SURVIVAL OF *SALMONELLA MONTEVIDEO* ON WHEAT STORED AT CONSTANT RELATIVE HUMIDITIES

by

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Introduction

Salmonella have long been recognized as a cause of gastro-intestinal upsets in man and animals. Various sources of Salmonella infection have been described, and new sources of the organisms are being recognized each year. The occurrence of Salmonella in wheat and other cereal grains has been reported by Silverstople, Plazikowski, Kjellander and Vahlne (1961), the U.S. Department of Health, Education and Welfare (USDHEW)(1964), and Foltz (unpublished data).

Human infections resulted in Sweden when infants were fed a Salmonella contaminated barley powder (Silverstople, et al. 1961). Animal infections due to Salmonella-contaminated cottonseed meal were described by the USDHEW (1965). In both cases the contamination level was very low, and the number of cells contaminating the product could not be determined.

Occurrence of Salmonella on the surface of cereal grain is an established fact, but information on the survival of these bacteria on cereal grains is lacking. The purpose of this research was to show the survival patterns of Salmonella montevideo cells on wheat stored at constant relative humidities ranging from 7 to 98%.

Review of Literature

Studies on the Survival of Dried Bacteria

Drying as a means of preserving bacterial cultures has been used for many years. De Ome (1944) studied the survival of Salmonella pullorum in relation to the temperature and relative humidity in the atmosphere. He prepared an aerosol of S. pullorum and measured the death rate as a function of temperature and relative humidity. He found the death rate increased as the relative humidity increased from 15 to 80%. The death rate at a constant relative humidity also increased as the temperature increased from 28 to 37 C. Cells atomized from distilled water showed a higher death rate than those atomized from a nutrient broth solution. Association with dust particles in the atmosphere increased the survival rate of S. pullorum.

Stamp (1947) dried S. typhi, S. typhimurium, S. typhisuis, S. gallinarum and S. paratyphi C in broth and found that cells held for 2 to 4 years over P_2O_5 maintained their viability and pathogenicity. The number of cells surviving dry storage ranged from 1.3 to 50% of the original.

Dunklin and Puck (1947) used aerosols of pneumococci, Group C hemolytic streptococci, and staphylococci to demonstrate the range of relative humidities most lethal to these organisms. They found the humidities around 50% were the most lethal for the test organisms. They also noted that 0.5% NaCl added to the suspending medium caused an increased death rate.

Two hundred forty-six strains of Salmonella were examined by Rhodes (1950) for their resistance to drying. She placed one loop

of horse serum and one loop of culture in a small tube and sealed several small tubes in a larger tube which contained P_2O_5 crystals. The cultures were sampled periodically for 14 years. At the end of the 14-year period 96% of the tubes contained viable cells.

In their work with S. typhimurium Fry and Greaves (1951) found that one of the most important factors to consider prior to drying was the suspending medium. They found that nutrient broth and nutrient gelatin worked well. They also found that 4 to 6-hr cultures were more sensitive to drying than 18 to 24-hr cultures. The cells that were dried were not more resistant to subsequent drying than freshly cultured cells.

Preservation of Salmonella by drying over P_2O_5 was investigated by Proom and Hemmons (1949). One hundred sixty-five strains were examined over a 3-year period and a high percentage of these organisms survived for 3 years.

Hinton, Maltman and Orr (1960) dried Staphylococcus cells for three days on a glass surface trying to simulate environmental conditions. They found the virulance decreased after drying when the cells were injected into mice.

In a similar study Maltman, Orr and Hinton (1960) tried to duplicate the natural air drying conditions of a hospital room. Staphylococcus pyogenes cells were allowed to set exposed to all the temperature and humidity variations that occurred in the room. Some cells were killed but others received sublethal damage. They showed the injured cells had a longer lag phase and required a longer time to produce coagulase.

Scott (1958) studied the survival of S. newport at different

relative humidities after the cells were dried in a papain digest of beef heart. The cultures were placed in small tubes and sealed in larger tubes which contained a saturated solution for the maintenance of constant relative humidity. The tubes were held at 25 C for 28 weeks at relative humidities ranging from 0 to 53%. He made viable counts periodically and found the maximum death rate was in the cells stored at 0% relative humidity and the minimum death rate in the cells held at 22% relative humidity.

McDade and Hall (1963) observed the survival of Staphylococcus aureus at temperatures of 3 to 6 C, 20 C, 30 C and 37 C and at relative humidities of 10 to 15%, 53 to 59% and 95 to 98% at each temperature. At 37 and 30 C the cells at 95 to 98% relative humidity increased in number while the cells at 10 to 15% decreased slowly and the cells at 53 to 59% decreased much more rapidly. At 20 C they reported a slight amount of growth in the cells held at 95 to 98% relative humidity, a very slow decrease in the cells at 10 to 15% and a very rapid decrease in the number of cells held at 53 to 59%. At 4 to 6 C, after a slight reduction in the first few days, the cells remained at a constant level at all three relative humidities.

In later work McDade and Hall (1964) studied the survival of several gram negative organisms exposed to environmental conditions. Escherichia coli, Pseudomonas aeruginosa, Salmonella derby, Proteus morgani and Proteus vulgaris were exposed to a controlled environment on glass, ceramic tile, rubber tile, and polished stainless steel surfaces. They held the temperature at 25 C and placed each set of test organisms on the surfaces at 11 and 53% relative humidity. A known number of cells were placed on the surface of a series of

each material. After a 48-day sampling period they found survival was better at 11% than at 53% relative humidity. They observed few viable cells after 24-hrs.

The previous discussion considered only the survival of organisms in atmospheres of varying relative humidities. Water controls the growth of microorganisms as well as their survival. When considering the amount of water in a growth medium relative humidity is not used since this term usually refers to the water in the surrounding atmosphere. Water activity (a_w) of a solution or growth medium is used as a measure of the water present. Water activity is numerically equal to its corresponding relative humidity expressed as a fraction i.e. $\frac{R.H.}{100}$. A growth medium with an a_w 0.96 would be in water vapor equilibrium with a relative humidity of 96% at a constant temperature.

In a comprehensive review of food spoilage microorganisms Scott (1957) noted the lower limit of growth for most gram negative bacteria was an a_w of 0.95.

Studies on the water requirements of 16 strains of Salmonella carried out by Christian and Scott (1953) showed little growth on synthetic media below an a_w of 0.95. In milk, meat and dried soup, they found a lower a_w would support growth of the strain tested.

Christian (1955) studied the influence of nutrition on the water requirements for growth of S. oranienburg. He found that the organisms grew only at a_w of 0.96 and above on minimal media. The addition of five amino acids and eight vitamins to the minimal media did allow the organism to grow at a_w 0.95 but no lower.

Mechanisms of Cell Death After Drying

The cause of cell death with special attention to water in the environment has been studied in detail by Webb (1960 a, 1960 b, 1961, 1963), Webb and Dumasia (1964) and Webb, Dumasia and Bhorjee (1965). They found relative humidities from 40 to 70% to be the most lethal to Escherichia coli, Serratia marcescens, Staphylococcus albus and Bacillus subtilus. In this relative humidity range partial metabolism occurs in the cells and there is a requirement for water. They believe water is removed from macromolecules of the cell i.e. proteins, deoxyribonucleic acid and ribonucleic acid. When water is removed these molecules are rearranged and the biological activity is altered or destroyed.

Salmonella in Dry Food Products

Salmonella have been a problem in food for many years. Until recently, however, animal feeds and grains had not been implicated in Salmonella transmission. In a Salmonella outbreak in a colony of laboratory animals reported by Griffin (1952), contaminated food was the cause of the disease. He felt fecal droppings in grain bins or bodies of mice in hay bales could enter the feed product. The Public Health Laboratory Services (1958 a, 1958 b) published the results of two studies in which they surveyed over 5000 samples for Salmonella. These samples included wheat, flour, offal, sourings, dust, wash water and rodent excreta. No Salmonella were found. They also stated that these data were only good for samples included in this study and may not reflect the occurrence of Salmonella in grain and grain products in other parts of the world.

Silverstople et al. (1961), while investigating the source of Salmonella in barley powder, found one lot of barley in a mill contaminated with S. gatuni.

Of 279 animal feeds sampled by the Communicable Disease Center USDHEW (1964) 1.1% of the grain components contained Salmonella. In a similar study, Allred et al. (1967) found 0.66% of 2629 grain samples contaminated with Salmonella. The serotype most prevalent in all feed components and finished feeds was S. montevideo. Foltz (unpublished data) examined 400 samples of wheat and found two samples contaminated with Salmonella. The serotypes isolated were S. muenchen and S. montevideo.

Food and Drug Administration reported to the Communicable Disease Center USDHEW (1968 c) the results of a 1-year study of numerous dried products. Salmonella were isolated from such products as macaroni, coconut and coconut products, dried milk, dried yeast, nuts and drugs and dietary articles in tablet and capsule form. No Salmonella were isolated from 52 samples of wheat, rice and other cereal grains.

Salmonella paratyphi B isolated from an outbreak of paratyphoid fever by Culley (1952), survived in flour for 7-weeks. He also noted that a water and flour mixture provided a good growth medium for the bacteria. S. paratyphi B also grew well in wet flour sacking material.

Salmonella typhimurium was isolated by Robinson (1966) from calves suffering from acute gastroenteritis. The same phage type of S. typhimurium was isolated from a bag of powdered buttermilk that was being used as feed.

Kunz and Ouchterlony (1955) isolated three Salmonella serotypes

from hospital patients suffering from severe intestinal upsets. All three serotypes were subsequently isolated from a special feeding formula given the patients. Dried yeast was found to be the material that contaminated the formula. Product and environmental samples from the yeast processing plant yielded the same three serotypes of Salmonella.

In a survey of various dried products Skovgaard (1964) isolated S. wien from a dried soup mix being imported into Norway.

Meister reported the results of a survey of milk drying plants to the Communicable Disease Center USDHEW (1968 b). Environmental and milk samples were collected from January 1967 through December 1967 and cultured for Salmonella. Salmonella organisms were isolated from 0.5% of the 12,047 milk samples and 7.5% of the 2,798 environmental samples. In a similar survey Garbe (1969) reported that 0.22% of 17,496 milk samples were contaminated with Salmonella and 4.8% of the environmental samples were contaminated.

Salmonella in eggs has been a problem for many years. Schnieder (1946) found viable S. montevideo organisms in high quality egg powder with a total moisture content of 2%. Solovey and Calesnick (1948) used Salmonella contaminated egg powder to show that viable organisms could survive the cooking process and remain a source of infection. They reported the number of viable cells after cooking was a function of the holding time and temperature before cooking and the time and temperature of the cooking process. McCullough and Eisele (1951 a, 1951 b, and 1951 c) reported experiments in which they fed varying amounts of Salmonella contaminated spray dried eggs to human volunteers. In all cases the dried organisms were still

capable of producing disease when ingested in sufficient numbers.

Recently other sources of Salmonella infection have become prominent. Semple, Parry and Graham (1961) isolated S. paratyphi from three cases of paratyphoid fever in England. The source of infection was traced to a shipment of desiccated coconut. Wilson and Mackenzie (1955) isolated S. typhi from one lot of dried coconut in Australia. Several cases of typhoid fever had led them to investigate dried coconut as a possible source of the organisms. In further studies they isolated S. senftenburg, S. paratyphi B, S. typhimurium, and other serotypes as well. Salmonella could survive the normal drying process for coconut.

Other workers have found Salmonella organisms in some previously unsuspected dried products. The Communicable Disease Center USDHEW (1967) reported results of a Food and Drug Administration survey of animal glandular products in 1966. Salmonella were isolated from such powdered products as thyroid, pancreatin, pituitaries, supra renal cortex, heart substance, granular pepsin, liver powder and desiccated liver. The Communicable Disease Center USDHEW (1968 a) reported the isolation of Salmonella from enzymatic drain cleaners. Salmonella organisms were isolated from 68 ingredients used in the production of enzymatic drain cleaners. Salmonella were also isolated from the 12 finished products examined. Bate and James (1958) found S. typhimurium in the dust bag of a vacuum floor polisher used in a children's ward of a hospital. The organism in the dust bag were the same phage type isolated from several children suffering from salmonellosis caused by S. typhimurium. Miura, Sats and Miyame (1964) collected 300 samples of chick fluff from commercial hatcheries and found over

50% of the samples contaminated with Salmonella. They held some of the samples for 1-year at room temperature in airtight plastic bags and were able to isolate viable Salmonella organisms up to 10^6 cells/g of fluff.

Materials and Methods

Standardization of Cell Suspensions

Growth and Harvesting of Cells: Salmonella montevideo cells used in this research were from a culture originally isolated from a sample of meat and bone meal obtained from the Kansas State University Department of Grain Science and Industry experimental feed mill. Cultures were grown on yeast extract enriched proteose peptone agar slants for 18 hr at 37 C. The cells were washed off of each slant with 5 ml of a 0.1% Tryptone (Difco) solution (Jayne-Williams 1963 and King and Hurst 1963). Suspensions from four tubes were combined to give each tube a volume of 20 ml prior to centrifugation. The cells were concentrated by centrifugation in an International model K size 2 centrifuge at 4500 RPM for 20 min. The supernatent fluid was discarded and the cells were resuspended in 10 ml of the 0.1% tryptone solution.

Spectrophotometric and Viable Count Analysis: A stock cell suspension was made by combining all centrifuged cells and diluting until an Optical Density (OD) reading ranging from 0.7 to 0.9 was obtained using a Bausch and Lomb Spectronic 20 spectrophotometer. Minimum absorbance of the tryptone solution was found to occur at 520 nm so this was the wave length of choice. The stock suspension was divided into five aliquots. Each aliquot was diluted to give five suspensions with Optical Densities ranging from 0.1 to 1.11. Optical Density readings above 0.4 were diluted further to give OD readings below 0.4 so that the linear function of OD vs cell number would be maintained (Society of American Bacteriologists, 1957).

The number of viable cells at each OD was determined by making serial dilutions in 0.1% tryptone so that final dilutions on Brilliant Green Agar (BGA) plates were 10^{-7}, 10^{-8}, and 10^{-9}. After the serial dilutions were made, 0.1 ml was removed from the appropriate dilution bottle and placed on the surface of a BGA plate. The cells were distributed over the surface of the plate with a sterile glass spreader. The plates were incubated for 24 hr at 37 C and were then counted. A linear regression plot (Fryer 1966) was obtained from these data (Table 3 and Fig. 12 Appendix).

Examination of Wheat for Salmonella

Culturing the Wheat: Ottawa variety of hard red winter wheat used in this research was provided by the Department of Grain Science and Industry at Kansas State University. Prior to contamination this wheat was checked for the presence of any naturally occurring Salmonella organisms. Ten 100 g samples of the wheat were placed in 330 ml of Brilliant Green Tetrathionate Broth (Difco). Each sample was incubated at 37 C. After 24, 48, and 72 hr incubation BGA plates were streaked from each sample. No Salmonella or Salmonella-like colonies appeared on any of the BGA plates. From these results it was assumed that the wheat sample was free of Salmonella.

Analysis for False Positives: To show that no Salmonella-like colonies would confuse results in the enumeration procedure, 10 10-g samples of the test wheat were placed in 90 ml dilution blanks of 0.1% tryptone. The samples were shaken for 5 min and 0.1 ml from each sample was spread over the surface of a BGA plate and incubated

for 24 hr at 37 C. No Salmonella-like colonies appeared on the plates. These data showed that there were no recoverable organisms in the wheat sample that would give false positive results and confuse the counts of S. montevideo in the subsequent research.

Contamination of the Wheat

Contamination Levels: Before the test wheat could be contaminated a method for obtaining a standard level of contamination in all samples was devised. Three 500-g samples of wheat were contaminated with 5 ml of a suspension of S. montevideo. One suspension contained approximately 5×10^{10} cells, another 5×10^{11} and the third 5×10^{12} cells. The suspensions gave theoretical contamination levels of 10^8, 10^9 and 10^{10} cells/g of wheat in the corresponding wheat sample. The wheat was stored in one half gallon mason jars. Immediately after inoculation the cells were distributed over the wheat by rotating the jars for 15 min. Following, mixing samples were removed from each jar and the number of viable S. montevideo cells/g of wheat was determined.

Enumeration Method: A 10-g wheat sample was placed in a 90 ml tryptone dilution blank. The cells were removed from the wheat by shaking the dilution bottle for 5 min. Dilutions were made so that the final dilutions on BGA plates were 10^{-4}, 10^{-5} and 10^{-6}. One tenth ml from the appropriate dilution blank was placed on the surface of the BGA plate and distributed over the surface of the plate with a sterile glass spreader. This procedure was repeated 5 times for each of the three samples. The plates were incubated for 24 hr at 37 C and counted with the aid of a Quebec colony counter. The wheat

sample contaminated with 10^{10} cells/g gave a recoverable viable count of 10^{6} cells/g. In the experimental work this level of contamination was used. This enumeration method was found to give consistent results and was used to enumerate the number of viable cells on the experimental wheat.

Constant Relative Humidity Chambers

Constant relative humidity chambers were prepared by coating the lids of one gallon wide mouth mason jars with Brewers Anaerobic Jar sealing clay to provide an air tight seal. Saturated salt solutions, contained in these jars, at 25 C gave the desired relative humidities (Robinson and Stokes 1955). The compounds used and their solubilities and corresponding relative humidities are in Table 1. The saturated solutions were prepared and 300 ml of the individual solutions were placed in one of 11 one gallon wide mouth jars with an air tight seal.

Table 1

	Compound	Solubility in 100 ml H_2O at 25 C	Relative humidity at 25 C
1.	$NaOH \cdot H_2O$	77.0 g	7.03 %
2.	$LiCl \cdot H_2O$	59.5 g	11.05 %
3.	$K(C_2H_3O_2) \cdot 5H_2O$	97.1 g	22.45 %
4.	$MgCl_2 \cdot 6H_2O$	79.0 g	33.00 %
5.	$K_2CO_3 \cdot 2H_2O$	82.2 g	42.76 %
6.	$Mg(NO_3)_2 \cdot 6H_2O$	58.6 g	52.86 %
7.	NH_4NO_3	90.2 g	61.83 %
8.	NaCl	31.7 g	75.28 %
9.	KCl	31.2 g	84.26 %
10.	KNO_3	33.4 g	92.48 %
11.	$K_2Cr_2O_7$	14.2 g	98.00 %

Experimental Materials and Methods

Contamination of Wheat: Eleven 500-g samples of wheat were prepared as previously described so the approximate viable count of S. montevideo cells per gram of wheat was 10^6 cells/g. After mixing to distribute the cells over the entire wheat sample, initial counts were made on five 10-g samples and the average was used as the initial viable count.

Storage of Wheat: After contamination the wheat was placed in sterile one quart wide mouth mason jars and each jar was then placed in one of the 11 constant relative humidity chambers. During storage the wheat was held at 25 C. Oxley (1948) found that the exchange of water on the surface of wheat kernels was very rapid and after an initial change in moisture the bran of the kernel remained at a constant moisture content. For this reason the wheat was not tempered before placing it in the constant relative humidity chamber.

Sampling: During the first 7 days the viable count was determined each day. Samples were taken every third day from day 7 to day 29. From day 29 to day 57 samples were taken every 7 days. All samples taken after day 57 were 14 days apart.

In each case a 10-g sample was removed with a sterile spoon and placed on sterile weighing paper. The viable counts were determined by the method discussed in the section on enumeration.

Identification of Cells: Confirmation that S. montevideo colonies were the only colonies appearing on the BGA plates was obtained by picking two colonies per plate and inoculating them into Triple Sugar Iron Agar (Difco) slants. These slants were incubated for 24 hr at 37 C and examined for reactions typical

of Salmonella. The slants giving typical Salmonella reactions were further tested with Salmonella polyvalent "H" antiserum and Salmonella "O" group C_1 antiserum. Salmonella montevideo is a member of the "O" group C_1. If the Triple Sugar Iron Agar slant reaction and the agglutination reactions were typical of S. montevideo, the colonies were assumed to be S. montevideo.

Final Moisture Content of Wheat

After the final samples were taken the moisture content of each wheat sample was determined using a MOTOMCO Moisture Meter Model 919. This instrument was checked against the dry oven method of determining moisture content of wheat and was found to be accurate within ±0.1%.

Results

After 28 weeks of storage final viable counts of S. montevideo were made. The number of viable cells appeared to be a direct function of the relative humidity of storage. As the relative humidity of storage increased from 7.03 to 98.00%, the number of surviving S. montevideo cells decreased. Survival patterns fell into three general groups.

The first group included those samples stored at 7.03, 11.05, 22.45 and 33.00% relative humidity. Cells on wheat stored at these humidities decreased from an initial viable count of 10^6 cells/g to a final count of approximately 10^4 cells/g of wheat after 28 weeks of storage. S. montevideo cells stored on wheat at 7.03% relative humidity (Fig. 1) showed a rapid decrease in viability from the initial 10^6 cells/g to approximately 5.5×10^4 cells/g after 7

weeks of storage. From the seventh week of storage to the twenty-eighth week of storage there was a slow decrease in the viable count from 5.5×10^4 to approximately 1.6×10^4 cells/g of wheat. Figure 2 shows the survival of S. montevideo cells on wheat stored at 11.05% relative humidity. The decrease in number of viable cells in this sample was very similar to that observed at 7.03% relative humidity. The viable counts of the sample held at 22.45% relative humidity exhibited the same type of initial decrease from 10^6 cells/g to about 5.5×10^4 cells/g after 7 weeks of storage (Fig. 3). Cell numbers then slowly decreased to a final viable count of 10^4 cells/g of wheat after 28 weeks of storage. The final viable count of S. montevideo cells on wheat held at 33.00% relative humidity (Fig. 4) was 4.3×10^3 cells/g of wheat. An initial decrease in numbers of viable cells was observed; however, the rate of decrease was slightly greater than the three previous samples, and there was no indication that the rate of decrease would level off as in the three previous samples.

The second group of samples exhibiting similar survival patterns for the test organism included those samples stored at relative humidities of 42.76, 52.86 and 61.83%. These samples also had an initial contamination level of 10^6 cells/g of wheat. This group of samples had a more rapid decline in viable cells than the previous group. Figure 5 shows the viable count of S. montevideo cells/g of wheat stored at 42.76% relative humidity. As in the previous samples, after 7 weeks of storage the viable count decreased from 10^6 cells/g of wheat to about 3.6×10^4 cells/g. In Fig. 6 the viable counts/g of wheat of the sample held at 52.86% relative

humidity are shown. The final count of viable S. montevideo cells/g of wheat in this sample was 10^3, and the rate of decrease was slightly greater than the two previous samples. The decrease of viable S. montevideo cells/g of wheat in the sample held at 61.83% relative humidity is shown in Figure 7. The final count/g of wheat in this sample was also lower than the two other samples in this group. The rate of decrease in viable cells/g of wheat was also greater than the samples previously discussed.

The remaining four samples were considered as a unique group since in all samples the viable count fell below the limits of detection by the previously discussed enumeration method before the end of the 28-week sample period. The relative humidities of these samples were 75.28, 84.26, 92.48 and 98.00%. After 22 weeks the viable S. montevideo count of the sample held at 75.28% relative humidity was below the limits of the enumeration method used (Fig. 8). There was a steady decline from 10^6 cells/g of wheat to this level during this 22-week period. Figure 9 shows the decline of the number of viable S. montevideo cells/g of wheat in the sample held at 84.26% relative humidity. After 8 weeks of storage at this humidity there was visible mold growth on the surface of some of the wheat kernels. The amount of mycelia increased during the remainder of the storage period. Viable counts of S. montevideo steadily decreased from 10^6 cells/g of wheat to below detectable levels after 16 weeks of storage at this humidity. As in the sample stored at 84.26% relative humidity, visible mold growth was evident in the sample stored at 92.48%. Mycelia were visible after 7 weeks of storage at this humidity (Fig. 10). Salmonella montevideo cells remained detectable at this relative

humidity for a longer period of time than the previous two samples. Viable counts of S. montevideo fell below detectable limits after 26 weeks of storage. The numbers of viable cells/g of wheat steadily decreased from 10^6 cells/g to numbers less than could be detected after 26 weeks. Molds were evident in the sample stored at 98% relative humidity (Fig. 11) after 5 weeks of storage. Viable counts of S. montevideo fell below detectable limits after 16 weeks of storage. This very rapid decrease in viable cells/g of wheat was very similar to that of the sample stored at 84.26% relative humidity.

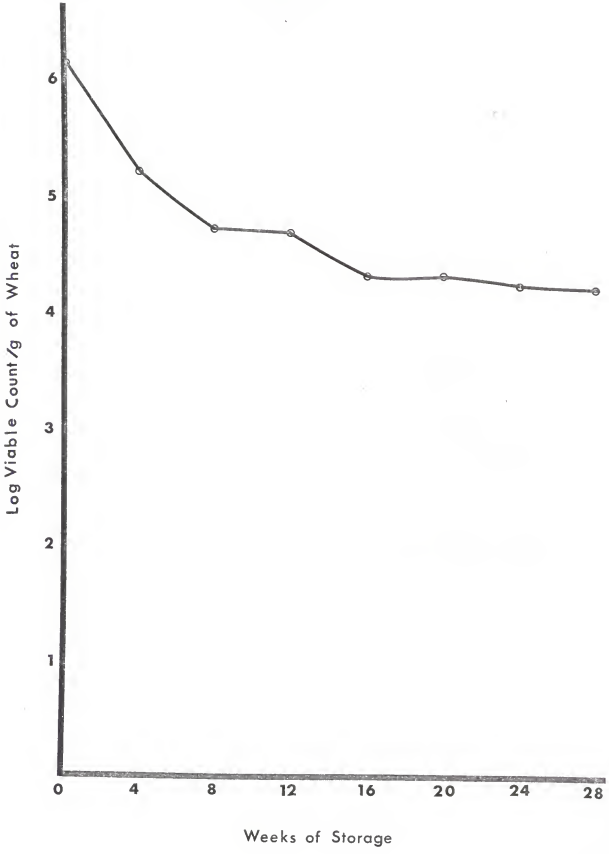
After 28 weeks of storage 100 g of wheat were removed from each sample of wheat and were cultured for S. montevideo as described in the materials and methods. Salmonella montevideo was isolated from all samples except the sample stored at 98% relative humidity. No other serotypes of Salmonella were isolated from any of the samples of wheat. Actual viable counts are found in the appendix (Tables 4-14).

The final moisture content of each wheat sample was determined, and the moisture content in relation to the relative humidity of storage is found in Table 2.

EXPLANATION OF FIGURE 1

Survival of S. montevideo on wheat stored at 7.03% relative humidity.

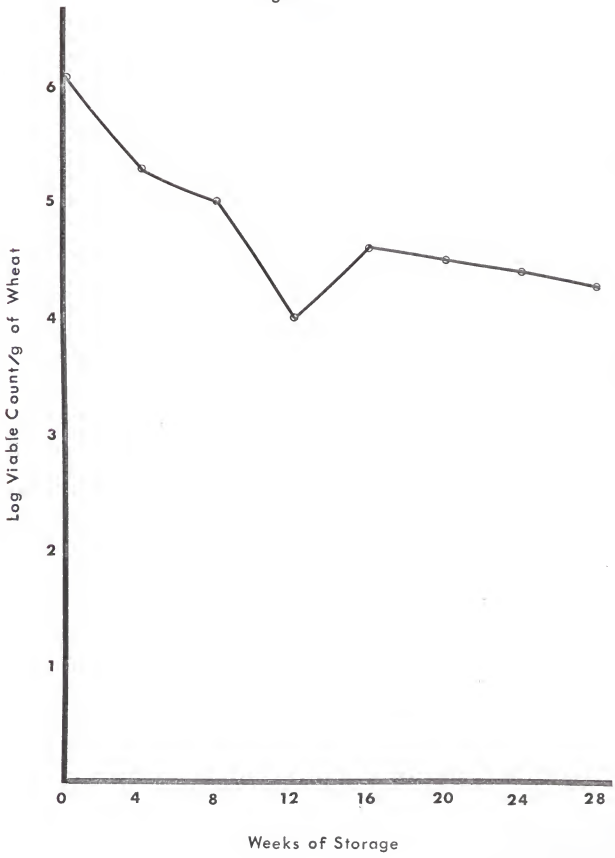
Figure 1


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 2

Survival of S. montevideo on wheat stored at 11.05% relative humidity.

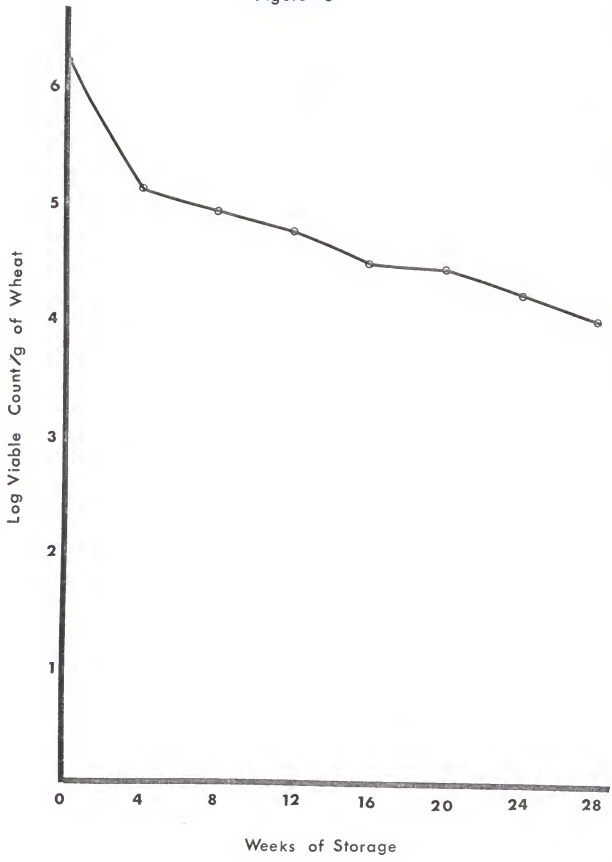
Figure 2


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 3

Survival of S. montevideo on wheat stored at 22.45% relative humidity.

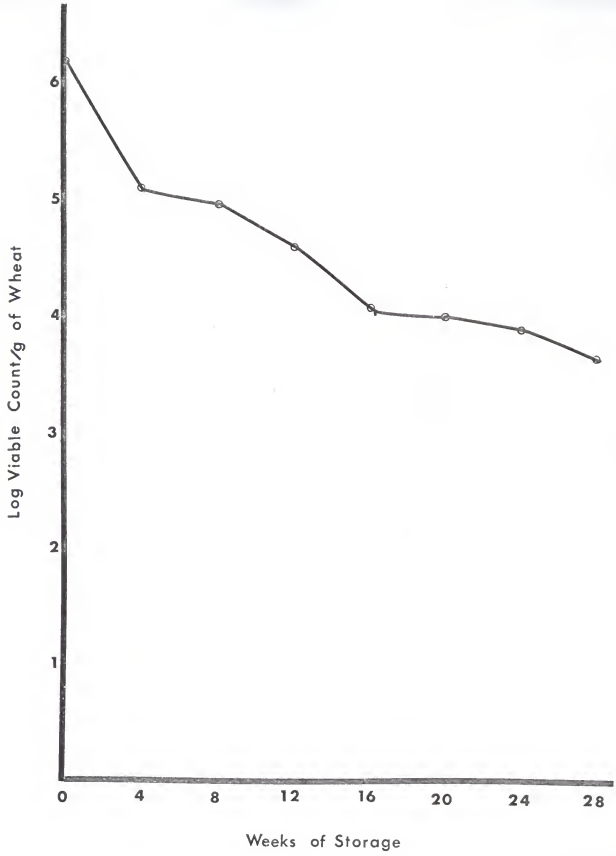
Figure 3


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 4

Survival of S. montevideo on wheat stored at 33.00% relative humidity.

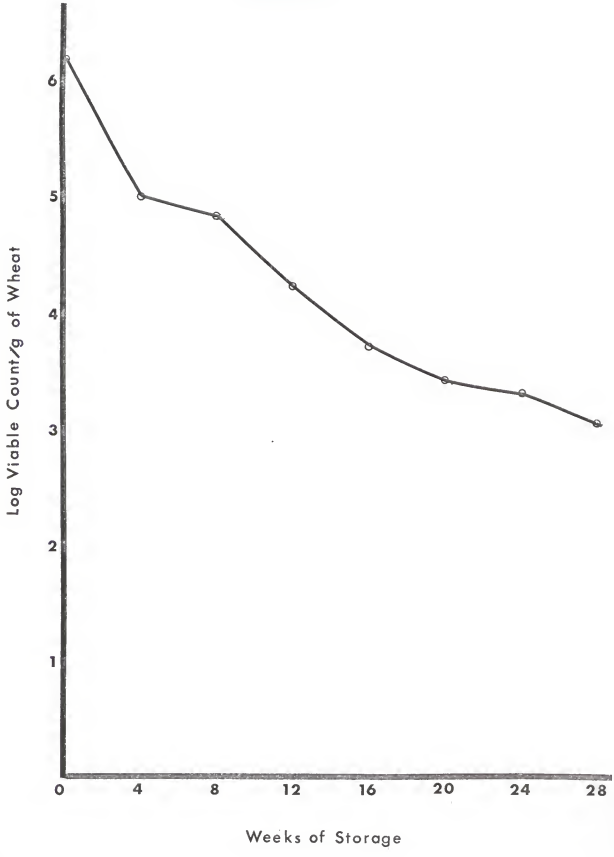
Figure 4


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 5

Survival of S. montevideo on wheat stored at 42.76% relative humidity

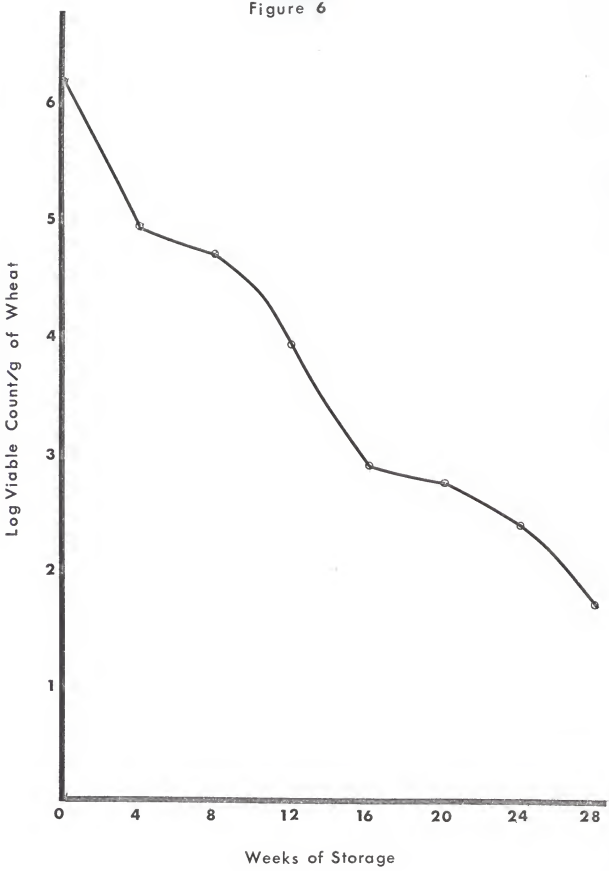
Figure 5


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 6

Survival of S. montevideo on wheat stored at 52.86% relative humidity.

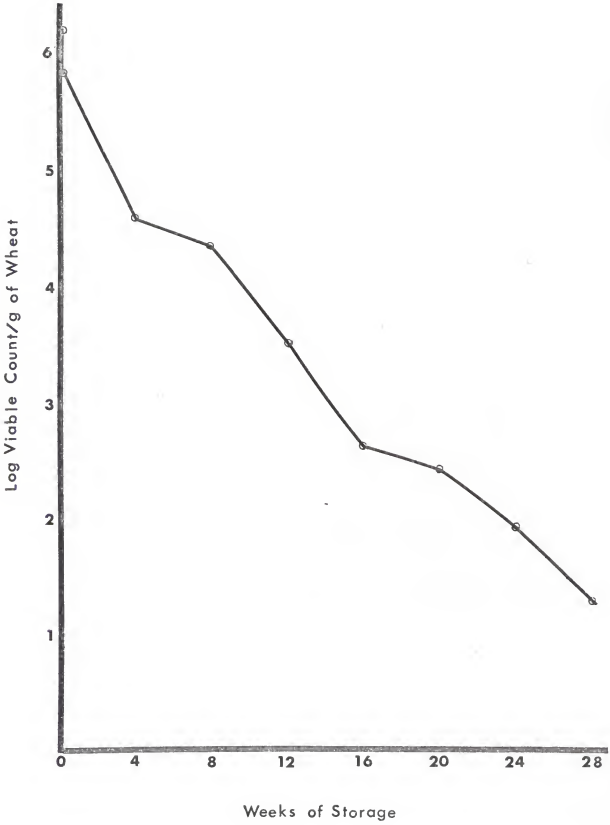
Figure 6


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 7

Survival of S. montevideo on wheat stored at 61.83% relative humidity.

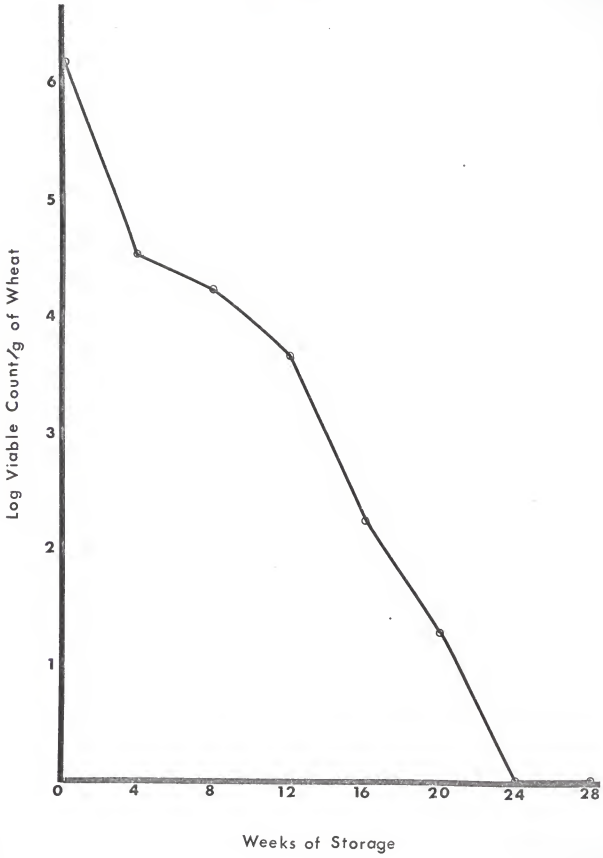
Figure 7


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 8

Survival of S. montevideo on wheat stored at 75.28% relative humidity.

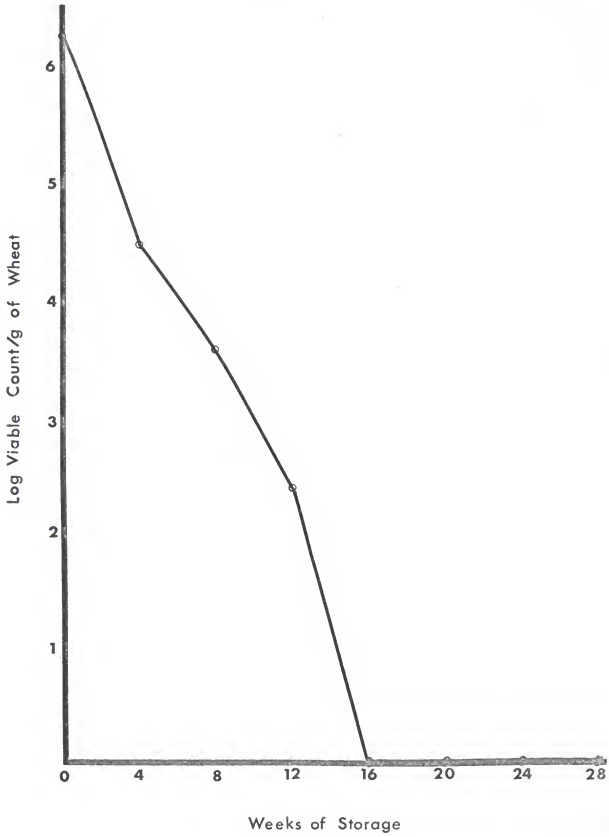
Figure 8


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 9

Survival of S. montevideo on wheat stored at 84.26% relative humidity.

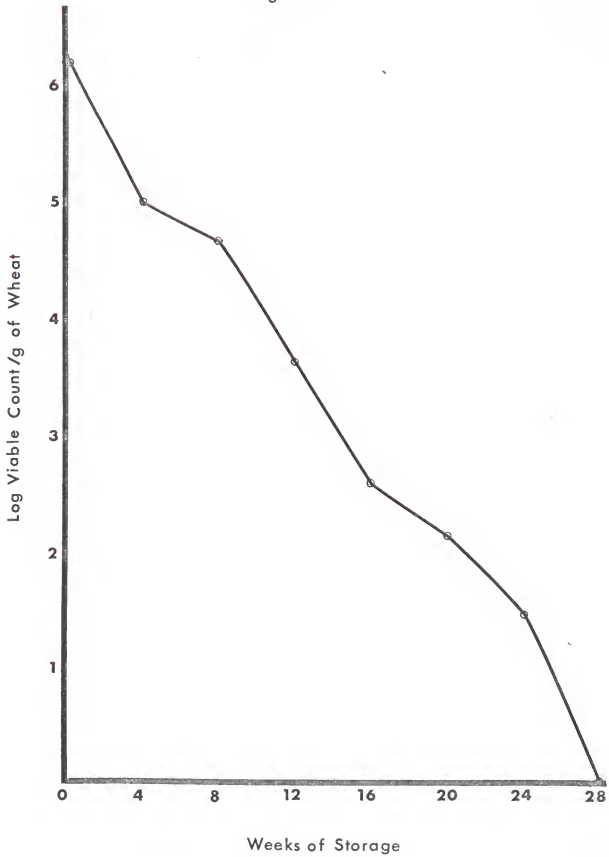
Figure 9


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 10

Survival of S. montevideo on wheat stored at 92.48% relative humidity.

Figure 10


Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

EXPLANATION OF FIGURE 11

Survival of S. montevideo on wheat stored at 98.00% relative humidity.

Figure 11

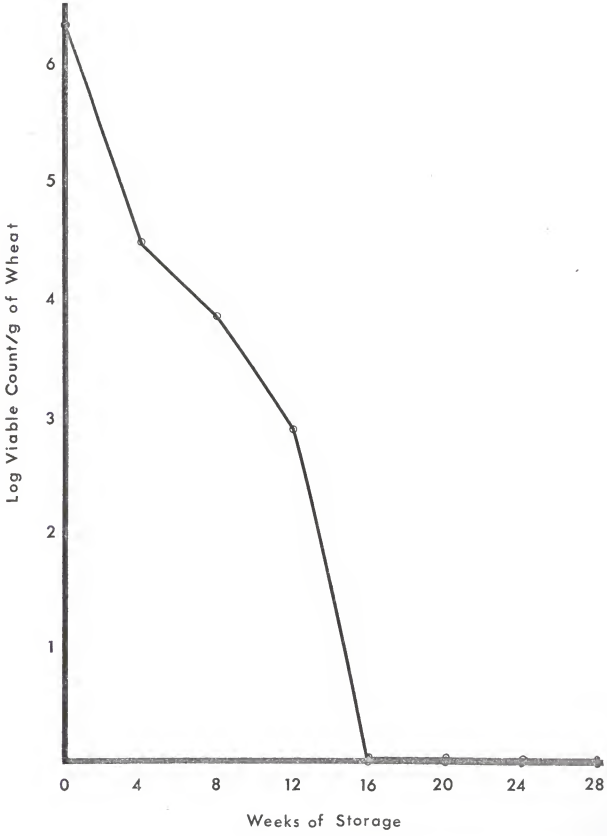

Log Viable Count/g of Wheat
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Weeks of Storage

Table 2

Relative Humidity of Storage: Wheat	Final Moisture Content: Wheat
7.03%	6.2%
11.05%	6.3%
22.45%	8.0%
33.00%	9.3%
42.76%	10.3%
52.86%	11.0%
61.83%	11.3%
75.28%	12.9%
84.26%	15.6%
92.48%	16.0%
98.00%	21.5%

Discussion

Enumeration of Salmonella from any material has presented a challenge to investigators for many years. A good method should combine accuracy and consistency. The method used in this research produced consistent results and met this criterion. The accuracy of any method is dependent upon a second generally accepted method to act as a standard. In Salmonella quanitation the Most Probable Number technique is the recommended method (Galton, Morris and Martin 1968). Since this method requires a greater number of samples and a larger quantity of sample material it was not used. The accuracy of the enumeration method used in this study then is still open to question since approximately 10^{10} viable S. montevideo cells/g of wheat were added and only 10^{6} viable cells/g of wheat could be recovered. Since spectrophotometric estimation of cell numbers will not distinguish live from dead organisms this may have been a source of error. Also since very young cells are much more susceptable to drying than are older cells this could also be an explanation of the difference in the approximate number of cells added to the wheat and the number that could be recovered. While shaking the dilution blanks removed a high percentage of the cells, placing the wheat and the diluting fluid in a food blender for a period of time might have been more effective. However for the purposes of this study, the enumeration method used was quite adequate. Viable counts that were obtained could be reproduced, and the rate that the viable counts decreased in each sample showed no irregular changes. Slight variations in the viable count/g of wheat could have been due to

inadequate distribution of the S. montevideo cells over the wheat sample. As the number of viable cells decreased there was a point where the counts semmingly approached zero. The presence of viable cells in all samples except the one held at 98% relative humidity demonstrated there were still some viable cells present after the number fell below the limits of the enumeration method. The lower limit of this technique was estimated to be less than 10 viable S. montevideo cells/g of wheat. It should be noted however that this estimation is based on the number of recoverable viable cells/g of wheat since some cells may not have been removed by shaking the dilution blank. Salmonella montevideo cells on wheat stored at different relative humidities showed survival patterns (Fig's. 1-11) similar to those of S. newport as reported by Scott (1958). Similar results were reported by McDade and Hall (1964) with S. derby and by DeOme (1944) with S. pullorum.

The growth of molds in the wheat samples at 84.26, 92.48 and 98% relative humidity was expected. Species present were Aspergillus spp, Penicillium spp, Alterneria spp and Fusarium spp. The presence of these fungi may have had an effect on the survival of S. montevideo. Metabolites of some of these fungi are toxic to bacteria, and the competition of the fungi for water may have decreased the survival of some S. montevideo cells. In the enumeration technique used, the presence of fungi did not complicate any of the counts. Fungi will grow on BGA plates, but development of colonies usually takes several days. Also these field and storage fungi grow a little better at temperatures around 25 C. Since fungi are normally present on most wheat kernels, there was no attempt to remove them. Also

to preserve natural storage conditions as closely as possible, no attempts were made to remove any of the normal bacterial flora. The presence of normal flora on Salmonella contaminated wheat may have an effect on the survival of these contaminating organisms. There was, however, no assay for this phenomenon in this study.

Wheat stored below 13% moisture is considered to be at a safe storage moisture level. Few fungi will grow at this moisture level, and no bacterial growth can occur. Growth is an important consideration; however survival of dangerous organisms must also be considered.

In this study S. montevideo survived 28 weeks of storage on 7 wheat samples held at relative humidities that resulted in equilibrium moisture contents of less than 13%. Wheat samples held at 52.86% relative humidity and less had an equilibrium moisture content of 11.1% or less (Table 2). Wheat stored at these moisture contents would hold for a very long period of time. As shown in this study, S. montevideo would also survive storage at these relative humidities. Certainly storage moistures of 6.2, 6.3 and 8.1% are rarely attained but a large amount of wheat is stored from 9 to 13% moisture. Survival of S. montevideo was the highest on samples held at equilibrium moisture contents of 6.2,6.3 and 9.3%. Salmonella montevideo survived the 28-week storage period in all samples except the sample held at 98% relative humidity. In samples held at higher atmospheric moisture levels, the viable counts of S. montevideo were lower than those samples held at lower humidities. In those samples held at 75.28, 84.24 and 92.48% relative humidities, the final number of surviving S. montevideo cells was very low, thus S. montevideo can survive on wheat in storage at constant relative humidities. Storage under

natural conditions where temperature and relative humidity may vary greatly was not studied but one may use these data to make inferences to contaminated wheat stored under natural conditions. In summer temperatures may reach above 40 C in a storage bin. At elevated temperatures bacteria are less resistant to drying than at low temperatures. In winter or when the relative humidity is quite low, and therefore the moisture content of the wheat is quite low, the survival of contaminating bacteria on wheat will be greater.

It should be noted that the incidence of Salmonella-contaminated grain is very low as reported by Allred et al. (1967), USDHEW (1964), Silverstople et al. (1961), Public Health Laboratory Services (1958 a) and Foltz (unpublished data). No attempts have been made to quantitate the number of Salmonella in any of these contaminated samples.

The route of Salmonella contamination of these grain samples has not been determined, but several avenues have been suggested. Contamination of grain by Salmonella could occur in a bin that was open to Salmonella-infected rodents or birds. Insects have also been implicated as a vector by which Salmonella can be carried from contaminated to clean grain (Husted 1969).

Silverstople et al. (1961) felt that Salmonella-contaminated barley was a source of an epidemic of salmonellosis in Sweden. Other outbreaks of salmonellosis caused by contaminated feed in animal cases and other contaminated ingredients in human foods. Contaminated grain probably plays a very small role in the complex Salmonella-cycle, but it should not be disregarded.

Acknowledgments

It is with a great sense of respect and gratitude that I acknowledge the guidance, the instruction, the encouragement and the friendship of Professor Vernon D. Foltz who has made my stay at Kansas State University both pleasant and rewarding.

I also wish to thank Dr. Robert Robinson of the Department of Grain Science and Industry for supplying the wheat used in this study.

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Literature Cited

Allred, J. N., J. W. Walker, V. C. Beal, and F. W. Germain. 1967. A survey to determine the Salmonella contamination rate in livestock and poultry feeds. J. Am. Vet. Med. Assoc. 151:1857-1861.

Bate, J. G. and U. James. 1958. Salmonella typhimurium infection dust-borne in a childrens ward. Lancet 2:713-714.

Christian, J. H. B. 1955. The influence of nutrition on the water relations of Salmonella oranienburg. Australian J. Biol. Sci. 8:75-82.

Christian, J. H. B., and W. S. Scott. 1953. Water relations of Salmonella at 30 C. Australian J. Biol. Sci. 6:565-573.

Culley, A. R. 1952. An outbreak of paratyphoid B fever in South Wales. Med. Officer 89:243-249, 257-262.

DeOme, K. B. 1944. The effect of temperature, humidity and glycol vapor on the viability of air-borne bacteria. Am. J. Hyg. 40:239-250.

Dunklin, E. W., and T. T. Puck. 1948. The lethal effect of relative humidity on air borne bacteria. J. Exptl. Med. 87:87-101.

Foltz, V. D. 1969. Unpublished data.

Fry, R. M., and R. I. N. Greaves. 1951. The survival of bacteria during and after drying. J. Hyg. 49:220-245.

Galton, M. M., G. K. Morris, and W. T. Martin. 1968. Salmonellae in foods and feeds review of isolation methods and recommended procedures. U.S. Public Health Service, CDC, Atlanta, Ga.

Griffin, C. A. 1952. A study of prepared feeds in relation to Salmonella infection in laboratory animals. J. Am. Vet. Med. Assoc. 121:197-200.

Hinton, N., J. R. Maltman, and J. H. Orr. 1960. The effect of dessication on the ability of Staphylococcus to produce disease in mice. Am. J. Hyg. 72:343-350.

Husted, S. W. 1969. Salmonella montevideo: Its effects on and transmission, the lesser rice weevil (Sitophilus oryzae) in wheat. Master's Thesis, Kansas State University.

Jayne-Williams, O. J. 1963. Report of a discussion on the effect of the diluant on the recovery of bacteria. J. Appl. Bacteriol. 26:398-404.

King, W. L., and A. Hurst. 1963. A note on the survival of some bacteria in different diluants. J. Appl. Bacteriol. 26:504-506.

Kunz, L. J., and O. T. G. Ouchterlony. 1955. Salmonellosis originating in a hospital a newly recognized source of infection. New England J. Med. 253:761-763.

Maltman, J. R., J. H. Orr, and N. A. Hinton. 1960. The effects of dessication on Staphylococcus pyogenes with special reference to implications concerning virulance. Am. J. Hyg. 72:335-342.

McCullough, N. B., and C. W. Eisele. 1951 a. Experimental human salmonellosis I. Pathogenicity of strains of Salmonella meleagridis and Salmonella anatum obtained from spray-dried whole egg. J. Infect. Diseases 88:278-289.

McCullough, N. B., and C. W. Eisele. 1951 b. Experimental human salmonellosis III. Pathogenicity of strains of Salmonella newport, Salmonella derby and Salmonella bareilly obtained from spray-dried whole egg. J. Infect. Diseases 89:209-213.

McCullough, N. B., and C. W. Eisele. 1951 c. Experimental human salmonellosis IV. Pathogenicity of strains of Salmonella pullorum obtained from spray-dried whole egg. J. Infect. Diseases 89:259-265.

McDade, J. J., and L. B. Hall. 1963. An experimental method to measure the influence of environmental factors on the viability and the pathogenicity of Staphylococcus aureus. Am. J. Hyg. 77:98-108.

Miura, S., G. Sato, and T. Miyamae. 1964. Occurrence and survival of Salmonella organisms in hatcher chick fluff from commercial hatcheries. Avian Diseases 8:546-554.

Oxley, T. A. 1948. The movement of heat and water in stored grains. Am. Assoc. Cereal Chem. Trans. 6:84-100.

Proom, H., and L. M. Hemmons. 1949. The drying and preservation of bacterial cultures. J. Gen. Microbiol. 3:7-18.

Public Health Laboratory Services. 1958 a. A search for organisms of the Salmonella group in wheat and flour. Monthly Bull. Min. Health Lab. Serv. 17:133-135.

Public Health Laboratory Services. 1958 b. Some observations on the bacteriology of flour. The possible role of flour in spreading enteric disease. Monthly Bull. Min. Health Lab. Serv. 17:135-146.

Rhodes, M. 1950. Viability of dried bacterial cultures. J. Gen. Microbiol. 4:450-456.

Robinson, R. A. 1966. Salmonellosis in young calves. New Zealand Vet. J. 14:33-39.

Robinson, R. A., and R. H. Stokes. 1955. Electrolyte Solutions. p. 510. Butterworth, London.

Scott, W. J. 1957. Water relations of food spoilage microorganisms. Adv. in Food Res. 7:83-127.

Scott, W. J. 1958. The effect of residual water on the survival of dried bacteria during storage. J. Gen. Microbiol. 19:624-633.

Scott, W. J. 1960. A mechanism causing death during storage of dried microorganisms, pp. 238-242 IN A. S. Parkes and A. U. Smith (eds.), Recent research in freezing and drying. Blackwell Scientific Publications, Oxford.

Schneider, M. D. 1946. Investigation of Salmonella content of powdered whole egg with not more than two percent moisture content II. General survey on occurance of species of Salmonella in high quality egg powder. Food Res. 11:313-318.

Semple, A. B., W. H. Parry, and A. J. Graham. 1961. Paratyphoid fever traced to dessicated coconut. Lancet 2:364-365.

Silverstople, L., U. Plazikowski, J. Kjellander, and G. Vahlne. 1961. An epidemic among infants caused by Salmonella muenchen. J. Appl. Bacteriol. 24:134-142.

Skovgaard, of V. 1964. Sallmonellafund i levaedsmidler. Nord. Vet. Med. 16:206-230.

Society of American Bacteriologists. 1957. Manual of microbiological methods. McGraw-Hill Book Co., Inc., New York.

Solowey, M., and E. J. Calesnick. 1948. Survival of Salmonella in reconstituted egg powder subjected to holding and scrambling. Food Res. 13:216-226.

Stamp, L. 1947. The preservation of bacteria by drying. J. Gen. Microbiol. 1:251-265.

Thomas, S. B., and P. B. Hobson. 1954. Coli-Aerogenes bacteria isolated from ears and panicles of cereal grains. J. Appl. Bacteriol. 18:1-18.

U.S. Department of Health, Education, and Welfare. 1964. Salmonella Surveillance, annual summary, 1963. Communicable Disease Center, Atlanta, Ga.

U.S. Department of Health, Education, and Welfare. 1965. Salmonella Surveillance, report no. 35. Communicable Disease Center, Atlanta, Ga.

U.S. Department of Health, Education, and Welfare. 1967. Salmonella Surveillance, report no. 59. Communicable Disease Center, Atlanta, Ga.

U.S. Department of Health, Education, and Welfare. 1968 a. Salmonella Surveillance, report no. 70. Communicable Disease Center, Atlanta, Ga.

U.S. Department of Health, Education, and Welfare. 1968 b. Salmonella Surveillance, report no. 71. Communicable Disease Center, Atlanta, Ga.

U.S. Department of Health, Education, and Welfare. 1968 c. Salmonella Surveillance, report no. 73. Communicable Disease Center, Atlanta, Ga.

U.S. Department of Health, Education, and Welfare. 1969. Salmonella Surveillance, report no. 80. Communicable Disease Center, Atlanta, Ga.

Webb, S. J. 1960 a. Factors affecting the viability of air-borne bacteria II. The effect of chemical additives on the behavior of air-borne cells. Can. J. Microbiol. 6:71-87.

Webb, S. J. 1960 b. Factors affecting the viability of air-borne bacteria III. The role of bonded water of protein structure in the death of air-borne cells. Can. J. Microbiol. 6:89-105.

Webb, S. J. 1961. Factors affecting the viability of air-borne bacteria V. The effect of desiccation on some metabolic systems of Escherichia coli. Can. J. Microbiol. 7:621-632.

Webb, S. J. 1963. The effect of relative humidity and light on air dried organisms. J. Appl. Bacteriol. 26:307-313.

Webb, S. J., and M. D. Dumasia. 1964. Bound water, inositol and the effect of X-rays on Escherichia coli. Can. J. Microbiol. 10:877-855.

Webb, S. J., M. D. Dumasia, and J. S. Bhorjee. 1965. Bound water, inositol and biosynthesis of temperate and virulant bacteriophages by air-dried Escherichia coli. Can. J. Microbiol. 11:141-150.

Appendix

Table 3. Optical density and viable counts of 0.1% tryptone suspensions of S. Montevideo.

Ml cell suspension		Ml Tryptone	Further dilution	O.D.	Final O.D.	Viable count
Set 1	0	10	none	0.00	0.00	0.0 cells/ml
	2	8	none	0.16	0.16	2.2×10^8 cells/ml
	4	6	none	0.30	0.30	7.3×10^8 cells/ml
	6	4	1-2	0.23	0.46	8.1×10^8 cells/ml
	8	2	1-2	0.30	0.60	11.8×10^8 cells/ml
	10	0	1-2	0.38	0.76	13.4×10^8 cells/ml
Set 2	0	10	none	0.00	0.00	0.0 cells/ml
	2	8	none	0.21	0.21	3.6×10^8 cells/ml
	4	6	none	0.40	0.40	5.2×10^8 cells/ml
	6	4	1-2	0.31	0.62	9.2×10^8 cells/ml
	8	2	1-3	0.25	0.75	10.0×10^8 cells/ml
	10	0	1-3	0.34	1.02	25.8×10^8 cells/ml
Set 3	0	10	none	0.00	0.00	0.0 cells/ml
	2	8	none	0.13	0.13	3.0×10^8 cells/ml
	4	6	none	0.27	0.27	6.4×10^8 cells/ml
	6	4	none	0.38	0.38	7.1×10^8 cells/ml
	8	2	1-2	0.27	0.54	10.5×10^8 cells/ml
	10	0	1-2	0.33	0.66	13.0×10^8 cells/ml
Set 4	0	10	none	0.00	0.00	0.0 cells/ml
	2	8	none	0.17	0.17	2.4×10^8 cells/ml
	4	6	none	0.33	0.33	7.3×10^8 cells/ml
	6	4	1-2	0.24	0.28	11.2×10^8 cells/ml
	8	2	1-2	0.32	0.64	12.1×10^8 cells/ml
	10	0	1-2	0.40	0.80	14.1×10^8 cells/ml
Set 5	0	10	none	0.00	0.00	0.0 cells/ml
	2	8	none	0.22	0.22	7.4×10^8 cells/ml
	4	6	1-2	0.22	0.44	8.4×10^8 cells/ml
	6	4	1-2	0.33	0.66	10.6×10^8 cells/ml
	8	2	1-3	0.29	0.87	17.6×10^8 cells/ml
	10	0	1-3	0.37	1.11	24.5×10^8 cells/ml

EXPLANATION OF FIGURE 12

Viable count of S. montevideo versus optical density.

Figure 12

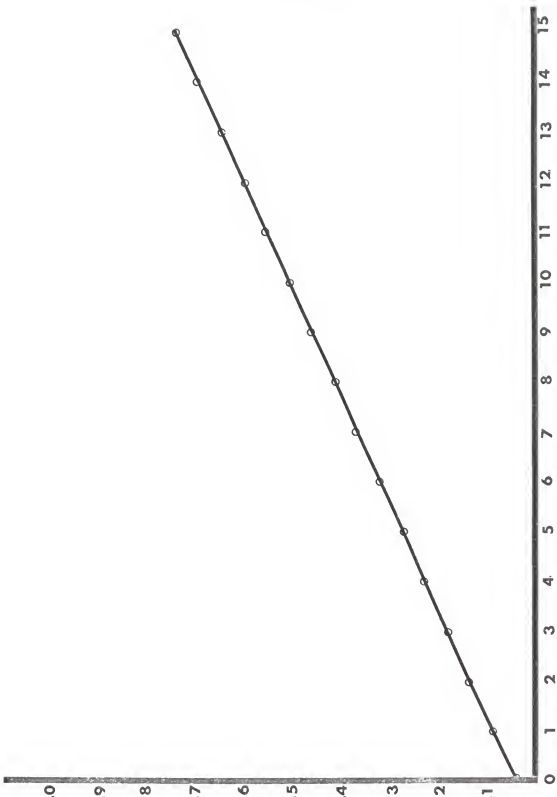
Optical Density at 520 mμ
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Viable S. montevidio / ml x 10^8

Table 4. Viable count and log viable count of S. montevideo on wheat stored at 7.03% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.4×10^6	6.15
1	2.4×10^5	5.38
2	1.7×10^5	5.23
3	1.9×10^5	5.28
4	1.7×10^5	5.23
5	1.75×10^5	5.24
6	8.3×10^4	4.92
7	5.6×10^4	4.75
8	5.5×10^4	4.74
10	5.5×10^4	4.74
12	5.1×10^4	4.71
14	3.0×10^4	4.48
16	2.0×10^4	4.30
18	2.1×10^4	4.32
20	1.95×10^4	4.29
22	1.78×10^4	4.25
24	1.70×10^4	4.23
26	1.63×10^4	4.21
28	1.57×10^4	4.20

Table 5. Viable count and log viable count of S. montevideo on wheat stored at 11.05% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.16×10^6	6.06
1	3.5×10^5	5.54
2	3.0×10^5	5.48
3	1.90×10^5	5.28
4	1.80×10^5	5.26
5	1.34×10^5	5.13
6	7.8×10^4	4.89
7	5.9×10^4	4.77
8	1.0×10^5	5.00
10	9.2×10^4	4.96
12	1.0×10^4	4.00
14	4.1×10^4	4.61
16	4.4×10^4	4.64
18	3.90×10^4	4.59
20	3.50×10^4	4.54
22	3.20×10^4	4.50
24	2.50×10^4	4.40
26	2.07×10^4	4.34
28	1.83×10^4	4.26

Table 6. Viable count and log viable count of S. montevideo on wheat stored at 22.45% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.57×10^6	6.20
1	3.20×10^5	5.50
2	1.70×10^5	5.23
3	1.05×10^5	5.02
4	1.30×10^5	5.11
5	1.10×10^5	5.04
6	9.40×10^4	4.97
7	5.60×10^4	4.74
8	8.50×10^4	4.93
10	5.30×10^4	4.72
12	5.80×10^4	4.76
14	3.10×10^4	4.49
16	3.00×10^4	4.48
18	2.70×10^4	4.43
20	2.90×10^4	4.46
22	2.60×10^4	4.42
24	1.90×10^4	4.28
26	1.20×10^4	4.08
28	1.01×10^4	4.00

Table 7. Viable count and log viable count of S. montevideo on wheat stored at 33.00% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.5×10^6	6.18
1	3.1×10^5	5.49
2	3.20×10^5	5.50
3	1.35×10^5	5.13
4	1.20×10^5	5.08
5	7.90×10^4	4.90
6	7.10×10^4	4.85
7	4.80×10^4	4.68
8	8.70×10^4	4.94
10	3.50×10^4	4.54
12	3.70×10^4	4.57
14	1.70×10^4	4.23
16	1.10×10^4	4.04
18	1.00×10^4	4.00
20	9.80×10^3	3.99
22	9.00×10^3	3.95
24	7.00×10^3	3.84
26	5.00×10^3	3.70
28	4.32×10^3	3.64

Table 8. Viable count and log viable count of S. montevideo on wheat stored at 42.76% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.5×10^6	6.18
1	5.30×10^5	5.72
2	3.30×10^5	5.52
3	1.20×10^5	5.07
4	9.80×10^4	4.99
5	1.1×10^5	5.04
6	4.1×10^4	4.61
7	3.60×10^4	4.56
8	7.00×10^4	4.84
10	1.60×10^4	4.20
12	1.80×10^4	4.26
14	6.20×10^3	3.79
16	5.00×10^3	3.70
18	4.80×10^3	3.68
20	3.60×10^3	3.56
22	2.80×10^3	3.45
24	1.95×10^3	3.29
26	1.36×10^3	3.13
28	1.07×10^3	3.03

Table 9. Viable count and log viable count of S. montevideo on wheat stored at 52.86% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.59×10^6	6.20
1	6.1×10^5	5.78
2	1.7×10^5	5.23
3	1.2×10^5	5.08
4	8.5×10^4	4.93
5	8.40×10^4	4.92
6	3.00×10^4	4.48
7	2.40×10^4	4.38
8	5.30×10^4	4.72
10	8.80×10^3	3.94
12	8.70×10^3	3.94
14	4.90×10^3	3.69
16	8.00×10^2	2.90
18	7.60×10^2	2.88
20	5.70×10^2	2.76
22	4.00×10^2	2.60
24	2.40×10^2	2.38
26	1.70×10^2	2.23
28	1.02×10^2	2.01

Table 10. Viable count and log viable count of S. montevideo on wheat stored at 61.83% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.59×10^6	6.20
1	4.70×10^5	5.67
2	1.90×10^5	5.28
3	1.10×10^5	5.04
4	4.10×10^4	4.61
5	4.50×10^4	4.65
6	1.50×10^4	4.18
7	1.40×10^4	4.15
8	2.40×10^4	4.38
10	1.10×10^4	4.04
12	3.30×10^3	3.52
14	1.50×10^3	3.18
16	4.40×10^2	2.64
18	3.60×10^2	2.56
20	2.70×10^2	2.43
22	1.50×10^2	2.18
24	90	1.95
26	70	1.84
28	20	1.30

Table 11. Viable count and log viable count of S. montevideo on wheat stored at 75.28% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.53×10^6	6.18
1	2.40×10^5	5.39
2	1.40×10^5	5.15
3	8.20×10^4	4.91
4	3.40×10^4	4.53
5	3.50×10^4	4.54
6	1.10×10^4	4.04
7	1.30×10^4	4.11
8	1.7×10^4	4.23
10	4.7×10^3	3.67
12	4.5×10^3	3.65
14	3.6×10^2	2.56
16	1.8×10^2	2.26
18	90	1.95
20	20	1.30
22	0	0.0
24	0	0.0
26	0	0.0
28	0	0.0

Table 12. Viable count and log viable count of S. montevideo on wheat stored at 84.26% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.85×10^6	6.27
1	1.70×10^5	5.23
2	1.10×10^5	5.04
3	4.90×10^4	4.69
4	2.90×10^4	4.46
5	2.0×10^4	4.30
6	6.80×10^3	3.83
7	5.80×10^3	3.76
8	3.70×10^3	3.57
10	1.60×10^3	3.20
12	2.30×10^2	2.36
14	80	1.90
16	0	0.0
18	0	0.0
20	0	0.0
22	0	0.0
24	0	0.0
26	0	0.0
28	0	0.0

Table 13. Viable count and log viable count of S. montevideo on wheat stored at 92.48% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	1.67×10^6	6.22
1	4.50×10^5	5.65
2	3.30×10^5	5.52
3	1.50×10^5	5.18
4	1.00×10^5	5.00
5	8.4×10^4	4.92
6	3.10×10^4	4.49
7	5.50×10^4	4.74
8	4.50×10^4	4.65
10	1.30×10^4	4.11
12	4.40×10^3	3.64
14	1.40×10^3	3.15
16	4.00×10^2	2.60
18	2.40×10^2	2.38
20	1.40×10^2	2.15
22	70	1.90
24	30	1.48
26	0	0.0
28	0	0.0

Table 14. Viable count and log viable count of S. montevideo on wheat stored at 98% relative humidity.

Week	Viable count per gram of wheat	Log of viable count
0	2.07×10^6	6.32
1	4.00×10^5	5.60
2	1.60×10^5	5.20
3	8.8×10^4	4.94
4	3.00×10^4	4.48
5	5.50×10^4	4.74
6	1.60×10^4	4.20
7	1.70×10^4	4.23
8	6.80×10^3	3.38
10	8.8×10^2	2.94
12	7.5×10^2	2.88
14	30	1.48
16	0	0.0
18	0	0.0
20	0	0.0
22	0	0.0
24	0	0.0
26	0	0.0
28	0	0.0

SURVIVAL OF SALMONELLA MONTEVIDEO ON WHEAT STORED AT CONSTANT RELATIVE HUMIDITIES

by

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B. S., Kansas State University, 1967

AN ABSTRACT OF A MASTER'S THESIS

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Eleven samples of Ottawa variety hard red winter wheat were inoculated with a standardized suspension of Salmonella montevideo cells so the resulting contamination level was 10^6 viable cells/g of wheat. The contaminated wheat samples were placed in constant relative humidity chambers held at 25 C. The relative humidities were 7.03, 11.05, 22.45, 33, 42.76, 52.86, 61.83, 75.28, 84.26, 92.48, and 98%. Constant relative humidity at 25 C was maintained with different saturated salt solutions in the sealed chambers. Periodic counts of viable S. montevideo cells/g of wheat were made over the 28-week sampling period. Viable counts of S. montevideo cells held on wheat at 7.03, 11.05, 22.45, and 33% relative humidity decreased from an initial 10^6 viable cells/g of wheat to a final count of approximately 10^4 viable cells/g of wheat in each sample. Salmonella montevideo cells held on wheat at 42.76% relative humidity decreased from the initial viable count of 10^6 cells/g of wheat to 10^3 viable cells/g after 28 weeks. The final viable counts of S. montevideo cells/g of wheat held at 52.86% and 61.83% relative humidity were 100 and 20 viable cells/g respectively. The viable counts/g of wheat of S. montevideo cells in the remainder of the samples fell below the level of detection of the enumeration method used before the end of the 28-week sampling period. After 22 weeks of storage at 75.28% relative humidity, S. montevideo could not be detected in the wheat with the enumeration method used. Viable counts of S. montevideo in the wheat samples held at 84.26, 92.48, and 98% relative humidity fell below the level of detection after 16,26 and 16 weeks respectively.

Culturing all samples after the 28-week storage period in an

enrichment broth revealed that all samples except the one held at 98% relative humidity still harbored viable S. montevideo although in some samples the numbers were very low.