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Effects of Sampling Procedures on *Salmonella* Recovery from Fresh Water Catfish

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ABSTRACT

To determine the effectiveness of different sampling procedures, 200 frozen catfish known to be contaminated with *Salmonella* were divided into 350 samples. Variables in sampling included anterior and posterior portions of fish, blending, immersion, swabbing, rinsing and incubation at elevated temperatures. The composite of blended anterior and posterior samples incubated at 43 C and immersion of whole fish incubated at 35 C showed the highest number of positive samples, 50% and 42%, respectively. The contact method of swabbing (14%) and rinsing (14%) were the least effective of the methods examined. The anterior (visceral cavity area) portions of the fish seemed to be more highly contaminated (38% positive) than the posterior portion (26% positive). These data show that the sampling procedure can greatly affect recovery of *Salmonella* from fresh water catfish. Overall levels of *Salmonella* were low and the hazards of cross-contamination with other foods seem remote.

Salmonella has been reported in fresh water or marine species of fish (*1,3,10,11,13,14*) and is generally associated with fecal contamination of water. Testing for *Salmonella* is usually initiated by either swabbing (*4,12,16*), rinsing (*7,8,21*) or the blending (*2,22*) of a sample in pre-enrichment or selective enrichment broths. A recent study of *Salmonella* detection on dressed frog legs by blending, immersion of whole legs, maceration with a Stomacher, and by rinsing showed no significant difference in recovery for the first three methods, whereas rinsing was significantly inferior (*2*). The present study compares the recovery of *Salmonella* from fresh water catfish as a function of sampling and sample incubation.

MATERIALS AND METHODS

In this study, 350 samples from 200 frozen catfish known to be contaminated with *Salmonella* were tested for the presence of *Salmonella* by various sampling procedures. Method I consisted of dividing a fish in half by severing it just behind the anal vent. The tail portion (posterior-IP) and the visceral portion (anterior-IA) were then weighed and blended separately in nine parts of lactose broth (LB). One-half of each homogenate was incubated at 35 C for 24 h and the remaining halves were combined and reblended for homogeneity. This composite sample was then halved and one-half incubated at 35 C for 24 h (composite-IC). Method IC served as the control method for this study. The other half of the composite homogenate was incubated at 43 C for 24 h (composite-IC43). Method II consisted of complete immersion and incubation of the entire fish in 1 part LB (w/v). Method III included shaking the entire fish in 1 part LB (w/v) for 1 min and removing it before incubation. Method IV consisted of swabbing the entire surface area of the fish using two dacron swabs moistened with LB and placing them in 20 ml of LB. The LB pre-enrichment broths for Methods II, III and IV were incubated at 35 C for 24 h. Ten samples were analyzed daily by each method. Pre-enrichment was followed by the selective enrichment of 1-ml portions of LB in 10 ml of tetrathionate (TET) and 10 ml of selenite-cystine broth (SEL). Brilliant green agar with sulfadiazine, bismuth sulfite agar and *Salmonella-Shigella* agar with 1% sucrose and 0.65% agar added, as recommended by Sperber and Deibel (*19*), were streaked from TET and SEL. Plates were incubated for 24 h at 35 C. Suspect *Salmonella* colonies as described in the *Bacteriological Analytical Manual for Foods* (BAM) (*22*) were picked from each plate to triple sugar iron agar slants and motility-indole-lysine (MIL) deeps (*23*). MIL is a modification of Ederer and Clark's (*9*) motility-indole-ornithine medium (MIO) with lysine substituted for ornithine. MIL was prepared by adding 1% trypticase and 0.2% agar to Falkow lysine broth (*23*). This medium allowed for biochemical differentiation of *Salmonella* from indole-positive *Edwardsiella*. Incubations were at 35 C for 24 h at each step of the isolation procedure. Suspect *Salmonella* isolates were further tested for methyl red, Voges-Proskauer reactions, utilization of citrate on Simmon's citrate agar and for acid production in 0.5% mannitol in purple broth base. Serological confirmation of *Salmonella* was performed with poly-O antisera using the slide agglutination method. All media used in this study were BBL with the exception of SS agar from Difco.

RESULTS AND DISCUSSION

The percentage of *Salmonella*-positive samples with different methods of sampling is given in Table 1. An analysis of variance was done to determine if there was a difference in the recovery rate of the methods. The F value obtained indicated a significant difference between the sampling methods. Duncan's new multiple range test was done to determine which methods were significantly different. The methods with different superscripts were significantly different. Method IC43 had the highest recovery rate, but was not significantly different from Method II. The effectiveness of the increased incubation temperature recommended by several workers (*5,6,15,17, 18,20*) is also applicable for *Salmonella* detection in catfish. No significant difference existed between Methods II, IA and IC (control). The control method was based on the procedure in the BAM manual (*22*) which calls for a 25-g portion from an unspecified area of the fish to be blended in nine parts of LB with incubation at 35 C. The present study indicates that a significant difference exists between the presence of *Salmonella* in

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TABLE 1. *Recovery of* Salmonella *from fresh water catfish sampled by body portion, homogenization, immersion, rinse, swab, and incubation at 43 C (50 samples each).*

Type of sample[1]			Positive (No.)	Positive (%)	Negative (No.)
IA[a]	-	Anterior portion (LB-homogenate)	19	(38)	31
IP[b]	-	Posterior portion (LB-homogenate)	13	(26)	37
IC[a]	-	Composite sample (IA & IP - LB-homogenate)	19	(38)	31
IC43[d]	-	Composite at 43 C (IA & IP - LB-homogenate)	25	(50)	25
II[d]	-	Whole fish (LB-immersed & incubated)	21	(42)	29
III[c]	-	Rinse (LB-rinse)	7	(14)	43
IV[c]	-	Swab (LB)	7	(14)	43

[1]a,b,c,d Sampling methods with different superscripts are significantly different.

the visceral cavity and the tail area of catfish. This could influence the *Salmonella* recovery rate if indiscriminate selections are made of portions of the fish. A higher incidence of *Salmonella* in the visceral cavity can be expected because of increased chances of contamination from the viscera and from increased handling during processing. The presence of *Salmonella* on the tail area would most likely result from cross-contamination during processing (*23*).

Maximum recovery of *Salmonella* from catfish, according to the present study, would be either by immersion of the whole fish or the visceral cavity portion of large fish in LB followed by incubation at 43 C. The statistical analysis indicates that recovery of *Salmonella* by incubation at 43 C was not significantly different than that obtained by Method II, although a higher recovery rate was noted at 43 C. Method II probably is more practical because of the simplicity in sample preparation and the minimal equipment needed for incubation. The sample can be collected and pre-enriched in the same plastic pouch and incubated in a dry-heat incubator, whereas a water bath is required for incubation at 43 C.

Results from a study by the U.S. Food and Drug Administration on the effect of sample methodology on *Salmonella* isolation from frog legs were in general agreement with those of this study (*2*). The methods the agency compared were immersion of whole frog legs, blending, use of the Stomacher and rinsing. The agency found no significant difference between the first three methods, but recovery was significantly lower with rinsing. The agency did not use 43 C as an incubation temperature.

With methods IA, IP, IC and IC43, *Salmonella* were detected in 41 (82%) of the 50 fish samples. Theoretically, with a *Salmonella*-positive IA or IP sample, one would expect the corresponding IC and IC43 samples to be positive. However, this only occurred with 11 of 19 IA-positive samples and with 9 of 13 IP-positive samples. This indicates that 20 (62.5%) of the 32 positive-IA and -IP samples had sufficient numbers of *Salmonella* to be recovered in the corresponding composite sample. Of the 25 positive-IC43 samples only 13 were positive as IC samples. Although the IC43 method had the highest recovery (25 positives), it missed 16 (33.3%) of the 41 positive fish. This indicates that although a large percentage (82%) of the fish were *Salmonella*-positive, the number of *Salmonella* present on each fish was at a relatively low level and unevenly distributed. In addition, the poor recovery (14%) obtained with contact methods (rinse and swab) indicates that very few *Salmonella* are dislodged from the surface area of the fish.

Salmonella in raw foods such as catfish, red meats, poultry and frog legs is of concern because of its cross-contamination potential to foods consumed raw and/or its survival in foods that receive marginal heat treatment. The poor recovery by contact methods (III and IV) coupled with low numbers indicated by Methods IA and IP (when compared to IC and IC43) negate catfish as a potential *Salmonella* hazard. The FDA had concluded this, based primarily on its 1977 study (*1*). In its opinion however, this was caused by the procedure used to process catfish. The procedure described was developed by this laboratory (*23*) and to our knowledge was in operation in only one plant. The procedure was part of a complete quality assurance program submitted to the FDA for approval to resume processing after being enjoined. This procedure was proven to be effective in producing *Salmonella*-free carcasses. Additional research (*23*) has shown that the presence of *Salmonella* in live fish is directly related to the level of *Salmonella* in the water. *Salmonella* was found to be continuously present in low numbers, but under certain conditions of increasing temperature, large stocking rate and increased fish body weight, the number of *Salmonella* in the water rapidly increased. The presence of *Salmonella* on the skin and in the viscera of live fish resulted in *Salmonella*-positive carcasses when normal processing procedures were used to dress catfish. Results of the present study indicate that the method of sampling can have a great effect on detection of *Salmonella* in catfish. Although *Salmonella* were present in a large percentage of the samples in this particular lot, methods are available (*23*) to reduce or eliminate *Salmonella* from dressed catfish.

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