MYCOPLASMAS

The mycoplasmas are small, Gram-negative, prokaryotes that lack cell walls and are bound only by a plasma membrane. They include all the organisms classified in the class Mollicutes, incorporating the families containing organisms which may be isolated from livestock, namely the Mycoplasmataceae (in which the genera *Mycoplasma* and *Ureaplasma* occur) and Acholeplasmataceae (containing the genus *Acholeplasma* which is of no known veterinary importance). The organisms grouped into these families have some of the smallest genomes recorded for prokaryotes. They lack the ability to synthesize peptidoglycan, which renders them insensitive to the deleterious effects of penicillin and its analogues, and susceptible to lysis by osmotic shock, detergents, alcohols, and specific antibody and complement.

The organisms are pleomorphic, varying in morphology from pear-shaped organisms to branched or helical filaments. Most of the species of these genera are facultative anaerobes and may be cultivated on cell-free media of varying complexity containing sterols and fatty acids, though some are more readily cultivated in cell cultures. The genera isolated from livestock can be distinguished from one another in several ways: *Acholeplasma* spp. do not require sterols for growth, while *Ureaplasma* spp. are distinguished by their ability to split urea into ammonia and carbon dioxide.[3] In addition, *Ureaplasma* spp. do not grow on the media commonly employed for the propagation of the mycoplasmas. Colonies of mycoplasmas on solid media are very small, tend to grow within the medium, and assume a 'fried egg' appearance. Mycoplasmas are also distinguished from all other prokaryotes by the fact that they are filterable through filters of 450-nm pore diameter. They are, however, superficially similar to the cell wall-defective L-phase variants of bacteria encountered as laboratory artifacts.

Most of the mollicutes are saprophytes, commensals or parasites, and some are pathogens of humans, animals and plants.[1] Pathogenic mycoplasmas are, with few exceptions, such as *Mycoplasma bovis*, host- and site-specific, and most are parasites of mucous membranes and joints. Infections by mycoplasmal species are almost invariably associated with diseases of the respiratory and urogenital tracts, mammary gland or eyes (**Table 1**). Of the many mycoplasmas that have been isolated from animal species, few have been shown without doubt to be pathogenic. Contagious bovine pleuropneumonia, contagious caprine pleuropneumonia, ovine keratoconjunctivitis, and mycoplasmal pneumonia of pigs, in particular, are highly contagious and of economic importance, whereas many of the other mycoplasmal infections, though ubiquitous, are more sporadic in occurrence, and most (e.g. those caused by *M. alkalescens*, *M. arginini*, *M. bovoculi*, *M. flocculare*, and *A. laidlawii* and *A. modicum*) are subclinical. Most pathogenic mycoplasmas are associated with chronic disease (**see Chapter 206: Bovine genital mycoplasmosis**).

Infectious keratoconjunctivitis of sheep is an important disease. Apart from *M. conjunctivae*,[1] other infectious agents may also cause this condition (**see Chapter 140: *Moraxella* spp. infections**). The lesions in the eyes progress from an initial acute hyperaemic stage to a mucopurulent

Table 1 Diseases caused by common *Mycoplasma* spp. infections in livestock

MYCOPLASMA SPP.	DISEASE	SPECIES AFFECTED
Mycoplasma mycoides mycoides SC	Contagious bovine pleuropneumonia	Cattle
Mycoplasma capricolum subsp. *capricolum*	Contagious caprine pleuropneumonia	Goats and sheep
Mycoplasma agalactiae	Mastitis	Goats and sheep
Mycoplasma hyopneumoniae	Mycoplasmal (enzootic) pneumonia of swine	Pigs
Mycoplasma hyorhinis	Pneumonia, arthritis, polyserositis	Pigs
Mycoplasma hyosynoviae	Arthritis	Pigs
Mycoplasma bovis	Infertility	Cattle
Mycoplasma bovigenitalium	Infertility	Cattle
Mycoplasma canadense	Mastitis	Cattle
Mycoplasma conunctivae	Keratoconjunctivitis	Sheep

keratitis which is followed by corneal ulceration and should be differentiated from those caused by photosensitization and other infectious agents.

Several mycoplasmas have been isolated from the lesions of sheep suffering from ulcerative balanoposthitis or vulvovaginitis, but the significance of their presence is unknown (**see Chapter 213: Ulcerative balanoposthitis and vulvovaginitis of sheep**).

It appears that the role played by ureaplasmal infections is increasing. *Ureaplasma diversum*, for instance, has been associated with vaginitis, endometritis, salpingitis, seminal vesiculitis, and abortions in cattle.[3]

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Contagious bovine pleuropneumonia

Synonyms: Péripneumonie contagieuse bovine (Fr.), lungsickness, longsiekte (Afrik.), Lungenseuche der Tiere (De.)

F THIAUCOURT, J J VAN DER LUGT AND A PROVOST*

Introduction

Contagious bovine pleuropneumonia (CBPP) is an acute, subacute or chronic disease of cattle and occasionally of water buffalo (*Bubalus bubalis*) caused by *Mycoplasma mycoides* subsp. *mycoides* Small Colony (MmmSC). The acute to subacute disease is characterized by a serofibrinous pleuropneumonia and severe pleural effusion. Persistent pulmonary sequestra result in the development of a chronic, often subclinical, carrier state in many recovered animals.

This disease was first described in 1564 by Gallo[18, 50] and has, with the exception of South America and Madagascar, occurred throughout the world at some time or another. It has been eradicated from North America and Australia. It is an economically important disease in Africa south of the Sahara,[39, 50] where it is present in certain countries in West, Central, East and southern Africa. In the latter region, it is currently endemic in Angola and the northern part of Namibia. The western part of Zambia is periodically infected from Angola and the eastern part is at risk, CBPP being widespread in Tanzania. It is present in some countries in the Near and Middle East, but its prevalence in other Asiatic countries is uncertain. Pakistan reported its last outbreak in 1997 and India in 1990.[50, 60] Sporadic outbreaks occur in southern Europe. The disease was detected in 1983 in Portugal after an absence of 30 years. Two regions, Entre-Douro e Minho and Beira Litoral, were particularly affected. Intensive test-and-slaughter policies resulted in a dramatic reduction in the number of outbreaks between 1993 and 1999, when the last case was detected. Outbreaks also occurred in Italy in 1990 to 1993, with cattle in two distinct regions being infected. The disease was eradicated after extensive slaughter-out policies had been adopted but the origin of the outbreaks was never identified.

The current presence of CBPP in parts of southern Africa and its present expansion in eastern Africa severely restricts economic development and the proper utilization of agricultural and land animal resources. Application of veterinary regulatory measures, which are essential to control the disease and prevent its spread, hamper proper land-use planning, and constant veterinary disease surveillance and costly annual prophylactic vaccination campaigns place high demands on the usually limited budgets of veterinary administrations.

Contagious bovine pleuropneumonia was introduced into South Africa from the Netherlands by a Friesian bull or bulls landed at Mossel Bay in 1853 in what is now the Western Cape Province.[46] From Mossel Bay the disease was disseminated rapidly by trek oxen; progressing in all directions along transport routes soon to reach the northern provinces. Within two years it had killed over 100 000 head of cattle.[26] Among its political effects was a major contribution to the Great Xhosa Cattle-killing Movement of 1856 to 1857 which resulted in the starvation of tens of thousands of Xhosas and the devastation of that nation.[46]

Namibia was infected in 1856 when a localized outbreak occurred at Warmbad in the south. Although the initial outbreak was contained by quarantine measures adopted by missionaries in the area, the infection reached the central cattle-raising districts in 1859 as a result of cattle introductions from either Botswana or South Africa. Very high mortalities resulted and the Herero people called 1860, 'otjipunga', the year of the lung. In 1861 CBPP moved across the Limpopo River to cause heavy losses among the cattle of the Matabele in southern Zimbabwe.[26]

Contagious bovine pleuropneumonia was introduced into Angola from the south, probably by infected cattle belonging to the Dorsland Trekkers who emigrated from South Africa and settled near Humpata in the Huila Province in the early 1880s. Its presence was confirmed there in 1888.[41] The extensive use of draught oxen is considered to be the cause

* Deceased

of its rapid spread throughout Angola, and by 1914 the whole country had been infected.

The introduction of CBPP into Zambia originated from Angola. Although eradicated in 1946, CBPP was again introduced from Angola into the Western Province of Zambia in late 1969.[57] This outbreak resulted in a 75 per cent morbidity rate and caused up to 68 per cent mortality in affected herds.[2] It was eradicated in 1976 but was reintroduced in 2000, again from Angola.

By the time rinderpest appeared in 1896 to 1897, CBPP was widespread in southern Africa. The effects of the rinderpest pandemic overshadowed those of CBPP and the disease killed many CBPP-infected cattle. It was not until the turn of the century that the persistence of lungsickness in the cattle populations of southern Africa again presented an obvious problem, and strict control measures were introduced in most countries of the region. These were, however, complicated by other events which occurred about that time, such as the South African War, the introduction of East Coast fever, the Herero war, and the human influenza pandemic of 1918.[26] The disease was eventually eradicated from Zimbabwe in 1904, from South Africa in 1924, and from Botswana in 1939. The latter country was unexpectedly reinfected in early 1995;[3] freedom from the disease occurring in 1997 after the slaughter of the entire cattle population in the infected zone. More than 300 000 cattle were slaughtered and compensated for by the Botswanan state in cash or with replacement cattle. These costly measures were mandatory in order to allow the country to resume its beef exports to Europe.

The disease still persists in northern Namibia[56] and in Angola, from where it periodically spreads into the Western Province of Zambia. In Namibia, the high-risk areas are the north-central districts, Kavango and Kunene. Although CBPP has not been diagnosed in the Eastern Caprivi of Namibia since it was eradicated in 1938,[4, 40] special attention is given to this area for the protection of Botswana. In 1997 Namibia commenced massive and regular vaccination campaigns in the north of the country in order to control CBPP. In Angola, the disease is endemic throughout the country; this represents a formidable threat for the rest of the region. The reintroduction of the disease into Tanzania in 1990 and its uncontrolled spread throughout the country pose a severe threat for the southern African region; on several occasions sporadic foci have been reported in north-eastern Zambia. The possibility that it has extended into the Democratic Republic of Congo cannot be ruled out, but the present situation is unknown because of the persistent civil strife.

The situation in West and Central Africa in recent years has been characterized by increased numbers of outbreaks of CBPP. This situation may be explained by the eradication of rinderpest from these regions with the consequent cessation of regular combined vaccination campaigns to control both rinderpest and CBPP. Recent CBPP vaccination campaigns have not included a large proportion of the cattle population, which consequently remain unprotected. In addition, a very low protection rate has been afforded by the vaccine which may have resulted from such factors as incorrect handling methods of the vaccine, leading to low dosage rates or immunologically ineffective material being administered.

Aetiology

Mycoplasma mycoides subsp. *mycoides* SC is a mollicute, a pleomorphic bacterium that lacks a protecting cell wall, and grows readily under aerobic and anaerobic conditions.

Various types of serum broth are used as culture media.[50] These should contain meat extract or peptone, or both, as the basic medium, as well as a fresh extract of baker's yeast and 10 to 20 per cent serum, preferably from horses. Various additives can be added, such as glucose, sodium pyruvate, DNA, sterol, and antibacterial and antifungal drugs in the case of primary isolation from a contaminated sample. Growth is relatively slow, and 24 to 48 hours are usually required before it is visible, but primary isolation may take longer or necessitate the making of subcultures if the original sample was not of adequate quality. Primary culture in liquid medium often yields the typical aspect of a whitish 'comet', a kind of cloud floating in the medium that is easily dispersed by agitation. Solid media are prepared by the addition of 1 per cent agar to the fluid medium, and MmmSC colonies have the typical 'fried-egg' appearance of mycoplasmal colonies. The colony size seldom exceeds 1 mm in diameter and, in order to visualize them, a stereomicroscope must be used.

Mycoplasma mycoides subsp. *mycoides* SC belongs to the so-called mycoides cluster,[17] a group of six mycoplasmal species or strains that are all ruminant pathogens. Based on 16S rRNA gene sequence analysis,[71] this group seems to be more related to *Spiroplasmas,* which are plant pathogens, rather than to the other animal and human mycoplasmal pathogens. The closest relatives of MmmSC are *Mycoplasma mycoides* subsp. *mycoides* Large Colony (MmmLC) and *Mycoplasma mycoides* subsp. *capri* (Mmc).[17, 64] These two subspecies are frequent pathogens of small ruminants and induce a 'contagious agalactia' syndrome characterized by the development of mastitis, arthritis, keratitis, and, in kids, pleuropneumonia and septicaemia. Some strains might have a specific pulmonary tropism and the disease caused by them has to be clearly differentiated from contagious caprine pleuropneumonia which is caused by *Mycoplasma capricolum* subsp. *capripneumoniae*, the former 'type F38'[31] (**see Chapter 202: Contagious caprine pleuropneumonia**). The distinction between MmmSC, MmmLC and Mmc has been difficult until recently but it can now be achieved by the use of specific monoclonal antibodies or specific polymerase chain reaction (PCR) techniques. *Mycoplasma mycoides*

subsp. *mycoides* SC strains were considered to be very homogeneous but recent evidence has shown that various genotypes can be distinguished by use of molecular techniques such as restriction analysis of whole DNA[48, 62] or southern blotting.[13, 34] African and European MmmSC strains can be distinguished by genetic[13, 69] as well as antigenic[24] differences, an indication that the outbreaks occurring since 1980 in France, Spain, Portugal and Italy were not due to the re-introduction of strains from Africa, but were more probably due to a resurgence of CBPP from a region or regions where it has never been completely eradicated since the previous century. A specific PCR allows the identification of T1 vaccinal strains.[33] These variations can be used as molecular markers but the link with possible variations of pathogenicity is not yet established. Within the 'mycoides cluster' MmmSC strains have the peculiarity of possessing a number of 'Insertion Sequences' which may account for a great part of the MmmSC genome variability.[13, 68]

Mycoplasma mycoides subsp. *mycoides* SC is susceptible to environmental factors and on average only survives outside the host for up to three days in tropical areas and up to two weeks in temperate zones. Cultures of MmmSC can be inactivated by ultraviolet radiation within a few minutes. The organism is inactivated within 60 minutes at 56 °C and within two minutes at 60 °C, but can survive more than 10 years in frozen, infected pleural fluid. The organism is inactivated by 1 per cent phenol solution in three minutes, 0,05 per cent formaldehyde solution in 30 seconds, and a 0,01 per cent mercuric chloride solution in one minute.

The serial passage of MmmSC in culture and in animals alters both its virulence and its pathogenicity; this formed the basis for the development of attenuated vaccinal strains.

Epidemiology

The three factors which are of greatest significance in the rate of spread of the disease are: closeness of contact, intensity of infection, and the number of susceptible animals.

Natural transmission of CBPP occurs by droplet infection from either cattle with clinical disease or from subclinical carriers which are actively excreting the organism to susceptible animals in close contact.[28] *Mycoplasma mycoides* subsp. *mycoides* SC occurs in great numbers in bronchial secretions, nasal discharges and exhaled air. Dissemination of infection therefore occurs most easily in closely stabled or trucked animals. Aerosols containing infected droplets may spread the disease over distances of 20 m or more. Even under extensive sub-Saharan African farming conditions, spread of the disease may be rapid, infection being facilitated by the general practice of kraaling the animals at night, and by the congregation of large numbers of animals at places such as watering troughs or markets.

Direct contact between susceptible and diseased animals appears to be mandatory for transmission. The infection is not transmitted to healthy animals introduced into crushes, stock sale yards or transport vehicles that have previously been occupied by infected cattle. Neither ingestion of infected fodder nor direct exposure to diseased organs of animals suffering from CBPP will cause transmission. *Mycoplasma mycoides* subsp. *mycoides* SC may also be present in the urine of cattle affected with severe disease; a 'urinary tract to nose' route of transmission or the spread of infection through aerosols of urine droplets thus appears to be possible.[38] The organism was also isolated[23] or detected by PCR in bull semen.[62] Therefore, direct sexual transmission and indirect transmission by frozen semen might occur; these methods of transmission, however, require further investigation.

Apart from clinical cases of the disease, there are a number of other ways in which the infection can be introduced or maintained. *Mycoplasma mycoides* subsp. *mycoides* SC can be present in the nasal passages of cattle for 40 days during the incubation period of the disease, before any serological response to it can be detected. Such animals may thus disseminate the infection to susceptible animals; they are suspected to play a very important role in the spread of the disease through commercial or familial cattle farming practices. Another manner in which infection may be introduced or maintained has long been proposed. During the course of the disease, pulmonary sequestra may develop in affected animals and these may persist in clinically recovered cattle for many months. They are comprised of necrotic tissue which becomes encapsulated by fibrous connective tissue and may harbour viable mycoplasmas for 12 months or longer. Such affected animals are known as 'lungers'.[26] The role of these lesions in the persistence and transmission of CBPP is, however, debatable.[73] Completely closed capsules are not likely to play any role in the dissemination of infection, but rupture of the capsule or reactivation of lesions in the surrounding lung tissue may lead to transmission of CBPP to susceptible animals. Under such circumstances, clinically recovered animals harbouring such lesions could be responsible for spreading the disease. Infected young calves usually develop arthritis particularly of the carpus and tarsus and until relatively recently it was thought that they did not develop pleuropneumonia. In the 1995 epidemic in Botswana, however, severe CBPP lesions were seen in the lungs of calves as young as three to six months of age.[16] In view of this it is hypothesized that in an endemic situation calves in the first few months of life are partially protected by a progressively waning colostral-derived passive immunity and therefore do not develop the typical disease, whereas those in an epidemic situation do not have this protection and, as a consequence, develop the fully fledged disease.

The intensity of infection is related to the concentration of infective organisms in a herd at a given time; it is highest during acute outbreaks but, from a transmission and control point of view, low intensities of infection are more

important. Low intensities occur during the early stages of an outbreak in a susceptible herd and result in a slow spread, a long incubation period in infected animals and a slow build-up of infection. Similarly, low intensities occur at the late stage of the infection or when antibiotic treatment has been applied only to clinically affected animals. These may result in an insidious mode of spread and many months may pass before epidemics become apparent.

The level of susceptibility of animals in a given herd varies considerably between individuals. Ten to 60 per cent of cattle may apparently be resistant, and as few as 8 per cent of a herd may develop clinical signs. Survivors in an outbreak have a substantial degree of resistance. Thus, in a closed-herd situation, even under African conditions, there is a tendency for the disease to disappear, albeit over a long period of time. In this situation newly born calves constitute a nucleus of susceptible animals. In Africa, movement of animals between herds is common and is the direct cause of many outbreaks. This can occur licitly through market transactions, dowry payments, cattle congregation at water points and pastures, or exchange of animals for reproduction or ploughing. It can also occur illegally through the marketing of sick animals in order to escape control measures, or by theft. Civil strife is a major cause of CBPP transmission as it results not only in reduced levels of control measures being applied but also uncontrolled animal movements.

Mycoplasma mycoides subsp. *mycoides* SC affects cattle and reportedly water buffaloes.[30] There are conflicting opinions as to whether there is a difference in susceptibility between *Bos indicus* and *Bos taurus* cattle breeds;[35] T1 vaccine is equally efficacious in both species.

Under natural conditions there is no evidence that clinical disease occurs in species other than cattle. In water buffaloes, the infection is abortive, and is followed by seroconversion, but transmission to susceptible cattle does not take place. In one instance, MmmSC could be isolated from a sequestrum in a water buffalo[51] but the role of this species in the transmission of CBPP is considered to be negligible. Similarly, MmmSC has been isolated from the lungs of naturally infected goats,[29] but this species seems to have a very limited susceptibility following experimental inoculation of the organism. Nevertheless, their role as reservoir is very unlikely as CBPP was eradicated from Botswana after the relatively recent outbreak there by slaughter of the cattle population only and not the goat and sheep populations. Previous reports of CBPP detection in wild animals by application of the complement fixation test (CFT) should not be taken into account as this technique is prone to cross-reactions with antibodies against related mycoplasmas, such as MmmLC. In an experiment, two African buffaloes (*Syncerus caffer*) were inoculated with MmmSC; both died after having shown high complement fixing antibody titres to the organism. *Mycoplasma mycoides* subsp. *mycoides* SC was isolated from lungs, liver and spleen in pure culture from one of them but neither had any lesion; the latter casts doubt on the real cause of their deaths.[59]

Pathogenesis

Infection via the respiratory tract causes bronchiolitis and pneumonia.[28] In this process MmmSC firmly attaches to the surface of epithelial cells of the respiratory system and is thus prevented from being eliminated in mucus secretions. This cellular adhesion, in which the surface of the mucous membrane is colonized, is central to the pathogenicity of the mycoplasma in the disease. In contrast to other *Mycoplasma* spp., such as *M. pneumoniae*, there is no proof as yet that this attachment occurs as a result of the production of an adhesin by the organism. The pathogenesis of lungsickness is poorly understood as its investigation has been hampered by the difficulty and cost of reproduction of CBPP in cattle and the absence of a reliable laboratory animal model. The attenuation of MmmSC strains by *in vitro* passage is proof that virulence genes do exist. These genes might not be a necessity for the growth of MmmSC and are capable of undergoing random mutations *in vitro,* thus altering their expression *in vivo*. However, no gene coding for a toxin has ever been identified. Multiplication of MmmSC in the lung results in an overwhelming local inflammation. The type of antigen that triggers this inflammation is not known, nor are the types of cells and cytokines involved, although there are some indications that alpha tumour necrosis factor (αTNF) may play a role. The lesions of CBPP in naturally infected cattle may be restricted to lung tissue simply because of its mode of transmission as experimental inoculation may result in the development of lesions at the site of inoculation and in other organs but not in the development of lesions typical of the natural disease. Subcutaneous inoculation of a pathogenic MmmSC strain results, after 10 to 25 days, in an invading local oedema known as a 'Willems reaction', the name being derived from the perpetrator of inoculation trials performed in 1852.[72] Inoculation into the peritoneal cavity induces a peritonitis.

The pseudo-capsule of MmmSC organisms, made of the carbohydrate, galactan, has been considered as a potential virulence factor[22] as it may play a role in attachment to target tissues. It may also contribute to the resistance of the organism to phagocytosis and induce the formation of autoantibodies to pneumogalactan. Other mycoplasmal components might also be involved, but their identification awaits the unravelling of MmmSC genome sequence and gene expression analysis. Differences in pathogenicity between European and African strains have been noticed but it is extremely difficult to achieve conclusive comparisons in such different environmental circumstances. The absence of a glycerol transporter in European strains might account for its lower virulence through a decreased production of hydrogen peroxide.[70] However, vaccine strains, such as T1, possess this glycerol transporter, and it is therefore probable

Figure 201.1 Typical stance of an animal suffering from contagious bovine pleuropneumonia: the head and neck are extended as a result of dyspnoea

that virulence is multifactorial and involves several genes.

The susceptibility of individual animals varies as it has often been observed that a certain percentage of a bovine population is naturally resistant to the disease. Similarly, post-vaccinal reactions may occur in a varying percentage of a population,[52] reflecting an exacerbated susceptibility of some animals. Additional factors may play a role in the pathogenesis of the disease. It is thought that external and internal parasite infestations might have an immunomodulatory effect, leading to a lower susceptibility, or that other intercurrent diseases impair a normal immune response. It is possible that animals in better physical condition exhibit an increased susceptibility to CBPP. A better insight into the pathogenesis of the disease will be obtained once the characterization of cells involved in, and the cytokines produced during the inflammatory process have been fully elucidated.

Clinical signs

The clinical signs in cattle have been reviewed and are summarized in the following paragraphs.[18, 21, 28, 50]

Under natural conditions the incubation period is rarely less than three to six weeks and may exceed three months. Under conditions of massive aerosol infection, the incubation period may be as short as 15 to 35 days. Cattle that have been in contact with animals suffering from CBPP should, however, be considered as being infectious for at least six months after exposure.

Clinical disease may be acute, subacute, or chronic. In the initial stages of an epidemic the disease tends to be acute, but as the epidemic progresses subacute and chronic cases predominate. In endemic areas the disease tends to be mostly subclinical and chronic. The typical clinical signs of CBPP are attributable to the pulmonary lesions.

Figure 201.2 Nasal discharge in a case of contagious bovine pleuropneumonia

Acutely affected animals have an elevated body temperature, are listless, and manifest signs of respiratory distress. They often grunt as if suffering from pain and occasional soft, moist coughs are heard which are aggravated by, and become more frequent on exercise or by percussion of the chest. As the disease progresses, coughing increases both in frequency and in intensity, the animal is reluctant to move, and stands with its head extended, mouth open, tongue

Figure 201.3 Serous pleural effusion flowing from an incision into the pleural cavity

protruded, and the elbows turned out (**Figure 201.1**). Contraction of the muscle of the abdominal wall occurs after each inspiration, while expiration is frequently followed by a characteristic grunt or groan. There is a mucoid discharge from the nostrils and frothy saliva accumulates around the mouth. The locality of the affected areas in the lungs is revealed by the presence of dull sounds during percussion. During the later stages of the disease the animals may show a nasal discharge which becomes mucopurulent (**Figure 201.2**), subcutaneous oedema of the lower parts of the chest and abdomen, and emaciation. In some affected animals, the difficulty in breathing is aggravated by the presence of large volumes of exudate in the thorax.

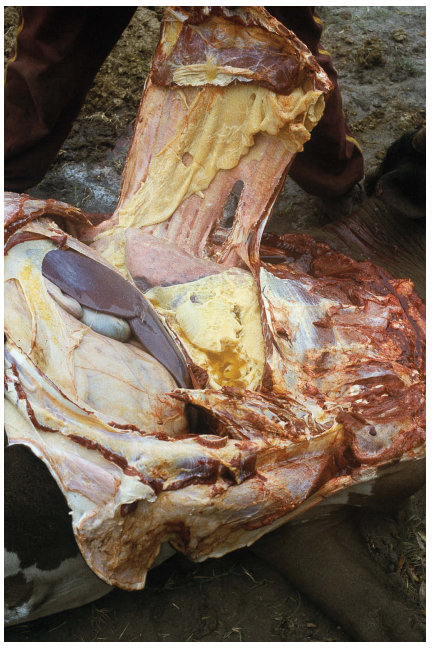
In subacute cases the lung lesions are more localized and less extensive and an infrequent cough is the only clinical sign. Usually the lesions resolve without the disease being noticed clinically.

The only clinical signs in chronic cases are emaciation and a cough, which commonly occurs when the animal rises.

In calves up to the age of six months, only signs of arthritis particularly of the carpal and tarsal joints may be present. Pulmonary lesions may or may not develop (**see Epidemiology, above**).

Once clinical signs are noticable, the course of the disease is usually two to three weeks; acutely affected animals, however, may die within one week as a result of the development of severe serofibrinous pleuropneumonia and/or a massive pulmonary infarct. In many cases the clinical signs gradually disappear, after which the general condition of affected animals improves rapidly. Severely affected animals may take many months to recover. Recovered cattle may harbour sequestra in their lungs in which the infection remains latent. Relapses may be precipitated by stress factors.

In water buffaloes, the clinical signs were absent on a a farm where five among 1 967 of them in contact with 2 539 cattle, had positive serum complement fixing titres. Only one buffalo exhibited a lesion in the lungs; this consisted of a typical sequestrum from which MmmSC was isolated.[54]



Figures 201.4 (top) and 201.5 (bottom) Extensive serofibrinous pleuritis of the parietal and visceral pleura

Pathology

At necropsy, cattle that die of chronic lungsickness are usually emaciated, with the most characteristic lesions occurring in the lungs and thoracic cavity.[11, 26, 28]

During the acute stage of the disease, there is often uni- or bilateral pleural effusion that may exceed 10 litres (**Figure 201.3**). The exudate is yellow to yellowish-grey, clear or turbid, contains pieces of fibrin, and is generally accompanied by a pronounced localized or diffuse, fibrinous parietal and visceral pleuritis (**Figures 201.4 and 201.5**). During the early stages of the disease, multiple small, well-circumscribed areas of consolidation are scattered throughout the lungs in some animals. Well-developed lesions manifested by a severe fibrinous pleuropneumonia in the lungs of acutely affected animals tend to be unilateral, involving a portion of a lobe, an entire lobe or more than one lobe (**Figure 201.6**).

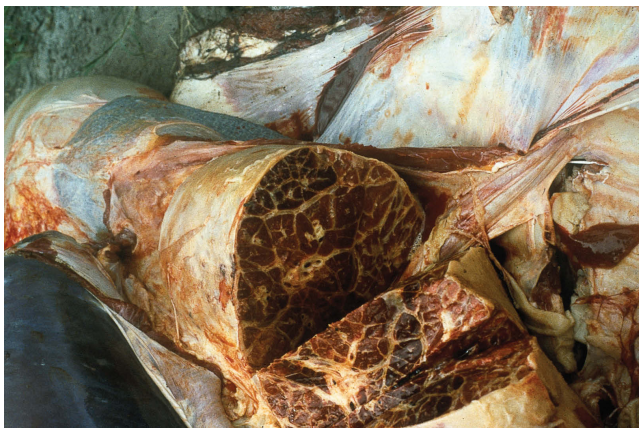


Figure 201.6 'Marbling' of the lung as a result of different stages (red and grey hepatization) of the pneumonic process and distension and 'beading' of interlobular septa

Figure 201.8 Pneumonic portion of a lung showing distension of interlobular septa

Figure 201.7 Lobules of the lung showing red and grey hepatization. These are separated from each other by distended interlobular septa

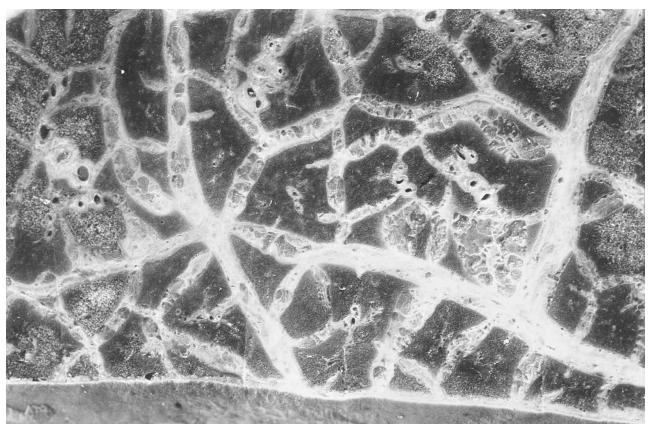


Figure 201.9 Closer view of prominent interlobular septa. Note lymphangiectasis in the septa

The pneumonic areas are characterized by 'marbling' caused by the intermingling of lobules which show varying stages of grey or red hepatization with relatively unaffected lobules (**Figures 201.6 and 201.7**). The 'marbling' is accentuated by the distension of interlobular septa and the interstitium surrounding the vessels and airways by a serofibrinous, sometimes blood-stained exudate (**Figure 201.8**). In more severely affected animals, pronounced haemorrhage may mask the septal involvement.

Copious amounts of a clear, yellowish fluid exude from the cut surface of the lung. The walls of the bronchi are thickened by oedema, and their lumens often contain fibrinous material. The interlobular septa and the parenchyma bordering areas of pneumonia may be oedematous. Multiple thrombi are found along the course of the lymphatics in the interlobular septa with subsequent irregular lymphangiectasis which often imparts a 'beaded' appearance to the septa (**Figure 201.9**). Infarcts, varying in size from about 10 to 300 mm, are frequently present in the affected lung tissue (**Figures 201.10 to 201.13**). These result from thrombosis of inter- or intralobular arteries and are manifested as well-demarcated areas of necrosis separated from the surrounding tissue by irregular, yellowish-grey reaction zones. The infarcts subsequently become sequestered from the adjacent parenchyma by granulation tissue, whereafter they are referred to as sequestra.

In chronic cases, one or more sequestra, which may vary from less than 10 to 300 mm in diameter and involve more than one lobe, are usually present in the lungs. In some animals, however, they may be very large and even involve a whole lobe. Over a period of three to four months smaller sequestra become replaced by fibrotic scars,[11] while the larger ones often persist for 12 months or longer. Recently formed sequestra are surrounded by a thin fibrous capsule, the necrotic content is firm, pinkish to yellowish-grey, and the original pulmonary architecture may still be discernible to some extent. In longer-standing cases the connective tissue thickens and a thin layer of a yellowish, semi-solid material may separate the firmer necrotic contents from the wall. In such sequestra the contents 'shell out' easily. Secondary bacterial infection of sequestra frequently occurs, resulting in a purulent lique-

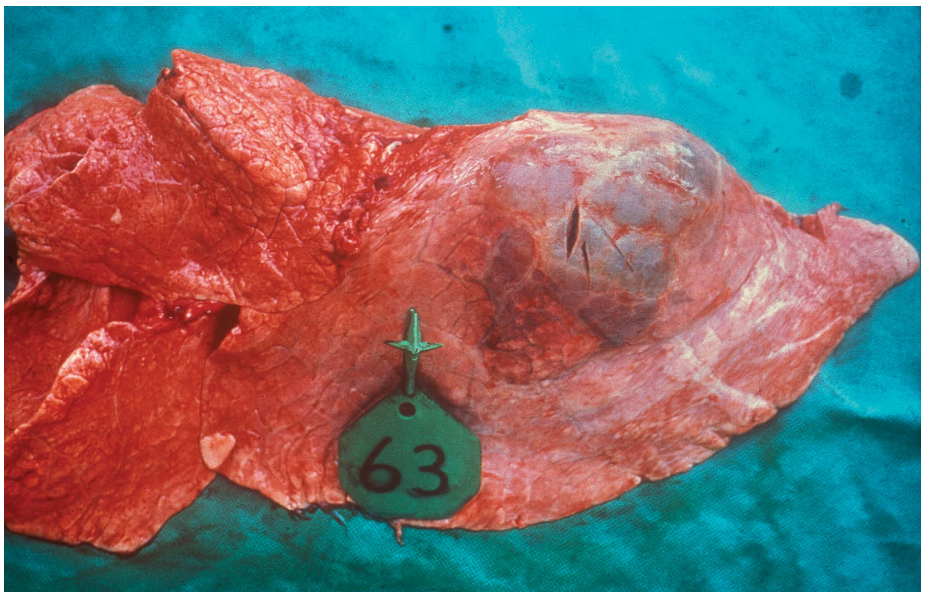


Figure 201.10 A pneumonic sequestrum protruding from the surface of a lung

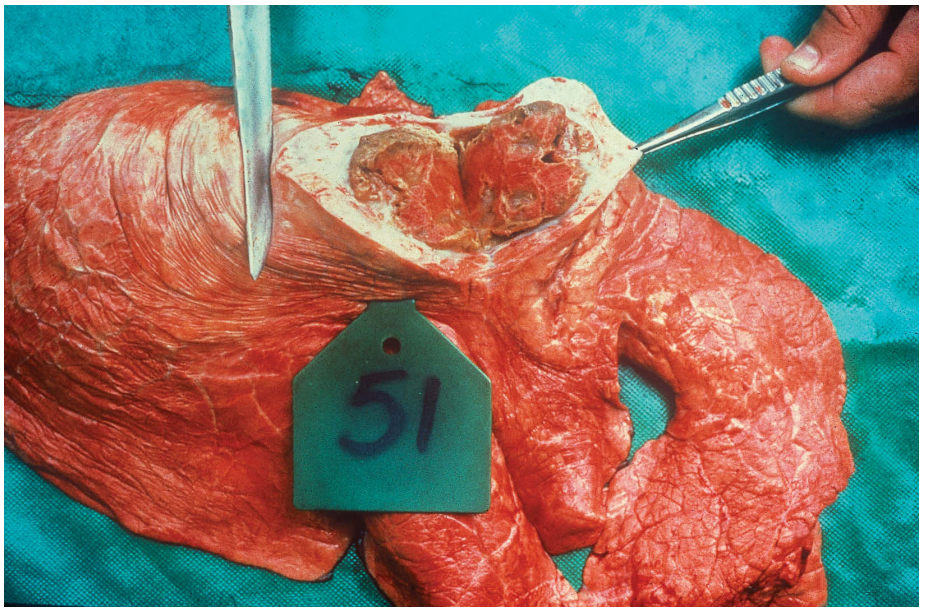


Figure 201.11 Incised encapsulated pulmonary sequestrum

fied content which, after rupture of the capsule, may escape into the bronchi.

In animals that have completely recovered, a process that may take many months, former infarcts are represented by fibrous scars, and areas of scar tissue are present in the healed lung and pleura. The latter is often thickened with fibrous adhesions remaining between the visceral and parietal pleural surfaces.

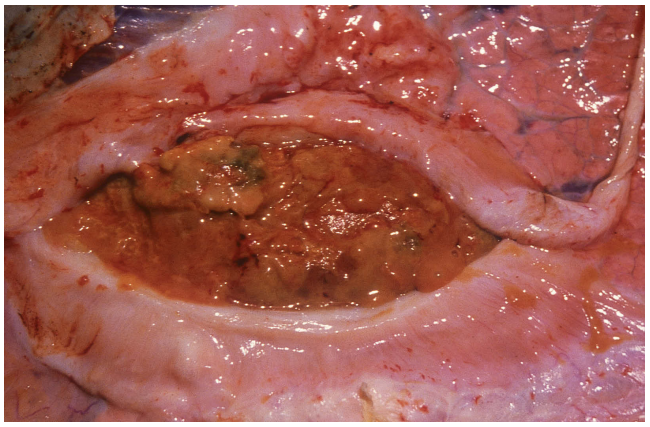
Lesions in other organs are often seen in acute cases of lungsickness. The mediastinal and bronchial lymph nodes are markedly oedematous, while necrotic foci of up to 40 mm in diameter may also be present in them.[28] In many cases, a serofibrinous pericarditis with copious amounts of exudate occurs.

Infarcts which are generally multiple, small and whitish are not uncommon in the kidneys,[11, 28, 37] where they probably result from occlusion of the renal arteries by septic emboli originating in the lungs.

A serofibrinous polyarthritis and tendosynovitis, affecting particularly the carpus and tarsus, is a common lesion in infected calves.[28, 67] The joints may remain swollen and thickened months after infection as a result of severe fibrosis of the joint capsule. Calves may also develop a vegetative, necrogranulomatous, valvular endocarditis and a myocarditis.[66] Myocardial lesions occur mainly in the atria.

'Occult' cases of CBPP, in which only the mediastinal lymph nodes are oedematous and there are no discernible lesions in the lungs, have been reported.[26, 74] The causative organism can be isolated from these lymph nodes.

Microscopically, one of the most characteristic features of the lesion in the lungs is the presence of different stages of the pneumonic process in adjacent alveoli or lobules, some



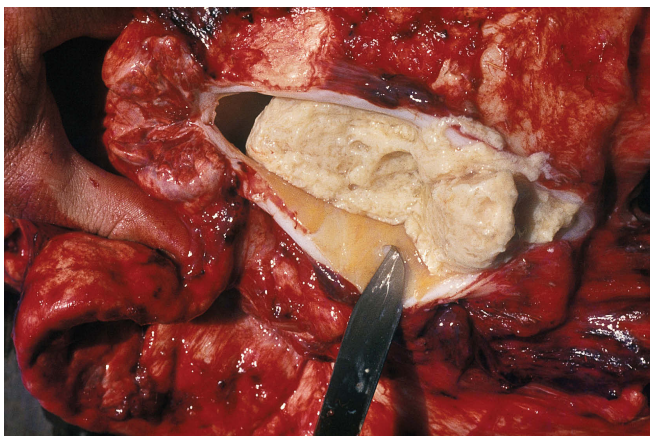


Figure 201.12 (top) and 201.13 (bottom) Well-encapsulated sequestra

being in an early stage while others are in later stages. Initially there is severe hyperaemia of the alveolar capillaries, after which the alveoli become flooded with serofibrinous exudate containing variable numbers of neutrophils, macrophages and red blood cells. As the lesions progress, the amount of fibrin increases, and in some areas numerous necrotic neutrophils fill the alveoli (**Figure 201.14**). The interlobular septa are widened by the accumulation of copious amounts of serofibrinous exudate and irregularly distended lymphatics. Necrotic neutrophils collect along the inner walls of the lymphatics and particularly along the margins of the septa where a distinct inflammatory zone is frequently discernible. A fibrinopurulent exudate similar to that in the alveoli is often found in and around bronchi and bronchioles of affected lobules. The bronchial mucosa and submucosa are oedematous and infiltrated by neutrophils and macrophages. The peribronchial and perivascular areas, and especially the lymphatics, are widely distended by an exudate of the same nature as that in the septa. As the lesions progress, these areas and the septa become thickened by fibrous connective tissue and by infiltrations of lymphocytes and plasma cells. In some parts of the lung the alveolar walls contain varying numbers of round cells and evidence of fibroplasia.

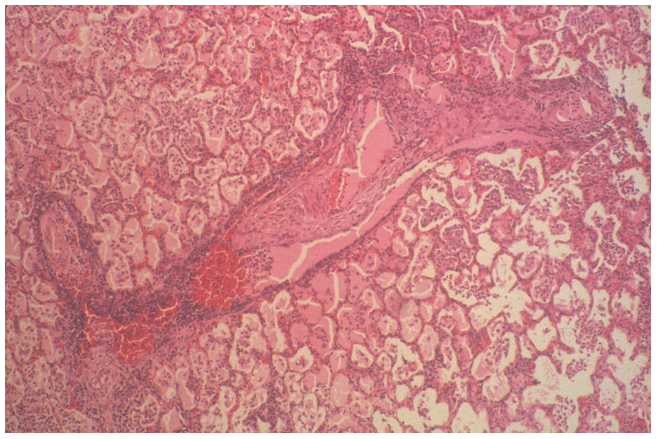


Figure 201.14 Serofibrinous pneumonia typical of contagious bovine pleuropneumonia

Vasculitis and thrombosis of intralobular and interlobular arteries and the lymphatics are frequently present in pneumonic parts (**Figure 201.15**). Involvement of the arteries culminates in necrosis of a lobule or part of a lobule, or in infarction of multiple lobules and intervening septa.[11] Initially a zone of necrotic inflammatory cells separates the infarcted areas from the surrounding tissue, but later granulation tissue followed by fibrosis at the edges of these lesions sequestrate the necrotic tissue from the neighbouring parenchyma. Follicle-like aggregates of lymphocytes may be evident in the fibrous capsule of some of the sequestra, as well as in the peribronchial and perivascular areas.

Microscopically, the lesions in lymph nodes are characterized by the hyperplasia of lymphoid tissue in the follicles and medullary cords; accumulation of oedematous fluid, fibrin and neutrophils in the subcapsular, cortical and medullary sinuses and trabeculae; vasculitis and thrombosis of blood vessels and lymphatics in the trabeculae; and necrosis of the lymphoid tissue.[11, 28]

Joint lesions in acute cases consist of hyperaemia, oedema and infiltration of predominantly lymphocytes and macrophages into the synovial membranes. Thrombosis of lymphatics and blood vessels, and fibrin deposition on the synovial surfaces also occur. In chronic cases the joint capsule is thickened by connective tissue and hypertrophy of the villi of the synovium in some areas.[67]

Diagnosis

Diagnosis based only on clinical signs is difficult, but necropsy findings do allow a presumptive diagnosis when lesions typical of the acute disease or sequestra are observed in the lungs. Diagnosis is much more difficult when only subacute cases are encountered. The introduction of, or contact with, new animals is always an important consideration which must be thoroughly investigated. Laboratory confirmation is essential.

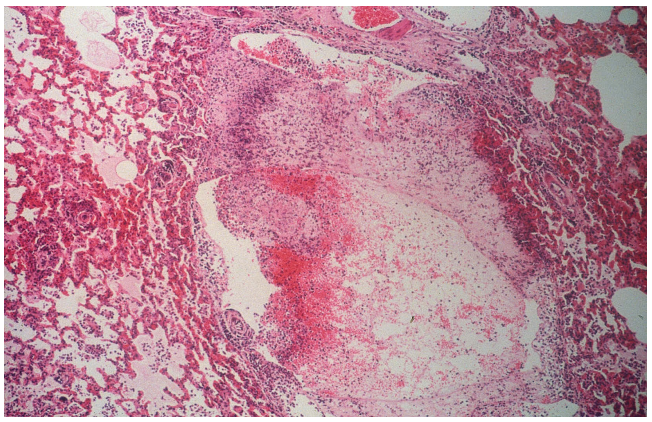


Figure 201.15 Vasculitis and thrombosis in a pneumonic part of the lung

Long considered difficult, the isolation and identification of MmmSC is now much easier.[45] Of primary importance is the quality of the sample to be submitted to the laboratory. When present, the pleural fluid is the sample of choice. One millilitre is adequate but it should be harvested aseptically. Ampicillin can be added to it to prevent the multiplication of contaminants. Another method of sampling pleural fluid is to allow a volume of it to be absorbed in sterile filter paper strips which are then allowed to dry before dispatch. Blocks of pneumonic lung tissue (100 mm^3 in size) can also be taken for laboratory investigation as well as small sequestra. From all cases, mediastinal and bronchial lymph nodes should be collected. These samples can be sent without refrigeration if transport is rapid, but the addition of ampicillin to a pleural fluid specimen and the maintenance at cold temperature of all specimens is recommended if transport to the laboratory takes several days. In addition, pieces of affected tissues should also be collected in 10 per cent buffered formalin for histopathological and/or immunohistological examination.

Fresh pleural fluid from an animal that did not receive any antibiotic treatment should yield a culture of MmmSC within two to three days. The organism is usually isolated in pure culture but classical mycoplasmal identification procedures require cloning steps to ensure its purity before identification. Identification is based on certain biochemical tests such as glucose fermentation (+), arginin hydrolysis (–), reduction of tetrazolium salts (+) and absence of a phosphatase activity. In addition, the growth inhibition test using hyperimmune serum to MmmSC should be positive. These tests, however, are not absolutely specific and do not allow a clear differentiation with related strains of the 'mycoides cluster', such as MmmLC. In an endemic area, this lack of specificity does not constitute a problem as MmmLC is rarely isolated from diseased bovine lungs. In a disease-free area the specific identification of MmmSC is of utmost importance. This specific identification can be achieved by use of immunobinding assays,[32] growth inhibition tests[53] using specific monoclonal antibodies, or by PCR. Various specific PCR systems have been developed.[7, 10, 42, 47] The first two that were established in 1994[7, 20] have been extensively used and evaluated. More recent tests have been described, but the multiplicity of PCR systems, which are difficult to standardize, may impede the diagnosis.

The direct detection of MmmSC can also be achieved without the need of cultivating the agent. Galactan, a major antigenic component of MmmSC, is elaborated by the organisms and can be detected in body fluids during and after the acute stage of the disease. The agar-gel precipitation test based on the Ouchterlony double immunodiffusion test is the test of choice for the detection of galactan. For this purpose, hyperimmune serum, preferably produced in sheep, is used.[25] The direct detection of MmmSC can now be obtained by immunodetection or by PCR directly from pleural fluids.[8] A preliminary centrifugation at low speed (500 xg) for 15 minutes allows inflammatory cells to be eliminated and a subsequent centrifugation at high speed, 12 000 xg for 20 minutes, allows the mycoplasmas to be harvested. Immunobinding or PCR tests can then be applied. Detection of MmmSC can also be achieved in formalin-fixed tissues by immunohistochemistry.[9, 55] Usually hyperimmune sera against MmmSC are used which enable the detection of mycoplasmas in affected tissues. Specific monoclonal antibodies can also be used but these reagents might not enable mycoplasmas engulfed in macrophages to be detected if their target is a surface-exposed membrane antigen.

The official serological test of the Office International des Epizooties (OIE) is the complement fixation test (CFT) based on the technique of Campbell and Turner[12] and adapted to microplate format. It has an excellent correlation between positive test results and active infection. Titres following an acute infection are usually very high. Antibodies can be detected rapidly, within eight days, but their persistence might be short, five to six months on average, and animals in which sequestra have developed might not be detected. One of the advantages of the CFT is that it does not detect antibodies that have developed as a result of vaccination with a live vaccine if blood samples are taken longer than three months after the injection. Therefore, the CFT can be used to monitor the prevalence of CBPP in vaccinated areas. The test has, however, several drawbacks. It is quite difficult to standardize between laboratories, especially if antigens from various sources are used, and its specificity is not absolute. The latter is important in disease-free areas[61] and for the calculation of the prevalence of CBPP in an area.

An additional serological test based on a competition enzyme-linked immunosorbent assay (cELISA) with a specific monoclonal antibody was developed in 1998.[32] It has a better specificity than the CFT but its sensitivity is comparable to that of the CFT. Its advantage is that a number of controls are included in each plate which allow the monitoring of internal quality controls over time. In June 2000, it was recognized by the OIE as an alternative test to the CFT. As is the case with the CFT, the cELISA does not detect antibodies in vaccinated animals, thus allowing prevalence studies in

vaccinated areas to be undertaken, and it also does not detect all infected animals.

A rapid, cow-side, diagnostic test was developed as long ago as 1954.[46] It is based on an inactivated, stained MmmSC antigen that can be used in an agglutination test. This test might be less sensitive and specific than the CFT and the cELISA but it has proved very useful for the rapid confirmation of acute cases in remote areas. This test detects mainly IgMs and therefore will detect antibodies formed quite soon after the infection.

All serological tests mentioned above must be used at the herd level as they fail to detect animals in the incubation period or some animals in the chronic stage in which sequestra are present. Consequently prevalence studies should be based on the herds which should be considered as the basic epidemiological unit. Those concerned with sampling frames for CBPP prevalence or incidence studies should take these facts into consideration.

A number of other tests have been developed. The passive haemagglutination test[14] has never been widely used because of its lack of specificity. Western blotting has been used in Portugal as a confirmatory test to the CFT in order to identify CBPP-infected herds as these by law have to be slaughtered. New ELISAs using recombinant antigens,[1] as well as new rapid diagnostic tests using polysaccharide-sensitized latex beads, are now being developed and evaluated. There is no doubt that additional tests which measure cellular immunity will be used in the near future in order to investigate whether animals are infected or if they are protected by vaccination.

Differential diagnosis

Acute CBPP must be differentiated from pneumonic pasteurellosis, in which *Mannheimia* (*Pasteurella*) *haemolytica* and (less commonly) *Pasteurella multocida* play major causative roles, and haemorrhagic septicaemia caused by *Pasteurella multocida* types B and E. Haemorrhagic septicaemia is often characterized by swellings of the laryngeal region and brisket, and has an acute to hyperacute course. In acute pneumonic pasteurellosis a serofibrinous pleural exudate and fibrinous pleuropneumonia are present. Smears from the affected lung or, in some cases, blood smears stained with Giemsa, Leishman or methylene blue stains will show the typical bipolar organisms and pure cultures of the organism involved, and can be isolated in most cases. Haemorrhagic septicaemia caused by *P. multocida* type E has been the cause of death of many cattle in the Caprivi district of Namibia. The acute serofibrinous pleuropneumonia that may result from aspirating foreign material into the lungs, often simulates CBPP very closely.

The sequestra in chronic cases of CBPP should not be confused with lung abscesses caused by pyogenic bacteria. Cultural and histopathological examinations will assist in distinguishing these lesions from a sequestra. The clinical signs of CBPP should be differentiated from those of East Coast fever[28] and Corridor disease, in which there is a marked pulmonary oedema. Bovine herpesvirus 1 infection (infectious bovine rhinotracheitis) generally causes lesions in the upper respiratory tract, but in some animals a bronchopneumonia may develop which is characterized by a necrotizing bronchitis and atelectasis and is often complicated by secondary bacterial infections such as pasteurellosis.

Control

The methods employed for the control of CBPP are determined by the specific epidemiological situation, animal husbandry methods applied, and the availability and effectivity of veterinary services in a specific country. Control methods used in southern Africa include stamping-out by slaughter when CBPP spreads into areas previously free of it, vaccination and movement restrictions in endemic areas, and quarantine and serological testing in specially designated disease control areas. Additional measures are enforced in non-infected countries in southern Africa in order to prevent the introduction and possible spread of the disease. The measures include the declaration of CBPP as a controlled disease, prohibition of the importation of cattle from infected countries, and quarantine and other control measures at frontiers.

Antibiotic treatment is not recommended because of the possible induction of chronic carriers and the emergence of resistant MmmSC strains. In addition, it may suppress the development of clinical signs and this may delay the recognition of the disease. Its effectiveness has not been adequately studied and will depend on many factors such as the type of antibiotic used, quality of the product, and proper administration of the correct dose and number of injections. Mycoplasmas are resistant to penicillins and rapidly become resistant to aminosides. These antibiotics should therefore not be used for the treatment of CBPP. Chloramphenicol should not be used in animals as it is restricted for use in humans. Antibiotics of the tetracycline family are regularly used for the treatment of CBPP in Africa, but others that are used are the macrolides, lincosamines and fluoroquinones which are active against MmmSC.[5, 10] These substances are efficient in alleviating the clinical signs in clinically affected animals, but there is some doubt as to their ability to reach organisms in all the affected organs and/or lesions in an animal suffering from CBPP, especially those in well-formed sequestra. Guidelines for the antibiotic treatment of cases of CBPP are therefore required before it can be recommended as a control measure in combination with the other procedures, such as vaccination and slaughter. Until these are introduced it is likely that antibiotic therapy will continue to be used to treat animals with respiratory signs; a ban on the use of such therapy is unlikely to be respected in many locations. Antibiotic therapy should, however, be used in the case of post-vaccinal reactions to

control the invading oedema at the site of the inoculation that develop in a small number of cases and which could jeopardize the faith of cattle owners in the efficacy of vaccination.

Vaccination is very important in the control of lungsickness in endemic areas, especially in countries where animal movement control is impractical. Attenuated vaccines against CBPP have been developed by *in vitro* multiplication or multiplication of the organism in heterologous hosts, such as embryonated hens' eggs.[58]

The efficacy of live vaccines is directly related to the virulence of the original strain of MmmSC used for their production. Attenuated virulent strains stimulate the best immunity,[36] but they also induce the most severe and undesirable local and systemic reactions which may even result in the death of the animal.[52] In the live attenuated vaccines currently in use a compromise between virulence, immunogenicity and safety has been obtained.[6] Until the 1950s it was commonly thought that, if vaccination were to be effective, a local lesion had to be produced at the site of inoculation. This situation was changed only 40 years ago at the insistence of cattle owners fearing post-vaccination reactions and mortality in their cattle, with the result that present vaccines cause only mild lesions, if any, at the injection site. Among the many vaccinal strains that were developed, only two are now used in Africa, the T1/44 and its streptomycin-resistant derivate, T1sr.[49] Vaccination failures occurring in East and southern Africa in 1994 had cast some doubts on the efficacy or the identity of the T1sr strain although it had been identified as the best choice by a panel of experts in 1990. The precise protection rate afforded by a vaccinal strain is very difficult to establish experimentally for several reasons: as experimental reproduction of CBPP is difficult in cattle and infection by intratracheal intubation may result in only few animals being infected, transmission experiments must be performed by contact between infected and uninfected animals. Therefore, large numbers of cattle must be used and the reproducibility of the rate of infection from one experiment to another is difficult to achieve. It is even more difficult to evaluate the protection afforded by a vaccine by observations in the field as a number of events might interfere with the efficacy of the vaccine, such as improper storage conditions of the freeze-dried product, use of an unsuitable diluent, and a reconstituted vaccine not being injected within a reasonable time. The question of identity of the T1sr strain has been resolved by the use of molecular epidemiology tools which allow its characterization and particularly its distinction from the less protective KH3J strain.[33, 63] In 1998 and 1999 the two strains, T1/44 and T1sr, were thoroughly re-evaluated.[65, 74] The experimental protocols used included the vaccination of naive cattle at the minimum dose recommended by the OIE, i.e. 10^7 live mycoplasmas per dose, and conducting the experiment in three different locations in Africa. The animals were challenged with virulent organisms three months after the vaccination. Results confirmed and re-established the existing knowledge on vaccine efficacy: a primary vaccination induces only a protection rate of 40 to 60 per cent, whatever the vaccine used. The duration of immunity is longer with the T1/44 strain than with the T1sr strain and revaccination after one year enhances the level of protection to 80 to 95 per cent whatever the strain used. It has therefore been re-established that a satisfactory protection by vaccination can be achieved only by repeated vaccinations, and those concerned with control strategies based on vaccination have to take this into account. The protection rate afforded by the vaccines was the same in all the locations, which is an indication that the genetic variations observed and used for molecular epidemiology are not linked to an ability of the pathogenic strains to evade the immune reaction following vaccination by T1. The recent findings that mycoplasmas are prone to frequent genetic variations and the elucidation of some of the underlying genetic mechanisms[15] call for the need for great care to be taken when producing batches of vaccine. Particular attention should be given to avoid cloning procedures that could be responsible for antigenic drifts in the final product.

The vaccinal strain T1sr is completely avirulent, but the T1/44 strain may induce a post-vaccinal reaction at the injection site in some animals. The number of reactors is unpredictable and the introduction of T1/44 in vaccination strategies has to be 'played by ear', which means that vaccinating teams must be prepared to treat the reactors with antibiotics, if required, two to three weeks after the vaccination. The number of reactors tends to be reduced when re-vaccinations are performed. Whatever the strain used, the seroconversion rate measured by CFT or cELISA is very variable, and there is no strict correlation between the test results and the protection obtained. Animals that do seroconvert should be protected from acquiring the disease but those that do not might also be protected. There is an urgent need for a test that allows the level of protection to be measured as it would permit the efficacy of the vaccination campaigns to be controlled. Such a tool, which was an important component for the control of rinderpest, is, however, not available for CBPP. The basis of protection for CBPP in cattle is not well understood, neither the nature of the MmmSC antigens that elicit protection nor the type of bovine immune response that leads to protection.[27] Recent work has established that CD4 T cells secreting gamma interferon may play an important role in the latter respect. The type of protective immune response has to be further characterized and the nature of the protective antigens determined before any new and more potent vaccine is developed.

Control strategies must therefore be defined with the currently available tools. The success of these control strategies is less dependent on technical issues than on the determination of veterinary services to implement them and on their ability to put them into practice in the field. This requires the strong support of the governments and an active participation of the cattle owners. Eradication

strategies also require the full co-ordination of neighbouring states as CBPP is a transboundary disease. A control strategy can be based on a slaughter-out policy, as was successfully applied in Botswana from 1995 to 1997. This requires not only a considerable investment for compensating the owners of slaughtered animals and for restocking purposes, but it also requires a strict control of animal movements to prevent reintroduction of the disease. Annual blanket vaccinations certainly produce a dramatic reduction of CBPP outbreaks, but they may fail to eradicate the disease if there is no way to control the reintroduction of infected cattle from neighbouring regions or countries. This was the situation prevailing in northern Namibia in 2001, and combined efforts of vaccination in that country and southern Angola are the only way to solve the problem. In Australia, blanket vaccinations were used to reduce the CBPP prevalence before regular serological testing and slaughtering procedures were put into practice; these eventually led to the eradication of CBPP in 1973.[44] Treatment with antibiotics might also be seen as an additional tool for the control of CBPP, but there is a lack of information on its long-term impact. Therefore, guidelines for such therapy cannot, at present, be formulated. Cost-effective control strategies should include the combined use of the different measures that are available, the choice of which will depend on local situations.

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