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.
A partially decomposed bovine foetus and placenta

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.

Severe placentitis revealing pale yellow foci in cotyledons and thickening of the intracotyledonary chorioallantois

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.
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 intercotyledonary 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.

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, spermatic 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

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.
Livestock Health, Management and Production › High Impact Diseases › Contagious Diseases › Brucellosis

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 spermatocoeles 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](image)

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 bcsps31 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 \textit{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 \textit{Brucella} spp., such as restriction fragment length polymorphism analysis based on the number of IS711 insertion sequences.

\textbf{Indirect diagnostic tests}

\textbf{Serological tests}

The same “smooth” \textit{Brucella} antigens are used in serological tests to detect antibodies induced by \textit{B. abortus}, \textit{B. melitensis} or \textit{B. suis}. These serological tests do not detect infections caused by \textit{B. ovis} and \textit{B. canis}. For these infections, “rough” \textit{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 \textit{Yersinia enterocolitica} serotype O:9 (YO9) is not uncommon in some areas, particularly in Europe. Since YO9 and \textit{Brucella} share a polysaccharide ‘O’ chain, \textit{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 \textit{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 \textit{Brucella} antigens occur on the surface smooth LPS and are to a large extent shared by all naturally occurring biovars of \textit{B. abortus}, \textit{B. melitensis}, \textit{B. suis}, \textit{B. neotomae}, \textit{B. ceti}, \textit{B. pinnipedialis} and \textit{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-\textit{Brucella} antibodies suggests exposure to \textit{Brucella} spp., but it does not indicate which \textit{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 \textit{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 \textit{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: 
http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.09_OVINE_EPID.pdf

A sound description of the different tests for the diagnosis of brucellosis caused by "smooth" \textit{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.

![Diagram of IgM and IgG responses over time with SAW and iELISA cut-offs]

**Outcome of SAW and ELISA tests performed at different times post-infection.**

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.