Mycoplasmas and the Dairy Industry

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Abstract

Mycoplasmal infections are of significant concern to dairy producers, and their concerns have energized changes in diagnosis, control, and prevention of the infections. Since management practices have significant impact on these infections, these practices need to be evaluated and adjustments made to coordinate them with the health measures to be applied.

In this presentation, a historical and current review of health measures applied to mycoplasmal infections is extended with a look forward to future needs in this area. Deficiencies in the application of control measures are highlighted, and opportunities for better interventions are surveyed.

Résumé

Les infections par les mycoplasmes sont un problème majeur pour les producteurs laitiers. Les préoccupations de ces derniers nous ont poussés à modifier nos méthodes de diagnostic, de lutte et de prévention contre ces infections. Puisque les pratiques de gestion du troupeau ont une grande influence sur les infections par les mycoplasmes, on doit évaluer ces pratiques et les adapter pour les coordonner avec les programmes de mesures sanitaires.

Dans cette communication, nous passerons en revue les mesures sanitaires passées et actuelles contre les infections par les mycoplasmes et examineront les besoins à combler dans ce domaine. Nous soulignerons aussi les faiblesses des mesures de lutte et les possibilités d'améliorer nos interventions.

Introduction

Mycoplasmal infections of cattle have gained the attention of producers and veterinarians in recent years. The lay press has given a high level of visibility to these diseases, and producers have responded with concerns and increasing demands on cattle health professionals. Studies have shown that some mycoplasma infections can be truly severe or economically important. In reality, these infections represent only a fraction of losses caused by the multiple disorders that are enhanced by modern intensive management practices. Thus, it is important to confront mycoplasmal diseases in the context of the management applied to the cattle. In the dairy industry, mycoplasma infections are now a concern for calf ranchers, heifer raisers, and milk producers, in each case with somewhat different clinical forms. This complexity of clinical forms has called for the unavoidable use of diagnostic aids, and compelled the evolution of these diagnostic tools. Treatment and prevention modes have also evolved, propelled in great measure by veterinarians that have found field solutions by trial and error.

This paper will present a historical perspective of mycoplasmal infections in the dairy industry, describe clinical forms and the principal agents involved, diagnostic approaches and their evolution, and some common and current control and prevention practices. Finally, an attempt will be made to describe future scenarios for mycoplasma infections in the dairy industry.

A Historical Perspective

Mycoplasmal mastitis was first recognized in Connecticut dairies in 1961.[8] Epizootic mastitis presentations were later recorded in California dairies,[9] followed by its recognition in most regions of the USA.[7] Worldwide distribution of infections is now accepted as fact.[13] In the recent past there has been clear evidence that mycoplasmal mastitis, particularly when caused by *Mycoplasma bovis*, was found with rapidly increasing incidence in midwestern dairies, while in the eastern USA the disease incidence had peaked and was leveling off (Ruegg P: Mycoplasma mastitis - Can you control it on your farm?; http://www.uwex.edu/milkquality/PDF/mycoplasmamastitis.pdf/). This pattern appears to also be followed by incidence reports on respiratory, joint, and middle ear *M. bovis* infections in dairy calves. The first reports of mycoplasmal mastitis involved *M. bovis*, and this pathogen has continued to be the most important mycoplasma, although it has become clear that multiple *Mycoplasma* species are involved in mastitis[10] as well as in calf diseases.[11] This fact currently poses a significant challenge to diagnostic labs. Finally, control strategies used against mycoplasmal diseases have evolved. Early attempts using aggressive culling had to give way to accommodation with production needs.[3,10] Control strategies now focus on minimizing *M. bovis* mycoplasmal mastitis while co-existing with asymptomatic tonsil, nasal and urogenital infections.

Mycoplasmal Agents and Clinical Forms

Mycoplasmal mastitis has been shown to be caused by seven possible species of pathogenic mycoplasmas.[10] The most frequently isolated and most pathogenic is *M. bovis*, followed by *M. californicum*. Other less pathogenic species that can be recovered from mastitis cases are *M. bovigenitalium, Mycoplasma* serogroup 7 (now considered bovine-adapted *M. capricolum*), *M. alkalescens*, *M. canadense*, *M. bovirhinis*, and *M. arginini*. Several species of *Acholeplasma* are commonly recovered from milk obtained under poor milking conditions and are not considered pathogenic. Importantly, all bovine *Mycoplasma* species tested could cause mastitis upon intracisternal inoculation, showing that the mammary gland is extremely susceptible to mycoplasmal infections.[4]

Pneumonia can be caused by several species of mycoplasma, notably *M. bovis* and *M. dispar*.[18] Polyarthritis cases have been associated with infections by *M. bovis*, *M. californicum*, *M.* serogroup 7, and *M. canadense,* and these species are presumed to more easily establish septicemic infections.[15] Middle ear infections in dairy calves have been associated primarily with *M. bovis* to date.[19]

Some studies have been made to determine if single or multiple strains of *M. bovis* are involved in disease production in a farm. As expected, calf ranches that collect newborn calves from multiple dairies have *M. bovis* outbreaks of pneumonia involving multiple strains of *M. bovis*, with two or more strains often recovered from the same calf.[5] In contrast, dairies may have a more limited repertoire of strains. Longitudinal evaluations of *M. bovis* isolates from a large dairy revealed that three strains co-existed on the dairy on initial observation, followed by only one of the strains on samplings done six months later. A year after the second sampling, the persisting strain was still detected, but three new strains were now also there (Rosenbusch R, personal communication). Such observations can be of increasing value, especially when correlated to other data on management, prevention, and control at the farm. A representative result from *M. bovis* strain evaluation is shown in Figure 1.

Diagnostic Tests

Early work done on mycoplasmal diseases in dairies relied on laborious isolation and species identification techniques using banks of well characterized antisera to identify the species of mycoplasma by serological means. These techniques were only used by a few specialized laboratories, and as these reagents became exhausted or had limitations, sero-identification of species became a service that no longer was available. As mycoplasmal diseases became more prevalent and recognized, demand for rapid and economical diagnostic results increased. Polymerase chain reaction (PCR) protocols have now become a standard feature, since they can yield the desired specificity, speed and price. Unfortunately, these protocols can only be developed and validated when sufficient genome sequence data becomes available for each of the species to be differentiated. Currently, most diagnostic laboratories in the USA can test by PCR for *M. bovis* and for mycoplasmas (using universal mycoplasma-specific primers).[2] PCR protocols specific for other bovine species are being developed and validated and are expected to be available over time. Some laboratories offer real-time PCR detection for *M. bovis*, giving the capability for same-day results.[6] This is a significant advantage for dairy operations that must make cow segregation and culling decisions at each milking. The availability of monoclonal antibodies specific for several bovine mycoplasmas has also been applied for diagnostic purposes. In the USA they are often used for the immunohistochemical detection of *M. bovis* in tissue sections.[1] This is an important diagnostic test that is applied in cases of mycoplasmal pneumonia, allowing the co-localization of specific lung lesions with large masses of *M. bovis* antigen.

Another important diagnostic capability is the application of arbitrarily primed PCR (AP-PCR) for molecular epidemiology of mycoplasmal outbreaks.[5] The test would see its best application in closed herds, or calf ranches using small-batch processing and all-

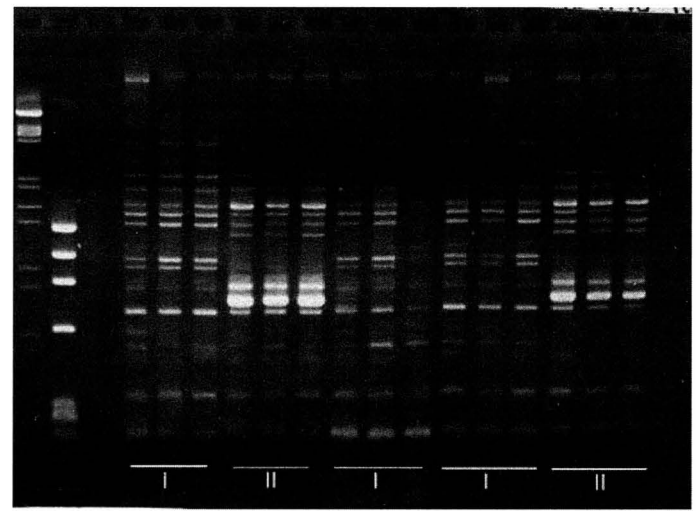

Figure 1. Arbitrarily primed polymerase chain reaction (AP-PCR) on genomic DNA from three clonal lineages of *Mycoplasma bovis* recovered from milk of five cows from a single dairy herd. Two different band patterns (I and II) identify two genetically distinct strains of *M. bovis*. A single strain of *M. bovis* was detected in each of the five cows.

in/all-out management. Most of the current use of AP-PCR is in the recognition of strain identification for the development of autogenous bacterins. Finally, tests for antimicrobial susceptibility of mycoplasma isolates are offered by a few labs.[14]

The value of any diagnostic test is impacted by the quality of the sample submitted. Bulk-tank milk samples are economical to process and are an important early warning signal of clinical infections. String and individual cow samples are more costly approaches that are needed if it is desired to clean an infected herd. And fresh cow milk samples are increasingly recognized as important in dairies that are not operated as closed herds.

Control and Prevention

The lifetime persistence of mycoplasmal infections was recognized in early work. Inappropriate management practices such as the use of the same intramammary canula in multiple cows led to explosive outbreaks of mycoplasmal mastitis and to the realization that ascending mammary infections are a primary route of transmission in the dairy cow.[10] Well validated milking hygiene protocols have evolved, and their use has significantly reduced the clinical incidence of mycoplasmal mastitis. Mycoplasma problems in calves have also been reduced by strict age segregation, pasteurization of discard milk fed to calves, and appropriate antimicrobial treatment. Still, all dairy-associated operations have evolved into very large and specialized units that often require the constant introduction of replacements into the herd. Costly quarantine and segregation protocols see minimal use, and the concept of all-in/all-out management is poorly applied.[17] All of this currently results in a transition period for the dairy industry, with many strategies still waiting to be applied for the control of mycoplasmal infections.

Some advances have been made in prevention of mycoplasmal diseases. In the USA, licensed *M. bovis* bacterins see increasing use, primarily in multi-dose protocols applied to very young dairy heifers. In contrast, a single dose high-antigen-load bacterin was shown to protect very young dairy calves.[12] The effectiveness of these products in older calves and cows is less well established, and this is a concern since immunity is presumed to be short-lived and possibly strain-specific, and risk of exposure is present at all stages of the dairy production enterprise.

Future Projections

It is difficult to envision that the USA will be free of *M. bovis* infections in the near future, since 8% of dairies tested positive for mycoplasmas on single bulk-tank testing done in 2002.[16] More realistically, the incidence of mycoplasmal disease may be reduced or confined to lowered pathogenicity species and strains by the application of improved control measures validated by better diagnostic tests. It is possible that modified-live mycoplasma vaccines would be used in specific circumstances to replace the current bacterins. It is also possible that increased regulatory limitations on antimicrobial use may result in increased clinical problems, particularly in the dairy calf. Since mycoplasma diseases are so intimately linked to intensive management practices, it is also important to try to project what the future management practices will be. Large dairies may shift to single lactation management, while niche dairies may focus on organic or recombinant dairy products. Each of these production systems will face specific challenges when confronted with mycoplasma infections. Future diagnostic tests may be able to discriminate virulent from avirulent strains of mycoplasma. As is occurring in other areas, diagnostic capabilities will probably be expanded to capture yet undescribed infectious agents, such as uncultivable organisms that escape detection at present.

Conclusions

Mycoplasmal infections impact the dairy industry at all levels, from birth to milk quality. There has also been evidence that incidence of some mycoplasma infections has been increasing, and is now recognized as a worldwide problem. The infections are complex, involving multiple species and strains within the species, and this has presented a challenge to diagnostic services. Molecular diagnostic methods have provided efficient diagnosis for a limited number of mycoplasma species, and challenges remain in full species differentiation and specialized diagnostic needs for the dairy bovine. Some infection control measures have been applied to significantly reduce clinical problems, yet many additional measures are still awaiting use. Prevention efforts are still in early stages of application, and this area can also see future advances. Projecting to the future, increased specialization of dairy production will probably result in very divergent types of mycoplasma infections. Diagnostic capabilities may expand to targeted recognition of virulence, and the detection of yet-undescribed infectious agents.

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