Animal By-Products Contaminated with Salmonella in the Diets of Lactating Dairy Cows

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ABSTRACT

As part of a total mixed ration, two rumen-fistulated dairy cows were fed meat and bone meal that had been artificially contaminated with Salmonella spp. Samples from the rumen, feces, and milk were taken 3 d/wk and cultured for salmonella. Rectal temperatures and rumen pH were also measured at the time of sample collection. Over the 2-mo study, salmonella were intermittently recovered from rumen contents, from feces, and from necropsy specimens of rumen contents, cecal contents, and mesenteric lymph nodes. No excretion of salmonella in milk was detected. An elevated rumen pH was associated with increased isolation of salmonella. No clinical illness was observed for either cow. Meat and bone meal that has been contaminated with low concentrations of salmonella is unlikely to result in clinical illness in healthy adult lactating cows. However, dairy producers should continue to be concerned about feed biosecurity and water contamination of animal by-products to prevent and control contamination by salmonella.

(Key words: salmonella, meat and bone meal)

Abbreviation key: MBM = meat and bone meal.

INTRODUCTION

The contamination of rendered animal products by salmonella and its effects on both animal and human health have been a concern for a number of years. A 1993 FDA survey of feed ingredients detected 56.4% of animal protein samples were positive for salmonella (13). Furthermore, several outbreaks of salmonellosis in cattle have been traced to contaminated feeds, such as an outbreak involving multiple herds in Manitoba, Canada in which several serotypes were traced to contaminated bone meal and an outbreak of Salmonella menhaden among cattle on eight dairies, which was associated with the consumption of animal fat in a TMR (17, 18).

Currently, 27% of the production of meat and bone meal (MBM) in the US is utilized by the dairy and beef industries as a source of bypass protein in the diets of these cattle to increase the intestinal supply of amino acids (16). Among Minnesota dairy producers, it is estimated that 20 to 30% of the rations of lactating dairy cows contains MBM (W. Olson, 1993, personal communication).

Previous attempts to determine the effects of Salmonella spp. have focused on salmonella inocula or inclusion of sludge contaminated with salmonella in the diets of calves or feedlot cattle (4, 5, 9). Those studies used various salmonella serotypes containing at least \(10^5\) organisms/L. The current study was an initial attempt to assess the clinical and microbiological effects of MBM contaminated with Salmonella spp. on lactating dairy cows and to determine whether the same serotypes of Salmonella spp. present in the MBM could be detected in the rumen contents, milk, or feces of these cows.

MATERIALS AND METHODS

Selection and Care of Cows

Two Jersey cows that were approximately 8 mo pregnant were purchased from a herd that was free of clinical signs of paratuberculosis, bovine virus diarrhea, and salmonellosis. The two cows were culture-negative for Salmonella spp. and Mycobacterium paratuberculosis. The bovine viral diarrhea virus could not be isolated from blood. To evaluate herd status, individual fecal samples from 10% of the lactating cows and calves in the herd were collected and cultured for Salmonella spp. For 1 wk, milk filters were also tested daily for Salmonella spp. All fecal samples and milk filters were culture-negative. Once cows had calved, they were housed in individual
rooms in the isolation unit of the College of Veterinary Medicine, University of Minnesota (St. Paul). A strict protocol was developed to prevent cross-infection between these cows and other cows in the unit. A student employee was assigned to clean and milk each cow using a portable milking machine. Each cow had a permanent rumen fistula inserted within a few days of calving to allow for the regular collection of rumen contents. Each cow was fed a TMR plus alfalfa hay and 600 g/d of contaminated MBM daily.

Clinical Examinations and Sampling

The MBM was supplied by a commercial rendering plant. An artificial inoculum of Salmonella spp. was added to the MBM to yield an estimated 1000 organisms/g of feed (Silliker Laboratories, Chicago Heights, IL). Common serotypes that are associated with feed were included in the inoculum preparation [Salmonella montevideo, Salmonella anatum, Salmonella cerro, Salmonella muenster, and Salmonella agona (14)]. Every 7 to 10 d cultures were taken on each batch of MBM prepared for cows in the study. All cows were monitored for clinical signs of illness (e.g., elevated temperature, pulse, or respiration rates and diarrhea) that could be compatible with salmonellosis. Ten grams of rumen contents, 10 g of feces, and 10 ml of milk were collected three times weekly to isolate salmonella. Rumen pH was determined at the time of sample collection. At the conclusion of the trial, each cow was euthanatized, and mesenteric lymph nodes and rumen and intestinal contents were collected aseptically for the isolation of salmonella.

Microbiology

Conventional qualitative microbiological methods used to isolate salmonella included 24-h enrichment in brilliant green tetrathionate broth followed by plating onto brilliant green and xylose lysine deoxycholate agars. Suspect colonies were examined for biochemical properties with triple sugar iron agar, urease, dextrose, and sulfur indole motility medium. Isolates that were positive for salmonella were tested with Poly O and Vi antisera (Difco Laboratories, Detroit, MI) for agglutination. Cultures that tested positive by both biochemical and agglutination tests were submitted to the National Veterinary Services Laboratory (Ames, IA) for serological identification.

RESULTS

Cows 1 and 2 were kept on the trial for 57 and 55 d, respectively (Table 1). Throughout the study, neither cow exhibited any clinical signs of salmonellosis. Although actual milk production was not measured, both cows appeared to be good producers, and no clinical mastitis attributed to salmonella was observed. All five of the salmonella serotypes added to the MBM were recovered in the rumen contents of the cows. Of the rumen samples, 11 of 24 (46%) were positive in cow 1, and all feces and milk samples were negative; 7 of 23 (30%) of the rumen samples were positive in cow 2. One fecal sample (4%) was positive for cow 2, and no milk sample was positive for either cow.

From necropsy results, S. cerro was cultured from the rumen and mesenteric lymph nodes of cow 1; cultures from cow 2 revealed S. anatum in both rumen and cecal contents but not in the associated lymph nodes. Salmonella anatum, S. cerro, and S. muenster were recovered from MBM samples. Most probable number estimations of this MBM revealed that the level of salmonella contamination ranged from 2900 to 11,000 organisms/kg of MBM.

An association between the rumen pH and isolation of salmonella from rumen contents was observed. Mean rumen pH was 6.8 when salmonella was isolated from rumen contents, but 6.5 from rumen samples that were culture-negative (P < 0.04). Furthermore, cow 1 had a mean rumen pH of 6.87, and cow 2 had a mean rumen pH of 6.33 (P < 0.01). No correlation between the isolation of salmonella and rectal temperature was observed.

DISCUSSION

Natural contamination of MBM by salmonella is low; most probable number estimates are less than 10 organisms in 100 g of feed (1, 6, 15). Low level contamination of MBM that is stored carefully in a dry

### Table 1: Serotypes of Salmonella spp. isolated by days on trial.

<table>
<thead>
<tr>
<th>Cow 1</th>
<th>Cow 2</th>
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<tbody>
<tr>
<td>Days on trial</td>
<td>Serotype</td>
</tr>
<tr>
<td>16</td>
<td>Salmonella anatum</td>
</tr>
<tr>
<td>19</td>
<td>S. anatum</td>
</tr>
<tr>
<td>23</td>
<td>Salmonella montevideo</td>
</tr>
<tr>
<td>28</td>
<td>S. anatum and Salmonella cerro</td>
</tr>
<tr>
<td>31</td>
<td>S. anatum</td>
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<tr>
<td>35</td>
<td>S. cerro</td>
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<tr>
<td>47</td>
<td>Salmonella agona</td>
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<tr>
<td>52</td>
<td>S. agona</td>
</tr>
<tr>
<td>54</td>
<td>S. cerro</td>
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</tbody>
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environment would be unlikely to result in rumen persistence among fed adult cows. However, the health of individual cows and proper feed storage cannot always be guaranteed, and proliferation may occur under inadequate storage conditions. Even in MBM lots with very low numbers of Salmonella spp., it is highly unlikely that bacteria are uniformly distributed, and there may be microcolonies that are unevenly distributed throughout the feed. In addition to animal by-products, other potentially contaminated feed sources, such as soybean meal supplements, may introduce salmonella into dairy herds (11).

All of the salmonella serotypes added to the MBM were recovered from rumen samples. However, only three of the five serotypes were recovered from the MBM directly. The absence of Salmonella spp. from all aseptically collected milk samples is reassuring for public health concerns; however, fecal contamination of the udder can readily lead to milk contamination under farm conditions. Persistent udder infections and salmonella excretion in milk have been documented with Salmonella dublin and Salmonella typhimurium (8, 10). Whether other salmonella serotypes can cause persistent mammary infection is unclear.

Although it is hazardous to draw conclusions from an experiment with only two cows, the significant difference in the mean rumen pH values between the positive and negative samples is of considerable interest. The rumen of a well-fed cow is an unfavorable environment for Salmonella spp. to persist and multiply. High concentrations of VFA and a low pH result in a marked reduction in salmonella numbers, and lower VFA concentrations have little or no inhibitory effects on growth (3, 12). Australian studies have shown that up to 45% of healthy cattle had Salmonella spp. in the rumen at slaughter (3, 12). The fate of these bacteria after being inoculated into the rumen depends on the dietary intake of the animal before and after inoculation. For example, although well-fed cattle maintained on alfalfa or grass hay rapidly eliminated Salmonella spp., cattle that were deprived of feed for 2 d or more had increased numbers of salmonella isolated, even when normal feeding was resumed (2).

Further studies are needed on the behavior of salmonella in the rumen, particularly during the periparturient phase and also in relation to other disease conditions. Because use of adult cows is expensive, dairy sheep may prove to be an acceptable alternative model.

The control of salmonellosis and reduced contamination of final food products are obviously multifaceted challenges. Because of the greater attention focused on preharvest food safety, it is necessary to scrutinize all links in the food processing chain to prevent contamination by salmonella. Even if Salmonella spp. can be completely eliminated from animal feedstuffs, as the FDA is demanding, or at least reduced to very low numbers, feedstuffs will have to be stored securely and handled properly to prevent recontamination from rodents, birds, and flies. The addition of organic acids, high temperature pelleting, or irradiation of these products may be additional ways to ensure feed safety (1, 7, 14). The economics of these procedures for the dairy industry await further analysis.

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REFERENCES


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