PEER REVIEWED

Case Report–Herd Investigation into the Role of *Pasteurella multocida* in an Outbreak of Mastitis

Michael J. Maroney, *DVM*; **Pamela L. Ruegg,** *DVM, MPVM, DABVP-Dairy*
Department of Dairy Science, University of Wisconsin, Madison, WI 53706
Address correspondence to Dr. Maroney

Abstract

An epizootic of clinical mastitis was investigated on a 225-cow dairy farm in Wisconsin. The authors were called to the farm because of a rising bulk tank somatic cell count (BTSCC) and an increased number of clinical mastitis cases refractory to treatment. The BTSCC had peaked at 974,000 in the month preceding the visit. During an initial herd visit, the investigators were able to obtain a herd history, inspect the facilities and observe the milking procedure. Management deficiencies were identified which could be contributing to the herd outbreak. Composite culturing of cows experiencing clinical mastitis indicated that *Pasteurella multocida* was present in 11 of 23 samples. Environmental sampling was unsuccessful in identifying a source for the outbreak. Recommendations for controlling mastitis were adopted and successful in reducing the spread of mastitis and lowering the BTSCC.

Résumé

Une épizootie de mammite clinique a été étudiée dans une ferme laitière de 225 têtes au Wisconsin. Les auteurs se sont rendus à la ferme en raison de l'augmentation du compte des cellules somatiques dans le lait du réservoir et du nombre de cas de mammite clinique résistants au traitement. Le compte des cellules somatiques avait plafonné à 974 000 le mois précédent la visite. Durant la première visite à la ferme, les enquêteurs ont pu obtenir de l'information sur le troupeau, inspecter les bâtiments et observer la méthode de traite. Des lacunes dans la gestion pourraient être impliquées dans l'épidémie. Les résultats de la culture d'échantillons composite de vaches atteintes de mammite clinique ont démontré la présence de *Pasteurella multocida* dans 11 des 23 échantillons. L'inspection des lieux n'a pas permis de déceler la source de l'épidémie. Des recommandations pour le contrôle de la mammite ont été adoptées et ont permis de réduire la progression de la mammite tout en diminuant le compte des cellules somatiques dans le lait de réservoir.

Introduction

Pasteurella spp are normal inhabitants of the bovine nasopharynx, and have been isolated from cases of pneumonia, abortion, mastitis, meningitis and septicemia.[10] *Pasteurella multocida* has been reported as a cause of mastitis, usually as sporadic cases refractory to treatment.[1,2,4,11] *P. multocida* has been described in herd outbreaks of mastitis in England and Australia.[7,8] In a survey of bovine mastitis in India, *Pasteurella multocida* was isolated in 0.8% of the 375 samples yielding bacteria.[6] It has been suggested that the organism can cause mastitis by hematogenous or lymphatic spread.[5] It has been isolated from the blood stream of a lactating cow during an episode of acute clinical mastitis.[12] This case report describes the role of *Pasteurella multocida* in a herd outbreak of mastitis and the measures taken to control it.

History

In September 2001, the owners of a dairy herd requested veterinary assistance when their bulk-tank somatic cell count (BTSCC) peaked at 974,000 cells/ml, and the herd experienced an excessive number of cases of clinical mastitis refractory to treatment. A herd visit was scheduled to review farm records, examine animal facilities and observe the milking routine. The herd was composed of 225 lactating Holstein cows housed in a two-row sand bedded freestall barn with slatted floors. Cows were milked twice a day in a double-10 parallel pit parlor. One owner was present at 90% of the milkings, assisted by one of six part-time employees. Non-lactating, post-parturient and antibiotic-treated cows were housed together with all ages of young stock

in a remodeled stanchion barn. A section of the old milk pipeline in this barn was used as a flat barn parlor for milking the post-parturient and antibiotic-treated cows.

Clinical and Laboratory Findings

During the farm visit, results from recent clinical mastitis cases were available. Milk samples were aseptically collected from affected glands prior to the administration of intramammary antibiotics. The report indicated that microbiological culture results of milk samples (n=5) obtained from cows exhibiting clinical mastitis yielded *Pasteurella* spp (n=2), *Streptococcus* spp (n=1) and no growth (n=2). A number of management deficiencies were noted during the farm visit, including inadequate milking system vacuum (12 mm Hg farm gauge), use of multiple-dose infusion vials to treat mastitis, lack of treatment records, use of a single cloth towel for drying teats of two cows, use of a teat dip not found on the National Mastitis Council (NMC) approved list, and substandard milking hygiene. Observation of the milking routine revealed that milking units were being attached to teats of glands that were secreting abnormal milk.

It was recommended that the milking system be analyzed during milking and the teat end vacuum set between 11 and 12 mm Hg. Since the BTSCC was approaching the legal threshold, use of a separate quarter milking unit was recommended to discard abnormal milk from cows with clinical mastitis refractory to treatment. Segregation was accomplished by creating a separate group for cows with mastitis. The milking procedure was changed to ensure that the preparation lag time was less than 90 seconds. An individual cloth towel was used on each cow to eliminate the potential transfer of pathogens on shared towels. A 0.5 % iodine predip and 1.0% iodine post-dip with 10% emollients was recommended. Microbiological surveillance was initiated by submission of bulk-tank milk samples on a monthly basis. The milk truck operator, when sampling milk for antibiotic residues, collected the bulk-tank sample. Sampling occurred from the top of the bulk-tank after ten minutes of agitation. Samples were then frozen until submission to the diagnostic laboratory. The farm was advised to collect samples for 3-5 days and submit them together for analysis as recommended by Farnsworth.[3] Culture of milk samples from cows diagnosed with clinical mastitis was also recommended. All cases of clinical mastitis were to be treated only with antibiotic preparations approved for intramammary infusion, and treatments were to be recorded.

P. multocida was cultured from almost half (48%) of milk samples (n = 23) obtained from cases of clinical mastitis in September and October (Table 1). After isolation of *P. multocida* from several cases of mastitis, an attempt was made to recover the organism from the environment and potential animal reservoirs (Table 2). Sample numbers were limited due to financial constraints. Environmental sites sampled included inflation liners and cleaning in place (CIP) cups in the parlor, inflation liners from the fresh/treated cow parlor, and calf milk-feeding pails. Animal reservoirs sampled in-

Table 1. Microbial culture results of milk samples submitted from September to December 2001.

Sample type	Clinical mastitis	Clinical mastitis	Clinical mastitis	WHC[a]
Date:	9/11/01	10/1/01	10/10/01	12/7/01
Results:[b]				
P. multocida	2 (40%)	8 (53%)	1 (33%)	3 (1.4%)
S. aureus	0	0	0	2 (0.9%)
Staph. spp[c]	0	0	0	57 (26.5%)
Strep. agalactiae	0	0	0	0
Strep. spp	1 (20%)	6 (40%)	0	2 (0.9%)
Enterococcus	0	0	0	3 (1.4%)
A. pyogenes	0	1 (6.7%)	0	1 (0.5%)
C. bovis	0	0	0	26 (12%)
E. coli	0	0	0	1 (0.5%)
Mycoplasma sp	0	0	0	1 (0.5%)
Contaminated	0	0	0	10 (4.6%)
NSG[d]	2 (40%)	4 (26%)	2 (66%)	102 (47%)
Total sample number	5	15	3	215

[a]Whole herd culture.
[b]Percent of samples submitted listed in parenthesis.
[c]Coagulase negative *Staphylococci* spp.
[d]No significant growth.

Table 2. Microbiological culture results of environmental and biological sampling.

Sample description	Number	Qualitative microbiological results
Parlor liner and CIP cups (pre-wash)	3	*E. coli*, alpha Streps, gram negative bacilli and mixed environmentals
Parlor liner and CIP cups (post-rinse)	1	No growth
Stanchion barn liner (pre-wash)	1	Bacillus, mixed environmentals
Stanchion barn liner (post-wash)	2	No growth
60 ml syringe (multiple use)	1	Bacillus
14 gauge hypodermic needle	1	Bacillus
Herbal remedy[a]	2	Mixed Bacillus
Sterile water	1	No growth
Teat skin[b]	2	alpha Streps, Bacillus and molds
Teat skin[c]	2	alpha Streps, Bacillus and molds
Nasal swabs (< 10 day old calves)	4	Bacillus, *Staph* sp, alpha Streps, coliforms
Buccal swabs (< 10 day old calves)	3	Mixed nasal flora
Calf milk-feeding pail	1	Mixed nasal flora, gram negative bacilli

[a]Excell 2130.
[b]Sample taken from cows previously culture-positive for *Pasteurella multocida* in milk.
[c]Sample taken from recently post-parturient cows.

cluded nasal swabs and buccal swabs from calves less than 10 days old. The nasal samples were also cultured for mycoplasma. Samples were collected from post-parturient and treated cows' teat skin. Additional samples submitted for culture included medicine and equipment used for multiple-dose intramammary treatments, including lids of vials, hypertonic saline, sterile water, an herbal remedy (Excell 2130), a syringe, and 14-gauge needles. The herbal remedy was labeled for oral use, but was infused intramammary on this farm as a treatment for mastitis. None of the environmental or biological reservoir samples revealed *Pasteurella multocida*. No *Mycoplasma* spp were isolated from any nasal swabs. When swabs were taken from liners that had been rinsed or sanitized, they revealed no growth.

Bulk-tank milk samples never yielded *Pasteurella multocida* (Table 3). Initial bulk-tank samples submitted for microbiological analysis indicated that the non-agalactiae *Streptococcus* and coliforms were too numerous to count. After milking procedures were modified these levels dropped to goal or moderate levels. A *Mycoplasma* sp was obtained from a bulk-tank sample in November. Due to the respiratory nature of these pathogens, bulk-tank milk was screened for bovine viral diarrhea virus (BVDV), and found to be negative.

After isolation of a *Mycoplasma* sp from the bulk-tank sample submitted in November, the decision was made to culture the entire lactating herd to identify any remaining *P. multocida* or *Mycoplasma* sp-infected animals (Table 1). Complete herd culture results indicated three cows with *P. multocida* and one cow with *Mycoplasma* sp mastitis were present in the herd.

Seven months after the initial herd visit, four cows previously cultured positive for *P. multocida* remained in the herd. The producer reported that all of these cows were "light" on one quarter. In April, milk samples were obtained from the functional quarters of these cows, but *P. multocida* was not identified. This process was

Table 3. Microbiological culture results of bulk-tank milk samples.

Bacteria	Goal Levels	8/29/01	10/11/01	11/8/01	11/9/01	11/13/01	11/17/01	1/30/02
S. aureus	<50	150[a]	0	0	0	0	0	0
Non-aureus Staph.	<300	2400	350	550	600	300	350	450
S. agalactiae	0	0	0	0	0	0	0	0
Non-agalactiae Strep.	500-700	2500	TNTC[b]	1000	145	115	200	550
Coliforms	<100	0	TNTC	200	5	50	10	400
Mycoplasma sp	Negative	Negative	Negative	Moderate	Negative	Negative	Negative	Negative
A. pyogenes	-----	-----	-----	-----	-----	-----	5400	550
Number of days in sample	-----	1	3	5	1	1	1	4

[a]Values reported as colony forming units per ml.
[b]Too numerous to count.

repeated in June, including samples of secretions from the affected quarters. *P. multocida* was not identified from any of these samples.

During the initial consultation, the owners reported that the problems began in June and worsened as the summer progressed. Examination of the BTSCC (Figure 1) suggested that subclinical mastitis problems began in April. The producers did not identify the initial changes in the BTSCC. The use of control charts to identify changes in the BTSCC has been described.[9] Control charts provide a framework to interpret variations within data. A set of rules is used to differentiate true changes (signals) in processes from normal background variation. In this instance, signals are based on changes in BTSCC. One rule for determining that the process has changed is when nine or more successive BTSCC values are above or below a predetermined average. The average BTSCC for January through March was 209,000 cells per ml. Beginning on March 28th, the BTSCC values began climbing above the mean and never returned below the 209,000 level until October 26th. This criterion of nine consecutive values above the average was met on April 12th, but the producers did not recognize the signal. Early recognition and intervention would have limited the impact on animal welfare and financial cost of this epizootic.

Discussion

The mechanism by which *P. multocida* infected the mammary gland and caused clinical mastitis was not elucidated during this investigation. However, it seems unlikely that *P. multocida* mastitis was spread solely by a hematogenous or lymphatic route. Wenz *et al* reported that cows with acute clinical mastitis and *Pasteurella* bacteremia had a grave prognosis.[12] Other potential mechanisms included introduction of bacteria into the mammary gland using multiple-dose treatment vials and syringes, or direct seeding from a sick calf when suckled. It is possible that after the indexed case(s) *P. multocida* mastitis was spread to herd mates during the milking or treatment process. Evidence supporting this theory is the fact that the control measures coincided with the epidemic subsiding; control measures were targeted towards the milking and treatment procedures and equipment.

The remodeled stanchion barn was a potential location for the origin of the index case(s). All ages of cattle, from newborns to dry cows, were housed in this facility. Newborn calves were allowed to wander freely throughout the facility for three days. Fresh cows were milked next to refractory cases of mastitis. After milking the treated and fresh cows, the same person fed and treated the preweaned calves.

Overcrowding of the prefresh pen could have been a contributing stress factor. During April and May, there was an increased number of calvings on the farm (Figure 2), which would have reduced space in the prefresh pen. This facility may have provided a time-place relationship for respiratory pathogens to gain access to fresh cows' udders, thus providing a reasonable scenario for the index case(s).

If the calving period presented the only exposure to *Pasteurella*, we would expect the calving dates of culture-positive cows to cluster in April and May, because the BTSCC increased at that time. When examining the month of calving for the known *Pasteurella* culture-positive cows, no clustering was apparent (Figure 3). There were no records indicating the reason for culling, however, the producer reported that 90% of the culls during the four months preceding our initial visit were for mastitis. Examination of the month of calving data (Figure 3) for the cows culled from June through Sep-

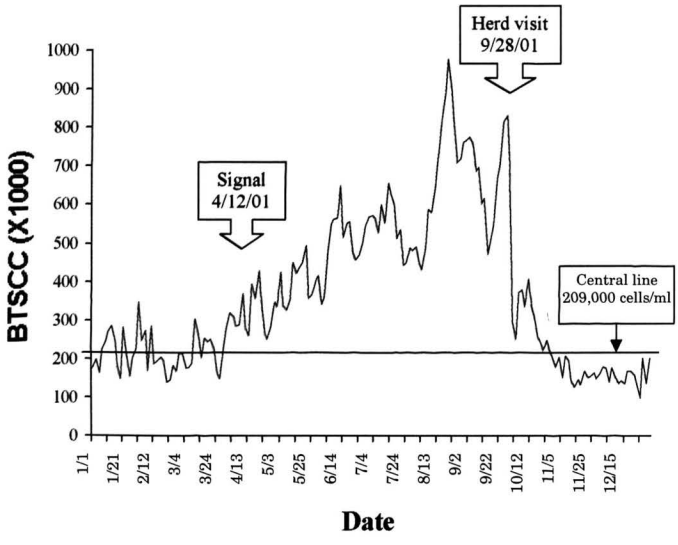

Figure 1. Bulk tank somatic cell counts during year 2001.

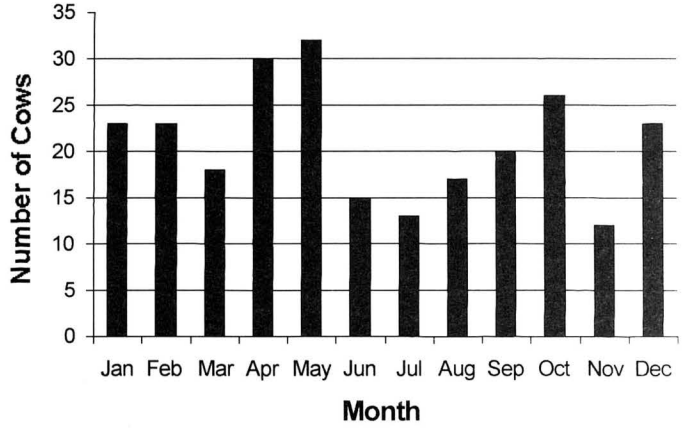

Figure 2. Number of calvings by month of year 2001.

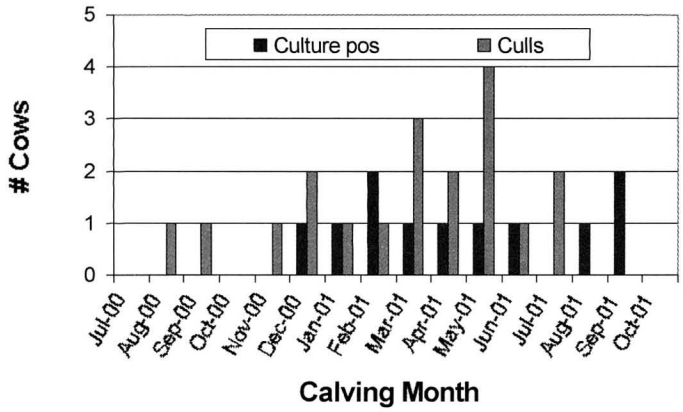

Figure 3. Month of calving for culled or *Pasteurella multocida* culture-positive cows.

tember shows a clustering from March through May. It is interesting to note that cows that calved from August of 2000 through February of 2001 were still at risk of being culled or culturing positive for *Pasteurella*.

Discussions with the producer one year after the initial herd visit indicated that control measures remained effective. The average BTSCC for September, October and November of 2002 were 175,000, 116,000 and 147,000 cells/ml, respectively.

Conclusion

Pasteurella multocida contributed to clinical mastitis during this epizootic. More information is needed to determine if *P. multocida* can behave as a contagious mastitis pathogen when the milking and treatment procedures are substandard. Implementation of industry standard practices for controlling mastitis was effective in curtailing this epizootic.

Acknowledgements

The authors would like to express their gratitude to Jennifer Keuning for her assistance facilitating the dairy advisory team, and Jill Makovec for her assistance in collecting milk samples.

References

1. Chakraborty AK, Sarmah BR, Boro BR: A case of clinical mastitis due to *Pasteurella multocida*. *Vet Rec* 103:313, 1978.
2. Chanda A, Mukherjee CR, *et al*: Clinical mastitis with *Pasteurella multocida* infection. *Indian Vet J* 66:571-572, 1989.
3. Farnsworth RJ: Microbiological Examination of Bulk Tank Milk. *Vet Clin North Am Food Anim Pract* 9(3):469-474, 1993.
4. Gupta RS, Joshi, DV Baxi, KK: Isolation of *Pasteurella multocida* from a case of bovine mastitis. *Indian Vet J* 54:769,1977.
5. *Laboratory Handbook on Bovine Mastitis*. Madison, National Mastitis Council Inc, 1999, pp 111-113.
6. Nagal KB, *et al*: Etiology of bovine mastitis in and around Palampur in Himachal Pradesh. *Indian J Anim Sci* 69:150-152, 1999.
7. O'Sullivan BM, Bauer J: Bovine mastitis caused by *Pasteurella multocida*. *Aust Vet J* 47:576,1971.
8. Pepper TA, Davies ET, Osborne AD: *Pasteurella septica (multocida)* mastitis in a dairy herd. *Vet Rec* 83:211-215, 1968.
9. Reneau JK, Kinsel ML: Records Systems and Herd Monitoring in Production-Oriented Health Management Programs in Food-Producing Animals, in Radostits OM (ed): *Herd Health Food Animal Production Medicine*, ed 3. Philadelphia, WB Saunders Co, 2001, pp 130-134.
10. Shewen PE, Bateman KG: Pasteurellosis in Cattle, in Howard JL (ed): *Current Veterinary Therapy III. Food Animal Practice*. Philadelphia, WB Saunders Co, 1993, pp 555-558.
11. Tabatabayi AH, Bolurchi M: Post surgical bovine mastitis due to *Pasteurella multocida*. *Vet Rec* 104:78, 1978.
12. Wenz JR, Barrington GM, *et al*: Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows. *J Am Vet Med Assoc* 219:976-981, 2001.

SelectVAC®, a proven preshipment health program from Pfizer, helps you produce healthier, in-demand, reputation calves.

SelectVAC provides powerful protection in four specific protocols—PreVAC, WeanVAC®, StockerVAC® and YearlingVAC®—that offer options to complement your program and goals.

You'll like what their reputation will do for yours.

SelectVAC calves can capture added value by:

- Improving health and reducing treatment costs, time and labor.
- Meeting buyer demand with verified health management documentation.
- Helping you develop valuable relationships with buyers.
- Commanding significantly higher sale prices than nonvaccinated calves[1].

Enrollment in SelectVAC is simple. Enroll online today at www.selectvac.com or contact your veterinarian or animal health supplier.

BEEF FRIENDLY TAKE TIME - OBSERVE LABEL DIRECTIONS

[1]Data on file, Pfizer Inc

Pfizer Animal Health

SelectVAC®

PROVEN PROGRAM. OPTIMUM HEALTH. HIGHER-VALUE CALVES.