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Evaluation of Methods for Recovery of *Salmonella* from Dairy Cattle, Poultry, and Swine Farms

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ABSTRACT

Current official methods for detection and isolation of *Salmonella* are mostly designed for foods. The objective of this study was to determine optimal methods for detection and isolation of *Salmonella* from animal and environmental samples of dairy, poultry, and swine farms. Preenrichment in lactose broth versus direct enrichment (no preenrichment) prior to selective enrichment in Rappaport-Vassiliadis, selenite cystine, and tetrathionate incubated at 35 and 42°C and in four differential/selective plating media (brilliant green, bismuth sulfite, Hektoen enteric, and xylose-lysine-tergitol 4 agar base) were evaluated for their ability to recover *Salmonella* from artificially contaminated samples. The effects of pH adjustments to samples on *Salmonella* recovery were determined. A pH adjustment of the enrichment broth to 6.8 ± 0.2 after addition of samples significantly improved recovery of *Salmonella.* The most effective medium combinations for isolation of *Salmonella* from farm samples depended on the type of samples. Generalizations of protocols for recovery of *Salmonella* from farm samples might result in poor recovery, increased recovery time, and increased sample processing costs.

An "on farm" management program for control of pathogens such as *Salmonella* is needed to minimize their transfer to foods, humans, and animals. To initiate such programs, reliable baseline data on the occurrence of *Salmonella* on dairy, poultry, and swine farms is necessary. To obtain and establish such data, accurate, efficient, and optimal detection methods are urgently required. Current official methods of the Food and Drug Administration *Bacteriological Analytical Manual* (FDA-BAM) *(5)* and the U.S. Department of Agriculture Food Safety and Inspection Services (USDA-FSIS) *(17)* for detection and isolation of *Salmonella* are mostly designed for food rather than farm samples.

Cultural procedures for recovery of *Salmonella* involve four or five basic steps: preenrichment, selective enrichment, selective plating, biochemical testing, and serological confirmation *(1, 4).* On the basis of published data, FDA-BAM and USDA-FSIS protocols, media recommended for isolation and identification of *Salmonella* include lactose broth (LB), buffered peptone water (BPW), and universal preenrichment broth (UB) for preenrichment; Rappaport-Vassiliadis (RV), selenite cystine (SC), and tetrathionate (TT) incubated at 35°C (TT35) and at 42°C (TT42) for selective enrichment; and bismuth sulfite (BS), brilliant green (BG), Hektoen enteric (HE), xylose-lysine desoxycholate (XLD), and xylose-lysine-tergitol 4 (XLT4) agars for selective plating.

Numerous studies have been conducted to compare and improve microbiological techniques for detection and isolation of *Salmonella* from foods. Andrews *(4)* reported that LB was the most widely used medium for preenrichment and was comparable or superior to other media. The International Standards Organization protocol, however, recommends BPW *(11).* Hoorfar and Baggesen *(10)* reported that BPW was more sensitive than UB for recovery of *Salmonella* from swine fecal samples; however, UB was more sensitive than BPW for poultry skin samples.

Early studies indicated that RV was the most productive selective enrichment broth for recovery of *Salmonella* from shrimp *(1),* milk *(19),* and high-moisture highly contaminated foods *(4, 12).* Hammack et al. *(8)* found that TT incubated at 35°C recovered the highest number of *Salmonella* from low–microbial load foods compared with RV or SC. Dusch and Altwegg *(6)* found that modified semisolid RV broth was the most sensitive and specific medium for recovery of *Salmonella* from stool specimens compared with standard medium HE and three other plating media. However, modified semisolid RV requires specific and careful handling in the laboratory. XLT4 had outstanding specificity and sensitivity comparable to HE. Other studies found that XLT4 recovered the highest number of *Salmonella* from various foods *(4)* and from chicken farm environmental samples *(15)* compared with BG, BS, HE, XLD, or Rambach agars. Mallinson et al. *(14)* found significantly fewer false-negative and false-positive *Salmonella* on XLT4 than on BS, HE, and XLD with various red and white meat samples.

Most studies have focused on the recovery of *Salmonella* from foods, with limited effort on animal and environmental farm samples. Therefore, the objective of our

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study was to compare, modify, and optimize methods for detection of *Salmonella* from animal and environmental samples of dairy, poultry, and swine farms.

MATERIALS AND METHODS

Experimental design. Experiments were conducted in two phases. In the preliminary study, we evaluated the effects of cellulase in LB and pH adjustment during direct enrichment on recovery of *Salmonella* from artificially contaminated dairy grain feed. The purpose of addition of cellulase was to break down plant materials, which could release and improve recovery of microorganisms. The efficacy of UB was compared with RV, SC, and TT with and without preenrichment in LB. In the second phase, we evaluated preenrichment versus a direct enrichment with no preenrichment (DE) step in four selective enrichment treatments (RV, SC, and TT incubated at 35°C and 42°C) in conjunction with four different plating media (BG, BS, HE, and XLT4) for the ability to recover *Salmonella* from various artificially and naturally contaminated animal and environmental samples. UB was dropped from the enrichment media because of poor and sporadic recovery of *Salmonella.* The experiments were conducted in 10 replications. Rainbow agar was initially included in the design as one of the differential plating media but was dropped because it did not appear to increase recovery and because of its prohibitively high cost.

Samples sources and collection. Environmental and animal samples were obtained from dairy, poultry, and swine farms at The University of Tennessee Experimental Station in Knoxville. Dairy environmental and animal samples included feeds (grain, silage, and total mix ration [TMR] from bunk feeder), fresh manure, bedding from barns, soil from pasture, trough water, milking parlor, flies, air, and hair swabs. Poultry environmental samples were feed remaining in the feeder, chicken litter, and droppings. Swine environmental samples included feed rations without and with antibiotic and feces from finisher pigs. All samples were transported under refrigeration to the laboratory and analyzed on the same day of collection.

Milking parlor and animals (hair) were sampled by swabbing areas (~10 by 10 cm^2) of floor, wall, window, or stall and hair around neck, respectively, with sterile sponges moistened with 10 ml of butterfield phosphate buffer (BPB, pH 7.2). The swab samples were placed in sterile bags for transport and analysis. Flies concentrated over feeders were captured with plastic bags and stored in a freezer (−18°C) overnight or until analyzed.

Air samples were collected in the calf barns and in the milking parlor by venting air through Millipore microfilters (0.45 μm) using a Gast vacuum pump (model 0322-V4BG18DX, Gast Co., Benton, Mich.). The pump was connected to a flow meter (model 65, Gilmont Instruments, Barrington, Ill.). The vacuum line was split into two outlets from the flowmeter and connected to two inlets that were attached to two vacuum flasks. The flasks were fitted with Nalgene Analytical Filter Funnels (type AF, Cat. No. 140) containing microfilters. The filters were moistened with BPB. The air flow was set to 37.56 standard liters per minute, and the pump was set with an automatic timer for 20 min (~750 liters). The filters were aseptically placed into sterile bags for transport and analysis.

Source and preparation of media and inocula. Except for Rappaport-Vassiliadis (Oxoid Inc., Ogdensburg, N.Y.), all culture media used in this study were Difco (Sparks, Md.) brand. The media were prepared according to the manufacturer's instructions or the FDA-BAM. Tetrathionate broth contained 0.1% brilliant green dye (10 ml/liter) and iodine-potassium iodide solution (20 ml/liter).

Freeze dried cultures of *Salmonella* Enteritidis (ATCC 13076), *Salmonella* Anatum (ATCC 9270), *Salmonella* Derby (ATCC 6960), *Salmonella* Heidelberg (ATCC 8326), and *Salmonella* Typhimurium (ATCC 14028) were obtained from the American Type Culture Collection (Rockville, Md.). The cultures were prepared and lyophilized according to the procedures of Hammack et al. *(8)* with modification. The cultures were lyophilized to obtain consistent inocula in all experimental sample units. Each culture was rehydrated and cultivated in 10 ml of brain heart infusion broth at 35°C for 24 h to produce a culture containing about 10^8 CFU/ml. The 24-h culture (50 ml) for each strain was centrifuged at 10,000 × *g* for 10 min. The pellets were suspended in and washed twice with 50 ml of BPB (pH 7.0). The washed cells were diluted in BPB to 10^{-6} to obtain about 10^2 CFU/ml inoculum. *Salmonella* was inoculated into samples to give target inocula of 1 to 2 CFU/g in experiments in phase I. For experiments in phase II, the diluted cells were prepared for lyophilization by mixing 50 ml of the 10^{-6} dilution with 50 ml of 20% (wt/vol) skim milk. The suspensions (2 ml) were pipetted into 5-ml ampules, frozen at −56°C in an ultralow-temperature freezer (Revco Scientific, Asheville, N.C.), and lyophilized for 24 ± 2 h on an automatic manifold freeze dryer (Labconco, Kansas City, Mo.). The lyophilized cultures were stored at −20°C prior to use. Samples were inoculated with lyophilized culture to give a target inoculum of 1 to 2 CFU/g (phase II). The inoculum level in samples was determined using the most-probable-number method *(8).*

Preparation of lactose broth with 1% cellulase. The stock solution was prepared by dissolving 1 g of cellulase from *Penicillium funiculosum* (Sigma Chemicals, St. Louis, Mo.) in 100 ml sterile distilled water. The solution was filtered with a 0.45-μm filter. The filtered stock solution (2.25 ml) was added to 225 ml of LB to obtain a final concentration of 0.01% in lactose broth.

Effects of cellulase and pH adjustment—phase I. To study the effect of cellulase on recovery, a grain sample (25 g) was weighed into stomacher bags and inoculated with *Salmonella* Enteritidis to provide about 1 to 2 CFU/g of sample. One set of samples was preenriched in 225 ml of LB and the other set in 225 ml of lactose broth plus cellulase.

For the pH experiment, a grain sample (5 g) was weighed into stomacher bags, inoculated with *Salmonella* Enteritidis (~1 to 2 CFU/g), and stored at 4°C for 2 weeks (these were termed "aged samples"). The aged samples were enriched by adding 45 ml of RV, SC, or TT for direct enrichment. The pH of one set of samples was adjusted to 6.8 ± 0.2 with 1 N HCl or NaOH, whereas the other set was not adjusted. The preenriched and enriched samples were processed further for isolation as described in the following sections.

Inoculation of samples. For solid samples, the lyophilized cultures were mixed by grinding with 100 g of nonfat dried milk in a mortar and pestle to a fine powder. Samples (25 g for preenrichment and 5 g for direct enrichment in stomacher bags) were inoculated with ground lyophilized cultures to provide an inoculum of about 1 to 2 CFU/g of samples. The inoculated samples were stored in a refrigerator (~4°C) for 2 weeks before preenrichment or direct enrichment. For wet samples, fresh manure and trough water, the lyophilized cultures were rehydrated with 2 ml of BPB and mixed. The volume was brought to 100 ml. The rehydrated cultures were inoculated into 25 g of manure or 25 ml of water in sterile plastic bags to provide inocula of about 1 to 2 CFU/g or ml of samples. The latter two samples were preenriched

TABLE 1. *Effect of pH adjustment during direct enrichment in selective broth on the recovery of* Salmonella *Enteritidis from artificially contaminated dairy grain feed (~1 to 2 CFU/g)*[a]

Selective enrichment	Differential plating[b]	Recovery (%)[c] pH not adjusted	pH adjusted
Rappaport-Vassiliadis	BG	30 EFGH	90 AB
	BS	50 CDEF	50 CDEF
	HE	0 H	70 ABCD
	XLT4	0 H	60 BCDE
Selenite cystine	BG	40 DEFG	100 A
	BS	70 ABCD	60 BCDE
	HE	18 FGH	18 FGH
	XLT4	0 H	11 GH
Tetrathionate incubated at 35°C	BG	0 H	90 AB
	BS	50 CDEF	100 A
	HE	20 FGH	30 EFGH
	XLT4	0 H	70 ABCD
Tetrathionate incubated at 42°C	BG	20 FGH	50 CDEF
	BS	30 EFGH	80 ABC
	HE	0 H	30 EFGH
	XLT4	0 H	30 EFGH

[a] The pH of sample solutions in selective enrichment broths was adjusted to 6.8 ± 0.2 with 1 N NaOH or HCl prior to incubation.
[b] BG, brilliant green; BS, bismuth sulfite; HE, Hektoen enteric; XLT4, xylose-lysine-tergitol 4 agar base.
[c] Percent positive *Salmonella* of 10 replications; values followed by unlike letters are significantly different ($P < 0.05$).

and enriched directly in a refrigerator without aging. Flies (10 individuals) were inoculated by soaking in the rehydrated cultures for 10 min, drained, and air dried on weighing boats at room temperature for 30 min prior to preenrichment and direct enrichment. Air and environmental swab samples were not inoculated with *Salmonella* culture.

Preenrichment. The studies in phase I showed that cellulase tended to reduce the recovery of *Salmonella.* Thus, in the experiments in phase II, samples were preenriched in LB without cellulase. Samples (25 g or 25 ml) were added to 225 ml of LB; the swab samples were preenriched in 50 ml of LB in bags and flies were preenriched with 10 ml of LB in test tubes. The samples in bags were mixed by hand-pummeling for 1 min. The pH of sample solutions was adjusted to 6.8 ± 0.2 with 1 N NaOH or HCl. The samples were allowed to stand at room temperature for 60 min prior to incubation at 35°C for 20 to 24 h. After incubation, a 1-ml aliquot was transferred to 10 ml of RV, SC, TT35, and TT42 in test tubes. The pH of sample solutions was again adjusted to 6.8 ± 0.2. RV and TT42 enrichment samples were incubated at 42°C, whereas SC and TT35 tubes were incubated at 35°C for 18 to 24 h before streaking onto selective plating media.

Direct enrichment. Samples (5 g) were directly enriched in 45 ml of RV, SC, TT35, and TT42. The water samples (25 ml) were enriched with 225 ml of broth, swab samples with 50 ml, and flies with 10 ml. The pH of sample solutions was adjusted to 6.8 ± 0.2 on the basis of the results of the preliminary studies (Table 1). Samples enriched in RV and TT42 were incubated at 42°C, and SC and TT35 at 35°C for 18 to 24 h before isolation onto selective plating media.

Isolation and identification of *Salmonella.* Upon incubation, a loopful of samples from each enrichment medium was streaked onto selective plating media (BG, BS, HE, and XLT4) and incubated at 35°C for 24 h; BS plates were incubated for 48 h. XLT4 was prepared from XLT4 agar base without addition of the supplement. Presumptive *Salmonella* colonies (three typical colonies) from each plate were inoculated into triple sugar iron slants and incubated at 35°C for 18 to 24 h. *Salmonella*-positive triple sugar iron reactions were followed with serological confirmation using polyvalent somatic (O) antisera (Difco). All confirmed isolates were subjected to API 20E strips for final confirmation.

Statistical analysis. Categorical data from the experiments were quantified to 1 for positive recovery and 0 for negative recovery of *Salmonella* and subjected to analysis of variance *(9).* The statistical design was a completely randomized design with split-plot treatment arrangement and 10 replications. The whole-plot experimental units were preenrichment or pH adjustment treatments, and the subplot experimental units were selective enrichment (RV, SC, TT35, and TT42) and selective plating (BG, BS, HE, and XLT4). All data were analyzed with the SAS Mixed Procedure *(13)* using SAS Software Release 8.2 (SAS Institute Inc., Cary, N.C.). Significant differences among means were determined by the Least-Squares Means method with the PDIFF (*P*-value for differences) option.

RESULTS AND DISCUSSION

Effects of cellulase and pH adjustment. Overall, addition of 0.01% cellulase into LB for preenrichment had no significant effect ($P > 0.10$) on the recovery of *Salmonella* Enteritidis from grain. In some medium combinations, cellulase tended to reduce recovery and was detrimental to the growth of the *Salmonella.* On the basis of these results, addition of cellulase was eliminated from treatments for preenrichment of samples in phase II and is not recommended for isolation of *Salmonella* spp. from sample types evaluated in this study. Addition of cellulase to a final concentration of 0.01 to 0.1% in LB was reported to improve the recovery of *Salmonella* from thickening agents *(2, 3).*

Adjustment of pH to 6.8 ± 0.2 of the selective enrichment broths (RV, SC, and TT) after addition of samples significantly improved recovery (100% recovery in some treatments) of *Salmonella* Enteritidis from mixed grain ($P < 0.0001$). In most medium combinations, pH adjustment doubled recovery of *Salmonella* Enteritidis (Table 1). Without pH adjustment, recovery was zero in 7 of the 16 treatment combinations. The best recovery without pH adjustment was 70% and occurred only in the SC-BG combination. The FDA-BAM clearly recommends pH adjustment; however, many laboratories omit this step to reduce equipment and processing time during analysis of samples. It is absolutely essential that pH adjustment be performed if *Salmonella* is to be reliably recovered.

Dairy hair and environmental samples. Preenrichment in LB did not affect isolation of *Salmonella* from hair swab samples ($P > 0.70$). In general, RV, SC, and BS were the best selective enrichment and plating media, respectively, for hair samples. Nevertheless, the optimal methods to isolate *Salmonella* from cow hair depended on the medium combinations. Preenrichment in LB followed by enrichment in RV, TT35, or TT42 and streaking onto BS (LB-RV/TT35/TT42-BS) resulted in the highest recovery (90%)

(Table 2). Direct enrichment in RV or SC prior to isolation on BS (DE-RV/SC-BS) gave 80% recovery of *Salmonella* and was not significantly different from preenrichment methods ($P > 0.05$). Therefore, direct enrichment could be used for faster results. Because the samples were not inoculated, these medium combinations were able to isolate *Salmonella* naturally present on the animal. The other medium combinations resulted in 70% or less positive *Salmonella*. Choice of differential plating medium/enrichment medium significantly affected recovery, with variations of 0 to 90% recovery in a given enrichment medium ($P < 0.05$).

The most effective methods to recover *Salmonella* from dairy feeds varied with the type of samples. Overall, direct enrichment was more effective then preenrichment ($P < 0.01$) to recover *Salmonella* from mixed grain samples. However, preenrichment in LB significantly increased recovery of *Salmonella* from silage and TMR samples ($P < 0.0001$). The pH of silage and TMR was generally less than 5 (TMR contains grain and silage). This stress might have caused injury to cells of the inocula during 2-week aging. Thus, the preenrichment might have resulted in resuscitation of the injured target *Salmonella* while suppressing competing microorganisms for recovery. Direct enrichment in TT incubated at 35°C followed by isolation on BS and enrichment in SC with BG plating were the most effective methods of recovering *Salmonella* from the grain samples (70 to 75% recovery) (Table 2). With the preenrichment technique, the highest recovery was 55%. Therefore, preenrichment in LB is not recommended for grain and mixed feed but is recommended for TMR and silage.

Several alternative medium combinations were effective for isolation of *Salmonella* from silage with 80% or higher recovery (Table 2). Because the alternative media were not significantly different, the choice would be dependent on laboratory sources. The highest recovery (100%), however, resulted from preenrichment in LB followed by selective enrichment in TT35 and isolation on XLT4 (LB-TT35-XLT4) or enrichment in SC and isolation on BS (LB-SC-BS). Without preenrichment, the highest recovery of *Salmonella* from silage was 60%. The most effective method (70% recovery) for isolation of *Salmonella* from TMR was preenrichment in LB followed by enrichment in TT35 and streaking on BS (Table 2). The other medium combinations resulted in 50% or less recovery. Without preenrichment in LB, recovery was zero in 9 of the 16 medium combinations, and the highest recovery was 50%, which occurred only in enrichment in TT35 followed by streaking on BS.

Overall, preenrichment in LB did not improve recovery of *Salmonella* from bedding ($P > 0.10$). The four selective enrichments generally had no significant effect on the isolation of *Salmonella* from bedding ($P > 0.40$). However, the most effective methods for recovery of *Salmonella* from bedding were dependent on the medium combination and most often occurred in direct enrichment methods (Table 2). Direct enrichment in RV followed by isolation on XLT4 or on HE recovered the pathogen from bedding in up to 100% of samples. Direct enrichment in RV or TT42 with isolation on BS and in SC with HE (70% recovery) was a less effective alternative method for bedding samples. Preenrichment of bedding samples in LB followed by any selective enrichment and plating media recovered only 60% of *Salmonella*. Thus, preenrichment in LB is not recommended for bedding.

Across all experiments, preenrichment in LB significantly decreased recovery of *Salmonella* from soil ($P < 0.0001$). The most effective selective enrichment was TT incubated at 42°C followed by plating media BS and HE. Direct enrichment in TT42 followed by isolation on BS, HE, or XLT4 (DE-TT42-BS/HE/XLT4) gave recoveries of 100% (Table 2). If preenrichment in LB was used, only the TT42 and BS combination was effective (90% recovery) for isolation of *Salmonella* from soil. Alternative methods for isolation of *Salmonella* from soil samples, with recoveries ranging from 80 to 90%, were DE-RV-BG or HE, DE-TT42-BG, and DE-SC-BS. Recoveries for other medium combinations were 70% or less; therefore, they are not recommended for isolation of *Salmonella* from soil samples.

Preenrichment in LB did not significantly increase recovery of *Salmonella* from cow manure ($P > 0.40$). The most effective enrichment for recovery of *Salmonella* from manure samples were RV, SC, and TT42; plating media included BS and XLT4. XLT4 was reportedly the most effective plating media for isolation of *Salmonella* from manure drag samples *(15)*. Direct enrichment in SC or TT42 followed by streaking on XLT4 was as effective as preenrichment in LB followed by enrichment in RV or TT42 with plating on BS for isolation of *Salmonella* from manure (100% recovery) (Table 2). This means that preenrichment is not needed for these samples; thus, 1 day of lab work to complete analysis can be eliminated. These results partly agreed with those reported by June et al. *(12)* for highly contaminated foods. However, they incubated TT at 43°C and postulated that the elevated temperatures inhibited competitive microflora. Hammack et al. *(8)* found that TT35 recovered the greatest number of *Salmonella* from low–microbial load foods. Gay et al. *(7)* also used direct enrichment in TT and in selenite-F broth incubated at 37°C followed by streaking on BG as the standard method for isolating *Salmonella* from different types of dairy fecal samples. Direct enrichment in RV followed by plating on BS or XLT4 gave 90% recovery in our laboratory for isolation of *Salmonella* from manure samples. Several other medium combinations enabled recovery of *Salmonella* from manure of up to 80%, including preenrichment in LB followed by enrichment in RV with plating on BG, or by enrichment in SC or TT42 with plating on HE or XLT4, and medium combinations from direct enrichment treatment (Table 2). Because there are many alternative effective medium combinations for recovery of the microorganism from cow manure samples, the choice of method will depend on the laboratory resources and time constraints. Our laboratory generally avoids the use of selenite cysteine broth when possible because of the toxicity of medium components and increased disposal costs.

The best technique for recovery of *Salmonella* from

TABLE 2. *Recovery (%)*[a] *of* Salmonella *from artificially contaminated dairy hair and environmental samples (~1 to 2 CFU/g)*[b] *using preenrichment in lactose broth or direct enrichment in selective media in conjunction with four selective media*

Enrichment[c]	Selective plating[d]	Hair (LB)	Grain (DE)	Silage (LB)	TMR (LB)[e]	Bedding (DE)	Soil (DE)	Manure (DE)	Water (LB)	Air (LB)[b]	MP (DE)[b]	Flies (LB)
RV	BG	10 CD	60 ABC	60 BC	40 ABC	30 BC	80 ABC	30 C	100 A	0 D	30 CDEF	10 DE
	BS	90 A	50 ABCDE	90 AB	50 AB	70 AB	50 CD	90 AB	100 A	80 AB	80 A	40 ABCD
	HE	20 CD	35 CDEF	80 ABC	0 D	100 A	90 AB	70 AB	100 A	80 AB	60 ABC	40 ABCD
	XLT4	20 CD	30 DEFG	80 ABC	10 CD	100 A	70 ABCD	90 AB	100 A	10 D	60 ABC	70 A
SC	BG	40 BC	70 A	80 ABC	40 ABC	50 BC	60 BCD	70 AB	80 ABC	0 D	40 BCDE	10 DE
	BS	60 AB	65 AB	100 A	20 BCD	60 ABC	80 ABC	80 AB	70 BCD	80 AB	20 DEF	40 ABCD
	HE	30 BCD	18 FG	80 ABC	40 ABC	70 AB	70 ABCD	80 AB	70 BCD	0 D	70 AB	30 BCDE
	XLT4	10 CD	6 G	90 AB	0 D	20 C	50 CD	100 A	50 D	40 C	60 ABC	50 ABC
TT35	BG	0 D	40 BCDEF	50 C	40 ABC	20 C	40 D	70 AB	80 ABC	0 D	0 F	70 A
	BS	90 A	75 A	90 AB	70 A	50 BC	70 ABCD	70 AB	60 CD	100 A	70 AB	40 ABCD
	HE	0 D	25 EFG	90 AB	20 BCD	30 BC	70 ABCD	60 BC	90 AB	40 C	0 F	40 ABCD
	XLT4	40 BC	35 CDEF	100 A	20 BCD	60 ABC	50 CD	80 AB	100 A	0 D	20 DEF	60 AB
TT42	BG	10 CD	35 CDEF	90 AB	20 BCD	30 BC	90 AB	60 BC	100 A	0 D	10 EF	0 E
	BS	90 A	55 ABCD	80 ABC	50 AB	70 AB	100 A	60 BC	100 A	60 BC	50 ABCD	40 ABCD
	HE	0 D	15 FG	90 AB	10 CD	30 BC	100 A	80 AB	100 A	40 C	0 F	20 CDE
	XLT4	10 CD	15 FG	90 AB	20 BCD	20 C	100 A	100 A	100 A	10 D	20 DEF	0 E

[a] Percent positive *Salmonella* of 10 replications; values in a column followed by different letters are significantly different ($P < 0.05$). Data are presented only for the LB (lactose broth preenrichment) or DE (direct enrichment with no LB preenrichment) method on the basis of the best recovery method; exceptions are discussed in "Results."

[b] Air and milking parlor (MP) swab samples were not artificially contaminated with inoculum.

[c] RV, Rapport-Vassiliadis; SC, selentine cystine; TT35 and TT42, tetrathionate incubated at 35°C and 42°C, respectively.

[d] BG, brilliant green; BS, bismuth sulfite; HE, Hektoen enteric; XLT4, xylose-lysine-tergitol 4 agar base.

[e] TMR, total mix ration obtained from cow feeder contains both grain and silage.

trough water was preenrichment in LB ($P < 0.0001$), whereas RV, TT35, and TT42 were superior overall to SC ($P < 0.05$). There was no significant difference among plating media BG, BS, HE, and XLT4 ($P > 0.50$). Without preenrichment in LB, most medium combinations did not recover *Salmonella* from water, and the highest recovery was 30%. After preenrichment in LB, the most effective medium combinations for recovery of *Salmonella* from trough water (100% recovery) were enrichment in either RV or TT42 followed by isolation on either BG, BS, HE, or XLT4 (Table 2). Enrichment in TT35 followed by plating on BG, HE, and XLT4 recovered 80, 90, and 100%, respectively, of the microorganism from water samples. Enrichment in SC was only effective (80% recovery) with BG plating. Recovery of *Salmonella* from trough water using other medium combinations following preenrichment in LB ranged from 50 to 70% (Table 2). The poor recoveries using direct enrichment methods might have been due to either residual chlorine (unlikely), osmotic shock, or competitive microflora. *Salmonella* cells injured by chlorine or osmotic shock might have had time to recover in LB but died in direct enrichment because of the toxicity of selective ingredients in the media. Chlorine levels in trough water were generally below detectable limits when sampled. In the sample preparation, we did not filter the water samples as recommended by the standard method because the cloudy trough water was difficult to pass through a 0.45-μm Millipore filter. Instead, we used 25-ml water samples for 225 ml of enrichment broth. Trough water was heavily contaminated with grain and other debris left by animals.

Recoveries of *Salmonella* from air were significantly improved ($P < 0.0001$) by preenrichment in LB. Overall, the best selective plating medium was BS ($P < 0.05$). After preenrichment in LB, several medium combinations recovered the microorganism from air, with up to 80 to 100% recovery (Table 2). Without preenrichment, the highest recovery was 60%, and most of the medium combinations failed to detect the pathogen in the air samples. The best method to recover *Salmonella* from air was preenrichment in LB followed by enrichment in TT35 and isolation on BS (100% recovery). The second alternative medium combinations effective in recovering *Salmonella* (80% recovery) after preenrichment were enrichment in either SC or RV, followed by isolation on BS and enrichment in RV with isolation on HE (Table 2). Because the air samples were not spiked, these medium combinations were able to isolate the organisms naturally present in the air at the farm.

Preenrichment in LB did not affect ($P > 0.75$) recovery of *Salmonella* from milking parlor environments (floor, wall, window, and stall). The most effective methods for isolating *Salmonella* from the milking parlor were dependent on medium combinations. Overall, the best media were RV and TT42 for selective enrichment and BS and XLT4 for selective plating. The best medium combinations (90% recovery) for isolating *Salmonella* from milking parlor environmental samples were preenrichment in LB followed by enrichment in TT42 and isolation on XLT4 (LB-TT42-XLT4). Preenrichment in LB followed by enrichment in RV and streaking on BS (LB-RV-BS) gave 80% recovery and was as effective as direct enrichment in RV with isolation on BS (DE-RV-BS) (Table 2). Therefore, preenrichment in LB prior to enrichment in RV and isolation on BS is not recommended because it did not improve on 80% recovery. Direct enrichment in SC and TT35 followed by isolation on HE and BS, respectively, were able to recover the pathogen in 70% of assays (Table 2), which was not significantly different from that of the preenrichment method. Thus, direct enrichment techniques are preferred for milking parlor samples because they reduce assay time. The other medium combinations gave 60% or less recovery of *Salmonella*. *Salmonella* was naturally present in milking parlor samples because the swab samples were not inoculated.

Preenrichment methods gave higher percent recovery of *Salmonella* from flies than direct enrichment ($P < 0.01$). The most effective selective enrichment and plating media were RV or TT35 and BS or XLT4, respectively ($P < 0.05$). Recovery of *Salmonella* from flies, however, was dependent on medium combinations. Direct enrichment in RV followed by plating on BS (DE-RV-BS) gave the greatest recovery (80%) of the pathogen. Preenrichment in LB followed by enrichment in RV and plating on XLT4 or enrichment in TT35 with isolation on BG gave 70% recovery (Table 2). After preenrichment in LB, Olsen and Hammack *(16)* used parallel enrichment in TT incubated at 43° and RV followed by isolation on BS, HE, and XLD to isolate *Salmonella* from naturally contaminated household flies. They found that 4 (18%) of the 22 pool sample were positive for *Salmonella,* which was lower than the recovery in our study.

Poultry farm samples. Overall, preenrichment in LB gave lower recovery of *Salmonella* from chicken ration than direct enrichment methods ($P < 0.005$), whereas the four selective plating media had no significant effect on the recovery. BG plating, overall, had lower recovery than BS, HE, or XLT4. The most effective recovery methods (80 to 100% recovery) depended on medium combinations including preenrichment in LB. The most effective methods (100% recovery) were direct enrichment in SC followed by plating on BS, HE, or XLT4 or direct enrichment in TT35 with plating on HE or XLT4 (Table 3). Preenrichment in LB followed by enrichment in TT35 and plating on BS also gave 100% recovery. Direct enrichment in RV with plating in HE gave 90% recovery (Table 3) and was as effective as preenrichment in LB followed by enrichment in TT42 and plating on BS or XLT for recovery of *Salmonella* from chicken ration. Several direct- and preenrichment methods gave 80% recovery, including DE-RV-BS, DE-SC-BG, DE-TT35-BG (Table 3), LB-RV-HE, LB-SC-BS, and LB-TT42-HE. RV was comparable to or better than other selective enrichments for recovery of *Salmonella* from highly contaminated foods *(12)* and has been adopted by AOAC International. In our study, this method gave good recovery but was not as good as SC and TT35 for poultry farm samples.

Recovery of *Salmonella* from both chicken litter and droppings was generally enhanced with preenrichment in

TABLE 3. *Recovery (%)*[a] *of* Salmonella *from artificially contaminated poultry and swine farm samples (~1 to 2 CFU/g) using preenrichment in lactose broth or direct enrichment in selective media in conjunction with four selective plating media*

		Poultry (chicken)			Swine		
Enrichment[b]	Selective plating[c]	Ration (DE)	Litter (LB)	Droppings (LB)	Feed (DE)[d]	A-feed (LB)[d]	Feces (DE)
RV	BG	60 BC	20 DE	70 ABC	50 ABCD	50 BCDE	60 BCD
	BS	80 ABC	60 BC	100 A	50 ABCD	60 BCD	70 ABCD
	HE	90 AB	90 AB	100 A	50 ABCD	30 DEF	90 AB
	XLT4	70 ABC	80 ABC	90 AB	10 D	20 EF	100 A
SC	BG	80 ABC	10 E	90 AB	80 AB	50 BCDE	80 ABC
	BS	100 A	60 BC	100 A	90 A	0 F	60 BCD
	HE	100 A	80 ABC	90 AB	30 CD	50 BCDE	80 ABC
	XLT4	100 A	70 ABC	90 AB	60 ABC	70 ABC	80 ABC
TT35	BG	80 ABC	50 CD	50 C	90 A	60 BCD	80 ABC
	BS	60 BC	50 CD	100 A	40 BCD	40 CDE	40 D
	HE	100 A	100 A	40 C	60 ABC	100 A	80 ABC
	XLT4	100 A	90 AB	60 BC	50 ABCD	50 BCDE	90 AB
TT42	BG	50 C	60 BC	90 AB	50 ABCD	70 ABC	90 AB
	BS	70 ABC	20 DE	100 A	70 ABC	100 A	80 ABC
	HE	10 D	70 ABC	90 AB	60 ABC	80 AB	90 AB
	XLT4	70 ABC	100 A	90 AB	30 CD	80 AB	50 CD

[a] Percent positive *Salmonella* of 10 replications; values in a column followed by unlike letters are significantly different ($P < 0.05$). Data are presented only for the LB (lactose broth preenrichment) or DE (direct enrichment with no LB preenrichment) method on the basis of the best recovery method; exceptions are discussed in "Results."
[b] RV, Rapport-Vassiliadis; SC, selenite cystine; TT35 and TT42, tetrathionate incubated at 35°C and 42°C, respectively.
[c] BG, brilliant green; BS, bismuth sulfite; HE, Hektoen enteric; XLT4, xylose-lysine-tergitol 4 agar base.
[d] Feed and A-feed, swine finishing ration without and with antibiotic, respectively.

LB ($P < 0.01$). Nevertheless, some direct enrichment methods were also effective (80 to 100% recovery). Enrichment in TT35 with plating on HE or in TT42 with plating on XLT4 following preenrichment were the two most effective methods (100% recovery) for isolation of *Salmonella* from chicken litter (Table 3). Direct enrichment in RV with isolation on XLT4 or in TT35 with isolation on HE gave lower recovery (90%) but was not significantly different from preenrichment in LB followed by enrichment in TT35 with plating on HE or enrichment in TT42 with plating on XLT4 for recovery of *Salmonella* from litter ($P > 0.05$). Tate et al. *(18)* were able to recover 38.7% of *Salmonella* from naturally contaminated environmental broiler flock samples using direct enrichment in TT incubated at 41°C followed by isolation on XLD. They increased the recovery to about 46% by replacing XLD with XLT4. In addition to two medium combinations of preenrichment in LB (Table 3), direct enrichment in RV with plating on BS or in SC or TT35 with isolation on BG also enabled 80% recovery of *Salmonella* from chicken litter.

The most effective methods (100% recovery) for isolating *Salmonella* from chicken droppings included several medium combinations of preenrichment (Table 3) and one direct enrichment technique (enrichment in TT42 with plating on HE). Following preenrichment in LB, selective enrichment in RV, SC, TT35, or TT42 followed by isolation on BS gave 100% recovery of *Salmonella.* Most of the other medium combinations of preenrichment recovered 90% of *Salmonella* in the chicken dropping samples (Table 3). Direct enrichment in TT42 with plating on XLT4 gave 90% recovery. Direct enrichments in RV, SC, TT35, or TT42 followed by plating on BG and other plating media were the least effective medium combinations but still gave up to 80% recovery for isolation of *Salmonella* from chicken droppings.

Swine farm samples. Preenrichment in LB did not improve ($P > 0.05$) recovery of *Salmonella* from the swine feed ration without antibiotic but did increase ($P < 0.001$) recovery of *Salmonella* from the swine feed ration containing antibiotic. The latter is likely because of resuscitation of injured cells. Similar to samples of dairy and poultry farms, the most efficient recovery methods for *Salmonella* from swine farm samples were dependent on medium combinations. There were 6 medium combinations of preenrichment (LB-RV-BS, LB-TT35-BS/HE, and LB-TT42-BS/HE/XLT4) and 3 medium combinations of direct enrichment treatments (Table 3) effective for recovery of *Salmonella* from feed without antibiotic (80 to 90% recovery). In addition, one medium combination from preenrichment (LB-SC-BS) and one from direct enrichment (DE-TT42-BS) treatments could serve as alternative methods because their 70% recovery did not significantly differ ($P < 0.05$) from those of medium combinations with 90% recovery. Although preenrichment in LB increased recovery overall from feed with antibiotic, there were two medium combinations of direct enrichment with recovery of 100%, including enrichment in RV or in TT42 followed by isolation

TABLE 4. *Methods recommended for recovery of* Salmonella *from farm samples on the basis of sensitivity, reliability, cost, safety, and convenience*

Sample type	Preenrichment[a]	Selective enrichment[b]	Differential plating[c]
Animal hair	—	RV	BS
Grain, cattle	—	TT35	BS
Silage	LB	TT35	XLT4, BS
TMR	LB	TT35	BS
Bedding (wood shavings)	—	RV	HE, XLT4
Soil	—	TT42	BS, XLT4
Manure	—	TT42	XLT4, HE
Trough water	LB	TT42	BS, XLT4
Air	LB	TT35	BS
Surfaces (wall, fans, floor, equipment)	—	RV	BS
Flies	—	RV	BS
Chicken ration	—	TT35	HE, XLT4
Chicken litter	—	RV	HE, XLT4
Chicken droppings	—	TT42	HE, XLT4
Swine feed with antibiotic	—	RV	BS
Swine feed without antibiotic	—	RV	BS
Swine fecal	—	RV	XLT4, HE

[a] LB, preenrichment in lactose broth.
[b] RV, Rappaport-Vassiliadis; TT35 and TT42, tetrathionate incubated at 35°C and 42°C, respectively.
[c] BS, bismuth sulfite; HE, Hektoen enteric; XLT4, xylose-lysine-tergitol 4 agar base.

on BS. Direct enrichment in TT42 with plating on BG also gave 90% recovery. With the preenrichment method, two medium combinations had 100% recovery (LB-TT35-HE and LB-TT42-BS) and two combinations had 80% recovery (LB-TT42-HE/XLT4) (Table 3). Because recovery ranged from 0 to 100% depending on medium selection, choice of one of several combinations giving high recovery is important but easy to accomplish.

Preenrichment in LB decreased recovery of *Salmonella* from swine feces overall compared with direct enrichment methods ($P < 0.01$). However, four of the most effective methods (100% recovery) were from preenrichment methods (LB-RV-BS/XLT4 and LB-TT35/TT42-BS) and only one from a direct enrichment method (DE-RV-XLT4) (Table 3). Preenrichment in LB followed by enrichment in RV and plating on XLT4 was as effective as direct enrichment in RV followed by plating on XLT4. All other medium combinations with recovery of 80 to 90% were of the direct enrichment method, except for LB-SC-XLT4. Because direct enrichment saves 1 day and reduces medium requirements, it is recommended for swine feces.

CONCLUSIONS

The results of these experiments indicated that protocols for isolation and identification of *Salmonella* from animal and environmental farm samples could not be generalized. In addition to poor recovery, generalization of protocols for farm samples has several disadvantages. Preenrichment of samples that could be directly enriched in selective broth might delay the results and increase labor and culture medium costs. Convenience and incubation space are also important considerations for many laboratories. A final consideration is to avoid use of media with components such as selenite when choices are equally effective because of toxicity and disposal constraints. Therefore, it is essential that each type of farm sample has its own protocol in order to obtain consistent and reliable isolation of *Salmonella*; however, this can be done and still meet the above requirements. Table 4 shows a summary of several multiple specialized enrichment and plating protocols that we have selected for use in our laboratory as our standard operational procedure on the basis of reliable and consistent recovery, convenience, avoidance of toxic components such as selenium where possible, and minimization of labor and culture medium costs.

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