Salmonella Viability in Frozen Bovine Feces

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Introduction

Salmonellosis is a common problem in food animals and may present a serious zoonotic health hazard to people. Researchers and clinicians studying enteric disease often evaluate fecal samples. To detect the presence of salmonellae, large-scale screening of animals and their environment is often necessary. Large sample numbers and/or delayed testing of samples can produce critical sample problems. Cryoprotectants, such as glycerine, have been shown to reduce freezing damage in purified microbial samples. Since their protective capabilities in tissue and fecal samples are questioned due to a lack of penetration and microbial contact, the clinician is left with the uncertainty of how many organisms will be lost in the freezing and storage process and what diagnostic value frozen samples retain. We present results of the effect of freezing bovine fecal samples, without cryoprotectants, containing a known amount of *Salmonella typhimurium,* at -20°C and -70°C on the ability to recover the original salmonella inoculum during a one-month monitoring period.

Materials and Methods

Fresh bovine feces were obtained from a feedlot at the U.S. Meat Animal Research Center, Clay Center, Nebraska. The feces were assayed and found free of salmonellae before it was inoculated with the test organism. *Salmonella typhimurium* was expanded in trypticase soy broth and feces were inoculated with 1,000,000 colony forming units (cfu's) per gram of feces. There was a total of 55, 10-gram samples. Twenty-five of the samples were frozen at -20°C and 25 of the samples were frozen at -70°C. Five samples (controls) were evaluated for the test inoculum immediately post inoculation. The frozen samples were tested in replicates of 5 for each temperature at 1, 2, 7, 14, and 28 days post inoculation.

Frozen samples were thawed, mixed with 90 ml of Butterfield's buffer, and placed in a stomacher (Tekmar, Cincinnati, OH) for 1 minute. One ml samples were removed from the stomached samples and serial dilutions were made in Butterfield's buffer. One hundred µl of the appropriate dilutions were inoculated onto EF-18 plates (QA Laboratories Ltd., Toronto, Canada) and incubated at 43°C for 20 hours before counting salmonella colonies. Controls were evaluated the same as frozen samples.

Data were evaluated on SAS-PC (SAS Institute, Inc., 1987, Cary, North Carolina) by analysis of variance using Dunnett's T- and Scheffe's mean- comparison tests. The Dunnett's T-test was used to evaluate significance of difference between the control salmonella count means and all stored sample salmonella count means. Scheffe's mean-comparison test was used to evaluate differences in salmonella count means at each time/temperature comparison.

Results

There was approximately 85% loss in salmonella viability after 24 hours of storage at both temperatures, $P<.0001$. There was no difference in the viability means at 24 hours for the 2 storage temperatures, $P>.05$. For all sample-storage times after 24 hours, viability in samples stored at -20°C was significantly less than samples stored at -70°C, $P<.01$. After the drop in viability at 24 hours, there was no significant change in prolonged storage of samples at -70°C, $P>.05$. At -20°C, prolonged sample storage caused a continual drop in viable salmonellae, $P<.0001$ (Fig. 1).

Conclusions

The data show that after 24 hours, freezing caused a precipitous drop in the number of viable salmonellae. Holding samples at -20°C was associated with a continual decrease in number of viable organisms. This decrease in viability may limit the diagnostic potential of samples held for extended periods of time. Storage beyond 24 hours at -70°C appeared to have no effect on

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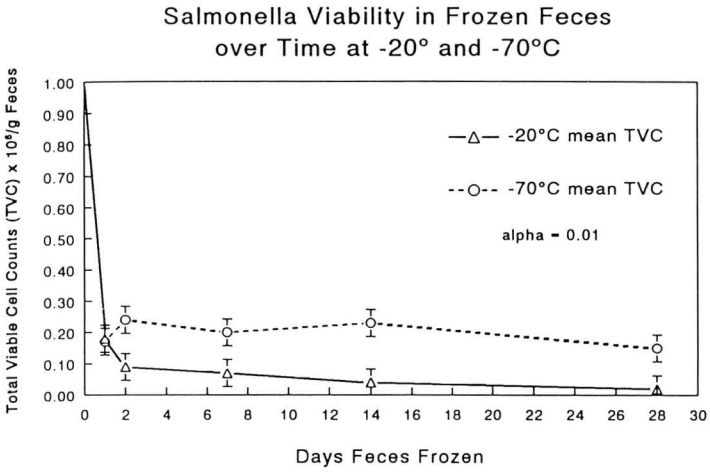
Figure 1. This graph demonstrates the effects of 2 different temperatures on salmonellae in bovine feces over a 28-day period. Viability counts taken after 1 day of storage were essentially identical for the 2 temperatures and appear superimposed on each other.



the viability of salmonellae that survived the initial freezing insult. These findings suggest that, if it is necessary to freeze and store fecal samples prior to evaluation, they should be frozen at -70°C. Even at this temperature, numerous salmonellae will be destroyed and the results obtained should be considered as presumptive only. If salmonellae are present in sufficient numbers, they can be detected even after freezing. However, enumeration of the cfu's per gram of feces would be misleading.

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