

Survival of Pathogenic Bacteria in Liquid Manure Storages

Final Report

**Project 03/14
for Ontario Pork
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R I D G E T O W N • O N T A R I O

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Executive Summary

This study set out to measure die-off rates of bacteria in liquid manure storages on swine farms and to establish which criteria, if any, have the biggest impact on these die-off rates. Between July, 2003 and July 2004, liquid manure samples were collected from 28 swine farms in southwestern Ontario. Four samples per storage were collected. The samples were analyzed for levels of manure nutrients and for *E. coli* and *Salmonella*. All of the storages served barns with a recent history of *Salmonella* presence (at least in individual animals or pens), so the prevalence of *Salmonella* was expected to be high. At each site, measurements were made of manure depth in storage, temperature of manure, and the approximate age of manure in the storage. The main findings of the study:

- Even though the farms were selected based on a high probability of finding *Salmonella*, only 40.9% of the samples tested positive for the presence of *Salmonella*.
- Numbers of *Salmonella* present were rather low. For the 45 samples testing positive, the geometric mean density was 1.36 organisms per mL (using the Most Probable Number analysis). The highest count was 427 MPN/mL.
- There was no significant difference in *Salmonella* counts between the four visits to the farms - roughly representing different seasons of the year.
- Despite the fact that there were differences in manure temperatures and dry matter levels, there was no significant difference in *Salmonella* numbers (log transformed) between covered and uncovered storages.
- There was no significant relationship between *Salmonella* numbers (log transformed) and either manure depth or manure age.
- There was a relationship between *Salmonella* detected in animals in the barn (from pen manure and from individual animals' fecal or blood samples) and *Salmonella* in the manure storage.
- High levels of *E. coli* in the stored manure did not prove to be a good predictor of the presence or density of *Salmonella*.
- Concentrations of any of the commonly-measured manure nutrients bore no relation to the density of *Salmonella* organisms in the manure.
- There was a significant difference in *E. coli* densities between farms and between visits, but not between storage types.
- There was no statistically significant relationship between the log density of *E. coli* and any of the following: manure depth, manure age, dry matter, NH₄, TKN, K, P, or manure temperature.

This study was not able to establish the rate of die-off of *Salmonella* or *E. coli* in manure storages. The most likely reason for this was that fresh manure was added regularly to all of the storages in the study. However, it did establish that in those manure storages where *Salmonella* was present, the counts were very low.

Survival of Pathogenic Bacteria in Liquid Manure Storages

Introduction

Liquid manure systems are used on most swine farms in Ontario. Based on economics, labour and ease of materials handling, liquid manure offers advantages over the bedded “solid” manure systems. Unfortunately, the risk of surface water or groundwater contamination is greater with liquid than solid manure. One of the concerns with liquid manure systems is their potential to contaminate water resources with pathogenic organisms (e.g. bacteria).

There have been advances in the understanding of how manure “treatment” systems affect pathogen levels. The anaerobic manure storage, commonly used on swine farms, may be viewed as a form of manure treatment. These storages actually have the potential to destroy pathogens - at least, they do not support the growth of pathogens, and there is an overall decrease in levels. Even though science is making gains at understanding the survival characteristics of a range of pathogenic organisms, there is still information lacking in the area of actual die-off of bacteria in anaerobic liquid manure storages. Storage practices, perhaps in conjunction with spreading management practices, may be effective at reducing pathogen levels.

Literature Review

In a study dealing with *Cryptosporidium* and *Giardia* in livestock manure storages and surface water, Fleming et al (1999) found that conditions in the manure storages did not appear to cause a complete die-off of these organisms. While these are pathogenic, they are protozoa, possibly with different survival characteristics than bacteria.

Cryptosporidium oocysts (the part of the life cycle that is most easily transmitted in the environment) were present in 37% of the swine manure samples collected. Of these, 86% were “viable” (i.e. capable of causing further infection in a host).

In a report commissioned by the Walkerton Inquiry, Goss et al (2002) summarized research results dealing with bacteria in manure storages. They found that bacterial populations can change significantly during storage. Survival rates appeared to be influenced by levels of dissolved oxygen (aerobic vs anaerobic conditions) and temperature. Temperature did not affect all organisms in the same way – it helped kill some but prolonged the life of others.

A literature review aimed at characterizing pathogens in livestock manure storages was completed in Ontario in 2003 (Ghimire et al 2003). The results of a wide range of studies from around the world were summarized. Some of the findings:

- pathogenic organisms deemed to be of greatest concern (i.e. prevalent in manure, pathogenic to humans, transmitted in the environment via manure) are *Salmonella*, *Campylobacter*, *E. coli* 0157:H7, *Cryptosporidium* and *Giardia*
- pathogen survival times vary greatly, depending on the organism and the environmental conditions - under certain conditions, some pathogens may survive in the environment for only a few days while under other conditions, they may survive for over a year
- pathogen survival time in manure storages is increased with lack of aeration, lack of self-heat generation, low ambient and storage temperature, and high solids content in the slurries
- though information is available on the pathogen content of fresh feces, much less information is available on pathogens in stored manure
- initial loading of pathogens affects survival, so studies where pathogens were inoculated at high rates may overestimate the real risk of manure pathogens (Ghimire et al 2003)

Objectives

A study was designed that involved an on-farm research project, over the course of a full calendar year, to:

1. measure die-off rates of bacteria in liquid manure storages on swine farms;
2. assess the importance of length of storage period, temperature of manure, and other management options on the survival of pathogens.

Project Setup

General

This was set up as an on-farm study. It took place over a full calendar year, starting in the summer of 2003. This allowed for sampling on each farm at four occasions, once per season. The expectation was that this would provide differences in manure temperatures that could affect bacteria survival.

Other anticipated sources of variability included:

- age of animal - i.e. differences in pathogen levels between feeder pig manure and sow manure
- covered storages (under barn) versus open storages - the covered storages would typically have a higher average temperature, would have less opportunity for aeration, and would have no exposure to sunlight

- age of manure in the storage - there may be a significant die-off the longer the manure is stored

At each of the four farm visits, two manure samples were collected. One sample was analyzed for levels of the two bacteria selected for the study. The other sample was submitted for nutrient analysis - including pH, dry matter, total Kjeldahl nitrogen (TKN), total phosphorous (P), total potassium (K), and ammonium-nitrogen (NH₄-N). The assumption was that the relative concentrations of manure nutrients might help point out differences in manure that could account for changes in bacteria levels.

Selection of Bacteria

Early in the planning for this project, there was a desire to test for levels of at least three bacteria: *E. coli*, *Salmonella* and *Listeria*. *Listeria* was initially included because its survival characteristics are different from *Salmonella*. For a variety of reasons, the list was shortened to *E. coli* and *Salmonella*, considered to be the two most critical organisms. *E. coli* would serve as a commonly-used indicator bacteria. Though it is not normally pathogenic, it is easily measured and is present in large numbers in all samples. Most studies on bacteria survival include *E. coli*, so it would be useful for comparison with past studies.

Salmonella was chosen because it is the pathogen of greatest concern on swine farms. There was some feeling initially that *Salmonella* was not a good choice because it is not present in many herds. A recent literature review (Ghimire et al 2003) suggests that this is an important organism to include in the test and that the expected prevalence in swine manure samples would be in the range of 11% to 21%. The relationship between presence and numbers of *Salmonella* to numbers of *E. coli* was considered to be very important to this study.

Site Selection

The original concept was to find 20 farms testing positive for *Salmonella* in the stored manure. Two options were developed to achieve this number. The first approach involved targeting farms with an identified recent history of *Salmonella* in the herd. The second involved sampling manure from a large number of farms (at least 80 farms) and screening out the farms testing positive for *Salmonella*. The first of the two approaches was deemed the more efficient and was therefore chosen.

The herd health status for several swine farms in Ontario is tracked as part of a Sentinel Herd Study, carried out by Dr. Bob Friendship, Ontario Veterinary College, University of Guelph. This study was able to identify *Salmonella*-positive farms - i.e. farms where at least a portion of the herd had recently tested positive for *Salmonella*. The fact that individual animals had tested positive for *Salmonella* did not guarantee that it would be found in measurable amounts in the manure storage. Therefore, to boost the chances of finding 20 farms testing positive, the sample number was increased to 28 farms. A further

advantage of using the Sentinel Herd farms was that historical data on herd health was available for the farms, if needed.

The Sentinel Herd study involved farms from across the province. The pathogen study, however, was limited to southwestern Ontario. This did not prove to be a problem, as a large portion of the swine population in Ontario is in the study area. The sites selected covered a region from Windsor (in the west) to Wingham (in the north) to the Guelph and Jarvis areas. Once farms were identified, the livestock producers were approached to see if they would participate in the study. All agreed to take part.

One of the initial assumptions was that if all the study farms were feeder pigs (as opposed to dry sows, farrowing, weanling, farrow to finish, etc.), it would help eliminate a potential source of variability. Also, feeder pig manure represents a greater percentage of the total swine manure spread in Ontario. It proved too difficult to be so restrictive, however. The result was a representative cross-section of the swine industry in Ontario. Test sites included farrow to finish, farrow to wean, wean to finish and finishing barns.

Some of the farms had covered storages, some had uncovered storages. Some had wet/dry feeders and some had dry feeders. A set of farm survey/data sheets was developed to capture the many management details that were deemed to be relevant to the study. Copies of these data sheets are attached as Appendix 1, 2 and 3. Information collected included: size of herd, herd management (e.g. "all-in, all-out", wet/dry feeders, manure additives, spreading dates, dimensions and capacities), typical use of antibiotics and any special cases of drug use and general herd health status.

Just as biosecurity was considered to be critical, so also was confidentiality considered to be vital for the study. The health status of a herd is private information, and the study cooperators were assured of confidentiality. No names of producers will be found in this report.

Sampling Schedule

The goal was to sample at each site four times over the year. This would allow for sampling in each season. It would also allow for sampling manure of varying "ages" (i.e. length of time in storage).

The sampling schedule needed to consider not only the season of the year, but also the wishes of the livestock producers, availability of labour to collect the samples, and weather (e.g. avoiding times of ice cover in storages).

Sampling was carried out by University of Guelph staff located at both the Ridgetown and Guelph campuses. Ridgetown College staff collected samples from 18 farms in the Windsor to Wingham and Stratford areas. Dr. Bob Friendship's staff, in OVC at the Guelph campus, sampled 10 farms in the Wellington, Grey, Oxford and Haldimand-Norfolk regions.

Sampling Procedure

Every effort was made to follow the biosecurity protocols established by the livestock producers in the study. The minimum level of precautions consisted of the following steps:

- # The vehicle used did not travel beyond a point at the farm designated by the cooperating farmer.
- # Those farms requiring that no previous farms had been visited that day (i.e. by the sampler) were scheduled as the first visit of the day.
- # Prior to entering the farm, sampling equipment was sanitized using a strong bleach solution and fresh water rinse. This was done in all cases, usually prior to leaving the previous farm.
- # The person carrying out the sampling wore freshly laundered coveralls and new plastic disposable boots (over sanitized rubber boots) and gloves for each visit.
- # All sampling was carried out without actually entering the barn.
- # Following sampling, coveralls, gloves and boots were placed in covered plastic bins and removed from the site.

The procedure used to collect the manure samples was as follows:

1. Using a sampling pole and bottle holder, manure sub-samples were collected from three locations around the storage, if possible, and at up to three depths (i.e. the top 1/3, mid depth and the bottom 2/3 of the storage). The bottle was inverted then lowered into the manure. When the bottle was at the desired depth, the sampler was surged up and down, thus filling the bottle at that depth. The sample was raised to the surface and manure was poured into a sanitized pail (using a clean pail and sampler bottle at each site). Sampling continued until all sub-samples were collected and poured into the pail.
2. The manure temperature was measured and recorded.
3. The composite manure sample was stirred using a sanitized stir stick or clean disposable cup to ensure thorough mixing. Using the disposable cup, a manure sample was transferred to the labeled sterile bottles - one for nutrients and one for pathogen testing. The use of this composite sample was meant to represent a typical sample of manure that was agitated prior to spreading on the land.
4. Sample bottles were placed into a cooler with adequate cold packs to maintain the temperature just above freezing for delivery to the lab.
5. Excess manure was put back into the storage - i.e. left on site.

In addition to the sample collection, manure depth in the storage was measured during each visit.

Sample Analysis

Samples were delivered to the Laboratory Services Division and the Food Microbiology Lab, University of Guelph, on the day of sampling. Between the time of sampling and delivery, samples were stored in a refrigerated cooler (at approximately 4°C). All manure samples were tested for *Salmonella* and *E. coli*, as well as pH, dry matter, total N, P, K and ammonium-N. Following is a brief description of the test procedures:

- Dry Matter: samples weighed wet, dried in 80°C oven for 24h, weighed dry, dry matter calculated
- K: modified Kjeldahl digestion, digestate analyzed by atomic absorption
- N: modified Kjeldahl digestion, digestate analyzed using colorimetric method
- NH₄-N: KCl extraction, extract analyzed using colorimetric method
- P: modified Kjeldahl digestion, digestate analyzed using colorimetric analysis
- pH: saturated paste method using pH electrode
- NO₃-N: KCl extraction, extract analyzed using colorimetric method

The *Salmonella* test involved a “presence/absence” screening step. The method used was the Health Protection Branch (Health Canada) method MFHPB-20, April 1998 - “Isolation and Identification of *Salmonella* from Foods”. Only those samples testing positive were examined further. The next step for the positives was an enumeration using a “Most Probable Number” (MPN) analysis. This test was the Laboratory Services Division Method MID-149 - Most Probable Number Method for the Enumeration of *Salmonella* in Poultry - Revision No. 0 (2001/01/31).

E. coli numbers were measured using Laboratory Services Division Method MID-104 - Enumeration of Coliforms and *Escherichia coli* using the Most Probable Number Method - Revision No. 1 (98/09/01). Samples were prepared using the procedures designed to measure *E. coli* in compost samples.

No further tests were performed to identify the serotypes of either the *Salmonella* or *E. coli* organisms. Results were recorded in a standard computer spreadsheet and the statistical analysis was performed using a commercial statistics computer program. A convention was adopted to handle those cases where numbers were reported as below the Lower Detection Limit of the lab procedure. A value of one half the Lower Detection Limit was entered and used for the statistical analysis - acknowledging that the actual value was somewhere between zero and the detection limit.

Results and Discussion

Sampling Dates

The first sampling began July 30, 2003 and was completed August 27 for the “Guelph” sites. The “Ridgetown” sites were sampled from September 16, 2003 to October 20, 2003. The wide time period from the start of sampling to completion of the first round was due to delays in getting all of the cooperators lined up. It took longer than expected to identify sites in the study region that tested positive for *Salmonella* in 2003, and then to get the approval from the owners to allow the study of their farm.

In general, the split in sampling responsibilities created some difficulties in matching up sampling dates. Staff at Guelph had difficulty scheduling the visits during target times due to other commitments. There was a staff turnover issue at Guelph that led to delays in the third site visits (i.e. for the sites handled by Guelph staff).

The second sampling was started on October 15, 2003 and completed on December 5, 2003 for the Guelph sites. The Ridgetown sites were sampled starting December 4, 2003 and completed December 16, 2003. Again, this had a wider sampling period than desired. Sampling was completed by early December to avoid frozen tanks (which would likely have prevented sampling, at least in the un-covered storages).

The third sampling was started March 25, 2004 and completed on April 1 for the Ridgetown sites and started May 10, 2004 and completed on June 17, 2004 for the Guelph sites (for reasons stated above). Sampling was delayed until late March for the Ridgetown sites to reduce the chance of frozen storages. Even with this delay, some sites were still frozen. This required breaking the ice to get a sample.

The final sampling was started July 6, 2004 and completed July 9, 2004 for the Ridgetown sites. The Guelph sites were sampled from July 5 to July 9, 2004.

Farm Characteristics

As mentioned the farms were located in a wide geographic area in southwestern Ontario. A breakdown of farm types:

- a) farrow to finish = 9 farms
- b) farrow to wean or farrowing only = 7
- c) finishing 12 (1 farm also includes weaner pigs)

The average numbers of sows, feeder pigs and weaner pigs on the farms housing these animals was 396, 1090 and 897, respectively. All farms had liquid manure systems. About half the farms used a system of “All-In, All-Out”, where animals are moved into the room all at once and they are also all removed at about the same time. Typically, the room is empty for a few days during a decontamination phase. Also, depending on the type of manure storage system, this may be a time when gutters are emptied. The alternative to All-In, All-Out is Continuous housing, where animals enter and leave the barn at staggered times and a room may contain a range of sizes of animals.

The study included seven instances where two storages were sampled from one farm operation. Typically, one of the storages served a sow and/or farrowing barn and one served a feeder/finisher barn. The two types of animals were owned by the same farm operation and had similar breeding lines. However, they were often located at different sites. There was no direct contact between manure storages or barns. They were deemed to be separate herds for the purpose of this study. This group of seven farms (i.e. 14 manure storages) will be discussed later.

There was another “special case” that involved two of the sites in the study. On these two farms, an extra sample was collected from a second storage serving the barn. It is a fairly common practice to have two storages, where one is located beneath the barn. This may be emptied periodically into a long-term storage outside. On the two sites where the extra storage was sampled, only the samples from the long-term storages were used in the statistical analysis that follows. The extra two samples were not included in the experimental design for the study, as they represent manure from the same group of animals. Because it was so convenient to collect these extra samples, they were included simply to get a snapshot of die-off over time. Typically manure in the long-term storage would be several weeks older than manure in the short-term storage. These results will be discussed briefly later.

Storage Characteristics

The initial intent was to have half of the manure storages uncovered and half covered. In the end, 11 of the 28 tested were covered and the remainder had no cover. Five of the covered storages were situated below the barn (i.e. under slatted floor systems). Of the uncovered storages, two were earthen storages (i.e. sloping sides, earth sides and floor) and the remainder were concrete tanks with vertical sidewalls. The average storage depth was 3.41 m, and the range was 2.4 m to 6.1 m. Most farms reported that they target the fall to empty the storage and spread manure. Typically, tanks were also emptied in the spring or summer.

Manure nutrients

Manure nutrient data for the study is summarized in Table 1. The mean values are in line with typical values for Ontario farms. For example, the NMAN database of 924 swine manure samples gives the following averages for manure having a dry matter in the range of 0 to 18%: 0.40% N, 2648 mg/L NH₄-N, 0.13% P, 0.17% K, and 3.8% DM (OMAF 2003).

Table 1 - Manure nutrient characteristics for the 28 farms (total of 4 visits)

	Mean	SD	Min.	Max.	Count
pH	7.57	0.39	6.5	8.6	109
TKN (% - As Is)	0.33	0.17	0.024	0.85	109
NH ₄ -N (mg/L)	1724	730	174	3860	109
P (%)	0.14	0.13	0.005	0.70	109
K (%)	0.17	0.077	0.052	0.35	109
DM (%)	2.73	2.19	0.27	12.28	109

Salmonella

This was not a *Salmonella* “prevalence” study. By selecting farms with a recent history of outbreak of *Salmonella*, we were hoping to have up to a 100% occurrence of the organism. We did not achieve this. Of the 110 samples submitted for analysis (i.e. four visits, 28 farms, two missing samples), 45 tested positive for *Salmonella* (40.9%). For this group of 45 samples, the geometric mean density was 1.36 MPN/mL. This is a rather low number and suggests that either numbers were initially low or there was a great deal of die-off or dilution in the storage. The highest count was 427 MPN/mL.

There were 9 (of the 45 values) where the reported density of *Salmonella* was below the lower detection level (of 0.3 MPN/mL). A value of one half the lower detection limit (i.e 0.15) was assumed for each of these samples.

Figure 1 shows the numbers of farms responsible for various numbers of positive tests for *Salmonella*. Of the 28 manure storages, seven tested negative for *Salmonella* all four times. Seven farms tested positive only once, six had two positives, six had three positives and on two farms, all four samples tested positive. Two samples were missing from this data set - one from a farm with no other positives and one from a farm with one positive test.

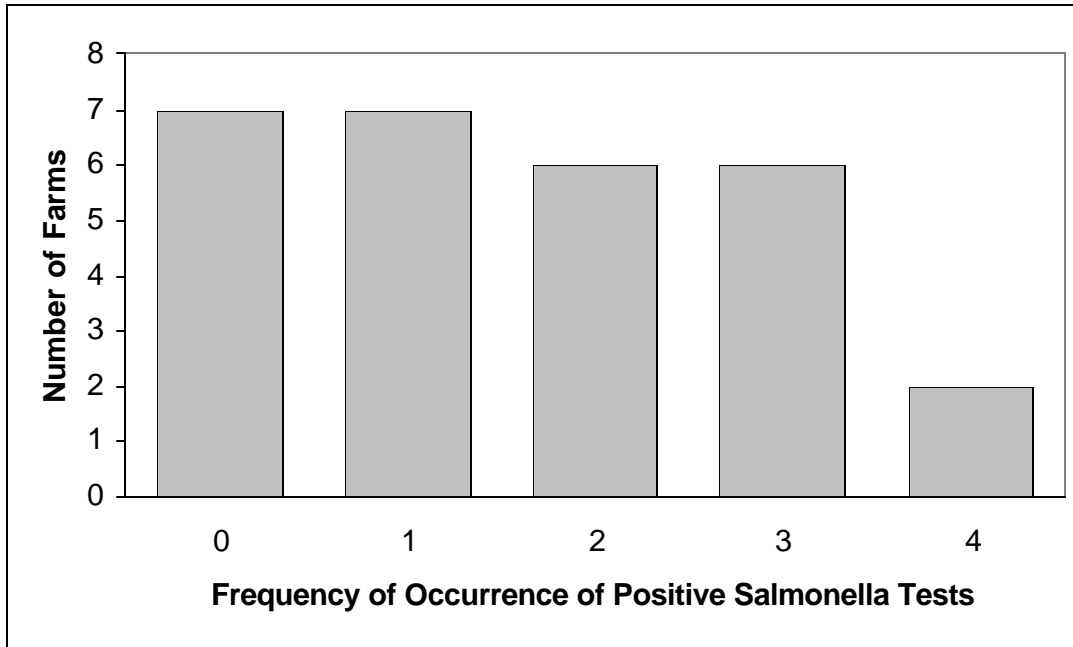


Figure 1 - Numbers of farms (of 28 total) vs number of positive tests for *Salmonella* (maximum 4) during the study

No clear pattern of occurrence emerged. A discussion of *Salmonella* results follows, divided into six topic areas. In the statistical analysis, sometimes only the 45 positive tests are considered. However, part of the analysis includes all test results. For this, all “Negative” tests were considered to have a density of 0. To perform log transformations, each density (whether zero or non-zero) was first increased by 1. Thus, the large number of negative tests for both storage types exerted an influence on the analysis. The analyses performed in this way will be identified as such.

a) Time of Year - There were four visits to each farm and these were roughly three months apart. The number of manure samples testing positive for each visit is shown in Figure 2. The third visit yielded the most positive tests (i.e. 15 out of 27 samples). However, there was no significant difference in the log of *Salmonella* densities between the various visits to the farms ($P = 0.94$). When the month of sampling was converted into Season (i.e. winter, spring, summer and fall), there was also no difference in densities. Unfortunately, no samples were collected in the winter (Dec 21 to Mar 21). During this period, there was a high likelihood of freezing in the uncovered storages (making sampling much more difficult).

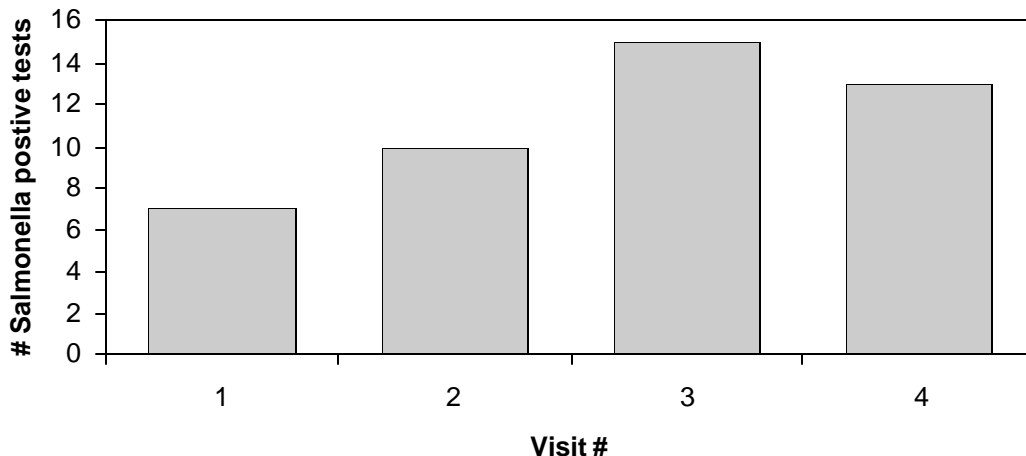


Figure 2 - Number of manure samples (out of 28 maximum) testing positive for *Salmonella* for each of the four farm visits between fall 2003 and summer 2004

b) Storage Type - As mentioned earlier, 11 of the 28 storages were covered. This was expected to lead to a difference in temperatures and/or dry matter levels of stored manure, which could affect the survival of pathogens.

Some temperature data were missing due to malfunctioning probes. Of the 83 temperature readings available, there was a significant difference between covered and open storages ($P=0.09$). The mean temperature for covered tanks was 17.2°C , and for open storages was 13.9°C . When the temperatures for only the second visit (late fall - cooler temperatures) were examined, there was a significant difference ($P=0.000$). The temperature of manure in covered storages (about half were under the barn) ranged from 5.9 to 18.1°C (average = 13.0°C). For the uncovered storages, the temperature ranged from 1.2 to 6.6°C (average = 3.6°C).

Manure dry matter is another variable that could affect pathogen survival and that could be affected by the presence or absence of a storage cover (i.e. different amounts of precipitation into the storage). The dry matter levels were significantly different. The average manure dry matter level for the covered storages was 3.62% . For uncovered storages, the average was 2.13% .

Despite the fact that there were differences in manure temperatures and dry matter levels, there was no significant difference in the log of *Salmonella* numbers between covered and uncovered storages ($P = 0.66$, $n=110$). This analysis used all the data (i.e. using $\log(\text{Salmonella}+1)$). When only the positive test results were considered, the differences between storage types were still not significant ($P = 0.21$, $n=45$).

c) Depth of Manure - Depth of manure was used as a way to represent the relative age on manure. This was thought to impact the die-off of organisms. It would also give an index of the amount of dilution of pathogens in the liquid manure. Unfortunately, fresh manure was frequently added to each of the storages. This could potentially introduce new organisms on a regular basis. Though it is not uncommon in Ontario to add manure only every several months, none of the farms in this study used that particular management option. For this study, age is taken to mean the maximum age of manure in the storage (in months). It mainly considers the date when the storage was most recently emptied.

There was no statistically significant relationship between *Salmonella* density (log-transformed) and either the manure depth or the manure age (using all results, whether testing positive or not).

d) Farm - Because several farms had no detection of *Salmonella*, it was expected that there would be significant differences between farms in the log-transformed *Salmonella* densities. Even so, 25 of the 28 sites were not significantly different from each other. Because there were so many “negative” occurrences and because the “positives” had fairly small densities, there were not large differences between mean densities of *Salmonella*.

The selection of farms in this study was based on a measured recent history of *Salmonella* in the herd. This was established through a Sentinel Herd program, run by Dr. Bob Friendship, University of Guelph. This program continued through the duration of the manure storage study. As such, additional information was available on the status of certain herds in the study. During the spring of 2004, fecal samples and blood samples were collected from individual animals and manure samples were collected from pens to gauge whether or not *Salmonella* was present in the herd. Only the farms with finishing pigs were sampled on these visits (Friendship 2004). The results (presence/absence) were compared with results from the manure storage samples for the spring, 2004 sampling.

The degree of agreement for these 15 results is shown in Table 2. In 12 of the 15 cases, both methods agreed either that *Salmonella* was present or that it was not. In the two cases where the animals tested negative but the storage tested positive, it is conceivable that *Salmonella* was present in the animals but had since cleared up. The fact remains that sampling directly from the animal gives an indication of the health status of individual animals. Sampling from the manure storage, however, represents an average, both throughout the barn population and over the time that manure is being stored, with adjustment for the die-off of the bacteria over time.

As mentioned earlier, there were seven farms where two storages from the same farm operation (but for a different group of livestock) were sampled. Of the four manure samples per storage and seven farms, there were five cases where both samples from the same farm tested positive for *Salmonella*, 11 where both tested negative and 11 where one tested positive and one negative (and one sample was missing). *E. coli* and nutrient

numbers showed no relationship. It seemed reasonable to conclude that the second storage (in each case), even though owned by the same farm operation, represented a separate herd.

Table 2 - Degree of agreement between occurrence of *Salmonella* in the swine herd and occurrence in the manure storage (for a selected group of herds sampled in the spring, 2004 - data supplied by Dr Bob Friendship, OVC, University of Guelph)

	Number of Herds Testing Positive	Number of Herds Testing Negative
Stored Manure Testing Positive	4	2
Stored Manure Testing Negative	1	8

There were two cases where additional samples were collected at the farm from a second manure storage. These numbers have not been used in the analysis, as they are basically duplicates from the same herd. However they represent manure samples of different ages and may help shed light on die-off rates. On one farm, there was no *Salmonella* detected in either storage over the four visits. On the second farm, six samples tested positive but the densities were quite low and each storage tested positive once when the other gave a negative test. There was some agreement on nutrient concentrations, once the concentrations were adjusted for different Dry Matter concentrations. Because of small numbers of data, these analyses did not yield much useful information.

e) Numbers Relative to *E. coli* Numbers - *E. coli* is frequently used as an indicator organism and is present in large numbers in a manure storage. It seemed reasonable to expect that there would be some correlation between occurrence and densities of *E. coli* and *Salmonella*. In fact, there was a significant relation between the two ($P = 0.032$). However, the correlation was rather weak ($r = 0.21$). The data points and straight line model are shown in Figure 3. High levels of *E. coli* did not prove to be a good predictor of the presence or density of *Salmonella* in the manure. There were numerous manure samples containing high levels of *E. coli* and no *Salmonella*. However, where *E. coli* numbers were less than 100 MPN/mL (there were several occurrences), no *Salmonella* was detected.

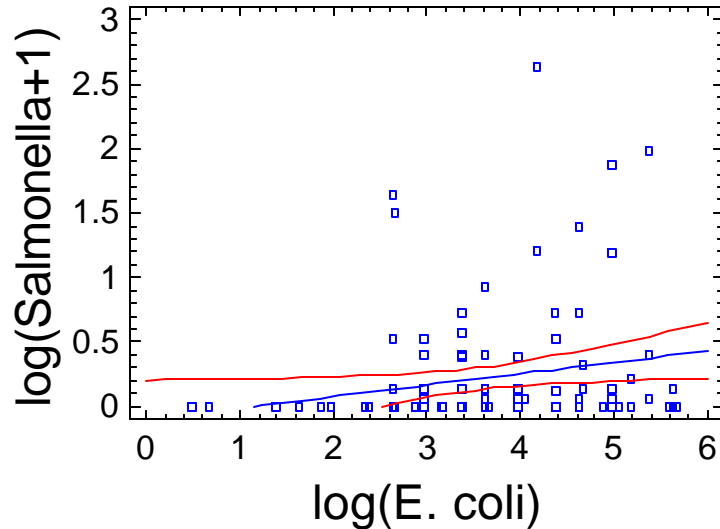


Figure 3 - Densities (MPN/mL) of *Salmonella* vs *E. coli* (log transformed) showing the best fitted line and 95% confidence limits

f) Numbers Relative to Nutrients, etc. - Manure nutrient statistics were reported earlier (see Table 1). A regression analysis was performed to test for a straight line relationship between the various nutrients and the log-transformed density of *Salmonella* (increased by 1 to include all 110 results). There was no significant linear relationship for TKN, NH₄-N, P, K, pH or Dry Matter. In other words, concentrations of any of the commonly-measured manure nutrients could not be used to predict the density of *Salmonella* organisms in the manure.

E. coli

As expected, *E. coli* was detected in each of the manure samples. Also as expected, the densities of organisms followed a log-normal distribution. The geometric mean density for all 110 samples was 3777 MPN/mL (median = 4270). The lowest count was 3.1 and the highest was 462,000 MPN/mL.

There was a significant difference in densities between farms. The geometric mean density ranged from 67 to 156,000 MPN/mL. There was a significant difference between visits ($P = 0.015$). *E. coli* densities were higher in the second and third visits (late fall to early spring) than in the first visit (late summer, early fall).

E. coli numbers behaved in the same manner as *Salmonella* when comparing storage types. The geometric mean density for covered storages was 6520 MPN/mL, which was not significantly higher than that for uncovered storages (i.e. 2630 MPN/mL; $P=0.06$).

There was no statistically significant relationship between the log density of *E. coli* and any of the following: manure depth, manure age, dry matter, NH_4 , TKN, K, P, or manure temperature. There was, though, a significant relationship with pH ($P = 0.000$). It was not a strong relationship, however (correlation coefficient = -0.40), as shown in Figure 4.

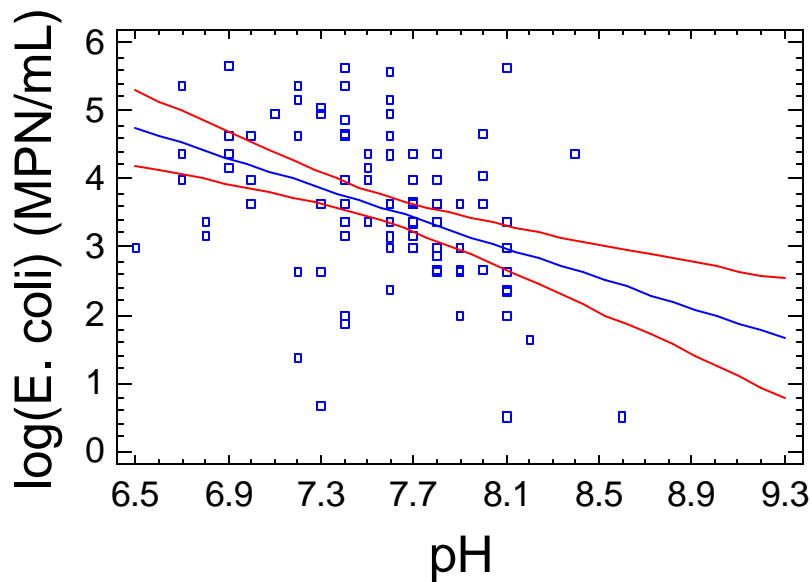


Figure 4 - Relationship between the log of *E. coli* density (MPN/mL) and manure pH

Summary

Between July, 2003 and July 2004, samples from liquid manure storages were collected from 28 swine farms in southwestern Ontario. Four samples per storage were collected, spread over the 12-month period. Nine of the storages represented farrow to finish operations, seven represented farrowing only, and 12 represented finishing barns (included one weaner operation). The samples were analyzed for levels of manure nutrients and for the bacteria: *E. coli* and *Salmonella*. All of the storages served barns with a recent history of *Salmonella* presence (at least in individual animals or pens), so the prevalence of *Salmonella* was expected to be high. At each site, measurements were made of manure depth in storage, temperature of manure, and the approximate age of manure in the storage. Eleven of the storages were covered and 17 were uncovered (including 15 concrete tanks and two earthen storages). The main findings of the study:

- As expected, there was a range of values for manure nutrients, and they were typical of Ontario farms. Average values were: pH = 7.57; TKN = 0.33% (as is); NH₄-N = 1724 mg/L; P = 0.14%; K = 0.17%; Dry matter = 2.73%.
- Even though the farms were selected based on a high probability of finding *Salmonella*, only 40.9% of the samples tested positive for the presence of *Salmonella*.
- Numbers of *Salmonella* present were rather low. For the 45 samples testing positive, the geometric mean density was 1.36 organisms per mL (using the Most Probable Number analysis). The highest count was 427 MPN/mL.
- Seven storages tested negative for *Salmonella* on all four visits and two tested positive all four times.
- There was no significant difference in *Salmonella* counts between the four visits to the farms - roughly representing different seasons of the year.
- Despite the fact that there were differences in manure temperatures and dry matter levels, there was no significant difference in *Salmonella* numbers (log transformed) between covered and uncovered storages.
- There was no significant relationship between *Salmonella* numbers (log transformed) and either manure depth or manure age.
- There was a relationship between *Salmonella* detected in animals in the barn (from pen manure and from individual animals' fecal or blood samples) and *Salmonella* in the manure storage. Out of 15 such comparisons carried out at the spring (2004) sampling, four were both positive, eight were both negative, and three were not in agreement (barn sample results were supplied from a separate study at the same sites).
- High levels of *E. coli* in the stored manure did not prove to be a good predictor of the presence or density of *Salmonella*.
- Concentrations of any of the commonly-measured manure nutrients bore no relation to the density of *Salmonella* organisms in the manure.

- The geometric mean density of *E. coli* was 3777 MPN/mL. The highest count was 462,000 MPN/mL.
- There was a significant difference in *E. coli* densities between farms and between visits, but not between storage types.
- There was no statistically significant relationship between the log density of *E. coli* and any of the following: manure depth, manure age, dry matter, NH₄, TKN, K, P, or manure temperature.

This study was not able to establish the rate of die-off of *Salmonella* or *E. coli* in manure storages. The most likely reason for this was that fresh manure was added regularly to all of the storages in the study. However, it did establish that in those manure storages where *Salmonella* was present, the counts were very low.

Future studies to establish die-off of *Salmonella* in storage would have a higher chance of success if they included only farms with confirmed positives, more frequent sampling, and restricted additions of fresh manure.

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- Dr Bob Friendship, Ontario Veterinary College, University of Guelph for assistance in project design, in selecting sites, in manure sampling and for sharing pertinent data from the Sentinel Herd project to enhance the results from this study.

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Appendix 1

**Survival of Pathogenic Bacteria in Liquid Manure Storages
Research Project Conducted for Ontario Pork – 2003-2004
Data Sheet**

Farm Number:

	Visit 1	Visit 2	Visit 3	Visit 4
Date				
Pathogens sample code				
Nutrient sample code				
Manure Temperature				
Manure Depth				
* Age of Manure				

* Age of manure will be based on how often storage is emptied. If storage is full and it is emptied every 6 months than it will be up to 6 months old. If it has just been recently emptied then it is likely mostly fresh manure.

Other Information such as spreading dates, antibiotic use, disease outbreaks etc.

**Survival of Pathogenic Bacteria in Liquid Manure Storages
Research Project Conducted for Ontario Pork – 2003-2004
Farm Information**

Farm number: _____

Farm Type: Finishing _____ Farrow to Finish _____ Farrowing _____
 Farrow to wean _____ Weaner Barn _____

Barn Capacity Number of:
 Finishing pigs _____ Sows _____ Weaner Pigs _____

Barn Management: All in all out _____ Continuous Flow _____
Other _____

Feeder Type: Wet/Dry _____ Dry Feeders _____
 Floor Feed _____ Other _____

Waterer used: Wet/Dry Feeder _____ Water Bowls _____
 Hanging Water Nipples _____ Other _____

Manure Additives: Yes _____ No _____ Name _____

Feed Additives for controlling manure odours: _____

Feed Used: _____

Antibiotics Used: Names : _____

When?: _____

Herd Health Status: _____

Manure Spreading Dates: _____

Appendix 3

**Survival of Pathogenic Bacteria in Liquid Manure Storages
Research Project Conducted for Ontario Pork – 2003-2004
Farm Information**

Storage Type: Covered _____ Open _____ Under Barn _____
Earthen _____ Other _____

Storage Dimensions:

Manure System:

All manure stored under barn _____

Partial storage under barn plus outside storage _____

Limited storage under barn (gutters) with outside storage _____

Other _____

Barn Floor Type: Fully Slatted _____ Partial Slats _____

Barn Layout: Single room _____ Several Rooms _____
Other _____

Ceiling Height: _____

Barn Dimensions: _____

Other Information: