Case report: Management of *Klebsiella* spp mastitis on a dairy farm

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Abstract

Mastitis caused by *Klebsiella* spp is an emerging issue associated with contaminated environments, often with poor response to therapy. This case report highlights 42 cows with 50 cases of clinical mastitis caused by *Klebsiella* spp. Eight cases of clinical mastitis were from 4 cows with repeat cases. The mean days-in-milk (DIM) at diagnosis was 135; 38% of cows were within the first 100 DIM. Cows diagnosed with mastitis had a 65% decrease in milk production. Severity scores assigned to each case were 24% mild, 33% moderate, and 43% severe. The majority of cows were either culled (38%) or euthanized (19%) as a result of the mastitis. Many of the cows that developed clinical mastitis had been good producing animals with low somatic cell counts prior to the case. Environmental control and cow hygiene are key to prevention of mastitis.

Key words: mastitis, *Klebsiella pneumoniae*, lipopolysaccharide (LPS)

Résumé

La mammite causée par Klebsiella spp est un nouvel enjeu associé aux environnements contaminés et qui souvent ne répond pas très bien à la thérapie. Cette étude de cas cible 42 vaches avec 50 cas de mammite clinique causée par Klebsiella spp. Huit cas de mammite clinique provenaient de quatre vaches avec récidive. Le nombre moyen de jours en lait au diagnostic était de 135 et 38% des cas survenaient avant 100 jours en lait. La production de lait a chuté de 65% chez les vaches diagnostiquées avec la mammite. Le score de sévérité assigné à chaque cas était léger dans 24% des cas, modéré dans 33% des cas et sévère dans 43% des cas. La plupart des vaches ont été soit réformées (38%) ou soit euthanasiées (19%) en raison de la mammite. Plusieurs des vaches qui ont développé la mammite clinique étaient des animaux productifs avec un comptage de cellules somatiques bas avant l'épisode. Le contrôle de l'environnement et l'hygiène des vaches sont essentiels pour prévenir la mammite.

Introduction

Mastitis caused by *Klebsiella* spp, considered to be an emerging problem, is caused primarily by *K. pneumoniae* or occasionally by *K. oxytoca*. For *Klebsiella* spp, prevention of exposure is key to infection control.³³ Intermittent fecal shedding of *Klebsiella* spp from healthy cows allows for environmental contamination.^{15,16,27} In herd and environmental surveys, these bacteria have been isolated from 81% of fecal samples collected from healthy cows. Once in the environment, *Klebsiella* spp populates bedding material by fecal contamination, regardless of bedding type.^{11,27}

Clinical mastitis (CM) caused by *Klebsiella* spp is caused by a profound immune response to lipopolysaccharide (LPS) from the outer cell membrane of the organism.³ Intramammary (IMM) infections with coliform bacteria result in the release of LPS and activation of the immune system, leading to high levels of the pro- and anti-inflammatory cytokines in milk.⁴ Milk cytokine concentrations are greater after IMM infection with *Klebsiella* spp than *E. coli*, and systemic cytokines also persist much longer in *Klebsiella* spp-infected cows than in *E. coli* infections.² This case report describes the case management of the first case of CM caused by *Klebsiella* spp diagnosed in 42 cows on a 400-cow dairy farm from July 1, 2013 to October 1, 2014.

Clinical Report

Herd Information

The surveyed herd is a university teaching and research herd that consists of approximately 400 lactating Holstein (90%) and Jersey (10%) cows. The cows were housed in a naturally ventilated 4-row freestall barn, and fed a total mixed ration (TMR) twice daily. Freestalls had a mattress base and were bedded with recycled manure solids generated on site. The manure solids did not undergo further processing, and were approximately 35% dry matter when added to the freestalls. The farm protocol was to manually clean the backs of the freestalls 3 times per day, with new bedding added every 2 to 3 days.

Cows were on a regular vaccination schedule, which included vaccination with a J5 bacterin^a given at dry-off and 30 ± 3 days pre-calving. Pregnant heifers were given the J5 bacterin at 60 ± 3 days pre-calving and 30 ± 3 days pre-calving. All lactating cows were again vaccinated 30 ± 3 and 90 ± 3 days-in-milk (DIM).

Any cow identified as having mastitis was examined by the veterinary team. As this is a research and teaching herd, animal treatments were directed by farm treatment protocols and administered by veterinary students as part of their training. Following physical examination, a severity score was assigned based on systemic and local (udder) clinical signs using parameters adapted from Wenz et al.²⁹ Initial therapy was instituted per the farm's treatment protocol based on the severity score (Table 1). A milk sample was collected aseptically and cultured using NMC guidelines as a guide for IMM therapy.¹⁷ Milk samples from new cases of mastitis were cultured the same day by the veterinarian diagnosing the mastitis case. Intramammary antibiotic therapy was withheld pending milk culture results. For CM cases where the culture result yielded "no growth" or a gram-negative organism, the cow did not receive IMM antibiotics per the farm's treatment protocol.

Clinical Case Summary

From July 1, 2013 to October 1, 2014, milk samples from 287 cows and 354 quarters were evaluated for mastitis and submitted for culture. Forty-two cows were diagnosed with 50 cases of *Klebsiella* spp mastitis over this time period, which accounted for 14% of all mastitis cases. The majority of *Klebsiella* spp cases were diagnosed in the summer months, especially July. The culture outcome from the 354 quarter

Table 1. Treatment protocol for systemic therapy in mastitis cases.

Mild	•No systemic therapy indicated. Reevaluate in 24 hours
Moderate	Ceftiofur ^b – 1 mg/lb (2.2 mg/kg) subcutaneously or intramuscular every 24 hours for 3 days As needed: Flunixin meglumine ^c – 1 mg/lb (2.2 mg/kg) intravenously every 24 hours Oral fluids – 10 to 15 gallons water plus 1 pound alfalfameal orally
Severe	Ceftiofur – 1 mg/lb (2.2 mg/kg) subcutaneously or intramuscular every 24 hours for 3 to 5 days Flunixin meglumine – 1 mg/lb (2.2 mg/kg) intravenously every 24 hours as needed Hypertonic saline 7.2% ^d – 5 mL/kg intravenously via rapid infusion Oral fluids – 10-15 gallons water orally immediately following hypertonic saline administration As needed: Calcium gluconate 23% ^e – 250 to 500 mL intravenously given as a slow infusion Calcium chloride/calcium sulfate bolus ^f – 1 bolus orally

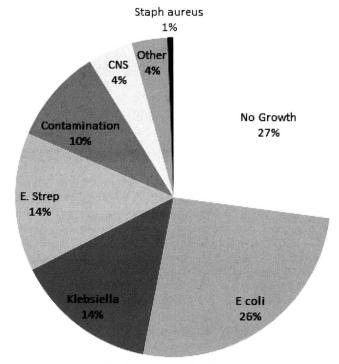


Figure 1. Culture results by organism for 354 clinical mastitis samples collected from July 1, 2013 to October 1, 2014.

milk samples are shown in Figure 1, and cases of mastitis and *Klebsiella* spp mastitis are presented by month in Figure 2.

The distribution of first-cases per cow by lactation group, severity, and outcome are shown in Table 2. Of the 42 cows identified with *Klebsiella* spp, 4 (9.5%) were first-lactation cows, 14 (33.3%) were second-lactation cows, and 24 (57.2%) were third and greater lactation cows. Days-in-milk on the day of mastitis diagnosis ranged from 2 to 478, with a mean of 142 (median = 141). There were 16 cases in the first 100 DIM, 13 between 101 to 200 DIM, and 13 between 201 to 300 DIM.

Severity scores for the first clinical *Klebsiella* spp cases diagnosed in 42 cows included 10 (24%) mild cases, 14 (33%) moderate cases, and 18 (43%) severe cases. Of the 42 cows, 18 (43%) remained on the farm and returned to production, 16 (38%) were culled due to low milk production as a result of clinical mastitis, and 8 (19%) were euthanized or died on-farm. Six of the cows that died were originally diagnosed with severe mastitis. One cow was originally classified as a moderate case, but clinical signs worsened 48 hours after initial diagnosis, and 1 cow was classified as a moderate case of mastitis, but also had a concurrent right displaced abomasum. That cow was euthanized due to surgical complications.

Somatic cell count (SCC) data from DHIA tests was available for 37 cows prior to mastitis diagnosis. Twenty-three cows (62%) had a linear score below 4.0 before mastitis, with a median linear score of 2.6 for all cows with SCC data prior to the first case. Following mastitis diagnosis, SCC data from

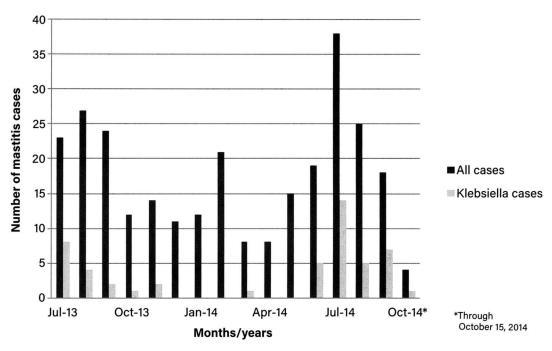


Figure 2. Total number of cases and cases of mastitis caused by Klebsiella spp by month from July 1, 2013 to October 15, 2014.

Table 2. Case numbers of mastitis for lactation number, severity score, and outcome categorized by days-in-milk for each case of *Klebsiella* spp mastitis.

		Days-in-milk				Totals		
		1-100	101-200	201-300				
Lactation number	1	1	0	3		4		
	2	6	3	5		14		
	3+	9	10	5		24		
Severity score	1	3	3	4		10		
	2	5	3	6		14		
	3	8	7	3		18		
Outcome	Retained	7	5	6		18		
	Sold	4	6	6		16		
	Died	5	2	1		8		

DHIA test were available for 20 cows. There were 6 cows (30%) below a linear score of 4 after the case of mastitis, with a mean and median linear score of 3.7 and 3.1, respectively (range 0.3 to 7.7).

Mean test-day milk production prior to mastitis diagnosis was 88 lb (40 kg), with a range of 46 to 118 lb (20.9 to 53.6 kg) per day. Individual daily milk weight records were available for 22 of the 42 cows diagnosed with mastitis (Figure 3). The mean and median milk production for the 3 days immediately prior to the mastitis event was 71.8 lb (32.6 kg) and 69.1 lb (31.3 kg), respectively, with a range of

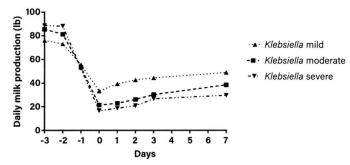


Figure 3. Mean daily milk production (lb) for cows diagnosed with their first case of *Klebsiella* spp mastitis by severity score.

12.8 to 117.2 lb (5.8 to 53.3 kg). Mean daily milk production was 25.3 lb (11.5 kg) on the day the cows were diagnosed with mastitis, a 65% decrease compared to the 3-day average previous milk production. The range of daily milk production change on the day of mastitis diagnosis was +0.9 lb to -83.2 lb (+0.4 kg to -37.8 kg).

Of the 22 records available, 15 represent cows which remained in the herd (Figure 4), and the remaining 7 are from cows that were culled. Of the animals remaining in the herd, their 3-day average milk production prior to mastitis was 80.2 lb (36.5 kg). On the day of mastitis diagnosis, their average milk production was 31.4 lb (14.3 kg). The 3-day average for milk production post-mastitis was 49.6 lb (22.5 kg), and averaged 58.0 lb (26.3 kg) 7 days post-mastitis. The 7 cows culled from the herd had a 3-day pre-mastitis milk production average of 88.1 lb (40 kg), but averaged only 10.3 lb (4.7 kg) on the day of mastitis diagnosis. During the 3 days

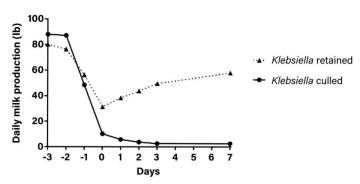


Figure 4. Daily milk weights (lb) 3 days before and 7 days after mastitis for cows culled (n=7) and retained (n=15) for cows diagnosed with their first case of *Klebsiella* spp clinical mastitis.

post-mastitis, average milk production was 2.6 lb (1.2 kg), and averaged 2.4 lb (1.1 kg) 7 days after mastitis diagnosis.

Cows on this dairy were not grouped by lactation, stage of lactation, or milk production after leaving the fresh pen at approximately 20 to 30 DIM. Cow-pen location within the barn on the day of mastitis diagnosis was relatively equally distributed throughout the barn. However, a higher percentage of cows (8 of 42 cases; 19%) diagnosed with *Klebsiella* spp were located within the fresh pen. Forty percent of the cows diagnosed with *Klebsiella* spp were within the first 100 DIM, which was similar to cows diagnosed with *E. coli* mastitis, where 46% of those cases were diagnosed in the first 100 DIM.

This herd was aggressively vaccinated with J5 bacterin. Despite vaccination, 83 cows were identified with E. coli mastitis over the period of this clinical report. Of these 83 cows, only 1 cow (1%) died and 14 (15%) were sold as a result of the mastitis. This is significantly lower than the 57% removal rate for the cows with Klebsiella spp mastitis (P<0.0001, Chi square).

Daily milk records and clinical severity scores were available for 37 of the cows diagnosed with a first case of *E. coli* mastitis (Figure 5). Cows diagnosed with mild or moderate *E. coli* mastitis averaged 86.5 lb (39.3 kg) of milk 3 days prior to diagnosis. On the day of mastitis diagnosis, cows

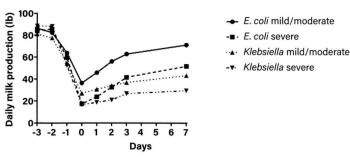


Figure 5. Mean daily milk weights (lb) 3 days before and 7 days after diagnosis with mild/moderate or severe *Klebsiella* spp or *E. coli* mastitis.

with mild and moderate mastitis caused by *E. coli* produced an average of 36.4 lb (16.5 kg) of milk. These cows averaged 62.9 lb (28.6 kg) and 71.5 lb (32.5 kg) of milk 3 and 7 days after mastitis diagnosis. Cows diagnosed with severe mastitis caused by *E. coli* averaged 84.4 lb (37.8 kg) of milk 3 days prior to mastitis diagnosis. On the day of mastitis diagnosis, they averaged 19.5 lb (8.8 kg) of milk production, 43.3 lb (19.7 kg) 3 days after mastitis diagnosis, and 51.9 lb (23.6 kg) 7 days after mastitis diagnosis.

Outcome measures were compared between cows diagnosed with *E. coli* and *Klebsiella* spp. Cows diagnosed with *E. coli* and *Klebsiella* spp mastitis had similar odds of developing severe clinical mastitis (OR: 0.86; P=0.72; 95% CI 0.38-1.94). However, if diagnosed with *Klebsiella* spp mastitis, cows were 5.5 (P<0.0001; 2.3-12.5 95% CI) times more likely to be culled and 19 (P=0.0003; 2.32-160 95% CI) times more likely to die or be euthanized on-farm.

Discussion

This case report highlights the disease process, case management, environmental considerations, and clinical outcomes of 42 cows with mastitis caused by *Klebsiella* spp. Much of the available literature for the treatment of *Klebsiella* spp mastitis is merged with the treatment and management of coliform mastitis. Due to the use of treatment protocols, these cases of mastitis were treated in a similar manner. Each cow was assessed by 1 of the authors as part of clinical instruction to senior veterinary students.

The potential severity of mastitis caused by *Klebsiella* spp compared to other mastitis pathogens has long been known, ^{21,22} but the prevalence of herds dealing with this pathogen appears to be increasing. ²² Clinical mastitis caused by a gram-negative organism is associated with the release of LPS from the outer membrane of the bacteria and the accompanying immune response of the host. Free LPS present in milk, and potentially blood, is recognized through the chauffeuring actions of CD-14 and LPS binding protein of free LPS to toll-like receptor (Tlr)-4 on the surface of monocyte lineage and other cells. Tlr-4 binding of LPS results in an upregulation of pro- and anti-inflammatory cytokines, such as IL-8, IL-10, IL-1ß, and TNF- α , leading to the clinical signs associated with the infection. ^{2,3,4}

Variation in severity between the different coliform agents may lie within differences in the lipid A structure of the LPS molecule. Studies of the lipid A structure of various gram-negative mastitis-causing bacteria have shown that pathogenic *E. coli* has 6 acyl groups within its lipid A structure, while *Klebsiella pneumoniae* have 7 and *Serratia marcesens* have 5 acyl groups. A reduction of 1 acyl group has shown to cause a 100-fold reduction in LPS biological activity.^{5,6,14} One research group has demonstrated reduced lethality of *Klebsiella pneumoniae* with mutant genes that have decreased acylation capabilities of LPS compared to wild-type organisms in a murine pneumonia model.⁷ This

suggests that bacterial organisms with an increased number of acyl groups in their lipid A structure may have an increased ability to stimulate cytokine production following binding with Tlr-4, and/or have increased resistance to the killing effects of cationic antimicrobial proteins. 5,7 The symmetric structure of the acyl groups also confers pathogenicity. Low pathogenic *E. coli* have more symmetric acyl groups in the lipid A fraction, where highly pathogenic *E. coli* have less symmetry of the acyl groups of the lipid A.

Most cows with *Klebsiella* spp identified in this report were healthy, high producing cows. The average test-day production of the cows affected with mastitis in this report was higher than the herd average, which was likely due to a high percentage (38%) of the cases occurring in the first 100 DIM, and none occurring >300 DIM, similar to a report by Oliveira et al.¹⁹ During this same time period, 46% of all the *E. coli* cases diagnosed on the farm occurred within the first 100 DIM.

There were an additional 8 cases of Klebsiella spp mastitis in 4 cows. These 4 cows were eventually culled from the herd due to low productivity. Two cows were diagnosed with clinical mastitis in an adjoining quarter within 2 days of the initial case of mastitis. The remaining 2 cows accounted for 6 additional cases of Klebsiella mastitis. The first cow had 3 diagnoses of mastitis, in the same quarter, with 10 months between the first and second diagnosis, and 1 month between the second and third diagnosis. The second cow was diagnosed with 5 episodes of Klebsiella spp mastitis. This cow had 3 separate quarters involved, with the left hind diagnosed 3 times with mastitis. There were 44 days and 350 days between the cases, including a dry period. The other 2 cases in this cow were in adjacent quarters, and diagnosed within 7 days of the case involving the left rear quarter. These 8 cases were both new cases and persistent infections. However, to fully evaluate if they were truly persistently infected, PCR of the isolate would have been prudent.

Klebsiella spp accounted for 14% of clinical mastitis cases in this herd. Incidence rates of 5.3% to 8% were reported in other field reports of clinical mastitis. 16,23 The majority of literature sources related to Klebsiella spp mastitis indicate that the organism originates from the environment, but at least 1 report suggested that outbreaks of Klebsiella spp mastitis could be spread through a contagious route.¹⁹ In an attempt to define the cause of the outbreak, the herd described in the current report implemented a quarter-level culturing program for cows with sub-clinical mastitis, defined as cows that had DHIA linear scores >4 for 2 consecutive tests and were positive on the California Mastitis Test. Klebsiella spp was isolated from only 1.9% of the 976 quarter milk samples included in this program, indicating that persistent IMM infections were not the underlying cause of this herd's outbreak. Additionally, these data support our premise that not treating clinical Klebsiella spp cases with IMM antibiotics would not lead to the development of persistent IMM infections. Of course, the use of advanced diagnostic modalities, such as DNA sequencing, will provide further insight into the relatedness of mastitis isolates. The authors are currently engaged in an investigation to further answer this question.

In the current study, 43% of the first cases of *Klebsiella* spp mastitis in 42 cows were classified as severe, compared to 31% and 32.6% published by Roberson et al and Oliveira et al, respectively. Differences in severity may be due to differences in scoring rubric used by Roberson et al, 20 or interpretation of clinical signs. Additionally, the number of cases classified as severe could simply be related to more hot weather months during the time frame of this report. Six of the 8 cows that died of *Klebsiella* spp mastitis were classified as severe cases. Furthermore, only 4 of 18 cows classified as severe cases remained in the herd.

Decreased milk production was the most notable clinical sign of these mastitis cases. Many cows were identified by farm staff due to a sharp decline in milk production and the presence of a firm, hot affected quarter. Mean daily milk weight of cows on the day of diagnosis was 35% of previous milk production. This is comparable to data from a challenge model reported by Bannerman et al. In that report, cows infected with *Klebsiella* spp had a 60% decrease in milk production compared to saline controls.³

Treatment of Mastitis Cases

Treatment of individual cows presented in this case report was based on daily physical exams, utilization of inhouse milk culturing, and use of on-farm records, including individual daily milk weights. On initial exam, every case of mastitis was given a severity score and treatment based on severity score, except for IMM antibiotic therapy, which was withheld until culture results were available. The farm treatment protocol specified parenteral ceftiofur be administered to cows with moderate and severe mastitis severity scores, starting at the initial exam prior to knowing the culture outcome. The severity scoring system used in the evaluation of these cows has been shown to have better specificity and accuracy for predicting bacteremia than scoring systems that utilize local (milk and udder) clinical signs only.³⁰

In a 2001 study, 23% of cows with moderate and 48% of severe cases of mastitis had bacteremia. ²⁹ The bacteremia is believed to be related to leukopenia and a decrease in host defenses. Bacteremia was associated with lower survival rates when a gram-negative mastitis organism is involved. ²⁹ However, culling rates in cows with and without bacteremia were not different. ²⁹ Erskine et al reported a decrease in the culling and death rates in cows with severe coliform mastitis when given ceftiofur systemically. ¹⁰ Treatment with systemic ceftiofur was not effective in altering the outcome of mild cases of clinical mastitis. ³¹

Flunixin meglumine was also administered to cows in the present study with moderate and severe mastitis scores. Administration was based on the clinical signs of fever and/ or udder swelling. Per farm protocol, fever was defined as

a rectal temperature of 103.5°F (39.7°C) or greater. In most cases of moderate to severe *Klebsiella* spp mastitis, the affected quarter was severely swollen, and NSAID therapy did not reduce swelling.

Some literature has shown a benefit to using flunixin as part of the treatment regime for coliform mastitis; the benefits of flunixin are mostly limited to fever reduction and increased rumen motility; 9,28 however, a 2012 report showed that flunixin increased dry matter intake and milk production. Other reports showed that flunixin did not improve milk production in cows with acute coliform mastitis. There was no difference in survival rates between cows affected with toxic mastitis that were treated with flunixin only, flunixin plus isotonic fluids, and isotonic fluids only. 13

Cows with severe mastitis scores were also administered hypertonic saline and calcium. The hypertonic saline coupled with water via an orogastric tube was given to correct dehydration and hypovolemia due to endotoxic shock. Calcium was given to cows with severe mastitis to correct subclinical hypocalcemia.^{21,22,25}

A study using a 5-day course of ceftiofur as an IMM infusion showed a bacteriologic cure rate of 57% and 19% for treated and control cows, respectively.²³ These results were in mild and moderate cases of mastitis, not severe cases. Following publication of this manuscript,23 the dairy in this report implemented a 5-day IMM ceftiofur treatment comparison to no IMM treatment in cows with moderate or severe Klebsiella spp mastitis. While only a small clinical observational study, after 20 cows were enrolled, the proportion of cows that survived, were culled, or died was nearly identical. Further intramammary treatment of *Klebsiella* spp mastitis cases in this herd was not continued due to the poor response to IMM antibiotics. Further research is needed to evaluate the impact of IMM treatment on cows that are more severely affected. A study using intramammary amoxicillin had microbiological cure rates of 27% and 36% at days 7 and 36 post-treatment, respectively.21 A Wisconsin study of 51 farms reported an odds ratio of 1.31 (CI = 0.45-3.82) for bacteriologic cure rate for 49 treated cases. 19 In this paper, the severity scores and clinical cure rate for Klebsiella spp were not reported. Based on the results of our work in this herd and the fact that gram-negative clinical mastitis is the result of LPS release, the authors do not feel that IMM antibiotics are prudent for controlling the clinical signs associated with coliform mastitis.

Klebsiella spp and the Environment

Approaches to controlling environmental mastitis pathogens such as *Klebsiella* spp involve reducing the exposure of teat ends to vectors harboring infectious doses of bacteria. Feces is the most implicated source of *Klebsiella* spp in the cow's environment.¹⁵ In a report by Munoz and Zadoks, the prevalence of *Klebsiella* spp in feces was 81%, with fecal shedding in healthy cows being transient in nature.¹⁶ Historically, *Klebsiella* spp were associated with the use of sawdust

bedding, but have been found on freestall surfaces without bedding, sand, and recycled manure solids.¹⁵

Due to the acute nature of *Klebsiella* spp mastitis cases, recommendations in the current case were focused on prevention of new mastitis cases, with most recommendations directed toward the cow's environment and reduction of fecal contamination. These recommendations included: frequent removal of all soiled bedding material; movement of dry bedding material from the front of the stall to the area under the rear of the cows; frequent cleaning of cross-overs and traffic areas; and cleanliness of the milking center including holding areas. In practice, many of these recommendations were not accomplished in this herd on a regular basis for a variety of reasons.

The farm described in this report utilized recycled manure solids as the bedding source. A study by Sorter et al showed that daily removal of bedding was superior to removal of fecal material only. In that study, the authors showed a 10-log reduction of *Klebsiella* spp in the bedding material.²⁶ When bedding material is not removed from stalls, the moisture content increases and freestall cleanliness decreases, which leads to increased colony forming units of *Klebsiella* spp.³⁴ Composting of manure solids prior to placement into the freestall was another option; however, a reduction in *Klebsiella* spp has not been reported in the literature.⁸

In the herd represented in the current report, the organism was ubiquitous due to the use of manure solids bedding. There was no clear association of pens with clinical cases, with the exception that the fresh pen had more cases of *Klebsiella* spp mastitis. Here, immune suppression in the periparturient period is a likely contributing factor. Additionally, clinical cases were uncommon in first-lactation animals in this herd. The majority of *Klebsiella* spp mastitis cases occurred during the summer months. The farm utilized fans and sprinklers for heat abatement, which increased the amount of water in the environment. There was no slope to the pen floors, thus standing water was normal in the alleys, especially at the feedbunks where sprinklers were mounted. Water troughs have also been implicated as a source of *Klebsiella* spp in the environment.²⁷

Converting to sand bedding had been strongly recommended as an alternative to the recycled manure solids in this herd. This conversion was not without trade-offs, with disposal of sand-laden manure as the top issue. Since the farm's current manure storage facility does not handle sand-laden manure, current disposal options for the farm would include hauling manure onto fields daily, installing a sand settling lane and utilizing the recovered sand as bedding, or composting the sand-laden manure for a nominal fee. The financial constraints of adding sand-handling facilities was deemed too great and those plans were not pursued. Additionally, the authors are aware of many dairy operations that bed with sand, but still have *Klebsiella* spp mastitis problems, so changing bedding would not completely eliminate the risk.

Based on the results in this clinical report and the clinical experiences of others, it is imperative that strategies to prevent new IMIs is more cost-effective than treatment.

This case report summarized 1 herd's struggles with CM associated with *Klebsiella* spp. This herd was later utilized for a USDA licensing trial of a *Klebsiella* spp bacterin against bacterial siderophore receptor and porin proteins. Efficacy data was recently published,¹² and the commercial product is now available.¹

Conclusions

Treatment and control of mastitis caused by *Klebsiella* spp is difficult and requires a multimodal approach. A thorough physical exam and understanding of all clinical signs associated with endotoxemia is paramount to treating cows with *Klebsiella* spp mastitis. Correction of dehydration, prevention of bacteremia, and reduction in fever are the key considerations. However, due to the severity of clinical cases and poor success of clinical therapy, prevention strategies must be at the forefront of herd control of *Klebsiella* mastitis. Due to the ubiquitous nature of the *Klebsiella* bacterium in the dairy environment, facilities and cow hygiene are the key to prevention.

Endnotes

- ^a Enviracor J-5 Bacterin, Zoetis Animal Health, Florham Park, NI
- ^bExcenel[®], Zoetis Animal Health, Florham Park, NJ
- ^c Flunixin meglumine, VetOne, MWI, Boise, ID
- d Hypertonic saline 7.2%, VetOne, MWI, Boise, ID
- e Calcium gluconate 23%, VetOne, MWI, Boise, ID
- ^f Bovikalc®, Boehringer Ingelheim Animal Health, Dublin, GA
- g Blood Agar Plate, Remel Products, Lenexa, KS
- ^hMacConkey Agar plate, Remel Products, Lenexa, KS
- i Gorden PJ, unpublished data
- j KLEBVax™ SRP®, AgriLabs, St. Joseph, MO

References

- 1. Anderson KL, Smith AR, Shanks RD, Davis LE, Gustafsson BK. Efficacy of flunixin meglumine for the treatment of endotoxin-induced bovine mastitis. *Am J Vet Res* 1986;47:1366-1372.
- 2. Bannerman DD. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. *J Anim Sci* 2009;87:10-25.
- 3. Bannerman DD, Paape MJ, Hare WR, Hope JC. Characterization of the bovine innate immune response to intramammary infection with *Klebsiella pneumoniae*. *J Dairy Sci* 2004;87:2420-2432.
- 4. Bannerman DD, Paape MJ, Hare WR, Sohn EJ. Increased levels of LPS-binding protein in bovine blood and milk following bacterial lipopolysaccharide challenge. *J Dairy Sci* 2003;86:3128-3137.
- 5. Bardoel BW, van Strijp JA. Molecular battle between host and bacterium: Recognition in innate immunity. *J Molecular Recognition* 2011;24:1077-1086. 6. Caroff M, Karibian D. Structure of bacterial lipopolysaccharides. *Carbohydrate Research* 2003;338:2431-2447.

- 7. Clements A, Tull D, Jenney AW, Farn JL, Kim S-H, Bishop RE, McPhee JB, Hancock REW, Hartland EL, Pearse MJ, Wijburg OLC, Jackson DC, McConville MJ, Strugnell RA. Secondary acylation of *Klebsiella pneumoniae* lipopolysaccharide contributes to sensitivity to antibacterial peptides. *J Biological Chem* 2007;282:15569-15577.
- 8. Cole KJ, Hogan JS. Short communication: Environmental mastitis pathogen counts in freestalls bedded with composted and fresh recycled manure solids. *J Dairy Sci* 2016;99:1501-1505.
- 9. Dascanio JJ, Mechor GD, Grohn YT, Kenney DG, Booker CA, Thompson P, Chiffelle CL, Musser JMM, Warnick LD. Effect of phenylbutazone and flunixin meglumine on acute toxic mastitis in dairy cows. *Am J Vet Res* 1995;56:1213-1218.
- 10. Erskine RJ, Bartlett PC, VanLente JL, Phipps CR. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. *J Dairy Sci* 2002;85:2571-2575.
- 11. Godden S, Bey R, Lorch K, Farnsworth R, Rapnicki P. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. *J Dairy Sci* 2008;91:151-159.
- 12. Gorden PJ, Kleinhenz MD, Ydstie JA, Brick TA, Slinden LM, Peterson MP, Straub DE, Burkhardt DT. Efficacy of vaccination with a *Klebsiella pneumoniae* siderophore receptor protein (SRP®) vaccine for reduction of *Klebsiella* mastitis in lactating cattle. *J Dairy Sci* 2018;101:10398-10408.
- 13. Green MJ, Green LE, Cripps PJ. Comparison of fluid and flunixin meglumine therapy in combination and individually in the treatment of toxic mastitis. *Vet Rec* 1997;140:149-152.
- 14. Llobet E, Campos MA, Gimenez P, Moranta D, Bengoechea JA. Analysis of the networks controlling the antimicrobial-peptide-dependent induction of *Klebsiella pneumoniae* virulence factors. *Infect and Immunity* 2011;79:3718-3732.
- 15. Munoz MA, Ahlstrom C, Rauch BJ, Zadoks RN. Fecal shedding of *Klebsiella pneumoniae* by dairy cows. *J Dairy Sci* 2006;89:3425-3430.
- 16. Munoz MA, Zadoks RN. Patterns of fecal shedding of *Klebsiella* by dairy cows. *J Dairy Sci* 2007;90:1220-1224.
- 17. National Mastitis Council. *Laboratory handbook on bovine*. Madison, WI. 1999.
- 18. Oliveira L, Hulland C, Ruegg PL. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. *J Dairy Sci* 2013;96:7538-7549.
- 19. Oliveira L, Ruegg PL. Treatments of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. *J Dairy Sci* 2014;97:5426-5436.
- 20. Roberson JR. Treatment of *Escherichia coli* and *Klebsiella*: Mild to moderate cases, in *Proceedings*. North Am Vet Conf , 2003.
- 21. Roberson JR, Warnick LD, Moore G. Mild to moderate clinical mastitis: Efficacy of intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin, and frequent milk-out treatment versus no treatment *J Dairy Sci* 2004;87:583-592.
- 22. Schukken Y, Chuff M, Moroni P, Gurjar A, Santisteban C, Welcome F, Zadoks R. The "other" gram-negative bacteria in mastitis: *Klebsiella, Serratia,* and more. *Vet Clin North Am Food Anim Pract* 2012;28:239-256.
- 23. Schukken YH, Zurakowski MJ, Rauch BJ, Gross B, Tikofsky LL, Welcome FL. Noninferiority trial comparing a first-generation cephalosporin with a third-generation cephalosporin in the treatment of nonsevere clinical mastitis in dairy cows. *J Dairy Sci* 2013;96:6763-6774.
- 24. Seydel U, Oikawa M, Fukase K, Kusumoto S, Brandenburg K. Intrinsic conformation of lipid A is responsible for agonistic and antagonistic activity. *European J Biochem* 2000;267:3032-3039.
- 25. Smith BP, ed. *Large animal internal medicine*. 4th ed. London: Mosby Elsevier, 2008;1872.
- 26. Sorter DE, Kester HJ, Hogan JS. Bacterial counts in recycled manure solids bedding replaced daily or deep packed in freestalls. *J Dairy Sci* 2014;97:2965-2968.
- 27. Verbist B, Piessens V, Van Nuffel A, De Vuyst L, Heyndrickx M, Herman L, Van Coillie E, De Vliegher S. Sources other than unused sawdust can introduce *Klebsiella pneumoniae* into dairy herds. *J Dairy Sci* 2011;94:2832-2839.
- 28. Wagner SA, Apley MD. Effects of two anti-inflammatory drugs on physiologic variables and milk production in cows with endotoxin-induced mastitis. *Am J Vet Res* 2004;65:64-68.

- 29. Wenz JR, Barrington GM, Garry FB, McSweeney KD, Dinsmore RP, Goodell G, Callan RJ. Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows. *J Am Vet Med Assoc* 2001;219:976-981.
- 30. Wenz JR, Garry FB, Barrington GM. Comparison of disease severity scoring systems for dairy cattle with acute coliform mastitis. *J Am Vet Med Assoc* 2006;229:259-262.
- 31. Wenz JR, Garry FB, Lombard JE, Elia R, Prentice D, Dinsmore RP. Efficacy of parenteral ceftiofur for treatment of systemically mild clinical mastitis in dairy cattle. *J Dairy Sci* 2005;88:3496-3499.
- 32. Yeiser EE, Leslie KE, McGilliard ML, Petersson-Wolfe CS. The effects of experimentally induced *Escherichia coli* mastitis and flunixin meglumine administration on activity measures, feed intake, and milk parameters. *J Dairy Sci* 2012;95:4939-4949.
- 33. Zadoks RN, Griffiths HM, Munoz MA, Ahlstrom C, Bennett GJ, Thomas E, Schukken YH. Sources of *Klebsiella* and *Raoultella* species on dairy farms: Be careful where you walk. *J Dairy Sci* 2011;94:1045-1051.
- 34. Zdanowicz M, Shelford JA, Tucker CB, Weary DM, van Keyserlingk MAG. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. *J Dairy Sci* 2004;87:1694-1701.

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