

Transmission of *Salmonella montevideo* from Contaminated to Clean Wheat by the Rice Weevil^{1,2}

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ABSTRACT

Sitophilus oryzae (L.) retained *Salmonella montevideo* internally and externally for at least 1 week after being in contaminated wheat for 7 days, and for at least 5 weeks after being in contaminated wheat for 14 and 21 days. Contamination of clean wheat by the rice weevils was greater after they had been exposed to *S. montevideo*-

contaminated wheat for 14 and 21 days. Rapid decrease in viable *S. montevideo* cells in wheat over a 21-day period apparently did not affect the ability of the weevils to pick up the organism internally or externally. Adult rice weevils were effectively surface-sterilized by washing in 70% ethyl alcohol followed by 1% mercuric chloride.

Various *Salmonella* and stored-grain insects have been problems in food sanitation for many decades, but relationships between them have not been studied extensively. Much has been written on dissemination of *Salmonella* by flies and cockroaches. In Australia, Mackerras and Mackerras (1949) reported that a few cockroaches carried *Salmonella bovis-morbificans* in a hospital where there was an epidemic of infantile gastroenteritis caused by that *Salmonella* organism. Greenberg et al. (1963) revealed that 12 types of *Salmonella* were recovered from flies around a slaugh-

terhouse in Mexico. Of the stored-product insects, large numbers of bacteria were isolated from larvae and adults of confused flour beetles, *Tribolium confusum* Jacquelin duVal, (Van Wyk et al. 1959); Harein and de las Casas (1968) isolated bacteria from the granary weevil, *Sitophilus granarius* (L.).

This study was concerned with the transmission of *Salmonella montevideo* by the rice weevil, *Sitophilus oryzae* (L.), as it seemed likely that *S. montevideo* also could be picked up and transmitted by stored grain insects.

MATERIALS AND METHODS.—Rice weevils were reared in 1-qt jars containing *Salmonella*-free 'Ponca' hard red winter wheat with approximately 13.6% moisture content. The jars were kept in a chamber (83×95×80 cm) at 27° ±1°C and 70–75% RH. Relative humidity was controlled by using a pan of water partially covered with a cardboard strip, and was periodically measured with a Bendix aspirated psychrometer. Moisture content of the wheat was determined with a Motomco electronic moisture tester, the accuracy of which was monitored by the air oven method.

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The wheat used in subsequent studies was cultured for *Salmonella* prior to addition of *S. montevideo*. No *Salmonella* were found. The wheat also was cultured for any organisms that may have given confusing colonies on brilliant green agar plates (*Pseudomonas*, *Proteus*, *Citrobacter*, *Arizona*, or *Providencia*). None of these organisms were present.

Wheat samples of 200 g in quart jars were inoculated with *S. montevideo* grown on proteose peptone agar slants and harvested for use after growth for 18 hr at 37°C. The bacteria were scraped from the slants and suspended in sterile 0.1% tryptone (Difco). The suspension approximated 200 times the Nephelometer no. 1 turbidity. Each jar containing 200 g of wheat was inoculated by adding 5.0 ml of *S. montevideo* suspension. The jars were immediately rolled on a U. S. Stoneware roller to distribute the *Salmonella* organisms over all kernels. The jars of wheat were then placed in the rearing chamber for at least 24 hr before insects were added.

Tests were conducted to determine whether rice weevils could transfer *S. montevideo* from contaminated to clean wheat and the length of exposure to the contaminated wheat necessary to enable transmission to clean wheat. Fifteen adult weevils (0-9 days after emergence) were placed on 7 g of contaminated wheat in each of 27 plastic boxes.

Weevils were retained in 9 boxes for 1 week, in 9 other boxes for 2 weeks, and 9 more for 3 weeks. They are referred to as the 1st, 2nd, and 3rd "sets," respectively. The weevils were removed from 3 of the boxes of each set after the designated time. Weevils were checked for presence of *S. montevideo* externally and in the digestive tract to determine the success of weevil contamination.

The remainder of the weevils from each set (6 boxes/set) were then taken from the boxes of contaminated wheat and placed in boxes of clean wheat. One week later, weevils from 3 boxes of each set were removed and checked for *S. montevideo*. The wheat from which the weevils were removed was also tested for *S. montevideo*. The weevils in each of the 3 remaining boxes in each set were removed and transferred to another box of clean wheat every 7th day. The wheat from which they were taken was tested for *S. montevideo* each time the weevils were transferred. This procedure was continued as long as bacteria were recovered in the wheat, to determine how long adult weevils could continue to transmit bacteria to clean wheat.

To test viability of *S. montevideo* cells on wheat over 21 days and effect on the ability of the rice weevil to pick up bacteria, two 1-gal jars with 500 g of wheat were contaminated with a heavy suspension of 18-hr-old *S. montevideo* cells, and 500 newly emerged adult weevils (0-7 days) were added 5 hr later. Ten g of wheat and 40 weevils were removed 24 hr after the wheat was inoculated and checked for *Salmonella* organisms. The number of viable *S. montevideo* per gram of wheat was determined in each jar, and the average for the 2 jars was recorded. This procedure was repeated every 3rd day until the 21st day.

Procedure for enumerating the number of viable *S. montevideo* cells per gram of wheat involved pouring 10 g of wheat into a 90-ml dilution bottle of sterile water and shaking it for 2 min. Ten-fold dilutions were made from the original dilution and duplicate 0.1-ml portions from each dilution were spread on the surface of brilliant green agar plates which

were incubated at 37°C for 24 hr and then counted. Two representative colonies were picked from each plate and inoculated into Triple Sugar Iron Agar (Difco) slants. After 24 hr incubation at 37°C, cultures typical of the *Salmonella* group were further tested against antisera. Polyvalent *Salmonella* H antiserum was first used to screen suspect cultures. If agglutination occurred the culture was tested with *Salmonella* O group C₁ antiserum. Since *S. montevideo* is a member of O group C₁, agglutination in the poly H antiserum and in the O group C₁ antiserum was used as the criterion for identification in this study.

While conducting these experiments it was necessary to develop a surface-sterilizing technique so that *S. montevideo* could be recovered from the digestive tract of the weevils without possible contamination from the exterior parts of the insect. After several modifications the following technique was found effective.

1. Instruments and glassware were sterilized in an autoclave at 18 lb pressure for 20 min.

2. All work surfaces were washed with a 1% mercuric chloride solution, including the microscope.

3. Weevils were removed with forceps from the culture jar and placed in a sterile petri dish (100×15 mm). Forceps were flamed 3 times over an alcohol burner before and after handling insects. The weevils were killed by placing them in a freezer for at least 1 hr.

4. After 1 hr, 10 of the weevils were placed in a vial containing proteose peptone broth and labeled as the control.

5. The mouthparts and anus of each of the remaining weevils were covered with Vaseline petroleum jelly. This was done after killing the insects to prevent possible *S. montevideo* inside the insect from coming out during or after surface-sterilization.

6. Weevils were placed in a petri dish containing 70% ethyl alcohol for at least 5 min.

7. Weevils were removed from the alcohol, placed in a petri dish, and covered with filter paper. The filter paper was covered with 1% solution of mercuric chloride with Tergitol for at least 15 min.

8. The mercuric chloride solution containing the insects was poured through a funnel containing filter paper. Sterile water was poured over the weevils twice to wash off the mercuric chloride.

9. Weevils were removed gently from the filter paper to avoid breaking the exoskeleton, then placed in sterile water and carefully agitated for 5 min. The water and insects were poured into another funnel with filter paper.

10. Weevils were removed from the funnel and put on dry filter paper. Ten weevils were placed in a proteose peptone broth vial to test for effectiveness of surface-sterilization. The remaining weevils were placed in a petri dish containing Tissuemat (paraffin-like wax with a melting point of 56.5°C). The Tissuemat was melted and the dorsum of each weevil was embedded in it.

11. Weevils were covered with sterile water for easier dissections.

12. Dissecting needles and forceps were flamed 3 times before and after dissections.

13. Digestive tracts were severed near the anus and close to the head. After removing at least $\frac{2}{3}$ of the tract of each weevil, 10 tracts were placed in each proteose peptone broth vial. Usually the tracts were removed in pieces exposing more of the contents to

Table 1.—Transmission of *Salmonella montevideo* by rice weevils (RW) from contaminated to clean wheat.

Weevil exposure to contaminated wheat (days)	<i>Salmonella</i> present externally (E) or in digestive tract (DT) after RW removal from contaminated wheat						<i>Salmonella</i> present in clean wheat after RW transferred from contaminated wheat, then retransferred to clean wheat every 7 days														
	Control		Transferral to clean wheat for 7 days		Transferral 5 times to clean wheat (7 days/transferral, 35 days total)		I ^a			II			III			IV			V		
	E	DT	E	DT	E	DT	1	2	3 ^b	1	2	3	1	2	3	1	2	3	1	2	3
7	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	+	+	-	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
21	+	+	+	-	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-

^a Represents consecutive transfers from contaminated wheat.

^b Represents replicates.

the broth. The vials were placed in a 37°C chamber for 24–72 hr.

14. Brilliant green agar plates were streaked from each vial after 24, 48, and 72 hr and incubated for 24 hr at 37°C. Suspect colonies were picked and inoculated into Triple Sugar Iron Agar slants which were incubated for 24 hr at 37°C. Cultures with typical *Salmonella* reactions in the Triple Sugar Iron Agar slants were screened with polyvalent *Salmonella* H antiserum. Those cultures agglutinating in poly H antiserum were then tested with *Salmonella* O group C₁ antiserum. Those cultures agglutinated by O group C₁ antiserum were assumed to be *S. montevideo* since *S. montevideo* is a member of O group C₁.

15. The petri dish containing the remains of the weevils was autoclaved after removal of the used Tissuemat. Tissuemat was placed in a jar of mercuric chloride for 1 hr and then discarded.

RESULTS AND DISCUSSION.—Weevils tested immediately after removal from contaminated wheat were positive for *S. montevideo* both externally and internally for each of the 7-, 14-, and 21-day groups (Table 1). Each group of weevils was positive externally even after being in clean wheat for 7 days, but only weevils in the 14- and 21-day groups tested positive externally and internally after 5 weekly transfers to clean wheat. That appeared to indicate a direct relationship between the length of time weevils were in contaminated wheat and their ability to retain the bacteria.

Table 1 indicates also the ability of contaminated weevils to contaminate clean grain. Some weevils exposed to contaminated wheat for 7 days transmitted the bacteria to clean wheat for only 7 days, while others exposed for 14 days transmitted them for 28 days to clean wheat. The tests also indicated that presence of the bacteria externally or inside the weevils did not always result in wheat contamination.

In the viability test of *S. montevideo* on wheat, the number of live cells in the contaminated wheat 24 hr after inoculation was 2.7×10^6 cells/gm. After 21 days this dropped to 8.8×10^4 cells/g. Rice weevils tested positive for the bacteria externally and in the digestive tract throughout the 21-day period in both jars.

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