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Bovine brucellosis

Synonyms: Contagious abortion; besmetlike misgeboorte (Afrik.)

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Introduction

Bovine brucellosis is a highly contagious disease caused by *Brucella abortus*, a bacterium which occurs intracellularly in its mammalian host. Apart from causing characteristic mid- to late-term abortion and infertility in cows, *B. abortus* also occasionally causes orchitis and inflammation of the accessory sex glands in bulls. Other livestock and wild animal species, though of varying susceptibility, are sometimes infected.[69] Bovine brucellosis is also an important zoonosis.[6] In some countries, particularly in southern Europe and western Asia, where cattle are kept in close association with sheep or goats, infection and abortion can also be caused by *Brucella melitensis*.[152] Occasionally, *Brucella suis* may cause an infection in cattle but has not been reported to cause abortion.[62]

By visiting the OIE (Office International des Epizooties) website[122] information on the worldwide brucellosis situation, as well as those animal diseases that have been included in the two official OIE lists of diseases due to their implications for international trade or public health, can be obtained. This information is regularly updated and is based on the emergency of the situation and on monthly and annual reports sent to the Central Bureau of the OIE by national veterinary administrations and other official sources.

In sub-Saharan Africa, brucellosis is an important disease in both humans and livestock. In general, the assessment of the relative occurrence of brucellosis is restricted to few published studies based on serological surveys and it is considered to be the highest in pastoral production systems in arid and semi-arid areas.[104] The surveillance and control of brucellosis in sub-Saharan Africa is rarely implemented outside southern Africa. The rate of infection in humans is virtually unknown and public awareness is extremely low. Hence, the impact of brucellosis in terms of public health and social importance is rarely correctly addressed.[104]

It is suspected that bovine brucellosis was introduced into southern Africa with cattle imported from Europe,[77] but there is also a possibility that it was introduced into the subcontinent much earlier during the migration of people and their cattle herds from other African countries.[101] The first reliable record of its existence in South Africa was that of Gray in 1906 when he reported a serious outbreak of abortion among cattle near Johannesburg.[77] Its presence was finally confirmed by Hall in 1913 when he isolated *B. abortus* from the stomach of an aborted bovine foetus.[77] Outbreaks of abortion thought to be bovine brucellosis were first observed in Zimbabwe in 1906, and the presence of brucellosis was confirmed in that country in 1914. According to an obituary notice in *The Veterinary Record* of 1957, Bevan, in Zimbabwe, was the first to show that *B. abortus*-infected cattle could transmit the pathogen to humans and that goats were not the source of the infection.[5]

The disease has a relatively high prevalence in southern Africa, especially in intensively farmed areas, and it is the most important bacterial cause of abortion on the subcontinent. It has an important economic impact on the beef and dairy cattle industries, especially as in 1990, 14,7 per cent of the herds in South Africa were known to be infected and the losses to cattle farmers exceeded R300 million per annum.[10]

Aetiology

Brucella abortus is a small, Gram-negative, non-sporulating, non-encapsulated coccus, coccobacillus or short rod, 0,6 to 1,5 µm in length and 0,5 to 0,7 µm in width.[4, 42] The organism is not acid-fast but does resist decolorization by weak acids and thus stains red with Stamp's modification of the Ziehl-Neelsen stain.[4, 142]

Most wild strains are fastidious and slow-growing, and require carbon dioxide (5 to 10 per cent) supplementation for primary isolation at an optimal growth temperature of 36 to 38 °C. *Brucella abortus* strain 19 is an attenuated strain of

* Deceased

reduced virulence which is used for the production of a live vaccine (**see Control, below**). It grows well in a normal atmosphere at 37 °C.[4] Complex media, containing serum, are required for the growth of *B. abortus* and, although most strains grow on sheep blood agar, the colonies may not be as distinctive as when grown on serum dextrose agar. Growth, on primary isolation, is seldom clearly visible before 48 hours of incubation at which stage the colonies are usually 0,5 to 1,0 mm in diameter. The use of selective media, such as Farrell's medium, may substantially enhance the chances of isolation by inhibiting the growth of contaminants.[63] The growth rate of *B. abortus* may, however, be markedly retarded by selective media and for this reason such cultures should be incubated for five days or longer.[4] Smooth colonies on a clear growth medium, such as serum-dextrose agar, are convex, entire-edged, have a smooth, shiny surface, and are pale yellowish-brown when viewed under transmitted light.[4, 142] Considerable variation in colour and surface texture are found in the rough strains. Smooth forms are often markedly pathogenic whereas the rough variants are usually less so.

There is no single test by which *B. abortus* may be identified with absolute certainty, but a combination of growth characteristics, colonial and cellular morphology, staining properties, agglutinating antisera and biochemical reactions will allow an accurate identification.[4] Eight biovars (biotypes) are recognized (biovars 1, 2, 3, 4, 5, 6, 7 and 9) which may be differentiated by phage typing, mono-specific antisera, biochemical reactions and growth inhibition tests.[4] There are no proven differences in the pathogenicity of field strain biovars.[113] Biovar 7 is reported to be a mixed culture and, because no authentic isolate of it has been made for many years, it is expected to be deleted from the list[4] (**see the introduction to *Brucella* spp. infections**). Ninety per cent of the isolates typed in South Africa are biovar 1 and 10 per cent biovar 2.[21] In Zimbabwe, *B. abortus* biovar 1 has been isolated.[108]

Epidemiology

No accurate figures are available on the prevalence of brucellosis in cattle in southern Africa, as most reports are based on non-representative laboratory results.[104]

In South Africa, since 1968, when compulsory calfhood vaccination was introduced, the control scheme has made a considerable impact on the prevalence of the disease. The prevalence has decreased on a national scale from 10,5 per cent in 1976 to 6 per cent in 1979,[24] 1,9 per cent in 1984/5,[64] and 1,4 per cent in 1988/9.[8] The variation in prevalence is demonstrated by a survey of 90 per cent of the dairy and beef herds in the Eastern Cape Province and Karoo between 1985 and 1989, which revealed a prevalence of less than 0,3 per cent.[86] The prevalence of brucellosis in about 5 000 adult beef cows slaughtered at a large KwaZulu-Natal abattoir in 1981/2 was estimated to be less than 1,5 per cent.[20] Although these cows were drawn from all the provinces of South Africa, they originated mostly from KwaZulu-Natal.[7] In 2001, South Africa reported 339 bovine brucellosis outbreaks to the OIE and the destruction of 5 320 animals, whereas in 2000, 323 bovine brucellosis outbreaks were reported and 1443 infected animals were slaughtered.[122]

A prevalence of 4,5 per cent in dairy cattle and an overall prevalence of 1,4 per cent in cattle herds tested in Zimbabwe have been reported, even though generally, intensive management systems employed in Zimbabwe seem to favour the spread of bovine brucellosis.[102] Cattle in the communal areas of Manicaland Province were free of brucellosis before 1985 while the prevalence was high in intensively managed herds in the Midlands and Mashonaland. However, with the establishment of dairy cooperatives in resettlement areas in Zimbabwe in 1980, cattle of unknown disease status were mixed and often grazed together on communal pastures. As a result of this practice the prevalence of brucellosis in one settlement scheme in Manicaland Province rose from 0,7 per cent in 1986 to 3,3 per cent in 1988. In Zimbabwe the prevalence of bovine brucellosis in the communal areas also varies from province to province. The prevalence is lowest in the Mashonaland provinces and highest in those in Manicaland and Matebeleland. The patchy distribution of brucellosis in the provinces (some herds are free of the disease, while others have a high prevalence) may be explained by the fact that some communal herds have been kept closed whereas other cattle owners have purchased infected animals to upgrade their stock.[102] Zimbabwe reported 13 outbreaks involving 42 animals in 2002. Vaccination is reported to be implemented.[122]

The disease is reported to be widespread in Zambia[52] and Malawi,[149] but its prevalence in those countries is unknown. In 2000, Malawi did not provide any information to the OIE, whereas Zambia reported a small number of outbreaks (ten outbreaks involving 34 animals) as well as the vaccination of 617 animals.[122] The estimated prevalence of brucellosis in the commercial farming areas of Namibia is 0,5 per cent.[7] No information on the number of brucellosis cases in Namibia and Mozambique in 2001 is available. However, vaccination of 96 389 animals in Namibia and 416 in Mozambique has been reported. In 2001, 12 outbreaks involving 17 animals were reported in Botswana and 42 675 animals were vaccinated.[122]

Cattle usually become infected after ingesting contaminated feed or water or licking an infected placenta, calf or foetus, or the genitalia of an infected cow soon after it has aborted or calved, at which time very large numbers of *B. abortus* are present, particularly in the placenta lochia.[1, 113] Animals may also become infected by inhaling organisms or through the conjunctiva.[113] Calves may acquire infections *in utero* or they may become infected after ingesting infected colostrum or milk. Although some will rid themselves of the infection within a few months, others may remain infected for life[113] and may spread the disease at their first and subsequent parturitions.[6]

Although infected animals usually abort only once, subsequent calves are carried to full-term although they may be infected. Approximately 2,5 to 9 per cent of heifers born of seropositive cows may be latently infected but serologically negative until the middle of their first gestation or even later, when, for the first time, antibodies to *B. abortus* may be detectable or abortion may occur.[6, 33, 57, 93, 94, 113, 154]

There appear to have been no controlled studies showing that bulls are more resistant to *B. abortus* than heifers and cows. Bulls may become infected *in utero* or during early calfhood by the oral route and retain the infection into adult life.[133] In bulls, the testes and accessory sex glands may be affected and reveal inflammatory changes. Infected bulls may shed brucellae in their semen, seminal fluid and urine, and therefore in infected herds they should always be viewed with suspicion,[129] particularly if artificial insemination using their semen is contemplated.[103] The risk of introducing the disease into a herd through embryo transplantation is probably not significant.[30, 143]

Brucella abortus is sensitive to pasteurization temperatures and its survival outside the host is largely dependent on environmental conditions. It may survive in an aborted foetus in the shade for up to eight months, for two to three months in wet soil, one to two months in dry soil, three to four months in faeces, and for eight months in liquid manure stored in tanks.[6, 113] Generally, removal of infected animals from contaminated premises for one month is sufficient to prevent infection, provided the facilities have been properly disinfected.[6] Large numbers of organisms are shed from the reproductive tract when infected cows abort. In those cows that lactate following abortion, milk, including colostrum, is an important source of infection, and bacteria may be excreted intermittently in milk throughout the lactation period. Urine and faeces of infected cattle are less important sources of the bacterium. The fluid in hygromas caused by *B. abortus* infection, may contain large numbers of organisms but, because they are restricted to the lesion, they do not seem to be important in the spread of the disease.[6, 58] There is a reduction in the numbers of organisms shed in the months following calving and abortion, and cows usually eventually become non-infective until the next pregnancy when there is again a rapid increase of brucellae organisms in the reproductive tract.[147] During subsequent pregnancies there is invasion of the gravid uterus and allantochorion,[51] but abortion rarely recurs. Ninety per cent of infected cows remain chronically infected; the infection may persist for life during which the infection is confined to the udder and lymph nodes.[113]

A contaminated environment or equipment used for milking or artificial insemination are further sources of infection. Permanent calving camps and lush pastures, particularly if they are wet and muddy, may play a very important role in the spread of the disease.[49, 113]

Although *B. abortus* has been isolated from ixodid ticks and their eggs in Brazil, ticks probably do not play an important role in the transmission of the disease.[72] The transmission of brucellosis by ticks, fleas or mosquitoes from an infected herd to a non-infected herd has never been proved. *Brucella abortus* infection in sheep and goats may occasionally cause them to abort, but the infection does not spread in these species and they are apparently not a real danger to cattle unless there is close association between the species.[98] Horses become infected particularly by ingestion of *B. abortus*-contaminated feed. In this species the organisms localize in bursae, tendons and joints and they are thus an unlikely source of infection for cattle.

Several species of wildlife — African (Cape) buffalo (*Syncerus caffer*), hippopotamus (*Hippopotamus amphibius*), zebra (*Equus burchelli*), eland (*Taurotragus oryx*) and impala (*Aepyceros melampus*) — have tested serologically positive for brucellosis (**see Chapter 148: Brucellosis in wildlife**), but these species are probably not of great importance in the epidemiology of bovine brucellosis in southern Africa. This is possibly because of the relatively infrequent contact between cattle and wildlife.[39, 40, 56, 88] There are few records of abortions in wildlife in southern Africa due to brucellae, although *B. abortus* biovar 1 has been isolated from the cotyledons of pregnant African buffalo at slaughter.[74] Although serological surveys have revealed up to 23 per cent positive reactors in African buffalo in the Kruger National Park in South Africa,[9, 79] these animals probably do not constitute a significant source of infection for cattle because of the strict control measures to prevent the spread of foot-and-mouth disease across the boundaries of the park and from adjoining private nature reserves.[19] In the USA *B. abortus* has been isolated from bison *(Bison bison)* and elk or wapitic *(Cervus elaphus)* and in Italy from chamois *(Rupicarpra rupicarpra)* (**see Chapter 148: Brucellosis in wildlife**).

The typing of *B. abortus* isolates may yield important epidemiological information, as it allows sources of infection to be traced, particularly in countries where a number of biovars are present.[47]

In humans brucellosis or undulant fever caused, among other *Brucella* spp., by *B. abortus* is chiefly an occupational disease, occurring most often in veterinarians, stock inspectors, abattoir workers, laboratory personnel and farmers who become infected by contamination of abraded or intact skin or mucous membranes, or by inhalation during contact with infected cattle, foetuses or foetal membranes, and calves.[6, 163] Humans may also become infected after ingesting unpasteurized dairy products containing field strains of *B. abortus* or after inadvertent self-inoculation with *B.abortus* strain 19 vaccine.[6] After an incubation period of 5 to 30 days or longer, a mild, self-limiting or severe, prolonged disease may follow. Patients typically experience a fever that may be intermittent or irregular, hence the name undulant fever. Other common symptoms include chills, depression, weakness, headache, joint pain, generalized aches and sweating.[163]

Papular to pustular skin rashes which are sometimes evident on the arms of veterinarians following obstetric procedures have been attributed to allergy to brucellae, but sensitivity to other pathogens including *Salmonella* Dublin, *Salmonella* Typhimurium and *Listeria monocytogenes*, have also been incriminated.[6]

Pathogenesis

The establishment of infection is influenced by the size of the infective dose, virulence of the bacteria, and the resistance, age, sex and reproductive status of the animal.[50, 113, 147]

Brucella abortus readily penetrates mucous membranes, such as those of the pharynx and alimentary tract, and survives and multiplies particularly in cells of the reticuloendothelial system.[61, 147] After penetration, the organisms are phagocytosed by neutrophils and macrophages which carry them to the regional lymph nodes where they multiply and induce a lymphadenitis which may persist for months. Multiplication of the organism here may be followed by a bacteraemia which may persist for several months, resolve itself, or be recurrent for at least two years in 5 to 10 per cent of animals. Recurrence occurs particularly during pregnancy. During the bacteraemic phase, organisms are carried intracellularly in neutrophils and macrophages, or free in the plasma and localize in various organs, especially the gravid uterus, udder and supramammary lymph nodes. Localization may also occur in other lymph nodes and the spleen, and in bulls in the testes, and male accessory sex glands.[25, 61, 113] Occasionally bacterial localization occurs in synovial structures causing a purulent tendovaginitis, arthritis or bursitis.[58, 65, 87]

Localization of the infection in the endometrium of the gravid uterus and in foetal membranes of cattle appears to be the result of the special affinity of the organism for erythritol,[51, 89, 147, 155] elevated levels of which occur in the placenta and foetal fluids from about the fifth month of gestation. The chorionic epithelium becomes parasitized and infection extends to the placental stroma, blood vessels and ultimately, to the foetus.[61, 147] There is considerable variation in the uterine and placental lesions in both natural and experimental *B. abortus* infections and foetuses that become infected late in gestation may be aborted without any grossly recognizable placental lesions. Depending on the severity of the placentitis, abortion, premature birth or the birth of a viable or non-viable calf may result.[61, 87, 113]

The abundance of erythritol in the pregnant uterus results in the massive multiplication of *Brucella* organisms in this organ. The growth of most strain 19 *B. abortus* organisms, however, is generally inhibited by the presence of erythritol,[60] but tolerance to erythritol by some strain 19 variants may be the cause of occasional persistent infections and abortions.[45]

In the pregnant animal brucellae replicate in the placental trophoblast during middle and late gestation after the cells have actively begun secreting steroids. The mechanism leading to abortion after midgestation in brucellosis is not known. Infected trophoblasts produce cortisol, a steroid hormone not produced in the non-infected placenta. This production, coupled with increased levels of oestrogen and prostaglandin synthesis and decreased production of progesterone, mimics the hormonal changes occurring at term in non-brucella-infected cattle and leads to the initiation of parturition.[61]

Up to 35 per cent of cows may be resistant to infection with *B. abortus* because their macrophages have a greater ability to kill *B. abortus* than that possessed by susceptible cows. The level of macrophage function, which is reduced in susceptible cows, plays a role in the establishment of chronic infections.[76] This enhanced macrophage-killing activity is significantly greater in cows that are genetically resistant to infection, including that caused by *Mycobacterium bovis*, *S.* Dublin and *S.* Typhimurium as well as *B. abortus*.[132] The bovine *nramp1* gene, the homologue of the murine tuberculosis resistance gene, has been identified as a major candidate for controlling the *in vivo* resistant phenotype to *Brucella* infection. It has been demonstrated in a murine macrophage cell line transfected with the resistance- and susceptibility-associated alleles of the bovine *nramp1* gene, that these alleles critically affect the control and replication of *B. abortus*.[16]

Phagocytes have, on the one hand, developed antimicrobial defence mechanisms, such as oxidative burst, acidification of phagosomes, or fusion of phagosomes with lysosomes, to eliminate pathogens, while on the other, facultative intracellular bacteria have developed strategies counteracting the host cell defences, resulting in intramacrophagic survival. Recent studies have revealed that caveolae or lipid rafts anchored in the membrane of macrophages are implicated in the entry of brucellae into murine macrophages and mediate an endocytic pathway avoiding fusion with lysosomes.[111] It has been shown that in human macrophages phagosomes rapidly acidify to a pH of 4,0 to 4,5 following *B. suis* infection and that this early acidification is crucial for intracellular replication as neutralization results in bacterial elimination.[131] In addition, if the phagosomal membrane is disrupted, then *B. suis* fails to multiply intracellularly.[90] These results highlight the necessity of an intact, acidic phagosome as a predominant replicative niche for brucellae in macrophages; it is called the 'brucellosome'.[91] A series of genes are involved in the adaptation of brucellae to three major stress conditions within the phagosome, i. e. acid stress, starvation and low oxygen tension.[92]

Long-term residence of brucellae in the phagosomal compartment of host macrophages is essential for their ability to produce disease in both natural and experimental hosts. Brucella infections inhibit spontaneously occurring apoptosis in human monocytes, thus preventing host cell elimination. This might represent a strategy for brucella development in infected hosts.[75] Studies with *Brucella*

mutants suggest that stationary-phase physiology is critical for their successful long-term residence in host macrophages,[135] and reveal striking parallels between the strategies employed by rhizobiae to establish and maintain intracellular residence in their plant host and those used by the brucellae during their long-term survival in the phagosomal compartment of host macrophages.[96]

Cytokines such as IFN-γ, TNF-α, IL-2, IL-10 and IL-12 control the intracellular growth of brucellae.[14, 59, 73] Among these cytokines, the most important is IFN-γ which strongly activates macrophages and induces an enhanced intracellular killing of brucellae.[14, 109, 164] In contrast to what is observed in murine macrophages, *B. suis* does not induce the production of TNF-α in human macrophages.[32]

In non-phagocytic cells, such as Hela epithelial cells, the *Brucella* bacterium initially interacts with compartments of the early endocytic cascade, then rapidly segregates from this intracellular pathway and associates with the autophagocytic cascade.[128] During the late stages of infection, brucellae proliferate within the endoplasmic reticulum of host cells. They replicate extensively intracellularly[54, 55, 128] without inducing obvious damage to the infected cell, and therefore seem to promote the survival of the cells for their own benefit.[73] Eventually, in the bovine pregnant uterus, this extensive replication does lead to cell necrosis and acute inflammation, and to the release of huge numbers of bacterial cells from both the trophoblasts and foetal tissues.[61]

Clinical signs

The length of the incubation period of bovine brucellosis varies considerably. The incubation period has been variously defined, *inter alia* as the period between exposure and abortion.[28] In bulls this period is even more imprecise as serological evidence of infection may be equivocal or lacking, and clinical signs may be absent. The length of the incubation period is also affected by the size of the infective dose, and the age, sex, stage of gestation, and immunity of the infected animal.[49] In cows that do eventually abort, the usual length of the incubation period varies according to the time at which infection occurred. Cows infected at service abort after an average interval of 225 days, whereas those infected at seven months' gestation abort about 50 days later.[148] Congenitally infected calves may remain sero-negative for at least 18 months, after which they may manifest clinical signs. The longest recorded 'incubation period' in a cow is nine years.[94]

The abortion rate in infected herds is dependent on many factors and varies according to the susceptibility of the pregnant animals, management practices, the severity of the challenge, the period for which the herd has been infected, and various environmental factors such as the quality of pastures which may affect cattle density, the climate and the topography.[49, 51, 113] In fully susceptible herds, abortion rates vary from 30 to 70 per cent, but in South Africa it seldom exceeds 50 per cent. Increased public awareness, veterinary intervention, improved management practices and vaccination have all contributed to making the disease in these herds assume a more insidious, chronic form. In such herds, which are often closed, very few or no abortions occur and the disease is almost impossible to recognize clinically.[49] Abortions typically occur at approximately five to seven months' gestation, although some occur earlier or later. Weak, full-term calves that often die shortly after birth are sometimes encountered. About 20 per cent of infected animals do not abort, while 80 per cent of animals that abort as a result of *B. abortus* infection, do so only once.[6] The placenta is not consistently retained after abortion but when it is retained metritis is common. Early abortion may result in a considerable reduction in the milk yield.[51] Infection of the udder is clinically inapparent and the organ appears to be normal when palpated.[106]

In bulls, an acute to chronic, uni- or bilateral orchitis, epididymitis, and seminal vesiculitis occasionally occur. The scrotal circumference may be normal or severely increased.[23] Strain 19 vaccination may also cause orchitis.[22]

Uni- or bilateral hygromas (**Figure 144.1**), especially of the carpal joints, may be evident in some animals in chronically infected herds,[64, 87] or may occasionally follow inoculation of heifers with strain 19.[44] A progressive, erosive, non-suppurative arthritis of the stifle joints has been reported in young cattle from brucellosis-free herds that were vaccinated with strain 19 vaccine.[161]

Pathology

Irrespective of the route of infection, the organism provokes a regional lymphadenitis which is characterized by reticuloendothelial cell and lymphoid hyperplasia, as well as the infiltration of large numbers of mononuclear cells and some neutrophils, and a few eosinophils and plasma cells.[87] Other

Figure 144.1 Hygroma on the carpal joint of a bull infected with *Brucella abortus*

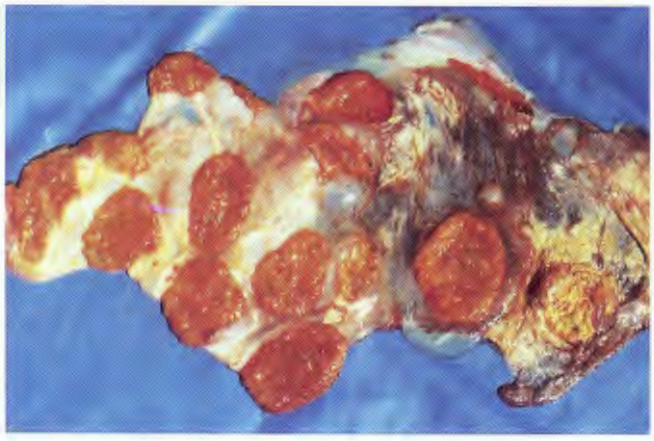

Figure 144.2 Note the mottled appearance of the cotyledons in the placenta of a cow infected with *Brucella abortus*

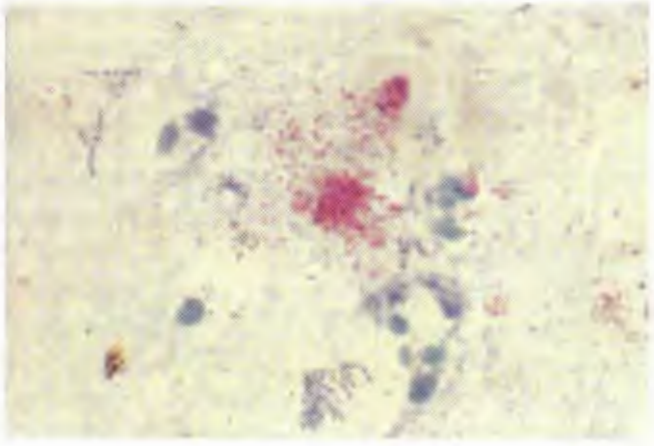

Figure 144.3 Large intracytoplasmic colony of *Brucella abortus* (Stamp's stain)

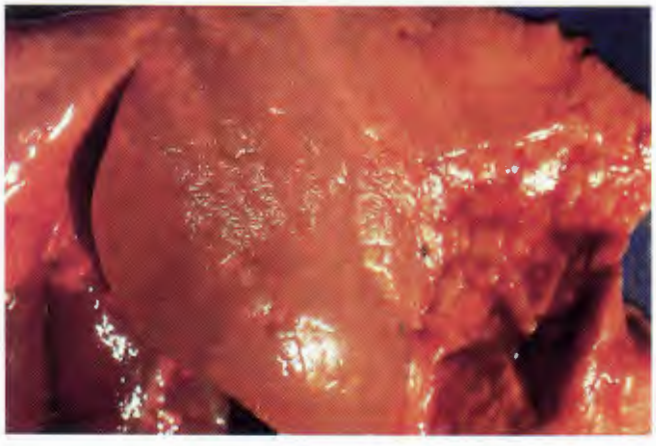

Figure 144.4 Bronchopneumonia in a foetus infected with *Brucella abortus*

lymph nodes in the body and the spleen may be affected later in the course of the infection but to a lesser degree.[61]

There is considerable variation in the severity of the uterine lesions at abortion. As the disease progresses lesions advance from an acute (mild to a severe) to chronic endometritis. Microscopically, the endometrium is infiltrated by lymphocytes and plasma cells, and some neutrophils. Microgranulomas may be scattered in the endometrium.[61, 87]

The chorion is not uniformly affected and large parts may appear quite normal. The lesions in and at the periphery of the cotyledons, as well as those in the intercotyledonary area vary in extent, appearing to be most severe adjacent to cotyledons. The affected cotyledons, or parts of them, are covered by a sticky, odourless, brownish exudate, and are yellowish-grey as a result of necrosis (**Figure 144.2**). Parts of the intercotelydonary placenta are thickened, oedematous, yellowish-grey and may contain exudate on the surface. Microscopically, the stroma of the chorion is infiltrated by numerous mononuclear cells and some neutrophils. Some chorionic villi are necrotic, while a fibrinopurulent exudate and desquamated necrotic chorionic epithelial cells are accumulated between the villi. Many of the chorionic epithelial cells are packed with numerous intracytoplasmic bacteria (**Figure 144.3**). Vasculitis, sometimes accompanied by thrombosis, may be evident in the chorion.[61, 87]

Some aborted foetuses have varying degrees of subcutaneous oedema and blood-tinged fluid in the thoracic and abdominal cavities, while the abomasal content is sometimes turbid, bright yellow and flaky. In some foetuses, greyish-white foci of pneumonia of 1 mm or larger in diameter, may be present, particularly in the apical lobes (**Figure 144.4**). A fibrinous pleuritis sometimes accompanies the pneumonia. The liver is usually enlarged, discoloured orange-brown and its surface may have a slightly uneven appearance. Many foetuses show no gross changes. Microscopically most aborted foetuses reveal a multifocal bronchopneumonia, characterized by the accumulation of cellular debris, neutrophils and macrophages in the lumen of the bronchi and bronchioli, patchy desquamation of bronchial epithelial cells, and a mild to moderate infiltration of mononuclear cells and some neutrophils in the alveolar septa. Vasculitis of some of the pulmonary vessels may be seen. Isolated small foci of necrosis or microgranulomas are often found in the liver, but may also occur in the lymph nodes, spleen and kidneys. In most aborted foetuses it is not possible — or very difficult — to demonstrate organisms in tissue sections, notwithstanding that they may have been specially stained for brucellae.[61] However, it is easy to demonstrate the organisms in smears made from the abomasal content or wall that have been stained with Stamp's modification of the Ziehl-Neelsen stain.[4, 6]

The udder in infected ruminants does not show any gross lesions, although the supramammary lymph nodes may be somewhat enlarged.[61, 81] Microscopically, infection of the udder is characterized by a lymphoplasmacytic and histiocytic interstitial mastitis while the regional lymph nodes show lymphoid hyperplasia, medullary plasmacytosis and sinus histiocytosis.[61, 87]

Acute orchitis is characterized by multifocal or diffuse necrosis of the testicular parenchyma, and a focal, necrotizing epididymitis may occur. Microscopically the seminal

epithelial cells are necrotic and desquamate; large numbers of organisms are present in them while numerous leukocytes, particularly neutrophils, and fibrin occur in the affected tubuli and interstitial tissues. In the chronic stage, spermatic granulomas develop in the testicular parenchyma and epididymis in response to dead sperm.[61, 87]

In horses, infection may localize in the bursa between the nuchal ligament, the atlas and axis, causing poll evil. Fistulous withers caused by *B. abortus* is characterized by inflammation of the bursa between the nuchal ligament and the dorsal spines of the thoracic vertebrae. Chronic draining sinuses are formed in both conditions.[49]

Brucellae in cattle may localize in the carpal and other bursae and induce the formation of hygromas in which large numbers of the pathogen may be found.[6, 64, 87]

Diagnosis

In order to develop sensitive and specific diagnostic tests and more efficacious vaccines and to explore new therapeutic approaches in both human and animal brucellosis, it is important to assess both innate and acquired specific immune responses directed against brucellae. Because the replicative niche of brucellae is the macrophage phagosome[92] and the non-phagocytic trophoblast of the pregnant uterus,[54, 55, 128] antibodies, while contributing to the protection against brucellae if present when infection occurs (for example after vaccination), are not as effective as that provided by mediated immunity particularly in the case of virulent strains because of their ability to survive intracellularly.[12, 13]

The humoral immune response is principally directed against the O-PS moiety of the smooth lipopolysaccharide (S-LPS).[118] The *Brucella* antiprotein antibody response is often delayed when compared to the anti-S-LPS response and is limited to animals that develop an active brucellosis infection.[95] Although there is evidence for a protective role of humoral antibodies directed either against LPS,[36, 85] or outer membrane proteins,[35, 85] the antibody response of cattle against *B. abortus* has been extensively used for the serological diagnosis of bovine brucellosis.[4, 11, 118]

Cellular immune responses contribute to the control of virulent and attenuated (e.g. vaccine strains) brucellae, more than the humoral response does.[12, 13] It has been shown for many intracellular bacteria and protozoa that IFN-γ activates the microbial killing activity of macrophages.[14, 107, 109, 164] Murine macrophages infected with brucellae produce cytokines such as TNF-α and IL-12.[165, 167] IL-12 seems to be critical in the mouse model because its depletion induces an exacerbation of the infection as well as an inhibition of the IFN-γ production.[164, 166] IFN-γ is an important component of the type 1 cellular responses referred nowadays as Th1 or T1 cell responses.[107] These responses are principally characterized by the production of T1 cytokines, i.e. IFN-γ and IL-2, by CD4+ T helper cells and CD8+ T cytotoxic cells as well as IgG_{2a} secretion by plasmatocytes.[107] For extracellular bacteria or toxins, a T2 or Th2 cell response will be induced. This response is principally characterized by the production of T2 cytokines, i.e. IL-4, IL-5 and IL-10, by CD4+ T helper cells as well as IgG_1 and IgE secretion by plasmatocytes.[107] According to the triggering mechanism, a precursor T helper cell will differentiate into either a Th1 or a Th2 cell.[41] Live brucellosis vaccines are more effective than inactivated vaccines because the latter tend to induce a T2 response.[14, 165] Therefore, it is important to identify bacterial proteins that induce a T1 mediated response, and it is critical to determine how the host first recognizes and then eliminates brucellae. However, although inducing a cellular immune response, few of these individual proteins induce host protection.[122]

Although current knowledge emanating from immunity studies in bovine brucellosis is incomplete, parallels between the bovine and murine immune systems allow for some extrapolation of immunity to the disease in cattle.[160]

Other T lymphocyte subsets, such as γδ T lymphocytes, may be important as mediator (secretion of IFN-γ and IL-2) or lytic effector cells in innate and acquired immunity. In humans, a particular subset of γδ T cells impairs intracellular multiplication of *B. suis* in monocytes through soluble factor release and contact-dependent cytotoxic effects.[59, 124] Bovine γδ T cells from cattle infected with *Mycobacterium bovis* or vaccinated with a killed *Leptospira borgpetersenii* adjuvanted vaccine, recognize specific antigens and produce IFN-γ as part of an acquired immune response which could play a role in protection.[110, 134]

Because of the variable incubation period and the often subclinical nature of the disease in most animals, a definitive diagnosis should be based on the isolation and identification of *B. abortus* and on positive serological results based on the detection of antibodies in blood, milk, whey, vaginal mucus, or seminal plasma.[4, 6, 11]

The isolation of *B. abortus* is mandatory when dealing with specific groups or herds of animals when there is doubt about their infection status. When a herd is known to be infected and where its environment is infected, serological reactors are viewed in a different light from those in a herd in which reactions might be due to the use of strain 19 vaccine. It is difficult to prescribe strict cut-off points when assessing serological results that will apply in all cases when strain 19 vaccine has been used. It is preferable to interpret serological test results according to the known infection status of the herd. As stated by Brinley Morgan:[28]

> I have found in my experience over the past twenty-five years working with *Brucella* spp. that you are invariably wearing at least two hats. One is where you are in a very clean environment where you can take an awful lot of chances, and the other hat is where you are working in an infected environment where you cannot afford to take any chances at all.

Nevertheless, failure to isolate *B. abortus* in a herd may result in difficulty in interpreting serological results.

When detecting rare events, such as the occurrence of brucellosis at the end of an eradication programme, the emphasis is no longer put on intrinsic values of a test, but its positive predictive value, which relates to the clinical utility of the result.[66] The aim of an eradication programme is not a 'zero seropositivity' situation, but the absence of infection, even if seropositivity is encountered. Indeed, the actual epidemiological situation (true incidence and prevalence rates) has to be taken into consideration: when tests are applied it must be borne in mind that the predictive values of tests vary according to the progress of the eradication programme and that criteria other than positive serology are important in an eradication programme.[71]

Diagnostic tests for bovine brucellosis are subdivided into three groups — tests for the demonstration of *B. abortus* organisms, those which detect immunoglobulins, and those dependent on allergic reactions to *B. abortus*.[4, 6] The 'ideal' diagnostic test should detect infection early, during the long and variable incubation period; not be influenced by the presence of 'non-specific' antibodies; detect carriers; and differentiate between responses to vaccination and those due to field infection.[28] There is no single available test that completely covers all these requirements.[121]

The tests described below are standard tests that are applied worldwide. Minimal requirements to ensure quality control of the reagents for the demonstration of *B. abortus*, the methods employed for the serological and allergic diagnosis of bovine brucellosis can be found in standard laboratory textbooks.[4, 6] A technical description of these tests can also be found in an OIE publication.[122]

Tests for the demonstration of *B. abortus*

Microscopic examination Using Stamp's modification of the Ziehl-Neelsen stain,[142] *B. abortus* stains red against a blue background in tissue sections and smears (**Figure 144.2**). However, this colour reaction is not specific for *Brucella* spp. as *Coxiella burnetii, Chlamydophila abortus* and *Nocardia* spp. are also weakly acid-fast. *Nocardia* spp. can be differentiated from these organisms on morphological grounds, but it is extremely difficult to differentiate *C. abortus* and *C. burnetii* from *Brucella* spp. beyond any doubt.[6]

Culture and typing The specimens of choice include foetal membranes, lungs, stomach content, liver and spleen of aborted foetuses and full-term calves; and from live cows uterine discharge, milk or colostrum.[4]

The supramammary lymph node is the most suitable specimen from carcasses of adult animals, but the retropharyngeal, mandibular, iliac, or the prescapular and parotid lymph nodes may also be collected. Other specimens which may be submitted include uterus, milk, udder tissue, fluid aspirated from hygromas, male accessory sex glands and testes.[46, 80, 113] Isolation may also be attempted from the semen or seminal plasma of infected bulls.[49]

The supramammary lymph nodes are preferred for isolation of *B. abortus* from animals that have been slaughtered and a 90 per cent recovery rate from infected animals may be achieved. Recovery of *B. abortus* on culture from infected adult cows may approach 100 per cent using the supramammary, parotid, mandibular and sub-iliac nodes.[46] Recovery of the pathogen from infected heifers is best achieved by the culture of the mandibular lymph nodes, and this probably reflects the primary route of infection through the conjunctiva or the nasal or oral mucosa.[46] When specimens of large groups of cows (five or more) must be cultured, collection of supramammary lymph nodes should be sufficient.[60]

In order to get valuable epidemiological information, isolated brucellae have to be typed (species and biovar) according to the standard methods described in textbooks.[4, 6]

Polymerase chain reaction Numerous polymerase chain reaction (PCR)-based assays have been developed for the identification of members of the genus *Brucella*.[52] Other circumstances require the identification of the *Brucella* spp. involved. For epidemiological trace-back, strain-specific identification is helpful. Several strategies have been explored to differentiate between *Brucella* spp. and biovars, including locus specific multiplexing, e.g. AMOS-PCR based on *IS711* insertion sequence, allowing the differentiation of the vaccine strain 19 and RB51,[26] and PCR-RFLP at the *omp2* locus.[37] Unfortunatelly, up to the present, no reproductible and robust technique allows for the differentiation between strains belonging to the same biovar.[27]

Initially, these assays were performed on purified DNA obtained from cultured organisms. As far as the identification of brucellae from field samples and food products, namely milk and cheese, is concerned, advancement in sample processing by removing polymerase inhibitors is needed before implementation of such techniques in diagnostic laboratories.[27]

Tests for the detection of specific immunoglobulins

The serological diagnosis of brucellosis began in 1897 with the development of an agglutination test by Wright.[159] Problems such as positive serological reactions resulting from, for example, exposure to cross-reacting micro-organisms, were encountered. Improvements of existing tests and development of new serological tests have since then taken place.[121] At present, several serological tests are used worldwide. The outer membrane of smooth *Brucella* spp. strains is composed of phospholipids, proteins and S-LPS. Most of the serological tests, particularly those using whole-cell suspensions as antigen, such as the slow (tube) agglutination test (SAT), the Rose Bengal test (RBT), the complement fixation test (CFT), most enzyme-linked immunosorbent assays (ELISA) and the milk ring test (MRT) have been developed to detect antibodies directed against the O-PS moiety of the S-LPS.[4, 6, 11]

These tests are suitable for use in surveys, large-scale campaigns and in control/eradication programmes of the disease as well as for trading purposes.[6, 11] Inexpensive,

rapid and simple screening tests with high sensitivity followed by more specific confirmatory test(s) in case of positivity in the screening test, are used in control programmes. If two or more tests are used, the interpretation (parallel or serial) of the results has to be done according to the epidemiological context and issues, such as the country, region and herd disease status and the demonstration of the presence of *B. abortus*. An epidemiological inquiry and a risk factor analysis also need to be addressed.[71]

The worldwide use of strain 19 vaccine, which induces a persisting antibody response, led to the development of tests that could solve or at least reduce the problem of the interference of vaccination in order to differentiate vaccinated animals from infected ones.[116, 121] This problem has led to the development of the RB51 vaccine that shows negligible interference in classical serological brucellosis tests.[140]

Reliance on serological tests alone for the diagnosis of brucellosis can be misleading and thus other tests, such as the brucellosis skin test, and a sound proficient epidemiological inquiry have to be implemented.[99, 137]

In acute bovine brucellosis an increase in the level of IgM in the serum is the first evidence of antibody response. IgG soon becomes the predominant antibody and usually persists for as long as the animal remains infected while the IgM levels wane. Of the two subclasses of IgG in the serum, IgG_1 is the most abundant and predominant agglutinating and complement-fixing antibody.[6, 117]

In South Africa serum samples are screened with the RBT, while the CFT is used as the confirmatory test. The SAT is sometimes used as a supplementary test and has some value in detecting IgM, the persistent and often predominant immunoglobulin resulting from vaccination with strain 19 vaccine.[6, 28, 117] The MRT is also used to screen and monitor bulk milk samples.

Rose Bengal test The RBT is a modification of the plate agglutination test. The antigen, which has been stained with Rose Bengal stain, is buffered at a pH of 3,65.[4, 6] At this level of activity, 'non-specific' agglutinins are destroyed and IgG, normally the most abundant antibody in the serum of infected animals, agglutinates strongly.[6, 28] Equal volumes (30 µ) of test serum and antigen are mixed, shaken for four minutes and viewed over an X-ray viewer and any degree of agglutination is recorded as positive.[4]

The test is inexpensive and easy to perform. False negative results are rare and are usually obtained during the more chronic stages of the disease. Despite improved specificity at an acid pH, a high percentage of false positive reactions occur usually due to the presence of IgM as a result of strain 19 vaccination.[1] This test is prescribed for international trade in cattle by the OIE.[11]

Complement fixation test This test is regarded throughout the world as being the confirmatory test for the serological detection of infected animals. It has been modified, standardized and adapted to a microtitre system.[4, 11] Unlike the SAT, the titres do not wane as the disease becomes chronic.[6, 137]. Results are expressed in International Units (IU) and a cut-off point of 20 IU has been defined[11] which is rigorously applied where strain 19 vaccine has not been used for several years, as is the case in most of the European Union (EU), the USA and Australia. Its strict application in countries enforcing compulsory strain 19 vaccination (such as South Africa) may often be problematic and sometimes leads to unacceptably large numbers of false positives, because vaccination induces serological titres. As a consequence, considerable expertise and experience are needed to certify herds or individual animals free of brucellosis when they are classified as positive by the test. As a rule, vaccine titres tend to decline faster than those due to infection with wild strains.The decline in titres is also dependent on the vaccine dose. Although the CFT is useful when differentiating calfhood vaccination titres from those due to wild-strain infections, there may be difficulty in distinguishing vaccine reactions from those caused by wild strains when animals have repeatedly been vaccinated (although calfhood vaccination alone is considered to be adequate) or after they have become sexually mature.[18] Haphazard vaccination of heifers and vaccination of adult animals may result in much confusion in the interpretation of laboratory results, and it is therefore essential that accurate records of vaccination and birth dates be kept to allow correct interpretation of the results of the CFT. In the UK persistent reactors in brucellosis-free herds occurred in less than 0,5 per cent of vaccinated heifers,[28] but 16 per cent of cows in brucellosis-free herds in New Zealand have been shown to develop CFT titres which persisted for at least 12 months after strain 19 vaccination.[18] In South Africa, the use of low-dose vaccination of sexually mature heifers causes few persistent titres.[21] This test is prescribed for international trade in cattle by the OIE.[11]

Slow (tube) agglutination test In a number of countries the SAT was and still is used as a screening test for eradication purposes. It is considered to lack specificity and some authors discourage its use, at least for trading purposes.[121] Non-specific agglutination in sera is decreased by the addition of EDTA with no reduction in *B. abortus* agglutination titres of sera from infected cattle.[67, 100] The SAT-EDTA is a very specific test and particularly useful in detecting new infections as early as two weeks after infection, as demonstrated in experimental conditions,[70] but its usefulness in herds that are chronically infected is more limited because some infected animals will be classified as negative by this test because the infection is in a chronic phase.[137] In South Africa the SAT is still very usefully employed as a supplementary test for indicating the levels of serum IgM, the predominant immunoglobulin after vaccination with strain 19 vaccine.[82–84]

Indirect enzyme-linked immunoabsorbent assay Indirect enzyme-linked immunoabsorbent assay (iELISA) is more sensitive for detecting antibodies to *Brucella* spp. than are the RBT, SAT and CFT, but great care must be exercised in animals vaccinated with strain 19 vaccine.[31, 121, 144] Recently, an iELISA test has been developed and validated in South Africa. It has been suggested that this test could replace not only the currently used confirmatory CFT test, but also the two in-use screening tests, namely the RBT and SAT.[127] The iELISA has been used successfully throughout Europe in strategies aimed to substantiate and to maintain the status of 'brucellosis-free countries'.[70, 71] This test is prescribed for international trade in cattle by the OIE.[11]

Competitive enzyme-linked immunoabsorbent assay The basis of this test is the use of a selected monoclonal antibody (MAb) that competes with low affinity antibody. The competitive enzyme-linked immunoabsorbent assay (cELISA) using a MAb specific for one of the epitopes of the *B. abortus* O-PS has been shown to have higher specificity than the iELISA.[119, 136, 145] The cELISA was reported to be able to eliminate cross-reaction problems in serological tests, induced by strain 19 vaccination or infection with cross-reactive bacteria. Unfortunately, the cELISA only partially solves the problem. Indeed, persistent competing antibodies have been observed after *Y. enterocolitica* O:9 infections,[71, 118] and vaccination with strain 19.[1] However, residual antibody activity due to vaccination or cross-reactive infection was less persistent than with the other tests.[1] This test is prescribed as an alternative test for international trade in cattle by the OIE.[11]

Fluorescence polarization assay The fluorescent polarization assay (FPA) is a simple and rapid technique for measuring antigen/antibody interaction and may be performed in a laboratory setting or in the field.[120] The mechanism of the assay is based on random rotation of molecules in solution. A fluorochrome-labelled antigen of small molecular weight (a fragment of the O-polysaccharide (O-PS) of *B. abortus* S-LPS, for example), is added to serum or other fluid to be tested. If antibody is present, attachment to the labelled antigen will cause its rotational rate to decrease and this decrease can be measured. The FPA has received very promising reports.[120] This test is prescribed as an alternative test for international trade in cattle by the OIE.[11]

Milk ring test The milk ring test (MRT) is used to detect antibodies in milk. The development of a positive reaction is dependent on two reactions: (i) fat globules in the milk are aggregated by milk antibodies (fat-globule agglutinnins); and (ii) stained *Brucella* cells (antigen), which are added to the milk, are agglutinated by the *Brucella* antibody/fat globule complexes which rise to form a coloured cream layer at the top.[4, 6] This is a sensitive screening test used on bulk milk samples either to detect infected animals on a herd basis or to monitor clean herds. It should be carried out at least quarterly to ensure that all animals that come into lactation are screened. The sensitivity of the MRT is somewhat reduced when it is applied to large herds with few reactors but this loss in sensitivity in large herds of 150 or more animals can be counteracted to some extent by decreasing the ratio of antigen to milk in the test.[4] Despite its reduced sensitivity in large herds, the MRT has been very successfully used to monitor the brucellosis-free status of dairy herds.[6, 49] In the EU, after a positive MRT performed on a bulk milk sample has been obtained, the cows which had supplied the milk are individually tested by serology in order to detect those that are infected.[69]

Factors that may cause false positive results include a high prevalence of mastitis; a high proportion of cows in early or late lactation; recent (within three to four months) vaccination with strain 19 vaccine; and souring of milk. Milk samples may be preserved for testing by adding 0,5 ml of a formalin solution (prepared by mixing 7,5 ml of 37 per cent formaldehyde with one litre of distilled water) to a 10 ml milk sample. The duration and temperature at which samples are stored (in particular excessive heating such as storing for longer than five minutes at 45 °C) may cause false negatives. Pasteurized milk cannot be effectively tested by the MRT.[4]

Several countries have replaced the MRT by a milk iELISA. Although this technique has not been standardized, it is prescribed for international trade in cattle by the OIE.[11]

Test to demonstrate an allergic reaction to *B. abortus*

A skin test for the diagnosis of brucellosis has been used in extensively managed herds in New Zealand.[99] The sensitivity of the test was considered to be low at the animal level and its specificity exceeded 99 per cent, and thus it was claimed that this test is a useful low-cost method of identifying infected herds rather than individual animals.[4] This test has recently been re-evaluated in the EU because of the emergence of 'False Positive Serological Reactions' (FPSR), i. e. non-specific reactions in all serological tests, that have emerged throughout Europe.[71] These have been documented in Belgium and France since 1990, affecting up to 15 per cent of the herds tested in some regions that are free of brucellosis. *Yersinia enterocolitica* O:9 infections have been shown to be responsible for these FPSR.[71, 157] Experimental studies have shown that the brucellosis skin test is the only test that is able to discriminate between *Y. enterocolitica* O:9 and *B. abortus* infections, beyond any doubt.[71, 137] It is now a recommended herd test for brucellosis by the OIE [11] and as an official test in the EU (Directive 64/432), particularly when monitoring is made difficult due to aspecific brucellosis serological reactions. Calfhood vaccination using strain 19 may complicate interpretation of the brucellin test by inducing prolonged sensitivity to brucellae allergens.[137]

A diagnosis of bovine brucellosis based on *in vitro* antigen-specific IFN-γ production, which can be regarded as an

in vitro correlate of the brucellosis skin test, has been developed.[155] Unfortunately, this test has been shown to be less specific than the brucellosis skin test.[71]

Differential diagnosis

Numerous infectious agents may cause foetal loss and abortion in cattle. A multidisciplinary approach in terms of diagnostic tests is necessary to make a definitive diagnosis.[78] Microscopic examination of smears or histopathological sections, particularly of the placenta, stained by the modified Ziehl-Neelsen method, may present difficulties in differentiating *B. abortus* morphologically from *Coxiella burnetii* or *Chlamydophila abortus*. *Brucella abortus* can be distinguished from *C. burnetii* and *C. abortus* by fluorescent antibody techniques and serological tests.[42]

Brucella abortus may cross-react serologically with *Escherichia coli* serogroup 0:157, *Y. enterocolitica* serovar O:9, *Salmonella* serotypes of the Kaufmann-White group N, *Francisella tularensis*, *Pseudomonas maltophilia*, and *Vibrio cholera*[43] because the immunodominant O-chain of S-LPS of these bacteria contains antigenic motives (epitopes) that may be detected in serological tests for brucellosis using whole *B. abortus* cells or S-LPS extracts.[158] Such FPSR induced by these organisms are probably not of great significance in the early phase of eradication campaigns but when the prevalence of the disease has been reduced to a very low level, then this phenomenon may jeopardize the success of the eradication programme.[71]

Control

Treatment

Cattle suffering from bovine brucellosis are generally not treated. *Brucella* spp. may undergo L-transformation when exposed to certain antibiotics, such as penicillin and oxytetracycline.[6, 15] The effect that such cell wall deficient forms have in preventing serological detection and the resultant creation of carrier animals requires further investigation.[15]

Vaccination

In 1906 Bang observed that cattle could be protected against brucellosis by vaccinating them with live cultures. Since then three strains of *B. abortus* have been used for the preparation of vaccines and have been studied extensively: strain 19, a smooth strain, used as a live attenuated vaccine;[29, 116] strain 45/20, as a rough killed vaccine;[105] and, more recently, strain RB51, as a rough live attenuated vaccine.[140]

The minimal requirements for vaccine production have to be followed and each batch of live vaccine must conform to the minimum standards set by the OIE.[122] These include viability, pathogenicity and ability to immunize guinea pigs and/or mice against challenge with a virulent strain of *B. abortus*.[11]

Strain 19 Because of its relative safety, potency, practicality of production and convenience of use in cattle, strain 19 remains the most acceptable and the most widely used vaccine against bovine brucellosis.[116] It was first described by Buck in 1930. After being kept one year in the laboratory after primary isolation from the milk of a Jersey cow, the strain was attenuated in guinea pigs.[29]

Strain 19 differs from other *B. abortus* biovar 1 strains in its requirement for carbon dioxide and sensitivity to thionine blue, penicillin and Safranin O. It is the only *Brucella* sp. strain that is inhibited by erythritol.[4, 6] The mutation in the erythritol catabolism genes has been determinated.[139]

Vaccination with strain 19 vaccine increases resistance to *B. abortus* but does not induce absolute immunity, and vaccination with it is not curative, i.e. if an animal is infected, vaccination will not cure the infection.[114] The increase in resistance following vaccination has been termed 'relative immunity' since it is estimated to be only about 70 per cent effective against field challenge by preventing unrestricted multiplication of *B. abortus* in the uterus and mammary glands.[6, 116]

The main disadvantage of strain 19 vaccination is the induction of post-vaccinal antibodies that are detected in serological tests. At present, there is no single individual test that can be used to discriminate between antibodies induced by vaccination and those induced by infection, although newer tests (or combinations of tests) have been developed to reduce this problem.[121]

Strain 19 vaccine must be stored correctly and the cold chain maintained to retain its full potency. Lyophilization, however, has proved successful in the preservation of the vaccine.[6] On reconstitution, lyophilized vaccine should be used on the same day, preferably within three hours.

Strain 19 and RB51 are the only brucellosis vaccines currently allowed for use in South Africa. Traditionally, the dose of strain 19 vaccine administered subcutaneously to heifer calves at four to eight months of age contains 5×10^{10} viable *Brucella* cells.[6, 116] The calfhood strain 19 vaccine produced by Onderstepoort Biological Products in South Africa contains $4–12 \times 10^{10}$ viable cells per dose, and a single dose of this vaccine administered to heifers at the age of five months generally provides a life-long relative immunity, although virtually all of them will have lost their serum antibody titres by the age of 16 to 18 months.[2, 116] The vaccination of heifers practically eliminates the occurrence of abortions in a herd.[116] A gradual reduction in the prevalence of bovine brucellosis may be expected when 80 per cent of the female population has been vaccinated and this vaccination pressure ('herd immunity') must be maintained for optimal results.[116]

A policy of using a reduced-dose vaccine containing $3 \times 10^{8-9}$ organisms/dose in heifers of 4 to 12 months of age, has been shown to provide the same degree of protection as the classical dose and has therefore been the only vaccine available in the USA and in Europe.[6, 116] Its use in the USA,

however, has been prohibited since 1996 when the RB51 vaccine was approved for use.[140]

Administration of strain 19 vaccine into the conjunctival sac (one or two doses of $5–10 \times 10^{10}$ at four and eight months of age, respectively) results in good protection and the almost complete elimination of serological reactions in heifers and adults.[112, 115, 130]

In the event of an outbreak, the vaccination of adult cattle with strain 19 vaccine may be advantageous, particularly in large dairy herds and in herds where a large proportion of the animals have not previously been vaccinated.[116] Mature cows inoculated with a $3–10 \times 10^8$ organisms/dose are protected for at least 12 months, although most (90 to 95 per cent) of the vaccinated animals lose their CFT titres within six months. Five to ten per cent of animals may, however, remain serologically positive for 8 to 12 months or longer and strain 19 organisms may be isolated from their milk.[3, 13, 60] It has been shown that the removal of infected cows and the stage of gestation at vaccination will affect the efficacy of strain 19 in cows vaccinated with a low dose; this may explain the variation in strain 19-induced protection.[48, 50] The disadvantages of vaccinating adult cows include the development of vaccine reactions, a positive MRT, and the stigma attached to vaccinated adult animals because of their association with positive herds.

In South Africa adult vaccination is restricted by law to individual farms and may only be given when written permission by the State to do so has been given. However, this practice may complicate the interpretation of serological results for a considerable period.[19]

In order to be able to determine infected animals from vaccinated ones, besides use of the CFT, the following epidemiological criteria are taken into account:

- the herd is closed and has been closed for at least two years;
- the herd is closed except for introductions from herds certified free of brucellosis;
- there is no evidence of spread of infection within the herd;
- all cows that aborted were bacteriology negative for *B. abortus*;
- the lymph nodes of animals with high titres, and which have been slaughtered, are negative for wild strains of *B. abortus* and/or have yielded strain 19 on culture;
- the foetal membranes and uterine discharges of cows exhibiting high titres are negative on culture;
- milk samples from reactors yielded strain 19 and/or are negative for the wild strain of *B. abortus*; and
- the possibility of infection having been introduced from a neighbouring infected herd or infected wildlife is ruled out.

Strain 19 vaccination may occasionally cause orchitis in bulls.[23, 150] Because it has also been found in the semen of vaccinated bulls, its use in males has been restricted or prohibited.[116] Uni- or bilateral hygromas, especially of the carpal joints may occasionally follow inoculation of heifers with strain 19 vaccine.[161] A progressive, erosive, non-suppurative arthritis of the stifle joints has been reported in young cattle in brucellosis-free herds that had been vaccinated with strain 19 vaccine.[44] Systemic reactions following vaccination are rare and temperature reactions varying from 40,5 to 42 °C, lasting for two to three days, may occur in addition to swelling at the injection site.[23] Abortion in less than 1 per cent of vaccinated cows in late pregnancy has also been reported.[116] There is no evidence of the spread of strain 19 to unvaccinated cattle, although the organism can be excreted in milk for two to three months in vaccinated cows after calving or abortion.[116] Erythritol-tolerant isolates of strain 19 have been isolated from milk and aborted foetuses in New Zealand and South Africa.[18, 21]

Although strain 19 organisms may be intermittently present in the milk of vaccinated cows, there is no evidence of infection in humans who have ingested milk containing them. However, undulant fever in humans has been reported after accidentally being injected with the vaccine.[6, 163]

Strain 45/20 In order to avoid post-vaccinal antibodies, rough vaccine strains have been developed and tested. *Brucella abortus* smooth strain 45/0 was isolated in 1922, and after 20 passages in guinea pigs a rough derivative named strain 45/20 was obtained. Strain 45/20, when first used as a live vaccine, was inclined to lose its rough characteristics and revert to the smooth form and therefore it has only been further used as a killed vaccine.[146] Strain 45/20 has been used with success in an inactivated vaccine incorporating an oil adjuvant,[51] but this, however, is not as protective as strain 19 vaccine in animals inoculated when less than nine months of age. Two consecutive vaccinations, 6 to 12 months apart, with strain 45/20 vaccine, are usually recommended. Large unsightly granulomas may develop at the site of injection following inoculation of some batches of strain 45/20.[51] This vaccine is no longer used.[141]

Strain RB51 Since 1996, *B. abortus* rough strain RB51 has been the official vaccine used in the USA for the prevention of brucellosis in cattle.[141] Certain other countries, mainly in Latin America, have officially approved the vaccine, but their vaccination protocols differ.[138] In 2002 Spain and Portugal received limited approval to conduct field trials with it before its possible approval in all the EU member states. RB51 was approved for use in South Africa in 2002.

This vaccine strain is a rough rifampicin-resistant mutant of *B. abortus* strain 2308, a smooth virulent *B. abortus* biovar 1 strain.[140] It is very stable and no reversion to smoothness has been described *in vivo* or *in vitro*. The genetic mutations that are responsible for the roughness and the attenuation of strain RB51 have been identified[153] in the *wboA* gene encoding a glycosyltransferase, an enzyme essential for the synthesis of O antigen.[68]

The organism behaves as an attenuated strain in a variety

of animals including guinea pigs in which it is rapidly cleared from the tissues and does not induce abortions. It is efficient in preventing *Brucella* infections in mice.[141] Although producing low levels of O-chain,[37] strain RB51 does not usually induce the production of O-chain antibodies in cattle that can be detected in the classical brucellosis serological tests regardless of their age, the dose they received and the frequency of injections.[141]

More than five million heifers have been vaccinated subcutaneously with a dose of 1–3,4 × 10^{10} cells without noticeable side-effects.[141] This vaccine has been found to have reduced abortifacient effects in cows, but placentitis was reported when used at a full dose.[125, 151] However, pregnant cows can usually be safely vaccinated with a reduced dose of 10^9 cells.[126]

Few experimental studies have been conducted in cattle to compare the vaccine potency of strain RB51 with that of strain 19. Strain RB51 vaccine has not been reported to induce a higher degree of protection than strain 19 vaccine.[34] Although, under field conditions, strain RB51 vaccine has been reported to be superior to strain 19 vaccine,[97] more information is needed in order to assess the intrinsic value of different strain RB51 vaccination protocols in cattle. An advantage of RB51 vaccination is that antibodies induced by it are not detected by the currently prescribed serological tests for brucellosis. Non-pregnant adult animals can therefore be vaccinated annually, thus boosting the individual and herd immunity.

BS2 vaccine A smooth strain of *B. suis*, biovar 1 (strain 2) has been in use as an oral vaccine to control brucellosis in cattle, sheep, goats and pigs in China since 1971.[162] This vaccine protects cattle against *B. abortus*, is safe if administered orally, does not induce persistent antibody titres and can be administered without having to restrain the animal.[162] Although it has been used widely in China for more than 20 years, its use is not recommended by the OIE.[11]

Management of control programmes

The planning and management of control or eradication programmes for brucellosis have been investigated in many countries, and by the World Health Organization.[6] In the EU, a task force for monitoring disease eradication in the member states was created in 2000 with the objective, as far as bovine brucellosis is concerned, of expanding eradication schemes and improving the cost-benefit ratio of eradication programmes co-financed by the EU (Council Decision 90/424/EEC). Strategies based on the prevention of the spread of the disease between animals, the monitoring of brucellosis-free herds and zones, the elimination of infected animals by test and slaughter, strict 'one-way' movement of infected and suspect cattle, mass immunization to reduce the infection rate, and supporting specific education and training programmes, have all received attention.[6, 49]

In South Africa, bovine brucellosis is listed as a State-controlled disease, and its control and eradication are given high priority by the Directorate of Animal Health. The control and eradication of bovine brucellosis in South Africa is broadly based on a test-and-slaughter policy, the vaccination of heifer calves, and the prohibition of the sale and movement of infected cattle. All sera are first screened with the RBT and those that are negative are usually not tested further. All sera that test positive are subjected to the CFT. In order for a herd to qualify for accreditation in the official South African Bovine Brucellosis Scheme, all animals must be CFT-negative in the final two tests. Herds with a prevalence of 10 to 20 per cent have been cleared of the disease while incurring losses that were still economically acceptable.[81] Good progress towards achieving eradication has been made in some regions in South Africa; in 1975 the prevalence in KwaZulu-Natal was estimated at 9,0 per cent and this was reduced to 0,6 per cent by 1988.[8] In 2001 South Africa reported 339 bovine brucellosis outbreaks to the OIE and the destruction of 5 320 animals.[122]

It is not appropriate to be prescriptive when recommending a strategy for the control or eradication of brucellosis that will be applicable or practical in all circumstances. Broadly based national strategies and policies will invariably differ in time according to the situation in each instance. The approaches to be adopted when attempting to eradicate the disease will vary markedly according to whether one is dealing with an infected herd or an uninfected (vaccine reactions) herd. Application of control measures on individual farms are also frequently affected by a variety of other factors. These include the infection rate in the herd; duration of infection, the abortion rate, whether an extensive or intensive farming system is being practised, rainfall, whether it is a beef or dairy herd, vaccine cover, the availability of, or necessity for, replacement stock, the financial situation of the producer, his proximity to a veterinary laboratory, neighbourhood cooperation, milk and slaughter quotas, slaughter prices of dairy cows, the availability of camps and suitable calving facilities, availability of veterinary guidance, and the handling facilities necessary to collect blood samples from large groups of cattle.[6, 49]

The following principles have been found to be crucial to the successful control or eradication of brucellosis:[49]

- reliable veterinary services,
- reliable diagnostic techniques,
- close cooperation and joint effort by the owner, laboratory, veterinary services and veterinarian,
- good records,
- education of the farming community and public awareness,
- established procedures for handling the disease,
- controlled movement of cattle, and
- permanent marking of infected and vaccinated animals.

Vaccination in conjunction with other measures can be used effectively in the control or eradication of brucellosis

and, in general, it is recommended that all heifer calves be immunized where the prevalence of brucellosis in an area, region, province or country is high, and that cessation of immunization with strain 19 vaccine only be considered when the prevalence of the infection is reduced to 0,2 per cent or less.[6, 150] At that time, a test-and-slaughter, management and hygiene programme should be implemented.

The reduction in numbers and the rapid elimination of all infected animals are essential in the control or eradication of brucellosis.[6] All calves in infected herds should be regarded as potential sources of infection. The 'two-year breakdown syndrome' (that is those previously infected herds which have apparently been clear for about two years and in which a 'breakdown' occurs after this period) has been ascribed to latently infected calves which remain serologically negative until at least mid-gestation or later during their first pregnancy.[51]

Cows should calve in an environment which can be thoroughly cleaned and disinfected with 2,5 per cent formalin. Wet and well-grassed calving camps should be avoided, and vehicles used for transporting infected animals should be disinfected after use. Isolation of cows prior to and after parturition contributes significantly to the control of brucellosis,[6] and, in addition, cows that abort should be isolated immediately and tested serologically soon after they aborted. If negative, they should be retested two to three weeks later. Animals testing positive should be culled. Foetuses, placentas and discharges must be disposed of, preferably by incineration.

It is important to maintain regular blood testing in infected herds.[48] Serological tests at intervals of two to three months and bacteriological examination of aborted foetuses, placentas, lochia and milk are often of great benefit. Milk from negative animals should be tested regularly and at short intervals by the MRT. All bulls from infected herds should be viewed with suspicion and the diagnosis should be based on clinical examination (including palpation of the seminal vesicles and ampullae), serological and allergic tests, and on bacteriological examination of semen.[129, 137]

It is important to prevent, or, in outbreaks, at least limit the spread of the disease. Depending on circumstances on a farm, the availability of spare camps can be advantageous, particularly where it is deemed necessary to split the herd into two or more groups. The two-herd system, based on the segregation of weaner heifers and adults, can be highly effective in extensive farming operations. It is often not possible to cull all infected animals at the outset of an eradication campaign, but it is vitally important to separate them from the 'negative' herd. The creation of another 'suspect' group of animals, and the effective separation of cows from four months' gestation onwards, may be beneficial. Replacement stock should be purchased from herds certified free of brucellosis.[49]

Whatever strategy is to be adopted, the owner must be fully aware of the implications of the proposed control programme. The cooperation of neighbouring farmers may be of assistance in this respect. In dealing with an infected herd, the need for decisive action is important. The 'negative' herd must be maintained as such and any contact with infected animals avoided. All bacteriologically or serologically positive animals must be permanently identified. Undoubtedly an element of 'over-kill' is built into a somewhat uncompromising and apparently ruthless approach in these programmes, but the long-term benefits will far out-weigh this short-term disadvantage. The economic implications of a test-and-slaughter eradication policy must also be taken into account.[6]

If brucellosis is present in wildlife (**see Chapter 148: Brucellosis in wildlife**), the concern of the livestock owner is to prevent the reintroduction of the infection into his or her livestock, particularly in regions or states that are 'officially brucellosis free' because of the implications for pre-movement testing for domestic animals.[19, 69]

Decision-making to determine the importance of brucellosis control relative to other public and animal health concerns also needs to be addressed.[104]

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