

## CHAPTER 2.8.5.

# PORCINE BRUCELLOSIS

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### SUMMARY

*Brucellosis in pigs is caused by Brucella suis, a bacterial infection that, after an initial bacteraemia, causes chronic inflammatory lesions in the reproductive organs of both sexes, with occasional localisation and lesions in other tissues. The species Brucella suis consists of five biovars, but the infection in pigs is caused by B. suis biovars 1, 2 or 3. The disease caused by biovars 1 and 3 is similar, while that caused by biovar 2 differs from 1 and 3 in its host range, its limited geographical distribution and its pathology. Biovar 2 is rarely pathogenic for humans, whereas biovars 1 and 3 are highly pathogenic causing severe disease. Porcine brucellosis is of widespread occurrence; generally, however, the prevalence is low, with the exception of South America and South-East Asia where the prevalence is higher. In some areas, B. suis infection has become established in wild or feral pigs – diagnostic methods recommended for wild and feral pigs are the same as for domestic pigs. Various biovars of B. suis cause infections in animals other than pigs, such as reindeer, caribou, hares and various murine species, and occasionally in cattle and dogs. Brucella suis infections in animals other than pigs are dealt with in an Appendix at the end of this chapter.*

*Signs of disease in sows include infertility and abortion at any stage of gestation, and birth of dead or weak piglets. In boars, the most prominent sign is orchitis, and the secondary sex organs may be affected. Brucella suis may be present in the semen, sometimes in the absence of clinical signs. Transmission during copulation is more common than is the case with brucellosis in ruminants. In both sexes, bones and especially joints and tendon sheaths may be affected, causing lameness and sometimes paralysis. Pigs are susceptible to artificial infection with B. abortus and B. melitensis, but reports of natural disease in pigs being caused by either of these organisms are rare. In humans, the infection is usually confined to those who are occupationally exposed to pigs, and to laboratory workers. The capability of B. suis to colonise the bovine udder with subsequent shedding in milk, has the potential to be a serious human health risk.*

**Identification of the agent:** *Brucella suis is readily isolated from live pigs by culture of birth products, and from carcasses by culture of lymph nodes and organs. Selective media are available for culture of contaminated samples. In nature, B. suis occurs invariably in the smooth phase – the appearance on solid medium is typical of smooth brucellae. Biovars of porcine origin agglutinate with monospecific A antiserum, and not with M antiserum. Definite identification of species and biovars may be effected by phage typing, molecular and biochemical tests, preferably carried out in specialised laboratories.*

**Serological tests:** *To date, none of the serological tests has been shown to be reliable in routine diagnosis in individual pigs. The indirect and competitive enzyme-linked immunosorbent assays (ELISAs), as well as the Rose Bengal test (RBT), complement fixation test (CFT) and fluorescence polarisation assay (FPA) are the prescribed tests for international trade purposes. The allergic skin test and the buffered plate agglutination test (BPAT) are also useful for identifying infected herds. The procedures for all the tests are the same as those described in Chapter 2.4.3 Bovine brucellosis.*

**Requirements for vaccines and diagnostic biologicals:** *Brucella suis strain 2 vaccine has been used for immunising pigs in China (People's Rep. of). Confirmation of the results obtained in China is required before strain 2 vaccine can be recommended for general use. In other countries, experimental work has shown that B. melitensis Rev.1 vaccine is superior to B. suis strain 2 in protecting sheep against B. melitensis. Sufficient data is not available to conclude if B. abortus strain RB51 vaccine is efficacious in protecting swine against exposure to B. suis. In practice, no*

product has yet found general acceptance. Preparation, testing and use of an established allergen, brucellin is described.

## A. INTRODUCTION

Porcine brucellosis is an infection caused by biovar 1, 2 or 3 of *Brucella suis*. It occurs in many countries where pigs are raised. Generally, the prevalence is low, but in some areas, such as South America and South-East Asia, the prevalence is much higher. Porcine brucellosis may be a serious, but presently unrecognised, problem in some countries. *Brucella suis* biovar 1 infections have been reported from feral pigs in some of the southern States of the United States of America (USA), and in Queensland, Australia. In both countries, a number of human infections have been reported from people who hunt and handle material taken from feral pigs (21, 22).

The disease is generally transmitted by consumption of feed contaminated by birth and/or abortion products and uterine discharges. Pigs will readily eat aborted fetuses and placental membranes. Transmission during copulation also occurs frequently, and this has implications for those practising artificial insemination.

In pigs, as in ruminants, after the initial bacteraemia, *B. suis* colonises cells of the reproductive tract of either sex. In females, placentas and fetuses are invaded, while in males, invasion occurs in one or more of the following: testis, prostate, epididymis, seminal vesicles, and/or bulbo-urethral glands. In males the lesions, which are most often unilateral, start with a hyperplasia that may progress to abscess formation; the final stage is characterised by sclerosis and atrophy. Arthritis may occur in various joints, and sometimes spondylitis occurs.

The most common manifestation of brucellosis in female pigs is abortion, occurring very early or at any time during gestation. Vaginal discharge is not often evident, and, in chronically infected herds, infertility rather than abortion is the most relevant clinical sign of the disease. In males, brucellosis is more likely to be persistent, with lesions in the genital tract often leading to interference with sexual activity, which can be temporary or permanent. The boar may excrete brucellae in the semen without any apparent abnormality in the sex organs or interference with sexual activity.

In both sexes, there may be swollen joints and tendon sheaths, lameness and, occasionally, posterior paralysis. A significant proportion of both male and female pigs will recover from the infection, often within 6 months, but many will remain permanently infected.

Brucellosis caused by *B. suis* biovar 2 differs from infection caused by biovars 1 and 3 in its host range, its distribution, and in its pathology. In general, the geographical distribution of biovar 2 has historically been in a broad range between Scandinavia and the Balkans (2). The prevalence in wild boars appears to be high throughout continental Europe (1, 3, 12, 14, 15). In recent outbreaks in Europe, wild boars have been implicated as the source of transmission of biovar 2 to outdoor reared pigs (12). In addition to wild boars, the European hare (*Lepus capensis*) is also considered as a reservoir for *B. suis* biovar 2 and has been implicated as a possible source of transmission to domestic livestock (2, 14, 24). *Brucella suis* biovar 2 causes miliary lesions, particularly reproductive tissues, that often become purulent. To date, biovar 2 has rarely been reported as the cause of human brucellosis. However, biovar 2 infection has been reported in two immuno-compromised hunters, who had been extensively exposed through gutting or skinning boars or hares (13).

The most common *B. suis* biovars (1 and 3) are serious human pathogens and adequate precautions are needed when handling and disposing of potentially infective material. This is especially so in the laboratory after culture has greatly increased the number of organisms present. Laboratory manipulation of the cultures or contaminated material from infected animals must be done under strict biosafety conditions to safely handle this dangerous zoonotic agent. Biosafety containment level 3 is recommended (see Chapter 1.1.2 Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities).

The classification, microbiological and serological properties of the genus *Brucella* and related species and biovars are given in the Chapter 2.4.3 Bovine brucellosis.

## B. DIAGNOSTIC TECHNIQUES

As far as biovars 1 and 3 are concerned, culture methods are at least as sensitive as serology (6). Biovar 2 appears to be highly sensitive to selective media and could be more difficult to isolate (Garin-Bastuji & Blasco, unpublished data). As the product of almost all pig-raising enterprises passes through abattoirs, surveillance methods (serology and culture) can be applied effectively at this point. In many areas, traditional village pig breeding is now accompanied by the development of larger commercial units, thereby increasing the use of

artificial insemination. Whereas artificial insemination using brucellosis-free boars can be a valuable aid in the control of porcine brucellosis, the inadvertent use of infected semen could, obviously, cause incalculable damage.

## 1. Identification of the agent

Optimal samples for bacteriologic culture and methods for processing of samples are similar to those described for bovine brucellosis in Chapter 2.4.3 Bovine brucellosis. Standard and selective media used for other species of brucellae are suitable for *B. suis* (see Chapter 2.4.3 Bovine brucellosis). The addition of serum is not essential, but basal medium containing 5% serum is a satisfactory medium, both for isolation, maintenance of cultures and typing. The addition of CO<sub>2</sub> to the atmosphere is not required.

In nature, *B. suis* invariably occurs in the smooth form and colonies are morphologically indistinguishable from other smooth brucellae, described in Chapter 2.4.3 Bovine brucellosis.

Biovars 1, 2 and 3 of *B. suis* are all A surface antigen dominant, and growth may be presumptively identified by slide agglutination with the monospecific A antiserum. Confirmatory identification of species and biovar should be performed in a specialised reference laboratory. The OIE Reference Laboratories for brucellosis are listed in the Table given in Part 3 of this *Terrestrial Manual*.

Confirmation of species and biovar depends on phage tests, production of H<sub>2</sub>S (only biovar 1 produces H<sub>2</sub>S), and growth in the presence of dyes. However, some strains of *B. suis* biovar 1 are atypical in that they grow on media containing 20 µg/ml of basic fuchsin. Most strains of *B. suis* are inhibited by safranin O at a concentration of 1/10,000, whereas *B. suis* usually reacts more rapidly in the urease test than either *B. abortus* or *B. melitensis*. Oxidative metabolic tests are supplemental tests that can be used for distinguishing *B. suis* from other smooth *Brucella* species.

Molecular genetic techniques using the polymerase chain reaction (PCR) and specific primers are available that can identify *B. suis* and other species of *Brucella* (see chapter 2.4.3 Bovine brucellosis). Some of these techniques can distinguish biovars of *B. suis* (5, 7).

## 2. Serological tests

None of the serological tests used for the diagnosis of porcine brucellosis are reliable for diagnosis in individual pigs.

The major antigen involved in the serological tests currently available is the smooth lipopolysaccharide (LPS). The OPS moiety of this molecule contains epitopes that cross-react with those existing in the corresponding LPS from *Yersinia enterocolitica* serotype O:9 (16). Therefore, available serological tests are unable to distinguish between antibodies raised to these two infections. *Y. enterocolitica* O:9 infection in pigs is not uncommon in some areas (1, 26). Studies have suggested that the sensitivities and specificities of the Rose Bengal test (RBT), the indirect and competitive enzyme-linked immunosorbent assay (I- and C-ELISAs), and the fluorescent polarisation assay (FPA) are similar (20). With the exception of acute stages of infection (<9 weeks) (16), the use of the FPA (18) or C-ELISA has been reported to eliminate cross-reactivity with *Y. enterocolitica* but this should be confirmed in additional field studies performed in various epidemiological situations. Swine serum may sometimes also contain nonspecific antibody, thought to be of the IgM isotype, further reducing the specificity of conventional tests, especially the serum agglutination test (SAT). Also, swine complement interacts with guinea-pig complement to produce a pro-complementary activity that reduces the sensitivity of the complement fixation test (CFT). Sensitivity levels may be low for the CFT; therefore caution should be taken when interpreting test results from individual animals. For international and other trade, e.g. purchasing boars, the disease status of the herd and of the area in which the herd is situated are of more importance than tests on individual animals.

### • Reference sera

The OIE reference standards are those against which all other standards are compared and calibrated. For the RBT and CFT, please refer to Chapter 2.4.3 Bovine brucellosis for antigen standardisation and test protocols. A porcine reference standard for ELISAs and FPA has been developed and will be available to national laboratories in the near future<sup>1</sup>.

### a) Enzyme-linked immunosorbent assay (prescribed tests for international trade)

Indirect and competitive ELISAs have been developed for the diagnosis of brucellosis in individual pigs and for screening large numbers of sera. These techniques promise to be more efficient than any of the tests mentioned above. At this time the indirect ELISA and the reference sera for this test are not commercially available.

<sup>1</sup> Obtainable from the OIE Reference Laboratory for Brucellosis at the Ontario Laboratories (Fallowfield), Canadian Food Inspection Agencies, 3851 Fallowfield Road, Nepean, Ontario K2H 8P9, Canada.

- **Indirect ELISA**

A method for the I-ELISA is described in detail in Chapter 2.4.3 Bovine brucellosis, however, antibody specific for porcine IgG conjugated with horseradish peroxidase (HRPO) has to be used. The I-ELISA may be used as a screening test. Before being used for international trade, the cut-off of I-ELISA should be properly established using the appropriate validation techniques (see Chapter 1.1.4 Principles of validation of diagnostic assays for infectious diseases) and the test should be standardised against the above-mentioned Standard.

- **Competitive ELISA**

C-ELISA procedures for detection of porcine antibody to *Brucella* sp. (18) are identical to the procedures used for bovine antibody to *B. abortus* described in Chapter 2.4.3. This assay is capable of eliminating some reactions due to *Y. enterocolitica* O:9 and in some situations other cross-reacting antibody, such as IgM, will not compete well. Before being used for international trade, the cut-off of C-ELISA should be properly established using the appropriate validation techniques (see Chapter 1.1.4 Principles of validation of diagnostic assays for infectious diseases) and the test should be standardised against the above-mentioned Standard

**b) Fluorescence polarisation assay (a prescribed test for international trade)**

The FPA for detection of porcine antibody to *Brucella* sp. is essentially the same as that described for cattle (for more details see Chapter 2.4.3); an example serum dilution used is 1/25 for the tube test and 1/10 for the plate test (18). It is a simple technique for measuring antigen/antibody interaction and may be performed in the laboratory or in the field. Lyophilised porcine sera tend to increase background activity in this assay. The FPA may be used as a screening and/or confirmatory test. Before being used for international trade, the cut-off of FPA should have been properly established using the appropriate validation techniques (see Chapter 1.1.4 Principles of validation of diagnostic assays for infectious diseases) and the test should be standardised against the above-mentioned Standard.

**c) Rose Bengal test (a prescribed test for international trade)**

The preparation and standardisation of RBT antigen and the method of performing the test are described in Chapter 2.4.3 Bovine brucellosis. The *B. abortus* antigens are appropriate for testing swine sera in RBT and will identify antibody against all three biovars of *B. suis*. The RBT may be used as a screening test, bearing in mind its lack of specificity for discriminating reactions caused by brucellosis from those caused by infections by *Y. enterocolitica* O:9 and other cross-reacting bacteria.

**d) Complement fixation test (a prescribed test for international trade)**

The preparation and standardisation of CFT antigen and the method of performing the test are described in Chapter 2.4.3 Bovine brucellosis. The *B. abortus* antigens are appropriate for testing swine sera in CFT and will identify antibody against all three biovars of *B. suis*. The CFT has a reduced sensitivity for diagnosing *B. suis* infection and is not capable of eliminating all reactions caused by *Y. enterocolitica* O:9. It may be used as a complementary test.

### 3. Other Tests

**a) Allergic (hypersensitivity) tests**

Brucelin-INRA is an sLPS free cytosolic extract from rough *B. melitensis* B115. This preparation does not stimulate the formation of antibodies that would be reactive in BBAT, CFT or ELISAs. The product has been developed for use in ruminants, but is also effective for confirming the disease at the herd level in pigs. A rough strain is used in its preparation, thereby avoiding the presence of sLPS. The preparation, standardisation and testing, of Brucelin-INRA is described in detail in Chapter 2.4.3 Bovine brucellosis. As a diagnostic agent in pigs 0.1 ml of the allergen is injected intradermally into the skin at the base of the ear or preferably next to the base of the tail. The latter appears more practical and less hazardous. The reaction is read after 48 hours. A positive reaction shows erythema of non-pigmented skin and an oedematous swelling. In severe reactions, there may also be some necrosis.

## C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

Numerous attempts have been made to develop a vaccine to immunise pigs against *B. suis*, but none has been found fully effective. Only one immunogen -*B. suis* strain 2 (S2) vaccine- has been reported suitable after

extensive field use in south China (People's Rep. of) (17, 27)<sup>2</sup> but experiments under strictly controlled conditions are not available. To date, it does not appear to have been used elsewhere in pigs, probably because it has been shown that this vaccine does not confer adequate protection in sheep against *B. melitensis* (25). Similarly, the *B. abortus* strain RB51 vaccine has been reported as inefficacious in protecting swine against exposure to *B. suis* (23).

## APPENDIX: *BRUCELLA SUIIS* INFECTIONS IN ANIMALS OTHER THAN PIGS

### 1. Rangiferine brucellosis

*Brucella suis* biovar 4 causes serious disease in reindeer or caribou (*Rangifer tarandus* and its various subspecies) throughout the Arctic region, including Siberia, Canada and Alaska (16, 19). Some of these animals are domesticated, others are wild and migratory. *Rangifer tarandus* is very susceptible to *B. suis* infection, which causes fever, depression and various local signs, such as abortion, retained placentas, metritis, sometimes with blood-stained discharge, mastitis, bursitis and orchitis. In the Arctic region, *B. suis* biovar 4 constitutes a serious zoonosis (8). Transmission to humans may be by direct contact or through consumption of milk and other inadequately heated products from reindeer. Bone marrow, which is considered to be a special delicacy in this region, is also a source of human infection.

The methods already described for isolating and identifying *B. suis* in samples taken from pigs are equally applicable to *B. suis* biovar 4 in samples taken from reindeer. Biovar 4 grows well on all the usual media used for the culture of *Brucella*. It reacts positively with both anti-A and -M monospecific sera. For serology, the tube agglutination test has been reported to be satisfactory, with titres of 1/20 or greater being considered to be diagnostic. The CFT has also been used but the clinical interpretation of these tests in reindeer has not been established.

Vaccination of reindeer with *B. abortus* S19 vaccine, or alternatively with *B. abortus* 45/20 adjuvant vaccine, has been tried experimentally without any clear-cut result. In the case of S19, the reaction to vaccination was rather severe and immunity in the vaccinated animals could only be demonstrated against challenge with very small doses of *B. suis* biovar 4. Gall *et al.* (9) compared several serological tests and found that the specificity values for the BPAT and CFT using reindeer/caribou sera was lower than the I-ELISA, C-ELISA and the FPA, while sensitivity values were similar for all tests.

### 2. *Brucella suis* infection in other nonporcine species

There are two different types of epidemiological situation with regard to *B. suis* infection in other nonporcine species. In the first case, *B. suis* infection occurs in animals that are not the natural host of the particular infection through the ingestion of contaminated materials or by co-habitation with infected natural hosts. For example, Arctic foxes and wolves may contract *B. suis* biovar 4 from reindeer; dogs and rodents, such as rats and mice, may acquire other *B. suis* biovars by cohabitation with infected hosts. Cattle and horses may become infected by cohabitation or interaction with infected swine (4). The infecting bacteria are invariably the well defined biovars of the natural host species.

In the second case, wildlife species that are natural hosts for *B. suis* or *B.-suis*-like infections become infected. One example is the so-called murine brucellosis of the former USSR, where small rodents are infected with *B. suis* biovar 5. Other similar situations have been reported from Queensland, Australia and from Kenya. In all three cases, *B. suis* strains with different characteristics were involved, and at least one of them was difficult to classify.

Brucellosis caused by *B. suis* biovar 2 in the European hare (*Lepus capensis*) (24), is characterised by the formation of nodules, varying in size from that of a millet seed to a cherry or even larger; these often become purulent. Such nodules may occur in almost any location, sometimes subcutaneously or intramuscularly, in the spleen, liver or lung and in the reproductive organs of either sex. The bodily condition of the hare may be surprisingly unaffected. Other species may also become infected by cohabitation with *B. suis* biovar 2 infected swine, wild boars or hares. Gutting or skinning wild boars in cattle sheds could be a route of transmission to cattle (11).

Serological investigations in nonporcine species are usually carried out for screening purposes. In these particular circumstances, specificity is more important than sensitivity. Here the CFT can be recommended, although the RBT, may be also useful because of its simplicity. The indirect and competitive ELISAs appear to be very useful for epidemiological sero-surveys in wild boars as both are more sensitive and specific than the RBT and CFT. The

2 Obtainable from the National Institute for the Control of Veterinary Products and Pharmaceuticals, Ministry of Agriculture, 30 Baishiqiao Road, Beijing 100081, China (People's Rep. of), or from VLA Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom. Supply from the Weybridge laboratory needs the prior permission of the World Health Organization.

test has also been used successfully on blood samples that are in poor condition. When poor quality samples are tested on other tests, the results may be uninterpretable. Another advantage of the ELISAs is that if serum is not available, it is possible to test meat juice samples (10). However, in nonporcine species the interpretation of serological results may be problematic. Where positive or equivocal serological results are encountered, a bacteriological investigation should be conducted.

For bacteriological investigations in situations such as these, where the infecting organisms may have unusual characteristics, it is advisable to duplicate the culture on selective media by culture on plain medium supplemented with 5% serum, and to broaden the investigation by incubating the cultures in an atmosphere containing 10% CO<sub>2</sub>. Colonies resembling *Brucella* can be tentatively identified by Gram staining, by slide agglutination tests with monospecific anti-A and -M sera, and by anti-rough *Brucella* serum (Chapter 2.4.3 Bovine brucellosis) *Brucella suis* biovar 5 is unusual in that it reacts with anti-M monospecific serum, and not with anti-A monospecific serum. Further identification is best carried out in a specialised laboratory.

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**NB:** There are OIE Reference Laboratories for Porcine brucellosis (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: [www.oie.int](http://www.oie.int)).