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Salmonella in Dairy Cattle

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KEYWORDS

• *Salmonella* • Dairy cattle • *Salmonella* Dublin • Diagnostic tests • Prevention • Public health

KEY POINTS

- *Salmonella* Dublin, the host adapted serotype in cattle, has the ability to establish lifelong infection in cattle, characterized by an asymptomatic carrier status with intermittent periods of bacteremia and shedding.
- Enteric, septicemic, and reproductive diseases are all possible manifestations of *Salmonella* infection, with pneumonia being a common manifestation of *Salmonella* Dublin infection in calves.
- Definitive diagnosis is based on detection of the organism through aerobic culture of feces or detection of genetic material from the bacteria via polymerase chain reaction techniques.
- Fluid therapy is the mainstay of treatment for cattle with enteric salmonellosis; antimicrobial therapy remains controversial.
- Larger herd size, crowded husbandry, free stall housing, and purchase of replacement animals contribute to an increased propensity for exposure to contaminated manure, the major source of infection on dairies.

INTRODUCTION

As an infectious, contagious pathogen *Salmonella* is probably rivalled by only bovine viral diarrhea virus in its ability to cause such a variety of clinical problems in dairy cattle. Enteric, septicemic, and reproductive diseases are all possible manifestations of *Salmonella* infection and, although reproductive losses are only of concern in sexually mature females, enteric disease can be seen in susceptible cattle at any age from true neonates through adulthood. The increasing prevalence in recent years of the host adapted serotype *Salmonella enterica* serotype Dublin, conventionally referred to by the abbreviated title of *Salmonella* Dublin, has added a new, and highly challenging,

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facet to salmonellosis on many modern dairies. The ability to establish lifelong infection, characterized by an asymptomatic carrier status, with intermittent periods of bacteremia and intermittent shedding, challenges control of this serotype. Enteric infection with other non–host-adapted serotypes, particularly in calves, can also be associated with true bacteremia, sepsis, and high mortality rates. No current discussion of bovine salmonellosis could be complete without acknowledging the increasing public health concern regarding its relevance as an important zoonosis, the risk that contaminated dairy and dairy beef products can pose to human health, and, just as important, the reality that increasing antimicrobial resistance among zoonotic enteric pathogens such as *Salmonella* brings the use of antimicrobials by veterinarians and producers under ever stricter scrutiny.

ETIOLOGY AND TAXONOMY

Salmonella is a genus of gram-negative, facultative anaerobic bacteria that belong to the family of Enterobacteriaceae. There are 2 recognized species within the genus: *S enterica* and *Salmonella bongori*. *S enterica* can be further divided into 6 subspecies, *S enterica* subspecies *enterica* being the most relevant in dairy cattle.[1] More than 2500 serovars (serotypes), differentiated by their antigenic composition, have been identified. Serovars are based on the somatic (O), flagellar (H), and capsular (Vi) antigens.[2] Most human and veterinary diagnostic laboratories have phenotypically divided *Salmonella* isolates into serogroups based on detection of the O lipopolysaccharide and H flagellar antigens, historically by agglutination methods.[2,3] Although these traditional serotyping techniques have formed the basis of human and veterinary diagnostic practice for salmonellosis for several decades, they are labor intensive and time consuming, typically taking at least 48 hours.[4]

With the advent of more advanced molecular diagnostic methods, genetic approaches to serotyping are beginning to supercede traditional tests. In general, these methods use 1 of 2 types of targets for serotype determination, the first are indirect targets, which use random surrogate genomic markers known to be associated with certain serotypes, and the second method uses direct targets requiring the use of highly specific genetic determinants of a particular serotype.[5] The latter typically involve the *rfb* gene cluster responsible for O somatic group antigen synthesis[6] and the *fliC* and *fliB* genes encoding the 2 flagellar antigens of *Salmonella.*[7] Genomic sequencing is becoming increasingly common for the identification and serotyping of *Salmonella* isolates.[4,5] The hope is that, with diminishing costs and continued refinement, more rapid, accurate genoserotyping will improve diagnostic and surveillance efforts for both public health and veterinary purposes.[8]

Most commonly, clinical bovine isolates have been divided by their O antigens, and serovars are further grouped into serogroups assigned to an early letter of the alphabet (eg, A, B, C, D, and E).[9] By current convention, *Salmonella* isolates are referred to by their serovar/serogroup classification (eg, *S enterica* subspecies *enterica* serovar Typhimurium, is abbreviated to *Salmonella* Typhimurium). Despite the diversity of serovars, relatively few are of clinical importance among cattle. The majority of cattle isolates are *Salmonella* of types B, C, and E, which are non–host specific, or *Salmonella* Dublin (type D), which is the host-adapted serovar in cattle.[9]

PREVALENT SEROTYPES IN DAIRY CATTLE

The isolation of *Salmonella* from the feces of dairy cows or calves as well as the environment on dairy farms is increasingly common. As part of the United States Department of Agriculture's National Animal Health Monitoring System (NAHMS) Dairy 2007

study,[10] fecal samples were collected from approximately 30 healthy cows on each of 121 dairy operations across 17 states. Forty percent of the dairy operations had at least 1 cow that was *Salmonella* positive on fecal culture. Of the roughly 3800 healthy cows sampled, 14% were fecal culture positive. Compared with the Dairy 1996 NAHMS study,[11] the percentage of *Salmonella*-positive operations had doubled and the percentage of positive cows had more than doubled.[10] For the 2007 study, when environmental sampling was performed in conjunction with individual cow sampling, the number of dairies with a positive *Salmonella* culture increased to nearly 50%.[10] Within the 2007 study, the most frequently isolated *Salmonella* serotypes included *Salmonella* Cerro*, Salmonella* Kentucky*, Salmonella* Montevideo, and *Salmonella* Muenster. These serotypes fall within groups K, C3, C1, and E, respectively.

In a comprehensive study of more than 800 dairy herds in the northeastern United States in 2009, fecal samples were collected from female dairy cattle for *Salmonella* culture based on a suspicion of clinical disease.[12] *Salmonella* was found in 11% of the dairy herds monitored for approximately 1 year over the course of the study. The herd-level incidence rate was approximately 9 positive herds per 100 herd-years; however, just 17% of the positive study herds accounted for more than 70% of the clinical *Salmonella* cases.[12] The predominate serotype identified was *Salmonella* Newport, accounting for 41% of the cases, followed by *Salmonella* Typhimurium, accounting for nearly 20% of cases.[12] Clustering of disease among herds was consistent with another US prevalence study that found that 25% of the enrolled dairy farms accounted for more than 75% of the *Salmonella*-positive fecal and environmental samples.[13] In this study, sampling of conventional and organic herds on 5 occasions over a period of 1 year resulted in detection at least 1 *Salmonella*-positive fecal sample on more than 90% of farms (100/110). Serogroup E1 was the most commonly identified serogroup in fecal samples, although serogroup B was the most common isolate across farms, with 43% of fecal-positive farms having at least 1 serogroup B isolate.

Data from a more recent 2013 study demonstrated that of the nearly 1800 *Salmonella* isolates identified at the National Veterinary Services Laboratory from clinical and nonclinical case submissions, the most common serotype was *Salmonella* Dublin (18%) followed by *Salmonella* Cerro (16%) and *Salmonella* Typhimurium (13%).[14] A retrospective study of *S enterica* isolates submitted to the Wisconsin Veterinary Diagnostic Laboratory from 2006 to 2015 parallels the findings from the National Veterinary Services Laboratory. Of the nearly 5000 isolates identified, *Salmonella* Dublin was the most prevalent serotype identified, accounting for a total of 1153 isolates (23% of total). Along with Dublin, *Salmonella* Cerro (16%), Newport (14%), Montevideo (8%), Kentucky (8%), and Typhimurium (4%) comprised the top 6 most commonly isolated sertotypes.[15] The emergence of *Salmonella* Dublin as one of the most commonly isolated serotypes is of major concern for the dairy industry. As the host-adapted strain of *Salmonella* in cattle, animals infected with *Salmonella* Dublin can become chronic, subclinical carriers that have the potential to shed large numbers of organisms into the environment. These carriers also play an important role in maintaining infection within a herd by shedding not only in feces, but also in milk and colostrum.

PATHOGENESIS

Salmonella infections are well-known for their association with clinical signs of enterocolitis, septicemia, and abortion in dairy cattle.[9] Pneumonia is an increasingly common manifestation of *Salmonella* Dublin infection in calves[16,17] and worth bearing in mind when dealing with mild, moderate, or severe respiratory disease on heifer rearing facilities. Whether or not this merely represents hematogenous localization of the

organism to the lungs in much the same way that is seen with septic arthritis, for example, or a more specific organ tropism for the lungs by this serovar is uncertain. However, personal observations by one of the authors and many others suggest that this particular clinical manifestation of *Salmonella* Dublin infection is increasingly common during the late nursing and postweaning period.

Salmonella infection is most commonly transmitted by fecal–oral contamination from other livestock, rodents, birds, or by feeding contaminated protein source animal byproducts.[1] Given the increased frequency with which the organism can be isolated on dairy farms, from both symptomatic and asymptomatic cattle, it is reasonable to assume that fecal–oral spread from other cattle is the most common means of spread on modern dairies. Older literature establishing that aerosol transmission was possible in closely confined, penned calves would also seem to be currently relevant with respect to the spread of certain *Salmonella* serotypes, especially *Salmonella* Dublin, on endemic heifer rearing facilities.[18,19] In both calves and adults, those factors that determine pathogenicity and whether or not clinical disease is seen include virulence of the serotype, dose of inoculum, degree of immunity (passive or adaptive) or previous exposure of host to the serotype, and other stressors currently affecting the host.[20] The organism will less frequently penetrate ocular or nasal mucous membranes.

The most detailed studies of the pathogenesis of bovine salmonellosis infection come from the literature describing enteric infection via the oral route, mainly in calves.[21–23] Once ingested, *Salmonella* attaches to mucosal cells and is capable of destroying enterocytes. Attachment is increased if gastrointestinal stasis is present or the normal flora has been disturbed or is not yet established, as is the case in neonates.[1] The organism penetrates through the enterocytes to the lamina propria of the distal small intestine and colon, where they stimulate an inflammatory response or are engulfed by macrophages and neutrophils.[1] Once salmonellae have gained entry to mononuclear phagocytes, they can be rapidly disseminated throughout the body. Salmonellae have a predilection for lymphoid tissues, invading through M-cells, and are found in the highest numbers in the Peyer patches and mesenteric lymph nodes. From here, the organism often enters the lymphatics and may eventually lead to bacteremia.[9,23] Experimental studies have also shown that oral exposure can lead to infection and systemic dissemination via pharyngeal lymphoid tissue (tonsils) without the need for true enteric infection.[24] Salmonellae are capable of surviving and multiplying in numerous host tissues, often as facultative intracellular bacteria in macrophages and reticuloendothelial cells.[1] These characteristics guard them against the hosts' normal defense mechanisms and potentially facilitate true bacteremia.

The virulence mechanisms of salmonellae are, therefore, composed of their ability to invade the intestinal mucosa, locate to and multiply within the lymphoid tissues, and to evade host defense mechanisms. Enterocolitis caused by *Salmonella* spp. is due to inflammation with subsequent maldigestion and malabsorption, and to a lesser extent from secretory mechanisms.[9,23] Inflammation in the colon leads to the commonly observed fresh blood in the feces of both adults and calves. The diarrhea caused by *Salmonella* spp. is principally mediated by the host inflammatory reaction to the infection.

To establish infection, enteropathogens such as *Salmonella* must first be able to overcome those host factors that resist colonization of the gut, principle among these being a fairly dense gut microbiota,[25] which secrete a variety of bacteriocins, antibiotics, and colicins that hinder enteropathogen growth.[26] There is increasing evidence that many enteropathogens, including salmonellae, are not able to colonize the gut in

the face of a normal microbiota[25] and hence factors that negatively influence this key component of resistance are important in the predisposition to enteric disease. Once *Salmonella* density reaches a critical threshold (about 10^8 colony-forming units per gram in the case of *Salmonella* Typhimurium in mice), then a sufficient number of organisms can invade the gut epithelium by first docking with and then invading the epithelial cells.[25] At a molecular level, *Salmonella* Typhimurium does this by specific bacterial adhesins for attachment and then a secretion system that injects a cocktail of bacterial toxins (the type III secretion system) that enables the bacterium to reach the lamina propria.[27] Damaged gut cells are expelled into the lumen, as part of the host defense system, giving rise to some of the clinical signs of salmonellosis and a profound inflammatory response is initiated via interleukin-18 within 10 to 18 hours after infection.[27]

There are molecular reasons that underscore the clinical observation that differences in pathogenicity between serotypes exist. Some strains of *Salmonella* Dublin and *Salmonella* Typhimurium, for example, have a virulence plasmid (carrying the SpV gene) that facilitates survival of the organism within phagocytes, partly perhaps explaining the increased association of these 2 serotypes with clinical disease in calves and adults. The ease with which genes can be transferred between *Salmonella* and other members of the *Enterobacteriaceae* also provides a rational explanation for the transfer of antimicrobial resistance.[20]

Precise and eloquent experimental data on the mechanisms by which *Salmonella* infection can lead to reproductive loss and abortion are hard to find. Clinically, abortions are most common when serotypes B, C, or D are involved and it makes intuitive sense that abortion in cattle infected with *Salmonella* spp. could arise through several different mechanisms. Septicemia could lead to seeding of the fetus and uterus, causing fetal infection and death.[1,9] The fact that diagnostic post mortem investigations of aborted fetuses can often recover the organism from fetal samples supports this possibility. Endotoxemia leading to inflammatory mediator release might also cause luteolysis secondary to prostaglandin release. High fevers or hyperthermia could also play a role in prostaglandin release or cause abortion through more direct fetal injury. Cows may abort at any stage of gestation, but expulsion of the fetus is most common at 5 to 9 months of gestation.[1,9]

DIAGNOSTICS

Live Animal

A definitive diagnosis of *Salmonella* infection in the live animal involves detection of the organism, most commonly by aerobic culture. Although a clinical history of febrile illness accompanied by hemorrhagic enteritis and anorexia may be suggestive in either calves or adults, there is a sufficient differential diagnosis list in both age groups that diagnostic sampling must be performed. When reproductive losses are encountered in pregnant cattle, unless there are concurrent cases of bloody diarrhea, the clinical signs are even less definitive for salmonellosis and the differential list even longer. For hemorrhagic enteritis in adults, the differential list principally includes winter dysentery and bovine viral diarrhea virus infection; in calves, depending on age, such a presentation merits consideration of several viral (rotavirus, coronavirus), protozoal (*Cryptosporidium*, *Eimeria*), and bacterial causes (*Escherichia coli*, *Clostridium perfringens*). However, one should not rely on the presence of blood in the stool; many cases of enteric salmonellosis present without this clinical finding. Remarkable variation in clinical severity will occur based on serovar virulence, host immunologic status, and inoculating dose. In calves, death may occur owing to septicemia before diarrhea

becomes obvious or a significant clinical abnormality. In large free stall dairies, it is increasingly common to encounter *Salmonella* infection as an endemic challenge with clinical presentations that are highly variable, ranging from the classic textbook description of reproductive losses and enteric disease in adult cattle through to lower impact problems with fevers of unknown origin, little to no diarrhea, and only modest consequences in terms of appetite and milk yield reduction.

Because *Salmonella* organisms are easily and rapidly out competed by other fecal gram negatives, the majority of diagnostic laboratories use enrichment media such as tetrathionate or selenite broth to improve the chances of *Salmonella* growth and then plate these enriched samples onto selective media such as brilliant green or xylose lysine deso-oxycholate agar.[28] Veterinarians working in the field are advised to contact their local diagnostic laboratory for assistance with sample handling, processing, and submission before investigating either individual or group problems with enteric disease suspicious for *Salmonella* infection. It is frequently worthwhile to place samples directly into enrichment media before submission to improve the chances of positive culture and to keep samples chilled until they arrive at the diagnostic laboratory.

Disadvantages of fecal culture include the fact that shedding can be sporadic, even in true infections (certainly when one considers the sensitivity of bacterial culture) and that, in the face of an ongoing outbreak, one can occasionally encounter clinically normal calves and adults who shed the organism but never develop any clinical signs.[20] The latter situation may still provide useful information, however, both from the perspective of deciding which animals merit treatment but also from the broader standpoint of identifying an enteric pathogen that should never be trivialized or considered a commensal. However, the general pattern is that subclinically or persistently infected cattle shed low numbers of organisms, whereas clinically ill or acutely infected animals may excrete higher numbers in feces.[17] When the clinical suspicion of *Salmonella* is high, a single negative culture is not sufficient to rule out infection. As mentioned, fecal samples should be submitted to qualified diagnostic laboratories that are equipped to culture enteric pathogens and with careful attention to sample handling.[9] Although culturing of individual cow fecal samples is the most common method used to assess individual and herd *Salmonella* status, it can be expensive and time consuming, especially in larger herds. In a study comparing individual, pooled, and composite fecal samples, it was found that composite fecal sampling was more sensitive at the sample level than the other 2 methods, primarily because of the increased number of cattle sampled indirectly through this method.[29] Hence, if one is merely trying to obtain a yes or no answer or identify and track specific serovars, or antimicrobial susceptibility patterns over time, composite fecal samples are typically collected from areas on dairy operations where manure accumulates from a majority of adult animals, such as holding pens, alleyways, and lagoons.[29]

Newer techniques for diagnosing *Salmonella* are based on detection of genetic material from the bacteria, that is, polymerase chain reaction (PCR) techniques.[30,31] These techniques are generally thought to be more sensitive than culture, but have the disadvantage that subsequent serotyping is not always possible.[17] Both species-specific (*S enterica*) and individual serotype PCR tests are available at some, but not all, veterinary diagnostic laboratories within the United States. There are 2 main PCR methods: the traditional PCR and the real-time PCR. In the traditional PCR method, the test result is qualitative (yes or no). In real-time PCR, a threshold cycle (Ct-value) gives a quantitative value of DNA in the sample; the Ct-value is inversely correlated with the starting concentration of the target DNA; hence, the lower the Ct number the more *Salmonella* DNA there will be in the sample. At the current point in time, only a few veterinary diagnostic laboratories offer both species-specific and

serotype (usually *Salmonella* Dublin) assays for use with biological samples such as feces, milk, or tracheal and bronchoalveolar lavage fluid. The advantage is a quicker turnaround time and the potential for greater sensitivity, although parallel cultures are still necessary for in vitro antibiograms to be performed to aid treatment decisions (Dr Keith Poulsen, Wisconsin State Veterinary Diagnostic Laboratory, personal communication, 2017). It is possible that, in the near future, PCR assays may become used for environmental samples although these can contain so many potential PCR inhibitors and out-competing organisms that sensitivity and specificity may be lost.[32] The use of PCR methodology to investigate contamination of milk is also of increasing relevance, potentially for veterinarians, but also from the public health perspective. Certain serovars, notoriously *Salmonella* Dublin, but also to include *Salmonella* Typhimurium and Newport, can be found in the milk or colostrum of infected lactating animals.[9] Although conventional pasteurization should kill the organism, there is an understandable desire for food safety reasons to use highly sensitive methods to detect the organism after harvest.[33]

Although fecal culture remains the gold standard at most laboratories, blood culture, a culture of transtracheal wash or bronchoalveolar lavage fluid, and joint fluid may all be useful choices for individuals experiencing bacteremic salmonellosis. The propensity for bacteremia in neonatal calves with salmonellosis makes aseptically obtained aerobic blood cultures a particularly useful diagnostic sample to consider in valuable animals.[1,9] Culture of these nonfecal samples is far less likely to be diagnostically valuable in adults, although PCR methods on such samples may potentially improve sensitivity in the future.

Post Mortem Sampling

Although gross post mortem findings of severe, diffuse, fibrinonecrotic ileotyphlocolitis with watery, often bloody content are highly suggestive, they are neither consistent enough or definitive for enteric *Salmonella* infection in calves or adults.[20] However, in both calves and adults, necropsy material can provide excellent diagnostic material for the definitive diagnosis of *Salmonella* infection. In all age groups, it is advised to obtain numerous samples from the gastrointestinal tract (ileum, cecum, colon), mesenteric lymph node, and gall bladder (bile is a particularly useful sample), as well as lung tissue, especially when consideration of *Salmonella* Dublin is warranted, as increasingly is the case. Because veterinarians are rarely only interested in the diagnosis of *Salmonella* infection during a field necropsy, one may need to take multiple samples from such sites and handle the samples specifically as described to enhance the chances of a positive *Salmonella* culture. Culture remains the most common method used by most diagnostic laboratories to confirm *Salmonella* infection in post mortem samples.

Samples from abortion cases that may have been caused by *Salmonella*, should include fluid or tissue from both the dam and the fetus. Most *Salmonella*-associated abortions are in the last trimester so there will be a fetus to work with, preferably relatively fresh depending on the delay before the fetus is discovered. Samples from the dam might include milk or colostrum, serum, and feces. Feces and milk can be screened via culture or PCR, whereas the serum sample can be used for *Salmonella* Dublin serology (described elsewhere in this article). Providing the fetus is not severely autolyzed, heart blood, abomasal contents, and intestinal or biliary samples might be useful but diagnostically veterinarians are all too commonly challenged by the "freshness" of an abortus. As is true of many enteritis investigations, with abortion cases veterinarians are typically attempting to submit samples that might reveal one of many possible infectious etiologies and it may be simpler to submit the entire fetus if this can be done in a timely manner.

Environmental Sampling

Environmental sampling on dairy farms and heifer rearing facilities has largely been a research tool rather than a clinically applicable procedure. However, quite a lot of information has been learned regarding areas of large free stall facilities where positive *Salmonella* cultures can often be repeatedly obtained either in herds with or without known clinical disease.[34,35] Not surprisingly, areas of high traffic use and density and where sick cows and cows soon to calve are located are frequently discovered to yield positive cultures.[34] Just as was discussed under individual cow fecal sampling, veterinarians are advised to seek the input of the laboratory to which they are going to submit samples before obtaining on-farm environmental specimens. The use of buffered peptone water or more specific enrichment broths before submission may improve chances of *Salmonella* being isolated from heavily contaminated samples.[34] Drag swabs, milk filters, and even absorbent socks worn over shoes, as have been used for environmental sampling in poultry houses, can be used.

Diagnostic Testing for Salmonella Dublin

Proof of current infection with *Salmonella* Dublin can be achieved via conventional culture with serotyping or PCR methodologies if available.[9,17] In addition, both in the United States and several countries in Europe it is also currently possible to use an enzyme-linked immunosorbent assay (ELISA) to measure the level of antibodies directed against O-antigens from *Salmonella* Dublin in blood and milk. In this way, one can measure the humoral immune response as an indicator of current or previous infection.[36,37] Some laboratories report the ELISA result as a semiquantitative percentage value, giving an optical density reading referable to a standard set of controls. In addition, ELISA tests can also be used for individual or bulk tank milk sample screening,[38] and have come to be used quite extensively in countries such as Denmark, where active surveillance programs for this serovar are in effect.[17,39] Sensitivity for the serum ELISA is considerably higher than fecal culture for the identification of *Salmonella* Dublin infected cattle,[17] and as a diagnostic test the serum ELISA is reported to perform best when used in animals between 3 and 10 months of age (**Box 1**).[36]

TREATMENT

Fluid therapy is the mainstay of treatment for cattle with enteric salmonellosis.[40] The type of fluid and route of administration is based on the severity of clinical signs

Box 1
***Salmonella* diagnostic testing options**

- Individual animal fecal culture using enrichment and selective media.
- Composite fecal sampling.
- *Salmonella* polymerase chain reaction (feces, milk, tracheal or bronchoalveolar lavage fluid).
- Blood, transtracheal wash, bronchoalveolar lavage, or joint fluid culture when bacteremia is suspected in calves.
- Culture of post mortem samples: gastrointestinal tract, mesenteric lymph node, bile, and lung.
- Environmental cultures.
- *Salmonella* Dublin enzyme-linked immunosorbent assay: serum or milk.

and the economic value of the animal. In calves with acute, severe diarrhea showing signs of hypovolemic shock, intravenous fluid therapy using a balanced electrolyte solution, such as lactated Ringers, is necessary.[20,40] In severely depressed or comatose animals, resuscitative fluids, such as hypertonic saline, are indicated. If administered, hypertonic saline, dosed at 2-4 mL/kg, should always be followed with isotonic crystalloids or water to replace the "borrowed" water from the intracellular space. Dextrose supplementation can be a critical part of the intravenous fluid therapy plan for calves with salmonellosis, not only because of poor feed intake, but because of the increased risk of hypoglycemia that may accompany septicemia. Calves that are ambulatory, have a suckle, and are only moderately dehydrated can often be managed with oral fluids.[40] Calves and even adult cattle can develop severe metabolic acidosis with peracute *Salmonella* infections and intravenous bicarbonate-rich fluids should be considered when profound depression or shocklike signs accompany diarrhea. Oral electrolyte solutions have proven to be helpful in correcting mild to moderate dehydration; however, depending on the degree of bowel inflammation, fluid absorption and digestion may be altered. Fluid therapy for adult cattle in the field setting can prove to be more challenging owing to the sheer volume of fluid needed in cases of severe dehydration. Hypertonic saline followed by at least 10 gallons of oral electrolytes or water, either consumed voluntarily or given by orogastric tube, is a highly efficient method of fluid resuscitation in adult cattle.

In valuable calves or adults, colloids (plasma or hetastarch) are often indicated as a result of hypoproteinemia secondary to albumin loss from the gastrointestinal tract. Synthetic colloids, such as hetastarch, are a more reasonably priced option, but only augment colloidal pressure. Plasma has the added benefit of immunoglobulins and acute phase proteins, which provide therapeutic benefits in septic or inflammatory conditions.[9]

Antimicrobial therapy for the treatment of salmonellosis was, is, and probably always will be, controversial. Of utmost concern is the potential for the creation of antibiotic-resistant strains of *Salmonella* that may present a risk to humans or animals in the future. Although antimicrobial therapy may aid in clinical recovery, it has also been criticized as failing to limit fecal shedding or to impart a positive effect on the duration of fecal shedding. In truth, this criticism is largely extrapolated from research in other species. In cattle, the effect of prior antibiotic use on fecal shedding may be age variable, with research identifying that the risk of fecal shedding after antibiotic treatment is greater for adults and heifers than in calves.[41] However, the risk of true bacteremia in calves with enteric salmonellosis is substantial, justifying the use of antimicrobials in patients of this age.[1] Bacteremic spread of the organism can result in concurrent disease in multiple organs, such as pneumonia, arthritis, and meningitis. The presence of these clinical infections should always merit antimicrobial administration. The comparative risks for such systemic complications in adults are less than in calves, making the routine use of antimicrobials in mature animals less justifiable.

If possible, antimicrobial selection should be based on culture and susceptibility of the *Salmonella* isolate. The dilemma faced by practitioners is frequently that real-time decisions regarding antimicrobial use and selection have to be made in advance of any definitive microbiologic data. Some guidelines regarding *Salmonella* susceptibility can be provided, however. According to the NAHMS 2007 study, isolates were found to be most resistant to tetracycline, streptomycin, ampicillin, and ceftiofur, but were frequently sensitive to aminoglycosides, fluoroquinolones, and trimethoprim-sulfas.[10] To the US readership, these lists will not provide much comfort because of restrictions on antimicrobial use under the current Animal Medicinal Drug Use Clarification Act. Fluoroquinolones and certain sulfonamides may not be used extra-label in the United

States. Additionally, there is a voluntary ban on the use of aminoglycosides, such as gentamicin and amikacin, in food-producing animals because of long-term tissue residues. As of 2012, the extra-label use of ceftiofur in regard to dose, route, and frequency of administration is also prohibited. Owing to the facultative intracellular nature of the organism, it is also worth bearing in mind that antimicrobial penetration into the cell can be limited, even for antimicrobials that show in vitro efficacy. When chosen, antibiotic therapy should be continued for at least 5 to 7 days in cases of acute or peracute salmonellosis.[9] Appropriate withdrawal times should be observed for all antimicrobial usage and Animal Medicinal Drug Use Clarification Act guidelines followed at all times. For questions regarding extended withdrawal times and extra-label use of antimicrobials, US readers are advised to contact the Food Animal Residue Avoidance Database.

In addition to crystalloid fluid therapy, colloid administration when indicated by hypoproteinemia, and responsible, legal, and signalment appropriate selection of antibiotics, the third and final component of therapy for salmonellosis is antiinflammatory use. The inflammatory cascade triggered by local or systemic infection with *Salmonella* is a critical component of the pathogenesis of this organism and culminates in many of the clinical signs observed. Direct endotoxin-mediated effects alongside the host systemic inflammatory response are major components of many calf and adult *Salmonella* infections that can be mitigated, at least in part, by the use of nonsteroidal antiinflammatory drugs.[1] Cattle may be dosed with flunixin meglumine at 1.1 mg/kg of body weight intravenously every 24 hours and then tapered to 0.5 mg/kg every 24 hours, or the medication discontinued after the patient stabilizes.[9] Label use of flunixin meglumine includes dosages of up to 2.2 mg/kg in the United States. Prolonged administration of nonsteroidal antiinflammatory drugs, particularly at the higher dose or in the face of dehydration, can lead to abomasal ulceration and renal papillary necrosis.[1,9,20] In rare circumstances, some clinicians elect to administer "shock" doses of corticosteroids, but this measure would be uncommon in either general or referral practice. Soluble prednisolone sodium succinate would be the preferred agent in such circumstances.

PREVENTION AND CONTROL

Adult Cows

From both the literature and personal experience, it seems that not only are herd epidemics becoming more common, but perhaps more worryingly the disease has become endemic on an increasing number of facilities. Endemicity is obviously problematic with any serovar, but is inevitable when the herd prevalence of *Salmonella* Dublin infection increases. Frequently, the disease becomes a cyclical problem responsible for a spectrum of illness that varies from the more classic presentations described through to milder illness perhaps characterized by fever, looser than normal stool, and mild production loss. Depending on the interaction of general cow health, other concurrent stressors, climatologic stress, and the level of fecal–oral challenge at any one time, adult cows may or may not become clinically ill. Transition cow management becomes an important factor in whether or not new infections are acquired and subsequently result in clinical illness in the late dry and early lactation period, a time when cattle may be at their most susceptible to infectious disease.[9]

As with any fecally–orally spread organism, control strategies are broadly speaking simple to describe, but not necessarily so easy to put into place for many dairies. Larger herd size, crowded husbandry, and free stall housing all contribute to an increased propensity for exposure to contaminated manure, and although purchased

feedstuffs are still occasionally incriminated as a means by which new *Salmonella* infections are introduced onto farms, as are rodent and bird populations, the major source of infection are other cattle shedding the organism in their feces. The high likelihood of feces being contaminated with *Salmonella* organisms on many diaries should mitigate against the spreading of manure on fields that are to be used for forages, or common use equipment for manure handling and feed distribution. Evidence suggests that heating of manure to greater than 45°C for more than 3 days, alongside aeration of composted manure using straw, markedly and significantly reduces the number of *Salmonella* organisms, although it is uncertain how practical this information is to larger dairies with modern large-volume manure handling systems.[42] Peculiarly, and perhaps rather worryingly, 1 study looking at risk factors for increased antimicrobial resistance among *Salmonella* isolates on dairy farms identified the use of composted manure for bedding as a significant problem.[43] The most directly applicable research regarding modern manure handling systems and survival of *Salmonella* organisms under natural rather than laboratory conditions demonstrated that a multiple-drug–resistant strain of *Salmonella* Newport survived for less than 24 hours in a compost pile at 64°C, but would survive for more than 4 months and more than 9 months in an effluent lagoon and field soil, respectively.[44]

Once salmonellosis has been confirmed in adult cattle, there are a number of further investigative and control measures that may be implemented. These measures do not differ according to serotype, but there are some specific challenges concerning the host adapted serovar *Salmonella* Dublin that will be discussed in a later section.

It is prudent to consider the possible source(s) of the infection. Although commodities, especially protein feed sources, and wild bird and rodent populations have been incriminated in many texts over the years, it seems quite uncommon these days for a single point source event to have introduced the infection onto a dairy de novo. Environmental sampling of feed, water, and storage facilities can be helpful in identifying contamination in this regard, but if, as is commonly the case on larger dairies, management continues to purchase replacement animals or expand from other herds, it seems inevitable from prevalence data that the infection will be introduced via infected cattle and their feces. In all probability, many "new" outbreaks are likely surges in clinical disease and new infections in a herd where the infection already existed but hitherto had remained subclinical. Factors in transition cow management that reduce immunologic competence or increase exposure risk, are likely to contribute to the onset of clinical disease in such circumstances.

The isolation of affected animals and strict attention to hygiene are pieces of advice routinely given but difficult to implement on large dairies. The numbers of affected animals can be overwhelming and lactating cows have to be milked at least twice a day, requiring them to walk and congregate in frequently trafficked areas and holding pens for the parlor into which they release enormous numbers of organism whenever they defecate. Avoidance of common use equipment for manure handling and feed distribution have already been mentioned, but should be in place on well-managed dairies anyway. Sick, transition, and maternity animals should never be housed together, but unfortunately are for convenience on many occasions; this condition merely ensures exposure of the most susceptible animals to those most likely to be contagious.

Cleaning and disinfection of the environment are also important, but again somewhat intimidating in the context of a larger dairy. Proper cleaning and disinfection of the environment and equipment after a *Salmonella* outbreak can, however, be critically important in decreasing the risk of disease transmission to both cattle and humans. Cleaning is defined as the removal of all visible debris and is arguably the most important step in decontamination of animal environments. Even the best disinfectants will

be minimally effective when used in the presence of organic matter, such as feces and bedding material.[1] Not only does cleaning remove the physical barrier between disinfectants and the organism, but it also removes a majority of the organisms so that fewer need to be killed by the disinfectants. This is especially helpful with fecally–orally spread infections like *Salmonella*. where the infectious dose is relatively high (often in the order of 10^6–10^8 organisms[9,17]). Livestock trailers, maternity and calf pens, feeding equipment, and other areas suspect of being contaminated with *Salmonella* should be the main focus for cleaning and disinfection. Although high-power washing can be quite helpful in removing organic debris, its use is not recommended because of the risk of cross-contamination of the environment, and splashing and aerosolization of contaminated material, which can lead to human and animal infection.[9,45] Power washing also fails to remove biofilm, which is an essential and vital component to proper cleaning. In place of power washing, hand-held foamers can be used to apply alkaline detergent and acid rinses for cleaning. The Wisconsin Veterinary Diagnostic laboratory has formulated a cleaning and disinfecting protocol specifically for premises with confirmed *Salmonella*, which can be found at www.wvdl.wisc.edu. A recent paper examining disinfection efficacy against several common bacterial pathogens in a large animal hospital environment showed an approximately 90% reduction in colony-forming units per milliliter of *S enterica* when either an accelerated hydrogen peroxide or peroxy monosulfate disinfectant product was used via a mist application technique, provided adequate cleaning was performed first.[46]

As with antimicrobial drugs, disinfectants have a spectrum of activity that can be highly variable between disinfectant classes.[1] Examples of disinfectants commonly used in veterinary medicine include bleach (sodium hypochlorite), quaternary ammonium, phenols, and peroxides. Bleach is rapidly inactivated by organic debris, but has a broad spectrum of activity. Quaternary ammonium has moderate activity in organic debris and is effective against gram-negative bacteria, such as *Salmonella*. The principle advantage of phenols is better activity in organic debris. Peroxides are increasingly used for environmental disinfection, footbaths, and environmental misting and fogging,[1,46] and are perceived as being more environmentally friendly than chemicals such as phenols and bleach. Chlorine dioxide is a powerful oxidant as well as disinfectant, and it can be used to remove and prevent biofilm formation. Its use in the dairy industry is becoming more common. Current recommendations from the Wisconsin State Veterinary Diagnostic Laboratory are for its use in solution at 250 ppm. Although rarely done on farm, the effectiveness of environmental cleaning and subsequent disinfection for *Salmonella* control can be assessed by postdisinfection sampling.

Ongoing efforts at animal isolation and environmental hygiene will be important because shedding of *Salmonella* will continue for many weeks after the initial cases have seemingly resolved. With respect to control, shedding continues periodically for the life of the animal in the case of *Salmonella* Dublin. Once *Salmonella* has been identified on a farm, veterinarians and management should increase awareness of the public health risk among workers and revisit personal hygiene, protective clothing, and appropriate disinfectant footbath use for employees. If time and labor resources are limited, then concentrating cleaning and disinfection efforts toward high-risk groups (transition cows, maternity pen) and high use traffic areas may be a reasonable compromise.

Inevitably, the identification of *Salmonella* infection in adult cows or calves will lead to a conversation about vaccine use as a preventative strategy. Many farms have at one time or another tried a commercially available or autogenous *Salmonella* vaccine as an adjunct component of control. The safety and efficacy of autogenous products are questioned by many academicians, but individual experiences are sometimes

compelling, at least in the short term in the face of an outbreak. As with other infectious contagious diseases such as infectious bovine keratoconjunctivitis, when any vaccine product is used during an outbreak it is impossible to know whether improvement was associated with vaccine use or natural immunologic exposure and protective antibody responses. The most commonly used product in the United States currently for the control of salmonellosis in adults is a siderophore receptor/porin vaccine derived from *Salmonella* Newport (*Salmonella* Newport Bacterial Extract, Zoetis Animal Health, Parsippany, NJ). It is administered to dry cows as an initial 2 injection series and boostered annually. It can, however, be given at any stage of lactation or to heifers. It will not prevent infection, but has been associated with an amelioration in disease severity. It does result in demonstrable antibody levels in colostrum when administered twice during the dry period, although the protective effect of these antibodies against challenge postnatally in calves at this time is unknown.[47] The efficacy of other gram-negative core vaccines to prevent or decrease *Salmonella* disease, such as the J5 product (Enviracor, Zoetis Animal Health) or Endovac-bovi (Immvac, Columbia, MO), which are specifically marketed for protection against coliform mastitis, is highly debatable.

The maintenance of good general health, excellent hygiene, and particular attention to the well-being of late gestation and early lactation animals are all critical components of *Salmonella* control. A closed herd is ideal, but rarely achieved, making exposure to the organism inevitable on most dairies. Prompt diagnosis, treatment, and isolation are important during an outbreak in adult cattle and environmental sampling to include bulk tank milk and high-risk housing areas should now be considered a routine part of disease prevention and surveillance.

Calves

Many of the important components of adult cow control programs mentioned in the previous section overlap with specific measures recommended for calves. An article in a previous volume of this journal provided an excellent review of control measures specific to calves.[20]

As in adult herds, endemic disease is increasingly common among calves. Commercial heifer rearing facilities that manage preweaned calves from as young as a few hours of age onward, sourced and transported from multiple farms of origin, create a high-risk environment for the acquisition and spread of neonatal salmonellosis. Adequate passive transfer, although imperative for rearing healthy calves, is not an absolute guarantee for protection from *Salmonella* infection. Fecal–oral transmission is a prime means of spread for enteric and septicemic *Salmonella* infection in calves, but one must be mindful of the risk posed by other secretions such as colostrum, unpasteurized milk, and respiratory secretions, especially in the case of *Salmonella* Dublin.

Hygiene, isolation, and treatment principles for calves, calf housing, and personnel working with calves are very similar to those discussed in the adult section. Special consideration should be given to fecal contamination of milk, milk replacer, colostrum, feeding equipment, and starter rations as a means of cross-infection. Periodic environmental sampling of equipment such as nipple feeders, buckets, and housing can be valuable tools to trouble shoot outbreaks and improve quality control and prevention efforts. Milk and colostrum are effective enrichment media for *Salmonella*, so sampling these sources should be done “as fed” rather than as initially mixed or prepared.[9] The increased availability of colostrum pasteurizers has added a very helpful tool to control not only *Salmonella* Dublin, but also other serotypes that can also be found in colostrum. Maternity area hygiene and management are extremely important

in the control of neonatal salmonellosis. Decreasing the postpartum exposure to the dam reduces the chances of immediate infection. A rather alarming recent publication has identified that true vertical transmission in newborn calves is documented with several serovars common to cattle in the United States.[48] If further studies confirm this finding, it would add yet another serious challenge to the control of salmonellosis in calves.

Because exposure of calves to *Salmonella* is very likely in the commercial dairy environment, management efforts should be directed toward limiting dose and maximizing health and disease resistance in the young replacement animal population. There are no revelations within this advice, but just as occurs with adult cattle, the degree to which farms are able to dedicate personnel and time may only be prioritized in the midst of, or immediately after, an outbreak of clinical disease. Prompt diagnosis, separation, and treatment are important, but group housing of calves can quickly create a "perfect storm" for contagious disease spread. As with adults, vaccination and immunization with modified live or killed (autogenous or commercially available) products is often part of the control and prevention measures instituted. There is very little evidence to support effective control of *Salmonella* infection in calves via passive transfer from immunized dams with any type of vaccine although the siderophore/porin product mentioned in the previous section in adults will stimulate colostral antibody.[47] *Salmonella* is predominantly cleared by cellular immune responses and humoral antibody alone may not provide satisfactory protection. Vaccine use in calves is best considered when management efforts at control and prevention have already been put in place, or if these have been implemented but found to make little difference in the pattern or severity of disease. Autogenous products derived from a specific serovar isolated from clinical cases must be used very carefully owing to the risk of anaphylactic reactions, and only from reputable biologic manufacturers. Similarly, caution is advised regarding modified live vaccine use in calves owing to the potential for adverse reactions. Killed vaccines have performed inconsistently in the small number of trials carried out in the past in calves.[49,50]

COMMENTS REGARDING *SALMONELLA* DUBLIN CONTROL

The increasing prevalence of *Salmonella* Dublin infection in the US dairy industry[14,15] and its unique status as the host adapted serovar of *S enterica* subspecies *enterica* in cattle merit some more specific attention. For readers who wish more, and a greater in-depth discussion of this serovar, we refer you to the excellent primary sources and review paper authored by Dr Liza Nielsen from Denmark who, together with her international collaborators, has published a great deal of excellent work, particularly as it applies to disease impact as well as control and surveillance strategies.[17,36,38,39,51–53]

Within the European community, especially within the Scandinavian countries, there are currently several active surveillance and certification programs that are designed to control, and potentially eradicate *Salmonella* Dublin infection in cattle herds. It is doubtful whether the immediate future holds much promise for such coordinated efforts within the US dairy industry, but there are undoubtedly useful lessons to be learned from experiences in other countries. All of the control measures described in this article for adults and calves can be applied to *Salmonella* Dublin infection, just as they can to other serovars. However, the serologic response to *Salmonella* Dublin, and the ability to measure that as a potential surrogate marker of the carrier status, opens up possibilities for identification and control.

Currently within the United States, the serologic test for *Salmonella* Dublin is available commercially through the Animal Health Diagnostic Center at Cornell University

and can be applied to either blood (serum) or bulk tank milk samples. It is important to recognize that a single time point positive test result does not confirm the carrier status, but indicates an antibody response owing to previous exposure, current infection, or passively derived antibody in a calf less than 3 months of age.[17] Repeated individual animal sampling at specified intervals can be used during surveillance programs to identify animals that are likely to be carriers based on the persistence of an ELISA positive result with a high optical density reading.[17,36,39] Using the data generated by Nielsen as a guide, the Animal Health Diagnostic Center at Cornell University categorizes a carrier as any animal that has 3 strong positive serum ELISA results over an 8-month period (Dr Belinda Thompson, personal communication).

From the currently available literature it does not seem to be possible to predict or estimate what percentage of infected calves or adults will go on to become true carriers, although the number is probably quite low. In herds classified as being endemic for *Salmonella* Dublin in Denmark, the seroprevalence is highly variable but may only be at 15% of the whole herd, with a higher proportion of infection in young stock compared with adults.[17] Reinfection of previously infected and seemingly recovered animals also seems to be possible when individuals are followed over long periods of time. Some of these subsequent infections may also result in the development of carrier status (Dr Belinda Thompson, personal communication).

Bulk tank samples can be used for periodic milking herd surveillance, or, if applied to selected milking groups, to identify whether *Salmonella* Dublin has been introduced into a herd or is present in a particular population of cattle within the herd.[17] From epidemiologic data, it seems that the risk of becoming a carrier after infection is greater for calves and for adults infected around the time of calving.[54] Another study shows that *Salmonella* Dublin infection in endemic herds can be reduced when an individual employee was dedicated to colostrum administration to newborn calves and calving cows were moved into a specific maternity pen before calving.[51]

A number of epidemiologic investigations in endemic *Salmonella* Dublin herds in Scandinavia have identified risk factors and important control points for eradication of infection.[51,54–57] Many of the risk factors and management tools demonstrated to improve control of *Salmonella* Dublin infection are intuitively sensible and relevant to other *Salmonella* serovars. Improving the likelihood of control is associated with avoiding cattle purchases from other farms and ensuring good calving area management and individual calf-rearing practices with solid, not permeable, barriers between calves.[51] Aggressive culling programs are not practical in situations where prevalence is high and may only become reasonable once new calf infections are serologically proven to decline to very low, or absent, levels.[17,56] It may be difficult for some producers and heifer rearers to instigate all of the management changes and practices that have been successful in European countries, but readers are directed to information available through the Animal Health Diagnostic Center at Cornell University website for very helpful guidelines concerning control of *Salmonella* Dublin.[58]

In the United States, there is a commercial live *Salmonella* Dublin vaccine (Intervene D, Boehringer Ingelheim Vetmedica, St. Joseph, MO) that is being used as a component of *Salmonella* Dublin control on many farms. The product is administered parenterally to newborn calves to stimulate an immune response before initial exposure to the pathogen. The goal is to prevent the serious health consequences of natural infection as well as the development of the carrier status in what is the most susceptible population of animals within endemic herds. However, when given according to label instructions the product will interfere with serologic testing, giving a false-positive result at up to 8 months of life.[9] Furthermore, the product can be associated with fatal anaphylactic reactions in some recipient calves. These reactions

seem to be more common in endemic herds than in naïve ones.[9] This product can stimulate colostral antibody production when given to dry cows and was not associated with any adverse reactions when given to late pregnant animals.[59] The vaccinated cohort in this study were from a farm with no clinical history of salmonellosis in recent years.[59] Whether this colostral antibody might provide protection against neonatal infection is currently unknown.

Herd Biosecurity?

Biosecurity Recommendations

- Maintain a closed herd.
- If purchasing cattle, ensure a negative serologic test from individual animals or a negative bulk tank milk test from the herd of origin within the last 6 months.
- Maintain separate maternity and sick cow pens.
- Have separate equipment for feed and manure handling.
- Dedicate personnel to solely work with high-risk or sick cattle versus neonates.

PUBLIC HEALTH CONCERNS WITH *SALMONELLA* AND THE DAIRY INDUSTRY

Salmonellosis not only can cause severe disease in cattle, but also poses a significant zoonotic risk. Farm workers, calf handlers, and their families are clearly at risk of becoming infected by *Salmonella* spp. during outbreaks of clinical illness, but the risk of exposure goes far beyond farm workers or veterinarians with direct animal contact during outbreaks of disease. Asymptomatic shedding of *Salmonella*, a characteristic of *Salmonella* Dublin infection, but also an issue with many other common bovine serovars such as Newport and Typhimurium, creates risk for people in direct contact with the animal, its feces, or milk.[9,60,61] However, the majority of human salmonellosis cases do not derive from direct animal contact, but are instead acquired through foodborne exposure.[62] So-called nontyphoidal salmonellosis is one of the leading causes of acute bacterial gastroenteritis in humans in the United States, responsible for an estimated 1.4 million cases of illness annually.[63] The predominant risk for zoonotic salmonellosis from cattle lies in exposure to contaminated meat from beef, which would include dairy beef and cull dairy cows, typically via fecal contamination of the carcass at the time of slaughter.[63–65]

Although *Salmonella* mastitis is extremely uncommon, shedding of the organism in milk is not, and its presence has been documented in bulk tank milk in several studies.[66–69] A positive bulk tank or milk filter sample may represent fecal contamination, true lactational shedding, or a combination of both. Conventional pasteurization should kill the organism, provided effective temperature and duration are reached. It is important to consider the diagnostic procedure performed to identify the *Salmonella* in bulk tank or milk filter samples when interpreting these studies. Studies using PCR[66,67,69] rather than culture will detect a greater prevalence of *Salmonella*-contaminated samples because of genomic material from both live and dead organisms in the sample. Side-by-side comparisons of conventional culture and PCR using the same samples have been performed and show that approximately one-quarter (2.6% vs 11.2%) of those bulk tank samples that are PCR positive for *S enterica* will be positive by culture. True “dairy” products actually account for only a small percentage of human salmonellosis in the United States, and many of these outbreaks are due to the consumption of raw milk and raw milk products.[67,70]

Bacterial antimicrobial resistance represents an important current and future problem in infectious disease public health. Concerns regarding zoonotic *Salmonella* infections have been amplified in recent years by the emergence of multiple drug-resistant

strains of several *S enterica* serovars associated with cattle.[71–74] It is generally accepted that antimicrobial-resistant bacteria are produced, maintained, and disseminated as a result of selection pressure introduced by the use of antimicrobial drugs.[75] Suspected principal foci of selection pressure include use of antimicrobials for the treatment of humans and in food-producing animals for treatment or prevention of disease and growth promotion.[75,76] Modern molecular methods combined with other conventional techniques such as pulse field gel electrophoresis can be used to investigate the origins of foodborne human enteric disease and the role of antimicrobial use in cattle with the occurrence of multiple drug-resistant *Salmonella* infection in humans. At this point in time, there are few published studies establishing such links from "farm to fork."[73] A recent extensive systematic literature review of 858 publications on the effect of antimicrobial use in agricultural animals on drug-resistant foodborne salmonellosis in humans from 2010 to 2014 concluded that, although antibiotic use in cattle increased the likelihood of colonization in the host, there were no studies that traced antimicrobial-resistant *Salmonella* in humans back to the farm.[76]

The antimicrobials of choice for treating bacterial gastroenteritis in humans are generally the fluoroquinolone, ciprofloxacin, for adults and the cephalosporin, ceftriaxone, for children.[63,77] At issue today is whether the veterinary analogs of these drugs may be responsible for the emergence of antimicrobial resistance in foodborne pathogens like *Salmonella*. The mechanism by which *S enterica* typically acquires antimicrobial resistance to fluoroquinolones differs quite markedly and, importantly, from that by which resistance to cephalosporins develops. Specifically, fluoroquinolone resistance is usually acquired through clonal dissemination of *Salmonella* isolates with mutations in chromosomally encoded resistance genes. Cephalosporin resistance usually is obtained via independent acquisition of mobile genetic elements via plasmids and transposons.[78] Further work is needed in this area to determine whether there is a connection between veterinary use of ceftiofur and the emergence of ceftriaxone resistance in *Salmonella* spp.[63] Although ceftriaxone-resistant *Salmonella* Typhimurium has been documented in cattle,[73] other larger studies have demonstrated little to no resistance to this particular third-generation cephalosporin in cattle sourced serovars despite more common resistance to other cephalosporins.[72,79] Although it is now 8 years old, interested readers are directed to the excellent review of antimicrobial resistant *Salmonella* in dairy cattle by Alexander and colleagues.[79] In a more recent publication, a significant decrease was observed in antimicrobial resistance among dairy cattle *Salmonella* isolates in the northeastern United States.[71]

Many practitioners and diagnostic laboratories will be very familiar with the wide variety of antimicrobial sensitivity patterns demonstrated by different *S enterica* serovars obtained from individual animal and environmental samples. Certain serovars seem to be more commonly associated with greater in vitro resistance than others. The paper by Cummings and colleagues[71] demonstrated a decrease in resistance trends between 2004 and 2011. It was postulated that this might have been related to an increase in the prevalence of the serovar Cerro in fecal samples from their study population. The biggest concern arises with serovars that have historically been more common in dairy cattle and that are associated with human disease outbreaks, such as Newport and Typhimurium. In particular, several human foodborne outbreaks caused by *Salmonella* Typhimurium DT104 of dairy or beef origin that are characteristically resistant to the antibiotics ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline have been reported.[63,80]

An interesting and rigorously investigated example of zoonotic multiple drug resistant *Salmonella* from cattle is provided by the Wisconsin experience with *Salmonella* Heidelberg over the last 2 years. Since 2015, the Wisconsin State Veterinary

Diagnostic Laboratory (WVDL), in conjunction with human and veterinary health organizations throughout Wisconsin, have been tracking a multidrug-resistant strain of *Salmonella* Heidelberg, a Group B serovar (Dr Keith Poulsen, WVDL personal communication). As of November 2016, there were 12 confirmed human infections from 7 different Wisconsin counties. Upon questioning, more than 90% of the infected individuals reported purchasing Holstein bull calves from livestock dealers or sale barns. During 2015 and 2016, the WVDL also isolated several multidrug-resistant *Salmonella* Heidelberg isolates from calves located mostly in Wisconsin. Pulse-field gel electrophoresis and whole genome sequencing of isolates indicated that the human and bovine isolates were very closely related. This strain of *Salmonella* Heidelberg is highly pathogenic and multidrug resistant. Only 1 antimicrobial drug is an effective treatment option for human cases and no effective, legal (United States) options exist for cattle (Dr Keith Poulsen, WVDL, personal communication). As the application of modern molecular techniques becomes more commonplace, it is probable that diagnostic and surveillance efforts will place food animal species and production methods under greater scrutiny with respect to zoonotic enteric diseases. Increased awareness, rigor, and possibly limitations regarding antimicrobial use in food animals should not be surprising outcomes.

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