take the contraceptive pill. These relatives have at least one family member with venous thrombosis who carries the mutation. Should we offer them screening? Perhaps they should first be told about the benefits and risks of knowing and asked about the acceptability of other forms of contraception. The advice to be screened might be firmer if other first degree family members are found to have had deep vein thrombosis at young ages or without clear risk factors (which would suddenly make the family into one with a tendency to multiple thrombosis), or if the index patient is a homozygote or carries combined thrombogenic defects. The clinical situation of the patient and the other family members will continue to direct the doctor's advice, rather than the mere presence or absence of a mutation.

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Grand Rounds—Hammersmith Hospital

A case of laboratory acquired brucellosis

A rare condition, but laboratory transmission is a risk

Series edited by:
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Brucellosis is now rare in Britain, and most cases are imported or contracted in a laboratory setting. We present the case of a veterinary scientist who contracted the condition while working on the products of conception from animals.

Case history

A 24 year old man who was studying immunology at a postgraduate medical school presented to this hospital's casualty department in July 1995 with a 17 day history of high fevers, night sweats, dry cough, and myalgia. He also complained of pain and discharge from a lower left molar tooth. He had no medical history and was taking no regular drug treatment. A travel and occupational history showed that he had lived in Himachal Pradesh in northern India until March 1995, when he moved to London. He had previously qualified as a veterinary surgeon, before studying for a microbiology degree between 1993 and 1995. During this time he had performed regular experiments on the products of conception from cattle and sheep to investigate possible infectious causes of abortion.

On examination at admission the patient had a fever of 38.5°C, a solitary cervical lymph node measuring 1 cm × 0.5 cm in size, a resting tachycardia of 110 beats per minute, and a palpable splenic tip. Examination of his mouth showed pus discharging from the lower left third molar tooth.

Initial investigations showed raised inflammatory markers (erythrocyte sedimentation rate 34 mm in the first hour and C reactive protein concentration of 84 U/l) and white cell count of 4.8 × 10^9/l. Three blood films for malaria parasites were negative. Standard liver function tests yielded abnormal results (with a raised serum alkaline phosphatase concentration of 405 U/l, a raised γ-glutamyltransferase concentration of 410 U/l, and a raised serum aspartate aminotransferase concentration of 248 U/l); the serum bilirubin concentration was at the upper end of the normal range, at 17 μmol/l.

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The serum angiotensin converting enzyme concentration was raised, at 113 U/l.

Serological tests for *Legionella* spp, *Chlamydia* spp, *Mycoplasma* spp, *Coxiella* spp, and hepatitis A, B, C, and E suggested no active or previous infection. There was no growth from cultures of urine, stool, and tooth pus. A Paul Bunnell test was negative and a Heaf test was grade 1 when read after 48 hours. Chest radiography was unremarkable, while abdominal ultrasonography confirmed the clinical findings of splenomegaly.

Table 1 shows the results of serological tests for brucella, and a Gram negative coccobacillus was grown from blood cultures on serum dextrose agar. Subsequent dye testing confirmed that the organism was *Brucella melitensis* biotype 3 (table 2).

Brucellosis, complicated by a dental abscess, was diagnosed. The affected tooth was extracted, and the patient was initially treated with rifampicin and doxycycline. Subsequent sensitivity testing showed resistance to rifampicin. His antibiotic treatment was therefore changed to co-trimoxazole and doxycycline so that he could complete a six week course of treatment. Medical follow up at four weeks showed that the patient had no fever, his IgM titres had fallen, inflammatory markers were lower, and standard biochemical liver function tests yielded normal results.

Table 1—*Brucella serological testing*

Test	Titre
Direct agglutination:	
B abortus	1:20
B melitensis	<1:20
ELISA IgG:	
B abortus	1:640
B melitensis	1:80
ELISA IgM:	
B abortus	1:320
B melitensis	1:160

Comment

Brucellosis is a zoonosis, a disease of both wild and domesticated animals that is transmissible to humans. *Brucella* spp are small, Gram negative coccobacilli. Six species exist, of which four cause disease in man: *B abortus*, *B melitensis*, *B suis*, and *B canis*, with the animal reservoirs being cattle, sheep and goats, pigs, and dogs respectively.[1]

Infection may result from inhalation or direct inoculation of infected material or ingestion of unpasteurised milk; incubation is between two and eight weeks. Symptoms include fever (which may become undulant if left untreated), malaise, anorexia, headache, and backache. Examination frequently shows nothing abnormal, apart from fever, but lymphadenopathy, splenomegaly, or hepatomegaly are found in some cases. Complications of brucellosis can affect any organ, and, apart from abscess formation, can occur anywhere in the body. Complications include osteomyelitis, lymphocytic meningitis, pneumonia, and lung abscesses. The main cause of mortality, however, is endocarditis.

The bacteria enter lymphatic vessels and replicate in regional lymph nodes before haematogenous dissemination to the reticuloendothelial system. As a facultative intracellular pathogen, the organism can multiply in phagocytic cells. This ability is aided by the production of adenine and guanosine monophosphate, which suppress the myeloperoxidase-hydrogen peroxide-halide system of neutrophils, factors which inhibit phagosome-lysosome fusion in macrophages and the production of superoxide dismutase. An effective immune response depends on cell mediated immunity—committed T cells secreting cytokines that activate the bactericidal mechanisms of macrophages and attract inflammatory cells leading to granuloma formation.[2] *Brucella* spp have interesting interactions with cytokines derived from macrophages that may ultimately lead to the development of novel therapeutic approaches.[3] Phagocytosis of many intracellular pathogens—for example, *Mycobacterium tuberculosis*—by macrophages leads to transcription activation of the gene for tumour necrosis factor and to secretion of this factor, which initiates a complex cascade of host defence mechanisms. *Brucella* spp actively inhibit such secretions from human monocytic cells, and this may be a critical mechanism, which allows infection to become established.[4] The regulation of the cytokine cascade in brucellosis is complex, with a mixed T helper 1 and 2 phenotype being documented in animal studies.[5] The pathogen may be able to influence development of the T helper 1 phenotype that is associated with elimination of intracellular organisms.

Table 2—*Results of dye testing*

Controls	Thionin	Fuchsin	Methyl violet	Pyronin
B abortus	–	+++	+++	+++
B melitensis	+++	+++	+++	+++
B suis	+++	–	–	–
Test strain	+++	+++	+++	+++

Diagnosis depends on a keen awareness of possible infection and a thorough occupational and travel history. Bacterial culture may take more than 30 days, but serological testing with the serum agglutination test or enzyme linked immunosorbent assay (ELISA) can lead to a more rapid diagnosis.[6] If the organism can be isolated then species identification requires testing of biochemical reactions. Tests based on polymerase chain reaction have been developed but do not yet have a diagnostic role.

In 1986 the World Health Organisation recommended six weeks of treatment with doxycycline and rifampicin for uncomplicated infection; subsequent trials established that other regimens including streptomycin and doxycycline were at least as effective.[7] Single agent treatment leads to a relapse rate of 5-40%, which is thought to be due to inadequate bacterial killing rather than the development of resistance.

In animals brucellosis is a chronic infection resulting in abortion and sterility. A large bacterial load is present in milk, urine, and the products of pregnancy. Humans contract infection via direct contact with infected animals, their carcasses, or unpasteurised milk. Not surprisingly, high risk occupations include animal husbandry, veterinary science, abattoir work, meat inspection, and laboratory science. Human to human transmission is extremely rare. In the United Kingdom the Central Veterinary Laboratory coordinates spot checks of milk and serological testing of all cattle every two years and before importation. About 20 cases a year are reported to the Centre for Disease Surveillance and Control in Colindale, north London, of which most are due to imported *B melitensis* or laboratory accidents.[8] Laboratory acquired disease, as in this case, can be prevented by strict adherence to the precautions listed for biosafety level 3.

Discussion

JMBH: There are several resemblances between brucellosis and tuberculosis, although transmission in brucellosis is always from animals to humans. The granulomatous nature of the disease explains the high serum angiotensin converting enzyme concentration. There are clear lessons here for people working with infected material.

JS: Are you sure that the laboratory was the route of transmission in this case?

JMBH: The patient listed all the organisms he had been exposed to in his laboratory work, and this accelerated the diagnosis quite considerably.

JS: Is consumption of unpasteurised milk the usual route of transmission in the part of India where he had lived (Himachal Pradesh)?

PA: No, only nomads drink unpasteurised milk in that part of the world.

WAL: A recent patient of ours presented with acute orchitis and fever, and biopsy of the testis showed granulomas. He had not travelled abroad for six years; inquiry then showed that his mother had been sending him unpasteurised goats' milk from Portugal. Recently

his wife had been diagnosed as having the chronic fatigue syndrome, and his daughter had had an illness resembling glandular fever, and both these conditions were proved serologically to be brucellosis. Clearly, the infection can be imported into the country in more than one way.

JMBH: Low gastric acidity is important in killing *Brucella* spp and protects from infection from unpasteurised milk products. People who consume these foods should be advised to avoid acid reducing drugs.

JC: Although there are some biochemical similarities between the cell wall lipopolysaccharide of the *Brucella* species and some other Gram negative organisms—for example, *Escherichia coli*—the disease process is very different. Despite the clinical similarities with mycobacterial infections, little is known about the pathogenic mechanisms of *Brucella* spp. As there is clearly a population at risk who could be targeted for vaccination, why has more not been done to develop a vaccine?

PA: A live attenuated vaccine is used only in animals in Britain and United States. However, it is used in high risk groups such as veterinary surgeons in some countries with high levels of infection. This vaccine can cause clinical disease in a proportion of those exposed, so interest in the development of a subunit vaccine has increased. Cell wall constituents have been investigated, but so far the cytokine response and resistance to challenge with live organisms has been disappointing.[9]

JSF: Although the details of cytokine responses in *Brucella* species remain to be elucidated, the potential role of cytokines as treatment has been investigated in animal models. Pretreatment with interleukin 1, a cytokine with many functional similarities to the tumour necrosis factor, depressed the growth of *B abortus* in a murine model. However, this effect was seen only if the cytokine was administered before infection, and interleukin 1 was not of benefit in chronically infected animals.[10] Treatment with interferon gamma, which has been reported to be of benefit in the treatment of patients with other intracellular infections, may be a more promising approach in brucellosis, but more data are needed.[11] In addition to biological treatments, new drugs such as azithromycin that penetrate well into the macrophages may prove to have a useful role in decreasing both the length of treatment schedules and the frequency of relapse of infection.

CP: The patient was serologically positive for two *Brucella* species, but you eventually identified only one. Is this a serological cross reaction?

TR: Yes, there is marked cross reactivity, particularly between *B abortus* and *B melitensis*, and it is often not possible to decide on the infecting species on serological testing alone.

JC: It also cross reacts with other organisms such as in tularaemia, so it is not that species specific.

SRB: If you have been infected with one species are you immune to subsequent infection with another?

TR: Immunity is not complete, and reinfection does occur after natural infection. There are appreciable laboratory hazards to people working in microbiology, and many of the cases that arise in this country in the indigenous population are acquired in the laboratory. This is a practical problem for the microbiology laboratory because we receive many specimens labelled as PUO [fever of unknown origin], in which the diagnosis is far from apparent in the initial stages of the illness.

JS: Do you not treat everything labelled as PUO as if it were potentially dangerous?

TR: There would be practical problems if we were to treat everything as if it were brucellosis, as we see perhaps only one case every couple of years. Of course, that one case is potentially very serious with regard to laboratory transmission. We naturally take precautions with all potentially infective samples.

The *BMJ* welcomes grand rounds from other hospitals.

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ANY QUESTIONS

Naval gunners were, apparently, issued with asbestos lined masks to protect them from any blow back. Is mesothelioma a recognised occupational hazard of naval gunnery?

Antiflash clothing including hoods or masks has been issued to naval gunners over many years and did contain asbestos. The majority of asbestos textiles were manufactured using chrysotile asbestos and this is the type of asbestos likely to have been used. Chrysotile asbestos is sometimes contaminated with amphibole fibres, and occasionally crocidolite has been used in the manufacture of asbestos textiles although there are no references to its use in flame proof or flash proof clothing. [1]

Generally speaking, while these textiles containing asbestos remain in good condition the release of fibres will be minimal although as they become worn small numbers of fibres may be released.[2 3] The aluminium coating on more modern fire fighting clothing in fact seems to prevent the release of asbestos completely as well as improving the fire retardant properties of the cloth.

Despite this evidence of a potential risk of mesothelioma for naval gunners there do not seem to have been any documented cases of mesothelioma attributed to this antiflash clothing. Cases of mesothelioma among shipyard workers, stevedores handling asbestos, and among merchant seamen, some with no readily identifiable exposure to asbestos, have been reported.[4]—JEREMY BEACH *is an occupational physician at the Institute of Occupational Health in Birmingham*

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