

Salmonella on U.S. Beef Cow-calf Operations NAHMS Beef 2017 Study

Information Brief

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INTRODUCTION

Salmonella bacteria have been isolated from nearly all vertebrates, and Salmonella infections are associated with animal and human disease. Typical signs of Salmonella infection in cattle and humans include fever and diarrhea. Severe cases can result in death. In calves, the Dublin serotype can also cause respiratory disease, as well as fever and diarrhea, often leading to economic losses for producers. Shedding of Salmonella in cattle can occur in the absence of clinical signs, sometimes for extended periods. Adult cattle are more likely to be asymptomatic carriers, intermittently shedding Salmonella bacteria in their feces (Grünberg, 2020).

In some cases, foods of animal origin have been implicated as the source of human illness caused by Salmonella. Salmonellosis in humans is typically self-limiting, and most people recover within one week. Antimicrobials are not necessary for treating most Salmonella infections in humans and animals; however, Salmonella resistance to antimicrobials is problematic because it can complicate treatment options in severe infections. Resistance to ciprofloxacin or ceftriaxone in Salmonella is a concern because of their frequency of use in human salmonellosis cases requiring antibiotics.

NAHMS BEEF 2017 STUDY

The U.S. Department of Agriculture's National Animal Health Monitoring System (NAHMS) conducted the Beef 2017 study in 24 of the Nation's major cow-calf States (Figure 1). Operations in these States accounted for 86.6 percent of the U.S. beef cow inventory and 78.9 percent of all U.S. operations with beef cows. All operations in these States with one or more beef cows as of January 1, 2017, were eligible to be selected for inclusion in the study. One of the study objectives was to describe the occurrence and antimicrobial resistance (AMR) of Salmonella on U.S. beef cow-calf operations.



Figure 1. States/regions that participated in the NAHMS Beef 2017 study

BIOLOGICAL SAMPLING

For the Beef 2017 study, up to 12 composite fecal samples (samples taken from different sites and combined) were collected per operation, of which 6 were from adult cow areas and 6 were from calf areas. The adult cow samples were collected from up to six selected areas on a farm (near a water source, feeding grounds, calving pens, open fields, pasture, or similar). Adult cow feces from six sites within each area were collected and combined into a composite sample. For example, if one of the composite samples was from an area near a water source, that sample consisted of feces combined from six sites near that water source. If calves were also present on the operation, the same process sampled up to six areas where calves were known to congregate, with each composite sample consisting of calf feces from six sites within each area.

All the composite fecal samples were tested for *Salmonella*. The USDA Agricultural Research Service's Bacterial Epidemiology and Antimicrobial Resistance (ARS BEAR) lab received the samples to be cultured for *Salmonella* spp. Samples were pre-enriched in buffered peptone water (BPW, Becton Dickinson) before screening for *Salmonella* using the BAX[®] *Salmonella* Real-time PCR (Dupont) according to the manufacturer's instructions. Aliquots of the BPW enriched PCR positive samples were transferred to additional enrichment broths (Hajna and Tetrathionate, followed by Rappaport-Vassiliadis, all Becton Dickinson). Aliquots of enrichment broth were plated on Xylose-Lysine-Tergitol-4 (Becton Dickinson) and BG Sulfa (Becton Dickinson) agar plates to isolate *Salmonella*. Isolated colonies consistent with *Salmonella* were then streaked to ensure pure growth and inoculated into triple sugar iron and lysine iron agar slants (Becton Dickinson) for biochemical confirmation. All confirmed *Salmonella* isolates were sent to the National Veterinary Services Laboratories (NVSL) for serotyping and antimicrobial susceptibility testing (AST).

The serotyping process used the xMAP *Salmonella* serotyping assay and classical serotyping using the White-Kauffmann-Le Minor classification scheme (Grimont and Weill, 2007). Antimicrobial susceptibility testing was performed using the National Antimicrobial Resistance Monitoring System's (NARMS) gram-negative CMV4AGNF AST plates (Thermo Fisher Scientific), according to Sensititre[™] protocols. The interpretation of the results used the 2012 NARMS retail meat breakpoints (NARMS, 2020).

This information brief includes results from the NAHMS Beef 2007–08 and Beef '97 studies. For these studies, up to 40 fresh fecal samples from individual fecal pats from adult cows were cultured for *Salmonella* from each operation. Further details of the methodology are published elsewhere (Dargatz et al., 2000; USDA, 2012). The use of the BAX PCR in the 2017 study was the only difference in culture and isolation methods from the 2007–08 and 1997 studies.

SALMONELLA PRESENCE AND SEROTYPES

The Beef 2017 study collected 1,057 fecal samples from 136 cow-calf operations. *Salmonella* was isolated from 11 samples (1.0 percent of samples positive) from 6 different operations (4.4 percent of operations positive). NAHMS Beef studies since 1997 have revealed a low prevalence of *Salmonella* on cow-calf operations (Table 1).

by study year							
		Number an	d Percentage				
	Operatior	IS	Samples				
Study	No. Positive / No. Sampled	Pct.	No. Positive / No. Sampled	Pct.			
Beef '97	21 / 187	11.2%	70 / 5,049	1.4%			
Beef 2007–08	16 / 173	9.2%	31 / 5,793	0.5%			
Beef 2017	6 / 136	4.4%	11 / 1,057	1.0%			

Table 1. Number and percentage of operations and samples positive for Salmonella,
by study year

The genus *Salmonella* is categorized by species, subspecies, and serotype designations. There are two species (*enterica* and *bongori*). The *enterica* species is further subdivided into six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae, and indica,* designated by Roman numerals I, II, IIIa, IIIb, IV, and VI, respectively). All serotypes of *Salmonella* can be recognized by antigenic formula, consisting of subspecies names written in Roman numerals followed by O (somatic) antigens, H (flagellar) antigens (phase 1), and H antigens (phase 2, if present). Most serotypes of the *enterica* subspecies receive a name (for example, Typhimurium), but some, such as I 4,[5],12:i:- are recognized only by antigenic formula. Most serotypes of subspecies other than *enterica* are identified only by antigenic formula (for example, IIIb 61:-:1,5, [7]). Most *Salmonella* found in humans and livestock in the United States are *Salmonella enterica* subsp. *enterica*. Two isolates from the NAHMS cow-calf studies, one from the 2017 study and one from the 2007–08 study, were subspecies other than *enterica*.

The *Salmonella* serotype most frequently found in the Beef 2017 study was Give (Table 2). Each serotype in the Beef 2017 study was found on just one operation. Two of the samples each had 2 isolates of different serotypes, resulting in a total of 13 isolates from 11 samples. Of these, seven isolates (53.8 percent) came from a single operation, another two isolates (15.4 percent) came from a single operation, and the remaining four isolates came from four different operations.

Across the three NAHMS cow-calf studies, only nine serotypes were in more than one study, and none of the serotypes were in all three studies. There does not appear to be a dominant *Salmonella* serotype on cow-calf operations. However, this may be due to the overall very low prevalence of *Salmonella* on cow-calf operations.

	Number and Percentage of Isolates						
	Beef '97		Beef 2	Beef 2007–08		Beef 2017	
	(n=78)		(n=	(n=34¹)		(n=13²)	
Serotype	No.	Pct.	No.	Pct.	No.	Pct.	
Give	1	1.3			4	30.8	
Cerro	17	21.8			3	23.1	
Havana					2	15.4	
Typhimurium	3	3.8			1	7.7	
Bareilly	1	1.3			1	7.7	
I 4,[5],12:b:-					1	7.7	
IIIb 21:z10:e,n,x,z15					1	7.7	
Oranienburg	17	21.8					
Bredeney	7	9.0					
Anatum	8	10.3	1	2.9			
Mbandaka	4	5.1	1	2.9			
Newport	3	3.8	2	5.9			
Heidelberg	2	2.6					
Montevideo	2	2.6	6	17.6			
Muenchen	2	2.6					
Muenster	2	2.6					
Rubislaw	1	1.3	1	2.9			
l 6,7:k:-			3	8.8			
Braenderup			2	5.9			
Meleagridis			2	5.9			
I 3,10:-:1,w			2	5.9			
All others	8 ³	10.3	14 ⁴	41.2	NA ⁵	NA ⁵	

Table 2. Number and percentage of *Salmonella* isolates by serotype, and by study year

¹Two isolates were cultured from each of two samples.

²Two isolates were cultured from each of two samples.

⁴Three untypable isolates are included here, as well as serotypes with one isolate each not found in other NAHMS cow-calf studies (Javiana, Lawndale, Oukam, Saugas, - - - - -).

⁵All isolates from Beef 2017 appear in the table.

Cells greyed out denote serotypes not found in the given study

 ³Serotypes with one isolate each not found in other NAHMS cow-calf studies included Enteritidis, Infantis, Java, Litchfield, Newington, Pomona, Poona, and Thompson.
⁴Three untypable isolates are included here, as well as serotypes with one isolate each not found in other NAHMS cow-calf

ANTIMICROBIAL SUSCEPTIBILITY

None of the *Salmonella* isolates from the Beef 2017 study were resistant to the 14 tested antimicrobials. Similarly, in the Beef 2007–08 study, none of the *Salmonella* isolates were resistant to any of the tested antimicrobials. The Beef '97 study observed some resistance to ampicillin, gentamicin, streptomycin, sulfamethoxazole, tetracycline, and ticarcillin (Table 3). Additionally, none of the *Salmonella* isolates from the three NAHMS cow-calf studies showed resistance to ciprofloxacin or ceftriaxone, antibiotics important for the treatment of human cases of salmonellosis.

Antimicrobial	Beef '97 (n=78) Pct.	Percent Isolates Study Beef 2007–08 (n=34) Pct.	Beef 2017 (n=13) Pct.
Amikacin	0.0	0.0	
Amoxicillin-Clavulanic Acid	0.0	0.0	0.0
Ampicillin	1.3	0.0	0.0
Apramycin	0.0		
Azithromycin			0.0
Cefoxitin		0.0	0.0
Ceftiofur	0.0	0.0	
Ceftriaxone	0.0	0.0	0.0
Cephalothin	0.0		
Chloramphenicol	0.0	0.0	0.0
Ciprofloxacin	0.0	0.0	0.0
Gentamicin	2.6	0.0	0.0
Meropenem			0.0
Kanamycin	0.0	0.0	
Nalidixic Acid	0.0	0.0	0.0
Streptomycin	11.5	0.0	0.0
Sulfamethoxazole/Sulfisoxazole ¹	11.5	0.0	0.0
Tetracycline	2.6	0.0	0.0
Ticarcillin	1.3		
Trimethoprim-Sulfamethoxazole	0.0	0.0	0.0

Table 3. Percentage of resistant Salmonella isolates, by study year and by antimicrobial

¹Sulfisoxazole replaced Sulfamethoxazole in 2007–08 and 2017.

Cells greyed out denote antimicrobials not tested in the given study

The percentage of *Salmonella* isolates susceptible (or intermediate) to all antimicrobials tested was 87.2 percent in the Beef '97 study, 100.0 percent in Beef 2007–08, and 100.0 percent in Beef 2017 (Figure 2).

Of the three isolates resistant to three or more antimicrobials in the Beef '97 study, only two would be considered multi-drug resistant (defined as resistant to at least three antimicrobial classes). The other isolate was resistant to gentamicin-streptomycin-sulfamethoxazole, which consists of only two antimicrobial classes (aminoglycosides and sulfonamides). Of the two multi-drug resistant isolates, one (*Salmonella* Oranienburg) was resistant to tetracycline-streptomycin-sulfamethoxazole, and the other (*Salmonella* Heidelberg) was resistant to ampicillin-gentamicin-streptomycin-sulfamethoxazole-ticarcillin.

Figure 2. Percentage of *Salmonella* isolates, by study year and by number of antimicrobials to which the isolate was resistant



CONCLUSION

The Beef 2017 study found few *Salmonella* isolates, with only 1.0 percent of samples across 4.4 percent of operations being *Salmonella*-positive. This finding, combined with the low *Salmonella* prevalence in previous studies, indicates that *Salmonella* is not very common on U.S. beef cow-calf operations. Repeated sampling of these same operations over time could identify more positive operations. There did not appear to be a dominant *Salmonella* serotype found on cow-calf operations across the three NAHMS cow-calf studies. However, this is likely due to the overall very low prevalence of *Salmonella* on cow-calf operations. The 2017 and 2007–08 studies found no resistance to any antimicrobials tested. The Beef '97 study found a low level of antimicrobial resistance, with only 10 of 78 isolates showing any antimicrobial resistance, and only 2 isolates were multi-drug resistant. These results suggest that antimicrobial-resistant *Salmonella* are uncommon on U.S. beef cow-calf operations.



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