Chapter 1
Introduction and Overview

R. Martin Roop II and Clayton C. Caswell

Abstract Bacteria in the genus *Brucella* are important human and veterinary pathogens, and they require a variety of metals to support their physiology and metabolism. Mammals employ both metal limitation and metal intoxication as defenses against invading pathogens, and correspondingly the metal acquisition and detoxification systems of *Brucella* strains play essential roles in their virulence.

Keywords *Brucella* · Zoonosis · Metals · Metal toxicity

1.1 *Brucella*

The genus *Brucella* currently consists of 11 recognized species of Gram-negative bacteria. Five of these—*B. melitensis*, *B. abortus*, *B. suis*, *B. canis* and *B. ovis*—are important veterinary and human pathogens. Molecular studies have shown that all *Brucella* strains are closely related at the genetic level. The separate 'species' designations have been retained, however, because these bacteria can be subdivided into distinct phenotypic groups (e.g., species and biovars within these species) that display different host specificities and virulence properties. These distinctions are important for understanding the epidemiology and pathogenesis of *Brucella* infections (reviewed in Whatmore 2009).

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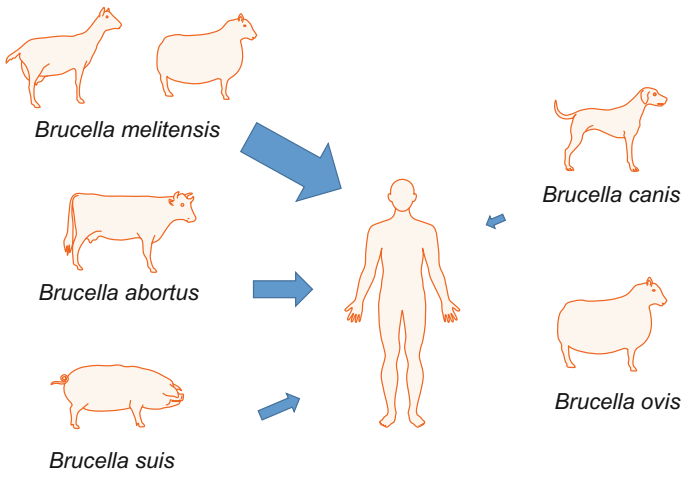

Fig. 1.1 Natural hosts of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. canis* and *B. ovis* and zoonotic potential of these strains. The size of the arrow pointing from the natural hosts to humans indicates the relative propensity of each *Brucella* spp. to cause human disease

1.1.1 B. melitensis, B. abortus and B. suis

Brucella melitensis, *B. abortus* and *B. suis* cause abortion and infertility in goats and sheep, cattle, and swine, respectively (Atluri et al. 2011). These bacteria are highly infectious in their natural hosts where they can produce chronic, often life-long infections. Agricultural communities worldwide devote tremendous resources annually to prevent and control food animal brucellosis (Godfroid et al. 2014). *B. melitensis*, *B. abortus* and *B. suis* can also be readily transmitted to humans via the consumption of unpasteurized dairy products or direct contact with infected animals, where they produce a serious, chronic debilitating febrile disease (Fig. 1.1). Human brucellosis represents a major public health problem in areas of the world where the disease is not effectively controlled in food animals, and in fact, this disease is considered to be one of the world's leading zoonotic infections (Pappas et al. 2006).

B. melitensis, *B. suis* and *B. abortus* strains also have characteristics that make them attractive as agents of biowarfare or bioterrorism (Valderas and Roop 2006). Specifically, they have low infectious doses via the aerosol route, the disease they produce in humans is difficult to treat with antibiotics, and there is no vaccine that can be safely and effectively used to prevent human brucellosis. Historically, *B. melitensis* and *B. suis* strains were included in the bioweapons arsenals of several countries before the global movement to ban the use of these weapons in the late 1960s and early 1970s. Today, many countries still tightly regulate the possession of *B. melitensis*, *B. suis* and *B. abortus* strains due to their potential use in bioterrorism.

1.1.2 *B. canis and B. ovis*

B. canis causes abortion and infertility in dogs, and canine brucellosis is a serious concern in kennels (Wanke 2004). This bacterium can also be transmitted from dogs to humans as a zoonotic agent (Fig. 1.1). Although the reported incidence of human disease caused by *B. canis* is low compared to that caused by *B. melitensis*, *B. abortus* and *B. suis*, it has been proposed that many human *B. canis* infections likely go unrecognized (Krueger et al. 2014). *B. ovis* causes epididymitis is sheep and is an important cause of infertility in rams worldwide (Gouletsou and Fthenakis 2015), but these strains are not known to cause human disease.

1.1.3 *Other Brucella Species*

Limited information is available regarding the importance of the remaining *Brucella* species as pathogens. *B. microti*, for instance, produces severe and sometimes fatal disease in both wild rodents (Hubálek et al. 2007) and experimentally infected mice (Jiménez de Bagüés et al. 2010), and was recently isolated from a wild boar (Rónai et al. 2015). But it is unclear how common and widespread *B. microti* infections are in wild rodents (Hammerl et al. 2015) or other wildlife, and disease in humans or domestic animals associated with this strain has not been reported. Similarly, although *B. ceti* and *B. pinnipedialis* strains are routinely isolated from marine mammals (Foster et al. 2007), overt clinical signs appear to be uncommon in the animals from which these strains are isolated (Nymo et al. 2011; Guzmán-Verri et al. 2012). *B. ceti* strains, have however, been isolated from a

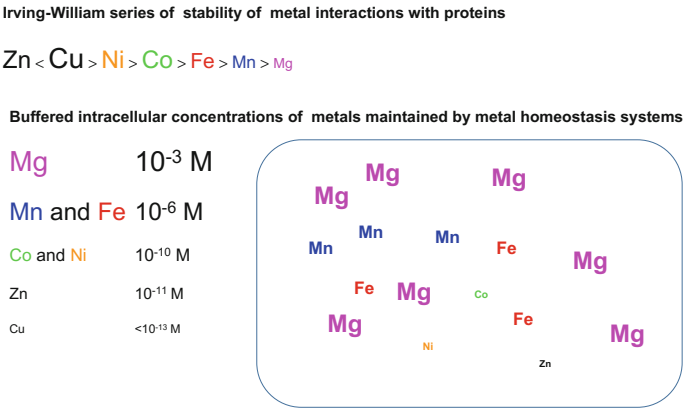

Fig. 1.2 Inverse correlation of the Irving-Williams series of stability of metal interactions with proteins (Irving and Williams 1948) and the buffered set points of intracellular metal concentrations maintained by metal homeostasis systems (Foster et al. 2014)

limited number of human infections (reviewed in Whatmore et al. 2008), suggesting that these strains have the potential to be zoonotic pathogens. Information regarding the prevalence, natural host range, pathogenicity and zoonotic potential of *B. neotomae* (Stoenner and Lackman 1957), *B. inopinata* (Scholz et al. 2010), *B. papionis* (Whatmore et al. 2014) and *B. vulpis* (Scholz et al. 2016) is even more limited, since only a few isolates of these strains have been characterized.

1.2 Biological Functions of Metals and the Importance of Metal Homeostasis in Living Cells

Copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), magnesium (Mg), nickel (Ni) and cobalt (Co) serve as important micronutrients for living cells. It has been estimated that approximately 50% of all enzymes, and 1/4–1/3 of proteins in general, require metal co-factors for their activity (Waldron et al. 2009). Metals can play either catalytic or structural roles in protein function. Zn in the active site of the enzyme carbonic anhydrase, for instance, directly participates in the interconversion of CO_2 and HCO_3^-, a reaction that maintains cytoplasmic pH balance in both prokaryotic and eukaryotic cells (Supuran and Scozzafava 2007). The interaction of specific amino acid residues with Zn also coordinates the proper folding of the large family of eukaryotic proteins known as 'Zn finger' proteins (Klug 2010) which perform a variety of different biological functions. The redox activities of Fe and Cu make proteins containing these metals essential components of electron transport chains (Liu et al. 2014), and the Fe incorporated in heme plays a critical role in O_2 transport in mammals (Fujiwara and Harigae 2015).

Despite the fact that they are essential micronutrients, metals can also be toxic when their levels exceed those required to meet the physiologic needs of the cell (Summers 2009). To prevent metal toxicity, both prokaryotic and eukaryotic organisms have evolved finely-tuned homeostasis systems that tightly control the intracellular levels of metals (Waldron and Robinson 2009; Foster et al. 2014). These systems are typically comprised of metal importers and exporters, metal chaperones, and metal storage and detoxification proteins, and the expression of the genes that encode these proteins is tightly regulated in response to cellular metal levels. Metal toxicity occurs primarily for two reasons. First, the affinity of metals for proteins follows a scale known as the Irving-Williams series, with Cu and Zn having the highest affinity and Mg having the lowest (Waldron and Robinson 2009), and the intracellular levels of the high affinity metals must be maintained at lower levels that those of the lower affinity metals to avoid the higher affinity metals displacing the lower affinity metals in proteins or enzymes where the latter are essential for protein function, or dedicated metallochaperones must be in place to ensure that proteins acquire the proper metal. As shown in Fig. 1.2, these metal homeostasis systems 'buffer' the intracellular levels of the individual metals to ensure that their concentrations are inversely proportionate to their potential toxicity

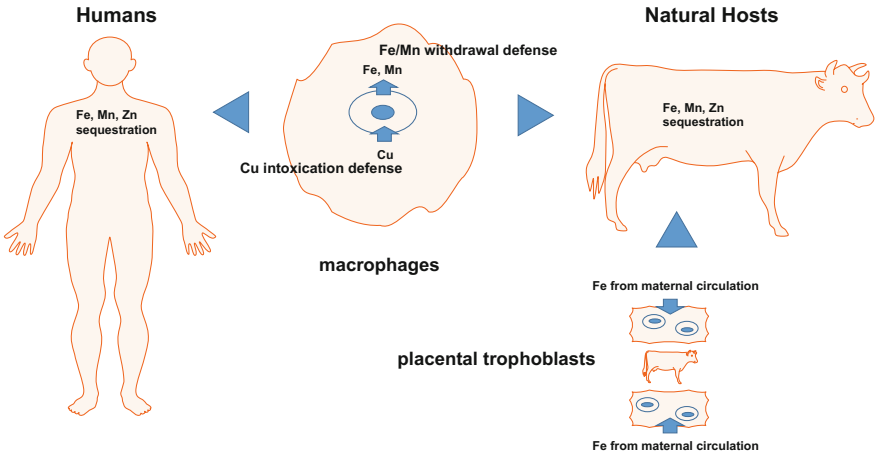

Fig. 1.3 Homeostasis systems and immune defenses that potentially influence the availability of metals to the brucellae during residence in the two key cell types they inhabit in their natural hosts and in humans

(Foster et al. 2014). The second reason for metal toxicity is that Fe reacts with reactive oxygen species such as the superoxide ion (O_2^-) to form highly reactive hydroxyl radicals which can damage proteins, nucleic acids and lipids (Imlay 2013). Consequently, Fe homeostasis systems also play important roles in oxidative defense, and the genes that encode the components of these homeostasis systems are often responsive to oxidative stress in addition to their regulation in response to cellular levels of the corresponding metal (Faulkner and Helmann 2011).

1.3 Metal Homeostasis in *Brucella* Strains

Early studies of the nutritional requirements of *Brucella* strains during in vitro cultivation determined that Fe and Mg were essential micronutrients (Gerhardt 1958). Because it is very difficult to remove contaminating metals from media components and culture vessels, however, these earlier studies underestimated the importance of other metals in the physiology of these bacteria. More recent studies employing genetically defined mutants and genome analysis have not only confirmed the importance of Fe and Mg as micronutrients for *Brucella* strains, but have also shown that Mn, Zn, Cu, Ni and Co play critical roles in their basic physiology (Roop et al. 2012).

Brucella strains live in close association with their mammalian hosts (Roop et al. 2009), where they reside predominantly as intracellular pathogens. Their capacity to survive and replicate in host macrophages underlies their ability to cause chronic infections, and their extensive intracellular replication in placental trophoblasts plays

an important role in their capacity to induce abortion in their natural hosts. Mammals not only tightly control the levels of metals in their tissues to avoid toxicity, but they also employ Fe, Mn and Zn deprivation and Cu intoxication as mechanisms to limit the replication of microbial pathogens (Hood and Skaar 2012) (Fig. 1.3). It is also notable in this regard that one of the important roles that placental trophoblasts play during pregnancy is to provide Fe from the maternal circulation to the developing fetus (Carter 2012; de Oliveira et al. 2012). Thus, it is not surprising that metal homeostasis systems have been shown to play critical roles in the virulence of *Brucella* strains in both experimental and natural hosts (Roop 2012). The following chapters will review the information that is currently available regarding the role that metal homeostasis plays in the biology and virulence of *Brucella* strains.

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