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Short communication

Association between an outbreak strain causing mycoplasma bovis mastitis and its asymptomatic carriage in the herd: A case study from Idaho, USA

V. Punyapornwithaya [a], L.K. Fox [a,*], D.D. Hancock [a], J.M. Gay [a], J.R. Alldredge [b]

[a] Department of Veterinary Science, Washington State University, Pullman, WA 99163-6610, USA
[b] Department of Statistics, Washington State University, Pullman, WA 99163-3144, USA

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ABSTRACT

The objective of this study was to determine the association between mycoplasma mastitis and colonization of mycoplasma organisms at body sites of asymptomatic carriers. The investigation was done in a dairy herd with a first outbreak of mycoplasma mastitis. Milk and swab solution specimens from accessible mucosal surfaces of body sites from cows and replacements were sampled at quarterly intervals (Herd Samplings 1–4). Samples were cultured and *Mycoplasma* spp. were isolated, speciated and fingerprinted. During Herd Sampling 1 two cows with mycoplasma bovis mastitis were identified and all swabbing solutions of body site samples from 18 of 84 cows and 36 of 77 replacements were positive to *Mycoplasma bovis* and fingerprinted as the same strain. A case of clinical *M. bovis* mastitis developed during Herd Sampling 3. During Herd Samplings 2–4, 4 lactating cows and 12 replacements were positive to *M. bovis* at various body sites with 4 different strains. Three isolates of *Mycoplasma californicum* were found from swabbing solutions of three cows during Herd Samplings 3 and 4. Only one strain of *M. bovis* caused mastitis although four strains were isolated from body sites of animals. Isolation of *M. bovis* from a body site never preceded mastitis. No lactating cow developed mastitis during Herd Sampling 4 although some animals were colonized with the organism. It appears that during the initial outbreak of *M. bovis* mastitis colonization of body sites by the outbreak strain may be common. However, the prevalence of colonization subsides and colonization does not appear to precede mastitis.

1. Introduction

Mycoplasma spp. have been considered contagious mastitis pathogens and thus the transmission of *Mycoplasma* spp. has been postulated to occur primarily during milking time and can be controlled by the use of proper milking time hygiene practices (Fox and Gay, 1993; Gonzalez and Wilson, 2003). Despite implementation of contagious mastitis control methods the prevalence of mycoplasma infection has increased, suggesting that transmission may more commonly occur from pathways other than those associated with milking (Fox et al., 2003). The mucosal surfaces of eyes, nasal cavities, ears and vestibular fossa are the colonization sites for *Mycoplasma* spp. in dairy cattle (Fox et al., 2005) and may be associated with mastitis (Biddle et al., 2005). Transmission of *Mycoplasma* spp. from extramammary sites of asymptomatic carriers, to the mammary gland, has been hypothesized. Such internal transmission may account in part for the inability of herds to eradicate mycoplasma mastitis despite employment of excellent milking time hygiene practices.

In this paper we describe the epidemiology of an outbreak of mycoplasma mastitis in a herd that had no known history of this disease. The association between the

* Corresponding author. Tel.: +1 509 335 0786.
E-mail address: fox@vetmed.wsu.edu (L.K. Fox).

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outbreak strain and asymptomatic carriage of *Mycoplasma bovis* mastitis by lactating cows and their replacements was made.

2. Materials and methods

2.1. Herd history

During a 20-year period the University of Idaho (UI) dairy herd of approximately 80 Holstein lactating cows had no history of mycoplasma mastitis based upon cultures of both bulk tank milk and milk from mammary quarters of cows with clinical mastitis. Thus the herd had been considered mycoplasma mastitis free, a naïve herd, but then reported their first case of mycoplasma mastitis that was initially detected through microbiological culture of bulk tank milk on August 3, 2004. One cow with clinical mastitis (Case 1) was identified as having an intramammary infection with *Mycoplasma* spp. from milk culture by the Washington Animal Disease Diagnosis Laboratory Pullman, WA. This cow was culled from the herd before the investigation began and no samples were obtained by our laboratory. Bulk tank milk culture results were negative to *Mycoplasma* spp. during the next 3-week period after the infected animal was removed. Approximately 2 weeks thereafter a bull calf was diagnosed with mycoplasma pneumonia and was culled. *Mycoplasma* spp. was again isolated from a bulk tank milk sample on September 22, 2004. Replacements born at UI herd, aged 4–6 months, were routinely transported to a state dairy institutional herd, raised, and returned just prior to first parturition. The last batch of replacements returned to the herd approximately 2 months before the first case of mycoplasma mastitis developed.

2.2. Definitions

A cow with clinical mastitis from which *Mycoplasma* spp. was identified in milk was considered a case. Cows are animals that were born and raised at the UI herd and never left the premises. Replacements included: (1) calves and heifers that were born and raised at the UI herd and never left the premises, (2) calves and heifers born at the UI herd but partially raised at the institutional herd, and (3) primiparous cows that were purchased from the institutional herd. The outbreak refers to the time when the second case of mycoplasma mastitis was found which was associated with a high prevalence of colonization in both replacements and cows. Sampling periods were the time that all animals in the herd were sampled and were conducted at quarterly intervals (Herd Samplings 1–4) for 1 year. Exposed animals were raised partially or entirely at the institutional herd. All other animals were considered restricted.

2.3. Sampling scheme

Milk and body site samples were collected quarterly with the start of the outbreak. All animals present in the herd were sampled once during each sampling period. The collection of milk samples and other body site samples was done as described (Biddle et al., 2005). In brief, composite milk samples were collected aseptically from lactating cows. Swabs, moistened by mycoplasma enrichment media, were in contact and rotated over the mucosal surfaces of the right and left eyes, the right and left nasal cavities, the right and left ears, the vestibular fossa and vaginal wall (vulvovaginal tract) from all animals which included calves, young heifers, heifers, dry cows and lactating cows. Swabs were returned to the mycoplasma enrichment media tubes after collection.

2.4. Microbiological culturing of samples and examination

Milk samples were vortexed, and 100 μL of milk were inoculated into 10 mL of mycoplasma enrichment medium. The mycoplasma enrichment media tubes from both milk samples and body site samples were incubated at 37 °C, 10% CO_2, for 4 days. A 100 μL portion of incubated media was plated on modified Hayficks agar for 10 days, then examined with a 15× dissecting microscope, to identify colonies with the distinctive "fried egg" appearance (Hogan, 1999). Results were considered positive if any mycoplasma colonies were seen and negative if there was no evidence of growth of *Mycoplasma* spp.

2.5. Species and strain identification

Pulsed field gel electrophoresis (PFGE) of chromosomal digests was used to fingerprint the mycoplasma isolates as described (Biddle et al., 2005). In brief, mycoplasma samples were centrifuged at 1200 × *g* at 4 °C for 10 min to pellet the microorganism. The supernatant was discarded, and pellet was suspended in 200 μL of buffer solution. 10 μL of proteinase K was added, and the DNA was embedded in 200 μL of agarose. Plugs were cast and lysed. Chromosomal DNA was digested with the restriction enzyme SalI. Electrophoresis was performed at 14 °C for 20.2 h at a setting of 6 V/cm of gel and a linear pulse ramp of 1–12.9 s. After electrophoresis, gels were stained with ethidium bromide for 30 min, washed twice in distilled water, and then photographed under UV light. Isolates were considered to be the same strain when chromosomal digestion produced the same number of bands of the same size (Fig. 1). Samples were speciated by the method outlined previously (Tang et al., 2000).

2.6. Statistical analysis

Proportional data were arranged into 2 × 2 contingency table. The χ^2-test was used to test:(1) differences in proportions of colonization at any body site between replacements and cows, (2) differences in proportions of colonization at any body site between exposed animals and restricted animals and, (3) differences in proportions of colonization at multiple body sites between replacements and cows and (4) differences in proportions of colonization at multiple body sites between exposed animals and restricted animals. In cases where a contingency table cell had a value less than 5, the Fisher's exact test was used. Statistical analysis was done using PROC FREQUENCY (SAS version 9.1, SAS institute, Inc., Cary, NC, USA). Level of significance is set at $\alpha = 0.05$.