

*The effect of stress on the excretion of Salmonellae by pigs is examined by means of an experiment. The implications of this experiment for understanding the path of Salmonellae from domestic animals to man is discussed.*

## **SALMONELLA EXCRETION IN JOY-RIDING PIGS**

*Leslie P. Williams, Jr., D.V.M., Dr.P.H., and Kenneth W. Newell, M.D., D.P.H.*

### **Introduction**

**A**T the APHA Meeting of the Epidemiology and Occupational Health Sections in 1965, some of the problems and questions relating to Salmonella excretion in market swine were reviewed and presented.<sup>1</sup> A number of workers over a period of years observed that when swine were examined on the farm, the Salmonella infection rate, as measured by rectal swabs, was low. However, if swine were similarly examined in holding pens at the slaughterhouse, or by cecal or rectal swabs taken after slaughter, the observed infection rate was often from 30 to 80 per cent.<sup>2-6</sup>

Proposed explanations of these findings fall into two groups:

1. The excretion rate of Salmonella on farms is low because the rates of infection are low. Although salmonellae may be found in the animals' feed, they are usually present in small numbers which may be less than the infecting dose. The feeding of various antibiotics in the feed may also influence the infection rate.

Salmonellae are found in swine in holding pens and at slaughter because the animals infect each other in the trucks and in the holding pens. They have many opportunities for contact with each other at these times, and are also exposed to a Salmonella-contaminated environment.

2. Infection of swine with salmonellae occurs frequently at the farm from feed and other sources. However, infection cannot be assessed by means of rectal swabs because the num-

bers of organisms are too small or they are not present in the rectum.

When swine are disturbed by transport, overcrowding in holding pens, and by rough handling before slaughter, cecal contents reach the rectum and a hidden or masked salmonellae infection becomes ascertainable. Cross infections also occur with greater frequency at these times. This increase could be considered to be a result of stress.

The evidence for either of these two explanations is meager<sup>1</sup> and may be difficult to obtain with studies of cross-sectional design. We have shown that many of the salmonellae found in the holding pens and at slaughter were in the feed, and that these frequently differed from those serotypes found in the holding pen environment.<sup>7</sup> However, the interpretation of findings such as these rested upon a stress hypothesis which was unsubstantiated. This paper describes an experiment that may help to clarify this issue. We have called it the experiment of the joy-riding pigs because it is based upon a confidence trick played upon a pig population.

### **Materials and Methods**

The plan was to select a part of a group of pigs approaching the age of slaughter. Our knowledge of the Salmonella content of the ingredients of the feed would lead us to consider that these pigs were being fed salmonellae.

Using rectal swabs, we would examine this group of pigs on the farm and find their salmonellae excretion rate by this method. We would then take a truck that had been cleaned and made free from salmonellae, and load these pigs upon it as if they were going to be slaughtered. However, rather than taking them to slaughter, we would give them a joy ride through the countryside and end the trip, not at the slaughterhouse but back at their home farm. We would examine them again at this time because, if the experiment had been successful, the pigs would have had a similar stress situation to pigs bound for slaughter. We then intended to repeat this experiment on the same pigs at a later date, but this time deliver them to slaughter.

For this experiment sterile, cotton swabs were used to collect rectal, truck, and cecal swabs. When the cecum was examined it was incised with a sterile knife and the swab was introduced without touching the edge of the incision. Swabs were placed immediately in tetrathionate for transport and incubation and the cultural methods used were those of Galton (1961).<sup>8</sup>

The Louisiana farm selected housed about 300 pigs. The concrete-floored pens held from 15 to 30 pigs per pen lot. The pens were covered and were washed each morning, insuring good sanitation. The pigs were fed stale bread, rolls, cakes, and other bakery items (including their wrappers) which had been run through a hammermill. Each pig received one-half pound of wheat bran per day and a 35 per cent protein-pelleted commercial supplement. This supplement contained soybean meal, cottonseed meal, digester tankage, wheat middlings, vitamins, trace minerals, and salt. It was medicated with 67.5 grams of streptomycin and 13.5 grams of penicillin per ton.

Six samples of each of the following ingredients of feed from the manufac-

turer showed no salmonellae: cottonseed meal, soybean meal, and the premix. Six of seven samples of the tankage (350 pounds per ton of supplement) showed salmonellae of the following serotypes: *S. oranienburg*, *lexington*, *anatum*, *derby*, *eimsbuettel*, *infantis*, and *st. paul*. Four samples of the complete supplement were negative for salmonellae.

The 20 pigs selected for this study had been purchased as feeders. After four months of feeding, each weighed approximately 190 pounds. The day of the experiment was a pleasant, clear one with a temperature of 70-78° F. The truck was a stake-bed, single-axle farm truck with solid sides and a slat tailgate. The truck was cleaned with live steam, allowed to dry, and sprayed with Wes-codyne (West Chemicals, New York) at a strength of two ounces to two gallons of water (125 ppm). Ten swabs were taken from the truck, following a 15-minute contact time, and all were negative for salmonellae.

The pigs were first rectal-swabbed in their pens. None of these swabs showed any salmonellae. The pigs were then loaded on the truck and were given a 60-mile joy ride. The truck was then parked in the shade for half an hour, and later was taken for another 90 miles before the pigs were returned to the farm. The total joy ride lasted for three and three-quarter hours. On their return the pigs were once again rectal-swabbed, and six pigs (30%) were shown to be excreting salmonellae (all *S. anatum*). Ten swabs of the truck all contained *S. anatum*.

Fourteen days later the same pigs, truck, and methods were used again. The day was cooler with a temperature of 39-50° F. On this occasion ten preparatory swabs of the truck were all negative for salmonellae. One rectal swab from one of the same study pigs at the farm (5%) was found positive for *S. anatum*. The pigs were taken to the slaughterhouse by truck—a four-

five-mile ride of 20 minutes' duration. Rectal swabs of the pigs on the truck, parked at the slaughterhouse, yielded four *Salmonella* positives (25%), all *S. anatum*. Six of the ten truck swabs taken after the ride contained salmonellae which were *S. newington*, *S. derby*, or *S. anatum*.

The pigs were put on the scale, weighed, and then placed in the crowding runway prior to slaughter. It was clean and dry except for two small wet spots. Pigs were held in the runway for half an hour. The runway was sampled while the pigs were in it and three of four swabs (75%) were positive for salmonellae; these included the serotypes *S. bredeney*, *typhimurium*, *newington*, and *derby*.

Cecal swabs taken after slaughter indicated that six of 20 pigs (30%) were infected with one or more of four *Salmonella* serotypes, including *S. norwich*, *derby*, *anatum*, and *newington*.

## Discussion

The findings in this experiment showed that pigs do differ in their rectal salmonellae excretion patterns, preceding and following the stress and disturbance of transport. It is clear from other experiments that transport disturbs animals.<sup>9-12</sup> Weight, pituitary, and adrenal changes have been described in disturbed pigs and rats. That these abuses also can result in changes in *Salmonella* excretion has not been previously shown in a longitudinal study.

If these changes occur generally and if this experiment is valid, there appear to be two main implications. The first specifically relates to pigs and to the evidence upon which many *Salmonella* investigators base their zoonotic cycle. The measurement of salmonellae infection in an undistributed animal by rectal swabs is inefficient and does not indicate *Salmonella* infection in a meaningful way. Hypotheses built upon this

evidence must be open to question. This experiment supports the explanation in Group 2—that feed source infection occurs on the farm, but is not measurable in the undisturbed animal.

However, it is not reasonable to state that, because of this, all pigs are infected on farms and that the control of *Salmonella* infections in pigs is necessarily restricted to feed control. In a second similar experiment on the same farm, similar methods were used but the pigs (because of chronic respiratory troubles in the herd) in addition had been fed large doses of sulfathiazole, arsanilic acid, streptomycin, and penicillin for two months. No salmonellae could be found by rectal swabs on the farm, rectal swabs during and after the joy ride, on the farm prior to slaughter, after arrival at the abattoir, or by cecal swabs after slaughter.

The second implication is a wider one. If other animals and man behave in a similar way to these pigs under study, then the evidence we use to judge the pathogenicity of enterobacteriaceae and the relationship of enteric infection to gastrointestinal illness may also be invalid. The greater frequency of isolation of an agent from a group of persons with diarrheal disease (as measured by fecal examination or rectal swabs), when compared with the isolation of the same agent in a nondiarrheal control group, has in the past been used to describe the pathogenicity of an agent. This may be an invalid method of judgment. A change in bowel action as a result of internal or external stress, a change in diet, or nonspecific causes, may result in detectable excretion of an agent in an infected individual whose infection was unascertainable by the usual methods prior to this change.

The hypothesis that man and the agents of man may behave in this way is supported by the work of Gangarosa and his co-workers (1966).<sup>13</sup> These authors showed that the judgment of human excretion of *Vibrio cholerae*,

biotype El Tor, could be altered by a "preparatory purge."

The excretion patterns of joy-riding pigs may therefore be important. Possibly, it should result in a re-examination of our concepts of enteric agents and their relationship to disease, as well as to our understanding of the path of salmonellae from domestic animals to man.

#### REFERENCES

1. Williams, L. P., Jr., and Newell, K. W. Patterns of Salmonella Excretion in Market Swine. *A.J.P.H.* 57:466-471, 1967.
2. Galton, M. M.; Smith, W. V.; McElrath, H. B.; and Hardy, A. B. Salmonella in Swine, Cattle and the Environment of Abattoirs. *J. Infect. Dis.* 95:236-245, 1954.
3. McDonagh, V. P., and Smith, H. G. The Significance of the Abattoir in Salmonella Infection in Bradford. *J. Hyg.* 56:271-279, 1958.
4. Leistner, L.; Johantges, J.; Deibel, R. H.; and Niven, C. F., Jr. The Occurrence and Significance of Salmonellae in Meat, Animals and Animal By-Product Feeds. *Proc. 13th Research Conf., Circular 64, American Meat Institute Foundation, Chicago, Ill. (July 9-20).* 1961.
5. Hanson, R.; Rogers, R.; Emge, S.; and Jacobs, N. J. Incidence of Salmonella in the Hog Colon as Affected by Handling Practice Prior to Slaughter. *J. Am. Vet. M. A.* 145:139-140, 1964.
6. Shotts, E. B., Jr.; Martin, W. T.; and Galton, M. M. Further Studies on Salmonella in Human and Animal Foods and in the Environment of Processing Plants. *Proc. 65th Ann. Meet. U. S. Livestock Sanitary Assn., Minneapolis, Minn. (Oct. 30-Nov. 3).* 1961, Trenton, N. J. MacCrellich and Quigley Co., 309-318, 1962.
7. Williams, L. P., Jr. The Relationship of Feed and Environment to the Recovery of Salmonella from Market Swine. (Unpublished thesis.) Tulane University School of Medicine. New Orleans, La., 1964.
8. Galton, M. M. Laboratory Procedures for the Isolation of Salmonella from Human and Animal Food Products. *Proceedings of 65th Annual Meeting of U. S. Livestock Sanitary Association, Minneapolis, Minn. (Oct. 30-Nov. 3).* 1961, Trenton, N. J. MacCrellich and Quigley Co., 434-440, 1962.
9. Juskiewicz, T., and Jones, L. M. Effects of Chlorpromazine and Ascorbic Acid in Rats During Simulated Transportation Conditions at Normal and High Temperatures. *Am. J. Vet. Res.* 22:544-548, 1961.
10. ————. The Effects of Chlorpromazine on Heat Stress in Pigs. *Ibid.* 553-557.
11. Dymsha, H. A.; Miller, S. A.; Maloney, J. F.; and Foster, H. L. Equilibration of the Laboratory Rat Following Exposure to Shipping Stresses. *Lab. Animal Care* 13: 60-65, 1963.
12. King, N. B.; Gale, C.; Smith, B. S.; Hamdy, A. H.; Sanger, V. L.; Pouden, W. D.; and Klosterman, E. W. Stress Factors in Shipping Fever. *Vet. Med.* 53: 67-72, 1958.
13. Gangarosa, E. J.; Saghari, H.; Emile, J.; and Siadat, H. Detection of *Vibrio cholerae* Biotype El Tor by Purging. *Bull. Wld. Hlth. Org.* 34:363-369, 1966.

Dr. Williams is Associate Professor, College of Veterinary Medicine and Biomedical Sciences, Department of Microbiology, Colorado State University, Fort Collins, Colo. 80521. He was formerly Assistant Professor, Division of Epidemiology, Department of Tropical Medicine and Public Health, Tulane University School of Medicine. Dr. Newell is presently Director, Division of Research in Epidemiology and Communications Science, WHO, Geneva. He was formerly Professor of Epidemiology, Division of Epidemiology, Department of Tropical Medicine and Public Health, Tulane University School of Medicine, New Orleans, La. 70112

These investigations were supported by Research Grant 5 RO1 CC-00094-03 from the National Communicable Disease Center, Department of Health, Education, and Welfare, Public Health Service.

This paper was originally presented before a Joint Session of the Conference of Public Health Veterinarians and the Epidemiology and Occupational Health Sections of the American Public Health Association at the Ninety-Fourth Annual Meeting in San Francisco, Calif., November 2, 1966. It was planned to publish this paper as part of a supplement to the *Journal*. However, due to lack of financial support this was not possible. The authors resubmitted the paper for publication in June, 1969.