ACUTE ABDOMINAL SYNDROME IN NEONATAL CALVES: THE ROLE OF CLOSTRIDIUM PERFRINGENS

by

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TABLE OF CONTENTS

INTRODUCTION

Acute abdominal syndrome in neonatal calves (2 to 30 days of age), seen primarily in beef calves, is characterized by acute onset of abdominal tympany or bloat, colic, lassitude, depression or sudden death. It has been recognized in association with abomasitis, hemorrhage, abomasal erosions, and abomasal ulceration with or without diarrhea. The problem has been recognized for over 30 years and was most often seen as a sporadic, isolated case in a herd. This syndrome is not considered to be highly contagious but has been reported with an increasing incidence over the last 15 years. Its economic significance is realized as death losses and decreased weight gains associated with illness during the preweaning period.

The etiology and pathogenesis have yet to be satisfactorily elucidated. Many etiologies have been suspected and investigated, but research has focused most often on dietary changes and environmental stress. Other current theories proposed to explain the pathogenesis include mycotic infection, low immune status associated with copper deficiency, and Clostridium perfringens. Different bacterial species have been isolated from abomasal lesions and ingesta of those calves. Most often the bacteria have been considered secondary, opportunistic infections or postmortem saprophytic invaders. Many investigators consider Clostridium perfringens to belong to one of these categories.

This study was undertaken to gain information regarding the nature and development of lesions of acute abdominal syndrome in neonatal claves. Calves presenting with this syndrome clinically were included in a study to ascertain the possible role of Clostridium perfringens in neonatal calves with this syndrome. Experimental calves were inoculated intraruminally with Clostridium perfringens types to reproduce the clinical syndrome and abomasal lesions.

Literature Review

Acute Abdominal Syndrome In Neonatal Calves

Introduction

Acute abdominal syndrome in neonatal calves (2 to 30 days of age) is characterized by acute onset of abdominal tympany or bloat, colic, anorexia, lassitude, and depression with or without diarrhea or sudden death. It has been recognized in association with abomasitis, abomasal hemorrhage, abomasal erosions and ulceration.[1-14] This problem has been reported for over 30 years and was most often seen as a sporadic case in most herds. During the period from 1970-1980 some herds in Wyoming and Nebraska experienced a 40% incidence in abomasal tympany and a 2-10% morbidity and mortality associated with abomasal ulceration in calves.[2-6,8] This syndrome is not considered to be contagious but economically significant because of death losses and decreased weight gains associated with illness during the preweaning period.

Clinical Description

Acute abdominal syndrome in neonatal calves may be peracute, acute or subacute to chronic.[1-14] It usually occurs in the neonatal calf ($\leq$ 1 month of age) but may occur in older calves up to 6 months of age.[1-8,11-16] Calves as young as 2 days of age have been seen with the problem, so if infectious, the incubation period would appear to be very short. A definitive etiology is yet to be ascertained.

The peracute form strikes with no history of apparent illness. Owners report calves to be doing quite well, being top calves in terms of weight gain and appearance,[7,8] and often from heavy milking dams. Calves are often found dead in the early morning with varying degrees of bloat.[2-7] The peracute and acute forms

are the most common and the most characteristic of the syndrome in neonatal calves. The acute form may have diarrhea preceding abdominal tympany, colic, anorexia, depression or other signs.[17-20] Some cases have occurred in herds with a history of neonatal enteritis.[17-20] Some animals from these herds have a history of diarrhea the first week of life, recovery, then development of acute abdominal syndrome one or two weeks later. Death is generally attributed to shock, toxemia and peritonitis in cases with perforated abomasal ulcers or abomasitis,[2-6] or to hypoxia and suffocation in calves with severe tympany.[7]

The subacute and chronic forms may be prolonged manifestations of the acute form where calves with recurrent tympany, anorexia, colic and other signs have been found to have abomasal lesions at necropsy.[21-23] These animals appear to have periodic episodes of diarrhea.

The course of acute abdominal syndrome is unpredictable. It varies from sudden death with no premonitory signs in the peracute form to 11 days or longer in calves given medical treatment for tympany, diarrhea, electrolyte disorders, and dehydration that eventually succumb or are euthanatized. The clinical pathologic changes for this syndrome have not been reported.

Practitioners, researchers, and producers have noted a seasonal tendency in calves with acute abdominal syndrome. Affected calves are most often reported during the mid-winter to late spring months.[3-7,17] This may only be true because more producers tend to have most of their calf crop in these months.

Gross Lesions

Necropsy findings include abomasitis, and presence of sand, hair balls (trichobezoars), or straw in the abomasum and rumen. A grayish-to-black fluid is

often found in the rumen and abomasum. This fluid may contain brown flecks of digested blood and milk curd.[2-6] Abomasal lesions vary from abomasitis, abomasal erosions, and different degrees of abomasal ulceration.[1-23] Two types of abomasal ulceration have been observed at neuropsy: true perforating ulcers and severe linear tears that appear as "blow-outs" from severe abomasal tympany.[2-6,8] Peritonitis may or may not be present; if present, it usually accompanies abomasal ulceration.[2-6,8-11] Four unique characteristic abomasal ulcer lesions may be found: 1) ulcers with slight hemorrhage, 2) ulcers with marked hemorrhage, 3) perforation with acute circumscribed peritonitis, and 4) perforation with acute diffuse peritonitis.[24] Some animals exhibit non-perforating ulcerative abomasitis, with varying degrees of abomasal and rumen tympany which may be quite severe.[22,23] Severe distension of the abdominal cavity due to large amounts of gas in the rumen, abomasum, and small intestine with marked subcutaneous gas accumulation and subcutaneous crepitation have been reported in some calves.[2-7] The mucosal surfaces of rumen, abomasum, small and large intestine appeared to be normal in this instance and death was attributed to suffocation due to severe bloat.[7] Some calves have exhibited a few small (3 mm) foci of hemorrhage on the rumen mucosa.[17] In animals with abomasal ulcers, they are usually located within the midpart of the fundus and on the greater curvature of the abomasum.[14] However, ulcers may be found near the pyloric region, too.[13]

Animals with this syndrome may have a profuse diarrhea varying from greyish white to brown in color.[18] Some are reported to have a catarrhal enteritis with enlarged but not congested mesenteric lymph nodes.[17] Ulcerative lesions with perforation in the jejunum and generalized peritonitis have been found in some cases.[17] In this instance, the gastric mucosa was noted to have numerous blisters along the folds of the abomasum, but there was no inflammatory reaction around or

beneath these foci. Duodenal ulcers have also been reported.[3]

Other organs and tissues were without lesions except for the finding of petechiae in heart muscle, kidneys, tracheal mucosa, thymus, and on the serosal surface of the gastrointestinal tract in some cases.

Microscopic Lesions

Histopathologic examination of abomasal lesions most often reveals varying degrees of abomasitis as evidenced by hyperemia, hemorrhage, edema, desquamation, and necrosis of epithelium.[2-6] Mild nonspecific cellular invasions and microthrombi have been noted in some cases.[2-6] Penetrating foreign bodies have been found in some calves.[16,22] In animals with severe tympany and no apparent gross lesions, foci of hemorrhage were found in some of the viscera.[7]

Some investigations have found hyphae within the central necrotic areas of ulcers.[11,17] In direct mounts from these lesions, numerous branching aseptate hyphae, 2.0-7.5 μm wide were seen amongst the tissue debris, which resembled the assimilative hyphae seen in cultures of mucoraceous fungi. The abomasal and ruminal content were found to contain plant debris in some cases, and numerous spores of saprophytic fungi were also found.[17] From the surface of the lesions large numbers of nonseptate hyphae could be seen penetrating the underlying tissues and in some places invading the blood vessels of the corium. The mucous membrane at these sites of ulceration showed varying degrees of necrosis, in some places quite severe but in other sections of the same lesion contours of normal glandular structures could also be found.[17] Histologically, the glands at the margin of the ulcer were smaller in height, and there was hyperemia of the lamina propria. A mild lymphocytic and fibroplastic reaction was noted in the submucosa,

muscularis, and serosa along the perforating ulcer edges.[12] Marked submucosal edema was found throughout the abomasum.[11,17] Polymorph leukocytic reaction and infiltration with hemorrhage and cellular debris were seen with the muscularis mucosae of the abomasum.[11,17] The muscularis mucosae was also edematous in some sections and the muscular layer of the stomach wall beneath the lesion was usually thickened. The blood vessels in these layers were congested.

Direct gram staining of tissues has revealed numerous gram positive bacilli or rods from abomasal lesions or mucosa in other cases with no evidence of hyphae.[2-6] In these investigations no hyphae were observed under histopathalogic examination utilizing H & E and Grocott stains.

Etiology

The etiology of acute abdominal syndrome with abomasal lesions is not known. Many theories have been put forth to explain mechanisms of injury or initiating factors which may contribute to the development of this condition but the etiology is yet to be elucidated.

Theories proposed to explain the pathogenesis of the abomasal lesions include dietary changes, in particular the addition of coarse roughage feeds and associated traumatic injury to the mucosa,[1,11,14,16-25] pica secondary to chronic enteritis,[1,25] abomasal bezoars,[1,14,16,18-20] environmental or physical stress,[1,3,26,27] hyperacidity,[28] vitamin E deficiency,[26] lactic acidosis,[29] mycotic infection,[11,17] bovine virus diarrhea (BVD),[8] and low immune status associated with copper deficiency.[3-6,10]

Various microorganisms have been isolated from affected abomasal tissues in neonatal calves including Escherichia coli, Pseudomonas, Proteus, Streptococcus,

Staphylococcus, Salmonella, Clostridium perfringens, Absidia and Mucor.[2-7,9,11,14,17] Most of these were considered to be postmortem invaders from the alimentary tract[17] or secondary opportunistic infections occurring after abomasal injury, stress,[1,3,26,27] or associated with copper deficiency.[3-6,10] In one case where hyphae were found within an abomasal ulcer, numerous blisters were found along the abomasal folds from which C. perfringens was cultured; the investigators considered this to be an insignificant post mortem saprophytic invader,[17] even though it was only cultured from these lesions and was not present in the abomasal content. C. perfringens type A was hypothesized to be the cause of sudden death in 10 Holstein replacement and feeder calves, 6-8 weeks of age, which had severe abdominal tympany because no other pathogen was isolated.[7] However, this was not considered to be an enterotoxemia because high concentration of exotoxin was not found, and there was no intravascular hemolysis, or lesions in the mucosal surfaces of the gastrointestinal tract. Perforating abomasal ulcers and abomasal tympany in range calves, 1-12 weeks of age, had C. perfringens isolated from 12 cases and E. coli from 3 cases.[2] Reports from practitioners in central Nebraska and Wyoming indicated a reduced incidence in abomasal ulcers and tympanitis in calves from herds vaccinated against C. perfringens types C and D,[2,9-10] and in which cows were vaccinated against E. coli.[3-5]

It is thought that stress ulceration of the gastric mucosa is the result of stimulation of gastric secretion via neural and hormonal pathways.[27,29,31] During stress in laboratory animals, mast cells in the gastrointestinal tract were observed to degranulate, indicating liberation of histamine.[27,31,32] Mast cells are distributed all over the body, especially in the gastric mucosa, and stress causes degranulation and reduction in their number, faster and more complete in the

mucosal than in the submucosal or subcutaneous mast cells.[32] It has been shown that endogenous corticosteroids degranulate the mucosal mast cells and liberate histamine.[33] In man, many gastric erosions result from steroid-mediated mechanisms.[27] Cortisone and ACTH reduce gastric mucus secretion which limits mucosal protection. Further, steroids decrease cell renewal in the gastric mucosa.[28] Mucosal lesions including ulceration have been produced experimentally with histamine injected intraperitoneally.[34] Macroscopic examination of the gastric mucosa in laboratory animals subjected to linear rocking motion at 200 to-and-fro movements per minute induced various grades of mucosal hemorrhage, erosion and ulceration.[27] Superficial abrasion and ulceration were of pinpoint, elongated or diffuse type involving mostly the body of the stomach. Deep ulcers and perforations were not observed. Blood histamine levels were found to be significantly increased in those animals with stress-induced ulcers. Hypersecretion of gastric juice was also significantly increased in stressed animals, but there was no direct correlation between the increase in the total volume of gastric juice and the degree of ulceration.[27]

Some cases of acute abdominal syndrome in calves have been noted after periods of weather stress.[2-6] All forms of stress are associated with increased endogenous steroid secretion; by activation of the hypothalamus to release ACTH, there is subsequent increased secretion of corticosteroids.[4-6,27,28] Because calves usually nurse less during storms, it was concluded that this behavior during the storm would result in their being hungry afterwards, and this along with the dam's over-abundant milk supply would lead to engorgement with rich milk. This could then induce gut stasis and a mild abomasitis, which in turn could lead to proliferation of certain bacteria and fermentation. Under these circumstances, C. perfringens is proposed to be an excellent opportunist.[2-6]

Histamine levels in blood were inconclusive and much higher than expected, ranging from 0.28-5.02 µg/ml in calves with this syndrome and abomasal ulceration.[3] However, these investigators attributed those high histamine levels to hemolyzed blood samples. In this study, C. perfringens was isolated from abomasal lesions and fluid in 13 cases. Alpha- and theta-toxins produced by strains of C. perfringens have been shown to produce morphological changes of mast cells caused by both phospholipase C (phosphatidyl-choline: choline phosphohydrolase E.C. 3.1.4.3) (α-toxin) and theta-toxin (θ_A- and θ_B-toxin). Both α-toxin and θ-toxin are capable of causing histamine release from mast cells.[35] The release has been shown to be dependent on dose and temperature and has a slow time course. The action of α-toxin is stimulated by Ca^{++}. Zinc appears to be essential for enzymatic activity; Zn^{++} stimulates the release by phospholipase C and free calcium ion is required to bind the enzyme to substrate.[35,36] Theta-toxin is also stimulated by free Ca^{++} but release of histamine caused by θ-toxin was inhibited by Zn^{++}[35]. Cow's milk is high in Ca^{++} and has low levels of Zn^{++}[37]; since calves affected with this syndrome are suckling cows or being given milk as a large part of their diet, this would supply adequate substrate for C. perfringens proliferation and α- and θ-toxin production, thereby enhancing conditions for ulceration.

Bovine virus diarrhea (BVD), a DNA togavirus, is known to cause erosions and ulcerations of the upper respiratory and gastrointestinal tract, the acute disease occurring most commonly in young cattle from 8 months to 2 years of age.[1] Experimental and natural cases have been reported in very young calves,[38] but rarely will calves under 6 months be affected with the acute disease.[1] Colostral antibody against BVD has been shown to be protective from 105-230 days, or longer with high initial levels. In beef calves, the disease has been shown to occur most often a few weeks or months after weaning from 6 to 10 months of age.[1]

BVD has been implicated in neonatal calf enteritis.[38] Calves may become infected in utero or postnatally via the dam's milk. In utero infection may terminate in abortion, cerebellar hypoplasia, birth of a weak calf, or clinically inapparent infection. Clinical signs in neonatal calves may include fever, leukopenia, nasal discharge, and diarrhea of varying severity. Death from severe dehydration may occur in 18 to 96 hours, or chronic diarrhea may persist for weeks. Erosions of the digestive tract, primarily of the oral and nasal mucosa, lips, tongue, gums, and hard palate, typical of fatal mucosal disease, are occasionally seen.[38] Experimental BVD infection in calves either in utero via intravenous administration of BVD virus to the calf's dam, 6-25 days prepartum, or by nasal or oral inoculation of calves with BVD virus, some permitted and some deprived of colostral feedings, resulted in diarrhea of varying severity.[39] Lesions in calves experimentally infected with BVD includes hemorrhagic enteritis, moderate lymphoid depletion - especially in the spleen, subserosal visceral and endocardial hemorrhages, and ulcers and erosions on the tongue, buccal mucosa, lips and gums.[39]

One report of abomasal ulcers in young calves (4 to 6 weeks of age) in Wyoming herds with a mortality of 5-10% of the annual calf crop implicated BVD as the causative agent.[8] Calves exhibited signs of sudden death and acute onset of colic, bloat, depression, and if left untreated, progressed to coma and death within 30 minutes after onset of clinical signs. Necropsy revealed that 65-70% of the dead calves had ruptured abomasal ulcers. The remainder had at least one ulcer, usually about 2 cm. in diameter. In most cases, the ulcers were located on the greater carvature of the abomasum. Since calves had been vaccinated with C. perfringens type C and D toxoid, enterotoxemia was considered unlikely. No pathogenic agents were isolated from postmortem samples (bacteriology methods and materials were not included in this report) and BVD fluorescent antibody (FA) tests were not

performed on calf tissues; blood samples from unvaccinated cows that had lost their calves to abomasal ulcers had BVD titers of $\geq$ 1:128. Instituting BVD vaccination programs in those herds were said to greatly decrease or eliminate the incidence of calf abomasal ulceration.[8] Calves with acute abdominal syndrome and a history of enteritis, from Nebraska and Wyoming herds, had BVD fluorescent antibody tests performed on tissue;[2,3] all FA's were negative (calves in these herds were also tested negative by FA for rota-, corona-, and IBR virus). Virus isolations on all samples taken from affected calves were negative.[2,3] Because neonatal calves with clinical signs of acute abdominal syndrome have been found to be FA negative for BVD, and experimental inoculation either in utero or by nasal or oral routes with live BVD virus in neonatal calves failed to produce abdominal tympany, colic, anorexia, depression or sudden death nor did it produce abomasal ulceration. Therefore, it is unlikely that BVD is the primary etiology of this syndrome.

The incidence and pathology of abomasal ulcers in range calves exhibiting abomasal tympany in Nebraska and Wyoming has been correlated to copper deficiency and secondary bacterial infection.[3-6,10] Use of injectable copper compounds for brood cows in certain herds experiencing annual ulcer problems in calves, in which low copper levels were diagnosed, was reported to elminate abomasal ulcers.[3-6,10] Bacterial isolates from abomasal content of lesions in affected calves were believed to have gained access to the upper alimentary tract via reflux action of duodenal contents. The bile salts, acids, lysolecithins, potential bacterial toxins, and sepsis in the refluxed material were thought to damage the surface gastric cells, causing mucosal ischemia, including intravascular coagulation with microthrombotic occlusions of the mucosal vessels, and making the cells susceptible to erosion or ulceration. Bile or its component acids exhibit

antibacterial activity depending on the concentration of bile and the susceptibility of a given organism.[40] In general, bile acids have been shown to inhibit gram-postive organisms, but usually have little affect against gram-negative organisms. This would tend to preclude duodenal reflux of content and bacteria as the route of entry for gram positive bacteria cultured from abomasal ulcers and lesions. Low copper levels in calves were hypothesized to cause derangement of elastin cross linkages, thereby compromising the integrity of the abomasal mucosa and its microvasculature, and to cause decreased leukocyte cytochrome oxidase activity contributing to decreased neutrophil function and increased susceptiblity to infection. In Nebraska, the low serum and liver copper concentrations were related to molybdenum excess.[6] Copper-deficient calves are believed to be immunologically inferior to calves with normal copper levels.[3-6,10,41] It has been shown that alveolar macrophages from copper-deficient cattle had no in vitro bacteriostatic nor bacteriocidal activity; in contrast, those with adequate copper levels killed 40-60% of the bacteria in in vitro challenge,[42] and presumably this may be true of macrophages associated with the digestive tract. Because many digestive functions of the abomasum depend on the integrity of the microvascular system of its mucosa and this is dependent on the integrity of elastin within these vessels, reduced cross linkages within the elastin related to copper deficiency was considered to be the primary etiology contributing to the development of abomasal ulcers in copper deficient calves.[3-6,10] Researchers studying copper-depleted cattle have found that cytochrome oxidase activity of the mucosa of the duodenum and jejunum was decreased,[43] the villi of the small intestine were atrophied,[44] and neutrophil function (candidacidal activity) was decreased.[45] However, in none of these studies was abomasal ulceration found in copper-deficient calves or adult cattle at necropsy.[43,44,46] The researchers in the Nebraska and Wyoming study

felt that compromised integrity of the epithelial cells of the digestive tract, an increase in susceptibility to infections, and a decrease in leukocyte function may be the link between abomasal ulcers and copper deficiency in cattle.[3-6,10] Clostridium perfringens was isolated from abomasal content and lesions in these studies alone or with other bacteria, but was considered to be an opportunistic invader associated with engorgement on rich milk after a period of anorexia during weather stress and immunological compromise associated with copper deficiency;[3-6,10] toxin neutralization testing in mice was not done in those cases.

Acute abdominal syndrome in neonatal calves may be related to pathophysiology associated with suckling.[2,3,7] The clinical history of calves affected with this syndrome often mentions that they are vigorous nursers of milk, and calves which "gulp down" their milk or feed were the ones at highest risk of dying with this problem.[7,8] Death regularly occurred within 6-8 hours after the last feeding.[7] Calves fed whole milk and calf starter pellets twice daily beginning the first day of life and housed in individual hutches were found to have this syndrome between 6-8 weeks of age, when they were being weaned. C. perfringens type A was isolated in large numbers from abomasum, duodenum, and ileum in calves in this group which died suddenly and had severe distension of the abdominal cavity. Possibly calves that develop this syndrome have esophageal groove dysfunction which allows abnormal amounts of milk to consistently enter the neonatal rumen, thus, providing substrate and an anaerobic environment for ingested C. perfringens to proliferate. This abnormal fermentation could allow large numbers of C. perfringens to survive, enter the abomasum, and colonize the abomasal mucosa creating areas of abomasitis. In man, certain Campylobacter sp., microaerophilic to anaerobic gram negative bacilli, have been found on gastric epithelia or within ulcerated tissues in almost all patients with active chronic

gastritis, duodenal ulcer or gastric ulceration,[47] and have also been reported in gastric ulcers of ferrets.[48] Gastritis associated with pernicious anemia, immunologically mediated in man and affecting the proximal stomach, has rarely been associated with Campylobacter-like organisms. This rarity is considered to indicate that the organisms probably do not develop secondary to gastritis and provides indirect evidence of those organisms as causative agents in chronic active gastritis.[48] This same mechanism may apply to calves with acute abdominal syndrome and abomasal ulceration where C. perfringens has been isolated from affected calves' abomasal and rumen content, and abomasal lesions. Of particular significance is the isolation of toxigenic C. perfringens from ruminal content collected by sterile nasogastric tube, and in those calves which succumbed, its presence from the abomasal content and lesions.

Clostridium perfringens type A has been isolated with other bacteria from abomasal ulcers and abomasitis in mature cattle.[49] In that case, C. perfringens type A appeared to be associated with inflammatory lesions, capillary dilatation, and cell shedding in the mucosa. Perforating abomasal ulcers in one study in suckling calves (4-6 weeks of age) was associated with C. perfringens, E. coli, Streptococcus, and Staphylococcus aureus.[14] Those ulcers were in the midpart of the fundus, and on the greater curvature of the abomasum; several calves had abomasal trichobezoars. Studies of the microflora in the rumen of healthy and bloating calves, 4-135 days of age, indicated that C. perfringens is rarely isolated.[50] The distribution of C. perfringens and E. coli in the feces of scouring and healthy calves was studied in herds experiencing neonatal enteritis in Montana.[51] Three calves with scours and one with no diarrhea were necropsied in that study. C. perfringens was isolated from the abomasum and upper small intestines in calves with diarrhea but a few organisms were also found in the

abomasum in the calf with no diarrhea. These findings concluded that C. perfringens is not normally present in the rumen and abomasum of neonatal calves, but may be isolated in herds experiencing neonatal enteritis. Under normal circumstances, vegetative cells of C. perfringens do not survive in the abomasum.[52] The action of C. perfringens and its toxins appeared to be responsible for the tympany (C. perfringens is a prolific gas forming organism), abomasitis, and abomasal ulceration seen in neonatal calves with this syndrome from which it was cultured in ruminal and abomasal content and lesions.[2-7,9,14] Decreased incidence of abomasal tympany and ulceration in neonatal calves from herds having a history of these problems after instituting C. perfringens vaccination programs support the theory that this organism may be the inciting agent in this syndrome.[3-6,10] Further investigations of calves with abomasal ulcers, abomasitis, and acute tympany may reveal other contributing factors to this syndrome, but C. perfringens should not be ignored as a serious pathogen in these cases, and indeed, may be the primary etiologic agent involved in its pathophysiology.

References

1. Blood DC, Henderson JA, Radostits OM, et al. Diseases of the alimentary tract II. In: Blood DC, Henderson JA, et al, eds, Veterinary medicine: A textbook of the disease of cattle, sheep, pigs, and horses, 5th ed. Philadelphia: Lea and Febiger, 1979; 197-199, 452-458.
2. Johnson JL, Hudson DB, Bohlender RE. Perforating abomasal ulcers and abomasal tympany in range calves, in Proceedings. 24th Am Assoc Vet Lab Diag 1981; 203-210.
3. Johnson JL, Lilley CW, Hamar DW, et al. Diagnostic observations of abomasal tympany in range calves, in Proceedings. 3rd Int Symp World Assoc Vet Lab Diag, Vol 2, Ames, Iowa; 1983; 485-491.
4. Lilly CW, Hamar DW, Johnson JL, et al. Factors associated with abomasal ulcers in beef calves, in Proceedings. 87th Ann Conv, Nebraska Vet Med Assoc, 1984; 70-77.
5. Lilly CW, Hamar DW, Johnson JL, et al. The association of copper and colon bacteria with abomasal ulcers in beef calves, in Proceedings. 87th Ann Conv, Nebraska Vet Med Assoc., 1984; 4-11.
6. Lilly CW, Hamar DW, Gerlach, et al. Linking copper and bacteria with abomasal ulcers in beef calves. Vet Med 1985; 80:85-88.
7. Berkhoff GA, Braun RK, Buergelt CD, et al. Clostridium perfringens type A associated with sudden death of replacement and feeder calves, in Proceedings. 23rd Am Assoc Vet Lab Diag 1980; 45-52.
8. Toombs RE. Abomasal ulcers in calves associated with BVD virus infection. Norden News 1982; 3:35.
9. Waas W. Abomasal ulcers. Mod Vet Pract 1986; 67:111-112.

10. Anonymous. Abomasal ulcers. Agr Pract 1985; 6:33.

11. Wray C, Thomlinson JR. Abomasal ulceration in calves. Vet Rec 1968; 83:80-81.

12. Rooney JR, Watson DF, Hoag WG. Abomasal ulceration and perforation - report of two cases. The North American Veterinarian 1956; 37:750-752.

13. Leudke AJ, Hokanson JF, Dunne HW. Perforating abomasal ulcer in a calf. J Am Vet Med Assoc 1956; 128:206-208.

14. Tulleners EP, Hamilton GF. Surgical resection of perforated abomasal ulcers in calves. Can Vet J 1980; 21:267-274.

15. Cheli R, Mortellaro CM. Left dislocation of the abomasum in the calf as pathology during the weaning period. Bovine Practioner 1976; 11:89.

16. Degen B. Pathological and histological investigation of the pathogenesis of pyloriz ulcers in veal calves. Fachbereich Veterinarmedizin, Freie Universitat, Berlin. 1982; 1-111.

17. Gitter M, Austwick PKC. The presence of fungi in abomasal ulcers of young calves: A report of seven cases. Vet Rec 1957; 69:924-928.

18. Wood DR. Diet in calf scouring. Vet Rec 1968; 82:269.

19. O'Brien JDP. Ulceration of the abomasum associated with calf scour and diet texture. Vet Rec 1968; 82:363.

20. Wood DR. Ulceration of the abomasum associated with calf scour and diet texture. Vet Rec 1968; 82:446.

21. Groth W, Berner H. Studies on abomasal ulcers in calves fed milk substitutes and intensively reared. Zbl Vet Med A 1971; 18:481-498.

22. Keindorf HJ. Abomasitis in the calf. Monatshefte fur Veterinarmedizin 1974; 29:606-607.

23. Buratto L. Ulcerative abomasitis of the veal calf. Obiettivi e Documenti Veterinari 1984; 5:15-19.

24. Fox FH. Abomasal ulcers. In: Ambstutz HE (ed), Bovine Medicine and Surgery, 2nd ed. Santa Barbara, California: American Veterinary Publications, Inc., 1980; 667-668.

25. Fox FH. Abomasal disorders. J Am Vet Med Assoc 1965; 147:383-388.

26. Smith HA, Jones TC, Hunt RD. The digestive system, In: Smith HA, Jones TC, Hunt RD, ed. Veterinary pathology, 4th ed. Philadelphia: Lea and Febiger, 1972; 1204.

27. Singh GB, Sharma JN, Kar K. Pathogenesis of gastric ulceration produced under stress. J Path Bact 1967; 94:375-380.

28. Robbins SL. Pathologic Basis of Disease. Philadelphia: W.B. Saunders Co. 1974; 916.

29. Tasker JB, Roberts SJ, Fox FH, et al. Abomasal ulcers in cattle - recovery of one cow after surgery. J Am Vet Med Assoc 1958; 133:365-368.

30. Jensen R, Pierson RE, Braddy PM, et al. Fatal abomasal ulcers in yearling feedlot cattle. J Am Vet Med Assoc 1976; 169:524-526.

31. Shay H. Stress and gastric secretion. Gastroenterology 1954; 26:316-324.

32. Rasanen T. A mucosal bleeding mechanism in the upper part of the gastroingestinal tract. Gastroenterology 1963; 44:168-177.

33. Rasanen T. Mucosal mast cells of rat stomach; influence of ACTH, cortisone, and growth hormone. Gastroenterology 1960; 38:70-75.

34. Zaidi SH, Mukerji B. Experimental peptic ulceration. Part I. The significance of mucous barrier. Indian J Med Res 1958; 46:27-37.

35. Strandberg K, Mollby R, Wadstrom T. Histamine release from mast cells by highly purified phospholipase C (alpha-toxin) and theta-toxin from Clostridium perfringens.Toxicon 1974; 12:199-208.

36. Smith LDS. Virulence factors of Clostridium perfringens Rev Infect Dis 1979; 1:254-262.

37. Lindamood J, Kristofferson T. Composition and nutritional value of milk and milk producers. In: Ohio Cooperative Extension Service Dairy Guide The Ohio State University, 1978; D6 204:1-8.

38. Lambert G, McClurkin AW, Fernelius AL. Bovine viral diarrhea in the neonatal calf. J Am Vet Med Assoc 1974; 164:287-289.

39. Lambert G, Fernelius AL, Cheville NF. Experimental bovine viral diarrhea in neonatal calves. J Am Vet Med Assoc 1969; 154: 181-189.

40. Floch MH, Gershengoren W, Elliott S, Spiro HM. Bile acid inhibition of the intestinal microflora - a function for simple bile acids? Gastroenterology 1971; 61:228-233.

41. Sanders DE. Copper deficiency in food animals. Compend Cont Ed Pract Vet 1983; 5:S504-S410.

42. Parish S. Alveolar macrophage function in secondary copper deficient cattle. Proc 5th Annual Vilestera Conf. for Food Animal Vet Med 1984; p. 21.

43. Suttle NF, Angus KW. Experimental copper deficiency in the calf. J Comp Path 1976; 86:595-608.

44. Mills CF, Dalgarno AC, Wenham G. Biochemical and pathological changes in tissues of Friesian cattle during the experimental induction of copper deficiency. British J Nutr 1976; 35:309-331.

45. Boyne R, Arthur JR. Effects of selenium and copper deficiency on neutrophil function in cattle. J Comp Path 1981; 91:271-276.

46. Spratling FR. Complex nutritional deficiency in a group of calves. Br Vet J 1976; 132:557-567.

47. O'Connor HJ, Axon ATR, Dixon MF. Campylobacter-like-organisms unusual in type A (pernicious anemia) gastritis. Lancet 1985; 1:111.

48. Fox JG, Edrise BM, Cabot EB, et al. Campylobacter-like organisms isolated from gastric mucosa of ferrets. Am J Vet Res 1986; 47:236-239.

49. Al-Mashat RR, Taylor DJ. Bacteria in enteric lesions of cattle. Vet Rec 1983; 112:5-10.

50. Jayne-Williams DJ. The Bacterial flora of the rumen of healthy and bloating calves. J Appl Bacteriol 1979; 47:271-284.

51. Lozano EA, Catlin JE, Hawkins WW: Incidence of Clostridium perfringens in neonatal enteritis of Montanta calves. Cornell Vet 1970; 60:347-359.

52. Niilo L, Moffah RE, Avery RJ. Bovine enterotoxemia II. Experimental reproduction of the disease. Can Vet J 1963; 4:288-298.

Isolation of Clostridium perfringens from neonatal calves with ruminal and abomasal tympany, abomasitis, and abomasal ulceration

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[a]Swinnex®-HA 0.45 µm filter unit, Millipore Corp, Bedford, Mass.

[b]1:250 Trypsin, Difco Laboratories Inc., Detroit, Mich.

[c]American Type Culture Collection, Rockeville, Md.

[d]Model 513 pH/blood gas analyzer, Instrumentation Laboratory Inc, Lexington, Mass.

[e]SMA 12/60, Technicon Instruments Corp, Tarrytown, NY.

[f]Technical Bulletin No. 50 - UV, Sigma Chemical Co, St. Louis, Mo.

[g]Beckman/Spectrametrics Corp, St. Louis, Mo.

SUMMARY

Eight neonatal calves, 2-21 days old, were referred to Kansas State University for clinical examination or necropsy with suspected abomasal displacement or intestinal obstruction after acute onset of abdominal tympany, colic, depression, or death. Routine hematology and serum chemistry analysis revealed no consistent changes. Necropsy examination indicated abomasal distension with varying degrees of abomasitis, hemorrhage, and ulceration, but no evidence of displaced abomasum or obstructed intestines. Ruminal contents collected via stomach tube or at necropsy and abomasal contents collected at necropsy were cultured anaerobically. Clostridium perfringens was isolated from all samples, and based on toxin neutralization tests in mice, seven were Type A and one was Type E. Copper concentrations in serum and tissue samples were within normal limits. It appears that acute abdominal syndrome in neonatal calves is unrelated to copper deficiency, and C perfringens, particularly Type A, may have a significant etiological role.

Abomasal ulcers, erosions, and abomasitis with or without bloat have been reported in neonatal and weanling calves in association with many suspected etiologies. Theories proposed to explain the pathogenesis include dietary changes, in particular the addition of coarse roughage feeds and associated traumatic injury to the mucosa,[1,2] pica secondary to chronic enteritis[1], abomasal bezoars,[1,2-4] environmental or physical stress,[1,5-7], hyperacidity,[8] vitamin E deficiency,[5] lactic acidosis,[9] mycotic infection,[10,11] and low immune status associated with copper deficiency.[6,12,13] Various microorganisms have been isolated from affected abomasal tissues in neonatal calves including Escherichia coli, Pseudomonas,

Proteus, Streptococcus, Staphylococcus, Salmonella, and Clostridium perfringens, and Absidia and Mucor associated with Phycomycoses.[10,11,14-17] Most of these were considered to be postmortem invaders from the alimentary tract or secondary opportunistic infections occurring after abomasal traumatic injury,[10] stress,[5-7] or associated with copper deficiency.[6,12,13,18]

The cause of sudden death involving 10 Holstein replacement and feeder calves, 6-8 weeks of age, was hypothesized to be caused by C perfringens type A because no other pathogen was isolated.[16] However, this was not considered to be an enterotoxemia because high concentration of exotoxin was not found, and there was no intravascular hemolysis, or lesions in the mucosal surfaces of the gastrointestinal tract. C. perfringens was isolated from 12 cases and E coli from 3 cases of perforating abomasal ulcers and abomasal tympany in range calves, 1-12 weeks of age.[14] Reports from practitioners in central Nebraska and Wyoming indicated a reduced incidence in abomasal ulcers and tympanitis in herds vaccinated against C perfringens types C and D[14,18] and herds in which cows were vaccinated against E coli.[6,13] Use of injectable copper compounds for brood cows in certain herds experiencing annual ulcer problems in calves, in which low copper levels were diagnosed, was reported to eliminate abomasal ulcers.[6,12,13] In this report, we describe the diagnosis of an acute abdominal syndrome in neonatal calves characterized by ruminal and abomasal tympany, abomasitis, and abomasal ulceration, and the isolation of Clostridium perfringens from ingesta or lesions in calves with normal copper levels.

Materials and Methods

Animal History - Neonatal calves, referred to KSU College of Vet. Med. from Jan. 1, 1985 - July 31, 1985, were included in the study if they presented with an acute abdominal syndrome characterized by abomasal and ruminal tympany, abomasitis, or abomasal ulceration after acute onset of abdominal tympany, colic, depression, or death. Owner history, obtained for each calf admitted for clinical evaluation or for necropsy, age, and clinical signs were used as criteria for including a calf in this study. Eight calves 2-21 days of age were included. All were from within a 60 mile radius of the KSU campus.

Some of these calves were noted to have had diarrhea within a few days after birth from which they recovered, but they suddenly became ill in 1-2 weeks. Two calves were observed to be doing fine in the evening and found dead the following morning. Owners reported calves to be kicking at their abdomens, rolling or throwing themselves down, acting depressed, and to be exhibiting various degrees of bloat and odontoprisis. Two of the 6 calves presented for clinical examination were reported to have had pasty, yellowish diarrhea for 2 days before the onset of the abdominal syndrome. Two of the six had been treated with various antibiotics and for relief of the abdominal tympany. These animals came from herds in which other calves of similar age had been scouring in the past 1 or 2 weeks. Four of the affected calves came from herds having vaccination programs for brood cows, which included E coli, Corona and Rotavirus vaccine, and 7-way Clostridium; the other 4 calves came from herds with no vaccination program for brood cows. Three of the calves were of dairy breeds and all others were beef calves.

Clinical Signs - Clinical signs of acute abdominal syndrome in neonatal calves were recorded for cases as they became available.

Examination of gut contents for C perfringens exotoxins - Ruminal samples were obtained from 6 live calves via sterile nasogastric tube for anaerobic culture. Contents of the rumen and abomasum were collected from calves presented for necropsy, and any lesions observed in these organs were cultured for anaerobic bacteria. Ruminal or abomasal contents were clarified by centrifugation at 12,750 x g for 20 minutes, and the supernatant was filtered through a 0.45 μm membrane filter[a], and injected intraperitoneally into mice to test for exotoxin.[19]

Isolation of C perfringens - All samples were initially cultured anaerobicially in chopped meat carbohydrate (CMC) - starch broth.[20,21] After 4 to 6 hours of anaerobic incubation at 45°C, cultures were streaked onto blood agar and incubated anaerobically for 24 hours at 37°C. Bacterial colonies tentatively identified as C perfringens, based on their double zone hemolysis on the surface of blood agar medium, were picked and subcultured in anaerobic BHI broth. Species confirmation was based on gram stain, lack of motility, lecithinase reaction on egg yolk agar, fermentation products profile in anaerobic peptone-yeast extract-glucose broth, and other standard biochemical tests.[22-23] The standard Kirby-Bauer antimicrobial susceptibility testing was conducted for all the isolates of C perfringens.

Typing of C perfringens-Isolates of C perfringens were typed based on toxin-antitoxin neutralization tests in mice. Colonies of C perfringens grown on blood agar were transferred to CMC-starch broth and incubated anaerobically at

37°C for 4 to 6 hours to obtain optimum toxin production during the logarithmic phase of growth.[20,21] Cultures were then refrigerated for 3 hours at 4°C, centrifuged at 12,750xg for 10 minutes, and pH was adjusted to 6.8-7.2 with 10 N NaOH. The supernatant from each culture was divided into two aliquots and one aliquot was incubated at 37°C for 45 minutes after mixing with 0.1% trypsin.[b] Both aliquots were held at room temperature (25°C) for 30 minutes after the addition of type specific antitoxin. Swiss-Webster (ICR strain) mice weighing 20-30 g were injected intraperitoneally with 0.5 ml of each sample. Control mice received 0.5 ml of horse serum (0.2 ml) combined with plain or trypsinized clinical isolate supernatant (1.0 ml).[20] Mice tested for C perfringens type C received cell-free culture filtrate, plain or trypsinized, filtered into sterile vials after passage through 0.45 µm membrane filters.[a] All mice were observed for 3 days to identify specific neutralization of the toxin by monovalent antitoxins. Fifty mice were used in antitoxin-toxin neutralization testing for each clinical isolate of C perfringens. Also known strains[c] of C perfringens types A, C, D, and E were included in the testing as postitive controls.

Clinical Pathology - Venous blood collected via the jugular vein from live calves was evaluated for routine hemogram and analyzed for acid-base, blood gases, serum chemistry, and serum copper concentration. Blood pH, Po_2, Pco_2 bicarbonate, base excess, and CO_2 concentration were determined with a pH/blood gas analyzer.[d] A semiautomatic analyzer[e] was used to measure serum creatinine, glucose, inorganic phosphate, calcium, albumin, total protein, chloride, alkaline phosphatase, urea nitrogen, potassium, and sodium. Serum sorbitol dehydrogenase (SDH) activity was measured by the UV kinetic method.[f] Serum and liver tissue samples collected at necropsy were analyzed for copper concentration by atomic

spectrochemical analysis, utilizing quantititive plasma atomic emission-direct current plasma technique.[g]

Pathology - Gross and histopathologic examinations were done on all calves admitted for necropsy or that died after being hospitalized for clinical evaluation and treatment. A complete examination of the gastrointestinal tract was done for evidence of erosions, ulcers, congestion, hyperemia, discoloration, or gas accumulation. Samples were collected at necropsy for bacteriology and clinical pathology, as previously described. Histopathology was performed on all tissues with lesions collected at necropsy.

Results

Eight calves, 2-21 days of age, 5 of beef breeds and the remainder of dairy breeds, had clinical signs of acute abdominal syndrome - acute onset of abdominal tympany, colic, depression, or death (Table 1). Two calves (257, 523) were presented for necropsy after sudden death. Calves 755 and 313, which were admitted for medical therapy, one in a moribund condition (313), died shortly after treatment was initiated. A fifth calf (69) admitted for medical treatment, which included antimicrobial therapy, balanced electrolyte replacement with fluid therapy, nursing care, and surgical exploration of the abdomen and abomasum, died after 11 days. Three calves (206, 447, 495) were released after hospitalization and medical treatment. Calves 206 and 495 completely recovered after 2-6 days of medical care, and were reported to be doing well by their owners one month after discharge from the hospital. Calf 447 was released to the owner who refused further therapy after the first day; this calf's final outcome was not ascertained.

TABLE 1 - Clinical signs, lesions, and isolation of Clostridium perfringens from neonatal calves with acute abdominal syndrome.

Calf Number	Breed	Age (Days)	Clinical signs/lesions*	Clostridium perfringens type†
69+	Beef	16	Depression, bloat, ulceration (3+)	A
206	Dairy	21	Depression, bloat, diarrhea	A
257+	Beef	6	Sudden death, bloat, ulceration (4+)	A
313+	Beef	4	Depression, bloat, diarrhea, ulceraton (1+)	E
447	Beef	21	Depression, bloat	A
495	Beef	14	Depression, bloat, diarrhea	A
523+	Dairy	2	Sudden death, bloat, ulceration (1+)	A
755+	Dairy	11	Depression, bloat, diarrhea, ulceration (3+)	A

*Degree of abomasal ulceration or erosion at necropsy, graded from mild (1+) to perforate (4+).

†C. perfringens isolated from ruminal or abomasal content or from abomasal lesions.

+Calf presented for necropsy or died after admission for medical care.

Clostridium perfringens was isolated from all samples of ruminal content collected by nasogastric tube from live calves and in samples of ruminal and abomasal content collected from calves at necropsy (Table 2). Intraperitoneal injection of clarified samples caused death of mice within 1-3 days, indicating the presence of lethal exotoxin. Based on toxin-antitoxin neutralization tests in mice, seven isolates were C perfringens type A and one was type E (Table 1). Antibiogram testing of C perfringens clinical isolates from the rumen and/or abomasal content indicated susceptibility to penicillin, ampicillin, amoxicillin, methacillin, cephalosporin, nitrofurazone furazolidone, amikacin, tetracyclines, lincomycin and erythromycin, but resistance to gentamicin, neomycin, kanamycin, streptomycin, trimethoprim-sulfamethoxazole, triple sulfa, and polymyxinß.

Routine hematology, blood gas, and serum chemistry revealed no consistent changes (Table 3). However, 3 of 6 calves had systemic acidosis as evidenced by low blood pH, low bicarbonate, and base deficits. Serum and liver copper concentrations from affected calves were within normal limits (Table 4).[28-31]

Histopathology from calves presented for necrospy or that died after initiation of therapy, showed varying degrees of abomasitis, abomasal erosions, and abomasal ulceration (Fig 1). The abomasum was most often affected in the fundic region and lesions were either diffuse or focal. Perforate ulcers were either circular or linear, and in some histopathologic sections, scattered large colonies of bacilli were seen within the submucosa and muscularis along with a dense infiltration by neutrophils and macrophages. Submucosal edema with "coffee ground" hemorrhages on the mucosa, lymphatic channels dilatation, and various degrees of inflammatory cell infiltration were consistent findings in all calves (Fig 2).

TABLE 2 - Biochemical characteristics of clinical isolates of Clostridium perfringens.

Characteristic	Clinical isolate number								ATCC strain*	Uninoculated medium
	69	206	257	313	447	495	523	755		
Morphology†	G + rod	G + rod	G + rod	G + rod	G + rod	G + rod	G + rod	G + rod	G + rod	---
Hemolysis on blood agar	double zone	double zone	double zone	double zone	double zone	double zone	double zone	double zone	double zone	---
Lecithinase	+	+	+	+	+	+	+	+	+	---
Motility	-	-	-	-	-	-	-	-	-	---
Indole production	-	-	-	-	-	-	-	-	-	---
Gelatin liquefaction	+	+	+	+	+	+	+W	+	+	---
Chopped meat digestion	+	+	+	+	+	+	+	+	+	---
Fermentation products ‡	A,p,B	A,p,B	A,p,B	A,p,B	A,p,B	A,p,B	A,p,B	A,p,B	A,p,B	---
Esculin hydrolysis	+	+	+	+	+	+	+	+	+	---
Starch hydrolysis	+	+	+	+	+	+	+	+	+	---
SUGAR FERMENTATION										
Peptone yeast (PY) extract (Final pH)ᵩ	6.30	5.95	5.91	5.82	5.58	5.98	5.84	5.79	5.75	7.15
PY-fructose	+ (4.76)	+ (4.59)	+ (4.51)	+ (4.54)	+ (4.55)	+ (4.56)	+ (4.64)	+ (4.47)	+ (4.39)	(6.63)
PY-glucose	+ (4.76)	+ (4.52)	+ (4.58)	+ (4.54)	+ (4.58)	+ (4.46)	+W (4.69)	+ (4.47)	+ (4.47)	(6.92)
PY-lactose	+ (4.82)	+ (4.66)	+ (4.57)	+ (4.57)	+ (4.55)	+ (4.71)	+W (5.60)	+ (4.73)	+ (4.44)	(6.77)
PY-maltose	+ (4.71)	+ (4.56)	+ (4.55)	+ (4.57)	+ (4.59)	+ (4.60)	+ (4.55)	+ (4.51)	+ (4.52)	(6.81)
PY-mannose	+ (4.87)	+ (4.54)	+ (4.60)	+ (4.54)	+ (4.58)	+ (4.55)	+ (4.63)	+ (4.55)	+ (4.44)	(6.79)
PY-mannitol	- (5.72)	- (5.75)	- (5.82)	- (5.61)	- (5.98)	- (5.91)	- (5.70)	- (5.85)	- (5.58)	(7.15)
PY-melibiose	- (5.83)	- (5.76)	- (5.63)	- (5.67)	- (5.89)	- (5.72)	- (5.58)	- (5.68)	- (5.51)	(6.92)
PY-ribose	+ (5.12)	+ (4.80)	+ (4.93)	+ (4.87)	+ (4.77)	+ (4.77)	+ (4.83)	+ (4.70)	+ (4.87)	(6.57)
PY-starch	+ (4.75)	+ (4.56)	+ (4.63)	+ (4.59)	+ (4.60)	+ (4.57)	+ (4.57)	+ (4.50)	+ (4.59)	(6.98)
PY-sucrose	+W (4.73)	+W (4.58)	+W (4.55)	+W (5.32)	+W (4.60)	+W (4.60)	+W (4.59)	+W (4.49)	+W (4.47)	(7.07)
PY-xylose	+W (5.35)	+W (5.49)	+W (5.25)	+W (5.23)	+W (5.32)	+W (5.37)	+W (5.12)	+W (5.25)	+W (5.45)	(6.28)

*American type culture collection no. 12916.
†No spores were detected in gram stained smear.
+W = weakly positive.
‡ Grown in peptone-yeast extract-glucose broth, A = acetic acid (> 30 mM), p = propionic acid (< 2 mM), B = butyric acid (> 10 mM).
ᵩ Numbers in parentheses indicate final pH.

TABLE 3 - Serum chemical, hemogram findings, and blood gas analysis in neonatal calves with an acute abdominal syndrome.

Measurement	Calf no. 69	206	313	447	495	755	Normal values[24-27]
SERUM CHEMICAL VALUES							
Glucose (mg/dl)	80.0	105.0	75.0	84.0	99.0	83.0	60 to 150
Urea nitrogen (mg/dl)	70.0	13.0	110.0	20.0	13.0	49.0	10 to 40
Creatinine (mg/dl)	2.5	1.1	3.5	1.8	1.1	2.0	1.0 to 2.0
Sodium (mEq/l)	149.0	149.0	131.0	139.0	131.0	106.0	132 to 152
Potassium (mEq/l)	6.0	3.4	7.0	7.4	7.7	7.2	3.9 to 6.9
Chloride (mEq/l)	115.0	109.0	108.0	100.0	101.0	74.0	94 to 112
Calcium (mg/dl)	10.4	8.4	7.9	9.2	11.5	8.9	9.7 to 12.4
Inorganic phosphate (mg/dl)	9.2	4.8	11.8	12.9	---[a]	5.7	4.0 to 7.0
Total protein (g/dl)	9.2	4.3	5.2	6.8	7.2	5.3	5.0 to 6.5
Alkaline phospatase (I.U.)	125.0	163.0	416.0	427.0	168.0	152.0	0 to 488
Total CO_2 (mEq/l)	21.0	27.0	8.0	17.0	18.0	19.0	21.2 to 32.2
Albumin (g/dl)	3.2	2.7	2.9	3.0	3.2	3.1	3.03 to 3.55
Sorbitol dehydrogenase (I.U.)	10.3	26.0	17.3	9.0	12.5	10.8	4.3 to 15.3
HEMOGRAM VALUES							
Packed cell volume (%)	50.5	31.7	28.9	41.1	30.0	26.7	35.9 $\pm$ 3.8
Total plasma protein (g/dl)	9.7	4.7	7.0	7.0	7.4	5.9	5.5 to 7.5
Fibrinogen (mg/dl)	150.0	300.0	1000.0	400.0	900.0	900.0	500 to 700
Hemoglobin (g/dl)	17.0	11.6	9.5	12.5	10.6	9.3	11.2 $\pm$ 2.5
White blood cells ($10^3/\mu l$)	21.0	18.0	11.9	16.8	19.1	23.5	10.7 $\pm$ 3.1
Platelet	Normal	Decreased	Normal	Normal	Normal	Normal	500 (200-800)$10^3/\mu l$
Poikilocytes	---	---	4+	---	2+	---	---
Anisocytosis	---	---	3+	1+	2+	---	---
Spherocytes	---	---	---	---	Few	---	0
Toxic Granulocytes	---	2+	---	2+	---	1+	0
ACID-BASE AND BLOOD GASES							
pH	7.239	7.369	6.904	7.245	ND	7.344	7.35 $\pm$ 0.05
PCO_2 (mm of Hg)	50.5	65.6	49.0	50.2	ND	41.5	42.8 $\pm$ 3.28
PO_2 (mm of Hg)	26.8	28.3	18.0	23.8	ND	25.0	---
Base excess (mEq/l)	-5.3	+1.9	-21.0	-4.8	ND	-1.9	0 to +3
Bicarbonate(mEq/l)	20.8	28.9	9.7	21.0	ND	21.8	20 to 30
Total CO_2 (mEq/l)	22.4	30.9	10.2	22.5	ND	23.1	22 to 32

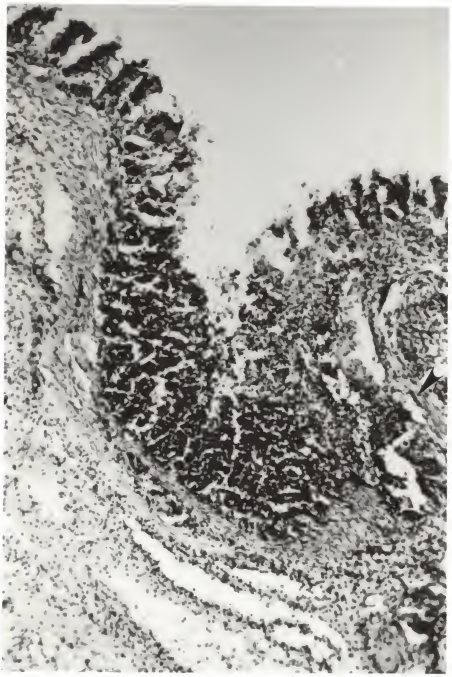
[a]Too hemolyzed.
ND = not determined.

TABLE 4 - Serum and liver copper concentrations in calves with acute neonatal abdominal syndrome.

Calf No.	Serum (µg/ml)	Liver (µg/g)
69	---	120.0
206	0.91	---
257	---	160.0
313	1.10	---
487	1.10	---
495	3.20	---
523	---	140.0
755	3.00	---
Normal values[28-31]	0.60 to 3.20	40.0 - 250.0
Toxic values[29-30]	>3.30	>300.0
Deficient values[28-31]	<0.60	<37.0

Fig 1 - Gross appearance of the abomasal mucosa from calf no. 755. Arrow indicates area of extensive submucosal ulceration. Note the scattered coffee ground hemorrhage over the surface with multiple abomasal erosions.

Fig 2 - Photomicrograph of abomasal ulcer from calf 755. Arrows indicate disruption of the muscularis mucosa with mucosal necrosis and inflammatory cell infiltration. The edematous submucosa also reveals a microthrombus in a lymphatic and lymphatic dilatation. H & E stain, X200.



Discussion

Clostridium perfringens has been implicated as a major cause of gas gangrene and food poisoning in man and in enteric infections and wound sepsis of animals.[1,32] Certain fatal enterotoxemias in animals have been attributed to specific antigenic types.[1,32] C perfringens produces four major lethal toxins (Table 5), which were the basis for defining 5 types (A to E).[32,33] In addition to production of four major lethal exotoxins, each type also produces other extracellular substances such as enterotoxin, collagenase, hyaluronidase, and deoxyribonuclease, which also contribute to its pathogenic behavior.[32,34] Transmission is either by introduction into a wound or by ingestion. Enterotoxemia, particularly with types B and C, is thought to be acquired by young animals from carrier adults.[20] Type D enterotoxemia is associated with dietary change, e.g., passage of large quantities of starch into the duodenum when animals overeat or sudden changes from a roughage to a high grain diet, and intestinal stasis.[1,32,34] C perfringens type A is the primary cause of gas gangrene[35], and enterotoxin formed by certain strains is responsible for food poisoning in man.[20,32] Type A strains have occasionally been implicated in an enterotoxemic condition of nursing lambs, alpacas, and kid goats characterized by icterus, hemoglobinuria, anemia, and intravascular hemolysis,[20,36] but this has rarely been confirmed in calves.[32,36] In calves diagnosed with type A enterotoxemia, diarrhea and rapidly developing depression were the main symptoms, but no characteristic gastrointestinal lesions were reported.[37,38] Type E is believed to rarely cause a form of enterotoxemia in calves and rabbits characterized by profuse diarrhea and death.[32]

C perfringens enteric infections are believed to begin either as an opportunistic commensal of the intestinal tract where certain conditions have

TABLE 5 - Major lethal exotoxins of Clostridium perfringens types.

Type	Exotoxins			
	alpha	Beta	Epsilon	Iota
A	++	-	-	-
B*	+	++	+	-
C	+	++	-	-
D	+	-	++	-
E	+	-	-	++

*Not reported in North America.
++ = Produced as predominant toxic fraction.
+ = Produced in lesser quantities.
- = Not produced.

allowed overgrowth and production of toxins, or as an ingested fecal contaminant in feeds that have escaped gastric degradation to proliferate in the intestinal tract.[20,32] Toxigenic C perfringens strains, most frequently type A, have been isolated from feces, the intestinal tract, and from multiple sites in cadavers (explained as postmortem proliferation in tissues and effusions).[20,32,36-39] Isolation of C perfringens from necropsy specimens, either untyped or type A, therefore, has usually been discounted due to its innocuous prevalence.[32,36-39] Examination of gut contents taken at slaughter from adult cattle on an "all-grain" diet have indicated the presence of C perfringens in ruminal, abomasal, ileal, and colon contents, with the primary site of isolation being the ileum.[39] No toxigenic strains were found to inhabit the abomasum in that study, and the presence of C perfringens in the upper alimentary tract may have been due to pyloric sphincter relaxation with subsequent mixing of intestinal content with that in the abomasum and forestomachs after death. In experimental inoculation of C perfringens type D into the rumen of sheep, it was shown that at least 90% of the inoculum was destroyed in the adult rumen with the mean half-life of the bacteria being 1.6 hours in the rumen.[40] The recovery of C perfringens Type D from the duodenum (by duodenal cannulae) in those sheep inoculated intraruminally was much smaller than that from the rumen, suggesting that considerable numbers of organisms also must have been destroyed during the passage from rumen to mid-duodenum.[40]

Studies of the microflora in the rumen of healthy and bloating calves, 4-135 days of age, indicated that C perfringens is rarely isolated.[41] The distribution of C perfringens and E coli in the feces of scouring and healthy calves was studied in herds experiencing neonatal enteritis in Montana.[42] Three calves with scours and one with no diarrhea were necropsied in that study. C perfringens was isolated from the abomasum and upper small intestines in calves with diarrhea but a few

organisms were also found in the abomasum in the calf with no diarrhea. These findings concur that C perfringens is not normally present in the rumen and abomasum of neonatal calves or adult ruminants, but may be isolated in herds experiencing neonatal enteritis. Under normal circumstances, vegetative cells of C perfringens do not survive in the abomasum.

Clostridium perfringens type A has been isolated with other bacteria from abosmasal ulcers and abomasitis in mature cattle.[43] In that case, C perfringens type A appeared to be associated with inflammatory lesions, capillary dilatation, and cell shedding in the mucosa. Perforating abomasal ulcers in suckling calves (4-6 weeks of age) have been associated with C perfringens, E coli, Streptococcus, and Staphylococcus aureus.[15] Those ulcers were in the midpart of the fundus, and on the greater curvature of the abomasum; several calves had abomasal trichobezoars, which along with other stressors were thought to have contributed to the development of the ulcers. The incidence and pathology of abomasal ulcers in range calves exhibiting abomasal tympany in Nebraska and Wyoming was studied, and correlated with copper deficiency and secondary bacterial infection.[12-14,18] Bacterial isolates from abomasal content or lesions in those calves were believed to have gained access to the upper alimentary tract via reflux action of duodenal contents. The bile salts, acids, lysolecithins, potential bacterial toxins, and sepsis in the refluxed material were thought to damage the surface gastric cells, causing mucosal ischemia, inducing intravascular coagulation with microthrombotic occlusions of the mucosal vessels, and making the cells susceptible to erosion or ulceration. Low copper levels in the calves were hypothesized to cause derangement of elastin cross linkages, thereby compromising the integrity of the abomasal mucosa and its microvasculature, and to cause decreased leukocyte cytochrome oxidase activity, contributing to decreased neutrophil function and

increased susceptibility to infection. C perfringens was isolated from abomasal content and lesions in these studies alone or with other bacteria, but was considered to be an opportunistic invader associated with engorgement on rich milk after a period of anorexia during weather stress and immunological compromise associated with copper deficiency. Toxin neutralization testing in mice to determine if preformed lethal toxin was present with isolates of C perfringens was not done in those cases.

Calves in our study had normal serum and tissue copper concentration, had no history of weather related stress, and C perfringens, primarily Type A, was consistently isolated from ruminal and abomasal contents or abomasal lesions. Antemortem isolation of toxigenic C perfringens was also demonstrated in calves sampled by sterile nasogastric tube. We suspect that pathophysiology associated with suckling may play a significant role in the development of the syndrome. Possibly these calves had esophageal groove dysfunction which allowed abnormal amounts of milk to consistently enter the neonatal rumen, thus, providing substrate and an anaerobic environment for ingested C perfringens to proliferate. This abnormal fermentation could allow large numbers of C perfringens to survive, enter the abomasum, and colonize the abomasal mucosa creating areas of abomasitis. In man, certain Campylobacter sp., microaerophilc to anaerobic gram negative bacilli, have been found on gastric epithelia or within ulcerated tissues in almost all patients with active chronic gastritis, duodenal ulcer, or gastric ulceration,[44] and have also been reported in gastric ulcers of ferrets.[45] Gastritis associated with pernicious anemia, immunologically mediated in man and affecting the proximal stomach, has rarely been associated with Campylobacter-like organisms. This rarity is considered to indicate that the organisms probably do not develop secondary to gastritis and provides indirect evidence of those organisms as causative agents in

chronic active gastritis.[45]

The calves in the present study with a history of sudden death were often described by owners as being top calves compared with the rest of the herd, and from heavy milking dams. Calves being bottle fed were usually noted to be vigorous nursers. Possibly this behavior could contribute to spill-over of milk into the neonatal rumen. Certainly calves with a history of diarrhea may have been somewhat immune compromised, but in our clinical experience most calves appear to be healthy and normal up until the onset of this syndrome. Copper deficiency was not found in the calves we studied, but may be a contributing factor in regions having molybdenum excess associated copper deficiency or primary copper deficient soils. The action of C perfringens bacteria and its toxins appeared to be responsible for the tympany (C perfringens is a prolific gas forming organism), abomasitis, and abomasal ulceration seen in these calves. The reported decreased incidence of abomasal tympany and ulceration in neonatal calves after instituting C perfringens vaccination programs in herds having a history of these problems[6,13,14,18] supports our theory that this organism may be the primary etiologic agent in this syndrome.

Anaerobic incubation of abomasal contents or samples from abomasal lesions in CMC-starch at 45°C initially, followed by further incubation at 37°C after transfer to other media, enhances isolation of C perfringens and inhibits other bacterial growth. This procedure helps to isolate C perfringens in almost pure culture and prevents its being lost or obscured by other bacteria, which may overgrow it on certain media. This technique allowed us to consistently isolate C perfringens from abomasal ulcers and lesions. Isolation of toxigenic C perfringens from the ruminal contents of live calves presented with this syndrome establishes the presence as significant, and also indicates its serious implications in sudden

death of calves. Further investigations of calves with abomasal ulcers, abomasitis, and acute tympany may reveal other contributing factors to this syndrome, but we believe C perfringens should not be ignored as a serious pathogen in this syndrome, and indeed, may be the primary etiologic agent involved in its pathophysiology.

References

1. Blood DC, Henderson JA, Radostits OM, et al. Diseases of the alimentary tract II. In: Blood DC, Henderson JA, et al, eds, Veterinary medicine: A textbook of the disease of cattle, sheep, pigs, and horses, 5th ed. Philadelphia: Lea and Febiger, 1979; 197-199, 452-458.
2. Wood DR. Diet in calf scouring. Vet Rec 1968; 82:269.
3. O'Brien JDP. Ulceration of the abomasum associated with calf scour and diet texture. Vet Rec 1968; 82:363.
4. Wood DR. Ulceration of the abomasum associated with calf scour and diet texture. Vet Rec 1968; 82:446.
5. Smith HA, Jones TC, Hunt RD. The digestive system, In: Smith HA, Jones TC, Hunt RD, ed. Veterinary pathology, 4th ed. Philadelphia: Lea and Febiger, 1972; 1204.
6. Johnson JL, Lilley CW, Hamar DW, et al. Diagnostic observations of abomasal tympany in range calves, in Proceedings. 3rd Int Symp World Assoc Vet Lab Diag, Vol 2, Ames, Iowa; 1983; 485-491.
7. Singh GB, Sharma JN, Kar K. Pathogenesis of gastric ulceration produced under stress. J Path Bact 1967; 94:375-380.
8. Tasker JB, Roberts SJ, Fox FH, et al. Abomasal ulcers in cattle - recovery of one cow after surgery. J Am Vet Med Assoc 1958; 133:365-368.
9. Jensen, R, Pierson RE, Braddy PM, et al. Fatal abomasal ulcers in yearling feedlot cattle. J Am Vet Med Assoc 1976; 169:524-526.
10. Gitter M, Austwick PKC. The presence of fungi in abomasal ulcers of young calves: A report of seven cases. Vet Rec 1957; 69:924-928.

11. Wray C, Thomlinson JR. Abomasal ulceration in calves. Vet Rec 1968; 83:80-81.

12. Lilly CW, Hamar DW, Johnson JL, et al. Factors associated with abomasal ulcers in beef calves, in Proceedings. 87th Ann Conv, Nebraska Vet Med Assoc, 1984; 70-77.

13. Lilly CW, Hamar DW, Johnson JL, et al. The association of copper and colon bacteria with abomasal ulcers in beef calves, in Proceedings. 87th Ann Conv, Nebraska Vet Med Assoc., 1984; 4-11.

14. Johnson JL, Hudson DB, Bohlender RE. Perforating abomasal ulcers and abomasal tympany in range calves. in Proceedings. 24th Am Assoc Vet Lab Diag 1981; 203-210.

15. Tulleners EP, Hamilton GF. Surgical resection of perforated abomasal ulcers in calves. Can Vet J 1980; 21:267-264.

16. Berkhoff GA, Braun RK, Buergelt CD, et al. Clostridium perfringens type A associated with sudden death of replacement and feeder calves, in Proceedings. 23rd Am Assoc Vet Lab Diag 1980; 45-52.

17. Waas W. Abomasal ulcers. Mod Vet Pract 1986; 67:111-112.

18. Anonymous. Abomasal ulcers. Agr Pract 1985; 6:33.

19. Sterne M, Batty I. Examination of specimens: procedures. In: Sterne M, Batty I, eds. Pathogenic clostridia, 1st ed. Boston: Butterworths, 1975; 72-84.

20. Smith LDS, Williams BL. The pathogenic anaerobic bacteria, 3rd ed. Springfield, Illinois: Charles C. Thomas Publishing Co. 1984; 94-136.

21. Holdeman LV, Cato EP, Moore WEC. Anaerobe laboratory manual, 4th ed., Blacksburg, VA: Virginia Polytechnic Institute and State University, 1977; 141-145.

22. Smith LDS, Hobbs J. Genus III Clostridium, In: Buchanan RE, Gibbons NE, eds. Bergey's Manual of Determinative Bacteriology. Baltimore, Md: Williams and Wilkins Co. 1974; 551-572.

23. Sutter VL, Washington II JA. Susceptibility testing of anaerobes. In: Lennette EH, Spaulding EH, Truana JP, eds. Manual of Clinical Microbiology, 2nd ed. Washington, D.C: American Society of Microbiology, 1974; 436-438.

24. Kaneko JJ. Clinical biochemistry of domestic animals 3rd ed. San Francisco: Academic Press, 1980; 792-795.

25. Rosenberger G. Clinical examination of cattle 1st ed. Philadelphia: W.B. Saunders Co. 1979; 126-145.

26. Schalm OW, Jain NC, Carrol EJ. Veterinary hematology. 3rd ed. Philadelphia: Lea and Febiger, 1975, 122-144.

27. Coles EH. Veterinary clinical pathology. 3rd ed Philadelphia: W.B. Saunders CO., 1980; 18, 298-299, 317, 320.

28. Claypool DW, Adams FW, Pendell HW, et al. Relationship between the level of copper in the blood plasma and liver of cattle. J Anim Sci 1975; 41:911-914.

29. Underwood EJ. Trace mineral elements in human and animal nutrition. 4th ed. New York: Academic Press, 1977.

30. Bulgin MS, Maas J, Anderson BC, et al. Death associated with parenteral administration of copper disodium edetate in calves. J Am Vet Med Assoc 1986; 188:406-409.

31. Matrone G, Shields GS, Olwin JH, et al. Copper deficiency and toxicity: Acquired and inherited in plants, animals, and man. Parkridge, NJ: Noyer Publication, 1971; 11, 94.

32. Niilo L. Clostridium perfringens in animal disease: A review of current knowledge. Can Vet J 1980; 21:141-148.

33. Carter GR. Diagnostic procedures in veterinary bacteriology and mycology. 3rd ed. Springfield, Illinois: Charles C. Thomas, Publication, 1984; 178-188.

34. Kadis S, Montie TC, Ajl SJ. Bacterial protein toxins, Microbial Toxins. Vol IIA. New York: Academic Press, 1971; 109-158, 159-187.

35. Marasanova LP. The role of C perfringens of the A,B,C,D,E,F types in the etiology of gas gangrene. Zurnal Mikrobiologii Epidemiologii I Immunobiologii 1971; 48:116-121.

36. Jubb KVF, Kennedy PC, Palmer N. Pathology of Domestic Animals. 3rd ed, Vol 2. New York: Academic Press Inc. 1985; 56, 61-62, 84-85, 149-183.

37. Vance HN. Clostridium perfringens as a pathogen of cattle. A literature review. Can J Comp Med Vet Sci 1967; 31:248-250.

38. Niilo L. Diagnostic problems of diseases caused by Clostridium perfrigens, in Proceedings. 22nd Am Assoc Vet Lab Diag, 1979; 33-52.

39. Vance HN: A survey of the alimentary tract of cattle for Clostridium perfringens. Can J Comp Med Vet Sci 1967; 31:260-264.

40. Bullen JJ, Scarisbriek R, and Maddock A. Enterotoxemia of sheep: the fate of washed suspensions of Clostridium welchii type D introduced into the rumen of normal sheep. J Path Bact 1953; 65:209-219.

41. Jayne-Williams DJ. The Bacterial flora of the rumen of healthy and bloating calves. J Appl Bacteriol 1979; 47:271-284.

42. Lozano EA, Catlin JE, Hawkins WW: Incidence of Clostridium perfringens in neonatal enteritis of Montanta calves. Cornell Vet 1970; 60:347-359.

43. Al-Mashat R R, Taylor DJ. Bacteria in enteric lesions of cattle. Vet Rec 1983; 112:5-10.

44. O'Connor HJ, Axon ATR, Dixon MF. Campylobacter-like-organisms unusual in type A (pernicious anemia) gastritis. Lancet 1985; 1:111.

45. Fox JG, Edrise BM, Cabot EB, et al. Campylobacter-like organisms isolated from gastric mucosa of ferrets. Am J Vet Res 1986; 47:236-239.

Experimental reproduction of abdominal tympany, abomasitis and abomasal ulceration in neonatal calves with Clostridium perfringens type A

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[a]American Type Culture Collection, Rockeville, MD.

[b]Model 513 pH/blood gas analyzer, Instrumentation Laboratory Inc., Lexington, Mass.

[c]SMA 12/60, Technicon Instruments Corp., Tarrytown, N.Y.

[d]Technical Bulletin No. 50-UV, Sigma Chemical Co., St. Louis, MO.

[e]Beckman/Spectrametrics Corp., St. Louis, MO.

[f]Swinnex®-HA 0.45 µm filter unit, Millipore Corp., Bedford, Mass.

[g]1:250 Trypsin, Difco Laboratories Inc, Detroit, Mich.

SUMMARY

The role of Clostridum perfringens type A in the etiology of an acute abdominal syndrome in neonatal calves characterized by abomasal and rumen tympany, abomasitis, and abomasal ulceration was investigated. Eight calves, 4 to 12 days of age, were inoculated with C perfringens type A (ATCC toxigenic type A culture or toxigenic type A culture isolated from rumen or abomasal content in a clinical case with this syndrome). Blood samples were collected from all calves before and after inoculation for blood gas, serum chemistry, serum copper analysis, and ruminal fluid for isolation of C perfringens. Calves were monitored daily for clinical abnormalities, and were either sacrificed or redosed within 4-7 days depending on the severity of their clinical signs. Routine histopathology and anaerobic culture of the abomasum and rumen were performed after necropsy.

Intraruminal inoculation of C perfringens type A into healthy calves induced anorexia, depression, bloat, diarrhea, and death in some calves. Copper concentrations in serum were within normal range. Necropsy examination revealed varying degrees of abomasitis, petechial to ecchymotic hemorrhages, and pin point to nearly perforate ulcers in the abomasum. Seven of those calves also had multiple rumen trichobezoars. None of these findings were seen in control calves at necropsy. In these calves acute abdominal syndrome was unrelated to copper deficiency, and C perfringens type A given intraruminally was able to reproduce the clinical syndrome.

Acute abdominal tympany, colic, depression or sudden death have been reported in neonatal calves with abomasal ulcers, abomasitis, and varying degrees of abomasal and rumen tympany.[1-11] Many suspected etiologic agents have been

reported: dietary changes, in particular the addition of coarse roughage feeds and associated traumatic injury to the mucosa,[1,2] pica secondary to chronic enteritis,[1] abomasal bezoars,[1,2] environmental or physical stress associated with secretion of ACTH and endogenous steroid release,[1,3,12] mycotic infection,[4,5] and low immune status associated with copper deficiency.[3,6-8] Various microorganisms have been isolated from affected abomasal tissues, peritoneal fluid contaminated with ingesta from perforate abomasal ulcers, and abomasal fluid, including: Escherichia coli, Pseudomonas, Proteus, Streptococcus, Staphylococcus, Salmonella, and C perfringens, and mycoses associated with Absidia and Mucor.[4,5,9-12] With the exception of mycotic agents[4,5], all others have been considered to be either postmortem invaders from the alimentary tract or secondary opportunistic infections occurring after abomasal traumatic injury,[4] stress,[3,13] or associated with copper deficiency.[3,6-8,12]

C perfringens type A has been isolated from abomasal, duodenal, and ileal content in calves 6-8 weeks of age, which died after acute onset of abomasal and rumen tympany.[11] Because no other pathogenic bacteria was isolated from these calves, it was concluded that this organism was associated with the deaths. This organism has been isolated with other bacteria from abomasal ulcers and abomasitis in mature cattle.[14] C perfringens was also isolated from abomasal linings of 12 calves (aged 2 days to 8 weeks) presented for necropsy after having abomasal tympany, abomasal rupture, perforating abomasal ulcers (single or multiple), and duodenal ulcers.[9] Secondary findings in those calves included presence of sand, dirt, trichobezoars, and straw in the ingesta, which was a grayish-black fetid fluid. The purpose in the present study was to examine the role of C perfringens type A in the etiology of this acute abdominal syndrome in neonatal calves having normal serum copper levels, and to study the pathophysiology of the syndrome in calves.

Materials and Methods

Production of disease - Ten healthy bull calves, 4 to 12 days old, were obtained from local herds after having received colostrum their first three days of life. Calves were housed in a barn with controlled ventilation and were tethered indivdiually and kept on grooved rubber mats with access to hay, water, and calf pellets, but unable to contact other calves. Calves were fed whole milk at 8% of their body weight each day in divided feedings twice daily. Ruminal contents were collected via sterile nasogastric tube for anaerobic culture and pH determination. Calves were inoculated intraruminally with either sterile distilled water (2 control calves) or C perfringens type A culture (8 experimental calves). Calves were inoculated intraruminally 2 hours before their morning feeding with 20 hour old C perfringens type A culture grown in anaerobic BHI broth. Calves were given dosages in the range of 1×10^9 - 2×10^{10} CFU per dose. C perfringens strain 26 (ATCC 3624[a]) toxigenic type A culture or toxigenic type A culture isolated from rumen or abomasal content in a clinical case with this syndrome were used for intraruminal inoculation (Table 1). Depending on clinical signs, calves were either necropsied or redosed within 3-11 days after the initial intraruminal inoculation.

Clinical signs - Clinical signs, body temperature and manure consistency and color were recorded daily.

Clinical pathology - Blood was collected via the jugular vein from all calves for serum chemistry, routine hemogram, and for analyses of serum copper, acid-base and blood gases before and after experimental inoculation. Blood pH, PO_2, PCO_2, bicarbonate, base excess, and CO_2 concentration were determined with a pH/blood gas analyzer.[b] A semiautomatic analyzer[c] was used to measure

serum creatinine, glucose, inorganic phosphate, calcium, albumin, total protein, chloride, alkaline phosphatase, urea nitrogen, potassium, and sodium. Serum sorbitol dehydrogenase (SDH) activity was measured by the UV kinetic method.[d] Serum samples were analyzed for copper concentration by atomic spectrochemical analysis, utilizing quantitative plasma atomic emission - direct current plasma technique.[e] Blood gas analysis was done daily, and serum chemistry analysis was done on day 0, 4, 7, and 10 after inoculation.

Pathology - Gross and histopathologic examination were done on all calves which were necropsied either after euthanasia or death. All calves were necropsied within 15-80 minutes after death. A complete examination of the gastrointestinal tract was done for evidence of erosions, ulcers, congestion, hyperemia, discoloration, or gas accumulation. Ruminal and abomasal contents, and any lesions in the abomasum were collected for cultural examination. Histopathology was performed on all lesions detected at necropsy, and sections of heart, liver, lung, lymph node, rumen, abomasum, small intestine, large intestine, kidney, and pancreas were examined.

Examination of gut contents for C perfringens exotoxins - Ruminal and abomasal contents collected at necropsy were examined for exotoxin. Briefly, ruminal or abomasal contents were clarified by centrifugation at 5,000 x g for 20 minutes, and the supernatant was filtered through a 0.45 µm membrane filter[f] if adequate clearing had not occurred (water clear), and injected intraperitoneally into mice to test for exotoxin.[15]

Isolation of C perfringens - All samples were cultured anaerobially in chopped meat carbohydrate (CMC) - starch broth.[16,17] After 4 to 6 hours of anaerobic incubation at 45°C, cultures were streaked onto blood agar and egg yolk agar and incubated anaerobically for 24 hours at 37°C. Bacterial colonies

tentatively identified as C perfringens, based on their double zone hemolysis on the surface of blood agar medium and their lecithinase reaction on egg yolk agar, were picked and subcultured in anaerobic BHI broth. Species confirmation was based on gram stain, lack of motility, lecithinase reaction on egg yolk agar, fermentation products profile in anaerobic peptone-yeast extract-glucose broth and other standard biochemical tests.[18,19]

Typing of C perfringens - Isolates of C perfringens were typed based on toxin-antitoxin neutralization tests in mice. Colonies of C perfringens grown on blood agar were transferred to CMC-starch broth and incubated anaerobically at 37°C for 4 to 6 hours.[16,17] Cultures were then refrigerated for 3 hours at 4°C, centrifuged at 12,750 x g for 10 min, and pH adjusted to 6.8 - 7.2 with 10 N NaOH. The supernatant from each culture was divided into two aliquots and one aliquot was incubated at 37°C for 45 minutes after mixing with 0.1% trypsin.[g] Both aliquots were held at room temperature (25°C) for 30 minutes after the addition of type specific antitoxin. Swiss-Webster ICR albrino mice, weighing 20-30 g were injected intraperitoneally with 0.5 ml of each sample. Control mice received 0.5 ml of horse serum (0.2 ml) combined with plain or trypsinized culture supernatant (1.0 ml).[16] Mice tested for C perfringens type C received cell-free culture filtrate, plain or trypsinized, filtered into sterile vials after passage through 0.45 µm membrane filters.[f] All mice were observed for 3 days to identify specific neutralization of the toxin by monovalent antitoxins. Known strains[a] of C perfringens types A, C, D and E were included in the testing as positive controls.

Results

Production of disease - Calves were placed into one of three groups based on the severity of clinical signs and characteristics of lesions found at necropsy (Table 1). All experimental calves had episodes of depression, bloating, and diarrhea. Two calves died (X-97, X-99) after 3 and 7 days postinoculation, respectively.

Clinical pathology - Blood gas analysis revealed varying degrees of acidosis (base deficit) during the first 6 days of postinoculation (Table 2). Serum chemical values showed no consistent changes in calves through day 10 postinoculation (Table 3). Serum copper concentration was also unaffected (Table 4). Because the blood gas and serum chemical values were not affected by the severity of clinical signs and lesions observed, values of all 8 calves were averaged.

Gross and histopathology - Consistent gross findings in experimental calves included diffuse to patchy hyperemia, particularly on the edges of abomasal folds, the abomasal folds often appeared to be edematous. Foci of intense hyperemia surrounded areas of erosion and ulceration (Fig. 1). Two calves had erosive and hyperemic foci at the pylorus. Petechial to ecchymotic hemorrhages were found in multiple foci in the abomasum of many calves (Fig. 2). Three calves had abomasal gas distention at necropsy (X-92, X-96, X-99) which varied from moderate to severe (Fig. 3). Some calves had small (2-3 mm) indistinct whitish foci on the abomasal surface characterized as catarrhal exudate, which histologically appeared to represent small areas of sloughed cells (Fig. 4). Seven of the eight experimental calves had ruminal trichobezoars. Several calves had mesenteric lymph nodes that appeared to be enlarged or swollen and edematous. Calves in group one appeared to exhibit the most severe lesions and those in group 2 had mild to moderate

Table 1 - Experimental production of abdominal tympany, abomasitis, and abomasal ulceration in neonatal calves inoculated intraruminally with Clostridium perfringens type A.

Group	Calf Number	Age* (Days)	Concentration of inoculum	C perfringens culture	Clinical Signs/lesions†
1	X-92	8	8×10^9	257A	Depression, bloat, intermittent diarrhea; abomasal tympany, hyperemia, ulceration (3+), ruminal trichobezoars
	X-96‡	9	1×10^{10}	257A	Depression, bloat, diarrhea, abomasal tympany, hemorrhage, hyperemia, ulceration (3+), ruminal trichobezoars
	X-97	10	1×10^{10}	ATCC	Depression, bloat, diarrhea, death; catarrhal abomasitis, hyperemia, ulceration (3+), ruminal trichobezoars
	X-99	10	4×10^{10}	69A	Depression, bloat, diarrhea, death; abomasal tympany (6-7x normal size), hyperemia, hemorrhage, ulceration (3+), ruminal trichobezoars
2	X-91	7	1×10^9	ATCC	Diarrhea, depression, bloat; abomasal edema and some catarrhal surface exudate, ulceration (1+), ruminal trichobezoars
	X-93	5	8×10^9	ATCC	Depression, intermittent diarrhea and bloat; abomasal hyperemia and some edema of mucosal folds, erosion at omasal-abomasal groove, ulceration (2+), ruminal trichobezoars
	X-94	11	3×10^9	ATCC	Depression, bloat, intermittent diarrhea, abomasal hyperemic foci and pyloric erosion, ulceration (1+)
	X-95‡	11	6×10^9	ATCC	Mild depression, intermittent diarrhea; abomasal hyperemia of mucosal folds, edema at pylorus, ulceration (1+), ruminal trichobezoars
3	X-100	5	50 ml	Distilled water	Healthy calf; small foci of hyperemia at pylorus.
	X-1622	9	100 ml	Distilled water	Healthy calf; no gross lesions.

*Age of calf when first inoculated.
†Degree of abomasal ulceration or erosion at necropsy, graded from mild (1+) to perforate (4+).
‡Reinoculated on day 7.

TABLE 2 - Blood acid-base and gases, packed cell volume, plasma protein, and fibrinogen concentration in experimental calves inoculated intraruminally with Clostridium perfringens type A.

Sampling days	pH	PCO_2 (mm of Hg)	PO_2 (mm of Hg)	Base excess (mEq/L)	Bicarbonate (mEq/L)	Total CO_2 (mEq/L)	Packed cell volume (%)	Plasma protein (g/dl)	Fibrinogen (mg/dl)
0	7.375	49.6	27.3	3.8	28.0	29.5	33.4	6.5	537.5
1	7.331	46.7	26.9	-0.4	24.5	25.9	32.0	6.2	550.0
2	7.291	48.6	28.8	-2.0†	23.5†	22.5†	32.0	6.3	512.5
3	7.271	48.5	25.6	-3.7†	22.0†	23.5†	33.5	5.8	462.5
4	7.258	47.2	26.0	-5.0†	20.9†	22.4†	31.6	6.0	575.0
5	7.308	50.4	25.2	-0.9	24.3	25.9	31.0	5.9	420.8
6	7.314	47.1	28.1	-1.6†	23.2†	24.6†	30.4	5.7	433.3
7	7.328	50.5	32.4	0.2	23.5	27.1	33.4	6.0	462.5
8	7.350	51.0	27.3	2.4	27.1	28.6	30.8	5.6	383.3
9	7.364	51.0	30.1	3.3	27.9	29.5	33.3	5.7	441.7
10	7.376	51.2	31.2	4.3	28.9	30.4	31.1	5.5	375.0
11	7.351	51.5	27.6	2.7	27.5	29.0	30.5	5.8	500.0
Normal values	7.24-27			0 to +3.0	20.-30.0	22. to 32.0		5.5-7.5	500-700.0
SEM	.029	2.5	2.8	2.1	1.6	2.0	2.1	0.3	78.7

*Least square means and SEM of 8 calves.

†Significantly different from zero day sample ($P<0.05$).

TABLE 3 - Serum chemical values in experimental calves inoculated intraruminally with Clostridium perfringens type A.

Item	Sampling days 0	4	7	10	SEM	Normal[24-27] values
Glucose (mg/dl)	108.4	102.5	90.4	109.8	9.6	60-150
Urea nitrogen (mg/dl)	0.9	2.6	1.2	0.9	0.7	10-40
Creatinine (mg/dl)	1.12	1.29	0.94	0.91	0.18	1.0-2.0
Chloride (mEq/L)	101.0	101.7	102.6	100.3	1.3	94-112
Sodium (mEq/L)	141.4	134.8†	135.8†	138.2	1.3	132-152
Potassium (mEq/L)	5.0	5.7	4.9	5.0	0.3	3.9-6.9
Calcium (mg/dl)	10.8	9.9	10.1	10.1	0.3	9.7-12.4
Inorganic phosphate (mg/dl)	7.7	8.1	6.8	7.0	0.4	4.0-7.0
Total protein (g/dl)	6.3	6.0	6.0	5.7	0.3	5.0-6.5
Alkaline phosphatase (I.U.)	268.9	226.4	238.8	172.8	57.3	0-488
Total CO_2 (mEq/L)	27.4	20.8†	22.3†	27.3	0.2	21.2-32.2
Albumin (g/dl)	3.2	3.3	3.3	3.3	0.1	3.03-3.55
Sorbitol dehydrogenase (I.U.)	8.5	11.0	9.4	13.3	1.6	4.3-15.3

*Least square means and SEM of 8 calves.

†Significantly different from zero day sample ($P<0.05$).

TABLE 4 - Serum copper concentrations in experimental calves.

Calf number	Serum (µg/ml)
X-91	1.60
X-92	2.60
X-93	2.10
X-94	2.00
X-95	2.00
X-96	2.30
X-97	2.20
X-99	1.40
X-100*	2.10
X-1622*	0.86
Normal values[20-23]	0.60 to 3.20
Toxic values[21-23]	>3.30
Deficient values[20-23]	<0.60

*Control calves.

Fig 1 - Abomasum with hyperemia surrounding an ulcerative foci from calf X-99.

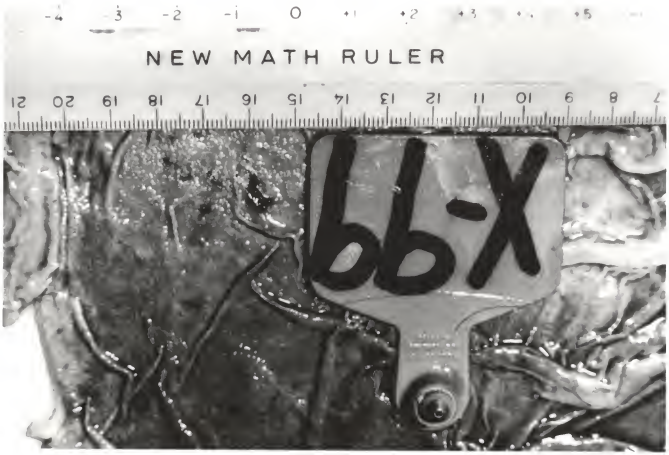
NEW MATH RULER
bb-X

Fig 2 - Multiple foci of petechial to ecchymotic hemorrhages located in the abomasum of calf X-96.

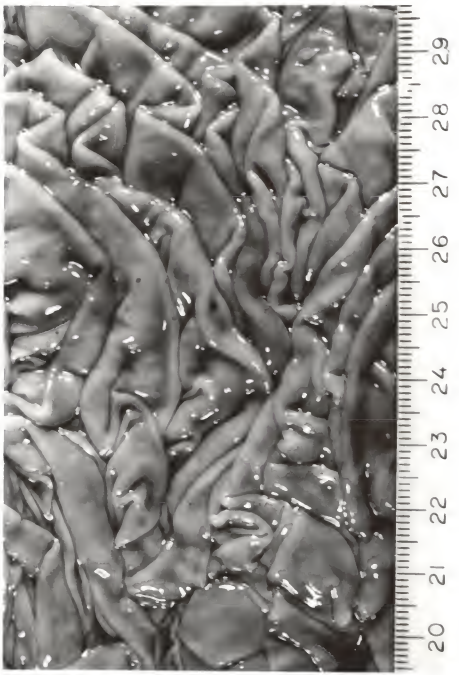
20
21
22
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Fig 3 - Ruminal and abomasal tympany at necropsy of calf X-99 4 days after intraruminal inoculation of a clinical isolate of C perfringens toxigenic type A.

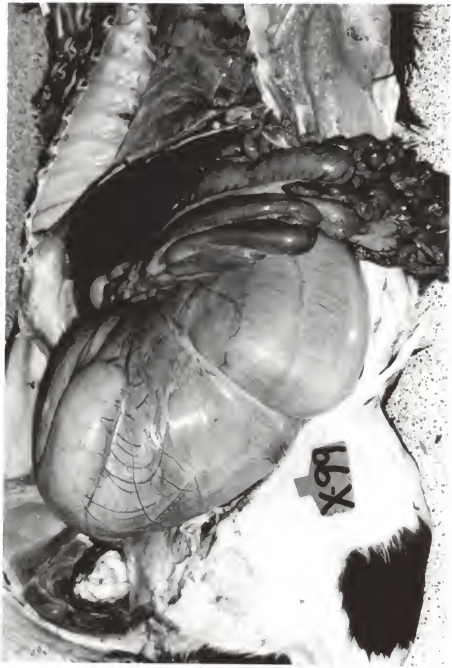
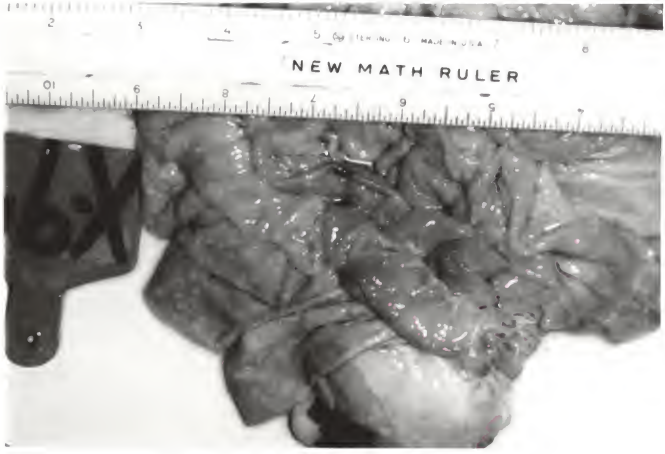

X-99

Fig 4 - Catarrhal abomasitis characterized by multiple foci (2-3 mm) of whitish exudate on the abomasal mucosal surface of calf X-97.


NEW MATH RULER

lesions. Group 3 control calves had no visible lesions.

Histopathology of experimental calves consistently revealed mild to marked, multifocal edema of the abomasal submucosa that had a distinct and prominent inflammatory component in some calves. Cell aggregates on epithelial mucosal surfaces were observed in some calves. These aggregates represented sloughed epithelium in some and inflammatory cells in others. Mucosal hemorrhages, submucosal edema, lymphatic dilatation, and varying degrees of inflammatory cell infiltration were consistent findings in all calves (Figs. 5-7). In calves not necropsied immediately after death it was sometimes difficult to differentiate antemortem from postmortem changes.

Examination of gut contents for C perfringens and its exotoxins - C perfringens was not isolated from the rumen contents of calves cultured before inoculation. C perfringens was isolated from all samples of ruminal and abomasal content collected at necropsy. Intraperitoneal injection of clarified samples caused death of mice within 1-3 days, indicating the presence of lethal exotoxin. Based on toxin-antitoxin neutralization tests in mice, all isolates were C perfringens type A.

Discussion

The inoculation of C perfringens type A culture and supernatant fluids into isolated segments of rabbit, lamb, and calf small intestine or given orally has been studied by several investigators.[28-34] These researchers have shown that C perfringens type A cultures given either orally in feed or by intraduodenal cannulae produced diarrhea in many, and death preceded by diarrhea and bloat in some cases in weaned ruminants.[28,29,31,32] C. perfringens type A enterotoxin studied by introduodenal cannulae, Thiry fistulae, and ligated intestinal loops in 4

Fig 5 - Abomasum with marked submucosal edema and mixed inflammatory cell infiltration in the submucosa of calf X-99.

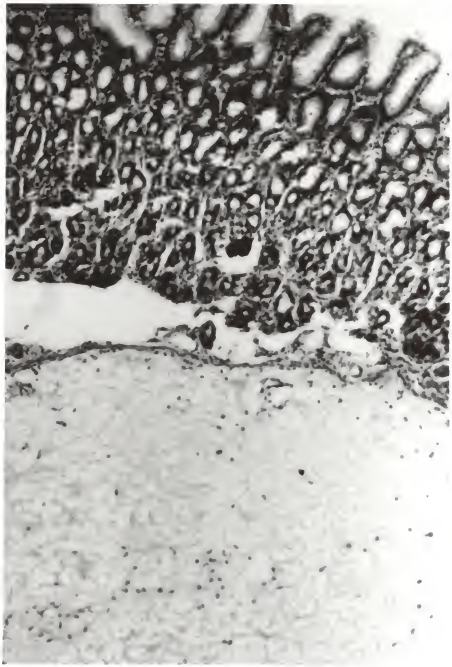


Fig 6 - Abomasum with perivascular inflammatory cell infiltration and a microthrombus (arrow) in the submucosa of calf X-99.

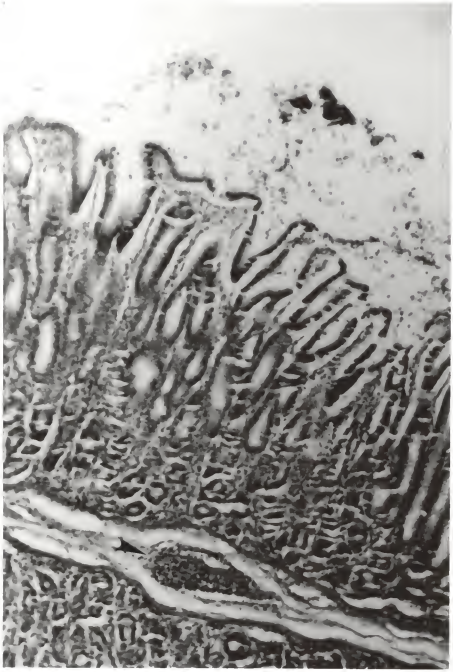
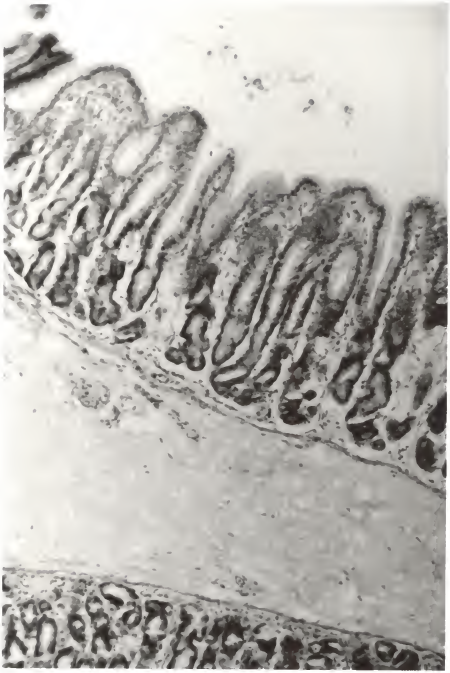


Fig 7 - Abomasum with submucosal edema, lymphatic dilation, and mild inflammatory cell infiltration in calf X-97.



to 6 month old calves demonstrated fluid accumulation and/or diarrhea as pathological responses within 0.5 - 5 hours after introduction.[31,32] The enterotoxin was not destroyed by the jejunal fluid and all of it was absorbed within 8 hours postinoculation.[31] The pathologic manifestations of vasodilation, increased capillary permeability, congested mesenteric lymph nodes and edema, and increased intestinal motility have been proposed as related to the mechanism of action of this toxin.[32,35] Mucosal lesions, including ulceration of the gastric lining, have been produced with histamine injections experimentally.[36] Alpha- and theta-toxins produced by strains of C perfringens have been shown to produce morphologic changes of mast cells, caused both by phospholipase C (α-toxin) and θ_A and θ_B-toxins (theta toxins), both of which are capable of causing histamine release.[37] Histamine levels in blood were inconclusive and much higher than expected, ranging from 0.28 - 5.02 µg/ml in calves with this syndrome and abomasal ulceration, C perfringens being isolated from abomasal lesions and fluid in 13 of those calves.[3] Intestinal loops inoculated with live cells also had gas accumulation and distension.[32] Calves which received intraduodenal C perfringens type A enterotoxigenic strains or supernatant fluids were observed to exhibit lassitude and abdominal discomfort as evidenced by frequent kicking at the abdomen and a slightly arched back.[32] These findings are compatible with the clinical signs and some of the pathology found in the experimental calves in the present study, particularly those receiving clinical isolates of C perfringens type A from calves exhibiting signs of acute abdominal syndrome and may indicate the presence of enterotoxin activity in the neonatal rumen and/or abomasum and intestine as well as the effects of alpha and theta toxins after intraruminal inoculation of C perfringens type A.

The enterotoxin of C perfringens type A has been shown to be present in

31-68% of the isolates from cattle.[38,39] Overall toxicity studies conducted by one group of researchers indicated that all C perfringens type A isolates should be considered potentially enterotoxigenic.[40] It has been shown that many strains of type A produce enterotoxin when the cells are sporulating, and when vegetative cells of this organism are ingested, they readily form spores in the intestine, a process that causes enterotoxin release.[41] The pathophyiology of C perfringens enterotoxin in the neonatal rumen has not been determined.

In experimental inoculation of C perfringens type D into the rumen of sheep, it was shown that at least 90% of the inoculum was destroyed in the adult rumen, the mean half-life of the bacteria being 1.6 hours in the rumen.[42] The recovery of C perfringens type D from the duodenum (by duodenal cannulae) in those 8 sheep inoculated intraruminally was much smaller than that from the rumen, suggesting that considerable numbers of organisms also must have been destroyed during the passage from rumen to midduodenum.[42] Studies of the microflora in the rumen of healthy and bloating calves, 4-135 days of age, indicated that C perfringens is rarely isolated.[43] In herds experiencing neonatal enteritis, C perfringens was isolated from the abomasum and upper small intestines in calves with diarrhea and a few organisms were also found in the abomasum of a calf with no diarrhea.[44] These findings suggest that C perfringens is not normally present in the rumen and abomasum of neonatal calves (none of the calves in this study had C perfringens present in pre-inoculation rumen fluid) or adult ruminants, but may be isolated in herds experiencing neonatal enteritis. Under normal circumstances, vegetative cells of C perfringens do not survive in the abomasum.[29]

C perfringens type A has been isolated with other bacteria from abomasal ulcers and abomasitis in mature cattle.[14] In this case C perfringens type A appeared to be associated with inflammatory lesions, capillary dilation, and cell

shedding in the mucosa. Perforate abomasal ulcers in suckling calves (1-12 weeks of age) have been associated with C perfringens, E coli, Streptococcus, Staphylococcus aureus and other bacteria.[3,6-10] These ulcers were in the midpart of the fundus, and on the greater curvature of the abomasum; several calves had abomasal trichobezoars, and these with other stressors were thought to have contributed to the development of the ulcers. Anaerobic incubation of abomasal contents or samples from abomasal lesions in CMC-starch at 45°C initially, followed by further incubation at 37°C after transfer to other media, enhances isolation of C perfringens and inhibits other bacterial growth.[16,17] This procedure helps to isolate C perfringens in almost pure culture and prevents its being lost or obscured by other bacteria which may overgrow it on certain media. This technique has allowed us to consistently isolate C perfringens from abomasal ulcers and lesions in neonatal calves.

The incidence and pathology of abomasal ulcers in range calves exhibiting abomasal tympany in Nebraska and Wyoming was studied, and correlated with copper deficiency and secondary bacterial infection.[6-9] Bacterial isolates from abomasal content or lesions in those calves were believed to have gained access to the upper alimentary tract via reflux action of duodenal contents. The bile salts, acids, lysolecithins, bacterial toxins, and sepsis in the refluxed material were thought to damage the surface gastric cells, causing mucosal ischemia and inducing intravascular coagulation with microthrombotic occlusions of the mucosal vessels, making them susceptible to erosion or ulceration. Bile or its acid components exhibit antibacterial activity depending upon the concentration of bile and the susceptibility of a given organism.[45] In general, bile acids have been shown to inhibit gram-positive organisms, but usually have little effect against gram-negative organisms. This would tend to preclude duodenal reflux of content

and bacteria as the route of entry for gram positive bacteria cultured from abomasal ulcers and lesions. Low copper levels in those calves were hypothesized to cause derangement of elastin cross linkages, thereby compromising the integrity of the abomasal mucosa and its microvasculature, and to cause decreased cytochrome oxidase activity of leukocytes, contributing to decreased neutrophil function and increased susceptibility to infection. C perfringens was isolated from abomasal content and lesions in those studies alone or with other bacteria, but was considered to be an opportunistic invader associated with engorgement on rich milk after a period of anorexia during weather stress and immunological compromise associated with copper deficiency. Toxin neutralization testing in mice to determine if performed lethal toxin was present with isolates of C perfringens was not done in those cases.

Calves in our study had normal serum copper concentrations, had no history of weather-related stress, and C perfringens type A, toxigenic ATCC or clinical isolate, given intraruminally, were consistently isolated from ruminal and abomasal contents or abomasal lesions at necropsy. These calves had signs of acute abdominal syndrome and abomasal lesions of varying severity after intraruminal inoculation of toxigenic strains of C perfringens type A. We suspect that pathophysiology associated with suckling may play a significant role in the development of this syndrome in neonatal calves. Possibly affected calves have esophageal groove dysfunction which allows abnormal amounts of milk to consistently enter the neonatal rumen, and thus provides substrate and an anaerobic environment for ingested C perfringens to proliferate. This abnormal fermentation could allow large numbers of C perfringens to survive, enter the abomasum, and colonize the abomasal mucosa creating areas of abomasitis. In man, certain Campylobacter sp., a microaerophilic to anaerobic bacilli, have been found

on gastric epithelia or within ulcerated tissues in almost all patients with active chronic gastritis, duodenal ulcer, or gastric ulceration,[46] and have also been reported in gastric ulcers of ferrets.[47] Gastritis associated with pernicious anemia, immunologically mediated in man and affecting the proximal stomach has rarely been associated with Campylobacter-like organisms. This rarity is considered to indicate that the organisms probably do not develop secondary to gastritis and provides indirect evidence of those organisms as causative agents in chronic active gastritis.[47] C perfringens may have a similar function in the etiology of acute abdominal syndrome in neonatal calves.

Calves with a history of sudden death have often been described by owners as being top calves compared with the rest of the herd, and from heavy milking dams. Calves being bottle fed are usually noted to be vigorous nursers. Possibly this behavior could contribute to spill-over of milk into the neonatal rumen. Certainly calves with a history of diarrhea may be somewhat immune compromised, but in our clinical experience most calves appear to be healthy and normal up until the onset of this syndrome. Copper deficiency was not found in the calves we studied, but may be a contributing factor in regions having molybdenum excess associated copper deficiency or primary copper deficient soils. Researchers studying copper depleted and deficient cattle have found that cytochrome oxidase activity of the mucosa of the duodenum and jejunum was decreased,[48] the villi of the small intestine were atrophied,[49] and neutrophil function (candidacidal activity) was decreased.[50] However, in none of these studies was bloat reported nor abomasal ulceration in copper-deficient calves or adult cattle present at necropsy.[48,49,51] We hypothesize that the action of C perfringens bacteria and its toxins may be responsible for the tympany (C perfringens is a prolific gas forming organism), abomasitis, and abomasal ulceration seen in these calves. The reported

decreased incidence of abomasal tympany and ulceration in neonatal calves from herds having a history of these problems after instituting C perfringens vaccination programs[3,6-8] supports our theory that this organism may be the primary etiologic agent in this syndrome. Isolation of toxigenic C perfringens from the ruminal contents of live calves presented with this syndrome establishes the presence as significant, and also indicates its serious implications in calves with sudden death. Further investigations of calves with abomasal ulcers, abomasitis, and acute tympany may reveal other contributing factors to this syndrome, but we believe C perfringens should not be ignored as a serious pathogen in these cases, and indeed, may be the primary etiologic agent involved in its pathophysiology.

References

1. Blood DC, Henderson JA, Radostits OM, et al. Diseases of the alimentary tract II. In: Blood DC, Henderson JA, et al, eds. Veterinary medicine: A textbook of the disease of cattle, sheep, pigs, and horses, 5th ed. Philadelphia: Lea and Febiger, 1979; 197-199, 452-458.
2. Wass WM, Thompson JR, Moss EW, et al. Diseases of the ruminant stomach: abomasal ulcers. In: Howard JL, ed. Current Veterinary Therapy: Food Animal Practice. Philadelphia: W.B. Saunders Co., 1981; 883-884.
3. Johnson JL, Lilley CW, Hamar DW, et al. Diagnostic observations of abomasal tympany in range calves, in Proceedings. 3rd Int Symp World Assoc Vet Lab Diag, Vol 2, Ames, Iowa; 1983; 485-491.
4. Gitter M, Austwick PKC. The presence of fungi in abomasal ulcers of young calves: A report of seven cases. Vet Rec 1957; 69:924-928.
5. Wray C, Thomlinson JR. Abomasal ulceration in calves. Vet Rec 1968; 83:80-81.
6. Lilly CW, Hamar DW, Johnson JL, et al. Factors associated with abomasal ulcers in beef calves, in Proceedings. 87th Ann Conv, Nebraska Vet Med Assoc, 1984; 70-77.
7. Lilly CW, Hamar DW, Johnson JL, et al. The association of copper and bacteria with abomasal ulcers in beef calves, in Proceedings. 87th Ann Conv, Nebraska Vet Med Assoc., 1984; 4-11.
8. Lilly CW, Hamar DW, Gerlach M, et al. Linking copper and bacteria with abomasal ulcers in beef calves. Vet Med 1985; 80:85-88.

9. Johnson JL, Hudson DB, Bohlender RE. Perforating abomasal ulcers and abomasal tympany in range calves. in Proceedings. 24th Am Assoc Vet Lab Diag 1981; 203-210.

10. Tulleners EP, Hamilton GF. Surgical resection of perforated abomasal ulcers in calves. Can Vet J 1980; 21:267-274.

11. Berkhoff GA, Braun RK, Buergelt CD, et al. Clostridium perfringens type A associated with sudden death of replacement and feeder calves. in Proceedings. 23rd Am Assoc Vet Lab Diag 1980; 45-52.

12. Waas W. Abomasal ulcers. Mod Vet Pract 1986; 67:111-112.

13. Singh GB, Sharma JN, Kar K. Pathogenesis of gastric ulceration produced under stress. J Path Bact 1967; 94:375-380.

14. Al-Mashat R R, Taylor DJ. Bacteria in enteric lesions of cattle. Vet Rec 1983; 112:5-10.

15. Sterne M, Batty I. Examination of specimens: procedures. In: Sterne M, Batty I, eds. Pathogenic clostridia, 1st ed. Boston: Butterworths, 1975; 72-84.

16. Smith LDS, Williams BL. The pathogenic anaerobic bacteria, 3rd ed. Springfield, Illinois: Charles C. Thomas Publishing Co. 1984; 94-136.

17. Holdeman LV, Cato EP, Moore WEC. Anaerobe laboratory manual, 4th ed., Blacksburg, VA: Virginia Polytechnic Institute and State University, 1977; 141-145.

18. Smith LDS, Hobbs J. Genus III Clostridium, In: Buchanan RE, Gibbons NE, eds. Bergey's Manual of Determinative Bacteriology. Baltimore, Md: Williams and Wilkins Co. 1974; 551-572.

19. Sutter VL, Washington II JA. Susceptibility testing of anaerobes. In: Lennette EH, Spaulding EH, Truana JP, eds. Manual of Clinical Microbiology, 2nd ed. Washington, D.C: American Society of Microbiology, 1974; 436-438.

20. Claypool DW, Adams FW, Pendell HW, et al. Relationship between the level of copper in the blood plasma and liver of cattle. J Anim Sci 1975; 41:911-914.

21. Underwood EJ. Trace mineral elements in human and animal nutrition. 4th ed. New York: Academic Press, 1977.

22. Bulgin MS, Maas J, Anderson BC, et al. Death associated with parenteral administration of copper disodium edetate in calves. J Am Vet Med Assoc 1986; 188:406-409.

23. Matrone G, Shields GS, Olwin JH, et al. Copper deficiency and toxicity: Acquired and inherited in plants, animals, and man. Parkridge, NJ: Noyer Publication, 1971; 11, 94.

24. Kaneko JJ. Clinical biochemistry of domestic animals 3rd ed. San Francisco: Academic Press, 1980; 792-795.

25. Rosenberger G. Clinical examination of cattle 1st ed. Philadelphia: W.B. Saunders Co. 1979; 126-145.

26. Schalm OW, Jain NC, Carrol EJ. Veterinary hematology. 3rd ed. Philadelphia: Lea and Febiger 1975; 122-144.

27. Coles EH. Veterinary clinical pathology. 3rd ed Philadelphia: W.B. Saunders CO., 1980; 18, 298-299, 317, 320.

28. Hauschild AHW, Niilo L, Dorward WJ. Experimental enteritis with food poisoning and classical strains of Clostridium perfringens type A in lambs. J Infect Dis 1967; 117:379-386.

29. Niilo L, Moffatt RE, Avery RJ. Bovine enterotoxemia II. Experimental reproduction of the disease. Can Vet J 1963; 4:288-298.

30. Hauschild AHW, Niilo L, Dorward WJ. The role of enterotoxin in Clostridium perfringens type A enteritis. Can J Microbiol 1971; 17:987-991.

31. Niilo L. Fluid secretory response of bovine thiry jejunal fistula to enterotoxin of Clostridum perfringens. Infect Immunity 1973; 7:1-4.

32. Niilo L, Dorward WJ. The effect of enterotoxigenic Clostridum welchii (perfringens) type A on the bovine intestine. Res Vet Sci 1971; 12:376-378.

33. Duncan CL, Strong DH. Experimental production of diarrhea in rabbits with Clostridum perfringens. Can J Microbiol 1969; 15:765-770.

34. McDonel JL, Demers GW. In vivo effects of enterotoxin from Clostrdium perfringens type A in the rabbit colon: binding vs. biologic activity. J Infect Dis 1982; 145:490-494.

35. Niilo L. Mechanism of action of the enteropathogenic factor of Clostridium perfringens type A. Infect Immunity 1971; 3:100-106.

36. Zaidi SH, Mukerji B. Experimental peptic ulceration. Part I. The significance of mucous barrier. Indian J Med Res 1958; 46:27-37.

37. Standberg K, Mollby R, Wadstrom T. Histamine release from mast cells by highly purified phospholipase c (alpha-toxin) and theta-toxin from Clostridium perfringens. Toxicon 1974; 12:199-208.

38. Tsai C, Torres-Anjel MJ, Riemann P. Characteristics of enterotoxigenic Clostridum perfringens type A isolated from cattle and chickens. J Formosan Med Assoc 1974; 73:501-510.

39. Wijewanta EA. Isolation of heat-resistant Clostridium perfringens from healthy cattle. Cornell Vet 1972; 62:26-31.

40. Genigeorgis C. Public health importance of Clostridium perfringens. J Am Vet Med Assoc 1975; 167:821-827.

41. Smith LDS. Virulence factors of Clostridium perfringens. Rev Infect Diseases 1979; 1:254-262.

42. Bullen JJ, Scarisbriek R, and Maddock A. Enterotoxemia of sheep: the fate of washed suspensions of Clostridium welchii type D introduced into the rumen of normal sheep. J Path Bact 1953; 65:209-219.

43. Jayne-Williams DJ. The Bacterial flora of the rumen of healthy and bloating calves. J Appl Bacteriol 1979; 47:271-284.

44. Lozano EA, Catlin JE, Hawkins WW: Incidence of Clostridium perfringens in neonatal enteritis of Montanta calves. Cornell Vet 1970; 60:347-359.

45. Floch MH, Gershengeren W, Elliot S, et al. Bile acid inhibition of the intestinal microflora - a function for simple bile acids? Gastroenterology 1971; 61:228-233.

46. O'Connor HJ, Axon ATR, Dixon MF. Campylobacter-like-organisms unusual in type A (pernicious anemia) gastritis. Lancet 1985; 1:111.

47. Fox JG, Edrise BM, Cabot EB, et al. Campylobacter-like organisms isolated from gastric mucosa of ferrets. Am J Vet Res 1986; 47:236-239.

48. Suttle NF, Angus KW. Experimental copper deficiency in the calf. J Comp Path 1976; 86:595-608.

49. Mills CF, Dalgarno AC, Wenham G. Biochemical and pathological changes in tissues of Friesian cattle during the experimental induction of copper deficiency. British J Nutr. 1976; 35:309-331.

50. Boyne R, Arthur JR. Effect of selenium and copper deficiency on neutrophil function in cattle. J Comp Path 1981; 91:271-276.

51. Spratling FR. Complex nutritional deficiency in a group of calves. Br Vet J 1976; 132:557-567.

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ACUTE ABDOMINAL SYNDROME IN NEONATAL CALVES: THE ROLE OF CLOSTRIDIUM PERFRINGENS

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

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ABSTRACT

An acute abdominal syndrome with clinical signs of abdominal tympany, colic, and death, not associated with displaced abomasum or obstructed intestines, in calves from 2 days to 12 weeks of age has been reported by several researchers. The gross pathology is usually confined to the abomasum with varying degrees of inflammation, hemorrhage and ulceration. The incidence is common in calves of beef breeds with morbidity and mortality approaching 2 to 10% in some herds. The theories proposed to explain the pathogenesis include dietary or environmental stress, mycotic infection, low immune status associated with copper deficiency, and Clostridium perfringens. The objective of our investigation was to study the possible role of C perfringens in the cause of neonatal acute abdominal syndrome. Eight neonatal calves, 2 - 21 days old, were referred to Kansas State University Veterinary Hospital for clinical examination or necropsy with suspected abomasal displacement or intestinal obstruction after acute onset of abdominal tympany, colic, depression, or death. Routine hematology and serum analysis revealed no consistent changes. Necropsy examination indicated abomasal distension with varying degrees of abomasitis, hemorrhage and ulceration but no evidence of displaced abomasum or obstructed intestines. Rumen contents collected via stomach tube or at necropsy and abomasal contents collected at necropsy were cultured anaerobically. Serum and samples of liver and kidney were analyzed for copper concentration. C perfringens was isolated from all samples and based on mouse toxin - antitoxin neutralization tests, seven were toxigenic type A and one was type E. Copper concentrations in serum and tissue samples were within normal range. Intraruminal inoculation of C perfringens type A (ATCC toxigenic type A or toxigenic type A from clinical cases), alone or in combination with types C, D

or E (ATCC strains) into healthy calves (4 - 12 days old) induced anorexia, depression, bloat, and diarrhea, and death in some calves. Necropsy examination revealed varying degrees of abomasitis, petechial to ecchymotic hemorrhages, and pin point to perforating ulcers in the abomasum. Many calves also had multiple rumen trichobezoars. It was concluded that acute abdominal syndrome in neonatal calves was unrelated to copper deficiency and C perfringens type A may have a significant role in the etiology of this syndrome.