Brucellosis

Author: Prof Jacques Godfroid


Licensed under a Creative Commons Attribution license.

TABLE OF CONTENTS

Introduction ........................................................................................................................................... 3

Epidemiology ........................................................................................................................................ 3

Brucella abortus .................................................................................................................................... 5
Brucella ovis .......................................................................................................................................... 7

Pathogenesis ........................................................................................................................................ 7
Smooth Brucella spp. (B. abortus, B. melitensis, B. suis) ...................................................................... 7
Brucella ovis .......................................................................................................................................... 9

Diagnosis and differential diagnosis .................................................................................................. 10
Clinical signs & pathology .................................................................................................................. 10
Brucella abortus .................................................................................................................................... 10
Brucella ovis .......................................................................................................................................... 14

Laboratory confirmation ..................................................................................................................... 17
Bacteriology and PCR based methods ................................................................................................. 17
Serological tests ................................................................................................................................... 18

Control / Prevention ........................................................................................................................... 26

Marketing and trade / Socio-economics ............................................................................................. 27
INTRODUCTION

Ten *Brucella* species are currently included in the genus *Brucella*. Each *Brucella* species has a preferred host species but can be transmitted to other species, including man.

The major clinical sign of acute brucellosis in pregnant females is abortion, which usually occurs only once. Acute clinical signs seen in males are orchitis and epididymitis. Chronic cases of brucellosis seen in livestock in the absence of control or eradication programmes and in wildlife are characterized by the presence of hygromas of the tarsal joint.

Vaccination is the cornerstone of control programmes in livestock and although the S19, RB51 (both in cattle) and Rev 1 (in sheep and goats) vaccines have been successfully used worldwide, they have drawbacks and the ideal brucellosis vaccine is still awaited. There is no vaccine available for pigs and wildlife. Animal brucellosis control strategies differ in the developed and the developing world. Most emphasis is put on eradication and on risk analysis to avoid the re-introduction of *Brucella* in the developed world. Information related to the prevalence of brucellosis is still scarce in the developing world and control programmes are rarely implemented.

Since there is no vaccine available for humans, prevention of human brucellosis relies on its control in the animal reservoir. At the animal/ecosystem/human interface it is critical to reduce opportunities for *Brucella* to jump host species as has already occurred in livestock, wildlife and humans.

EPIDEMIOLOGY

*Brucella* spp. are small, Gram-negative, non-sporulating, non-encapsulated cocci, coccobacilli or short rods, 0.6–1.5 µm in length and 0.5–0.7 µm in width. The organism is not acid-fast but does resist decolourization by weak acids and thus stains red with Stamp's modification of the Ziehl-Neelsen stain.

<table>
<thead>
<tr>
<th>Species</th>
<th>Biovars</th>
<th>Colony Morphology*</th>
<th>Preferential Host(S)</th>
<th>Pathogenicity In Humans**</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. melitensis</td>
<td>1-3</td>
<td>smooth</td>
<td>sheep, goat</td>
<td>high</td>
</tr>
<tr>
<td>B. melitensis</td>
<td>1-3</td>
<td>smooth</td>
<td>sheep, goat</td>
<td>high</td>
</tr>
<tr>
<td>B. abortus</td>
<td>1-6,9</td>
<td>smooth</td>
<td>cattle</td>
<td>high</td>
</tr>
<tr>
<td>B. suis</td>
<td>1,3</td>
<td>smooth</td>
<td>pig</td>
<td>high</td>
</tr>
</tbody>
</table>
**Species** | **Biovars** | **Colony Morphology*** | **Preferential Host(S)** | **Pathogenicity In Humans**
--- | --- | --- | --- | ---
2 | smooth | wild boar, hare | low
4 | smooth | reindeer, caribou, rodents | high
5 | smooth | | no

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>
B. neotomae | - | smooth | desert rat | moderate
B. ovis | - | rough | ram | no
B. canis | - | rough | dog | moderate
B. pinnipedialis | - | smooth | cetaceans | ?†
B. ceti | - | smooth | seals | ?
B. microti | - | smooth | soil, vole, fox | ?
B. inopinata | - | smooth | human | ?

*‘Smooth’ *Brucella* spp. harbour a smooth lipopolysaccharide, whereas ‘Rough’ *Brucella* spp. harbour a rough lipopolysaccharide
**Pathogenicity in humans
†Although some human cases have been described, the actual pathogenicity remains unknown.

Information related to the epidemiology of brucellosis per country or continent, can be found at the World Animal Health Information Database (WAHID) Interface: [http://web.oie.int/wahis/public.php?page=home](http://web.oie.int/wahis/public.php?page=home)

The WAHID Interface provides access to all data held within OIE’s new World Animal Health Information System (WAHIS). Disease timelines (2005-2011) can be retrieved from the database. The information reported for South Africa can be summarized as follows: the presence of *B. abortus* is confirmed but limited to certain zones, both in cattle and wildlife. Confirmed clinical infection has also been reported. *Brucella melitensis* has been confirmed on 2 occasions in 2007 and 2010, whereas the disease has not been reported at other times during the period 2005-2011. *Brucella suis* has never been reported. *Brucella ovis* clinical infection has been seen in rams (epididymitis) during the whole period.
This rest of the section will present the main features of *Brucella abortus* infection in cattle and wildlife as a prototype “smooth” *Brucella*. To a large extent, these features are shared with *B. melitensis* infection in small ruminants. However, *B. melitensis* infection is rarely found in wildlife. Specific features related to *B. ovis* (which is a “rough” *Brucella*) are addressed in a separate section.

**Brucella abortus**

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. Cattle usually become infected after ingesting contaminated feed or water or licking an infected placenta, calf or fetus, 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 placental lochia. Animals may also become infected by inhaling organisms or through the conjunctiva. 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 and may spread the disease at their first and subsequent parturitions.

Although infected animals abort only once, subsequent calves are carried to full-term but may remain infected. Approximately 2.5 - 9% 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.

There appears to be 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. 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, particularly if artificial insemination using their semen is contemplated. The risk of introducing the disease into a herd through embryo transplantation is probably not significant.

*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 fetus 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. Generally, removal of infected animals from contaminated premises for one month is sufficient to prevent infection, provided the facilities have been properly disinfected.

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. There is a reduction in the numbers of organisms
shed in the months following calving and abortion, and cows eventually become non-infective until the next pregnancy when there is again a rapid increase of *Brucella* organisms in the reproductive tract. During subsequent pregnancies there is invasion of the gravid uterus and allantochorion 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. 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.

**Video link:** [https://www.youtube.com/watch?v=Pm3EmU4KzGE&feature=youtu.be](https://www.youtube.com/watch?v=Pm3EmU4KzGE&feature=youtu.be)

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. 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. 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 buffalo (*Syncerus caffer*), hippopotamus (*Hippopotamus amphibius*), zebra (*Equus burchelli*), eland (*Taurotragus oryx*) and impala (*Aepyceros melampus*)] have tested serologically positive for brucellosis, 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. There are few records of abortions in wildlife in southern Africa due to *Brucella* spp. although *B. abortus* biovar 1 has been isolated from the cotyledons of pregnant African
buffalo at slaughter. Although serological surveys have revealed up to 23 per cent positive reactors in African buffalo in the Kruger National Park in South Africa, 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.

*B. melitensis* infection has only been seen in wildlife in sable antelope (*Hippotragus niger*) in 2007 in the Eastern Cape province.

**Brucella ovis**

*Brucella ovis* produces a disease unique to sheep and is one of the most common causes of epididymitis in rams and a rare cause of abortion in ewes and neonatal mortality in lambs. Low reproductive rates may occur in affected flocks. It is a non-zoonotic disease. The disease is spread primarily by infected rams. *Brucella ovis* may be excreted in the semen of infected rams even before the development of lesions. Clinically or subclinically infected rams may excrete *B. ovis* in their semen for years.

Infection may spread from ram-to-ram as a result of homosexual activity or venereally during coitus when non-infected rams mate with ewes which passively harbour the bacteria. Ewes transfer organisms mechanically from infected to non-infected rams when they are mated in succession by different rams during the same heat period.

**PATHOGENESIS**

**Smooth Brucella spp. (*B. abortus, B. melitensis, B. suis*)**

*Brucella* spp. readily penetrate mucous membranes, such as those of the pharynx and alimentary tract, and survives and multiplies particularly in cells of the mononuclear phagocytic system. 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 five to ten 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. Occasionally bacterial localization occurs in synovial structures causing a purulent tendovaginitis, arthritis or bursitis.

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, elevated levels of which occur in the placenta and fetal fluids from about the fifth month of gestation. The chorionic epithelium becomes
colonized and infection extends to the placental stroma, blood vessels and ultimately, to the foetus. There is considerable variation in the uterine and placental lesions in both natural and experimental *Brucella* spp. 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. The abundance of erythritol in the pregnant uterus results in the massive multiplication of *Brucella* organisms in this organ. In the pregnant animal, *Brucella* spp. replicate in the placental trophoblast during middle and late gestation after the cells have actively begun secreting steroids. The mechanism leading to abortion after mid gestation 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.

Up to 35% of cows may be resistant to infection with *B. abortus* because their macrophages have a greater ability to kill *B. abortus*. The level of macrophage function which is reduced in susceptible cows plays a role in the establishment of chronic infections. This enhanced macrophage killing activity is significantly greater in cows that are genetically resistant to infection including that caused by *Mycobacterium bovis*, *Salmonella Dublin* and *Salmonella Typhimurium* as well as *B. abortus*. 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 of the replication of *B. abortus*.

Phagocytes have developed antimicrobial defense 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 defenses, 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 *Brucella* spp. into murine macrophages and mediate an endocytic pathway avoiding fusion with lysosomes. It has been shown that human macrophage phagosomes rapidly acidify to a pH of 4.0–4.5 following *Brucella suis* infection and that this early acidification is crucial for intracellular replication as neutralization results in bacterial elimination. In addition, if the phagosomal membrane is disrupted, then *B. suis* fails to multiply intracellularly. These results highlight the necessity of an intact, acidic phagosome as a predominant replicative niche for *Brucella* spp. in macrophages; it is called the “brucelosome”. A series of genes are involved in the adaptation of *Brucella* spp. to three major stress conditions within the phagosome, i. e. acid stress, starvation and low oxygen tension.

Long-term residence of *Brucella* spp. in the phagosomal compartment of host macrophages is essential for their ability to produce disease in both natural and experimental hosts. *Brucella* spp. infections inhibit spontaneously occurring apoptosis in human monocytes, thus preventing host cell elimination. This might represent a strategy for the persistence of *Brucella* spp. in infected hosts. Studies with *Brucella* mutants
suggest that stationary-phase physiology is critical for their successful long-term residence in host macrophages, 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 *Brucella* spp. during their long-term survival in the phagosomal compartment of host macrophages.

Cytokines such as IFN-gamma, TNF-alpha, IL-2, IL-10 and IL-12 control the intracellular growth of *Brucella* spp. Amongst these cytokines, the most important is IFN-gamma which strongly activates macrophages and induces an enhanced intracellular killing of *Brucella* spp. 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. During the late stages of infection, *Brucella* spp. proliferate within the endoplasmic reticulum of host cells. They replicate extensively without inducing obvious damage to the infected cell, and therefore seem to promote the survival of the cells for their own benefit. 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.

**Brucella ovis**

Rams become infected after penetration by *B. ovis* of the mucous membranes of the prepuce, penis, nasal cavity or conjunctiva. This is followed by bacteraemia and localization of the infection in lymph nodes and organs such as the epididymis, ampullae, seminal vesicles, bulbo-urethral glands, spleen, liver and kidneys. Organisms are first excreted in the semen from between 31 and 45 days following exposure.

Gross lesions only develop in the genitalia. In rams the earliest lesions usually occur in the tail of the epididymis, but lesions in the head and/or body of the epididymis may develop later. Initially the bacteria cause degeneration and necrosis of the epididymal epithelium, resulting in leakage of semen into the interstitial tissues where it provokes a severe inflammatory reaction and the development of spermatic granulomas. Similar inflammatory changes may also occur in the vas deferens, ampullae, seminal vesicles, bulbo-urethral glands and testes.

Inflammatory cells (particularly neutrophils) in semen, and a decrease in the production and quality of sperm as a consequence of testicular degeneration, all lead to lower fertility or infertility. Infertility may also be caused by the total cessation of spermatogenesis or by obstruction of the epididymis by spermatic granulomas and the development of a spermatocoele. There is a direct relationship between the semen quality, extent of the epididymal lesions and the number of leukocytes present in the semen. The reduction in fertility is ascribed to both low spermatozoa counts and the high number of defective spermatozoa; defects of the spermatozoal head and neck being common.

In ewes organisms enter mainly through the vaginal mucosa. In pregnant ewes, the ensuing bacteraemia causes a placentitis that may result in abortion or the birth of lambs with reduced birth weights. However, despite induction of severe endometritis, *B. ovis* has a relatively low capacity to induce abortion in sheep.
After experimental infection, the uterus and the iliac and supramammary lymph nodes are the main target organs of *B. ovis* infection in ewes.

**DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS**

**Clinical signs & pathology**

*Brucella abortus*

The length of the incubation period of bovine brucellosis varies considerably. The incubation period has been defined inter alia as the period between exposure and abortion. 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. 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. Congenitally infected calves may remain seronegative for at least 18 months, after which they may manifest clinical signs. The longest recorded incubation period in a cow is nine years.

The abortion rate in infected herds is dependent on many factors and varies according to the susceptibility of the pregnant females, management practices, severity of the challenge, the period for which the herd has been infected, and environmental factors such as the quality of pastures which may affect cattle density, the climate and the topography. In fully susceptible herds, abortion rates vary from 30 to 70%, but in South Africa it seldom exceeds 50%. 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.

Abortions typically occur at approximately five to seven months of gestation, although some occur earlier or later.
Weak, full-term calves that often die shortly after birth are sometimes encountered. About 20% of infected animals do not abort, while >80% of animals that abort as a result of *B. abortus* infection do so only once. The placenta is not consistently retained after abortion but when it is, metritis is common.

Early abortion may result in a considerable reduction in the milk yield. Infection of the udder is clinically inapparent and the organ appears to be normal when palpated. In bulls, an acute to chronic, uni- or bilateral orchitis, epididymitis, and seminal vesiculitis occasionally occur. The scrotal circumference in these animals may be normal or severely increased. Strain 19 vaccination may also cause orchitis.

Uni- or bilateral hygromas, especially of the carpal joints may be evident in some animals in chronically infected herds, or may occasionally follow inoculation of heifers with strain 19.
Bilateral carpal hygromas in a cow

Carpal hygroma in a kudu

Bilateral carpal hygromas in an African buffalo

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.

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

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.

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. Parts of the intercotelydonary placenta are thickened, oedematous, yellow-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. Vasculitis, sometimes accompanied by thrombosis, may be evident in the chorion.

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, grayish-white foci of pneumonia of 1 mm or larger in diameter, may be present, particularly in the apical lobes. A fibrinous pleuritis sometimes accompanies the pneumonia.

A foetal lung showing areas of 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, bronchitis and bronchiolitis characterized by the accumulation of cellular debris, neutrophils and macrophages in the lumen of the bronchi and bronchioi, 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 Brucella spp. However, it is easy to
demonstrate the organisms in smears made from the abomasal content or wall and that have been stained with Stamp's modification of the Ziehl-Neelsen stain.

The udder in infected ruminants does not show any gross lesions, although the supramammary lymph nodes may be somewhat enlarged. 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.

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, spermatoc granulomas develop in the testicular parenchyma and epididymis in response to dead sperm.

**Brucella ovis**

The interval between the infection and the development of lesions in rams varies considerably, being anything from 50 to 250 days. In rams, the first detectable abnormality may be a marked deterioration in semen quality associated with the presence of inflammatory cells and organisms.

![Hot, swollen and oedematous testicle in a ram with ovine brucellosis](image)

The most consistent clinical sign is enlargement, particularly of the tail of the epididymis, which may be barely perceptible or up to a four- or fivefold increase in size. The head, body or the entire epididymis are less often affected. The lesions often occur unilaterally, but bilateral involvement is relatively common. Rams suffering from acute epididymitis are not usually systemically affected. The entire testis on the affected side may be hot, swollen and oedematous but only a localized swelling of the epididymis is detectable in animals that are less severely affected.
Clinically detectable lesions may be acute to chronic. Although chronic lesions may follow an acute epididymitis, they more commonly develop insidiously without clinical evidence of the acute phase.

![Enlargement of the entire epididymus in a ram with ovine brucellosis](image)

Chronic epididymitis is clinically characterized by enlargement and an increased consistency of the affected parts. As a result of fibrous adhesions, the mobility of the affected testis in the scrotum is often reduced. The marked increase in scrotal circumference caused by the epididymal and testicular lesions can be seen from a distance.

The testis is seldom primarily affected. In some cases the testis on the affected side may be slightly atrophied and have a somewhat softer consistency than normal, while in others with a severe, chronic epididymitis, the testis may be severely atrophied and firm. Affected rams may be sterile, or have reduced fertility. The degree of impairment depends on whether the lesions are uni- or bilateral, and on the course and severity of the lesions. The libido of affected rams remains unaffected.

Ewes abort very rarely as a result of *B. ovis* infection. In experimentally infected pregnant ewes, abortions may affect from none to about 30% of the inoculated animals. However, field reports suggest that as many as 50% of the pregnant ewes may abort.
Typical lesions in the affected epididymis include solitary or multiple spermatoceles and spermatic granulomas which contain a creamy fluid or inspissated, caseous material, thickening due to the presence of a low grade, non-purulent inflammatory response, and fibrosis of the interductal connective tissue and the tunica albuginea.

Spermatic granuloma in the epididymal tail of a ram with ovine brucellosis

In most cases fibrous adhesions form between the tail of the epididymis, the parietal tunica vaginalis and the distal pole of the testis.

Testicular atrophy, which is usually more severe in rams with widespread and severe adhesions, may accompany the epididymal lesions. In most cases, however, the changes in the testis are minimal and non-specific. Changes in the vas deferens and accessory sex glands may be similar to those in the epididymis.

Semen quality is determined by the extent and severity of the lesions in the epididymes, testes, and accessory glands. Poor semen quality is characterized by reduced density of the ejaculate (due to decreased numbers of spermatozoa), reduced motility and longevity of spermatozoa, an increase in the proportion of abnormal spermatozoa such as those with detached sperm heads, mid-piece abnormalities, bent tails, coiled tails, and tails tightly coiled around the heads of spermatozoa and the presence, in many cases, of varying numbers of leukocytes, particularly neutrophils.

The carcasses of aborted lambs are not autolyzed but are dehydrated, and they manifest a fibrinous peritonitis. The accompanying placentitis is characterized by a yellowish fibrinous exudate which is present particularly in the intercotyledonary areas. Histologically the lesions in the placentas are characterized by a multifocal suppurative inflammation. Foetal tissues manifest a suppurative bronchitis, bronchiolitis and bronchopneumonia.
Laboratory confirmation

Diagnostic tests can be applied with different goals: confirmatory diagnosis, screening or prevalence studies, certification, and, in countries where brucellosis has been eradicated, surveillance in order to avoid the reintroduction of brucellosis through importation of infected animals or animal products. Diagnostic methods include direct tests, involving microbiological analysis or DNA detection by polymerase chain reaction (PCR)-based methods and indirect tests, which are applied either in vitro (mainly to milk or blood) or in vivo (allergic test). The choice of a particular testing strategy depends on the prevailing epidemiological situation of brucellosis in susceptible animals (livestock and wildlife) in a country or a region.

Bacteriology and PCR based methods

Isolation of Brucella spp. or detection of Brucella spp. DNA by PCR is the only method that allows certainty of diagnosis. Biotyping provides valuable epidemiological information that allows tracing of infections back to their sources in countries where several biotypes are co-circulating. However, when one particular biovar is overwhelmingly predominant, classical typing techniques are of no use because they do not allow the differentiation of isolates belonging to the same biovar of a given biotype. In this context, new fingerprinting methods such as multiple locus variable (number of tandem repeats) analysis (MLVA), which measures the number of tandem repeats at a given locus and multi-locus sequence analysis (MLSA) can differentiate isolates within a given biovar. These methods are gaining wider acceptance and will in the coming years almost certainly be used as routine typing and fingerprinting methods for molecular epidemiological purposes.

New PCR techniques allowing identification and sometimes quick typing of Brucella spp (both the “Smooth” and “Rough” species) have been developed and are in use in certain diagnostic laboratories. The best validated methods are based on the detection of specific sequences of Brucella spp., such as the 16S-23S genes, the IS711 insertion sequence or the bcsA gene encoding a 31-kDa protein. As a general rule, brucellosis PCR techniques show a lower diagnostic sensitivity than culture methods, although their specificity is close to 100%. The best results have so far been obtained by combining culture and PCR detection on clinical samples. A description of the bacteriology methods and the PCR based tests can be found on the World Organization for Animal Health (Office International des Epizooties, OIE), website: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf.

For typing of Brucella spp., the multiplex AMOS PCR, named for its applicability to “abortus, melitensis, ovis, suis” species, is often used. This PCR and PCR protocols derived from it allow discrimination between Brucella species and between vaccine and wild-type strains. They do not, however, allow discrimination among all the biovars of a given Brucella species. The multiplex “Bruce ladder” PCR is the first method designed to identify and differentiate all of the known Brucella species and the vaccine strains in the same test.
The lack of PCR-based methods to discriminate among biovars within a species stimulated the development of other molecular typing techniques for *Brucella* spp., such as restriction fragment length polymorphism analysis based on the number of IS711 insertion sequences.

**Indirect diagnostic tests**

**Serological tests**

The same “smooth” *Brucella* antigens are used in serological tests to detect antibodies induced by *B. abortus*, *B. melitensis* or *B. suis*. These serological tests do not detect infections caused by *B. ovis* and *B. canis*. For these infections, “rough” *Brucella* antigens must be used.

These tests are derived from research done mainly on brucellosis diagnosis in cattle. To a large extent the characteristics of the different tests can be transposed to sheep and goats, except for the milk ring test, which is not an accepted test in these species because it generates too many false positive results.

In pigs, infection by *Yersinia enterocolitica* serotype O:9 (YO9) is not uncommon in some areas, particularly in Europe. Since YO9 and *Brucella* share a polysaccharide ‘O’ chain, *Brucella* spp. antigens used in serological tests react equally well with the surface smooth LPS of YO9 and are therefore unable to distinguish between antibodies to these two pathogens. Thus, as determined by the OIE, none of the conventional serological tests used for the diagnosis of porcine brucellosis is reliable for diagnosis in individual pigs.

Several studies of brucellosis serology have been performed in wildlife as well as in zoo collections, with the goal of assessing the presence or spread of *Brucella* spp. within different wild species and to classify species or individuals as exposed or non-exposed. Brucellosis serology is usually performed using the same antigens as in livestock serology because the immunodominant *Brucella* antigens occur on the surface smooth LPS and are to a large extent shared by all naturally occurring biovars of *B. abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. ceti*, *B. pinnipedialis* and *B. microti*. Most brucellosis serological tests have been directly transposed to wild species, without validation, from domestic livestock populations, where their use has quite often not been validated either.

In order to validate serological tests, results should be analysed according to the true infectious status of an animal. The presence of anti-*Brucella* antibodies suggests exposure to *Brucella* spp., but it does not indicate which *Brucella* species induced production of those antibodies. Moreover, seropositivity does not necessarily mean that the animals have current or active infection at the time of sampling. In fact, studies of experimental and natural infections indicate that nearly all animal species vulnerable to *Brucella* infection can lose their antibody titers. This means that the actual prevalence of brucellosis may be higher than that indicated by antibody screening. Therefore, the “gold standard” in brucellosis remains the isolation of *Brucella* spp. If brucellosis is
suspected in livestock or in wildlife because of positive serological results, attempts to isolate the organism are considered mandatory and should always be performed.

A sound description of the different tests for the diagnosis of ovine epididymitis (B. ovis) is available online on the OIE web site:

A sound description of the different tests for the diagnosis of brucellosis caused by “smooth” Brucella spp. is available online on the OIE web site:
http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf.

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.02_CAPRINE_OVINE_BRUC.pdf.

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.05_PORCINE_BRUC.pdf.

In the following section, only the primary benefits and shortcomings of the tests will be addressed. Sensitivities and specificities of indirect tests, as documented in the literature, are depicted in Table 2.
Table 2. Sensitivity and specificity of indirect tests for the diagnosis of cattle brucellosis as published in the literature.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serological tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT (SAW)/MAT</td>
<td>81.5</td>
<td>98.9</td>
</tr>
<tr>
<td>CFT</td>
<td>90-91.8</td>
<td>99.7-99.9</td>
</tr>
<tr>
<td>BAT</td>
<td>87</td>
<td>97.8</td>
</tr>
<tr>
<td>iELISA</td>
<td>97.2</td>
<td>97.1 - 99.8</td>
</tr>
<tr>
<td>cELISA</td>
<td>95.2</td>
<td>99.7</td>
</tr>
<tr>
<td>FPA</td>
<td>96.6</td>
<td>99.1</td>
</tr>
<tr>
<td><strong>Milk tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRT</td>
<td>88.5</td>
<td>77.4</td>
</tr>
<tr>
<td>FPA</td>
<td>76.9</td>
<td>100</td>
</tr>
<tr>
<td>iELISA</td>
<td>98.6</td>
<td>99.0</td>
</tr>
<tr>
<td><strong>Cellular tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin test</td>
<td>78-93</td>
<td>99.8</td>
</tr>
</tbody>
</table>

SAT : Slow Agglutination Test; SAW: Slow Agglutination of Wright; MAT: Micro Agglutination Test; CFT: Complement Fixation Test; BAT: Buffered Brucella Antigen Test, iELISA: indirect ELISA; cELISA: competitive ELISA; FPA: Fluorescence Polarization Assay; MRT: Milk Ring Test.
**Slow Agglutination Test or Slow Agglutination of Wright (SAT or SAW)**

The principle of this test is to detect agglutinating antibodies mainly of the IgM isotype directed against *Brucella* spp. At an optimum concentration of antigens and antibodies, large antigen-antibody complexes form and precipitate at the bottom of the test tube. This reaction is slow because, in contrast to the rapid agglutination tests, it requires an overnight incubation at 37°C. This technique can also be performed in small reaction volumes of 100 µl, without a change in performance (microagglutination test, - MAT). Reading of the result is facilitated by the addition of a dye that stains the bacterial cells. The relative lack of specificity and sensitivity of this test has often been presented as a major drawback. Nevertheless, this is a standardised and extremely robust test that has shown good results and has proven its efficacy in several countries now declared officially free of brucellosis. The specificity of the test is increased by treating the serum with a chelating agent such as EDTA. This treatment reduces cross-reactions due to IgM. On the other hand, serum agglutination activity is increased by the addition of anti-IgG antiserum (Coombs) that reveals incomplete or blocking antibody activity of IgG that have little agglutination activity. Although this test is no longer recommended by the OIE for bovine brucellosis diagnosis, it is still widely used in human brucellosis diagnosis.

**Buffered Brucella antigen tests**

The Rose Bengal (RB) and buffered plate agglutination (BA) tests are the well-known buffered *Brucella* antigen tests. These tests are rapid agglutination tests lasting 4 minutes and done on a glass plate with the help of an acidic-buffered antigen (pH 3.65 ± 0.05). This test has been introduced in many countries as the standard screening test because it is very simple and thought to be more sensitive than the SAT. These tests are “prescribed tests for trade” by the OIE.

**Complement fixation test**

The complement fixation test (CFT) allows the detection of anti-*Brucella* antibodies that are able to activate complement. Cattle immunoglobulins that can activate bovine complement are the IgG and the IgM. According to some literature this test is not highly sensitive but shows an excellent specificity. Because the test is difficult to standardize, it is progressively being replaced by ELISAs. This test is a “prescribed test for trade” by the OIE.

**ELISAs**

ELISAs are divided into two categories, the indirect ELISA (iELISAs) and the competitive ELISA (cELISAs). Most iELISAs use purified smooth LPS as antigen but a good deal of variation exists in the anti-bovine Ig conjugate used. Most iELISAs detect mainly IgGs or IgG sub-classes. Their main quality is its high sensitivity but are also more vulnerable to non-specific reactions, notably those due to YO9 infection. These cross-reactions seen in iELISAs motivated the development of cELISAs. The O-chain of the smooth LPS of *Brucella* contains specific epitopes that are not
shared with the LPS of YO9. Therefore, by using monoclonal antibodies directed against specific epitopes of the *Brucella* LPS, the development of more specific cELISAs has been possible. These tests are more specific, but less sensitive, than iELISAs. These tests are “prescribed tests for trade” by the OIE.

**Fluorescence Polarisation Assay**

The fluorescence polarisation assay (FPA) is based on a physical principle: how quickly a molecule spins in a liquid medium correlates with its mass. Molecules of small size spin faster and depolarize a polarized light beam more, while bigger molecules spin more slowly and, consequently, depolarize light less. FPA measures the degree of depolarization in milli-polarization units (mP). During the test, serum samples are incubated with a specific antigen of *B. abortus* labelled with fluorescein isothiocyanate. In the presence of antibodies against *Brucella* spp., large fluorescent complexes are formed. In negative samples, the antigen remains uncomplexed. These smaller molecules spin more quickly and therefore cause greater depolarisation of the light than do the samples positive for *Brucella* spp.

This test can be easily automated and is very quick, since after mixing the labelled antigen and serum, the reading is almost instantaneous. The test sensitivity seems slightly lower than that of iELISAs. The specificity varies between 98.8 and 99.0%. This test is already used in brucellosis control and certification programs in North America and in Europe. This test is a “prescribed test for trade” by the OIE.

**Milk tests**

These tests are prescribed by the OIE as tests to use in control and eradication programs but not for trade purposes.

**Milk Ring Test**

The test consists of mixing coloured *Brucella* whole-cell antigen with fresh bulk/tank milk. In the presence of anti-*Brucella* antibodies, antigen-antibody complexes form and migrate to the cream layer, forming a purple ring on the surface. In the absence of antigen-antibody complexes, the cream remains colourless. This test is not considered sensitive but this lack of sensitivity is compensated by the fact that the test can be repeated, usually monthly, due to its very low cost. This test is prescribed by the OIE for use only with cow’s milk.

**ELISAs and Fluorescence Polarisation Assay FPA**

These two tests, discussed above in the context of serum samples, can also be applied to milk samples. The sensitivity of these tests applied to milk is lower than when applied to sera. Before being applied to tank milk (which may represent a pool of milk produced by hundreds of cows), a validation of the detection on a pool of milk samples has to be checked. This lower sensitivity in
the case of tank milk can often be compensated by increasing the testing frequency. These tests are prescribed by the OIE for testing the milk of cattle and small ruminants.

**Skin test**

The skin test is an allergic test that detects the specific cellular immune response induced by *Brucella* spp. infection. The injection of brucellergene, a protein extract of a rough strain of *Brucella* spp, is followed by a local inflammatory response in a sensitised animal. This delayed-type hypersensitivity reaction is measured by the increase in skin thickness at the site of inoculation. This test is highly efficient in discriminating between true brucellosis cases and false positive serological reactions (FPSR). The skin test is highly specific but its weak sensitivity makes it an acceptable test for herds but not for individual certification. It cannot discriminate between infection and vaccination. This test is prescribed as an alternative test by the OIE.

**Strategic use of serological tests**

This section highlights the strategic use of certain serological tests in order to discriminate (1) between false positive serological reactions and true brucellosis and (2) between vaccination and infection.

Brucellosis is an infectious disease but animals are not always contagious. Indeed, excretion of *Brucella* spp. only occurs at certain times, mainly when abortion occurs. During an abortion, billions of *Brucella* spp. are excreted and this is a major source of infection for congeners and for professionals in contact with aborted materials. In order to avoid contamination from aborted material, it is important (1) to isolate pregnant heifers in their 6th month of pregnancy, given that brucellosis induces abortion usually in late pregnancy; and (2) to predict abortion and eliminate animals likely to abort, before they become a source of infection. Vaccination does not provide complete protection from exposure to *Brucella* spp. Therefore the key question to be addressed is: when a pregnant animal is infected, regardless of whether or not it has been vaccinated, is it possible to predict whether it will abort? Key factors that determine the answer to this question are the kinetics of antibody production and the type of antibodies produced.

**Kinetics of the immune responses in cattle**

Indirect diagnostic tests are based on the detection of immune responses induced by infection. These tests show different sensitivities and specificities depending on numerous variables, such as the infection dose and route, the presence of so-called "cross-reactive bacteria" antigenically similar to *Brucella* spp., the kinetics of the induced immune response and previous vaccination. Serology is the method of choice for screening in any sound control or eradication program. Strong humoral immune responses are induced after exposure. Humoral IgG responses persist after the peak of the response (3-4 weeks post-infection) and remain detectable over long periods of time (up to several years); in contrast, the IgM response is rapidly induced 2-3 weeks after
exposure and may disappear after a few months. The cell-mediated immune response (induced 3-4 weeks after exposure), as measured by the brucellosis skin test, is long-lasting and can be detected for several years. Thus, given the kinetics of the immune responses induced after infection, when the different tests are performed after exposure has a major impact on the results, as depicted below.

![Outcome of SAW and ELISA tests performed at different times post-infection.](chart)

According to the time point after infection at which sampling and testing occur, different serological results may be generated. Therefore, epidemiological information is extremely important for interpretation of the test results. IgG responses will be induced 1 or 2 weeks later than the IgM response but they will last for long periods of time, usually years. The intensity of the response is measured by serum antibody titers using SAT, which measures mainly IgM, and iELISA, which measures mainly IgG. The kinetics of production and disappearance of the principal immunoglobulin isotypes during infection, and the activity of these immunoglobulins in the different serological tests, will usually permit the distinction between acute and chronic infections. For example, the immune responses against B. abortus in cattle are rapid IgM production 2-3 weeks after experimental infection, followed by IgG production 3-4 weeks after experimental infection. Therefore, the following principles apply:

1. The concomitant presence of IgM (detected in an agglutination test) and IgG (detected in iELISA) suggests acute brucellosis, while chronic brucellosis is characterized by the presence of IgG alone.
2. A positive response in an agglutination test, which detects mainly IgM, is not indicative of brucellosis if it is not confirmed by a positive IgG response by iELISA within one week.
The SAT and the buffered agglutination tests (BAT -BAT/RB Rose Bengal) are commonly used as screening tests for the diagnosis of bovine brucellosis. However, the OIE and the EU have recently decided not to recommend use of the SAT because they consider it inferior to the other standard tests. The complement fixation test (CFT) is used as a confirmatory test after a positive agglutination reaction. This test is gradually being replaced by iELISAs and, more recently, by the FPA. All these tests must be standardized and should be performed according to validated standard operating procedures (SOPs) in accredited laboratories.

Serology and vaccination

For over 60 years, the B. abortus S19 vaccine has been used in cattle and the B. melitensis Rev.1 vaccine has been used in sheep and goats to prevent abortion and infertility caused by natural infection with virulent strains of these Brucella species. These vaccines, combined with serologic surveillance tests, have been instrumental in the success of the brucellosis eradication program. Conventional serologic tests for brucellosis detect antibody against the LPS antigens induced by vaccination with S19 or Rev. 1 or exposure to virulent field strains. Therefore, no single serologic test can differentiate, beyond any reasonable doubt, animals vaccinated with S19 or Rev. 1 and animals infected with virulent Brucella spp. field strains. Nevertheless, strategic use of tests to detect different isotypes of immunoglobulins provides useful information in order to differentiate vaccination from infection. Indeed, more than 90% of heifers vaccinated with S19 were classified negative by classical serological tests (i.e. SAT, RB and CFT) at 16 weeks post-vaccination, while they were still classified positive by iELISAs. Moreover, under experimental conditions, the kinetics of antibody production differs between vaccination and infection such that iELISAs can be used to predict abortion in heifers and thus allow their elimination before congeners can be contaminated.

Recently, a rough mutant of B. abortus, strain RB51, has been proposed for use as a vaccine for cattle of all ages. Although RB51 expresses low levels of the O side chain, naïve animals remain seronegative in surveillance tests following vaccination with RB51. This is a major advantage in a control program based on vaccination combined with serological testing. Unfortunately the efficacy of the RB51 vaccine in cattle is still questionable. RB51 has been shown to be non-protective in small ruminants. Currently, there is no vaccine available for humans, pigs or wildlife.
CONTROL / PREVENTION

Successful eradication programmes have always been costly, long, and hard to carry through. The difficulties in controlling and eradicating brucellosis stems from a variety of issues, the most important of which is the animal management conditions (extensive breeding, transhumance, co-existence of several livestock species, etc.). Most often, endemic areas are in countries with structural weaknesses, an aggravating circumstance since efficient use of current vaccines requires proficient veterinary services.

This requirement relates in part to some of the limitations of currently available brucellosis vaccines, and it seems likely that an ideal vaccine could greatly facilitate control and eradication. The ideal brucellosis vaccine should: (1), trigger a solid and life-long immunity; (2), protect against infection by *Brucella* species other than those typical of a given host; (3), be innocuous regardless of the physiological state of the animal; (4), be effective in a single dose; (5), not interfere with serological diagnostic tests; (6), not be virulent for humans or carry resistance to antimicrobial drugs; (7), not be shed in the environment; (8), be stable; and (9), be affordable. Indeed, some of these requirements have become apparent only after using the classical brucellosis vaccines for more than half a century. To what extent has those requirements been met and what approaches have been followed to solve some of the problems?

The two best vaccines developed in the past century (*B. abortus* S19 and *B. melitensis* Rev 1) are both attenuated (live) vaccines with a certain degree of residual virulence. Strain 19 is used in cattle and Rev 1 in goats and sheep, not only against *B. melitensis* but also against *B. ovis*. Both vaccines carry a smooth (S) lipopolysaccharide (S-LPS) with an O-polysaccharide similar to that of the wild type *Brucella*.

Limitations of the Rev 1 vaccine are the abortifacient effect if applied during pregnancy, interference in serological diagnosis, virulence for humans and resistance to streptomycin and tendency to dissociate into ineffective rough [R] mutants. These limitations can be partially overcome by vaccinating animals conjunctivally when they are less than 4 months old which reduces greatly the interference in serological diagnosis and avoids vaccine-induced abortions; a minimal personal protection makes Rev 1 vaccination safe; and there are well-established quality control protocols. Rev 1 has been crucial wherever *B. melitensis* eradication has been achieved and, moreover, vaccination with Rev 1 is economically sound. Since cattle may become infected with *B. melitensis* (and by some *B. suis* biovars), it has been suggested that Rev 1 could be used in these ruminants. However, the protective efficacy against *B. melitensis*, innocuousness and safety of Rev 1 in cattle is not known. *B. melitensis* infections in cattle can be controlled with the help of S19 but there is a paucity of studies with regard to *B. suis*.

With the exception of a handful of countries with favorable geographical and management conditions, all successful programs in cattle have used S19. Subcutaneously, standard S19 doses generate immune responses interfering in diagnostic tests and may induce abortions if applied during pregnancy and genital lesions in males. Moreover, a small proportion of animals may develop subclinical infections and shed the vaccine. Conjunctival vaccination with reduced doses when animals are less than 4 months old avoid the abortions as well as the serological interference and udder infections. It is not known whether this route
and doses make S19 safe in males, a point that would be worth investigating. Conjunctival vaccination is also adequate for vaccinating adult cattle since abortions and milk shedding are reduced to less than 1%.

Despite their limitations, S19 and Rev 1 have been successfully used in some developed countries to eradicate brucellosis. However, their use in eradication programmes poses the problem of distinguishing infected from vaccinated animals in serological tests. Although it is important to stress that this problem is of little or no significance in countries unable to implement testing and slaughtering programmes, this has been considered the major drawback of these vaccines.

*B. abortus* RB51 is an R mutant obtained by passage on media with rifampin and penicillin. It carries a mutation in the O-polysaccharide gene *wboA* but also other and unknown genetic defects. Concerning protection, controlled experiments show that RB51 is inferior to S19. RB51 does not elicit significant amounts of antibodies to the O-polysaccharide so that its interference in brucellosis tests that use smooth *Brucella* suspensions is minimal. However, the antibodies induced by RB51 are detected in tests apparently specific for Smooth *Brucella* spp. RB51 can induce abortions and can be excreted, and its use should be limited to non-pregnant animals. Since RB51 is more attenuated, it should be less dangerous than S19, and only very few human cases have been described. Although introduced over 12 years ago, no country using RB51 has eradicated cattle brucellosis although success has recently been suggested in the Azores, Portugal. However, such field observations are either contradictory or controversial because of the implementation of additional control measures and the absence of appropriate control groups. RB51 does not protect sheep against either *B. melitensis* or *B. ovis*.

Given the successful eradication of *B. abortus* and *B. melitensis* in some developed countries, it may be asked whether this research is necessary at all. The answer is that the organization and favorable environmental conditions found in these countries were a decisive factor in eradication. Those conditions are unlikely to be reached soon or even be possible in many areas where brucellosis is endemic and control and eradication can be facilitated only by an ideal brucellosis vaccine. Moreover, vaccines for reindeers, water buffaloes, yaks, camels, and swine, all susceptible animals that are important in the economies of many countries, are still missing.

**MARKETING AND TRADE / SOCIO-ECONOMICS**

In a study done in Kampala, urban residents who had no contact with livestock were at risk of being *Brucella* infected, an exposure attributed to consumption of raw milk products purchased from rural and peri-urban area. Since consumption of raw milk continues to be a major mode of exposure as demonstrated in several studies, pasteurization or boiling of milk and milk products is likely to reduce human infections. A survey conducted in Kenya showed that boiling of milk reduced the risk of exposure to *Brucella*. Other factors contributing to exposure included ignorance of risk of *Brucella* infection. In some cases, perceived enhanced nutritional qualities, taste, and health benefits have all been advocated.
as reasons for increased interest in raw milk consumption. Therefore, involvement of anthropologists and social workers will become increasingly important in successful control of human brucellosis.

According to the World Organisation for Animal Health (OIE), bovine brucellosis is a reportable zoonosis and is of considerable socioeconomic concern. It is of major importance in the international trade of animals and animal products. Because brucellosis has public health and international-trade implications, all member states of the OIE have an obligation of reporting.

**FAQ**

1. **What is brucellosis?**

   It is a contagious, costly disease of ruminant animals that also affects humans. Although brucellosis can attack other animals, its main threat is to cattle, sheep and goats, and swine. It can also affect wildlife.

2. **How serious is brucellosis?**

   Considering the damage done by the infection in animals such as decreased milk production, weight loss in animals, loss of young, infertility, and lameness, it is one of the most serious diseases of livestock. The rapidity with which it spreads and the fact that it is transmissible to humans makes it all the more serious.

3. **What disease agents cause brucellosis?**

   The disease is caused by a group of bacteria known scientifically as the genus *Brucella*. Three species of *Brucella* cause the most concern: *B. abortus*, principally affecting cattle; *B. suis*, principally affecting swine; and *B. melitensis*, affecting sheep and goats. Bacteria are shed in milk or via the aborted fetus, afterbirth, or other reproductive tract discharges.

4. **What are the clinical signs of brucellosis?**

   There is no effective way to detect infected animals by their appearance. The most obvious signs in pregnant animals are abortion or birth of weak calves. Milk production may be reduced from changes in the normal lactation period caused by abortions and delayed conceptions. Not all infected cows abort, but those that do usually abort between the fifth and seventh month of pregnancy. Infected cows usually abort once, but a percentage will abort during additional pregnancies, and calves born from later pregnancies may be weak and unhealthy. Even though their calves may appear healthy, infected cows continue to harbour and discharge infectious organisms and should be regarded as dangerous sources of the disease. Other signs of
Brucellosis include an apparent lowering of fertility with poor conception rates, retained afterbirths with resulting uterine infections, and (occasionally) enlarged, arthritic joints.

5. **How is brucellosis spread?**

Brucellosis is commonly transmitted to susceptible animals by direct contact with infected animals or with an environment that has been contaminated with discharges from infected animals. Aborted fetuses, placental membranes or fluids, and other vaginal discharges present after an infected animal has aborted or calved are all highly contaminated with infectious *Brucella* organisms.

6. **What is being done to fight brucellosis?**

In the developed world, programs to eliminate the disease from the country are implemented. Like other animal disease-eradication efforts, success of the program depends on the support and participation of livestock producers. In the developing world, the disease is at best controlled but often baseline information is missing.

7. **How do epidemiologists help fight brucellosis?**

Epidemiologists are specially trained veterinarians who investigate disease sources and the means of eliminating infection in affected herds and areas. Epidemiologists are concerned with disease in a group or population of animals and evaluate circumstances connected with the occurrence of disease. These veterinarians help eliminate brucellosis by identifying factors essential to its control and prevention.

8. **How costly is brucellosis to the livestock industry?**

In the US, annual losses from lowered milk production, aborted calves and pigs, and reduced breeding efficiency have decreased from more than $400 million in 1952 to less than $1 million today. Studies have shown that, if brucellosis eradication program efforts were stopped, the costs of producing beef and milk would increase by an estimated $80 million annually in less than 10 years.

9. **What is the basic approach to eradication?**

The basic approach has always been to test cattle for infection and send infected animals to slaughter. Identification of market animals for tracing, surveillance to find infected animals, investigation of affected herds, and vaccination of replacement calves in high-risk areas are important features of the current program.

10. **How is infection found in cattle?**
Two primary surveillance procedures are used to locate infection without having to test each animal in every herd. Milk from dairy herds is checked two to four times a year by testing a small sample obtained from creameries or farm milk tank for evidence of brucellosis. Cattle herds that do not produce milk for sale are routinely checked for brucellosis by blood-testing animals sold from these herds at livestock markets or at slaughter. In addition, it is sometimes required that adult cattle and bison are subjected to blood tests for brucellosis upon change of ownership even if sold directly from one farm to another.

11. Can brucellosis in animals be cured?

No. Repeated attempts to develop a cure for brucellosis in animals have failed. Occasionally, animals may recover after a period of time. More commonly, however, only the signs disappear and the animals remain diseased. Such animals are dangerous sources of infection for other animals with which they associate.

12. Can brucellosis be prevented?

The disease may be avoided by employing good sanitation and management practices. Replacement animals should be tested when purchased and retested after a 30- to 60-day isolation period during which they are kept separate from the remainder of the herd. These practices will allow detection of animals that were in the incubation period of the disease when acquired.

13. What about vaccination?

For cattle and bison in heavily infected areas or replacement animals added to such herds, officials recommend vaccinating heifers with an approved Brucella vaccine. The vaccine is a live product and must be administered only by an accredited veterinarian. For best results, female calves should be vaccinated when they are 4 to 6 months old. At the time of vaccination, a tattoo is applied in the ear; that tattoo identifies the animal as an "official vaccinate." The tattoo identifies the year in which vaccination took place.

14. Are all the Brucella species zoonotic?

No. The most dangerous ones are B. melitensis B. abortus, and B. suis. The vaccine strains S19, Rev. 1 and RB51 may also infect humans. Among the B. suis biovars, Brucella suis biovar 2 (which infects wildboars and hares in Europe) is not pathogenic for humans. Likewise, B. ovis is not pathogenic for humans. Brucella canis may occasionally infect humans.
15. Which humans are at risk for brucellosis?

Everyone is susceptible to the bacteria and may get the disease if exposed. People usually are infected in one of three ways: eating or drinking something that is contaminated with *Brucella*, breathing in the organism (inhalation) or having the bacteria enter the body through skin wounds. The most common ways to be infected include eating or drinking contaminated, non-pasteurized milk products and working with infected animals or their tissues.

16. What are the symptoms of brucellosis in humans?

Symptoms of brucellosis include irregular fevers of varying lengths, headache, weakness, swollen lymph nodes, excessive sweating, chills, weight loss and generalized aching. How soon do symptoms appear? The time period is highly variable, five to 60 days, but symptoms usually appear within one to two months. How is brucellosis spread? Direct person-to-person spread of brucellosis is extremely rare. Rare instances of infected mothers who are breast-feeding, sexual transmission and contaminated tissue transplantation have been reported. Typically, brucellosis is spread by eating non-pasteurized milk and other dairy products from infected cows and goats. People also may become infected when they work or handle blood, urine, discharges and aborted fetuses from infected cattle, pigs or goats. In this case, infection occurs through cuts and scrapes in the skin or breathing the bacteria in the air. When and for how long is a person able to spread the disease? It is unlikely that this disease could spread from person to person. How is a person diagnosed? Brucellosis may be diagnosed in a laboratory by looking at samples of blood or bone marrow. If you think you have brucellosis, contact your health-care provider.

17. How are humans treated?

Brucellosis can be treated with antimicrobial drugs, but treatment can be difficult. Doctors may prescribe doxycycline and rifampin in combination for six weeks to prevent recurring infection. Depending on the timing of treatment and severity of illness, recovery may take a few weeks to several months. Fewer than 2 percent of people who have the disease die.

18. Does past infection make a person immune?

It is unlikely that an individual will develop the disease again.

19. Should children or others be excluded from day care, school, work or other activities if they have brucellosis?

No. Infants, toddlers and school-age children should not be excluded unless the staff determines the child is unwilling or unable to participate in activities. They also should be excluded if the staff determines that they cannot care for the child without compromising their ability to care for the health and safety of the other children in the group. All others can attend work and other functions.
as long as they are well enough to do so. As always, good hand washing and respiratory
etiquette is recommended.

20. What can be done to prevent the spread of brucellosis among humans?

The control of the human disease depends on the removal of the disease in cattle, goats, swine
and other animals. Pasteurization of milk and milk products, including soft cheeses, which are
meant for human consumption is important to prevent disease. The certification of raw milk does
not eliminate the risk of transmission of *Brucella* bacteria.

REFERENCES

On line

   http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELLE.pdf
   brucellosis.
   http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.02_CAPRINE_OVINE_BRUC
   .pdf
   http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.09_OVINE_EPID.pdf

Books and book chapters

1. Techniques for the brucellosis laboratory. Alton GG, Jones LM, Angus RD, Verger JM. 1st
   - 3 volumes.
   - *Brucella abortus* infection. Godfroid J, Bishop GC, Bosman PP, Herr S.
   - *Brucellamelitensis* infection. Godfroid J, Garin-Bastuji B, Blasco JM, Thoen CO, Angus RD.
- *Brucella ovis* infection. Blasco JM, Garin-Bastuji B, Thoen CO, Gilsdorf MJ, Godfroid J.
- *Brucella melitensis* infection. Godfroid J, Angus RD, Thoen CO.
- Brucellosis in wildlife. Godfroid J.

   - Bovine brucellosis. Saegerman C, Berkvens D, Godfroid J, Walravens K.


**Literature**


