

Mycoplasmal mastitis in dairy cows in the Moghan region of Ardabil State, Iran

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ABSTRACT

Mycoplasmas are an important and economically significant cause of mastitis in dairy cows in various parts of the world. The organisms are highly contagious, with the main reservoir of infection originating from cows with subclinical mastitis. In 1998 the 1st cases of bovine mastitis due to *Mycoplasma bovis* were diagnosed in Ardabil State, Iran. An investigation was carried out with the aim of establishing the extent of mycoplasma infections in dairy cows in Ardabil State. Milk samples obtained from 80 cows with clinical mastitis were cultured in the laboratory for the presence of mycoplasmas. Similarly, 48 bulk-tank milk samples were examined for the presence of mycoplasmas. A modified Hayflick broth was used to isolate the mycoplasmas and an immunoperoxidase test used for the species identification of the isolates. *Mycoplasma bovis* was isolated from 39 (48.75 %) of the clinical mastitis samples and from 48 of the bulk-tank milk samples tested. This indicated that mycoplasma udder infections were more prevalent in dairy cows in Ardabil State than previously thought.

Key words: cow, mastitis, milk, mycoplasma.

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Mastitis in dairy cows caused by mycoplasmas was first reported in England in 1960 and in the United States of America in 1961. Subsequently the organisms have been reported in other countries such as Canada, Japan, Israel, Australia, New Zealand and many European countries. Mycoplasmas are now regarded as being an important and economically significant cause of mastitis in dairy cows in various parts of the world.

Different species of mycoplasmas have been shown to cause udder infections in dairy cows, with *Mycoplasma bovis* being responsible for most of the mastitis outbreaks. Characteristics of this disease are its highly infectious and contagious nature, complex clinical presentation, and extreme resistance to treatment^{5,8,14}. Mastitis caused by *Mycoplasma* species has highly variable signs, ranging from mild clinical cases to complete loss of milk production. The 2 characteristics of most *Mycoplasma* mastitis infections of individually affected cows are failure to respond to therapy, and spread of the infection from one quarter of the udder to another. Milk transfer during milking is the most common way *Mycoplasma* mastitis is spread between cows^{2,7,9,10}.

Mycoplasma species have been isolated

from the respiratory and urogenital tracts of asymptomatic, healthy cattle. Under certain circumstances *Mycoplasma* has been associated with pneumonia, ear and eye infections, vaginal infections and joint disease in cows and calves. It has been suggested that the spread of *Mycoplasma* spp. from an infected organ system to the mammary gland may occur. Infected cows may remain infected for life, regardless of therapy^{1,3,6,7}. Culture of bulk-tank and cows' milk samples is a useful procedure to determine the existence of *Mycoplasma*-infected cows in a herd^{1,8}. The milk of cows with clinical mastitis is often watery, tan to brown in colour, and often has a flaky or sandy sediment. The affected quarter of the udder is firm and appears swollen, as if the cow had recently freshened^{1,3,6,7}. Results of milk culture for *Mycoplasma* are reported after 3 and 7 days of being plated in the laboratory. A portion of the milk sample is placed in a pre-incubation broth for 48 hours, then plated onto a special medium and incubated in a low-oxygen (micro-aerophilic) environment^{1,5,7,12}.

Until 1998, *Mycoplasma* mastitis had not been recognized in the State of Ardabil or the Moghan region in spite of repeated attempts to culture organisms from milk samples of mastitis cases refractory to treatment. In 1998, however, 44 cases with classical signs of mycoplasmal mastitis, based on culture-positive milk samples,

were reported (Veterinary data 2002, Iran Veterinary Organization, Ardabil City). *M. bovis* was recovered from milk samples in these cases. A survey of bulk-tank samples and cows' milk samples was therefore initiated to determine the prevalence of mycoplasma infections in a random sample of Moghan dairy herds, and to establish whether the organism was significantly distributed in dairy cows in Ardabil State.

Four dairy farms, A, B, C, D, with >550 animals/herd were chosen for this study. Strict aseptic procedures were used when collecting milk samples to avoid contamination with bacteria present on the skin of the cow, on the hands of the sampler and in the barns. Sampling was monitored as follows: A, teats and udders were cleaned and dried before attempting to collect sterile samples; B, teats were grasped with one hand, and then vigorously scrubbed with cotton moistened with alcohol; C, the cap of each sterile, labelled vial was loosened and held at an angle under the udder; D, 2–3 streams were milked onto the floor; E, each vial was carefully opened and 2–3 streams of milk directed into it; F, neither the ends of the teats nor fingers were allowed to come into contact with the open end of the vial or the inside of the cap; G, caps were replaced and the samples refrigerated; H, quarter samples (1 quarter per vial) were collected from quarters affected with clinical mastitis; I, the farm name, date of collection and source of the sample were written on each vial.

Samples from bulk tanks on each farm were collected as follows:

The tank was agitated for at least 5 minutes. A sterile milk straw or sterile dipper was used to obtain each sample. A sterile vial was used to contain the sample and filled to about three-fourths. Samples were not collected from the valve at the bottom of tank. The samples were placed on ice or refrigerated immediately for transportation to the laboratory.

Milk samples were streaked over one-half of a plate and bulk-tank samples over an entire plate. Plates were examined for colonies under low power on a standard microscope and colonies were identified by their shape. Growth could be seen after

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3 days of incubation at 37 °C in a moist 10 % CO₂ incubator, but 7 days of incubation were needed for full development of colonies. Incubation was continued for 7 days before plates were considered negative^{1,7,12,13}. For pre-enrichment, 0.1 ml of each milk sample was diluted in 3 serial, 10-fold dilutions of mycoplasma enrichment broth (modified Hayflick broth, Hi-media Laboratories, Biomed, Mumbai, India) containing 15 % horse serum, DNA extract and fresh yeast.) The serial dilutions were incubated at 37 °C for 48 hours to allow for the enrichment of samples^{1,7}.

Selective inhibitors of bacterial growth were added to the mycoplasma broth and agar. Initial samples were processed using ampicillin, methicillin, bacitracin and thallium acetate^{1,7}. For improved control of bacterial contamination during enrichment, cefoperazone was added in addition to the above inhibitors throughout the rest of the study. For culture detection, enhanced growth in broth followed by culture on a mycoplasma agar medium⁷. Identification of the colonies was accomplished by an indirect immunoperoxidase test¹. Recommended antisera were used for each of the *Mycoplasma* spp.^{1,5}.

Mycoplasma bovis was isolated from 39 (48.75 %) of the clinical mastitis samples. The percentage of positive samples per farm are given in Table 1. *Mycoplasma bovis* was also isolated from 48 of the bulk-tank samples. No other *Mycoplasma* species were isolated from any of the samples.

This investigation was specifically aimed at determining whether *Mycoplasma* udder infections were of any significance in dairy herds in the Moghan region of Ardabil State. The possible role of other causative organisms was not included in the study. The findings of this study indicate that almost 50.0 % of the clinical mastitis cases were caused by *Mycoplasma bovis*.

It is likely that, using bulk-tank milk, the true occurrence of *Mycoplasma* is underestimated because cows that recover from an initial infection may stop shedding *Mycoplasma* organisms or shed the organisms in such low numbers that they go undetected in these samples. In addition, bulk-tank samples generally represent only healthy, untreated cattle within a herd, since milk from unhealthy or treated cattle is kept separately. Also, to ensure accurate screening of a dairy herd, routine sampling of bulk-tank milk is required^{11,12,13}.

There is no laboratory or epidemiological data available for this disease in Iran. Veterinary data in Iran from 2002 (Iran Veter-

Table 1: Percentage of clinical mastitis milk samples with

Farms	No. samples	No. samples positive	Percentage positive
A	49	28	57.14
B	14	5	35.71
C	9	4	44.44
D	8	2	25
Total	80	39	48.75

inary Organization, Tehran), showed that 96.84 % of dairies tested positive for *Mycoplasma* when a single bulk-tank milk sample was cultured using standard culturing methods. The species isolated most frequently was *M. bovis* (92 % of farms) followed by very small but virtually equal numbers of *M. californicum*, *M. alkalescens* and *M. bovisgenitalium*. States in the Western region had a greater percentage of farms with positive *Mycoplasma* cultures than farms in the Midwest, Northeast, and Southeast regions. In addition, large herds (500 animals or more) were more likely to have positive *Mycoplasma* cultures than medium-sized (100–499) or small herds (<100). All of the 23 States had at least 1 farm with a positive *Mycoplasma* bulk-tank milk culture. These data showed that *Mycoplasma* infections can be economically devastating to dairies because of the contagious nature of the organism and its resistance to therapy. Early detection and segregation or culling of *Mycoplasma*-infected cattle is essential in this area, and also throughout Iran. In this survey we described the need for an epidemiological approach to the investigation of disease problems in Iran.

The most effective method of preventing an outbreak of *Mycoplasma* mastitis is to screen all introduced cattle by collecting milk samples at freshening (heifers) or prior to mingling purchased cattle with the home herd.^{4,6}

In conjunction with the screening of newly introduced heifers and cows, bulk-tank milk should be cultured at least once a month. In herds with 500 or more cows, a bulk-tank culture may not be accurate enough to detect a single infected cow. In this case, culturing a sample from a smaller group of cattle (e.g. an individual pen sample) is recommended on a monthly basis.^{11,13} In herds that are expanding or when routine purchasing of heifers is carried out, screening of bulk-tank and/or pen samples twice a month should be considered. In addition to the screening of purchased cattle, and bulk-tank or pen sampling, the implementation of proper milking techniques is critical for minimising the spread of *Mycoplasma* in a parlour. Proper milking techniques that

minimise the spread of *Mycoplasma* include the use of gloves, minimising the amount of milk on hands and towels while moving from cow to cow, and using a 1 % iodine post-milking teat dip^{4,11}.

This investigation confirmed the importance of *Mycoplasma bovis* as a significant pathogen in dairy herds as reported in other parts of the world. The study also highlighted the importance of conducting surveys in dairy herds so as to determine the prevalence and significance of mycoplasmal infections.

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REFERENCES

- Anderson KL 1993 *Update on bovine mastitis*. W B Saunders, Philadelphia
- Bayouni A, Faruer T B, Bushnell B, Olivera A 1988 Enzootic mycoplasmal mastitis in a large dairy during an eight-year period. *Journal of the American Veterinary Association* 192: 902–905
- Blood D C, Radostits O M, Henderson J A 1989 *Veterinary medicine* (7th edn). Baillière Tindall, ELBS, London
- Carrol E J 1977 Environmental factors in bovine mastitis. *Journal of the American Veterinary Association* 10: 1143–1147
- Chimi A 1996 *Veterinary bacteriology and bacterial disease*. Nashr Publication, Tehran
- Hale H H, Helmboldt C F, Plastring W N, Atula E F 1962 Bovine mastitis caused by a *Mycoplasma* species. *Cornell Vet.* 52: 582–591
- Jasper D E 1981 Bovine mycoplasmal mastitis. *Advances in veterinary science and comparative medicine*. Academic Press, New York
- Jasper D E, Boothby T T, Thomas C B 1987 Pathogenesis of bovine *Mycoplasma* mastitis. *Israel Journal of Medical Sciences* 6: 625–627
- Louise ruhne H, Thewley D, Nelson F C 1976 Bovine mastitis in Ontario due to *Mycoplasma agalactiae* subsp. *bovis*. *Canadian Journal of Comparative Medicine* 40: 142–147
- Mackie D P, Ball H J, Logan E 1986 *Mycoplasma californicum* mastitis in the dry dairy cow. *Veterinary Record* 4: 350–351
- Philpot W N 1979 Control of mastitis by hygiene and therapy. *Journal of Dairy Science* 62: 168–175
- Schalm A W, Jain N C, Carrol E J 1971 *Bovine mastitis*. Lea & Febiger, Philadelphia
- Thomas C B, Willeberg P, Jasper D E 1981 Case control study of bovine *Mycoplasma* mastitis in California. *American Journal of Veterinary Research* 42: 511–515
- Tabatabaye A H, Firoozi R 2001 *Disease of animals due to bacteria*. Tehran University Press, Tehran