RESEARCH REPORT

PORK SAFETY

Title: Colonization Pattern of *Salmonella typhimurium* DT104
NPB #98-194

Investigator: Paula J. Fedorka-Cray

Institution: USDA-ARS-RRC

Date Received: 8/8/2000

ABSTRACT

Salmonella typhimurium definitive phage type (DT) 104 has a unique antimicrobial resistance pattern (R-type) with multiple resistance observed for ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamides (Su), and tetracycline (T) (ACSSuT). Recovery from humans and animals has increased over the past several years and has become the predominant phage type in a number of regions world-wide. Although dissemination appears to be widespread, factors influencing this occurrence have not been defined. We challenged pigs with either a wild-type penta-resistant *S. typhimurium* DT104 (Group R) or a pan-sensitive *S. typhimurium* DT104 (Group S) by commingling challenged pigs (n=2/group) with sentinel pigs (n=13/group) for each group. Control pigs (Group C) were not exposed to *Salmonella*. Pigs were monitored for signs of clinical illness and rectal shedding of *Salmonella* was also monitored. Pigs from each group were necropsied at weeks 1, 2, and 3 post-challenge. Tonsil, liver, cecum and cecal contents, ileocolic lymph nodes (ICLN) and ileocolic junction (ICJ) were collected aseptically for qualitative bacteriology. Cecal contents were also evaluated quantitatively. Rectal shedding of *Salmonella* in sentinel pigs was sporadic in Group S (average 9% positive for days 0 thru 6). For Group R, rectal shedding increased over time (27% and 100% positive on days 1 and 6 respectively) in sentinel pigs exposed to the resistant strain and averaged 32% positive over the period evaluated (days 0 through 6). Levels of *Salmonella* being shed into the environment were determined for challenged pigs and sentinel pigs by group on day 3. Feces (n=1) from Group S had 1.4 $\log_{10}$ CFU/g *Salmonella* while feces (n=2) from Group R pigs contained 1.7 $\log_{10}$ CFU/g. *Salmonella* levels were too low to quantify in fecal composites from sentinel pigs in either Group R or S on day 3. The percent *Salmonella* positive tissues recovered from Group S was 50, 40 and 8% for necropsy at weeks 1, 2 and 3, respectively. The percentage *Salmonella* positive tissues recovered from Group R was 76, 64 and 56% for necropsy at weeks 1, 2 and 3, respectively. Over the course of the

study 35 and 65% of the tissues were positive for Group S and R, respectively. Levels of *Salmonella* in the cecal contents declined over time in Group S from 2.2 $\log_{10}$ CFU/g at week 1 to undetectable levels for weeks 2 and 3. Levels of *Salmonella* in the cecal contents persisted in Group R and were 1.8, 1.5 and 1.7 $\log_{10}$ CFU/g *Salmonella* for weeks 1, 2 and 3, respectively. This suggests that acquisition of multiple resistance has altered the colonization potential of the strain and may explain the dissemination of the organism among many different species. Further studies are needed to determine the factors affecting colonization of *Salmonella* with different antimicrobial resistance patterns in order to develop strategies for altering colonization.

INTRODUCTION

Salmonella typhimurium definitive phage type (DT) 104 was first identified in humans in England and Wales in 1984.[1] This strain has a unique antimicrobial resistance pattern (R-type) with multiple resistance observed for ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamides (Su), and tetracycline (T) (ACSSuT). The number of isolations in the UK from humans rose slowly from 1984 to 1990 then more rapidly so that by 1993, DT104 with R-type ACSSuT accounted for over 80% of the isolations.[2] In 1995, R-type ACSSuT DT104 accounted for over 87% of *S. typhimurium* isolates recovered from humans with 26.4% and 6.2% of these isolates having additional resistance to trimethoprim and ciprofloxacin, respectively.[2,3]

In the animal population in the UK, DT104 was first recovered in 1989 from cattle. Only one herd was identified. Since that time, isolations have continued to rise and now account for the majority of *S. typhimurium* isolates. Recovery has also been documented from sheep, pigs, poultry, goats, rabbits, dogs, seabirds, rodents, porpoises, cats, horses, and animal feed.[1,2,4,5] Contact with ill farm animals, particularly cattle, is implicated as a primary factor for transmission.[2,6] Long-term carriage has also been observed in all species, particularly in cats and cattle.[7,8] Illness associated with DT104 from humans and animals has now reached epidemic proportions in the UK.

Antimicrobic resistance in *Salmonella* species is most often plasmid mediated. This is advantageous as removal of the selective pressure typically results in a reversion to susceptibility. Although DT104 harbors a 60-Mda plasmid, the resistance genes for R-type ACSSuT DT104 are chromosomally integrated.[1] However, it is also worrisome as removal of the selective pressure is expected to have no effect on resistance. Additionally, DT104 appears to have the ability to acquire additional resistance as described above, rendering treatment with other drugs useless.[9] Of importance in this observation is the fact that susceptibility to the newest class of antimicrobics, fluoroquinolones, has been compromised decreasing from 99% susceptible (1% resistant) to ciprofloxacin in 1994 to 94% susceptible (6% resistant) in 1995.[10] Resistance to ciprofloxacin is chromosomally encoded.

Invasiveness of DT104 in humans does not appear to differ from other *Salmonella*e, however, an increase in severe illness is noted with 36% of 105 patients in one case control study requiring hospitalization.[2] DT104 is very resistant to heat treatment in contrast to other *Salmonella*e, having the ability to survive boiling temperatures.[5] It is also resistant to desiccation and chemical disinfectants.[5]

Severe illness is noted in cattle and illness (ranging in severity) is also noted in other animal species. Following resolution of clinical disease, asymptomatic carriage can occur. Contact with ill animals (regardless of species) and consumption of pork sausages, chicken, and meat paste have been identified as significant risk-factors in case control studies in the UK.[2,6,11] Additionally, it has been suggested that person-to-person transmission may also play a significant role in transmission.

Multiple drug resistance in the U.S. in *Salmonella* species is increasing. Prior to 1986, DT104 was not recognized in the U.S. In the Pacific Northwest, from 1986 to 1991, 13% of *S. typhimurium* isolates of bovine origin were R-type ACSSuT compared to 64% for the period 1992 to 1995.[12] A rise in the human isolations in the Pacific Northwest was also observed with only 2 of 46 isolates identified from 1989 and 80 of 188 (42.5%) identified in 1994.[12] Phage typing of a small number of isolates to date indicates that all are type DT104. Interestingly, no tendency for an increase in the annual number of reported animal or human *S. typhimurium* cases has been evident in the Pacific Northwest.[13] This suggests that DT104 may soon become the predominant strain in the region.

For the period July 1, 1994, through June 30, 1995, the Centers for Disease Control (CDC) serotyped 3,923 *Salmonella* of which 976 (24.9%) were *S. typhimurium*. Approximately 28% or 275 of these *S. typhimurium* have the R-type ACSSuT. Thirty isolates representing 10 different states were randomly selected for phage typing. Phage type 104 has been confirmed for 25 of the 30 isolates. CDC is now projecting that approximately 80% of the R-type ACSSuT *S. typhimurium* are DT104.[14]

From an animal disease standpoint, there is a paucity of information regarding the pathogenesis of disease induced by DT104. Additional characterization of the epidemiology and transmission is required particularly in swine in which clinical disease is inapparent. Other than first screening for antimicrobial resistance followed by phage typing, there is no rapid test available to detect DT104. Virulence attributes, other than recognition of the 60-Mda plasmid, also need to be defined.

OBJECTIVE

The objective of this study was to assess the colonization patterns of both a pan-sensitive strain and a wild-type penta-resistant strain of *Salmonella typhimurium* DT104 with respect to tissue distribution, shedding patterns, and length of carriage.

PROCEDURES

Forty early weaned pigs (14 days of age), mixed breed and mixed sex, were randomly allotted to one of three groups; Group R (penta-resistant strain, n=15), Group S (pan-sensitive strain, n=15) and Group C (controls, n=10). Pigs were raised in isolation as described previously [15] and were allowed four weeks to acclimate to the isolation rooms. Rectal swabs were collected from each pig and cultured for *Salmonella* upon arrival and again prior to challenge.

The challenge cultures were prepared by streaking tryptic soy agar (TSA) plates with *Salmonella* obtained from -90°C frozen stock cultures and incubating overnight. Several colonies from the TSA plate were used to inoculate 5 ml Luria Bertani (LB) broth which was incubated stationary overnight at 37° C. A 1% inoculum was transferred to 250 ml of fresh LB broth and incubated at 37° C and 150 rpm for approximately 3.5 h. The culture was centrifuged at 10,000 g for 20 minutes and the pellet was resuspended in 30 ml phosphate buffered saline (PBS, pH 7.2). The culture was adjusted to an optical density of approximately A600 1.1, and final concentration was determined by plate counts on TSA. At six weeks of age, two pigs each from Groups R and S were marked for future identification and were challenged intranasally with 1ml of 10^{11} CFU/ml penta-resistant *Salmonella typhimurium* DT104 (wild type swine isolate; Group R) or with 1 ml of 8 X 10^{8} CFU/ml pan-sensitive *Salmonella typhimurium* DT104 (Group S). Following challenge, the two pigs were commingled with the 13 remaining sentinel pigs in each of their respective groups. The third group (Group C) of pigs were not exposed to *Salmonella* and remained as controls.

Post challenge (PC), pigs were observed twice daily for clinical signs of infection. Rectal swabs and rectal temperatures were obtained daily for the first week PC. Levels of *Salmonella* shedding in the feces was determined on day 3 PC by pooling feces collected via fecal loop from the 13 commingled sentinel pigs from Groups R and S. Levels of fecal shedding were also determined on the two pigs which were challenged in Groups R and S.

Pigs were necropsied weekly for three weeks PC. Tonsil, liver, cecum and cecal contents, ileocolic lymph nodes (ICLN) and ileocolic junction (ICJ) were collected aseptically for qualitative bacteriology. Cecal contents were also evaluated quantitatively. Bacteriologic culture was performed as described previously[16,17,18]. Briefly, all rectal swabs, fecal pools and tissues were enriched in Tetrathionate broth (Difco Laboratories, Inc., Detroit, MI) for 48 h at 37° C after which time an aliquot was transferred to Rappaport R-10 broth (Difco) for secondary enrichment (24 h at 37° C). Samples were streaked onto xylose-lysine-tergitol-4 (XLT4; Difco) agar plates and incubated 24 h at 37° C. Colonies with appearance typical of *Salmonella* were inoculated into triple sugar iron and lysine iron agar slants for biochemical confirmation. Positive isolates were serogrouped by agglutination using *Salmonella* O antiserum (Difco) and were sent to the National Veterinary Services Laboratory, Ames IA for definitive serotyping.

RESULTS

All pigs were culture negative for *Salmonella* prior to challenge. *Salmonella* was not recovered by rectal swab or tissue culture from any of the control pigs over the course of the study. Rectal temperatures of pigs in all three groups were elevated (> 103° F) on day 0 and remained elevated throughout the period of temperature recording. However, overt clinical disease was inapparent in that pigs appeared healthy, had normal feed intakes, and grew over the course of the study.

One of the two challenge pigs from Group S (challenged intranasally with the pan-sensitive strain) died the day following challenge (day 1). Results from this pig have been excluded

as other factors may have contributed to fatal coutcome. Sentinel pigs in this group were exposed to one versus two challenged pigs shedding *Salmonella* into the environment. Over the course of the study, the pig that died was the only pig among all groups exhibiting even mild morbidity.

Rectal shedding of *Salmonella* in sentinel pigs was sporadic in Group S (average 9% positive for days 0 thru 6). For Group R, rectal shedding increased over time (27% and 100% positive on days 1 and 6 respectively) in sentinel pigs exposed to the resistant strain and averaged 32% positive over the period evaluated (days 0 through 6*).* Levels of *Salmonella* were determined for challenged pigs and sentinel pigs by group on day 3. Feces (n=1) from Group S had 1.4 $\log_{10}$ CFU/g *Salmonella* while feces (n=2) from Group R pigs contained 1.7 $\log_{10}$ CFU/g. *Salmonella* levels were too low to quantify in fecal composites from sentinel pigs in either Group R or S on day 3.

The percentage of *Salmonella* positive tissues declined over time in both Group R and S. However, levels of recovery remained higher in the sentinel pigs from the resistant group (Group R). The percent *Salmonella* positive tissues recovered from Group S was 50, 40 and 8% for necropsy at weeks 1, 2 and 3, respectively. The percentage *Salmonella* positive tissues recovered from Group R was 76, 64 and 56% for necropsy at weeks 1, 2 and 3, respectively. Over the course of the study 35 and 65% of the tissues were positive for Group S and R, respectively.

Levels of *Salmonella* in the cecal contents declined over time in Group S. At week 1, cecal contents had 2.2 $\log_{10}$ CFU/g *Salmonella*, however, levels were too low to quantify at weeks 2 and 3. Levels of *Salmonella* in the cecal contents persisted in Group R and were 1.8, 1.5 and 1.7 $\log_{10}$ CFU/g *Salmonella* for weeks 1, 2 and 3, respectively.

CONCLUSIONS

Although the dose and number of pigs challenged differed among Groups R and S, the levels of *Salmonella* shed into the environment by day three were comparable in both groups (1.41 $\log_{10}$ CFU/g *Salmonella* for Group S versus 1.7 $\log_{10}$ CFU/g for Group R) indicating that sentinel pigs had equal opportunity in both group for exposure to their respective *Salmonella* strains. Previous experiments have demonstrated the comparability of exposing sentinel pigs to infected pigs versus direct challenge.[18] Although overt clinical disease was not apparent, colonization of both strains occurred. Had the experiment continued beyond three weeks, the expectation would have been for low to no *Salmonella* being recovered from Group S while, in all probability, recovery of *Salmonella* would have continued to occur in Group R. This suggests that acquisition of multiple resistance has altered the colonization potential of the strain and may explain the dissemination of the organism among many different species.

Information from this first report defining the colonization and carrier state of *Salmonella typhimurium* DT104 in pigs will assist the industry in adopting standards for removal of pigs from the herd once DT104 has been identified. Further studies are needed to determine the factors affecting colonization of *Salmonella* with different antimicrobial resistance patterns in order to develop strategies for altering colonization.

REFERENCES

1. Threlfall, E.J., J.A. Frost, L.R. Ward, and B. Rowe. 1994. Epidemic in cattle and humans of *Salmonella typhimurium* DT104 with chromosomally integrated multiple drug resistance. Vet. Rec. 134:577.

2. Wall, P.G., D. Morgan, K. Lamden, M. Ryan, M. Griffin, E.J. Threlfall, L.R. Ward, B. Rowe. 1994. A case control study of infection with an epidemic strain of multi-resistant *Salmonella typhimurium* DT104 in England and Wales. Commun. Dis. Rep. 4:R130-R135.

3. Wall, P.G., E.J. Threlfall, L.R. Ward, and B. Rowe. 1996. Multiresistant *Salmonella typhimurium* DT104 in cats: a public health risk. Lancet 348:471.

4. Anonymous. 1993. *Salmonella* in animal and poultry production 1992. Ministry of Agriculture. Fisheries and Food. Welsh Office, Agricultural Department, Scottish Office, Agriculture and Fisheries Department.

5. Munro, D. 1996. Personal communications.

6. Wall, P.G., D. Morgan, K. Lamden, M. Griffin, E.J. Threlfall, L.R. Ward, and B. Rowe. 1995. Transmission of multi-resistant strains of *Salmonella typhimurium* from cattle to man. Vet. Rec. 136:591-592.

7. Low, J.C., G. Hopkins, T. King, and D. Munro. 1996. Antibiotic resistant *Salmonella typhimurium* DT104 in cattle. Vet. Rec. 138:650-651.

8. Wall, P.G., S. Davis, E.J. Threlfall, L.R. Ward, and A.J. Ewbank. 1995. Chronic carriage of multidrug resistant *Salmonella typhimurium* in a cat. J. Small Animal Practice 36:279-281.

9. Frost, J.A., E.J. Threlfall, and B. Rowe. 1995. Antibiotic resistance in *Salmonella*s from humans in England and Wales: the situation in 1994. PHLS Microbiol. Digest 12:131-133.

10. Threlfall, E.J., J.A. Frost, L.R. Ward, and B. Rowe. 1996. Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. Lancet 347:1053-1054.

11. Nichols, G.L. and J. DeLouvois. 1995. The microbiological quality of raw sausages sold in the UK. PHLS Microbiol. Digest 12:236-242.

12. Besser, T., J. Gay, C. Gay, and R. Collins. 1995. Increasing prevalence of *Salmonella typhimurium* DT104 in animals and humans in Washington state. 76th Annual Meeting Conf. Workers in Animal Dis., Chicago, IL, A104.

13. Besser, T.E., M. Goldoft, and C.C. Gay. 1996. Emergence of *Salmonella typhimurium* DT104 in humans and animals in the Pacific Northwest. INCDNCM, Seattle, WA.

14. Angulo, Fred. 1996. Personal communications.

15. Fedorka-Cray, P.J., D.L. Harris and S.C. Whipp. 1997. Isolated weaning as a method to raise swine culture negative for *Salmonella*. Vet. Med. April, pp.375-382.

16. Gray, J.T., P.J. Fedorka-Cray, T.J. Stabel, and M.R. Ackermann. 1995. Influence of inoculation route on the carrier state of *Salmonella choleraesuis* in swine. Vet. Microbiol. 47:43-59.

17. Gray, J.T., T.J. Stabel, and P.J. Fedorka-Cray. 1996. Effect of dose on the immune response and persistence of *Salmonella choleraesuis* infection in swine. Am. J. Vet. Res. 57:313-319.

18. Gray, J.T., P.J. Fedorka-Cray, T.J. Stabel, and T.T. Kramer. 1996. Natural transmission of *Salmonella choleraesuis* in swine. Appl. Environ. Microbiol. 62:141-146.