Salmonella Typhimurium DT104 Situation Assessment

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Executive summary

Food safety and antibiotic resistance are two aspects of the threat *Salmonella* Typhimurium DT104 poses to the US. This report focuses more on the food safety aspect of DT104. The Food and Drug Administration (FDA) regulates antibiotic use and the issue of antibiotic resistance is considered in greater detail by the Interagency Working Group on Antimicrobial Susceptibility Monitoring chaired by FDA, Center for Veterinary Medicine (CVM). This report is particularly concerned with DT104 as a foodborne pathogen and the potential for mitigation of the risk from this source.

Salmonella Typhimurium DT104 (DT104) is an emerging foodborne pathogen detected in several countries worldwide including: the United States, the United Kingdom (UK), Canada, Germany, France, Austria, and Denmark. Of particular concern with DT104 is the presence of a multiple antimicrobial resistance pattern (R-Type) to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). Molecular studies have demonstrated that in this strain resistance genes are chromosomally encoded. This is important because removal of the selective pressure from antibiotics is not expected to reverse resistance, as it might with extrachromosomal mediated resistance. Also of concern is the increasing trend of additional resistance of DT104 to trimethoprim and ciprofloxacin (a fluoroquinolone) which has occurred in the UK. In the US, DT104 R-type ACSSuT has been isolated from poultry, swine, cattle, and other domestic and wild animals. However, no DT104 R-type ACSSuT isolates in the United States have shown resistance to trimethoprim or ciprofloxacin.

In the UK, DT104 was first detected in 1984. The number of reported human isolates increased from 259 in 1990, to 4,006 in 1996. This rapid increase in DT104 isolates exceeds the increase in *Salmonella* Enteritidis cases in the previous decade, and evidence suggests that the rate of increase is continuing. DT104 is now the second most prevalent strain of *Salmonella* isolated from humans in England and Wales.

In the US, the number of sporadic *Salmonella* isolates from humans that were Typhimurium did not increase dramatically between 1990 (8,510 isolates) and 1996 (9,501 isolates), but the proportion of *Salmonella* Typhimurium isolates that are R-type ACSSuT increased from 9% in 1990, to 33% in 1996. Although all geographic areas had cases, cases were more common in the west. The Centers for Disease Control and Prevention (CDC) was involved in four outbreak investigations of *S.* Typhimurium R-type ACSSuT infections, three on the west coast and one in the northeast. Each of these investigations implicated unpasteurized dairy products. The source of DT104 in two of the outbreaks was traced to dairy farms.

DT104 is an animal pathogen and a foodborne pathogen of humans. The mortality rate for clinically affected cattle with this strain is reported to be 40-60% in some outbreaks in the UK. The case fatality rate in humans may be higher than with other

Salmonella infections. In a study of 83 DT104 cases in the United Kingdom, 41% of patients were hospitalized and 3% died. In contrast, the case-fatality rate for other nontyphoid *Salmonella* infections was approximately 0.1%. In the United States, *S.* Typhimurium R-type ACSSuT infections have been more likely to cause bacteremia and have been associated with longer hospitalizations. No increase in mortality has been observed.

Recent antibiotic use in humans and animals is a predisposing factor for *S*. Typhimurium DT104 infection. This has been observed in other enteric bacterial pathogens as well. Killing the competitive gut flora may allow unrestricted growth of a pathogen. In addition, the use of antibiotics to treat infection prolongs the carrier state of DT104 in humans and animals. Both of these features indicate a need to educate physicians, veterinarians, and the public about the potential adverse consequences of indiscriminate antibiotic use.

Currently, the best prospect for minimizing human illness is a strategy of barriers to introduction and multiplication of DT104 throughout the farm-to-table continuum. No unique control methods are presently available for *S.* Typhimurium DT104 in humans or animals because little is known about the epidemiology of DT104. Control measures that are effective against other types of *Salmonella* spp. Will reduce the likelihood of transmission of *S*. Typhimurium DT104.

Because of the need for a farm-to-table risk reduction strategy for DT104, an interagency team of scientists from FSIS, CDC, APHIS, ARS, and FDA should be equipped with resources to: 1) Facilitate studies in order to develop appropriate risk reduction measures at all segments of the farm-to-table continuum, 2) Communicate research projects and needs to avoid duplication and encourage collaboration, and 3) Develop a coordinated process to investigate DT104 human and animal outbreaks from the point of illness to the source.

Introduction

Food safety and antibiotic resistance are two aspects of the threat *Salmonella* Typhimurium DT104 poses to the US. This report focuses more on the food safety aspect of DT104. The Food and Drug Administration (FDA) regulates antibiotic use and the issue of antibiotic resistance is considered in greater detail by the Interagency Working Group on Antimicrobial Susceptibility Monitoring chaired by FDA, Center for Veterinary Medicine (CVM). This report is particularly concerned with DT104 as a foodborne pathogen and the potential for mitigation of the risk from this source.

For the purpose of this report *Salmonella enterica* serotype Typhimurium definitive type 104 (DT104) refers to *S.* Typhimurium organisms as identified by phage typing procedures. A clone of DT104 has an antimicrobial resistance pattern (R-Type) to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). This bacterial strain most commonly includes a 60 megadalton (MDa) plasmid (epidemic strain) that does not code for antibiotic resistance. It should be noted that not all definitive type DT104 isolates are R-type ACSSuT and not all R-type ACSSuT *Salmonella* Typhimurium are definitive type 104. The relationship between *S*. Typhimurium, R-type ACSSuT, and definitive type 104 needs better quantification.

Recent reports of increasing incidence of a multiple antibiotic resistant strain of *S.* Typhimurium DT104 in humans and cattle have posed a major emerging public health issue of international concern.[1,2,3] Although most of the well-known occurrences were originally reported in the UK, other European countries such as Germany, France, Austria and Denmark have subsequently reported outbreaks.[2] There is a growing emergence and concern in the US as well.[3,4]

A dramatic increase in the total number of human isolations of a multiple antibiotic resistant strain of *S*. Typhimurium DT104 is accompanied by an emerging complexity of multiple antibiotic resistance patterns.[3,5] A recent study in England and Wales indicates a progressive increase in the total number of *S*. Typhimurium DT104 isolations from 259 in 1990 to 4006 in 1996.[6] The R-type ACSSuT pattern with resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines increased from 39% of the DT104 isolates in 1990 to 98% in 1995. Further, a R-type pattern with additional resistance to trimethoprim (ACSSuTTm) that was first reported in 1992 increased from <2% in 1993 to 24% of DT104 isolates in 1996. A third R-type with additional resistance to ciprofloxacin (ACSSUTCp) increased from 1% in 1993 to 14% of DT104 isolates in 1996. This growing drug resistance pattern will complicate the options available in the treatment of salmonellosis.

S. Typhimurium DT104 in Animals

United Kingdom

The *S.* Typhimurium DT104 R-type ACSSuT strain has an extensive reservoir base that encompasses a wide range of species with potential to transmit infection to humans directly and indirectly.[1,7] During the early 1990s in the UK, the prevalence of *S.* Typhimurium DT104 of R-type ACSSuT increased in cattle, calves, swine, sheep and poultry (Table 1). The prevalence of this strain increased in cattle, calves, swine and sheep, accounting for 70%, 72%, 36% and 70% of *Salmonella* isolates respectively in 1995. The most dramatic increase in prevalence for this strain occurred in poultry, which increased from 4% of *Salmonella* isolates in 1993 to 52% in 1995. The Laboratory of Enteric Pathogens in the UK during 1991-1995 received 110 *Salmonella* isolates from cats, 78 (71%) were serotype Typhimurium and 40 (51%) of those were DT104 R-type ACSSuT.[8]

Table 1. Prevalence of *S.* Typhimurium DT104 in Other Countries

Country	Year	Type of Animal	*S.* Typhimurium isolates that are DT104 (%)
United Kingdom (National Veterinary Diagnostic Lab)[a]	1993	adult cattle	45 (96/211)
		calves	45 (300/663)
		swine	13 (32/252)
		sheep	42 (18/43)
		poultry	4 (10/220)
	1995	adult cattle	70 (368/528)
		calves	72 (970/1347)
		swine	36 (101/283)
		sheep	70 (86/122)
		poultry	52 (174/334)
Netherlands[b]	1996		18
Germany[b]			>30
Canada[b]	1986-1996		15

[a]Data from *Salmonella in Livestock Production*, 1995, Ministry of Agriculture Fisheries and Food, Veterinary Laboratory Agencies, UK. [b]Data from the International Workshop on Multidrug resistant *S.* Typhimurium DT104 May 1, 1997, CDC.

United States

Retrospective evidence from banked *Salmonella* isolates in Washington state indicate that *S*. Typhimurium DT104 R-type ACSSuT has been present in cattle in the US since at least 1993 (personal communication, Dan Rice, Washington State University). This strain has also been isolated from a wide variety of other animals in the US including pig, sheep, chicken, turkey, horse, goat, emu, cat, dog, elk, mouse, coyote, ground squirrel, raccoon, chipmunk and several species of birds.[4]

Currently in the US there are three sources of animal prevalence information on *S*. Typhimurium DT104 R-type ACSSuT: 1) The National Antimicrobial Susceptibility Monitoring System, 2) The National Veterinary Services Laboratories (NVSL), and 3) Washington State University investigations. Differences related to the design of these studies must be kept in mind when interpreting and comparing this information. None of these studies represent solely a random sampling of *Salmonella* isolates from animals in the US and therefore can not be considered representative of a national prevalence. The majority of isolates available for testing from the NVSL and from Washington State are clinical specimens submitted from veterinarians, thus livestock producers who are less likely to utilize the services of a veterinarian are excluded from the database. The National Antimicrobial Susceptibility Monitoring System has both clinical and non-clinical isolates. Farms from which several isolates were submitted for analysis may skew the interpretation of the data and overestimate the overall prevalence of *S*. Typhimurium. The Washington State study reports data on cattle only, whereas the other two sources include a variety of species.

All three sources tested isolates by first identifying *S*. Typhimurium by serotyping. Antibiotic susceptibility testing was then performed to identify the ACSSuT resistance pattern. Finally, *S*. Typhimurium isolates demonstrating R-type ACSSuT were phage typed to identify DT104. Phage typing has not been completed for all isolates reported. The R-type pattern ACSSuT is considered a good marker for the phage type DT104 in the US and is the test more commonly applied in the US since most laboratories are equipped to perform it. An *S*. Typhimurium isolate with the ACSSuT R-type pattern is not, however, always phage type DT104 and DT104 isolates with resistance patterns other than R-type ACSSuT will not be detected.

The National Antimicrobial Susceptibility Monitoring System is an ongoing interagency program to provide descriptive data on the extent and temporal trends of antimicrobial susceptibility of *Salmonella* isolates from human and animal populations. The 1995 baseline study for the animal portion of this program tested 1,041 animal *Salmonella* isolates for antibiotic susceptibility. The animal isolates came from healthy and ill animals throughout the US. In 1996, the number of animal *Salmonella* isolates tested was 1,922. The species distribution changed from 1995 to 1996 making comparison of results between years difficult. Animal isolates included in this program came from multiple sources such as NVSL, the National Animal Health Monitoring System (NAHMS), the National Animal Disease Center, the Food

Safety and Inspection Service (FSIS) the National Poultry Improvement Plan, and research projects of collaborators.

Though the types of species and their distribution are similar from year to year, the sources of the isolates and exact proportions of types of animals vary. Caution in making direct year to year comparisons of the results is advised. The proportion of *S.* Typhimurium isolates which were R-type ACSSuT in 1995 was 10.2% (14/137), in 1996, 10.5% (45/427). The proportion of *S.* Typhimurium R-type ACSSuT isolates which are DT104 in the National Antimicrobial Susceptibility Monitoring System studies are 64% (9/14) for 1995 and 22.2% (10/45) for 1996.

The NVSL study included *Salmonella* isolates from various species and from clinically ill and healthy animals submitted to NVSL from October 1996 through mid February 1997. The proportion of *S*. Typhimurium R-type ACSSuT among *S*. Typhimurium isolates (26%) in the study is higher than in the National Antimicrobial Susceptibility Monitoring System 1996 study (10.5%). Possible explanations for the differences are the inclusion of more healthy animals in the National Antimicrobial Susceptibility Monitoring System study than in the NVSL study, differing proportions of the various species included in the two studies, and that many samples were collected much longer before submission in the animal segment of the National Antimicrobial Susceptibility Monitoring System.

Washington State University investigators recognized outbreaks of salmonellosis in dairy herds in the Pacific Northwest over the past several years associated with the R-type ACSSuT strain of *S*. Typhimurium.[4] In a retrospective analysis of antibiotic resistance patterns of isolates submitted to the diagnostic lab at Washington State University, none of 44 *S*. Typhimurium isolates submitted prior to 1986 had the R-type ACSSuT. Between 1986 and 1991, 13% of 83 *S*. Typhimurium isolates were identified as R-type ACSSuT and this proportion increased to 64% of 51 isolates between 1992 and 1995. The reported annual number of animal *S*. Typhimurium cases in the Pacific Northwest has not shown an increasing trend. This possibly indicates a competitive advantage for phage type DT104 R-type ACSSuT,[9] resulting in a reduction in other serotypes of *Salmonella* while DT104 increased. Sixty of the isolates were phage typed and 95% (57/60) were DT104.

Additional prevalence information for *S*. Typhimurium DT104 in cattle will be available in the future. NAHMS conducted a national survey of dairy operations in 1996. A stratified random sample of dairy operations from 20 selected states that represent 83% of US dairy cows was chosen for the study. Fecal samples were obtained from 100 farms and 100 livestock markets (cull dairy cows) and tested for *Salmonella* spp. including *S*. Typhimurium DT104. Information was collected which will allow evaluation of herd-level and animal-level risk factors for *Salmonella* shedding. In August 1997 NAHMS began collecting fecal samples from beef cows on 200 operations across the US. These will be cultured for the presence of *Salmonella* spp. including *S*. Typhimurium DT104.

Demographic, Geographic and Seasonal Distribution

Little information is available on the demographic distribution of the *S.* Typhimurium DT104 T-type ACSSuT strain in animals other than cattle. In the UK in 1995, a similar proportion of *Salmonella* isolates among calves (72%) were DT104 compared to adult cattle (70%) (Table 1). Calves were more severely affected than adult cattle in a case-control study conducted in the U.K.[1] Investigators in the northwest US however, have reported that clinical disease occurred predominantly in cows in the immediate post calving period and less commonly in young calves.[4] Outbreaks of DT104 in cattle in the US have occurred primarily in dairy cattle herds with only a few incidents in beef cow-calf herds (Besser, 1997, Workshop Minutes). In the northeast US, eight outbreaks on dairy farms of *S.* Typhimurium resistant to ampicillin, chloramphenicol and tetracycline (testing did not include sulfonamides or streptomycin) were reported from 1996 through May 1997 (personal communication, Dr. Pat McDonough, Cornell University). Multiple age groups of cattle were affected in these outbreaks in the northeast US.

DT104 is present in many areas of the US. Northern regions of the country, especially the northwest, may have higher prevalences than other regions (Dan Rice, written communication, Workshop Minutes). Whether this is due to differences in active surveillance or real differences is unknown. In a study of cull dairy cattle at five slaughter establishments widely separated across the United States all *S.* Typhimurium DT104 were obtained from cull dairy cows at the western establishment. These samples constituted 88.6% (31/35) of the *S.* Typhimurium examined from that location.[10]

Seasonality has been identified as a factor in the distribution of *S.* Typhimurium DT104 in cull dairy cattle. Thirty of the 31 (96.8%) *S.* Typhimurium identified as DT104 were isolated during the winter sampling period.[10] In a case-control study of cattle conducted in the UK, cases were more common in the summer months, associated with the calving season, than the winter months.[1]

Clinical Characteristics and Duration of Shedding

DT104 appears to cause a higher morbidity and mortality rate among infected animals than other nontyphoid *Salmonella* infections.[11] The mortality rate for clinically affected animals with this strain is reported to be 50-60% in some outbreaks in the UK (Workshop Minutes). In a case-control study of cattle in the UK, a 40% case fatality rate was reported, with a higher rate among calves than among adult animals.[1] Clinical signs of DT104 in cattle included pyrexia (fever), depression and mental dullness, decreased milk production, anorexia, dehydration with scleral injection, increased saliva production, and diarrhea progressing to dysentery.[1] Animals may be asymptomatic carriers of DT104 and shed large numbers of organisms for up to 18 months following an outbreak.[1,8] Cats have been reported to shed the organism up to 14 weeks and symptomatic cats are reported to shed large numbers of organisms from the mouth.[22]

Risk Factors

A case-control study of risk factors for *S*. Typhimurium DT104 infection was reported in 1996.[1,12] The study population included all cattle herds that submitted specimens to the diagnostic laboratory services of the Ministry of Agriculture, Fisheries, and Food Veterinary Investigation Centres and Scottish Agricultural Colleges from May 1994 to October 1995. Statutory reporting of all *Salmonella* is mandated for all food animal species and some other species under the Zoonosis Order, 1989 and the Poultry Breeding Flocks and Hatcheries Order, 1993. *Salmonella* isolates are serotyped under the Kauffman White Scheme and phage typed according to PHLS schemes. Case herds were herds in which *S*. Typhimurium DT 104 R-type ACSSuT was isolated from at least one bovine on the premises. Group B *Salmonella* isolated from bovines during standard diagnostic investigation were evaluated for resistance to ampicillin, chloramphenicol, tetracycline, and trimethoprim to identify the epidemic strain. Control herds were selected randomly from all non-case submissions during the same time as the case herds. Data were collected via personal interviews at the farm by Veterinary Investigation Officers. Outbreaks were more prevalent in dairy herds than in beef herds, mixed dairy and beef herds, and calf herds. Outbreaks were more prevalent in larger herds and during the summer which corresponds to the calving season for dairy herds in the UK. The within-herd prevalence ranged from 0.1 to 100.0 percent, but the median prevalence was only 4%. The most frequently reported clinical signs were watery diarrhea, anorexia, and poor condition. Exposure variables containing two to eight levels of exposure were analyzed for association between *S*. Typhimurium DT 104 infection and exposure. Statistically significant risk factors were identified using univariate and multi variable analyses. Odds ratios and 95% confidence intervals, adjusted for two potentially confounding variables (geographic location and the season in which the laboratory diagnosis was made), were reported. There was no indication that herd-size was considered as a confounder.

Five of the ten risk factors that were statistically significant in the univariate analysis were included also in the multivariable analysis (Table 2). Three additional risk factors were statistically significant in the univariate analysis, but were excluded from the multivariable analysis due to a high percentage of missing data points (Table 2). A total of 28 odds ratios were reported for the 10 risk factors because multiple levels of several risk factors were analyzed. Given that 28 odds ratios were reported, of which 5 were statistically significant, at least one of the 5 could have been significant by chance alone.

Table 2. Case-control Studies of Risk Factors for Salmonella Typhimurium DT104 Infection of Cattle in Great Britain.[a]		
Risk Factors	**Odds Ratio**	**95% C.I.[b]**
Dealer herds[c]	14.25	1.14 to 177.1
New herd additions within 14 days preceding the index case[c]	2.58	1.36 to 4.92
Confinement housing[c]	2.79	1.24 to 6.29
Water source = main[c]	2.01	1.37 to 2.94
Sick animals and difficult calvings housed together[c]	1.51	1.06 to 2.16
High feral cat density[d]	1.35	1.09 to 1.65
Wild bird access to feed storage facilities[d]	1.67	1.11 to 2.51
Purchase cattle from dealer herds[d]	3.9	1.62 to 9.36

[a] These results are from Evans, 1996 and Evans and Davies, 1996. The study included 656 cases and 505 controls.
[b] C.I. - confidence interval
[c] Results of multivariable statistical analysis
[d] Results of univariate statistical analysis

As with other enteric bacterial pathogens, recent antibiotic use is a risk factor for infection with *S*. Typhimurium DT104. In the US, *S*. Typhimurium with resistance to ampicillin, chloramphenicol and tetracycline (streptomycin and sulfonamides were not included in resistance testing), caused three cat associated outbreaks, one in a veterinary hospital in New York and two in veterinary hospitals in California between 1996-1997. A predisposing event in these cat outbreaks was prophylactic antibiotic therapy (personal communication, Dr. Pat McDonough, Cornell University). Recent antibiotic use may kill competitive gut flora allowing unrestricted growth of the pathogen.

Current control methods in animals

Unique control methods are presently not available for *S.* Typhimurium DT104 because little is known about the epidemiology of DT104. The same methods that are effective against other types of *Salmonella* spp. i.e. good production practices, should help control harborage and transmission of DT104. These generally rely on decreasing exposure and increasing immunity of susceptible hosts. *S.* Typhimurium DT104 has been isolated from a wide range of species including livestock, companion animals, and wildlife. The widespread distribution of DT104 makes effective controls potentially more problematic than the control of

S. Enteritidis phage type 4, which has a reservoir mainly confined to poultry. Some of the risk factors associated with the spread of DT104 among cattle were listed in Table 2. Further studies on the spread of DT104 are needed.

Of particular importance from a control point of view in cattle is the role of asymptomatic carriers. Following rccovery from illness, *S*. Typhimurium DT104 has been found in fecal samples of clinically normal cattle for varying periods up to 18 months.[1,13] In one instance when a carrier was introduced to another healthy group, it triggered infection leading to clinical signs in the healthy group. Identifying clinically ill herds as a source of asymptomatic shedders may be one beneficial control measure to minimize the spread of DT104.

Flock or herd exposure is decreased through cleaning and disinfecting premises, controlling rodents, and minimizing contamination of feed. Cleaning and disinfecting premises and controlling rodents are most effective in closed environments. Once introduced, it is difficult to consistently eliminate *Salmonella* spp. from the environment. Oosterom showed that reduction but not elimination of *Salmonella* spp. in swine was possible through an integrated program of rodent control, cleaning and disinfecting, and pelletizing feed.[14,15]

In contrast Wray reported that *Salmonella* spp. were isolated from the environment of six of nine dairy farms after cleaning and disinfecting.[16] The *Salmonella* Enteritidis Pilot Project isolated *S.* Enteritidis in approximately 50% of the layer houses after cleaning and disinfecting.[17] In a few cases *S.* Enteritidis was isolated from houses that had previously tested negative. This project also showed that rodent control in layer houses decreased the probability of the houses being contaminated with *S.* Enteritidis.

Numerous reports have shown that although animal feed is frequently contaminated with *Salmonella* spp, the *Salmonella* spp. in feed are not necessarily the same as the *Salmonella* spp. isolated from animals. Veldman described a survey of poultry feed in which 10% of 360 samples of poultry feed in the Netherlands were contaminated with *Salmonella* spp.[18] No *S.* Enteritidis was isolated despite an ongoing outbreak of *S.* Enteritidis in poultry flocks at the time.

Research to control salmonellosis has focused on the use of bacterins to increase immunity. Davies reported that a killed bacterin was effective in controlling *S.* Typhimurium in cattle.[19] Hassan reported that a modified live bacterin prevented infection of virulent *S.* Typhimurium in chickens.[20] The Salmonella Enteritidis Pilot Project showed that layer hens vaccinated with a killed *S.* Enteritidis bacterin were less likely to lay *S.* Enteritidis contaminated eggs.

S. Typhimurium DT104 in humans

United Kingdom

S. Typhimurium DT104 R-type ACSSuT was first detected in humans in the UK in 1984. Initially, most DT104 isolates were associated with foreign travel and few DT104 isolates were multidrug resistant. In the late 1980s, the number of human isolates of DT104 in the United Kingdom began to increase remarkably and an increasing proportion of the isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT). In the 1990s, the number of reported human isolates of DT104 R-type ACSSuT increased from 259 in 1990, to 4,006 in 1996. This rapid increase in DT104 R-type ACSSuT isolates exceeds the increase in *S.* Enteritidis cases in the previous decade, and there is evidence that the rate of increase is continuing. By 1995, *S.* Typhimurium DT104 R-type ACSSuT had become the second most commonly isolated *Salmonella* from humans, second only to *S.* Enteritidis phage type 4. In 1996, DT104 R-type ACSSuT accounted for more than 10% of human *Salmonella* isolates in the United Kingdom, and caused an estimated 30,000-300,000 human infections.[5]

Of additional concern is the increasing proportion of human DT104 R-type ACSSuT isolates which are resistant to antibiotics important in the treatment of life-threatening invasive *Salmonella* infections. The proportion of DT104 isolates resistant to trimethoprim increased from <2% in 1993 to 24% in 1996, while the proportion resistant to ciprofloxacin increased from 1% in 1993 to 14% in 1996.

Other non-US countries

The first known human *S.* Typhimurium DT104 isolate was identified in Canada in 1970 (Workshop Minutes). *S.* Typhimurium DT104 R-type ACSSuT has been reported from many European countries, including Denmark, Germany, France and Italy. Although precise information on the incidence of DT104 is not available, several countries have reported increases similar to the United Kingdom. In addition, the increase in reported *S.* Typhimurium DT104 occurred at approximately the same time. Molecular studies indicate that the organism in these additional countries is very similar, if not identical, to the organism in the United Kingdom (personal communication, John Threlfall, PHLS, London).

United States

Preliminary data suggests that DT104 may have emerged in the United States at approximately the same time as in the United Kingdom. Each year in the United States, there are an estimated 800,000 - 4 million cases of salmonellosis of which approximately 40,000 are culture-confirmed and reported to the Centers for Disease Control and Prevention (CDC). *Salmonella* isolates are not routinely forwarded to CDC from state health departments. However, special studies were conducted in selected counties in 1980, 1985, 1990, and 1995 in which all *Salmonella* isolates were forwarded to CDC for antimicrobial susceptibility testing. The proportion of *S.*

Typhimurium isolates in the selected county studies which were R-type ACSSuT increased from 2% in 1980, to 4% in 1985, to 9% in 1990, and to 12% in 1995 (workshop minutes). The proportion of those R-type ACSSuT which were DT104 was 0% in 1980, 25% in 1985, 50% in 1990, and 85% in 1995.

In 1995, all 50 states participated in the National *Salmonella* Antimicrobial Resistance Study where they sent every tenth isolate to CDC. Twenty-eight percent (275/976) of the *S.* Typhimurium isolates were R-type ACSSuT, and approximately 85% of those isolates were DT104. Although all geographic areas had cases, cases were more common in the west.

In 1996, isolates from 14 state and local health departments participating in the National Antimicrobial Susceptibility Monitoring System were sent to CDC. Thirty-three percent (110/304) of *S.* Typhimurium isolates were R-type ACSSuT, and approximately 85% of those isolates were DT104. Cases were more common in the west. To date, no DT104 R-type ACSSuT isolates in the United States have shown resistance to trimethoprim or ciprofloxacin.

Clinical features in humans

The clinical features associated with infection with DT104 R-type ACSSuT may be more severe than other *Salmonella* infections. In a study of 83 DT104 cases in the United Kingdom, 41% of patients were hospitalized and 3% died. In contrast, the case-fatality rate for nontyphoid *Salmonella* infections is approximately 0.1%. In the United States, *S.* Typhimurium R-type ACSSuT infections have been more likely to cause bacteremia and have been associated with longer hospitalizations; further investigations are ongoing (Workshop Minutes).

Risk factors for infection

Consumption of food items contaminated with *S*. Typhimurium DT104 and direct contact with infected animals are important risk factors in the transmission cycle. Often these are foods of animal origin such as beef, pork or poultry. In a case-control study of sporadic DT104 infections in the United Kingdom in 1993, infection was associated with eating chicken and pork sausages.[21] In 46 outbreak investigations of DT104 infections conducted in the United Kingdom from 1992 to 1996, 78% (n=36) of the outbreaks were attributed to foodborne transmission and 15% (n=7) were associated with direct contact with farm animals (personal communication, Patrick Wall, PHLS, London). A variety of food items were implicated.

Epidemiological investigations in the United States are ongoing. Preliminary analysis of a national case-control study of sporadic *S.* Typhimurium R-type ACSSuT infections did not clearly implicate specific food items. There was an association between infections and direct contact with animals with diarrhea or visiting farms. Such contact, as in the United Kingdom, may explain perhaps 10% of cases and represents a higher proportion of illness associated with animal contact compared to other *Salmonella* infection. In addition, a high proportion of persons infected with *S.*

Typhimurium R-type ACSSuT reported taking antibiotics, particularly penicillin-type antibiotics (amoxicillin, ampicillin, etc) compared to controls, indicating the need to educate physicians and the public about the potential adverse consequences of widespread antibiotic use among humans.

There have been five outbreaks of human illness identified in the United States (two in California, one each in Washington, Nebraska, and Vermont). Four of these outbreaks involved consumption of unpasteurized dairy products or contact with dairy cattle.

The presence of a large, cat population residing indoors as pets in close association with humans could be an important epidemiological factor in the spread of this disease to humans. Cats have been reported to shed *S.* Typhimurium DT104 in feces for twelve weeks or longer after recovery from an acute illness.[22] The cycle may be perpetuated in a barn setting where cats contaminate animal feeds.

The association between ill animals and *S*. Typhimurium DT104 infections in humans has also been described in Washington State where a higher proportion of persons with *S*. Typhimurium DT104 infections lived in counties with a large number of dairy cattle compared to persons with other *Salmonella* infections. Field investigations of *S*. Typhimurium DT104 outbreaks on dairy farms in Washington and New York provide anecdotal reports that a higher proportion of these farm workers had recent diarrheal illness compared to outbreaks of other *Salmonella* spp. on other dairy farms. CDC has been involved in four outbreak investigations of *S.* Typhimurium R-type ACSSuT infections, three on the west coast and one in the northeast. Each of these investigations have implicated unpasteurized dairy products and two of the investigations have been traced to dairy farms.

Current control methods in humans

No unique control methods are presently available for *S.* Typhimurium DT104 in humans because little is known about the epidemiology. Control measures that are effective against other types of *Salmonella* spp. may reduce the likelihood of transmission of *S*. Typhimurium DT104. Controlling *Salmonella* spp. in humans relies on decreasing exposure through hygienic processing of products and proper preparation and storage of cooked products. Specific measures include thoroughly cooking foods of animal origin, avoiding cross contamination of other foods, avoiding consumption of unpasteurized dairy products, and educating food handlers. Even in the absence of adequate control methods in animals, instituting control methods at food preparation can still minimize the number of human outbreaks. [23] More research is needed on risk factors for foodborne illness from *S.* Typhimurium DT104.

Current economic impact

The following estimated numbers of US *S.* Typhimurium DT104 cases are based on several assumptions that must not be over interpreted. Some data from sentinel projects, which may not be generalizable, are extrapolated to make national assumptions. Percentages of *Salmonella* surveillance isolates identified as a particular serotype or subtype are based on small numbers that may not be stable. The following two methods provide estimates of the *S.* Typhimurium DT104 burden in the US.

1. US estimates of the total number of human *Salmonella* infections range from 800,000 to 4,000,000 per year.[24] Approximately 24% of the 4024 *Salmonella* isolates in the 1995 National *Salmonella* Antimicrobial Resistance Study and 24% of the 39,032 *Salmonella* isolates reported to CDC in 1996 were *S.* Typhimurium. Approximately 33% of *S.* Typhimurium isolates are of R-type ACSSuT. Approximately 93% of the *S.* Typhimurium isolates with R-type ACSSuT are in the DT104 complex. These percentages produce a cumulative multiplier (24% * 33% * 93%) of 7.4%. Multiplying 0.074 by the 800,000 to 4,000,000 estimated *Salmonella* infections per year, yields an estimated range of 59,200 to 296,000 *S.* Typhimurium DT104 cases per year.

2. Similar estimates are obtained using a different technique. The sequential surveillance artifact for *Salmonella* has been estimated at 39:1; in other words, for each reported culture confirmed case, thirty-nine people were initially infected.[25] There are approximately 10,000 culture confirmed cases of *S.* Typhimurium annually. Assuming the general *Salmonella* artifact applies to *S.* Typhimurium, and there is no a priori reason to believe otherwise, we estimate 390,000 people are infected with *S.* Typhimurium per year. Using the previous percentage estimates of R-types and definitive types, an estimate of 120,000 *S.* Typhimurium DT104 cases per year is obtained. This value lies within the range derived from the first method.

Cost of illness estimates are based on the number of foodborne cases and deaths, number of cases with secondary sequela, the associated costs of medical care, lost productivity, and other costs such as special education and residential-care costs.[26] There is equivocal evidence on whether human infection with DT104 is more severe than infection with other non-typhoidal *Salmonella*. A study in the UK showed that 78% of 46 outbreaks of DT104 were of foodborne origin. (personal communication, Patrick Wall, PHLS, London) Using the above estimates, 46,000 to 231,000 *foodborne* cases of DT104 occur per year. Fifty-eight to 128 of these may result in death.[26] Two *S.* Typhimurium DT104 costs of illness estimates can be obtained[26]:

Using a $5 million (1995 dollars) statistical life value estimated from wage-risk studies, the financial burden of foodborne non-typhoidal *Salmonella* has been estimated at $4.8 – 12.2 billion per year. Using the above 0.074 multiplier and a $5 million (1995 dollars) statistical life value estimated from wage-risk studies, the financial burden of *S.* Typhimurium DT104 infection on the US population is estimated to be $360 to 900 million per year.

A human capital approach, increased by a willingness-to-pay multiplier, estimates the value of a statistical life, depending on age, to range from roughly $15,000 to $1,979,000 in 1995 dollars. Using the human capital approach, the financial burden of foodborne non-typhoidal *Salmonella* has been estimated at $0.9 – 3.5 billion per year. Using the above 0.074 multiplier, the financial burden of *S.* Typhimurium DT104 infection on the US population is estimated to be $67 to 260 million per year.

Comparison to other priority pathogens

The priority rating process provides a relative public health ranking of foodborne pathogens associated with beef at slaughter.[27] Although the following relative priority ranking applies specifically to beef, this does not imply that beef is the primary foodborne vehicle for DT104. Epidemiologic investigations are needed to define the major DT104 risk factors. The following assumptions were made in completing the priority rating model to determine the relative ranking of DT104. These assumptions are largely reflective of knowledge about *Salmonella* spp. in general, not *Salmonella* Typhimurium DT104 in particular.

- All demographic groups susceptible to infection.
- Less than 10^6 estimated cases per year.
- Low current consumer concern.
- 0.5-5% case fatality rate.
- 3 – 7 day duration of infection.
- Multiple gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea) likely in infected people.
- Moderate extra-intestinal symptoms in the general population.
- Rarely detected with current organoleptic slaughter inspection procedures.
- Agent can multiply on product to reach infectious dose.
- Moderate infectious dose (10^2 to 10^5).
- Low prevalence on carcasses (0.1 to 0.5% positive).

Using the priority rating method, *S.* Typhimurium DT104 ranked fifth, below the other priority pathogens *E. coli* O157:H7, *Salmonella* spp. (excluding DT104), *Listeria monocytogenes*, and *Campylobacter* (Table 3). This ranking places *S.* Typhimurium DT104 in the upper tier of beef associated foodborne agents of concern.

Table 3. Priority rating for six beefborne pathogens	
Agent/Condition	**Priority Rating (max=90)**
E.coli 0157:H7	65
Salmonella spp.	63
Listeria	61
Campylobacter	55
*S.*Typhimurium DT104	44
Cysticercus bovis	40

Research Gaps

Limited information concerning the prevalence and distribution of DT104 in humans and animals in the US is available. There are few analytic studies to date in the literature which address risk factors and transmission of DT104 in the US. Because of the need for a farm-to-table risk reduction strategy for DT104, an interagency team of scientists from FSIS, CDC, APHIS, ARS, and FDA should be equipped with resources to: 1) Facilitate studies in order to develop appropriate risk reduction measures, 2) Communicate research needs and work in progress to avoid duplication and encourage collaboration, and 3) Develop a coordinated process to investigate DT104 human and animal outbreaks from the point of illness to the source to identify risk factors for the reduction of the organism at all segments of the farm-to-table continuum.

Future research concerning *S.* Typhimurium DT104 in the areas of surveillance, analytic epidemiology, microbiology and pharmacology, should address the following objectives.

Surveillance

Human:

- determine and monitor national incidence, prevalence and distribution of sporadic infections
- develop an outbreak surveillance system
- test *Salmonella* isolates for flouroquinolone, trimethoprim, and apramycin/aminoglycoside resistance

Animal:

- determine and monitor national prevalence and distribution of infection by species and by clinical status

- develop an outbreak surveillance system
- conduct carcass and product testing
- test Salmonella isolates for flouroquinolone, trimethoprim, and apramycin/aminoglycoside resistance

Analytic Epidemiology

Human:
- Risk factor studies to determine: role of antibiotic use, behaviors associated with infection, demographic risk factors
- Transmission studies to determine: role of specific foods in transmission, live animal (pets, livestock) to human transmission, waterborne transmission, person-to-person transmission, restaurant versus home transmission
- Identify and evaluate control measures: determine effectiveness of education and other control programs

Animal:
On Farm
- Risk factor studies to determine: demographic risk factors; role of animal movement both on farm and livestock markets, sales, trucks; role of antibiotics; role of farm management factors
- Transmission studies to determine: role of environmental contamination eg. water, feed, sludge, rodents, wildlife; role of direct animal to animal and human to animal transmission; watershed contamination.
- Identify and evaluate control measures: determine effectiveness of improved biosecurity, sanitation and hygiene measures in preventing transmission; effectiveness of potential methods to increase animal immunity (bacterins, vaccine, feed type, competitive exclusion), quality assurance programs.

Processing
- Evaluate the effectiveness of HACCP systems in decreasing carcass contamination with DT104
- Evaluate the effectiveness of procedures such as irradiation, carcass washing, steam pasteurization, etc. in reducing DT104

Microbiology
- Standardize lab diagnostic procedures
- Examine usefulness of various subtyping techniques (PFGE, Plasmid profiles, PCR)
- Establish reference database of molecular subtyping information
- Determine specific organism characteristics such as: heat and acid tolerance, infective dose, ecology, survivability in the environment in various media, etc.
- Evaluate potential for DT104 to acquire new resistance (in-vitro studies)
- Determine significance and role of Copenhagen variant
- Evaluate the genetic relatedness of DT104 isolates

Pharmacology

- Evaluate the effect of antibiotic use on infection, shedding, etc.

Conclusions

Salmonella Typhimurium DT104 is an emerging pathogen detected in several countries worldwide including: the United States, the United Kingdom, Canada, Germany, France, Austria, and Denmark. Illness has been associated with the consumption of pork sausages, chicken, unpasteurized dairy products, a brand of meat paste, and direct contact with ill animals. Much additional information is needed about the epidemiology of DT104 in the US.

Of particular concern with DT104 is the presence of a multiple antimicrobial resistance pattern (R-Type) to ampicillin, chloramphenicol., streptomycin, sulfonamides, and tetracycline (ACSSuT). Molecular studies have demonstrated that in this strain resistance genes are chromosomally encoded. This is important because removal of the selective pressure from antibiotics is not expected to reverse resistance, as it might with plasmid mediated (non-chromosomal) resistance. Also of concern is the increasing additional resistance of DT104 to trimethoprim and ciprofloxacin (a fluoroquinolone) which has occurred in the UK. To date, no DT104 R-type ACSSuT isolates in the United States have shown resistance to trimethoprim or ciprofloxacin.

Pathogen reduction efforts in the production segment of the farm-to-table continuum will be difficult with DT104. DT104 has a broad reservoir base in domestic, companion, and wild animals. A significant rate of morbidity has been observed in infected livestock and poultry and a significant rate of mortality has been identified in cattle. Producers will be more willing to pursue control when their livelihood is affected. On the other hand, control efforts must be targeted to a wide range of animals. Further study is needed on the ecology of DT104 on farms to identify effective controls.

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