Impact of Prototheca Mastitis on Bulk Tank Somatic Cell Count and Standard Plate Count

Rubén N. González, DVM, MPVM, PhD Gary J. Bennett, DVM Sharon A. Sickles, PhD Debra St. Hilaire, BS Daryl V. Nydam, DVM Quality Milk Promotion Services College of Veterinary Medicine Cornell University Ithaca, NY 14850

Introduction

Prototheca spp are achlorophyllic algae that are ubiquitous in soil and aqueous habitats.¹⁻⁶ *Prototheca zopfii*, mainly, and *P. wickerhamii* have been reported as etiologic agents of intramammary infections (IMI) in cows.^{1,2,4,7,8} Prototheca generally behaves as an environmental mastitis pathogen, but it can be spread from cow to cow during milking.^{4,8}

This is a preliminary report about the impact of Prototheca mastitis on prepasteurized milk quality in two northern New York State dairy herds.

Material and Methods

Dairies

Dairy A was a closed herd of 60 milking cows housed in a tiestall barn with a round the barn pipeline milking system. Cows were milked twice a day. The barn had a concrete floor, part of it with rubber mats, and hay was used for bedding. Mastitis control procedures included both premilking and postmilking teat dipping (0.5% chlorhexidine) applied with a dipper cup. When the weather allowed, milking cows were turned out into a pasture surrounding the barn. Nonlactating cows were housed in the same barn as the milking cows, but had a separate exercise corral next to the barn. Before starting the study, monthly bulk tank milk (BTM) SCC (somatic cell count) range was 200,000-300,000 cells/ml while SPC (standard plate count) range was 5,000-10,000 CFU/ml.

Dairy B was an open herd of 209 milking cows with the majority housed in a four row tiestall barn. The remaining cows were in a freestall housing adjacent to the tiestalls. A lauricidine postmilking teat dip was applied with a dipper cup. Bedding consisted of chopped hay for the tiestalls and sand for the freestalls. Dry cows were housed separately from the milking cows in a freestall facility with sand bedding. The BTMSCC had increased from 260,000 to 780,000 cells/ml before the initiation of the study. At the same time, the SPC had increased from 7,000 to 260,000 CFU/ml.

Milk, environmental and fecal samples

Milk samples were obtained from milking cows during a series of whole-herd mastitis screening surveys and from BTM. To determine the source of *Prototheca* on farms, samples were obtained from all possible water sources, bedding, feed, pasture sites, the dry cow lot, teat dip jugs, teat dip dippers, manure, intramammary treatments, and from fecal samples of randomly selected cows.

Bacteriologic diagnosis

Milk samples were plated on sheep blood agar and potato dextrose agar (PDA) plates, and incubated for 48-72 hours at 37°C and 30°C, respectively. Environmental and fecal samples were plated on selective *Prototheca* isolation medium (PIM)⁷ and incubated at 30°C for 72 hours. Presumptive identification of *Prototheca* was based upon morphology of the organisms as seen on culture and smears stained with Gram or methylene blue. The API 20 C system (bioMérieux Vitek, Inc., Hazelwood, Missouri) was used to identify species.

Results

At the beginning of 1996, Dairy A experienced a rapidly rising SPC which reached 40,000 CFU. A screen-

ing BTM yielded pure *Prototheca*, while the SPC and SCC performed at the milk plant reached 180,000 CFU/ml and 350,000 cell/ml, respectively. When the herd was sampled for the first time, 26 of 53 milking cows (49%) showed IMI due to *Prototheca*. A month later, the prevalence in the herd increased to 50% (28 of 56 milking cows). During the period January 1996-December 1997, the SPC ranged from 9,000 to 190,000 CFU/ml and the SCC from 120,000 to 660,000 cell/ml, reflecting the chronicity of *P. zopfii* IMI.

In July 1995, during a whole herd mastitis screening survey, Prototheca was cultured for the first time from 16 (7%) of the 229 cows at Dairy B. However, at that time, the BTM sample cultured negative for Prototheca. In April 1997, SPC and SCC performed at the milk plant reached 260,000 CFU/ml and 780,000 cell/ml, respectively. When a whole-herd mastitis screening survey was performed a week after those results, 46 cows (22%) showed P. zopfii IMI. The owner eliminated 40 of those Prototheca-infected cows from the herd. The SPC for the BTM sample was 230,000 CFU/ml, 210,000 of which were P. zopfii. Four months later, during a new whole-herd survey, 45 of 192 cows (29%) had Prototheca IMI, 39 of which were newly infected. During the period April 1997-December 1997, ranges were 45,000-600,000 CFU/ml and 280,000 to 940,000 cell/ml for SPC and SCC, respectively.

At both dairies, the dry cow lot, splash/puddle areas, fecal samples (from cows with and without *Prototheca* IMI), bedding and some inflations cultured *Prototheca*. Two *Prototheca* species were isolated on Dairy A, *P. zopfii* and *P. stagnora*, of which only *P. zopfii* was isolated from milk. At Dairy B, only *P. zopfii* was isolated from the environment and milk.

Discussion

Naturally occurring cases of *Prototheca* have been diagnosed at QMPS for approximately 35 years. *Prototheca* are frequently isolated from cow composite milk samples during whole herd mastitis screening surveys performed by QMPS personnel or from quarter or composite milk samples submitted to our laboratories by veterinarians and dairy farmers. Isolation of *Prototheca* has generally been made from herds with 1-3 infected cows. *Prototheca* seems to be quite ubiquitous in some dairy environments, especially in splash/

puddle manure-contaminated areas. The chronicity of protothecal IMI occurs because of the lack of an effective treatment as well as the absence of spontaneous recovery.^{4,8}

In both farms, cows with either clinical or subclinical IMI did not show signs of systemic illness. Some infected glands were swollen and hard. As described previously,⁴ several cows became intermittent shedders of *P. zopfii*. Milk production seemed to be decreased while SPC dramatically increased in both dairies and was the main concern in meeting the standards for prepasteurized milk.

The definite source of *Prototheca* and/or predisposing risk factors, as well as the mode of transmission in these herds have not been satisfactorily identified. Bedding or food materials have been suggested as the source of *Prototheca* mastitis outbreaks.^{3,5} The isolation of *Prototheca* from milk and fecal samples suggests that these organisms can be brought into dairy herds through the purchase of animals with infected mammary glands or as residents of digestive tracts of animals of different ages. Furthermore, manure contamination of teats could provide easy access for *Prototheca* into the mammary gland.

Acknowledgments

The field work of Thomas Fife, and the skilled technical assistance of Phyllis Weybrecht and Andrea Walsh, Quality Milk Promotion Services, Cornell University, are gratefully appreciated.

References

1. Anderson KL, Walker RL. Sources of Prototheca spp in a dairy environment. J Am Vet Med Assoc 1998; 193:553-6. 2. Costa EO, Carciofi AC, Melville PA, et al. Prototheca sp outbreak of bovine mastitis. Zentralbl Veterinarmed (B) 1996; 43:321-4. 3. Frank N, Ferguson CC, Cross RF, et al. Prototheca, a cause of bovine mastitis. Am J Vet Res 1969; 30:1785-90. 4. González RN. Prototheca, yeast and Bacillus as a cause of mastitis. Proc 35th Annu Meet Natl Mastitis Council 1996, pp 82-92. 5. Lerche M. Einen durch algen (Prototheca) hervorgerefene mastitis der kuh. Berl Munch Tieraerztle Wochenschr 1952; 65:64-9. 6. Pore RS, Barnett EA, Barnes WC, et al. Prototheca ecology. Mycopathologia 1983; 81:49-56. 7. Pore RS, Shahan TA, Pore MD, Blauwiekel R. Occurrence of Prototheca zopfii, a mastitis pathogen, in milk. Vet Microbiol 1987; 15:315-23. 8. Spalton DE. Bovine mastitis caused by Prototheca zopfii: A case study. Vet Rec 1985; 116:347-9.

SEPTEMBER, 1998 217