

***Research Progress in
Hemorrhagic Bowel Syndrome
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Hemorrhagic Bowel Syndrome (HBS, also known as Jejunal Hemorrhage Syndrome, bloody gut, or dead gut) is a newly emerging, highly fatal intestinal disease of adult dairy cows in the United States. HBS is characterized by sudden, progressive, and occasionally massive hemorrhage into the small intestine, with subsequent formation of clots within the intestine that create obstruction. Affected segments almost inevitably die, releasing toxins into the cow's bloodstream and abdominal cavity.

Successful treatment of this disease is difficult. Anecdotal reports exist of successful treatment with fluids, laxatives, anti-inflammatory drugs, antibiotics, and surgery; however, it appears that such treatment successes are quite rare. With or without treatment, death of affected cattle usually occurs within several hours to 1-2 days after the onset of clinical signs. At the CSU Veterinary Teaching Hospital, only about 10% of cows affected with HBS have survived.

The disease is seen most commonly in adult dairy cows early in lactation. Thus, factors specific to fresh cow management, nutrition, or the physiologic stress of peak lactation could play a role in the development of this disease. Occasional cases occur in late lactation or the dry period, and heifers are rarely affected. Although HBS usually occurs as a sporadic disease on most dairies, several cows in a herd may be affected in a relatively short period of time (i.e. "clusters" of cases). Anecdotal reports exist of dairies that struggle with this disease on virtually a continual basis.

Research on potential causes: Clostridium perfringens type A is a bacterial organism that has been associated with HBS. This organism has been documented to cause disease in a variety of birds and mammals, including cattle. The primary disease-causing factor for this organism is a potent, lethal toxin called alpha toxin that is released from the organism during rapid growth. The alpha toxin acts as an enzyme that destroys cell membranes. It is lethal to a variety of cells, including intestinal cells and red blood cells.

Some strains of *C. perfringens* type A also carry the beta2 toxin gene; these strains are designated as **A+beta2**. Beta2 toxin is also lethal for intestinal cells. The distribution of *C. perfringens* strains that carry this toxin gene is not well described, but has been identified in the intestine of both healthy and diseased cattle, horses, pigs, sheep, and goats. One or both of these organisms may be isolated from a cow with HBS.

The association between these bacteria and HBS is based on these observations: (1) affected cows have positive fecal cultures for these organisms, (2) *C. perfringens* type A and/or type A+beta2 can be isolated from the affected segments of intestine in cows affected by HBS, (3) there is microscopic evidence of intestinal necrosis associated with heavy intestinal growth of one or both of these organisms, and (4) other enteric pathogens

associated with hemorrhagic enteritis have, to date, been rarely identified in tissues or intestinal contents of affected cows. Reduced monthly incidence of HBS has occurred following administration of an autogenous *C. perfringens* vaccine to adult cows on certain dairies but data from controlled studies are not available for evaluation of such vaccines on the incidence of, or survival rate for, this disease.

Despite these observations, there are many reasons why we hesitate to consider *Clostridium perfringens* as the sole pathogen causing HBS. *Clostridium perfringens* is widely distributed in the environment, and it is considered to be part of the normal bacterial flora of the intestine of most mammals. In the past, veterinary microbiologists have been reluctant to consider *C. perfringens* type A as an important disease-causing pathogen of livestock because this organism can be readily cultured from the intestine of healthy cattle. This organism proliferates rapidly in the intestine after death, making isolation from necropsy specimens of questionable diagnostic significance. If the lethal toxins of *C. perfringens* can be demonstrated in intestinal contents and/or blood of diseased animals, it is considered more likely that the organism is causing disease, rather than simply acting as a part of the normal gut flora. Until recently, the toxins produced *in vivo* by *C. perfringens* in HBS cases had not been identified, and the genotype and density of colonization of *C. perfringens* isolated from HBS cows and normal dairy cows had not been compared.

Members of the Integrated Livestock Management Program have recently completed research that investigated these specific questions. We have found that *Clostridium perfringens* types A and A+B₂ can be isolated from multiple sites of the intestinal tract of HBS cows at a significantly higher rate than unaffected herd mates (cows with LDA). In addition, alpha and beta₂ toxin production can be demonstrated in the intestine of HBS cows, but not control herd mates with LDA.

It is unclear at present whether proliferation of, and toxin production by, *C. perfringens* occurs as part of the primary insult to the intestine, or if proliferation occurs secondary to another disease process or triggering factor. Hemorrhage into the intestine from another cause could, in theory, initiate secondary proliferation of the ubiquitous *C. perfringens*, as this organism multiplies rapidly when cattle ingest large quantities of soluble protein or carbohydrate. Once the organism proliferates, however, the toxins that it releases during rapid growth could contribute to the degradation of the intestinal wall that is so characteristic of HBS.

An alternate investigation of HBS, headed up by investigators at Oregon State University, focused on characterizing the role of *Aspergillus fumigatus*, a mold (fungus) that can be found in livestock feeds. Genetic material of this fungal agent can be detected in the blood and intestine of affected cows. A research project involving dairy cows with HBS and dairy cows that have died of other gastrointestinal diseases (the control group) is currently being conducted in Wisconsin, Minnesota, and Oregon. The rates of isolation of *C. perfringens*, *Salmonella*, and bovine viral diarrhea virus are being compared among cows of these two groups. The rate of detection of *Aspergillus fumigatus* DNA by polymerase chain reaction in the tissues is also being compared. The DNA of this fungal

organism was present in the tissues of a significantly greater proportion of cows with HBS than of cows that died of another GI disease. No statistical disparity was found among the two study groups for the presence of *C. perfringens*, although the authors indicated that future data may produce different results on this issue. To date, BVDV and Salmonella have been isolated from only a small fraction of cows affected with HBS.

Aspergillus fumigatus is clearly associated with HBS, and there are currently two hypotheses regarding its participation in this disease: (1) As a primary contributor to the intestinal lesion, or (2) As an agent that impairs the cow's immune system, thereby facilitating or inciting whatever disease process triggers HBS. Anecdotal reports suggest that the incidence of HBS can be reduced on dairies following the introduction of a feed supplement (Omnigen AF®) into the ration. Controlled studies on the efficacy of this product for HBS prevention are pending. When fed to immunosuppressed sheep and cows, this product has recently been demonstrated to improve certain indicators of immune function in neutrophils, a type of white blood cell that plays a critical role in an animal's defense against infection.

Preventive strategies for HBS remain somewhat speculative, given that our understanding of this disease is incomplete. It may be best to first consider all proposed causes or risk factors (e.g. bacteria, fungi, reduced host disease resistance) and take measures to mitigate these potential risk factors. In so doing, one should consider: 1) identifying and correcting management and environmental factors that might impair cow immunity, 2) performing a careful partial budget analysis of the cost of specific preventive measures, and 3) deciding upon which specific corrective measure(s) might be most justified for a particular dairy.

The authors currently recommend a thorough analysis of transition and fresh cow management to identify problems with cow comfort, nutrition, and disease control that might impact disease resistance during peak lactation. Ration formulation and feed management should be reviewed, focusing on effective fiber and soluble carbohydrate content to limit potential dietary influences on gut flora, assessment of feed bunk and pen management to maintain consistent feed and nutrient intake, and a review of commodity handling and silage management to limit spoilage and mold formation. Since these critical areas impact numerous facets of cow health other than HBS, identification and correction of problems in these areas will likely provide an overall benefit to cow health. Potential use of feed additives or vaccines directed against specific, potential contributory pathogens (i.e. *Aspergillus* and *Clostridium perfringens*) should be considered carefully, with the costs of the proposed interventions and their potential efficacy weighed against the costs of the disease.

Current studies at CSU concentrate upon a comparison of the characteristics of *C. perfringens* isolated from HBS cows with characteristics of the same organism isolated from normal cows. Preliminary genetic analysis of isolates taken from HBS cases indicates that these bacteria appear to be genetically diverse. However, toxin production is greater in *C. perfringens* isolated from animals with HBS than in the same organism isolated from herd mates without HBS. Variation in the amount of alpha toxin produced

is known to occur among different isolates of *C. perfringens* type A. What induces these changes in *C. perfringens* in cows with HBS? Is it the presence of blood in the intestine? A feed change? A signal from *A. fumigatus*? It is apparent that both *Aspergillus fumigatus* and *Clostridium perfringens* types A and A+beta2 can be demonstrated in the tissues and/or blood of cows affected with HBS. Do these bacterial and fungal agents act together or independently? These questions are under investigation, and hopefully, greater understanding of the pathogenesis of this troublesome disease is forthcoming. The funding provided to the ILM by dairy producers has helped us make progress in our research on HBS, and the authors wish to express their sincere appreciation for that support.