

Brucellosis in humans and animals



**Food and Agriculture
Organization
of the United Nations**



**World Organisation
for Animal Health**



**World Health
Organization**

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Preface

These *Guidelines* are designed as a concise, yet comprehensive, statement on brucellosis for public health, veterinary and laboratory personnel without access to specialized services. They are also to be a source of accessible and updated information for such others as nurses, midwives and medical assistants who may have to be involved with brucellosis in humans.

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The executive editors have drawn on the expertise of contributors who are acknowledged experts in their field and who understand the difficulties of dealing with this disease under the suboptimal conditions which still apply in many of the areas in which brucellosis remains an important economic and public health problem. We are grateful for their outstanding contributions and for the constructive comments of the many other experts who have advised on the text. Further, we wish to thank both the Swiss and the Italian Ministry of Foreign Affairs for their financial support.

MJ Corbel, SS Elberg and O Cosivi (editors).

Abbreviations

| | |
|-------|--|
| 2-ME | 2-mercaptoethanol |
| BCV | <i>Brucella</i> chemical vaccine |
| CF | complement fixation |
| CFT | complement fixation test |
| CIEP | counter-immunoelectrophoresis |
| CNS | central nervous system |
| CSF | cerebrospinal fluid |
| CT | computed tomography |
| DNA | deoxyribonucleic acid |
| DTT | dithiothreitol |
| ELISA | indirect enzyme-linked immusorbent assay |
| IU | international unit |
| LPS | lipopolysaccharide |
| MRT | milk ring test |
| NMR | nuclear magnetic resonance |
| PCR | polymerase chain reaction |
| RBT | Rose Bengal plate test |
| RES | reticuloendothelial system |
| SAT | serum agglutination test |
| S-LPS | smooth lipopolysaccharide |
| SMZ | sulfamethoxazole |
| TMP | trimethoprim |

1. Introduction

Brucellosis, also known as “undulant fever”, “Mediterranean fever” or “Malta fever” is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It affects people of all age groups and of both sexes. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs. It is an important human disease in many parts of the world especially in the Mediterranean countries of Europe, north and east Africa, the Middle East, south and central Asia and Central and South America and yet it is often unrecognized and frequently goes unreported.¹ There are only a few countries in the world that are officially free of the disease although cases still occur in people returning from endemic countries.

It is a zoonosis and the infection is almost invariably transmitted to people by direct or indirect contact with infected animals or their products. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs.

Expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling partly account for brucellosis remaining a public health hazard. Expansion of international travel which stimulates the taste for exotic dairy goods such as fresh cheeses which may be contaminated, and the importation of such foods into *Brucella*-free regions, also contribute to the ever-increasing concern over human brucellosis.

The duration of the human illness and its long convalescence means that brucellosis is an important economic as well as a medical problem for the patient because of time lost from normal activities. Prompt diagnosis and treatment with antibiotics has greatly reduced the time a patient may be incapacitated. Nevertheless, there are many regions where effective diagnosis or treatment is not available and/or where programmes for the detection and prevention of the infection in humans and animals are not adequately carried out. In these areas, the animal disease remains a constant threat to human welfare, particularly for those in the most vulnerable socioeconomic sections of the population.

¹ World animal health data, including brucellosis in animals and humans, are contained in Handistatus II and are also available in a hardcopy publication entitled World Animal Health. This information is collected from Veterinary Services of OIE, FAO and WHO Member Countries by the OIE Central Bureau, Paris, France, using a joint annual questionnaire and can be accessed through the following address: <http://www.oie.int>

The disease can be insidious and may present in many atypical forms. In many patients the symptoms are mild and, therefore, the diagnosis may not be even considered. Indeed it should be noted that even in severe infections differential diagnosis can still be difficult. The application of well-controlled laboratory procedures and their careful interpretation can assist greatly in this process.

While there is still a need for technical advances in some areas, it is important to note that the basic scientific information and methods required for the control of brucellosis in ruminants are at hand. Even where brucellosis in animals is not under control there are measures that can be taken to prevent human infection and to treat infected persons.

Intersectoral cooperation in support of primary health care approaches plays an important role in the control of brucellosis and may contribute to the development of appropriate infrastructures in areas of animal production, food hygiene, and health care. On the other hand the prevention and control of brucellosis needs supportive action from various sectors, including those responsible for food safety and consumer education.

Emphasis in this document is placed on fundamental measures of environmental and occupational hygiene in the community and in the household as well as on the sequence of actions required to detect and treat patients.

2. Clinical manifestation

Brucellosis is essentially a disease of animals, especially domesticated livestock, caused by bacteria of the *Brucella* group with humans as an accidental host. In other words it is a zoonosis. On genetic grounds the *Brucella* group can be regarded as variants of a single species which for historical reasons is identified as *Brucella melitensis*. However, for practical purposes this approach is considered unsatisfactory and six main “species” are distinguished: *B. abortus*, *B. suis*, *B. melitensis*, *B. neotomae*, *B. ovis*, *B. canis*. Strains isolated from marine mammals fall into at least three groups distinct from these and may be designated as new “nomen species”.

The differentiation of these variants is of practical importance as the epidemiology and, to a lesser extent, the severity of the disease in humans, is influenced by the type of organism and its source. Thus *B. abortus* is normally associated with cattle, *B. melitensis* with sheep and goats, *B. suis* with swine (although biovars 4 and 5 are specifically associated with reindeer and rodents respectively). *B. ovis* causes an infection specific for sheep and has not been conclusively implicated in human disease, *B. suis* biovar 5 has only been isolated on a few occasions from rodents and *B. canis* is usually associated with disease in dogs but occasionally causes human brucellosis. *B. neotomae* has been isolated on few occasions and has never been implicated in human disease.

The human disease usually manifests itself as an acute febrile illness which may persist and progress to a chronically incapacitating disease with severe complications. It is nearly always acquired directly or indirectly from animal sources, of which cattle, sheep, goats and pigs are by far the most important. In these natural hosts, the infection usually establishes itself in the reproductive tract, often resulting in abortion. Excretion in genital discharges and milk is common and is a major source of human infection.

The clinical picture is not specific in animals or humans and diagnosis needs to be supported by laboratory tests. Effective treatment is available for the human disease but prevention is the ideal, through control of the infection in animals and by implementation of hygienic measures at the individual and public health levels.

2.1 The disease in humans

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as may signs referable to almost any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized infection or a non-specific syndrome resembling the “chronic fatigue syndrome”. The disease is always caused by infection with a *Brucella* strain and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigens.

Evidence in support of the diagnosis includes:

- A history of recent exposure to a known or probable source of *Brucella* spp. This includes common host species, especially cattle, sheep, goats, pigs, camels, yaks, buffaloes or dogs; consumption of raw or inadequately cooked milk or milk products, and, to a lesser extent, meat and offal derived from these animals. In addition, the resistance of the organism and its high infectivity make environmental contamination a probable hazard, although this is always difficult to prove. Occupational exposure and/or residence in an area in which the infection is prevalent, also raise the probability of the diagnosis.
- Isolation of *Brucella* spp. from the patient.
- Demonstration by validated polymerase chain reaction (PCR) of the presence of *Brucella* genetic material in blood or other tissue sample.
- Demonstration by a validated serological method of *Brucella* antigen in blood or other tissue sample.
- Demonstration of a rising antibody titre in any serological test for brucellosis in the absence of exposure to any known source of cross-reacting antigens.
- Demonstration of a high sustained IgG antibody titre in the agglutination, complement fixation or ELISA tests with standardized antigens.

Susceptibility to brucellosis in humans depends on various factors, including the immune status, routes of infection, size of the inoculum and, to some extent, the species of *Brucella*. In general, *B. melitensis* and *B. suis* are more virulent for humans than *B. abortus* and *B. canis*, although serious complications can occur with any species of *Brucella*.

Common routes of infection include direct inoculation through cuts and abrasions in the skin, inoculation via the conjunctival sac of the eyes, inhalation of infectious aerosols, and ingestion of infectious unpasteurized milk or other

dairy products. Blood transfusion, tissue transplantation and sexual transmission are possible but rare routes of infection.

The disease is acute in about half the cases, with an incubation period of two to three weeks. In the other half, the onset is insidious, with signs and symptoms developing over a period of weeks to months from the infection. The clinical manifestations are varied and nonspecific. They include fever, sweats, fatigue, malaise, anorexia, weight loss, headache, arthralgia and back pain. Commonly, patients feel better in the morning, with symptoms worsening as the day progresses. The desire to rest can be profound, and depression is pervasive. If untreated, the pattern of the fever waxes and wanes over several days ("undulant fever"). Table 1 reports symptoms and signs in 500 patients with brucellosis due to *B. melitensis*.

Table 1. Symptoms and signs in 500 patients with brucellosis due to *B. melitensis*.

| Symptoms and signs | Number of patients | % |
|--------------------------------------|--------------------|-----------------|
| Fever | 464 | 93 |
| Chills | 410 | 82 |
| Sweats | 437 | 87 |
| Aches | 457 | 91 |
| Lack of energy | 473 | 95 |
| Joint and back pain | 431 | 86 |
| Arthritis | 202 | 40 |
| Spinal tenderness | 241 | 48 |
| Headache | 403 | 81 |
| Loss of appetite | 388 | 78 |
| Weight loss | 326 | 65 |
| Constipation | 234 | 47 |
| Abdominal pain | 225 | 45 |
| Diarrhoea | 34 | 7 |
| Cough | 122 | 24 |
| Testicular pain/epididymo-orchitis | 62 | 21 ^a |
| Rash | 72 | 14 |
| Sleep disturbance | 185 | 37 |
| Ill appearance | 127 | 25 |
| Pallor | 110 | 22 |
| Lymphadenopathy | 160 | 32 |
| Splenomegaly | 125 | 25 |
| Hepatomegaly | 97 | 19 |
| Jaundice | 6 | 1 |
| Central nervous system abnormalities | 20 | 4 |
| Cardiac murmur | 17 | 3 |
| Pneumonia | 7 | 1 |

Adapted from MM Madkour. *Brucellosis Overview*. In: Madkour's Brucellosis, 2nd edition. Springer, Berlin

^a Among 290 males

Brucella species are facultative intracellular pathogens that can survive and multiply within phagocytic cells of the host. The mechanisms by which *Brucella* evades intracellular killing are incompletely understood. Nevertheless, *Brucella* organisms ultimately become sequestered within monocytes and macrophages of the reticuloendothelial system (RES), such as lymph nodes, liver, spleen and bone marrow. Brucellosis is a systemic infection that can involve any organ or tissue of the body. When clinical symptoms related to a specific organ predominate, the disease is termed “localized”. Commonly, localization involves organs of the RES.

Although humoral antibodies appear to play some role in resistance to infection, the principal mechanism of recovery from brucellosis is cell-mediated. Cellular immunity involves the development of specific cytotoxic T lymphocytes and activation of macrophages, enhancing their bactericidal activity, through the release of cytokines (e.g. gamma interferon and tumour necrosis factor) from specifically committed helper T lymphocytes. Coincident with the development of cell-mediated immunity, the host usually demonstrates dermal delayed-type hypersensitivity to antigens of *Brucella*.

2.1.1 Osteoarticular complications

Bone and joint involvement are the most frequent complications of brucellosis, occurring in up to 40% of cases. A variety of syndromes have been reported, including sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis, and tenosynovitis. *Brucella* sacroiliitis is especially common. Patients present with fever and back pain, often radiating down the legs (sciatica). Children may refuse to walk and bear weight on an extremity. Early in the disease, radiographs and bone scintigrams can appear normal, but, in time, computed tomography (CT) or nuclear magnetic resonance (NMR) scans may show narrowing of the intervertebral disc space. Vertebral osteomyelitis is readily apparent through radionuclide scans showing destruction of the vertebral bodies. The lumbar vertebrae are involved more often than the thoracic and cervical spine. Paravertebral abscesses are less common in brucellosis than in spinal tuberculosis. A post-infectious spondyloarthropathy involving multiple joints has been described, and is believed to be caused by circulating immune complexes.

2.1.2 Gastrointestinal complications

Brucellosis, especially when due to *B. melitensis*, is often foodborne, and unpasteurized milk or dairy products, such as cheese, are common vehicles of transmission. Foodborne brucellosis resembles typhoid fever, in that systemic symptoms predominate over gastrointestinal complaints. Nevertheless, some patients with the disease experience nausea, vomiting, and abdominal discomfort. Rare cases of ileitis, colitis and spontaneous bacterial peritonitis have been reported.

2.1.3 Hepatobiliary complications

The liver is commonly involved in brucellosis, although liver function tests can be normal or only mildly elevated. The histological changes in the liver are variable, but disease caused by *B. abortus* may show epithelioid granulomas that are indistinguishable from sarcoidosis lesions. A spectrum of hepatic lesions has been described in cases due to *B. melitensis*, including scattered small foci of inflammation resembling viral hepatitis. Occasionally larger aggregates of inflammatory cells are found within the liver parenchyma with areas of hepatocellular necrosis. In other cases, small, loosely formed epithelioid granulomas with giant cells can be found.

Despite the extent of hepatic involvement, post-necrotic cirrhosis is extremely rare. Hepatic abscesses and chronic suppurative lesions of the liver and other organs have been described in cases due to *B. suis*. Acute and chronic cholecystitis have been reported in association with brucellosis.

2.1.4 Respiratory tract complications

Aerosol inhalation is a recognized route of transmission of brucellosis, especially common in abattoirs where infected animals are slaughtered. A variety of pulmonary complications have been reported, including hilar and paratracheal lymphadenopathy, interstitial pneumonitis, bronchopneumonia, lung nodules, pleural effusions, and empyema. *Brucella* organisms are rarely isolated from expectorated sputum.

2.1.5 Genitourinary complications

Orchitis and epididymitis are the most frequent genitourinary complications of brucellosis in men. Usually unilateral, *Brucella* orchitis can mimic testicular cancer or tuberculosis. Although *Brucella* organisms have been recovered from banked human spermatozoa, there have been a few reports implicating sexual transmission. Renal involvement in brucellosis is rare, but it too can resemble renal tuberculosis. In women, rare cases of pelvic abscesses and salpingitis have been reported.

2.1.6 Pregnancy and breastfeeding

Brucellosis during the course of pregnancy carries the risk of spontaneous abortion or intrauterine transmission to the infant. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for *B. abortus*. Although erythritol is not present in human placental tissue, *Brucella* bacteremia can result in abortion, especially during the early trimesters. Whether the rate of abortions from brucellosis exceeds rates associated with bacteremia from

other bacterial causes is unclear. In any event, prompt diagnosis and treatment of brucellosis during pregnancy can be lifesaving for the fetus.

Very rare human-to-human transmission from lactating mothers to their breastfed infants has been reported.

2.1.7 Cardiovascular complications

Infective endocarditis is the most common cardiovascular manifestation, and it is said to be the most common cause of death from brucellosis. Endocarditis is reported in about 2% of cases, and can involve both native and prosthetic heart valves. The aortic valve is involved more often than the mitral valve. Aneurysms of the sinus of Valsalva and other vascular structures appear to be most common when infection is caused by *B. suis*. Mycotic aneurysms, usually involving the middle cerebral artery, can be a neurological complication of infective endocarditis. Treatment of endocarditis caused by *Brucella* species usually requires a combination of antimicrobial therapy and valve replacement surgery.

2.1.8 Neurological complications

Neurobrucellosis refers to a variety of neurological complications associated with brucellosis. Direct invasion of the central nervous system occurs in about 5% of cases of *B. melitensis* infection, and meningitis or meningo-encephalitis are the most common manifestations. *Brucella* meningitis can be acute or chronic. It often occurs late in the course of disease, but it can be the presenting manifestation. Analysis of cerebrospinal fluid (CSF) usually reveals an elevated protein content, normal or low glucose concentration, and a lymphocytic pleocytosis. *Brucella* organisms are rarely isolated from CSF, but specific antibodies can be demonstrated in the CSF and serum. Other CNS manifestations of brucellosis include cerebral vasculitis, mycotic aneurysms, brain and epidural abscesses, infarcts, haemorrhage, and cerebellar ataxia. Peripheral nerve complications include neuropathy/radiculopathy, Guillain-Barré syndrome, and a poliomyelitis-like syndrome.

Brain scans (e.g. CT, magnetic resonance imaging) are usually normal in meningitis, but can be useful for detecting space-occupying lesions and the integrity of the epidural space. Basal ganglia calcification has been reported in some patients with neuro-brucellosis.

2.1.9 Cutaneous complications

A variety of skin lesions have been reported in patients with brucellosis, including rashes, nodules, papules, erythema nodosum, petechiae, and purpura.

Cutaneous ulcers, abscesses, and suppurative lymphangitis appear to be more common with *B. suis*. Occasionally, epistaxis, gingivorrhoea, haematuria, and cutaneous purpura occur in association with severe thrombocytopenia, which has been ascribed to hypersplenism, bone marrow haemaphagocytosis, and/or anti-platelet antibodies.

2.1.10 Ophthalmic complications

Although uncommon, a variety of ocular lesions have been reported in patients with brucellosis. Uveitis is the most frequent manifestation, and can present as chronic iridocyclitis, nummular keratitis, multifocal choroiditis or optic neuritis. Since *Brucella* organisms have not been isolated from the structures of the eye in humans, many of these lesions are considered to be late complications, possibly immunologically mediated. Consequently, the usual treatment for ocular complications is steroids.

2.1.11 Chronic brucellosis

Perhaps no aspect of the disease elicits more controversy than chronic brucellosis. This is due, in part, to the lack of a universally accepted definition. Most authorities agree that the term “chronic brucellosis” should be reserved for patients whose clinical symptoms persist for 12 months or more from the time of the diagnosis. Using this criterion, patients fall into three categories: (1) relapse, (2) chronic localized infection, and (3) delayed convalescence.

Relapse is defined as the recurrence of characteristic signs and symptoms (with or without a positive culture) occurring at some time after the completion of a course of treatment. Patients with relapse characteristically have objective signs of infection, such as fever, and persistently elevated titres of IgG antibodies in their serum. Most relapses occur within six months after therapy is discontinued, and relapse is not usually due to the emergence of antibiotic resistant strains, although this has been seen after monotherapy with rifampicin or streptomycin. Therefore, relapse can usually be treated by repeating the course of therapy with the same drugs.

Chronic localized infection is defined as the recurrence of characteristic signs and symptoms (with or without a positive blood culture) caused by the failure to eliminate a deep focus of infection, such as osteomyelitis, or deep tissue abscesses. Patients with localized infection have also objective signs of infection, such as fever, although symptoms may recur intermittently over long periods of time. As is the case with patients with relapse, localized infection is characterized by persistent elevation of IgG antibodies in the serum. Unlike relapse, chronic localized brucellosis may require surgical intervention to drain foci of infection in addition to antimicrobial therapy.

Delayed convalescence is defined as the persistence of symptoms, without objective signs of infection, such as fever, in patients who have completed a course of therapy, and in whom titres of antibodies have declined or even disappeared. The etiology of delayed convalescence is unknown, but psychological studies of some patients suggest a high incidence of personality disorders, often predating the onset of brucellosis. In any case, patients with delayed convalescence do not appear to benefit from repeated courses of antimicrobial therapy.

2.1.12 Childhood brucellosis

Once considered rare in children, it is now recognized that brucellosis can affect persons of all ages, especially in areas where *B. melitensis* is the predominant species. The course of infection and the incidence of complications appear to be similar regardless of the age of the patients.

KEY POINTS ON THE DISEASE IN HUMANS

- Human brucellosis usually presents as an acute febrile illness.
- Most cases are caused by *B. melitensis*.
- All age groups are affected.
- Complications may affect any organ system.
- The disease may persist as relapse, chronic localized infection or delayed convalescence.

2.2 The disease in animals

Brucellosis is a sub-acute or chronic disease which may affect many species of animals. In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male (Fig. 1 and 2). Clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of *Brucella* spp. either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibody or cell-mediated immune responses.

Brucellosis is a disease of many animal species but especially of those that produce food: sheep (especially milk-producing), goats, cattle and pigs and,

on a more localized scale, camels, buffaloes, yaks and reindeer. Five of the six currently recognized *Brucella* species cause infection and clinical signs in one or more animal hosts (see Table 2). Four of these also cause human disease: *B. melitensis*, *B. suis*, *B. abortus* and *B. canis* in descending order of pathogenicity. The recently recognized types associated with marine animals may also have the capacity to cause human disease.

The *Brucellae* are somewhat host-specific but cross-species infections occur, especially with *B. melitensis*. Infections in many wildlife species have been reported but those that obviously affect population fecundity and result in human infections are quite rare. *B. melitensis* infections in dairy herds, however, have severe economic and public health implications.

Infections in sheep and goats are highly contagious because of the pathogenicity of *B. melitensis* and because of close contact caused by the density of the flocks or herds, the commingling of those of different owners and heavy exposure in housing. Animal-to-animal transmission occurs as a result of the large number of organisms shed in the environment.

Humans are often infected due to direct animal contact or ingestion of contaminated dairy products. Human cases may be a useful indicator of the presence of disease in animal populations and may be the only source of information for surveillance. It is important, however, to determine if the infection was acquired locally or elsewhere, and, if food products are implicated, to establish whether these were locally produced or imported (see Section 8).

Characteristic but not specific signs of brucellosis in most animal hosts are abortion or premature births and retained placenta. In some areas, abortion is relatively uncommon. In some parts of Africa, hygromas and abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. abortus* biovar 3. There is lowered milk production due to premature births. Interference with fertility is usually temporary and most infected animals will abort only once and some are unaffected. The udder is often permanently infected, especially in the case of cows and goats. Shedding of organisms in milk is frequent. Localized infections in sheep result in orchitis or epididymitis in the case of *B. melitensis* and *B. ovis*. In goats, cattle, swine and dogs similar complications may follow infection with *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* respectively. Arthritis may also be a rare sign in *B. melitensis*-infected sheep and goats. In horses, local abscess formation in bursae may be the only clinical sign and infection in this species is often asymptomatic. Camels infected with *B. melitensis* shed the organisms in milk and in some countries this is a serious public health problem. Clinical signs of brucellosis in camels appear to be very rare.

The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated animals and numbers of

organisms shed are much greater. The bacteria are found in tissues and fluids associated with pregnancy, the udder and the lymph nodes which drain the relevant areas.

Most infections result from ingestion of bacteria either from diseased animals or contaminated feedstuffs. However, infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural breeding transmits infection in swine and dogs and, to a lesser extent, sheep and goats. Persistent bacteraemias are also more common in the first two species. Bacteraemia occurs during the course of infection in other species but is usually intermittent and of short duration.

KEY POINTS ON THE DISEASE IN ANIMALS

- Brucellosis infects many species, especially cattle, sheep, goats, pigs.
- Different *Brucella* types infect different species preferentially.
- Brucellosis presents typically as abortion in animals.
- Diagnosis can only be confirmed by laboratory tests.

Table 2. Animals affected by *Brucella* spp.

| HOST | <i>B. abortus</i> | <i>B. melitensis</i> | <i>B. suis</i> | <i>B. canis</i> | <i>B. ovis</i> |
|------------------|-------------------|----------------------|----------------|-----------------|----------------|
| Cattle | + | + | +(rare) | – | – |
| Buffaloes | + | + | – | – | – |
| Bison | + | – | – | – | – |
| Sheep | +(rare) | + | +(possible) | – | + |
| Goats | +(rare) | + | – | – | – |
| Swine | +(rare) | +(rare) | + | – | – |
| Dogs | + | + | +(rare) | + | – |
| Camels | +(rare) | + | – | – | – |
| Caribou/Reindeer | – | – | +(biovar 4) | – | – |
| Elk | + | – | – | – | – |
| Horses | + | +(rare) | +(rare) | – | – |
| Rodents | +(rare) | +(rare) | +(biovar 5) | – | – |

3. Epidemiology

3.1 Epidemiology of brucellosis in humans

3.1.1 Reservoirs of infection

Brucellosis is a zoonotic disease, hence the ultimate sources of infection are infected animals. The key species are the major food-producing animals: cattle, sheep, goats, pigs. Others, including bison, buffalo, camels, dogs, horses, reindeer and yaks are less important, but they can be very significant local sources of infection in some regions. Recently, the infection has also been identified in marine mammals, including dolphins, porpoises and seals, and these may present an emerging hazard to persons occupationally exposed to infected tissues from them.

The risk of disease and its severity is to a significant extent determined by the type of *Brucella* to which an individual is exposed. This will be influenced by the species of host animal acting as source of infection.

B. melitensis is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. The organism is normally associated with infection in sheep and goats, but other species, including dogs, cattle and camels can be infected. In some countries, particularly in the Middle East, *B. melitensis* infection of cattle has emerged as an important problem. Contrary to some traditional views, *B. melitensis* remains fully virulent for man after infecting cattle. The bovine infection presents a particularly serious problem because of the large volume of infected milk that can be produced by an individual animal and because of the extensive environmental contamination that even single abortions or infected births can produce.

B. abortus is the most widespread cause of infection, but associated with much less human disease. Infection in man is often sub-clinical and, where disease does occur, it is usually less severe than that caused by *B. melitensis* or *B. suis*. Cattle are by far the most common source of *B. abortus* but bison, buffalo, camels, dogs and yaks are important in some areas.

B. suis has a much more restricted occurrence than *B. melitensis* and *B. abortus*. It is locally important as a source of human infection which can be as severe as that produced by *B. melitensis*. The sources and virulence of the organism vary with its biovar (subtype defined by laboratory tests).

Biovars 1, 2, and 3 are associated with pigs and also, in the case of biovar 2, with hares. This variant has a low pathogenicity for humans but biovars 1 and 3 are highly virulent and can cause severe disease. Biovar 4 is associated with infection of caribou and reindeer in Alaska, Canada and Northern Russia. It is infrequently reported as a cause of human disease. Naturally acquired human cases of biovar 5 infection have not been reported.

B. canis is a widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have usually been mild.

Brucella infection occurs in many species of wild animals but these are rarely implicated as sources of human disease.

3.1.2 Transmission of brucellosis to humans

The possible means of acquisition of brucellosis include: person-to-person transmission, infection from a contaminated environment, occupational exposure usually resulting from direct contact with infected animals, and foodborne transmission.

3.1.2.1 Person-to-person transmission

This is extremely rare. Occasional cases have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of transmission.

Of more potential significance is transmission through blood donation or tissue transplantation. Bone marrow transfer in particular carries a significant risk. It is advisable that blood and tissue donors be screened for evidence of brucellosis and positive reactors with a history of recent infection be excluded. Transmission to attendants of brucellosis patients is most unlikely but basic precautions should be taken. Laboratory workers processing samples from patients run a much greater risk.

3.1.2.2 Infection from a contaminated environment

This is difficult to document but probably occurs more frequently than is recognized. Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market places, especially if abortions occur. Inhalation brucellosis may then result from exposure to contaminated dust, dried dung etc. Contact infection may also result from contamination of skin or conjunctivae from soiled surfaces. Water sources, such as wells, may also be contaminated by recently aborted animals or by run-off of rain water from contaminated areas.

Brucella spp. can survive for long periods in dust, dung, water, slurry, aborted fetuses, soil, meat and dairy products. The precise duration of

survival is dependent on many variables such as the nature of the substrate, number of organisms, temperature, pH, sunlight, the presence of other microbial contaminants. Some examples are given in Table 3.

3.1.2.3 *Occupational exposure*

Certain occupations are associated with a high risk of infection with brucellosis. These include people who work with farm animals, especially cattle, sheep, goats and pigs: farmers, farm labourers, animal attendants, stockmen, shepherds, sheep shearers, goatherds, pig keepers, veterinarians and inseminators are at risk through direct contact with infected animals or through exposure to a heavily contaminated environment. Infection may occur by inhalation, conjunctival contamination, accidental ingestion, skin contamination especially via cuts or abrasions, and accidental self-inoculation with live vaccines.

The families of farmers and animal breeders may also be at risk as domestic exposure may be inseparable from occupational exposure when animals are kept in close proximity to living accommodation. In some areas, the animals are kept in the yards of houses and may even be brought inside, especially in severe weather. In the case of recently aborted animals, this has resulted in infection of entire households. The use of dried dung as a fuel may also import infection into households. It should be noted that brucellosis often presents as clusters of cases in a family or tribal group, usually relating to a common infected food source, and often follows an outbreak in animals.

Children can be particularly at risk as they may adopt newborn or sick animals as pets. In some areas they may be the only group presenting with acute symptoms, as older members of the community are likely to be immune or chronically infected.

Persons involved in the processing of animal products may be at high risk of exposure to brucellosis. These include slaughtermen, butchers, meat packers, collectors of fetal calf serum, processors of hides, skins and wool, renderers and dairy workers. Direct and environmental contamination may present hazards through inhalation, ingestion, mucous contamination and skin contact or penetration.

Staff employed in the maintenance of farm premises, factories or plants used for processing animal products are often overlooked as occupationally exposed groups but may be at considerable risk from environmental contamination.

Laboratory staff involved in culturing *Brucella* are at particular risk. In some countries in which brucellosis is no longer endemic, this potential hazard may be overlooked or considered no longer relevant. Nevertheless, the performance of diagnostic procedures on patients with unsuspected imported disease may lead to culture of organisms which are not correctly identified until laboratory-acquired infection raises the level of suspicion. The use of

rapid identification gallery test systems has caused *Brucella* strains to be misidentified as *Moraxella* spp, with serious consequences for the staff. Inhalation of aerosols generated by manipulation of cultures presents the greatest hazard, especially if breakage of containers occurs during such processes as centrifugation.

The preparation and use of live vaccines is also hazardous as strains such as *B. abortus* S19 and *B. melitensis* Rev 1 are not completely avirulent for humans. The rough vaccine strain *B. abortus* RB 51 appears to be of low pathogenicity but still presents a potential hazard through accidental injection and is rifampicin-resistant. The use of virulent strains to prepare diagnostic antigens should also be avoided where possible.

3.1.2.4 Foodborne transmission

This is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unheated milk is the main source of infection for most populations. Cow, sheep, goat or camel milk contaminated with *B. melitensis* is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms. Butter, cream or ice-cream prepared from such milk also presents a high risk. Soft cheeses prepared from sheep or goats milk by addition of rennet are a particularly common source of infection in Mediterranean and Middle Eastern countries. The cheese-making process may actually concentrate the *Brucella* organisms, which can survive for up to several months in this type of product. Such cheeses should be stored in cool conditions for at least six months before consumption. Hard cheeses prepared by lactic and propionic fermentation present a much smaller risk. Similarly, yoghurt and sour milk are less hazardous. *Brucella* dies off fairly rapidly when the acidity drops below pH 4, and very rapidly below pH 3.5. Equipment used in the transport or processing of infected milk or other raw material may contaminate uninfected products unless good hygienic practice is observed.

Meat products are less frequently associated with infection, mainly because they are not usually eaten raw. However, this is a not unknown practice among butchers and abattoir workers. Muscle tissue usually contains low concentrations of *Brucella* organisms but liver, kidney, spleen, udder and testis may contain much higher concentrations. In some countries, dishes prepared from these organs may be eaten raw or undercooked. Fresh blood, either alone or mixed with fresh milk, may also be drunk and presents an obvious potential hazard.

In many countries, the consumption of “health foods” has become fashionable. These often include unpasteurized milk or milk products and may pose a particular risk. There is often considerable resistance to accepting that such “healthy” products can be dangerous. Raw vegetables may be contaminated by infected animals and present a hazard. In endemic areas, tourists consuming “ethnic” food products may be particularly at risk.

Persons with achlorhydria resulting from disease or through consumption of antacids or H₂ antagonists may have an increased risk of acquiring brucellosis through ingestion of contaminated foods.

Individuals with immunodeficiency states resulting from disease or treatment with immunosuppressive agents may also be at increased risk of severe brucellosis, although this is difficult to quantify.

3.1.3 Seasonal factors

In countries with temperate or cold climates there is a marked seasonal variation in the incidence of acute brucellosis, with most cases occurring in the spring and summer. This coincides with the peak period for abortions and parturitions among farm animals and hence for the highest level of exposure of those attending the animals and consuming their milk. The seasonal effect is more obvious for ovine/caprine brucellosis than for bovine brucellosis, possibly because of the longer lactation period in cattle.

In tropical and subtropical areas, where animal breeding extends throughout the year, there is no seasonal influence on the incidence of brucellosis.

3.1.4 Age and sex distribution

In industrialized countries and in those others in which food hygiene prevents foodborne brucellosis, the disease is very largely occupational and the majority of cases are males between the ages of 20 and 45 years. In these situations, the disease is usually caused by *B. abortus* or *B. suis*. In countries or areas where *B. melitensis* is prevalent, the practices followed in marketing and distributing sheep and goat milk products in particular make the enforcement of hygienic measures very difficult. In this situation the whole population is at risk and many cases occur in women and children. In nomadic societies, the adults have often been exposed to infection at an early age and do not manifest acute disease, although many may have sequelae from chronic infection. Under such conditions children account for a high proportion of acute cases and brucellosis is largely a paediatric problem.

3.1.5 Travel-acquired brucellosis

Tourists or business travellers to endemic areas may acquire brucellosis, usually by consumption of unpasteurized milk or other dairy products. Travellers may also import infected cheeses or other dairy products into their own countries and infect their families or social contacts by this means. Imported cases now account for most of the acute brucellosis cases seen in North America and Northern Europe.

3.1.6 Bio-terrorism

B. melitensis and *B. suis* have been developed experimentally as biological weapons by state sponsored programmes. Their relative stability in aerosol form, combined with low infectious dose make them suitable agents for this purpose. *Brucella* could be used to attack human and/or animal populations. The impact is likely to be greatest in those areas in which the disease is not endemic. The organism can be obtained from natural sources in many parts of the world. Health and veterinary authorities should be aware of this potential source of infection.

Table 3. Survival periods of *B. abortus* or *B. melitensis* in various substrates.

| Medium | Temperature or environment | Survival |
|-----------------------------|----------------------------|-------------|
| <i>B. abortus</i> | | |
| Solid surfaces | <31 °C, sunlight | 4–5 hours |
| Tap water | –4 °C | 114 days |
| Lake water | 37 °C, pH 7.5 | <1 day |
| Lake water | 8 °C, pH 6.5 | >57 days |
| Soil – dried | ~20 °C | <4 days |
| Soil – wet | <10 °C | 66 days |
| Manure | summer | 1 day |
| Manure | winter | 53 days |
| Farm slurry animal waste | ambient-temperature tank | 7 weeks |
| Farm slurry animal waste | 12 °C tank | >8 months |
| <i>B. melitensis</i> | | |
| Broth | pH>5.5 | >4 weeks |
| Broth | pH 5 | <3 weeks |
| Broth | pH 4 | 1 day |
| Broth | pH <4 | <1 day |
| Soft cheese | 37 °C | 48–72 hours |
| Yoghurt | 37 °C | 48–72 hours |
| Milk | 37 °C | 7–24 hours |

KEY POINTS ON THE EPIDEMIOLOGY OF BRUCELLOSIS IN HUMANS

- Cattle, sheep, goats and pigs are the main reservoirs of *Brucella*.
- Transmission to humans occurs through occupational or environmental contact with infected animals or their products.
- Foodborne transmission is a major source of infection, with cheese made from raw milk and unpasteurized milk presenting a high risk.
- Brucellosis can be a travel-associated disease.
- Blood or organ/tissue transfer are possible sources of infection.
- Person-to-person transmission is extremely rare.

3.2 Epidemiology of brucellosis in animals

This will vary with the host species affected. For cattle, infection is usually caused by *B. abortus*. However, *B. melitensis* and rarely *B. suis* can also establish themselves in cattle and the mode of transmission is then similar to that for *B. abortus*. These infections are particularly dangerous to humans because of the high virulence of most *B. melitensis* and *B. suis* strains and of the large numbers of bacteria that are excreted by these animals.

In cattle and other Bovidae, *Brucella* is usually transmitted from animal to animal by contact following an abortion. Pasture or animal barn may be contaminated and the organisms are probably most frequently acquired by ingestion but inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The use of pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays little role in the epidemiology of bovine brucellosis. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection.

In sheep and goats, *B. melitensis* is nearly always the infecting species. *B. ovis* can also infect sheep but is of little significance in relation to human disease. The mode of transmission of *B. melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays a greater role. The transmission of disease is facilitated by commingling of flocks and herds belonging to different owners and by purchasing animals from unscreened sources. The sharing of male breeding stock also promotes transfer of infection between farms. Transhumance of summer grazing is a significant promoting factor in some areas as is the mingling of animals at markets or

fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection.

Swine brucellosis is transmitted by direct contact with recently aborted sows, by ingestion of contaminated food or exposure to a contaminated environment. However, sexual transmission is particularly important. Brucellosis may be introduced on to farms through the communal use of boars or by purchase of infected animals.

For all species, embryo transfer is safe provided that recommended procedures are followed.

B. canis can be a major problem in dog breeding kennels. Transmission is by contact with recently aborted animals or with food or environment contaminated by abortions or excreta. Sexual transmission is also an important means of spread and males can excrete the organisms in large numbers in their semen. Urinary excretion also occurs and is a potential hazard to humans. However, in some countries where *B. canis* is present in the dog population, overt human disease caused by this organism seems to occur infrequently.

It should be remembered that dogs can acquire infection with *B. abortus*, *B. melitensis* or *B. suis* from aborted ruminants or swine, usually by ingesting fetal or placental material. They can then excrete these bacteria and may present a serious hazard to humans and domestic livestock.

B. suis biovar 4 causes brucellosis in caribou and reindeer. The epidemiology is similar to that of bovine brucellosis. Transmission to people can occur through the usual routes. However, ingestion of raw or undercooked reindeer bone marrow has also been implicated as a source of human infection.

In cattle, sheep, goats and swine, susceptibility to brucellosis is greatest in sexually mature animals. Young animals are often resistant, although it should be noted that latent infections can occur and such animals may present a hazard when mature.

Breed may also affect susceptibility, particularly in sheep. The milking breeds seem to be the most susceptible to *B. melitensis*. Breed differences in susceptibility have not been clearly documented in cattle although genetically determined differences in susceptibility of individual animals have been demonstrated. Polymorphism of the natural resistance associated monocyte protein (NRAMP) gene has been shown to influence substantially susceptibility to brucellosis in cattle and pigs. However, management practices are far more important in determining the risk of infection. Latent or inapparent infections can occur in all farm animal species. These usually result from infection in utero or in the early post-natal period. Such animals can retain the infection for life and may remain serologically negative until after the first abortion or parturition. Latent infection has been estimated to occur in

the progeny of about 5% of infected cows. The extent of the problem in other species is not known, but latency has been documented in sheep.

Acquired immunity has a substantial effect on susceptibility. Vaccination of cattle with *B. abortus* strain 19 or RB 51, or sheep and goats with *B. melitensis* Rev 1 can reduce susceptibility a thousand fold or more to the homologous species. *B. abortus* strain 19 does not protect cattle against *B. melitensis*. However, there is little information on the use of Rev 1 vaccine in cattle. The efficacy of this vaccine against the *B. melitensis* strains prevalent in some areas has also been questioned. Vaccines must be obtained from a reliable, internationally approved source. It is possible that strains of *B. melitensis* exist which can circumvent the immunity induced by this vaccine. However, it is at least as probable that variations in vaccine quality have affected protection rates. For the present, Rev 1 vaccine is the most effective vaccine available against *B. melitensis* and in many countries has given very good results. Its use is recommended when uncontrolled *B. melitensis* infection exists in ruminant populations.

KEY POINTS ON THE EPIDEMIOLOGY OF BRUCELLOSIS IN ANIMALS

- *B. abortus* causes most brucellosis in cattle, but *B. melitensis* and *B. suis* can also cause bovine infection.
- *B. melitensis* is the main cause of brucellosis in sheep and goats and *B. suis* in swine.
- Transmission occurs by direct contact and environmental contamination following abortion.
- Sexual transmission and/or artificial insemination are also important.
- Seronegative latent infections can occur.

4. Diagnosis

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme in animals. *Brucella* represents a risk to personnel handling it in the laboratory. Attention must be paid to the local legal requirements for handling *Brucella* and it is essential that certain minimum standards of laboratory safety are adhered to. These are specified in Section 6. For information on the international standards of diagnostic tests, please also refer to the *Manual of diagnostic tests and vaccines for terrestrial animals*, OIE, 2004

4.1 Diagnosis in humans

The diagnosis of human brucellosis cannot be made solely on clinical grounds due to the wide variety of clinical manifestations of this disease, and it is essential to perform bacteriological and serological tests. However, all physicians dealing with a febrile patient living in an endemic area or recently travelled to a country where brucellosis is endemic (“travel-associated disease”) must be aware of the possibility that the patient could be infected with *Brucella*. For this reason, correct clinical history taking is essential to orientate the diagnosis, and the need for some very basic questions (profession, food ingested, contact with animals and travel to endemic areas) must be emphasized. Moreover, a rapid screening test must be performed. The Rose Bengal plate test can be used as a sensitive rapid screening test but the results should be confirmed by bacteriological and other serological tests. Should the screening test prove negative in the face of a history and clinical presentation, it is advisable to check the result using additional tests. Careful observance of these practices will help to avoid delayed diagnosis.

4.1.1 Bacteriological diagnosis

The only conclusive evidence of *Brucella* infection is the recovery of the bacteria from the patient. Although *Brucella* can be isolated from bone marrow, cerebrospinal fluid, wounds, pus, etc., blood is the material most frequently used for bacteriological culture. Concentrating and lysing the leukocyte fraction before culture is reported to improve the isolation rate.

The system of blood culture that is recommended is the biphasic method of Castaneda which uses both solid and liquid medium in the same container. This limits the need for subculture and thus reduces the risk of laboratory-acquired infection. Serum dextrose broth with corresponding solid phase is often recommended but *Brucella* will grow on most high quality peptone based media used for blood culture. Selective medium is not necessary for culture of human blood samples taken with aseptic precautions. Incubation should be performed in air supplemented with 5% CO₂. The newer semi-automatic methods (BACTEC 9204 and Bac/Alert) shorten considerably the time taken for detection; the presence of *Brucella* can be detected with these methods by the third day of incubation. However, limited published data exist for a significant number of blood cultures that would permit comparison of these and traditional methods. It should be noted that earlier systems (BACTEC NR 730) failed to detect an appreciable number of samples which were positive by conventional blood culture systems.

Conventional Castaneda blood cultures are seldom positive before the fourth day of incubation. The majority of blood cultures are positive between the seventh and 21st day, and only 2% are positive after the 27th day. For this reason, incubations should be carried out for at least 45 days before rejecting a blood culture as negative for *Brucella*. Periodic tipping of the broth-blood mixture in Castaneda bottles over the solid phase should minimise the need for subculture. However, if no growth has appeared after one week it is recommended that the liquid be subcultured on to a solid medium, and that smears be prepared and stained with the modified Ziehl-Neelsen method of Stamp. This process can distinguish *Brucella* from the possible Gram positive cocci and bacilli skin contaminants, and from artifacts present in media inoculated with blood.

In Mediterranean countries, for unknown reasons, *B. abortus* is rarely isolated from human cases. In Spain, for example, over 98% of 2107 isolates from humans examined in one study were *B. melitensis*. The percentage of positive blood cultures in patients with fever can be as high as 86.5%. In patients either with low fever or without fever, the percentages fall to 75% and 28.5% respectively. This also holds true for relapsed patients. However, it has been shown in two extensive studies that 31.8% and 41.9% isolations of *B. melitensis* were from patients without fever.

A presumptive identification of *Brucella* isolates at genus level can be made on the basis of colonial morphology, appearance of smears stained with the methods of Gram and Stamp, and the results of oxidase and slide agglutination tests with *Brucella*-specific antisera. Alternatively, if a validated molecular identification method is available, such as PCR with primers for the genus-specific insertion sequences *IS 711* or *IS650*, or sections of the *16S-23S rRNA*, *BCPS31* and *omp 2a* genes, this may be used. The presumptive *Brucella* isolate should be submitted to a reference laboratory for a precise identification at species and biovar level, as this can provide very valuable epidemiological information.

Recently, some authors have proposed PCR-based assays for the direct detection of *Brucella* organisms in blood. However, more experience is needed before deciding whether this can replace the traditional blood cultures. Before any such assay is introduced into the routine laboratory tests, it must be validated for sensitivity, specificity and reproducibility. Before implementing any such procedures, it is essential to institute efficient containment procedures to prevent contamination of samples with bacterial DNA or amplified replicons from the laboratory environment.

4.1.2 Serological diagnosis

4.1.2.1 *Antigens and immunoglobulins of diagnostic significance*

The major *Brucella* antigens that are useful for diagnosing human brucellosis are the smooth (S) lipopolysaccharide (LPS) of the outer cell membrane and the internal (cytosolic) proteins. As in the case of other Gram-negative bacteria in the S-phase, the surface of *Brucella* is an outer membrane containing S-LPS which is exposed to the environment. The LPS is the immunodominant antigen but is also the molecule carrying the epitopes that may cross-react with other Gram-negative bacteria including *Yersinia enterocolitica* O:9, *Escherichia coli* O:157, *Francisella tularensis*, *Salmonella urbana* O:30, *Vibrio cholerae*, and others.

The serum (tube) agglutination test (SAT), or micro-titre plate variants of this, using heat/phenol-killed whole S-cells, detects antibodies to the S-LPS. Antibodies reacting against S-LPS can also be detected by other tests – e.g. enzyme-linked immunosorbent assay (ELISA) – when they are adapted to use extracts which contain S-LPS. Since this is the immunodominant antigen, antibodies to proteins are detected using S-LPS-free cytosolic protein preparations. An important point in the use of the cytosolic proteins is that a significant serological cross-reactivity with the above mentioned bacteria has never been found (although cross-reactivity with bacteria such as *Ochrobactrum* that are closely related genetically to *Brucella* is possible). Therefore, these proteins can be used to distinguish infections caused by *Brucella* from those caused by bacteria cross-reacting at S-LPS level.

The human immune response to *Brucella* is characterized by an initial production of IgM isotype antibodies followed after a longer period by the secretion of IgG isotype antibodies. The ELISA using S-LPS can be used to measure the evolution of immunoglobulin isotypes following infection and after treatment. A good correlation has been found between ELISA-IgM and serum agglutination titres. In one study, after treatment the titre of IgM antibodies appeared to decline faster than that of IgG antibodies. However, between 25% and 50% of patients with acute brucellosis presented IgM antibodies one year after treatment. Among these patients, 85% had high titres of IgG 18 months after clinical recovery, and in patients suffering a relapse, there was a concomitant increase in IgG but not IgM. IgA titres

roughly paralleled IgG titres. In contrast, in a different study using SAT and 2-mercaptoethanol (2-ME) or dithiothreitol (DTT) agglutination, patients successfully treated for brucellosis had a rapid decline in 2-ME resistant (IgG) and sensitive (IgM) agglutinins. However, low levels of 2-ME sensitive (IgM or IgA) agglutinins, as measured by SAT, could remain in the serum for long periods of time. It should be noted that reduction tests are not totally specific for IgM as they can degrade IgA as well. Therefore their results must be interpreted with caution. ELISA is to be preferred for detecting specific isotypes. The apparently different results of the 2-ME and ELISA-IgG are partly due to this effect and partly to the detection in the latter test of small amounts of antibodies of the IgG class that do not agglutinate. Since it is important to correlate the titres of antibody to *Brucella* with the clinical course of infection, one must be aware of the antibody class that is measured by the individual tests.

Theoretically, in acute brucellosis, the first and principal immunoglobulin isotype is IgM. Subsequently there is a switch to IgG isotype synthesis in patients who have not received treatment. The initial IgM response may not be seen in patients with a slow insidious onset of disease, in those seen late in the course of the disease or in those with relapses. The titres of agglutinins (IgM, IgA and IgG), should decline after successful treatment; if they do not, it is necessary to evaluate the patient for the possibility of a relapse or chronic focal disease. IgG and IgA titres increase in relapses.

4.1.2.2 Serological tests that detect antibodies against S-LPS

The RBT is currently the recommended rapid screening test, but the results should always be confirmed by other tests detecting agglutinating and non-agglutinating antibody and by bacteriological culture, particularly in areas where there is a high incidence of animal brucellosis. The sensitivity of RBT is over 99%, but it can give false positive reactions with sera from patients infected with *Y. enterocolitica* 0:9 or other cross reactive organisms and from healthy individuals that have had contact with *S-Brucella* without developing disease.

The SAT is a very useful test for the diagnosis of human brucellosis when it is performed with a standardized antigen preparation, and titres which can be expressed in International Units (IU) can be correlated well with clinical stages of infection. To make this test more informative, an agglutination in parallel should be performed using as diluent phosphate buffer containing 2-ME at a final concentration of 0.05 M or DTT at a final concentration of 0.005 M, which destroys the agglutinating activity of IgM (and IgA).

The problem of defining an SAT titre indicative of active infection has yet to be solved. In general, each patient produces an individual response and it is not possible to predict the behaviour of this in each case, nor to explain why some patients develop high agglutinin titres, while others have only low values during the disease. For example, in a study of 238 brucellosis patients,

it was found that, if a titre of 1/80 (100 IU) was to be considered as diagnostic, 29.2% of the patients would be considered negative; furthermore, in the same study it was found that 3.4% of the sera had a titre of 1/10 (12 IU) or below. As a guide, titres of 160 (200 IU) or more have a clear diagnostic value as long as the patient presents signs and symptoms of the disease. Some authors consider that in areas with a high incidence of animal brucellosis the diagnostic titre should be higher than this as many asymptomatic individuals will have titres at this level. In these circumstances the value of the SAT is severely limited. Although seroconversion is highly indicative of infection it is seldom seen in practice, because serum samples are rarely taken at a sufficiently early stage of infection.

A good correlation between the results of IgG ELISA and Coombs tests has been reported. However, the Coombs test (and IgG ELISA) remains positive longer than other agglutination tests. The titres of Coombs tests are usually very high when infection with *Brucella* has been present for a long time before the diagnosis is made. This may be summarized as follows: in acute brucellosis, Coombs titres are usually 4 to 16 times higher than SAT titres, whereas in patients with a long period of evolution without treatment the titres are 16 to 256 times higher.

Although immunofluorescence and radioimmunoassay have also been used in the past, the most suitable method for studying the immunoglobulin isotype distribution is the indirect ELISA. The analysis of data shows that ELISA is useful for measuring IgG antibodies and that it is possible to replace the Coombs test by an indirect ELISA with S-LPS and anti-IgG conjugates. However, although ELISA with S-LPS is a very promising test, several problems, including standardization, variable quality of commercial reagents and interpretation of results, particularly when based on optical density readings alone, cause problems of inter-laboratory comparability and need to be resolved by the establishment of standard reference materials.

The routine use of the complement fixation test (CF) is not recommended in small laboratories because of its technical complexity (much greater than that of SAT and ELISA) and because of the problems encountered in its standardization. However, this test is useful. Experience has shown that: (i) the CF and SAT are positive in 91.7% of cases; (ii) CF titres are higher than SAT titres after the 4th or 5th month of illness; (iii) a negative CF result with a significant SAT titre occurs in approximately 4.6% of patients, generally in the initial days or weeks of illness; and (iv) a negative SAT result with high CF titres occurs in approximately 3.7% of cases, generally corresponding to chronic illness or to patients who have recovered.

4.1.2.3 *Serological tests that detect antibodies against cytosolic proteins*

Antibodies to cytosolic protein antigens of *Brucella* have been studied by counter-immunoelectrophoresis (CIEP), ELISA and western blotting. By CIEP it has been found that sera of those patients with 2-ME resistant antibodies

and high Coombs titres also produce a greater number of precipitation lines and higher titres of antibodies to proteins. Obviously, the responses of those patients had longer evolution times and this is interpreted to mean that the number of precipitation lines increases as the disease evolves without diagnosis and treatment. The ELISA studies have shown that, while antibodies to S-LPS may appear in persons that have had contact with *S-Brucella* but have not developed clinical disease, the antibodies to selected proteins are indicative of an active infection. In a series of patients with persistent infection or relapse it was found that titres of anti-protein antibodies remained elevated, whereas in patients who recovered anti-protein antibodies disappeared. Qualitatively similar results have been obtained with western blotting. The shortcomings of these methods include their non-quantitative and rather subjective interpretation, the lack of validation data, and the non-availability of reference reagents.

4.1.3 Diagnosis of *Brucella* meningitis and meningoencephalitis

In such cases, ideally, *Brucella* should be cultured from CSF. However, bearing in mind that in most cases the routine cultures give negative results, it is mandatory to perform serological tests on CSF. In brucellosis that is not affecting the central nervous system, patients do not develop antibodies in the CSF. In contrast, in those patients in whom neurobrucellosis develops, the CSF contains low titres of antibodies against S-LPS and cytosolic proteins. These antibodies can be easily detected by RBT and CIEP tests respectively. The CSF is usually clear and its analysis reveals an increase of IgG and a lymphocytic pleocytosis.

4.1.4 Intradermal tests

The development of delayed hypersensitivity to intradermally administered *Brucella*-specific antigens is an indication of past exposure to infection but does not indicate its current significance. Although used in the past in some countries, the intradermal test is not recommended for diagnosis. The use of undefined and unstandardized antigen preparations may also provoke antibodies which interfere with subsequent serological tests.

4.1.5 Conclusion

A correct serological diagnosis of human brucellosis can be made with a test that uses S phase, whole cells. Recommended tests are RBT, SAT alone or with 2-ME or DTT reduction, Coombs antiglobulin, CFT and ELISA. The results of a combination of tests such as SAT and Coombs antiglobulin can be used to assess the stage of evolution of the disease at the time of diagnosis. The ELISA, with a conjugate of the appropriate IgM or IgG specificity and S-LPS, could replace established tests but requires further standardization

and validation. Other methods can be useful but are less specific and have not been adequately evaluated.

KEY POINTS ON THE DIAGNOSIS OF BRUCELLOSIS IN HUMANS

- In acute brucellosis, isolation of *Brucella* from blood or other tissues is definitive.
- Culture is often negative, especially in long-standing disease.
- Serology is the most generally useful diagnostic procedure approach.
- The RBT, tube agglutination and ELISA procedures are recommended.
- Methods which differentiate IgM and IgG can distinguish active and past infection.
- False positive serological reactions may occur.
- Skin test reactions indicate past exposure not active infection.

4.2 Diagnosis in animals

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk.

Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens. The isolation of *Brucella* is definitive proof that the animal is infected, but not all infected animals give a positive culture and the methods and facilities that must be employed are not always readily available. The detection of antibody or a hypersensitivity reaction provides only a provisional diagnosis, but in practice is the most feasible and economic means of diagnosis. False positive reactions to serological tests can occur through a number of factors, including vaccination, and this must be borne in mind when interpreting results. Similarly, dermal hypersensitivity only indicates previous exposure to the organism, not necessarily active infection, and may also result from vaccination.

Vaccination is an extremely important and effective facet of most control strategies but has the disadvantage that its use may confuse diagnosis by stimulating the production of hypersensitivity or antibodies detectable by serological tests. Antibody titres may persist for a prolonged period in a small proportion of vaccinated animals and this proportion increases with age at vaccination. To reduce this problem, in cattle vaccination is usually employed in young animals below the age of six months, but may be used in adults if a reduced dose is given, especially by the intraconjunctival route. There is currently no widely available test that is able to distinguish vaccinated from infected animals, although some tests are under evaluation.

It is of utmost importance that the use of vaccination is strictly controlled, that it is used at the correct age, that vaccine of sufficient quality is used and that vaccinated animals are correctly identified. If this is not the case, correct serological diagnosis is confused. The vaccination programme can be suspended when the prevalence of the disease reaches a very low level, when the disadvantages of vaccination outweigh any benefit that it may bring on the basis of cost-benefit and cost-effectiveness analysis.

4.2.1 Bacteriological methods

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme. It should be noted that all infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing.

4.2.1.1 Stained smears

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Koster's methods. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia* may superficially resemble *Brucella* (Fig. 6).

4.2.1.1 Culture

Brucella may most readily be isolated in the period following an infected abortion or calving, but isolation can also be attempted post-mortem.

Brucella are excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media. It is of the utmost importance that faecal and environmental contamination of the material is kept to a minimum to give the greatest chance of successfully isolating *Brucella*. If

other material is unavailable or grossly contaminated, the contents of the fetal stomach will usually be otherwise sterile and are an excellent source of *Brucella*.

In some circumstances it may be appropriate to attempt the isolation of *Brucella* post-mortem. Suitable material includes supramammary, internal iliac and retropharyngeal lymph nodes, udder tissue, testes and gravid uterus.

Milk samples should be allowed to stand overnight at 4 °C before lightly centrifuging. The cream and the deposit are spread on to the surface of at least three plates of solid selective medium. Placental samples should be prepared in the field by selecting the least contaminated portion and cutting off pieces of cotyledon. In the laboratory, the portions should be immersed in alcohol which should be flamed off before cutting with scissors or scalpel and smearing the cut surface on three plates of selective medium. Other solid tissues can be treated in a similar manner, or, ideally, they should be macerated mechanically following flaming before plating out. The tissues may be ground manually or homogenised in a blender or stomacher with a small proportion of sterile water. Fetal stomach contents are collected, after opening the abdomen, by searing the surface of the stomach with a hot spatula and aspirating the liquid contents with a Pasteur pipette or syringe.

Bacterial colonies may be provisionally identified as *Brucella* on the basis of their cultural properties and appearance, Gram staining, and agglutination with positive antiserum (Fig. 4 and 5). If available, a PCR-based molecular identification method may be used. Definitive identification of suspect colonies can only be made using techniques available at *Brucella* Reference Centres.

4.2.2 Serological methods

The detection of specific antibody in serum or milk remains the most practical means of diagnosis of brucellosis. The most efficient and cost-effective method is usually screening all samples using a cheap and rapid test which is sensitive enough to detect a high proportion of infected animals. Samples positive to screening are then tested using more sophisticated, specific confirmatory tests for the final diagnosis to be made (Fig. 5 and 6).

It is absolutely essential that only internationally recognized tests using antigens standardized against the 2nd International anti-*B. abortus* Serum are used. Appropriate quality control sera should be included with each batch of tests, and tests should be repeated if the quality control criteria are not met.

Serological results must be interpreted against the background of disease incidence, use of vaccination and the occurrence of false positive reactions due to infection with other organisms. As with all laboratory based diagnosis,

it is imperative to correctly identify the “audit trail” of individual animal identity, sample number and test result so that there is complete certainty of the linkage between animal and result.

4.2.2.1 *Rose Bengal plate test (RBT)*

The RBT is one of a group of tests known as the buffered *Brucella* antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH. The RBT and other tests such as the buffered plate agglutination tests and the card test play a major role in the serological diagnosis of brucellosis worldwide (Fig. 7).

The RBT is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The test is an excellent screening test but may be over-sensitive for diagnosis in individual animals, particularly vaccinated ones. The procedure can be automated but this requires custom-made equipment.

4.2.2.2 *ELISA tests*

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal and human diseases. Although in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution. It should be noted, however, that although the ELISAs are more sensitive than the RBT, sometimes they do not detect infected animals which are RBT positive. It is also important to note that ELISAs are only marginally more specific than RBT or CFT.

4.2.2.3 *Serum agglutination test (SAT)*

The SAT has been used extensively for brucellosis diagnosis and, although simple and cheap to perform, its lack of sensitivity and specificity mean that it should only be used in the absence of alternative techniques.

4.2.2.4 *Complement fixation test (CFT)*

The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good laboratory facilities and trained staff. If these are available and the test is carried out regularly with good attention to quality assurance, then it can be very satisfactory.

It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from infected animals do not fix complement. This is due to the presence of high levels of non-complement fixing antibody isotypes competing for binding to the antigen. At higher dilutions these are diluted out and complement is fixed. Such positive samples will be missed if they are only screened at a single dilution.

In other cases, contaminating bacteria or other factors in serum samples fix or destroy complement causing a positive reaction in the test, even in the absence of antigen. Such “anti-complementary” reactions make the test void and a CFT result cannot be obtained.

4.2.3 Supplementary tests

Many other serological tests have been employed. Some, such as the Rivanol or 2-ME test, are variations of the SAT and, although more specific, share many of its disadvantages. At present, the use of such procedures in the place of the standard test is not advised.

4.2.3.1 *Milk testing*

In dairy herds, milk is an ideal medium to test as it is readily and cheaply obtained, tests can be repeated regularly and give a good reflection of serum antibody. Milk from churns or the bulk tank can be screened to detect the presence of infected animals within the herd which can then be identified by blood testing. This method of screening is extremely effective and is usually the method of choice in dairy herds.

4.2.3.2 *Milk ring test*

The milk ring test (MRT) is a simple and effective method, but can only be used with cow's milk. A drop of haematoxylin-stained antigen is mixed with a small volume of milk in a glass or plastic tube. If specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring at the top of the column of milk. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd. Non-specific reactions are common with this test, especially in brucellosis-free areas. The milk ELISA is far more specific than the MRT.

4.2.3.3 *Milk ELISA*

The ELISA may be used to test bulk milk and is extremely sensitive and specific, enabling the detection of single infected animals in large herds in most circumstances.

4.2.3.4 *Fluorescence polarization assay*

This technique, which requires special reagents and reading equipment, is claimed to have advantages in sensitivity and specificity over other methods. Evaluation has been limited however, and the procedure is not widely available. Further information is required before its overall value can be assessed.

4.2.3.5 *Intradermal test*

This procedure, using a standardized antigen preparation such as Brucellin INRA or Brucellergene OCB, can be used for monitoring the status of herds in brucellosis-free areas. It is sensitive and specific but false positive reactions can occur in vaccinated animals.

4.3 Remarks on the diagnosis of brucellosis other than cattle

The procedures described above are primarily intended for the diagnosis of brucellosis in cattle. However, the bacteriological methods are also applicable to the diagnosis in all other species. The serological procedures require some modification for individual species as follows:

4.3.1 **Sheep and goats**

The RBT is useful for screening sheep and goat sera for antibodies to *B. melitensis*. An antigen suspension adjusted for highest sensitivity against a panel of control sera is recommended. The test is less sensitive than in cattle, however, and may not detect some infected animals. It is best used in combination with the complement fixation test.

The SAT using 5% sodium chloride as diluent has been widely used but has low sensitivity and specificity.

The micro-agglutination variant has higher sensitivity and specificity. It is only recommended in situations when more sophisticated tests are not available. The antigen is standardized to give 50% agglutination with a 1:650 dilution of the second International Standard for *B. abortus* antiserum.

It should be noted that agglutination methods are particularly sensitive to non-specific agglutinins and cross-reacting antibodies. They tend to be more sensitive in the acute phases of infection and are severely affected by vaccination. They should only be used if no alternative is available. The CFT is superior to agglutination methods but its sensitivity and specificity are limited and it should be regarded as a complementary rather than confirmatory

test. It may be used for screening if automated methods are available. Sera should be inactivated at 62 °C for 30 min. Vaccination produces seroconversion in the CFT but, in that case, antibody titres decline much more rapidly than those resulting from infection. ELISA is reported to give superior results to other tests in sensitivity and specificity, but experience is limited. The MRT is not suitable for use on sheep or goat milk but ELISA can be used. The CFT is also usable on whey samples but is technically demanding and no longer recommended. It should be understood that no currently available serological test can be considered reliable for the detection of ovine or caprine brucellosis at the level of individual animals. Diagnosis and control should be applied at the herd/flock level.

The intradermal test for delayed hypersensitivity to *Brucella* antigens is useful as a flock or herd test. However, it is affected by vaccination. A purified antigen preparation which contains a mixture of *Brucella* proteins free of smooth LPS, should be used to avoid compromising serological tests. It is useful for monitoring the status of brucellosis-free flocks, especially if vaccination is not practised.

4.3.2 Pigs

Serological tests are much less satisfactory for detecting pigs with brucellosis than for diagnosis of the disease in cattle, sheep and goats. Testing should be done on a herd basis. Non-specific agglutinins and cross-reacting antibodies engendered by intercurrent infections with *Escherichia coli* O157, *Salmonella*, *Yersinia enterocolitica* O9 and other organisms are common. The RBT is useful for screening large numbers of sera. The SAT is not recommended. The CFT gives results comparable with the RBT. ELISA offers the highest sensitivity and specificity of all currently available serological tests.

The intradermal test, using a defined antigen preparation is the most reliable diagnostic procedure for pigs on both an individual or herd basis. When infection is detected, it should be dealt with by slaughter of the herd as many infected animals are likely to remain undetected.

4.3.3 Camels, buffalo, reindeer, yaks

The serological tests used for cattle are applicable to these species. Camel sera for testing in the CFT should be inactivated at 60–62 °C for 30 min. The Rivanol test has been recommended for examining buffalo sera. The ELISA has not been widely evaluated for most of these species but is potentially useful subject to adequate standardization.

4.3.4 Dogs

B. abortus, *B. melitensis*, and *B. suis* infection in dogs can be diagnosed using the procedures described for cattle, except for ELISA, which has not been widely assessed in dogs. For *B. canis* infection the most reliable procedure is isolation of the organisms. As persistent bacteraemia is common, blood culture is a useful procedure. Serological tests are less satisfactory. They must use antigens prepared from *B. canis* or *B. ovis* strains as the surface antigens of smooth *Brucella* spp. do not cross-react with these. ELISA is probably the most useful procedure but is not widely available.

KEY POINTS ON THE DIAGNOSIS IN ANIMALS

- Culture of *Brucella* from abortion material, milk or tissues collected at autopsy provides a definitive diagnosis.
- Serology is usually the most practicable method.
- Cattle: the RBT is recommended for screening; ELISA or complement fixation are recommended for confirmation of infection in individual animals. Screening of milk samples by milk ring test or ELISA is useful for surveillance.
- Sheep, goats and pigs: no single serological test is reliable for confirmation of infection in individual animals. Serological tests should be used on a herd or flock basis. Similarly, the skin test is useful for screening at the herd or flock level, especially if vaccination is not used.
- A “rough-specific” antigen must be used for *B. canis* serology.

5. Treatment of human brucellosis

The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time. This should be within the context of general medical supervision and, for severely ill patients, is best carried out in hospital if circumstances permit. Antibiotic treatment should be implemented at as early a stage as possible, even in patients who appear to be showing a spontaneous improvement. In those patients with complications, additional treatment, including in some cases surgical intervention, will be necessary.

Uncomplicated acute brucellosis almost invariably responds well to appropriate antibiotic treatment. Patients and their families should be reassured that full clinical and bacteriological recovery is usual in human brucellosis.

A variety of antimicrobial drugs have activity in vitro against *Brucella* species; however, the results of routine susceptibility tests do not always correlate with clinical efficacy. Consequently, beta-lactam antibiotics, such as penicillins and cephalosporins, and macrolide antibiotics, such as erythromycin, are associated with unacceptably high rates of relapse when used to treat patients with brucellosis. Although newer macrolides, such as azithromycin and clarithromycin are more active in vitro than erythromycin, they have not shown superiority over current regimens for treatment of patients with brucellosis, and their role in therapy remains to be determined.

5.1 Treatment of uncomplicated brucellosis in adults and children eight years of age and older

5.1.1 Tetracyclines

Tetracycline (500 mg every six hours orally) administered for at least six weeks has long been the standard treatment of human brucellosis. Doxycycline (a long acting tetracycline analogue) is now the preferred drug because it can be given once or twice daily, and is associated with fewer gastrointestinal side effects than tetracycline. Doxycycline is given in a dose of 100 mg every 12 hours orally and is administered for a period of six weeks.

5.1.2 Aminoglycosides

Because the rate of relapse when tetracycline or doxycycline are given alone remains between 10–20%, most authorities recommend an amino-glycoside to be given in addition to the tetracyclines for the first two to three weeks of therapy.

Streptomycin (1 g/day intramuscularly) administered for two to three weeks has long been the aminoglycoside of choice when used in combination with tetracycline or doxycycline. Although synergy between the two drugs is difficult to prove using routine in vitro assays, bacterial killing studies have shown that *Brucella* species undergo a more rapid rate of killing by the combination than by either drug alone.

Gentamicin is more active in vitro against *Brucella* species than streptomycin and, when administered as a single daily dose, is associated with few adverse side-effects. Although gentamicin, in a dose of 5mg/kg/day intravenously or intramuscularly, administered for 7 to 10 days in combination with doxycycline administered for six weeks, yielded good results in one study, experience with this regimen is too limited to justify its use over doxycycline plus streptomycin. Unfortunately, no direct study comparing the results of doxycycline plus streptomycin versus doxycycline plus gentamicin has yet been published. Until additional experience is gained using gentamicin in place of streptomycin, the optimal dose and duration of therapy remain unknown.

5.2 Principal alternative therapy

Rifampicin is active in vitro against *Brucella* species, is remarkably lipid soluble, and it accumulates within eukaryotic cells. In order to provide a completely oral regimen with which to treat brucellosis, the combination of doxycycline (200 mg/day orally) plus rifampicin (600–900 mg/day orally), with both drugs administered for six weeks, was recommended by the WHO Expert Committee in 1986. This regimen has generally been found to be of similar efficacy to doxycycline plus streptomycin for patients with uncomplicated brucellosis. Caution is advised when considering this regimen for patients with complications, such as spondylitis. An analysis of various treatment regimens concluded that overall the regimen of doxycycline plus streptomycin was likely to be the most effective. In addition, some data have been reported indicating that rifampicin might enhance the plasma clearance of doxycycline, thus yielding subtherapeutic levels – a possible explanation of treatment failures with this regimen.

5.3 Secondary alternative therapy

Fluoroquinolones. Fluoroquinolone antibiotics have greater activity in vitro against *Brucella* species than the parent drug nalidixic acid. In addition, they are well absorbed after oral administration, and they achieve high concentrations within phagocytic cells. Although the minimum bactericidal concentration of quinolones is reported to be approximately four times the minimum inhibitory concentration, a lack of bactericidal activity was found at pH levels comparable to those found within cells. In addition, when quinolones were used as monotherapy in experimental animals and humans infected with *Brucella*, the rates of relapse were unacceptably high. Therefore, quinolones should always be used in combination with other drugs, such as doxycycline or rifampicin.

Trimethoprim/sulfamethoxazole (TMP/SMZ, co-trimoxazole). TMP/SMZ in a fixed ratio of 1:5 (80 mg TMP/400 mg SMZ) is more active in vitro against *Brucella* species than either drug alone. Although initial studies with TMP/SMZ reported good results, prospective, controlled, comparative trials demonstrated that the drug was associated with an unacceptably high rate of relapse. Consequently, TMP/SMZ should always be used in combination with another agent, such as doxycycline, rifampicin or streptomycin.

5.4 Treatment of complications of brucellosis

5.4.1 Spondylitis

Osteo-articular complications of brucellosis are common, occurring in up to 40% of cases in some series of patients. Some manifestations, such as sacroiliitis, do not appear to require special treatment. In contrast, spondylitis and osteomyelitis with related complications, such as para-vertebral and epidural abscesses, may require prolonged therapy, such as the continuation of doxycycline for eight weeks or more. Surgical drainage is rarely necessary.

5.4.2 Neurobrucellosis

The treatment of central nervous system complications of brucellosis poses a special problem because of the need to achieve high concentrations of drugs in the CSF. Since tetracyclines and aminoglycosides do not penetrate the blood/brain barrier well, it is recommended that drugs which achieve this, such as rifampicin or co-trimoxazole, be added to the standard regimen of doxycycline plus streptomycin. The optimal duration of treatment for neurobrucellosis has not been determined, however, most authorities recommend a minimum of six to eight weeks, and possibly longer, depending on the clinical response.

5.4.3 *Brucella* endocarditis

Although death from brucellosis occurs in less than 1% of cases, the complication most frequently leading to a fatal outcome is infective endocarditis. The treatment of *Brucella* endocarditis poses special problems because of the need to achieve bactericidal concentrations of drugs within the valvular vegetations. In addition, delays in making the diagnosis often result in progressive valve damage. For these reasons, both antimicrobial chemotherapy and surgical replacement of the damaged valve are often necessary. The combination of doxycycline plus an aminoglycoside results in rapid killing of the bacteria, and rifampicin or co-trimoxazole are used for their ability to penetrate cell membranes. Prolonged therapy is recommended (at least eight weeks), and therapy should be continued for several weeks after surgery when valve replacement is necessary.

5.5 Treatment of brucellosis during pregnancy

If promptly diagnosed, antimicrobial therapy of pregnant women with brucellosis can be life-saving for the fetus. Pregnant women and nursing mothers pose special problems with regard to the selection of appropriate drugs. All drugs cross the placenta in varying degrees, thus exposing the fetus to potential adverse drug effects. Tetracyclines are contraindicated in pregnancy owing to the potential for permanent staining of fetal dentition, and the susceptibility of pregnant women to drug-induced fatty necrosis of the liver and pancreatitis. The teratogenic potential of many drugs, such as the fluoroquinolones, rifampicin, and co-trimoxazole, are simply unknown. Fetal toxicity has been reported in pregnant women treated with streptomycin; however, there are no reports of toxicity with gentamicin. Consequently, the optimal therapy for brucellosis during pregnancy has not been determined with certainty. Co-trimoxazole has been used in individual cases with reported success. Another alternative is rifampicin therapy for at least 45 days depending on the clinical outcome.

5.6 Treatment of brucellosis in children less than eight years of age

The optimal treatment for brucellosis in neonates and children less than eight years of age has not been definitively determined. Tetracyclines are contraindicated because of the potential for permanent staining of deciduous teeth and inhibition of bone growth. Doxycycline binds less to calcium than

other tetracyclines, and may pose less of a risk, however, there are no studies to confirm this with certainty. Consequently, aminoglycosides, co-trimoxazole, and rifampicin are the drugs generally recommended. Co-trimoxazole and rifampicin are not recommended by the manufacturers for use in young children, and the rates of relapse are high when either agent is used alone. Satisfactory results have been reported with TMP/SMZ (8/40 mg/kg/day twice daily orally) administered for six weeks plus streptomycin (30 mg/kg/day once daily intramuscularly) administered for three weeks or gentamicin (5 mg/kg/day once daily intravenously or intramuscularly) administered for 7 to 10 days. Alternatives include TMP/SMZ plus rifampicin (15 mg/kg/day orally) each administered for six weeks, or rifampicin plus an aminoglycoside. Until additional experience is obtained with these regimens, it is not possible to define the therapy of choice.

5.7 Post-exposure prophylaxis

With increasing use of live *Brucella* vaccines to immunize cattle (*B. abortus* strain 19 and RB 51) and sheep and goats (*B. melitensis* strain Rev 1), the problem of accidental self-inoculation by veterinarians is widespread. The majority of vaccine needle-stick injuries cause puncture wounds, but usually little vaccine is injected. However, a potential risk of infection remains and it is advisable to supplement local wound care and tetanus toxoid (when indicated) with a six-week course of doxycycline. It should be noted that *B. abortus* RB 51 is resistant to rifampicin. In contrast, splashing the eyes (conjunctival inoculation) with live *Brucella* vaccines is a very effective method for transmitting brucellosis. Consequently, for vaccine accidents involving the conjunctival route, local eye care and one or two drugs administered for the full six-week course is recommended. In addition, serum should be tested for antibodies to *Brucella* as soon after the accident as possible, to provide a baseline for follow-up in case symptoms occur.

5.8 Vaccines and immune system stimulants

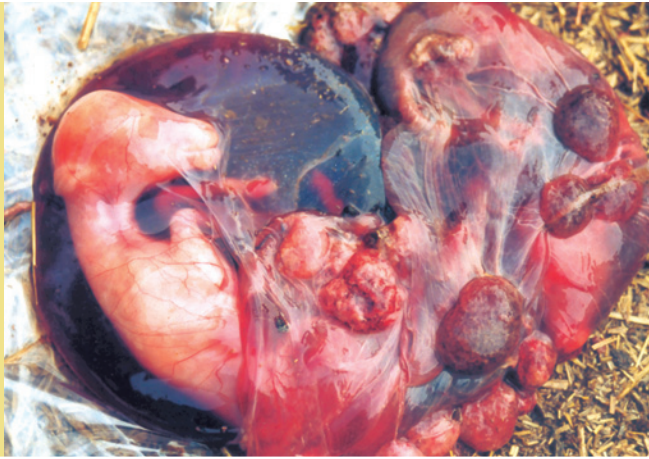
There is no convincing evidence of benefit from administering *Brucella* vaccines or antigen preparations, nor for the use of immune system modulators, such as levamisole, in the treatment of human brucellosis. Caution should be exercised in the use of anti-inflammatory agents to deal with local complications. Where possible, specialist advice should be sought.

KEY POINTS ON TREATMENT OF BRUCELLOSIS IN HUMANS

- The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time.
- Treatment of uncomplicated cases in adults and children eight years of age and older: doxycycline 100 mg twice a day for six weeks + streptomycin 1 g daily for two to three weeks.

OR

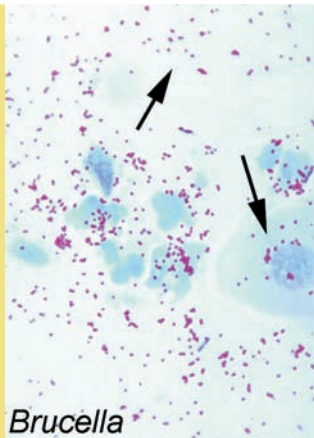
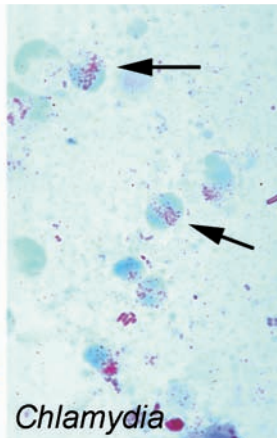
- Doxycycline 100 mg twice a day for six weeks + rifampicin 600–900 mg daily for six weeks.

**Figure 1**

Brucellosis may produce abortion in goats or sheep at about the fourth month of pregnancy. *Brucella melitensis* is a major problem in many countries.

Figure 2

Epididymitis (tail of epididymides) in a bull infected by *B. melitensis*.

*Brucella**Chlamydia***Figure 3**

Stamp stain (modified Gimenez method) of vaginal swabs from aborted ewes. Note the differences between *Chlamydophila abortus* and *Brucella melitensis*.

Figure 4

Culturing: *Brucella* can be transmitted easily during laboratory work. Bacteriological analyses should always be performed under adequate protection in safety hoods.



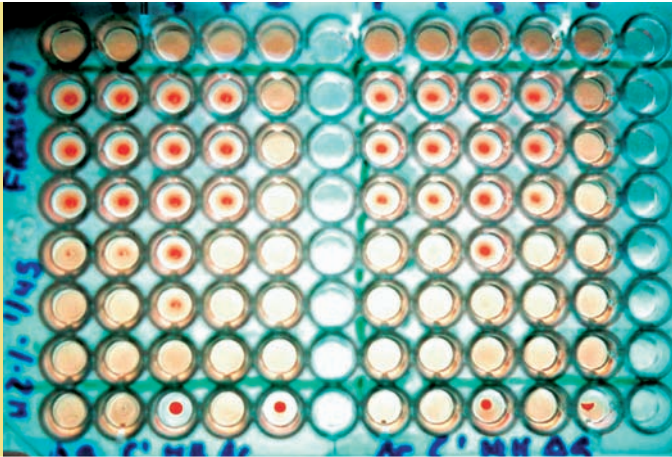


Figure 5

Complement fixation test is probably the most widely used serological test for the diagnosis of brucellosis in animals.

Figure 6
Indirect ELISA.
Alternative screening
(or confirmatory) test.

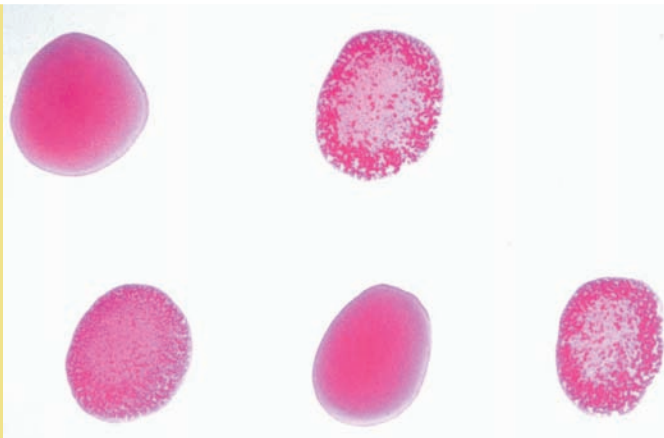
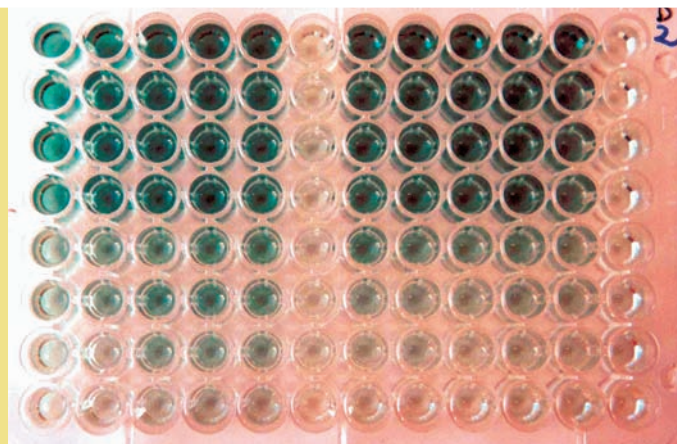


Figure 7

Rose Bengal plate test.
The most widely used
screening test.

Figure 8
A technician
taking organs for
bacteriological
culture.



6. Prevention of human brucellosis

As the ultimate source of human brucellosis is direct or indirect exposure to infected animals or their products, prevention must be based on elimination of such contact. The obvious way to do this – elimination of the disease from animals – is often beyond the financial and human resources of many developing countries. The technical and social difficulties involved in eradicating *B. melitensis* from small ruminants has even taxed the resources of some developed countries. In many situations there is little alternative but to attempt to minimize impact of the disease and to reduce the risk of infection by personal hygiene, adoption of safe working practices, protection of the environment and food hygiene. The lack of safe, effective, widely available vaccines approved for human use means that prophylaxis currently plays little part in the prevention of human disease.

In industrialized countries and others where animal husbandry is practised under settled conditions, the main sources of infection are:

- 1) occupational exposure;
- 2) ingestion of contaminated food products.

Under conditions of nomadic or migratory husbandry or on small traditional farms, the differentiation of sources of infection is far less clear-cut and all sections of the population may be exposed to infection by direct contact with animals or from contaminated food.

Although the practice of even elementary hygienic precautions can be difficult for populations living under primitive conditions, especially in arid or semi-arid areas, the observance of some basic measures can considerably reduce the risk of brucellosis.

6.1 Occupational hygiene

The groups in which the occupational risk of infection is greatest include those whose work brings them in direct contact with infected animals or their products. These include farmers, stockmen, shepherds, goatherds, abattoir workers, butchers, dairymen, artificial inseminators, veterinarians and those involved in the processing of viscera, hides, wool and skins. Persons involved in the maintenance of buildings or equipment used for these purposes may also be at risk. An additional important category includes laboratory workers who may be exposed to contaminated specimens and to *Brucella* cultures, either during the course of diagnostic procedures or vaccine production, for example. The production and use of live vaccines also carries some risk.

6.2 Personal hygiene

All persons carrying out high-risk procedures, which includes contact with animals suffering from or suspected of having brucellosis, should wear adequate protective clothing. This includes an overall or coat, rubber or plastic apron, rubber gloves and boots and eye protection (face shield, goggles or respirator). The risk of infection is greatest when dealing with aborting animals or those undergoing parturition but hazardous activities also include contacts with infected animals in other circumstances like shearing, dipping, clinical examination, vaccination and treatment, and the disinfection and cleaning of contaminated premises.

The work clothes should be reserved for this purpose and retained on the premises. They should be disinfected after use either by heat treatment (boiling or steaming), by fumigation with formaldehyde or by soaking in a disinfectant solution of appropriate concentration (iodophor, phenolic soap, chloramine or hypochlorite). Particular attention should be given to the disinfection of footwear to ensure that infection is not transferred outside the premises or into the house or tent.

Ideally, operatives should have access to full washing or showering facilities. As a minimum, the hands should be rinsed in a 1% chloramine solution (or other approved disinfectant), washed in soap and water and then treated with an emollient cream. Any superficial injuries such as cuts or scratches should be treated with an antiseptic, e.g. tincture of iodine, and covered with a bandage or self-adhesive dressing.

Eye protection is particularly important as conjunctival contamination carries a high risk of infection. Should any infectious material enter the eye, it should be removed under clean or aseptic conditions away from the working area. The eye should be thoroughly rinsed with running water and chloramphenicol or tetracycline eye drops or ointment applied.

Respiratory contamination is also a high risk in heavily infected environments. Inhalation of dust or aerosols derived from dried excreta or tissues released at abortion, parturition or slaughter should be prevented by the use of suitable respirators. The filters, which must be capable of retaining bacteria, should be changed regularly and the equipment itself disinfected by chemical or moist heat treatment.

Ideally, staff should be kept under medical surveillance with periodic serological examinations. It is strongly recommended that new staff provide a baseline blood sample before starting work. Any that develop clinical disease should be treated promptly. Young people under 18 years of age and pregnant women should be excluded from high risk occupations.

6.3 Farm sanitation

Farm workers, and animal attendants in particular, should wear adequate protective clothing when contact with infected animals is probable or if the environment is likely to have been contaminated by excreta, abortions or parturition products from animals with brucellosis. This is particularly important when dealing with animals that are aborting or giving birth, when the shedding of *Brucella* organisms will reach maximum levels.

Aborted fetuses, placentae and contaminated litter should be collected in leak-proof containers and disposed of preferably by incineration. Deep burial in freshly slaked lime at sites away from water courses is an acceptable alternative. Any area in which an abortion or infected parturition has occurred should be washed down with an approved disinfectant (hypochlorite, iodophor or phenolic disinfectant at recommended working strength).

Farm implements used for handling contaminated material should be disinfected after use by immersion in a suitable disinfectant (iodophor, phenolic soap or dilute caustic soda).

Dung should be cleared daily and stored in a secluded area until rendered safe by natural decay (this will probably require about one year) or else burnt or soaked in disinfectant before disposal. Liquid manure can remain infected for long periods, especially at low temperatures. Destruction of *Brucella* organisms can be hastened by addition of calcium cyanamide or xylene but the material should still be stored for at least six months.

Vehicles entering or leaving infected premises should pass through shallow troughs of disinfectant, or over straw or plastic foam soaked in an approved disinfectant. Premises that have held *Brucella*-infected animals should not be re-stocked until at least four weeks have elapsed between cleaning and disinfection. Maintenance workers (e.g. builders, plumbers, electricians) should not be allowed on to premises that have not been decontaminated.

Buildings should be maintained in a condition which prevents ready access to vermin. Rodent control measures should be enforced and insect infestation kept to a minimum by the use of fly screens, light traps and insecticides.

6.4 Prevention of brucellosis under nomadic or migratory conditions

Even in some developed countries sheep and goat raising is often practised under semi-nomadic conditions. These do not readily lend themselves to the hygienic measures indicated for farms. The situation is even more difficult

when animal husbandry is practised on a completely nomadic basis in arid or semi-arid conditions. Under these circumstances it is rarely possible to follow hygienic practices to the extent required to prevent infection. However, steps can be taken to reduce the impact of the disease by educating the population in the nature of the disease and its mode of transmission. In such societies, most of the adult population will already have been exposed to brucellosis and will presumably have some degree of immunity. The disease therefore makes its greatest impact on children who should be prevented from having contact with newborn animals or those that have recently aborted or given birth. Although cultural traditions can be difficult to change, the consumption of raw milk, blood or uncooked meat or offals should be discouraged.

Vaccination is often the only measure that can be applied to such populations. However, fully satisfactory vaccines are not currently available.

6.5 Hygienic precautions in meat processing establishments and rendering plants

Sheep, goats and cattle infected with *B. melitensis* or *B. abortus* and pigs infected with *B. suis* are particularly dangerous at the slaughter stage. During the bacteraemic phase of the disease, the bacteria are widespread in the tissues. The mammary glands, uteri and testes may be particularly heavily infected. Animals that have recently aborted or given birth may also have extensive external contamination.

Cattle infected with *B. melitensis*, especially if pregnant or in milk, may shed enormous numbers of bacteria on opening the uterus or udder and present a severe risk to abattoir workers. It is advisable to dry off such animals before slaughter or, if this is not practicable, to incinerate the whole carcass.

If animals are known to be infected with *Brucella*, they should be slaughtered at abattoirs designated for that purpose, where the staff have been specially trained and equipped to deal with the risk. The slaughtermen should wear full protective clothing including waterproof overalls or aprons, boots, respirators and goggles or face shields. Rubber gloves must be worn and chainmail guards should be used to protect against accidental cuts. Eating, drinking and smoking must be prohibited in the working area. Adequate facilities for disinfection of protective clothing, implements and for personal washing should be provided.

If specially designated abattoirs are not available, the slaughter of infected animals should take place at the end of the working day, after slaughter of healthy animals has been completed. Tissues that are likely to be heavily infected, such as udder and genitalia, should be destroyed.

Full cleaning and disinfection of the premises and the equipment must be performed at the end of each working day. Animal tissues and refuse for disposal should be retained in leak-proof containers such as plastic bags. It is recommended that such material be incinerated.

Entry to the premises should be restricted to employees. Young persons under the age of 18 and pregnant women should not be allowed access. If possible, staff should be recruited from individuals known to have serological evidence of previous exposure to *Brucella*.

The staff should be kept under medical surveillance and antibiotic therapy implemented for any who develop symptomatic brucellosis. All employees and especially women of childbearing age must be apprised of risks associated with *Brucella* infection. Information regarding conditions affecting immune status (e.g. pregnancy, immunosuppressive drugs, neoplasms, etc.) should be provided to workers. Personnel should be encouraged to self-identify to the organization's medical authority/health care provider so they may receive appropriate medical counselling and guidance. Education in safe and hygienic working practices and containment practices should be ongoing and is especially important for new staff.

6.6 Safety measures in the laboratory: precautions required in handling materials that may contain pathogenic *Brucellae*

Brucellae fall into WHO Risk Group 3, i.e. pathogens that pose a high risk to the worker involved, but only a low risk to the community. Brucellosis is in fact one of the most easily acquired laboratory infections. The degree of risk varies, not only with the virulence of the organism, *B. melitensis* and *B. suis* being the most dangerous for humans, but also with the numbers of bacteria in the material being handled. Blood samples and biopsy material for either serological or bacteriological diagnosis will rarely contain *Brucellae* in sufficient numbers to present a significant risk to personnel handling them but should still be handled with care at Biosafety level 2. Normally these will be dealt with in general diagnostic sections along with samples that may contain other human pathogens (Fig. 8). However, after *Brucellae* have grown in culture, dangerous numbers of organisms are present and strict precautions are required. At the same time Biosafety level 3 facilities, practices and procedures are required. The same applies when handling birth products from animals. Clotted blood samples present little risk and milk samples only a slight risk. Membranes, fetal tissues and fluids may contain up to $>10^9$ *Brucella* cells per gram, and similar numbers may be encountered in handling cultures grown in the laboratory. The precautions described below

apply to handling this dangerous material. For a fuller treatment of the subject, the *WHO Laboratory Biosafety Manual* (3rd ed.) should be consulted; it contains much valuable information on biosafety in the laboratory and a bibliography of the subject.

6.6.1 Physical requirements for a laboratory handling pathogenic *Brucellae*

When handling cultures and other potentially high-titered materials such as membranes, fetal tissues and fluids Biosafety level 3 is prudent. A separate room is required with only one entrance; a biohazard notice prohibiting the entry of unauthorised persons should be prominently displayed at the entrance. Ideally, the room should have a double-door entrance designed to provide an airlock. The ventilation should be arranged to maintain the pressure within the room at a slightly lower level than its surroundings. Air from the room should be discharged to the exterior, well away from air intakes and opening windows, otherwise it must be sterilized by filtration or heat treatment. The walls should be impermeable and all windows sealed to allow disinfection and fumigation; it should be safeguarded against infestation with rodents or insects. The room must have a properly installed and tested Class II or III biological safety cabinet. The air exhaust from the cabinet should be so arranged as to avoid interference with the air balance in the room or within the cabinet when it is switched on. The room should have a sink, an autoclave and enough incubator space for all culture requirements. Hand washing facilities must be provided near the exit.

6.6.2 Biological safety cabinets

See the *WHO Laboratory Biosafety Manual*, 3rd edition. For appropriate and useful information on selection and use of biological safety cabinets.

6.6.3 General precautions

Since brucellosis has been documented as one of the most frequently acquired laboratory infections the importance of using appropriate biosafety practices and facilities cannot be over emphasized. Each laboratory should have written procedures addressing use of equipment (especially equipment that may generate aerosols); disinfection of equipment and contaminated materials, handling and processing samples; spill containment and clean-up; and waste handling. These procedures should be clearly and concisely written, easily accessible and rigorously followed. As previously mentioned, Biosafety level 3 is appropriate for handling *Brucella* cultures or infected membranes, fetal tissues and fluids.

6.6.4 Measures for specific laboratory processes

In the production of antigens, strains of low virulence such as *B. abortus* strain 1119-3¹, strain 19^{1,2} or strain 99² should be used; they have the additional advantage that they do not require added CO₂ for growth.

Centrifuges may cause dangerous aerosols, especially when tubes containing virulent bacteria break. Glass tubes should not be used for virulent materials, instead polycarbonate tubes with tightly fitting screw-capped lids are recommended. If virulent material has to be centrifuged, the containers should be loaded and unloaded inside the biohazard cabinet. The continuous-flow type of centrifuge should not be used for virulent strains. Electric homogenisers, stomachers, sonicators and similar appliances should be used inside the cabinet.

Except for grossly contaminated materials, direct culture is the preferred method for bacteriological diagnosis rather than the inoculation of laboratory animals. If the inoculation of potentially dangerous materials is required, only needles that lock to the barrel of the syringe should be used. Needles should not be re-capped before disposal. The operators should wear protective clothing and respirators.

6.6.5 Health and medical surveillance

All persons working with virulent *Brucellae* should be kept under close clinical and serological surveillance. In some countries prophylactic immunization is offered to those at special risk. However, the vaccines currently available are of uncertain efficacy and some may cause unacceptable reactions.

6.7 Prevention of foodborne brucellosis

For the general population which does not have direct contact with animals, the greatest potential source of brucellosis is through consumption of unpasteurized milk and dairy products. Meat may also be a significant source of infection, especially in cultures where the consumption of raw or undercooked meat products is favoured.

6.7.1 Milk and milk products

Milk from infected cattle, sheep, goats, buffalo, yaks, camels and reindeer can contain large number of *Brucella* organisms. Because quite large volumes

¹ Available from USDA National Veterinary Services Laboratories, P.O.Box 844, Ames, Iowa 50010, USA.

² Available from Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, England.

may be consumed or concentrated into other products, such as cream or cheese, it presents a particularly serious hazard.

Soft cheeses prepared from fresh milk may concentrate large numbers of *Brucella* organisms. The preparation of such products from untreated milk should be strongly discouraged. If local customs make this difficult to achieve, the cheese should be stored for six months before being released for consumption. Hard cheeses which may undergo propionic as well as lactic fermentation are usually much less hazardous because of the acidification. Unpasteurized whey left over from cheese making could transmit infection if fed to animals. It may also contaminate containers used to transport other materials unless these are decontaminated before use.

Rennet used in cheese making can also serve as a source of infection if prepared from the stomachs of *Brucella*-infected animals.

Butter, sour milk, sour cream and yoghurt also undergo acidification processes which will drastically reduce the *Brucella* content. However, the acidity has to fall below pH 3.5 for reliable killing of the bacteria.

Ice cream prepared from infected milk may be particularly hazardous, especially as milk from different sources may be blended to make the product. All milk and cream used for this purpose should be heat treated.

Boiling or high temperature pasteurization will kill *Brucella* in milk. Ideally all milk produced in areas in which brucellosis is present should be pasteurized. If pasteurization facilities are not available, the milk should be heated to a minimum temperature of 80–85 °C and the temperature held at that level for at least several minutes, or boiled. This should apply to all milk for human consumption, whether to be drunk without further processing or to be used for making other food products.

6.7.2 Meat

Muscle tissue is unlikely to contain more than low concentrations of *Brucella* organisms and their numbers are further reduced if the meat is stored correctly before consumption. Kidney, liver, spleen, udder and testes may contain much larger numbers. None of them present a serious hazard from brucellosis if thoroughly cooked. However, in some cultures, raw or undercooked meat may be eaten through choice. This practice and the consumption of fresh blood, either alone or mixed with milk, should be discouraged.

The handling and preparation of infected meat and offal without proper hygienic precautions may also lead to the contamination of other foods.

Drying, salting and smoking are not reliable methods for killing *Brucella*. Similarly, the organisms survive well under refrigeration or deep freeze conditions. It is strongly recommended that all meat products are thoroughly cooked before consumption.

6.8 Vaccines*

Safe and effective vaccines for the prevention of human brucellosis are not generally available. However, vaccination has played a significant role in the prevention of the disease, in conjunction with other measures, in the former USSR and China. Two live attenuated vaccine strains have been employed extensively in heavily infected areas.

B. abortus strain 19-BA was used from 1952 onwards in the former USSR. The vaccine was administered as a dose of 1×10^9 cells by skin scarification (epicutaneous route). Protection was effective for up to one year but with maximum efficacy at five to six months after vaccination. Accordingly, vaccination was usually timed to anticipate the season of peak incidence of disease in animals. In general, the vaccine was well-tolerated in healthy adults when given by the epicutaneous route. Local reactions manifested as hyperaemia and induration occurred in 76% of those immunized, whereas general reactions characterized by headache, lethargy and mild pyrexia, occurred in 3 to 7% of vaccinates. The frequency of general reactions was much greater in those showing evidence of previous exposure to *Brucella*.

Epidemiological studies showed that the vaccine was effective in reducing morbidity in high-risk areas, with a 5 to 11-fold reduction in reported cases of acute brucellosis. However, the vaccine did induce hypersensitivity, especially with repeated doses and there were numerous contra-indications to vaccination.

In China, the live attenuated strain *B. abortus* 104M has been used. This was administered as a dose of $7\text{--}10 \times 10^9$ viable cells given by the epicutaneous route. This strain is appreciably more virulent than *B. abortus* 19-BA and serious reactions may follow subcutaneous injection. The indications for use are similar to those for the 19-BA strain. Care must be taken to avoid vaccinating individuals who may have been sensitized by previous exposure to vaccine or natural infection.

These live vaccines are not currently available from sources whose production and quality control procedures would meet international standards. Their availability and use is now quite restricted. More emphasis in recent years has been on the development of non-living vaccines based on sub-cellular fractions. Two of these have received fairly extensive study.

* See also *Manual of diagnostic tests and vaccines for terrestrial animals*, OIE, 5th ed.

A peptidoglycan fraction (PI) obtained as the phenol-insoluble residue of lipid extracted cells of *B. melitensis* M15 was developed in France. This was subsequently prepared from *B. abortus* strain 19 cells. It has been used in occupationally exposed groups, particularly laboratory workers. Two doses of 1 mg each are given subcutaneously, separated by a two-week interval. The vaccine is non-toxic and rarely elicits generalized reactions. It is very weakly allergenic and does not cause severe sensitization. However, it is reported to result in enhanced lymphocyte proliferation responses to *Brucella* antigens which correlate with immunity. The protective response was reported to last up to two years. Although used in about 2000 individuals over nearly two decades, evidence of efficacy from controlled clinical trials is not available. The vaccine is not at present in production.

Another sub-cellular fraction, “*Brucella* chemical vaccine” (BCV), was developed in Russia. It is extracted from cell wall preparations of *B. abortus* strain 19-BA with 0.1N acetic acid and comprises a protein-polysaccharide complex. The vaccine is given in doses of 1 mg by intramuscular injection and stimulates only mild local and general reactions. It does not produce severe hypersensitivity responses even in previously exposed individuals. Protective immunity is comparable with that given by the live 19-BA strain. Studies involving the use of 75 000 doses in Kazakhstan indicated an efficacy of 79.6% for BCV and 76.6% for the live vaccine. However, BCV was at least 2–4 fold less likely to induce cutaneous hypersensitivity than the live vaccine. Repeated doses could be given after one year without risk of serious hypersensitivity reactions. This vaccine would appear to merit further evaluation under a wider range of conditions.

Other vaccines are currently under development, including live attenuated strains with defined attenuating mutations and LPS-protein conjugate vaccines.

6.9 Public health aspects

From a public health point of view, the main sources of brucellosis are either food-related or are dependent on contact with infected animals either in an occupational or recreational contact. Person-to-person transmission is not a significant problem except through blood or organ transfer which should be subject to proper control. Airborne or contact infection through environmental contamination may be a significant problem when infected animals pass through densely occupied areas, e.g. on the way to market. Appropriate measures should be taken to address these problems. A key means of achieving this is through education of the population, and especially those directly involved in the animal and food industries.

All measures should be integrated into adequately designed and effectively implemented control programmes. Close collaboration between public health and veterinary services as well as other relevant agencies is fundamental in order to meet the targets.

6.9.1 Public health education

Food safety is one of the principal pillars on which protection of human health resides. Humans are infected by *Brucella* mainly through inappropriately prepared and/or preserved food of animal origin.

There is no lack of scientific knowledge on the systems, technologies and procedures with which to implement safe food preparation and consumption. Conversely, there is a huge gap in knowledge among the population, especially in developing countries, on the significance of safe handling, cooking and preserving food. Furthermore, food processing plant owners are often uninterested in, or even fail to apply correctly, the known rules of food safety (see Annex 1).

Foodborne diseases, including brucellosis, cause considerable morbidity in populations in many parts of the world, having a major impact principally on young children and the elderly.

Other than human suffering, foodborne diseases cause substantial economic losses. These include loss of income and manpower, medical care costs, loss of food due to inadequacy of processing or spoilage. Therefore, public health education should be included among the essential activities to be performed within the framework of brucellosis control programmes or even as an independent activity. Health education is a difficult and extremely complex task. It cannot be regarded as effective if specific considerations referring to the community are not taken into account. These include: culture, beliefs, traditions, educational level, social status, occupation, age, etc. Hence, health education programmes should be aimed at targeted social groups. These should include physicians, veterinarians and farmers who may not be fully aware of the problem. They should be directed not only at specific measures but should also emphasize the responsibility of individuals for safeguarding and improving their own health and that of the community. The key objectives are to enable individuals to define their own problems and needs; to understand what can be done to deal with these problems using their own resources and external support and to decide on appropriate action. This is best achieved in the context of a detailed knowledge of the social and environmental background.

Elements of health education and methodology are referred to in Annex 2.

6.9.2 Community participation

Health programmes are unlikely to succeed if community participation is not an integral part of the structure and execution of these programmes at local level. Laws, regulations and veterinary policy measures alone will not bring the desired results. The whole community needs to be involved through health education in schools, in the workplace and in the population at large.

Firstly, the higher the level of self-reliance and social awareness, the more individuals and families will accept responsibility for protecting their animals and themselves from disease hazards transmitted directly, through food of animal origin, or through environmental vectors or fomites. The relevant community education programmes should concentrate on what people can do for themselves to improve their own health situation.

Secondly, community members should be involved in planning the programmes that will affect them personally. Local residents know local social structures, local situations, local resources and local needs.

Thirdly, community members should be fully involved as participants in the implementation of health programmes in their communities. They have the important advantages of speaking the local dialect, of knowing how to reach people and animals and of enjoying social acceptance. Annex 3 refers to community groups to be identified for participation in health education campaigns.

There is no single model for promoting community participation. The degree of community involvement in zoonoses control programmes will vary from situation to situation, and is often strongly influenced by social, cultural, political and economic factors. Only guiding principles can be provided which might be applicable in different settings, provided that the will exists to begin and sustain the efforts. The general public, especially communities in endemic areas, has to be made aware of the danger to health and of the economic importance of zoonoses and foodborne diseases. As far as possible, full use should be made of the mass media. All available means of informing each community should be used but an effective method is discussion in small groups. In such discussions, the health worker (educator) suggests some kind of concrete action, for example, formation of working committees soon after the discussions. Such committees have proved to be extremely useful in the initial early phases of several control programmes.

6.9.3 Training of health workers and school teachers on public health education

As far as possible, the health educators should be drawn from the community in which they will be working. Everyone involved directly or indirectly in a control programme against zoonotic and foodborne diseases must carry out public health education. It is, therefore, essential that this subject have an

important place in staff training. Such training should be planned and preferably imparted by a specialist who should also advise on the selection of appropriate educational methods, the preparation of educational material suited to local conditions, and finally to the various phases of the programme.

Most of the health educators selected will already be professionally active and frequently good field experts. Therefore, on training courses academic lessons should be cut drastically and other teaching techniques preferably used that secure more active participation. Problem solving, case studies, working in small groups and role playing are examples of such techniques.

The school represents the most important learning situation for a large and significant group of the population. Effective instruction of children will have an influence not only on their own lives but also on the next generation. Children are influenced primarily via two channels, parents and teachers. They need to convey the need to develop a healthy lifestyle and the acquisition of healthy habits. It should be remembered that many school age children and young people in endemic areas do not attend school on a regular basis. Therefore, health education needs to extend beyond the school environment to reach educationally deprived groups which often include those at high risk.

The workplace is another important location for health education. Workers in the food industry and the managers and owners of food preparation facilities should be instructed in the potential causes of foodborne disease and the means of avoiding them.

For further information on strategies which can be adopted in education programmes see Annex 3.

KEY POINTS ON PREVENTION OF BRUCELLOSIS IN HUMANS

- The prevention of human brucellosis is based on occupational hygiene and food hygiene.
- Vaccination is not generally recommended.
- All dairy products should be prepared from heat-treated milk.
- Consumption of raw milk or products made from raw milk should be avoided.
- Meat should be adequately cooked.
- Special precautions should be taken by laboratory workers.
- Physicians and health workers should be aware of the possibility of brucellosis.
- Public health education should emphasize food hygiene and occupational hygiene.

7. Prevention, control and eradication of animal brucellosis

The justifications for prevention of the introduction of brucellosis into populations of animals are the same as those for the control of the disease in populations which are already infected: economic benefits and the protection of public health.

Brucellosis is a zoonosis with a strong correlation between animal and human diseases. While public health measures such as pasteurisation and education have varying degrees of success, it remains primarily a veterinary responsibility to control brucellosis, including application of principles of epidemiology and animal husbandry. Intersectoral collaborative strategies for control and prevention of brucellosis are reported in Annex 7*.

7.1 Prevention

It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them. For brucellosis, the measures of prevention include:

- Careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from *Brucella*-free herds or flocks. Pre-purchase tests are necessary unless the replacements are from populations in geographically circumscribed areas that are known to be free of the disease.
- Isolation of purchased replacements for at least 30 days. In addition a serological test prior to commingling is necessary.
- Prevention of contacts and commingling with herds or flocks of unknown status or those with brucellosis.
- If possible, laboratory assistance should be utilized to diagnose causation of abortions, premature births, or other clinical signs. Suspect animals should be isolated until a diagnosis can be made.
- Herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBT.
- Proper disposal (burial or burning) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly.

* For more information on the sanitary standards for international trade of animals and their products, see *Terrestrial animal health code*, OIE, 14th ed.

- Cooperation with public health authorities to investigate human cases. Animal brucellosis, especially when caused by *B. melitensis*, can often be identified through investigations of cases in humans.

7.2 Control

The aim of an animal control programme is to reduce the impact of a disease on human health and the economic consequences. The elimination of the disease from the population is not the objective of a control programme, and it is implicit that some “acceptable level” of infection will remain in the population. Control programmes have an indefinite duration and will need to be maintained even after the “acceptable level” of infection has been reached, so that the disease does not re-emerge. In many countries, methods for the control of brucellosis are backed by governmental regulation/legislation. In others, no authorities exist. Therefore, the procedures for management of infected herds and flocks may vary widely. Nevertheless, certain principles apply, namely: 1) the reduction of exposure to *Brucella* spp. and 2) the increase of the resistance to infection of animals in the populations. These procedures may be further classified under the general categories of test and isolation/slaughter, hygiene, control of animal movement, vaccination.

7.2.1 Test and isolation/slaughter

There are no pathognomonic signs of brucellosis in animals at individual level; the occurrence of abortion storms in naive herds/flocks is usually a strong indicator of infection. Therefore, serological (and sometimes allergic) tests are the usual method of identifying possible infected animals. Bacteriological procedures are useful for confirming test results and for epidemiological studies.

The decision about slaughter of test-positive animals is made after regulatory, economic and prevalence factors are considered. In most cases, test and slaughter of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (e.g. 2%). Retention of positive animals is less hazardous if the remaining animals have been vaccinated but should only be considered as a last resort. The isolation of test-positive animals is essential, especially during and after parturition.

The immediate slaughter of test-positive animals is expensive and requires animal owner cooperation. Compensation is usually necessary. Furthermore, the application of test and slaughter policies is unlikely to be successful with brucellosis of sheep and goats where the diagnostic tests are less reliable than in cattle. Test and slaughter is also unlikely to be successful in cattle if the remainder of the herd is unvaccinated, especially in large populations. Repeated herd or flock tests are necessary to further reduce the incidence of brucellosis and to confirm elimination.

7.2.2 Hygiene

The goal in the application of hygiene methods to the control of brucellosis is reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g. commingling of herds or flocks), patterns of commerce, prevalence of clinical signs, type of facilities, and degree of dedication of the owners of animals, will also determine success. Owners are often poorly informed about disease transmission and recommendations, such as separation of parturient animals, can be difficult or impossible to implement.

Antibiotic treatment of known infected animals, or of those which are potentially exposed to them, has not been commonly used and it should be ruled out as an option in the control of brucellosis. A limited number of studies have shown rapid reductions in the incidence of brucellosis when the herd or flock was treated but this procedure is considered to be restricted in practice. Treatment has been used in animals of special breeding value, but because of the uncertain outcome it is not generally recommended.

7.2.3 Control of animal movement

This may be regarded as an aspect of hygiene. However, it is essential in any programme to limit the spread of brucellosis. Animals should be individually identified by brand, tattoo or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests.

In practice, it is much more difficult to control the movement of camels and small ruminants kept under nomadic or semi-nomadic conditions than that of beef or dairy cattle kept under intensive conditions. The owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries.

7.2.4 Vaccination

There is general agreement that the most successful method for prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B. abortus* strain 19 have proven to be superior to all others. The non-agglutininogenic *B. abortus* strain RB51 has been used in the USA and some Latin American countries, with encouraging results. The source and quality of the vaccines are critical. The dosages and methods of

administration, especially with Rev.1, vary and these can affect the results. Consequently, whole herd or flock vaccination can only be recommended when all other control measures have failed. When applied, the vaccinated animals must be identified by indelible marking and continually monitored for abortions resulting from the vaccine. Positive serological reactors and secretors must be removed from the herd on detection.

It is often recommended that vaccination with strains 19 and Rev.1 should be limited to sexually immature female animals. This is to minimize stimulation of postvaccinal antibodies which may confuse the interpretation of diagnostic tests and also to prevent possible abortions induced by the vaccines. However, field and laboratory studies have demonstrated that conjunctival administration of these vaccines makes the vaccination of the herd or flock a practical and effective procedure. Rapid herd immunity is developed and application costs are minimized. The lowered dose results in lower antibody titres and these recede rapidly. Several diagnostic tests have been developed which are useful in differentiating antibody classes. Of these, the complement fixation test and ELISA are currently the most widely used.

Vaccination of animals usually results in elimination of clinical disease and the reduction in numbers of organisms excreted by animals which become infected. Furthermore, animal owners are more likely to accept vaccination as a method of control since they are accustomed to this form of disease control. In many countries, vaccination is the only practical and economical means of control of animal brucellosis.

The worldwide trend towards more animal commerce and larger populations, along with limited resources, have made the control of brucellosis very difficult in many countries. Evaluation of the procedures used for the prevention and control of animal brucellosis should be performed. This should include surveillance of animals and humans and investigations of outbreaks. Procedures, including case definition and diagnostic tests, should be standardized and should be flexible enough to allow modification when new information becomes available.

7.3 Eradication

Eradication means the elimination of a pathogenic agent from a country or a zone (i.e. part of the territory of a country with a distinct animal health status). A highly organized effort is needed to reach eradication in either a territory and in a population. Eradication is conceptually very different from control: it is neither a casual nor an automatic consequence of a control programme, no matter how well planned and implemented the control programme is. It is based on sanitary measures and on an organization of activities completely different from those implemented for a control programme.

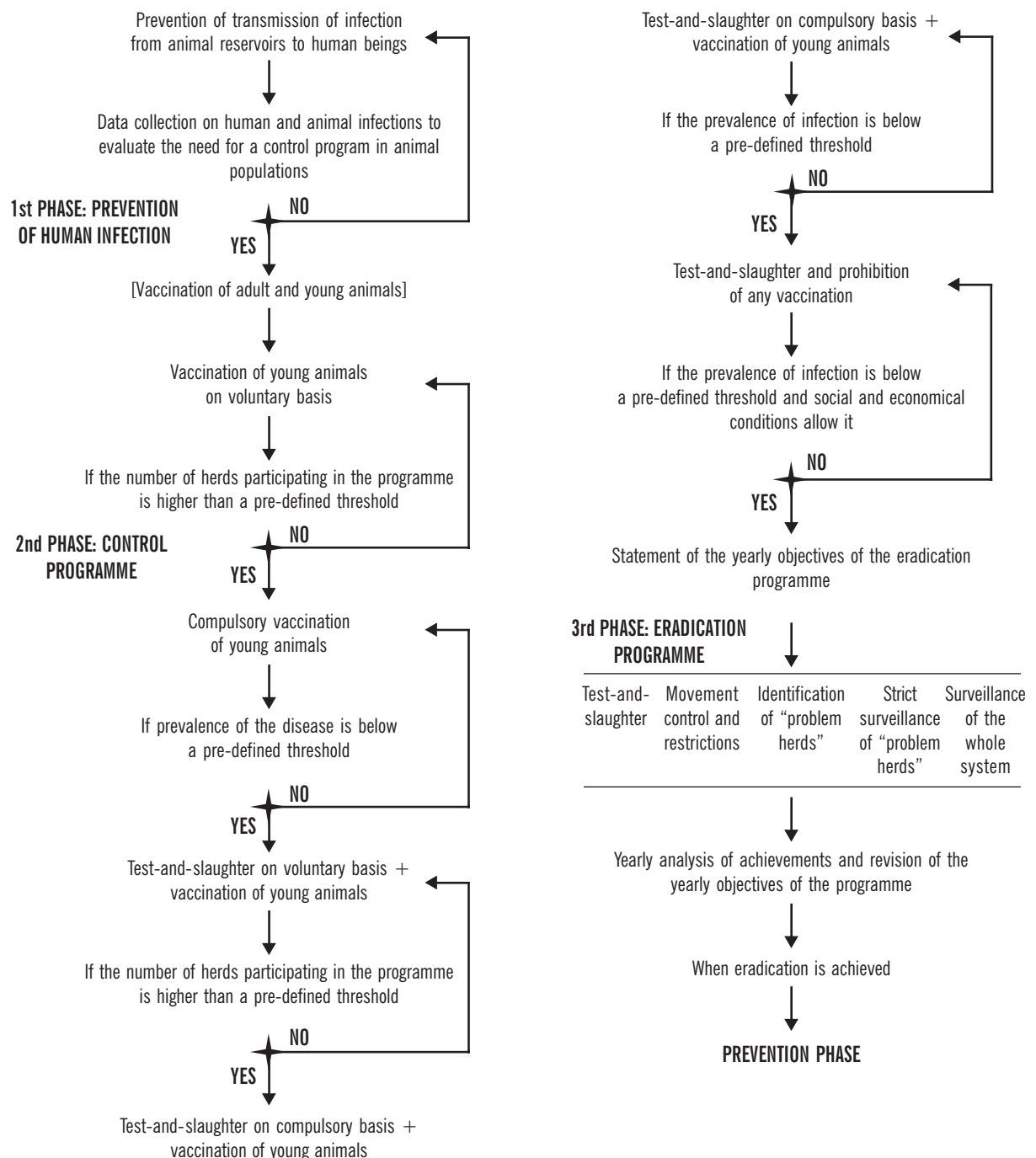
Crucial factors for the success of an eradication programme are the implementation of an effective surveillance system with adequate laboratory support, and the understanding and sharing of objectives for eradication by the decision-makers, farmers, and all other stakeholders. To keep an unaffected population free from an infection, prevention measures must be implemented to segregate an infectious organism from a geographical area and its human and animal populations. Adequate knowledge of the local human and animal populations and of the territory is essential.

The strategies described above for prevention and control can be applied for eradication; however, they are not mutually exclusive, on the contrary they can be arranged in a cascade as shown in diagram 1.

On a long-term basis, eradication programmes in general are more economically advantageous compared to control programmes. This advantage, however, cannot always be translated into practice. In fact, an eradication programme involves the mobilization of an amount of resources (financial and human) that may not be available or whose returns for the investment may require a time span longer than any decision-making authority can afford. Cost-benefit and cost-effectiveness analysis can be used to support decisions on control strategies. However, no in-depth analysis is possible in absence of epidemiological surveillance. There is also little doubt that very often failures of control and eradication efforts are due to the absence of an adequate epidemiological surveillance system sustaining both technical and political decision-making.

KEY POINTS ON PREVENTION, CONTROL AND ERADICATION OF ANIMAL BRUCELLOSIS

- Animal brucellosis is best prevented by careful herd management and hygiene.
- Vaccination is useful for prevention and control of infection.
- *B. abortus* strains 19 and RB 51 are recommended for prevention of bovine brucellosis.
- *B. melitensis* Rev 1 is recommended for prevention of *B. melitensis* infection in sheep and goats.
- Vaccine efficacy may be limited in the face of heavy exposure.
- Control and prevention schemes require effective collaboration between all sections of the community.
- Control programmes must be properly planned, coordinated and resourced.
- Education and information programmes are essential to ensure cooperation at all levels in the community.
- Eradication can only be achieved by test-and slaughter combined with effective prevention measures and control of animal movements.

Diagram 1: Steps to achieve the eradication of brucellosis

Source: Mediterranean Zoonoses Control Programme. *The MZCP Report on the Third Workshop on Human and Animal Brucellosis Epidemiological Surveillance in the MZCP Countries*, Damascus, Syrian Arabic Republic, 4–5 May 1998 (<http://www.mzcp-zoonoses.gr/pdfen/Brucellosis.pdf>, accessed January 2007).

8. Surveillance

Surveillance consists of the systematic collection, collation, analysis, interpretation and prompt dissemination of data on specific diseases or syndromes to those who need to know, for relevant action to be taken. The main purpose of a surveillance system is to determine the need for immediate or longer-term actions in response to diseases and to provide information to optimize the use of the available resources through data analysis, determination of priorities, design of alternative actions, and determination of their likely costs and benefits. In relation to brucellosis, an effective surveillance system, based on the collaboration between the human and animal health services, is a prerequisite of any control or eradication programme. The surveillance system must be adapted to the adopted strategy for coping with the disease: prevention of human disease, and prevention, control or eradication of the infection in the animal population. In addition, other factors that will also determine the type of surveillance system include husbandry systems, marketing methods, and capacities of veterinary services.

The surveillance programme should be planned as an organisation made up of various components including institutions, facilities, activities and procedures with a common mission.

Collection and management of information is a costly activity; therefore, only the minimal set of information needed should be collected; and only the most suitable routinely performed activities should be used to collect information. A surveillance programme can be described in terms of inputs, processing and analysis, and outputs.

Inputs of a surveillance programme include passively or actively collected data. In the case of passive collection of information, data supply the system as a consequence of current activities. Main sources of passively collected data are: peripheral public health services, peripheral veterinary services, hospitals, public health laboratories, veterinary laboratories, border health services, border veterinary services. Other sources of data include clinics, physicians, veterinary practitioners, and universities. The laboratory is one of the main sources of data on zoonotic diseases. However, because of biases related to collection of samples in the population, laboratory data may not represent the sanitary situation of the population. Nevertheless, laboratories have a central role in generating information and, without them, a constant monitoring of animal health status for disease prevention and control cannot be carried out.

The active collection of data include those which are actively sought and gathered on the basis of a specific dedicated programme. Active collection is suitable for ad hoc surveys, to evaluate the performance of passive collection

of data, to carry out pilot trials to evaluate if an emergent phenomenon deserves the implementation of a routine system of data collection. Ad hoc surveys are often the only possible way of collecting surveillance data when veterinary and health services do not have a strong infrastructure.

The following step in processing surveillance data is analysis, which aims at identifying and quantifying needs of health activities and evaluating their delivery. Indicators need to be identified to monitor progress.

The outputs of a surveillance system are, in general, technical reports on health conditions, resources available, their use and results obtained. Reports need to be adapted to the various recipient groups: peripheral collectors of data, intermediate and central technical people, decision makers, etc.

8.1 Surveillance in humans

The key to effective surveillance is the case definition, which includes the set of clinical and/or laboratory criteria that must be fulfilled in order to identify a person as a case. Individual reports of cases should be classified as suspected, probable or confirmed. Annex 8 includes the recommended standards for the surveillance of brucellosis in humans.

Monitoring of the number of cases reported by medical practitioners, clinics and hospitals can give an indication of the presence of the disease in a population. It is unlikely to give an accurate quantitative indication of the incidence as brucellosis is generally under-reported. Mild cases are particularly liable to be misdiagnosed or not reported.

Surveillance data can be actively collected by clinical and serological surveys on high-risk groups and on others, such as blood donors or pregnant women, who are accessible for examination. Surveys may also be conducted on patients admitted to hospital, military recruits and school children. Because of the absence of distinctive clinical signs, such studies have to depend on serological tests. These have to be interpreted with caution as exposure to cross-reacting organisms, including *Salmonella* O:30, *Escherichia coli* O:157, *Yersinia enterocolitica* O:9, may result in false positive reactions. Screening tests, such as the RBT, should be supported with more specific tests, such as IgG or IgA ELISAs, on at least a proportion of the samples.

Bacteriological screening of populations is not practical for surveillance purposes but cultures isolated from human patients should be identified to biovar level to enable possible tracking of sources and relation to outbreaks in animals.

The intradermal skin test has been widely used for epidemiological studies in some countries. This is even more difficult to interpret than serological tests and at best only gives an indication of past exposure. The specificity of the procedure is likely to be highly variable if inadequately standardized, and if unpurified *Brucella* preparations are used. These may also induce antibodies that interfere with subsequent serological tests. However, where other methods cannot be implemented e.g. through lack of laboratory facilities, intradermal testing with appropriate antigens may give a useful indication of level of exposure within a population.

8.2 Surveillance in animals

As for human brucellosis, a crucial factor is the case definition. The lack of any specific clinical presentation in animals makes the use of laboratory tests indispensable to define animal brucellosis cases. While the unit of reference for public health surveillance is the human case or cases (i.e. outbreak), for animal surveillance, the unit of reference is usually the infected herd or flock rather than the individual animal.

An animal brucellosis surveillance system may use data from diagnostic laboratory findings, outbreak/case investigations and slaughterhouse or animal marketing tests, or specially commissioned local or national surveys. These data can be used to ascertain flock or herd prevalence of a given population or area, and in infected flocks or herds, the prevalence of the disease in the flock or herd and to determine the incidence. An important use of incidence data is the evaluation of efforts to achieve control or elimination.

The following are methods for active and passive collection of data on animal brucellosis. Table 4 reports several recommended survey procedures according to the animal species under investigation.

- area tests (i.e. census) – the systematic testing of all animal herds or flocks within a geographical area;
- selected herd tests – the testing of animal populations considered to be higher risk: e.g. herds adjacent or commingled with infected herds;
- epidemiological investigations – the tracing of sources of animals which have been added to or sold from infected herds. It also includes attempts to locate sources of human cases of brucellosis. These are sometimes the first evidence of brucellosis in animals, especially for *B. melitensis*;
- surveys – tests of randomly selected herds to determine initial prevalence in an area or to monitor disease occurrence;

- tests of animals at slaughter or markets – This is a primary surveillance method in organized programmes where origins of positive animals can be located with reasonable confidence. The effectiveness lessens as the incidence in the population decreases. This form of surveillance is useful to determine initial prevalence;
- bulk milk ring tests – These are widely used to determine the prevalence of brucellosis in dairy cattle herds and to locate possible additional infected herds. Bulk milk (composite) samples are tested at least three to four times annually and individual cows are tested in herds where the ring test is positive. The test is very sensitive and false positive tests are common, especially where cattle herds are small or when cows have been vaccinated with strain 19.
- abortion investigations – In some countries abortions must be reported to authorities who are responsible for disease control.

Monitoring the males will indicate the presence of infection in the herd and drastically reduces workload.

The effectiveness and usefulness of any form of surveillance will depend upon the reliability and cooperation of animal owners and the availability and capabilities of diagnostic services.

KEY POINTS ON SURVEILLANCE BRUCELLOSIS IN HUMANS AND IN ANIMALS

- Continued surveillance is essential to monitor the presence/absence of brucellosis and the efficacy of control programmes.
- The key to effective surveillance is the case definition, reporting, analysis of data and dissemination of information for action.
- The surveillance programme must be designed according to the adopted control strategy.
- Human cases may be the first indication of infection in the animal population.

Table 4. Survey procedures recommended for the assessment of brucellosis epidemiology

| ANIMAL | SURVEY PROCEDURE |
|--|---|
| CATTLE | |
| <i>DAIRY HERDS</i> | <p>Milk ring tests to identify infected herds and establish the prevalence of infected herds in the various regions·Blood samples from positive herds to establish the prevalence of reactors in infected herds</p> <p>Culture of milk from positive herds to support the serological data and identify the causal <i>Brucella</i> species and biovar</p> <p>Culture of abortion material</p> |
| <i>BEEF CATTLE</i> | <p>Serological tests on blood samples from breeding females sent to abattoirs, followed by identification of any infected farms</p> <p>Blood samples from infected farms</p> <p>Culture of lymph nodes and abortion material</p> <p>Serological surveys on live animals on farms and at markets, shows, and fairs</p> |
| SHEEP, GOATS | <p>Serological tests on blood taken at abattoirs, followed by identification of any infected flocks</p> <p>Serological tests in flocks suspected of being infected</p> <p>Surveys using allergic tests on selected herds followed by blood testing of those found positive</p> <p>Culture of lymph nodes and abortion material and milk samples from positive herds</p> |
| PIGS | <p>Serological tests on blood taken at abattoirs, including non-breeding females and castrated males, followed by identification of the source of any infected animals</p> <p>Blood tests in infected herds</p> <p>Culture of lymph nodes collected at abattoirs</p> <p>Culture of abortion material</p> |
| FERAL OR WILD-LIFE SPECIES in contact with domestic animals | <p>Capture or shooting and serological examination, supported by isolation and identification of the organisms</p> |

Source: Joint FAO/WHO Expert Committee on Brucellosis. Sixth Report, Technical Report Series, No. 740, World Health Organization, Geneva, 1986.

9. Intersectoral collaboration

In the preceding sections, the zoonotic nature of brucellosis has been emphasized. This implies that the disease in man can only be prevented effectively by elimination of the animal reservoir. This necessitates a close interaction between the medical authorities concerned with public health authorities on the one hand and the veterinary authorities on the other. This collaboration is only the first step in establishing an effective control programme. For a successful outcome, all sections of the community need to be involved in the process and to lend their support. This extends from the individual citizens who need to be aware of the measures required to protect and improve their own health, through local to national political leaders who will need to find and commit the resources required to implement the programme. Within this structure, the provision of specialist expertise is the responsibility of the medical and veterinary authorities. They will be responsible for diagnosis, treatment and surveillance and for executing control and preventive measures. They will also need to provide the necessary information to those concerned in occupational and community education programmes. The importance of this interaction and collaboration between sectors cannot be over-emphasized. Specific examples of approaches which may be adopted are given in Annex 3.

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Annex 1

Five keys to safer food¹

- **Keep clean**

- Wash your hands before handling food and often during food preparation
- Wash your hands after going to the toilet
- Wash and sanitize all surfaces and equipment used for food preparation
- Protect kitchen areas and food from insects, pests and other animals

- **Separate raw and cooked**

- Separate raw meat, poultry and seafood from other foods
- Use separate equipment and utensils such as knives and cutting boards for handling raw foods
- Store food in containers to avoid contact between raw and prepared foods

- **Cook thoroughly**

- Cook food thoroughly, especially meat, poultry, eggs and seafood
- Bring foods like soups and stews to boiling to make sure that they have reached 70°C. For meat and poultry, make sure that juices are clear, not pink. Ideally, use a thermometer
- Reheat cooked food thoroughly

- **Keep food at safe temperatures**

- Do not leave cooked food at room temperature for more than 2 hours
- Refrigerate promptly all cooked and perishable food (preferably below 5°C)
- Keep cooked food piping hot (more than 60°C) prior to serving
- Do not store food too long even in the refrigerator
- Do not thaw frozen food at room temperature

- **Use safe water and raw materials**

- Use safe water or treat it to make it safe
- Select fresh and wholesome foods
- Choose foods processed for safety, such as pasteurized milk
- Wash fruits and vegetables, especially if eaten raw
- Do not use food beyond its expiry date

¹ *Prevention of foodborne disease: Five keys to safer food*. Geneva, Switzerland, World Health Organization, Department of Food Safety, Zoonoses and Foodborne Diseases (<http://www.who.int/foodsafety/consumer/5keys/en/index.html>, accessed January 2007)

Annex 2

Methodology for health education of the public

The content and form of the educational material and aids as well as means of communication have to be adapted carefully to the target populations and also to the health action about which information is to be prepared.

Most of the target populations in different parts of the world where brucellosis is endemic are illiterate or only partly literate. Written words, pamphlets and newspapers are therefore of little value in such situations. Fortunately, radio and television are spreading fast in various countries and portable radio sets are being carried even by nomads. These mass media are available for health education provided that the material is presented in a useful and interesting form.

Small discussion groups and lectures are extremely useful means of communication and could be followed immediately by such actions as formation of action committees or even by collection of diagnostic samples or immunization. Several audiovisual aids are available which could be used in conjunction with lectures or group discussion with great advantage. Others, such as posters and wall pictures, can be used on work premises to remind workers of various dangers or of precautions they have to take in handling potentially infected animals or products. It is important to enlist the aid of community leaders in the education campaign.

The content of the educational material including lectures, etc. has to be selected and prepared to suit the action to be supported and the beliefs and perceptions of the target populations. The topics treated in the rest of this section could each be the subject of educational communication, but each has to be broken down into very simple and easily comprehensible parts. Consideration of popular beliefs, however absurd they may appear, is important for the educator. If they are ignored, then resistance and lack of cooperation may follow. The correction of wrong beliefs should be done appropriately but in a gentle manner. The economic benefits expected to result from the control of brucellosis should be brought out fully in the educational material.

Many animal owners and patients do not like to have blood samples drawn for diagnostic or other purposes. Some object also to needle pricks for bleeding or injections. There are other real or imaginary fears of pain or injury resulting from health action. Many farmers do not cooperate for fear of immediate or future expense and others withhold cooperation simply due to ignorance. All these factors have to be taken into full consideration by the health educator in preparing his education aids and materials.

(Source: S.S. Elberg. *A guide to the diagnosis, treatment and prevention of human brucellosis* (unpublished document VPH/81.31. Rev.1). World Health Organization, Geneva, Switzerland, 1981)

Annex 3

Public health education groups for community participation

The following groups, which are to be found in most communities, are important:

1. Local health and veterinary services. The personnel of these services are not only participants in community programmes, but serve at the same time as educators and promoters.
2. Local health committees and community health workers. This group is most important for community motivation and education in the course of their work.
3. Local religious bodies. They guide both the attitudes and the actions of the people in many countries. Their advocacy of health programmes is essential. They can often make such invaluable facilities as meeting halls, audiovisual equipment and communication networks available to community projects.
4. Local civic groups. Dedicated to community improvement, they bring together civic leaders and have resources in the form of personnel and funds that can be extremely helpful in community projects.
5. Local school and adult education groups. Located within the communities, they can reach entire families, have facilities and resources for group meetings, attract the respected educated people in their communities, and can play an invaluable part in health programmes.
6. Local practitioners of traditional medicine, birth attendants and midwives. Often respected by large segments of their communities, they should be involved in health programmes and actively participate in them, whenever possible.
7. Local police or local military units. Often anxious to participate actively in community service, these groups must be informed of, and involved in all programmes within their communities.

Both in rural and urban areas, community groups are all-important in the planning and implementation of health programmes. They provide the resources needed for adapting plans to local conditions, carrying out tasks at little or not cost, and overcoming constraints. They must be informed in their approach and informative about their role in achieving the aims of the programme.

Annex 4

Table A.1 Characters differentiating the nomen species and biovars of *Brucella*

| Growth in presence of 1 in 50 000 (20 mg/ml) | | | | Agglutination by monospecific serum† | | | Preferred natural host | | |
|--|---------------------|-----------------------------|-----------------------------|--------------------------------------|---------|---------------|------------------------|------|---|
| Species | Biovar and biogroup | CO ₂ requirement | H ₂ S production | Urease | Thionin | Basic fuchsin | A | M | R |
| <i>B. melitensis</i> | 1 | – | – | + | + | + | – | + | – |
| | 2 | – | – | + | + | + | + | – | – |
| | 3 | – | – | + | + | + | + | + | – |
| <i>B. abortus</i> * | 1 | (+) | + | + | – | + | + | – | – |
| | 2 | (+) | + | + | – | – | + | – | – |
| | 3 | (+) | + | + | + | + | + | – | – |
| | 4 | (+) | + | + | – | (+) | – | + | – |
| | 5 | – | – | + | + | + | – | + | – |
| | 6 | – | – | + | + | + | + | – | – |
| | 9 | – | + | + | + | + | – | + | – |
| <i>B. suis</i> | 1 | – | + | + | + | (–) | + | – | – |
| | 2 | – | – | + | + | – | + | – | – |
| | 3 | – | – | + | + | + | + | – | – |
| | 4 | – | – | + | + | (–) | + | + | – |
| | 5 | – | – | + | + | – | – | + | – |
| <i>B. neotomae</i> | – | – | + | + | – | – | + | – | – |
| <i>B. canis</i> | – | – | – | + | + | (–) | – | – | + |
| <i>B. ovis</i> | – | + | – | – | + | (–) | – | – | + |
| ' <i>B. maris</i> ' # | 1 | + | – | + | + | + | + | or – | – |
| | 2 | – | – | + | + | + | + | or – | – |

+ Positive; (+) usually positive,
– Negative, (–) usually negative.
* Former *B. abortus* biovars 7 and 8 are no longer regarded as valid
† A: abortus; M: melitensis; R: rough
‡ *B. abortus*, biovar 3 grows in the presence of 1 in 25 000 thionin; biovar 6 does not.
'*B. maris*' includes several distinct types and each may be accorded nomen species status

Table A.2 Oxidative metabolism of species of *Brucella*

| Species | Amino acids | | | | | | | |
|----------------------|--|--------------|------------------|------------|--------------|--------------|--------------|--|
| | L-alanine | L-asparagine | L-glutamine acid | L-arginine | DL-citulline | L-lysine | DL-ornithine | |
| <i>B. melitensis</i> | + | + | + | – | – | – | – | |
| <i>B. abortus</i> | + | + | + | – | – | – | – | |
| <i>B. suis</i> | v* | v* | v* | + | + | v* | + | |
| <i>B. neotomae</i> | v | + | + | – | – | – | – | |
| <i>B. canis</i> | v | – | + | + | + | + | + | |
| <i>B. ovis</i> | v | + | + | – | – | – | – | |
| <i>B. 'maris'</i> | – | – | + | – | ND | – | – | |
| Carbohydrates | | | | | | | | |
| | L-arabinose | D-galactose | D-ribose | D-xyllose | D-glucose | i-erythritol | | |
| <i>B. melitensis</i> | – | – | – | – | + | + | | |
| <i>B. abortus</i> | + | + | + | v | + | + | | |
| <i>B. suis</i> | v* | v* | + | + | + | + | | |
| <i>B. neotomae</i> | + | + | v | – | + | + | | |
| <i>B. canis</i> | v | v | + | – | + | v | | |
| <i>B. ovis</i> | – | – | – | – | – | – | | |
| <i>B. 'maris'</i> | ND | v* | + | + | + | + | | |
| + | positive | | | | | | | |
| – | negative | | | | | | | |
| v | variation between strains | | | | | | | |
| v* | variation between biovars of some assistance in classification | | | | | | | |
| ND | no data | | | | | | | |

Table A.3 Lytic activity of phages for smooth (S) and rough (R) *Brucella* species

| Phage group | Phage strain | <i>abortus</i> | | <i>suis</i> | | <i>melitensis</i> | | <i>neotomae</i> | | <i>canis</i> | | <i>ovis</i> | | <i>maris</i> | |
|-------------|-----------------|----------------|----|----------------------------|---------------------------|-------------------|----|-----------------|----|--------------|----|-------------|----|--------------|----|
| | | S | R | S | R | S | R | S | R | S | R | S | R | S | R |
| 1 | Tb | L | NL | NL | NL | NL | NL | NL or PL | NL | NL | NL | NL | NL | NL | NL |
| 2 | Fi 75/13 | L | NL | PL | NL | NL | NL | L | NL | NL | NL | NL | NL | L | L |
| 3 | Wb | L | NL | L | NL | V | NL | L | NL | NL | NL | NL | NL | L | L |
| 4 | BK ₂ | L | NL | L | NL | L or PL | NL | L | NL | NL | NL | NL | NL | L | L |
| 5 | R/C | NL | L | NL | NL | NL | NL | NL | NL | NL | NL | L | L | NL | NL |
| 6 | Iz ₁ | L | NL | bg 1, 4: L bg 2,3,5: PL | bg 1,4: V bg 2,3,5: NL | L or PL | V | L | PL | NL | NL | NL | NL | L | L |
| 7 | Np | L | NL | NL | NL | NL | NL | L | NL | NL | NL | NL | NL | ND | ND |

L confluent lysis
 PL partial lysis, single plaques or growth inhibition
 NL no lysis
 V variable, some strains lysed
 RTD routine test dilution
 ND no data
 bg biogroup

Annex 5

Bacteriological examination for presence of *Brucella*

- **Procedure**

Blood (5–10 ml) or other specimen:

- a) Liquid medium (50–100 ml per bottle, and containing 1–2% sodium citrate when blood is to be studied)
OR
Liquid – solid combined medium (biphasic or Castañeda culture)
Incubation at 37 °C until growth appears
- b) Subculture from I on to agar medium
Incubate as above allowing 4–5 days for colony formation if necessary
- c) Colonies examined for “Smooth-Rough” quality and by slide agglutination test in anti-*Brucella* serum
- d) Confirm the presumptive evidence of *Brucella* isolation by standard taxonomic tests on subculture

- **Culture media**

Both solid and liquid media may be used. Both may be prepared from similar ingredients. A good quality peptone is essential for the basal medium. Trypticase Soy Broth (BBL®), Bacto-tryptone® (Difco Laboratories GmbH), Tryptic soy (Gibco), Tryptone Soya Broth (Oxoid Ltd) and Trypticase soy (bioMérieux) are all suitable. To prepare liquid medium, sterile equine or bovine serum (which must be free of *Brucella* antibodies) and dextrose are added to the autoclaved peptone solution cooled to 56 °C, to give final concentrations of 5% v/v and 1% w/v, respectively. For solid medium, agar to 1.5% final concentration is added to the peptone solution before autoclaving.

Selective media: modified Brodie and Sinton liquid medium

To prepare this, antibiotics are added to the serum dextrose broth to give final concentrations as follows:

| | |
|-----------------------|----------|
| bacitracin: | 25 u/ml |
| cycloheximide: | 100 u/ml |
| polymyxin B sulphate: | 6 u/ml |
| nalidixic acid: | 5 µ/ml |
| vancomycin: | 20 µ/ml |

amphotericin B: 1 u/ml
D-cycloserine: 100 u/ml

Selective media: Farrell's medium

This solid selective medium is prepared from serum dextrose agar by addition of antibiotics to the molten medium cooled to 56°C, to give the following concentrations:

bacitracin: 25 u/ml
cycloheximide: 100 µg /ml
polymyxin B sulfate: 5 u/ml
vancomycin: 20 µg/ml
nalidixic acid: 5 µg/ml
nystatin: 100 u/ml

- **Biphasic medium**

To prepare a biphasic medium of Castañeda type, either non-selective serum dextrose broth and serum dextrose agar can be used to form the liquid and solid phases. If a selective medium is required the antibiotics for the modified Brodie and Sinton medium are included in both phases. The bottles are prepared as follows.

In a 125 ml flat-sided bottle place 12–14 ml melted agar medium (above, to which 2.5% agar has been added) to cover the longer side of the bottle, autoclave and leave to rest on the longer side until it hardens. When the medium has hardened, add aseptically to the bottle 15 ml sterile liquid medium with 1–2% sodium citrate. The bottle now has a solid medium on one side and a liquid medium on the bottom. The specimen is introduced into the liquid phase, the bottle incubated in the upright position. Every 24–48 hours the liquid is washed over the agar surface for a few minutes, the bottle returned to the upright position and further incubated. Eventually *Brucella* colonies will grow on the agar as well as in the liquid phase.

- **Incubation**

Incubation conditions must allow for the possibility of *B. abortus* that requires 10–20% additional CO₂ in the air. Cultures therefore must be made in duplicate at the beginning, one set incubated in air, the other set incubated in the presence of additional CO₂, until it is observed that the colonies have grown in air alone. At this point it is no longer necessary to provide additional CO₂ to the cultures. Most positive cultures become evident within one to two weeks. However, it is advisable not to discard cultures as negative until four to six weeks have elapsed.

Annex 6

Serological tests

Serum agglutination test

The test is performed in clear glass or plastic tubes of approximately 1–2 ml total volume by placing 0.8 ml of phenol saline (0.5% [w/v] phenol in 0.15 M sodium chloride) into the first tube and 0.5-ml volumes of phenol saline in the remaining tubes of a series of five or ten tubes. A volume of 0.2 ml serum is added to the first tube, mixed, and then 0.5 ml is transferred to the next tube. Further volumes of 0.5 ml are transferred to subsequent tubes to give a series of doubling dilutions. An equal volume of standard *B. abortus* agglutination suspension, diluted to working strength in phenol saline, is then added to each tube, and the tubes are incubated at 37 °C for 20 hours.

The tests are read against opacity standards prepared by diluting the working strength antigen 1 in 4, 2 in 4, and 3 in 4, to correspond to 25%, 50% and 75% agglutination. Phenol saline is used as the 100% control, and the undiluted working strength antigen as the 0% control. The results are scored as the degree of agglutination (1+ = 25%, 2+ = 50%, 3+ = 75%, 4+ = 100%) over the serum dilution. In each set of tests, a positive control serum calibrated against the International Standard for *B. abortus* antiserum (ISABS) must be included. This enables the results to be expressed in IUs and permits tests that have been performed in different laboratories to be compared. Titres have to be interpreted in the light of the patients' history and occupational background.

For cattle, titres equivalent to 50 IU or more for unvaccinated animals and 100 IU or more for vaccinates are regarded as indicative of infection.

Microagglutination methods using a stained antigen may be performed in microtitre plates instead of tubes.

2-mercapto-ethanol agglutination test

The 2-mercapto-ethanol test is carried out by diluting 0.1 ml of serum in 0.4 ml of 0.15 M sodium chloride (physiological saline) and adding 0.5 ml of 0.2 M 2-mercapto-ethanol in saline. The serum is then reduced by incubation at 37 °C for one hour, followed by serial doubling dilutions in saline. Volumes of 0.5 ml of standard SAT antigen diluted to working strength in saline (without phenol) are then added to each tube, and the test is subsequently performed as for the standard test. It is more closely correlated with active infection but no more sensitive than the standard SAT.

The test is useful in the examination of human sera as it may help to differentiate IgM agglutinins resulting from recent infection or exposure to cross-reacting antigens from the IgG agglutinins associated with active or long-standing infection.

Coombs antiglobulin agglutination test

The serum agglutination test is performed according to the recommended procedure. Following incubation at 37 °C, the cells are deposited by centrifugation, preferably in a refrigerated centrifuge at 4 °C. The supernatant liquid is then discarded and the cell deposit washed by resuspension in 0.15 M sodium chloride followed by centrifugation. This process is performed at least twice. The cell deposit is then finally re-suspended in 0.5 ml volumes of anti-human IgG serum diluted to working strength. The tubes are then re-incubated at 37 °C overnight. Agglutination is then scored as for the agglutination test. A human positive serum control and a saline negative control should be included in the series.

If IgA antibodies are to be detected, a broad specificity anti-human immunoglobulin reagent should be used. This test has now been largely superseded by the ELISA.

Rose Bengal plate test (RBT)

Serum samples may be screened using the Rose Bengal plate agglutination test or card test. Serum (0.03 ml) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter. The mixture is agitated gently for four minutes at ambient temperature, and then observed for agglutination. Any visible reaction is considered to be positive. The test is very sensitive and positive samples should be checked by the CFT or by an IgG specific procedure such as ELISA. False-negative reactions occur especially in the early stages of acute infection. The RBT can be used in all animal species but positive results should be confirmed by a quantitative test. False positive results occur in vaccinated animals. False negative results are common in sheep, goats and pigs.

Complement fixation test (CFT)

Numerous variations of this exist but, whichever procedure is selected, the test must use an antigen that has been prepared from an approved smooth strain of *B. abortus*, such as strain 99 or 1119-3, and standardized against the second ISABS. Antigen for the CFT can be prepared by special procedures or antigen that has been prepared for the standard agglutination test can be used after diluting the stock suspension 1 in 200 in CFT buffer before

standardization. The packed cell volume of the concentrated antigen suspension for CFT should approximate 2% before standardization against the second ISABS, as described below. The phenol concentration must not exceed 0.5%. The appearance of the antigen when diluted 1 in 10 in phenol saline must be that of a uniform, dense, white suspension with no visible aggregation or deposit after incubation at 37 °C for 18 hours. It must not produce anti-complementary effects at the working strength for the test. The antigen should not be frozen.

A convenient procedure is the microtitration method. All dilutions are made in a buffer prepared from a stock solution of sodium chloride (42.5 g), barbituric acid (2.875 g), sodium diethyl barbiturate (1.875 g), magnesium sulphate (1.018 g), and calcium chloride (1.147g) in 1 litre of distilled water and diluted by addition of four volumes of 0.04% gelatine solution before use (CFT buffer). The indicator system is a 3% suspension of fresh sheep red blood cells (SRBC) sensitized with an equal volume of rabbit anti-sheep RBC serum diluted to contain five times the minimum concentration required to produce 100% lysis of the SRBC in the presence of a 1/30 dilution of fresh guinea pig complement.

The latter is independently titrated to determine the minimum concentration required to produce 100% lysis of a sensitized SRBC suspension; this is defined as the unit of complement. The standard *B. abortus* agglutination test antigen should be diluted in a CFT buffer to a concentration that gives 50% fixation of complement (1.25 units) at a dilution of 1/200 of the second ISABS. The test sera are diluted in equal volumes of CFT buffer and inactivated by heating at 58 °C for 50 minutes.

Test procedure

- a) Using standard 96-well U-bottom micro-titre plates, volumes of 25 μ l of diluted test serum are placed in the wells of the first and second rows, and 25 μ l unit volumes of CFT buffer are added to all wells except those of the first row.
- b) Serial doubling dilutions are then made by transferring 25 μ l volumes of serum from the second row onwards.
- c) Volumes of 25 μ l of antigen, diluted to working strength and 25 μ l of complement at 1.25 units strength, are added to each well. Control wells containing diluent only, serum+complement+diluent, antigen+complement+diluent, complement+diluent, are set up to contain 75 μ l total volume in each case.
- d) The plates are incubated at 37 °C for 30 minutes with agitation for the initial 10 minutes, or at 4 °C overnight.

- e) Volumes of 25 μ l of sensitized SRBC suspension are added to each well, and the plates are re-incubated at 37 °C for 30 minutes with occasional agitation for the first 10 minutes.
- f) The results are read after the plates have been left to stand at 4 °C for two to three hours to allow unlysed cells to settle.

The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis. Results should always be expressed in IUs, calculated in relation to those obtained in a parallel titration with a standard serum calibrated against the ISABS. In general, sera giving positive fixation at a titre equivalent to 20 ICFTU/ml or greater, are considered to be positive. However, for human sera, the history should be taken into account when interpreting the results of this test.

Indirect ELISA

Numerous variations of this indirect ELISA have been described. Commercial tests are available. Only those ELISAs that use *B. abortus* or *B. melitensis* smooth lipopolysaccharide (LPS) are recommended. Tests are performed in 96-well, flat-bottomed, polystyrene microplates. The choice of microplate will have a slight effect on assay performance in terms of background activity observed. Low to medium protein-binding microplates give the lowest background activity using LPS antigen.

The antigen-coating buffer is 0.05 M carbonate-bicarbonate buffer, pH 9.6, composed of NaHCO₃ (2.93 g), Na₂CO₃ (1.59 g), and NaN₃ (0.2 g) in 1 litre distilled or deionised water. The conjugate and test sera diluent buffer is 0.01 M PBS, pH 7.2, + 0.05% (v/v) Tween 20 composed of Na₂HPO₄ (1.21 g), KH₂PO₄ (0.20 g), NaCl (8.00 g), and KCl (0.20 g) in 1 litre distilled or deionised water + the addition of 0.50 ml Tween 20/litre. The wash buffer is 0.002 M PBS, pH 7.4 + 0.05% Tween 20.

The conjugate used should be a polyclonal antibody specific for both heavy and light chains of human IgG and conjugated to horseradish peroxidase. The substrate system is 4.4 mM H₂O₂ and 3.6 mM 2,2'-Azino-bis-(3 ethylbenzothiazoline-6-sulphonic acid) (ABTS) in 0.05 M phosphate/citrate buffer, pH 4.5, composed of 0.2 M Na₂HPO₄ (25.7 ml), 0.1 M citric acid (24.3 ml), and distilled/deionized water (50 ml); adjust the pH if necessary). The enzymatic reaction-stopping solution is 4% sodium dodecyl sulphate or 0.1 M NaN₃ in distilled/deionized water.

Test procedure

- a) LPS antigen is diluted in coating buffer to a concentration determined by checker-board titration, usually approximately 1 μ g/ml, and dispensed to all

microwells in 100 μ l volumes. The microplates are then incubated at 37 °C for two hours or at 4 °C overnight. As this is a solid-phase ELISA technique, the microplate wells require intervening washes between each assay step to remove unbound or unreacted reagents. From three to four wash cycles using the washing buffer, are sufficient. Prior to addition of the next reagent, the plates should be inverted and slapped onto a lint-free absorbent surface to discharge any residual contents.

- b) Test sera and controls are diluted 1/200 in diluent buffer and applied to appropriate wells in 100 μ l volumes. The plates are covered or sealed and placed on an orbital plate shaker and incubated at 37 °C for one hour with continuous shaking. Wash the plate as above.
- c) The enzyme conjugate is diluted in diluent buffer and applied to all wells in 100 μ l volumes. The plates are covered or sealed and placed on an orbital plate shaker and incubated at 37 °C for one hour continuous shaking. The optimal dilution of conjugate should be such that when reacted with the strong positive control under standard conditions, it will result in an average absorbance value of between 1.0 and 1.4 absorbance units (see step f). A known positive Reference Serum should be used. Wash the plate as above.
- d) Fresh substrate/chromogen solution is prepared by adding 60 μ l of a 3% H_2O_2 stock solution to 12 ml of phosphate/citrate buffer containing 3.6 mM ABTS. The substrate/chromogen solution is applied to all wells in 100- μ l volumes. The plates are transferred to an orbital plate shaker and incubated at 37 °C for precisely 15 minutes with continuous shaking. After 15 minutes incubation, the stopping solution is applied to all wells in 100- μ l volumes and the plate is shaken briefly on the plate shaker to ensure thorough mixing. All wells now contain a total volume of 200 μ l.
- e) The colour development is read with a microplate photometer using a 405 or 414 nm interference filter.
- f) The data may be expressed in a number of different ways, but it is recommended that test serum reactivity be expressed as per cent positivity of a standardized strong positive control serum. The strong positive control serum should be such that, when prediluted in negative serum, it exhibits an antibody activity that lies on the linear portion of the dose/response curve of the original high-titred serum, just below the plateau phase.

Annex 7

Intersectoral collaboration strategies for control and prevention of brucellosis

1. Strategies

The implementation of any control programme requires collaboration between many sectors of the community if it is to be successful. Development of prevention and control programmes should involve the participation of the community from the outset to provide a basis for efficient, effective and economical control. The elaboration of comprehensive national programmes should be based on the requirements of community programmes. Strategies in the development of such programmes are outlined below.

- To prevent the spread of infection amongst animals, and monitoring of brucellosis-free herds and areas.
- To carry out mass immunization to reduce the rate of infection in specified herds and areas.
- To control the spread of infection, implementation of non-specific measures in addition to specific measures, or as an alternative in areas where specific measures are not available.
- To eliminate infected animals by test and slaughter in order to develop brucellosis-free herds and areas.
- To inform and educate the general public, and train professional personnel (Annex 4, Table A.1).

These strategies are not mutually exclusive and the most effective programmes will combine all these elements.

2. Methods to be used in the field

Implementation of a programme would involve the application of various technologies applicable to field conditions. These will include:

- Analysis of the epidemiological situation (see details in Section 8, Table 4)
- Selection of herds/flocks and areas for action;
- Protection of low-prevalence and brucellosis-free areas (further details are given in the *OIE International Zoo-Sanitary Code*, 1997).

3. Planning, management and implementation of prevention and control measures

3.1 Steps initiated and sustained by the community

- Designation of a responsible person(s)
- Allocation of resources
- Annual assessment of the community programme(s)
- Identification of any further specific measures to be taken in collaboration with local government, and discussion of plan of action for epidemiological assessment and schemes for prevention and control
- Requesting intersectoral cooperation between different national services

3.2 Implement community – initiated schemes with the assistance of the government

- Elaboration of guidelines on simple decision-making processes
- Formulation of local plans of action, including:
 - epidemiological surveys,
 - education and information media on personal hygiene,
 - immunization programmes,
 - animal replacement schemes.

3.3 Development of various activities and services, in collaboration with peripheral governmental services

- Diagnostic services
- Vaccine provision in conjunction with use of cold chain
- Animal waste disposal or rendering
- Report on the progress of the programme to the central government

3.4 Comprehensive national programmes

- Establishment of an inter-ministerial committee
- Designation of a national programme directorate
- Preparation of guidelines for:
 - community activities
 - supporting services
 - comprehensive national plan
- Review and improvement of national rules and regulations
- Formulation of the countrywide programme of brucellosis control
- Institutional framework

- Mobilization of resources
- Programme implementation harmonizing the separate phases including the following aspects:
 - financial resources
 - geographical coverage
 - technologies
 - manpower
- Programme monitoring, periodic evaluation, and review.

All groups within the community, from ministerial to individual citizen level, must be kept informed of the status of the programme if it is to succeed.

3.5 International cooperation

In relation to the technical aspects of prevention and control of brucellosis, WHO, jointly with FAO and OIE, would wish to encourage and support programmes incorporating the above-mentioned international strategies on brucellosis control. Advice on human brucellosis may be sought from WHO. FAO and OIE can advise on the agricultural and international trade aspects of animal brucellosis.

Table A.4 Health education topics in the control of brucellosis

| Group | Topic | Expected action |
|--|--|--|
| Livestock breeders | <ul style="list-style-type: none"> • Concept of brucellosis • Characteristics of the disease • Damage done to human health • Damage done to animal production • Legislation backing the measures taken by the control agencies | <ul style="list-style-type: none"> • Collaboration with the measures of prevention and control of brucellosis carried out by public health and animal health services |
| | <ul style="list-style-type: none"> • Concept of brucellosis • Characteristics of the disease • Damage done to human health • Affecting species • Means of transmission to man • Preventive measures such as use of protective clothing, personal hygiene, environmental health | <ul style="list-style-type: none"> • Application of the recommended measures in order to prevent the disease |
| Personnel that work in direct contact with animals (shepherds, milkers, farmer, abattoir workers, inseminators, veterinarians) | | |
| General population | <ul style="list-style-type: none"> • Concept of brucellosis and its importance as a zoonosis • Ways of transmission to man • Symptomatology in man • Methods of prevention, especially related to milk or fresh cheese consumption | <ul style="list-style-type: none"> • Positive attitude with respect to the care of their own health and acknowledgment of brucellosis as a human disease |
| | | |

Source: Joint FAO/WHO Expert Committee on Brucellosis. Sixth Report. Technical Report Series, No. 740, WHO, Geneva, 1986.

Annex 8

Recommended standards for surveillance, prevention and control of human brucellosis (A23)

General introduction

Brucellosis is a widespread zoonosis mainly transmitted from cattle, sheep, goats, pigs and camels through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products (especially unpasteurized milk and soft cheese). In endemic areas, human brucellosis has serious public health consequences. Worldwide, *Brucella melitensis* is the most prevalent species causing human brucellosis, owing in part to difficulties in immunizing free-ranging goats and sheep. In countries where eradication in animals (through vaccination and/or elimination of infected animals) is not feasible, prevention of human infection is primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety. In most countries, brucellosis is a notifiable disease.

Causal agent and main modes of transmission

- *Causal agent.* *Brucella abortus*, biovars 1–6, 9; *B. melitensis*, biovars 1–3; *B. suis*, biovars 1,3 and 4; *B. canis*. *B. suis* biovar 2 and *B. maris* infections have rarely been described. Infected animals (mainly cattle, sheep, goats, pigs and less commonly dogs and other animals) and their products are the reservoirs and sources of infection.
- *Main modes of transmission.* Ingestion, direct contact through breaks in the skin and airborne infection (laboratories and abattoirs), primarily affecting consumers of raw milk and derivatives, farmers, butchers, veterinarians and laboratory personnel. The incubation period is highly variable, usually 2–4 weeks, can be 1 week to 2 months or longer.

Clinical description and recommended case definition

Clinical description. Brucellosis may present with acute or insidious onset, with continued, intermittent or irregular fever of variable duration, profuse sweating, fatigue, anorexia, weight loss, headache, arthralgia and generalized aching. Abscess formation is a rare complication. *Brucella* endocarditis and neurobrucellosis cause most deaths.

Laboratory criteria

Presumptive diagnosis

- Rose Bengal test (RBT) for screening; positive tests to be confirmed by one of the tests mentioned below under *Confirmatory diagnosis* below;
- Standard agglutination test (SAT).

Confirmatory diagnosis

- Isolation of *Brucella* spp. from blood or other clinical specimen.
- A presumptive laboratory diagnosis based on detection of agglutinating antibodies (RBT, SAT) combined with detection of non-agglutinating antibodies through:
 - ELISA IgG test;
 - Coombs IgG.

PCR and new rapid tests such as the lateral flow assay are yet to be accredited.

Case classification (humans)

- *Suspected*: a case that is compatible with the clinical description and is epidemiologically linked to suspected/confirmed animal cases or contaminated animal products.
- *Probable*: a suspected case with presumptive laboratory diagnosis.
- *Confirmed*: a suspected or probable case with confirmatory laboratory diagnosis.

Surveillance

- *Rationale for surveillance*: surveillance is a key element for management of prevention and control programmes.
- *Recommended types of surveillance*: early case-based reporting by health care providers or laboratories to upper levels of the public health sector and to appropriate levels of the animal health sector; in endemic countries where investigation of all reported cases may not be feasible, a representative proportion of reported cases should be investigated routinely.

Recommended minimum data elements

Case-based data

- Case classification.
- Unique identifier, age, sex, geographical information and occupation.

Aggregated data reporting

- Number of cases by case classification (probable/confirmed), age, sex, geographical area, occupation.

Recommended data analyses, presentation, reports

Graphs: number of probable/confirmed cases by month.

Tables: number of probable/confirmed cases by age, sex, month, and place.

Maps: number of probable/confirmed cases by place.

Performance indicators for surveillance

- Completeness and timeliness of reporting.
- Proportion of suspect, probable and confirmed cases.
- Number of investigations compared with number of cases.

Control activities

Case management

Doxycycline 100 mg twice a day for 45 days + streptomycin 1 g daily for 15 days. The main alternative therapy is doxycycline 100 mg twice a day for 45 days + rifampicin 15mg/kg/day (600–900mg) for 45 days. Experience suggests that streptomycin may be substituted with gentamicin 5mg/kg/daily for 7–10 days, but no study directly comparing the two regimes is currently available. Optimal treatment in pregnant women, neonates and children under 8 years has not yet been determined; for children there is experience with trimetoprim/sulfamethoxazole (co-trimoxazole) in combination with an aminoglycoside (streptomycin, gentamicin) or rifampicin.

Prevention

- Education to avoid consuming unpasteurized milk and milk derivatives.
- Barrier precautions for hunters and professionals at risk (butchers, farmers, slaughterers, veterinarians).
- Careful handling and disposal of afterbirths, especially in cases of abortion.
- Serological or other testing of animals; immunization of herds/flocks may be envisaged; elimination of infected herds/flocks.

Epidemics

Conditions under which epidemics may occur

Distribution of incriminated produce, usually raw milk or cheese from an infected herd/flock.

Management of epidemics

Identify common vehicle of infection; recall incriminated products, stop production and distribution unless pasteurization is introduced.

Drug-resistance monitoring. Not applicable.

Performance indicators for control activities. Number of new cases per 100 000 population over time.

Other aspects

Special considerations/other interventions. The most successful method for prevention and control of brucellosis in animals is vaccination. Control activities to be coordinated and shared between the public health and animal health sectors, who should ensure joint administrative arrangements to facilitate immediate cross-notification of cases, as well as coordination of joint investigations, control, and public health education programmes.

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