Enteric Diseases in the Ruminant

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As with most animal species, the neonate is more susceptible to enteric upset than mature animals. The mature ruminant has the benefit of a densely populated fore-stomach that may aid in the elimination of pathogens introduced orally. Enteric diseases are the greatest cause of illness and death in calves (NAHMS, 1996). Greater than 50% of dairy calves may experience diarrhea caused by enteric diseases (Franklin et al., 1998). Producers often treat calves with antibiotics therapeutically even though most of the causative agents for diarrhea are not responsive to antibiotics. Additionally, most commercial milk replacers contain sub-therapeutic levels of antibiotics in an attempt to prevent enteric diseases. There are a number of diseases that deserve mention in this text but time constraints limit the scope of the text to some of the more economically relevant organisms. The emergence or persistence of these pathogens and mechanisms of control will be discussed.

Hemorrhagic bowel syndrome

Hemorrhagic bowel syndrome (HBS) is also known as bloody gut or jejunal hemorrhage syndrome and is characterized by large blood clots in the intestine. These clots result in blockage and distinct enlargement of this segment of bowel. Clinical signs associated with HBS include a dramatic drop in milk production, clotted fecal blood, loss of appetite, dehydration, abdominal distention, depression, weakness and an extremely high fatality rate (APHIS, 2003, Dennison et al., 2002). The exact cause of HBS is not currently known; however, *Clostridium perfringens* Type A seems to play a role in the disease. Since this organism is a common environmental and GI tract inhabitant, it seems unlikely to be involved in the primary disease process, but has been isolated from 85% of the cases in one study (Dennison et al., 2002). Recently investigators at Oregon State University have implicated *Aspergillus fumigatus* as also playing a role in this disease (Puntenney et al., 2003). However, to this author's knowledge, Koch's Postulate has not been fulfilled in proving or disproving this common environmental fungus as a primary or secondary etiological agent in HBS. A study on steers fed *Aspergillus fumigatus* contaminated feed did demonstrate rapid presence of *A. fumigatus* DNA in the blood of cattle consuming infected feed, but no report on HBS accompanied this data. The role that mycotoxins may play during the pathogenic phase of mycotic agents is usually overlooked in defining mechanisms of disease. Cole and coworkers (1977) reported herd deterioration with symptoms similar to malnutrition and protein deficiency with some mortality when moldy silage was fed. Subsequent testing with a chloroform extract of *A. fumigatus* culture fed to calves revealed hemorrhagic enteritis in the small intestine and the large intestine (to a lesser extent). Intake in a calf dosed with this extract dropped to 25% of the pretreatment level. Examination of the lungs also showed patchy interstitial thickening of alveolar walls. Mycotoxins including Fumigaclavine and tremorgens were found in the silage. These and other identified mycotoxins from *A.*

fumigatus have been implicated with immunosuppression, abortion and other health problems (Cole et al., 1977; Pitt and Hocking, 1997).

Clostridium perfringens

As mentioned above, *Clostridium perfringens* is a commensal organism in the intestinal tract of most animals. In the ruminant, the acid environment of the abomasum,
normal gut peristalsis, and the limited quantities of fermentable substrate in the intestinal tract prevent overgrowth of the organisms under normal circumstances.
Several conditions favor overgrowth of *C. perfringens* in the digestive tract: 1) oral antibiotic therapy, 2) a diet excessive in protein and readily fermentable carbohydrates, 3) abrupt feeding changes that disturb the normal intestinal flora, and 4) an injury to the small intestinal mucosal layer.

In the course of Clostridial intestinal overgrowth, some strains of *C. perfringens* produce a toxin that causes increased intestinal permeability. This aids in the absorption of the organism into the bloodstream.

Cryptosporidium

Cryptosporidium is a protozoal organism that can infect a variety of animals causing scours, weight loss, and dehydration. Cryptosporidium was first detected in mice in 1907 and not identified in cattle until 1971. Cryptosporidiosis is not currently recognized as a major cause of death in calves but does represent a potential economic loss for the dairy farmer. It is found most commonly to be a problem in calves less than 3 weeks old and prevalence is higher during the summer months. *C. parvum* is a species commonly found in cattle that also can infect humans. Because of this, press reports suggested that an outbreak in humans in Milwaukee, Wisconsin in 1993 originated from the runoff of nearby dairy farms (USDA:APHIS, 1993). It is estimated that 22% of preweaned heifers are shedding Cryptosporidium and the percentage of farms with this protozoan is estimated to be greater than 90%.

Salmonella

The genus Salmonella contains more than 2,400 serotypes and all are pathogenic in certain species. Salmonellosis can adversely affect adult ruminants and has been associated with poor performance and reduced milk yields (Quinn, 2002; Robinson et al., 1992). In 1990, dairy and beef operations were identified as important vectors for human Salmonella outbreaks (Bean and Griffin, 1990). A study in 1995 examined 1,063 dairy operations and 6,861 fecal samples from heifer calves (Losinger et al., 1995). Interestingly, the investigators found that only 7.4% of the farms and 2.1% of the fecal samples tested positive for Salmonella. When examining the variables involved in management that may be correlated with Salmonella shedding, the use of medicated milk replacers from 24-h of age to weaning had the strongest association with reducing Salmonella shedding. However, this is a two-edged sword as there are also multiple suggestions of medicated milk replacers being involved in selection of drug-resistant bacteria (Martel and Coudert,1993).

E. coli

Enteric colibacillosis primarily affects the newborn calf. Diarrhea develops within the first few days of life, and the incidence and severity of infection increases under intensive management conditions (Quinn et al., 2002). The mature ruminant is comparably resistant to *E. coli* enteric disturbance although again, stress can lead to infection. Additionally, fecal exposure of the teat may lead to coliform mastitis which can be life-threatening depending on the strain of bacteria infecting the dam.

The past 20 years, *E. coli* O157:H7 has emerged as a major concern as a food-borne pathogen in humans producing hemorrhagic colitis-hemolytic uremic syndrome. Cattle have been targeted as a major reservoir for *E. coli* O157:H7 and outbreaks have been traced to undercooked hamburger, raw milk, and exposure to live animals carrying the organism. Contaminated fruits, vegetables and water have also been found to harbor this organism. In 2002 NAHMS conducted a national study of U.S. dairy operations collecting 3,733 samples from 96 dairy operations. Of these samples 4.3% were culture positive for *E. coli* O157 with June being the peak month for cows to shed these organisms. The west region also had the highest percentage of positive cows with 7.6%.

Although the 2002 report indicated a more than 3-fold increase in the percentage of positive cattle, this increase is likely due to improved culture methods rather than an actual increase in prominence in ruminants (NAHMS, 2002). The actual impact of O157:H7 on ruminant health is a subject of debate. However, it has been shown that management practices that increase stress increase the concentrations and subsequent shedding of *E. coli* O157:H7. As with many zoonotic organisms, health officials would like to see reductions in the incidence of isolation of human pathogens from livestock in an attempt to lower human exposure.

Campylobacter

Campylobacter is currently recognized as the leading cause of food-borne illness in humans in the U.S. Recent data suggests that there are between 2 and 4 million cases per year in the U.S. (FDA/CFSAN, 2003; Mead et al., 1999). The high prevalence of this organism in causing disease has probably always been present, but the development of improved methods of detection of Campylobacter in feces has made the organism easier to detect. The prevalence of Campylobacter in cattle has been reported to range from 5-68%. Causes for this variation include; 1) method of detection, 2) age of the animal, 3) season, and 4) whether the sample was taken from the feces of directly from the intestine (Atabay and Corry, 1998; Beach et al., 2002; Garcia et al., 1985; Stanley et al., 1998; Wesley et al., 2000). The failure of one trial to detect a higher incidence of isolation of *C. jejuni* or *C. coli* from culled cows compared to clinically healthy cows leads one to question the role of Campylobacter in ruminant enteric disease (Wesley et al., 2000). Campylobacter may produce diarrhea in neonatal calves and other strains of this genus may cause abortion, infertility and early embryonic death in adult cattle.

Control Methods for Bacterial Pathogens

There is little doubt that antimicrobials benefit the producer by lowering the incidence of disease and death, and improving animal performance. A review of the literature conducted in 1996 indicated that in 12,153 livestock and poultry trials, the addition of antimicrobial growth promotants to the diet increased production 72% of the time (Rosen, 1996). Today more than 50% of all antibiotics produced are used in animal feeds. However, because of the potential risk to human health many consumer groups, government officials, and researchers promote the elimination of sub-therapeutic doses of antibiotics in animal feeds, in an attempt to reduce or eliminate the antibiotic resistant bacterial populations. Fecal shedding of antibiotic resistant bacteria is one of the primary mechanisms of transfer of antibiotic resistance.

Antibiotic Use in Dairy Applications

Although direct evidence for a link between sub-therapeutic antibiotic use and human infection is lacking, several studies have implicated infections in humans to multiple drug resistant strains of bacteria in animals. An epidemic of resistant *Salmonella heidelberg* in Connecticut was traced to calves on a dairy farm (Lyons, et al., 1980). Eighteen people in the Midwest became ill when infected with multiple-drug-resistant *Salmonella newport* traced to a beef herd in South Dakota (Holmberg et al., 1984). Separate outbreaks of *Salmonella typhimurium* in Arizona and Canada were traced to multiple-drug-resistant bacteria in raw milk (Tacket et al., 1985; Bezanson et al., 1983). Finally, an outbreak of *S. newport* in 45 patients in California was linked to beef from dairy farms (Spika et al., 1987).

Even the perception that contaminated dairy products or meat from ruminant animals could endanger humans may hinder consumption. Therefore, the dairy industry must be proactive in searching for alternatives to antibiotics. A frequent area of use of sub-therapeutic antibiotics in the dairy industry is in the formulation of milk replacers.

As mentioned earlier, medicated milk replacers represent a conundrum for the industry. The benefits in improving helath and production are well documented, but there may also be a downside. Martel and Coudert (1993) suggested that the emergence of antibiotic resistant organisms was greater in young calves compared with older cattle, reasoning that drug use is greater with young calves because of greater susceptibility to bacterial diseases compared to older animals. In addition, the transfer of antibiotic resistance from cows receiving antibiotics to nursing calves has also been examined. Development of antibiotic resistant microorganisms was investigated in calves fed milk from cows treated for mastitis (Wray et al., 1990). Calves that received milk from cows treated with antibiotics for mastitis developed bacteria that were resistant to streptomycin, whereas calves in the group fed milk replacer without antibiotics did not shed antibiotic resistant organisms into the environment.

Other studies have reported the existence of antibiotic resistant organisms in calves. Hariharan et al. (1989) isolated *E. coli* that was resistant to potentiated sulfonamide (a combination of trimethoprim and sulfamethoxazole) from fecal samples of calves with diarrhea. Also, all the *E. coli* isolates were resistant to tetracycline, and most were resistant to neomycin and ampicillin as well. A summary of studies from several countries indicates that multiple-drug resistant *E. coli* is widespread in calves with enteritis (National Research Council, 1999).

Microbiology of the neonate
The newborn animal is most susceptible to enteric pathogens due to the development of the neonatal gastrointestinal microflora. Ruminants, like other mammals, are born devoid of indigenous intestinal microorganisms. During transport through the vagina some bacteria are acquired by the neonate. This route may provide the initial inoculum of the newborn gastrointestinal tract, since predominant bacteria in the vagina include lactic acid bacteria. After passage through the birth canal, the neonate may become contaminated and subsequently colonized by fecal organisms such as *Escherichia coli*.

These events may help to explain the initial microflora of the neonate described by Smith (1965). In his work, *E. coli, Clostridium perfringens* and streptococci are described as the first organisms to be found in the neonate, followed closely by lactobacilli. Lactobacilli are slower to grow than the other organisms, but it has been documented that a noticeable *Lactobacillus* population corresponds to a drop in stomach pH (Newman and Jacques, 1995). One threat to the neonatal ruminant stems from the fact that the initial gastrointestinal tract pH is near neutrality. Since the microbial population is in transition and extremely sensitive at this time, it is susceptible to colonization from pathogenic bacteria. For this reason, greater than 60% of calf deaths are attributed to diarrhea or other digestive problems (NAHMS, 1996). These problems occur despite the fact that most commercial milk replacers contain sub-therapeutic levels of antibiotics in an attempt to prevent enteric diseases.

Mannan oligosaccharide and calf and ruminant production
Mannan oligosaccharide (MOS) is a complex carbohydrate consisting of polymers of mannose and mannose derivatives. Complex carbohydrates have risen to a prominent research topic with the realization that distinct carbohydrate structures can have specific biological activities. The biological diversity of these compounds can be easily demonstrated by examining the difference between alpha and beta-bonded 1-4 glucose units. When these two glucose units are bound in the alpha configuration, the compound, amylose, is easily degraded by starch degrading enzymes found in saliva. Conversely, beta-bonded 1-4 glucose represents cellobiose, a compound that is not degraded by any mammalian enzyme system. This example only looks at the difference in biological activity of the same two glucose molecules bound together at the same site with only a difference in the type of bond between these glucose units. One need only imagine the diverse nature of carbohydrate chemistry to see that the opportunities for novel compounds with unique biological activity. Carbohydrates and oligosaccharides are also now being utilized for their role in nutrition and immunity.

Carbohydrates are important surface entities of many animal cells. They project from the cell surface and form the antigenic determinants of certain cell types. Bacteria (including pathogens) recognize these sugars and have receptors which allow them to attach, colonize and, in the case of pathogens, cause disease in the animal. Mannose specific lectins (protein fimbriae on the bacterial surface), are utilized by many gastrointestinal pathogens as a means of attachment to the gut epithelium (Mirelman and Ofek, 1986). One way to prevent pathogens from causing disease is to prevent them from attaching to the epithelial cells in the gut. A complex sugar called mannan oligosaccharide has been successfully used to prevent this attachment by providing the bacteria a mannose-rich receptor that serves to occupy the binding sites on the bacteria and prevent colonization.

Several studies have been conducted examining the role of mannans and their derivatives on binding of pathogens to epithelial cells in the GI tract. E. coli with mannose-specific lectins did not attach to mammalian cells when mannose was present (Salit and Gotschlich, 1977). Spring and coworkers (2000) used a chick model to demonstrate that MOS could significantly reduce the colonization of Salmonella and E. coli. Animal trials in other species show similar benefits in reducing pathogen concentrations. In a number of calf trials, calves receiving MOS in milk replacer formulations had lower fecal coliform and E. coli concentrations than calves receiving unsupplemented milk replacer (Table 1).

Table 1. Effect of MOS supplementation on fecal bacterial populations.

Population	Control (log CFU/g)	Mos Supplementation (log CFU/g)	Reference
E. coli	6.1	3.2	Jacques and Newman, 1994
E. coli	7.2	5.8	Nippei, 1996
Coliforms	6.9	4.7	Newman, et al., 1993

Reductions in fecal scouring and improved fecal consistency have also been noted in a number of trials (Dildey, et al., 1997; Nippei, 1996; Heinrichs, 2001). In addition, reductions in pathogen concentrations do not seem to be limited to the pre-ruminant. In a field trial situation using heifers, the number of Salmonella positive heifers was dramatically reduced when MOS was added to the diet. Unlike many feed additives, MOS is not affected by heat. In laboratory tests, heating MOS to 121^{o}C for 20 minutes had no adverse effects on any of the parameters examined.

Summary

Enteric disease in ruminants represents a two-fold situation. Many human pathogens are considered commensal or non-pathogenic strains in cattle. Because of this, the producer is being asked to control these organisms at the farm level in an attempt to prevent them from entering the human food chain. There is also the probability that many of these organisms can cause sub-clinical performance problems. A number of studies have examined improvements of farm hygiene and management practices as mechanisms to reduce the risk of enteric disease and the subsequent spread of the pathogen into the environment and (possibly) into the human food chain. Improving the health and immune status of the animal through nutritional sources has also proven to be an area prime for pioneering research.

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