J. Dairy Sci. 100:1–12
https://doi.org/10.3168/jds.2016-11419

Antimicrobial resistance patterns of bovine *Salmonella enterica* isolates submitted to the Wisconsin Veterinary Diagnostic Laboratory: 2006–2015

J. R. Valenzuela,* A. K. Sethi,† N. A. Aulik,‡ and K. P. Poulsen§[1]
*Wisconsin Veterinary Diagnostic Laboratory, Madison, WI 53706
†Department of Population Health Sciences, University of Wisconsin-Madison, Madison 53726
‡Department of Pathobiological Sciences, and
§Medical Sciences Department, University of Wisconsin-Madison, Madison 53706

ABSTRACT

Salmonellosis on the dairy continues to have a significant effect on animal health and productivity and in the United States. Additionally, *Salmonella enterica* subspecies *enterica* causes an estimated 1.2 million cases of human illness annually. Contributing to the morbidity and mortality in both human and domestic animal species is emergence of antimicrobial resistance by *Salmonella* species and increased incidence of multidrug-resistant isolates. This study describes serotype distribution and the antimicrobial resistance patterns for various *Salmonella* serotypes isolated from bovine samples submitted to the Wisconsin Veterinary Diagnostic Laboratory (WVDL) over the past 10 yr. *Salmonella* serotyping and antimicrobial susceptibility testing data were obtained from the laboratory information management system at WVDL. Data from accessions were limited to bovine samples submitted to the WVDL between January 2006 and June 2015 and those that had both a definitive serotype and complete results for antimicrobial susceptibility testing. A total of 4,976 isolates were identified. *Salmonella enterica* ser. Dublin was the most prevalent serotype identified among bovine samples submitted to the WVDL, accounting for a total of 1,153 isolates (23% of total isolates) over the study period. Along with Dublin, *Salmonella enterica* ser. Cerro (795, 16%), Newport (720, 14%), Montevideo (421, 8%), Kentucky (419, 8%), and Typhimurium (202, 4%) comprised the top 6 most commonly isolated serotypes during that time. Overall, resistance of bovine *Salmonella* isolates in the study population remained stable; although, decreases in resistance were noted for gentamicin, neomycin, and trimethoprim sulfamethoxazole during the study period. All isolates remained susceptible to enrofloxacin. These data show that antimicrobial susceptibility for bovine *Salmonella* has changed in the population served by WVDL in the past 10 yr. This information is important for understanding *Salmonella* disease ecology in Wisconsin. Our findings are also relevant for animal and public health by improving informed antimicrobial use, new drug development, and regulation of their use in food animals.

Key words: bovine, salmonella, antimicrobial resistance, dairy, Wisconsin

INTRODUCTION

Salmonella causes significant disease in food animals and those animals are also potential sources of zoonotic human infection (Mandal and Brennand, 1988). Common clinical manifestations of *Salmonella* infection in cattle include diarrhea, pneumonia, abortion, and death. Infection in dairy cattle is also associated with decreased milk production (Nielsen et al., 2012, 2013; O'Doherty et al., 2015). Salmonellosis can have a substantial economic effect on dairies in the United States, with some financial loss estimates ranging in the billions of dollars annually (Peters, 1985; Huston et al., 2002). Although cattle can be infected by several *Salmonella enterica* serotypes, the majority of infections are attributed to serovar Dublin, which tends to cause more systemic infections, and serovar Typhimurium, more often causing enteritis in young calves (Costa et al., 2012).

Salmonella enterica is also of significant public health interest with zoonotic potential. The majority of infections in humans are associated with products derived from food animals and contaminated produce, which has been linked to domestic and feral animal populations (Jay-Russell et al., 2014; Haack et al., 2016). Most cases of *Salmonella* in humans are likely to be self-limiting; however, severe illness has been seen in children, the elderly, and immune-compromised patients, which

Received May 8, 2016.
Accepted October 30, 2016.
[1]Corresponding author: keith.poulsen@wvdl.wisc.edu

require antibiotic treatment (CDC, 2013b). Annually, the United States has approximately 100,000 infections with a resistant strain of non-typhoidal *Salmonella* (CDC, 2013b). Human salmonellosis costs the US economy approximately $2 to $4 billion through loss of work, health care costs, and morbidity (Foley and Lynne, 2007).

Effective antimicrobial stewardship is contingent, in part, on ongoing surveillance of antimicrobial resistance trends in food products, animals, and people. This information can better inform public policy as well as clinical practice regarding appropriate antimicrobial use. Trends in antimicrobial resistance of *Salmonella* isolates from food animals are of public health concern given the potential for the spread of resistant microorganisms to people. Thus, the objective of our study was to describe the epidemiology and antimicrobial resistance patterns for bovine *Salmonella enterica* serotypes isolated from samples submitted to the Wisconsin Veterinary Diagnostic Laboratory (**WVDL**) over the last 10 yr.

MATERIALS AND METHODS

Data Collection

Data were retrospectively collected on all bovine *Salmonella enterica* isolates identified at the WVDL between January 1, 2006, and July 30, 2015, using the Laboratory Information Management System (UVIS, Ross Group Inc., Dayton, OH). Antimicrobial susceptibility testing (**AST**) data were queried from antimicrobial resistance databases at the WVDL Madison and Barron locations (Sensititer, Thermo Fisher Scientific, Waltham, MA). The WVDL is 1 of 3 laboratories that provide testing and diagnostic services to veterinarians and producers in the state. Approximately 90% of the total caseload is from the state of Wisconsin, which is dominated by the dairy industry. *Salmonella enterica* is typically associated with such clinical presentations as calf and adult cow diarrhea, abortion workups, and septicemia in both adult cows and calves. The organism is also often associated with nonspecific herd health issues or endemic disease problems, in particular involving *Salmonella enterica* ser. Dublin. Sample types included feces submitted for routine diagnostics and tissues harvested during necropsy. Milk was excluded from our study samples because it is unknown how often *Salmonella enterica* is shed in milk, but it is thought to be rare and attributed mostly to postcollection contamination (Nielsen, 2013; Van Kessel et al., 2013). Diagnostic specimens are required to be submitted by veterinarians for interpretation of results for the purposes of disease management.

Due to the financial constraints of submitting clients, WVDL only fully serotypes and provides sensitivity for 1 *Salmonella* serotype isolated from a single accession, which can include multiple animals or tissues. All *Salmonella* isolates are serogrouped to confirm that all *Salmonella* isolated from a single accession or animal are of the same serogroup, and then one of the *Salmonella* isolated is fully serotyped. These accessions are considered single accessions, as just 1 serogroup of *Salmonella* was identified. If an accession or animal had more than 1 serogroup, each serogroup would have 1 isolate serotyped and susceptibility assessed. These accessions are considered multiple accessions, as more than 1 *Salmonella* serotype was identified. Each serotype would be given a unique identification number composed of the original accession number followed by an animal and specimen number. In rare cases, the same accession or animal might have had more than 1 isolate fully serotyped and susceptibility performed even if the serotype was the same. For these accessions, only those isolates with unique AST results were included.

All serotyping was performed from isolated colonies following the Kauffman-White classification system. Identification of *Salmonella* Dublin using real-time reverse transcription PCR (**RT-PCR**) was introduced at the laboratory in the summer of 2014. Dublin isolates with high (>35) cycle threshold ($\mathbf{C_T}$)-positive RT-PCR values were not included in this data set because they are generally not able to be successfully cultured to be subject to AST. Low (<35) C_T-positive RT-PCR samples were automatically cultured, serotyped, subject to AST, and included in this study. The internal success rate is estimated to be 70 to 75% for successful post-PCR culture of samples with C_T lower than 35 for both the *Salmonella* species and *Salmonella* Dublin multiplex PCR.

Isolates that were identified as *Salmonella* Bardo were counted as *Salmonella* Newport based on recent recommendations by the Centers for Disease Control and Prevention on interpretation of *Salmonella* serotypes, which describes this pair as genetically indistinguishable (CDC, 2013a). The year of the finalized date was used for analysis for accessions spanning change of the calendar year.

Antimicrobial Susceptibility

Isolates were included if they were fully serotyped, using standard operating procedures reviewed and accredited by the American Association of Veterinary Laboratory Diagnosticians, and subject to AST performed using the bovine or porcine MIC format plate with tulathromycin [Sensititer BOPO6F (post-2007) or BOPO1F (pre-2007), Thermo Fisher Scientific, Oak-