

Laboratory findings associated with abomasal ulcers/tympany in range calves

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Abstract. The etiology of abomasal ulcers/tympany was investigated in 48 animals from 36 ranches in Wyoming and Nebraska. Results indicate that subclinical trace mineral deficiencies of copper and/or selenium exist in the range cattle in west central Nebraska and Wyoming. Etiological agents most frequently incriminated by bacteriologic cultures and/or histopathic examination were *Clostridium perfringens* and *Campylobacter* species. Histopathologic evaluation of abomasums revealed 31 of 38 cases contained abundant gram-positive bacteria associated with the damaged abomasal mucosa. *Campylobacter-like* organisms were demonstrated in 9 of 38 cases using the modified Dieterle stain. *Clostridium perfringens* was isolated in 14 of 38 cases, and *Campylobacter jejuni* was recovered from 5 of 38 cases.

The occurrence of abomasal ulcers/tympany in the range calves of western Nebraska and Wyoming has been an increasing problem for several years. The morbidity commonly reported in western Nebraska, eastern Wyoming, Montana, and Alberta, Canada, is approximately 10-20%.^{9,12,15} Geographically, the north central region of the United States, along the eastern slopes of the Rocky Mountains and Alberta, Canada, has the highest prevalence of the disease. This condition usually affects the faster-growing calves nursing heavier milk-producing cows. Numerous environmental stresses and etiological agents have been reported as associated with this syndrome.

The cause or causes of abomasal ulcers is complex and often obscure. Stresses reported to be associated with this condition are environmental, nutritional, physical, genetic, hyperacidity, lactic acidosis,¹¹ and coarse rations. Bacterial isolations from cases include *Clostridium perfringens*,^{2,7,12,19} *Escherichia coli*, *Streptococcus*, *Staphylococcus*, and *Salmonella* species.¹² *Campylobacter* species have been reported as associated with gastric ulceration in man and ferrets¹⁹ and have recently been isolated from 2 cases of abomasal ulcers in neonatal calves.⁸ Fungi such as *Absidia* and *Mucor* species have also been recovered. Bovine virus

diarrhea virus (BVDV), rinderpest virus, and bovine malignant catarrhal fever virus have also been associated with abomasal ulceration and perforation.^{12,13} Phenylbutazone at a daily dose of 2 g has been reported to cause abomasal ulcers as early as the fifth day of treatment.²⁵ This report records the laboratory findings associated with abomasal ulcers/tympany in range calves in Nebraska and Wyoming.

Materials and methods

Animals. Thirty-six cattle ranches were selected in west central Nebraska (12) and Wyoming (24) on the basis of past history of incidence of abomasal ulcers/tympany in calves. Specimens were tested for infectious agents at the Wyoming State Veterinary Laboratory (WSVL), Laramie, Wyoming, and at the Veterinary Science Laboratory, West Central Research & Extension Center (VSL-WCREC), University of Nebraska, North Platte, Nebraska. Forty-eight different animals and/or tissues were examined. Thirty-eight animal samples were correlated as to histopathology and bacteriology (Table 4).

Bacteriology. Abomasum, rumen, and small intestine contents were cultured on blood agar plates aerobically at 37 C and observed daily for 3 days. Isolation for *Campylobacter* species was attempted on Blaser and/or Skirrow media in a microaerophilic atmosphere^{3,20} Identification of *Campylobacter jejuni* was accomplished by direct examination of colony morphology, using darkfield microscopy, and biochemical tests including catalase production, nalidixic acid susceptibility, hippurate hydrolysis, and growth at 41 C. Isolation attempts for *Clostridium perfringens* utilized an anaerobic system.^a

Virology. Virus isolations were performed using primary bovine spleen cells. Fluorescent antibody (FA) staining of frozen tissues for BVDV, rotavirus, and coronavirus was done using conjugates provided.^b Intestinal contents were examined by negative contrast electron microscopy for viral particles.²⁷

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Table 1. Hepatic trace elements (ppm wet weight) and abomasal pH values in calves with perforating ulcers.

| Case no. | State* | Age (wk) | Sex | pH | Cu | Zn | Fe | Se |
|----------|--------|----------|-----|-----|------|-------|-------|------|
| 848 | N | 6-8 | M | -† | 19.3 | 41.7 | 68.3 | 0.03 |
| 1038 | N | 4 | M | - | 23.8 | 48.9 | 154.0 | - |
| 2348 | W | 8 | F | - | 10.8 | 82.4 | 91.6 | - |
| 2607 | W | - | M | 6.0 | 43.4 | 67.3 | 72.8 | - |
| 2620 | N | - | F | 5.0 | 66.0 | 55.3 | 117.0 | 0.05 |
| 2963 | W | 12 | M | 5.5 | 6.9 | 60.4 | 97.0 | - |
| 2985 | W | 2 | F | 5.0 | 54.0 | 109.0 | 125.0 | - |
| 3112 | N | 3-4 | M | 7.0 | 16.7 | 43.6 | 127.0 | - |
| 3115 | N | 8-12 | M | 6.0 | 24.3 | 29.9 | 97.3 | - |
| 3117 | N | 6 | M | 4.0 | 40.4 | 46.6 | 121.0 | - |

* N = Nebraska, W = Wyoming.

† - = no data.

Histopathology. Tissues were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 5 µm thickness. Sections were routinely stained with hematoxylin and eosin (HE), Gram,¹⁶ and modified Dieterle stains.²⁴

Analytical methods. Trace element determinations were done on liver samples^c by inductively coupled plasma-atomic emission spectrometry.²¹ Deficient values were determined by utilizing ranges suggested by Puls.¹⁸

Blood serum selenium concentrations were determined by spectrophotofluorometer, and serum copper was analyzed by flame-made atomic absorption spectrophotometry.²⁶

Hay fed during the last trimester was analyzed for total protein and trace elements.^d Analysis was done by energy dispersive x-ray fluorescence for magnesium, aluminum, silicon, phosphorus, sulfur, chlorine, molybdenum, potassium, calcium, manganese, iron, copper, zinc, lead, chromium, nickel, arsenic, selenium, and bromine. Deficient values were determined by comparison with reported ranges for nutritional requirements of beef cattle.^{1,23} The pH of the abomasal fluid was determined using pH strips.^c

Results

Gross examination. Abomasal lesions were found in 35 calves. Perforating abomasal ulcers were present in both the fundic and pyloric regions of 10 calves, with the greatest number of lesions occurring in the pylorus. Linear or abomasal line ulcers were present

in the mucosa and submucosa of the longitudinal plicae in the abomasal fundus of 15 calves. Lesions of abomasal rupture and tympany occurred only in the fundic region of 10 calves.

The pH readings were recorded for 19 animals. The Nebraska calves generally had a lower pH (8 of 10, pH 4-5), whereas only 3 of 9 Wyoming calves had pH values between 4 and 5, and one calf had a value of 5.5. Five of 9 Wyoming calves had pH values between 6 and 7, and 2 of 10 Nebraska calves were in that range (Tables 1-3).

Stomach content varied; both states reported various degrees of abomasitis with the abomasum containing sand, dirt, hairballs, and grayish-brown to yellow fluids. There was a greenish fetid fluid in 4 Wyoming animals.

Histopathology. The most common finding was mucosal ulceration with occasional perforation. Inflammation and fibrosis of various degrees occurred in the peritoneum in these cases. Thirty-one of the 38 cases had gram-positive bacteria located in the damaged mucosa. *Campylobacter-like* organisms were demonstrated in 9 cases, using the modified Dieterle stain (Table 4).

Bacteriology. *Clostridium perfringens* was the most common organism recovered from abomasal lesions

Table 2. Hepatic trace elements (ppm wet weight) and abomasal pH values in calves with abomasal tympany and/or abomasal rupture.

| Case no. | State* | Age (WW) | Sex | pH | Cu | Zn | Fe | Se |
|----------|--------|----------|-----|-----|------|-------|-------|------|
| 1122 | N | 4 | F | -† | 68.0 | 42.6 | 94.4 | - |
| 1782 | N | 1 | M | 4.0 | 21.1 | 145.0 | 125.0 | 0.04 |
| 1783 | N | 3 | - | - | 40.0 | 39.6 | 43.1 | 0.04 |
| 2026 | N | 1 | M | 4.0 | 71.4 | 73.2 | 73.5 | 0.06 |
| 2621 | N | 3 | F | - | 24.2 | 109.0 | 49.5 | 0.25 |
| 2622 | N | 2 | F | 4.5 | 29.0 | 71.6 | 36.2 | 0.39 |
| 2624 | N | 6 | - | - | 23.0 | - | - | - |
| 3116 | N | 6-8 | - | - | 27.5 | 48.5 | 73.8 | - |
| 3118 | N | - | - | 4.5 | 50.3 | 53.6 | 66.2 | - |
| 3395 | N | 6-8 | - | - | 69.1 | 33.3 | 62.0 | 0.16 |

* N = Nebraska.

† - = no data.

Table 3. Hepatic trace elements (ppm wet weight) and abomasal pH values in calves with abomasitis and linear ulcers.

| Case no. | State* | Age (wk) | Sex | pH | Cu | Zn | Fe | Se |
|----------|--------|----------|-----|-----|-------|-------|-------|------|
| 841 | N | 1 | —† | — | 32.0 | — | — | — |
| 945 | N | 3 | M | — | 30.2 | 78.9 | 39.5 | — |
| 1819 | W | — | — | — | 43.0 | — | — | — |
| 1838 | W | — | — | — | 89.0 | — | — | — |
| 2499 | W | 6 | M | 7.0 | 54.0 | 25.1 | 186.0 | — |
| 2567 | W | — | — | — | 43.0 | — | — | — |
| 2598 | W | 2 | M | 5.0 | 220.0 | 205.0 | 153.0 | — |
| 2620 | W | — | — | — | 32.0 | — | — | 0.07 |
| 2625 | N | 4 | M | 5.0 | 87.0 | 74.3 | 75.5 | 0.04 |
| 2751 | N | 7 | F | 4.5 | 36.8 | 29.0 | 96.1 | 0.10 |
| 2753 | W | 6 | M | 6.5 | 11.5 | 37.3 | 151.0 | — |
| 2786 | W | 6 | M | 7.0 | 32.2 | 94.6 | 98.3 | — |
| 2787 | W | 6 | F | 7.0 | 60.4 | 183.0 | 156.0 | — |
| 3114 | N | 6–8 | F | — | 29.7 | 50.2 | 76.0 | — |
| 3647 | W | 12 | — | 4.5 | 19.8 | 40.8 | 69.8 | — |

* N = Nebraska, W = Wyoming.

† — = no data.

(14 of 38 cases). *Campylobacter jejuni* was isolated in 5 of 38 cases (Table 4). Other organisms isolated were *E. coli* (10), alpha-hemolytic streptococcus (10), and *Pseudomonas*, *Bacillus*, and *Proteus* species. One of 10 of the *E. coli* isolates was positive for K99 pilus antigen.

Virology. Electron microscopic examination of feces from 3 animals and isolation attempts on tissues from 7 animals were negative for viruses. The FA test results were 3 of 37 animals were positive for BVDV, 1 of 30 was positive for coronavirus, and 8 of 25 were positive for rotavirus.

Trace elements. Trace element concentrations in liver tissue varied from state to state and from ranch to ranch. Trace element analyses showed 6 of 14 livers from Wyoming calves and 12 of 21 livers from Nebraska calves were deficient in copper (35 ppm wet weight or less). Six of 10 livers from Nebraska and 1 of 1 liver from Wyoming were deficient in selenium (Tables 1-3).

Feed analysis. The primary finding in the analysis of Nebraska hay was high molybdenum. Other trace mineral inadequacies were low selenium, low zinc, and high iron. One Wyoming ranch submitted 4 feed samples, and all showed improper copper : molybdenum ratios (less than 3: 1) and high iron and low zinc values. Copper values were in the low normal range, and 1 sample contained toxic levels of molybdenum.

Discussion

Abomasal ulcers are generally classified as line ulcers or wall ulcers. The abomasal wall ulcers are subdivided into hemorrhagic and perforating. Wall ulcers occur both in the fundic and pyloric regions, whereas abomasal line ulcers occur in the longitudinal plicae or folds of the fundus.¹⁰

The etiology of abomasal ulcers is obscure. Many

chemicals, microorganisms, mineral deficiencies, etc., have been associated with this condition. *Clostridium perfringens* is the most commonly isolated organism^{7,8,12,13,17,19} Most outbreaks of abomasal ulcers/tympy occur during or shortly after a period of weather-induced stress in the faster-growing calves nursing on the heavier milk-producing cows.^{9,12} Extreme weather variation plays an important role in changing abomasal flora. Calves usually do not nurse or they nurse poorly during bad weather and may engorge themselves after bad weather, leading to proliferation of certain bacteria that cause fermentation.^{14,15} Abomasal pH values in this study suggest that lactic acidosis in these perinatal calves was not an important factor as has been reported in feedlot calves.¹⁰

Previous studies implicated *Clostridium perfringens* Types A and E as causative agents of abomasal ulcers but showed no relationship of this condition to copper deficiency as determined by blood serum copper levels.¹⁹ *Clostridium perfringens* was incriminated in other reports, but the data showed a high correlation between abomasal ulcers and copper deficiency as determined by hepatic copper concentrations.^{14,15} *Clostridium perfringens* is the most frequently isolated bacterial agent from Nebraska calves, and on 2 occasions, BVDV was recovered.^{12,13}

Bovine virus diarrhea virus has been isolated from calves with line or linear abomasal ulcers causing hemorrhagic abomasitis and from calves with perforating abomasal ulcers.^{12,13} The majority of the viruses demonstrated in this study were from calves with abomasal tympany. One calf positive for rotavirus had a perforating ulcer.

Trace minerals interact closely in animals. Deficiencies in copper and/or selenium may contribute to the overall pathogenesis of abomasal ulcers/tympy in neonatal calves.^{14,15,17} Excessive levels of zinc, molyb-

Table 4. Types of abomasal lesions and bacterial isolations in range calves.

| Case no. | Mucosal GMB-D* | Mucosal erosions | Mucosal ulcers | Peritoneum inflammation | Peritoneum fibrosis | Bacteria G + B* | Bacteria CAMP-L* | <i>Clostridium perfringens</i> culture | <i>Campylobacter jejuni</i> culture |
|----------------|----------------|------------------|----------------|-------------------------|---------------------|-----------------|------------------|--|-------------------------------------|
| 679 | +† | - | - | + | - | - | - | - | - |
| 863 | + | - | - | + | - | + | - | - | - |
| 965 | - | - | - | - | - | + | - | + | - |
| 1035 | - | - | - | - | - | + | - | - | - |
| 1331 | - | - | - | - | - | + | - | - | - |
| 1540 | - | + | - | - | - | + | - | - | - |
| 1750 | - | + | + | + | + | + | - | - | - |
| 1781 | + | + | + | - | - | + | - | - | - |
| 1782 | - | - | - | - | - | + | - | - | - |
| 1783 | + | + | - | - | - | + | - | - | - |
| 1819 | + | + | - | - | - | + | - | + | - |
| 1838 | + | + | - | - | - | + | - | + | - |
| 2090 | + | + | - | - | - | + | + | - | - |
| 2315 | - | - | - | - | - | + | - | - | + |
| 2348 | + | + | + | + | - | + | + | + | + |
| 2427 | + | + | + | + | - | + | + | - | - |
| 2499 | - | - | - | - | - | - | - | - | - |
| 2567 | + | - | + | + | - | + | + | - | + |
| 2598 | + | + | + | - | - | + | + | + | - |
| 2620 | + | + | + | + | + | + | - | + | - |
| 2622 | + | + | + | - | - | + | - | - | - |
| 2624 | + | + | - | - | - | +/- | - | + | - |
| 2625 | + | + | - | - | - | +/- | - | - | - |
| 2660 | + | + | - | - | - | + | + | + | - |
| 2748 | + | + | - | - | - | - | - | - | - |
| 2751 | + | + | - | - | - | + | - | - | - |
| 2786 | + | + | - | - | - | - | - | - | - |
| 2787 | - | + | - | - | - | - | - | - | - |
| 2963 | + | + | + | + | - | + | + | + | - |
| 2985 | + | + | + | + | + | + | - | - | + |
| 3112 | + | + | + | + | + | + | - | + | - |
| 3114 | - | - | - | - | - | + | - | + | - |
| 3115 | + | + | - | - | - | + | + | - | - |
| 3116 | + | + | - | - | - | + | + | - | - |
| 3118 | + | + | - | - | - | + | - | + | - |
| 3395 | + | + | - | - | - | + | - | + | - |
| 3652 | + | - | - | - | - | + | - | + | + |
| 4174 | + | + | - | - | - | + | - | - | - |
| Total positive | 28 | 27 | 11 | 10 | 4 | 31 | 9 | 14 | 5 |

* GMB-D = disruption of gastric mucosal barrier, G+B = gram-positive bacilli, CAMP-L = *Campylobacter*-like.

† + = positive findings, - = negative findings.

denum, or sulfur will reduce the availability of copper.²³ Copper may play a role in abomasal disease through its influence on the immune system^{4,6,9} The cuproenzyme cytochrome oxidase activity is significantly decreased in copper-depleted rats, sheep, and cattle.¹⁴ In copper-depleted cattle, cytochrome oxidase activity of the mucosa of the duodenum and jejunum was decreased,²² villi of the small intestine were atrophied,¹⁷ and neutrophil function (candidacidal activity) was decreased.⁴ Copper deficiency induced by an excess of either molybdenum or iron impairs neutrophil phagocytic function more than does a diet with low levels of copper.^{5,6}

Eighteen of 35 livers analyzed had copper levels be-

low 35 ppm wet weight. The majority of these low levels were caused by secondary copper deficiencies. Selenium levels were 0.07 ppm wet weight or below in 7 of 11 livers. Two of these livers were also deficient in copper. Trace mineral deficiencies are area problems and may vary from year to year. In Wyoming and western Nebraska, trace mineral deficiencies are consistent findings in abomasal ulcers/tympany. Correction of these deficiencies has led to increased animal productivity and reduction in abomasal ulcers/tympany in range calves.

This study concurred with previous reports from Nebraska and Wyoming implicating *Clostridium perfringens* as the major bacterial agent in abomasal ul-

cers/tympany.^{7,12-14,19} The isolation of *Campylobacter jejuni* supports the findings in a previous report⁷ in which *Campylobacter* sp. was associated with abomasal ulceration; however, the relationship between *Campylobacter* and neonatal abomasal ulceration is undetermined. The exact etiology of the abomasal ulcer/tympany syndrome continues to be an enigma because of multiple factors associated with this disease.

Sources and manufacturers

- a. Forma Scientific, Marietta, OH.
- b. National Veterinary Services Laboratory, USDA/APHIS, Ames, IA.
- c. Diagnostic Laboratory, Michigan State University, Lansing, MI.
- d. Department of Agronomy, University of Nebraska, Lincoln, NE.
- e. pHydron Strips, Micro Essential Laboratory, Brooklyn, NY.

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