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Mycoplasma and Prototheca Mastitis Management

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Introduction

Normally the three most prevalent contagious mastitis pathogens considered are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma sp.* *Streptococcus agalactiae* and *Mycoplasma sp.* are more rare, with less than 5% of operations affected (APHIS, 2008)¹. However, *Mycoplasma sp.* is increasing in prevalence and more than 21% of large dairies, greater than 500 cows, may be affected in the U.S. (APHIS, 2003)². For this reason, mycoplasma mastitis appears to be an emerging mastitis problem (Fox et al., 2003)³. Recent reports also attest to its global significance, affecting areas previously unreported, as in Prince Edward Island, Canada (Olde Riekerink et al., 2006)⁴, Northern Greece (Filioussis et al., 2007)⁵, Saudi Arabia (Al Abdullah, 2006)⁶, France (Arcangioli et al., 2011)⁷, Belgium (Passchyn et al., 2011)⁸, Mexico (Miranda-Morales et al., 2008)⁹ and Iran (Ghazaei, 2006)¹⁰. Given that mycoplasma mastitis seems to be associated with larger herds (APHIS, 2008; Fox et al., 2008; Thomas et al., 1981)^{1,11,12}, and that herd size has been increasing over time, it would seem logical that mycoplasma mastitis has become more prevalent.

Two primary dairy herd risk factors for mycoplasma mastitis as stated by Jasper (Jasper, 1981)¹³ are introduction of diseased animals to the lactating herd and problems with milking time procedures, most notably inadequacies in hygiene. Recent reports (Punyapornwithaya et al., 2010 and 2011)^{14,15} suggest that failures in milking time hygiene have not been associated with mycoplasma mastitis occurrence, but that exposure to cattle with non-mastitis mycoplasma infections might be the nidus. Thus the thrust of this review will be to discuss the previous evidence that would indicate that mycoplasma mastitis was principally a contagious mastitis pathogen transmitted at milking time and the more recent evidence that describes the potential threat that symptomatic and asymptomatic carriage of *Mycoplasma sp.* by cattle has on outbreaks of mycoplasma mastitis. A brief description of diagnosis of mycoplasma mastitis will also be addressed.

Prototheca mastitis was perhaps first reported by John McDonald (1984)¹⁶. Since that time there have been sporadic reports of prototheca mastitis and its frequency of occurrence was deemed sufficient so as to be described in the National Mastitis Council Laboratory Handbook on Bovine Mastitis (Hogan, et al. 1999)¹⁷. Recently the frequency of cases and scientific reports has appeared to have increased, but this increase may be a function of the new awareness of the agent and or enhanced diagnostic abilities.

Diagnosis

The simple genome and fastidious growth requirements of *Mycoplasma sp.* can be associated with their slow replication and difficult identification in many mastitis diagnostic laboratories. Given the difficulties in culture of *Mycoplasma sp.* it has been speculated that mycoplasma mastitis is not a routine procedure used in the diagnosis of mastitis.

Diagnosis of mycoplasma mastitis is most commonly done using microbiological procedures (Gonzalez and Wilson, 2003)¹⁸. For presumptive identification of *Mycoplasma sp.* from milk, it is advised that milk be cultured on modified Hayflick's media, incubated at 37°C

under 10% CO₂ for 7-10 days (Hogan et al., 1999)¹⁷. Other methods such as immuno-detection of specific antigens and nucleic acid detection (PCR technology) have been established (Fox et al. 2005)¹⁹. The PCR technology is promising and is undergoing testing (Boonyayatra et al., 2012a)²⁰ and thus culture based techniques are still the most common method for the detection. The advantage to the non-cultured based systems is that they can discriminate between species. *Mycoplasma bovis* is generally regarded as the pathogenic strain affecting cattle, although other strains need to be considered (Fox et al., 2012)¹⁹. False positive results are possible, given when other mycoplasma like organisms, mollicutes, can be confused with the pathogenic strains. The most common non-pathogenic mollicutes are *Acholeplasma sp.* The *Acholeplasma sp.* can be very effectively distinguished from *Mycoplasma sp.* by the digitonin and nisin tests using a culture based system (Boonyayatra et al., 2012b)²¹. Yet it should be kept in mind that *Acholeplasma sp.* are capable of causing mastitis (Counter, 1978)²².

Mycoplasma sp. isolation can be easily overlooked by mastitis diagnostic laboratories since primary isolation of mastitis pathogens is usually done using blood agar plates, 5% CO₂, for 1-2 days. Relatively few laboratories may be equipped to detect this pathogen group (Biddle et al., 2005)²³. Indeed, Nicholas et al. (Nicholas et al., 2007)²⁴, argued that given the difficulty in isolation of *Mycoplasma sp.* that mycoplasma mastitis was often under diagnosed. Thus situations described in this review might be representative of a greater problem than is apparent.

Prototheca sp. are algae organisms that will grow on blood agar but often can be confused with yeast, and are better suited for culture on Sabouraud Dextrose Agar and Potato Dextrose Agar (Hogan et al., 1999)¹⁷. *Prototheca sp.* is microscopically visible under a wet mount without the aid of stains.

Dissemination of *Mycoplasma sp.* Within and Between Cattle

Experimental infection

Perhaps one of the first reports on the effect of experimental inoculation of cattle with disease causing *Mycoplasma sp.* on infection was done by Jain and coworkers (Jain et al., 1969)²⁵. In this study, four Holstein-Fresian cows received inoculations with different strains of mollicutes. Two of the four, were strains from cases of mastitis, one was isolated from the vagina and believed to be *M. bovis genitalium* and the third believed to be *A. laidlawii*. One of the strains from a case of mastitis, when inoculated into mammary quarters of 3 cows, caused intramammary infection in the inoculated quarters and also caused infection in non-inoculated quarters. Moreover, that strain of *Mycoplasma sp.* was found in the blood, nasal fluid, mucosal surface of the eye, vagina and rectum, within days after the inoculation was made. Also, one inoculated cow gave birth to a female calf, and that calf shed the mastitis strain, as it was found in the mucosal surface of her nose, eye, and vagina. In one cow, the pathogen spread only to a mammary quarter not inoculated on the homo-lateral side of the gland. A systemic antibody response was observed in response to this infection. An attempt to inoculate the non-infected mammary half was successful, despite the antibody response. The authors clearly demonstrated that the infection could spread from organ to organ, and that the systemic antibody response might not be sufficient to prevent and/or clear the infection. Jain and coworkers (Jain et al., 1969)²⁵ suggest that there is a complex method of spread within the body that might not only be hematogenous, but involve the lymphatic system. They note that there was marked enlargement of the supramammary lymph nodes, and that other lymph nodes were often involved. In total, these authors provided excellent findings of the nature of the response to

intramammary mycoplasma mastitis, and immune response after the establishment of infection. They established that mycoplasma could migrate to different body sites after establishing infection in the mammary gland and that previous antibody response was not sufficient to prevent new infections. The possible development of vaccines to prevent the disease would have to consider augmentation of arms of the immune system other than the antibody mediated component.

Natural infection

Within cow and between cow dissemination of *Mycoplasma sp.* that cause mastitis has also been seen in a study reported more recently. Djordjevic and coworkers (2001)²⁶ reported on the diversity and dissemination of *Mycoplasma species* bovine group 7 associated with disease on 3 related dairy herds. *Mycoplasma species* bovine group 7 have not been classified into a specific species (Manso-Silva et al. 2007)²⁷. Apparently the characteristics of this mycoplasma group have not been well defined, and some of the known characteristics seemed to be shared sufficiently with other species, thwarting this group's precise taxonomic classification. Manso-Silva et al. (2007)²⁷, suggest that *Mycoplasma species* bovine group 7 be classified as a subspecies of *M. capricolum*. This research group has more recently indicated other designations which are pending. Djordjevic and coworkers (2001)²⁶ indicate that *Mycoplasma species* bovine group 7 have been isolated from various body sites of healthy and diseased cattle. Specifically, they found that *Mycoplasma species* bovine group 7 have been found to cause mastitis, polyarthritis and pneumonia in cattle, as well as having been associated with aborted fetuses from 3 different dairies over 15 months. These dairies were managed and owned by one operator. They examined the heterogeneity of the *Mycoplasma species* bovine group 7 isolates by genetic analysis, and compared the heterogeneity to isolates obtained from other research groups at different global locations. Isolates from one farm that experienced the abortion outbreak were indistinguishable. These isolates were obtained from various body sites including joints, pericardial fluid, lymph node, stomach, and spleen; and from milk. Isolates from fetal tissue were similarly indistinguishable. Isolates from milk and joints, 18 months after the initial outbreak, were also indistinguishable. The authors demonstrated that nucleic acid fingerprinting analysis could readily distinguish source of isolates as those obtained from world-wide colleagues had significant heterogeneity with those in the Australian outbreak. This report clearly demonstrates the ability of a single clone of bovine *Mycoplasma sp.* could affect and cause disease in multiple body sites, with a single strain causing disease over several months in cattle of all ages. Thus the earlier work of Jain et al. (1969)²⁵ which found multiple body site dissemination after involving the mammary gland using an experimental model was confirmed in this field study (Punyapornwithaya et al., 2010)¹⁴ using molecular biology and fingerprinting.

Biddle and coworkers (2005)²³ reported on internal dissemination of *Mycoplasma sp.* in 7 cows with clinical mastitis. *Mycoplasma sp.* isolates were fingerprinted similar to that by Djordjevic and coworkers (2001)²⁶. Milk samples were collected daily for 28 days. Ante mortem swabbing solution samples were collected from the mucosal surfaces of the nares, eye, ear, vagina, and rectum at weekly intervals for 4 weeks. Then postmortem samples were collected from all cows. Tissue samples were taken from: mammary parenchyma and supramammary lymph node, pharyngeal tonsil, retropharyngeal tonsil, primary bronchus, tertiary bronchus, lung parenchyma, sinus, nasal turbinate, pleura, bronchial lymph node specimens, vaginal, vestibular fossa, and suburethral diverticulum specimens, conjunctiva, urinary bladder, knee joint, hock joint, pericardial sac, mesenteric lymph node, spleen, and auditory tube.

Swabbing solutions and tissue samples were incubated for isolation of *Mycoplasma sp.* The fingerprint patterns of all isolates were compared. Within cow, all the mammary parenchyma samples had the same fingerprint as those causing mastitis. The respiratory system had the most heterogeneity. Although 90% of ante mortem sample isolates of the respiratory system had the same fingerprint as the mammary gland isolates, less than 25% of the postmortem samples were similar. All of the urogenital isolates had the same fingerprint as the mastitis isolates, and greater than 90% of all other isolates had the same fingerprint as the milk isolates. The data from the study clearly confirmed that *Mycoplasma sp.* that cause mastitis can disseminate through out the body to other tissue sites. It also demonstrates that other isolate types can be present, but save for the respiratory system, the large majority of isolates are the same type. What this study and that of Djordjevic and coworkers (2001)²⁶ do not show is the direction of dissemination. It is not clear if the *Mycoplasma sp.* moves from the gland to other tissue sites, or if the nidus of colonization or infection initially occurs at an extramammary site. Nor does it show how cow to cow transmission might occur, whether infection starts with a respiratory disease in one cow, the agent is transmitted to another, where it is carried asymptotically at an extramammary site and then transferred to the mammary gland where it causes mastitis. Data from Jain et al. (1969)²⁵ using an experimental model indicates dissemination can occur from the mammary gland to an extramammary site.

In field studies there is evidence that suggests that mycoplasma mastitis outbreaks can originate from asymptomatic carriers. In one study the evidence indicates that an outbreak of mycoplasma mastitis originated during the dry period. *Mycoplasma bovis* mastitis affected 16 of 99 cows and appeared to start with thirteen dry cows and one periparturient cow (Jackson and Boughton, 1991)²⁸. An explanation for cow to cow, udder to udder, transmission during the dry period is difficult. Mackie and coworkers (2000)²⁹ describe an outbreak of mycoplasma mastitis in periparturient cows with a mixed infection of *M. californicum* and *M. canadense*. Given that the urogenital tract appeared to be colonized by both pathogens, they suspect that the nidus of the mastitis outbreak was not an initial intramammary infection. Their data would indicate that asymptomatic carriage coincided, perhaps preceded, intramammary infections. The research group previously had reported on an outbreak of mastitis that appeared to involve systemic transfer of the mycoplasma agent (Mackie and coworkers 2000)²⁹. Jasper et al., (1966)³⁰ also noted that in one herd with a mycoplasma outbreak cows were also suffering from mycoplasma arthritis, suggesting that the disease agent was systemically transferred. Additionally, some cows had no signs of clinical mastitis but shed *Mycoplasma sp.* and leukocytes in milk in abnormally high levels.

Wilson and coworkers (2007)³¹ reported on an outbreak of mycoplasma disease in first lactation cows in a closed commercial dairy herd. The investigation began with the presentation of a first lactation Holstein cow to the Veterinary Diagnostic Laboratory with polyarthritis. The cow died and a postmortem exam indicated the involvement of *Mycoplasma sp.* An examination of the herd and an interview with the herd owner indicated that 8 other first lactation cows had been affected with arthritis, three of the 9 cows affected had died of pneumonia, and 3 had been infected with clinical mastitis. *Mycoplasma sp.* was found to be associated with the affected animals. The outbreak did not appear to have spread beyond the 9 first lactation animals. Similarly it was reported that cases of mastitis and arthritis that appeared to be connected (Houlihan et al., 2007)³². In one herd of 120 milking cows, the arthritis was the prominent feature, although an abortion with *M. bovis* was noted as well as nasal discharge of some arthritic cows. Clinical cases of mastitis due to *M. bovis* followed the arthritis. In the second herd, an

organic dairy of 84 cows, mastitis was the most prominent feature with some cows affected with arthritis. Cows that failed to resolve were culled, and that control method appeared to be sufficient.

There is sufficient evidence to indicate that mycoplasma mastitis outbreaks are generally associated with other forms of disease by this agent (Houlihan et al., 2007, Wilson et al. 2007, Jasper et al., 1966; Mackie and Finlay, 2000; Mackie et al., 1986)^{32,31,30,29,33}. Wilson et al. (2007)³¹, reported that it appeared that lameness preceded the mastitis problem, suggesting internal transmission of the disease from one organ site, in this case seemingly extramammary, to another organ site, mammary. The findings of the clinical report by Wilson and coworkers (2007)³¹ also indicate that the agent may be endemic to the herd given that the outbreak was in a closed herd, and infected a select group of related animals. In aggregate, findings indicate that lactating cattle may be the reservoir of the multi-site infections. But there is evidence that calves may be diseased or asymptomatic carriers of the agent.

Mycoplasma sp. is carried in calves, and it appears the nares might be the most readily colonized site. Bennett and Jasper (1977)³⁴ examined the nasal prevalence of replacement cattle in herds with and without mycoplasma mastitis. They were almost six times as likely to find the agent in the nasal passage of apparently healthy calves of mycoplasma mastitis herds.

Mycoplasma sp. was isolated from 6% of calves in non-mycoplasma mastitis herds. This could suggest that the agent was more likely to be present in asymptomatic carriers, calves, and that carriage was a function of a prevalence of disease in the herd. Moreover their findings suggest that herds that fed milk were more likely to have carrier calves. Latter, Springer and coworkers (1982)³⁵ reported that 80% of herds, 19% of calves, carried *Mycoplasma sp.* in their nares in herds without apparent problems with respiratory disease. Thus replacement animals of dairies could be asymptomatic carriers of the agent, or they themselves be diseased with the mycoplasma mastitis agent.

Hum et al. (2000)³⁶ reported on an outbreak of mastitis polyarthritis and abortion caused by *Mycoplasma species* bovine group 7 in 3 dairy herds that were centrally managed. Each herd had approximately 1000 lactating cows. Calves were removed from dams within 24 h of birth and fed colostrum. Calves from all 3 farms were co-mingled on one farm at 3 days of age. Mycoplasma disease in the form of polyarthritis was first noticed in the calves, at approximately 2 weeks of age. Approximately a third of all calves at the rearing site were diseased and most were culled or died. An increased abortion rate was reported at one dairy and suspected to be caused by *Mycoplasma species* bovine group 7. Milk samples from cows with clinical mastitis and bulk tank milk samples were found to have *Mycoplasma sp.* bovine group 7. Later (Djordjevic et al., 2001)²⁶ it was found that fingerprints of the *Mycoplasma species* bovine group 7 isolates were found to be indistinguishable, suggesting all isolates were from the same clone. However, *Mycoplasma sp.* were never isolated from colostrum samples. Hum et al. (2000)³⁶ suggest that the source of the outbreak was the colostrum fed to calves, and that the organism was resident in the herd causing disease in other animals. Given the early age that the calves became diseased suggests that this might have been the case. Yet given the fact that the colostrum did not appear to contain the disease agent, and that the agent was implicated in causing abortions and thus infecting the reproductive tracts, an alternative explanation is possible. It could be that the *Mycoplasma species* bovine group 7 was disseminated to a calf or calves during parturition and spread to other calves in the calf rearing unit. Regardless, calves were implicated as carriers of disease and the agent appeared to be disseminated in the adult population as well.

Punyapornwithaya and coworkers (2010)¹⁴ reported on a mycoplasma mastitis outbreak in a herd with no history of the disease. A calf with pneumonia was culled a month before the first cow with mycoplasma mastitis was discovered. Almost coincident with the first case of mycoplasma mastitis was a second case of mycoplasma pneumonia in a calf. *Mycoplasma bovis* was isolated from the lung tissue of that second calf. Both calf and cow were culled. Shortly thereafter a second cow was found to have *Mycoplasma bovis* mastitis and an investigation was initiated. Swabbing solution samples were collected from accessible mucosal surfaces (nares, eye, ear, and vagina) from all animals on the farm. Additionally, milk samples were collected from all lactating cows. One hundred and sixty three animals were sampled and *Mycoplasma bovis* was isolated from 72 animals, primarily the nares (n=57). Calves were slightly more likely to carry the clone of *Mycoplasma bovis* endemic to the herd than cows as determined by swabbing solution cultures. One cow was found to have mycoplasma mastitis at the first whole herd sampling. The fingerprint patterns of electrophoresed chromosomal digests were indistinguishable, suggesting the same clone was carried by all animals. The herd was sampled as described at quarterly intervals for a year. During that time, two new cases of mycoplasma mastitis were identified, but the percentage of animals with perceptible carriage of *Mycoplasma sp.* was less than 10%, and most often the *Mycoplasma sp.* identified appeared to be different than the original. No new cases of mycoplasma mastitis were found during the last 6 month period. Carriage and disease by this agent seemed to “run its course” and had almost disappeared from the herd. *Mycoplasma sp.* was not cultured from the swabbing solutions of mucosal sites from the cows which prior to their development of mycoplasma mastitis. Thus asymptomatic carriage did not appear to precede mastitis. In total this case report reinforces the concept that the mycoplasma agent causing mastitis can colonize multiple extramammary body sites, stay resident for several months in a closed herd, and that carriage may not pose a threat to the herd as evidenced by the limited number of outbreak mastitis cases.

Two recent articles present evidence that the calf may develop mycoplasma mastitis and act as a reservoir for the disease in lactating cattle. Roy et al., (2008)³⁷ indicate that a 7 week old heifer calf on farm on the University of Montreal’s veterinary service was found to have septic arthritis of the left tarsus. Upon full physical exam it was noted that the right rear mammary gland was inflamed. *Mycoplasma bovis genitalium* was isolated from a mammary gland inflamed quarter, but not from the tarsus. *Mycoplasma arginini* was isolated from the nares but no other body sites were positive for *Mycoplasma sp.* The dam of the calf did not appear to suffer from mastitis, nor did mycoplasma mastitis seem to affect other cows in the herd as determined from weekly cultures of bulk tank milk samples. The calf was kept in the herd and the septic arthritis and the mastitis resolved without treatment. The authors conclude that infected calves could play a role in the epidemiology of mastitis in a herd, although apparently in this case the disease did not pose a threat to the calf or herd mates. The findings are clear that calves can act as carriers of the mycoplasma mastitis agent.

In the second recent report (Fox et al. 2008)¹¹, it was found that intramammary infections with *Mycoplasma sp.* occur in Holstein heifer calves prior to puberty. Three heifers on two dairies with mammary nodules were investigated. The attending veterinarian identified these nodules on the calves at the time of vaccination and removal of supernumerary teats. Lacteal fluid from mammary gland quarters with nodules had *Mycoplasma sp.* The agent was also isolated from organ tissue aseptically collected from calves after euthanasia. Strains were identified as *Mycoplasma bovis* and were fingerprinted. Swabbing solutions from accessible body site mucosal surfaces of the dam of the heifers, or a herd mate of the dam in one instance

where the dam had been culled, as well as milk and blood samples, were collected. Samples from the respiratory system and the mammary tissue of one calf had the same chromosomal digest fingerprint pattern. This pattern was also indistinguishable from the milk sample from a cow in the herd with mastitis. In the second herd, both the dam's blood and milk sample were positive for *M. bovis*, the same strain as found in the calf's mammary gland. In aggregate the data indicate that *M. bovis* maybe carried in cows and calves in a herd. Hematogenous dissemination is indicated by the presence of the agent in the blood of the dam of one calf. The dam in this case was shedding *Mycoplasma bovis* in the milk although her mammary gland seemed healthy and functional, her 305 day mature equivalent production for the lactation was 12,274 kg of milk and her somatic cell count was consistently lower than 100,000. Since the dam was a symptomatic carrier, and the manifestation of the problem in the calf occurred at an early age, it maybe most plausible that the reservoir of the agent was the cow. Similarly, in the other herd, a cow with mastitis was found to be infected with the same clone of *M. bovis* as a calf, and that clone had colonized or infected several body sites of the calf. Again, it is more plausible that the older animals were the reservoir for the agent in this herd. The data from this study also indicate that not all isolates of *Mycoplasma sp.* associated with the samples collected were of the same fingerprint. Thus several strains of *Mycoplasma sp.*, some with the ability to cause mastitis, may be present on a herd simultaneously. Lastly, heifer calves may present at the time of pubertal health and vaccine check, with mammary nodules infected with *Mycoplasma bovis*. Such infections may pose a risk to the herd at a time when those heifers come into lactation.

All of these studies beg the question, how can mycoplasma mastitis be better controlled? The data presented herein indicates that the disease causing agent can remain in the herd for months and be asymptotically carried by cows and calves. In a study of 18 herds (Punyapornwithaya et al., 2012, in press)³⁸ with a new case of mycoplasma mastitis, we attempted to identify the control points that could be applied prevent an outbreak from erupting. The majority of herds were ostensibly free of mycoplasma mastitis within 1 month. No evidence suggested an advantage of preferential culling in control of mycoplasma mastitis as Fast Recovery herds, herds that were apparently cleared mycoplasma mastitis, were split evenly between preferential and non-preferential culling, and all Slow and Non-recovery herds, herds that took longer than 1 month to clear mycoplasma mastitis, culled cows preferentially. There was little variation between herds with respect to MTH practices and thus no specific practice was linked to time to clearance. Thus testing and preferential culling of cows with mycoplasma intramammary infections did not appear to hasten the apparent clearance of mycoplasma mastitis from the herd as determined by BTM cultures. It would seem that dairy operators and their veterinary practitioners should consider the importance of culling cows with mycoplasma mastitis as part of a control strategy. The importance of monitoring the mycoplasma mastitis status by culturing bulk tank milk is recommended.

In a follow up study we examined the role of a hospital pen for control of *M. bovis* clinical mastitis (Punyapornwithaya, et al., 2012)³⁸. The overall incidence rate of mycoplasma mastitis in the hospital pen approached 100 fold greater than the incidence rate in the milking pens. The hospital pen was created to isolate mycoplasma mastitis cows and keep them from infecting the non-infected cows. It appeared that 3 new episodes of mycoplasma mastitis were created when cows with true mycoplasma mastitis infections were added to the hospital pen and transmitted their pathogens to cows which had clinical mastitis cases, and therefore suspect mycoplasma mastitis cows, but were initially free of the disease. The lag in time between

collection of a sample and the culture result, 10 d, accounted for increased exposure to the incident cases of mycoplasma mastitis. Although it is possible that cows had subclinical mycoplasma intramammary infections prior to their entry into the hospital pen and then developed clinical mastitis, the evidence from our study suggested that new cases of mycoplasma mastitis occurred in the hospital pen and that the addition of cows with clinical mycoplasma mastitis appeared to be the nidus for additional infections in the hospital pen. Three months after creation of the hospital new cases of mycoplasma mastitis subsided suggesting that establishment of the hospital pen may have contributed to control of this disease. But future studies enrolling more herds should be directed to determine if formation of a hospital pen contains the spread of mycoplasma mastitis and/or accelerates transmission of this disease.

Prototheca sp. Epidemiology and Control

Prototheca sp. that cause mastitis most commonly belong to the species *wickerhamii* or *zopfii*, with the latter being the most prevalent. *Prototheca sp.* that cause mastitis have been generally regarded as an environmental mastitis pathogen, with several reports indicating it can be found on dairies in fecal matter, water sources, and bedding (Anderson et al., 1988; Costa et al., 1997; Saccabarozzi et al., 2008)^{39,40,41}. Moreover, the occurrence of *Prototheca sp.* in the environment may not be more prevalent on dairy farms that have experienced an outbreak than those that have had no *Prototheca sp.* mastitis (Anderson et al., 1988)³⁹. Thus it would seem logical that the recommendation for the control of prototheca mastitis would be keeping the cow's environment clean and dry. *Prototheca sp.* are not susceptible to common antibiotics as they are not bacteria. It has been suggested that cows with intramammary infections of *Prototheca sp.* should be culled as the infections cannot be successfully treated and teat cups maybe contaminated with the pathogen (A. Lichtenwalner, personal communication, 2011)⁴². Additionally *Prototheca sp.* have been found to be slightly resistant to disinfectants, and data suggest that dilute solutions of disinfectants associated with backflush would fail to kill the agent (A. Lichtenwalner, personal communication, 2011)⁴². This data and the effectiveness of culling of cows with chronic prototheca mastitis to control the disease in aggregate could suggest that *Prototheca sp.* should also be classified as a contagious mastitis pathogen. Given that the cow does not appear to be a primary reservoir for the disease, it may be best to approach the control of *Prototheca sp.* that cause mastitis as an environmental problem.

Conclusion

Mycoplasma mastitis might be difficult to diagnose and remains a problem affecting dairy cattle worldwide. Recent reports establish that replacement animals can act as asymptomatic, or perhaps symptomatic, carriers of a strain that causes mastitis in the herd. Although not conclusive, the data suggests that the strains associated with mastitis are passed on to the calf from the dam, as seen in Djordjevic and coworkers (2001)²⁶ and Fox et al. (2008)¹¹. Carriage of mycoplasma by cows and calves maybe greatest during the outbreak, diminish over time, and thus may not be a threat to cause further disease, mastitis (Punyapornwithaya et al., 2010)¹⁴. The role of isolation of mycoplasma cows either through segregation such as a hospital pen or culling, did not seem to hasten the abatement of new cases of mycoplasma mastitis. Moreover, other control strategies for the disease were not readily apparent. It may be that the

asymptomatic carriage is often most prevalent and that intervention strategies fail to isolate a requisite number of animals that would reduce the nidus of infection.

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