Cell. Mol. Life Sci. 63 (2006) 2229–2236
1420-682X/06/202229-8
DOI 10.1007/s00018-006-6311-4

Cellular and Molecular Life Sciences

Brucella as a biological weapon

G. Pappas [a, *], P. Panagopoulou [a], L. Christou [b] and N. Akritidis [c]

[a] Institute for Continuing Medical Education of Ioannina, Velissariou 15-19, 45221 Ioannina (Greece),
Fax: +30 26510 49045, e-mail: gpele@otenet.gr
[b] Internal Medicine Department, University Hospital of Ioannina, (Greece)
[c] Internal Medicine Department, General Hospital 'G. Hatzikosta' of Ioannina (Greece)

Online First 9 September 2006

Abstract. *Brucella* has traditionally been considered a biological weapon. It was the subject of extensive offensive research in the past, and still belongs to category B pathogens on most lists. Its propensity for airborne transmission and induction of chronic debilitating disease requiring combined antibiotic regimens for treatment, its abundance around the world and its vague clinical characteristics defying rapid clinical diagnosis are some of the characteristics that apply to the pathogen's weapons potential. Yet minimal mortality, availability of treatment options, protracted inoculation period and the emergence of new, more virulent potential weapons means that its inclusion among agents of bioterrorism is nowadays mainly of historical significance. Nevertheless, in the interest of literacy and of avoiding panic, physicians and the public both should be aware of the most common zoonosis worldwide.

Keywords. Brucella, brucellosis, bioterrorism.

Introduction

Brucellosis is an ancient disease. It remains the most common anthropozoonosis worldwide, inducing an often chronic, often incapacitating disease with low mortality. Its significance as a potential agent of bioterrorism was acknowledged early, and the pathogen remains on the category B biodefense research list of both the Centers for Disease Control and Prevention (CDC) [1] and the National Institute of Allergy and Infectious Diseases (NIAID) [2]. It is also invariably included on the non-stratified lists of potential biological weapons of other organizations, such as the World Health Organization (WHO), the North Atlantic Treaty Organization (NATO), and the Biological and Toxin Weapons Convention (BTWC). Certain epidemiological, microbiological and clinical parameters of the pathogen render it an attractive agent for malicious use. We will analyze these parameters, along with other aspects that emerge following a hypothetical deliberate release of the agent.

* Corresponding author.

The pathogen

Brucella belongs to the genus of a-proteobacteria and consists of seven species: *B. melitensis, B. abortus, B. suis* and *B. canis* are known to induce human disease, while *B. neotomae* and *B. ovis* are not virulent to humans. *B. pinnipediae* and *B. cetaceae* are marine species pathogens discovered recently and provisionally named [3] that may also be human pathogens [4]. The genome of *B. melitensis* [5], *B. abortus* [6] and *B. suis* [7] has been fully decoded, and extended active research on the significance of various proteins expressed by the bacterium will probably allow for better understanding of the unique pathogenetic processes involved in human brucellosis [8]. Brucellosis is principally a zoonosis, a common cause of abortions in sheep and goats (*B. melitensis*), cows (*B. abortus*) and pigs (*B. suis*). *B. canis* is a canine pathogen, *B. ovis* is also a sheep pathogen, and *B. neotomae* is found in rodents. Furthermore though, other species, including wildlife, can serve as the reservoir of *Brucella*, although the prevailing subtypes in wildlife may be of minimal human importance [9], as in France. That is not the case in United States though, where *B. abortus*-infected bisons were recently slaughtered in certain Midwestern states,

raising concerns about the cost effectiveness and political correctness of the whole procedure [10]. *Brucella* does not form spores, but it is still significantly environmentally resistant.

History

Brucellosis is an ancient disease. It induced disease in a significant number of inhabitants of Pompei [11], and is possibly the cause, in a bizarre form of 'divine bioterrorism', of the fifth plague of Egypt, which decimated Egyptian cattle. Yet the disease is also described as incapacitating equine species, and others have suggested that the fifth plague was in fact anthrax (the sixth plague, which is a more accurately describes human and animal anthrax disease, is suggested by some to be smallpox). At the end of the 19th century, brucellosis was prevalent among British troops stationed in Malta. And although it is tempting to assume that locals deliberately infected troops by offering raw goat milk, we know that raw milk was considered an excellent tool for strengthening patients supposedly suffering from typhoid. Sir David Bruce, a British Army officer, was the first to isolate the organism and along with his coworkers subsequently managed to trace the epidemiology back to goat milk. He developed the first serum agglutination test for to diagnose brucellosis [12]. By the beginning of World War II, the medical and veterinary aspects of the disease had been extensively outlined, and brucellosis emerged as an attractive candidate in the still premature biowarfare industry. The attractiveness of *Brucella* was based on certain combat parameters of the era: an agent that could caused a protracted incapacitating disease with minimal mortality would mean that most of the enemy's troops would be sidelined by illness, and a significant percentage of non-infected army members would be needed to care for them (this percentage would be higher and implicated for a longer period than the one needed for dealing with dead bodies, had a more lethal pathogen been used). Practically every major national program for offensive biological weapon development dealt with *Brucella. Brucella* was one of the agents with which Japan experimented in the infamous 731 Manchuria Unit before and during World War II. In the United States, *B. suis* was the first agent weaponized in 1952, and extended field testing with *B. suis*-filled bombs took place thereafter [13]. Soon though, other, more potent weapons were targeted. In the former Soviet Union, *Brucella* was one of the agents developed for offensive purposes by Biopreparat, the extensive Soviet biological weapons program. Ken Alibek, a former deputy director who relocated in United States in 1992 stated that untreatable, antibiotic-resistant forms had been developed, the agent was weaponized both in dry and liquid forms, production capability ranged at the level of 100 tons of bacteria and the means to deliver the pathogen had been extremely sophisticated. As with other agents developed by the Soviet Union, extended field testing was performed on the island of Vozroshdeniye, in the midst of the Aral Sea [14]. Despite its historical significance and attractiveness in the era of traditional combat situations, by the end of the 20th century interest in *Brucella* gradually waned: it is characteristic that Alibek states that *Brucella* was dropped from the Soviet program in favour of *Burkholderia pseudomallei*, which was considered more potent. Still, as with other aspects of the Biopreparat program, questions about the subsequent whereabouts of the resistant strains developed remain.

Epidemiology

Brucellosis, particularly caused by *B. melitensis*, remains the commonest zoonotic disease worldwide, and moreover seems to be relocating and re-emerging in recent years [15]. Middle Eastern countries as Syria, Iran and Iraq Figure prominently on a list of endemic countries, but new foci that have emerged include all the former communist Asian republics, such as Kazakhstan, Kyrgizstan and especially Mongolia. The situation is slowly improving in the European Union, although the disease is still endemic in Greece, Spain, Portugal and southern Italy. International travel and the importation of exotic food from endemic areas account for a limited number of cases reported annually from brucellosis-free industrialized countries. The same stands for the United States, where most cases appear in states neighboring Mexico, in patients with Hispanic origin and related to importation of infected dairy products from the still endemic Mexico [16]. North Africa remains an endemic area, while the situation in sub-Saharan Africa cannot be adequately evaluated; furthermore, other infectious disease-related priorities exist in these countries. Three important bioterrorism-related aspects emerge from the current global disease status. First, *Brucella* can be easily obtained practically anywhere in the world, in contrast with agents such as smallpox, and thus its use as a biological weapon could be kick-started rather easily. In that vein one has to question the rationale behind widely circulated reports about Iraq obtaining *Brucella* strains from a US firm at the end of the 1980s: brucellosis was already endemic in Iraq during that period, so strains could easily be isolated from naturally occurring human cases. Second, one has to note that certain endemic areas coincide with areas where active foreign army operations have been evolving; thus, a naturally occurring case in a US soldier in Iraq could, at least initially, raise concerns about possible deliberate release. In this context, brucellosis was related to the development of Gulf War Syndrome (see following sections). Containment of naturally occurring

disease in these areas does not seem feasible at present, since the disease is related to overall socioeconomic status and political factors. The emergence of brucellosis in Kosovo and Bosnia-Herzegovina in recent years after lengthy political unrest and extended military operations and the mechanisms of disease trafficking in the Balkans is a typical example [14]. The third important aspect is that of awareness: brucellosis being a rare disease in the developed world, many physicians and infectious disease specialists are not familiar with its characteristics, leading to delayed diagnoses or false alarms [17]. More on this follows in upcoming sections.

Pathogenesis

As already stated, *Brucella* was initially attractive as biowarfare partly due to its ability to induce chronic disease. The pathogenesis of brucellosis is unique, and animal models often cannot accurately reproduce events evolving during human infection. *Brucella* is a Gram-negative pathogen, yet its surface lipopolysaccharide induces far smaller immune response comparing with other Gram-negative bacteria. Brucellae have a propensity for invading the reticuloendothelial system, practically hiding inside macrophages and non-professional phagocytes. In there, they reside in specialized compartments with acidic environments, and multiply using parts of the cytoskeleton, without interrupting cell cycle and function [18]; on the contrary they are apoptosis inhibitors, thus creating a frame for eternal survival and replication. Immune response is partly muted by certain *Brucella* factors, inhibition of tumor necrosis factor-alpha (TNF-α) being a prominent event. Cellular immune responses predominate [19], although antibody production serves as a diagnostic tool. It has been long postulated that the outcome of the disease reflects the equilibrium developed between the bacterium and the human immune response, and that relapses and chronic disease should also be viewed in this context.

Transmission to humans

One important bioterrorism-related characteristic of *Brucella* is the small inoculum needed to induce human disease, traditionally described in the levels of 10–100 microorganisms. The commonest means of *Brucella* transmission to humans is by consuming unpasteurized dairy products as milk and soft cheese. Direct contact through skin abrasions with infected animal tissues (as in slaughterhouse workers) is also implicated, but the most important means of transmission in the context of bioterrorism importance is airborne transmission. *Brucella* can be easily aerosolized, and when in air, can be easily transmitted through the airways and induce disease, while staying for a protracted period in this virulent form. Characteristically, brucellosis is considered the commonest laboratory-acquired infection worldwide [20], and thus certain isolation and experimental procedures should be performed in Biosafety Level 3 (BSL3), and laboratory workers should be informed ahead of time about the diagnostic possibility of brucellosis in order to implement appropriate diagnostic precautions. Its propensity for aerosolization and easy spread is another of the important bioterrorism-related characteristics of the bacterium. Using *Brucella* as a biological weapon through the food chain could be feasible, but would result in localized clusters of cases: one would have to intervene at a post-industrial level, since pasteurization kills the pathogen. The potential for such an approach in order to induce massive disease is obviously low, but should also be entertained by policy makers of the area, and is beyond the scope of this review.

The inoculation period is relatively protracted, especially when compared with other pathogens considered as potential biological weapons, ranging 9–60 days. This alone could be a drawback in the context of biowarfare, since a deliberate release would not lead to a sharp outbreak curve, but would rather induce a smooth curve of gradual increase, and subsequent decrease over a period of 1–2 months. Thus, more time would be allowed for authorities to diagnose and intervene, and less public unrest and health authorities' burden would be created [21]. Since deliberate release of a pathogen theoretically aims, at least partly, at social disruption, *Brucella* is by far an unsuitable agent. Still, certain approaches to bioterrorism risk argue that penetration of infectious sequelae deep in time might be more important for social disruption in long term [22], and brucellosis should be re-evaluated in this context. The possible existence of an inverse relationship between microbial inoculum and inoculation period should also be further studied.

Inapparent-to-apparent infection ratios cannot be adequately calculated, owing to discrepancies in different series. A genetic predisposition seems to exist [G. Pappas et al., unpublished data].

Clinical characteristics

Brucellosis can cause practically any clinical syndrome [23], and in endemic areas the tagline 'everything is *Brucella* until proven different' might actually be useful. The commonest syndrome presented is one of a flu-like illness, with fever that may be protracted (often presenting as fever of unknown origin [24]), arthralgia, myalgia, fatigue and malodorous perspiration. The propensity for reticuloendothelial system invasion leads often to hepatomegaly, splenomegaly and lymphadenopathy. Uncompli-

cated disease is readily responsive to antibiotic treatment. Complications reported are abundant, the commonest being arthritis (either peripheral or, often, sacroiliitis) and spondylitis [25], which can be debilitating and difficult to treat [26]. The propensity of the pathogen for granuloma formation can lead to abscess formation in various sites. Epididymo-orchitis [27], mild hepatitis, rashes and ascites [28] are often reported. The most serious complications of the disease are neurobrucellosis, which can present in various forms [29], and endocarditis, which is the main cause of mortality (altogether very low) and often requires surgical intervention [30]. Respiratory complications in brucellosis are more usual than generally thought; yet there is no connection between airborne transmission, the probable route of transmission after deliberate release and emergence of respiratory complications, as outlined in a large series of cases [31]. Laboratory characteristics include cytopenia of varying range and severity [32], mild increases in serum aminotransferase levels and relative lymphocytosis.

In pregnancy brucellosis is related to an increased risk of spontaneous abortion [33], while in childhood the disease is generally thought to be more benign [34]. The relatively few data on brucellosis in immunocompromised patients [35] suggest that clinical severity is not enhanced in this population.

Chronic brucellosis is an entity much talked about, but inadequately understood. By the traditionally accepted definition, the disease is chronic when exhibiting a course of more than 6 months. Yet chronicity can present as frequent relapses, residual disease after treatment and sometimes as persistent behavioral changes accompanied by ill-defined neurological syndromes, weight loss and fatigue, in the absence of any laboratory evidence of brucellosis relapse. This syndrome is familiar to brucellosis specialists; some argue that its nature is autoimmune. It is a syndrome strikingly familiar to chronic fatigue syndrome (CFS), an equally vaguely described syndrome developed by Gulf War veterans after the first Gulf War. Brucellosis was endemic in the battlefield area, and fear of deliberate exposure to *Brucella* aerosols from the Iraqi army was also prominent. Thus, brucellosis was one of the first diagnoses entertained in the approach of CFS. No significant evidence has emerged, yet one should remember the long-standing hypotheses on the relationship between *Brucella* and demyelinating syndromes [36]. Advances in diagnostic options might help to more clearly define and understand the exact nature of chronic brucellosis, and decisively outline the presence or not of any etiological relationship with CFS.

The clinical presentation of brucellosis being protean, in the event of a deliberate release a clinical diagnosis might not be easily achieved. Most patients would experience a constellation of symptoms also pointing towards other pathogens, such as *Francisella tularensis, Coxiella burnetii* and several viruses. Yet establishing of a clinical diagnosis would be largely related to clinicians' awareness of the disease, which in turn is influenced by the effectiveness of educational programs on bioterrorism and the endemicity of the agent in the targeted area. A recent educational US program including a multiple-choice questionnaire outlined this fact: 15% of participating physicians wrongly diagnosed brucellosis in a patient presenting with severe pneumonia. A co-resident of the patient had died 4 days earlier with hemoptysis and dyspnea [37], and the physicians thus attributed to brucellosis a predominantly respiratory distress course and the capacity for person-to-person transmission (more worrying, though, was the fact that only 15% accurately diagnosed pneumonic plague, which was the correct diagnosis). Although most imported cases in the developed, brucellosis-free world can be accurately traced to travel in, or importation of food from, endemic areas [38], this relationship is not always evident. Even in endemic areas, a minority of patients (14%) could not readily identify the source of the infection [39]. A diagnosis of brucellosis in a patient from a non-endemic area in the absence of specific risk factors for acquisition of the disease should lead at least to enhanced awareness for the following days, in order to readily identify an evolving trend and respond adequately and rapidly.

Diagnosis

Isolating the organism remains the gold diagnostic standard, although blood culture positivity is reported to vary widely [40], and bone marrow aspiration and culture, considered by some as extremely sensitive [41], remains an invasive, painful procedure. Moreover, in the context of an outbreak, the traditionally protracted period needed for species culture and identification (ranging from 3 days to 6 weeks), means that other diagnostic procedures should be sought.

Serology, in the form of various agglutination tests targeting surface antigens, and enzyme-linked immunosorbent assay (ELISA), targeting other bacterial antigens [42], is extremely useful; sensitivity and specificity are well above 85% for both approaches. Drawbacks of serum agglutination tests include false-negative results of varying etiology (delayed seroconversion, blocking antibodies, prozone phenomenon) and cross-reaction-induced false positive results, and the inability to serologically follow patients up due to protracted persistence of increased antibody titers. ELISA is more sensitive, and a diagnostic procedure of choice for cerebrospinal fluid specimens in neurobrucellosis, but evolution of antibody titers in follow-up and detection of relapses are still troublesome.

Various polymerase chain reaction (PCR) assays have been developed but clinical studies are limited [43].

Moreover- real-time PCR (rtPCR) is now emerging as an important diagnostic tool [44, 45]. Specific PCR assays for field detection of significant bioterror pathogens have also been developed by the military [46].

rtPCR may offer a rapid (less than an hour), exquisitely sensitive and specific diagnosis in a deliberate release outbreak, although traditional serology might be more suitable as a diagnostic tool in such a situation: the trend would be rapidly recognized. (Even when taking into account false negatives, the majority of patients will seroconvert, and even in an endemic area, high titers could not be attributed to previous contact with the pathogen. On the other hand, false positives would be extremely unlikely, and should be entertained only as a possible problem in a deliberate release of *F. tularensis*, where a false positive diagnosis of brucellosis might steer response in a wrong direction). Microorganism isolation for further characterization and recognition of any genetic modifications would of course remain paramount in the overall response.

Treatment

Various principles apply to brucellosis treatment: the organism hides inside macrophages which requires antibiotics with adequately intracellular penetration. Moreover, these antibiotics need to be active in the acidic environment where the bacteria reside. The optimal treatment is a combination regimen, since monotherapy has been traditionally associated with an increased percentage of treatment failure and relapse [46, 47]. Duration of treatment also matters [48], and 6-week regimens are associated with an acceptable percentage of relapses. The World Health Organization (WHO) endorses regimens that combine doxycycline, 100 mg b.i.d., and rifampicin, 600–1200 mg daily, for 6 weeks, or doxycycline for 6 weeks and streptomycin, 15 mg/kg daily, for 2–3 weeks. The latter combination is considered superior [49], but demands parenteral administration. Gentamicin can adequately replace streptomycin, at a dose of 5 mg/kg for 5–7 days. Alternatives include trimethoprim-sulfamethoxazole in various combinations, and combinations including ofloxacin or ciprofloxacin. Quinolone-containing regimens are generally adequate, but cost-effectiveness and the possibility of community resistance are issues to be considered [50]. Triple or quadruple protracted regimens should be used in serious complications, in conjunction with invasive procedures, as indicated. Rifampicin and trimethoprim-sulfamethoxazole are the mainstays of treatment in pregnancy and pediatric populations, respectively. Future options may incorporate adjuvants aiming at altering the acidic intracellular environment or new antibiotics [51].

The development of a vaccine for brucellosis suitable for humans would be an ideal solution to the problems of inadequate veterinary control of animal disease, inadequate epidemiological study of human disease and inadequate antibiotic treatment. The absence of such a vaccine underlines the absence of interest in a common, albeit usually non-fatal, zoonosis, at least in areas with adequate scientific and financial tools for such development and the still incomplete knowledge about important steps of the molecular pathogenesis of brucellosis.

Numerous vaccines have been tested in the past; none of them have gained wide acceptance [52]. An intradermally administered vaccine derived from *B. abortus* 19 strain has been used extensively in the Asian Republics of the former Soviet Union, causing a 5–11 fold reduction in the annually reported cases of human brucellosis. Still, the vaccine offers limited protection of short duration and requires booster doses. Moreover, an increased number of hypersensitivity reactions were reported, with 76% local reactions and 3–7% generalized adverse effects [53].

Another vaccine used in the same area, similar to the previous but administered intramuscularly, appeared to evoke minimal reactions and similar protection. Its reported efficacy after 75,000 doses performed in Kazhakstan reached 79%. Strains of *B. abortus* 84-C and 104-M have been utilized for intradermal injection or inhalation in the former Soviet Union and in China, respectively [54]. The vaccines were considered effective but of high risk for serious adverse reactions. The French experience with a vaccine utilizing a phenol-insoluble peptidoglycan fraction of *B. melitensis* strain M-15 raised questions in the past about its efficacy. The vaccine was administered in two subcutaneous doses and supposedly offered protection for a 2-year period [55]. Efficacy of other vaccines that could be considered for humans has been proven in animals: the various preparations include a lipopolysaccharide-protein conjugate, a purified protein antigen L7/L12, Cu-Zn SOD, and glyceraldehyde-dehydrogenase. Theoretical vaccine targets for the future include the RB51 strain (although it induces minimal human disease, which is rifampicin resistant) [56], purE mutants (that still have significant residual virulence), rfbK mutants of *B. melitensis*, Omp 19, Omp 28, and the cytoplasmic protein BP-26 [57].

Yet even if developed, the efficacy of a human vaccine in the setting of a deliberate release outbreak would be minimal. Sufficient prophylaxis in such a case would demand pre-emptive vaccine administration, since following exposure, and despite the prolonged incubation period of the disease, antibody production would not be brisk enough. Antibiotics would be the only option in such a case. The use of antibiotic prophylaxis for asymptomatic persons exposed to *Brucella* is an inadequately studied issue [58];

most data derive from accidental laboratory exposure. In general, the most prudent approach would be to follow up for seroconversion, and subsequently treat, even when no symptoms appear. With this strategy, prophylaxis administration would be minimized, and persons at risk of developing brucellosis (the ones seroconverting) would be adequately treated. Another option would be to withhold antibiotics in the absence of any symptoms, even for persons exhibiting seroconversion. But the insidious course of the disease would leave these patients at risk of developing brucellosis for practically the rest of their life.

Environmental implications

A deliberate release outbreak of brucellosis would obviously have profound environmental effects, their range depending on characteristics of the targeted area. Areas whose economy is largely based on animal productivity would suffer the most, due to massive loss of livestock and diminished trade in dairy products in the future. Even though the overall financial burden of health services would be lower than most other potential biological weapons [59], the long-term effect on the targeted region's economy would be profound, more so when taking into account environmental pollution, which has the potential for secondary airborne community outbreaks.

Attack scenarios

The art of developing attack scenarios, and in that manner outlining problems that might emerge in a real-life situation, has been inadequately explored. This is particularly important since the same attack would have different outcomes in different targeted areas, depending on numerous characteristics. Even the public response would be different, depending on awareness, and endemicity. A traditional attack scenario [60] projected minimal fatalities in a *Brucella* attack under 'optimal' circumstances. Yet when a similar scenario was transcribed in an endemic area, the projected outcome was far less morbid [61]. In this latter scenario, an attack in an endemic city of 100,000 inhabitants would result in only two deaths, and the level of social disruption would be minimal, due to public awareness of the pathogen.

Conclusions

The importance of *Brucella* as a biological weapon may only be historical nowadays, due to its minimal mortality and protracted inoculation period. Yet our times have taught us that everything is possible. Educating physicians and public alike, walking the fine line between inadequate awareness and fear of a 'nasty bug', might help in limiting unnecessary interventions, in determining areas of research that need to be addressed, and in creating a web of response that is adequate yet not overly restrictive for scientists.

1 Centers for Disease Control and Prevention. Emergency Preparedness and Response. Bioterrorism agents/diseases. Accessed. June. 29, 2006, at http://www.bt.cdc.gov/agent/agentlist-category.asp
2 National Institute of Allergy and Infectious Diseases Biodefense Research. NIAID Biodefense Agenda for CDC Category B and C Priority Pathogens. Accessed June 29, 2006, at http://www3.niaid.nih.gov/Biodefense/Research/categorybandc.pdf
3 Pappas, G., Akritidis, N., Bosilkovski, M. and Tsianos, E. (2005) Brucellosis. N. Engl. J. Med. 352, 2325–2336.
4 Sohn, A. H., Probert, W. S., Glaser, C. A., Gupta, N., Bollen, A. W., Wong, J. D., Grace, E. M. and McDonald, W. C. (2003) Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. Emerg. Infect. Dis. 9, 485–488.
5 DelVecchio, V. G., Kapatral, V., Redkar, R. J., Patra, G., Mujer, C., Los, T., Ivanova, N., Anderson, I., Bhattacharyya, A., Lykidis, A. et al. (2002) The genome sequence of the facultative intracellular pathogen *Brucella* melitensis. Proc. Natl. Acad. Sci. USA 99, 443–448.
6 Sanchez, D. O., Zandomeni, R. O., Cravero, S., Verdun, R. E.,Pierrou, E., Faccio, P., Diaz, G., Lanzavecchia, S., Aguero, F., Frasch, A. C. et al. (2001) Gene discovery through genomic sequencing of *Brucella* abortus. Infect. Immun. 69, 865–868.
7 Paulsen, I. T., Seshadri, R., Nelson, K. E., Eisen, J. A., Heidelberg, J. F., Read, T. D., Dodson, R. J., Umayam, L., Brinkac, L. M., Beanan, M. J. et al. (2002) The *Brucella* suis genome reveals fundamental similarities between animal and plant pathogens and symbionts. Proc. Natl. Acad. Sci. USA 99, 13148–13153.
8 Michaux-Charachon, S., Jumas-Bilak, E., Allardet-Servent, A., Bourg, G., Boschiroli, M. L., Ramuz, M. and O'Callaghan, D. (2002) The *Brucella* genome at the beginning of the post-genomic era. Vet. Microbiol. 90, 581–585.
9 Godfroid, J. and Kasbohrer, A. (2002) Brucellosis in the European Union and Norway at the turn of the twenty-first century. Vet. Microbiol. 90, 135–145.
10 Pope, C. Feel safer now? Huffington Post. Accessed June 29, 2006, at http://www.huffingtonpost.com/carl-pope/feeling-safer-now_b_15589.html
11 Capasso, L. (2002) Bacteria in two-millennia-old cheese, and related epizoonoses in Roman populations. J. Infect. 45, 122–127.
12 Vassallo, D. J. (1992) The corps disease: brucellosis and its historical association with the Royal Army Medical Corps. J. R. Army Med. Corps 138, 140–150.
13 Christopher, G. W., Agan, M. B., Cieslak, T. J. and Olson, P. E. (2005) History of U. S. military contributions to the study of bacterial zoonoses. Mil. Med. 170, Suppl. 39–48.
14 Alibeck, K. (1999) Biohazard. Hutchinson, London.
15 Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E. V. (2006) The new global map of human brucellosis. Lancet Infect. Dis. 6, 91–99.
16 Doyle, T. J. and Bryan, R. T. (2000) Infectious disease morbidity in the US region bordering Mexico, 1990–1998. J. Infect. Dis. 182, 1503–1510.
17 Center for Disease Control and Prevention (2000) Suspected brucellosis case prompts investigation of possible bioterrorism-related activity – New Hampshire and Massachusetts, 1999. MMWR 49, 509–512.

18 Gorvel, J. P. and Moreno, E. (2002) *Brucella* intracellular life: from invasion to intracellular replication. Vet. Microbiol. 90, 281–297.
19 Yingst, S. and Hoover, D. L. (2003) T cell immunity to brucellosis. Crit. Rev. Microbiol. 29, 313–331.
20 Yagupsky, P. and Baron, E. J. (2005) Laboratory exposures to brucellae and implications for bioterrorism. Emerg. Infect. Dis. 11, 1180–1185.
21 Pappas, G., Akritidis, N. and Tsianos, E. V. (2005) Attack scenarios with Rickettsial species. Implications for response and management. Ann. N.Y. Acad. Sci 163, 451–458.
22 Casadevall, A. and Pirofski, L. A. (2004) The weapon potential of a microbe. Trends Microbiol. 12, 259–263.
23 Colmenero, J. D., Reguera, J. M., Martos, F., Sanchez-De-Mora, D., Delgado, M., Causse, M., Martin-Farfan, A. and Juarez, C. (1996) Complications associated with *Brucella* melitensis infection: a study of 530 cases. Medicine (Baltimore) 75, 195–211. Erratum in: (1997) Medicine (Baltimore) 76, 139.
24 Saltoglu, N., Tasova, Y., Midikli, D., Akhsu, H. S., Sanli, A. and Dundar, I. H. (2004) Fever of unknown origin in Turkey: evaluation of 87 cases during a nine-year-period of study. J. Infect. 48, 81–85.
25 Solera, J., Lozano, E., Martinez-Alfaro, E., Espinosa, A., Castillejos, M. L. and Abad, L. (1999) Brucellar spondylitis: review of 35 cases and literature survey. Clin. Infect. Dis. 29, 1440–1449.
26 Pappas, G., Seitaridis, S., Akritidis, N. and Tsianos, E. (2004) Treatment of *Brucella* spondylitis: lessons from an impossible meta-analysis and initial report of efficacy of a fluoroquinolone-containing regimen. Int. J. Antimicrob. Agents 24, 502–507.
27 Akritidis, N., Mastora, M. and Pappas, G. (2002) Genitourinary complications of Brucellosis. Infect. Med. 19, 384–386.
28 Akritidis, N. and Pappas, G. (2001) Ascites caused by brucellosis: a report of two cases. Scand. J. Gastroenterol. 36, 110–112.
29 Shakir, R. A., Al-Din, A. S., Araj, G. F., Lulu, A. R., Mousa, A. R. and Saadah, M. A. (1987) Clinical categories of neurobrucellosis. A report on 19 cases. Brain 110, 213–223.
30 Hadjinikolaou, L., Triposkiadis, F., Zairis, M., Chlapoutakis, E. and Spyrou, P. (2001) Successful management of *Brucella* mellitensis endocarditis with combined medical and surgical approach. Eur. J. Cardiothorac. Surg. 19, 806–810.
31 Pappas, G., Bosilkovski, M., Akritidis, N., Mastora, M., Krteva, L. and Tsianos, E. (2003) Brucellosis and the respiratory system. Clin Infect. Dis 37, e95–e99.
32 Sevinc, A., Buyukberber, N., Camci, C., Buyukberber, S. and Karsigil, T. (2005) Thrombocytopenia in brucellosis: case report and literature review. J. Natl. Med. Assoc. 97, 290–293.
33 Khan, M. Y., Mah, M. W. and Memish, Z. A. (2001) Brucellosis in pregnant women. Clin. Infect. Dis. 32, 1172–1177.
34 Lubani, M. M., Dudin, K. I., Sharda, D. C., Ndhar, D. S., Araj, G. F., Hafez, H. A., al-Saleh, O. A., Helin, I. and Salhi, M. M. (1989) A multicenter therapeutic study of 1100 children with brucellosis. Pediatr. Infect. Dis. J. 8, 75–78.
35 Moreno, S., Ariza, J., Espinosa, F. J., Podzamczer, D., Miro, J. M., Rivero, A., Rodriguez-Zapata, M., Arizzabalaga, J., Mateos, R. and Herrero, F. (1998) Brucellosis in patients infected with the human immunodeficiency virus. Eur. J. Clin. Microbiol. Infect. Dis. 17, 319–326.
36 Murrell, T. G. and Matthews, B. J.(1990) Multiple sclerosis – one manifestation of neurobrucellosis? Med. Hypotheses 33, 43–48.
37 Cosgrove, S. E., Perl, T. M., Song, X. and Sisson, S. D. (2005) Ability of physicians to diagnose and manage illness due to category A bioterrorism agents. Arch. Intern. Med. 165, 2002–2006.
38 Al Dahouk, S., Nockler, K., Hensel, A., Tomaso, H., Scholz, H. C., Hagen, R. M. and Neubauer, H. (2005) Human brucellosis in a nonendemic country: a report from Germany, 2002 and 2003. Eur. J. Clin. Microbiol. Infect. Dis. 24, 450–456.
39 Pappas, G., Siozopoulou, V., Saplaoura, K., Vasiliou, A., Christou L, Akritidis, N. and Tsianos, E. V. (2006) Health literacy in the field of infectious diseases: the paradigm of brucellosis. J. Infect. Mar. 10 [Epub ahead of print].
40 Al Dahouk, S., Tomaso, H., Nockler, K., Neubauer, H. and Frangoulidis, D. (2003) Laboratory-based diagnosis of brucellosis – a review of the literature. Part I: Techniques for direct detection and identification of *Brucella* spp. Clin. Lab. 49, 487–505.
41 Gotuzzo, E., Carrillo, C., Guerra, J. and Llosa, L. (1986) An evaluation of diagnostic methods for brucellosis – the value of bone marrow culture. J. Infect. Dis. 153, 122–125.
42 Al Dahouk, S., Tomaso, H., Nockler, K., Neubauer, H. and Frangoulidis, D. (2003) Laboratory-based diagnosis of brucellosis – a review of the literature. Part II: serological tests for brucellosis. Clin. Lab. 49, 577–589.
43 Navarro, E., Casao, M. A. and Solera, J. (2004) Diagnosis of human brucellosis using PCR. Expert Rev. Mol. Diagn. 4, 115–123.
44 Queipo-Ortuno, M. I., Colmenero, J. D., Baeza, G. and Morata, P. (2005) Comparison between LightCycler Real-Time Polymerase Chain Reaction (PCR) assay with serum and PCR-enzyme-linked immunosorbent assay with whole blood samples for the diagnosis of human brucellosis. Clin. Infect. Dis. 40, 260–264.
45 Vrioni, G., Priavali, E., Pappas, G., Gartzonika, C., Kostoula, A., Boboyanni, H., Pappa, C., Stafanou D. and Levidiotou, S. (2005) Real-time PCR assay for detection of *Brucella* DNA in clinical samples from patients with suspected brucellosis. J. Chemother. 17, Suppl. 26.
46 Pappas, G., Akritidis, N. and Tsianos, E. (2005) Effective treatments in the management of brucellosis. Expert Opin. Pharmacother. 6, 201–209.
47 Solera, J., Martinez-Alfaro, E. and Espinoza, A. (1997) Recognition and optimum treatment of brucellosis. Drugs 53, 245–256.
48 Solera, J., Geijo, P., Largo, J., Rodriguez-Zapata, M., Gijon, J., Martinez-Alfaro, E., Navarro, E., Macia, M. A. and Grupo de Estudio de Castilla-la Mancha de Enfermedades Infecciosas (2004) A randomized, double-blind study to assess the optimal duration of doxycycline treatment for human brucellosis. Clin. Infect. Dis. 39, 1776–1182.
49 Solera, J., Martinez-Alfaro, E. and Saez, L. (1994) [Meta-analysis of the efficacy of the combination of rifampicin and doxycycline in the treatment of human brucellosis.] Med. Clin. 102, 731–738.
50 Falagas, M. E. and Bliziotis, I. A. (2006) Quinolones for treatment of human brucellosis: critical review of the evidence from microbiological and clinical studies. Antimicrob. Agents Chemother. 50, 22–33.
51 Pappas, G., Solera, J., Akritidis, N. and Tsianos, E. V. (2005) New approaches to the antibiotic treatment of brucellosis. Int. J. Antimicrob. Agents 26, 101–105.
52 Schurig, G. G., Sriranganathan, N. and Corbel, M. J. (2002) Brucellosis vaccines: past, present and future. Vet. Microbiol. 90, 479–496.
53 Young, E. J and Corbel, M. J. (1989) Brucellosis: Clinical and Laboratory Aspects, CRC Press.
54 Deqiu, S., Donglou, X. and Jiming, Y. (2002) Epidemiology and control of brucellosis in China. Vet. Microbiol. 90, 165–182.
55 Hadjichristodoulou, C., Voulgaris, P., Toulieres, L., Babalis, T., Manetas, S., Goutziana, G., Kastritis, I. and Tselentis, I. (1994) Tolerance of the human brucellosis vaccine and the intradermal reaction test for brucellosis. Eur. J. Clin. Microbiol. Infect. Dis. 13, 129–134.
56 Ashford, D. A., di Pietra J,. Lingappa, J., Woods, C., Noll, H., Neville, B., Weyant, R., Bragg, S. L., Spiegel, R. A., Tappero, J.

and Perkins, B. A. (2004) Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51. Vaccine 22, 3435–3439.
57 Ko, J. and Splitter, G. A. (2003) Molecular host-pathogen interaction in brucellosis: current understanding and future approaches to vaccine development for mice and humans. Clin. Microbiol. Rev. 16, 65–78.
58 Bossi, P., Tegnell, A., Baka, A., Van Loock, F., Hendriks, J., Werner, A., Gouvras G., Task Force on Biological and Chemical Agent Threats, Public Health Directorate, European Commission, Luxembourg (2004) Bichat guidelines for the clinical management of brucellosis and bioterrorism-related brucellosis. Euro Surveill. 9, E15–E16.
59 Kaufmann, A. F., Meltzer, M. I. and Schmid, G. P. (1997) The economic impact of a bioterrorist attack: are prevention and postattack intervention programs justifiable? Emerg. Infect. Dis. 3, 83–94.
60 World Health Organization (1970) Health Aspects of Chemical and Biological Weapons: Report of a WHO Group of Consultants. World Health Organization, Geneva, Switzerland.
61 Pappas, G. and Akritidis, N. (2001) A scenario of bioterrorism in the Balkans. Pharmacother 21, Suppl. 1, 47.