# Mycoplasma Mastitis and Prevention

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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). 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). 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), Northern Greece (Filioussis et al, 2007), Saudi Arabia (Al Abdullah, 2006), France (Arcangioli et al., 2011), Belgium (Passchyn et al., 2011), Mexico (Miranda-Morales et al., 2008) and Iran (Ghazaei, 2006). Given that mycoplasma mastitis seems to be associated with larger herds (Pinho, 2012APHIS, 2008; Fox et al., 2008; Thomas et al., 1981), and that herd size has been increasing over time, it would seem logical that mycoplasma mastitis has become more prevalent. This would seem a most important risk factor given that the Western USA has the largest dairy farms in the nation (APHIS 2009) and is the fastest growing dairy area, especially when considering cows per dairy herd.

Two primary dairy herd risk factors for mycoplasma mastitis as stated by Jasper (Jasper, 1981) are (1) introduction of diseased animals to the lactating herd and (2) problems with milking time procedures, most notably inadequacies in hygiene. Recent reports (Punyapornwithaya et al., 2010 and 2011) 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.

## 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). 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% $CO_2$ for 7-10 days (Hogan et al., 1999). Other methods such as immuno-detection of specific antigens and nucleic acid detection (PCR technology) have been established (Fox et al. 2005). The PCR technology may have promise (Justice-Allen et al., 2012, Pinho et al., Boonyayatra et al., 2012a) , but 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). 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). Yet it should be kept in mind that *Acholeplasma sp.* are capable of causing mastitis (Counter, 1978).

*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% $CO_2$, for 1-2 days. Relatively few laboratories may be equipped to detect this pathogen group (Biddle et al., 2005). Indeed, Nicholas et al. (2007) 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.

### 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 (1969). 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. bovigenitalium* and the third believed to by *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) 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 the 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 that 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) 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). 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), 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) 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) which found multiple body site dissemination after involving the mammary gland using an experimental model was confirmed in a field study (Punyapornwithaya et al., 2010) using molecular biology and fingerprinting.

Biddle and coworkers (2005) 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). 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) 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 asymptomatically at an extramammary site and then transferred to the mammary gland where it causes mastitis. Data from Jain et al. (1969) 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 bovigenitalium* mastitis affected 16 of 99 cows and appeared to start with thirteen dry cows and one periparturient cow (Jackson and Boughton, 1991). An explanation for cow to cow, udder to udder, transmission during the dry period is difficult. Mackie and coworkers (2000) 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). Jasper et al., (1966) 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. Pinho et al. (2012) recently reported that from a survey of farms in Portugal, 20% of cows with mycoplasma mastitis intramammary infections had negative California Mastitis Test scores. This would indicate that even when diseased, a noticeable minority of cows with mycoplasma mastitis have subclinical infections.

Wilson and coworkers (2007) 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). 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). Wilson et al. (2007) 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) 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) 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) 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 can be diseased with the mycoplasma mastitis agent.

Hum et al. (2000) 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) 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) 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) 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) 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 bovigenitalium* 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), 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 cells/ml of milk. Since the dam was a symptomatic carrier, and the manifestation of the problem in the calf occurred at an early age, the most plausible explanation is 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 asymptomatically carried by cows and calves. In a study of 18 herds (Punyapornwithaya et al., 2012, in press) 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). 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.

**Recent cases: A report.** It should be noted that the author has recent experience with two herds with mycoplasma mastitis outbreaks. The first herd was the Washington State University herd of 150 cows which had a history of being mycoplasma mastitis free for 25 years as determined by bulk tank milk cultures. When a first bulk tank culture appeared positive, milk samples were collected and cultured from all cows currently with clinical mastitis and all cows with recent cases of clinical

mastitis. None of these milk cultures identified cows with mycoplasma mastitis. Next, milk samples were collected from all lactating cows and then cultured; a cow with subclinical mycoplasma mastitis was identified. This cow was kept with the milking herd for approximately 10 days and then isolated; her milk was discarded after identification. Following identification of the infected cow, nasal swabbing solutions were collected from all animals on the dairy farm and it was determined that 54/353 (15.3%) of all replacements and adult cows were shedding the same strain of *M. bovis* as that causing subclinical mastitis. Yet no cases of mycoplasma disease were noted and no additional cases of mastitis developed. The herd appeared free of mycoplasma mastitis when the cow with the subclinical case was culled.

In a second recent outbreak in a commercial herd of approximately 500 cows, 47 of 166 clinical mastitis cases were determined to be due to *M. bovis*. This *M. bovis* mastitis outbreak lasted 2 months. During the outbreak cows were culled from the commercial herd shortly after they were identified. At the beginning of the outbreak calves and cows suffered from polyarthritis and thick nasal discharge. A joint tap sample and a nasal swab sample had *M. bovis*. The outbreak was deemed to have passed when bulk tank milk sample cultures were negative for one month and when no new cases of clinical mycoplasma mastitis occurred during that time. A last report from this herd suggested that after a hiatus, new cases of clinical mycoplasma mastitis appeared but not nearly as severe as the initial outbreak.

There was a dramatic difference in the adherence to milking time hygiene techniques between to the two herds. The WSU herd has a history of excellent contagious mastitis control procedures as evidenced by low milk somatic cell counts (mean less than 150,000 cells/ml) and no *Streptococcus agalactiae* and low prevalence of *Staphylococcus aureus,* consistently less than 3%. Contrarily, the commercial herd had a bulk tank somatic cell count that often exceeded 300,000 cells/ml and had a history of episodes of contagious mastitis. Clearly the *M. bovis* strain in the WSU herd was transmissible, having spread to more than 15% of the herd as evidenced by asymptomatic carriage in the nasal passages. However no new cases of mastitis developed. In the commercial herd the contagious mastitis control was specious and spread occurred amongst the lactating herd in the form of mastitis; no changes in milking time hygiene protocols were made and culling appeared to control the spread. Although it is difficult to make firm conclusions based on these two herd case histories, it does appear that in both herds the strain of *M. bovis* was transmissible and it seems that a difference in the outcome might have been tied to milking time hygiene techniques and contagious mastitis control practices. In the WSU herd the infected animal was culled, although by the time of culling the agent had spread to all age groups of animals on the farm and was carried asymptomatically by more than 15% of the herd. Thus it could be argued that perhaps culling was an unnecessary control strategy in the WSU case, since widespread dissemination had already occurred. In the commercial herd where the owner/operator made no changes to contagious mastitis control procedures with the exception of selected culling of mycoplasma mastitis cows, transmission eventually diminished but was likely not eliminated. Perhaps culling mycoplasma mastitis animals helped reduce transmission. But there is more to the control of the dynamics of *M. bovis* disease and colonization in a herd than simply culling mastitis cases. Our experience in an earlier outbreak where culling cases of clinical mycoplasma mastitis and other mycoplasma diseases was that culling could not have possibly stayed ahead of transmission. In that case, the outbreak first manifested clinically as pneumonia in calves, later as mastitis in cows and as arthritis in calves, affecting a total of 8 animals clinically, but more than ten fold that number of animals as evidenced by asymptomatic carriage of the strain causing clinical disease (Punyapornwithaya et al., 2010).

A previous report questioned the importance of culling as a universal *M. bovis* mycoplasma mastitis control procedure (Punyapornwithaya et al., 2012). Clearly in both herds transmission of *M. bovis* occurred extensively before culling would have had an effect. In the WSU herd, excellent milking time hygiene could have contributed significantly to arresting the spread of the agent and isolated the case of mastitis to a single individual. But also clearly the agent had likely spread to all rearing areas on the farm as a significant number were asymptomatically shedding it nasally. In the commercial herd, transmission had occurred to both calves and cows and caused disease other than mastitis, and such transmission preceded the implementation of the culling strategy.

So what arrested transmission of *M. bovis*? Although apparently the transmission on both dairies had been widespread, affecting calves and cows, the agent was only detected in a minority of animals. Either more were asymptomatically affected but shedding the agent at levels below detection or most animals were not susceptible to carriage and/or disease. If the latter was true, then culling of cows could have reduced the exposure of the agent to other susceptible animals. Yet it is also possible that over time the infecting strain had changed genetically. This was seen in a more detailed case report (Punyapornwithaya et al., 2010). Alternatively, it maybe that specific immunity had developed within the animals in the herd, or perhaps a combination of induced *M. bovis* genetic alteration by immune reactions accounted for the cessation of the outbreak.

## 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) and Fox et al. (2008). Carriage of mycoplasma by cows and calves may be greatest during the outbreak, diminish over time, and thus may not be a threat to cause further disease, mastitis (Punyapornwithaya et al., 2010). 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 in some illustrated cases. However, culling as the primary method of mycoplasma mastitis control appeared to be the most effective strategy in other reports. The initiation of an outbreak that follows the importation of cattle that occurs in herds deemed to have good to excellent milking time hygiene may be due to the fact that these intervention strategies fail to isolate a requisite number of asymptomatic carrying animals that would reduce the nidus of infection. Perhaps bovine immunity against the agent develops and is sufficient to contain the outbreak over time. *Mycoplasma sp.* in general, *M. bovis* in particular, are highly transmissible agents and the primary methods of control should focus on maintaining overall cattle health through nutrition and environment, and reducing the exposure to new strains by careful selection of how and when cattle are imported into the herd.

## References

Al-Abdullah, H. A., and E. A. Fadl. 2006. Mycoplasma mastitis in dairy cattle herds in Saudi Arabia. Vet. Rec.159:88-89.

APHIS, 2003. Mycoplasma in bulk tank milk in U.S. dairies. APHIS Info Sheet # N395.053, Fort Collins, CO.

APHIS. 2008.  Prevalence of contagious mastitis pathogens on U.S. dairy operations, 2007.  APHIS Info Sheet. #N533.1008  Fort Collins, CO.

APHIS. 2009.  Dairy 2007: Part IV:  Reference of dairy cattle health and management practices in the United States.  APHIS Publication  #N494 0209  Fort Collins, CO.

Arcangioli, MA, M Chazel, E Sellal, et al. 2011. Prevalence of Mycoplasma bovis udder infection in dairy cattle: preliminary field investigation in southeast France.  N Z Vet J 59:75-78

Bennett, R. H., and D. E. Jasper. 1977.  Nasal Prevalence of Mycoplasma bovis and IHA Titers in Young Dairy Animals. Cornell Vet. 67:361-373.

Biddle, L.K., Fox, M. A. Evans, and C.C. Gay. 2005. Pulsed-field gel electrophoresis patterns of Mycoplasma isolates from various body sites in dairy cattle with Mycoplasma mastitis. J. Am. Vet. Med. Assoc. 227:445-459.

Boonyayatra, S., L.K. Fox, T.E. Besser, A. Sawant, J.M. Gay and Z. Ravivi. 2012. A PCR assay and PCR-Restriction fragment length polymorphism combination identifying the 3 primary Mycoplasma species causing mastitis. J. Dairy Sci.  95:196-205.

Boonyayatra, S., L.K Fox, J.M Gay, A. Sawant and T.E. Besser. 2012.  Discrimination between Mycoplasma and Acholesplasma species of bovine origin using digitonin disc diffusion assay, nisin disc diffusion assay and conventional polymerase chain reaction.  J. Vet. Diag. Invest. 24:7-13

Counter, D.E. 1978. A severe outbreak of bovine mastitis associated with Mycoplasma bovigenitalium and Acholeplasma laidlawii. Vet Rec. 103:130-131

Djordjevic, S. P., W. A. Forbes J. Forbes-Faulkner, P. Kuhnert, S. Hum, M. A. Hornitzky, E. M. Vilei, and J. Frey. 2001. Genetic diversity among Mycoplasma species bovine group 7: Clonal isolates from an outbreak of polyarthritis, mastitis, and abortion in dairy cattle. Electrophoresis 22:3551-3561.

Filioussis, G., G. Christodoulopoulos A. Thatcher, V. Pertidou, and E. Bourtzi-Chatzopoulou. 2007. Isolation of Mycoplasma bovis from bovine clinical mastitis cases in Northern Greece. Vet. J. 173:215-218.
Fox, L. K., D. D. Hancock A. Mickelson, and A. Britten. 2003. Bulk tank milk analysis:  Factors associated with appearance of Mycoplasma sp. in milk. J. Vet. Med. B. 50:235-240.

Fox, L.K., J.H. Kirk, and A. Britten. 2005.  Mycoplasma mastitis: A review of transmission and control.  J. Vet. Med. 52:153-160.

Fox, L. K., F. J. Muller, M. L. Wedam, C. S. Schneider, and M. K. Biddle. 2008. Clinical Mycoplasma bovis mastitis in prepubertal heifers on 2 dairy herds. Can. Vet. J. 49(11):1110-11102.

Ghazaei C. Mycoplasmal mastitis in dairy cows in the Moghan region of Ardabil State, Iran. J S Afr Vet Assoc 2006;77:222-223.

Gonzalez, R.N. and D. J. Wilson. 2003. Mycoplasma mastitis in dairy herds. Veterinary Clinics of North America: Food Animal Practice. 19:199-221.

Hogan, J. S., R. N. Gonzales, et al. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Inc., Madison, WI.

Houlihan, M. G., B. Veenstra, M. K. Christian, R. Nicholas, and R. Ayling. 2007. Mastitis and arthritis in two dairy herds caused by Mycoplasma bovis. Vet. Rec. 160:126-127.

Hum, S., A. Kessell, S. Djordjevic, R. Rheinberger, M. Hornitzky, W. Forbes, and J. Gonsalves. 2000. Mastitis, polyarthritis and abortion caused by Mycoplasma species bovine group 7 in dairy cattle. Aust. Vet. J. 78(11):744-750.

Jackson, G. and E. Boughton. 1991. A mild outbreak of bovine mastitis associated with Mycoplasma bovigenitalium. Vet Rec. 129:444-446.

Jain, N. C., D. E. Jasper, and J. D. Dellinger. 1969. Serologic Response of Cows to Mycoplasma Under Experimental and Field Conditions. American Journal of Veterinary Research. 30(5):733-742.

Jasper, D. E. 1981. Bovine Mycoplasmal Mastitis. In: Cornelius, CE and Simpson, BF, eds. Advances in veterinary sciences and comparative medicine. New York: Academic Press, 1981. p. 121-157.

Jasper, D. E., N. C. Jain, and L. H. Brazil. 1966. Clinical and Laboratory Observations on Bovine Mastitis Due to Mycoplasma. J. Am. Vet. Med. Assoc. 148(9):1017-1029.

Justice-Allen, A., J. Trujillo, G. Goodell, and D. Wilson. 2011. Detection of multiple *Mycoplasma* species in bulk tank milk samples using real-time PCR and conventional culture and comparison of sets sensitivities. J. Dairy Sci. 94-3411-3419.

Mackie, D. P., H. J. Ball, E. F. Logan. 1986. Mycoplasma californicum mastitis in the dry dairy cow. Vet. Res. 19:350-351.

Mackie, D. P., D. Finley, N. Brice, and H. J. Ball. 2000. Mixed mycoplasma mastitis outbreak in a dairy herd. Vet. Rec. 147:335-336.
Manso-Silván, L., X. Perrier, and F. Thiaucourt. 2007. Phylogeny of the Mycoplasma mycoides cluster based on analysis of five conserved protein-coding sequences and possible implications for the taxonomy of the group. Int. J. Syst. Evol. Microbiol. 57:2247-58.

Miranda-Morales, RE, V Rojas-Trejo, R Segura-Candelas, et al. Prevalence of pathogens associated with bovine mastitis in bulk tank milk in Mexico. Ann N Y Acad Sci 2008;1149:300-302.

Nicholas, R., R. Ayling, L. McAuliffe. 2007. Mycoplasma mastitis. Vet. Rec. 160:382:383.

Olde Riekerink, R. G., H. W. Barkema, S. Veenstra, D. E. Poole, R. T. Dingwell and G. P. Keefe. 2006. Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. Can. Vet. J. 47:567-572.

Passchyn, P, S Piepers, L De Meulemeester, et al. 2012. Between-herd prevalence of Mycoplasma bovis in bulk milk in Flanders, Belgium. Res Vet Sci 92 (2):219-220.

Pinho, L., G. Thompson, M. Machado, J. Carvalheira.  2012.  Management practices associated with the bulk tank milk prevalence of *Mycoplasma spp*. in dairy herds in Northwestern Portugal.

Preventive Veterinary Medicine.  Epublication, ahead of print
(http://www.sciencedirect.com/science/article/pii/S0167587712002115)

Punyapornwithaya, V., L. K. Fox, D. D. Hancock, J. M. Gay, and J. R. Alldredge. 2010. Association between an outbreak strain causing Mycoplasma bovis mastitis and its asymptomatic carriage in the herd: A case study from Idaho, USA. Prev. Vet. Med. 93(1):66-70.

Punyapornwithaya, V, LK Fox, DD Hancock, JM Gay, JR Wenz, JR Alldredge. 2011. Incidence and transmission of *Mycoplasma bovis* mastitis in Holstein dairy cows in a hospital pen: A case study. Prev Vet Med 98:74-78.

Punyapornwithaya, V., L.K. Fox, D.D. Hancock, J.M. Gay, J. R. Alldredge. Time to clearance of mycoplasma mastitis:  The effect of management factors including milking time hygiene and preferential culling. Canadian Veterinary Journal, in press.

Roy, J., D. P. Francoz, and O. Labreque, O. 2008. Mastitis in a 7-week old calf caused by Myocplasma bovigenitalium. Vet. J. 176:403-404.

Springer, W. T., R. W. Fulton, H. V. Hagstad, S. S. Nicholson, and J. D. Garlton. 1982. Prevalence of mycoplasma and chlamydia in the nasal flora of dairy calves. Vet. Micro. 7:351-357.

Thomas, C. B., P. Willeberg, and D. E. Jasper. 1981. Case-control study of bovine mycoplasmal mastitis in California. Am. J. Vet. Res. 42(3):511-515.

Wilson, D. J., R. T. Skirpstunas, J. D. Trujillo, K. B. Cavendar, C. V. Bagley, and R. L. Harding. 2007. Unusual history and initial clinical signs of Mycoplasma bovis mastitis and arthritis in first-lactation cows in a closed commercial dairy herd. J. Am. Vet. Med. Assoc. 230:1519:1523.

# Notes: