Policy Forum

Perspectives for the Treatment of Brucellosis in the 21st Century: The Ioannina Recommendations

Javier Ariza, Mile Bosilkovski, Antonio Cascio, Juan D. Colmenero, Michael J. Corbel, Matthew E. Falagas, Ziad A. Memish, Mohammad Reza Hasanjani Roushan, Ethan Rubinstein, Nikolaos V. Sipsas, Javier Solera, Edward J. Young, Georgios Pappas[*]

Brucellosis is probably the commonest anthropozoonotic infection worldwide [1–3], but remains in various aspects an enigma in the 21st century [4]. *Brucella melitensis* remains the major cause of human disease worldwide, followed by *B. abortus* and *B. suis*, while rare but persisting cases of *B. canis* human infection and disease by novel *Brucella* pathogens of marine mammals have also emerged. The disease is re-emerging as a significant cause of travel-related disease [5] and represents an index of poor socioeconomic status (Figure 1). Its treatment is largely based even today on the principles applied half a century ago by pioneer researchers [6] and few modifications have been made in the following years, despite the emergence of new antibiotic classes and different therapeutic approaches [7].

The World Health Organization (WHO) issued recommendations for the treatment of human brucellosis in 1986 [8], suggesting the use of doxycycline, 100 mg twice daily for six weeks combined with either rifampicin, 600–900 mg daily for six weeks, or streptomycin, 1 g daily for 2–3 weeks. During the following years, a number of clinical studies assessed the efficacy of different regimens. Furthermore, reports from various regions of the world revealed that the WHO-recommended regimens have not been universally applied in clinical practice. More importantly, these regimens still allow for a small, albeit significant percentage of therapeutic failures, most commonly in the form of relapses, ranging from 5% to 15% of uncomplicated cases. Although

Summary Points

- Brucellosis remains the commonest anthropozoonosis worldwide, and its treatment remains complex, requiring protracted administration of more than one antibiotic.

- In November 2006, a consensus meeting aimed at reaching a common specialist statement on the treatment of brucellosis was held in Ioannina, Greece under the auspices of the International Society of Chemotherapy and the Institute of Continuing Medical Education of Ioannina.

- The author panel suggests that the optimal treatment of uncomplicated brucellosis should be based on a six-week regimen of doxycycline combined either with streptomycin for 2–3 weeks, or rifampicin for six weeks. Gentamicin may be considered an acceptable alternative to streptomycin, while all other regimens/combinations should be considered second-line.

- The development of a common global therapeutic language for human brucellosis, and future, properly conducted clinical trials would definitely solve controversies regarding the disease.

these relapses are usually mild and can be treated successfully with the same regimens, they represent a significant morbidity factor. Risk factors for relapse have been assessed [9,10], but it remains unclear what is the best regimen to be used in their presence.

Another controversial subject in brucellosis treatment has been the duration of therapy: If the treatment is prolonged, the risk of relapse progressively decreases. Obviously, defining an acceptable relapse rate based on the duration of the schedule used is somewhat arbitrary.

Javier Ariza is with the Servicio de Enfermedades Infecciosas, Hospital de Bellvitge, Universidad de Barcelona, Barcelona, Spain. Mile Bosilkovski is with the Clinic for Infectious Diseases and Febrile Conditions, Clinical Center, Skopje, Former Yugoslav Republic of Macedonia. Antonio Cascio is with the Scuola di Specializzazione in Malattie Infettive, Dipartimento di Patologia Umana, Universita di Messina, Messina, Italy. Juan D. Colmenero is with the Infectious Diseases Service, Carlos Haya University Hospital, Malaga, Spain. Michael J. Corbel is with the Division of Bacteriology, National Institute for Biological Standards and Control, Hertfordshire, United Kingdom. Matthew E. Falagas is with the Alfa Institute of Biomedical Sciences (AIBS), Athens, Greece, and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts, United States of America. Ziad A. Memish is with the Department of Infection Prevention and Control, King Fahad National Guard Hospital, Riyadh, Saudi Arabia. Mohammad Reza Hasanjani Roushan is with the Department of Infectious Diseases, Yahyanejad Hospital, Babol Medical University, Babol, Iran. Ethan Rubinstein is with the Section of Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada. Nikolaos V. Sipsas is with the Pathophysiology Department, Laikon General Hospital and Medical School, National and Kapodistrian University of Athens, Athens, Greece. Javier Solera is with the Servicio de Medicina Interna, Hospital General Universitario, Albacete, Spain. Edward J. Young is with the Medical Services, Veterans Affairs Medical Center, Houston, Texas, United States of America. Georgios Pappas is with the Institute of Continuing Medical Education of Ioannina, Ioannina, Greece, and the Working Group on Zoonoses, International Society of Chemotherapy, London, United Kingdom.

[*] To whom correspondence should be addressed. E-mail: gpele@otenet.gr
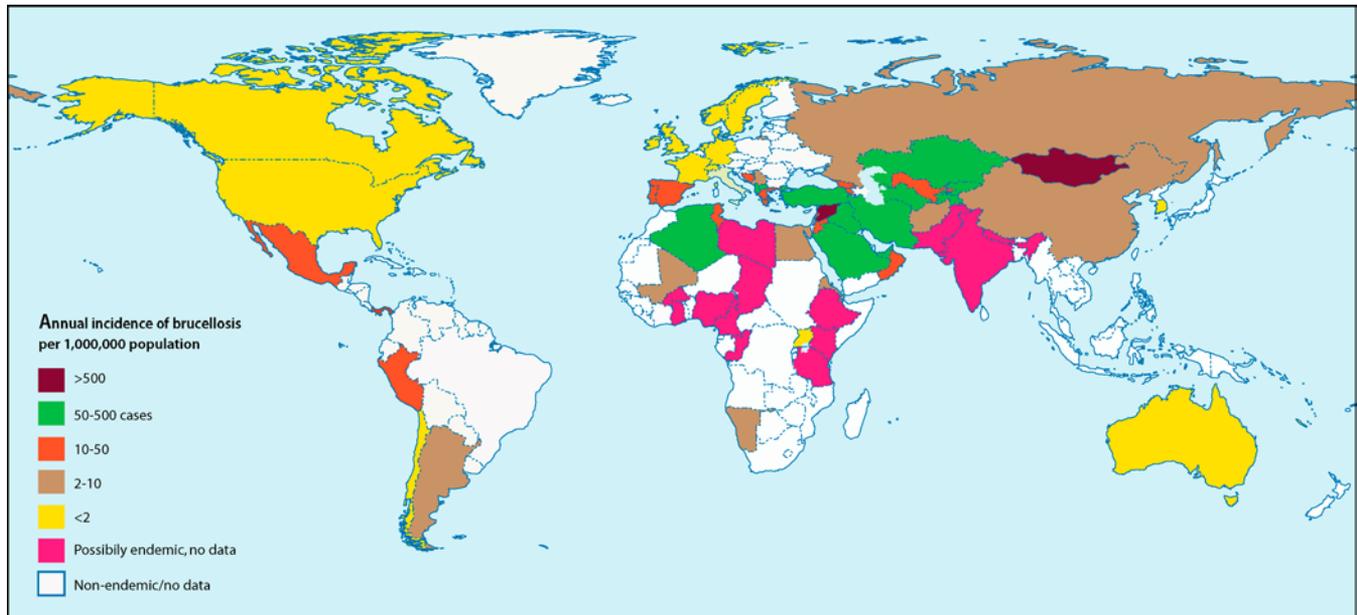
Figure 1. The Global Incidence of Human Brucellosis

Reproduced from: Gutierrez Ruiz C, Miranda JJ, Pappas G (2006) A 26-year-old man with sternoclavicular arthritis. PLoS Med 3(8): e293. doi:10.1371/journal.pmed.0030293 Derived from: Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV (2006) The new global map of human brucellosis. Lancet Infect Dis 6: 91-99.

In an era of rapid emergence of antimicrobial resistance, controversies regarding the prolonged use of antibiotics with established activity against Brucella pose special problems. In some endemic regions, especially in the developing world, brucellosis and tuberculosis coexist in the same communities. So for example, for those medications active against tuberculosis that are also used for the treatment of brucellosis, such as rifampicin, there is an increased potential in the community for emergence of mycobacterial resistance to these antimicrobials because of their use against brucellosis.. The public health significance of such resistance may be far larger than the cumulative brucellosis morbidity [11]. Finally, it should be kept in mind that the majority of human and animal brucellosis cases occur in resource-deprived countries of the developing world. Therefore, the cost of the new, sophisticated antimicrobials should be taken into account when designing an effective therapy for a large number of patients.

Development of the "Ioannina Recommendations"

In November 2006, the 1st International Meeting on the Treatment of Human Brucellosis was held in Ioannina, Greece, co-organized by the Institute of Continuing Medical Education of Ioannina and the International Society of Chemotherapy, as part of the latter's Disease Management Series. The aims of the meeting were to: review the current situation of the disease worldwide; summarize the existing knowledge regarding the pathogenesis of risk factors for, and natural history of, brucellosis; review recent basic science and clinical developments in the field of diagnosis and treatment of brucellosis; and set the background for the production of a statement on the current optimal diagnosis and treatment options of human disease.

Selected experts were assigned to review all existing literature data, each on a specific aspect of human brucellosis treatment, and present them by analyzing the strength of evidence regarding each particular treatment option; a final session was held in order to set up the framework for the development of a common statement offering clinical perspectives for the 21st century. The statement was developed as a draft and re-circulated among the authors until a final form approved by all authors was reached. In developing the present therapeutic recommendations, guidelines of the Infectious Disease Society of America

have been used (Table 1). The current recommendations are summarized in Table 2.

A second position paper on the optimal diagnosis of the disease is currently under preparation.

Efficacy of the WHO-Recommended Regimens

A meta-analysis performed in 1995 [12] evaluated the efficacy of the two WHO-recommended regimens and concluded that the efficacy of the doxycycline-streptomycin (DOX-STR) regimen was superior to that of the doxycycline-rifampicin (DOX-RIF) regimen, as already suggested by previous studies [13–15]. Even if subsequent randomized therapeutic trials were to be added to a future meta-analysis, the slight superiority of DOX-STR is obvious. The superior efficacy of the DOX-STR regimen was further supported by pharmacokinetic data (lower doxycycline serum levels induced by co-administration of rifampicin) [16,17] and resistance-related data: prolonged administration of rifampicin for brucellosis may increase the resistance of *Mycobacterium tuberculosis* to this compound in endemic areas. Moreover, experimental data suggested that the development of mycobacterial resistance to rifampicin may lead to

Table 1. Infectious Diseases Society of America–United States Public Health Service Grading System for Ranking Recommendations in Clinical Guidelines

Category, Grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation for use; should always be offered
B	Moderate evidence to support a recommendation for use; should generally be offered
C	Poor evidence to support a recommendation; optional
D	Moderate evidence to support a recommendation against use; should generally not be offered
E	Good evidence to support a recommendation against use; should never be offered
Quality of evidence	
I	Evidence from ≥1 properly randomized, controlled trial
II	Evidence from ≥1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1 center); from multiple time-series; or from dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

doi:10.1371/journal.pmed.0040317.t001

development of resistance to other antimicrobials as well [18].

On the other hand, two recent studies have shown that the DOX-RIF regimen is preferred by both clinicians [19] and patients [20], even when they are aware of the relative superiority of the alternative regimen. The fact that DOX-RIF is an all-oral regimen may allow for better implementation in clinical practice in areas with less well-developed health infrastructure, because it eliminates the need for parenteral administration of streptomycin as part of the DOX-STR regimen. Furthermore, streptomycin shortage in numerous areas of the world may hamper the implementation of the DOX-STR regimen. Although evidence-based medicine would suggest that the DOX-STR regimen should always be offered, particularly in patients with complicated or serious forms of the disease, convenience, which can be translated into better adherence and overall success rates, forces the authors to suggest that DOX-RIF could be considered as an acceptable first-line regimen as well.

Regarding potential side-effects of these combinations, the authors suggest that the ototoxicity and nephrotoxicity of the aminoglycoside-containing regimens should be more thoroughly investigated in future randomized trials. The authors stress the need for inclusion of a tetracycline antibiotic in any therapeutic regimen for adult brucellosis. Various trials have been performed with combinations omitting such a compound, often reporting adequate results; yet understanding the pathophysiology of the disease

renders the inclusion of tetracyclines imperative.

Monotherapy

Monotherapy for brucellosis has generally been considered inadequate due to unacceptably high relapse rates. In older studies the use of tetracyclines as monotherapy was associated with varying relapse rates, ranging from 2% to 39% [21–26]. Nevertheless, these studies have major drawbacks: they were not randomized, they used doubtful diagnostic criteria, and the duration of treatment and adequacy of follow-up were widely varying. However, these criticisms do not apply to the excellent randomized comparative therapeutic trial performed by Montejo and colleagues [24]. This study showed that doxycycline monotherapy (100 mg twice daily for six weeks) is associated with relapse rates similar to the WHO-recommended regimen of DOX-RIF. The use of either trimethoprim-sulfamethoxazole (TMP-SMX) [24–31] or rifampicin [26,32] as monotherapy has also been reported in the past in adult brucellosis patients, with similarly varying results and similar reservations concerning the adequacy of the study design. A well-designed trial with TMP-SMX used for 45 days demonstrated a relapse rate of 46% [33].

Newer antibiotics such as ciprofloxacin and ceftriaxone have been tested as monotherapy in brucellosis in the past years, with disappointing results [34,35].

In the absence of definite data from randomized trials, the authors concluded, although not unanimously, that the available studies cannot convincingly support the use of

monotherapy in human brucellosis. The authors however strongly recommend carefully designed prospective randomized clinical trials assessing the efficacy and safety of tetracycline monotherapy in patients with low relapse risk according to published data on relapse risk factors [9,10]. However, future single-regimen trials with other antibiotic classes are not justified.

The Doxycycline-Gentamicin Regimen

Non-comparative studies on the efficacy of the combination of doxycycline and gentamicin (DOX-GENT) in brucellosis usually used shorter schedules of aminoglycosides than those of the standard regimens (i.e., 5–7 days for gentamicin) [12,36–38] with failure/relapse rates ranging from 10% to 20% (the latter when doxycycline treatment duration was also shortened), which exceeded failure rates during the WHO-recommended treatment by about 5%. In the only randomized trial available of doxycycline administered for six weeks in combination with gentamicin for seven days, relapse rates were at least comparable to those reported for the WHO-recommended regimens [36].

The authors consider the DOX-GENT combination to be an adequate regimen for the treatment of human brucellosis that offers advantages over DOX-STR, given the wider availability of gentamicin and the sparing of streptomycin, a valuable anti-tuberculosis agent. However, the duration of gentamicin treatment should be further evaluated in future randomized control trials, as the

Table 2. The recommendations of Ioannina on the Optimal Treatment of Brucellosis Without Serious Complications in Adults

Treatment Regimen	Dose	Recommendation Validation	Comments
DOX-STR	DOX: 100 mg twice daily orally for 6 weeks; STR: 15 mg/kg daily intramuscularly for 2–3 weeks	AI	Considered the "gold standard."
DOX-RIF	DOX: as above; RIF: 600–900 mg daily for 6 weeks, one morning dose	AI	Convenience of the regimen overcomes slight drawbacks concerning the pharmacokinetics of the combination and the overall outcome.
DOX-GENT	DOX: as above; GENT: 5 mg/kg daily parenterally in 1 dose for 7 days	BI	May be considered the preferred alternative regimen. Duration of GENT administration may need modification for optimal result (different studies suggest that it may be shortened to 5 days or extended to 14 days).
TMP-SMX-containing regimens	TMP-SMX: 800 + 160 mg twice daily for 6 weeks	CII	Recommendation referring to three-drug regimens containing DOX.
Quinolone-containing combination regimens	Ofloxacin: 400 mg twice daily for 6 weeks; ciprofloxacin: 500 mg twice daily for 6 weeks	CII	Ofloxacin or ciprofloxacin may be used alternatively as second or third agents in combination regimens containing DOX.

doi:10.1371/journal.pmed.0040317.t002

authors were divided on whether gentamicin administration should be prolonged.

The aminoglycoside-containing combinations raise concerns about the applicability of a parenteral regimen in resource-deprived countries, because of the need for an adequate health infrastructure (for example walk-in clinics, staffed with experienced personnel, to deliver intramuscular injections daily) to eliminate the necessity of hospitalization, which would increase the overall treatment cost.

Although there are no brucellosis-specific data, the existing data support the feasibility of once-daily aminoglycoside dosing [39]. Interventions with gentamicin-encapsulated microspheres have shown promise in animal models and may prove useful alternative options in the future [40].

Fluoroquinolone-Containing Regimens

Two recent reviews focused on fluoroquinolone-containing regimens for the treatment of brucellosis [41,42]. Both reviews, although using different approaches, conclude that combinations including fluoroquinolones can be acceptable alternatives, but do not recommend them as first-line options for human brucellosis treatment. According to the first review, the few proper randomized trials existing on the subject do not indicate the superiority or even non-inferiority of fluoroquinolone-containing regimens. The second review reports a cumulative response rate of above 85%, which is an adequate response; however, the currently higher cost of fluoroquinolone-containing regimens and the risk of enhancing the development of overall fluoroquinolone resistance in the community argue against wide use of fluoroquinolones for human brucellosis. The authors feel that in the future, if older fluoroquinolones become less expensive, further properly designed randomized trials may explore their potential in combined brucellosis treatment. There are no data, apart from sporadic case reports, on the efficacy of the newer fluoroquinolones in brucellosis, but in view of their unique efficacy against respiratory pathogens the authors do not recommend their routine use in brucellosis. Therefore, these antibiotics should be administered only in the context of properly designed prospective clinical trials.

TMP-SMX-Containing Regimens

TMP-SMX has been a popular choice, and was included in various combination regimens around the world, due to its significantly lower cost (compared to other antimicrobials included in treatment regimens for brucellosis), which rendered it the most cost-effective drug against brucellosis in certain developing world counties. Data on its interaction with rifampicin suggest a potential beneficial increase in the latter's serum levels [43], but a synergistic effect has not been proven [44,45]. TMP-SMX combination with rifampicin has proven successful in many reports of pediatric brucellosis. In adults, a recent randomized study suggested an adequate (but not superior) response rate comparable to the WHO-recommended regimens, although the treatment duration was extended [46]. Past studies have produced contradicting results, with relapse rates ranging from 0% to 30% (TMP-SMX combinations with tetracycline) [25,47].

TMP-SMX has been extensively used in triple combinations in some countries. The overall reported response rate was above 90% for the various TMP-SMX-containing combinations, although the absence of doxycycline from the regimen was related to an unacceptably high percentage of treatment failures (M. Bosilkovski, unpublished data). These data were derived from clinical studies which included patients with severely complicated disease; therefore extrapolation of these conclusions to patients with uncomplicated brucellosis is not easy. At present, the routine use of a triple regimen containing TMP-SMX cannot be advocated. However, the authors suggest that, depending on the regional overall failure rates of the WHO-recommended regimens, TMP-SMX may be used as an additional third (and only third) antibiotic.

One important issue regarding the use of TMP-SMX in brucellosis

treatment is the development of resistance in *B. melitensis*. Previous studies have suggested that TMP-SMX use has been associated with increasing *B. melitensis* resistance rates, sometimes reaching 62% [48,49]. Resistance rates may vary over time, reflecting the antibiotics' overall use for brucellosis [50]. If a DOX/TMP-SMX combination is to be considered for a future clinical trial, the problem of resistance to TMP-SMX should be seriously taken into account. (The well-known dissociation between in vitro activity and in vivo activity—i.e., therapeutic failure in vivo—in brucellosis refers only to drugs with in vitro activity and not in vivo, but not vice versa. This means that if in vitro activity is absent, for example as with most beta-lactams, clinical failure is guaranteed.) In future studies, isolated strains should be carefully studied for resistance to TMP-SMX and the other agents active against Brucella, and the relation of overall antibiotic use to the development of resistance should be analyzed in every region.

Other Tetracyclines and Related Compounds

A number of Italian studies have investigated the use of minocycline instead of doxycycline in the treatment of brucellosis. Early studies from the 1980s used minocycline either as monotherapy or in combination with intravenous rifampicin for short periods (2–4 weeks); both antimicrobials were delivered initially intravenously and subsequently orally. All of the above studies reported very low relapse rates, and were summarized in a recent article [51]. However, these data should be interpreted cautiously because the studies were retrospective and not comparative. The potential superiority of minocycline over doxycycline could be attributed to its increased bioavailability and the increased area under the curve of free minocycline compared to free doxycycline, as well as minocycline's greater lipophilicity, which allows for better tissue penetration [52]. The authors stress that, given wide anecdotal reports of lower adherence to minocycline treatment (compared to doxycycline), a combination including minocycline would have to prove far superior compared to doxycycline to merit further consideration.

Regarding oxytetracycline, at present it might be considered as a less convenient, in terms of multiple daily doses, but adequate alternative to doxycycline.

Tigecycline is the first glycylcycline antibiotic, a new member of the tetracycline family with chemical structure similar to that of minocycline. The drug has the ability to overcome the two major resistance mechanisms of tetracycline—drug-specific efflux pump acquisition and ribosomal protection—and is active against many gram-positive and gram-negative organisms [53]. The authors believe that the potential role of this new compound in the treatment of brucellosis may be hampered by problems such as the cost and the need for parenteral administration; a future clinical trial using this compound would need to show substantial superiority regarding efficacy and treatment duration in order to overcome these reservations. Future studies should also explore a possible role of tigecycline in the therapy of serious clinical forms of the disease requiring intravenous antibiotics.

Antibiotics Not Suitable for Brucellosis Treatment

Numerous other antibiotic classes have been clinically tested in the treatment of brucellosis. Azithromycin has been shown to be inadequate in a small but well-randomized trial [54]. Data from literature from Russia suggest a potential role for meropenem [55], yet the cost and importance of this agent for the treatment of more serious infections lead the authors to advocate against further clinical trials with this agent. However, its use might be considered in an individual hospitalized case as an alternative or as salvage therapy.

Special Situations

Treatment of brucellar spondylitis varies widely; one recent attempt at a meta-analysis of the existing data concluded that what matters for the outcome is the duration of treatment, and not the specific (recommended) regimen used [56]. However, many of the authors believe that some principles do apply in the treatment of brucellar spondylodiscitis: aminoglycoside-containing regimens may be superior to rifampicin-containing ones [57]; magnetic resonance imaging (MRI) of the spine should always be performed when there is clinical suspicion of spinal involvement irrespective of the rachideal level potentially affected; and the spine (especially if there is cervical involvement) should be immobilized in order to avoid devastating neurological complications. Although the previously mentioned meta-analysis did not conclude that a streptomycin-containing regimen may be superior, the authors suggest that the outcome of spondylitis may potentially be improved when such a regimen is used. According to certain authors this approach may be successful (in patients who have spondylitis only and not associated paravertebral or epidural abscess) even if duration of treatment is limited to the six weeks' therapeutic course used in uncomplicated brucellosis [58]. At present though, the available data support a longer duration of treatment of not less than three months [56,59]. Another important subject for future properly designed clinical trials would be the utility of MRI in the follow-up of these patients, since conflicting data exist on the subject, i.e., should treatment be continued until resolution of spinal MRI findings or not.

Neurobrucellosis is a term encompassing a wide spectrum of central nervous system clinical manifestations. Although central nervous system involvement is probably more common than originally considered, the paucity of therapeutic data preclude any recommendations to be offered for the time being.

Childhood brucellosis will be discussed in a separate statement currently being developed.

There are no randomized trials for brucellosis in pregnancy. The most extended series support the use of TMP-SMX alone or in combination with rifampicin [60]. The potential for adverse effects on the fetus from antimicrobials makes brucellosis in pregnancy a key situation where monotherapy trials are needed. For example, patients without risk factors for relapse or those without focal disease may be treated with rifampicin monotherapy (arguably the safer of all available antibiotics for brucellosis in pregnancy) until delivery. The advantage of minimizing the risks for fetal health is counterbalanced by the disadvantage of higher relapse rates;

however, in case of postpartum relapse, the patient can be treated with a standard regimen.

Brucellar endocarditis is a notorious complication with high mortality that has been reported fewer than 200 times in the literature; therefore no randomized data are available. Although a report has suggested that certain factors may allow for conservative treatment [61], the selection of antimicrobials remains empirical; in the majority of reported cases surgical intervention was necessary. The authors suggest that through a large, international database of brucellosis cases more endocarditis cases could be recorded, and the risk factors, pathophysiology, natural history, and clinical characteristics of this complication could be better characterized. This information would enable the design of an effective therapeutic approach.

Developing a Common Language

One of the major obstacles in the clinical research on brucellosis is the absence of properly designed and randomized therapeutic trials. Standards applying to typical bacterial infections cannot be easily implemented in brucellosis research; the concept of microbiological cure is vague in brucellosis. Moreover, the concept of relapse is in itself vague as well: is relapse a clinical failure? And if so, what is the required period of follow-up after treatment completion? The pathogen's virulence may also vary in different regions of the world: this variation is not only because of different *Brucella* strains (*B. melitensis* versus *B. abortus*, etc.), but also to different *B. melitensis* biovars that are endemic in each country. Important issues that should be addressed in the future include the optimization of treatment adherence, the monitoring for potential adverse effects, the importance of expert consultation in complicated disease, and the importance of a good relationship between patients and physicians, which is essential for a better prognosis, especially in cases of chronic brucellosis.

Regarding the nature of chronic disease: All attempts to categorize the disease have been hampered by the lack of agreement on terminology and the arbitrary nature of the designations. Nevertheless, many published works divide patients into acute versus chronic disease, often based on clinical complaints alone, or on some arbitrary number of days of symptoms. Although this classification does not usually affect the regimens used in various antibiotic treatment studies, the development of a common terminology is of paramount importance in order to better design effectively large, international, multicenter clinical studies. ∎

References

1. Corbel MJ (1997) Brucellosis: an overview. Emerg Infect Dis 3: 213-221.
2. Young EJ (1995) An overview of human brucellosis. Clin Infect Dis 21: 283-289.
3. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV (2006) The new global map of human brucellosis. Lancet Infect Dis 6: 91-99.
4. Ariza J (2002) Brucellosis in the 21st century. Med Clin (Barc) 119: 339-344.
5. Memish ZA, Balkhy HH (2004) Brucellosis and international travel. J Travel Med 11: 49-55.
6. Magoffin RL, Spink WW (1951) The protection of intracellular brucella against streptomycin alone and in combination with other antibiotics. J Lab Clin Med 37: 924-930.
7. Solera J, Martinez-Alfaro E, Espinosa A (1997) Recognition and optimum treatment of brucellosis. Drugs 53: 245-256.
8. [No authors listed] (1986) Joint FAO/WHO expert committee on brucellosis. World Health Organ Tech Rep Ser 740: 1-132.
9. Solera J, Martinez-Alfaro E, Espinosa A, Castillejos ML, Geijo P, et al. (1998) Multivariate model for predicting relapse in human brucellosis. J Infect 36: 85-92.
10. Ariza J, Corredoira J, Pallares R, Viladrich PF, Rufi G, et al. (1995) Characteristics of and risk factors for relapse of brucellosis in humans. Clin Infect Dis 20: 1241-1249.
11. Al-Hajjaj MS, Al-Kassimi FA, Al-Mobeireek AF, Alzeer AH (2001) Progressive rise of Mycobacterium tuberculosis resistance to rifampicin and streptomycin in Riyadh, Saudi Arabia. Respirology 6: 317-322.
12. Solera J, Martinez-Alfaro E, Saez L (1994) Meta-analysis of the efficacy of the combination of rifampicin and doxycycline in the treatment of human brucellosis. Med Clin (Barc) 102: 731-738.
13. Colmenero Castillo JD, Hernandez Marquez S, Reguera Iglesias JM, Cabrera Franquelo F, Rius Diaz F, et al. (1989) Comparative trial of doxycycline plus streptomycin versus doxycycline plus rifampin for the therapy of human brucellosis. Chemotherapy 35: 146-152.
14. Cisneros JM, Viciana P, Colmenero J, Pachon J, Martinez C, et al. (1990) Multicenter prospective study of treatment of *Brucella melitensis* brucellosis with doxycycline for 6 weeks plus streptomycin for 2 weeks. Antimicrob Agents Chemother 34: 881-883.
15. Ariza J, Gudiol F, Pallares R, Viladrich PF, Rufi G, et al. (1992) Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus streptomycin. A randomized, double-blind study. Ann Intern Med 117: 25-30.
16. Colmenero JD, Fernandez-Gallardo LC, Agundez JA, Sedeno J, Benitez J, et al. (1994) Possible implications of doxycycline-rifampin interaction for treatment of brucellosis. Antimicrob Agents Chemother 38: 2798-2802.
17. Garraffo R, Dellamonica P, Fournier JP, Lapalus P, Bernard E, et al. (1987) Effects of rifampicin on the pharmacodynamics of doxycycline. Pathol Biol (Paris) 35: 746-749.
18. Marianelli C, Ciuchini F, Tarantino M, Pasquali P, Adone R (2004) Genetic bases of the rifampin resistance phenotype in *Brucella* spp. J Clin Microbiol 42: 5439-5443.
19. Pappas G, Siozopoulou V, Akritidis N, Falagas ME (2007) Doxycycline-rifampicin: Physicians' inferior choice in brucellosis or how convenience reigns over science. J Infect 54: 459-462.
20. Pappas G, Siozopoulou V, Saplaoura K, Vasiliou A, Christou L, et al. (2007) Health literacy in the field of infectious diseases: the paradigm of brucellosis. J Infect 54: 40-45.
21. Hall WH (1990) Modern chemotherapy for brucellosis in humans. Rev Infect Dis 12: 1060-1099.
22. Farid Z, Miale A Jr, Omar MS, Van Peenen PF (1961) Antibiotic treatment of acute brucellosis caused by *Brucella melitensis*. J Trop Med Hyg 64: 157-163.
23. Feiz JM, Sabbaghian H, Sohrabi F (1973) A comparative study of therapeutic agents used for treatment of acute brucellosis. Br J Clin Pract 27: 410-413.
24. Montejo JM, Alberola I, Glez-Zarate P, Alvarez A, Alonso J, et al. (1993) Open, randomized therapeutic trial of six antimicrobial regimens in the treatment of human brucellosis. Clin Infect Dis 16: 671-676.
25. Mousa AR, Elhag KM, Khogali M, Marafie AA (1988) The nature of human brucellosis in Kuwait: study of 379 cases. Rev Infect Dis 10: 211-217.
26. Lulu AR, Araj GF, Khateeb MI, Mustafa MY, Yusuf AR, et al. (1988) Human brucellosis in Kuwait: a prospective study of 400 cases. Q J Med 66: 39-54.
27. Padrino JM, Roces A, Zubieta AJ, Morillas L, Castillo A (1986) Treatment of osteoarticular brucellosis with trimethoprim-sulfamethoxazole. Evaluation of 18 cases. Rev Clin Esp 178: 51-53.
28. Velasco AC, Gutierrez A, Rodriguez Noriega A (1978) Treatment of brucellosis with trimethoprim-sulfamethoxazole: evaauation of the results in 40 patients. Proceedings of the 1st Mediterranean Congress of Chemotherapy, Madrid 765-768.
29. Navarro-Martinez A, Solera J, Corredoira J, Beato JL, Martinez-Alfaro E, et al. (2001) Epididymoorchitis due to Brucella mellitensis: a retrospective study of 59 patients. Clin Infect Dis 33: 2017-2022.
30. Rodriguez-Torres A, Landinez R, Abad R (1984) Evaluation of the treatment of human brucellosis. Dev Biol Stand 56: 587-592.
31. Al-Rawi ZS, Al-Khateeb N, Khalifa SJ (1987) Brucela arthritis among Iraqi patients. Br J Rheumatol 26: 24-27.
32. Shehabi A, Shakir K, el-Khateeb M, Qubain H, Fararjeh N, et al. (1990) Diagnosis and treatment of 106 cases of human brucellosis. J Infect 20: 5-10.
33. Ariza J, Gudiol F, Pallares R, Rufi G, Fernandez-Viladrich P (1985) Comparative trial of co-trimoxazole versus tetracycline-streptomycin in treating human brucellosis. J Infect Dis 152: 1358-1359.
34. Doganaqy M, Aygen B (1992) Use of ciprofloxacin in the treatment of brucellosis. Eur J Clin Microbiol Infect Dis 11: 74-75.
35. Lang R, Dagan R, Potasman I, Einhorn M, Raz R (1992) Failure of ceftriaxone in the

treatment of acute brucellosis. Clin Infect Dis 14: 506-509.

36. Solera J, Espinosa A, Martinez-Alfaro E, Sanchez L, Geijo P, et al. (1997) Treatment of human brucellosis with doxycycline and gentamicin. Antimicrob Agents Chemother 41: 80-84.

37. Solera J, Geijo P, Largo J, Rodriguez-Zapata M, Gijon J, et al. (2004) A randomized, double-blind study to assess the optimal duration of doxycycline treatment for human brucellosis. Clin Infect Dis 39: 1776-1782.

38. Hasanjani Roushan MR, Mohraz M, Hajiahmadi M, Ramzani A, Valayati AA (2006) Efficacy of gentamicin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in humans. Clin Infect Dis 42: 1075-1080.

39. Hatala R, Dinh T, Cook DJ (1996) Once-daily aminoglycoside dosing in immunocompetent adults: a meta-analysis. Ann Intern Med 124: 717-725.

40. Lecaroz C, Blanco-Prieto MJ, Burrell MA, Gamazo C (2006) Intracellular killing of *Brucella melitensis* in human macrophages with microsphere-encapsulated gentamicin. J Antimicrob Chemother 58: 549-556.

41. Falagas ME, Bliziotis IA (2006) Quinolones for treatment of human brucellosis: critical review of the evidence from microbiological and clinical studies. Antimicrob Agents Chemother 50: 22-33.

42. Pappas G, Christou L, Akritidis N, Tsianos EV (2006) Quinolones for brucellosis: treating old diseases with new drugs. Clin Microbiol Infect 12: 823-825.

43. Malhi R, Uppal R, Sharma PL (1992) Drug interaction between rifampicin, isoniazid and cotrimoxazole in rabbits. Hum Exp Toxicol 11: 105-107.

44. Farrell W, Wilks M, Drasar FA (1977) The action of trimethoprim and rifampicin in combination against Gram-negative rods resistant to gentamicin. J Antimicrob Chemother 3: 459-462.

45. Alvarez S, DeMaria A Jr, Kulkarni R, Klein JO, McCabe WR (1982) Interactions of rifampin and trimethoprim in vitro. Rev Infect Dis 4: 390-401.

46. Roushan MR, Gangi SM, Ahmadi SA (2004) Comparison of the efficacy of two months of treatment with co-trimoxazole plus doxycycline vs. co-trimoxazole plus rifampin in brucellosis. Swiss Med Wkly 134: 564-568.

47. Al-Rawi TI, Thewaini AJ, Shawket AR, Ahmed GM (1989) Skeletal brucellosis in Iraqi patients. Ann Rheum Dis 48: 77-79.

48. Memish Z, Mah MW, Al Mahmoud S, Al Shaalan M, Khan MY (2000) Brucella bacteraemia: clinical and laboratory observations in 160 patients. J Infect 40: 59-63.

49. Kinsara A, Al-Mowallad A, Osoba AO (1999) Increasing resistance of Brucellae to co-trimoxazole. Antimicrob Agents Chemother 43: 1531.

50. Carrillo C, Franco V, Bazan L, Adachi J, Tolmos J, et al. (1995) In-vitro susceptibility of *Brucella melitensis* from 1967 to 1994 in Peru [abstract 4225]. 19th International Congress of Chemotherapy; 16-21 July 1995; Montreal, Canada.

51. Cascio A, Scarlata F, Giordano S, Antinori S, Colomba C, et al. (2003) Treatment of human brucellosis with rifampin plus minocycline. J Chemother 15: 248-252.

52. Saivin S, Houin G (1988) Clinical pharmacokinetics of doxycycline and minocycline. Clin Pharmacokinet 15: 355-366.

53. Rubinstein E, Vaughan D (2005) Tigecycline: a novel glycylcycline. Drugs 65: 1317-1336.

54. Solera J, Beato JL, Martinez-Alfaro E, Segura JC, de Tomas E, et al. (2001) Azithromycin and gentamicin therapy for the treatment of humans with brucellosis. Clin Infect Dis 32: 506-509.

55. Maletskaia OV (2002) Efficacy of some new antibiotics in treating experimental brucellosis. Antibiot Khimioter 47: 13-17.

56. Pappas G, Seitaridis S, Akritidis N, 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.

57. Solera J, Lozano E, Martinez-Alfaro E, Espinosa A, Castillejos ML, et al. (1999) Brucellar spondylitis: review of 35 cases and literature survey. Clin Infect Dis 29: 1440-1449.

58. Ariza J, Gudiol F, Valverde J, Pallares R, Fernandez-Viladrich P, et al. (1985) Brucellar spondylitis: A detailed analysis based on current findings. Rev Infect Dis 7: 656-664.

59. Colmenero JD, Reguera JM, Fernandez-Nebro A, Cabrera-Franquelo F (1991) Osteoarticular complications of brucellosis. Ann Rheum Dis 50: 23-26.

60. Khan MY, Mah MW, Memish ZA (2001) Brucellosis in pregnant women. Clin Infect Dis 32: 1172-1177.

61. Reguera JM, Alarcon A, Miralles F, Pachon J, Juarez C, et al. (2003) Brucella endocarditis: clinical, diagnostic, and therapeutic approach. Eur J Clin Microbiol Infect Dis 22: 647-560.

www.plosmedicine.org