Eurosurveillance

BICHAT GUIDELINES* FOR THE CLINICAL MANAGEMENT OF BRUCELLOSIS AND BIOTERRORISM-RELATED BRUCELLOSIS

P Bossi, A Tegnell, A Baka, F Van Loock, J Hendriks, A Werner, H Maidhof, G Gouvras

Task Force on Biological and Chemical Agent Threats, Public Health Directorate, European Commission, Luxembourg

Corresponding author: P. Bossi, Pitié-Salpêtrière Hospital, Paris, France, email: philippe.bossi@psl.ap-hop-paris.fr

Interest in *Brucella species* as a biological weapon stems from the fact that airborne transmission of the agent is possible. It is highly contagious and enters through mucous membranes such as the conjunctiva, oropharynx, respiratory tract and skin abrasions. It has been estimated that 10-100 organisms only are sufficient to constitute an infectious aerosol dose for humans. Signs and symptoms are similar in patients whatever the route of transmission and are mostly non-specific. Symptoms of patients infected by aerosol are indistinguishable from those of patients infected by other routes. Regimens containing doxycycline plus streptomycin or doxycycline plus rifampin are effective for most forms of brucellosis. Isolation of patients is not necessary. Trimethoprim-sulfamethoxazole and fluoroquinolones also have good results against *Brucella*, but are associated with high relapse rates when used as monotherapy. The combination of ofloxacin plus rifampicin is associated with good results. Even if there is little evidence to support its utility for post-exposure prophylaxis, doxycycline plus rifampicin is recommended for 3 to 6 weeks.

Euro Surveill 2004; 9 (12)
http://www.eurosurveillance.org/em/v09n12/0912-237.asp

Introduction

Brucellosis (also named Mediterranean fever, Gibraltar fever, Malta fever, Cyprus fever, undulant fever and typhomalarial fever) is a zoonotic infection caused by bacteria from the *Brucella sp*. Currently, of the six main biovars of *Brucella*, four are associated with moderate-to-significant human pathogenicity [1]. These biovars are found in goats and cattle (*B. melitensis*), swine (*B. suis*), cattle and bisons (*B. abortus*) and dogs (*B. canis*). *B. melitensis* occurs more frequently in humans than any of the other types and is the most virulent, pathogenic and invasive species, followed by *B. suis*, *B. abortus* and *B. canis*. There is an epidemiological link with consumption of raw milk from cattle infected with *B. abortus* and human cases of brucellosis [1]. Abortions are common in animals infected with any *Brucella species*, but not in similarly infected pregnant women [1,2].

The infection occurs worldwide in both humans and animals, but is most common in the Mediterranean countries of Europe and Africa, the Middle East, India, Central Asia, Mexico, and Central and South America [2].

Transmission of brucellosis to humans is usually the result of ingestion, direct contact via cuts or abrasions of the skin, mucous membranes or inhalation [1,2]. Risk factors for infection include handling of infected animals (tissues and body fluids such as blood, urine, vaginal discharges, aborted animal foetuses and especially placentae), ingestion of contaminated animal products such as meat, raw or unpasteurised milk and milk products [1]. Persons at high risk are those working (farmers, veterinarians, abattoir workers) with infected agricultural animals such as cattle, pigs, sheep, camels and goats. *Brucella* is one of the most common organisms acquired due to laboratory exposure usually by aerosolisation [3]. Airborne infection has also been reported in pens and stables of infected animals. Outbreaks of brucellosis are usually reported after inhalation of aerosols. Rare cases have occurred from accidental self-inoculation of the *B. abortus* vaccine strain 19 and the *B. melitensis* animal vaccine strain Rev-1 [4,5].

Person-to-person transmission usually does not occur, with the exception of the very rare cases reported following blood exposure, primary exposure to infected tissues or after sexual contact.

Brucellosis and bioterrorism

Interest in *Brucella species* as a biological weapon stems from the fact that airborne transmission of the agent is possible (e.g. human airborne transmission during abortions of infected animals, aerosolisation in laboratories). It is highly contagious, as it can enter through mucous membranes such as the conjunctiva, oropharynx, respiratory tract and skin abrasions. It has been estimated that only 10-100 organisms are needed to constitute an infectious aerosol dose for humans [2]. *Brucella* is sensitive to exposure to heat and most disinfectants but can survive in the environment for up to two years under specific conditions, becoming a continuing threat to both humans and animals. In 1954, *B. suis* became the first agent weaponised by the United States (US). Several other countries have or are suspected to have weaponised this agent, including the United Kingdom (UK), although to our knowledge, this agent has never been used as a biological weapon [6]. Nevertheless, *Brucella*, particularly *B. melitensis* and *B. suis*, is considered one of the agents of lesser threat. The incubation period is rather long, many infections are asymptomatic and the mortality is low. However, the agent might be used more as an incapacitating agent, as the disease is associated with a high morbidity combining a protracted illness.

Microbiological characteristics

Brucella is a small, Gram negative aerobic coccobacillus that grows extra- and intracellularly within macrophages. It lacks capsule, flagellae, endospore or native plasmid. The genus *Brucella* contains six species with multiple biotypes that vary in terms of biochemical reactions, host specificity and pathogenicity for humans [1]. The species affecting humans are mentioned above, while *B. ovis* and *B. neotomae* are not infectious for humans.

The principal virulence factor of *Brucella* is the cell-wall lipopolysaccharide. Strains with smooth lipopolysaccharide are

more virulent and more resistant to intracellular destruction by polymorphonuclear leukocytes.

Clinical features

Signs and symptoms are similar in patients whatever the route of transmission and are mostly non-specific (TABLE I). Symptoms of patients infected by aerosol are indistinguishable from those of patients infected by other routes [2]. Physical examination is usually without abnormalities A high degree of suspicion is imperative in order to establish the diagnosis. The incubation period of brucellosis is highly variable, from one to 60 days, up to several months, with an average of 1-2 months. The illness may be mild and self-limited or severe. Onset of the disease may be abrupt or insidious (50%). Brucellosis is a systemic infection that can involve any organ or organ system. It is generally characterised by fever, which may be continuous, intermittent or irregular [1]. Fever, which is the most common symptom (90%-95%), may be associated with other symptoms such as weakness, profuse sweating (40%-90%), chills, diffuse or localised arthralgias (20%-40%), malaise (80%-95%), weight loss and generalised pain (40%-70%) [2,7-9]. Neuropsychiatric complaints, which include headache, depression and irritability, are frequent. Gastrointestinal symptoms such as anorexia, nausea, vomiting, abdominal pain, diarrhoea or constipation are also frequent (up to 70% of cases). Rare cases of ileitis, colitis or peritonitis have been reported with *B. melitensis* [1]. The liver is involved in the great majority of cases as shown by a mild increase of liver function enzymes. Non-caseating epithelioid granulomas indistinguishable from the ones seen in sarcoidosis can be found in liver biopsy. Hepatic abscesses and acute cholecystitis have also been reported. Cough and chest pain are present in 15%-25% of infected patients with a normal chest radiograph [1]. Lung abscesses, nodules and pleural effusions have been reported. Lymphadenopathies are present in 10%-20% of patients and 20%-70% experience splenomegaly and/or hepatomegaly (10%-30%). Cutaneous manifestations may include ulcerations, petechiae, purpura, and erythema nodosum. Osteoarticular complications of brucellosis are common (up to 40%) and include sacroileitis, which represents the most frequently involved joint, peripheral joint arthritis, bursitis, osteomyelitis and particularly vertebral osteomyelitis or spondylitis (6%) [1,10,11]. Arthritis of peripheral joints usually involves large joints such as the hips, knees and ankles. Osteoarticular complications are usually due to *B. melitensis*. Other complications such as epididymo-orchitis, meningitis (manifested by lymphocytic pleocytosis, elevated protein and normal-low glucose in the CSF), encephalitis, seizures and neuropathies are less frequent (<2%). Severe behavioural changes of unknown aetiology occur in some patients. Endocarditis occurs in approximately 2% of patients [1]. Other cardiovascular complications include myocarditis, pericarditis and aneurysms of the aorta and cerebral vessels [1].

In brucellosis, routine laboratory tests are commonly non-specific: mild elevation of serum lactate dehydrogenase and alkaline phosphatase [2]. Haematological abnormalities may include anaemia, neutropenia and thrombocytopenia [12].

The case fatality rate is very low in untreated patients (less than 2%). It is usually due to *B. melitensis* endocarditis or meningitis. Nearly all patients respond to antibiotic treatment, with fewer than 10% manifesting relapses. The systemic symptoms may last for weeks or months. Most of the patients recover within a year, even without antibiotic treatment.

Diagnosis

The diagnosis of brucellosis should be considered in a patient with a chronic fever of unknown origin. Case definitions of brucellosis are listed in Tables 2 and 3.

It is definitively established by culture of the organism, which can be most frequently recovered from blood or bone marrow specimens. Positive culture may require 4 to 7 days to grow *Brucella* (up to 40 days). Blood cultures are positive in 10%-90% of patients and are not helpful for the initial diagnosis of the disease. More rapid detection of the organisms may be performed on BACTEC blood cultures [13].

Standard serological diagnosis using the tube agglutination test confirms the diagnosis when a single titre $\geq$160 is found or a four-fold rise in the antibody titre is noted between the onset of illness and convalescent-phase serum, collected 14-21 days after. ELISA and western blot are also available, but these methods lack standardisation. PCR may demonstrate the presence of the organism in some cases [2].

Treatment

A six-week regimen containing doxycycline plus streptomycin or doxycycline plus rifampicin are effective for most forms of brucellosis [2,14,15] (TABLE IV). Isolation of patients is not necessary. Patients with endocarditis, spondylitis or meningoencephalitis may need to be treated for longer. Endocarditis requires doxycycline plus streptomycin plus rifampicin and may nevertheless require valve replacement. Trimethoprim-sulfamethoxazole and fluoroquinolones also have good results against *Brucella*, but are associated with high relapse rates when used as monotherapy. The combination of ofloxacin plus rifampicin is associated with good results [15].

Even if there is little evidence to support its utility for post-exposure prophylaxis, doxycycline plus rifampicin is recommended for 3 to 6 weeks [14,15].

There is currently no authorised human *Brucella* vaccine in the European Union or the US. There are some limited clinical data on a live, attenuated vaccine-candidate strain from the former Soviet Union and China. Due to the frequency of adverse events and the short duration of immunity, it seems that it is no longer used [16]. Sub-unit vaccine candidates have also been studied but evidence of efficacy is inconclusive. The potential to develop human *Brucella* vaccine is limited by the small market potential, which will probably restrict development efforts to national defence agencies only [16].

In conclusion, brucellosis has been reported as a possibility after a bioterrorist act. The agent is stable and could be transmitted by the airborne route. But the disease is associated with low mortality and has a comparatively limited impact.

References

1. Young E. An overview of human Brucellosis. *Clin Infect Dis* 1995; 21: 282-90
2. Hoover D, Friedlander A. Brucellosis. In Zajtchuk R, ed. Textbook of Military Medicine: Medical Aspects of Chemical and Biological Warfare. Washington, DC: US Department of the Army, Surgeon General, and the Borden Institute 1997: 513-21
3. Fiori PL, Mastrandrea S, Rappelli P, Cappuccinelli P. *Brucella abortus* infection acquired in microbiology laboratories. *J Clin Microbiol* 2000; 38: 2005-6
4. Spink W, Thompson H. Human brucellosis caused by *Brucella abortus* strain 19. *JAMA* 1953; 153: 1162-5
5. Blasco J, Diaz R. *Brucella melitensis* Rev-1 vaccine as a cause of human brucellosis. *Lancet* 1993; 342: 805

6. Suspected Brucellosis case prompts investigation of possible bioterrorism-related activity-New Hampshire and Massachusetts, 1999. *MMWR* 2000; 49: 509-12
7. Mousa A, Elhag K, Khogali M, Marafie A. The nature of human brucellosis in Kuwait: study of 379 cases. *Rev Infect Dis* 1988; 10: 211-7
8. Buchanan T, Faber L, Feldman R. Brucellosis in the United States, 1960-1972: an abattoir-associated disease. *Medicine* (Baltimore) 1974; 53: 403-13
9. Gotuzzo E, Alarcon G, Bocanegra T et al. Articular involvment in human brucellosis: a retrospective analysis of 304 cases. *Semin Arthritis Rheum* 1982; 12: 245-55
10. Mousa A, Muhtaseb S, Almudallal D, Khodeir S, Marafie A. Osteoarticular complications of brucelllosis: a study of 169 cases. *Rev Infect Dis* 1987; 9: 531-43
11. Ariza J, Gudiol F, Valverde J et al. Brucellar spondylitis: a detailed analysis based on current findings. *Rev Infect Dis* 1985; 7: 656-64
12. Franz D, Jahrling P, Friedlander A et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA* 1997; 278: 399-411
13. Rich M, Bannatyne R, Memish Z. Direct urease test on BACTEC blood cultures: early presumptive diagnosis of brucellosis in an area of endemicity. *J Clin Microbiol* 2000; 38: 1706
14. The European Agency for the Evaluation of Medicinal Products/CPMP guidance document on use of medicinal products for treatment and prophylaxis of biological agents that might be used as weapons of bioterrorism. July 2002; www.emea.eu.int
15. www.afssaps.sante.fr
16. Schurig G, Sriranganathan N, Corbel M. Brucellosis vaccines: past, present, future. *Vet Microbiol* 2002; 90: 479-96
17. Commission decision of 19 March 2002. Case definitions for reporting communicable diseases to the Community network under decision N° 2119/98/EC of the European Parliament and the Council. *Official Journal of the European Communities.* OJ L 86, 3.4.2002; 44
18. Amending Decision N°2119/98/EC of the European Parliament and of the Council and Decision 2000/96/EC as regards communicable diseases listed in those decisions and amending decision 2002/253/EC as regards the case definitions for communicable diseases. *Official Journal of the European Union.* OJ L 184, 23.7.2003;35-9

* **BICHAT**, the European Commission's Task Force on Biological and Chemical Agent Threats, has developed this set of guidelines that may be the basis of national authorities' guidance, and may also be used directly by clinicians, general practitioners and specialists when confronted with patients infected by agents that may be due to deliberate release of biological agents. Ref. Bossi P, Van Loock F, Tegnell A, Gouvras G. Bichat clinical guidelines for bioterrorist agents. *Euro Surveill.* 2004; 9(12)

http://www.eurosurveillance.org/em/v09n12/0912-230.asp

Editorial note: *These clinical guidelines were reviewed by the Task Force and by two experts designated by each Member State of the European Union. This review was completed at the end of February 2003. The revised guidelines were submitted to the Health Security Committee which approved them in April 2003 and agreed their publication in a widely disseminated journal so as to allow access to as large an audience as possible. The editorial process of Eurosurveillance also introduced modifications that improved the contents of these guidelines.*

TABLE 1

Summary of clinical and biological description of brucellosis

Clinical picture
Incubation period: from 1 to 60 days Symptoms non-specific similar, regardless of the route of transmission • fever: continuous, intermittent or irregular • weakness, profuse sweating, chills, diffuse or localised arthralgia, malaise, weight loss • neuro-psychiatric complaints (headache, depression, irritability) frequent • gastrointestinal symptoms (anorexia, nausea, vomiting, abdominal pain, diarrhoea or constipation • cough and chest pain are rare
Physical examination
• usually normal • lymphadenopathy, splenomegaly and/or hepatomegaly (10%-30%)
Complications
• osteoarticular (sacroileitis, peripheral joint arthritis, bursitis, osteomyelitis and spondylitis) • epididymo-orchitis • meningitis, encephalitis, seizures • endocarditis, myocarditis, pericarditis and aneurysms (aorta and cerebral vessels)
Diagnosis
• Demonstration of a specific antibody response (>160 or 4-fold rise between acute and convalescent phase) • Demonstration by immunofluorescence of *Brucella sp.* in a clinical specimen • Isolation of *Brucella sp.* from a clinical specimen
Management
• Antibiotics (see Table 4) and supportive care • Spondylitis and endocarditis complications need long treatment courses and possibly surgical treatment • Post-exposure prophylaxis: doxycycline and rifampicin (see Table 4) • No approved human vaccine available

TABLE 2

Case definitions of suspected or confirmed cases of brucellosis

Possible case
• **NA**
Probable case
• A case that is epidemiologically linked to a confirmed case or • A case that has supportive serology (isolated high titre)
Confirmed case

• A clinically compatible illness that is laboratory confirmed
  • isolation of *Brucella sp.*
  • ≥4-fold rise in *Brucella* agglutination titre between acute and convalescent phase

NA: Not applicable

Source: [17,18]

TABLE 3

Case definitions of brucellosis due to deliberate release

Suspected deliberate release
o Large-scale outbreak of brucellosis, especially in areas where *Brucella* is not endemic o Clusters of cases in unusual settings, such as: • where *Brucella* is not endemic • without a travel history to endemic areas • without relevant epidemiological history (food consumption, sexual contact, etc) • without a history of occupational or laboratory exposure • where the cases are linked in time and place, especially *geographically related groups* of illness following a wind direction pattern

TABLE 4

Recommendations for treatment and post exposure prophylaxis of brucellosis

		Treatment of suspected or confirmed clinical cases (6 weeks)	Post-exposure prophylaxis (3-6 weeks)
Adults **Pregnant women** It is recommended, when possible, to stop breastfeeding.	**First line treatment**	- Doxycycline: 100 mg IV bid followed by 100 mg bid per os **and** - Rifampicin: 10-15 mg/kg/day in 1 or 2 doses, followed by 600-900 mg per os once daily **or** - Gentamicin: 3-5 mg/kg IV once daily or 1.5-2.5 mg/kg twice daily (max 2 weeks) **or** - Streptomycin: 1 g IM once or twice daily (max 2 weeks)	- Doxycycline: 100 mg bid per os **and** - Rifampicin: 600-900 mg per os once daily
	Second line treatment or first line treatment as an alternative for pregnant women	- Trimethoprim (6-8 mg/kg/day) + sulfamethoxazole (40 mg/kg/day) IV in 1 or 2 divided doses followed by Trimethoprim (6-8 mg/kg/day) + sulfamethoxazole (40 mg/kg/day) per os in 1 or 2 divided doses **and** - Rifampicin: 10-15 mg/kg/day in 1 or 2 doses, followed by 600-900 mg per os once daily	- Not recommended
Children	**First line treatment** **In children > 8 years**	- Doxycycline: . > 45 kg: idem adult . < 45 kg: 2.2 mg/kg IV twice daily followed by 2.2 mg/kg per os twice daily **and** - Rifampicin: 10-15 mg/kg/day in 1 or 2 doses, followed by 10-15 mg/kg per os in 1 or 2 doses daily **or** - Gentamicin: 1-2.5 mg/kg IV 3 times daily (max 2 weeks) **or** - Streptomycin: 15 mg/kg IM once or twice daily (max 2 g/d and max 2 weeks)	- Doxycycline: . > 45 kg: idem adult . < 45 kg: 2.2 mg/kg per os twice daily **and** - Rifampicin: 10-15 mg/kg per os in 1 or 2 doses daily
	First line treatment **In children < 8 years**	- Trimethoprim (6-8 mg/kg/day) + sulfamethoxazole (30-40 mg/kg/day) IV in 2 divided doses followed by Trimethoprim (6-8 mg/kg/day) + sulfamethoxazole (30-40 mg/kg/day) per os in 1 or 2 divided doses **and** - Rifampicin: 10-15 mg/kg/day in 1 or 2 doses, followed by 10-15 mg/kg per os in 1 or 2 doses daily	- Trimethoprim (6-8 mg/kg/day) + sulfamethoxazole (30-40 mg/kg/day) per os in 1 or 2 divided doses **and** - Rifampicin: 10-15 mg/kg per os in 1 or 2 doses daily

Source: [14]