasteurized. Rohrbach et al. (1992) conducted a survey by collecting milk samples and having families answer a questionnaire. *Salmonella* were recovered from 26 (8.9%) of 292 bulk tank samples collected. Based on answers received in the questionnaire, 68 (35%) of 195 producers admitted drinking raw milk from the bulk tank. West et al. (1988) went further to see the effects of consuming raw bulk tank milk. Stool swabs were submitted from 165 people from 39 families. Five families were infected with *Salmonella* species and all lived on infected farms. The five families contained 35 individuals, and of these members, 22 (62.9%) who drank raw bulk tank milk tested positive for *Salmonella*. These results are substantial evidence that infection can be transferred from animal to human. The cost of human infection for mild cases has been estimated to be roughly $221 each, and the total cost for mild cases is estimated to be approximately $400 million annually in the United States (Roberts, 1988).

Previous studies have been done on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* species, individually or grouped with other pathogens, but there is not a published study dedicated to these multiple pathogens in any dairy environment over time at multiple locations. By conducting the study in this way, relationships may also be observed between the pathogens. This study evaluated the presence of the *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* species at various locations throughout the farm. By identifying the sources and

reservoirs, proper measures can perhaps be taken to control critical points of contamination and allow for HACCP-like standards to be implemented on the farm.

Chapter III

Materials and Methods

Facilities

The University of Tennessee Dairy Experiment Station (Figure 1) was established in 1929 and is a 249-hectare farm located on rolling to steep topography. Based on soil maps, the soils are mostly clay and silt loams of Huntington, Talbott, Emory, Maury, and Egam soil classification over a karst limestone base. Big Rock Creek provides drainage for the farm and passes within 200 meters of the milking facility as it flows north into the city of Lewisburg. Based on data collected by Hollon and Owen (1981), the average rate of flow over 2 years was 0.43 m^3/s with a maximum flow of 1.31 m^3/s and a minimum flow of 0.025 m^3/s.

The facilities consist of a milking parlor, calf and maternity barns, feed storage buildings, vertical silos, free stall barns, and feeding areas for 375 head of Jersey cattle. There are 180 lactating animals on the farm. The facilities have no active water wells; cattle drink water from a municipal source. During the summer months, a temperature sensor activates a mister to cool the cattle while they are eating silage. The area around the facilities is entirely concrete with cattle gaps located around the farm that allow runoff from the sprinklers and rainfall to be collected in a sump located beneath the loafing area. The total area of the concrete around the buildings is 0.71 hectares. Manure deposited on the concrete is scraped into the sump daily. From the sump, the waste is

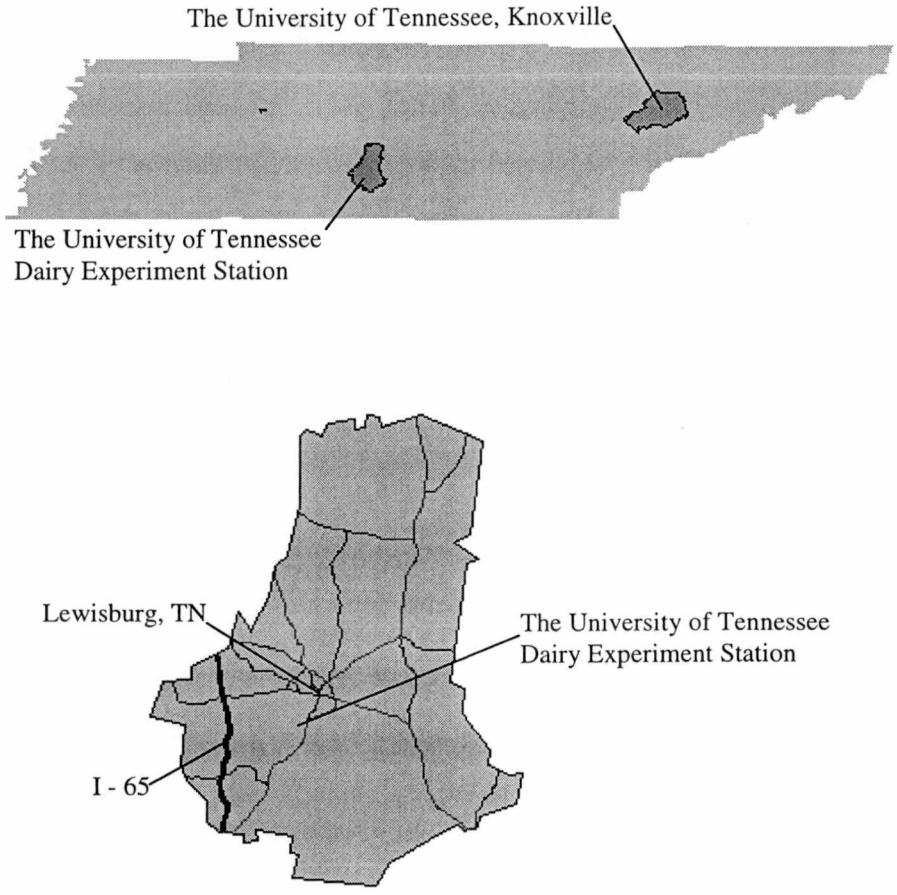

Figure 1 – Location of The University of Tennessee Dairy Experiment Station

pumped to a horizontal screw press liquid-solids separator (Model KP-6R, Vincent Corporation, Tampa, FL) where the resulting separator solids are piled for approximately one month, allowed to go through a heat cycle, and later used as bedding for the free stall barns. The separated liquid flows by gravity into a holding pond. When the holding pond is full, the liquid is pumped approximately 50 m and distributed via impact sprinklers onto a nearby pasture planted in fescue and Bermuda grass. This area is often used as a calving lot.

Sampling collection and storage

Samples were collected monthly from The University of Tennessee Dairy Experiment Station beginning in December 1999 and continuing through November 2000, generating 12 months of data. Seventy-five grams or milliliters of each sample were needed to conduct all the required tests. Samples were collected from Big Rock Creek from upstream and downstream of the loafing area on the farm to determine possible contamination of the stream from the farm (Figure 2). Freshly separated liquids and solids were collected from the liquid-solids separator, because this could possibly be used to determine cattle shedding patterns. Free stall bedding was collected because of its contact with the cow's udder and the possibility of pathogen transfer to the udder from the bedding; however, collection occurred before the bedding was placed in the free-stalls and contaminated by fresh manure. Pumped liquid from the holding pond was taken from pipe discharge points, before it contacted the ground. Bulk tank milk was collected to give a representative sample of the herd. A freshly fed silage sample was collected from the troughs before the animals were allowed access to it to determine if the silage

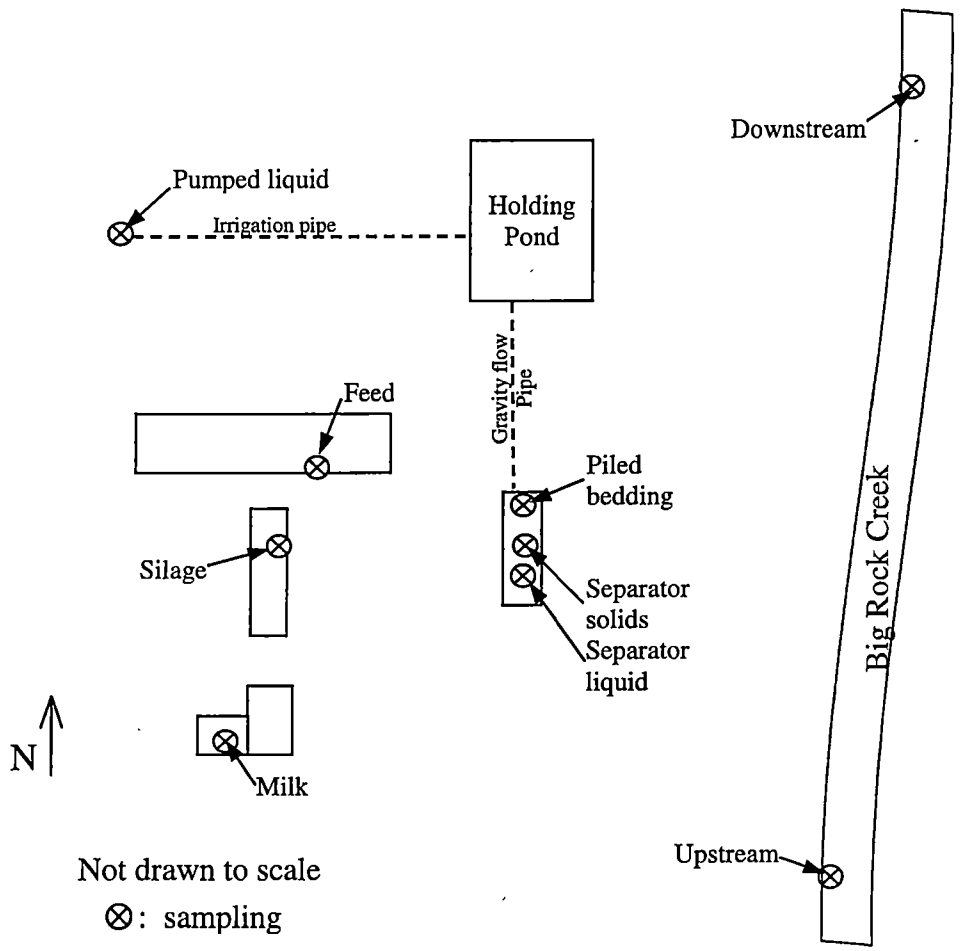

Figure 2 – Location of sample collection sites on The University of Tennessee Dairy Experiment Station.

had been properly stored and fermented. Feed samples were collected from the feed hopper for testing, because it was brought onto the farm from another location. After noticing positive samples in the pumped liquid, three soil samples from the topsoil of the irrigated pasture were collected in September and November.

Liquid samples were collected in glass bottles sterilized in bleach, and solid samples were collected in sterile resealable sandwich bags. Grab samples of all the solid samples were taken with disposable rubber gloves that were changed between samples, and the liquid samples were taken by placing the bottle in the liquid stream and allowing it to fill. Three samples were collected at each location every month during the study period. All samples were transported to the lab in an insulated cooler with an electric cooling unit to keep the temperature at 4 °C.

Meteorological data were taken each month from a weather station located on the farm. The station recorded total solar radiation, rainfall, average air temperature, relative humidity, wind speed and direction, and the maximum and minimum temperatures at 30-minute intervals. At the end of every 24-hour period, the data were summarized. These data were used to determine seasonal changes in pathogen presence.

Comparative sampling plan

It is of value to know the extent of pathogen presence throughout the area and off the farm. If pathogens are present throughout the off –farm area, the positive results found on the farm have less meaning. This would only show that the environment, in general, contains pathogens.

To determine if the public is in contact with pathogens in their everyday lives, samples were taken within the city limits of Lewisburg, Tennessee (Figure 3). Lewisburg has approximately 10,000 residents based on the 2000 Census Report and is located approximately 5 km north of the UT Dairy Experiment Station. Samples taken from the area included a soil sample from the local little league baseball park consisting of the top 4 cm of soil. Stream samples were taken up- and downstream of the municipal wastewater treatment plant. The treatment plant is located downstream from the dairy experiment station on Big Rock Creek. A water sample was also taken from Snake Creek, downstream of the Lewisburg Industrial Park. These samples were collected and tested to evaluate other instances of bacteria in a public area off of the farm. Three samples were collected at each location in

June, September, and November.

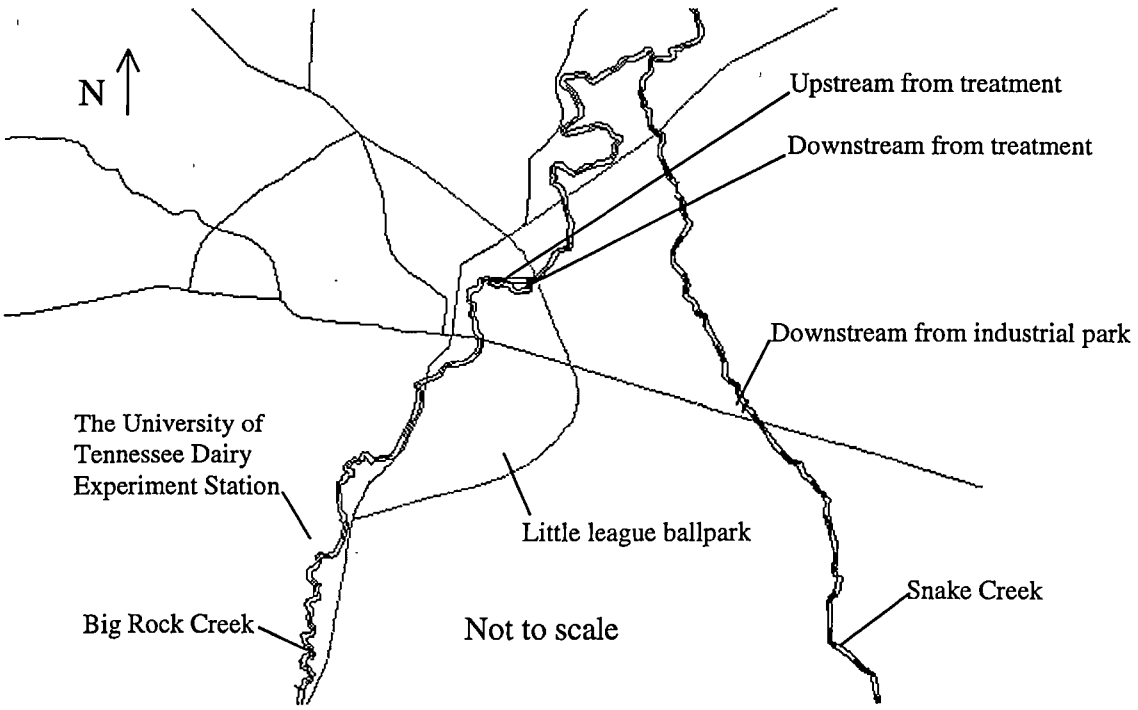

Figure 3 – Lewisburg area sampling locations

Microbiological analyses

The collected samples were taken to The University of Tennessee Food Science and Technology Lab for analysis. Two different media were used in the isolation of *Listeria monocytogenes* and *Salmonella* to ensure that the pathogens would be accurately identified.

Escherichia coli O157:H7

Twenty-five gram sample units were enriched with 225 ml of 0.1% peptone diluent and blended thoroughly. This mixture was then streaked onto MacConkey Sorbitol Agar (SMAC) (Difco Laboratories, Detroit, MI) with selective cefixime and tellurite supplement for the isolation of *Escherichia coli* O157:H7 and incubated for 48 hours at 35 °C. To test for coliforms, the mixture was streaked on Violet Red Bile 4-methylumbellifery-B-D-glucuronide (VRB-MUG)(Difco) and incubated for 48 hours at 35 °C. After incubation, the VRB-MUG plates were placed under UV light and checked for a positive result by fluorescence of the colonies. Colonies from the SMAC plates were placed on TSA slants, incubated at 35 °C for 24 hours, and then stored at 4 °C until confirmation. A coagulation test (Oxoid, Columbia, MD) was done on the SMAC colonies to confirm for *Escherichia coli* O157:H7.

Listeria monocytogenes

Enrichment of the samples was done by adding 25 g of the sample unit to 225 ml of Modified Listeria Enrichment Broth (LEB) (Difco). The broth was then incubated at 30 °C for 48 hours. After enrichment, the samples were streaked on Modified Oxford Media (MOX) (Difco) and PALCAM (Difco) agar plates and incubated at 35 °C for 24 hours. A characteristic colony was picked from each MOX and PALCAM plate and

placed on trypticase soy agar (TSA) slants in test tubes. These tubes were incubated at 35 °C for 48 hours and then stored at 4 °C until confirmation. Biochemical tests were done to determine the species using catalase, mannitol, CAMP with *Staphylococcus aureus*, motility medium (Difco), xylose, and rhamnose.

Salmonella

Enrichment of samples was accomplished by adding 25 g of the sample unit to 225 ml of RV (Oxoid) broth and incubating for 24 hours at 35 °C. To isolate the colonies, the broth was streaked on Hektoen (HE) (Difco) and incubated for 24 hours at 35 °C. The broth was streaked on XLT (Difco) plates and incubated for 48 hours at 35 °C. Characteristic colonies from each plate were picked from the plates and used to inoculate triple sugar iron agar (TSI) (Difco) slants. A polyvalent O agglutination test (Difco) was also used to test the colonies to ensure that they were Salmonella.

Statistical analyses

Statistical analysis to determine differences in locations was performed using chi-square test with SAS® software (SAS, Cary, NC). The results from the test were not significant ($P>0.05$), so a simple correlation was done for each pathogen with SAS to determine any significant ($P<0.05$) relationships between the locations. A simple correlation ranges from negative one to positive one to identify positive and negative relationships. A simple correlation was also done to determine if there was a relationship between average monthly temperature and positive isolates.

Chapter IV

Results and Discussion

Determination of a positive location was based on the presence of the pathogen in any of the three sampling units collected from that location each month. In cases where two media were used, if either plate showed growth, the sample for that month was considered positive for the pathogen of interest.

Listeria monocytogenes

During the 12-month testing period, all of the sampling locations tested positive at least once, except for the bedding and silage sampling location (Figure 8 on page 31). Overall, 30 (9%) of the total 324 samples collected on the farm tested positive for *Listeria monocytogenes*. Based on the data shown in Figure 4, there appeared to be more positive isolates identified in the colder months than when the temperature began to rise. A simple correlation performed using SAS® software identified a significant ($P<0.05$) negative relationship (-0.65) on a scale of negative one to positive one between the temperature and the number of isolates yielded. This observation agrees with the findings of Lovett et al. (1987) who observed the peak incidence of *Listeria monocytogenes* during cold weather months.

Milk was found positive for *Listeria monocytogenes* in one (3%) sample during the 12-month testing period. While this is lower than studies done on milk by Lovett et

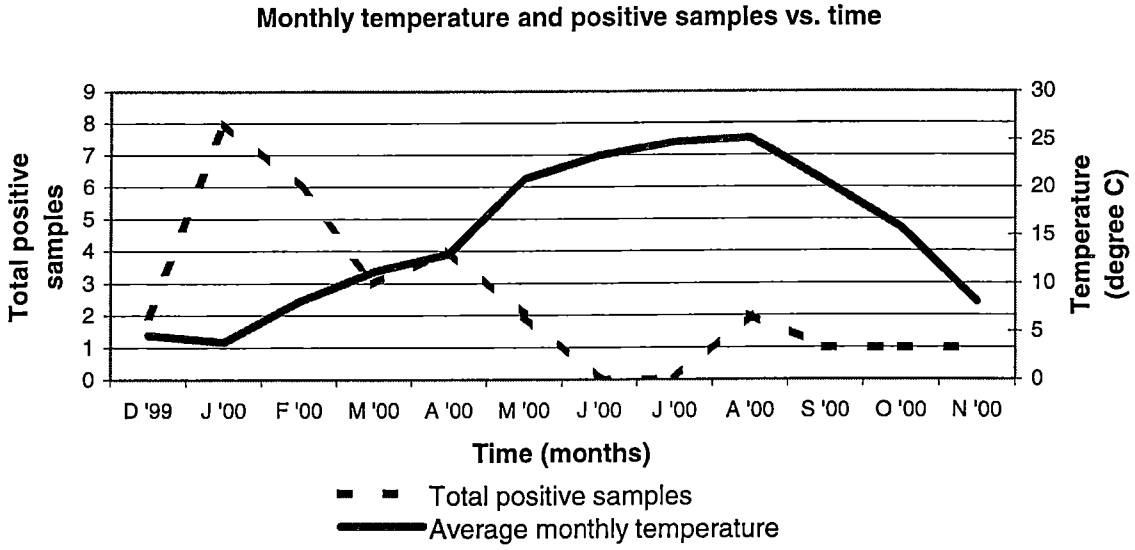

Figure 4 – Comparison of average monthly temperature and positive samples collected throughout the 12-month testing period for *Listeria monocytogenes*.

al. (1987) and Slade et al. (1988) with 4.2% and 5.4%, it is higher when compared to studies done by Farber et al. (1988) and Steele et al. (1997) who reported 1.3% and 2.7%. More samples should be collected to get a more accurate representation of pathogen presence.

No silage samples were found to be positive for *Listeria monocytogenes* during the testing period, which agrees with Husu et al. (1990). However, the lack of positives is in direct contrast to studies done by Ryser et al. (1997), Perry and Donnelly (1990), and Fenlon et al. (1989), that showed positives ranging from 12.5% to 33.3%. This contrast can perhaps be attributed to the small number of samples collected in this study, or to good management of the silage. Feed tested positive for *Listeria monocytogenes* in one (3%) of the 36 samples collected.

Collection locations associated with manure consistently yielded more positive isolates than other locations, as shown in Figures 5, 6, and 7. The pumped liquid and separator liquid contained 11 (31%) positives in 36 samples collected at each location. The fresh separator solids yielded positive isolates in four (11%) of the 36 samples. These results are a higher percentage of positives than the study done on dairy cattle conducted by Husu (1990) who found *Listeria monocytogenes* in 6.7% of the fecal samples. A simple correlation done using SAS found a significant ($P<0.05$) positive relationship (0.75) between positive samples in separator solids and separator liquids. This relationship was the only one of significance found by the simple correlation. Based on the results showing no pathogens in silage and a high concentration in the pumped liquid, a possible method for reducing *Listeria monocytogenes* from the holding pond in

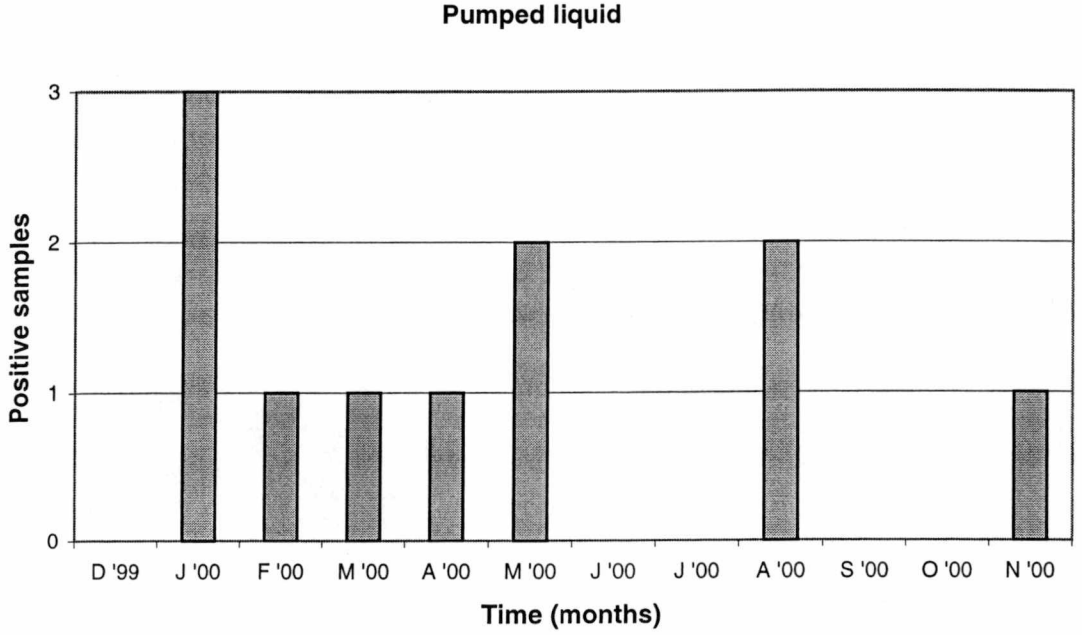

Figure 5 - *Listeria monocytogenes* positive results for each month at the pumped liquid sampling location (# positives/3 samples).

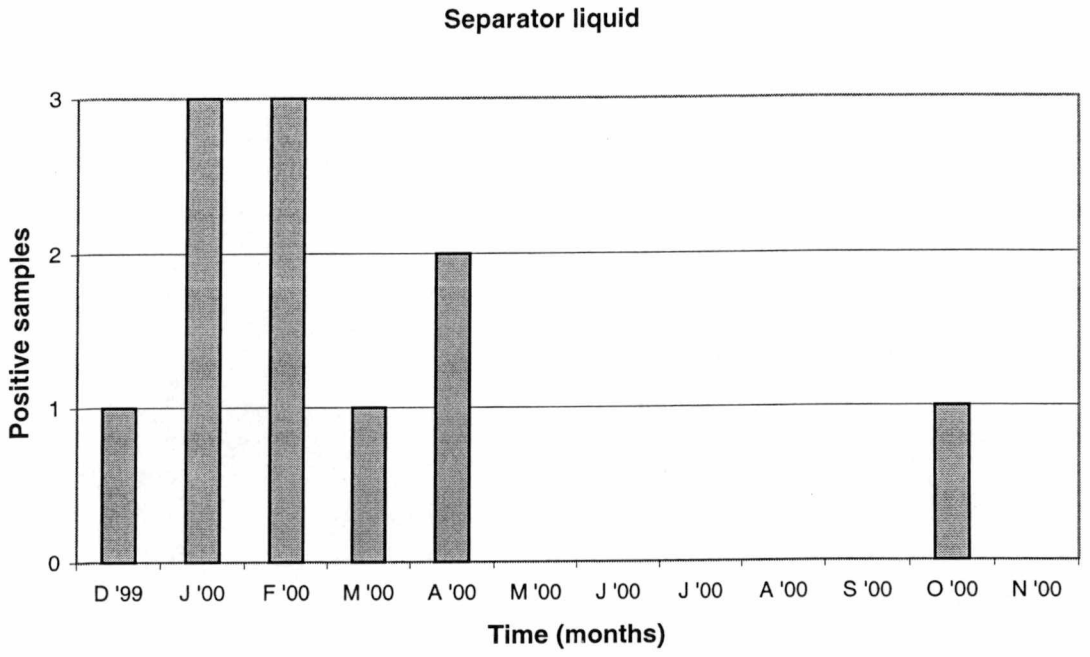

Figure 6 - *Listeria monocytogenes* positive results for each month at the separator liquid sampling location (# positives/3 samples).

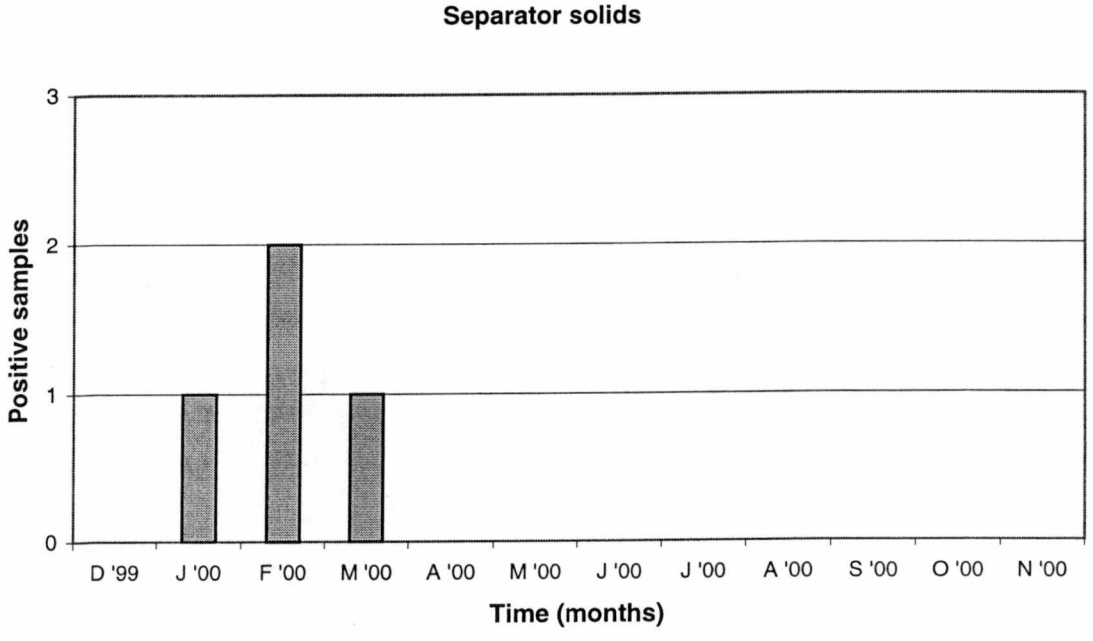

Figure 7 - *Listeria monocytogenes* positive results for each month at the separator solids sampling location (# positives/3 samples).

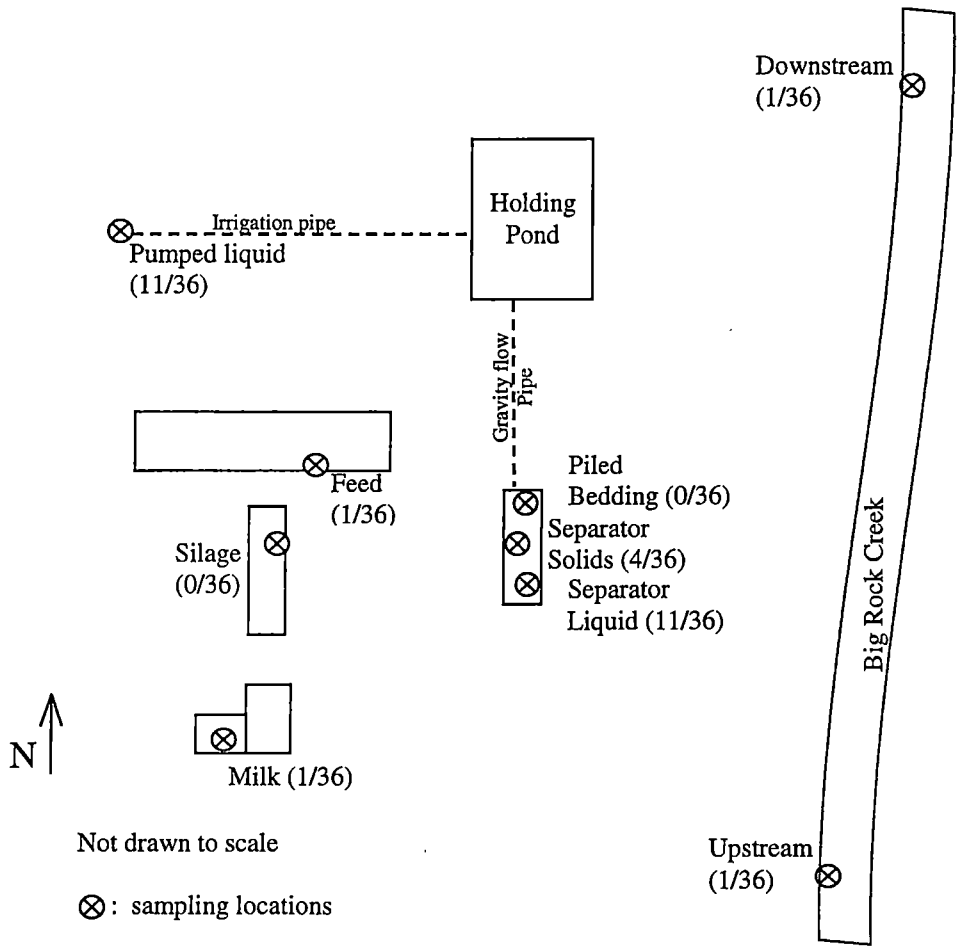

Figure 8 – *Listeria monocytogenes* positive results for each sampling location throughout the 12-month period (# positives/36 samples)

the future could be to irrigate silage corn with the holding pond liquid and proper fermentation should kill or significantly reduce pathogen levels that would have been present in the holding pond water.

Downstream and upstream samples each yielded one positive sample for September and January, respectively. From the six samples that were collected in September and November, one sample collected from the irrigated pasture soil in November tested positive for *Listeria monocytogenes*, suggesting that pasture irrigation may also be an acceptable method for reducing pathogen presence.

Salmonella

Over the 12-month period, every sampling location yielded at least one positive sample. The pumped liquid samples contained the highest number of isolates, and the milk samples contained the smallest number of isolates. Overall, 154 (48%) of the 324 total samples were positive for *Salmonella.* There did not appear to be a seasonal trend in the collection of *Salmonella* from samples (Figure 9).

Salmonella was found in milk (Figure10) in one of the three samples collected in January and in July. Based on three samples a month for twelve months, *Salmonella* was present in two (6%) of the 36 samples collected. This presence is higher than in other studies of a larger scale, such as McManus and Lanier (1987) and McEwen et al. (1988a), but can perhaps be explained by the small number of samples collected.

Samples collected above and below the farm showed that only in February was *Salmonella* isolated in the downstream samples (Figures 11) and not in the upstream samples (Figure 12). The pathogen was not present in either downstream or upstream

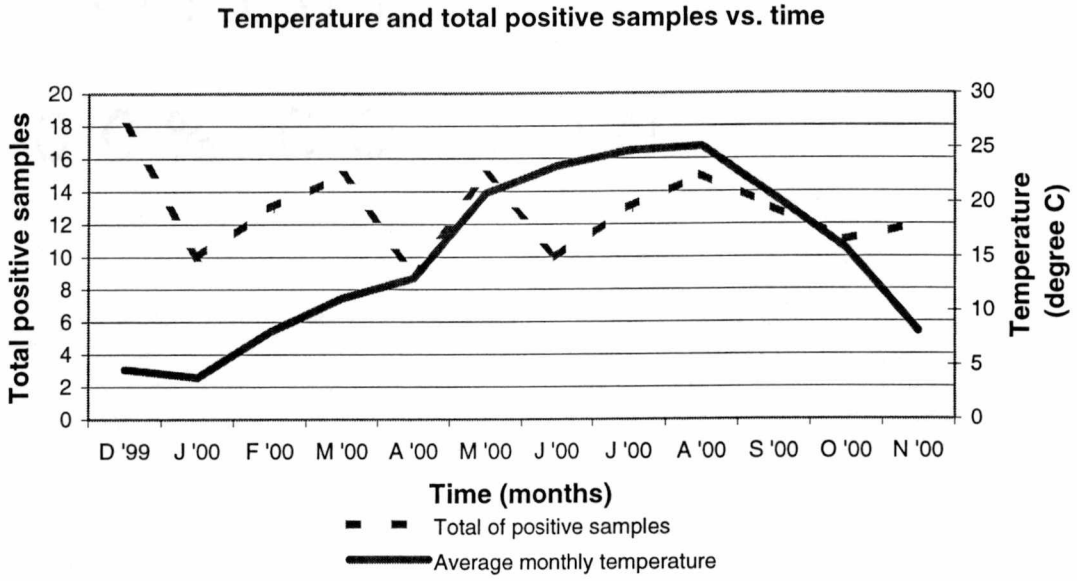

Figure 9 - Comparison of average monthly temperature and positive samples collected throughout the 12-month testing period for *Salmonella.*

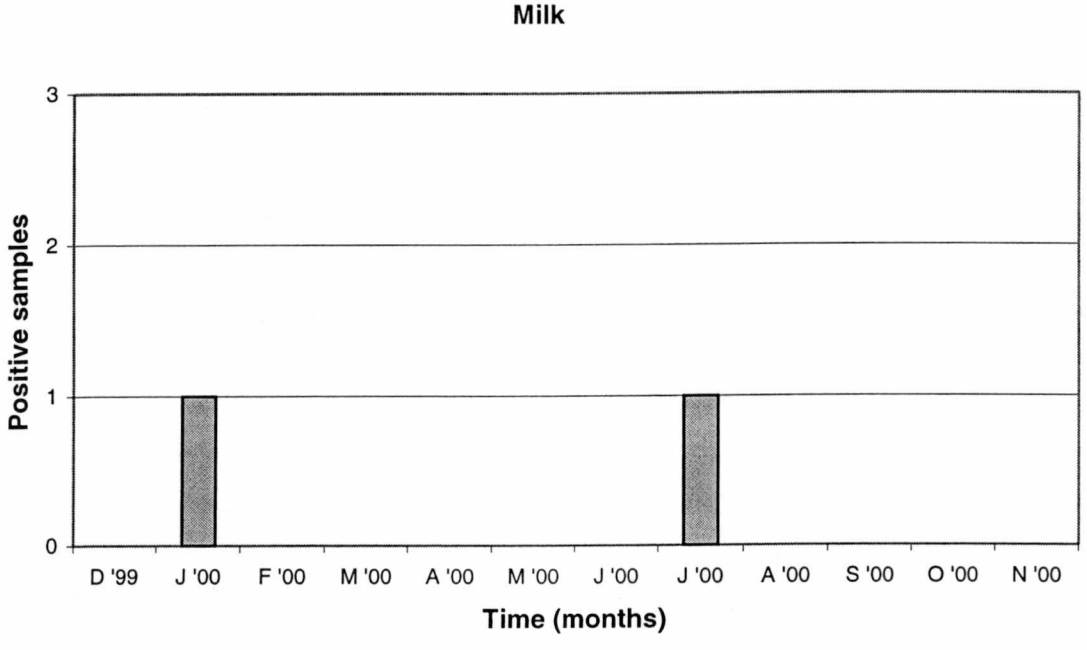

Figure 10 – *Salmonella* positive results for each month at the milk sampling location (# positives/3 samples).

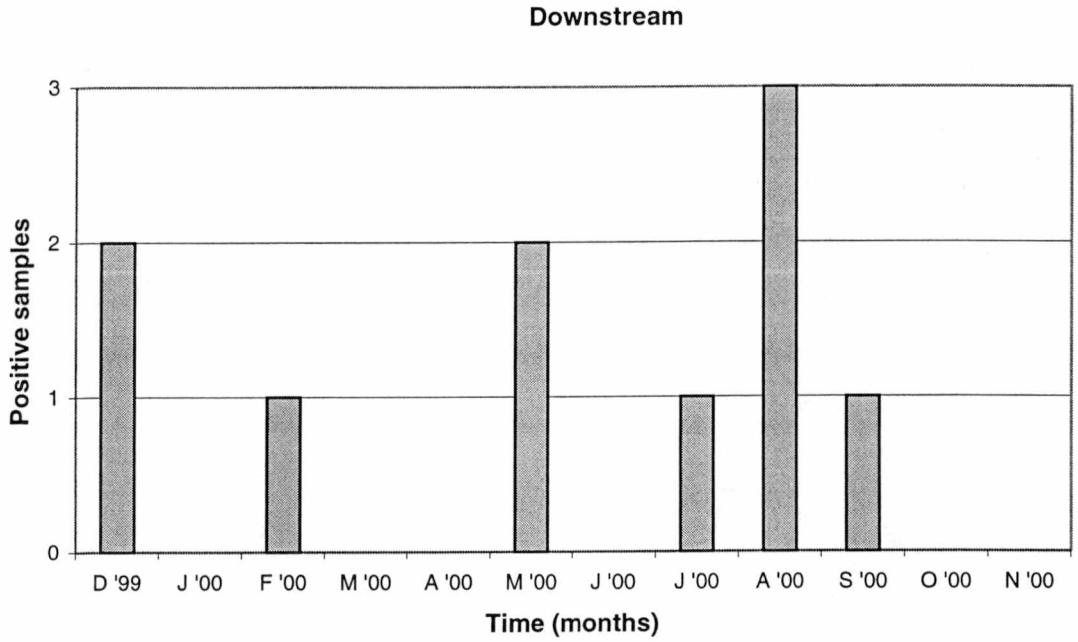

Figure 11 – *Salmonella* positive results for each month at the downstream sampling location (# positives/3 samples).

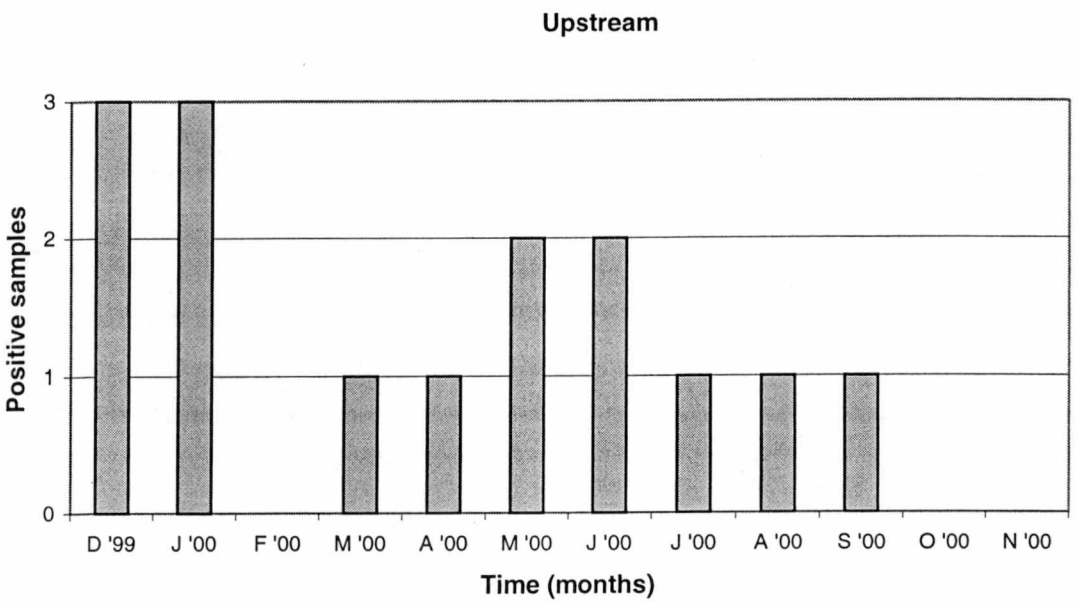

Figure 12 – *Salmonella* positive results for each month at the upstream sampling location (# positives/3 samples).

samples in October and November. At the downstream location, *Salmonella* was isolated from ten (28%) of the 36 samples. Fifteen (42%) of the 36 upstream samples resulted in positive isolation of *Salmonella*. These results are similar to those of Smith et al. (1978) who found the pathogen in 39% of the samples collected from streams and rivers.

The separator liquid samples (Figure 14) yielded 26 (72%) positive isolates from the 36 possible samples. June was the only month to return no positive samples. The pumped liquid (Figure 13) showed significant contamination in every month but February, having all three samples isolate *Salmonella*. The results showed that 34 (94%) of the 36 samples tested for the pathogen were positive. These results suggest a possible incubation of the pathogen in the holding pond before it is discharged onto the pasture.

Every month yielded at least one positive sample from separator solids shown in Figure 15. Collected separator solids tested positive for *Salmonella* in 29 (81%) of the 36 samples. This percentage is similar to that of separator liquid suggesting that the pathogen does not favor one medium over the other (Figure 19). The bedding samples (Figure 16) tested positive in 21 (58%) of the 36 samples. Based on the results presented here, composting the solids in a more uniform manner instead of allowing them to remain in a pile until they are needed might decrease the concentration of pathogens. *Salmonella* was present in silage (Figure 17) in nine (25%) of the 36 samples tested. There appears to be a seasonal trend with most of the isolates collected in the cooler months. The feed (Figure 18) collected from the farm resulted in 22% positive isolation. These results are lower than the results found by McChesney et al. (1995) that found 56.4% of the animal protein samples and 36% of the plant protein samples positive for *Salmonella*. No *Salmonella* isolates were recovered from the irrigated pasture soil.

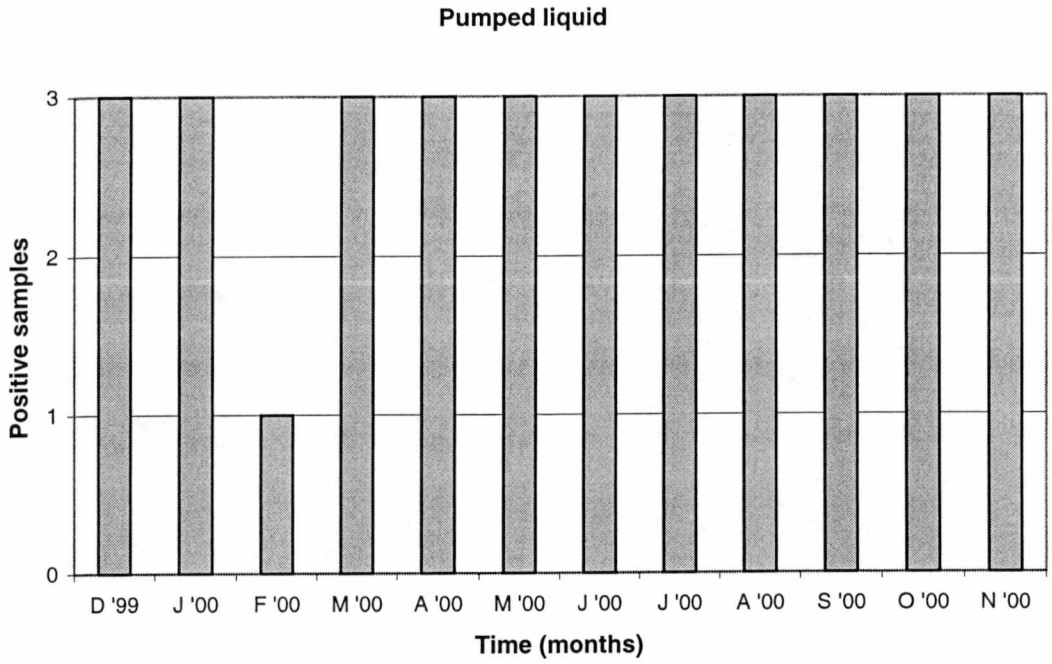

Figure 13 – *Salmonella* positive results for each month at the pumped liquid sampling location (# positives/3 samples).

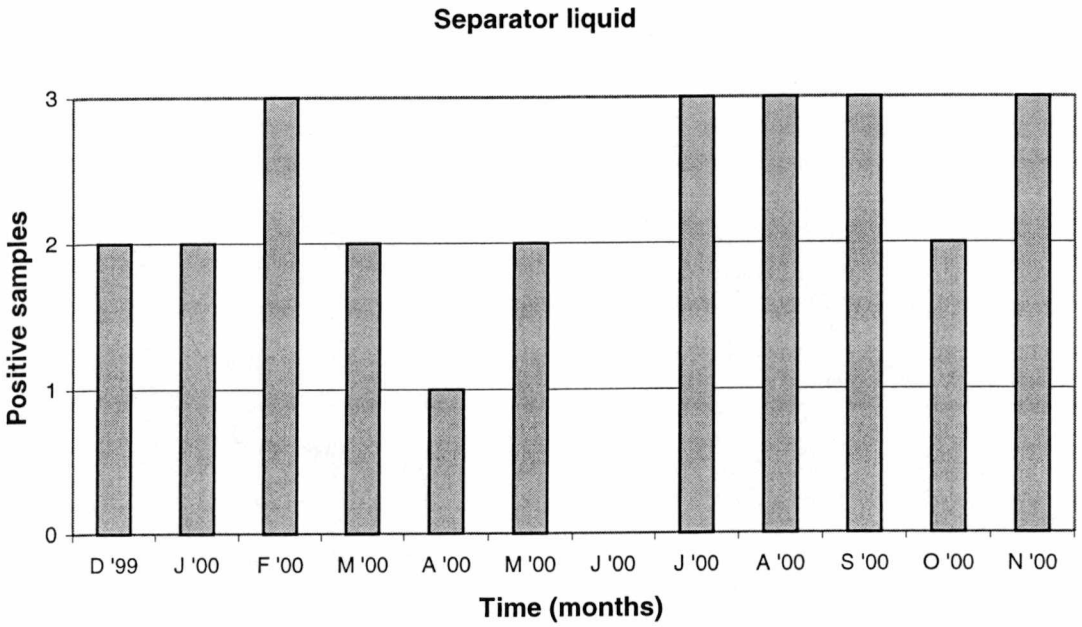

Figure 14 – *Salmonella* positive results for each month at the separator liquid sampling location (# positives/3 samples).

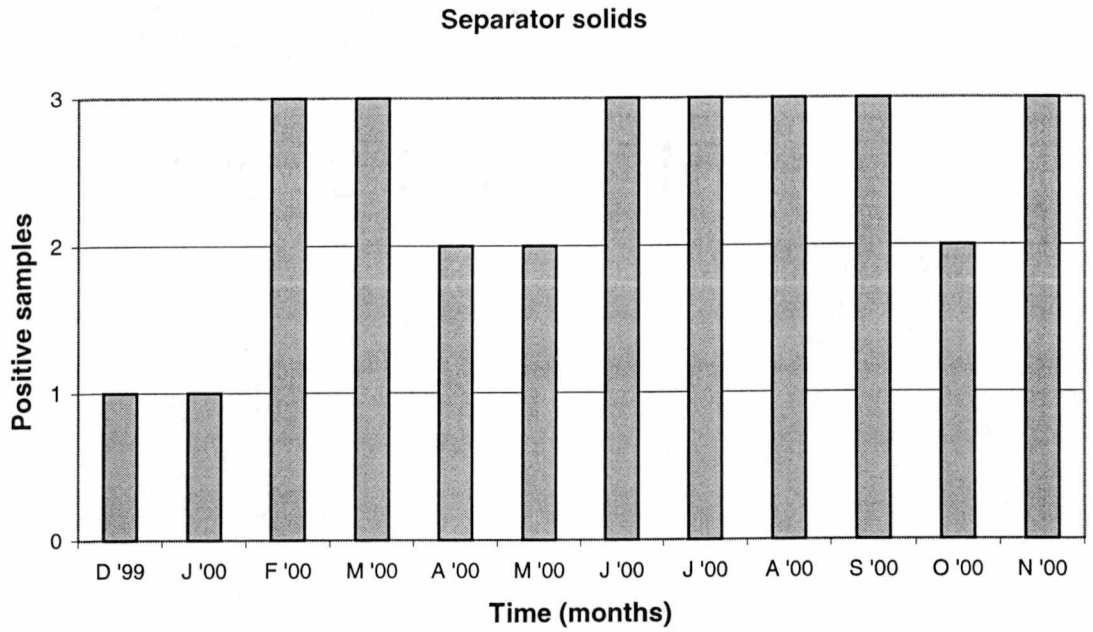

Figure 15 – *Salmonella* positive results for each month at the separator solids sampling location (# positives/3 samples).

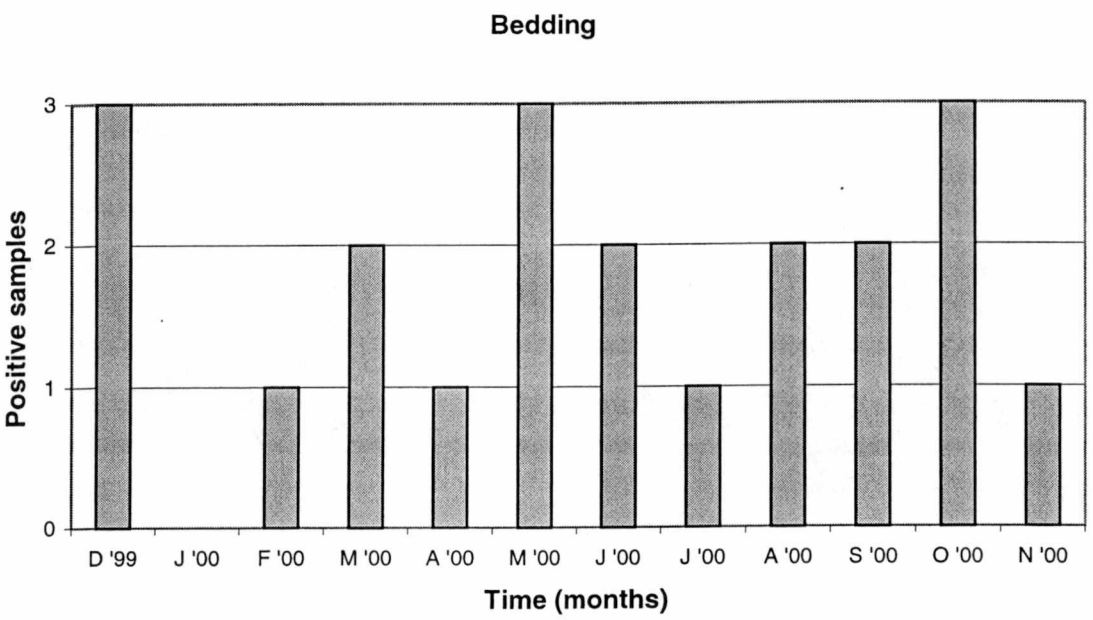

Figure 16 – *Salmonella* positive results for each month at the bedding sampling location (# positives/3 samples).

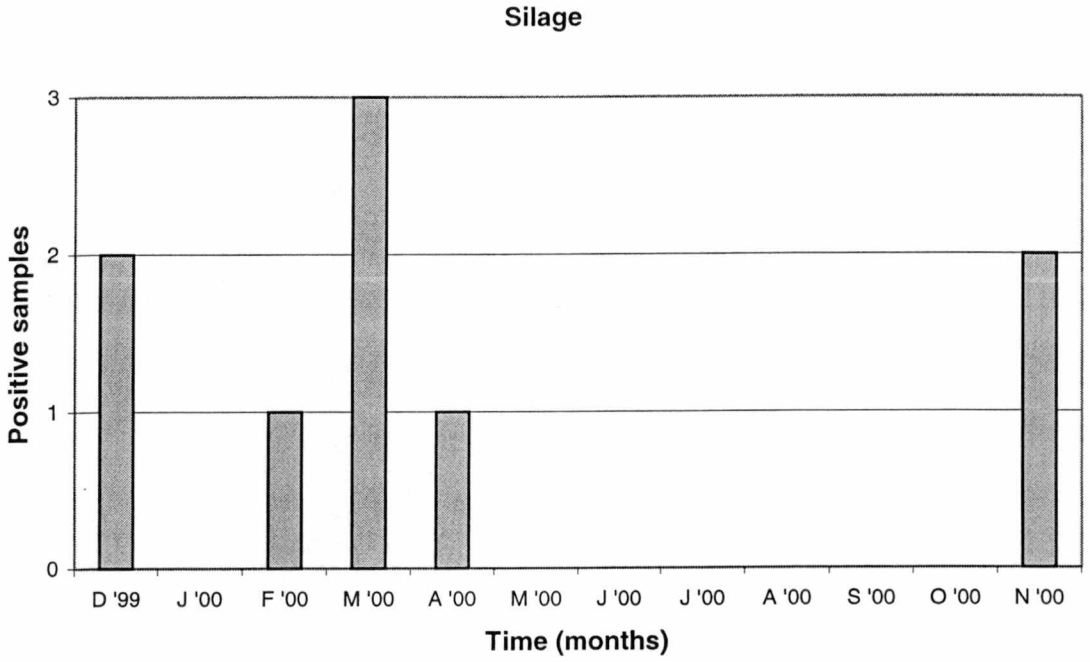

Figure 17 – *Salmonella* positive results for each month at the silage sampling location (# positives/3 samples).

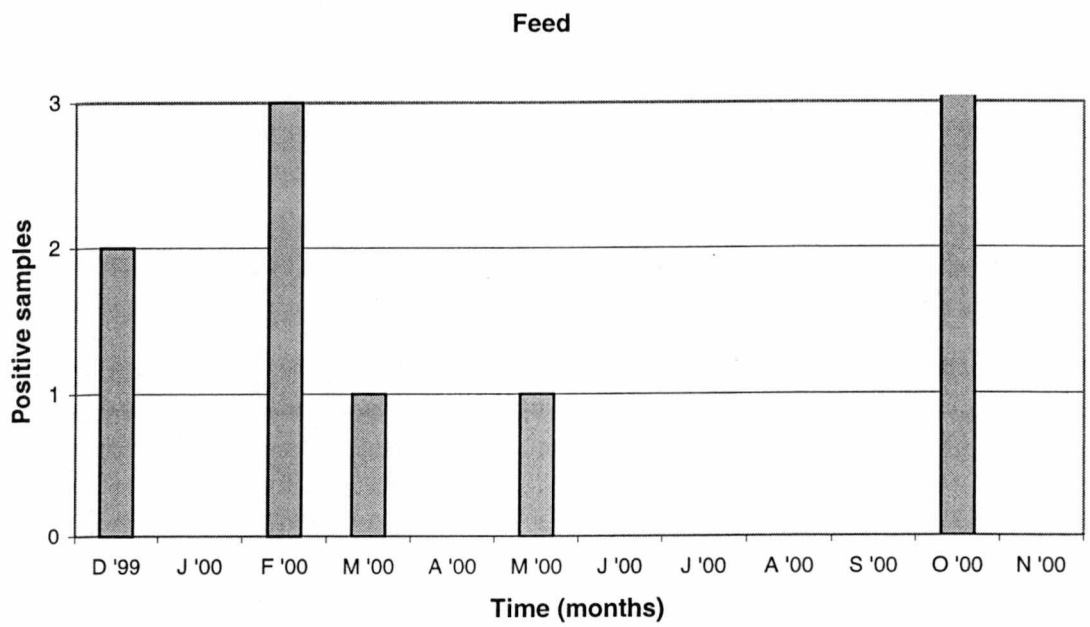

Figure 18 – *Salmonella* positive results for each month at the feed sampling location (# positives/3 samples).

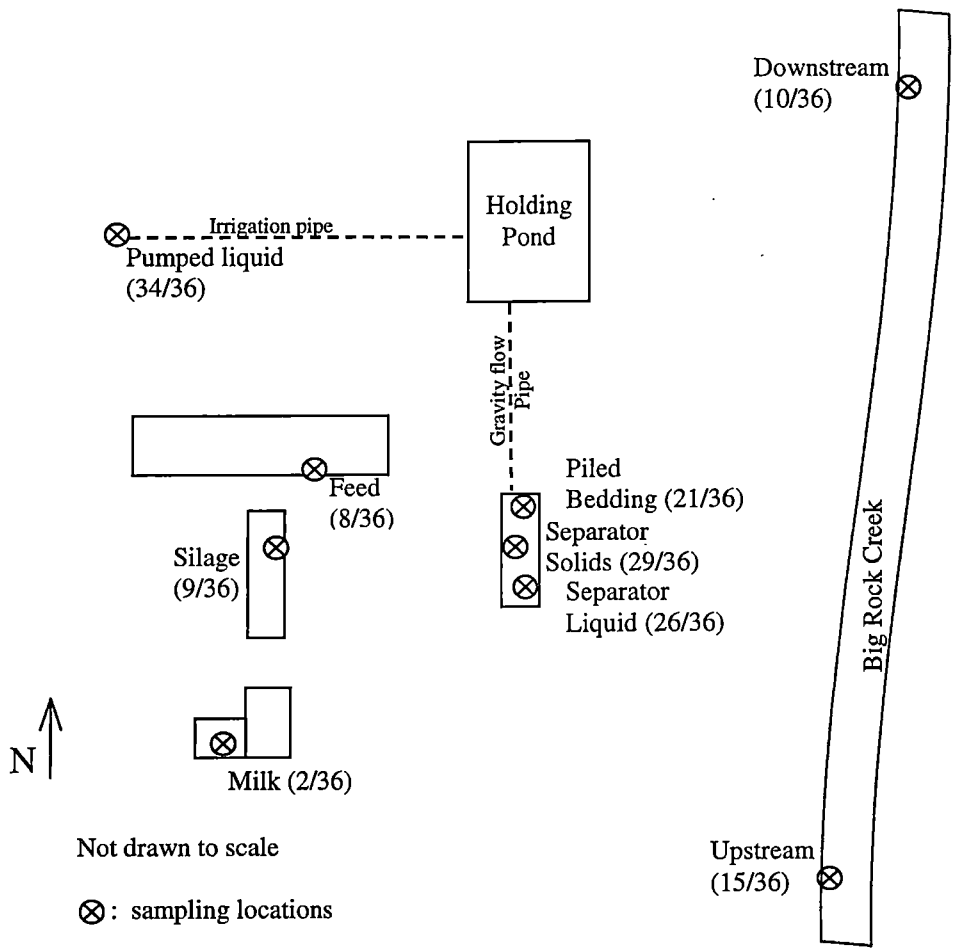

Figure 19 - *Salmonella* positive results for each sampling location throughout the 12-month period (# positive/36 samples).

Escherichia coli

Based on the fluorescence of colonies, pumped liquid, separator liquid, and separator solids were positive every month for coliforms. Bedding was positive five months with 33% of the samples fluorescing. Milk plates showed fluorescence in April, May, and July showing a 20% occurrence of positive samples, while feed, silage, down-, and upstream samples showed no occurrences of coliforms during the12-month test period. A soil sample taken from the irrigated pasture tested positive one time in September. After serological testing, all samples that were tested for *Escherichia coli* O157:H7 were negative. The lack of positive isolates for *Escherichia coli* O157:H7 is not surprising considering the limited scope of this study. The study done by Hancock et al. (1997b) found only 1.41% positive isolates out of 12,664 samples collected. In order to get a better idea of the extent of isolates on the farm, a study with a larger scope should be conducted.

Comparative samples

Figures 20 and 21 exhibit the results of the off-farm samples. The samples collected from the little league baseball park soil resulted in one positive *Salmonella* isolate. *Listeria monocytogenes* was not isolated from any of the nine soil samples collected from the park.

Each of the environmental stream samples collected from Big Rock Creek and Snake Creek produced one positive sample of *Listeria monocytogenes* for the month of September. The comparative stream samples showed an increase in the number of isolates of *Salmonella* below the wastewater treatment plant release from those above the

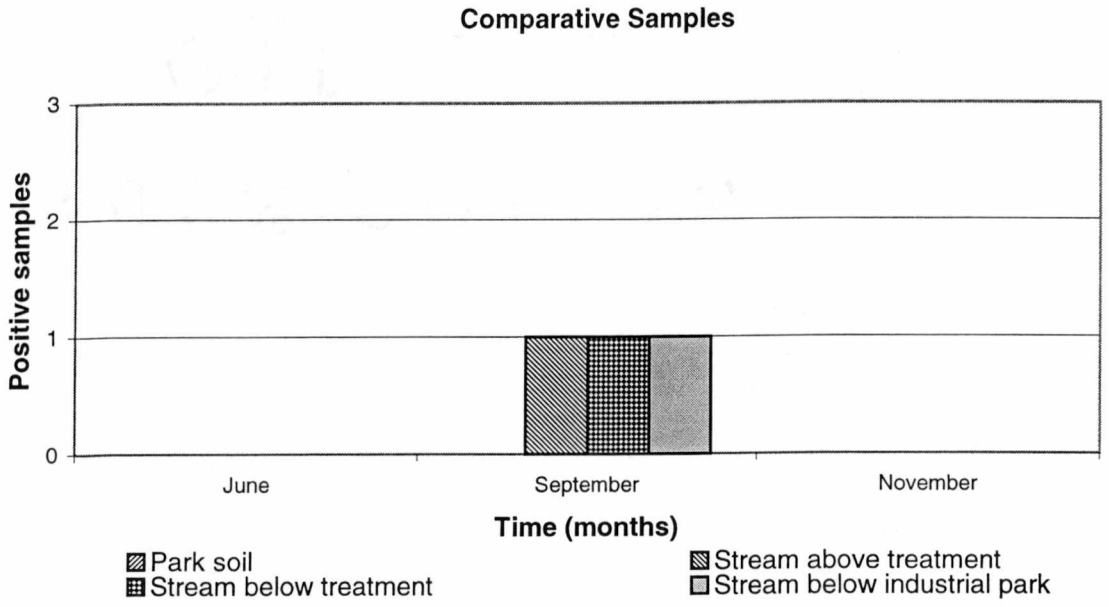

Figure 20 – *Listeria monocytogenes* positive results at each comparative sampling location during the testing period (# positive/3 samples).

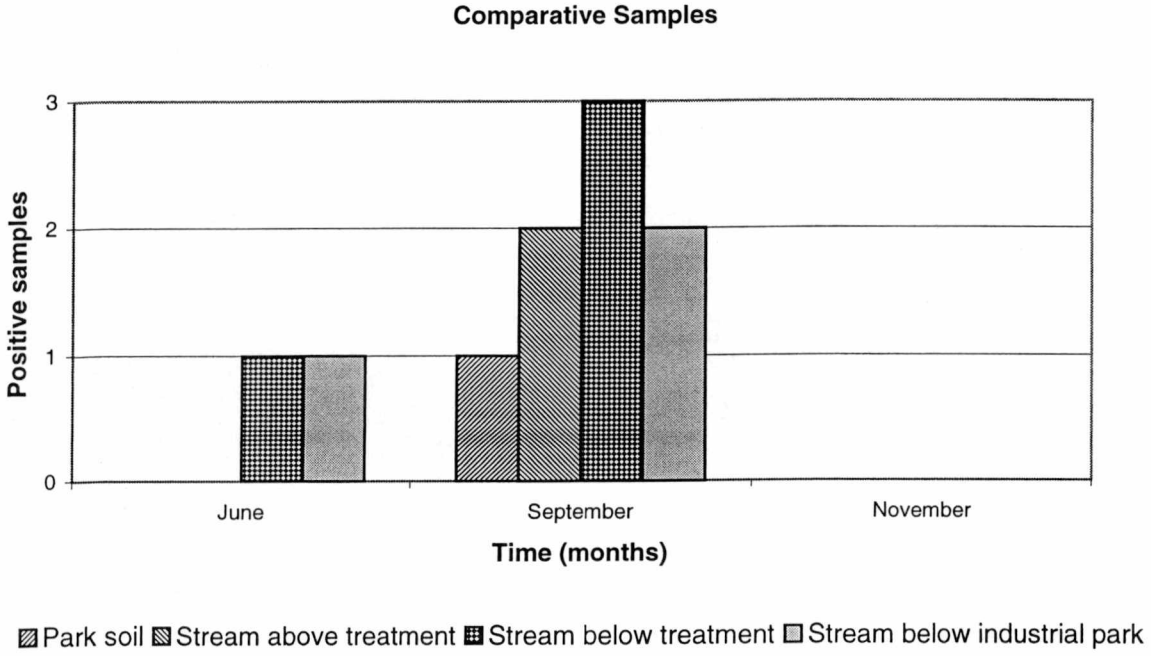

Figure 21 – *Salmonella* positive results at each comparative sampling location during the testing period (# positive/3 samples).

treatment plant by 22%. Isolates were positive in four (44%) of the nine samples collected below the wastewater treatment outlet and only two (22%) were positive above the outlet. These data are consistent with the results of a study done by Smith et al. (1978). In that study, *Salmonella* was isolated from 39% of the samples, and Smith et al. suggested that sewage effluent was a greater source of microbial pollution than farm effluent. Three (33%) of the nine samples collected below the industrial park were positive for *Salmonella*. No positive isolates were found in November from any of the streams on or off the farm.

Both of the soil samples collected from the irrigated pasture were negative for *Salmonella*, whereas, the samples collected from the little league baseball park resulted in one positive isolate. However, the irrigated pasture soil yielded a positive *Listeria monocytogenes* sample in November, and none of the park soil tested positive for the pathogen.

Samples collected below the waste treatment outfall provided similar *Salmonella* results (44%) to downstream (27%) and upstream (42%) samples taken on the farm. Three (33%) of the nine samples collected below the industrial park were positive for *Salmonella*.

Chapter V

Conclusions

Salmonella were found at every location on the farm at least once during the 12-month study; however, as might have been expected, the pathogen was more concentrated in samples collected from the pumped liquid, separator liquid, and separator solids with 58% of the positive samples collected from these locations. These locations should be considered possible control points for *Salmonella*; however, the two times that soil samples were collected from the pasture irrigated with holding pond water no *Salmonella* were found. Further research should be done, but pasture irrigation of holding pond water appears to be a method of reducing S*almonella* presence. Another possible method for reducing *Salmonella* from the holding pond in the future could be to irrigate silage corn with the liquid and then to ensure proper silage fermentation. Based on the results from this study, silage tested positive five of the tested months, while pumped holding pond liquid had positive samples every month. While the silage collected had not been irrigated with holding pond water, proper fermentation of the silage might reduce the pathogen levels that were present in the pumped liquid samples. While positive samples were collected from the stream at both locations on the farm resulting in 22 (31%) of the total stream samples being positive, positive samples were also collected from streams sampled away from the farm. The positive results from the stream samples show that the wastewater treatment facility (44%) and the industrial park (33%) were similar to the results collected from the on-farm samples.

Listeria monocytogenes was not as prevalent as *Salmonella*, but there was a marked difference in the concentrations at the different locations. Both the pumped liquid and separator liquid exhibited higher incidences of *Listeria monocytogenes* than any of the other locations, containing 73% of the samples that tested positive; one positive sample was collected from the irrigated pasture soil. These results suggest that by setting up control points at these locations the spread of the pathogen could possibly be reduced. When the isolates collected from the pumped liquid are removed from the total number of positives, the percentage of positive samples decreases from 9% to 7%. Each of the stream sites tested, both on the farm and off the farm, yielded one positive sample for the 12-month period. This leads to the conclusion that pathogens may be present in the stream before it reaches the farm. Lack of positive samples for silage is not consistent with other studies done; perhaps, the sampling size was not large enough in scope to obtain comparable results.

No *Escherichia coli* O157:H7 positive samples were found in any of the samples collected. Based on other studies of a much larger scale, the absence of the pathogen in the relatively small number of samples collected in this study is not surprising. To be able to better identify positive samples, better methods of isolation should be used than the methods that are currently in use.

Figure 22 shows the total positive samples collected at each location throughout the 12-month period. These results suggest it may be possible to implement or develop a strategy for on-farm control points similar to the HACCP system that may reduce, but not eliminate pathogens. Possible suggestions for reducing the pathogen presence on the farm are composting the separated solids to reduce pathogen populations in the bedding

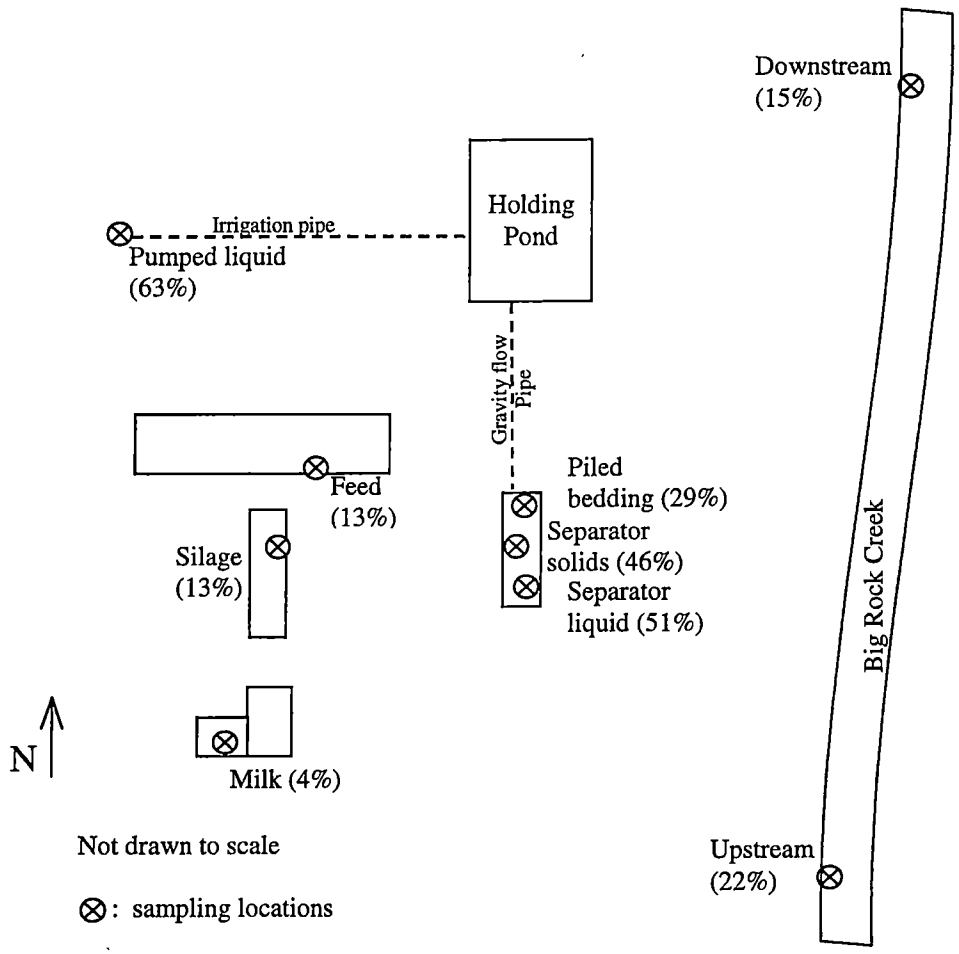

Figure 22 – Total positive samples for all pathogens at each location on the farm.

and applying the holding pond water to pasture or silage that will be properly fermented to reduce the pathogen concentration.

The farm environment inherently contains pathogens. Cows, workers, birds, and other mammals that have contact with the farm are carriers of pathogens. Pathogen control can possibly be accomplished in chicken houses, because those are controllable, enclosed environments. To remove all pathogens will be impossible, but by controlling the sources and reservoirs, such as composting of separator solids and irrigation of silage crops, pathogen concentrations can be reduced on the farm and over time pathogen populations in critical locations could be reduced and possibly eliminated.

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Vita

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