PEER REVIEWED

Case Report - Jejunal Hemorrhage Syndrome of Dairy Cattle

M.A. Kirkpatrick, *DVM, MS*[1]; **L.L. Timms,** *MS, PhD*[2]; **K.W. Kersting,** *DVM, MS*[3]; **J.M. Kinyon,** *BS, MS*[4]
[1]*Pharmacia Animal Health – Dairy Technical Services, Kuna, ID 83634*
[2]*Department of Animal Science - Dairy Extension, College of Agriculture, Iowa State University, Ames, IA 50011*
[3]*Department of Veterinary Diagnostic and Production Animal Medicine (VDPAM) - Food Animal Medicine and Surgery, College of Veterinary Medicine, Iowa State University, Ames, IA 50011*
[4]*Department of Veterinary Diagnostic and Production Animal Medicine (VDPAM) - Bacteriology, Clinical Microbiology, College of Veterinary Medicine, Iowa State University, Ames, IA 50011*

Abstract

In the last three years veterinary practitioners from Iowa, Minnesota and Wisconsin have reported, with increased frequency, a peracute, segmental hemorrhagic enteritis in mature dairy cattle. Based on these reports, clinicians at Iowa State University have begun to suspect Jejunal Hemorrhage Syndrome (JHS) as a new emerging disease syndrome.

The morbidity rate for this disease has been sporadic, and mortality approaches 85-100% due to the peracute nature and severity of this disease. There are frequently no prodromal signs and the mature cow is found dead, or an individual cow is found down and in systemic collapse. Clinical signs include sternal recumbency, diaphoresis, enophthalmia and signs of shock due to occlusion of the small intestine. Ballotment of the standing cow in the lower right abdominal area can elicit a pronounced fluid slosh, due to backup of ingesta and fluid behind the occlusive lesion. Signs of abdominal pain include bruxism, vocalization, treading and kicking at the abdomen. At necropsy, segmental lesions localized to the jejunum are observed. These areas consist of frank hemorrhage and immediate clotting, forming a functional occlusion of the small intestinal lumen. Necrosis of the lumen may or may not be apparent.

In April 1999, the Veterinary Diagnostic and Production Animal Medicine Department at the Iowa State University College of Veterinary Medicine was asked by a northeastern Iowa veterinarian to investigate recurring sporadic peracute death losses. Examination of affected cattle, production records, rations and postmortem results led investigators to conclude that a variant of *Clostridium perfringens*, specifically type A, should be considered further as a possible cause of this disease syndrome.

Recommendations for investigation of a problem herd are discussed. Specific items of increased risk in this particular herd included an association between increased milk production and death loss, an increased incidence rate associated with higher soluble carbohydrate feeding rates, and disease following re-introduction of culture-positive alfalfa haylage. There appear to be gut motility aberrations, evidenced by intussusceptions in tandem with Jejunal Hemorrhage Syndrome lesions.

Résumé

Depuis les trois dernières années, les vétérinaires praticiens de l'Iowa, du Minnesota et du Wisconsin rapportent plus fréquemment une entérite hémorragique segmentée suraiguë chez des vaches laitières adultes. Suite à ces rapports, les cliniciens de l'Iowa State University soupçonnent le syndrome hémorragique jéjunal (JHS) comme nouveau syndrome de maladie.

Le taux de morbidité pour cette maladie a été sporadique et la mortalité atteint les 85-100% en raison de la nature suraiguë et de la sévérité de la maladie. Il n'y a fréquemment pas de signes prodromiques et la vache adulte est trouvée morte ou en état de décompensation. Les signes cliniques incluent le décubitus sternal, la diaphorèse, l'énophtalmie et des signes indiquant un choc suivant l'occlusion du petit intestin. La succussion dans la région abdominale droite de la vache debout peut engendrer un bruit de liquide dû à l'accumulation de l'ingesta et de fluides derrière la lésion d'occlusion. Les

signes de douleur abdominale incluent le bruxisme, la vocalisation, le piétinement et les coups de pattes à l'abdomen. À la nécropsie, on observe des lésions segmentées localisées au niveau du jéjunum. Ces zones hémorragiques avec coagulation immédiate entraînent l'occlusion nette de la lumière du petit intestin. Une nécrose de la paroi peut ne pas être évidente.

En avril 1999, le département de diagnostic vétérinaire et de médecine de production animale du collège de médecine vétérinaire de l'Iowa State University fut saisi d'une demande par un vétérinaire du nord-est de l'Iowa pour enquêter sur l'apparition sporadique des pertes animales suraiguës. L'examen du bétail affecté, des dossiers de production, des rations et des résultats post-mortem ont conduit les chercheurs à conclure qu'une souche de *Clostridium perfringens*, plus spécifiquement du type A, devrait être considérée comme une cause possible de ce syndrome de maladie.

Des recommandations pour l'examen d'un troupeau à problème sont discutées. Parmi les facteurs qui pourraient augmenter le risque dans ce troupeau en particulier, on retrouve l'association entre l'augmentation de la production de lait et la mortalité, une incidence plus élevée associée avec un taux d'ingestion alimentaire plus grand d'hydrates de carbone solubles et le déclenchement de la maladie suite à la réintroduction de foin de luzerne avec culture positive. On a observé des aberrations au niveau de la motilité du système digestif comme en font foi les intussusceptions en parallèle avec des lésions associées au syndrome hémorragique jéjunal.

Introduction

In the last three years veterinary practitioners from Iowa, Minnesota and Wisconsin have reported with increased frequency a peracute, segmental hemorrhagic enteritis in mature dairy cattle. *Clostridium perfringens* is the principal cause of Clostridial enteric disease in domestic animals and is divided into five phenotypes (A, B, C, D and E) based on production of four major toxins: alpha, beta, epsilon and iota (α, β, ε, and ι). *Clostridium perfringens* type A produces α toxin; type B produce α, β, and ε toxins; type C produces α and β toxins; type D produces α and ε toxins; and type E strains produces α and ι toxins. *Clostridium perfringens* type A is the most common, the most variable in its toxigenic properties and the most confusing organism with respect to potential pathology.[26] Much of this confusion relates to the ease of isolation of this organism from tissues, effusions and intestinal tracts of cadavers within hours of death. It grows rapidly on culture, and may mask the presence of other organisms.[26]

C. perfringens type A is found in the intestinal tract of warm-blooded animals and the environment, and can be characterized into two varieties.[12] The "classic" variety produces dermonecrotic alpha toxin (lecithinase capable of destroying cell membranes) and is associated with anaerobic cellulitis and gas gangrene.[4,12,28] Alpha toxin is also known as a powerful hemolysin of cattle and mouse red blood cells, resulting in intravascular hemolysis.[12]

The second, or enterotoxigenic, variety of *C. perfringens* type A is characterized by *C. perfringens* enterotoxin production (CPE) and is capable of causing human enteritis.[5,25,26] CPE differs from classical exotoxins in that it is produced intracellularly by sporulating cells of *C. perfringens* type A. The exotoxin causes an efflux of cellular fluids *via* the cellular pore-forming nature of the protein toxin. It is resistant to digestive enzymes, but does not cause necrotizing lesions; rather, there is an outpouring of fluid into the lumen of the small intestine, resulting in diarrhea.[26]

C. perfringens type A is implicated as the cause of enterotoxemia in lambs, and tympany, hemorrhagic enteritis, abomasitis and abomasal ulceration in calves in the western United States. It also causes necrotic enteritis in domestic chickens, necrotizing enterocolitis and villous atrophy in suckling and feeder pigs, hemorrhagic gastroenteritis in dogs, and necrotic enteritis of foals.[3,5,11,26,28,33]

Bovine enterotoxemia has classically been a disease of young calves and cattle in the feedlot. Feedlot enterotoxemia has been described as a disease caused by *C. perfringens* types C and D. The most frequent basis for this diagnosis is reddened or hemorrhagic areas in the small intestine.[15] If not a primary agent, *C. perfringens* type A could play a secondary role in various disease conditions.[15] Introduction of polymerase chain reaction (PCR) techniques has raised serious questions about whether *C. perfringens* types C and D are involved at all.[9,33] Daube *et al* (1996) reported that Clostridial isolates from suspected enterotoxemia cases are all *C. perfringens* type A.[9]

The role of enterotoxin in bovine enteritis has been examined through inoculation of enterotoxigenic *C. perfringens* type A into ligated gut loops of experimental calves. The enterotoxigenic strains produced fluid efflux, gas and edematous, congested mesenteric lymph nodes, as well as slight edema of the mesenteric attachment of the ileum, thus demonstrating its pathogenesis in the neonatal bovine.[27] Additional investigation into the C. *perfringens* type A enterotoxin suggests the capability of the enterotoxin to bind to presynaptic nerve endings, inhibiting neuromuscular transmission.[31] This could be an important pathogenic consideration, as any disruption of intestinal motility could be considered a risk factor for the presentation of disease by *C. perfringens* type A.[10]

Recent isolations of *C. perfringens* type A strains from Jejunal Hemorrhage Syndrome cases (JHS) have

demonstrated a variant that expresses Beta2 toxin. Of 47 cases in adult dairy cattle, 25 isolates yielded the Beta2 toxin.[17,18,23,24] Beta2 toxin was first found in association with *C. perfringens* type A in necrotic enteritis of swine and enterocolitis of horses in Europe, and this particular toxin is known to create inflammation of the small intestine, with loss of mucosa.[12,14,17,18,20,23,24]

Based on practitioner and producer reports from northeastern Iowa, southeastern Minnesota and southwestern Wisconsin, as well as reports from across the United States during 1999, clinicians at Iowa State University have begun to suspect JHS as possibly a new emerging disease syndrome. Presentation of this syndrome has been sporadic, affecting only individual dairies and individual mature cows within dairies. Based on practitioner reports, morbidity rates of 1-2% of the mature cow population would be typical, with mortality approaching 85-100% due to the peracute nature and severity of this disease.

Clinical signs

Based on cases attended by the authors and the referring veterinarian, clinical signs of JHS are peracute. Frequently the producer will see no prodromal signs and find a mature cow dead. Sometimes an individual cow may be found recumbent and in systemic collapse. Clinical signs of these animals include:

- Sternal recumbency
- Vocalization
- Diaphoresis
- Bruxism
- Enopthalmia
- Shock, as evidenced by pale mucous membranes and poor capillary refill time.
- If the cow is standing, ballotment of the lower right abdomen can elicit a pronounced fluid slosh due to the backup of ingesta and fluid behind the occlusive lesion.
- Rectal examination may reveal signs of constipation, followed by evidence of melena or frank hemorrhages and clots within the rectal vault. Dilated intestinal loops may also be palpable.

Rule-outs

The most frequent diagnostic rule-outs for the indicated signs are Salmonellosis (*Salmonella kentucky*); abomasal ulceration and hemorrhage; and abomasal displacement, volvulus and compromise. Rule-outs for the cause of sudden death include intestinal volvulus or intussusception, acute peritonitis, traumatic pericarditis or abomasal volvulus. In general, *Salmonella* cases have a slightly longer survival "window" than do peracute cases of JHS. The veterinary practitioner is encouraged to obtain a fecal sample to culture for *Salmonella* sp. prior to making a diagnosis of JHS. Due to the ubiquitous nature of *C. perfringens* type A, a positive fecal culture for this organism should not be considered diagnostic. While a causal link has not been established between *C. perfringens* type A and JHS, a practitioner collecting samples for culture should focus on sampling the lesion site, keeping in mind that overgrowth can occur early following death.

Clinical chemistry

Due to the peracute nature of this syndrome, there is often little time to perform clinical chemistry analysis. A condition that could mimic JHS is intussusception, which is characterized by hemoconcentration with increased packed cell volume and total protein. The complete blood count (CBC) could show a neutropenia with a strong left shift. Serum chemistry could show hypocalcemia, hypochloremia, metabolic alkalosis, hyponatremia, hypokalemia and hyperglycemia.[1,7]

Post-mortem findings

Segmental lesions localized to the jejunum have been observed. These areas consist of frank hemorrhage and immediate clotting, forming a functional occlusion of the small intestinal lumen. Necrosis of the lumen may or may not be apparent. Some cases have also presented intussusceptions immediately anterior to the area of segmental hemorrhage and clotting. It is unclear which lesion presents first, or whether one contributed to the other. It is possible that the presence of an intussusception could indicate intestinal hypo- or hyper-motility, resulting in the slowing of ingesta flow and allowing Clostridial growth and sporulation.[10]

Bacterial isolations

Suspect JHS cases presented to the Iowa State University Diagnostic Laboratory for culture have consistently yielded C. *perfringens* type A in high numbers. Impression smears of the lesion sites have also yielded high numbers of gram-positive rods, indicative of the presence of a Clostridial species. The isolation patterns found in these samples would suggest that this organism be considered for further research as a possible pathogen causing this syndrome. However, this organism is ubiquitous and can be found as a part of the gut flora of warm-blooded animals.[12] Overgrowth following death can be rapid and may mask another potential pathogen.

Additional cultures have yielded *Salmonella* species of variable typings from the lesion sites. No consistency in *Salmonella* sp. has been seen from case to case. Presence of segmental occlusive hemorrhagic lesions may provide *Salmonella* sp. an opportunistic environment. Fluorescent antibody work examining for presence of bovine viral diarrhea virus (BVDV) in the tissue samples has been negative to date.

Treatment efforts

Due to its peracute nature, this syndrome should be considered a medical emergency. Cattle found alive are extremely compromised. Even considering this compromise, the use of intravenous calcium has been beneficial. Additional therapy has included flunixin meglamine[a] (1.1 mg/kg IV) or isoflupredone[b] (20 mg IM for an adult cow) for control of pain and shock, along with intravenous (IV) fluid therapy. Some practitioners have attempted to flush the clot from the intestinal mass using oral fluid therapy or oral administration of mineral oil, along with parenteral and oral antibiotics. Results have been variable, with some cows expelling a significant amount of blood clots and surviving. Affected cattle frequently remain compromised; it is likely that salmonellosis may complicate the animal's recovery.

Affected cattle are extremely poor candidates for surgical intervention, and often may not even survive transportation. Surgical intervention has included intestinal resection and anastamosis or, alternatively, manual massage of the affected area to breakdown the offending clot. The post-operative prognosis should be considered extremely guarded due to metabolic compromise, and potential re-occurrence of the lesions post-surgery.[8]

Case Study

History

In April 1999, the Veterinary Diagnostic and Production Animal Medicine Department at the Iowa State University College of Veterinary Medicine was asked by a northeastern Iowa veterinarian to investigate recurring, sporadic peracute death losses in a dairy herd of Brown Swiss cows. Previous efforts centering on treatment, autogenous vaccine usage and ration manipulation to halt occurrence of the syndrome had been unrewarding. Production data for this 140-cow herd were: 21,824 lb of milk rolling herd average; 23,488 lb of milk 305 Day ME; 65.9 lb of average milk per day per lactating cow; 78 lb of management level milk; 4.24% test-day fat; and 3.57% test-day protein.

The dairyman reported one-to-two undiagnosed, sporadic deaths in the herd each year for the last 20 years. In the two years preceding the investigation, the incidence of sudden deaths greatly increased, with the owner and veterinarian reporting 30 deaths due to enteritis. The increased death rate was coincidental with expansion efforts which doubled the herd size from 70 to 140 head. Cow numbers were increased from within the herd, and no outside cattle were purchased. A freestall barn was built to house the increased number of cows, and the tie-stall barn was converted to a parlor. Total mixed ration (TMR) feeding was also adopted. All animals were administered a 7-way clostridial bacterin/toxoid[c] at 10-12 months of age; booster vaccinations were given annually prior to freshening. Since the outbreak of deaths, an autogenous *C. perfringens* type A bacterin/toxoid was administered to each lactating cow every 60 days, and the 7-way clostridial bacterin/toxoid was administered quarterly.

Clinical findings

Prior to our farm visit, the producer was requested to submit samples of the lactational TMR for Penn State particle separator testing and wet chemistry analysis, realizing that a truly representative TMR sample might be impossible to obtain.[19] Individual components used in the TMR were also submitted for examination, as the effect of a TMR mixer makes it impossible to evaluate individual components. Further ration analysis was performed by review and re-calculation of the ration parameters submitted by the herd nutritionist. The producer was performing monthly herd testing of milk production, milk components and somatic cell counts.[d] Current and previous records were obtained from AgSource Cooperative Services and evaluated against the death loss record to determine if any production or nutritional parameters could be contributing to disease outbreak. Following the pre-visit evaluation, an on-site field investigation with the herd owner, veterinarian, nutritionists and dairy cooperative field representative was arranged.

Diet was typical of rations fed in northeast Iowa and was fed as a single-group TMR. Dry matter intake was 53 lb/head/day when calculated across all lactations and days-in-milk (DIM). Table 1 shows the daily amounts of ration components presented to the herd in a TMR on an "as-fed" basis. To determine if there was variance between the ration offered the cows and the composition recommended by the nutritionist, the TMR ration was submitted for wet chemistry analysis and compared against the original nutritional recommendations. Table 2 shows the ration parameters as determined by computer calculation and wet chemistry analysis.

Physical form (amount of long-fiber fraction) of the ration was evaluated through the use of the Penn-State Particle Separator box.[19] Results were consistent with particle separator tests performed by Iowa State University Extension Service during Iowa Dairy Days in

[a]Banamine®, Schering-Plough Animal Health, Union, NJ 07083
[b]Predef® 2X, Pharmacia Animal Health, Kalamazoo, MI 49001
[c]Fortress® 7, Pfizer Animal Health, Exton, PA 19341
[d]AgSource Cooperative Services, PO Box 930230, Verona WI, 53593.

Table 1. Daily amounts of ration components presented to the herd in a total mixed ration on an "as-fed" basis.

Feed	As-fed basis lb/head/day
2nd cutting alfalfa haylage	18
Corn silage	30
2nd cutting alfalfa hay	6.5
High moisture corn	12.8
Whole cottonseed	5.5
Linseed meal	1.8
Wet corn gluten	18
Roasted soybeans	2
Mineral mix	1.9
Water	4
Total	100.5

Table 2. Ration parameters as determined by computer calculation and wet chemistry analysis.

Ration determinations	Calculated dry matter basis	Wet chemistry dry matter basis
Moisture	49.20%	45.30%
Dry matter	50.80%	54.70%
Crude protein	17.18%	17.85%
ADF (acid detergent fiber)	20.19%	18.43%
NDF (neutral detergent fiber)	35.95%	34.29%
Calcium	0.82%	1.08%
Phosphorous	0.53%	0.66%
Magnesium	0.28%	0.35%
Potassium	1.22%	1.56%
Ash	7.97%	7.53%
Fat	4.64%	4.91%
Protein solubility		33.55%
TDN (total digestible nutrients)	74.26%	74.55%
NFC (non-fiber carbohydrate)	34.58%	35.42%
NEL (net energy for lactation) Mcal/cwt	77.00	77.47
NEG (net energy for gain) Mcal/cwt	53.00	52.37
NEM (net energy for maintenance) Mcal/cwt	81.00	80.66

February 1998. The average northeast Iowa TMR particle separator test results are shown in Table 3, and compared to the TMR fed to the herd and the 24-hour refusals. All TMR particle separator analysis determinations were performed in three replicates to ensure consistency. Of particular concern was the evaluation of the TMR 24-hour refusals. The increased percentage of long-fiber-length particles was indicative that cows were preferentially consuming the high-caloric, small-particulate matter instead of the long-fiber fraction.

Samples of individual feedstuffs used in the TMR were visually evaluated to determine the producer's level of feedstuff management. All feed products were well preserved and in good condition. There was very little soil or manure contamination. The product of most concern was a sample of ensiled high-moisture corn, which appeared to be of extremely fine consistency. Given the high moisture content of this product, a high rate of ruminal fermentation should result. Conversely, it is possible for the starch-like component to escape ruminal fermentation and pass quickly into the intestinal tract. The high-moisture corn was shaken using Fisher Scientific brass sieves (#4, #8, #16 and pan). The results were 32% remaining on the #4 sieve, 37% on #8, 13% on #16, and 17% in the pan. Although concerned about the product, we chose to rule this out as a risk factor because of the thorough mixing of the product throughout the TMR, and the overall low levels of total-ration non-fiber carbohydrate (NFC) level, which was determined to be 35.42%.

Since the onset of the increased death rate, the producer kept detailed records of all cows that had died, days-in-milk at death, lactation number and milk production. Additional records were obtained through AgSource Cooperative Services. No clear trends emerged, as milk production of affected cows ranged from 50-120 lb of milk daily, and DIM ranged from 10-455. The producer expressed the opinion that all affected cows were "aggressive eaters" (high dry-matter consumption).

Because milk production is a function of ration energy density and consumption levels, management-level milk production was evaluated in relation to monthly

Table 3. TMR particle separator results.

Sample character	Avg NE Iowa TMR	Subject herd TMR	Subject herd TMR refusals
Long fiber length	8.70%	11.10%	23.40%
Medium fiber length	34.50%	36.80%	35.30%
Short particles	56.90%	52.10%	41.30%

death loss records. Figure 1 shows the herd management-level milk production in relation to deaths caused by JHS. This "standardizes" every current record for DIM, lactation, season of freshening and breed. This measurement allows the producer to compare one test period to another, and to determine the effects of their management and ration expertise on the production level.[30] Figure 1 shows a trend of increased deaths due to JHS as management-level milk increases. Increased milk production could be a potential risk factor in the presentation of JHS.

The heifer population of the cow herd is known for lower overall dry-matter consumption and greater milk production persistency than their greater-than-first-lactation herd mates. Figure 2 shows the "early" milk production of the first, and greater-than-first, lactation individuals compared to occurrences of deaths. "Early" is defined by AgSource Cooperative Services to denote cows that are 1-100 DIM when the record is obtained. There appears to be no association between milk production of the first-lactation heifers and death loss. However, in greater-than-first-lactation cows, there was an association between increased milk production and increased death losses. In this investigation, there have been no deaths in the first-calf heifers.

High carbohydrate levels or low fiber levels may predispose lactating cows to subclinical ruminal acidosis, defined by a rumen pH of < 5.5. Suspect pH levels for subclinical ruminal acidosis are 5.8 or less.[13,29] During low rumen pH, ruminal volatile fatty acid (VFA) proportions change; acetate levels drop, while proprionate levels increase. Since acetate is the precursor to butterfat synthesis, this can result in lowered butterfat in relation to milk protein. Figure 3 shows the monthly DHIA butterfat and milk protein tests in relation to occurrences of JHS. No decrease in herd butterfat levels could be associated with peaks in deaths due to JHS. A confounding factor is that DHIA reporting is a once-monthly snapshot that could miss a transient, whole-herd acidosis event occurring during another part of the month. Of additional concern is that a specific population of cattle (first-calf heifers or fresh cows) may be affected by acidosis that is masked by butterfat percentages produced by the balance of the herd. Figure 3 did not lend evidence that a whole-herd acidosis event triggered presentation of JHS.

To achieve better sensitivity through increased sample frequency, researchers analyzed the bulk-tank pickup records for herd butterfat and milk-protein percentages. Figure 4 represents the JHS events compared to bulk-tank records for butterfat and milk protein. The initial herd visit, and the first and second JHS events, are represented by vertical lines. Records at the time of JHS incidents did not reveal any characteristic whole-herd butterfat decline in relation to milk protein. Whole-herd masking could again be present, maintaining the possibility that ***individual*** acidosis could still play a part in the sporadic presentation of JHS.

To minimize the whole-herd masking effect, herd data were partitioned into sub-populations of DIM and

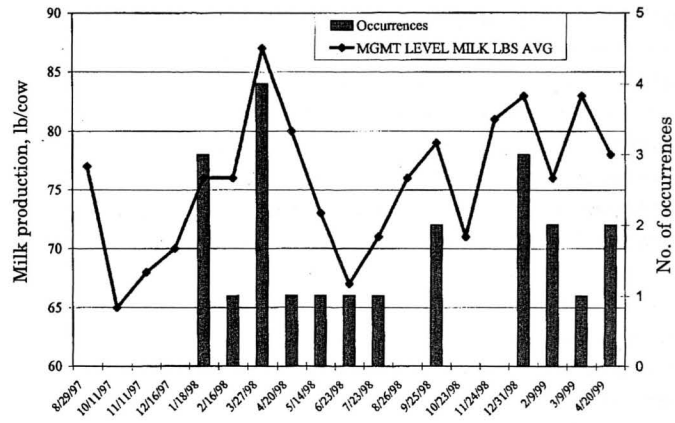

Figure 1. Herd management level milk (standardized 150-day milk) vs. occurrences of death.

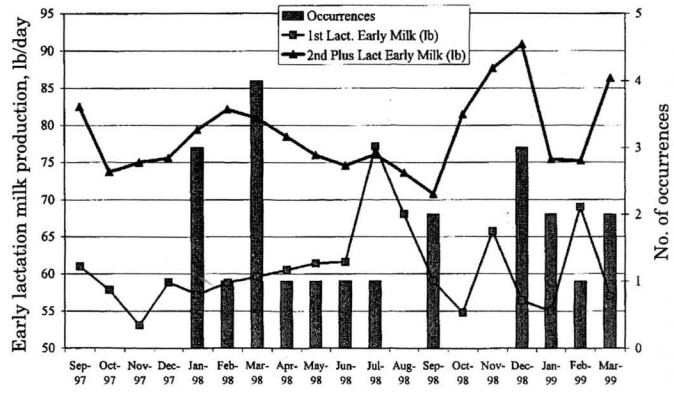

Figure 2. Early milk production of first lactation and greater-than-first-lactation individual vs. occurrences of death.

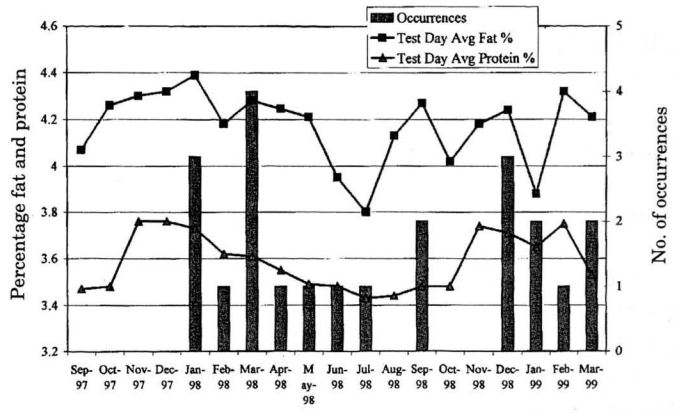

Figure 3. Butterfat and milk protein vs. occurrences of death.

lactation one, compared to the greater-than-first lactation members of the herd (Table 4). The hypothesis of interest was that cows less than 50 DIM, or first-lactation heifers, might be more sensitive to the effects of acidosis as characterized by butterfat depression. There was no evidence in the partitioned data of butterfat levels lower than milk protein which would indicate a protein/butterfat inversion due to subclinical ruminal acidosis.[34] Likewise, there was no evidence to suggest acidosis was a trigger event to the presentation of JHS in any other examined partition. The only abnormality noted was an elevated fat percentage in the 0-50 day population, suggestive of fat mobilization due to ketosis around the freshening event.

Current and previous herd data were then evaluated using scatter plots with milk protein and butterfat percentages on the X and Y axes, respectively. A straight line was placed on the graph to indicate the threshold where butterfat levels equal milk protein. This line represents a ratio equal to 1.0, where butterfat is divided by milk protein. Individual cows below this line were considered to be technically inverted and could be considered at risk of having subacute ruminal acidosis (SARA). Table 5 shows the herd demographics of first-lactation individuals and greater-than-first lactation cows. In both current and previous tests, the greater-than-first-lactation individuals accounted for approximately 66% of the herd. Of the inversions, 77% were incurred by greater-than-first-lactation cows in both current and previous herd tests. Greater-than-first-lactation individuals appeared to be at greater risk of being technically inverted.

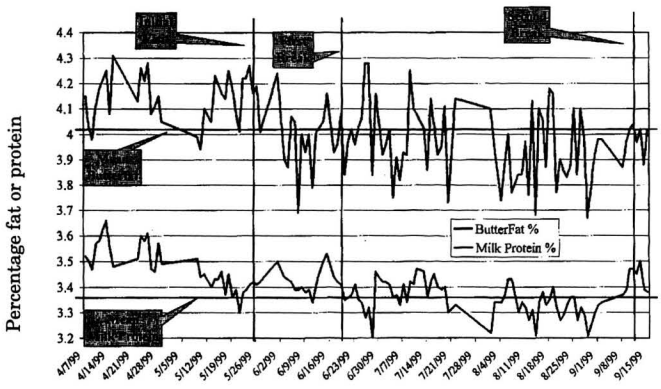

Figure 4. Bulk-tank records for butterfat and milk protein vs. occurrences of death.

Table 6 shows the days-in-milk and milk-production distributions of individuals with technical butterfat inversions. A broad range of milk production was noted for all lactations with technical inversions. In first-lactation cows, inversions were seen only in individuals greater than 250 days-in-milk. Greater-than-first-lactation cows demonstrated inversions in a range of 38 to 436 DIM. This mimics the pattern of DIM distribution of the deaths attributed to JHS in this particular herd. While interesting, it must be remembered that cows with technical inversions can be considered only at risk for SARA. Definitive diagnosis of this condition depends on demonstrating a ruminal pH of 5.5 or less.[13,29]

Diagnostic testing

Prior to the field investigation, the referring veterinarian collected post-mortem tissues and submitted them on three different occasions to diagnostic laboratories at South Dakota State University, University of Wisconsin and Iowa State University (ISU) for culture. Isolations at all three laboratories yielded high numbers of *C. perfringens* type A. Samples submitted to the ISU Diagnostic Laboratory from other dairies experiencing this syndrome also yielded *C. perfringens* type A.

Table 4. Average butterfat and milk protein percentages for current and previous test days partitioned by days-in-milk and by lactation.

	Test day				Previous test day			
Herd	Protein		Fat		Protein		Fat	
partitioning	%	SEM	%	SEM	%	SEM	%	SEM
0-50 Days-in-milk	3.36	0.0957	4.83	0.4821	3.62	0.2120	4.37	0.2140
51-100 Days-in-milk	3.34	0.0751	3.86	0.3558	3.27	0.0522	4.19	0.2463
101-150 Days-in-milk	3.49	0.0960	4.51	0.2994	3.45	0.0619	3.80	0.1949
151-200 Days-in-milk	3.58	0.0702	4.26	0.1782	3.69	0.0504	4.38	0.1009
>200 Days-in-milk	3.68	0.0322	4.03	0.0943	3.88	0.0291	4.47	0.0810
First-lactation cows	3.57	0.0521	4.31	0.2548	3.75	0.0475	4.48	0.1154
2nd-plus lactation cows	3.58	0.0370	4.20	0.1067	3.77	0.0366	4.32	0.0742

Table 5. Distribution by lactation of all cows within the herd, and cows identified as having a butterfat-to-milk protein ratio of <1.0.

May Test Day

	Number	% Herd	Number inverted	Percent inverted	% Lact. inverted	Total % inverted
Lact 1	41	34%	5	23%	12%	
Lact >1	80	66%	17	77%	21%	
Total	121		22			18%

April Test Day

	Number	% Herd	Number inverted	Percent inverted	% Lact. inverted	Total % inverted
Lact 1	39	33%	3	23%	8%	
Lact >1	78	67%	10	77%	13%	
Total	117		13			11%

Management / prospective investigation

With the potential routes of pathogenesis determined, steps were taken to monitor future disease outbreaks:

- *Establishment of a rolling bank of TMR samples* – the producer saved a gallon plastic bag of TMR daily and placed it into a freezer. A total of 10 samples was saved, with the producer removing the oldest sample for disposal on day 11. When the syndrome next struck, there would be 10 days of feed samples available for evaluation of functional fiber amounts and/or sorting.
- *Keep a log of daily feed intake* – as both production and the above-mentioned risk factors are potentially linked to dry matter consumption, we attempted to determine if aberrations in dry matter consumption could lead to the onset of this syndrome. The producer elected not to maintain this record.
- *Submit the next case or fatality* to the ISU Veterinary Teaching Hospital for treatment and/or evaluation.
- *Submit samples from all ensiled feeds* for Clostridial culture as per the isolation procedure and for mycotoxin analysis.
- *Submit several herd fecal samples* to examine for presence of gastrointestinal parasites leading to small intestine motility aberrations.

Table 6. Distribution of days-in-milk and milk production for cows identified as having a butterfat-to-milk protein ratio of <1.0.

First-lactation cows (n = 5)

	Days-in-milk	Milk lb	Butterfat %	Milk protein %
Mean	343	43	3.20	3.78
Median	356	35	3.00	3.80
SE Mean	24.04	9.72	0.15	0.12
Minimum	256	16		
Maximum	398	67		

Greater-than-first-lactation cows (n = 17)

	Days-in-milk	Milk lb	Butterfat %	Milk protein %
Mean	238	67	3.02	3.57
Median	260	59	3.10	3.60
SE Mean	26.52	6.45	0.14	0.06
Minimum	38	16		
Maximum	436	118		

Clostridium culture and isolation

A standardized protocol was developed at the Iowa State University Veterinary Diagnostic Laboratory for isolation of *C. perfringens* type A from feed, haylage, silage and feces, as follows:[22]

- *Direct Culture:* Moisten swab with sterile saline if sample is very dry, and then swab through material. Inoculate and streak for isolation on blood agar, anaerobic blood agar, anaerobic 4% blood agar and incubate at 95°F (35°C). Evaluate the cultures at 24 and 48 hr for *C. perfringens* double zone hemolysis. Isolations are inoculated in chopped meat broth for transportation for toxin analysis and genotyping. If direct isolation fails to detect colonies of *C. perfringens* the laboratory starts the next step of isolations.

- *Straight enrichment:* Place 1 gram of sample in 10ml chopped meat media and incubate anaerobically at 113°F (45°C) for 24 to 48 hr. Streak for isolation on blood agar, anaerobic blood agar, anaerobic 4% blood agar and incubate at 95°F (35°C) and check for characteristic growth pattern of *C. perfringens* at 24 to 48 hr.
- *Heat shock isolation:* Place 1 gram of sample in 10ml chopped meat media and hold at 176°F (80°C) for 10 min. Cool to room temperature and streak for isolation on blood agar, anaerobic blood agar, anaerobic 4% blood agar and incubate at 95°F (35°C) and check for characteristic growth pattern of *C. perfringens* at 24 to 48 hr.
- *Heat shock enrichment:* Same technique as straight enrichment, with the exception that inoculated tubes are held for 24 hr at 113°F (45°C) in an anaerobic environment. The tubes are then held at 176°F (80°C) for 10 minutes and processed according to the above instructions.
- *Toxin analysis and typing:* All isolations were submitted to Dr. Glenn Songer at The University of Arizona.

Initial laboratory results

No ensiled materials had detectable amounts of aflatoxin, ochratoxin, vomitoxin, zearalenone or T-2 toxin. Fecal flotations on mixed samples from the herd were negative for parasite eggs. Efforts to isolate *Clostridium* spp. from the corn silage and high-moisture corn were negative. An alfalfa haylage sample was obtained on the day of the field investigation from the vertical silo (old crop), which was the last of that particular batch. A new crop sample from the same structure was obtained for culture 45 days following ensiling (new crop). Both old and new crop samples yielded *C. perfringens* type A. At this point the silo structure came into question as a possible contaminant of the alfalfa haylage product.

Given the results of contaminated old and new crop alfalfa haylage, we requested the producer to consider putting up haylage in a plastic storage bag. Since this is an item of new manufacture, it could be considered relatively sterile prior to entry of the haylage. Due to the lack of storage alternatives, the producer was willing to do this. This product was also sampled for culture 45 days after filling. Isolation proved difficult, and no *C. perfringens* type A cultures were isolated until the final heat shock enrichment step was performed. On isolation, very low numbers of colonies were present.

Due to the number of positive isolations obtained from alfalfa haylage samples, the investigation team decided to sample haylage from other area dairies. Six samples were obtained from a variety of structures including bunkers, upright silos and plastic bags. Four of the six samples returned positive isolations of *C. perfringens* type A, with one isolation genotyped positive for Beta2 toxin production. The sampled dairies had no history or current cases of JHS.

Further disease outbreaks

When the producer finished feeding the old crop alfalfa haylage, the nutritionist recalculated the ration to include more long-stem hay to counter the loss of haylage. No deaths were reported during the 3.5 weeks that the herd was on the modified ration. As alfalfa haylage became available, the producer reverted back to the original ration with haylage, and within 1.5 weeks of the ration change four cattle were affected overnight on June 21, 1999. Two of the affected cows were found dead, and the two surviving cattle were transported to the Iowa State University Veterinary Teaching Hospital, where both were dead on arrival. Post-mortem examinations were immediately performed by the ISU Pathology Department. Necropsies revealed segmental hemorrhaging, clotting, subsequent intestinal blockage and intestinal intussusceptions in both cows. Cultures were performed on the isolated tissues, revealing large numbers of *C. perfringens* type A colonies by direct isolation method. No Beta2 toxin expression was found in the *C. perfringens* type A isolations and no *Salmonella* species were isolated.

The 10-day TMR sample bank was evaluated using the Penn State Particle Separator.[19] Figure 5 focuses on the top pan (long-stem fiber – Penn State particle separator) percentages of TMR samples collected prior to the June outbreak. There is evidence that on Days 4 and 2 prior to the event, long-stem fiber levels dropped to approximately 6%. Either the hay was processed too finely, or there was a change in the total amount placed in the ration. Combined with sorting by the cows, the ration could have entered an area of carbohydrate risk. Addi-

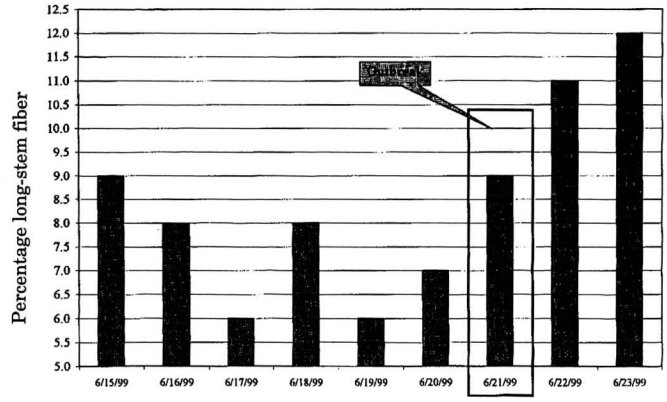

Figure 5. Top pan (long-stem fiber—Penn State particle separator) percentages of TMR samples collected prior to the June outbreak.

tionally, the timing of this outbreak was suggestive that resumption of haylage feeding presented an increased risk due to Clostridial contamination.

On September 13, 1999 the producer again experienced a disease break, with two animals affected. The producer immediately treated the cows orally with 2 gallons of mineral oil and 75 million units of procaine penicillin G. The cows also were administered 6 gm of ampicillin sodium intravenously. Both animals were transported to ISU on the third day following the break. One cow was dead on arrival, and the other was euthanized due to the extremely poor prognosis. The Pathology Department of the ISU College of Veterinary Medicine performed both postmortem examinations, which noted segmental hemorrhage, clotting and blockage. *C. perfringens* type A was again isolated on direct culture, along with a Group B *Salmonella* spp. in both animals.

At this time the producer had not maintained the rolling feed sample bank, making the 10-day samples of TMR unavailable. Prior to the disease break, the producer had run out of corn silage. He refilled the bunker and sealed it. One week later he opened the new corn silage bunker and within two days the outbreak occurred. During this disease break, the producer continued to feed alfalfa haylage from the upright silo sources. Following the outbreak, the vertical silo haylage was nearly exhausted and the TMR was then shifted to using haylage from the plastic bag source, which yielded low culture levels of *C. perfringens* type A. While feeding this source the producer had no further JHS breaks for a period of eight months.

Simultaneous to the second outbreak in the field investigation herd, there were reports of JHS in both large and small dairies across northeast Iowa, southeast Minnesota and southwest Wisconsin surrounding the harvest period of September through November 1999. All breaks during this period were associated with feeding new crop corn silage. As fermentation times progressed, the number of reports decreased.

Results and Discussion

Based on previous isolations of *C. perfringens* type A (by South Dakota State University, University of Wisconsin and ISU), the sporadic and peracute presentation of the syndrome, presentation of a segmental hemorrhagic enteritis and the association with ration changes, the investigators elected to more fully investigate the possibility that *C. perfringens* type A could be a contributor to this syndrome.

Investigators identified possible disease mechanisms based on known Clostridial enteritis presentations. In humans, presence of *C. perfringens* type A causes food poisoning through the activity of *C. perfringens* enterotoxin. In this instance, the risk factor is the presence of the causative organism itself. A second possible mechanism is represented by lamb enterotoxemia disease (overeating disease) caused by C. *perfringens* type D growth and sporulation. Presence of the bacteria in the small intestinal lumen is insufficient to cause disease, and it can only be replicated by direct intestinal injection of the live organism with Dextrin. Once present, ingestion of lush, rapidly growing pasture or cereal crops, or heavy grain feeding in feedlots causes *C. perfringens* type D to proliferate rapidly, sporulate and produce high amounts of toxins, thus causing clinical expression of the disease syndrome.[2] The major risk factor is consumption of high amounts of fermentable carbohydrates. The third model of disease considered was disruption of intestinal motility leading to ingesta stasis, leading in turn to Clostridial overgrowth and sporulation.

For *C. perfringens* to cause disease, three elements are necessary:[8]

1. *C. perfringens* must be present in the intestinal tract.
2. There must be an abundance of nutrients, especially carbohydrates, for organism growth and sporulation.
3. There must be at least a partial slowdown or stoppage of intestinal tract movement brought about by ingesting a particularly large amount of feed, allowing the toxins of *C. perfringens* to accumulate and be absorbed in the gut.

Carbohydrate consumption

The first possible mechanism for Clostridial disease identified the consumption of large amounts of fermentable carbohydrate as a potential risk factor. Milk production is a highly energetic process dependent on ready availability of dietary carbohydrates, protein, rapid ruminal fermentation and high dry matter intake. Presentation of excess carbohydrates, either through over-feeding or preferential sorting by the cow, can be detected through ration evaluation, diagnosis of SARA via rumenocentesis[13,29] or through milk protein/butterfat inversions.[34]

On examination of the diet, investigators concluded that the ration did not appear to have any gross calculation errors, which was supported by wet chemistry analysis. The non-fiber carbohydrate was not excessive, and the net energy of lactation was well within lactating dairy limits. Adequate fiber is critical to normal function of the rumen in providing rumen "scratch factor" that stimulates contractions and a grain particulate "trap" to assist rumen fermentation. In this particular case the acid and neutral detergent fiber appeared adequate at 20.19 and 35.95%, respectively. Additional analysis of TMR physical form using the Penn

State particle separator verified adequate fiber levels, with long- and medium-fiber material occupying 11.1 and 36.8% of the ration, respectively.[19]

TMRs can be preferentially sorted by the cow to gain access to the grain portion of the diet. In severe cases, preferential sorting can lead to SARA. The investigators were concerned that the long-fiber fraction of the ration moved from 11.1 to 23.4% when the feed refusals were compared to the original TMR using Penn State particle separator analysis.[19] On a herd basis this may not be clinically apparent, but an individual with high dry matter intake and who is sorting could be considered at risk of SARA, and possibly JHS, if carbohydrate consumption is a mechanism of risk.

Another possibility for excess carbohydrate consumption was evident during the June outbreak of JHS. TMR samples from the TMR banking procedure were evaluated using the Penn State particle separator.[19] On Days 4 and 3 prior to the outbreak, the long-fiber fraction of the ration dropped to 6.0% of the total ration. These low levels, combined with sorting by the cows, could have placed the herd at additional risk of developing SARA, and possibly JHS.

Milk protein/butterfat inversions can be indicative of SARA.[34] Due to the relationship of this syndrome to carbohydrate consumption, investigators chose to evaluate this as a possible risk indicator for JHS. The herd was evaluated using monthly DHIA records, bulk-tank pickups and individual data partitioned by lactation, days-in-milk and by individuals. The most interesting information from technical inversions came from evaluation of individuals. The greater-than-first-lactation cattle represented 66% of the lactating herd, yet had 77% of the inversions. They also displayed inversions from 38 to 436 DIM. This mimics the JHS presentation in this herd, as the death pattern was limited to greater-than-first-lactation cows ranging from 10-455 DIM. While this finding is not conclusive, it leaves open the possibility that individual subacute ruminal acidosis is a trigger event.

Evaluation of butterfat levels compared to milk protein on a herd and sub-group basis did not demonstrate abnormal risk of either SARA or potential JHS. Although this is an important diagnostic technique, the researcher is constrained by the "snapshot" characteristic of this determination. Carbohydrate overconsumption and SARA can be transient events. It is possible that without daily butterfat or protein determinations we lack the diagnostic sensitivity to associate milk protein/butterfat inversions with an increased risk of JHS.

The most interesting piece of evidence that JHS might be associated with feeding practices or carbohydrate ingestion was shown in Figure 1, which showed association of increased death rates with increased management-level milk. Maximal milk production is a product of carbohydrate consumption and dry matter intake, both of which could be considered as possible risk factors for JHS in this particular herd. Additionally, milk production can be broken down into sub-categories of risk:

1) Presentation of starch (energy). Milk production is related to the presentation of carbohydrates in grain form, and if the energy density of the consumed ration is high it may predispose the affected cow to ruminal acidosis. Energy-dense rations may represent further risk through predisposition of the animal to intestinal Clostridial growth, sporulation and toxin production.
2) Dry matter intake by the cow. Milk production follows energy intake, which follows feed intake.[6,35] Dry matter intake may be a risk factor due to starch consumption levels, or to some as-yet undescribed decrease of intestinal motility related to high ruminal fill rates.
3) Alfalfa haylage consumption. Removal of this product due to a shortage following the field investigation caused an immediate 5 lb (2.27 kg) decrease in milk per cow. One month later the product became available and resulted in a milk-production rebound of the lost 5 lb (2.27 kg). The positive *C. perfringens* type A isolations in this product may present an element of risk from presence of the organism.

Dry matter intake as a risk factor for JHS in this herd was further supported by data in Figure 2 which examined death occurrence rate compared to early milk production of both first-lactation and greater-than-first-lactation individuals. While examination of first-lactation individual milk production showed no association with the death occurrence rate, the greater-than-first-lactation cows did show a production trend that matched the death occurrence rate. This figure was supported by the fact that no first-lactation individuals had been affected with JHS. First-lactation cows are unique due to their increased lactational persistency with lower dry matter intake, compared to older herdmates.

Additional support for carbohydrate intake as a JHS risk factor was demonstrated by the September disease outbreak, in which the herd was fed corn silage that had only been allowed to ferment for one week. The ensiling process consists of bacterial fermentation of plant sugars, with conversion to organic acids and subsequent preservation of the fermented feedstuff. Feeding this product before complete fermentation would offer a product high in soluble carbohydrates that could place the cow at risk of SARA or possibly JHS. This observation was not limited solely to the herd of investigation. The

ISU clinicians were made aware of herds in northeast Iowa, southeast Minnesota and southwest Wisconsin with similar presentations of JHS associated with the early feeding of corn silage. As fermentation times progressed, the reports of JHS diminished by November 1999.

Presence of C. perfringens type A

Because *C. perfringens* type A would have to be present to cause disease, the fermented feedstuffs were submitted for bacterial culture. Alfalfa haylage samples were positive for *C. perfringens* type A on all samples from the subject herd, while the corn silage and high-moisture corn were negative. Because the producer had runout of alfalfa haylage at the time of the field investigation, it was replaced with dry hay. Following ensiling, new alfalfa haylage was placed back in the ration, and within 10 days of the ration change another outbreak occurred. Confounding this observation was the discovery that Clostridial contamination of alfalfa haylage was not unique to this herd. Alfalfa haylage samples from six dairies in northeast Iowa yielded *C. perfringens* type A isolations in four of the locations, with one sample positive for Beta2 toxin production.

While *Clostridium* contamination is not unique to this herd, the owner elected to explore the hypothesis that Clostridial contamination of the alfalfa haylage was elevated and might be due to a contaminated silo structure. The owner placed alfalfa haylage within plastic bags stored on the ground. These structures were sampled following fermentation and found to have very low numbers of *C. perfringens* colonies, and only then after progression through the full shock sporulation technique. The herd was placed on this source of haylage following the September outbreak and experienced eight months free of JHS cases.

Disruption of gut motility

This mechanism was considered a possible risk factor associated with individual high levels of dry matter consumption. We have no direct evidence of a link between dry matter intake and disruption of gut motility, other than the owner's observation that the "aggressive eaters" were predisposed. We do, though, have evidence of gut motility disruption and the presentation of JHS as presented by the two cows submitted from the June 1999 outbreak. Presence of intussusceptions in both animals from one dairy on one day was highly unusual, and points to the possibility of some form of intestinal motility aberration. This abnormal intestinal motility could take the form of either hypo- or hypermotility. In both animals, the intussusception was located directly anterior to the JHS site in the jejunum.

An additional consideration concerning intussusceptions was presented in a retrospective analysis of intussusception in cattle: 336 cases (1964-1993).[7] Analysis of small intestinal intussusception indicated the Brown Swiss breed had an adjusted odds ratio of 4.18, significantly higher than those of the reference group. The Holstein, Jersey, and other dairy breeds had adjusted odds ratios of 1.00, 0.65 and 0.48, respectively. This indicates that the Brown Swiss breed is particularly predisposed to intestinal motility aberrations, and may have increased risk factors with respect to presentation of both intussusceptions and JHS. While there was no evidence to indicate to investigators which lesion presented first, there is information to suggest that enteritis is associated with intussusception.[16,21] Conversely, the increased risk factors for intussusception in Brown Swiss cattle suggest the breed itself is at greater risk. The most important point is that it appears likely that some form of intestinal motility aberration is associated with clinical JHS, and begs the question whether alpha toxin could disrupt neuromuscular transmission and contribute to this syndrome.[31]

We considered other conditions that might contribute to presentation of JHS, including intestinal parasitism and presence of mycotoxins. When evaluated, these items were not found. An aggressive Clostridial vaccination program was pursued by the owner for control of JHS, including the quarterly administration of a commercial 7-way bacterin-toxoid and an autogenous *C. perfringens* type A bacterin-toxoid every 60 days. There was no apparent remission in case incidence rate with use of these vaccines. Conversely, the herd became sensitized to the commercial 7-way product; reduced dry matter intake and a dramatic drop in milk production occurred following administration.

Conclusions

We were unable to identify a cause-and-effect relationship between *C. perfringens* type A and presence of JHS. The data, though, did suggest new avenues for further investigation. This includes possible association between increased milk production levels and increased risk of JHS; onset of JHS with increased soluble carbohydrate levels, as evidenced by the September 1999 outbreak with fresh corn silage; presence of disease following the re-introduction of *C. perfringens* type A-positive alfalfa haylage; and the possible role played by intestinal motility aberrations in the syndrome pathogenesis. The wide range of investigational items would suggest JHS is a true syndrome whose presentation may not be solely dependent on presence of a causative organism, but on the combination of a range of conditions.

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