FATE OF SELECTED BACTERIAL PATHOGENS AND INDICATORS IN FRACTIONATED POULTRY LITTER DURING STORAGE

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SUMMARY

A study of broiler litter re-utilization potential was conducted with the goal of determining if storage of litter significantly reduced potential pathogens to levels safe for re-utilization. Litter from four broiler houses was separated into a fine fraction for fertilizer use and a coarse fraction for use as a supplement to wood shavings in growing subsequent flocks of birds. Fractions and whole litter were stored in indoor piles for four months with periodic analysis for culturable pathogenic and indicator bacteria. Significant reductions in microbial concentrations occurred in a majority of samples tested during four months of storage (in most cases to below detection limits of approximately 30 CFU/g dry weight). Poultry feed was found to be one possible source of litter contamination.

Key words: Bacteria, fractionation, indicators, litter, pathogens, poultry, storage
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DESCRIPTION OF PROBLEM

The poultry industry is extremely important to the economy of several states in the United States. Georgia, Alabama, Mississippi, North Carolina, Virginia, Arkansas, Maryland, and Delaware derive more than 25% of their agricultural income from the poultry industry. The intensive nature of poultry production leads to the generation of large amounts of waste in the form of poultry litter. The amount of litter produced in these concentrated poultry production areas often exceeds the limits that can be safely applied to the land area available for litter application. When these application limits for litter are exceeded, environmental degradation often results [1].

Litter consists of a manure carrier, usually wood shavings, added to the growing house to absorb manure and facilitate its removal at the end of the growing period (i.e., the time required to grow one or more flocks of birds). It also contains other constituents such as soil and feathers. The product remaining at the end of the growing period is high in nitrogen content, so it is useful as a fertilizer and as a ruminant feed additive [1].

Fractionation of this litter would reduce litter removal and wood shaving costs to the broiler grower. A fine fraction (<0.83 mm) could serve as a fertilizer, and a coarse litter fraction (>3.33 mm) could be reintroduced into houses to supplement wood shavings. This processing would improve litter re-utilization economics and result in increased litter recycling [1] and a reduction in environmental degradation.

Litter contains a diverse population of microorganisms, some of them potentially pathogenic to humans, poultry, or both. One concern is that the use of recycled litter as a fertilizer may transfer the potentially pathogenic microorganisms to the crop environment and then to the human consumer. A wide variety of viruses may also be found in and transmitted by litter, including avian pathogens. Therefore, this study is concerned with both human and avian pathogens. In the case of exclusive human pathogens, this study examines the fate of these organisms in the general broiler environment as it relates to the ultimate quality of broilers for human consumption.

Numerous bacterial species have been isolated from litter, including those belonging to the genera of Salmonella, Staphylococcus, Pseudomonas, Escherichia [2], Campylobacter [3], and Clostridium [4]. Other microorganisms which may be found in animal environments include Yersinia spp., Klebsiella spp., Listeria spp. [5], Aeromonas spp., fungi, and bacterial fecal indicators such as coliforms and fecal streptococci [6].

Viruses have also been isolated from poultry and their litter. Using electron microscopic techniques, Collins et al. [7] found picornaviruses, parvoviruses, and caliciviruses in chickens exhibiting symptoms of "wet litter" disease. Reoviruses have been isolated from chicks and implicated in malabsorption syndrome [8]. Infectious bursal disease and Marek's disease (Avian leucosis) are recognized diseases caused by viruses in chicken flocks throughout the country. Rotaviruses have been isolated from turkeys exhibiting signs of enteritis [9]. Other viruses isolated from poultry include adenoviruses [10], reticuloendotheliosis virus [11], astroviruses [12], avian influenza viruses [13], and infectious nuclear polyhedrosis virus [14]. These findings indicate the variety of viruses present in the bird's environment.

The goal of this study was to determine the survival of bacterial pathogens and indicators in fractionated litter during storage under conditions designed to simulate typical farm separation and storage.

MATERIALS AND METHODS

SAMPLE PREPARATION

Broiler litter samples were collected from either single or multi-flock houses in the North Georgia area using plastic containers and utensils sanitized with a solution of 600 ppm sodium hypochlorite (Mayo Chemical Company, Smyrna, GA). Single flocks of broilers were grown on litter from Houses #1 and #2, while multiple flocks were grown on litter from Houses #3 and #4. Houses #1, #3, and #4 had dirt floors; House #2 had a concrete floor. All houses were curtain ventilated. Whole litter was separated into coarse and fine fractions using a drum separator (designed and built for this study) with pore sizes of 3.33 mm or #6 mesh screen (coarse fraction), and 0.83 mm or #20 mesh screen (fine

fraction). All exposed separator surfaces and sampling equipment were sanitized between samples with the sanitizer solution described above. Samples were separated by house number and stored in a broiler house at the University of Georgia (UGA) Agricultural Experiment Station, Athens, GA in separate, sanitized, numbered 4 x 4 x 4 ft. plywood open-top bins divided by plywood partitions into four 2 x 2 x 4 ft. sections. Each section was filled to a depth of approximately two feet with either whole, coarse, or fine litter fractions. The method of separation and storage was designed to simulate as closely as possible the typical litter separation and piled storage conditions on a farm.

Litter subsamples were collected initially and at time intervals of 2, 4, 8, and 16 wk of storage using sterile equipment. Samples were collected from each type of litter (whole, coarse, and fine) in the center of each bin section at a depth of about six inches. Researchers collected samples of feed from each of the four houses at the same time as initial litter samples using the same sampling techniques described above. Unfractionated whole feed and typical wood shaving samples were subjected to the same microbial analyses as litter samples. All subsamples (approximately 100 g each) were stored in sterile plastic bags until all microbiological analyses were completed, usually within 24 hr. Subsamples of whole litter of approximately 500 g were delivered for viral analysis.

MICROBIOLOGICAL ASSAYS

Litter samples of 10.0 g wet weight were each weighed directly into sterile glass jars with screw-top blender attachments. Researchers added 50 mL of 1.0% buffered peptone-water (BPW) and blended the sample for twenty seconds at highest speed (approximately 15,000 rpm) in an Oster blender (Sunbeam/Oster, Schaumburg, IL). The peptone-water surfactant and blending method were determined by preliminary research to be optimum for the recovery of bacteria from litter samples [15]. Litter samples of approximately 5 g were dried in a vacuum drying oven at 105°C for approximately four hours to determine percentage moisture content so that microbial numbers were expressed on a dry weight basis.

The assays used in determining the microbial populations present in the litter samples were primarily those of Standard Methods for the Examination of Water and Wastewater, 17th Edition [16], with modifications as specified and using prepared media (Difco Laboratories, Detroit, MI).

MOST PROBABLE NUMBER (MPN) METHODS

MPN methods [17] using multiple tube dilution of litter samples in enrichment media followed by streaking onto confirmatory media were used for isolation and enumeration of *Staphylococci* [16], *Salmonella* [18, 19, 20, 21, 22, 23], and *Listeria monocytogenes* [24]. A statistically MPN of bacteria per milliliter was determined based upon confirmation of growth of bacteria in a combination of tubes.

MEMBRANE FILTRATION METHODS

Membrane filtration methods [25] used a filtration funnel and funnel holder (Nalgene Co., Rochester, NY) and a stainless steel manifold with dual moisture traps attached to a vacuum pump. Filters of 0.45 μm pore-size (Micron Separations, Inc., Westborough, MA) were transferred after filtration to the surface of appropriate selective agars in small 60 mm x 11 mm petri dishes containing about 2.5 mL agar per plate for microbial group enumeration.

SPREAD PLATE METHOD

Heterotrophic bacteria were enumerated using the spread plate method of Standard Methods for the Examination of Water and Wastewater, 17th Edition [16] on plate count agar.

TEMPERATURE MONITORING

Temperature was monitored in piled litter (in bins) at a depth of approximately one foot using type T thermocouples connected to a multiple channel recorder (Monitor Labs, Inc., San Diego, CA) for thirty days. Ambient temperature was also concurrently monitored until interior pile temperature became consistent with ambient temperature.

RECOVERY FROM BELOW DETECTION LIMITS

The possibility existed that litter microbes, once they dropped below the detec-

tion limit, might recover when provided with an appropriate environment and nutrients. Therefore, litter samples for which microbes were below detection limits were placed in 1.0% BPW and allowed to incubate at 37°C for 48 hr. At that time appropriate dilutions were again tested for the same microbes previously assayed (using techniques described above in the microbiological assays section).

STATISTICAL ANALYSES

Curves of microbial concentration and physical parameter data generated during litter storage were fitted with linear and non-linear (second and third-order polynomial) regression lines. Correlation coefficients and coefficients of determination were tested for significance at a $P < .05$ level.

RESULTS AND DISCUSSION

Concentrations of microorganisms recovered from litter were not found to be consistently different among houses or among litter fractions. Results from Houses #1 through #4 for all litter fractions will be discussed together. Data for survival of microorganisms recovered in litter from Houses

#1 through #4 during storage are shown in Tables 1 through 4. Data for recovery of viruses from litter, recovery of microorganisms from feed, changes in litter temperature, and changes in litter moisture content appear below.

SURVIVAL OF FECAL INDICATOR ORGANISMS DURING STORAGE

Fecal indicator microorganisms recovered from litter included total coliforms, fecal coliforms, and *Escherichia coli*.

Concentrations of total coliforms recovered from litter decreased from a range of $\log_{10} 3$ to 8 to $\log_{10} 1$ to 5 during four to sixteen weeks of storage. Concentration reductions for total coliforms were significant for nine of twelve samples tested.

Fecal coliform concentrations recovered from litter decreased from a range of $\log_{10} 2$ to 5 to below the detection limit of 30 CFU/g dry weight during two to sixteen weeks of storage. Reductions in concentrations of fecal coliforms were significant for seven of twelve samples tested.

E. coli concentrations recovered decreased from a range of $\log_{10} 3$ to 8 to $\log_{10} 1$

TABLE 1. Microorganisms recovered from broiler litter (House #1) during storage ($\log_{10}$ CFU/g dry weight)

MICROBIAL GROUP OR SPECIES	LITTER FRACTION	INITIAL MICROBIAL CONCENTRATION[A]	TOTAL MEAN REDUCTION (2–16 WK)
Total coliforms	Whole	6.34 ± 1.58	2.85
	Coarse	4.31 ± 4.31	1.52**
	Fine	4.40 ± 4.45	2.20
Fecal coliforms	Whole	5.07 ± 4.65	3.65**
	Coarse	4.93 ± 4.12	4.31
	Fine	3.43 ± 3.57	2.01*
Escherichia coli	Whole	5.28 ± 5.27	1.84
	Coarse	3.63 ± 3.30	1.55
	Fine	3.42 ± 1.15	1.52
Heterotrophic bacteria	Whole	9.67 ± 9.61	+0.33
	Coarse	10.26 ± 9.99	0.37**
	Fine	10.03 ± 9.92	+0.03**
Aeromonas hydrophila	Whole	3.69 ± 3.52	2.27**
	Coarse	3.76 ± 2.98	2.33
	Fine	1.42 ± 0^{B}	0

[A]Mean ± one standard deviation

[B]Detection limit

*Statistically significant at $P < .05$; **Statistically significant at $P < .01$

TABLE 2. Microorganisms recovered from broiler litter (House #2) during storage (log₁₀ CFU/g dry weight)

MICROBIAL GROUP OR SPECIES	LITTER FRACTION	INITIAL MICROBIAL CONCENTRATION[A]	TOTAL MEAN REDUCTION (2–16 WK)
Total coliforms	Whole	8.52±7.05	2.85
	Coarse	7.26±6.77	3.53**
	Fine	7.65±5.33	3.82*
Fecal coliforms	Whole	5.21±4.45	3.78*
	Coarse	5.74±5.88	4.31**
	Fine	3.92±3.59	2.52
Escherichia coli	Whole	8.37±6.89	3.00*
	Coarse	6.44±0	3.07**
	Fine	7.29±6.90	3.91
Heterotrophic bacteria	Whole	10.12±9.66	0.99
	Coarse	9.91±9.64	+1.15**
	Fine	10.22±9.35	+0.77**
Yeasts & Molds	Whole	5.31±4.76	1.93**
	Coarse	6.23±4.15	3.57**
	Fine	5.03±4.34	2.37**
Aeromonas hydrophila	Whole	3.96±2.86	2.53**
	Coarse	4.53±4.37	3.11
	Fine	4.35±4.30	2.93
Pseudomonas aeruginosa	Whole	3.15±2.61	+0.12**
	Coarse	4.44±0	3.01*
	Fine	3.49±2.94	2.09**
Yersinia enterocolitica	Whole	4.73±4.66	3.30**
	Coarse	4.34±3.73	2.91*
	Fine	4.24±3.26	2.84**
Campylobacter jejuni (MPN)	Whole	3.12±3.26	2.82**
	Coarse	1.60±1.33	1.30**
	Fine	3.55±3.61	3.25*

[A]Mean ± one standard deviation

*Statistically significant at P < .05; **Statistically significant at P < .01

to 5 during three to sixteen weeks of storage. Concentration reductions for *E. coli* were significant for seven of twelve samples tested.

SURVIVAL OF HETEROTROPHIC BACTERIA, AND YEASTS AND MOLDS IN LITTER DURING STORAGE

Concentrations of heterotrophic bacteria recovered from litter decreased significantly during sixteen weeks of storage for three of the twelve samples tested.

Concentrations of yeasts and molds recovered from litter during storage decreased from a range of log₁₀ 5 to 6 to log₁₀ 1 to 4 during ten to sixteen weeks of storage. Reductions in concentrations of yeasts and molds were significant for all nine samples tested.

SURVIVAL OF PATHOGENS IN LITTER DURING STORAGE

Aeromonas hydrophila concentrations recovered from litter decreased during storage from a range of log₁₀ 3 to 4 to below the detection limit of 30 CFU/g dry weight during one to four weeks of storage. Reductions in concentrations of *A. hydrophila* were significant for five of twelve samples tested.

TABLE 3. Microorganisms recovered from broiler litter (House #3) during storage (log₁₀ CFU/g dry weight)

MICROBIAL GROUP OR SPECIES	LITTER FRACTION	INITIAL MICROBIAL CONCENTRATION[A]	TOTAL MEAN REDUCTION (2–16 WK)
Total coliforms	Whole	5.67±3.15	4.23**
	Coarse	3.73±2.57	2.31**
	Fine	3.83±0	2.41**
Fecal coliforms	Whole	4.64±3.78	3.20
	Coarse	4.87±4.11	3.45**
	Fine	2.91±2.58	1.49*
Escherichia coli	Whole	5.37±4.10	3.93*
	Coarse	3.37±1.93	1.95**
	Fine	3.38±2.15	1.96**
Heterotrophic bacteria	Whole	9.13±8.35	0.84
	Coarse	11.06±10.05	2.19**
	Fine	10.99±0	1.99
Yeasts & Molds	Whole	6.12±6.26	3.90**
	Coarse	5.50±4.65	3.26**
	Fine	5.45±4.17	3.18**
Aeromonas hydrophila	Whole	3.88±3.73	2.44*
	Coarse	4.44±4.55	3.00
	Fine	3.37±3.09	1.93*
Pseudomonas aeruginosa	Whole	4.70±3.80	3.26*
	Coarse	3.86±3.75	2.42*
	Fine	3.42±2.61	1.98
Yersinia enterocolitica	Whole	5.85±5.48	4.41*
	Coarse	4.95±5.07	3.51**
	Fine	4.01±3.11	2.57*

[A]Mean ± one standard deviation

*Statistically significant at P < .05; **Statistically significant at P < .01

Pseudomonas aeruginosa concentrations recovered from litter decreased during storage from a range of log₁₀ 2 to 4 to below the detection limit of 30 CFU/g dry weight during four to sixteen weeks of storage. *P. aeruginosa* concentration reductions were significant for five of nine samples tested.

Yersinia enterocolitica concentrations recovered from litter decreased during storage from a range of log₁₀ 4 to 6 to below the detection limit of 30 CFU/g dry weight during two to sixteen weeks of storage. *Y. enterocolitica* concentration reductions were significant for all nine samples tested.

Campylobacter jejuni concentrations recovered from litter decreased during storage from a range of log₁₀ 1 to 3 to below the detection limit of 2.0 MPN/g dry weight during four to sixteen weeks of storage. *C. jejuni* concentration reductions were significant for all six samples tested.

STATISTICAL ANALYSES OF MICROBIAL CONCENTRATION CHANGES DURING STORAGE

Generally, significant reductions in microbial concentrations occurred in litter during the storage period. Of the samples tested, 72% (sixty-three of eighty-eight) showed significant (P < .05) microbial reductions according to coefficients of determination generated by polynomial regression analysis. Many litter samples not showing statistically significant microbial concentration re-

TABLE 4. Microorganisms recovered from broiler litter (House #4) during storage ($\log_{10}$ CFU/g dry weight)

MICROBIAL GROUP OR SPECIES	LITTER FRACTION	INITIAL MICROBIAL CONCENTRATION[A]	TOTAL MEAN REDUCTION (2-16 WK)
Total coliforms	Whole	6.45±6.60	5.01**
	Coarse	5.88±5.89	4.45**
	Fine	3.95±3.92	2.53**
Fecal coliforms	Whole	5.91±4.75	4.47**
	Coarse	1.43±0[B]	0
	Fine	1.43±0[B]	0
Escherichia coli	Whole	6.02±5.93	4.58**
	Coarse	5.58±5.57	4.15**
	Fine	3.47±0	2.04
Heterotrophic bacteria	Whole	9.07±8.48	+0.80
	Coarse	10.24±10.08	1.71**
	Fine	10.37±9.53	1.95
Yeasts & Molds	Whole	5.58±5.36	1.93**
	Coarse	5.71±5.79	4.28**
	Fine	5.69±5.76	4.26**
Aeromonas hydrophila	Whole	4.46±4.17	3.00**
	Coarse	1.43±0[B]	0
	Fine	1.43±0[B]	0
Pseudomonas aeruginosa	Whole	3.91±2.30	2.47**
	Coarse	2.20±2.27	0.77
	Fine	3.21±2.33	1.78
Yersinia enterocolitica	Whole	6.32±6.37	4.88**
	Coarse	4.62±4.12	3.19**
	Fine	4.03±3.75	2.60**
Campylobacter jejuni (MPN)	Whole	3.53±3.66	3.23*
	Coarse	3.63±3.59	3.20**
	Fine	3.56±3.70	3.00*

[A]Mean ± one standard deviation

[B]Detection limit

*Statistically significant at P < .05; **Statistically significant at P < .01

ductions did, however, show substantial mean concentration reductions during storage (Tables 1 through 4). Differences in microbial concentrations recovered from litter were generally non-significant among houses.

MICROORGANISMS BELOW DETECTION LIMITS IN LITTER (NON-CULTURABLE)

Microorganisms which were below their detection limits in litter throughout the time period tested for all houses included *Salmonella* spp. (detection limit = 0.2 MPN/g dry weight), *Listeria monocytogenes* and *Staphylococcus aureus* (detection limits 2.0 MPN/g dry weight), and *Clostridium perfringens* and fecal streptococci (detection limits 30 CFU/g dry weight). Those microorganisms not shown in the data for a particular house were below specified detection limits in litter samples from that house (*i.e.*, non-culturable).

MICROBIAL CONCENTRATIONS IN FEED AND WOOD SHAVINGS

Concentrations of microorganisms recovered in feed from the four broiler houses were

substantial (up to $\log_{10}$ 7 total coliforms). It appears that feed may be a source of microbial contamination in these broiler houses, although the feed may have become contaminated after introduction into the broiler houses through contact with unclean surfaces. Other theoretical sources of microbial contamination in the house environment include airborne materials (e.g., dust, feathers, etc.), soils, and human carriers. Concentrations of microorganisms recovered from wood shavings were negligible in comparison to broiler litter (i.e., below detection limits).

RECOVERY OF VIRUSES FROM LITTER

Litter samples were analyzed for enteric viruses using scanning electron microscopic (SEM) analysis at the University of Georgia, College of Veterinary Medicine, Athens, GA using a protocol developed at Dr. Pedro Villegas' laboratory in the University's Poultry Disease Research Center. Samples of whole litter at time zero (and Houses #1 and #2 at five months) were examined for viruses. Coronaviruses and rotaviruses were found in the whole litter from Houses #1 and #2, respectively, at time zero of storage. However, no evidence of viruses was found in these two houses after a litter storage period of about five months, indicating that their survival is limited to the first few months of storage. No evidence of viruses was found in Houses #3 and #4 at time zero, so no further analyses were attempted on these samples.

TEMPERATURE AND MOISTURE CONTENT CHANGES DURING LITTER STORAGE

Temperature in coarse and fine litter fractions peaked after about five days during storage (up to 43°C) and then decreased to about 20 to 25°C until about fifteen days, when it dropped to ambient temperatures of about 10 to 20°C. Whole litter appeared to maintain a lower temperature (maximum of about 32°C after about five days) during storage, but retained this temperature for a longer period of time until about seventeen days when it also dropped to ambient temperature. Stored litter temperature readings were taken in the late winter (February). Changes in temperatures of ambient storage during the year may change the rate and amount of microbial concentration reductions.

Temperature changed significantly with storage time for whole litter and fractions when the data curves are fitted by third-order polynomial regression analysis ($R^2 = 0.891**$, $0.893**$, and $0.647**$ for whole, coarse, and fine litter, respectively [** = $P < .01$]).

Moisture content decreased significantly for litter fractions, but not for whole litter ($R^2 = 0.893*$ and $0.807*$ for coarse and fine fractions, respectively, vs. $R^2 = 0.319$ for whole litter [* = $P < .05$]). The reduction found in litter moisture from a mean of about 20% to about 15% may have been adequate to significantly reduce the Gram-negative pathogenic bacteria recovered which are generally more susceptible to desiccation.

RECOVERY OF INJURED MICROORGANISMS FROM LITTER (i.e., FROM BELOW DETECTION LIMITS)

No microorganisms were cultured from litter samples in which microbes were below detection limits after placing these samples in 1.0% BPW, incubating at 37°C for 48 hr, then plating on appropriate media. This finding indicates that recovery from below detection limits to substantial numbers is unlikely under normal conditions.

CONCLUSIONS AND APPLICATIONS

1. Fractionation of litter followed by storage for sixteen weeks in piles simulating farm storage conditions yielded statistically significant or substantial (although non-significant) microbial concentration reductions for a majority of samples tested.
2. Viruses initially found in litter were not found after five months of storage.
3. Initially elevated litter temperatures of 30 to 40°C and gradual litter desiccation appeared sufficient to significantly reduce most microbial concentrations during two to sixteen weeks of storage.

4. Broiler litter re-utilization potential was improved physically by fractionation and micro-biologically by significant or substantial microbial concentration reductions during two to sixteen weeks of litter storage.

5. Litter fractionation and storage appear to be of potential benefit to the goals of sustainable agriculture by providing products that can be more safely and economically re-utilized in an agricultural environment and by reducing the risks of environmental contamination.

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17. MPN Methods. Dilutions of 1.0, 0.1, and 0.01 mL of litter homogenates were tested, except in the case of Salmonella spp., in which case dilutions of 10, 1.0, and 0.1 mL of litter homogenates were used. Sterile 9-mL blanks of appropriate enrichment media were prepared and appropriate transfers performed to achieve desired dilutions using sterile disposable pipettes. Transfers to confirmatory media by streaking were made using a flamed platinum-iridium loop. The standard method [16] used for enumeration of Salmonella spp. was modified by using pre-enrichment of 1.0% buffered-peptone-water [18] for 24 hr incubated at 37°C followed by selective enrichment in tetrathionate-BG broth containing 1:100,000 brilliant green dye, again incubated at 37°C for 24 hr [16]. Presumptive confirmation was made by streaking onto a variety of media including bismuth sulfite, brilliant green with and without the addition of novobiocin [19], XLBG [20], XLT4 [21] (all incubated at 37°C for 24 hr), and modified semisolid Rappaport-Vassiliadis medium [22], incubated at 42°C for 24 hr. A more sensitive delayed secondary enrichment method was also employed when no Salmonella spp. were found initially [23]. However, none of these methods isolated any Salmonella from litter samples in this study. Final confirmation, while rarely necessary and never positive, was made by streaking onto triple sugar iron (TSI) agar and lysine iron agar (LIA) slants at 37°C incubated for 24 hr. Campylobacter jejuni and Staphylococcus aureus were enumerated according to Standard Methods for the Examination of Water and Wastewater, 17th Edition [16]. C. jejuni was isolated and enumerated using Oosterom's enrichment medium and Butzler's agar confirmatory medium [16]. S. aureus was isolated and enumerated using Staphylococcus enrichment broth and Lipovitellin-salt-mannitol agar confirmatory medium [16]. L. monocytogenes was isolated and enumerated using Listeria enrichment broth and Oxford formulation confirmatory agar medium, according to the method of Varabioff [24].

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25. **Membrane Filtration Methods.** Either 0.1, 1.0, or 10.0 mL of appropriate dilutions of litter homogenates made in sterile 99-mL blanks of 0.1% buffered peptone-water (BPW) were filtered. Sterile 1.0 or 10.0 mL plastic disposable pipettes or micropipette tips were used for transfers and discarded between samples and replicates of samples. Aseptic technique was observed throughout the procedures. The filter apparatus was sterilized by autoclaving and rinsed with sterile buffer between duplicate samples and when necessary to insure even distribution of the microorganisms on the filter. Clostridium perfringens isolation was attempted using the method of Bisson and Cabelli [26]. However, C. perfringens isolation proved unsuccessful using this method. The method in Compendium of Methods for the Microbiological Examiantion of Foods, Second Edition [27] was then used (which also proved unsuccessful in isolating C. perfringens). The methods for enumeration of Yersinia en-

terocolitica, Pseudomonas aeruginosa, yeasts and molds, fecal coliforms, and Klebsiella spp. were taken from Standard Methods for the Examination of Water and Wastewater, 17th Edition [16]. Aeromonas hydrophila was enumerated using the method of Rippey and Cabelli [28] utilizing mA agar, with confirmation on mannitol agar. Total coliforms were enumerated using the method of Freier and Hartman [29] with m-TMM medium and allowed the enumeration of E. coli through that species' unique expression of fluorescence on this medium.

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