Salmonella Current Concepts

John K. House, *BVMS, PhD*
Bradford P. Smith, *DVM*
Dept. Medicine and Epidemiology
University of California
Davis, CA 95616

Salmonella induces a wide spectrum of disease in cattle ranging from inapparent subclinical infections to acute fulminant bacteremia, endotoxemia, and death. The variable manifestations of disease reflect the virulence of different Salmonella serotypes and the influence of challenge dose and host immunity. Many salmonella infections reflect opportunistic infections of compromised hosts. Strategies to prevent and manage salmonella outbreaks should emphasize minimizing pathogen exposure and maximizing host immunity.

The Salmonella Pool

There are over 2,200 reported serotypes of Salmonella yet fewer than 2% of these account for approximately 80% of the disease reported in livestock, poultry, and humans.[1] In cattle, over 95% of salmonella associated with disease are in serogroups B, C, D, and E. There is significant homology between the serotypes isolated from livestock, poultry, and humans suggesting all species are exposed to a common pool of Salmonella. Epidemiological studies indicate significant transmission of Salmonella between species.[2-4] Human salmonellosis is commonly linked to the consumption of Salmonella contaminated beef, dairy, and poultry products.[5-8] Human transmission of Salmonella to livestock occurs sporadically when Salmonella infected individuals work with livestock[9,10] and extensively when Salmonella contaminated human effluent is released into waterways used to irrigate livestock forage crops.[11-15]

Disease outbreaks in livestock amplify environmental Salmonella contamination. Irrigation of crops with Salmonella contaminated waste water contaminates forages and watersheds maintaining the Salmonella challenge to the herd and disseminating Salmonella throughout the region.[16-19] Mammals, reptiles, birds, and insects also disseminate Salmonella within and between production units.[20-25] Cattle dying of salmonellosis are commonly rendered along with other by-products from the livestock and poultry industries, and are converted into animal feed. Although rendering is effective at killing Salmonella, post process contamination often leads to significant (50% of lots tested) Salmonella adulteration of rendered feed products.[26]

Adult Infections

Salmonella infections are most commonly acquired through fecal oral and oral oral contamination via the environment or fomites. The number of Salmonella required to produce clinical disease is dependent on the virulence of the serotype and immunity of the host. The infectious dose for healthy adult cattle is approximately 10^9-10^{11} Salmonella.[27,28] When immunity is compromised by concurrent disease, or physiological or dietary stress, the infectious dose may be several hundred Salmonella.[29]

It is estimated that between 5 and 20% of feed fed to dairy cows in the U.S.A. is contaminated with Salmonella.[30] Healthy adult cattle normally tolerate small numbers of Salmonella in feed and do not develop clinical disease.[30] Although the number of Salmonella in feed may initially be low, under appropriate moisture, temperature, and pH conditions Salmonella replicate approximately every 30 minutes.[31] The resultant increase in Salmonella numbers is exponential. Salmonella outbreaks often reflect a series of events that culminate in a large challenge dose and impaired host immunity.

Salmonellosis in adult dairy cows commonly occurs close to parturition and may be associated with inter-current disease.[32,33] Immunity is depressed and significant dietary changes occur in the periparturient period. The growth of Salmonella in the rumen following ingestion is influenced by dietary intake before and after the organisms are ingested.[34] Dry matter intake may be depressed as much as 50% for the four days prior to parturition.[35] The growth of Salmonella in the rumen is inhibited by high concentrations of volatile fatty acids and a low rumen pH (normal is 5.5-6.5).[36,37] Anorexia is associated with low concentrations of volatile fatty acids and a high rumen pH (approaching pH 7.5). Salmonella disappear rapidly from the rumen of regularly fed cows, but maintain or increase their numbers when feed intake is decreased or interrupted for one or more days.[34] Feeding after a period of starvation is associated with multiplication of Salmonella.[38,39] Following parturition, dairy cattle are fed a high energy production ration. Clinical and subclinical lactic acidosis are common at this time. Disruption of normal fermentation with the production of lactate favors the less fastidious Salmonella, which multiplies rapidly using the available substrate.[36] Qualitative dietary stress and dietary changes have been implicated as a predisposing risk factor in Salmonella outbreaks in dairy cattle and feedlot lambs.[40-42] The incidence of clinical disease may be reduced by manipulation of the ration formulation and adjustment of feeding practices.[41]

Salmonella shedding by clinically affected animals exponentially amplifies Salmonella contamination of the environment. Clinically affected animals may excrete 10^8 to 10^{10} Salmonella per gram of feces.[43] Considering cattle produce approximately 20-28 kilograms of feces per day,[44] clinically affected cows may shed over 10^{14} Salmonella each day. As environmental Salmonella contamination increases, the balance between challenge dose and herd immunity is tipped in favor of the pathogen. Clinically affected animals should be kept isolated from the remainder of the herd. On intensive dry lot dairies there are rarely adequate facilities to isolate clinically ill animals. Post partum "fresh cows" and sick cows ("hospital cows") are commonly housed and milked together to facilitate milk management. This practice effectively exposes cows to the largest challenge dose when they are most susceptible to infection.

Salmonella outbreaks commonly last several months. Resolution appears to reflect increasing herd immunity in response to Salmonella exposure. Despite resolution of clinical disease, Salmonella may continue to cycle through the herd and persist in the environment. Salmonella contamination of dairy and beef products continues even in the absence of clinical disease.[45]

Neonatal Infections

Immunity to Salmonella changes rapidly during the first 3 months of life. At 2 weeks of age the LD_{50} for some virulent strains is 10^5 organisms,[46] at 6-7 weeks 10^7, and at 12-14 weeks 10^{10} organisms.[47] In contrast, administration of 10^{10} Salmonella to 24-28 week old calves failed to induce clinical signs of disease.[47] Different age predilections, manifestations of disease, and virulence are observed between Salmonella serotypes and between different strains of the same serotype.[28,48]

Calves on endemically infected farms are commonly exposed to Salmonella in the first few days of life.[49] Salmonella exposure may occur via Salmonella contaminated colostrum or milk, surface contamination of teats and udder, personnel, equipment, or the environment. Chronically infected Salmonella carriers may shed 2.5×10^8 Salmonella in milk per day (25 kg of milk containing 10^5 Salmonella per ml).[27] Salmonella contamination of colostrum and milk from periparturient and sick cows is common on farms with endemic Salmonella infections.[45] Pooling colostrum is associated with poor passive transfer and increases the risk of exposing calves to Salmonella. Outbreaks of salmonellosis in calves are commonly associated with the feeding of unrefrigerated "hospital" milk. Many cows clinically affected with salmonella are shedding salmonella in milk during their illness. Maternity pen management also impacts the amount of environmental Salmonella contamination calves are exposed to at birth. Feeding utensils and personnel often play a significant role in transmitting Salmonella between calves.[50] Salmonella infects the salivary glands and is shed in saliva and nasal secretions.[51,52] Adequate cleaning and disinfection of feeding utensils is necessary to remove Salmonella contamination. Salmonella is sensitive to most disinfectants, but removal of contaminating organic debris is imperative as the activity of disinfectants is reduced by the presence of organic matter.[53]

Salmonella Vaccines

The observation that calves exposed to low doses of virulent Salmonella are protected against subsequent high dose virulent challenge[54,55] suggests prevention of salmonellosis via vaccination is possible. Salmonella vaccine studies in cattle have focused on Salmonella bacterins and attenuated modified live Salmonella.

Most of the Salmonella vaccines licensed for commercial use in the United States are formalin inactivated, aluminum hydroxide adjuvanted products. The reported efficacy of Salmonella bacterins ranges from good to ineffective.[54,56-62] The overall consensus of these reports is that vaccination of cattle with Salmonella bacterins provides partial protection against Salmonella challenge. The absence of controls limits the interpretation of empirical reports describing the application of these vaccines in herd Salmonella control programs.[63] Adverse reactions in the form of anaphylactic reactions are occasionally reported in cattle vaccinated with Salmonella bacterins.

There are a number of naturally occurring and genetically manipulated attenuated Salmonella strains that have been used to immunize cattle against salmonellosis. The most widely tested modified live salmonella vaccines in cattle are the genetically altered aromatic amino acid (aro) and purine (pur) auxotrophic mutants.[64-68] Comparative vaccine trials indicate modified live attenuated Salmonella vaccines provide greater protection against virulent Salmonella challenge than Salmonella bacterins.[58,61,69,70] Induction of protective immunity with modified live Salmonella vaccines is dose (size, number, and interval), route, and age dependent. The frequency and magnitude of adverse reactions are also dose, route, and age dependent. Protective immunity can be induced in young calves with lower inoculation doses than older calves and parenteral administration induces protective immunity with lower doses than enteral administration.[71] Following oral administration of attenuated modified live vaccines to calves, the vaccine strain may be isolated from tissues and feces for 14-21 days post vaccination. The capacity of modified live Salmonella vaccines to persist in the host is important for efficacy.[72-74] The extensive use of antibiotics on some commercial calf raising facilities may adversely impact the persistence and efficacy of modified live Salmonella vaccines.

Passive Protection via Colostral Transfer

The level of passive protection achieved via feeding calves colostrum from vaccinated cows is questionable. A number of reports suggest immune colostrum provides passive protection and others report no protective effect. The results of the different trials may partly be explained by the study designs employed. Immunization of pregnant cows with formalin-killed *Salmonella typhimurium* 7 and 2 weeks prior to parturition protected their calves against experimental *S. typhimurium* challenge in the first week of life.[75] Feeding colostrum at birth and then daily for the first 8 days of life reduced mortality more than feeding colostrum only at birth. No protective effect was observed when calves were challenged at 3 weeks of age.[54] Although the duration of immunity associated with colostral transfer may be short, calves are commonly exposed to Salmonella in the first week of life so colostral protection may be useful. The impact of colostral transfer on the development of acquired immunity to Salmonella has not been evaluated.

Diagnostic Tools

Salmonella infections in cattle are traditionally diagnosed by isolating Salmonella from feces or tissues of infected animals using a variety of enrichment media and selective plating techniques. The sensitivity of culture techniques is affected by the methods employed. Serotyping aids interpretation of Salmonella cultures. Virulent Salmonella serotypes like *S. dublin*, *S. typhimurium*, and *S. montevideo* are more likely to cause primary infections in healthy cattle. *Salmonella dublin* also commonly causes chronic Salmonella infections. Chronic infections with other Salmonella serotypes have been reported but are less common. Herd outbreaks involving multiple obscure Salmonella serotypes commonly reflect opportunistic infections of compromised cattle or a large challenge dose associated with heavy feed contamination. Isolation of Salmonella from livestock indicates a potential public health risk. The implications for herd and individual cow health are often less clear. Isolation of Salmonella from animals displaying clinical signs of salmonellosis suggests a causal relationship, however Salmonella may also be isolated from apparently healthy animals. To define the true Salmonella infection status of apparently normal cattle it is necessary to perform multiple cultures over a 3 - 6 month period to distinguish convalescent animals from chronically infected Salmonella carriers and passive carriers.

A number of highly sensitive PCR techniques have been developed to detect Salmonella in biological samples.[76-84] The high cost of PCR currently limits the practical application of this technology.

An alternative strategy for diagnosing Salmonella infections is to evaluate the hosts immune response to Salmonella antigens. A number of serological and other immunological tests have been developed to identify Salmonella infected cows. The enzyme linked immunosorbent assays (ELISA) have the greatest sensitivity. Serology may be used to evaluate the Salmonella infection status of herds or individual cows. The specificity of the test is determined by the plate antigen employed. Application of Salmonella LPS as a plate antigen provides a serogroup specific test. As a population management tool Salmonella ELISA serology has been used to identify Salmonella infected herds[85-89] and as an epidemiological tool to identify events in the production cycle associated with Salmonella exposure. Salmonella ELISA serology has also been used to identify individual *S. dublin* carriers.[27,90] In this capacity ELISA serology has been used to erradicate *S. dublin* from an endemically infected herd.[91] Calves younger than 12 weeks of age do not produce a strong antibody response to Salmonella LPS limiting the application of serology to older cattle.

The different diagnostic modalities; culture, serology, and PCR provide complementary information and are best applied together during the investigation of Salmonella disease outbreaks. Salmonella cultures allow identification of the specific Salmonella serotypes involved in the outbreak and provides an isolate for preparation of an autogenous bacterin. Cultures of the environment, feed, rodents, and water identifies sources and reservoirs of infection. Repetitive Salmonella cultures are financially limiting restricting the use of fecal cultures to define the infection status of the herd. ELISA serology provides an economical means of screening the population or cohorts of the population to determine what facilities or events are associated with Salmonella exposure. In the case of *S. dublin,* ELISA serology is also useful for identifying Salmonella carriers. If automated PCR techniques become available they will be useful for defining the Salmonella infection status of herds and will provide a means of monitoring the effectiveness of Salmonella control programs.

Treatment

Common clinical signs associated with "salmonellosis" include fever, diarrhea, anorexia, depressed mentation, and dehydration. Many of the clinical signs are associated with endotoxemia induced by the lipid A component of lipopolysaccharide. Systemic signs of endotoxemia include, fever, tachypnea, tachycardia, scleral injection, leukopenia / leukocytosis, weakness, and ruminal stasis. Some serotypes particularly *S. typhimurium* have a tendency to induce severe inflammation of the bowel mucosa resulting in dysentery, and passage of fibrin and mucosal casts. Fluid, electrolyte, and protein loss may progress rapidly and become life threatening. Fluid therapy should be instituted to correct fluid and electrolyte deficits, non steroidal anti-inflammatory drugs administered to block the effects of endotoxin, and antibiotics administered to treat the associated bacteremia. Controversy surrounding the use of antimicrobials for treating salmonellosis originates from the human literature. In contrast to human salmonellosis, bovine salmonellosis is more commonly associated with systemic infections. Antimicrobial selection should be based on the sensitivity of the organisms isolated. **High mortality despite treatment is most commonly associated with inadequate or inappropriate fluid therapy.**

Summary

Salmonella commonly behaves as an opportunistic pathogen of cattle. The determinants of outcome in the host pathogen interaction are host immunity and pathogen dose and virulence. Healthy adult cattle are resistant to salmonella infections, disease is commonly associated with compromised immunity due to nutritional stress, other infectious diseases, or intoxications. Control strategies should be directed at alleviating concurrent stressors, minimizing pathogen exposure, and maximizing host immunity.

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