Natural Resistance

Research uncovers genetic basis for non-black cattle having a natural resistance to Salmonella and E. coli.

by ***Heather Smith Thomas***

Disease caused by *Salmonella* and *E. coli* can be life-threatening in calves. Bacterial contamination of food with these pathogens is also a human food safety issue responsible for many illnesses in the U.S. each year. For instance, pathogenic strains of *Salmonella* cause approximately 40,000 documented cases of foodborne illness annually. Even though many cases of "food poisoning" are due to bacterial contamination of poultry products and fresh produce (vegetables and fruit), there are some cases caused by eating undercooked ground beef.

Much of this problem has been successfully addressed by better methods of handling carcasses at slaughter to prevent contamination from dirty hides, but some food safety issues still exist. Part of the problem is that *Salmonella* can be present in lymph nodes of cattle and some of these nodes may be incorporated into hamburger even when the carcasses are meticulously clean.

Finding ways to eliminate or reduce these bacterial infections in cattle is a challenge, but new research in genetics — discovering natural resistance in some cattle to these pathogens — has opened another option. As producers learn more about the genetics of cattle and gain the tools to find specific genes that affect various traits and differences in animals, they are developing the ability to test individuals and select for (or against) certain traits. Genetic tests now make it possible to discover whether certain animals carry genetic defects, and some new tests are also being developed to determine whether cattle possess certain desirable genes, such as genetic resistance to disease.

Dr. Steve Carlson and Tim Day, researchers at Iowa State University, recently became involved with PSR Genetics, a company that was founded five years ago in Scott City, Kan. PSR stands for Phenotype *Salmonella* Resistance. This company is based on a proprietary genetics platform that allows Carlson and Day to uncover various SNPs (single nucleotide polymorphisms).

"These are small genetic changes that can often lead to significant changes in the animal," explains Carlson. He was asked to serve as a consultant in the search for cattle that have SNPs that lead to resistance to *Salmonella* infection since this is a disease he has studied for most of his research career.

"In my earlier work with USDA (U.S. Department of Agriculture), I performed a number of infection experiments in cattle, using *Salmonella*," he says.

Dayna Harhay, a microbiologist/molecular biologist at the Meat Safety and Quality Research Unit at USDA's Meat Animal Research Center, Clay Center, Neb., sent Carlson various strains of *Salmonella* to use in creating experimental infections in 400-lb. calves. Carlson then sent lymph nodes from those calves to Harhay for her to check to see if *Salmonella* was present in those nodes.

"Some of the calves became very ill, but we would occasionally notice an animal that was hard to infect," Carlson says. "These individuals had more natural resistance and were always non-black. They might be Red Angus, Hereford, Shorthorn, red and white Holsteins, Piedmontese, Tarentaise, Salers, Simmental, etc. We haven't tested Charolais yet, but that would be another breed that could possibly exhibit resistance. This remarkable resistance seemed to be most common in the non-black breeds."

After this discovery, the new company was formed to find a SNP that leads to this disease resistance.

"Through trial and error and testing in the lab they found a SNP that did confer this resistance, so they asked me to oversee research in which live cattle were infected with *Salmonella*," Carlson explains. "I guided a number of experiments, which did indeed show significant and natural resistance through what they called the PSR SNP, which was uncovered in their genetic platform."

The company began looking at dairy cattle, since *Salmonella* in dairy animals can be a significant and insidious problem. Lactating cows may have diarrhea and go off feed. This infection generally isn't lethal in cows, but they can spread it to calves, and the diarrhea may become lethal in young animals.

"They couldn't find very many red and white Holsteins that had two copies of the PSR SNP, so the company began looking at beef cattle, where it seems to be more prevalent in the non-black cattle," says Carlson.

The first tests were in the lab, using blood cells. "*Salmonella* resistance is fairly easy to determine with blood cells, because *Salmonella* likes to invade these cells. This was a good way to screen for this trait, just using blood samples from cattle and counting the *Salmonella* bacteria invading these cells," he says. Any animals showing *Salmonella* in the cells were not resistant.

The next step was to infect cattle with virulent strains of *Salmonella*. "We found it very difficult to make the naturally-resistant animals sick, while the rest of their cohorts were succumbing to the disease. The naturally resistant individuals have two copies of this PSR SNP and are non-black," Carlson explains.

After being experimentally infected with a virulent strain, the cattle lacking any copies of the PSR gene were so ill they had to be euthanized within a few days. Cattle with one copy of the gene were susceptible, but resisted illness and death for twice as long.

Black cattle with two copies of the gene survived for up to 10 days. Non-black cattle with two copies of the gene were significantly more resistant; the researchers could not produce illness (nor any shedding in feces) in these animals, even when using 10 times the typical challenge dose of bacteria.

"The non-black genotype and phenotype is conferred through a gene called MCR. Non-black is a recessive phenotype; you must have two recessive alleles (one from each parent) for the animal to be non-black," Carlson says. Black is always dominant.

Tim Day (left) and Dr. Steve Carlson, researchers at Iowa State University, are investigating the genetic basis for non-black cattle having a natural resistance to *Salmonella* and *E.coli*.

Other researchers have also uncovered many interesting things about the MCR genes. The dominant MCR gene encodes a receptor needed for black pigmentation and also has other functions. The recessive MCR/MCR cattle are not only non-black, but, research suggests, metabolize antibiotics faster (thus shorter withdrawal time is needed) and also have an elevated pain threshold (they are less likely to go off feed when ill).

Salmonella deaths are not of huge economic importance in cattle. What may be more important is that cattle with less resistance may have the bacterium in their intestines and shed it and may have intermittent diarrhea.

"The company also did some research looking at less virulent strains of *Salmonella* and lower doses of the pathogen. Interestingly they found that these non-black cattle that have two copies of the PSR gene do not shed *Salmonella* when infected, and had very few bacteria that would colonize in the intestine," Carlson says. Therefore these resistant cattle would not pass the disease to susceptible cattle. The few *Salmonella* bacteria researchers found in the intestinal tract of resistant cattle tended to be inactive and unable to cause disease.

Food safety issues

The next thing the company wanted to investigate involved a phenomenon that has become evident during the past few years. Some researchers, primarily at Clay Center, Neb., found that at slaughter many cattle lymph nodes contain *Salmonella*, even in healthy animals.

"They found that up to 30% of carcasses contained infected lymph nodes," says Carlson. These numbers vary by season and region and may be even higher during summer in southern feedlot cattle. Interestingly, feedlot cattle generally have a higher incidence of contaminated lymph nodes than mature cull cattle.

"What probably happens in some cattle is they get a low-grade *Salmonella* infection in their intestines and the immune cells try to take care of it by engulfing the *Salmonella* bacteria and escorting them off to the lymph nodes where the immune system is supposed to kill the bacteria. But *Salmonella* is very good at resisting this defense," says Carlson.

Lymph nodes generally serve as a filter to collect bacteria, viruses and other infectious agents, where they are eventually destroyed by the lymphocytes. But certain bacteria, especially *Salmonella*, are able to evade this immune response by invading and surviving inside the immune cells and lymph nodes.

So this immune system tactic simply moves the *Salmonella* bacteria from the intestinal tract to the lymph nodes. "The problem with this translocation is that some of these lymph nodes get incorporated into hamburger when the animal is slaughtered and processed," Carlson says.

Lymph nodes around the gut are not a problem because they are discarded during slaughter, but others are located within the fat tissue of muscle cuts such as flank and chuck and could be a source of contamination for ground beef. Many of these lymph nodes are small and hard to sort out.

Carlson adds, "Beef packers are having a lot of trouble with this issue, since they can't effectively decontaminate these lymph nodes or efficiently cut them out. It is also difficult to irradiate them or chemically treat them."

The beef industry is currently struggling with this situation because the animals in question are not sick and a person would never suspect a problem.

Researchers don't know if this is a new problem — with new strains of *Salmonella* — or just something they weren't aware of in earlier years or if there are more cattle being marketed today that have poor resistance to *Salmonella*.

"The packers are having difficulty dealing with it even though they are trying very hard to mitigate this problem. There have been a few hamburger-associated outbreaks in the past few years and they've done a good job at instituting the recalls of that beef," says Carlson.

"There is some talk about the USDA possibly declaring *Salmonella* as an adulterant in beef. If that happens they might go to mandatory testing, which would be another problem for the beef packers to deal with," Carlson says.

This would be just one more challenge the beef industry doesn't need.

"PSR Genetics was able to acquire a couple of these problematic *Salmonella* strains from the Clay Center researchers and do experimental infections. What they found was that the non-black cattle with two copies of the PSR gene had no detectable *Salmonella* in their lymph nodes. The researchers sent the lymph nodes to Clay Center, Neb., for verification," Carlson says.

By contrast, all the other genotypes of cattle they worked with had some *Salmonella* in their lymph nodes. "There were three primary lymph nodes they checked, because they are fairly easy to get at and represent potential sites for contamination into hamburger," he says.

The next step was to look at *E. coli* because these two bacteria are closely related, and *E. coli* has become an increasingly prevalent problem in processed meat during the past 25 years — about the same length of time that black cattle have dominated the market for beef. The first major hamburger recalls coincide with this influx of black cattle.

"The company wanted to assess how their findings would extrapolate to intestinal colonization by *E. coli* 0157:H7. They did an experimental infection with cattle of these known genotypes, and found a marked reduction in the colonization of *E. coli* 0157 in the non-black cattle

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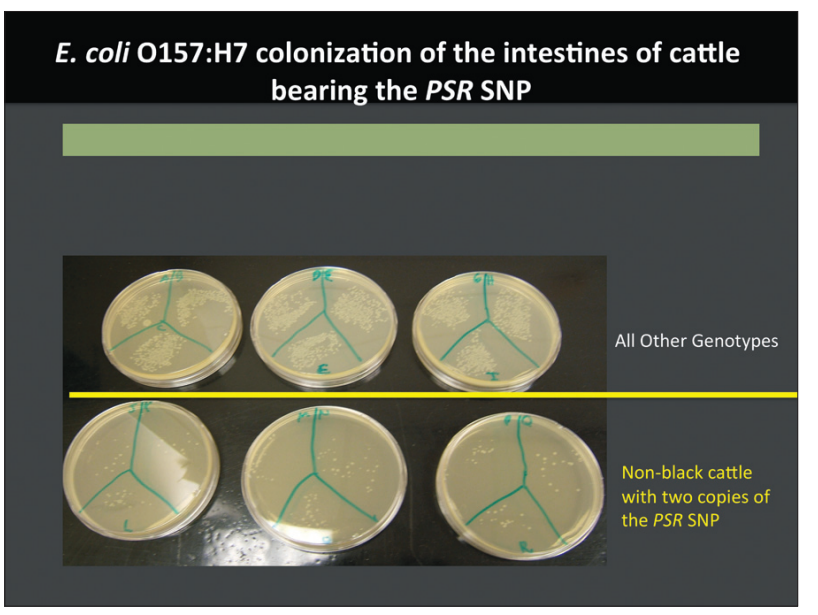
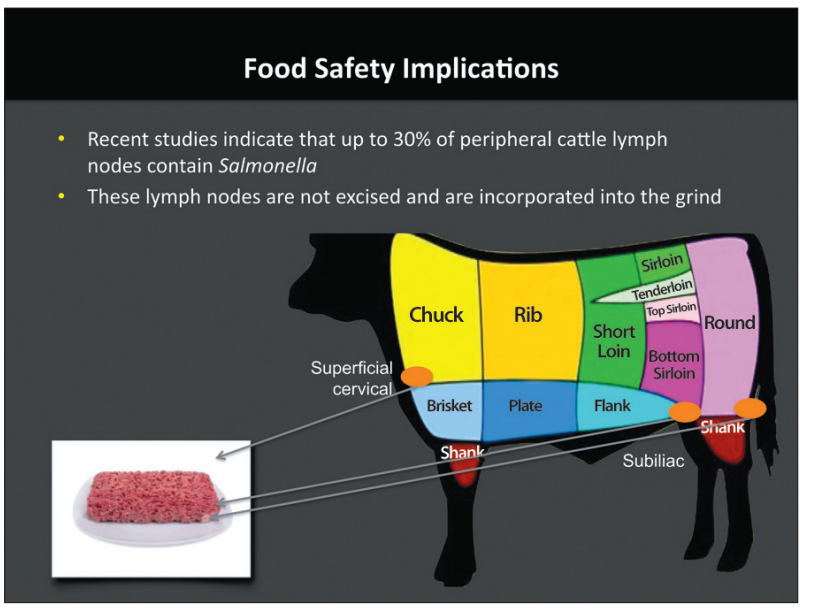
Difference between *E. coli* contamination and *Salmonella*

Feces on the hides of cattle are the primary sources of bacterial contamination, including *Salmonella* and *E. coli*. This problem is why efforts in the past several years to control surface contamination at harvest have reduced the incidence of *E. coli* in beef; the prevalence of *E. coli* O157:H7 in ground beef has declined more than 70% since 2001. By contrast, *Salmonella* contamination has stayed about the same — probably because of the recently discovered factor of *Salmonella* hiding in the lymph nodes.





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with two copies of the PSR SNP." These cattle have resistance to *E. coli* as well as to *Salmonella*.

"It's about a 15 to 20-fold drop in susceptibility. It's not as robust as what we see in the *Salmonella* data, but still a significant factor. On the *Salmonella* side, the company has a pretty good idea of how it works. On the *E. coli* side, this is something they are working on. They have also done some studies looking at related *E. coli*. There are a number of *E. coli* serotypes that behave similarly to 0157:H7, as far as being a nasty human pathogen, like 0145 and 0111. From some of the work they've done in the lab, it looks like it does extrapolate to those strains, as well," Carlson says.

A genetic test

"The next step is to develop a licensable genetic test that could be offered to cattle producers, using this as a food safety tool to minimize some of these food safety pathogens," he says. This test might be of major interest to producers who are trying to raise natural, healthy beef.

"The beef packers as a whole have some issues making this work, mainly because there are so many black cattle produced today. It's hard for packers to fully utilize this information just because of the preponderance of black cattle in the industry," Carlson explains.

The past couple of decades have seen a tremendous increase in the number of black cattle because of the popularity of black Angus.

"Currently the company is trying to get this genetic test finished and available to producers. It's been a bit of a struggle, however, because of the popularity of black cattle. Thus it will probably be more of a niche market, for other breeds and for small producers who want to market their animals on their own," says Carlson.

This test might be of interest to producers who are not raising black cattle in order to find out if their animals are resistant to two important food safety pathogens, information that would be a great marketing tool.

"The company is still working on getting this test to the marketplace. One of the reasons I was asked to participate was to assist them with making this happen. There are a number of producer groups who can help push this forward and will appreciate this kind of test," Carlson says.

Many beef producers in the U.S. have bred their cattle black during the past several decades. But this breeding choice narrows the gene pool when they look at all the beneficial traits that they might wish to select for in seedstock. Hybrid vigor is also a great tool. If they have more genetic variety, cattle tend to be hardier and healthier.

Producers need to make sure that they always have options for crossbreeding and complementary trait selection, and this genetic discovery about disease resistance highlights an important trait that non-black cattle may offer in the cattle industry's fight against disease and food safety issues. **HW**

More research needed

In the recent research that sampled lymph nodes from carcasses in slaughter facilities around the country, the hides were off and the researchers did not know the breed or the color of the animals. It would be interesting to do a study to identify red cattle in feedlots where researchers know there is *Salmonella* present in the environment — in feedlots where there are different color coats in the cattle. A study could look at *Salmonella* prevalence in these cattle and see what the different percentages of positive lymph nodes might be.

Dr. Steve Carlson, a researcher at Iowa State University, explains, "A few black cattle have two copies of the PSR gene and are partially resistant to *Salmonella*; there is a moderate level of resistance in these animals. The PSR gene does help, in black cattle, but helps the most in non-black cattle that have two copies of the PSR gene."

Another line of research the company is currently engaged in involves bovine respiratory disease (BRD). "They are not quite as far along with this project; they have not yet identified a genetic test. But it looks like they've identified a phenotype test in which they can test blood from an animal and predict whether it will come down with BRD following stresses such as weaning, transport etc. that often precipitate respiratory illness," says Carlson.

At this point most of the research on this front has been in the lab. Researchers have not done anything yet with live cattle, but that will hopefully be the next step if they can find a company to partner with them to expand this research. BRD is a much more expensive disease to study. **HW**