Preface

The ability to cost-effectively and rapidly sequence genomes, advances in computational biology and development of high-throughput technologies that facilitate dissection of the ever-expanding list of "omics" has revolutionized research approaches within the biological sciences. For example, the concept of reverse vaccinology has brought antigen identification into the genomics era, and bioinformatics tools can now be used to select and prioritize a list of candidate vaccine antigens from predicted pathogen proteomes for further testing. In addition, the ability to infer metabolic capacity based on pathogen genome sequences has resulted in the identification of several targets for chemogenomics, a discipline that may lead to the generation of novel chemotherapeutics. These two tangible outputs, candidate vaccine antigens and drug targets, have energized efforts in developing improved methods of pathogen control. In addition, development of genome-wide molecular diagnostic tools provides an opportunity to study pathogen genotypes and population dynamics, and should allow those all-important correlates with phenotype to be made. Thus, genomics technologies are now being used to develop a holistic approach to study the genetics of host and pathogen populations and their molecular responses to each other at a level of detail not previously possible. Application of genomics technologies to study the process of infection, disease, vaccination, and interventions leading to immunity is likely to result in building databases, which will help realize the holy grail of developing rational approaches for pathogen and disease control.

The scope for a book on genome mapping and genomics of animal-associated microbes is too huge. We decided to exclude viruses and nonpathogenic microbial associations and chose livestock as the target animals. We concentrated on selecting bacterial or protozoan pathogens of global and developing country significance. We also picked pathogens whose genome sequence has been available for a few years so that the impact of genomics in driving research would be more apparent to the reader. Of the six pathogens chosen, most affect ruminants, and three are vector-transmitted, increasing the complexity of their "animal" associations but providing another target for potential intervention.

We are grateful to the senior authors and co-authors of the chapters presented in this volume, as they have done a marvelous job in summarizing complex topics. They have provided excellent overviews of their pet pathogens as well as comparative aspects with related pathogens. Remarkable progress has been made in studying the organisms reported on here and the next few years promise exciting scientific breakthroughs in both basic and applied pathogen biology.

Baltimore, MD — Vishvanath Nene
Clemson, SC — Chittaranjan Kole
June 2008

1 *Brucella*

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1.1.1 Discovery of the *Brucella*

Examination of the skeletal remains of the Roman residents of Herculaneum (Naples, Italy) killed by the catastrophic volcanic eruption of Mt. Vesuvius in the late August AD 79 revealed vertebral bone lesions typical of brucellosis in more than 17% of the residents. Scanning electron microscopy of recovered cheese provided a likely explanation for the high incidence of the disease. The buried carbonised cheese, made from sheep's milk and found with the bones, revealed the presence of cocco-bacillary forms that were morphologically similar to *Brucella spp*. (Capasso 2002). Eighteen centuries later, Sir David Bruce isolated *Micrococcus melitensis* (now *Brucella melitensis*) from the spleen of a British soldier who died from a febrile illness (Malta fever) common among military personnel stationed on Malta, an island not far away from Herculaneum (Godfroid et al. 2005). For almost 20 years, brucellosis was thought to be a vector-borne disease. The zoonotic nature of the brucellosis was accidentally demonstrated in 1905 by isolating *B. melitensis* from goat's milk used for the production of soft cheese in Malta (Nicoletti 2002; Godfroid et al. 2005). It was believed that goats were not the source of infection since they did not become ill when inoculated with *Brucella* cultures. Although raw goat's milk had been used as an essential nutritional meal for hospitalized patients suffering from Malta fever, it was decided to ban it from hospitals. The public did not follow the same recommendation and consumed infected dairy products and remained exposed to the disease (Nicoletti 2002).

1.1.2 Species Discovery

In 1897, a Danish veterinarian, L.F. Benhard Bang, discovered Bang's bacillus or bacillus of abortion

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(*B. abortus*) the causative agent of Bang's disease (brucellosis in cattle). Bang's bacillus was not recognised as being related to *Micrococcus melitensis* (isolated by Bruce) until 1918, when Alice Evans in the Hygiene Laboratory of the U.S. Public Health Service (now the National Institutes of Health) showed the close relationship between the two organisms and renamed the genus *Brucella* to honour Bruce (Meyer and Show 1920; Bang 1933; Nicoletti 2002).

In 1914, Traum isolated *B. suis* from an aborted pig foetus in U.S. (Traum 1914; Nicoletti 2002). The description of isolates from cattle and swine led to the recognition of widespread distribution of the disease.

In 1953, a different strain, thought to be a rough *Brucella* mutant, was described in sheep in New Zealand by Buddle and in Australia by Simmons (Simmons and Hall 1953; Buddle 1956; Diaz et al. 1967). Although the Subcommittee on the Taxonomy of *Brucella* of the International Committee on Bacteriological Nomenclature was not satisfied that the organism was a member of the genus *Brucella* and advised further study, the species was eventually recognized as *B. ovis* (Diaz et al. 1967).

In 1957, Stoenner and Lackman isolated *B. neotomae* from desert wood rat (*Neotoma lepida*) in Utah, U.S. (Stoenner and Lackman 1957). Carmichael isolated *B. canis* in 1966 from beagles in the U.S. (Carmichael and Bruner 1968).

Brucellosis in marine mammals was first described in 1994 in the U.S. when a bacterial isolate from the aborted foetus of a bottlenose dolphin (*Tursiops truncatus*) was characterized as a nontypical *Brucella spp*. (Ewalt et al. 1994). Since 1994, several new *Brucella* species have been isolated from marine mammals (Ross et al. 1994; Foster et al. 1996). The zoonotic nature of marine brucellae and its ability to cause abortion in cattle were documented (Brew et al. 1999; Rhyan et al. 2001). The discovery of the marine *Brucella* has changed the concept of a land-based distribution of brucellosis and associated control measures to that of a land- and ocean-based approach for control and eradication.

As of 2006, eight *Brucella* species are recognized. Six of them infect terrestrial animals: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (Verger et al. 1987) and two infect marine mammals: *B. cetaceae* and *B. pinnipediae* (Verger et al. 2000). Within these species, seven biovars are recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis* (Verger et al. 2000); the remaining species have not been differentiated into biovars.

1.1.3 Zoonoses

Although *Brucella* was first isolated by Bruce in the nineteenth century, clinical conditions characteristic of brucellosis have been described by Hippocrates in 450 BC (Evans 1950). In 1751, Cleghorn, a British army surgeon stationed on the Mediterranean island of Minorca, described cases of chronic, relapsing febrile illness and cited Hippocrates's description of a similar disease (Hoover and Friedlander 1997). Marston, a British army surgeon working on the island of Malta, described the clinical characteristics (Malta fever) of his own infection in 1861 (Hoover and Friedlander 1997).

Brucella was discovered and isolated for the first time from humans in 1887 before it was recognised as an animal pathogen in 1905. The first recognised human case of brucellosis in the USA was in an army officer based in Puerto Rico in 1898 (Brown 1977; Nicoletti 2002). The zoonotic nature of *B. canis* was reported in 1975 in US (Blankenship and Sanford 1975; Munford et al. 1975). The zoonotic nature of marine brucellae was documented in 1999 in a case of a laboratory-acquired human infection (Brew et al. 1999).

B. suis was the first biological agent to be weaponised by the US in 1942 during its offensive biological warfare program. The agent was formulated to maintain long-term viability, placed into bombs and tested in field trials during 1944–1945 using animal targets (Hoover and Friedlander 1997). By 1967, the USA terminated its offensive program for the development and deployment of *Brucella* and other pathogens as biological weapons (Hoover and Friedlander 1997).

B. melitensis, *B. suis* and *B. abortus* are listed as potential bioweapons by the Centers for Disease Control and Prevention (Kaufmann et al. 1997; Kortepeter and Parker 1999), because of their virulence in humans. This is due to the highly infectious nature of all three species as they can be readily aerosolized. Moreover, an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza (Chain et al. 2005).

In comparison to abortions, orchitis, followed by persistent infections of supra-mammary lymph nodes and reticuloendothelial system in animals (Adams 2002), humans develop symptoms that start out as flu-like symptoms followed by undulant fever with severe cold sweats in between (Pappas et al. 2006a, b). In some affected individuals the disease could be fatal if untreated, while others can become permanently infected and suffer from fever and cold sweats, particularly when they are stressed. Brucellosis has also been associated with mild to severe cases of arthritis in adults and childen (Pourbagher et al. 2006).

1.1.4 Eradication Program

By the year 1922, several states in the USA had passed laws and regulations in an attempt to prevent introduction of the disease by cattle purchased from other states (Nicoletti 2002). The Cooperative State-Federal Brucellosis Eradication Program began in 1934 and cost about $3.5 billion by 1997. The program's Uniform Methods and Rules set forth the minimum standards for states to achieve eradication. A state is designated as brucellosis-free when none of the cattle in that state are found infected for 12 consecutive months under an active surveillance program. In 1956, there were 124,000 affected herds in the U.S.A., which corresponds to one in every eight cattle herds. By 1992, this number had dropped to 700 herds, and by 2000 there were only six known infected herds remaining in the entire U.S.A. Consequently, the number of human brucellosis cases in the USA has dropped from 6,321 in 1947 to about 100 per year by 1998, mostly acquired overseas or due to consumption of infected milk products from Mexico (Cook et al. 2002).

Infected wild life (bison, elk and feral swine) still remains a source of infection to domestic livestock three of the five brucellosis affected cattle herds disclosed in FY 2005 are due to wild life (Olsen and Stoffregen 2005).

In spite of the availability of very effective vaccines like *B. abortus* strains 19 and RB51, eradication of cattle brucellosis has not been accomplished in all the countries of the world. Most of North America has essentially eradicated the disease from their cattle. In Mexico, South America, Asia, Africa, Middle East and Caucuses States, the disease is highly prevalent, even though in many of these countries, there are 'test and slaughter' programs in place. Because of lack of indemnification, the programs have not been very effective. In countries like India, penal codes that prohibit slaughter of cows complicate the issue even further. From the long and successful efforts in the USA, one can conclude that eradication of brucellosis in cattle can be accomplished only when all the concerned parties get involved in finding a solution. It is basically a political disease; unless there is a strong political support for the indemnification of the farmer for the loss due to removal of infected animals, it is almost impossible to control this important zoonosis. The farmers, milk and milk products industry, breeding companies, consumers and the politicians must work together and find a practical eradication effort that is suitable for each country.

In this regard, the recent brucellosis control effort in Iran is worth careful consideration by other countries. The Iranian investigators effectively eradicated the disease from a set of large commercial dairy farms by monthly serological testing and slaughtering of all positive cows for a period of a year (personal communication, Kamran Afshar Pad, Veterinary Organization of Iran, 2005). This was followed by mass vaccination of the entire herd with regular doses of strain RB51. The investigators educated the commercial farmers to expect a certain level of abortion in the vaccinated animals. They established standard operating procedures for the safe removal of all aborted materials and the cows. They were able to attain what could be considered as disease-free status in these large commercial farms within 10–18 months; this process took 20 years for USA to accomplish. The commercial farms were willing to withstand the losses by having increased productivity and the lack of abortions. This approach would certainly have to be modified to address the very different economic conditions of a small-scale farmer, who would also be the primary target of such an eradication effort in many of the above countries having endemic brucellosis.

1.1.5 Vaccination

The first attempt at using a *Brucella* vaccine was performed in 1906 by Bang (Bang 1906). He demonstrated that the injection of live *B. abortus* protected