Treatment of Mycoplasma Infections: Susceptibility of Field Isolates and Outcome of Treatment; Recovered Cattle: Risk or Bonus?

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Abstract

A number of *Mycoplasma* species cause disease in cattle including *Mycoplasma mycoides* subspecies *mycoides* small colony type, the cause of the OIE list A disease, contagious bovine pleuropneumonia in Africa, and *Mycoplasma bovis*, one of the biggest causes of calf pneumonia world-wide. Symptoms include pneumonia, mastitis and arthritis, all of which adversely affect the economics of farming. Control of *M. bovis* is difficult because it has systems for evading the immune system and has developed resistance to a number of antimicrobials. While strategies can be deployed on the farm to reduce or possibly eliminate disease caused by *M. bovis*, these approaches can be time consuming, and costly in terms of drug treatment and testing, with no guarantees of success. Although some vaccine trials have seen an exacerbation of disease, vaccines are being developed, and some are in use in the USA, although data on their efficacy is sparse.

Introduction

A range of infectious agents, viruses and bacteria cause respiratory infections in cattle making diagnosis and treatment difficult. One group of often overlooked infectious agents are the wall-less mollicutes of which at least 15 different *Mycoplasma* species have been isolated so far from cattle, as well as the closely related *Acholeplasma* species, *Ureaplasma* species and the recently renamed *Eperythrozoon* species. *Mycoplasma* species are responsible for enormous economic losses world-wide, but most significant are: *Mycoplasma mycoides* subspecies *mycoides* small colony type (MmmSC) that causes contagious bovine pleuropneumonia (CBPP), an OIE List A disease, and *Mycoplasma bovis* which causes calf pneumonia, mastitis, arthritis, otitis media, keratoconjunctivitis, infertility, endometritis and abortion.[18] CBPP, which appears to have existed in the ancient world,[6] has been eradicated from many countries whilst *M. bovis,* which was not isolated until 1961, remains widespread. It may therefore be beneficial to compare our knowledge of these two organisms and the way they are treated.

Mycoplasma mycoides subspecies *mycoides* SC

Contagious bovine pleuropneumonia is rife in Africa affecting at least 29 countries with typical mortality rates of 10-70%. CBPP nearly disappeared from the African continent following an eradication campaign in the 1960s and 1970s.[11] In 1995 Botswana successfully eradicated the disease with a massive slaughter and compensation scheme. Elsewhere in Africa vaccination is ineffective at stopping the spread of disease. CBPP was eradicated from the USA in 1896 and from Europe in 1999; in Europe in the late 1980s and 1990s the disease was characterised by low morbidity and low or non-existent mortality with the majority of infected cattle showing chronic lesions. In Italy and Portugal a slaughter and compensation scheme successfully eliminated the disease, but the costs were very high. The cost of controlling the Italian outbreak of 1990-1993 was estimated at over 28 million ECUs. No estimates have been made for the enormous cost of the 15 year eradication campaign in Portugal.

The main signs of CBPP are respiratory distress and coughing, but animals may show dullness, anorexia, irregular rumination and lower milk yields. The gross pathology of lungs affected by CBPP is often described as pathognomic, and lesions are usually unilateral, with consolidation of the lung and the typical marbled appearance.[6] The gross pathology of severely affected lungs from cases of *M. bovis* calf pneumonia can be similar to that of CBPP so extra surveillance is necessary in at risk countries.

Mycoplasma bovis

Mycoplasma bovis is widespread across the world. It has previously been estimated to cost 144 million Euros per year across Europe.[16] In the USA the cost of *M. bovis* infections as a result of loss of weight gain and carcass value have been estimated at $32 million per

year and may be as high as $108 million per year with losses due to bovine mastitis.[26] Therefore one may consider that outside of Africa that *M. bovis* is one of the most economically important diseases affecting cattle.

The diagnosis of *M. bovis* mastitis, arthritis and even otitis media caused by *M. bovis* appears more common in the USA and Northern Canada than it does in the UK. This difference in diagnosis may be genuine or may be due to different levels of awareness of the effect of *M. bovis* by the diagnostician. In the UK approximately 25% of calf pneumonia is caused by *M. bovis*, but mastitis, arthritis and otitis media caused by *M. bovis* are also reported. However, *M. bovis* also causes other conditions including keratoconjunctivitis, infertility, endometritis and abortion.[18] Accurate diagnosis is clearly essential and a range of serological, cultural and molecular diagnostic tests are available.[18]

Characteristics of *Mycoplasma* species

Mycoplasmas are the smallest organisms capable of self-replication. *M. bovis* has a genome size of just 961 +/- 18.9kb;[29] while that of MmmSC's is 1211kb.[31] This small genome limits their range of metabolic activities and thus they are largely dependent on extracellular sources of amino acids, nucleic acid precursors and lipids making them totally reliant on their hosts for nutrients. The absence of specific cell wall-associated polymers also renders mycoplasmas resistant to the action of antimicrobials, such as penicillin and cycloserine, which act against cell wall synthesis.[24] Cell size ranges from 0.15 μm to over 1 μm in diameter and the small size and plasticity of cells enables them to pass through 0.45 μm bacteriological filters.

Structurally some differences exist between MmmSC and *M. bovis.* Bound to the outside of the cell membrane of MmmSC is a carbohydrate capsule which comprises 10% of the dry weight of the cell and is typically 20-40 nm in thickness,[7] whereas no capsule has been reported for *M. bovis.* MmmSC ferments glucose to produce acid, whereas *M. bovis* is non-fermentative and is able to oxidise pyruvate. In *M. bovis* thirteen different types of variable surface proteins (*vsp*'s) have been described,[10] which helps the organism evade the hosts immune system, but only recently has a single gene, the *Vmm* gene, which encodes for a phase variable lipoprotein been described in MmmSC.[21]

McAuliffe *et al*[14] described the formation of biofilms by *M. bovis* which are layers of cells adherent to a surface normally surrounded by a polysaccharide matrix. They may form on the surface of the lung, within a joint, in the oral cavity, in the intestinal tract or even in the environment. The formation of a biofilm may make cells 10-1000 times more resistant to antimicrobials and the host defences.[14]

MmmSC and *M. bovis* are undoubtedly spread via the respiratory tract; infected aerosol droplets are spread by close and repeated contact. For *M. bovis* the teat canal, milk or genital tract can all be routes of infection. A fetus may also become infected through the uterus. Fulfilling Koch's postulates for mycoplasma infections has always been difficult despite attempts at inoculating calves with large doses of laboratory cultured MmmSC or *M. bovis* directly into the airways, highlighting the importance of environmental factors in the pathogenesis of disease.

Molecular Typing

With such small genomes early workers anticipated that *Mycoplasma* species would only contain sufficient DNA to maintain their minimal cell.[30] However, this is not the case as the genome sequence of MmmSC has shown a coding density of just 87% which included several different insertion sequence (IS) elements, in total 73 full-length copies and 13 truncated copies of IS*1296*, IS*1634* and ISMmy*1*.[20] Analysis of IS*1296* elements identified a European clonal line distinct from African and Australian strains.[5] Other molecular methods used to analyse MmmSC isolates show few differences and the isolates appear homogeneous.

In contrast *M. bovis,* which has the same ISMmy*1* element as MmmSC, appears more heterogeneous. A number of different molecular types exist and by using amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE) methods McAuliffe *et al*[13] demonstrated two genetically distinct clusters in the UK. Miles *et al*[15] used insertion sequence profiling to show similar clustering. A single farm, or even cow can have a number of different molecular and antigen types of *M. bovis* simultaneously.

Antimicrobials

Antimicrobials are used widely for mycoplasma diseases, though often ineffectively, but have not been recommended for use in the control of CBPP because they are considered to hinder the control of the disease by suppressing the symptoms so that infected animals are not detected; these animals may then act as reservoirs of infection, aiding the spread of disease.[22] *M. bovis* diseases in cattle are often refractory to therapy. This may possibly due to the complex nature of diseases like calf pneumonia where viral infections are also frequently present. One may also hypothesize that it may be due to treatment not effectively targeting the *M. bovis* that appears to secrete itself throughout the body or possibly protect itself in biofilms.

The MIC and MMC values obtained for *M. bovis*

and MmmSC isolates gave similar values for danofloxacin, but differed markedly for the other test antimicrobials.[1,2] Oxytetracycline, spectinomycin, florfenicol and tilmicosin antimicrobials appeared relatively ineffective against *M. bovis in-vitro*, although they were more effective for MmmSC, with oxytetracycline having an MIC 6 dilutions lower than that obtained for *M. bovis,* and tilmicosin giving the lowest MIC_{50} value obtained.[1,2] However, the way in which MIC values relate to the effectiveness of antimicrobials in animals is a complex issue. Antimicrobials demonstrating little or no *in vitro* activity are unlikely to be effective clinically in aiding the body's defenses to eradicate infectious organisms. However, it is known that some classes of antimicrobials, particularly the macrolides, may be actively concentrated (up to 18 fold) in the phagolysosomes of cells. This may make them a more appropriate choice for chemotherapy than MIC or MMC data might suggest.[22] More recent MIC work on MmmSC tested 50 isolates against 21 antimicrobials, and tilmicosin still gave the lowest MIC values with an MIC_{90} of <0.06mg/ml (Ayling, unpublished data).

Stipkovits *et al*[27] reported some success in treating *M. bovis* pneumonia and arthritis in calves by treating the calves with valnemulin in the milk for three weeks. More recently they[28] compared enrofloxacin with valnemulin via the milk replacer and demonstrated an improvement in the condition of the treated infected calves in comparison with the untreated infected control group. Clinically calves appeared to respond more rapidly to valnemulin and it eliminated *M. bovis* from the lungs more effectively than enrofloxacin. Byrne *et al*[4] described the elimination of *M. bovis* mastitis from a dairy herd, using a combination of broad spectrum antibiotic treatment, segregation and some culling of affected cows, which relied on effective identification of infection. Advocin has also been used to treat arthritis caused by *M. bovis* (Nicholas, personal communication). A beef farm in the UK reduced losses caused by *M. bovis* by including antimicrobials in the feed, but this is expensive and is likely to lead to the development of antimicrobial resistance.

Preliminary work on CBPP demonstrated that the spread of mycoplasma from naturally affected cattle treated with danofloxacin to in-contact animals was significantly reduced, suggesting that antimicrobials may have a role in the control of this disease.[8]

Vaccines

The only proven vaccine effective against CBPP is a live broth culture of a live attenuated strain of $T_1$44 which has been used in Africa for over 50 years. However, this is known to cause adverse reactions in as many as 11% of treated animals.[9] In addition, it is reported that the vaccine can actually cause CBPP.[12] Improved vaccines are therefore required. Although several vaccines have been tested, to date all have resulted in exacerbation of the disease, so clearly new strategies are needed.

Some *M. bovis* vaccines are available in the USA, but none are available globally, or in Europe. Nicholas *et al*[17] reported a significant level of protection against a large virulent challenge in calves and it reduced the spread of *M. bovis* to internal organs, including the joints. The challenge and vaccine strain were from different sources, a UK isolate for the vaccine and a Hungarian isolate for the challenge, possibly indicating cross-protection between different antigen and molecular types. However, reports by some research workers developing *M. bovis* vaccines have resulted in exacerbation of the disease.[3,25] Currently, in the UK an autogenous vaccine is being tested on a beef farm with a history of *M. bovis.* Initial indications are that treatment is successful, with no losses, and animals are in a much improved condition although some low grade pneumonia persists.

Mycoplasma bovis Free Herds

Mycoplasma bovis is not ubiquitous but widely spread within the bovine population in enzootically infected areas,[18] which indicates that a farm can stay free of the disease. With a closed herd free of *M. bovis* the organism is unlikely to spontaneously appear on the farm. However bringing new stock, often from more than one source, onto a farm will always present a potential disease risk. However, effective testing and quarantine arrangements may prevent bringing costly diseases onto the farm. Testing and quarantine may be considered expensive and time consuming practice, but weighed against the losses and cost of a *M. bovis* infection or other diseases, it is worth reconsidering. Conversely, persistently infected herds remain a major disease threat to new replacement stock.

In Ireland, the dairy production department of the government agency Teagasc replaced their stock after BSE and formulated a strategy in relation to health status which included being *M. bovis* free.[19] By serological testing prior to purchase, *M. bovis* free stock was introduced to Teagasc and the herd has remained *M. bovis* free, providing proof that prevention is better than cure.

Mycoplasma bovis – Other Options for Disease Control

The options for controlling *M. bovis* infections appear limited, and without severe culling the chances of eliminating *M. bovis* are few. Early treatment is most effective; if signs are missed or treatment delayed then

opportunities for controlling the disease are quickly lost. Even if culling is carried out, the disease may be introduced when restocking unless prior testing is carried out.

However, the effect of improved animal husbandry practices combined with strategic antimicrobial treatment is not well documented. It may be possible to reduce the infection to a manageable level which is acceptable not only in terms of animal welfare but may also be financially advantageous. Actions to reduce contact between animals such as isolating affected cattle, reducing stocking density, increasing ventilation, moving stock outside, increasing the number of feeding troughs, reducing the number of cows being held in a group before milking, separation of animals into smaller groups, etc. may be beneficial. Affected cows should be milked last and all equipment, cloths, etc., cleaned thoroughly. Milk being fed to calves should be heat treated at 140°F (60°C) for 30 minutes to kill any *M. bovis* present. Use of disinfectants will ensure reduced survival levels in the barns and on feeding troughs.

Conclusion

In summary an infected animal represents a financial burden in terms of veterinary costs, ill thrift, etc. Early intervention can prevent spread of disease throughout a herd. Treated animals may appear cured, but they can relapse and remain as an infective carrier for the remainder of a herd. Increased awareness of the disease, implementation of disease prevention measures, quarantine and testing of new stock and the eventual introduction of a vaccine should reduce the impact of *M. bovis.*

Acknowledgements

We would like to acknowledge the work and support the mycoplasma team at VLA (Weybridge), and Defra for continuing financial support for mycoplasma work.

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