



## Mastitis Outbreak Due to *Mycoplasma californicum* and *Mycoplasma canadense* in a Commercial Dairy Herd in the State of Jalisco, México

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**ABSTRACT.** A commercial dairy herd with 282 cows in lactation suddenly began to have a problem with atypical clinical mastitis. The first 28 cases showed severe purulent mastitis with hard swollen mammary glands, but without systemic signs of illness. Some of the cows were lame. The cases were not responsive to antibiotics. The evidence suggested that milking machine malfunction and other management practices may have contributed to spread infection and to increase the number of clinical cases. Culture of milk of the affected cows and bulk tank milk yielded mycoplasma colonies that were identified as *Mycoplasma californicum* and *Mycoplasma canadense*. This is the first isolation and report of these mycoplasmas in México and Latin America. The 28 positive cows were segregated and culled; however, the owner did not follow the recommendations to sample all the milking and dry cows and to implement other preventive measures. Consequently, losses were much greater and 177 cows were culled.

**Key Words:** *Mycoplasma californicum*, *Mycoplasma canadense*, México, Isolation

**RESUMEN.** En un hato lechero comercial, con 282 vacas en lactación, repentinamente varias vacas empezaron a mostrar casos clínicos de una mastitis atípica. Los primeros 28 casos mostraron, una severa mastitis purulenta, la ubre estaba inflamada y dura; sin embargo las vacas afectadas comían normalmente sin mostrar signos clínicos de la enfermedad. Algunas vacas cojeaban al caminar. Los casos no respondieron a los antibióticos utilizados. La evidencia sugirió que el mal funcionamiento de la máquina ordeñadora y otras malas prácticas de manejo pudieron haber contribuido a dispersar la infección e incrementar el número de casos clínicos. El cultivo microbiológico de la leche de las vacas afectadas y de la leche del tanque de enfriamiento, permitió el aislamiento de bacterias que fueron identificadas como *Mycoplasma californicum* y *Mycoplasma canadense*. Siendo este el primer aislamiento y reporte de estos micoplasmas en México y en América Latina. Las 28 vacas positivas a micoplasma fueron segregadas y posteriormente desechadas; sin embargo, el dueño no siguió las recomendaciones, de muestrear a todas las vacas secas y en lactación, así como la de implementar otras medidas preventivas de manejo. Consecuentemente, las pérdidas se incrementaron y 177 fueron desechadas.

**Palabras clave.** *Mycoplasma californicum*, *Mycoplasma canadense*, México, Aislamiento

### INTRODUCTION

Bovine mastitis due to mycoplasmas was described in Europe in 1960.<sup>3</sup> And in 1962 mycoplasmal mastitis was first reported in the United States.<sup>4</sup> Subsequently, reports of herd epizootics have increased in frequency, geographic location and number of mycoplasma species involved. The largest series of herd epizootics was reported in 1979.<sup>11</sup> Five species were involved with cattle epizootics; these were in order of frequency: *Mycoplasma bovis*, *Mycoplasma californicum*, *Mycoplasma canadense*, *Mycoplasma atkalescens* and *Mycoplasma bovirhinis*.<sup>11</sup>

In Mexico sporadic instances of mycoplasmal mastitis

infection in bovines have appeared since 1984.<sup>5,6,7,10</sup> Reports of naturally occurring cases of mastitis, arthritis, and pneumonia have appeared; but the number of cases has been small implicating only *Mycoplasma bovis* as the etiologic agent.<sup>5,10</sup> In Latin America reports of mycoplasmal mastitis infection have been documented, involving to *M. bovirhinis*, *M. bovis* and *M. bovirhinis* as the etiologic agents.<sup>14,16,17</sup>

Mastitis produced by *M. californicum* and *M. canadense* have been isolated, reported and documented in the United States, Canada and Europe,<sup>3,11,12</sup> but not in México and Latin America.

The objective of this report is to document an outbreak



of bovine mycoplasmal mastitis produced by *Mycoplasma californicum* and *Mycoplasma canadense* on a commercial dairy in the State of Jalisco, México. Clinical, microbiological, immunological and epidemiological findings are presented.

## MATERIAL AND METHODS

Twenty-seven composite milk samples from milking cows, one-sample from a dry cow from one herd having an unusual kind of mastitis and one-sample from bulk tank milk were obtained through a veterinarian.

The isolation and identification of the implicated mycoplasmas were as follow: The 29 samples were enriched. A 200  $\mu$ l aliquot from the milk samples was added to a tube containing 3 ml of mycoplasma broth and incubated for 36 h at 37°C in an atmosphere of 10 % of CO<sub>2</sub>.<sup>9</sup> All the samples before and after enrichment were streaked in 0.1 ml amounts on modified Hayflick media and on blood agar plates as has been previously described.<sup>8</sup>

Species diagnosis was determined by the immunobinding, immunofluorescence and immunoperoxidase tests.<sup>2,8,9</sup>

**Case history.** A commercial dairy of 320 head with 282 lactating Holstein Friesen cows experienced signs of an unusual kind of mastitis toward the end of October 1991. The mastitis cases were characterized by marked reduction in milk yield and hard swollen mammary gland. The mastitic milk showed sandy or flaky sediments with some of the milk samples being seropurulent. Several cows showed clinical signs of arthritis although the affected cows were eating normally.

Apparently, cattle from different sources had been introduced into the dairy. The affected cows were treated with systemic and local antibiotics including ampicillin, streptomycin-penicillin, tetracycline and sulfonamides.

When the owner and his veterinarian observed that the cows were not responding to the treatment, they requested a local laboratory to prepare a vaccine for intramammary use to try to increase the local defenses of the lactating cows. After two and a half months and after the vaccination, the attack rate was 8.75 %. When the treatment and the local vaccination did not give the expected results. They decided to request assistance from other experienced veterinarian, who at the same time sought laboratory assistance from the Autonomous University of Tamaulipas and the National Institute of Forestry Agriculture and Livestock Research (INIFAP).

**Clinical findings.** Clinical examination of the affected cows revealed the following signs: painless and hard, swollen mammary glands, normal temperature (38.4°C), severe purulent mastitis and arthritis in 14 cows. There was a drastic drop of milk yield in all sick cows, but a normal appetite. The sample from bulk tank milk had a high somatic cell count ( $1 \times 10^6$ ). The mastitic milk samples also showed very high leukocyte counts ( $6 \times 10^6$ ) and sandy or

flaky sediments. The antimicrobial treatment consisted of ampicillin, streptomycin-penicillin, tetracycline and sulfonamides by local and systemic administration; however the antimicrobial chemotherapy was not effective.

**Microbiological findings.** After 24 h of incubation on blood agar plates, a great number of large, moist, gray colonies were present, they consisted of Gram negative rods that were classified as coliforms.

Colonies were present on the hayflick agar after 48 h of incubation, which ranged from 0.5 to 1 mm in diameter. The largest colonies had a central area surrounded by a peripheral zone, giving fried egg appearance. When heavy growth was seen, the individual colonies were primarily distinguished at the edge of the culture.

**Immunological findings.** When the immunobinding, immunofluorescent and immunoperoxidase tests were carried out on the mycoplasma agar plates and in the mycoplasma broth, the following results were obtained, all the samples were negative to the immunobinding test using monoclonal antibodies against *M. bovis*; however, the immunofluorescent and immunoperoxidase tests identified 7 samples as *M. canadense*, 21 samples were identified as *M. californicum*. The bulk tank milk sample was positive to both species of mycoplasmas.

Local antibodies of the IgG class against *M. californicum* and *M. canadense* were detected in milk-serum samples from the infected cows by the indirect immunofluorescent test using as antigen the isolated mycoplasmas.

**Epidemiological finding.** A case was defined as a cow that showed the following characteristics: hard, swollen and painless mammary gland, normal appetite, afebrile, mastitic milk showing sandy or flaky sediments, mastitis not responsive to chemotherapy with or without isolation of mycoplasmas or cows without the clinical signs, but with mycoplasma isolation from the milk.

*M. californicum* and *Mycoplasma canadense* were found to be the etiologic agents responsible for the disease outbreak. The isolated colonies of coliforms were considered contaminants due to the lack of good sanitary sampling practices.

When the owner was asked if he had purchased new replacements, the answer was negative; but during the dairy inspection, the same question was made to a caretaker and the answer was positive. He said that 4 to 6 weeks ago, some cows were introduced into the dairy. A review of the herd records showed a lack of basic information.

**Milking procedures.** The milking equipment and the animal health practices were evaluated and mismanagement practices were observed as follows: the administration of antibiotics as well as the vaccine into the mammary glands was done by using a single cannula and one syringe for all the sick and healthy cows. The cannula was not sterilized between each cow injection. In addition to that, the tests were not disinfected before the antibiotics or the vaccine were administered.

The cows were milked without any order, healthy and



sick cows were allocated in the same string. The udders were poorly-washed with cold water without any sanitizing solution and dried with pieces of news paper; consequently, many cows showed wet and dirty teats. When the teat cups were attached, many drops of water from the udder were falling into the teat cups.

**Milking equipment.** There were a combination of large and irregular low vacuum fluctuations. The daily cleaning routine of the milking equipment was done by using only cold water and without the appropriate detergents.

When laboratory help was requested, the attack rate was 8.75%. The attributable risk of the milking cows compared with dry cows was 6.9.

Control procedures were prepared and sent to the owner and both veterinarians; however, eight months later, when data about the outbreak were requested by the experienced veterinarian, he wrote, saying that the owner never put into practice the designed control procedures because they were very expensive, difficult to follow, and he would have to change the management practices and did not believe in the recommendations. When the owner realized about his mistake. In September, 1992, the secondary attack rate was 51.02 % and the rate of culling during this period of time of the outbreak was 60.41%. Early in 1993, the dairy was closed down.

**Control procedures.** A control program was designed rapidly and because of the long distance involved, the program was sent by fax and some comments were made by telephone to the owner and both veterinarians. The control program recommended was as follows: segregate the sick cows; they must be handmilked last; milkers' hands should be dipped in sanitizer between handling of cows; all the milking cows and dry cows must be tested for mycoplasma using composite milk samples; also must be cultured, at first cows at freshening and all new clinical cases; bulk tank milk samples weekly and later on a monthly basis; individual paper towels must be used for washing udders and the infected cows must be slaughtered.

The milking equipment dealer was to be contacted regarding improving the sanitation of the equipment by using the proper detergents and disinfectants in adequate concentrations and by repairing machine failures and faults detected during the inspection.

## DISCUSSION

Mycoplasmas are known to be the cause of bovine mastitis which in some areas could be a low prevalence disease; however, if mismanagement practices and malfunctioning equipment are associated, infection may spread and produce explosive outbreaks along with large losses.<sup>3,4,12</sup>

Several observations can be made regarding this outbreak. The owner, the veterinarian and the care takers were not aware of this unusual type of mastitis and because of

this, they neither established the control program that was designed nor gave the required attention when necessary.

Consequently the problem grew so much that it got out of control; and caused large losses.

The vacuum fluctuations, the inadequate-cleaning of the milking equipment, the mismanaging practices such as milking healthy and sick cows without segregation and washing the udders with water without sanitation solution, careless intramammary therapy and vaccination techniques and introduction of new cows without testing into the herd are all known factors that increase the risk and speed with which mycoplasmal mastitis spreads<sup>11,12,13</sup>

The presence of arthritis associated with mycoplasmal mastitis provides evidence that these bacteria cause disease elsewhere than in the udder. There is also the possibility that systemic spread occurs and some of the organs may act as reservoirs. Therefore introduction of new infected replacements into a herd may not always be required to spread the infection.<sup>11</sup>

The identification of two mycoplasmas in an outbreak is infrequent; but it is possible that several strains of mycoplasma may be found in milk from a single herd.<sup>2</sup>

This outbreak showed that rural practitioners and dairy owners should be aware of the clinical signs of this type of mastitis and its serious epidemiological and economic consequences.

Both the dairymen and the veterinarian should be prepared to promptly secure an accurate diagnosis and to take the proper steps to control whatever form of mastitis or concurrent mycoplasma infection is present in the herd.

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