Brief Communication Communication brève

Clinical *Mycoplasma bovis* mastitis in prepubertal heifers on 2 dairy herds

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Abstract — Findings of herd investigations of heifers with prepubertal mastitis are presented. *Mycoplasma bovis* was isolated from lacteal secretions and tissue samples of necropsied heifers; the same strain infected dams and herd mates. Vertical transmission is suggested in this first report of intramammary infections of *M. bovis* in peripubertal heifers.

Résumé — Mammites cliniques à *Mycoplasma bovis* chez des génisses prépubères dans 2 troupeaux laitiers. Ce travail présente les résultats de travaux de recherche sur des troupeaux de génisses atteintes de mammites prébubères. *Mycoplasma bovis* a été isolé à partir de sécrétions lactées et d'échantillons de tissus prélevés lors de la nécropsie; la même souche infectait également les mères et les autres membres des troupeaux. Une transmission verticale est suggérée dans ce premier rapport d'infections intramammaires à *M. bovis* chez des génisses péripubères.

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Two neighboring dairy herds of Holstein cattle in Sunnyside, Washington, USA, had cases of clinical mastitis in prepubertal calves. At the time of the investigation, approximately 650 cows were milked in Herd 1 and 750 in Herd 2. Cattle in both herds were housed in open lots and bedded with straw during periods of inclement weather. In both herds, calves were raised on the premises; preweaned calves were raised in hutches and then moved to group pens. The protocol regarding neonatal calf management was the same in both herds. Calves were separated from the dam within 1 h of birth. Calves were fed 4 L of unpasteurized colostrum by esophageal tube within 24 h of birth. Normally, the colostrum was from the dam of the calf; but the calf received either thawed, stored, pooled colostrum or fresh, pooled colostrum, depending on availability, in cases where the dam's colostrum was inferior, as determined by a colostrometer; from a mammary gland with mastitis; of insufficient volume; or otherwise deemed unacceptable. The servicing veterinary practitioners were the same for both herds and helped the dairy managers establish their neonatal calf management protocols.

The practitioner commonly inspected the mammary glands of 6-month-old replacement calves for supernumerary teats during routine examination and vaccination procedures. The mammary glands of 2 calves in Herd 1 and 1 calf of Herd 2 were of interest during the winter of 2004 and the spring of 2005, as palpation revealed mammary nodules. Secretions from the affected mammary quarters were collected into sterile test tubes, using aseptic techniques. A *Mycoplasma* sp. was cultured from secretions in the clinic's laboratory.

After discovery of the *Mycoplasma* sp. in the lacteal secretion of Calf 6 in Herd 1, the calf was euthanized and necropsied by the herd's veterinarian. Swabbing solutions of external mucosal surfaces (nares, eye, vagina, ear, and rectum) and tissue samples were collected from the calf immediately thereafter by personnel at Washington State University, as previously described (1). Tissue samples were placed in sterile bags (Whirlpaks; Nasco, Modesto, California, USA), kept on ice, and transported to the laboratory. A milk sample was also collected, using aseptic techniques, from Cow #987, which had clinical mastitis at the time of the 1st investigation. The dam of Calf #6 had been culled prior to the calf being sampled.

Eight months after the examination of Calf #6, a 2nd calf in Herd 1, Calf #2, was found to have a similar mammary nodule at a similar age. A necropsy was done on Calf #2 and samples were collected, as previously described (1). External mucosal surfaces of the dam of Calf #2, Cow #739, were swabbed and handled as previously described. Milk samples were also collected aseptically from Cow #739.

A 6-month-old calf (#670) on the neighboring farm (Herd 2) was found to have a similar nodule at the time of the 2nd sampling at Herd 1. Samples were collected from this calf after necropsy and handled as previously described. Additionally, a milk sample and a heparinized blood sample were obtained from Cow #389, the dam of Calf #670.

Cultures from milk, swabbing solutions, and tissue samples were all made by using the procedures outlined by Biddle et al (1). Briefly, samples were inoculated into mycoplasma broth enrichment medium and incubated for 4 d. Then, a 0.1-mL

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L 20 — DNA lambda ladder
L 19 — Farm 1 Calf 2 Rt Nasal Passage
L 18 — Farm 1 Calf 2 RF Mammary Tissue
L 17 — Farm 1 Cow 987 RR Milk
L 16 — Farm 1 Calf 6 RR Mammary Tissue
L 15 — Farm 1 Calf 6 RF Mammary Tissue
L 14 — Farm 1 Calf 6 Lt Eye
L 13 — Farm 1 Calf 6 3° Bronchi
L 12 — Farm 1 Calf 6 1° Bronchi
L 11 — Farm 1 Calf 6 Lung
L 10 — Farm 1 Calf 6 Lt Nasal Passage
L 9 — Farm 1 Calf 6 Rt Nasal Passage
L 8 — DNA lambda ladder
L 7 — BLANK LANE
L 6 — DNA lambda ladder
L 5 — Farm 2 Dam 389 Milk (Qt Composite)
L 4 — Farm 2 Dam 389 Blood
L 3 — Farm 2 Calf 670 Pustular Mass from LF Mammary Gland
L 2 — Farm 2 Calf 670 LF Mammary Fluid
L 1 — DNA lambda ladder

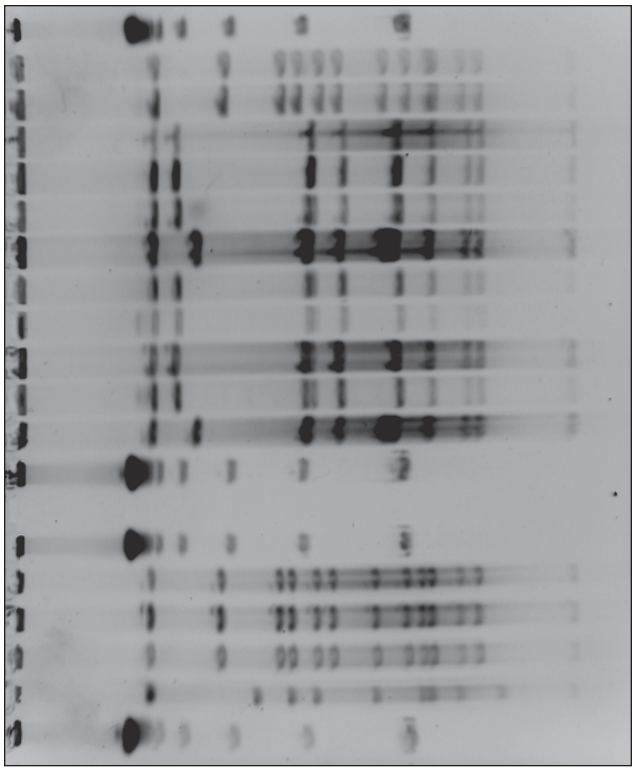

Figure 1. Pulsed field gel electrophoretogram of strains of *Mycoplasma bovis* isolated from cows and calves from 2 farms. Lane 1 to Lane 20, bottom to top, are labeled with the source of each isolate.

aliquot from each inoculated tube was transferred to modified Hayflick's agar plates and cultured for 10 d. All mycoplasma isolates were investigated for their genomic makeup (fingerprint) (1) by using pulsed field gel electrophoresis (PFGE), depicted in Figure 1. With PFGE, the bacterial DNA is isolated and then digested with restriction enzymes to produce different-sized fragments. Fragments of DNA of different sizes migrate at different rates and form different visible bands when the gel is stained. Presumably, isolates with different band sizes and numbers of bands have different, fingerprints and, thus, are of different strains. For example, in Figure 1, isolates in lanes 2 and 3 (L2 and L3) have different banding patterns and can be considered different strains. Isolates in L3 and L4 have indistinguishable banding patterns and thus are considered to be of the same strain. One of the same strain isolates was randomly selected, speciated by the Washington Animal Disease Diagnostic Laboratory in the College of Veterinary Medicine, Pullman, Washington, and identified as *M. bovis.*

In Herd 1, *M. bovis* was isolated from 1) the mammary parenchyma of the right rear and front quarters, and the lung, bronchi, nasal passages, and eye of Calf #6; 2) a composite milk sample from Cow #987; and 3) the nasal and parenchyma of the right front mammary quarter from Calf #2. *Mycoplasma bovis* was not isolated from Cow #739.

In Herd 2, *M. bovis* was isolated from the mammary gland tissue of the right rear quarter and from lacteal fluid expressed from the same quarter of Calf #670. Additionally, *M. bovis* was found in the milk and blood of Cow #389.

In Herd 1 almost all the mycoplasma isolates from Calf 6 appear to be of the same strain, as the number and positions of bands are aligned, except for the isolates from the nasal passage (L9) and the mucosal surface of the eye, L14 (Figure 1). Moreover, the predominant isolate from this calf had the same fingerprint as the mycoplasma isolate from the milk of Cow #987 with mastitis. The mycoplasma isolate from Calf #2, discovered more than 6 mo later, had a different fingerprint from that found associated with isolates of Calf #6 (Figure 1, L9 and L19), indicating multiple strains of this agent causing mastitis might emerge in a herd. In Herd 2, the isolates found associated with Cow #389 appeared to be the same as 1 of the isolates found in the left front mammary quarter of Calf #670. However, the fingerprints of these isolates did not match those of the isolate found associated with the lacteal fluid associated with that gland (Figure 1, L2 and L3).

Mycoplasma spp. cause several diseases in cattle, most notably otitis media (2), disorders of the urogenital tract (3), polyarthritis (4), pneumonia (5), and mastitis (5). Colonization of the internal organs by *Mycoplasma* spp. is common, especially the respiratory system.

Woldehiwet et al (6) found that 92% of the calves they sampled were colonized in the nares by *Mycoplasma* spp. They concluded that some of the *Mycoplasma* spp. that had colonized were commensal, while others were potentially pathogenic. However, it was not apparent what factors might affect the agent and induce the change from an asymptomatic to a diseased state.

The transmission of *Mycoplasma* spp. between different body sites has been reported, with dissemination presumed to be hematogenous, lymphatic, or through shared anatomical passageways. In 1 study, nearly half of all calves with mycoplasma

otitis also had mycoplasma pneumonia (7). In a field survey of *M. bovis* infections in calves and cows where the calves were descendents of cows with mastitis, infections started in the upper respiratory tracts of calves, as evidenced by isolation of a *Mycoplasma* sp. and the production of antibodies against the agent (5). Apparently, the agent spread to other animals, assumably via nasal discharge, through either direct contact or aerosol. Later, pregnant heifers were infected and, at calving, the *M. bovis* was widely disseminated within the body of the recently calved heifers and their newborn calves. The investigators suspected not only hematogenous spread within infected cattle, but vertical spread from dam to fetus. Wilson et al (8) suggested a connection between arthritis and mastitis by *M. bovis* in cattle in a closed commercial dairy herd. These reports suggested links between nonmammary mycoplasmal diseases and mastitis (5,8).

The 1st report of mycoplasma mastitis in prepubertal calves was in Quebec (9). In that report, it was suggested that mastitis in young replacement animals might play a role in the epidemiology of mastitis in dairy herds. Indeed, mastitis in dairy herds may be underestimated (10).

The findings of the current report indicate that the same strain of mycoplasma that causes mastitis in lactating cows was the cause of mastitis in young replacement calves. Both the 1st calf sampled and a herd mate of her dam were infected by mycoplasma of the same strain. Additionally, the strain that caused the mastitis in this calf was asymptomatically carried in her respiratory system. Roy et al (9) suggest a link between asymptomatic carriage by the dam and disease in the calf, where disease was manifested as both arthritis and mastitis. Our findings add strength to the argument that asymptomatic carriage by the dam can be a factor in the transmission of the mycoplasma disease agent to the offspring. The dam-calf pair in Herd 2 both had mastitis, but the dam at the time of sampling was asymptomatically infected with the mycoplasma strain, as elevation in the milk somatic cell count (SCC) did not occur until the subsequent lactation, her milk SCC never exceeded 100 000 cells/mL for the current lactation. During the following lactation, her milk SCC rose from fewer than 130 000 cells/mL at 100 d in milk, to 682 000 cells/mL at 220 d in milk, and to 1 123 000 cells/mL at 311 d in milk. She was never reported to have suffered clinical mastitis, but she was removed from the herd, with high SCC being given as the reason for culling.

Recovery of the same strain from a blood sample from Cow #389 indicates systemic involvement. Thus, it is tempting to suggest that the strain of *Mycoplasma* sp. was transferred from dam to calf while the cow was an asymptomatic carrier. In both herds, the strains appeared in the lactating herd and, logically, such transfer from adult to a replacement might contribute to transmission and future cases of this disease when those replacements mature and enter the lactating herd. Roy et al (9) suggested that transfer of the agent from the dam to the calf occurred through the feeding of colostrum or from contact with vaginal secretions or both. To better control mycoplasma mastitis, the determination of which cows are asymptomatically colonized with a *Mycoplasma* sp. might be helpful in identifying sources of colostrum that should not be fed to replacements. Additionally, such identification could be made to determine where dam and calf interactions might be best limited. Additionally, dairy practitioners should realize that mastitis in prepubertal heifers occurs, and that it may be reflective of a wider mastitis problem in the herd.

Authors' contributions

Dr. Fox wrote the manuscript and administers the laboratory where the work was completed. Drs. Muller and Wedam are the herds' veterinary practitioners and conducted the necropsies. Dr. Schneider is a researcher in bovine mycoplasma diseases and he assisted with the development of the manuscript. Ms. Biddle was a graduate student; she initiated the project while working with Drs. Muller and Wedam. CVJ

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