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The impact of *Salmonella* on feedlot and dairy cattle

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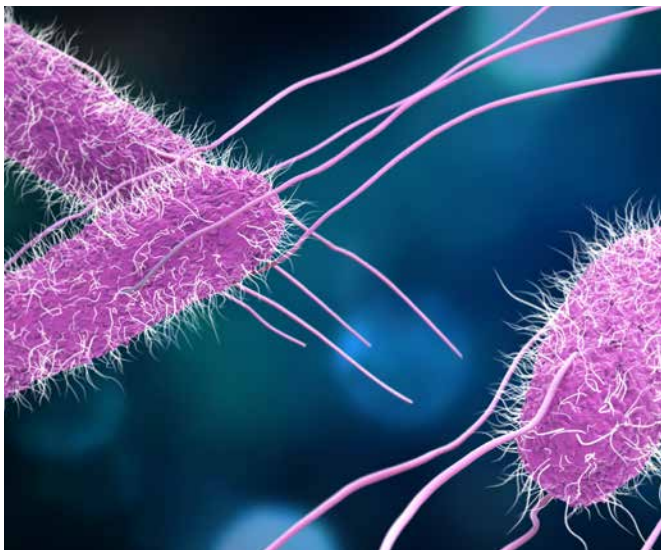


Significant economic loss resulting from *Salmonella*

Salmonella enterica (commonly referred to as *Salmonella*) is a leading cause of foodborne illness (Jajere, 2019). The Centers for Disease Control and Prevention (CDC) estimates that *Salmonella* causes over one million foodborne illnesses every year in the United States. The economic loss resulting from *Salmonella* foodborne illness was estimated by the United States Department of Agriculture (USDA) Economic Research Service (ERS) at $3.7 billion in medical expenses, loss of productivity, and cost of premature death (Foodborne Illness, 2015). Humans may contract *Salmonella* either by fecal-oral transmission or acute gastroenteritis following consumption of contaminated food. Fecal-oral transmission happens when a person is exposed to a human or animal that is shedding the pathogen. A few examples of potentially contaminated food sources are eggs, poultry, produce, pork and beef. According to the CDC, one in seven *Salmonella* outbreaks is attributed to beef (*Salmonella* and Food, 2020).

In the early 1990's, *E. coli* O157:H7, a Shiga-toxin-producing *E. coli* (STEC) caused one of the most infamous outbreaks of foodborne illness the beef industry has faced (Jack in the Box, 2017). After the outbreak, plants worked tirelessly to figure out how to reduce such catastrophic events from occurring. One of the major changes implemented at the plant level was declaring *E.coli* O157:H7 a zero-tolerance adulterant and implementing multiple Hazard Analysis and Critical Control Points (HACCP). The assumed route of contamination of carcasses for both *E.coli* and *Salmonella* has been via transmission from the hides of animals during processing (*Salmonella* White Paper, 2019). However, although HACCP has effectively reduced risk from STEC, reduction of *Salmonella* within foods, such as ground beef, has not had the same level of success (*Salmonella* White Paper, 2019). The USDA Food Safety and Inspection Service (FSIS) found that contamination of ground beef with *Salmonella* averages around 2.1% and little to no improvement has been achieved over the past few decades (FSIS, 2011). Research has shown that the major hurdle we face with *Salmonella* is the pathogen's ability to remain viable within the peripheral lymph nodes (LN) of cattle (Gragg et al., 2013). As peripheral LN are not typically removed prior to grinding of beef, they are the most common source of contamination in ground beef products (Laufer et al., 2015). All things considered, the best strategy for reducing risk during processing is to find solutions that reduce the likelihood of *Salmonella* getting into the lymphatic system from the gut and to reduce the amount of *Salmonella* being shed in the feces of animals that can contaminate their hides. These solutions are considered by the USDA to fall in the area of Pre-harvest Food Safety.

However, food safety concerns are not the only issue facing producers. The dairy industry is significantly impacted both in the calf and cow segments from *Salmonella*. Research has shown that losses from direct issues such as reduced milk yield, dead animals, treatment costs, and abortions, coupled with indirect economic losses such as sold cows and lower milk yield from replacement animals, all resulted in large gross margin losses, especially in poorly managed scenarios (Nielsen *et al.*, 2013). The economic impact of these losses coupled with the fact that this pathogen is a significant public health risk to farm employees, visitors,

and families makes *Salmonella* an important management focus.

Detection of *Salmonella*

The National Animal Health Monitoring System Dairy '96 study showed that 5.4% of milk cows shed *Salmonella*, while 18% of cows to be culled within one week were shedding the organism. Overall, 28% of dairy farms and 67% of cattle markets had animals that shed *Salmonella*. Additionally, a study by NAHMS of 68 large (≥1,000 head capacity) feedlots in 2011 found that 35.6% of pens and 60.3% of feedlots had one or more positive test samples for *Salmonella* (NAHMS, 2014). Overall prevalence of *Salmonella* cultured from fecal samples was 9.1%.

Production of milk and performance of dairy cows can be negatively impacted due to infections with *Salmonella*; however, rarely are there easy-to-detect signs of infection (Holschbach and Peek, 2018). This means that cattle can be sick and shedding *Salmonella* without any useful visual methods of detection. Although *Salmonella* is usually undetectable in feedlot cattle, younger calves are susceptible to infection from this pathogen, and can experience severe diarrhea, high fevers, and death. "As an infectious, contagious pathogen, *Salmonella* is probably rivaled by only bovine viral diarrhea virus in its ability to cause clinical disease, such as enteritis, septicemia, pneumonia, and reproductive losses" (Holschback and Peek, 2018).

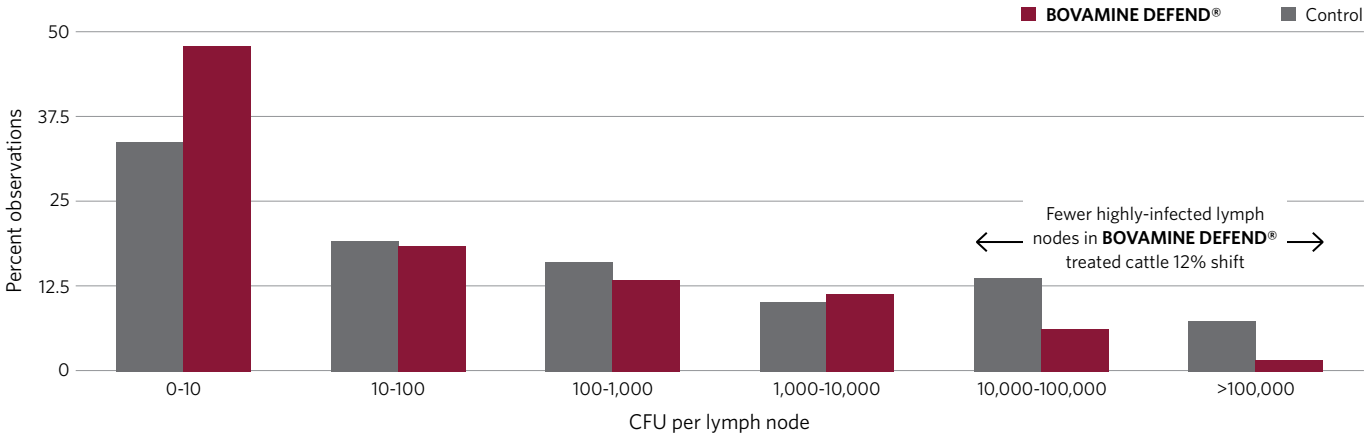
In addition to the risk of continued contamination to cattle actively shedding the pathogen, *Salmonella* can survive for long periods of time in the environment, and therefore incidental contamination of water, feed, and equipment are all possible sources of transmission (Murray, 1991).

***Salmonella's* path to lymph nodes**

Understanding how *Salmonella* is able to persist within cattle, how fast it can replicate, how the immune system responds to the pathogen, and how to kill it, is ultimately necessary to achieve an overall reduction of prevalence within cattle. Research has found that *Salmonella* can move from the mouth and into the GIT relatively unperturbed by the host (Kaiser et al., 2013). Therefore, if a large dose of *Salmonella* is consumed, then a large dose will inevitability end up in the GIT. Once in the GIT, *Salmonella* is able to replicate at a rapid rate. Research conducted with mice showed that the bacterial population could double within the intestine every 5.9 hours and migrate to cecal LN at a rate of 298 cells per day. Although this migration rate is surprisingly low, it still reflects this pathogen's ability to break through the natural gut barriers and end up in the lymphatic system (Kaiser et al., 2013). Breaking out of the GIT is a complex process which usually requires multiple failures of normal GIT physiology. In a healthy, unstressed animal, normal barrier protections cannot be overcome by one dose of one pathogen. But, when an animal and its GIT are experiencing multiple stressors, such as heat stress, dietary transitions, or depressed intake coupled with a pathogen challenge, the barrier functions can faulter. This compounding of stressors and the subsequent pathological responses lead to what is called "leaky gut syndrome (LGS)". Dr. Lance Baumgard, Professor of Animal Science at Iowa State University describes LGS as "the inability of the intestinal barrier to prevent unwanted molecules inside of the intestine from entering into the body" (Boylen, 2016). Repeated exposure of the GIT to virulent pathogenic bacteria, such as *E.coli* and *Salmonella*, can result in LGS, but sometimes it's the response of the host's immune system that result in LGS. In response to a detrimental shift in the microbiome in the GIT resulting from the aforementioned stressors, the host can mount an inflammatory response to protect the GIT from damage. Pro-inflammatory signaling molecules can be released and macrophages and neutrophils can be recruited to challenged areas. These cell types produce reactive oxygen species (ROS) that can cause additional local stress and damage. The inflammation caused by these types of immune responses opens intercellular channels through which *Salmonella* can pass. Once *Salmonella* exits the GIT and enters the portal blood system, it will be filtered into the lymphatic system and will end up in lymph nodes and the liver. In addition to breaking through the GIT during LGS, *Salmonella* may also get a "free ride" to the lymph nodes via other cells within the innate immune system (Holzer, 2020).

The innate immune system, which is one of two main systems the body has to combat disease, is the most immediate response the immune system has once an antigen or foreign material is detected. The innate immune system could be thought of as one of our body's first lines of defense against pathogens (Nicholson, 2016). There are many different players involved in the innate immune response, including dendritic cells and phagocytes which produce ROS and are toxic to *Salmonella* (Slauch, 2011). Dendritic cells have a unique role in this process as they sample the pathogen and bring it to T helper cells, which are located within LN. Once a T-helper cell determines how to best kill the pathogen, the adaptive immune response is activated. The adaptive immune response is associated with antigen-specific responses and is much more complex than innate responses (Nicholson, 2016). For the adaptive immune response to work, the pathogen much first be recognized. At this point, specific immune cells designed

Figure 1. Concentration of *Salmonella* within the subiliac lymph node (SLNs) for control and supplemented cattle from a commercial feedlot study. Total control SLNs was 191 and total treatment group SLNs were 150.



to attack that specific pathogen will be released. Upon this "attack" the adaptive immune system is also able to create a "memory" towards the specific pathogen to ensure future responses are efficient, this is why vaccinations are so effective (Nicholson, 2016). When dendritic cells take up *Salmonella* within the GIT and carry them to LN for sampling, the final step is to hand off the bacteria to phagocytes such as neutrophils or macrophages to engulf and destroy (Kaiser et al., 2013). This process is a perfectly designed system, unfortunately, *Salmonella* is able to combat phagocytes and actually thrive within LN (Holzer, 2020; Brichta-Harhay et al., 2012). So, *Salmonella* is arriving to the LN because of LGS where they are then able to survive and replicate. Basically, the immune system's process for activation of the adaptive system and its method to kill the pathogen ends up being the systems very own Achilles Heel!

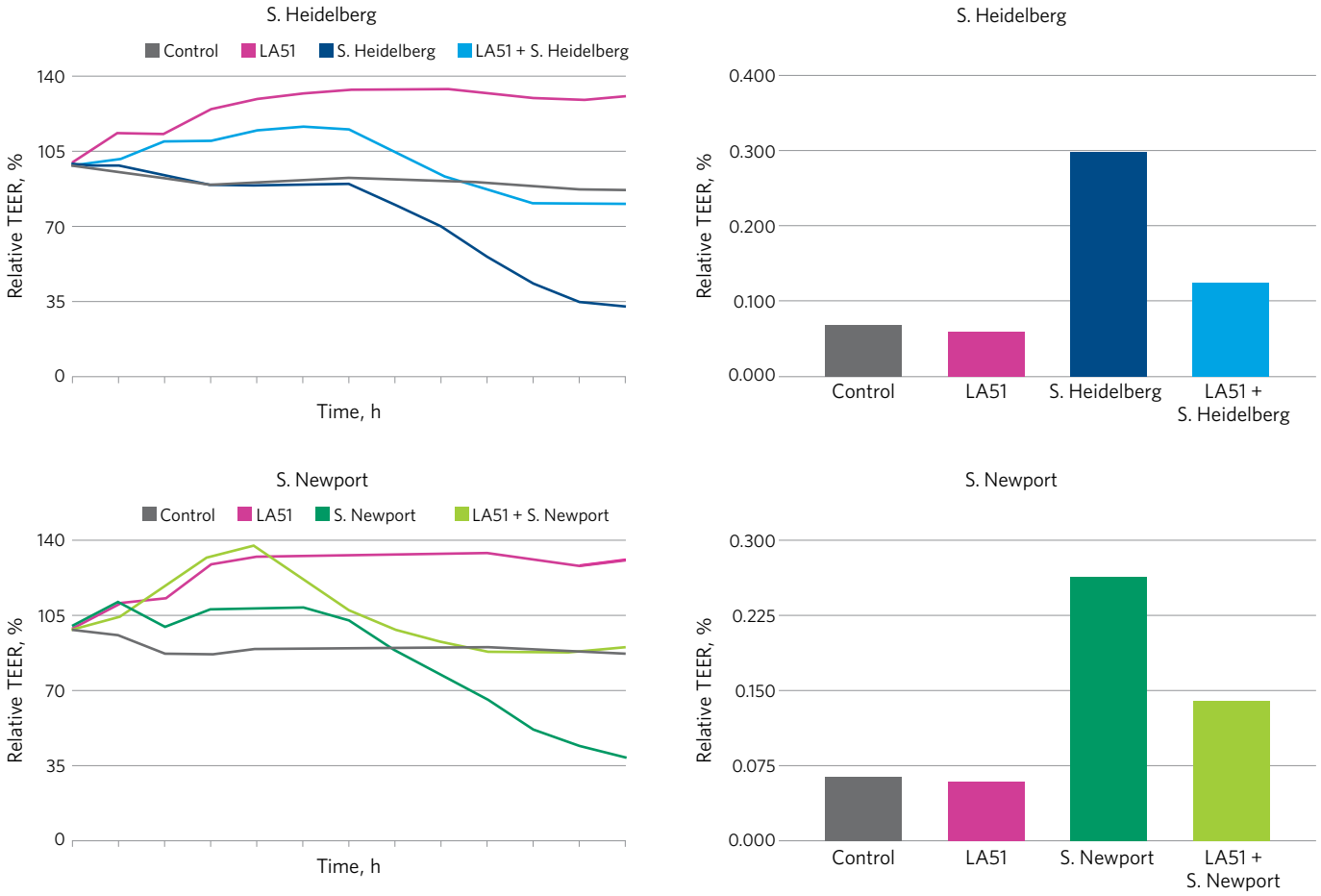
Probiotic approaches for reduction of *Salmonella*

Lactobacillus animalis (LA51), one of two primary LAB strains within **BOVAMINE®** brand products, has been shown to significantly improve the overall GIT health of both young, pre-weaned calves (Dick et al., 2013) as well as finished feedlot steers (Elam et al., 2003). A research trial conducted at Texas Tech University found that LA51 significantly reduced the prevalence and concentration of *Salmonella* in subiliac LN (Vipham et al., 2015); Figure 1. Due to the vast research that has been conducted both *in vitro* and *in vivo*, **BOVAMINE DEFEND®** is the only product in the Beef Industry Food Safety Council (BIFSCo) pre-harvest library as a product which has met the established requirements for listing. Using LA51 as a pre-harvest food safety intervention may also have benefits to the producer. Increased performance, reduced late-term deads, and overall health benefits to cattle are often observed and documented within our research trials. Recently, our Chr. Hansen research team has started working with LA51 within lab models to determine what specific modes of action are associated with this good bacteria's ability to reduce the prevalence of *Salmonella* in LN. Using a method called Transepithelial Electrical Resistance (TEER), researchers have discovered that when a monolayer of intestinal cells in a transwell is challenged with *Salmonella*, the electrical resistance of that layer drastically declines over time as the pathogen destroys the tight junctions between the intestinal cells. Thus, this leads to increased "gaps" between the cells which mimics the effect of LGS. When LA51 is added to the transwells that contain the intestinal cells and *Salmonella*, the electrical resistance is significantly greater as the integrity of the junctions between the intestinal cells improves. To measure the differences in integrity of intestinal junctions after the TEER analysis is complete, dextran molecules are added to the well and allowed to "settle" to the bottom of the well beneath the intestinal cells. The greater number of dextran molecules that are able to float through gaps in the intestinal cells to the bottom of the well, then the more porous the monolayer of intestinal cells. Wells that contain *Salmonella* plus LA51 always have a significantly improved cell barrier with less dextran moving through the well, which means that the barrier formed by intestinal cells, although under a *Salmonella* challenge, is stronger in the presence of LA51; Figure 2.

Conclusion

It is crucial that both the beef and dairy industry continue to find ways to reduce the prevalence of *Salmonella*. Over the past 20 years, amazing strides have been made to reduce the risks associated with *Salmonella* as a food safety concern. But more consistent reductions within the system need to be developed both for animal health and human health implications. The major challenge facing this goal is keeping *Salmonella* from entering the portal system and ending up in the lymph.

Figure 2. Transephitelial Eletrical Resistance (TEER) *in vitro* assay for measuring the paracellular intestinal permeability of small molecules and bar charts representing Fluorescein Isothiocyanate (FITC)-Dextran addition to wells post TEER.



Proper pen and nutritional management can help reduce some of these issues. Keeping pathogens from breaking through the GIT and supporting the immune system of these cattle are critical barriers that also need to be managed. One option for producers looking for an economical way to reduce pathogen prevalence while also supporting the overall health of cattle is Bovamine® brand products. *Salmonella* reduction will ultimately take producers and packers working together to develop and implement multiple approaches to the reduction of this pathogen during both the pre- and post-harvest. But producers must begin to think of *Salmonella* as an economic issue for their operation and not just a food safety concern before strides can truly be accomplished.

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