

Brucella: not your ‘typical’ intracellular pathogen



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Currently the genus *Brucella* consists of a group of bacteria that are genetically monospecific yet phenotypically diverse, and a recent genetic and phenotypic divergent group known as ‘atypical’ *Brucellae*. The host range is extremely varied and includes mammals, including humans, terrestrial animals and marine mammals, but now extends to reptiles and amphibians. Almost all *Brucella* species are zoonotic. The disease collectively termed Brucellosis leads to abortion and reproductive disease in animals, whereas human infection presents as a non-specific undulating fever accompanied by general malaise, chills, joint pain, muscle aches, genitourinary disease and adverse pregnancy outcomes. These Gram-negative coccobacilli invade and replicate in the host macrophages where they can limit the effects of the host immune system and antibiotic treatment. Due to the phenotypic and genotypic diversity and close relationship with *Ochrobactrum* species, the genus *Brucella* presents challenges for accurate identification and recognition of new species.

The disease, Brucellosis, affects animals and humans, causing abortions and reproductive disease in animals, and is known as undulating fever, Malta fever or Mediterranean fever in humans¹. The causative agent, *Brucella*, are facultative intracellular, small Gram-negative coccobacilli (Figure 1) 0.5–0.7 µm × 0.5–1.5 µm that survive in the phagocytic cells of the infected host and were first identified in 1887². Transmission occurs through direct contact with infected material via mucus membranes, broken skin, ingestion or inhalation³. Animals tend to recover but can continue to shed the bacterium into the environment, which makes control of the disease difficult¹. Brucellosis occurs in most parts of the world, although the disease is rare in Australia with cases usually attributed

to international travel in endemic countries or from contact with infected feral pigs particularly in the Eastern states of NSW and QLD. In these areas, hunting of feral pigs has been identified as the principal risk factor for human and dog brucellosis and can result in zoonotic transmission to veterinarians and household contacts⁴.

Brucella species tend to be host specific but can infect other hosts although the disease is usually self-limiting in a non-primary host. Currently, there are 12 species of *Brucella* and can be divided into classical *Brucellae* (*Brucella melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*), marine mammal (*B. ceti* and *B. pinnipedialis*) and recently identified species (*B. inopinata*, *B. microti*, *B. paponis* and *B. vulpis*). There are also additional strains, awaiting genus affiliation, isolated from human and animal sources. A selection of recent species exhibits different phenotypic traits and greater genetic diversity than those in the classical group and are designated ‘atypical’ *Brucellae* (*B. microti*, *B. inopinata* and *B. vulpis*).

Most *Brucella* species pose a significant zoonotic threat to humans most notably *B. melitensis*, *B. suis*, *B. abortus* and *B. canis*. Other *Brucella* species including those that affect marine mammals are also zoonotic⁵. To date only *B. ovis*, which causes reproductive failure and abortion in sheep, is not zoonotic^{6,7}.

The taxonomy of *Brucella* presents challenges. Traditionally, new species were named according to the host from which they were isolated, and biovars were assigned to reflect the diverse range of phenotypes within some species. Classical *Brucella* species are 90% homologous, which means that the genus is monospecific according to the designation of species as having greater than 70% homology using DNA-DNA hybridisation. In this case *B. melitensis* is the only species, and the rest are biovars⁸. However, a consensus

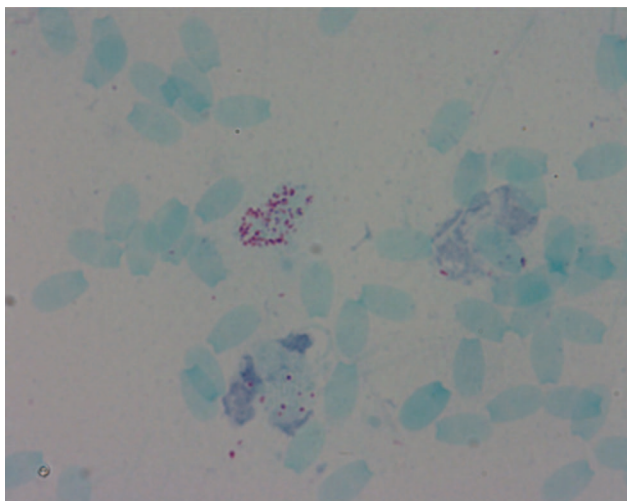


Figure 1. Modified acid fast stain of *Brucella ovis* in ram semen.

held by the scientific community has seen the traditional species names retained irrespective of the traditional taxonomic conventions⁹. Interestingly, application of molecular typing methods such as pulsed field gel electrophoresis, infrequent restriction site polymerase chain reaction, restriction fragment length polymorphisms, insertion sequence site testing, multilocus sequence analysis, variable number tandem repeat analysis and genome sequencing show clustering of genotypes that supports the classical designation of species and biovars^{10–13}.

Greater genetic diversity exists among the atypical *Brucella* clade than in the classical clade (Figure 2). Atypical *Brucellae* diversity has been attributed to the ability of these basal species to exchange DNA with each other and with other microbes in the environment using horizontal gene transfer¹⁵. The atypical group includes

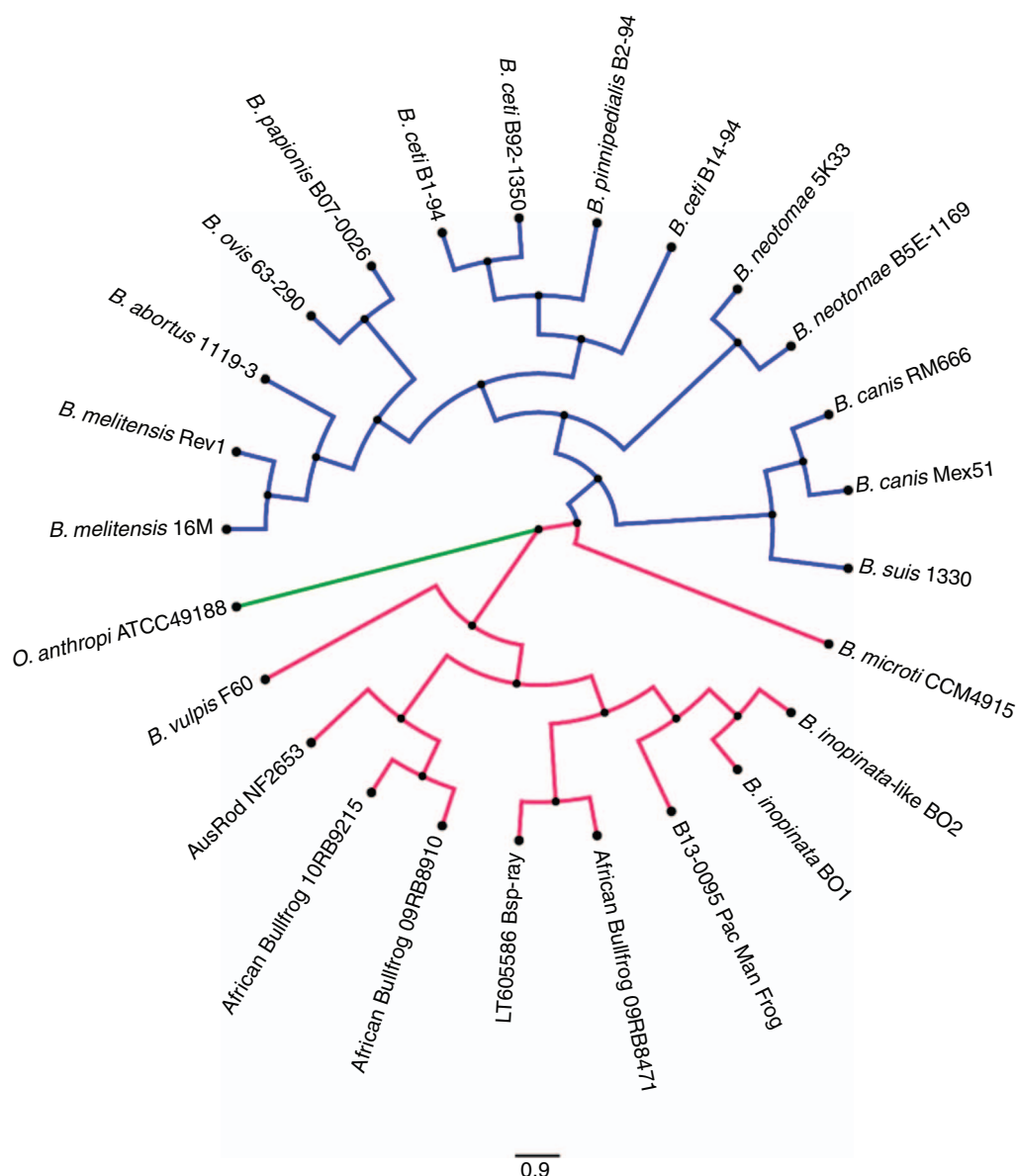


Figure 2. Phylogenetic placement of Classical and Atypical *Brucella* spp. determined by kSNP. A maximum likelihood SNP phylogenetic placement of the isolates was drawn using kSNP¹⁴. Blue indicates Classical; pink indicates Atypical.

designated species (as mentioned earlier) as well as candidate strains like BO2 from a human¹⁶, LT605586 from a bluespotted ribbontail ray¹⁷ and NF2653 from a rodent¹⁸. Recently, atypical *Brucella* species have been isolated from amphibians^{15,19–22}. Some *Brucella* isolates obtained from amphibians are most closely related to *B. inopinata* BO1 and *Brucella*-like BO2 strains based on whole genome analysis. Given their similarity to BO isolates, for which the animal reservoir has not yet been identified, amphibians might represent a possible source of these strains. Recently, *Brucella* from domestic marsh frogs in France were found to be more similar *B. microti* and not *B. inopinata*²³.

Isolation of frog *Brucella* sp. from Africa, Europe, Australia and America suggests that they may be widespread and highlight a need for a broader assessment of the presence of *Brucella* in amphibians worldwide. A major aspect of *Brucella* virulence is their capacity to replicate inside macrophages and escape the host immune system. Recently, *in vitro* and *in vivo* infection experiments with amphibian *Brucella* isolates found that isolates were able to invade and even multiply intracellularly in macrophages and survive in the murine host for up to 12 weeks¹⁵. Given the lack of definitive evidence and their proximity with strains associated with human disease, isolates from amphibians should be considered as potential zoonotic pathogens.

Diagnostics

Accurate identification of pathogens is essential for establishing dependable diagnosis, choosing a treatment, and understanding the source of infection. Conventional identification of *Brucella* is based on modified acid fast staining and phenotypic methods including phage typing and serology that differentiate the species and biotypes^{24,25}. These tests are usually done in a specialist laboratory because of the types of tests conducted and the biohazard of working with the organism. *B. melitensis* can be identified using matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectroscopy; however, further differentiation of species is not available using this technology without additional curation of the reference database²⁶. It is important to differentiate *Brucella* and *Ocbrobactrum* species (close genetic relative within the family *Brucellaceae*, a genus largely consisting of environmental bacteria occasionally infecting humans) as *Brucella* has a higher biosafety risk to hospital and laboratory staff and can have different treatment strategies. Due to their similarity, some routine commercial identification systems can mis-identify the two organisms as *B. melitensis* and *O. intermedium* have a 98.8% similarity according to the rRNA gene sequence²⁷. Atypical *Brucellae* further complicate laboratory identification as most members, including

amphibian isolates, are motile^{15,22}. Amphibian isolates also exhibit variant lipopolysaccharides (LPS), phage lysis, serum agglutination and dye sensitivities compared to classical *Brucella* and are often misidentified as *Ocbrobactrum*^{28,29}. Although, when the *Brucella* reference database is available, MALDI-TOF assays can correctly identify them as *Brucella*. The differences in LPS of atypical and classical *Brucella* could result in serological diagnostics being impaired especially for human infections.

Until recently the genus *Brucella* was considered to represent a genetically homogeneous group of bacteria associated with mammalian hosts. Recently, the situation has become more complex with a rapid increase in the number of novel, genetically divergent, *Brucella* being isolated from cold-blooded hosts. The zoonotic potential and pathogenicity of these *Brucella* sp. strains remains unknown. Further studies are required to gain insights on the bacterial carriage and characterisation of these isolates to understand their role in the evolution of the species from being soil bacteria that are characterised by motility and a broad metabolic activity to becoming highly virulent but host-specific clonal pathogens.

Conflicts of interest

The authors declare no conflicts of interest.

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Glanders: re-emergence of an ancient zoonosis



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Glanders, although known to be endemic in certain regions/ countries of the Old and New Worlds for centuries, had been largely overlooked as a threat to equine and human health until the disease re-emerged in the Middle East in 2004. The exponential growth in international horse movements,

both legal and illegal, mainly for performance purposes, has enhanced the risk of global spread of glanders in the Middle East and elsewhere. Ever since the First World War, the glanders bacillus has been recognised as a potential biological warfare agent.

The organism

Glanders is an OIE (World Organisation for Animal Health) listed notifiable disease caused by *Burkholderia mallei*, a Gram-negative, non-motile and non-spore-forming bacterium. Previously known as *Pseudomonas mallei*, it is genetically closely related to the agent of melioidosis, *Burkholderia pseudomallei*. It is an obligate pathogen of domestic equids. Glanders is one of the oldest recorded diseases of horses dating back to Aristotle (350 BC), and long recognised as a very important zoonotic disease of humans.

The incubation period of glanders varies from a few days to many months according to the route and level of exposure and