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Recent developments in the diagnosis and control of mycoplasma infections in cattle

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1. Introduction

It is a little known fact that mycoplasmas cause some of the most serious and economically most costly diseases of cattle. Contagious bovine pleuropneumonia (CBPP), the only bacterial disease classified by the Office International des Epizooties as a list A disease, is caused by *Mycoplasma mycoides* subsp. *mycoides* small colony (SC) variant. This mycoplasma was isolated just over a century ago but, while largely eradicated from Europe, it still presents immense problems in Africa. *Mycoplasma bovis* is a major, and often overlooked, cause of calf pneumonia, mastitis, arthritis and other conditions. In addition to these respiratory pathogens, *M. dispar*, *Ureaplasma diversum*, *M. bovirhinis* and, more recently *M. canis*, have been isolated from the lungs of pneumonic cattle though not all are believed to be primary causes of disease. Table 1 lists some comparative properties of important cattle mycoplasmas and the diseases that they cause.

2. Characteristics

Mycoplasmas belong to the class Mollicutes which also contain ureaplasmas, acholeplasmas, spiroplasmas, the newly classified haemoplasmas (formerly Eperythrozoon and Haemobartonella spp and other wall-less bacteria. They are characterised by small size (500-1100 kbp), lack of cell wall, extreme fastidious *in vitro* and tendency to form centred colonies on solid medium. While all live a parasitic existence colonising the mucosal epithelium and relying on the host to provide most nutritional requirements, the majority are commensals, though occasionally opportunistic invading lung tissue following bacterial or viral infections. Their lack of means that Mycoplasmas are inherently resistant to those antibiotics that target the cell wall; in addition they have higher mutation rates than conventional bacteria which means that they can rapidly develop resistance to other drugs including the oxytetracyclines and tylosin as has been seen in Europe recently (Ayling et al 2000; Thomas et al 2003). Conversely their lack of cell wall means that they are fragile in the environment; consequently successful infection is restricted to close and repeated aerosol transmission.

3. Contagious bovine pleuropneumonia

3.1 Distribution

In Africa CBPP, caused by *Mycoplasma mycoides* subsp. *mycoides* SC variant is found in at least 29 countries in sub Saharan Africa. Endemic infection extends throughout the pastoral herds of much of western, central and eastern Africa, with Angola and northern Namibia in

southern Africa. Newly-infected areas in the 1990s include much of Uganda, parts of Kenya, the Democratic Republic of Congo and most of Tanzania, where recently the disease has spread alarmingly; Rwanda (1994), Burundi (1997), Botswana (1995) and Zambia (1997) were recently reinfected. In Asia CBPP has been reported in recent times from India, Bangladesh and Myanmar.

CBPP was largely eradicated from Europe around the end of the 19th century. However, there have been outbreaks somewhere in Southern Europe during most decades of the 20th Century. A resurgence of CBPP occurred in the early 1980s with reports in southern France on a few occasions, between 1980 and 1984 while in Italy the disease reappeared in 1990 but was eliminated in 1993 (Nicholas et al 2000a). The last case recorded in Spain was in 1994. In Portugal the disease reappeared in 1983 after a 25 year absence; the number of cases has declined rapidly in recent years, from 2818 in 1996 to a single case in 1999 (Nicholas et al 2000a). No outbreaks have been reported in the present millennium.

3.2 Transmission/ clinical signs

Direct contact is the principal mode of transmission although wind-borne and indirect transmission cannot be excluded (Regalla et al 1996).

There is considerable variation in severity of signs observed in cattle affected by CBPP, ranging from hyperacute through acute to chronic and subclinical forms. Respiratory distress and coughing, evident on stimulation of resting animals, are the main signs of CBPP. The incubation period of the natural disease generally ranges from 1 to 2 months days. When control cattle were placed in contact with naturally affected affected cattle from a recent outbreak in Namibia, seroconversion was seen after 6 weeks, rose rapidly in the next two weeks by which time 40% of contacts had died (Hubschle, personal communication).

The early stages of CBPP are indistinguishable from any severe pneumonia with pleurisy. Animals show dullness, anorexia, irregular rumination with moderate fever and may show signs of respiratory disease. Coughing is usually persistent and is slight or dry. Sometimes fever goes up to 40 – 42 °C, and the animal prostrates with difficulty of movement. As the lung lesions develop, the signs become more pronounced with increased frequency of coughing and the animal becomes prostrate or stands with the back arched, head extended and elbows abducted. While classical respiratory signs may be evident in calves, articular localisation of the causative agent with attendant arthritis usually predominates.

The clinical signs observed in the acute form are much accelerated. The pathological signs are usually characteristic with marked pleural adhesion accompanied by exudative pericarditis (Regalla et al 1996). Affected animals may die within a week exhibiting classical respiratory signs.

In the subacute form, signs may be limited to a slight cough only noticeable when the animal is exercised. CBPP in Europe, unlike that caused in Africa where mortality rates are typically 10-70% in epizootics, is characterised by low morbidity and low or non-existent mortality with the majority of infected cattle showing chronic lesions; this is characteristic of endemic disease; the sub-acute form is most common in Africa (Regalla et al 1996). These differences are, in

part, due to the fact that European cattle are healthier in general, better fed, subjected to less physical stress, are often permanently housed throughout the year and probably experience strains of lower virulence than in Africa (Regalla et al 1996). In Italy, during the early 1990s, less than 5% of cattle in an infected herd showed clinical signs (Guadagnini et al 1991). The use of antibiotics and anti-inflammatory drugs may help to mask clinical signs but serious consideration is now being given to their use because of the ineffectiveness of current vaccines. In Africa, up to a third of cases that recover from acute disease become potential carriers. This figure was probably higher in Europe where there is a far more widespread use of antimicrobials.

3.3. Gross pathology

Lesions are confined to the lungs and thoracic cavity and are mostly unilateral. In a study in Portugal, 95% of lesions were restricted to a single lung (Egwu et al 1996) with the diaphragmatic lobes being more commonly affected than cranial lobes. Adhesions to the chest caused roughened pleural membranes are common. Many litres of straw coloured pleural fluid can be found in acute cases which makes ideal diagnostic material.. The interlobular septa are often distended and lungs show the typical marbled appearance with lung lobules showing great variations in colour from red, grey to yellow depending on the stage of inflammation. Associated lymph nodes undergo hypertrophy. In chronic cases the sequestrum is the main lesion type and consists of necrotic material surrounded by a fibrotic capsule ranging from 10 to 100 mm in diameter. Necrotic foci have been reported in the kidneys of affected cattle.

3.4 Diagnosis

While there are plenty of tools available for CBPP diagnosis including PCR techniques, there is no single test which can detect all infected animals. However approved serological tests, complement fixation test and the competitive ELISA, are poorly sensitive. A more sensitive and specific immunoblotting test has been developed and is presently used to confirm positive and negative samples but requires expert analysis and is does not lend itself to mass screening. The culture and identification of *M. mycoides* SC is still required though PCRs can facilitate diagnosis. An urgent requirement particularly in Africa is the development of rapid penside tests; latex agglutination tests have been used but require further evaluation (Nicholas et al 2000a)

3.5 Control

In Europe, apart from a brief period in the the second half of the 20th Century when vaccines were seriously considered in the Iberian pensinsula following the re-emergence of CBPP, stamping out has been the control method of choice. The chief detection tool was either abattoir surveillance and/or serological monitoring. Control in Africa, where practised, is based on 60 year old vaccine which is poorly protective, can cause severe adverse reactions and has residual virulence; movement control is difficult to enforce and antibiotics, officially frowned upon in some countries, are used widely though their effectiveness is difficult to assess. Recent studies, however, have shown that a fluoroquinolone, danafloxacin, may be able to prevent or inhibit the spread of mycoplasma from affected cattle to contact controls (Hubschle personal communication). It is difficult to see any improvement, and many expect a worsening, in the CBPP situation in Africa in the next 5 years as new vaccines appear a long

way off. A complicating factor appears to be the fact that the host may be mainly responsible for the lesions seen with this disease (Nicholas et al 2000a)

4. Mycoplasma bovis

4.1 Introduction

M. bovis was first isolated in 1961 in the USA from a case of severe mastitis in cattle (Hale et al 1962). It then appears to have spread via animal movements to, amongst many countries: Israel (1964), Spain (1967), Australia (1970), France (1974), Britain (1975), Czechoslovakia (1975), Germany (1977), Denmark (1981), Switzerland (1983), Morocco (1988), South Korea (1989), Brazil (1989) Northern Ireland (1993), Republic of Ireland (1994) and Chile (2000). (Nicholas 2002). Today, infection occurs in most European countries and the mycoplasma has been reported throughout the world.

The prevalence of *M. bovis* is almost certainly under reported as *Manheimia haemolytica*, *Pasteurella multocida* and *Haemophilus somnus* are invariably isolated first from pneumonic calves; in arthritis and mastitis where *M. bovis* is a primary and uncomplicated cause, it is often overlooked as few laboratories routinely monitor for mycoplasmas. However unlike these conventional bacteria, the occurrence of *M. bovis* in a herd is invariably linked to cases of disease in particular pneumonia, mastitis and/or arthritis (Pfutzner and Sachse 1996). *M. bovis* has also been associated with eye disease, endometritis, salpingitis, oophoritis, abortion and seminovesiculitis, all of which have been reproduced experimentally (Ruhnke 1994).

4.2 Economic losses

In the UK, it is estimated that up to 1.9m cattle are affected annually by respiratory disease which costs the cattle industry £54 million (Reeve-Johnson, 1999). Furthermore approximately 157,000 calves die annually as a result of pneumonia and related illnesses which would have a potential market value of £99m. Across Europe with approximately 90 million cattle this extrapolates to total losses of 576m euros. It is likely that *M. bovis* is responsible for at least a quarter to a third of these losses although this is likely to be an underestimate (Nicholas et al 2000b). In the USA, the cost caused by *M. bovis* as a result of loss of weight gain and carcass value have been estimated at \$32 million per year (Rosengarten and Citti 1999). The losses due to bovine mastitis caused by *M. bovis* may be higher than that for respiratory disease with estimates from the USA of up to \$108 million per year with infection rates of up to 70% of a herd (Rosengarten and Citti 1999).

4.3 Epidemiology

Contrary to popular belief, *M. bovis* is not ubiquitous but widely spread within the bovine population in enzootically infected areas. The infection is usually introduced to *M. bovis*-free herds by clinically healthy calves or young cattle shedding the mycoplasma and once established on multi-age sites becomes very difficult to eradicate. Its appearance on some farms suffering low grade respiratory disease can lead to increased morbidity and mortality (Gourlay et al 1989). Infected cattle can shed the mycoplasma via the respiratory tract for many months even years and act as a reservoir of infection (Pfutzner 1990). Contact animals become infected via the respiratory tract, the teat canal or genital tract; artificial insemination with infected semen is another common route (Pfutzner 1990).

Following its introduction into the North and South of Ireland in 1994 from mainland Europe, *M. bovis* has been consistently isolated from 13-23% of pneumonic lungs (Brice et al 2000; Byrne et al 2001). In France, *M. bovis* was isolated from 30% of calf herds with pneumonia (Le Grand et al 2001) while in Britain, about 20-25% of pneumonic herds contain animals with antibodies to *M. bovis* (Nicholas et al 2001). In the UK, significant antibody titres to *M. bovis* were detected in just under half of 55 pneumonic herds examined, of which only 7 herds had rising titres to viral pathogens: respiratory syncytial virus, infectious bovine rhinotracheitis or bovine viral diarrhoea virus (Nicholas et al 2001). Clearly other factors play a role in bovine respiratory disease such as concurrent viral and bacterial diseases as well as environmental factors but it is increasingly believed that *M. bovis* is the predisposing factor in the infectious process leading to invasion by other bacterial pathogens possibly by compromising host defences (Rebhun et al 1995; Poumarat et al 2001).

Tschopp et al (2001) confirmed the importance of *M. bovis* as an agent of respiratory disease in a recent field study in which 50% of over 400 calves introduced to infected fattening sites developed respiratory disease attributable to *M. bovis*; they also showed an 8% reduction in weight gain in the 55% of calves which became seropositive seven weeks after introduction with these calves requiring twice as many antibiotics as seronegative calves.

4.4 Disease course

A clinical study of endemic pneumonia, from which *M. bovis* and *Pasteurella multocida* were frequently isolated from the herd, showed that nearly half of dairy calves were shedding mycoplasmas at 5 days of age and over 90% by 4 weeks (Stipkovits et al 2001). Clinical disease in the calves, including up to 10% mortality as a result of severe serofibrinous pneumonia, was highest between 10-15 days. Surviving calves showed very poor weight gain and remained retarded; other signs included fever, depression, hyperpnoea, dyspnoea, nasal discharge, mild to continuous coughing and loss of appetite. In the UK, calf pneumonia usually begins in November and peaks around January before declining but deaths due to *M. bovis* continue to occur in some herds in the spring at pasture representing relapses because of unresolved lung lesions (Nicholas et al 2001).

Arthritis associated with *M. bovis* infection may be a sequel to either the respiratory or mastitic form of the disease (Pfutzner 1990). There is lameness, swelling of joints, slight elevation of temperature, failure of antibiotic treatment, and if severe, reduced consumption of feed and debilitation. This condition often arises within 2-3 weeks of housing and also following transportation of calves over long distances. A case of severe keratoconjunctivitis impairing vision in a number of bullocks involving *M. bovis* may also have been exacerbated by the stress of transport (Kirby and Nicholas 1996).

Pulmonary lesions in naturally infected calves comprise an exudative bronchopneumonia and extensive foci of coagulative necrosis surrounded by inflammatory cells. In studies involving experimental infections of gnotobiotic calves with *M. bovis*, significant pneumonia was induced involving up to 30% of the lung surface and was of sufficient severity to cause clinical respiratory disease in some calves (Thomas et al 1986). Distinctive areas of coagulative necrosis were prominent within the lesions. Chronic infections are often associated with a

lymphocytic “cuffing” pneumonia where there is marked hyperplasia of peribronchial lymphoid tissue causing stenosis of airway lumina and compression and collapse of adjacent pulmonary pyrenchyma. *M. bovis* antigen is mainly detected at the periphery of the areas of coagulative necrosis, in necrotic exudates and in close association with infiltrating macrophages and neutrophils (Rodriguez et al 1996).

4.5 Diagnosis

Clinical and pathological signs are not characteristic for *M. bovis* so laboratory diagnosis is necessary for identification. In a recent study Thomas et al (2002) showed that sampling by bronchoalveolar lavage was more predictive of lower respiratory airway pathogens, including *M. bovis*, than nasal swabs although clearly not as convenient. *M. bovis* grows well in a variety of media producing “centred” colonies within 3-5 days (Nicholas and Baker 1998). In an appropriate medium (such as Eaton’s) *M. bovis* produces films and spots and gives an orange colour to the broth. Other biochemical characteristics are shown in table 2 alongside other bovine mycoplasmas. Growth inhibition, film inhibition, fluorescent antibody or metabolic inhibitions tests can be used to identify the mycoplasma using hyperimmune rabbit serum (Poveda and Nicholas 1998).

Ball and Findlay (1998) described a sandwich ELISA for *M. bovis* in which specific monoclonal antibodies, fixed to the microplate, captured *M. bovis* antigen from the medium; the sensitivity of the test, similar to that of conventional culture diagnosis, can be improved by a short enrichment stage. *M. bovis* can be easily outgrown by opportunistic mycoplasmas like *M. bovirhinis* and acholeplasmas and occasionally antigenic variability of strains may make serological tests unreliable (Ayling et al 1997). For these situations, polymerase chain reaction (PCR) tests are very convenient. Early PCRs, based on 16S rRNA genes, also amplified *M. agalactiae* DNA (Pfutzner and Sachse 1996; Ayling et al 1997) but recent PCR developments based on the *uvrC* gene are more specific (Frey et al 1999). PCRs have been used to detect *M. bovis* directly in milk and nasal samples (Hotzel et al 1996) and even in preservative treated milk (Pinnow et al 2001).

Serological detection of *M. bovis* antibody is often a more reliable diagnostic method as antibody levels detected by ELISA remain high for many months. This is particularly true where antibiotics have been used extensively on the herd which severely hampers isolation; *M. bovis* is also difficult to isolate from chronically affected cattle (Nicholas 1997). Here, immunohistochemical techniques preferably using monoclonal antibodies may be valuable to visualise the mycoplasma antigens in the affected tissue (Adegboye et al 1995). The presence of specific antibodies also indicates that the infection is invasive as animals in which *M. bovis* is found only in the nasal passages rarely seroconvert. While a number of serological tests including indirect haemagglutination and film inhibition have been reported, these have been mostly superseded by the indirect ELISA using whole cell or chemically treated antigens (Nicholas et al 2000b); commercial tests are also available (Bommelli, Switzerland; Biovet, Canada). The use of the ELISA for accurately detecting antibodies in the milk has also been described and was capable of identifying infected individual udder quarters (Byrne et al 2000).

4.6 Treatment and control

Pfutzner (1990) stated that diseases due to *M. bovis* were resistant to any chemotherapy. In spite of this antibiotics are widely used sometimes correctly to reduce secondary bacterial infections but often ineffectively to treat the mycoplasma infections. Many *in vitro* studies have compared the susceptibility of *M. bovis* to a range of antibiotics (table 4). While it is clear that antibiotics which are ineffective *in vitro* are unlikely to be effective *in vivo*, those with strong activities *in vitro* will not necessarily perform well in the field for reasons which are unclear (Ayling et al 2000). Recent evidence suggests that *M. bovis* strains in Europe are becoming resistant to antibiotics traditionally used for treatment of mycoplasma infections in particular oxytetracyclines, tilmicosin and spectinomycin (Ayling et al 2000). The fluoroquinolones are still effective but their use in animals is controversial (Nicholas et al 2000b). Further field evidence of the ineffectiveness of antibiotics was provided by Haines et al (2001) who found *M. bovis* in the lungs and joints of 80% of cases of feedlot cattle that had failed to respond to antibiotic therapy; bovine viral diarrhoea virus and *Mannheimia haemolytica* were found in only 40 and 23% respectively of these cases.

The inability of chemotherapy to control *M. bovis* infections has focused attention on vaccination. Surprisingly there are no vaccines currently available although a quadrivalent inactivated vaccine containing respiratory syncytial virus, parainfluenza type 3 and 2 mycoplasmas, *M. dispar* and *M. bovis*, showed some protection against respiratory disease in the field (Howard et al 1987). A vaccine prepared with formalin inactivated strains of *M. bovis* and *Mannheimia haemolytica* taken from the target herd reduced losses from pneumonia and cost of treatment in newly introduced feedlot calves (Urbanek et al 2000). More recently a saponised inactivated vaccine was shown to be safe, highly immunogenic and protective against a strong experimental challenge of virulent *M. bovis* (Nicholas et al 2002). Vaccinated calves showed few respiratory signs while all unvaccinated calves developed signs of pneumonia. There was a statistically significant decrease in body weight gain in unvaccinated calves compared to vaccinates and a significant increase in lung lesions and rectal temperatures in unvaccinated calves. The vaccine also reduced the spread of *M. bovis* to internal organs including the joints.

The prophylactic use of antibiotics is generally undesirable but its use may be justified when calves are introduced to a site with a history of *M. bovis* infection in which high levels of mortality are being sustained. Nagamoto et al (1996) treated one group of introduced calves with leucomycin prior to the development of clinical signs. Untreated groups of calves showed mortality rates of up to 41% while all the calves in the treated group survived although coughing and nasal discharges were evident. Interestingly, while *M. bovis* antibodies were detected in non-treated groups after 2 months following introduction, antibody development was significantly delayed until over 8 months in the antibiotic treated groups. This suggests that antibody responses are rarely protective in mycoplasma infections.

Control of calf pneumonia should also include measures to reduce environmental stress and to ensure adequate housing with good circulation of air. Wherever possible consideration should be given to "all in, all out practices" to prevent older animals infecting younger ones. If this is not possible separation of calves from the adults is advisable at the earliest possible opportunity where endemic disease exists and where the particular farm husbandry permit.

5. Other mycoplasma pathogens

Several other mycoplasmas have been associated with disease in cattle. Most important of these and certainly most overlooked, because of its extreme fastidiousness, is *M. dispar* which has been shown to induce lesions in gnotobiotic calves. It is also found widely in the lungs of pneumonic cattle. The lack of good diagnostic tests for this mycoplasmas means its occurrence will remain under reported. Similarly the requirement for specialist medium for the growth of ureaplasmas also means that their prevalence is underestimated but studies have shown that *Ureaplasma diversum* is a cause of pneumonia, vulvovaginitis, conjunctivitis and infertility in cattle (Nicholas 2000b). The widespread occurrence of *M. canis* in pneumonic calves in the UK since its first isolation in 1995 indicates its successful colonisation of this host (Nicholas et al 2000b). Other countries reporting *M. canis* include The Netherlands, Belgium and Canada. Convincing proof of the role of *M. bovis genitalium* in reproductive disease is still lacking though its frequent isolation from cases of infertility, endometritis, “whites” and seminal vesiculitis coupled with its presence in frozen sperm must make it a serious candidate for this role (Nicholas 2002). *M. bovine* group 7 is the least studied of the *M. mycoides* cluster and causes arthritis, mastitis and, more recently, respiratory disease in cattle and occasionally small ruminants. It has been reported in France, Switzerland and Australia. Though not as serious as *M. mycoides* SC, the close relationship of *M. BG7* with the agent of CBPP may lead to diagnostic confusion in the event of its isolation in the UK. The development of new molecular techniques like DGGE (McAuliffe et al 2003) will help detect and identify mycoplasmas in days, rather than weeks, and enable a better understanding of their role in disease.

6. Conclusions

Contagious bovine pleuropneumonia (CBPP) is the most important livestock disease in Africa today affecting about 30 countries. New outbreaks have been seen in Zambia and Namibia. It must also still be considered a threat to cattle in Europe, having only just been eradicated from Portugal in 1999 and Italy in the mid 1990s. *Mycoplasma bovis* is a major, but often overlooked, pathogen causing respiratory disease, mastitis and arthritis in cattle. It is found worldwide and has spread into new areas, including Ireland and parts of South America, in the last decade. In Europe, it is responsible for at least a quarter to a third of all calf pneumonia although this may be an underestimate as few laboratories regularly monitor for mycoplasmas. Like all mollicutes, *M. bovis* is inherently refractory to certain groups of antibiotics because it does not possess a cell wall; moreover evidence is accumulating that strains of *M. bovis* are becoming resistant to antibiotics, including tetracycline, tilmicosin and spectinomycin, traditionally used for their control. Other mycoplasmas including *M. dispar*, *Ureaplasma diversum*, *M. bovis genitalium* and more recently, *M. canis* have been implicated to a greater or lesser extent in cattle disease. Mycoplasma diseases are extremely difficult to control as antibiotics are generally ineffective. While a vaccine is available for CBPP in Africa, it provides limited protective and can produce serious adverse reactions as well as retaining some virulence which can lead to outbreaks. No vaccines are presently available for *M. bovis* infections.

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Résumé

La péri-pneumonie contagieuse du bœuf (CBPP) est la plus importante des maladies de bétail en Afrique aujourd'hui. Cela touche environ 30 pays avec récemment de nouvelles épidémies en Zambie et la Namibie. Avec des vaccins inefficaces et l'incapacité de contrôler les mouvements de bétails, il est probable que la maladie continue à se répandre en Afrique dans l'avenir. La maladie doit également être considérée comme une menace pour le bétail en Europe étant donné qu'elle vient à peine d'être éradiquée au Portugal en 1999 et en Italie dans les années 90. *Mycoplasma bovis* est un pathogène d'une importance majeure, quoique souvent négligé, causant maladies respiratoires, mastite et arthrite chez le bétail. *M. bovis* se trouve dans le monde entier et s'est répandu dans de nouveaux endroits, tels que l'Irlande et certaines parties de l'Amérique du Sud dans la dernière décennie. En Europe, cette maladie représente un quart à un tiers des cas de pneumonie chez les veaux, bien que ceci puisse être une sous-estimation étant donné que les mycoplasmes ne sont pas régulièrement analysés en laboratoire. Comme tous les mycoplasmes, *M. bovis* est intrinsèquement réfractaire à certains groupes d'antibiotiques dû au manque de paroi cellulaire. De plus en plus de preuves montrent que *M. bovis* devient résistant aux antibiotiques, dont tétracycline, tilmicosine et spectinomycine, qui sont généralement utilisés contre cette maladie. D'autres mycoplasmes tel que *M. dispar*, *Ureaplasma diverum*, *M. bovis genitalium* et plus récemment *M. canis* ont été plus ou moins impliqués dans les maladies bovines. Les maladies mycoplasmes sont extrêmement difficiles à contrôler car les antibiotiques sont généralement inefficaces. Bien qu'un vaccin soit disponible contre la CBPP en Afrique, celui-ci offre une protection limitée, peut produire des sérieuses réactions néfastes, et retient une certaine virulence, ce qui peut mener à des épidémies. Aucun vaccin n'est actuellement disponible pour des infections de *M. bovis*

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