

Project Title:	Evaluation of and Factors that Influence the Site and Extent of <i>Salmonella</i> in Extra-Intestinal Tissue of Cattle
Principle Investigator(s):	G. H. Loneragen ¹ , T. S. Edrington ² , M. Bugarel ¹ , H. C. den Bakker ¹ and K. K. Nightingale ¹
Institution(s):	¹ Department of Animal and Food Sciences, Texas Tech University ² USDA, ARS, Food and Feed Safety Research Unit
Completion Date:	June 2015

Background

Salmonella continues to pose challenges for human and animal health. Researchers have reported it is not uncommon to recover *Salmonella* from peripheral lymph nodes (PLN) of feedlot cattle at slaughter. This observation is particularly so during certain times of the year and within more southern climates. It has been speculated that much of the *Salmonella* recovered from the PLN arrives there via regional afferent lymph vessels following transdermal infections. If so, the ecology and transmission dynamics of *Salmonella* among cattle populations is far more complex than previously believed.

Tremendously high concentrations of *Salmonella* have been observed in some PLN. At present, it is uncertain if *Salmonella* simply accumulates in PLN or it replicates within PLN. Moreover, a new serovar of *Salmonella*, *Salmonella* Lubbock, has recently been discovered. This offers the opportunity to explore common attributes that facilitate persistence within cattle and environmental niches. If so, it may point to opportunities for control. Lastly, researchers have observed increased diversity in the genes that encode resistance to the critically important drugs known as 3rd-generation cephalosporins (3GC).

The objectives of this study were to evaluate the role of stress hormones in the extent of *Salmonella* replication within extra-intestinal tissues of cattle (in particular, PLN), to perform whole-genome sequencing (WGS) on *Salmonella* isolates recovered from cattle samples (feces, hides and PLN) to explore common attributes that could confer a fitness advantage, and to perform WGS on a selection of non-type-specific *E. coli* isolates recovered from cattle feces that display a phenotype consistent with extended-spectrum beta-lactamase (ESBL) producing *E. coli* which are resistant to many cephalosporins.

Methodology

Fifteen Holstein steers were randomly assigned to one of three treatments that were: control, norepinephrine (45 µg/kg body weight i.m./d), or a synthetic catecholamine (200 mg Ractopamine/head/day). Steers were challenged on days 0, 2 and 4, with *Salmonella* Montevideo on the right side of the animal (8.4 x 10⁸/ml) and *Salmonella* Newport on the left side of the animal (7.6 x 10⁸/ml) intra-dermally. Steers were challenged using the ComforTen® Multiple Skin Test Device and challenge strains were applied medially and laterally above the knee and hock, as well as on both sides of the back, just below the shoulders and on each side of the abdomen. Steers were euthanized on day 7 and culture

and isolation was performed on sub-iliac, popliteal, and pre-scapular lymph nodes. In addition, 104 and 96 *Salmonella* and *E. coli* isolates, respectively were subjected to whole-genome sequencing.

Findings

Salmonella in PLN continues to be a challenge to the beef production industry. In the study described herein, a detectable difference was not seen among treatments in terms of concentration of *Salmonella* in the PLN. While it was not statistically significant, the concentration within animals treated with norepinephrine was always slightly above that observed in the control calves. If this difference was indeed biologically associated with treatment, then this study was underpowered to detect such a difference. Others have observed an association of epinephrine and bacterial replication, and an association of catecholamines with *Salmonella* growth rate in minimal media. Additional work is warranted to refine the model to determine if stress hormones are associated with bacterial replication and concentration in PLN.

In terms of WGS of *Salmonella*, researchers have generated a large sequence database to interrogate for characteristics that may be shared among serovars that offer a fitness advantage. This represents a form of convergence evolution in that an ecological niche creates an opportunity for emergence and persistence of isolates with a shared set of characteristics. If such characteristics can be identified, they will provide new insights into the complex ecology of *Salmonella* in cattle populations. Of importance, this information will also provide insights into likely areas of effective control.

Researchers identified 8 genes that have previously been associated with ESBL production. Moreover, these genes likely conferred the ESBL phenotype observed in the isolates in this study. The genes were not evenly distributed and some were only observed very rarely. These isolates were recovered from fecal samples on operations where 3GC use is relatively common and likely contributed to the broad diversity of ESBL genes. However, these data ought to inform development methods for surveillance on operations in which ESBL harborage is expected to be rare (e.g., feed-lots).

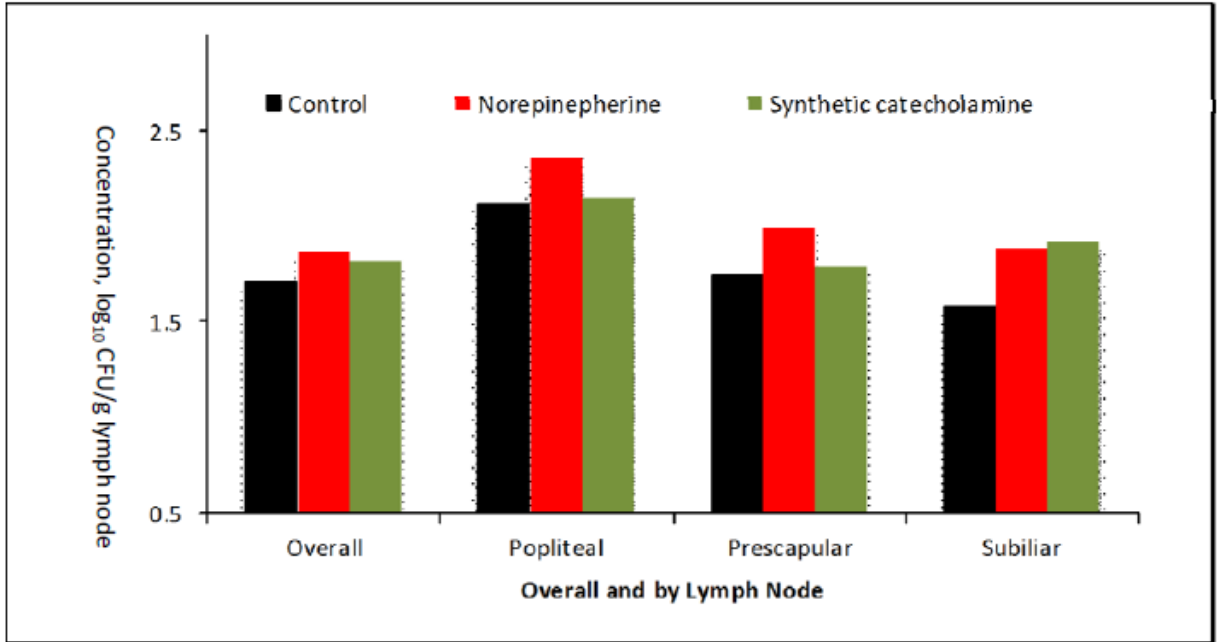


Figure 1. Concentration of *Salmonella* in peripheral lymph nodes recovered from cattle.

Table 1. Genes identified that have been previously associated with ESBL production.

ESLB family	ESBL gene	Number observed (of 96)
CTX-M-1 β -lactamases		
	<i>bla</i> _{CTX-M-1}	8
	<i>bla</i> _{CTX-M-11}	1
	<i>bla</i> _{CTX-M-15}	4
	<i>bla</i> _{CTX-M-32}	47
	<i>bla</i> _{CTX-M-57}	5
CTX-M-9 β -lactamases		
	<i>bla</i> _{CTX-M-27}	25
	<i>bla</i> _{CTX-M-65}	2
TEM β -lactamases		
	<i>Bla</i> _{TEM-168}	9
Combinations		
	<i>bla</i> _{CTX-M-1} , <i>Bla</i> _{TEM-168}	6
	<i>bla</i> _{CTX-M-32} , <i>Bla</i> _{TEM-168}	3
	<i>bla</i> _{CTX-M-32} , <i>Bla</i> _{CTX-M-27}	1