# e2 Brucella

A 30-year-old male returning from Cyprus made a virtual appointment with his primary healthcare provider (PHCP) complaining he had a temperature, night sweats, and backache with pain in his hips and he had been recording his temperature. The PHCP suggested he attend his clinic where he was eventually examined, and hepato-splenomegaly was noted. The patient was referred to hospital for further investigations of a pyrexia of unknown origin (PUO).

On admission, a chest and abdominal X-ray was taken with a series of blood tests: Full blood count, U&Es, LFTs and a T-spot. Chest X-ray was normal, the T spot was negative, the hematology results indicated an anemia with neutropenia and thrombocytopenia; biochemical results indicated slightly elevated transaminases and normal U&Es. The temperature chart he recorded while waiting for his PHCP appointment is shown in **Figure e2.1**. A blood culture yielded *Brucella*.

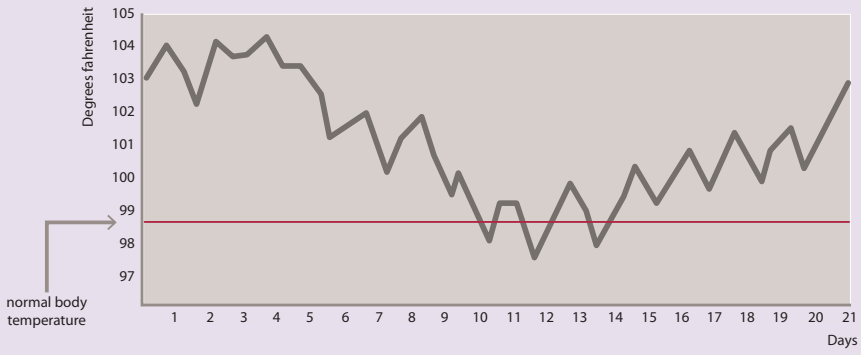


**Figure e2.1** The temperature chart of this patient demonstrating "undulant" fever.

## 1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

### CAUSATIVE AGENT

*Brucella* belongs to the Brucellaceae, along with *Mycoplasma* and *Ochrobacterium*, within the Family Rhizobiales. This Family includes soil organisms important in plant physiology and it lies within the alphaproteobacteria, which also includes *Rickettsia*, *Bartonella*, and *Erlichia*. *Brucella* has a similar lifestyle to these three Genera although, for *Brucella*, it is a **facultative intracellular lifestyle** not an obligate one. Original studies identified the genus *Brucella* as genetically homogeneous but phenotypically diverse. Several of the *Brucella* have two genomes, the result of either acquisition of a large plasmid or scission of a single genome. There is little genetic diversity between the various species but the diversity that does exist is mainly caused by recombination events between regions of VNTR (variable number of tandem repeats). A zero order Markov chain model divides the *Brucellae* into six groups: Groups 1, 3, and 5 include *B. melitensis*, *B. abortus*, *B. ovis*, *B. suis*, *B. canis*, and *B. neotomae*; Groups 2 and 4 include the more recent isolates from marine mammals; Group 6 includes other Genera, that is *Agrobacterium* and *Ochrobactrum*.

*B. abortus*, *B. canis*, *B. melitensis*, *B. neotomae*, *B. ovis*, and *B. suis* were originally isolated from animals. *B. abortus* and *B. melitensis* are found in a wide variety of animals (mainly cattle, sheep, goats, pigs (uncommon for *B. melitensis*), dogs, and camels (uncommon for *B. abortus*); *B. suis* is associated mainly with pigs, *B. canis* is found in dogs, *B. ovis* is found in sheep, and *B. neotomae* in rodents. All of the above (with the exception of *B. neotomae*) are zoonoses. *B. suis* has five biovars: biovars 1–3 are found in pigs whereas biovar 2 is also found in hares and biovar 4 in reindeer. Biovar 5 has not been linked with disease.

The more recently recognized *Brucella* spp. are *B. ceti* (porpoise, dolphins), *B. pinnipedalis* (seals), *B. microti* (rodents foxes), and *B. inopinata* isolated from a human case of breast abscess.

The closest relatives to *Brucella* are plant pathogens *Ochrobacter* and *Agrobacterium*. Other additional species have been isolated as yet without species names.

*Brucella* are gram-negative cocco-bacilli with a similar cell wall structure to other gram-negative bacteria. They are strict aerobes, non-motile, and do not produce spores. Typical colonies appear as small (~1 mm) moist, circular, convex, and translucent that become opaque, dry, and yellowish with prolongued culture. The GC content is 55–58 and DNA analysis suggests a single species but, because of the clinical utility of the difference in epidemiology and virulence, the separation into different species has been retained. They are oxidase positive (except *B. ovis* and *B. neotome*) and urease positive (except *B. ovis*).

Both *B. melitensis* and *B. suis* have been developed as bio-weapons owing to their low infectious dose (10–100 organisms) and environmental stability. Attacks could be targeted at both humans or at animals, in order to destroy a country's food sources.

## ENTRY INTO THE BODY

Transmission occurs to humans from close contact with animals, for example, vets dealing with animal obstetric problems or, more generally, from consumption of contaminated unpasteurized food: milk or cheese.

Laboratory acquired infection can also occur handling or processing infected specimens.

Entry into the body is via the mouth, usually by eating contaminated food, or by the conjunctiva, abraded skin or respiratory tract when in a laboratory or when dealing with an aborted animal.

It is not clear how *Brucella* penetrates the mucosal barrier, but it is likely that it enters the body (in the intestinal tract) via M cells in Peyer's patches by binding to the prion protein $PrP^C$ via *Brucella*'s Hsp60 protein. It is able to replicate within the epithelial layer but the mechanism of release into the lamina propria is not known.

## SPREAD WITHIN THE BODY

Once in the lamina propria, the organism moves to the regional lymph nodes or, if in the blood, to the spleen. It is taken up by the process of phagocytosis into polymorphs, macrophages, and dendritic cells and circulated round the body by the macrophages, lodging in various solid organs.

## PERSON-TO-PERSON SPREAD

This is rare but can occur via breast milk from an infected mother, by sexual transmission, or from human organ donation or blood products.

## EPIDEMIOLOGY

*Brucella* is a zoonosis with global spread. It is an occupational hazard for persons who work closely with animals: vets, abattoir workers, butchers, and farmers but also laboratory personnel handling human and veterinary specimens, as it is highly communicable. The most frequent *Brucella* species causing disease in humans is *B. melitensis* but the most widespread organism causing disease (humans and animals) is *B. abortus*.

*B. abortus* survives poorly in environmental water but can survive for weeks in wet soil or tap water and for months in animal slurry. *B. melitensis* survives for weeks on alkaline broth but less than one day in acid broth. In milk, it can survive for a day but in soft cheese for three days.

Globally, there are over half a million human cases annually. High incident rates occur in Kenya (203/100 000), Yemen (90/100 000), Greece (43/100 000), and Eritrea (21.8/100 000) (2020 statistics). In Europe, the incidence is 0.03/100 000 and in the US, the incidence is 0.02–0.09 /100 000 (2012 statistics). In the UK in 2015, there were 12 cases reported, all of whom were infected abroad. There were no cases of brucellosis in animals.

# 2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

## INNATE IMMUNITY

The innate immune response is involved in recognition, killing of foreign organisms, and activation of the complement system and the adaptive immune response. These functions are achieved by natural killer (NK) cells (killing), phagocytosis (killing and stimulation of the adaptive response) by neutrophils, macrophages, and dendritic cells, and secretion of cytokines which are the soluble signals orchestrating these functions. Both NK cells and neutrophils do not seem to be important for clearing *Brucella* as absence of the cells do not effect eradication of the organism. For neutrophils, killing of the organisms is more effective in their absence and, if present, activation of the neutrophils is linked to local pathology.

Initial recognition of pathogen associated molecular patterns (PAMPs) on *Brucella* is by Toll-like receptors (TLRs) exposed on cell surfaces. Both the Lipopolysaccharide (LPS) and a non-lipidated outer membrane protein (OMP16) bind to TLR4 and activate the TRIF-IRF pathway to stimulate the production of IFNγ. This cytokine acts in an autocrine fashion to stimulate the JAK-STAT1 pathway leading to production of IFN-activated proteins. *Brucella* DNA further activates production of IFN-γ by an unknown pathway. None of these pathways lead to clearing of the *Brucella* species. Lipidated Omp16 and 19 activate TLR2 through the IRAK-MAPK pathway leading to activation of NFκB and the production of pro-inflammatory cytokines. This pathway is relevant

to eradication of the organism. Cytosolic receptors NOD1 and NOD2 also detect the presence of *Brucella* and lead to activation of NFκB, although this pathway does not seem to be significant for eradication of *Brucella*. The net result is that IL-1, IL-6, IFNγ, and TNFα are produced but the innate response does not eradicate the organism although it may limit the number of bacteria and induce the nonspecific symptomatology.

The key cell in eventually controlling *Brucella* in humans is the macrophage. In the initial stage of infection, the organism can replicate in the macrophage and the presence of the organism induces a Th-1 response although later in infection a Th-2 response develops inhibiting the host's response to the organism.

After the development of the adaptive response, the macrophages then successfully eradicate the organism. The ability to kill the organisms within the macrophage in this stage of infection is due to activation of the macrophage by the cytokines IL-12, IFN-γ, and TNF-α. TNF-α secretion is increased by phagocytosis of opsonized bacteria, however, in the early stages of infection with *Brucella*, the organism inhibits the release of TNF-α as part of the anti-inflammatory response.

## ADAPTIVE IMMUNITY

The main link between the innate and adaptive response is the dendritic cells. *Brucella* is taken up into antigen presenting cells inducing cytokine production, particularly IL-2 and IL-12, with the result that clonal expansion of CD4 and CD8 cytotoxic T cells occurs. Antigen presentation to CD8 cells and B cells occurs leading to CD8 cytotoxic T cells and antibody producing B cells. A wide variety of antibodies directed against *Brucella* antigens are produced. In one study, antibodies against 34 different antigens covering OMPs, T4SS, cytosolic proteins and, as yet, uncharacterized proteins were detected from patients who were culture or Rose Bengal positive compared to naïve patients. A further, smaller set of antigens that were cross reactive, as they were also identified in naïve patients, were also detected together with a different smaller set of antigens present in culture-positive patients but not in the culture-negative /Rose Bengal positive patients.

Despite this large set of antigens from different structural and functional proteins found in *Brucella*, neither the cytotoxic nor the antibody response were sufficient enough to eradicate the organism.

In humans and mice, *Brucella* affects TLR2 signaling and down-regulates the maturation of the dendritic cells. Rough strains of *Brucella* have more OMPs in the cell wall and stimulate dendritic cell maturation more effectively leading to TNF-α and IL-12 secretion with high CD4 T-cell activation compared to smooth isolates. Activation by rough strains involves caspase-2 and TLR6. Key host proteins involved in recognition of Brucella are TLR2, TLR4, TLR6, and TLR9. Clearance of *Brucella* is associated with **MyD88** and IL-12, TNF-α, and IFN-γ.

The adaptive response is key for the clearance of *Brucella* from an infected animal or human. Relevant cells involved are B cells producing antibody and CD4, CD8 T cells producing IFN-γ and a Th-1 pro-inflammatory response. A subset of CD4 cells are memory cells necessary for activation if the individual is exposed to the same infection again, and the CD8 cells destroy *Brucella* infected cells. *Brucella* antigens bind to MHC1 and II thereby resulting in stimulation of CD8 and CD4 T cells. The key cytokine produced by the CD4 and CD8 cells is IFN-γ. Both CD4 and CD8 cells co-operate in the eradication of *Brucella* principally due to cell activation by IFN-γ and cytotoxicity of infected cells.

Humoral B-cell immunity against *Brucella* is less important than cytotoxic T-cell immunity. Antibodies are produced to *Brucella* antigens, mainly the LPS, either by direct activation of B cells or by binding to MHC II on B cells and thus involving T cells. Antibodies are also produced to Omp and polysaccharide antigens on the *Brucella* cell wall. Rather than aiding the eradication of *Brucella*, the humoral response may provide a safe haven inside B cells ensuring an established chronic infection.

## PATHOGENESIS

*Brucella* produces a number of chemicals that may aid the organism establishing itself in the host: urease may have some use during entry into the gastrointestinal (GI) tract via the acid stomach; cholyglycine hydrolase mitigates the effect of bile salts by inhibiting bacterial lysis; non-lipidated OMP19 inhibits proteases found in the respiratory tract increasing the chance of infection.

The organism has several adhesions, for example, SP29 (sialic acid binding), proteins with Ig-like domains, auto-transporters (BtaE) binding to the extracellular matrix and cellular ligands on epithelial cells and professional phagocytic cells (macrophages, dendritic cells, and polymorphs).

The LPS of *Brucella* has poor endotoxin activity and does not activate complement or macrophages. The two main differences compared to *E. coli* LPS are the long acyl chains (C19-29, cv *E. coli* C12–14) and the acyl chains being linked to the core by amide bonds. There is poor recognition by the host TLR2/4/5 and, consequently, *Brucella* does not activate macrophages or dendritic cells effectively. The organism remains in stasis owing to a metabolic block on replication caused by excess guanosine penta-phosphate. This phase persists until the organism gains entry to the macrophages where it replicates.

During infection, *Brucella* modulates the innate and the adaptive immune response of the host by regulation of the host Th-1 and Th-2 immune responses. *Brucella* inhibits the expression of PAMPs and decreases TLR responses by secreting proteins with TLR-like sequences. *Brucella* also stimulates a Th-1 response initially, by down-regulating the Th-2 response but, later in infection, *Brucella* can also down-regulate the Th-1 response by up-regulation of leukotriene B4 decreasing IFN-γ and IL-12 and increasing Th-2 responses.

Recognition of an organism by the rapid onset, broad-spectrum innate immune system is the first line of defense against the intruder. The system is activated by foreign antigens located on the organism and leads to a series of signaling cascades ending in activation of NFκB, the production of transcription factors and the release of pro-inflammatory cytokines necessary for an ongoing response against the organism. *Brucella* species, however, manage to avoid stimulating the immune system to a large degree. LPS generally is a key PAMP but the structure of the Lipid A component of LPS is not recognized by the immune system. Additionally, the structure of the polysaccharide of LPS inhibits complement activation and *Brucella* can also inhibit the maturation of dendritic cells with reduction of MHC Class II, the net result being no pro-inflammatory response. The second approach used by *Brucella* once it enters phagocytic cells is to inhibit the fusion of the phagosome with the lysosome and to replicate inside a *Brucella*-containing vacuole (BCV) which is related to the expression of Sar1 (a small GTPase) facilitating replication. A third stealth approach *Brucella* uses is to induce apoptosis in antigen-presenting cells thereby reducing the immune response, although in macrophages, the opposite occurs and apoptosis is inhibited maintaining its intracellular location (**Table e2.1**). *Brucella* also eliminates neutrophils and modulates the PPARg pathway leading to activation of M2 macrophages rather than the M1 macrophages inimical to *Brucella*. Also, *Brucella* effects the reduction of CD64 and thus a reduction in antigen processing and killing by the cell. This stage is achieved by production of a T4SS by *Brucella*, releasing inhibitory factors into the host cell cytosol preventing fusion and aiding the formation of the BCV.

The avoidance of the host immune system allows the organism to replicate in macrophages and disseminate throughout the body inducing a nonspecific illness presenting as a PUO. The actual method of disease inducing pathology is not clear, but replication and cell death in various body locations lead to the presentation of illnesses such as infective endocarditis, meningitis, and osteomyelitis. The host response is the formation of granuloma thereby limiting the local spread of the organism.

## 3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

The main symptoms of brucellosis are general and nonspecific, similar to a mild upper respiratory tract infection: fever, sweating, a feeling of tiredness with myalgia and arthralgia (typically lumbar and pelvic pain). This may be accompanied by a relative bradycardia and lymphocytosis. The spleen may be enlarged. Often, the primary diagnosis is of a PUO. As the illness progresses (in the absence of treatment), the temperature regresses and recurs giving an undulant pattern (**Figure e2.1**) (the disease was previously called Undulant Fever or Malta Fever).

**Table e2.1** Methods of stealth by *Brucella*

| |
|---|
| Lack of stimulation of TLRs |
| Metabolic block on replication during entry |
| Inhibition of expression of PAMPs |
| Down-regulation of Th-1 response |
| Inhibition of fusion of phagosome-lysosome |
| Replication in a BCV |
| Induction of apoptosis in APCs |

*B. melitensis* is the most virulent in humans with *B. abortus* causing mild infection and *B. suis* intermediate in virulence.

Additional symptoms referable to other organ systems can occur:

- neurology: meningitis, meningoencephalitis;
- orthopedics: arthritis sacroiliitis, back pain;
- cardiology: endocarditis, myocarditis;
- gastrointestinal: hepatitis, abscess;
- ophthalmology: uveitis;
- renal: epididimoorchitis;
- dermatology: erythema nodosum-like lesions.

In animals, abortion is a common problem, but not in humans.

## 4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

Because of the nonspecific nature of the presentation, a key factor in diagnosing brucellosis is taking an adequate history to elicit occupation, contact with animals, and consumption of risk-foods such as unpasteurized cheese or milk.

Laboratory diagnosis relies on culture, serology, and polymerase chain reaction (PCR). Specimens used for culture are usually blood, bone marrow, joint aspirate, CSF or a biopsy specimen. Several culture techniques exist to increase the rate of isolation and reduce the time to growth detection. *Brucella*, grown in a monophasic culture bottle, can take up to 6 weeks to become positive. Ruiz-Castaneda developed a biphasic culture method (RC) incorporating an agar slope and broth avoiding the need to subculture. Alternative methods are to allow the blood to clot and remove the serum by centrifugation, or to induce lysis of blood cells (as the *Brucella* organisms are facultative intracellular bacteria) followed by centrifugation to collect the bacteria. Comparison of culture techniques demonstrated isolation rates of 34%, 43%, and 24% for direct culture (RC), lysis centrifugation (LC), and clot culture (CC) respectively. Sensitivity of culture depends on the phase of the infection, either acute or chronic. In acute disease, the isolation percentage (and days to detection) of RC was 71% (7) compared to LC 90% (2) but for chronic disease the values were 3% (7) and 74% (3). Detection of *Brucella* is possible by current automated machines, but time of detection

can be worse than manual methods as *Brucella* produces little $CO_2$ which is the basis for growth detection in automated machines.

Molecular tests are also available for the detection of *Brucella* species such as probe-based real-time loop-mediated isothermal PCR. However, 16S PCR can be limited because of the close sequence homology of *Brucella* to *Ochrobacterium*.

There are a number of test formats for the detection of antibodies to *Brucella* spp. including competitive enzyme-linked immunosorbent assay (ELISA), complement fixation, serum agglutination, Coombs test, immunoprecipitation (*Brucella* proteins), lateral flow assay, and Rose Bengal assay. The problem with serologic assays for *Brucella* spp. is that not all species have the common antigen used for detection. First, *B. suis* does not express the smooth colony variant lipopolysaccharide (S-LPS) on the surface of this species. Second, there is a cross reaction with LPS from *Yersinia enterocolitica* O:9. Assays based on *Brucella* proteins do not have this cause of false positive reactions. A third problem is the presence of incomplete blocking antibodies giving false negative results. The use of *Brucella* Coombs Gel Test and *Brucella* CAPT (immune capture agglutination) mitigates this source of error. Many of these assays are complex and expensive and a study of the value of the Rose Bengal assay compared to the above assays demonstrated that none of the assays were 100% accurate but that the Rose Bengal assay was the most accurate of any of the other tests.

Detection of positive cases relies on a two-stage assay with a screening test, followed by a confirmatory assay or, alternatively, an acute test followed by a second test 2–4 weeks later, and determining a four-fold rise in antibody titer. A single titer of 1/160 suggests infection by *Brucella*.

*Brucella* is highly contagious, and specimens should be handled in Category 3 facilities. Infection of laboratory staff has been reported when correct procedures are not carried out.

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis for brucellosis is wide because of the nonspecific type of presentation and includes the following:

- neoplasia;
- mycobacterial disease;
- systemic fungal disease;
- rheumatic disease;
- collagen disease;
- urinary tract infections;
- infective endocarditis;
- leptospirosis;
- typhoid.

# 5. HOW IS THE DISEASE MANAGED AND PREVENTED?

## MANAGEMENT

The first-line antibiotic combination to treat brucellosis is doxycycline (200 mg stat followed by 100 mg OD for six weeks) and streptomycin (1 g IM for 3 weeks). Gentamicin can be used instead of streptomycin. An alternative combination is doxycycline plus rifampicin (900 mg OD PO) which is more convenient but has a higher relapse rate. Cotrimoxazole is also used as a first-line agent. More than one agent should be used as relapse is high although relapses are not generally due to antibiotic resistance. Treatment should be for 3–6 weeks depending on the drug regimen.

Second-line agents that can be used are chloramphenicol, fluoroquinolones, and tigecycline. Different regimens are used in special patient groups:

***Children***: rifampicin + cotrimoxazole. Doxycycline is less likely to cause tooth staining and could also be used.

***Pregnancy***: combinations of rifampicin/cotromixazole (not in first trimester) /gentamicin.

***Neurological***: doxycycline/cotrimoxazole/rifampicin.

***Cardiology-infective endocarditis***: gentamicin/rifampicin/doxycycline/cotrimoxazole for 4 weeks followed by rifampicin/doxycycline/cotrimoxazole for 10 weeks.

## PREVENTION

A "One Health" approach to *Brucella* infections is to control the disease in animals and avoidance of high-risk foods. *Brucella* is a highly contagious organism and when dealing with a patient, Personal Protective Equipment (PPE) should be used (mask, gloves, eye protection). Clinical specimens should be handled in the safety cabinet in a Category 3 Laboratory. Animal vaccines exist which are attenuated strains of *B. abortus* (S19 and RB51) but are not useful for humans. Both vaccine S19 and RB51 induces a Th-1 response with increased IFN-γ, TNF-α and antigen-activated CD4 and CD8 cells. RB51 vaccine also produces Th-17 cells with cytokines which are believed to act synergistically with Th-1 cytokines to provide protection. A human vaccine does not exist.

## SUMMARY

### 1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- A gram-negative cocco-bacillus in the alphaproteobacteria and is a facultative intracellular pathogen.
- Found in a wide variety of animals (cattle, sheep, goats, pigs, dogs, elk, and rodents etc.).
- Causes human and animal disease.
- *B. abortus*, *B. melitensis* and *B. suis* are the common ones causing human disease.
- Acquired from close contact with animals (Zoonosis) or consumption of animal products (e.g. cheese).
- Person-to-person spread is rare, e.g. breast milk from an infected mother.

### 2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- Binding to TLR2, TLR4, TLR9, NOD1, and NOD2 leading to NFκB activation in phagocytic cells.
- Development of a wide range of IgG antibodies to many *Brucella* proteins.
- Inability of immune response to eradicate the organism.
- *Brucella* has several mechanisms to avoid the immune response.
- The host responds by limiting its spread by forming a granuloma.

### 3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- Nonspecific symptoms of fever, lethargy, and arthralgia.
- Typically produces a characteristic pattern of the fever called an undulant pattern.
- Usual initial diagnosis is PUO.
- Can produce a number of clinical presentations depending on the body system affected.
- Complications include meningitis, arthritis, endocarditis abscess, uveitis, and epididymoorchitis.

### 4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- Taking an occupational and travel history is important.
- Laboratory diagnosis relies on culture, serology, and PCR.
- *Brucella* is highly contagious and requires Category 3 methods.
- Several different culture techniques used with lysis/centrifugation are the most effective.
- Many serologic test formats available including agglutination, ELISA, and Rose Bengal.
- Two-stage assay with screen and conformation or two assays to measure rise in titer.
- Very wide differential including TB, collagen disease, neoplasia, and systemic fungal disease etc.

### 5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- First-line agents are doxycycline + streptomycin or rifampicin or cotrimoxazole.
- Length of treatment is 6 weeks.
- Specific antibiotic combinations for patient group and clinical presentation.
- Prevention requires a "One Health" approach to the disease.
- Use of Category 3 facilities in laboratories.
- A human vaccine is not available.

## FURTHER READING

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