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The Veterinarian's Role in Diagnosis, Treatment, and Prevention of Multidrug Resistant *Salmonella typhimurium* DT104

David A. Dargatz, CEAH, USDA-APHIS-VS, Ft. Collins, CO
Scott J. Wells, CEAH, USDA-APHIS-VS, Ft. Collins, CO
Paula J. Fedorka-Cray, Richard Russell Research Center, USDA-ARS, Athens, GA
Judy Akkina, CEAH, USDA-APHIS-VS, Ft. Collins, CO

Overview of *Salmonella* and *Salmonella typhimurium* DT104 in the US

Clinical and subclinical *Salmonella* infections in livestock have long been a source of concern to industry from both the animal disease and human health perspectives (Robinson et al., 1992). *Salmonella* food poisoning in humans remains one of the major causes of gastroenteritis in the developed world (Cabello et al., 1992). Based on reports from the Centers for Disease Control and Prevention (CDC), *Salmonella* was the leading cause of foodborne illness outbreaks in the US between 1973 and 1987 (Bean and Griffin, 1990). In recent years, there has been a perception that the frequency of *Salmonella* infections in both humans and animals is increasing. This may be due in part to a growing awareness among producers and veterinarians, improved recovery and identification techniques for *Salmonella*, and the media publicity given human outbreaks of salmonellosis associated with food animal products (Robinson et al., 1992).

Financial losses resulting from *Salmonella* infections are considerable (Hogue et al., 1997). A 1996 Economic Research Service (ERS) report of foodborne *Salmonella* infections in humans estimated a total cost of $0.6 to $3.5 billion dollars annually in medical expenses and productivity losses (Buzby et al., 1996). Other costs to producers, which were not accounted for in this review, include treatment costs and livestock deaths resulting from *Salmonella* infections, increased cull rates, reduced feed efficiency and decreased weight gain.

Public and animal health agencies are becoming increasingly concerned about the occurrence of *Salmonella typhimurium* (definitive type [DT] or phage type) 104 that is resistant to at least five antimicrobics, ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. One of the concerns regarding this pathogen is that it is associated with higher hospitalization and mortality rates among people than for other *Salmonella* infections (Wall et al., 1994).

The pattern of DT104, including multidrug resistant (mr) DT104 (mrDT104), occurrence in the US is not well established and requires further investigation. Based on the evaluation of cattle *Salmonella* isolates which have been banked over time, it appears that mrDT104 has been present in the US since at least the early 1990's (T.E. Besser, personal communication). According to data accumulated by the National Veterinary Services Laboratories (NVSL) and reported by the US Animal Health Association, the percentage of *Salmonella* typhimurium isolates from clinically ill animals has increased slightly since 1990. Whether or not this increase is due to increases in mrDT104 is unknown. Estimates of national disease prevalence and/or incidence derived from diagnostic laboratory accessions should be interpreted with caution (Robinson et al., 1992). Some of the acknowledged limitations of the NVSL *Salmonella* data bank include the following:

- Most diagnostic laboratories accept specimens only from licensed veterinarians, thus, livestock producers who are less likely to utilize the services of a veterinarian are under-represented in the database.
- Often, no distinction is made between subgroups within a specific commodity (i.e., beef, dairy, veal and other subgroups as well as juveniles vs. adults).
- Seasonality of disease occurrence is difficult to determine because isolates are often batched over a period of time before being sent to a diagnostic laboratory.

- Information regarding the number of sources represented for any given year in which isolates were collected is not provided. Thus, a farm from which several isolates were submitted for analysis may skew interpretation of the data and overestimate the incidence of disease.
- Samples sent to diagnostic laboratories represent predominantly ill animals.
- Samples taken during disease outbreaks are more likely to be submitted to diagnostic laboratories than isolates from individual cases.

While the NVSL *Salmonella* database does not represent a random sample of *Salmonella* isolates in the US, it does represent the most comprehensive and consistent collection of data on *Salmonella* serotypes from clinical cases available on a nationwide basis at this time (Robinson et al., 1992). It should be noted that in the United Kingdom (UK), both human and animal *Salmonella typhimurium* DT104 isolation reports also come from diagnostic laboratories and therefore may not reflect the actual strain prevalence in the general population. Similar to the US situation, they represent the most consistent UK collection of data on serotypes from clinical cases.

What is *Salmonella typhimurium* DT104?

Salmonella typhimurium DT104 is a subpopulation of the *Salmonella* serotype typhimurium which reacts in a specific way when tested against a battery of bacteriophages (a type of virus which infects and, in some cases, kills a bacterial organism). The type(s) of phages capable of infecting and killing a bacteria are used as a means of classifying bacteria into "phagetypes" or "definitive phagetypes". Phagetyping can be used as a tool to distinguish between various strains of a serotype, such as *Salmonella typhimurium*, which can cause disease outbreaks.

In addition to phagetype characteristics, a hallmark of mrDT104 is the fact that isolates are resistant to at least five antimicrobial drugs: ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT). Some isolates of mrDT104 in the UK have shown additional resistance to trimethoprim (25 percent in 1996) (Threlfall et al., 1997). For fluoroquinolones the National Committee for Clinical Laboratory Standards (NCCLS) has determined that isolates with minimum inhibitory concentration (MIC) values less than 2 lg/ml are susceptible to fluoroquinolones and those with MIC values m4 lg/ml are considered resistant. In 1996 the MIC of some of UK human mrDT104 isolates shifted within the NCCLS susceptible range. Because the UK uses a lower MIC (.25 lg/ml) to define resistance, 14 percent of mrDT104 isolates were considered "resistant" in the UK in 1996 (Threlfall et al., 1997). Since fluoroquinolones (ciprofloxacin, etc.) are currently the drug of choice for treating highly invasive cases of *S. typhimurium* in humans this shift in MIC values for some isolates may pose serious public health implications if they continue toward resistance (Threlfall et al., 1996).

The genes encoding antibiotic resistance in mrDT104, with the exception of trimethoprim, have been chromosomally integrated as opposed to being plasmid-mediated (Threlfall et al., 1996). Chromosomal integration is a mechanism by which bacteria can retain resistance patterns permanently, even in the absence of the selective pressure of antibiotics. Therefore, withdrawal of antibiotic agents is unlikely to have any impact on decreasing resistance. This situation would most likely limit the choices for effective drug selection among available products (Threlfall et al., 1994).

Have there been any human or animal outbreaks of DT104 in the US?

To date, there have been five reports of localized mrDT104 outbreaks in the US. A joint outbreak investigation between the USDA and CDC was recently conducted in Vermont involving mrDT104 infections in humans and dairy cattle. Two human outbreaks of mrDT104 in California and one in Washington have implicated noncommercial/ homemade cheese as the vehicle of disease transmission. A 1996 outbreak of mrDT104 among elementary school children in Nebraska suggested possible associations with milk and/or animals (Hosek et al., 1997).

What are the reservoirs of DT104?

Reservoirs for mrDT104 in the US are not well established (Hosek et al., 1997). Unlike other *Salmonella* serotypes, such as *S. enteritidis*, which is associated almost exclusively with poultry and poultry products, mrDT104 has been isolated from a wide variety of animals and animal products (Threlfall et al., 1996).

In the UK, most of the DT104 isolates from animals have been from cattle or calves. *Salmonella* isolates from cattle and calves represent a large proportion of those submitted to the central laboratory and a relatively high proportion of these isolates (41 to 46 percent of *Salmonella* isolates) are DT104. The true prevalence of DT104 and mrDT104 among all animals (ill and healthy) is not known. DT104 also represented at least 22 percent of *Salmonella* isolates from sheep, pigs, and turkeys in the UK.

In the US, mrDT104 has been isolated from cattle, sheep, goats, pigs, wild birds, dogs, cats, mice, and horses (Besser et al., 1997; FRI Briefings, 1997; Gay et al., 1997). The 1996 Nebraska outbreak suggested possible associations with animal reservoirs; a kitten and a turtle (Hosek et al., 1997). However, further epidemiological studies are needed to fully elucidate the ecology and public health implications of this pathogen (Hosek et al., 1997).

How is mrDT104 transmitted?

Direct animal-to-animal transmission of mrDT104 is thought to occur via a fecal-oral route. Spread of infection is exacerbated by stress and overcrowded conditions during transport and in holding areas prior to slaughter at abattoirs (Wall et al., 1994). Indirect transmission of *Salmonella* can also occur by use of contaminated feed and water supplies, pasture contaminated by slurry or sewage, and wildlife vectors such as small mammals and birds (Radostits et al., 1994; Smith, 1990). Animal feeds that contain cereal contaminated with *Salmonella* or byproducts of meat processing (such as bone meal) have been documented as sources of infection among animals (Wall et al., 1994).

Human mrDT104 infections can occur directly by contact with ill farm animals (cattle and sheep have transmitted mrDT104 infections to humans) and indirectly by consumption of contaminated foods such as beef, poultry, sausage, salami, unpasteurized milk, and meat-paste (Wall et al., 1994; Hogue et al., 1997). Contamination of meat products usually occurs at slaughter processing plants where unsanitized equipment serves as a vehicle for transmission of mrDT104 from infected to noninfected carcasses (Wall et al., 1994). Epidemiological studies have not identified a unique food stuff as responsible for increasing the number of human cases of mrDT104 in the UK (Wall et al., 1994). It is likely that human mrDT104 outbreaks have a complex etiology associated with several different kinds of foods (Wall et al., 1995b). A portion of human infections may also be caused by direct contact with sick pets such as cats and dogs, which can also be infected with mrDT104 (Meslin, 1997). *Salmonella* has been shown to be shed in large numbers from the buccal cavity of symptomatic cats and their grooming habits can lead to contamination of the coat of the animal (Wall et al., 1996). Like humans, pets probably acquire mrDT104 infection through consumption of contaminated raw meat, milk, poultry, or poultry-derived products or contact with infected animals including wildlife.

What are the factors which increase the likelihood of human infection with mrDT104?

A clear association between human illness and contact with farm animals, particularly sick calves, was demonstrated by a 1995 study in the UK (Wall et al., 1995b). It is likely, therefore, that producers and livestock handlers on mrDT104 infected farms are at greater risk of mrDT104 infections than the general population. It is imperative that these individuals be aware of and implement the necessary steps to reduce the spread of this pathogen. Veterinarians will have an important role in alerting their clients of the potential risks to human health resulting from contact with mrDT104 infected animals.

Age (old or very young) and immune system compromise, are also factors which increase the likelihood of infection with *Salmonella*, including mrDT104. Occurrence of mrDT104 infections is higher among the elderly and young individuals of a population and these people are found to be at greater risk for developing serious complications requiring hospitalization (FRI Briefings, 1997). Other factors which increase the probability of mrDT104 infection include consumption of raw or improperly cooked animal products mentioned previously.

What are the signs of mrDT104 infection in animals?

In cattle, clinical mrDT104 infections usually present with fever, mental dullness, loss of condition, decreased milk production, anorexia, dehydration, and diarrhea progressing to dysentery (Evans and Davies, 1996; Wray and Davies, 1996). Abortion in cows associated with mrDT104 infections is rarely reported (Evans and Davies, 1996). Carrier animals in a herd may harbor subclinical infections which do not manifest any signs commonly associated with mrDT104 infections (Evans and Davies, 1996). Furthermore, cattle have been shown to shed the pathogen in their feces for up to eighteen months following an outbreak (Evans and Davies, 1996). A case report of a mrDT104 infection in a domestic cat described diarrhea, pyrexia and vomiting (Wall et al., 1995a). Specific information regarding clinical presentation of mrDT104 infections in other species is limited.

What are the signs and symptoms of mrDT104 infections in humans?

Clinical signs of a mrDT104 infection in humans may include diarrhea, fever, headache, nausea, bloody stool, and vomiting (Hosek et al., 1997). A study in the UK reported severe clinical signs including septicemia, which resulted in hospitalization among 41 percent of the patients and death in 3 percent of the patients (Wall et al., 1994). In most healthy adult individuals, however, mrDT104 infections result in symptoms which are less severe and usually self-limiting.

How is mrDT104 diagnosed?

The only way to definitively diagnose mrDT104 is to conduct laboratory tests on the feces of infected individuals. A positive blood culture would also be diagnostic. Such tests include culturing for *Salmonella*, serotyping of *Salmonella* isolates to determine if they are typhimurium, phagetyping, and antibiograms to determine the pattern of antibiotic resistance. However, there are several obstacles associated with these methods which can potentially hinder the identification of mrDT104 in an outbreak investigation. First, the process of serotyping and identification can take weeks. Second, the unusual R-type pattern associated with this epidemic strain would probably not be detected in routine diagnostic work on livestock problems in the US, as the use of chloramphenicol is illegal for use in food animals and is not commonly included in sensitivity testing of isolates from agricultural animals (Besser et al., 1997). Furthermore, *Salmonella* isolates in the US are currently not routinely phagetyped as they are is in the UK (Besser et al., 1997).

Other, more molecular-based diagnostic tools such as plasmid profile analysis, pulsed field gel electrophoresis (PFGE), and polymerase chain reaction (PCR), are also being investigated. PCR, which requires minute amounts of DNA, has been used to subtype *S. typhimurium* isolates in preliminary experiments and may prove to be vital in the diagnosis of carrier individuals, both human and animal (Cohen et al., 1994; Stone et al., 1995). Although none of these methods are currently available commercially, initial reports indicate that these methods hold great potential for fast, accurate diagnosis of mrDT104 in acute outbreaks as well as in herd monitoring/screening programs (Olsen et al., 1993; Hoorfar and Wedderkopp, 1995).

How is mrDT104 treated?

The use of antibiotics should be reserved for treating severe cases of mrDT104 in which there is evidence of systemic invasion. The indiscriminate use of antibiotics may lead to failure of therapy and further resistance. Antibiotic use in animals should be based on: the recommendation of a veterinarian, results from culture and sensitivity testing, and the diagnostic tests previously mentioned.

Since mrDT104 is resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, antibiotic treatment for this highly virulent strain poses a medical dilemma for physicians and veterinarians alike. Some have speculated that resistance to trimethoprim in this phagetype may have resulted from the earlier extensive use of this drug to treat mrDT104 infections of cattle in the UK (Threlfall et al., 1996). The shift in MIC values for fluoroquinolones for some mrDT104 isolates started to increase in 1994. In late 1993 fluoroquinolones were licensed for veterinary use in the UK, leading some to propose another causal link (Threlfall et al., 1996). However, the fluoroquinolone product was reportedly not launched by the sponsoring company and available for farm use until 1995 (Carnevale, 1998). This would seem to contradict the hypothesis that the shift in MIC for fluoroquinolones among mrDT104 isolates was caused by veterinary use in food animals. Nonetheless, in response to these concerns, the FDA implemented an amendment to the Animal Medical Drug Use Clarification Act (AMDUCA) in August of 1997 which prohibits the extra-label use of approved fluoroquinolones, among other drugs, in food producing animals in the US (FDA, 21 CFR, 1997).

In the case of a chronically infected cat, fecal shedding of mrDT104 persisted for 12 weeks until a 14-day course of parental enrofloxacin was administered (Wall et al., 1995a). Isolates from the kitten revealed an R-type ACSSuT-Tm resistance pattern (Wall et al., 1995a). This supports the necessity for culture and sensitivity testing on isolates from clinical cases. In cases of persistent shedding, post-treatment cultures are also necessary (a minimum of 4-5 times is recommended). Thus, veterinarians should consider the possibility of salmonellosis when treating cats with gastroenteritis and alert their clients to the zoonotic potential of their pets' infection (Wall et al., 1996).

What are effective measures to prevent mrDT104 infections?

Salmonella spp. are frequently found in many types of animals, animal food products, animal feeds and water sources. It is unlikely that eradication of *Salmonella* in domestic animals is possible in the foreseeable future (Wray & Davies, 1996). It is quite conceivable, however, that efforts to reduce and control the incidence of infections in animals will be successful. Experience with mrDT104 infections among cattle in Washington State suggests that management practices that are effective in reducing the risk of other *Salmonella* infections are also effective for mrDT104. Livestock producers, farmers, and veterinarians should pay particular attention to these

practices, so that they do not become infected or serve as vehicles of infection for other animals or people (Wall et al., 1994). Effective preventive measures include:

- Proper waste management. Common areas where animals congregate (e.g., around feed bunks and water sources) should have fecal material cleaned out periodically. Feces should be composted (Wray & Davies, 1996). Lagoons, if used, should be maintained far enough from the herd water source to prevent contamination.
- Good hygiene practices such as hand-washing with antimicrobial soap as well as the use of coveralls and boots which are designated for use exclusively with livestock will reduce the risk of infection. Coveralls and boots should be changed between farms, as well as between buildings or groups of animals within a herd.
- Footbaths containing bleach or bactericide disinfectant should be utilized by all farm workers and visitors to reduce travel of fecal material. Scrubbing boots with a brush using running water prior to dipping them in the bath will help reduce the amount of fecal material carried on the boots.
- Ideally, all farm workers should maintain a routine whereby groups of animals with calves are managed first, followed by older cattle. This measure will help prevent the spread of disease from adult animals to young, naive animals.
- Feed and water sources should be kept off the ground and in feedbunks to avoid fecal contamination. Mobile feedbunks help reduce the amount of fecal accumulation around the bunk.
- Purchase of replacement stock should be made from direct sources with a known herd health history.
- Newly purchased livestock should be quarantined in a separate pen/facility (preventing nose-to-nose contact) for a period of 4 weeks before introduction into the herd. On dairy operations, these animals should be milked using separate equipment or after resident cattle.
- Milking equipment such as bottles, nipples, hoses, bulk tanks, etc., should be properly sanitized between each use.
- Sick animals should be housed in a designated isolation area separate from calving areas and recently freshened cows.
- Cattle and calves, particularly pre-weaned calves, should not be housed in high densities.
- Access to feed storage areas by possible vectors, such as wild birds, and mammals, such as rodents, dogs, and cats, should be prevented.
- Pets, such as cats and dogs, should not be allowed access to unprotected food or food preparation areas.
- Pets should have limited contact with livestock and animal facilities.
- Prevention of *Salmonella* using vaccines has yielded mixed results and cannot be universally recommended at present (Wray and Davies, 1996).
- Consider limiting exposure of very young and elderly people to livestock, especially ill animals.

Control measures should include reducing the risk of infection in food animals, reducing the risk of contamination of animal products at all stages of food processing, reducing risk for farm workers and their families, and raising awareness of measures to prevent food poisoning among food handlers and the general public, such as avoiding the consumption of unpasteurized milk or products made with unpasteurized milk.

Where do we go from here?

The control of mrDT104 infections in animals and humans will only be achieved through the close collaboration of veterinary, medical, and environmental health professionals and the promotion of methods to reduce *Salmonella* contamination in animal feeds and foods for human consumption (Wray and Davies, 1996). A joint effort is currently underway between USDA-Animal and Plant Health Inspection Service (APHIS), USDA-Agricultural Research Service (ARS), USDA-Food Safety and Inspection Service (FSIS), CDC, and FDA to develop a collaborative agenda for the control and prevention of mrDT104 infections in humans and animals. This service will ultimately provide sound statistical data about mrDT104 to US veterinarians, producers and industry leaders. This project, as a part of the National Antimicrobial Susceptibility Monitoring System, includes ongoing monitoring of *Salmonella* isolates from humans and animals for antimicrobial susceptibility testing. This testing will provide descriptive data about the geographic and temporal trends of antibiotic resistance in *Salmonella* spp. and facilitate the identification of new resistance patterns as they emerge. Another goal of this interagency effort is to develop and implement a standardized method of phagetyping for monitoring purposes. As monitoring efforts continue, mrDT104 isolates will undoubtedly be found. Thus, more outbreaks in the future will be associated with mrDT104 simply because we are looking for the organism. The challenge will be to develop and evaluate on farm methods useful to control contamination of food products destined for human consumption.

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