Outbreaks of clinical mastitis caused by *Trichosporon beigelii* in dairy herds

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A dairy farm located in central New York (dairy A) had 45 milking cows that were kept in a 2-row tie-stall barn with a high line milking system that had 4 milking units. The barn had a concrete floor, and sawdust was used in the stalls for bedding. Before attaching the milking units, teats were massaged, and dust was removed with a dry cloth towel that was used for multiple cows. Mastitis control procedures consisted of teat dipping with a homemade chlorinated solution after milking and intramammary treatment of cows affected by clinical mastitis during lactation with a homemade combination antimicrobial product provided by a local veterinary practitioner. A commercial penicillin-dihydrostreptomycin combination antimicrobial was used for treatment at the beginning of the nonlactating period. Bulk-tank milk somatic cell count (SCC) ranged monthly between 300,000 and 400,000 cells/ml. Mean 305-day milk production was 7,100 kg (15,620 lb). During a 2-week period, the herd had 6 cases of clinical mastitis; affected cows were treated by the producer with the antimicrobial product provided by the veterinarian. All of these samples, including the 6 milk samples collected by the attending veterinarian, were submitted to the Quality Milk Promotion Services (QMPS) laboratory for microbiologic examination. The producer was advised to milk the affected cows last, improve sanitation, and not treat cows without veterinary supervision.

Several additional cows developed the same type of mastitis. The attending veterinarian collected 6 more composite milk samples; 3 samples were from cows known to have intramammary yeast infections, and 3 were from newly affected cows. Personnel from QMPS visited the farm and collected: 1) 2 cloth towels that had been used in different milking units; 2) 2 unused sawdust bedding samples; 3) 4 used sawdust bedding samples obtained from several stalls each (2 samples consisted of material obtained from the silo and 2 samples of total mixed ration that included corn silage and were taken from feeders on different days; 4) 2 samples of corn silage obtained from different sites in the area where the cows' udders usually rested; 5) 3 bottles that contained bulk-treatment antimicrobial preparations (1 bottle was empty, 1 bottle was opened and in use, and 1 bottle was unopened); 6) 14 swab specimens from teat-cup liners, which were obtained after milking units had been used to milk cows with yeast infections; and 7) 2 bulk-tank milk samples (the first sample was collected by QMPS personnel at the time of the farm visit, and the second sample was collected by the farmer 3 days afterwards). All of these samples, including the 6 milk samples collected by the attending veterinarian, were submitted to the QMPS laboratory for microbiologic examination.

Aliquots (0.01 ml) of quarter milk samples obtained from cows with clinical mastitis were examined by use of bacteriologic culture on tryptose soy agar plates that contained 5% sheep blood and 0.1% esculin as well as Sabouraud dextrose agar plates and incubated aerobically at 37 and 30°C, respectively.

Trichosporon beigelii is widely distributed in nature and is classically associated with white piedra, a mycosis that may involve the hair of the human body.

Intramammary infections caused by *T beigelii* may be fatal in cows; the prevalence in affected dairy herds may be high.

Affected cows may have hyperthermia, swelling of the udder, and substantially decreased milk production or agalactia.

Intramammary infections caused by yeast, including *T beigelii*, may also be associated with high bacterial counts in bulk-tank milk.

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Samples of bedding (used and unused), silage, and total mixed ration were placed into sterile blender cups to which sterile saline (0.9% NaCl) solution was added to facilitate grinding. Cloth towels were placed in a 2,000-ml beaker with 500 ml of sterile saline solution and left to soak for 1 hour. Towels were then taken out of the beaker with sterile forceps and discarded. Two 0.1-ml aliquots of each homogenized specimen (bedding, silage, total mixed ration, towel-soaking liquid) were transferred with sterile pipettes onto Sabouraud dextrose agar plates that contained 0.4 g of chloramphenicol/L and incubated aerobically at 37 and 30 C. Plates were examined 24 and 48 hours later for evidence of yeast and yeast-like organisms. Empty bottles that had contained bulk antimicrobial treatment were flushed with 10 ml of sterile saline solution that was injected through the disinfecting rubber stopper of the bottles by use of a syringe and sterile needle. Liquid was drawn out by use of a syringe with a needle, and 2 or 3 drops were streaked onto blood agar and Sabouraud dextrose agar plates. The same procedure was used for treatment preparations from in-use and unused bottles, except that flushing was not used. Teat-cup liner swab specimens were directly swabbed onto Sabouraud dextrose agar and blood agar plates. When recovered from milk, bedding, towel-soaking liquid, teat-cup liner swab specimens, or treatment bottles, isolates were streaked onto Sabouraud dextrose agar plates and incubated aerobically at 30 C for 48 hours. Isolates from these plates were then identified by use of a commercial microbial identification system in conjunction with examination of smears stained with methylene blue stain; positive result for uracil hydrolysis in broth at 37 C was also a criterion. Furthermore, yeast-like colonies on blood agar plates obtained from milk samples previously examined by use of bacteriologic culture performed by the farm veterinarian when he visited the farm for the first time were identified by use of the same procedures. For total yeast count, 10-g samples of bedding material were combined with 90 ml of sterile saline solution, and Sabouraud dextrose agar plates were inoculated in duplicate with 10-fold dilutions of the suspension. Colonies were counted after 48 hours of aerobic incubation at 30 C, and the mean of the 2 plate counts for each dilution was reported as CFU per gram of bedding. Bulk-tank milk samples were examined by use of bacteriologic culture on blood agar and Sabouraud dextrose agar. Yeast colonies were counted on Sabouraud dextrose agar plates after 48 hours of aerobic incubation at 30 C. Other microorganisms were also counted on the blood agar plates.

Trichosporon beigelii was isolated from the 6 milk samples, 1 of the 2 towels, the empty and in-use treatment bottles, and 5 of the 14 teat-cup liner swab specimens. However, the organism was not isolated from sawdust, silage, or samples of total mixed ration. Yeast-like colonies originally isolated by the farm veterinarian were found to be T beigelii as well. The 2 bulk-tank milk samples yielded standard plate counts of 66,000 and 75,000 CFU/ml. The counts were almost entirely attributable to T beigelii as well as a few environmental bacteria and Prototheca sp.

Because more cows were detected almost daily with clinical mastitis in at least 1 mammary quarter, the producer was suffering substantial financial losses. Furthermore, the milk plant informed him that the standard plate count was > 100,000 CFU/ml. At this time, the producer began to treat the cows with intramammary infusions he prepared with a variety of drugs such as amphotericin B, polymyxin B, miconazole, thiabendazole, cycloheximide, and amprolium. Neither the attending veterinarian nor QMPS personnel could determine how these drugs were obtained by the producer. Twenty-three of the 45 milking cows developed intramammary infections with T beigelii during a 40-day period. Several cows were hyperthermic (rectal temperature, 40 to 41 C [104 to 105.8 F]) for periods of 3 to 7 days. Two of the 23 lactating cows and 3 cows that had calved recently had intramammary infections that were attributable to Prototheca sp. Therefore, the farmer decided to sell the cows to slaughter and go out of business.

A second herd (dairy B), also located in central New York, consisted of 26 milking cows housed in a tie-stall barn with a concrete floor and stalls that were covered with rubber mats with chopped hay bedding on top of them. The barn had a high line milking system, with 3 milking units. Cows’ teats were washed with several common cloth towels and a solution of water with a quaternary ammonium-based sanitizer. Teats were not dried before attaching the milking units. Mastitis control procedures included teat dipping after milking by use of a 0.33% chlorhexidine product and treatment of lactating and nonlactating cows with commercial products that contained cephalosporin or cloxacillin. The bulk-tank milk SCC was 1,500,000 cells/ml, and the milk plant standard plate count had increased from 10,000 to 66,000 CFU/ml during a 5-week period. Mean 305-day milk production was 5,900 kg (12,980 lb). The producer consulted with veterinarians from QMPS, and a whole-herd mastitis screening survey was performed. Bacteriologic culture of composite milk samples from all milking cows in the herd was performed with blood agar medium, as described. Among cows with a variety of intramammary infections, 9 cows had infections caused by Streptococcus agalactiae. Intramammary antimicrobial treatment of all 4 quarters for the 9 cows was recommended. Within a week after treatment with cloxacillin, 7 of the treated cows developed severe acute mastitis with hyperthermia (rectal temperature, 41 C [105.8 F]), 3 cows died, and milk production dropped abruptly. Composite milk samples or secretions from the surviving 4 cows were examined by use of bacteriologic culture at the QMPS laboratory. T beigelii was isolated from samples from 3 of these cows. Candida rugosa and C krusei were isolated from the remaining cow. Bacteriologic culture of bulk-tank milk yielded almost pure cultures of T beigelii, C rugosa, and C krusei, which were responsible for a standard plate count of 50,000 CFU/ml.

The producer collected a sample of bedding material at random from several stalls. He also collected 2 towels that had been used during 1 milking and put them into sealed plastic bags to maintain the soil and moisture they possessed after the cows had

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been milked. Bedding material and towels were shipped refrigerated to the QMPS laboratory for microbiologic examination. Bacteriologic culture of towel specimens revealed several bacterial species, but yeast could not be recovered. Candida rugosa, C krusei, C pseudotropicalis, and Cryptococcus neoformans were isolated from bedding material. Two of the 4 cows that were infected with T beigelii ceased milk production. Because of our previous experience with dairy A, we advised the producer to sell the yeast-infected cows. He did so, and the mastitis outbreak ended; T beigelii was not cultured from further cows during several follow-up investigations.

A third dairy (dairy C), which was located in northeastern Pennsylvania, maintained a mean of 75 milking cows housed in a free-stall barn; chopped hay was used for bedding. Cows were milked in a double-8 herringbone milking parlor with automatic removal of milking units. Mastitis control procedures included washing the teats with a commercial chloride sanitizer without drying before attaching the milking units and dipping teats after milking with a 0.5% iodine solution. Cows with clinical mastitis during lactation were treated by intramammary administration of amoxicillin solution prepared by the producer with nonsterile saline solution. Selected cows received intramammary infusions with commercial cephalxin benzathine or penicillin-dihydrostreptomycin products at the beginning of the nonlactating period. Mean 305-day milk production was 7,200 kg (15,840 lb)/cow, and bulk-tank milk SCC had increased from 200,000 to 900,000 cell/ml after purchasing several cows and heifers as replacements. The producer requested a whole-herd mastitis screening survey from QMPS. Of the 75 cows from which samples were collected by QMPS personnel, 41 had intramammary infections caused by S agalactiae. The producer was advised to treat all the cows at the same time in all 4 quarters for 3 consecutive milkings. He did so, and samples were again collected from the whole herd 4 weeks later. Eight of the treated cows had developed intramammary infections caused by T beigelii. We were unable to obtain any amoxicillin solution prepared by the producer or containers used for the preparation. Two of these 8 cows developed clinical mastitis and were treated by the producer with a chloride solution and eventually eliminated from the herd because of low milk production. Because the bulk-tank milk SCC from the milk plant decreased to 600,000 cell/ml, the producer was not interested in continuing with a regular mastitis control program. Therefore, we were unable to complete follow-up on the T beigelii-infected cows.

A fourth dairy (dairy D), which was located in northwestern Pennsylvania, had 90 milking cows housed in a free-stall barn with sawdust used for bedding. Cows were milked in a double-6 parallel milking parlor. Milking procedures included dry massage with newspaper towels if teats were dirty and teat dipping after milking with a 0.5% chlorhexidine. Cows with clinical mastitis during lactation were treated with either pirlimycin or a mixed antimicrobial preparation provided by the herd veterinarian. The bulk-tank milk SCC for this dairy ranged between 220,000 and 290,000/ml, and 305-day milk production was 8,100 kg (17,820 lb)/cow. A county extension agent informed us that 7 cows previously treated for contagious mastitis developed clinical mastitis with severe swelling of the udders; some quarters became agalactic. The herd veterinarian suspected an outbreak of mastitis caused by Mycoplasma sp. Therefore, composite milk samples from 12 cows with clinical mastitis (including the 7 that had been treated for contagious mastitis) were collected and sent to the QMPS laboratory for microbiologic diagnosis. Trichosporon beigelii was isolated from 8 cows (5 of which belonged to the treated group). C rugosa was isolated from 2 cows (1 of which belonged to the treated group). 1 sample yielded Staphylococcus aureus, and the remaining sample yielded negative culture results. All samples yielded negative results for Mycoplasma sp.

Because cows with clinical mastitis had been treated with a homemade antimicrobial veterinary preparation, 1 bottle each from discarded, still in use, and unused antimicrobial preparations were received at the laboratory for analysis. We also requested several samples of used and unused bedding material but received only 1 sample that contained used bedding material. Trichosporon beigelii and C rugosa were isolated from discarded and still-in-use bottles, whereas bacteriologic culture of the unused antimicrobial treatment preparation did not yield any organisms. For bedding samples, culture yielded 500 CFU of C rugosa per gram, 300 CFU of T beigelii per gram, and 20 CFU of C albicans per gram. One of the 2 cows with intramammary infection caused by T beigelii and agalactic mammary quarters died, and the remaining cow was eliminated from the herd. The extension agent left his position and neither the owner nor the attending veterinarian were interested in a follow-up of the outbreak; thus, the remaining yeast-infected cows were lost to follow-up.

Yeast are always in the dairy environment as well as in the skin of the mammary gland.1,2 Trichosporon beigelii is a minor component of normal skin flora in humans,3 is widely distributed in nature,4,5 and has been isolated from soil, sawdust, water, and dairy cattle.6 This yeast is associated with the soft nodules of white piedra, a superficial mycosis that may involve the hair of any part of the human body.7 Although it has been stated that Trichosporon sp are a common cause of mycotic mastitis in the United States,8,9 we were unable to find support in the literature for this assertion. In veterinary medicine, yeast are seldom identified to species level and, to the best of our knowledge, this is the first report of T beigelii as a cause of bovine mastitis. Intramammary infections caused by T beigelii can be fatal to cows and have epidemic proportions with severe economic losses for affected herds. Seven cows died in the 4 herds described in this report, and prevalence of infection attributable to T beigelii ranged from 7 to 51%. This figure is similar to that reported for other herds with mastitis caused by yeast.10 Cows had clinical signs, including hyperthermia, similar to those often detected in cows with acute clinical mastitis caused by coagulase-negative staphylococci.11 The outbreaks in the 4 dairies we dealt with may have been traced...
either to antimicrobial preparations that were contaminated with yeast (a probable cause at dairy C) or faulty intramammary administration of antimicrobials (all 4 dairies). Yeast infections have been commonly associated with eradication programs for *S agalactiae*. This seems to have been the factor that sparked the outbreaks at dairies B and C and probably D. Intramammary infections caused by *T beigelii* also caused agalactia or a substantial decrease in milk production in affected cows. Furthermore, to the authors' knowledge, this is the first report of a possible association between intramammary infections caused by yeast in cows and high bacteria counts in bulk-tank milk (dairies A and B), because yeast isolated from cows (*T beigelii*, *C rugosa*, and *C krusei*) were also isolated from bulk-tank milk.

At dairy A, intramammary infections may have been transmitted from cow to cow at milking by 2 fomites (teat-cup liners and cloth towels) or may have been caused by intramammary treatment with the producer's homemade antimicrobial preparations, faulty treatment procedures, or both. The finding of *T beigelii* on teat-cup liners and a cloth towel used in udder preparation is consistent with spreading of the organism at milking. The potential risk for transmission of intramammary infections caused by yeast through contaminated milking units was reported by Yeh et al after they isolated several *Candida* species at the same time from cows with intramammary infections and teat-cup liners of milking machines. The opinion of Yeh et al as well as ours is in opposition to that of authors who stated that yeast infections did not spread from quarter to quarter or from cow to cow at milking. In humans, cloth particles have been found to serve as a substrate for yeast proliferation as well as a vehicle for the transmission of *T beigelii*. The producer at dairy A was informed about the differences between human yeast infections and intramammary infections of cows, that treatment failure of *T beigelii* in humans was not rare, and that the organism was resistant to amphotericin B. Nevertheless, he continued treating cows with a variety of drugs. Antifungal agents are generally toxic to the mammary gland and may cause more damage than the yeast infection itself. These reasons may have contributed to the dispersal of the herd. We also cannot exclude the possibility that a more virulent strain of *T beigelii* affected this herd, compared with the strain of the organism that affected the other 3 herds.

At dairy C, *T beigelii* may have entered the mammary glands by way of contaminated saline solution used to prepare the intramammary infusions for treatment of lactating cows. Similar observations were reported for intramammary infections caused by *C neoformans*. At dairy D, the origin of the outbreak could have also been associated with contamination of the sawdust used for bedding. We do not know if the sawdust was naturally contaminated with *T beigelii* and other yeast, or if it became contaminated with udder secretions from infected cows.

Yeasts may gain entrance into the mammary gland of dairy cows from the environment as a result of teat injuries, irritating teat dips, or tissue damage caused by a previous bacterial infection or may follow intramammary treatment. Although yeast may be cultured from milk samples from 1 or 2 cows in a typical herd during whole-herd mastitis screening surveys, outbreaks of clinical mastitis caused by yeast almost always develop after intramammary treatment for mastitis is performed by farm personnel. As in the 4 dairies we studied, this is most commonly seen when a bulk-treatment bottle is used and doses are drawn out of the bottle at different times. Old needles stuck through rubber stoppers make this more likely. Therefore, care and aseptic procedures during intramammary infusion of antimicrobials and a single-dose sterile treatment should be used. Although it has been reported that most intramammary infections caused by yeast, including *Trichosporon sp.*, are self-limiting and cows usually return to normal milk production in 1 to 2 weeks, this did not happen in the herds we studied. Cows infected with *T beigelii* died, had a substantial decrease in milk production, or became agalactic. Furthermore, several cows were eventually removed from dairies B and D, and the whole herd was dispersed from dairy A.

References

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<th>Route of administration</th>
<th>Remarks</th>
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