Salmonella/ Hemorrhagic Bowel Syndrome
"The Bloody Gut"

Chris Ashworth DVM
Monsanto Dairy Business
Fort Smith, AR 72908

Introduction

Salmonellosis and Hemorrhagic Bowl Syndrome **(HBS)** have been increasing in frequency on dairy farms in the High Plains in the past 3-5 years. This may be from an absolute incidence of the disease, but is more than likely occurring at a higher frequency due to the fact that there are more dairy cattle in this region.

For this talk, I will discuss them as two independent diseases. The main things both diseases have in common is a high death loss, as well as both diseases can occur in any age of animal.

Salmonellosis

Salmonella is a bacterium. It is not a normal inhabitant of a mammal's intestinal tract. Today there are over 2200 known serotypes (serovars) of these bacteria in the world. Many of the serotypes are named after locations in the world where these bacteria were first isolated. Salmonella is a gram negative bacterium that is also a facultative, intracellular parasite. This means that it can evade the immune system of the animal by hiding and continuing to live, within some cells of the animal's body.

Approximately six serovars of the bacterium make up the vast majority of Salmonella infections in cattle in North America. These would include *S. typhimurium, S. agona, S. Newport, S. Montevideo, S. Dublin,* and *S. anatum*.

Most serotypes are NOT host specific, meaning they can infect multiple mammalian species. However, in cattle one serovar, *S. Dublin,* is host specific for cattle. An issue with host adapted serovars is that they are found most often in their host species, where true long-term carriers can exist. This leads to massive outbreaks and continuous recontamination of the environment.

Non host-specific Salmonella infections, as a rule, will infect an animal for 3-16 weeks before the infection is cleared from the body. Sanitation plays a key role in slowing these types of infections and preventing them from spreading to other sites on the dairy, i.e from calving area to the calf hutches.

Salmonella infections are also important in that they can be zoonotic infections. This means the disease can be contracted from animals, back to man. Salmonella infections in humans are one of the most important food safety threats in the United States. It is estimated that 1.4 million cases of Salmonella occur each year in the U.S., with over 500 deaths. Young children, the elderly, patients receiving chemotherapy, or patients with

compromised immune systems are more prone to long-term infections and complications. In recent years, there is an increased concern for several serovars to have mutated and become multi-drug-resistant (**MDR**). These would include *S. typhimurium* and *S. Newport*.

Many diagnostic laboratories have the capability to culture and isolate Salmonella organisms. The bacterium is not always easy to isolate, especially if the animal has been treated with antibiotics prior to fecal or tissue cultures. Most laboratories will only have the capability to determine the serogroup, such as Type A, Type B, Type C, Type D, Type E. These laboratories will then need to submit the isolate to the National Veterinary Service Laboratory in Ames, IA for final speciation. For cattle, there are several common organisms that occur in each serogroup.

Clinical Signs of Disease

The clinical signs of Salmonella in cattle can be just about anything. It most often depends on the serotype infecting the animals. The most common clinical signs that I have seen are fever and diarrhea. Incubation time can be as short as 12 h, especially if calves are fed contaminated colostrum. Many times cows with a Salmonella infection will have high numbers of Salmonella in colostrum, prior to that animal breaking with clinical signs herself. This makes control of the disease difficult from the very beginning.

Diarrheic feces can be varied in color, odor, and consistency. Most often in calves a putrid, foul odor is present. Feces may or may not contain blood. In calves, with damage to the lining of the intestine, the animal will then start to move fluid from the body into the lumen of the intestine. This is where the water is coming from for the diarrhea to occur. The animal can become very dehydrated very quickly. Many of the animals that die of this condition, actually die of dehydration and fluid loss, not the bacterial infection itself. Rapid recognition of the disease and an aggressive fluid treatment program can save many of these calves.

Table 1. Most common Salmonella serotypes associated with commonly encountered serogroups.

Serogroup	Most common serotype	Most common host animal
B	*S. typhimurium*	All species
	S. agona	
C	*S. Newport*	Cattle, sheep
	S. Montevideo	Cattle
D	*S. Dublin*	Cattle
E	*S. anatum*	Cattle, horses

Source: National Veterinary Service Laboratory, USDA, Ames, IA

One of the many issues with a Salmonella infection is the fact that these bacteria are gram negative organisms, just like *E. coli*. These bacteria can release toxins that affect blood flow to certain tissues of the body. This also can explain the very elevated body temperatures, as well as the rapid heart rate in many of these animals.

Bacteria can move out of the intestine and into the blood stream of the animal, leading to further complications that may include microscopic blood clots throughout the body. This can lead to calves sloughing their ears and/or hooves. In my experience this tends to occur more with *S. Dublin* infections. In most of my experiences with *S. Dublin*, death rates are very high, and in most instances calves will have severe pneumonia. These animals tend to be greater than 60 days old, as well. In contrast, *S. typhimurium* tends to occur in much younger calves, 12 h to 45 d of age, with most infections occurring prior to 25 d of age.

In adult cows, we often times see diarrhea and or cows that appear listless, with elevated body temperatures and normal feces. Although Salmonella infections can occur at any time of the year, I have seem most massive outbreaks in adult dairy cows following severe extreme cold. In a prior position in a veterinary diagnostic laboratory setting, we would see large increases of sample submissions for Salmonella following the first arctic cold blast that would move out of Canada and across the US. Many of these adult cows are harboring Salmonella bacteria in their intestinal tracts with no clinical disease being shown. However, when a new stress or a severe stress comes into those animals' environments, the immune system is stressed and the disease then is allowed to become a clinical disease entity.

Abortions can occur with Salmonella infections. Most often once the bacteria has gained access to the blood stream, these bacteria can potentially be filtered out by the uterine-placental attachment and an infection can then begin at the level of the placental attachment, which results in fetal death and expulsion.

A second form of abortion can occur as well with Salmonella infections. This would occur when endotoxins from the Salmonella bacteria are released and/or damage to the intestinal lining by these bacteria allows the release of prostaglandins (**PGF**), leading to lysis of the corpus luteum, and therefore a subsequent abortion could occur.

Salmonella organisms can be found in many tissues in an infected animal. Salmonella are invasive organisms that penetrate ocular, nasal, oral, and intestinal membranes. It is important to remember that Salmonella organisms are shed in very high numbers in saliva. This is an issue as this allows for contamination of milk bottles, buckets, and worker's hands. Small children can be infected by allowing calves to suckle on their fingers and then placing their fingers in their mouth or eating without washing their hands.

In calves, Salmonella organisms are more readily available to attach to the intestinal cells if calves have not been fed or if the gut is static and not moving. Twenty years ago it was common to move calves long distances back to veal ranches and not feed those animal prior to movement, but to actually feed them electrolytes on arrival followed by milk 8-12 h later. This most likely leads to more Salmonella infections. Similar findings have been found in feedlot animals that are fasted prior to sending to

the slaughter house, and this is thought to play a role in the number of E.coli 0157:H7 organisms found in the intestinal tracts of those animals.

Where did the infection come from?

Salmonella infections tend to be cyclical events that reoccur every few weeks, especially in calves. More so if the environmental temperature has been extreme; greater than 95 °F or less than 15 °F in my experience.

Salmonella Dublin infections tend to be the most severe. In some operations, *S. Dublin* infections may occur for as long as 4-5 months once the infection gets established in a farm or calf ranch. In these types of outbreaks, the disease is maintained in the operation from carrier calves continuing to shed the organism and re-infecting the environment with large volumes of diarrheic fluid. This leads to environmental contamination of bedding, feeding equipment, contaminated calf housing, and employees that bring the organism to the calf with contaminated needles, bolus guns, drenching items, or even a contaminated calf milk mixing tank.

In adult cows, Salmonella is spread most commonly, in my opinion, when fresh cows and hospital cows are forced to share a common water tank. Infected cows with high numbers of the bacteria in their saliva contaminate an entire water tank. Feces in feedstuffs, as well as saliva in feed, are also methods of contamination that can lead to cows becoming infected with Salmonellosis. Anytime a medical instrument is placed into a cow's mouth or rumen and then used to treat another animal, the risk factor for a Salmonella infection increases.

Dairies that flush manure also have been shown to have a significantly higher risk of Salmonella infections. It is thought that this may be from cows drinking flush water or from cows lying down and grooming themselves after they have stood in flush water while barns were being flushed.

In a recent paper from Washington State University, they concluded that a common hospital-maternity pen provided a niche where a given Salmonella strain could persist on a dairy farm for protracted periods of time. Since periparturient cows have been shown to have immunosuppression during late gestation, parturition, and the early stages of lactation; this would also make these cows more susceptible to infection when exposed to sick animals and forced to eat feed and drink water with these animals. The movement of periparturient cows, which enter and leave a calving pen area and are continually replaced with new cows due to calve, helps maintain new infections in the fresh cows. This allows infected fresh cows to be moved out into high producing cow pens, when their infections are not noticed or are of a carrier state. This therefore allows high producing cows to also have increased exposure levels to Salmonella.

The exact epidemiology of Salmonella carriers with different serovars is not well characterized. We do know that there are major differences in the length of infections for the different serovars as well as how long each of these can persist in the environment. Salmonella bacteria have been noted to persist in a dairy for as long as nine years with DNA fingerprinting. It is not uncommon for Salmonella to persist in a lagoon area for over eight months.

Sanitation of premises, especially the hospital pen, periparturient pens, and fresh cow pens can not be over stated. Cleanliness and sanitation of instruments used to treat animals must be of the highest order of magnitude.

Several advances in the area of vaccinations have also been made. These advances include siderophore receptors and porins (**SRP**), which are highly specialized proteins found on the cell wall of gram-negative bacteria. These SRP proteins are found in all strains of Salmonella and; therefore, can have a significant impact on preventing infections from multiple strains of Salmonella.

When cattle are vaccinated with SRPs they produce antibodies to these SRPs. These antibodies attach to the SRPs on the cell wall of the Salmonella organism. This blocks the SRP's ability to move iron into the Salmonella bacterial cell, which leads to starvation of the Salmonella organism, resulting in death of the Salmonella organism. This vaccine, therefore, helps to control the subclinical infection of fecal shedding, as well as clinical cases, of Salmonella.

In conclusion, we must remember that we need to reduce the exposure of our farm employees to this organism as well, since it is an infection that can be spread from animals to man, leading to severe diarrhea, dehydration, weakness, and flu-like symptoms.

Hemorrhagic Bowel Syndrome
The Bloody Gut

Hemorrhagic Bowel Syndrome is a relatively new disease process, first described in the US in Idaho by Dr. Bruce Anderson in 1991. The condition was first described as occurring in five high producing Holstein cows. The condition was described as having sub-mucosal hematomas (blood clots) affecting the jejunum – the middle portion of the small intestine.

Incidence

The incidence of the disease appears to be increasing in some areas and some evidence suggests that it is responsible for 2 % of the deaths of adult dairy animals in the US. Many dairies have modified management to reduce the incidence of the disease. In 2005 an AVMA article noted that HBS had been observed on 9.1 % of operations during the preceding 5 years and on 5.1 % of operations during the preceding 12 mo. For individual cows with signs consistent with HBS, the third lactation was the median of the parity distribution and the median time between parturition and the onset of clinical signs was 104 d.

A Colorado State University study by Dr. Dennison noted in a retrospective study that in 22 clinical cases reviewed, age ranged from 2-8 years and the incidence occurred at a mean of 107.5 days post-parturition. Average milk production was 89.8 pounds. Two-thirds of the cases occurred in the cooler months of September through February.

Clinical Signs

The initial clinical signs of HBS are varied. In general, we see animals become depressed, with inappatence, a significant drop in milk production, watery to bloody diarrhea, and eventually shock. Animals may also kick at their abdomen as well as may have mild to moderate distention of the right side of the abdomen. Animals may become anemic. Death usually occurs within 48 h after clinical signs are first noted.

It is interesting to note in the Colorado State University study by Dr. Dennison, that only 7 of 15 cows had bloody feces. This study also noted that *Clostridium perfringenes* was isolated from fecal samples in 17 of 20 cows. Genotyping of the *C. perfringenes* in 10 cows revealed Type A in five cows and Type A with the b2 toxin gene in the remaining five cows. The dairy from which the *C. perfringenes*-positive cows had originated had vaccinated the cows with *C. perfringenes* types C & D toxoid.

In many cattle, up to 50 % of deaths may occur with the progression of the disease being very sudden to peracute, with no to few clinical signs at the time of death. Segments of the small bowel may be necrotic with a reddish-purple color and filled with blood.

Dr. M.A. Kirkpatrick noted in his investigations that an intestinal motility disruption and the presentation of HBS occurred in two cows. The presence of intussusceptions in both animals from one dairy on one day was highly unusual and points to the possibility that some form of intestinal motility aberration was possible. This abnormal intestinal motility could possibly take the form of either hypo- or hypermotility. In both animals the intussusceptions were located directly anterior to the HBS site in the jejunum.

Causative Agents

Dr. Neil Forsberg and Dr. Steven Puntenny proposed that a causative agent other than *C. perfringenes* was responsible for HBS. The use of real-time SybrGreen quantitative polymerase-chain reaction (**PCR**) analysis indicated that all HBS cows were infected with *Aspergillus fumigatus*. Samples from their control cows were negative. Multiplex PCR analysis of five clostridial toxin genes did not reveal a correlation with HBS. Specifically, clostridial toxin genes were detected in both HBS and control animals. *A. fumigatus* correlates closely with HBS and may play an important role in its etiology.

Moldy feed has been observed on many dairies which have experienced HBS. In humans, *A. spergillus fumigatus* has been described at pathogenic (disease causing), that causes intestinal bleeding and invasive aspergillosis in immunocompromized patients. Dr. Forsberg and colleagues has proposed that *A. fumigatus* plays a role in HBS.

A. fumigatus and *A. flavus* are the most pathogenic molds of the Aspergillus species. Pathogenicity of Aspergillus is attributed to three virulence factors:

1) production of iron (Fe)-sequestering siderophores,

2) secretion of complement and phagocytic-inhibitory lipids, and

3) secretion of proteases.

Aspergillus has been shown to infect the ruminant gut and multiple sites and to cause intestinal hemorrhage. Hemorrhagic lesions have also been noted in the reticulum, rumen, omasum, and the Peyer's patches.

Predisposing factors for fungal infections include:

1) feeding of moldy feed,
2) immunocompromising diseases,
3) acidosis,
4) antimicrobial therapy,
5) reflux of abomasal contents,
6) metabolic disturbances,
7) post-partum stress,
8) viral erosive diseases such as IBR,
9) anti-inflammatory treatment, and
10) abortion

A. fumigatus has been detected by DNA in feed samples, intestinal contents, and the intestinal wall as well as 3 of 5 lymph nodes from eight cows diagnosed with HBS. Local feeds were also tested for *A. fumigatus* and it has been determined that many are infected with *A. fumigatus*, even though the infection may not always be visible.

Proper storage and rotation of feed ingredients, especially ensiled feeds can help in reducing the incidence of HBS by reducing the potential exposure to *A. fumigatus*. Extreme care should be taken when feeding infected silages. The top layer of silage (3-8 in) may need to be discarded in order to reduce the number of fungal spores being introduced into the cow's digestive system, IF THIS CAN BE DONE WITHOUT POSING A SAFETY RISK TO EMPLOYEES.

Maturity of harvested forages is also important. Goals for moisture in corn silage and alfalfa haylages should be less than 70 %. Silage pits should be rapidly filled and packed. Packing and rapid covering of the entire silage pile is the single most important management tool that a dairy producer can perform to limit *A. fumigatus* infections in silages. When corn silage is ensiled properly and the pH reaches 4.0 or less, this limits the ability for *A. fumigatus* to establish itself.

A. fumigatus can also build up in feed bunks, feed lanes, and water troughs. These areas also need to be cleaned regularly. As environmental temperatures increase in spring and summer months, this becomes more critical as well.

References

AgriLabs Website. 2006. New SRP Technology: A better way to make vaccines. Accessed Feb. 27, 2006. http://agrilabs.com/newproductpops/popSRPTech.html

Anderson, B.C. 1991. 'Point Source' haemorrhage in cows. Vet. Rec. 128:619-620.

Baker, T. 2002. Be on the lookout for hemorrhagic bowel syndrome. Hoard's Dairyman, pg 776, November.

Berghaus, R.D., B. J. McCluskey, and R. J. Callan. 2005. Risk factors associated with hemorrhagic bowel syndrome in dairy cattle. JAVMA 226:1700-1706.

Cobbold, R.N., D.H. Rice, M.A. Davis, T.E. Besser, and D.D. Hancock. 2006. Long-term persistence of multi-drug-resistant Salmonella enterica serovar Newport in two dairy herds. JAVMA. 228:585-591.

Dennison, A.C., D. C. VanMetre, R. J. Callan, P. Dinsmore, G. L. Mason, and R. P. Ellis. 2002. Hemorrhagic bowel syndrome in dairy cattle: 22 cases (1997-2000). JAVMA 221:686-690.

Gay, J.M., and M.E. Hunsaker. 1993. Isolation of multiple Salmonella serovars from a dairy two years after a clinical salmonellosis outbreak. JAVMA 203:1314-1320.

Kirkpatrick, M.A., et. al. 2001. Jejunal Hemorrhage Syndrome of Dairy Cattle. *In:* Proc. Four-State Applied Nutrition and Management Conference. pp. 143-153.

Losinger, W.C., S. J. Wells, L. P. Garber, H. S. Hurd, and L. A. Thomas. 1995. Management factors related to Salmonella shedding by dairy heifers. J. Dairy Sci. 78:2464-2472.

Puntenney, S.B., Y. Wang, and N. E. Forsberg. 2003. Keeping them out of the rough: practical insights into Hemorrhagic Bowel Syndrome. *In*: Proc. Florida Dairy Business Conference.

Smith, B.P., et. al. 1996. Large Animal Internal Medicine. Salmonellosis. C.V. Mosby. Co Publisher. pp 894-899.

Wolfgang, D.R. 2005. Clostridium perfringenes and Hemorrhagic Bowel Syndrome. Pennsylvania State University Veterinary Extension Newsletter. July.