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Getahun E. Agga
U.S. Department of Agriculture, Agricultural Research Service, getahun.agga@ars.usda.gov

Terrance M. Arthur
terrance.arthur@ars.usda.gov, terrance.arthur@ars.usda.gov

John W. Schmidt
USDA-ARS, john.w.schmidt@ars.usda.gov

Rong Wang
U.S. Department of Agriculture, Agricultural Research Service, rong.wang@ars.usda.gov

Dayna M. Brichta-Harhay
USDA-ARS, dayna.harhay@ars.usda.gov

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Diagnostic Accuracy of Rectoanal Mucosal Swab of Feedlot Cattle for Detection and Enumeration of *Salmonella enterica*

GETAHUN E. AGGA, TERRANCE M. ARTHUR, JOHN W. SCHMIDT, RONG WANG, AND DAYNA M. BRICHTA-HARHAY*

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center P.O. Box 166, State Spur 18D, Clay Center, Nebraska 68933-0166, USA

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ABSTRACT

Cattle are noted carriers of the foodborne pathogen *Salmonella enterica*. The perceived need to decrease the potential human health risk posed by excretion of this pathogen has resulted in numerous studies examining the factors that influence *Salmonella* shedding in cattle. Fecal grab (FG) samples have been the predominant method used to identify cattle colonized or infected with *Salmonella*; however, FG sampling can be impractical in certain situations, and rectoanal mucosal swabs (RAMS) are a more convenient sample type to collect. Despite a lack of studies comparing FG and RAMS for the detection and enumeration of *Salmonella* fecal shedding, RAMS is perceived as less sensitive because a smaller amount of feces is cultured. In a cross-sectional study to address these concerns, paired RAMS and FG samples were collected from 403 adult feedlot cattle approximately 90 days prior to harvest. Samples were processed for *Salmonella* enumeration (direct plating) and detection (enrichment and immunomagnetic separation). In all, 89.6% of RAMS and 98.8% of FG samples were positive for *Salmonella,* and concordant prevalence outcomes were observed for 90.8% of samples. Mean enumeration values were 3.01 and 3.12 log CFU/ml for RAMS and FG, respectively. The sensitivity and specificity of RAMS were 91% (95% confidence interval [CI]: 87.5 to 93%) and 100% (95% CI: 48 to 100%), respectively, for *Salmonella* detection. Furthermore, RAMS *Salmonella* enumeration was substantially concordant ($\rho_c = 0.89$; 95% CI: 0.86 to 0.91) with FG values. We conclude that RAMS are a reliable alternative to FG for assessing cattle *Salmonella* fecal shedding status, especially for cattle shedding high levels of *Salmonella*.

Key words: Cattle; Concordance; Fecal grab; Rectoanal mucosal swab; *Salmonella*; Sensitivity

Salmonella enterica subsp. *enterica* (hereafter *Salmonella*) are important human enteric pathogens, and cattle are noted as reservoirs *(8, 17, 21, 27)*. Cattle feces are a substantial environmental source of *Salmonella* contamination, as well as a source of hide contamination, which may lead to contaminated carcasses at harvest, thus representing a potential risk to food safety *(2, 4, 13, 25)*. As such, practical methods for characterizing cattle *Salmonella* shedding status are important components of research efforts for understanding the cycle of *Salmonella* contamination in animal production settings and identifying critical control points to target to interrupt this cycle.

A considerable body of research exists examining cattle fecal shedding of *Salmonella*; however, the majority of studies conducted to date have involved the collection of fresh pen floor fecal pats to examine shedding at the cohort or herd levels *(17–19, 23, 33)*. Although cohort level sampling gives a measure of pathogen shedding at a group level, it does not allow for identification of members of a cohort that may be shedding at higher levels. Sampling at the individual animal level facilitates the identification of high shedders within a cohort, thus providing information needed for implementing possible mitigation strategies. Fecal grab (FG) sampling (i.e., manual collection of feces from the rectum) is a recognized method for assessing individual animal fecal shedding status *(16–18, 40)*. However, there are aspects of FG sampling that are not well suited for routine sample collection from large numbers of animals, including dwell time in squeeze chute required for sampling, failure to recover full sample amount when no feces are present in terminal rectum, shipping issues, and large laboratory space requirements for FG enrichment cultures.

As an alternative to FG sample methods, rectoanal mucosal swab (RAMS) sampling has been found to be a suitable method for examining cattle fecal shedding of pathogens. This sampling method previously has been shown effective for identifying cattle shedding *Escherichia coli* O157:H7 and, particularly, for cattle shedding high levels of this pathogen (designated super shedders) *(24, 36)*. Compared with FG, RAMS are easier to collect, transport, and process in the laboratory. In addition, it has been theorized that RAMS are more specific than FG samples for identifying organisms that colonize the rectal niche, such as *E. coli* O157:H7 *(24)*. A study conducted in human adult volunteers for the detection of *Salmonella* Typhimurium reported a moderate diagnostic utility of fecal swabs as

* Author for correspondence. Tel: 402-762-4343; Fax: 402-762-4149; E-mail: Dayna.Harhay@ars.usda.gov.

TABLE 1. *Prevalence and mean* Salmonella *concentration in fecal grab (FG) and rectoanal mucosal swab (RAMS) samples in feedlot cattle*

Sampling date	Lot	Lot size	Total sampled	% sampled	% RAMS positive	% FG positive	Mean (SD) RAMS (log CFU)	Mean (SD) FG (log CFU)
Oct. 2012			148	18.9	98.0	100	3.0 (1.4)	3.2 (1.5)
	A	230	51	22.2	98.0	100	2.6 (1.4)	2.6 (1.3)
	B	315	57	18.1	96.5	100	2.9 (1.4)	3.0 (1.5)
	C	239	40	16.7	100	100	3.6 (1.1)	4.0 (1.2)
Jan. 2013			105	26.4	87.6	100	1.9 (1.0)	2.0 (1.1)
	D	218	55	25.2	89.1	100	1.9 (1.0)	2.0 (1.1)
	E	179	50	27.9	86.0	100	1.9 (1.0)	2.0 (1.0)
May 2013			150	20.8	82.7	96.7	1.9 (1.1)	1.9 (1.1)
	F	93	34	36.6	76.5	94.1	1.6 (0.9)	1.6 (0.9)
	G	402	66	16.4	87.9	97.0	2.2 (1.2)	2.2 (1.1)
	H	225	50	25.2	80.0	98.0	1.7 (1.1)	1.7 (1.0)
Total/avg		237.6	403	23.5	89.6	98.8	2.3 (1.3)	2.4 (1.4)

compared with fecal samples *(26)*. RAMS sampling has been used previously to examine *Salmonella* shedding in calves and feedlot cattle *(5–7, 40)*; however, data evaluating the accuracy of RAMS in comparison with FG for identifying *Salmonella* shedders in cattle are lacking. Thus, the objective of this study was to evaluate the sensitivity and specificity of RAMS, in comparison with FG samples, for the detection of *Salmonella* shedding in individual feedlot cattle, as well as for identifying cattle shedding high levels of *Salmonella* within a cohort.

MATERIALS AND METHODS

Animals. Paired FG and RAMS samples were collected from 403 adult cattle from eight different lots at a commercial cattle feedlot operation (Table 1). Samples were collected from cattle in the U.S. southern high plains in October 2012 (fall), January 2013 (winter), and May 2013 (spring). Animals were sampled while they were restrained in a squeeze chute for the administration of a growth promoter implant, approximately 90 days preharvest.

RAMS and FG sample collection and *Salmonella* enumeration. RAMS samples were collected by using foam-tipped swabs (VWR International, Buffalo Grove, IL) and swabbing an area (3 by 5 cm) inside the anal canal of each animal. Swabs were immediately placed into a 15-ml conical tube containing 4 ml of tryptic soy broth (TSB)–PO_4 (TSB [Sigma, St. Louis, MO] 30 g, KH_2PO_4 2.13 g, K_2HPO_4 12.54 g/liter, final pH of 7.2), and gloves were changed in between each sample. RAMS samples were packed in coolers with ice packs and shipped to the laboratory for analysis within 24 h of collection. Upon arrival, swab samples were vortexed for 10 s, and debris was allowed to settle for approximately 5 min. Next, 0.5 ml was removed to a 2-ml cluster tube (Simport, Beloeil, Québec, Canada) for spiral plate analysis (50 μl per plate) on xylose lysine desoxycholate agar (Remel, Lenexa, MO) plus 4.6 ml/liter Tergitol, 15 mg/liter novobiocin, and 10 mg/liter cefesulodin (XLD_{tnc}) *(11, 37)*. Plates were incubated at 37°C for 24 h, and presumptive colonies characteristic of *Salmonella* were enumerated. Conical tubes with remaining sample were incubated for 8 h at 42°C and then held at 4°C until the next day for further processing *(37)*.

For each animal sampled, a FG sample was collected after RAMS sample collection by using a veterinary examination glove. FG samples were shipped to the laboratory for analysis in coolers with ice packs and were processed within 24 h of collection. For each sample, 10 g of feces was weighed and placed into filter bag with 90 ml of TSB-PO_4. Samples were then homogenized by hand, and 1 ml was removed to a 2-ml cluster tube for spiral plate analysis on XLD_{tnc}. Plates were incubated and presumptive colonies were enumerated, as described previously. The remaining sample was incubated for 2 h at 25°C, for 6 h at 42°C, and then held at 4°C until further processing, as described in the following *(11)*.

Salmonella concentration was calculated for each sample, log transformed, and reported as log CFU per milliliter for both FG and RAMS samples. Log CFU per milliliter was chosen as the unit of measurement as opposed to the more typical log CFU per gram for FG or log CFU per swab for the RAMS because of factors that prohibited accurate determination of the weight of feces collected. Predominant among these was the variation in swab weight as a result of the sample collection process (i.e., differing lengths of the swab handle, the top of which is broken off postcollection, once the swab is in the tube). As both sample types are resuspended in liquid growth media, use of the CFU per milliliter unit of measurement for each sample type facillitatated comparisons between them. For samples in which *Salmonella* was present after enrichment, but was below the limit of enumeration on direct plating assay (i.e., those that were prevalence positive but enumeration negative), half the value of the lowest observed nonzero count (i.e., 20 CFU/ml) was added to overcome zero counts before transforming the data to log CFU per milliliter for analysis, as described elsewhere *(1)*. The frequency distribution of all log-transformed enumeration values, as determined by FG or RAMS, was plotted and formed the basis for defining the shedding level of *Salmonella* high shedders.

***Salmonella* enrichment and confirmation.** All enrichments were subjected to immunomagnetic separation using anti-*Salmonella* beads (Dynabeads, Invitrogen, Carlsbad, CA). Recovered beads were transferred to 3 ml of Rappaport-Vassiliadis (Remel) broth, incubated at 42°C for 18 to 20 h, and then streaked onto XLD_{tnc} as previously described *(9)*. Plates were incubated at 37°C for 18 to 20 h, and one to three presumptive *Salmonella* isolates were selected per positive sample, both from prevalence and enumeration plates, for confirmation using a PCR assay to detect the *Salmonella* specific portion of the *invA* gene, as previously described *(34, 35)*.

Statistical analysis. *Salmonella* prevalence and percentage of high shedders (defined here as animals with fecal samples yielding

≥2.7 log CFU/ml), as determined by using RAMS and FG samples, were compared with McNemar's chi-square test, and FG was determined a priori the reference sample collection method. A statistically significant McNemar's chi-square test indicates an association between sample type and outcome (i.e., detection of *Salmonella* presence or detection of a *Salmonella* high shedder). Agreement between RAMS and FG samples for the detection of *Salmonella*-positive cattle and identification of high shedders was assessed by kappa or prevalence-adjusted bias-adjusted kappa (PABAK) *(14)* as appropriate. PABAK was calculated as (2 × observed agreement) − 1. As the prevalence of false-positive and true-negative sample outcomes was rare, PABAK, which corrects for biased or unstable kappa values due to high (>80%) or low (<20%) prevalence, was used for the final inference *(14)*. Interpretative criteria proposed by Landis and Koch *(28)* were used to interpret kappa and PABAK values as follows: <0 (poor); 0 to 0.2 (slight); 0.21 to 0.4 (fair); 0.41 to 0.6 (moderate); 0.61 to 0.8 (substantial); and 0.81 to 1.0 (almost perfect) agreement. To further evaluate diagnostic accuracy of RAMS for the detection of *Salmonella*-positive cattle and identification of high shedders, sensitivity, specificity, positive and negative predictive values, and area under the receiver operating characteristics (AU-ROC) were calculated using *diagt,* a user-written command in Stata *(38)*. The paired *t* test was used to compare mean log CFU per milliliter obtained from samples that were enumerable by both FG and RAMS ($n = 229$), and the concordance correlation coefficient *(31, 32)* and Bland-Altman's limits of agreement were used to evaluate the agreement between RAMS and FG sampling for the enumeration of *Salmonella*. All analyses were carried out using Stata, Version 13 *(39)* or Prism version 6.0f (GraphPad Software, Inc., www.graphpad.com) and Standards for Reporting of Diagnostic Accuracy Studies checklists were followed *(10)*. Statistical differences with $P < 0.05$ were considered significant for inference.

RESULTS

Descriptive statistics. The percentages of *Salmonella*-positive cattle and mean *Salmonella* log CFU per milliliter are shown in Table 1. All *Salmonella*-negative samples as determined by FG (1.2%) were from cattle sampled in May. *Salmonella* prevalence as determined by RAMS was higher in lots of cattle sampled in October (98%) than in those sampled in January (87.6%) or in May (82.7%). These observed differences reflect the higher mean *Salmonella* shedding levels observed at that sample point (3.2 log CFU/ml versus 2.0 or 1.9 mean log CFU/ml). The frequency distribution of all log-transformed enumeration values determined by FG or RAMS sampling is depicted in Figure 1. Based on the distribution, *Salmonella* high shedders were defined as those with fecal samples yielding ≥2.7 log CFU/ml. This is equivalent to shedding at $\geq 5.0 \times 10^3$ CFU/g or $\geq 2.0 \times 10^3$ CFU per swab, as determined by FG or RAMS sample, respectively.

Comparison of RAMS to FG for the detection of *Salmonella* in feedlot cattle. In all, 89.6% of RAMS and 98.8% of FG samples were positive for *Salmonella,* with 90.8% of samples with concordant outcomes (361 positive for *Salmonella* and 5 negative). Despite this high level of agreement, *Salmonella* prevalence as determined by FG was found to be significantly higher than that by RAMS by

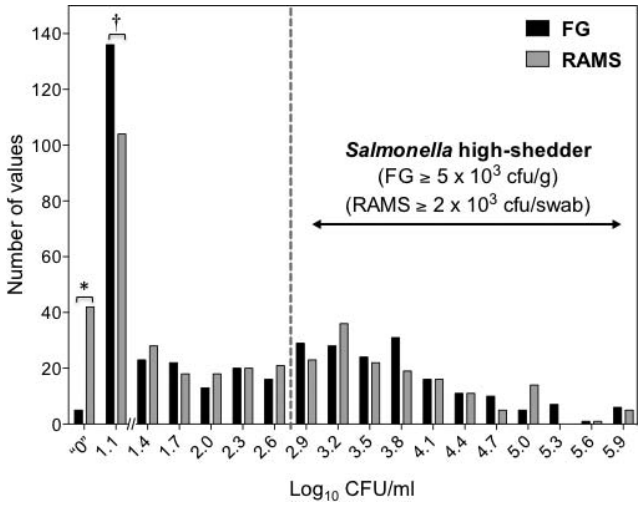

FIGURE 1. *Distribution of rectoanal mucosal swab (RAMS) and fecal grab (FG)* Salmonella *counts among sampled feedlot cattle. Bars represent the number of samples positive for* Salmonella *in the indicated log CFU per milliliter range. The break in the* x *axis denotes samples that were found positive for* Salmonella*, but below the limit of enumeration (†), and samples that were found negative for* Salmonella *(*). The dashed line demarcates the threshold value for samples indicative of potential* Salmonella *high shedders.*

McNemar's test ($\chi^2 = 37$; $P < 0.0001$). This indicates a significant association between sample type (FG or RAMS) and outcome (i.e., classifying cattle as positive for *Salmonella* shedding). The agreement of RAMS and FG samples was further assessed by kappa and was found to be low (20%), despite high, observed agreement (91%) between the sample types. This paradoxical result occurs because kappa is highly dependent on the prevalence of a condition in a population, and when used to evaluate sample sets with rare findings (in this case, the low occurrence of *Salmonella*-negative samples), the reliability of kappa breaks down. To address this, PABAK, which adjusts both for the bias and for high-low prevalence, was developed *(14)* and is a better measure of agreement. Using this test statistic, the agreement of RAMS and FG samples for the detection of cattle shedding *Salmonella* showed almost perfect agreement (PABAK value of 0.82). The results of the diagnostic accuracy parameters of RAMS, with respect to FG, are presented in Table 2. RAMS missed 9.2% (false-negative rate) of *Salmonella* shedders among FG-positive animals; however, it classified all FG-negative animals as negative (i.e., 0% false-positive rate). Overall diagnostic performance of RAMS for the detection of *Salmonella* was given by an AU-ROC value of 0.95 (95% confidence interval [CI]: 0.94 to 0.97), as shown in Figure 2.

Comparison of RAMS to FG for *Salmonella* enumeration in feedlot cattle. Direct plating enumeration of all paired samples showed that 71.9% of samples ($n = 403$) contained *Salmonella* at concentrations above the limit of detection (1.30 log or 20 CFU/ml). Of these, 79% were enumerated by both methods (229 of 290 paired samples with enumeration data), while 11.4 and 9.6% were enumerated either only by RAMS or FG samples,

TABLE 2. *Diagnostic characteristics of rectoanal mucosal swab (RAMS) with reference to fecal grab (FG) for the detection of* Salmonella *in feedlot cattle*[a]

	RAMS		
FG	Positive	Negative	Total
Positive	361	37	398
Negative	0	5	5
Total	361	42	403
Parameters	Estimate	95% CI[b]	
Observed agreement	90.8%		
Kappa	0.2	0.05–0.3	
PABAK[c]	0.82		
Prevalence (FG)	99%	97.0–99.6	
Prevalence (RAMS)	89.6%	86.2–92.4	
Sensitivity	90.7%	87.4–93.4	
Specificity	100%	47.8–100	
Positive predictive value	100%	99.0–100	
Negative predictive value	11.9%	4.0–25.6	
AU-ROC curve[d]	0.95	0.94–0.97	

[a] Positive, positive for *Salmonella* contamination; Negative, negative for *Salmonella* contamination.
[b] CI, confidence interval.
[c] PABAK, prevalence-adjusted bias-adjusted kappa.
[d] AU-ROC, area under the receiver operating characteristics curve.

respectively. Mean observed enumeration values were 3.04 log CFU/ml ($n = 257$, range 1.47 to 5.98) for RAMS and 3.15 log CFU/ml ($n = 262$, range 1.47 to 5.84) for FG. Comparison of mean log CFU per milliliter for samples enumerated by both methods showed that values for RAMS and FG were not significantly different (3.19 and 3.34 log CFU/ml, respectively; $P = 0.1276$), Substantial concordance ($\rho_c = 0.89$; 95% CI: 0.86 to 0.91) was observed between RAMS and FG. However, this value did not approach 1

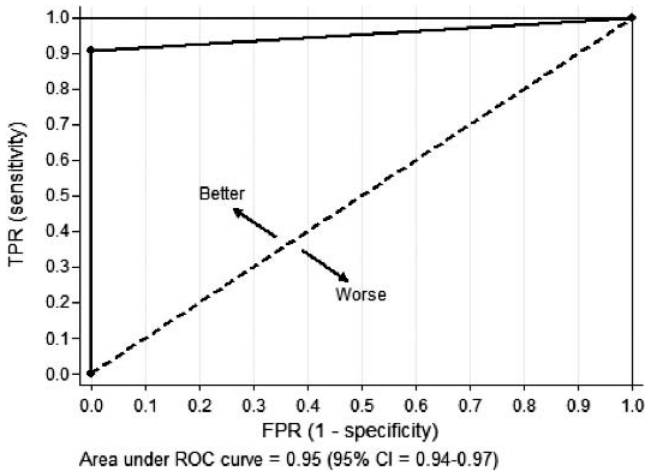

FIGURE 2. *Area under the receiver operating characteristics (AU-ROC) curve, plotted as false-positive rate (FPR) versus true-positive rate (TPR), for the diagnostic evaluation of rectoanal mucosal swab compared with fecal grab for the detection of* Salmonella *from feedlot cattle. The dashed line indicates chance line.*

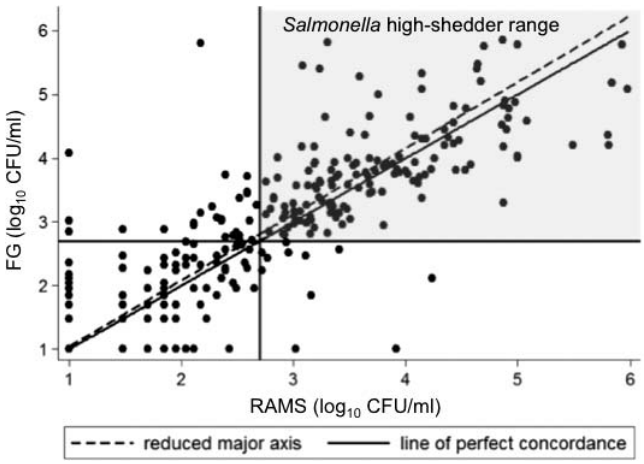

FIGURE 3. *Concordance plot of the log CFU per milliliter counts of* Salmonella *from feedlot cattle comparing fecal swab to fecal grab sampling. The horizontal and vertical lines and shaded area indicate samples categorized as high shedders (≥2.7 log CFU/ml). The slope of the reduced major axis is 1.038.*

owing to lack of perfect Pearson's correlation ($\rho = 0.89$) and from bias, i.e., when the proportion of animals classified as positive by FG was significantly higher than by RAMS as shown by the McNemar's χ^2 test previously mentioned *(14)* (C_b = 0.997). The concordance plot showing the relationship between RAMS and FG for *Salmonella* enumeration is shown in Figure 3. Overall, enumeration data from RAMS samples coincided with 84% of FG samples that identified cattle as high shedders ($n = 170$, FG samples $\geq$ 2.7 log CFU/ml). Further comparison of the performance of RAMS with FG for identifying high

TABLE 3. *Diagnostic characteristics of rectoanal mucosal swab (RAMS) with reference to fecal grab (FG) for the detection of* Salmonella *high shedders in feedlot cattle*[a]

	RAMS		
FG	HS	NHS	Total
HS	143	27	170
NHS	9	83	92
Total	152	110	262
Parameters	Estimate	95% CI[b]	
Observed agreement	86.3%		
Kappa	0.71	0.63–0.80	
PABAK[c]	0.73		
Prevalence (FG)	65.0%	59–70.7%	
Prevalence (RAMS)	58.0%	52.0–64.0%	
Sensitivity	84.1%	77.7–89.3%	
Specificity	90.2%	82.2–95.4%	
Predictive value positive	94.1%	89.1–97.3%	
Predictive value negative	75.5%	66.3–83.2%	
AU-ROC curve[d]	0.87	0.83–0.91	

[a] HS, high shedder; NHS, non–high shedder.
[b] CI, confidence interval.
[c] PABAK, prevalence-adjusted bias-adjusted kappa.
[d] AU-ROC, area under the receiver operating characteristics curve.

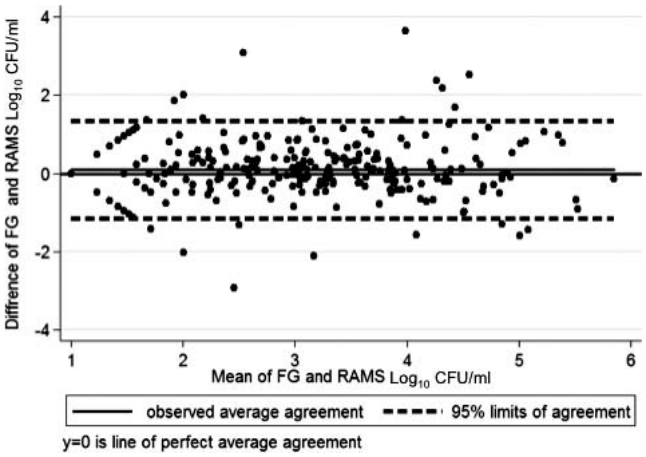

FIGURE 4. *Bland-Altman plot for the limits of agreement between fecal grab and fecal swab sampling methods for the enumeration of* Salmonella *from feedlot cattle*.

shedders is shown in Table 3 and Figure 3. The McNemar's chi-square test was not significant ($P = 0.59$), indicating the absence of significant association between sample type in classifying cattle as high shedders. In addition, the prevalence of high shedders was not very high (>80%) or very low (<20%), and as a result, the kappa value was not affected. In fact, both kappa and PABAK analyses resulted in similar values (0.71 and 0.73, respectively), indicating substantial agreement between RAMS and FG for the detection of high shedders (Table 3). Furthermore, RAMS showed moderate sensitivity (84.1%) and high specificity (90.2%) for identifying high shedders. Among the 42% of cattle sampled and found to be shedding high levels of *Salmonella,* the mean log CFU per milliliter was found to be the same for FG and RAMS samples at 3.8 (95% CI = 3.7 to 3.9). In keeping with this result, Bland-Altman's analysis of the discrepancy between the two methods revealed the bias to be close to zero (0.1423), further indication that both methods produce similar results (Fig. 4).

DISCUSSION

Salmonella carriage and shedding by asymptomatic cattle are major sources of contamination in feedlot settings, especially when the ability of *Salmonella* to survive for more than 190 days in the environment is considered *(3, 16, 41)*. The cycle of *Salmonella* contamination (from feces to hides and pen surfaces, to feed and water, then back to the bovine gastrointestinal tract) may contribute to the contamination of beef products via hide to carcass transfer of *Salmonella* in the dressing process at slaughter *(2, 12)*. The cycle of *Salmonella* contamination also may contribute to the presence of *Salmonella* in bovine peripheral lymph nodes, which can be incorporated in ground beef as a component of fat trim *(2, 20, 22)*. It has been suggested that early identification of high shedders so that they can be sequestered and possibly treated represents a viable intervention strategy to reduce shedding of pathogens into the environment and further dissemination to other animals *(15)*. Alternately, identification of cattle cohorts with an above-average number of high shedders prior to harvest could indicate the need for pen treatment and harvest strategies (yet to be defined) aimed at mitigating the risk of *Salmonella* entering the beef food chain. However, implementing specific control measures targeting high shedders requires a suitable sampling method that can be easily and affordably applied to test large numbers of animals *(29, 30)*.

Here, we evaluated the efficacy of RAMS as a facile sample collection method for the detection and enumeration of *Salmonella* fecal shedding in cattle. The data presented show that the overall diagnostic accuracy of RAMS in comparison with FG samples for detection of *Salmonella* shedding was high (ROC value of 95%; Fig. 2), with observed agreement of 90.8% and a calculated PABAK statistic of 0.82 (interpreted as almost perfect agreement; Table 2). The sensitivity and specificity of RAMS with respect to FG were found to be 90.7 and 100% respectively, with a positive predictive value of 100%, and a negative predictive value of 12%, although these high positive predictive value and low negative predictive value are the result of the overall high prevalence of *Salmonella* shedding detected in the populations tested here (99% as determined by FG samples). Despite the positive indicators of diagnostic accuracy, *Salmonella* prevalence as determined by FG was found to be significantly higher than that by RAMS as evaluated using McNemar's test ($\chi^2 = 37$; $P < 0.0001$), indicating a significant association between sample type and outcome (i.e., classifying cattle as positive for shedding *Salmonella*). Note that the majority of samples found positive by FG but not RAMS (89.2%; $n = 37$) were below the limit of detection of the direct plating enumeration methods used and that the estimated mean log CFU per milliliter for these samples was 1.07. As such, the observed significant difference in outcome is not surprising given that heterogeneous distribution of *Salmonella* in feces is likely more pronounced in cattle shedding lower levels, and in these instances, sampling a greater volume of feces increases the chance of detection.

Examination of the frequency distribution of the log-transformed enumeration values determined by both sample methods revealed a bimodal distribution (Fig. 1). Based on this distribution, we defined fecal samples yielding ≥2.7 log CFU/ml (equivalent to shedding at $\geq 5.0 \times 10^3$ CFU/g or $\geq 2.0 \times 10^3$ CFU per swab) as representative of cattle shedding high levels of *Salmonella* (designated high shedders). Comparison of direct plating of RAMS and FG samples for the identification of high shedders showed moderate concordance with an observed agreement of 86.3% (Table 3), in keeping with the concordance plot illustrating the relationship between RAMS and FG enumeration data (Fig. 3). Further examination of this subset of data showed that sensitivity and specificity of RAMS for identifying high shedders was 84.1 and 90.2%, respectively, with a mean log CFU per milliliter of 3.8 (95% CI = 3.7 to 3.9) for both sample types. The observed decrease in sensitivity and specificity of RAMS for identifying high shedders, in comparison with those values calculated for *Salmonella* detection, are, in part, a reflection of the unusually high *Salmonella* prevalence in the cattle populations tested, as stated previously, but are also a

consequence of the reality that RAMS and FG samples will rarely yield the same value (CFU per milliliter), because of sample-to-sample variation. Despite this variation, most samples identified by RAMS and FG to be in the high-shedder category were observed to be within the same log range (Fig. 3). Further evaluation using McNemar's chi-square test revealed no significant difference ($P = 0.59$), indicating the absence of an association between sample type and outcome. Also, as the prevalence of high shedders was neither high nor low, both kappa and PABAK analyses resulted in similar values (0.71 and 0.73, respectively), indicating substantial agreement between RAMS and FG for the identification of high shedders (Table 3). Finally, Bland-Altman's analysis of the discrepancy between the two sample methods revealed the bias to be close to zero (0.1423), further indication that both methods produce similar results, especially when *Salmonella* concentrations were in the range of log 2 to 4 CFU/ml (Fig. 4).

Although it is important to emphasize that the data collected in this study reflect shedding at a single time point and that no attempt to address questions of duration of high-shedding status were made, the results presented do suggest that RAMS fecal samples (evaluated using the methods described) may be used to reliably detect *Salmonella* shedding at levels greater than 1.3 log CFU/ml (equivalent to 1.9 log CFU per swab), and thus represent a practical alternative to FG sampling for assessing cattle *Salmonella* fecal shedding status. Accordingly, use of RAMS will aid research efforts geared at understanding the cycle of *Salmonella* contamination in animal production settings and identifying critical control points that will interrupt this cycle.

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