

CHAPTER 2.7.2.

CAPRINE AND OVINE BRUCELLOSIS (excluding *Brucella ovis*)

SUMMARY

Brucella melitensis (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis. Sporadic cases caused by *B. abortus* have been observed, but cases of natural infection are rare in sheep and goats. *Brucella melitensis* is endemic in the Mediterranean region, but infection is widespread world-wide. North America (except Mexico) is believed to be free from the agent, as are Northern and Central Europe, South-East Asia, Australia and New Zealand.

Clinically, the disease is characterised by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis, with excretion of the organisms in uterine discharges and in milk. Diagnosis depends on the isolation of *Brucella* from abortion material, udder secretions or from tissues removed at post-mortem. Presumptive diagnosis of *Brucella* infection can be made by assessing specific cell-mediated or serological responses to *Brucella* antigens.

Brucella melitensis is highly pathogenic for humans, causing Malta fever, one of the most serious zoonoses in the world. All infected tissues, cultures and potentially contaminated materials should therefore be handled at containment level 3.

Identification of the agent: Presumptive evidence of *Brucella* is provided by the demonstration, by modified acid-fast staining of organisms typical of *Brucella* in abortion material or vaginal discharge, especially if supported by serological tests. The polymerase chain reaction (PCR) methods provide additional means of detection. Whenever possible, *Brucella* spp. should be isolated using selective or non-selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes, spleen, uterus, testes and epididymes. Species and biovars should be identified by phage lysis, and by cultural, biochemical and serological criteria. Molecular methods have been developed that could also be used for complementary identification based on specific genomic sequences.

Serological and allergic skin tests: The buffered *Brucella* antigen tests (BBAT) and the complement fixation test (CFT) are usually recommended for screening flocks and individual animals. The serum agglutination test is not considered to be reliable for use in small ruminants. The indirect enzyme-linked immunosorbent assay (I-ELISA) and fluorescence polarisation assay (FPA) can also be used for screening purposes. For pooled samples, there are no useful tests such as the milk ring test for cattle. The brucellin allergic skin test can be used as a screening or complementary test in unvaccinated flocks, provided that a purified, lipopolysaccharide (LPS)-free, standardised antigen preparation is used. Results must then be interpreted in relation to the clinical signs, history, and results of serological or cultural examination.

Requirements for vaccines and diagnostic biologicals: *Brucella melitensis* strain Rev.1 remains the reference vaccine to immunise sheep and goats at risk of infection from *B. melitensis* and is the vaccine with which any other vaccines should be compared. Production of *Brucella* antigens or Rev.1 vaccine is based on a seed-lot system. Seed cultures to be used for antigens for serological and allergic skin tests and for vaccines should originate from reference centres. They must conform to minimal standards for viability, smoothness, residual infectivity and immunogenicity, purity, identity and safety, if applicable. Brucellin preparations for the intradermal test must be free of smooth lipopolysaccharide and must not produce nonspecific inflammatory reactions or interfere

with serological tests. Antigens for BBAT and CFT must be prepared from smooth strains of *B. abortus*, strain 1119-3 or strain 99. Antigens for I-ELISA are prepared from *B. abortus* strain 1119-3 or strain 99 or *B. melitensis* biovar 1 reference strain 16M or antigens prepared from different smooth *Brucella* strains. All antigens must comply with minimum standards for purity, sensitivity and specificity.

A. INTRODUCTION

Brucellosis in sheep and goats (excluding *Brucella ovis* infection) is primarily caused by one of the three biovars of *B. melitensis*. Sporadic infections caused by *B. abortus* or *B. suis* have been observed in sheep and goats, but such cases are rare. Pathologically and epidemiologically, *B. melitensis* infection in sheep and goats is very similar to *B. abortus* infection in cattle (see Chapter 2.4.3 Bovine brucellosis). In most circumstances, the primary route of transmission of *Brucella* is the placenta, fetal fluids and vaginal discharges expelled by infected ewes and goats when they abort or have a full-term parturition. Shedding of *Brucella* is also common in udder secretions and semen, and *Brucella* may be isolated from various tissues, such as lymph nodes from the head, spleen and organs associated with reproduction (uterus, epididymides and testes), and from arthritic lesions (2).

Brucella melitensis infection in domestic and wild susceptible species (see Chapter 2.4.3) is not rare when these species are reared in close contact with sheep and goats in enzootic areas. The manifestations of brucellosis in these animals are similar to those in cattle or sheep and goats.

The World Health Organization (WHO) laboratory biosafety manual classifies *Brucella* (and particularly *B. melitensis*) in Risk group III. Brucellosis is readily transmissible to humans, causing acute febrile illness – undulant fever – which may progress to a more chronic form and can also produce serious complications affecting the musculo-skeletal, cardiovascular, and central nervous systems. Infection is often due to occupational exposure and is essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of dairy products constitutes the main risk to the general public. There is an occupational risk to veterinarians, abattoir workers and farmers who handle infected animals and aborted fetuses or placentas. Brucellosis is one of the most easily acquired laboratory infections, and strict safety precautions should be observed when handling cultures and heavily infected samples, such as products of abortion. Specific recommendations have been made for the safety precautions to be observed with *Brucella*-infected materials (for further details see Chapter 1.1.2 Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities, and refs 1, 39, 94 and 95 of Chapter 2.4.3). Laboratory manipulation of live cultures or contaminated material from infected animals is hazardous, as is handling large volumes of *Brucella*, and must be done under containment level 3 or higher conditions, as outlined in Chapter 1.1.2, to minimise occupational exposure.

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

1. Identification of the agent

Refer to Chapter 2.4.3 Bovine brucellosis for the detailed agent identification procedure for *Brucella*.

2. Serological tests

In situations where bacteriological examination is not practicable, diagnosis of *Brucella* infection must often be based on serological methods (2, 21). In routine tests, anti-*Brucella* antibodies are detected in serum. The most widely used serum-testing procedures for the diagnosis of smooth *Brucella* infections in sheep and goats are the buffered *Brucella* antigen tests (BBAT), and the complement fixation test (CFT). The bulk milk ring test, which has been very useful in cattle, is ineffective in small ruminants.

In small ruminants, the BBAT and the CFT are the most widely used methods (20). The indirect enzyme-linked immunosorbent assay (I-ELISA) and the fluorescence polarisation assay (FPA) have shown similar diagnostic performance. All these tests are prescribed for international trade. The BBAT is not completely specific, but is adequate as a screening test for detecting infected flocks or for guaranteeing the absence of infection in brucellosis-free flocks. However, due to the relative lack of sensitivity of both tests, discrepancies between results obtained using the Rose Bengal test (RBT) and the CFT are not rare in infected sheep and goats (7). The results of the two tests should therefore be considered simultaneously to increase the likelihood of detecting infected individuals and to improve control of the disease in areas where it has not been completely eradicated (1, 5, 7). When, for practical or economic reasons, the CFT cannot be used simultaneously with the RBT in eradication programmes, it is recommended to improve the sensitivity of the RBT by using three volumes of serum and one

volume of antigen (e.g. 75 µl and 25 µl, respectively) in place of an equal volume of each. This simple modification increases RBT sensitivity and minimises the discrepancies between RBT and CFT results (7). Because antibodies induced after Rev.1 vaccination cannot be differentiated in both tests from those induced by *B. melitensis* infection, RBT and CFT results should be carefully interpreted according to the vaccination status in the flock. In addition, both tests are not specific enough to discriminate serological reactions due to *B. melitensis* from the false-positive reactions (FPSR) due to cross-reacting bacteria such as *Yersinia enterocolitica* O:9.

Good diagnostic results have been obtained in sheep and goats with indirect (I-) or competitive (C-) enzyme-linked immunosorbent assays (ELISAs) using various antigens, but generally the ELISAs that use antigens with a high content of smooth lipopolysaccharide (sLPS) are the most useful. The C-ELISA provides similar sensitivity to the classical tests, RBT and CFT, and the I-ELISA has greater sensitivity. Like these classical tests, both ELISAs are unable to differentiate *B. melitensis*-infected animals from those recently vaccinated with the Rev.1 vaccine (22) or infected with cross-reacting bacteria. Some of these ELISAs have potential advantages in sensitivity and/or specificity with respect to both BBAT and CFT (17). Preliminary C-ELISAs studies with a periplasmic protein from *B. abortus* (27) or *B. melitensis* (12) as antigen have been applied in sheep and reported to be promising in differentiating Rev.1 vaccinated from *B. melitensis* infected animals (11, 14).

- **Reference sera**

The OIE reference standards are those against which all other standards are compared and calibrated. For the BBAT and CFT, please refer to chapter 2.4.3. Bovine brucellosis for antigen standardisation and test protocols. A caprine reference standard for ELISAs and FPA for sheep and goat antibodies has been developed and will be available to national reference laboratories soon¹.

- **Production of cells**

Please refer to Chapter 2.4.3 Bovine brucellosis. *Brucella abortus* biovar 1 strains 99 or 1119 are the only strains recommended for the preparation of BBAT and CFT in sheep and goats.

a) *Brucella*-buffered antigen test (a prescribed test for international trade)

Please refer to Chapter 2.4.3 Bovine brucellosis.

- **Antigen production**

Please refer to Chapter 2.4.3 Bovine brucellosis. Note that RB antigen made with *B. abortus* is usually used to test for *B. melitensis*. The standardisation of RB antigen, as it is prescribed in Chapter 2.4.3, provides a sufficient sensitivity to the BBAT for international trade purposes. Moreover, it helps assure an adequate specificity in free areas where FPSR occur because of cross-reacting bacteria such as *Yersinia enterocolitica* O:9. However this standardisation is probably the main cause of the reduced sensitivity of some RB antigen batches and of the discrepancies with the CFT (7). Therefore, when RBT is used in eradication programmes in endemic areas, it could be advisable to adjust the RB antigen titre so that it is positive at a 1/45 OIEISS dilution and negative at a 1/55 dilution, without affecting the specificity of the test. The discrepancies with the CFT can also be minimised by using three volumes of serum and one volume of antigen (e.g. 75 µl and 25 µl, respectively) in place of an equal volume of each as mentioned in the standard test procedure.

- **Test procedure**

Please refer to Chapter 2.4.3 Bovine brucellosis.

b) Complement fixation test (a prescribed test for international trade)

- **Antigen production**

Please refer to Chapter 2.4.3 Bovine brucellosis. Note that CF antigen made with *B. abortus* is used to test for *B. melitensis*.

- **Test procedure**

Please refer to Chapter 2.4.3 Bovine brucellosis.

¹ Obtainable from the OIE Reference Laboratory for Brucellosis at Veterinary Laboratories Agency (VLA) Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom.

c) Enzyme-linked immunosorbent assays (a prescribed test for international trade)

Several variations of the I-ELISA have been described using different antigen preparations, antiglobulin-enzyme conjugates, and substrate/chromogens. Several commercial I-ELISAs are available but before being used for international trade, their respective cut-off 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 these tests should be standardised against the above-mentioned Standard.

The test method is described in Chapter 2.4.3 Bovine brucellosis

d) Fluorescence polarisation assay (a prescribed test for international trade)

The FPA for detection of caprine and ovine 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 (23–26). It is a simple technique for measuring antigen/antibody interaction. The FPA may be used as a screening and/or confirmatory test. Before being used for international trade, the FPA cut-off 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.

3. Other tests**a) Brucellin skin test (an alternative test for international trade)**

An alternative diagnostic test is the brucellin skin test, which can be used for screening unvaccinated flocks, provided that a purified (free of sLPS) and standardised antigen preparation (e.g. brucellin INRA) is used.

The brucellin skin test has a high sensitivity for the diagnosis of *B. melitensis* infection in small ruminants and, in absence of vaccination, is considered one of the most specific diagnostic tests (2, 5, 17, 20). This test is of particular value for the interpretation of FPSR due to infection with cross-reacting bacteria (FPSR affected animals are always negative in the skin test), especially in brucellosis-free areas.

Rev.1 vaccinated animals can react in this test for years (17). Therefore this test cannot be recommended either as the sole diagnostic test or for the purposes of international trade in areas where Rev.1 vaccine is used.

To obtain suitable results it is essential to use standardised brucellin preparations that do not contain sLPS, as this antigen may provoke antibody-mediated inflammatory reactions or induce antibodies that interfere with subsequent serological screening. One such preparation is brucellin INRA, which is prepared from a rough strain of *B. melitensis* that is commercially available².

- **Test procedure**

- i) A volume of 0.1 ml of brucellin is injected intradermally into the lower eyelid.
- ii) The test is read after 48 hours.
- iii) Any visible or palpable reaction of hypersensitivity, such as an oedematous reaction leading to an elevation of the skin or thickening of the eyelid (≥ 2 mm), should be interpreted as a positive reaction.

Although in the absence of vaccination the brucellin intradermal test is one of the most specific tests in brucellosis, diagnosis should not be made exclusively on the basis of positive intradermal reactions and should be supported by adequate serological tests. The intradermal inoculation of brucellin might induce a temporary anergy in the cellular immune response. Therefore an interval of 6 weeks is generally recommended between two tests repeated on the same animal.

b) Native hapten tests

The native hapten-based gel precipitation tests³ (as described in Chapter 2.4.3.) are also of interest in sheep and goats as they are very specific for discriminating the serological responses of infected animals (positive) from those induced in Rev.1 vaccinated animals (usually negative after a given time after vaccination).

² Brucellergène OCB®, Synbiotics Europe, 2 rue Alexander Fleming, 69007 Lyon, France.

³ The detailed procedure could be obtained from the Departamento de Sanidad Animal, Centro de Investigación y Tecnología Agroalimentaria/Gobierno de Aragón, Avenida Montañana 930, 50059. Zaragoza. Spain.

The optimal diagnostic sensitivity (around 90%) is obtained in the double gel diffusion (DGD) or reverse radial immunodiffusion tests for sheep and goats, respectively (14, 20).

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

C1. Brucellin

Please refer to Chapter 2.4.3 Bovine brucellosis.

C2. Vaccines

Brucella melitensis strain Rev.1 vaccine

The most widely used vaccine for the prevention of brucellosis in sheep and goats is the *Brucella melitensis* Rev.1 vaccine, which remains the reference vaccine with which any other vaccines should be compared. The RB51 vaccine is not effective in sheep against *B. melitensis* infection (16). In addition, other rough mutants defective in core and O-polysaccharide synthesis and export induce antibodies reacting in the I-ELISA with sLPS and are less effective than Rev.1 vaccine against *B. melitensis* infection in sheep (4). The Rev.1 vaccine is used as a freeze-dried suspension of live *B. melitensis* biovar 1 Rev.1 strain for the immunisation of sheep and goats. It should be normally given to lambs and kids aged between 3 and 6 months as a single subcutaneous or conjunctival inoculation. The standard dose is between 0.5×10^9 and 2.0×10^9 viable organisms. The subcutaneous vaccination induces strong interferences in serological tests and should not be recommended in combined eradication programmes (15, 22). However, when this vaccine is administered conjunctivally, it produces a similar protection without inducing a persistent antibody response, thus facilitating the application of eradication programmes combined with vaccination (15, 22). Care must be taken when using Rev.1 vaccine to avoid the risk of contaminating the environment or causing human infection. In many developing countries and endemic areas, vaccination of the whole population has to be considered as the best option for the control of the disease (6). However, Rev.1 vaccine is known to often cause abortion and excretion in milk when animals are vaccinated during pregnancy, either with a full or reduced dose (6). These side-effects are considerably reduced when adult animals are vaccinated conjunctivally (full dose) before mating or during the last month of pregnancy. Therefore, when mass vaccination is the only means of controlling the disease, a vaccination campaign should be recommended using the standard dose of Rev.1 administered by the conjunctival route when the animals are not pregnant or during the late lambing and prebreeding season (6).

The subcutaneous vaccination of young animals and the vaccination of adult animals, even at reduced doses, may lead to long-term persistence of vaccinal antibodies in a significant proportion of vaccinated animals that creates serious interferences in the serological diagnosis of brucellosis. As indicated above, conjunctival vaccination minimises these problems and thus it is the recommended method for combined eradication programmes. Therefore, the serological diagnosis of brucellosis should take into account the vaccinal state of the herd and the overall frequency distribution of antibody titres detected in the group of animals tested.

1. Seed management

a) Characteristics of the seed

Brucella melitensis biovar 1 strain Rev.1 original seed for vaccine production can be obtained commercially⁴. A European reference Rev.1 strain that possesses the characteristics of the Rev.1 original seed is also obtainable from the European Pharmacopoeia⁵.

Production of *Brucella* live vaccines is based on the seed-lot system described above (Section B.2) for BBAT and CFT antigens. Strains should be cultured in a suitable medium. Strain Rev.1 must conform to the characteristics of *B. melitensis* biovar 1, except that it should grow more slowly. Additionally, when incubated in air (atmospheres containing CO₂ alter the results) at 37°C, it should grow on agar containing streptomycin (2.5 µg/ml), and it should be inhibited by the addition to a suitable culture medium of sodium benzylpenicillin (3 µg [5 International Units (IU)]/ml), thionin (20 µg/ml) or basic fuchsin (20 µg/ml). Recently, polymerase chain reaction and molecular techniques have been used to further characterise the vaccine (3, 13). It must also conform to the characteristics of residual virulence and immunogenicity in mice of the original seed.

⁴ Obtainable from the OIE Reference Laboratory for Brucellosis at AFSSA, 94706 Maisons-Alfort, France.

⁵ Obtainable from the European Pharmacopoeia, BP 907, 67029 Strasbourg Cedex 1, France.

b) Method of culture

Serum–dextrose agar, and trypticase–soy agar, to which 5% serum or 0.1% yeast extract may be added, are among the solid media that have been found to be satisfactory for propagating the Rev.1 strain (2, 28). Rev.1 strain does not grow well on potato agar.

For vaccine production, Rev.1 may be grown under conditions similar to those described for S99 and S1119-3 (see Chapter 2.4.3), except that Rev.1 generally needs 3–5 days to grow, the phenol saline is replaced by a freeze-drying stabiliser, and the organisms are not killed but are stored at 4°C while quality control examinations are carried out as described below. Moreover, the specific requirements for Rev.1 vaccine production recommend that: each seed lot (i.e. the culture used to inoculate medium for vaccine production) should be no more than three passages removed from an original seed culture and that the harvest of a vaccine lot should be no more than three passages from a seed lot or an original seed. The original seed culture should always be checked for the absence of dissociation before use. The recommended method for preparing seed material is given in ref. 2. The following freeze-drying stabiliser (sterilised by filtration) is of proven value: enzymatic digest of casein (2.5 g); sucrose (5 g); sodium glutamate (1 g); distilled water (100 ml).

c) Validation as a vaccine

Numerous independent studies have confirmed the value of *B. melitensis* strain Rev.1 as a vaccine for protecting sheep and goats from brucellosis. Its virulence is unchanged after passage through pregnant sheep and goats. Abortions may result when the Rev.1 vaccine is inoculated into pregnant ewes or goats. The vaccine-induced abortions are not avoided using reduced doses, and doses as low as 10^6 , used either subcutaneously or conjunctivally, have been demonstrated to induce abortions and milk excretion of the vaccine strain (6).

A Rev.1 vaccine is efficient if it possesses the characteristics of the Rev.1 original strain, i.e. those of *B. melitensis* biovar 1 reference strain 16M (ATCC No. 23456), except those specific for the strain Rev.1 (2, 20), and if it proves to be satisfactory with respect to immunogenicity and residual virulence in the mouse model (9) (see below).

2. Method of manufacture (2, 28)

For production of *B. melitensis* strain Rev.1 vaccine, the procedures described above for antigens (2) can be used except that the cells are collected in a freeze-drying stabiliser and deposited by centrifugation. The yield from one fermenter run or the pooled cells from a batch of Roux flask cultures inoculated on the same occasion from the same seed lot constitutes a single harvest. More than one single harvest may be pooled to form the final bulk that is used to fill the final containers of a batch of vaccine. Before pooling, each single harvest must be checked for purity, cell concentration, dissociation and identity. The volume of the final bulk is adjusted by adding sufficient stabiliser so that a dose contains an appropriate number of viable organisms. After adjusting the cell concentration of the final bulk, tests for identity, dissociation and absence of contaminating organisms are conducted (see below).

3. In-process control

In-process checks should be made on the growth of Rev.1 vaccine from either solid or liquid medium to verify identity and to ensure purity and freedom from dissociation to rough forms during preparation of seed lots, single harvests, final bulks and the final (filling) lots. At least 99% of cells in seed lots and 95% of cells in final lots should be in the smooth phase.

Cell concentration should be estimated on the bulks and precisely determined on final lots. Immunogenicity and the residual virulence (50% persistence time or 50% recovery time) should also be determined on seed lots and final lots. If these tests have been done with good results on a representative batch of the test vaccine, it does not have to be repeated routinely on other vaccine lots prepared from the same seed lot and with the same manufacturing process.

4. Batch control

With freeze-dried vaccine, the control tests should be conducted on the vaccine reconstituted in the form in which it will be used.

a) Sterility (or absence of extraneous microorganisms)

The Rev.1 vaccine should be checked for bacterial and fungal contamination as prescribed in Chapter 1.1.9 Tests for sterility and freedom from contamination of biological materials.

b) Safety

The Rev.1 vaccine is a virulent product *per se*, and it should keep a minimal virulence to be efficacious (see Section C2.4.c in Chapter 2.4.3).

c) Potency

A Rev.1 vaccine is efficient if it possesses the characteristics of the Rev.1 original strain, i.e. if it is satisfactory with respect to immunogenicity, and residual virulence (10). Batches should also be checked for the number of viable organisms.

- **Identity**

The reconstituted Rev.1 vaccine should not contain extraneous microorganisms. *Brucella melitensis* present in the vaccine is identified by suitable morphological, serological and biochemical tests and by culture: when incubated in air at 37°C, Rev.1 strain is inhibited by addition to the suitable culture medium of 3 µg (5 IU) per ml of sodium benzyl-penicillin, thionin (20 µg/ml) or basic fuchsin (20 µg/ml); the strain grows on agar containing 2.5 µg per ml of streptomycin.

- **Smoothness (determination of dissociation phase)**

Please refer to Chapter 2.4.3 Bovine brucellosis.

Sometimes slight and difficult to observe differences, can be seen in the size of Rev 1 colonies. The small colonies (1–1.2 mm in diameter) are typical for Rev.1, but larger Rev. 1 colonies can appear depending on the medium used, the amount of residual moisture in the incubator atmosphere, and the presence or absence of CO₂. The frequency of variation in colony size occurs normally at a ratio of 1 large to 10³ small colonies. Both Rev.1 variants are of the S (smooth) type. To avoid an increase in this colony size variation along successive passages, it is important to always select small colonies for preparation of seed lots.

- **Enumeration of live bacteria**

Please refer to Chapter 2.4.3 Bovine brucellosis.

- **Residual virulence (50% persistence time or 50% recovery time) (8, 18)**

The same technical procedures indicated for 50% recovery time (RT₅₀) calculation of S19 vaccine (see Chapter 2.4.3) have to be applied for Rev.1, except that *B. abortus* S19 seed lot or batch to be tested (test vaccine) and the S19 original seed culture (used as a reference strain), respectively, are replaced by the corresponding *B. melitensis* Rev.1 seed lot or batch to be tested (test vaccine) and the *B. melitensis* Rev.1 original seed culture as the reference strain. For the reference original Rev.1 strain, RT₅₀ and confidence limits are around 7.9 ± 1.2 weeks. A given Rev.1 vaccine seed lot or batch should keep similar residual virulence to be acceptable.

If this test has been done with good results on a representative batch of the test vaccine, it does not have to be repeated routinely on other vaccine lots prepared from the same seed lot and with the same manufacturing process.

- **Immunogenicity in mice**

The same technical procedures indicated for immunogenicity calculation of S19 vaccine (see Chapter 2.4.3) have to be applied for Rev.1, except that *B. abortus* S19 seed lot or batch to be tested (test vaccine) and the *B. abortus* S19 original seed culture (used as a reference strain), respectively, are replaced by the corresponding *B. melitensis* Rev.1 seed lot or batch to be tested (test vaccine) and the *B. melitensis* Rev.1 original seed culture as the reference strain.

Conditions of the control experiment are satisfactory when: i) the response in unvaccinated mice (mean of Y) is at least of 4.5; ii) the response in mice vaccinated with the reference Rev.1 vaccine is lower than 2.5; and iii) the standard deviation calculated on each lot of six mice is lower than 0.8.

If this test has been done with good results on a representative batch of the test vaccine, it does not have to be repeated routinely on other vaccine lots prepared from the same seed lot and with the same manufacturing process.

d) Duration of immunity

It is accepted that subcutaneous or conjunctival vaccination with standard doses of Rev.1 confers a solid and durable immunity in sheep and goats. However, growing field evidence shows that the immunity conferred declines with time, and revaccination could be advisable in endemic areas.

The use of reduced doses of Rev.1 produces a less efficient immunity, while side-effects, such as antibody responses or induction of abortion, are not fully avoided.

e) Stability

Strain Rev.1 vaccine prepared from seed stock from appropriate sources is stable in characteristics provided that the in-process and batch control requirements described above are fulfilled, and shows no tendency to reversion to virulence. The lyophilised vaccine shows a gradual loss of viable count, but should retain its potency for the recommended shelf life. Allowance for this phenomenon is normally made by ensuring that the viable count immediately following lyophilisation is well in excess of the minimum requirement. Maintenance of a cold chain during distribution of the vaccine will ensure its viability.

f) Preservatives

Antimicrobial preservatives must not be used in live Rev.1 vaccine. For preparation of the freeze-dried vaccine, a stabiliser as described in Section C2.4.f of Chapter 2.4.3 is recommended.

g) Precautions (hazards)

Please refer to chapter 2.4.3. Bovine brucellosis. *Brucella melitensis* Rev.1, although an attenuated strain, is still capable of causing disease in humans. Accordingly, cell cultures and suspensions must be handled under appropriate conditions of biohazard containment (see Chapter 1.1.2). Reconstitution and subsequent handling of the reconstituted vaccine should be done with care to avoid accidental injection or eye or skin contamination. Vaccine residues and injection equipment should be decontaminated with a suitable disinfectant. Medical advice should be sought in the event of accidental exposure. The efficacy of the antibiotic treatment of infections caused by Rev.1 (a streptomycin-resistant strain) in humans has not been adequately established but data in mice suggest that if Rev.1 contamination occurs, a combined treatment with doxycycline plus rifampicin or gentamycin could be recommended (19).

5. Tests on the final product

a) Safety

See Section C2.4.b of Chapter 2.4.3.

b) Potency

For the freeze-dried vaccine, the potency must be determined on the final product. The tests are as described in Section C2.4.c of Chapter 2.4.3.

In order to assess the vaccine efficiency, a representative sample of previously seronegative animals vaccinated with each new vaccine batch should be bled 15–20 days after vaccination and the serum samples submitted to BBAT. If adequate and independently of the vaccination route used, more than 80% of vaccinated animals should be BBAT positive.

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NB: There are OIE Reference Laboratories for Caprine and ovine brucellosis (excluding *Brucella ovis*) (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).