



BRUCELLOSIS

GUIDELINES FOR ACTION IN THE EVENT OF A DELIBERATE RELEASE

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Note: Comments are welcome from healthcare, laboratory and public health professionals, and should be sent to DRcomments@hpa.org.uk. These guidelines may be subject to change as comments are received, so please ensure that you have the latest version, which is available through the HPA website.

[http://www.hpa.org.uk/deliberate accidental releases/biological](http://www.hpa.org.uk/deliberate_accidental_releases/biological)

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1. BACKGROUND

These guidelines are intended for healthcare, laboratory and public health professionals to guide clinical and public health action in the event of a deliberate release of *Brucella*.

1.1 Introduction

Brucellosis, also known as undulant fever or Mediterranean fever, is caused by bacteria of the genus *Brucella*. It is a highly transmissible zoonosis (human infection of animal origin) affecting a wide variety of mammals in which it predominantly causes abortion and epididymitis. Human-to-human transmission is rare. Human infections arise through direct contact with infected animals or their milk. Although *Brucella* species are widespread throughout the world, brucellosis is rare in the UK and almost all cases are acquired abroad. The control of human disease is affected by control of animal disease through vaccination, test and slaughter of infected herds and by pasteurisation of milk products. *Brucellae* represent a significant aerosol biohazard to laboratory workers and others at occupational risk such as veterinarians, abattoir workers and farmers.

1.1.1 Deliberate release of *Brucella*

Brucella is a potential biological warfare agent because it is highly infectious by the airborne route and could be used in an aerosolised form or as a contaminant of food, milk and water or by direct inoculation. The organism survives well in the environment and widespread contamination is possible. Human infection is rarely fatal but brucellosis can be a protracted debilitating illness often requiring prolonged antibiotic treatment and there are no effective human vaccines, although animal vaccines are available.

1.2 Epidemiology

The genus *Brucella* comprises a group of closely related bacteria that are variants of a single species, *B. melitensis*. For convenience these are classified into six 'nomen-species' that differ from one another by their preferred animal hosts:

<i>B. melitensis</i>	especially goats, sheep, camels	<i>B. canis</i>	dogs
<i>B. abortus</i>	especially cattle, camels	<i>B. ovis</i>	goats, sheep
<i>B. suis</i>	pigs	<i>B. neotomae</i>	desert wood rat

The nomen-species can be further subdivided into 18 biovars, based on a panel of cultural, biochemical and antigenic characteristics. The first three regularly cause human infection although there have been cases of *B. canis* in man (Lucero *et al* 2009). In most developed countries *B. abortus* has been eradicated from cattle and is now a rare cause of disease. With the exception of some cattle associated *B. abortus* cases in Northern Ireland all human cases in the UK are acquired abroad. Two further groups (*B. ceti* and *B. pinnipedialis*) have been described in marine mammals and human infection with marine-associated *Brucella* spp. has also been reported. The risk these new species present to health is uncertain and suitable precautions should be taken to avoid any risk of infection by people who handle or work with these animals.

1.2.1 Transmission

Human infections usually arise from direct contact with infected animals or their milk. Infection is through ingestion, inhalation or direct inoculation.

Ingestion:

- unpasteurised milk products including soft cheeses
- acidified milk products (e.g. yoghurt, buttermilk) are less hazardous but still pose a risk

Inhalation:

- aerosol contact, particularly with infected products of conception from animals
- aerosol release from laboratory accidents or in abattoirs

Direct contact or inoculation:

- entry via the conjunctiva, usually in veterinarians or farmers
- veterinarians using live attenuated vaccine (*B. abortus* S19 and *B. abortus* RB51)
- blood transfusion, bone marrow/organ transplant, sexual, transplacental, via breast milk

1.2.2 Infectious dose

Infectious dose is dependent on organism type, virulence, and host resistance. Based on laboratory infections the dose is probably <500 cfu. The infectious dose is probably lower by the respiratory route and Guihot *et al.* (2004) suggest that 10-100 bacteria would be sufficient.

1.2.3 Incubation period

The period between presumed infection and onset of symptomatic disease can vary considerably, it is typically 5-30 days, but may be up to 6 months. One study found the incubation period ranging from a few days to 24 months, with a median of 4 weeks (Al Dahouk *et al.*, 2007). The infection may persist for months without causing any symptoms.

1.2.4 Period of communicability

Person-to-person spread is rare, although mother to child transmission and sexual transmission have been documented.

1.3 Clinical Features

Clinical features can be diverse and the course of the disease is variable. There may be an acute or insidious onset. The major features are prolonged fever and debilitation, weight loss, general malaise, muscle and joint pain. Sweating is often prominent. Acute and chronic arthritis affecting large joints and acute and chronic spinal osteomyelitis are common. About a quarter of patients have a dry cough and a quarter will have hepatosplenomegaly. Around 10% patients have lymphadenopathy or orchitis. Up to 5% of patients present with a meningoencephalitic picture, with or without psychosis and 1-2% will develop endocarditis which is a poor prognostic feature if left untreated. *B. melitensis* and *B. suis* tend to cause more severe, acute disease than *B. abortus*. *B. suis* also has the propensity to induce abscess formation.

In the event of widespread release individuals exposed to a high dose will present with more severe disease after a shorter incubation period. Acute disease lasts for weeks, leading to chronic, relapsing infection that may last for years with malaise, depression and destructive arthritis/osteomyelitis. A substantial minority will only have self-limiting disease.

1.4 Mortality

Illness can be protracted and debilitating but brucellosis is rarely fatal in humans, less than 2% even without treatment. Only in those developing endocarditis is there significant mortality.

1.5 Organism Survival

Brucella species are related to soil organisms and have very prolonged survival in both hot and cold environments, particularly in moist conditions. Under favourable conditions they may survive for up to two years in the environment, thus constituting a risk to both animals and humans. Pastures and animal accommodation on farms can remain contaminated for months. However, *Brucellae* are very sensitive to direct sunlight, moderately sensitive to acid and can be destroyed by pasteurisation or cooking. They are also sensitive to common disinfectants used at the appropriate concentration and temperature.

1.6 Antimicrobial Sensitivities

Brucella spp. are sensitive *in vitro* to a wide range of antimicrobials including the tetracyclines, aminoglycosides, rifampicin, sulfa drugs, trimethoprim (and cotrimoxazole), macrolides, fluoroquinolones and cephalosporins. The *in vivo* sensitivity of the intracellular organism may be less impressive but resistance to antimicrobials does not usually occur in human infections and treatment failure or relapse is usually due to inadequate duration or dose of therapy.

2. CLINICAL PROCEDURES

2.1 Diagnosis and Collection of Samples

Brucellosis may be suspected in patients with a fever and a history of travel to an endemic area. Epidemiological history, including occupation, food consumed, and contact with animals, will help with the diagnosis.

Laboratory tests are used to confirm the diagnosis. *Brucella* organisms may be grown from samples of blood or bone marrow and blood can be tested for antibodies against *Brucella*.

2.1.1 Culture

Bacteraemia is constant but low grade, and the organisms are slow growing. At least two sets of blood cultures should be taken prior to the start of antibiotic treatment. Blood culture yield is lower in *B. abortus* (<30%, typically 5-10%) than *B. melitensis* (~60%) infections and is much lower in chronic than acute disease, so serology remains the mainstay of laboratory diagnosis.

It is essential that any laboratory processing blood and other cultures is aware that brucellosis is suspected because

- Extended incubation of blood cultures is required for at least 6 weeks (3 weeks in BacTec systems) and strains of *B. abortus* require 5-10% CO₂ for primary isolation
- The organisms represent a significant hazard to laboratory personnel and any sub-culturing or growth should be handled in containment level 3 facilities

Bone marrow culture has a higher diagnostic yield than multiple blood cultures, but should be reserved for situations of diagnostic uncertainty, particularly if the patient has received antimicrobials. The aspirate should be inoculated directly in blood culture bottles. Other tissues or fluids may be cultured including: aspirate or biopsy of enlarged lymph nodes when present, liver, CSF, or joint fluid.

2.1.2 Serological tests

Immunity to brucellosis is not complete and reinfection is common. The mainstay of diagnosis is the tube or standard agglutination tests (SAT), or its variant the microagglutination test (MAT). Whilst these tests may be performed in any suitable laboratory samples should be sent for confirmation to the Brucella Reference Unit (BRU) or the Veterinary Laboratory Agency (VLA) as appropriate. Conventional testing uses antigens of *B. melitensis* and *B. abortus* but serological responses do not completely distinguish between the two species. These tests will cross react with *B. suis* infections but not with *B. canis* infection. ELISA and microELISA techniques are preferred in some laboratories and have comparable results and problems.

Patients living in enzootic areas or exposed in their occupation may have background serological positivity, affecting the diagnostic specificity of individual titres. Separation of IgG and IgM responses is only partially successful at distinguishing acute and chronic infections. Biological false positive reactions occur after cholera vaccination, *Yersinia enterocolitica* infections and with some other Gram-negative infections including *Francisella tularensis* and some *Salmonella* spp. In a non-endemic area, biological false positive serological reactions are expected to be more common than true positives. Over-reaction to such reports can provoke inappropriate bioterrorist alerts (Greenblatt *et al.*, 1999).

In normal circumstances, 90% of patients with acute infection will have positive serological results at clinical presentation. Most of the 10% that are negative will seroconvert after a further 14 days, and the majority of the 90% that are positive at presentation will have further increase in titres after 14 days.

The pattern of prolonged serological positivity (months to years) varies both after treatment and without treatment, and is a matter for expert interpretation. Agglutinating antibodies persist for many

years after natural infection, while complement fixing antibodies typically persist for about a year.

Consultation with the reference laboratory in Liverpool is essential for interpretation of results for both acute diagnosis and for clinical follow-up.

2.1.3 Antigen detection/PCR

No clinical antigen detection techniques are commercially available, although an immunoassay for detection of circulating LPS in serum and in tissue has been described (Al-Shamahy *et al.*, 1998). PCR-based techniques are potentially useful for the diagnosis of brucellosis particularly in chronic cases; however these tests are not yet in routine clinical use (Nimri, 2003).

2.1.4 Histology/CSF

Affected tissues show non caseating granulomata on biopsy. CSF is usually lymphocytic with mildly raised protein. The main differential is tuberculosis.

2.1.5 Imaging

Chest x-ray is usually clear even in the presence of cough, although pneumonitis/pneumonia or, rarely, empyema, is possible.

Bone x-rays: subtle erosions are the first manifestations; more destructive changes including sclerosis and loss of joint space (spine) are late findings. Gross destruction of spine is more suggestive of TB.

Isotope scans are more sensitive and often show multiple hot spots in addition to highlighting clinically affected joints/bones. CT/MR scans are ancillary to the above.

2.1.6 Other

Inflammatory markers such as ESR and CRP may be normal or raised. Full blood count usually shows a low WBC (possibly with relative lymphocytosis) and there may be mild thrombocytopenia. In a small proportion there is pronounced thrombocytopenia which may present with bleeding. Mild disturbance of liver function tests is common. Adenosine deaminase levels are raised in the CSF of patients with neurobrucellosis, but this will not necessarily discriminate from the main differential diagnosis of tuberculosis.

2.1.7 Transport of samples

Strict procedures should be followed for the transport of samples of suspected brucellosis, both from the clinical environment to the laboratory, and from local laboratories onto the reference laboratory. These are outlined in section 3.5 *Brucella* spp cultures fall into category A for the purposes of transport. All samples should be transported as per UN 602 as described in "Appendix 1.2 Transport of infectious substances" in "Biological agents: Managing the risks in laboratories and healthcare premises." Advisory Committee on Dangerous Pathogens (ACDP), Health and Safety Executive (HSE) May 2005 accessed at <http://www.advisorybodies.doh.gov.uk/acdp/managingtherisks.pdf>

2.2 Treatment and Follow-up

2.2.1 Treatment

Treatment with antibiotics relieves symptoms, shortens the duration of illness and reduces complications. Combination therapy must be used as relapse occurs in 30% of adults treated with monotherapy alone. Repeat courses are recommended in cases of relapse and relapsed or chronic infection should be treated for 3 months. El Miedany *et al.* (2003) suggested that triple therapy with streptomycin, rifampicin and doxycycline would prevent relapse but this is not usual practice. A review and meta-analysis of 30 randomised controlled trials (Skalsky *et al.* 2008) also concluded that preferred treatment should be with dual (e.g. doxycycline and gentamicin) or triple (e.g. doxycycline with rifampicin and gentamicin) regimens.

The World Health Organization recommends treating adults with acute infection with doxycycline and rifampicin for at least six weeks - the dosage and length of treatment and alternative treatments are shown in the table below. A multicentre study confirmed that six weeks of a doxycycline containing regimen is superior to four weeks and that gentamicin can be substituted for streptomycin or rifampicin (Solera *et al.*, 2004).

Table 1: Recommended treatment for brucellosis

NB: For osteoarticular disease, endocarditis or meningo-encephalitis expert advice should be obtained.

	Antimicrobial agent	Duration
Adults	Doxycycline 100 mg PO twice daily	6 weeks
	<i>plus either</i> Rifampicin ⁽¹⁾ 600 mg PO daily	6 weeks
	<i>or</i> Gentamicin 5 mg/kg IV daily	2 weeks
Children over 12 ⁽²⁾	Doxycycline 2mg/kg PO twice daily	6 weeks
	<i>plus either</i> Rifampicin 10-15 mg/kg PO daily	6 weeks
	<i>or</i> Gentamicin 5 mg/kg IV daily	2 weeks
Children under 12	Co-trimoxazole (sulfamethoxazole 40mg/kg and trimethoprim 8mg/kg) PO daily	6 weeks
	<i>plus either</i> Rifampicin 10-15 mg/kg PO daily	6 weeks
	<i>or</i> Gentamicin 5 mg/kg IV daily	2 weeks
Women who are pregnant or breast-feeding	Rifampicin ⁽³⁾ 600 mg PO daily	6 weeks

⁽¹⁾ Female patients/contacts should be informed that if they receive Rifampicin as part of their treatment/prophylaxis this can interact with the contraceptive pill and therefore other forms of contraception may be necessary.

⁽²⁾ WHO recommend this treatment for children >8 years old. However, it is recognised that doxycycline should not be given to children less than 12 years of age, or to pregnant or breast-feeding women. Therefore the doxycycline combination treatment is only recommended for children over 12 years old.

⁽³⁾ In pregnancy, rifampicin alone is conventionally used, although experience in the Middle East suggests that combination with cotrimoxazole (2 tablets bd) is safe and effective (Khan *et al.*, 2000). Fluoroquinolones may be used as an adjunct in complicated cases or as a substitute for doxycycline in pregnant women. Expert advice should be obtained.

2.2.2 Follow-up

Follow-up is essential at 3 and 6 weeks to encourage patient adherence to the chemotherapy regimen. Further follow-up at 3 months, 6 months and 1 year is the minimum to detect relapse. The main indicators of success are:

- General patient well-being
- Weight
- Absence of fever
- Disappearance of any positive findings at presentation eg: lymphadenopathy, hepatosplenomegaly
- Improving serological picture (decline of antibody levels is slow over months to years)

Repeat blood cultures are not necessary unless there is suspicion of relapse or failure.

2.3 Infection Control Practice

2.3.1 Decontamination of exposed person

In the event of a known exposure to *Brucella*, the risk for re-aerosolisation is uncertain and is likely to depend on a number of variables, including the quantity of organisms on the surface; the type of surface and host factors. However, even low numbers of organisms could potentially lead to infection in any person having contact. An incident specific risk assessment will be required.

In situations where the threat of exposure to the organism exists, cleansing of skin and potentially contaminated fomites such as clothing, personal possessions or environmental surfaces should take place. Decontamination of exposed persons includes:

- Removal of contaminated clothing and possessions – it should be stored in labelled double plastic bags until exposure to *Brucella* has been ruled out.
- If brucellosis is confirmed, all contaminated material must be incinerated or autoclaved.
- Minimal handling of clothing and fomites to avoid agitation.
- Instructing exposed persons to shower thoroughly with soap and water- appropriate facilities will be provided at the scene as necessary.
- Instructing attending personnel to wear full PPE when handling contaminated clothing and other fomites.

2.3.2 Isolation of patients

There is no person-to-person spread therefore no isolation is required for infected patients.

- Standard Universal Precautions should be used for the care of patients with brucellosis – gloves, gowns, masks and hand washing.
- Single room placement for brucellosis patients is not necessary.
- Airborne transmission does not occur.
- Standard Universal Precautions should be maintained when patients are moved.

2.3.3 Cleaning, disinfection and Waste disposal

Contaminated environmental surfaces should be cleaned with hypochlorite solution (5,000ppm available chlorine).

2.3.4 Post-mortem procedures

The risk of acquiring brucellosis following contact with the body of a person who has died from the disease is negligible, because person-to-person transmission is very rare (vertical, sexual, iatrogenic – blood transfusion or transplant) and there is no evidence of autopsy transmission.

Autopsy examinations may be carried out with appropriate precautions – standard post-mortem examination PPE (e.g. disposable gown, apron, safety gloves) and as infected tissue may represent an aerosol hazard respiratory protection (FFP3 high efficacy air filter masks) should be worn. The same procedures should be followed as for open pulmonary tuberculosis. The Pathologist must be informed of the known or suspected diagnosis. Standard precautions for post-mortem examinations on patients infected with Containment Level 3 organisms are appropriate. Instruments should be autoclaved.

Similarly, body preparation should be carried out with normal control of infection procedures.

Standard precautions for the disposal of bodies infected with Containment Level 3 pathogens should be observed, and the undertaker should be informed. **Cremation** is the preferred method for disposal of the deceased. **Embalming** of bodies should not be undertaken because the body fluids are likely to contain large numbers of the causative bacteria and therefore the process of embalming exposes the embalmer to an unacceptable risk.

Pacemaker removal is permitted. Pacemaker should be treated with hypochlorite solution (10,000 ppm available chlorine), bagged and disposed of appropriately (not by incineration).

Table 2: Recommended prophylaxis after exposure to *Brucella* spp

Table 2	Antimicrobial agent	Duration
Adults	Doxycycline 100 mg PO twice daily <i>plus</i> Rifampicin 600 mg PO daily <i>or</i> Cotrimoxazole 960mg PO twice daily	3 weeks
Children over 12⁽²⁾	Doxycycline 2mg/kg PO twice daily <i>plus</i> Rifampicin 10-15 mg/kg PO daily <i>or</i> Co-trimoxazole (sulfamethoxazole 40mg/kg and trimethoprim 8mg/kg) PO daily	3 weeks
Children under 12	Co-trimoxazole (sulfamethoxazole 40mg/kg and trimethoprim 8mg/kg) PO daily <i>plus</i> Rifampicin 10-15 mg/kg PO daily	3 weeks
Women who are pregnant or breast-feeding	Rifampicin ⁽³⁾ 600 mg PO daily	3 weeks

⁽¹⁾ Female patients/contacts should be informed that if they receive Rifampicin as part of their treatment/prophylaxis this can interact with the contraceptive pill and therefore other forms of contraception may be necessary.

⁽²⁾ WHO recommend this treatment for children >8 years old. However, it is recognised that doxycycline should not be given to children less than 12 years of age, or to pregnant or breast-feeding women. Doxycycline combination treatment is only recommended for children over 12 years old.

⁽³⁾In pregnancy, rifampicin alone is conventionally used, although experience in the Middle East suggests that combination with cotrimoxazole (2 tablets bd) is safe and effective (Khan *et al.*, 2000). Fluoroquinolones may be used as an adjunct in complicated cases or as a substitute for doxycycline in pregnant women. Expert advice should be obtained.

2.4 Prophylactic treatment for persons exposed to brucellosis

There is supportive experimental evidence from animal and laboratory exposures to support the use of post-exposure prophylaxis (PEP). The usual combination is 200mg doxycycline plus 600mg rifampicin daily (see Table 2 for recommended prophylaxis). However, the need for prophylaxis must be balanced against the possibility of significant side-effects. Maley *et al.* (2006) reported significant side effects in six of seven laboratory staff exposed to brucella culture from a patient. They were given 3 weeks doxycycline/rifampicin prophylaxis and all reported nausea, vomiting and anorexia. One was admitted to hospital with fever and mild hepatitis and another developed minor facial

swelling and mild depression. Only four staff completed the full course. There were no sero-conversions and none developed brucellosis in 8 months of follow-up.

In a major incident information on how to access stocks of antibiotics for the initial treatment or prophylaxis can be found on the DH website at:

http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_081038

2.4.1 Immunisation

There is no vaccine available for humans in the UK.

2.4.2 Monitoring of exposed persons

Persons directly exposed should have a baseline serum sample stored for possible later comparative serology in the event of their developing symptoms. They should be aware that development of fever, malaise, unexpected loss of weight or joint/bone pain may be due to brucellosis and that they should mention this possibility if they attend any physician for care. Clinical incubation could be prolonged for up to six months.

2.5 Environmental Decontamination

The greatest risk to human health following a release of brucella occurs during the period in which the organism is airborne. The duration and scale of the infectious risk depends on the duration for which brucella remains airborne and the distance traveled. This depends on meteorological conditions and aerobiological properties of the dispersed aerosol. The residual risk is probably low, although there is a theoretical risk of re-aerosolisation early after an attack. In the event of a known release, an exposed zone will be defined according to the time and place of release in order to identify all persons exposed to primary aerosolisation. Expert advice will be provided to determine the time after release for which brucella is likely to remain airborne.

Decontamination of a large area in the event of widespread release is not feasible. Contamination of a small enclosed area should be dealt with by fumigation with formaldehyde gas when practicable.

2.6 Protection of Frontline Workers

This includes all emergency staff involved in management at the scene of a release, as well as those involved in treating patients.

2.6.1 Protective clothing

The overt release of *Brucella* will create an **exposed zone**, the area affected by primary aerosolisation will depend on the time and place of release. This exposed zone presents a high risk of infection. Any personnel entering this zone should wear a biologically-resistant suit with outer gloves and boots (for example a CR1, PRPS or gas-tight suit), and a correctly fitting high-efficacy particulate respirator of FFP3 standard **AT ALL TIMES**.

Healthcare workers will not normally be asked to enter this zone, but may be called into it to treat casualties, for example if an explosive device has accompanied the release of biological agent. In this case the appropriate protective clothing and equipment should be worn.

Exposed persons will normally be moved from the exposed zone, through decontamination, and into a place of safety (see section 4.3.1) for medical assessment and administration of prophylactic treatment. Frontline workers involved in decontamination, and others who have who have any contact with contaminated clothing and fomites should wear the appropriate protective clothing and equipment. Emergency staff who attend exposed persons after decontamination has been completed do not need to take any special precautions.

For healthcare workers involved in the management of hospitalised patients with all forms of brucellosis, standard Universal Precautions (gloves, gowns, masks and hand washing) provide sufficient protection, and mortuary staff should use similar barrier protection. More sophisticated countermeasures for airborne protection such as high-efficacy air filter masks are **not** required.

2.6.2 Antibiotic prophylaxis

Frontline workers entering the exposed zone and others in the exposed zone should be offered antibiotic prophylaxis – see Table 2 (section 2.4).

Decisions about who should receive prophylaxis should be taken on an individual basis according to duration and degree of potential exposure, and taking into account the availability and side effects of prophylactic treatments.

3. LABORATORY PROCEDURES

3.1 Risk Assessment

Brucella is a Hazard Group 3 pathogen and should be covered by existing risk assessments for handling such organisms in diagnostic microbiological laboratories. *Brucella* spp. are easily transmitted by aerosols, by ingestion and percutaneous inoculation. Cultures must be handled under containment conditions appropriate to Class 3 pathogens.

In the event of widespread release, the National Blood Service would need to be made aware of the potential risk of subclinical infection in exposed blood donors.

Brucellosis is a significant hazard for both human and animal populations, the latter also serving as a reservoir for further human infections. Introduction of brucellosis into British dairy herds would lead to a significant occupational hazard to farmers and veterinarians due to a recurrence of abortion storms in non-immune animals. In the event of widespread release, there should be early consultation and cooperation between the DPH/Health Action Team and their veterinary counterparts from DEFRA/VLA.

3.1.1 Receipt of samples

Samples should be labelled as "High risk" or "Danger of Infection" by the submitting staff and should be handled according to local protocols for such samples. All laboratory procedures should be performed by experienced, appropriately trained staff, in containment Level 3 facility using a Class 1 protective safety cabinet. Chain-of-evidence documentation should accompany specimens. In larger incidents this would only be required for several of the initial cases.

3.2 Isolation and Identification

Brucellosis is confirmed in man by isolating the organisms from blood or other tissue samples and by serological and other tests.

3.2.1 Culture

'Conventional' blood cultures rarely become positive in the first 7 days and should be incubated for at least 6 weeks before being discarded. Modern non-radiometric or other signalling systems (Bactec etc) are more sensitive and yield earlier positive results, but should still be retained for at least 3 weeks before being declared negative. Special media e.g. Castañeda medium increase yield but are not usually used. Lysis-centrifugation also increases yield but also increases the hazard to laboratory personnel. Agar culture dishes with positive growth should not be 'sniffed' by laboratory personnel.

The organism is a small Gram-negative cocco-bacillus and may be misidentified as a contaminant (eg. diphtheroids) if Gram-staining techniques are poor. The organism may be misidentified as *Moraxella phenylpyruvica* or *Ochrobactrum anthropi* by API 20NE strips used in most routine diagnostic practice (Batchelor *et al.*, 1992; Elsaghir & James, 2003; Robichaud *et al.*, 2004), or occasionally as *Haemophilus* spp. All suspect isolates of Gram-negative cocco-bacilli should be forwarded to the Veterinary Laboratory Agency Weybridge for confirmation.

3.2.2 Serological tests

The serological diagnosis of brucella is by the tube agglutination test or by ELISA. These tests are subject to significant prozone effects. There is great variation in purity and specificity of antigen used both between batch and sources, affecting especially the specificity as well as sensitivity. Patients with brucellosis have circulating IgA which blocks these tests at low dilutions, giving false negative results unless adequate serial dilutions of serum are employed. Standardisation of procedures and antigens is of paramount importance.

In a suspected infection, it is essential that sera are forwarded to the Brucella Reference Unit in Liverpool for confirmation, whether negative or positive results are obtained locally.

3.2.3 Confirmation

Consultation with the reference laboratory in Liverpool is essential for interpretation of results for both acute diagnosis and for clinical follow-up.

3.3 Waste Disposal

In the laboratory hypochlorite (5,000ppm available chlorine) disinfection is necessary for decontaminating surfaces. All waste containers should be autoclaved.

3.4 Reference Laboratory

All positive isolates and cultures should be sent to the Veterinary Laboratory Agency reference laboratory in Weybridge for confirmation. In addition samples may be sent directly if local laboratories lack the facilities for dealing with them. Samples for serology should be sent to the Brucella Reference Unit in Liverpool. All samples must be packaged appropriately, taking care to observe the procedures outlined below (see 3.5). The senders name and address should be clearly marked. The reference laboratory should be telephoned prior to sending to expect the sample.

Cultures or samples for isolation should be sent urgently to:

Department of Statutory and Exotic Bacterial Diseases
Veterinary Laboratory Agency Weybridge
New Haw, Addlestone
Surrey KT15 3NB
Tel: +44 0193 234 1111

Samples should be sent urgently to:

Clinical Microbiology and HPA Collaborating Laboratory
Brucella Reference Unit (BRU)
University Hospital Aintree
Lower Lane
Liverpool L9 7AL
Tel: +44 0151 529 4900

3.5 Transportation of samples with suspicion of *Brucella* spp.

Strict procedures apply for transport of samples to the laboratory. Biological agents, or materials that contain or may contain them, are allocated to UN Division 6.2 – infectious substances. Infectious substances are divided into Category A or Category B. Full details are given in Appendix 1.2 Transport of infectious substances in *Biological agents: Managing the risks in laboratories and healthcare premises*. ACDP HSE May 2005, available at <http://www.advisorybodies.doh.gov.uk/acdp/managingtherisks.pdf> and in the Department of Health's guidance, available at <http://www.dh.gov.uk/assetRoot/04/11/48/13/04114813.pdf>

Cultures of *Brucella* spp are Category A infectious substances capable of causing disease in humans or animals and are therefore assigned to UN2814 and must be packaged in accordance with UN Packaging Instructions PI620 (road/rail) /PI602 (air). P620 and P602 are identical specifications but given different codes in ADR and ICAO regulations respectively (for a full description of PI see <http://www.unece.org/>). Category A transfers should be individually requested through an approved courier. The service will be a next day, tracked door-to-door delivery, which must be signed for at collection and receipt.

Clinical samples are generally classified as Category B and are assigned to UN3373 (Diagnostic and Clinical specimens) and should be packaged in accordance with UN PI650. Clinical samples

may be posted.

Packaging must meet with UN performance requirements i.e. UN-type approved packaging for Class 6.2 substances. The packaging should consist of an inner package (watertight receptacle, watertight secondary packaging, an absorbent material in sufficient quantity to absorb the entire contents placed between the receptacle and the secondary packaging) and a rigid outer package of adequate strength for capacity, mass and intended use. Packages should be marked with the proper shipping name i.e. "Infectious substance affecting humans", the appropriate UN number (i.e. UN 2814), and the appropriate warning label (i.e. the danger sign for infectious substances).

The following procedures should be adopted for the transport of all specimens, and also all cultures for confirmation. These apply within hospitals and laboratories as well as for specimens sent to the reference laboratory:

- The primary container (bijoux or similar) should be screwed tight, labeled and placed in an intact plastic bag.
- A 'High Risk' label should be affixed to both specimen and request form. The latter should include any other relevant information and include adequate clinical details to indicate level of suspicion.
- Under no circumstances should the request form be placed in the same bag as the specimen.
- The bag should be sealed, using tape or heat sealer. Pins, staples and metal clips should not be used. A separate bag should be used for each specimen.
- Each specimen must then be placed in a leak-proof secondary container with sufficient absorbent material to absorb all the contents should leakage occur.
- Each specimen must be packaged individually - i.e. three specimens, three separate packages.
- The secondary container should be externally disinfected – e.g. by wiping with hypochlorite (1,000ppm).

3.5.1 Samples sent to the reference laboratory

- Secondary containers should be placed within a final outer tertiary packaging.
- This packaging **must** comply with the UN 602 standard packaging for the transport of infectious substances by air, road or rail.
- The package should be certified to this standard and carry the appropriate UN certification numbers on the tertiary packaging along with the following information:
- BIOHAZARD – danger of infection symbol Class UN 6.2.
- Instructions not to open if found.
- Telephone number of responsible person e.g. Consultant Microbiologist, Laboratory Manager.
- The container should be transported either by an approved courier for cultures (UN 2814) or by post for clinical samples (UN 3373), without delay, directly to the reference lab.

3.5.2 Samples sent within hospitals and laboratories

Samples should be transported according to local arrangements for High Risk specimens.

Precautions should include:

- Secondary containers should be placed in a good quality box, which is well taped up and clearly labeled "Pathological Specimen – Open only in Laboratory".
- Specimens should be transported by hand by a responsible person using the above packaging. Vacuum-tube systems should **not** be used for transportation of specimens within hospitals or laboratories.
- Extra care should be taken to ensure that laboratory records are kept to a high standard.

3.6 Protection of Laboratory Staff

All laboratory procedures must be performed in a Containment Level 3 facility using a Class 1 biological safety cabinet. Under these circumstances there is no indication for antibiotic prophylaxis for laboratory staff unless there is an inoculation injury or a spillage releasing aerosols. Any member of laboratory staff, working with specimens or cultures of brucella, who develops a febrile illness, should seek urgent medical attention.

4. PUBLIC HEALTH PROCEDURES

An incident specific risk assessment will be required.

4.1 Surveillance and detection of deliberate release of *Brucella*

A deliberate release may be overt with an announcement and/or confirmation by environmental sampling. However, it is also possible that a deliberate release may be covert and will not be identified until the first cases of disease arise.

Brucellosis is rare in England and Wales because of successful eradication from cattle. There are less than 10 human cases reported each year and these are acquired abroad. Therefore cases of brucellosis without foreign travel especially if in clusters or accompanied by a rise in clinical illness or abortions in cattle or other ruminants in the same area should be investigated to exclude deliberate release.

Close co-ordination with veterinary colleagues is essential. Disease in animals is usually asymptomatic, but can lead to abortion and stillbirths; infected animals could act as ongoing source of potential human infection. Incident managers should ensure that appropriate veterinary advice is taken.

4.2 Case Definitions

4.2.1 Suspected case

An illness characterised by acute or insidious onset with fever, night sweats, fatigue, malaise, anorexia, headache and arthralgia with no other cause.

If *Brucella* is suspected, serological specimens should be sent to the *Brucella* Reference Unit, Liverpool and microbiological samples to the Veterinary Laboratory Agency Weybridge. Consideration should be given to initiating empirical treatment pending results. Obviously the level of suspicion depends on local circumstances at the time – in the event of a known or suspected deliberate release the threshold for diagnosing *Brucella* should be lower.

4.2.2 Confirmed case

A case that clinically fits the criteria for suspected brucellosis, and in addition, definitive positive results are obtained on one or more pathological specimens by the reference laboratory.

4.3 Public Health Action

4.3.1 Procedure for handling exposed persons

Depending on the site and method of release, *Brucellae* may be dispersed over a wide area. Expert advice will be provided to define an exposed zone in time and space. All individuals who have been present in the exposed zone need to be identified. In the event of an overt release, some will still be at the scene when emergency services respond to the incident. This group will be decontaminated and then referred to health workers at a nearby place of safety for assessment (this will be a clinical area just outside the exposed zone and within the cordon that will be established at the scene of the incident). Others will have left the scene before emergency services arrive and will be identified later when they approach GPs and Emergency Departments after details of the incident have been made public. Procedures need to ensure that these individuals are appropriately decontaminated, receive prophylaxis, and have their details collected for follow-up.

4.3.2 Post-exposure prophylaxis

There are two groups of individuals for which post-exposure prophylaxis (doxycycline/ rifampicin for 3 – 6 weeks) is indicated: (1) Individuals present in the exposed zone and (2) Health care workers. If suspected or confirmed cases of brucellosis arise among persons who were outside but in close proximity to the exposed zone in time or space, the defined parameters of the exposed zone should

be reviewed with a view to extending post-exposure prophylaxis (2.4 Prophylaxis - PEP Table 2). Prophylaxis for other groups may be considered in the event of an incident. However, it is not advisable to give antibiotics to people who do not have a clear history of having been present at the time and site of release. It is inappropriate to provide antibiotics to large numbers of people who have not been exposed, but who are generally concerned or have non-specific mild illness. Rather than prophylaxis, increased surveillance, prompt investigation and treatment are encouraged.

4.3.3 Follow-up of exposed persons

After an overt release, a basic set of personal details needs to be collected from all persons present in the exposed zone.

4.3.4 Case finding

Once cases of brucellosis have been detected and a release is suspected, there should be enhanced surveillance for other cases with advice to health care workers and other individuals.

4.3.5 Preventing secondary spread

Person-to-person spread of *Brucella* spp. is rare therefore there is no specific treatment or advice required for secondary contacts. There is no requirement for quarantine of infected patients.

4.4 Epidemiological Investigations

A case of brucellosis in a person without foreign travel should be immediately reported to the HPA Centre for Infections (020 8200 6868, 24 hours). Cases should be investigated thoroughly to identify the source of infection and exclude deliberate release. If cases arise due to a covert release, or following an overt release but in people who have not been present in the exposed zone, it is important to collect some epidemiological details in addition to a basic set of personal details. This is in order to define or redefine the exposed zone and aid identification of others at risk of infection. Details should be as thorough as possible, whilst recognising that in the event of a large release with multiple exposed persons or cases, it may not be possible to collect comprehensive information from everyone.

The aim of epidemiological investigations may be:

- Following a covert release, to assist definition and ongoing review of the temporal and spatial parameters of the exposed zone so that post exposure prophylaxis can be distributed appropriately.
- Following an overt release, to guide review of the exposed zone if cases arise in persons who were not present within it.

5. LIST OF NATIONAL EXPERTS

Advice on *Brucella* including diagnosis, management and public health aspects can be obtained from:

Clinical advice

Dr NJ Beeching
Tropical and Infectious Diseases Unit
Royal Liverpool University Hospital
Liverpool L7 5XP
Tel: (+44) 0151 706 3835
Out of hours: (+44) 0151 706 2000
e-mail: nicholas.beeching@rlbuht.nhs.uk

Dr RC Spencer
Health Protection Agency
Level 8
Bristol Royal Infirmary
Marlborough Street
Bristol BS2 8HW
Tel: (+44) 0117 342 3242
Mobile: (+44) 07885 434000
e-mail: robert.spencer@ubht.swest.nhs.uk
bob.spencer@hpa.org.uk

Diagnostic laboratory and environmental advice

Dr M Rothburn or Dr R Cooke
Clinical Microbiology and HPA Collaborating Laboratory
Brucella Reference Unit (BRU)
University Hospital Aintree
Lower Lane
Liverpool L9 7AL
Tel: (+44) 0151 529 4900
e-mail: mike.rothburn@aintree.nhs.uk
richard.cooke@aintree.nhs.uk

Veterinary laboratory and clinical advice

Mrs Judy Stack
Department of Statutory and Exotic Bacterial Diseases (SEB)
Veterinary Laboratory Agency Weybridge
New Haw
Addlestone
Surrey KT15 3NB
Tel: (+44) 0193 235 7610
e-mail: j.a.stack@vla.defra.gsi.gov.uk

Public Health and **Out of Hours** contact details are held at HPA Centre for Infections by the 24 hour on call duty doctor; Tel: (+44) 020 8200 6868 or (+44) 020 8200 4400

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