Application of variable number of tandem repeats typing to describe familial outbreaks of brucellosis in Argentina

Nidia E. Lucero,¹ Marina Tenenbaum,² Nestor R. Jacob,³
Gabriela I. Escobar,¹ Pauline Groussaud⁴ and Adrian M. Whatmore⁴

Correspondence

Nidia E. Lucero
nidia@elsitio.net

¹Brucellosis Laboratory, National Laboratories and Institutes of Health Administration (ANLIS) 'Dr C. G. Malbrán', Avenida Velez Sarsfield 563, 1281 Buenos Aires, Argentina

²Department of Infectology, Pediatric Hospital 'Dr Ricardo Gutierrez', Gallo 1330, 1425 Buenos Aires, Argentina

³Department of Infectology, Hospital 'Dr Cosme Argerich', Margall 750, 1155 Buenos Aires, Argentina

⁴Brucellosis Reference Unit, Department of Statutory and Exotic Bacterial Diseases, Veterinary Laboratories Agency, Woodham Lane, Addlestone, Surrey, UK

Consumption of inadequately pasteurized dairy products is the most common means of transmission of brucellosis. This report describes two foodborne outbreaks that occurred in families infected after consumption of fresh home-made cheese bought in different Argentine provinces. High resolution variable number of tandem repeats (VNTR)-based analysis revealed two well-defined groups comprising essentially identical profiles and corresponding to the two different outbreaks. Similar clinical findings in members of the same family could indicate that the differential virulence of different bacterial clones, as indicated by VNTR data, could have influenced the course of the disease. We observed the importance of adequate treatment in early stages of the disease; combination therapy and extended treatment for 6 weeks or longer yielded significantly better results. The risk of the foodborne transmission of this zoonotic disease and disease prevention should be considered.

Received 12 November 2009

Accepted 12 February 2010

INTRODUCTION

Brucella spp. are mainly transmitted to humans through direct contact with infected animals, laboratory accidents or ingestion of contaminated dairy products. Airborne transmission has been considered a risk because of its use as a biological weapon (Yagupsky & Baron, 2005). However, consumption of inadequately pasteurized dairy products is the most common mode of transmission, especially via milk, soft cheese, butter and ice cream. Hard cheese, yoghurt and buttermilk are less hazardous since both propionic acid and lactic acid fermentation take place (Pappas *et al.*, 2005). Fresh cheese home-made by small scale local producers has been demonstrated to be an important route of transmission and a risk for public health (Mendez Martinez *et al.*, 2003; Castell Monsalve *et al.*, 1996). Such soft cheese is prepared by a method that does not kill the *Brucella*, which may persist in the finished product for up to 1.5 months in conditions of low acidity and temperatures between 11 and 14 °C (Elberg, 1981) or for 48–72 h at 37 °C (Corbel, 2006).

Abbreviations: CDC, Centers for Disease Control and Prevention; TMS, trimethoprim–sulfamethoxazole; VNTR, variable number of tandem repeats.

This particular type of production is generally not covered by normal sanitary controls. The epidemiological importance is further enhanced by the fact that there is often a demand for such cheeses from the general public in other towns who develop a taste for them.

Recently, variable number of tandem repeat (VNTR)-based typing schemes have been described demonstrating the utility of the technique as applied to the genus *Brucella* (Bricker *et al.*, 2003). Following this investigation, a number of different VNTR typing schemes were reported (Whatmore, 2009) that could be used for traceback analysis, to identify the origin of infections and/or to recognize human outbreaks that relate to a common source. On the basis of the more variable loci, these assays could also discriminate isolates originating from restricted geographical sources, indicating its potential as an epidemiological tool.

We present data on two brucellosis outbreaks that occurred in families infected after consumption of fresh home-made cheese bought in different Argentine provinces. We discuss the characteristics of the infection, patient history and the epidemiological tracing of *Brucella* transmissions. These outbreaks elicit many questions about the sanitary controls

for food products and education campaigns for the general public in order to limit the risk of infection.

METHODS

Index case family A. In August 2006 a 6-year-old girl, with a 3 month history of hip and knee arthralgias, was diagnosed as having Perthes' disease and this was followed up by the Orthopaedic Service of the Paediatric Hospital Dr R. Gutierrez, Buenos Aires. After 15 days of fever (39–40 °C) she was admitted to the Department of Infectology. The results of a physical examination were normal except for pain at the right hypocondrium. On admission, laboratory tests showed mild anaemia, a total leukocyte level of $10.4 \text{ cells } \mu\text{l}^{-1}$, haematuria and an increase in inflammatory markers glutamic pyruvic transaminase, glutamic oxaloacetic transaminase and alkaline phosphatase. Cytomegalovirus, Epstein–Barr virus, toxoplasmosis, and hepatitis A and B serology tests were negative.

Because brucellosis was suspected, a serum agglutination test, competitive ELISA and blood culture were performed. Her paternal uncle had been seen in the emergency room of another hospital after 2 weeks of fever and arthralgias in April. He was diagnosed with brucellosis, as was her paternal grandfather who in June had experienced 2 months of arthralgias, fever and sweating. Her maternal uncle complained of severe pain in his right knee and hip, had difficulty in walking and bending, and was admitted with brucellosis to another hospital in September. When the five members of the family were screened for brucellosis, only the girl's mother tested negative.

Index case family B. In March 2006, a 17-year-old boy was hospitalized in the intensive therapy unit of the Hospital 'Dr Cosme Argerich' with septic shock and acute liver failure, and was evaluated for liver transplantation. He had a 2 month history of abdominal pain, fever and night sweats. On admission, he had sensorial disorders, fever, jaundice and hepatosplenomegaly. His vital signs were blood pressure of 130/80 mmHg, axillary temperature of 39 °C and acid–base results pH 7.47, pCO_2 31 mmHg, pO_2 112 mmHg, HCO_3^- 22.5 mEq l^{-1} (mmol l^{-1}). A computed tomography (CT) brain scan was normal, but chest X-rays and CT showed bilateral and diffuse opacities. He was treated with 1 g amikacin daily and 2 g cefepime every 8 h. Fever, asthenia, myalgias, jaundice, low weight and headache persisted, and 18 days later a Gram-negative coccobacillus was isolated from the blood culture. Brucellosis was suspected and serological tests were performed. Because the patient tested positive the whole family was screened in a search for additional cases. His mother, approximately 40 years old, reported fever, severe abdominal pain and vomiting with a duration of over 1 month from the same date, while his two brothers, aged 16 and 14, and his sister, aged 19, had had fever, night sweats, headache, asthenia and abdominal pain as main symptoms and mild arthralgias. They tested positive, whereas his father tested negative.

Epidemiological data. Given these findings, patients were questioned about exposure to animals, consumption of the same contaminated food or travel to brucellosis endemic areas of the country. One family lived in the urban area of Buenos Aires and the other in San Juan province. The index-case patient of the first group and her family had eaten goat cheese, presumptively made from unpasteurized raw milk, bought by her paternal uncle at a farmhouse located in Catamarca province. The index-case patient from the second family had eaten homemade goat cheese bought in San Juan province and had shared it with his family.

Case definition. Case definition was considered probable (CDC, 1997) when a clinically compatible case was epidemiologically linked to a confirmed case or had supportive serology (i.e. a *Brucella* agglutination titre greater than or equal to 1:160 in one or more

serum specimens taken after the onset of symptoms) and when the clinically compatible case was confirmed by the laboratory.

Patient cultures. For blood cultures and cerebrospinal fluid routine isolation techniques in commercial liquid medium Hemo-Brucella (Britania), an in-house medium prepared in this laboratory containing BBL Brucella broth, 2.5 % sodium citrate and 3 % equine serum, or automated BacT/ALERT blood culture systems were used.

Microbiological methods. Colony morphology was studied by direct observation, acriflavine test and staining of colonies with crystal violet (Corbel & Banai, 2005; Alton *et al.*, 1988; Osterman & Moriyón, 2006). The strains isolated from patients were identified and typed by classical methods including: CO_2 requirement; agglutination pattern with monospecific anti-A, anti-M and anti-R sera; urease test; production of H_2S ; growth on dyes; erythritol, streptomycin and penicillin sensitivity; and lysis by Tb and R/C phages. Procedures described elsewhere were followed and typed *Brucella* strains of each species were included in all tests as controls.

VNTR typing. In order to confirm that the outbreaks had a common source, VNTR analysis was performed as described previously (Whatmore *et al.*, 2006). The relationships between isolates were examined by cluster analysis using the categorical coefficient and UPGMA implemented in Bionumerics version 5.1 (Applied Maths). Profiles of type strains were included as controls: *Brucella melitensis* biovar 1, 16M; *B. melitensis* biovar 2, 63/9; *B. melitensis* biovar 3, Ether; and vaccine strain, *B. melitensis* Rev.1.

Serological tests. The buffered plate agglutination test, Rose Bengal test, standard tube agglutination test and complement fixation were performed (Lucero & Bolpe, 1998) using antigens prepared at ANLIS 'Dr C. G. Malbrán' with *Brucella abortus* 1119-3 strain. Competitive ELISA was performed as previously reported (Lucero *et al.*, 1999), the antigen (S-LPS from *B. abortus* 1119-3) and the mAb were standardized and supplied by the Brucellosis Centre of Expertise and OIE (Office International des Epizooties) Reference Laboratory, Animal Diseases Research Institute, Canada. The test is considered positive when the percentage inhibition is >28 .

Therapy. The index case of family A was placed at first on 10 mg (trimethoprim)/50 mg (sulfamethoxazole) TMS kg^{-1} daily and 20 mg rifampicin kg^{-1} daily. Seven days later, due to TMS intolerance, the treatment was suspended and 48 h afterwards treatment was continued using 20 mg rifampicin kg^{-1} daily, with 7 mg gentamicin kg^{-1} daily added for 7 days. The total therapy lasted for 45 days. The index case of family B began with 300 mg rifampicin every 8 h, 100 mg doxycycline every 12 h and 160 mg (trimethoprim)/800 mg (sulfamethoxazole) TMS every 8 h for 8 weeks.

During this period of treatment the patients from family A received different schedules of antibiotics, but most of the cases received 1 g intramuscular streptomycin for 15 days and 100 mg oral doxycycline twice daily for 45 days. Case 4 in family A and all patients in family B were treated with 100 mg doxycycline every 12 h and 300 mg rifampicin every 8 h for 8 weeks.

RESULTS AND DISCUSSION

Clinical presentation, serological findings and treatment

The index case and members of family A had fever, arthralgias and osteoarticular pains as main symptoms. One member of this group presented deformity in the

knuckle joints of the right fingers, and arthralgias in left knee and elbow, with activity limitations of difficulty in walking, bending and standing. The main symptom of index case from family B was severe hepatic insufficiency, and members of his family had fever, asthenia, disorders triggering nausea, vomiting, abdominal discomfort and mild arthralgias (Table 1). Serological results of family A showed that the index case and case 1 and 3 had low titres 2 years after admission, while in family B only the index case presented persistent titres (data not shown). We observed the importance of adequate treatment in early stages of the disease, and combination therapy and extended treatment for 6 weeks or longer yielded significantly better results and minor relapses than shorter courses (Ariza *et al.*, 2007). During the 2 year follow-up period, no signs or symptoms of relapse were detected in the child or the 6 adults (A/1, A/3, B/5-6, B/8-9). Case 4 in family A and index case in family B presented high levels of antibodies 23 and 28 months after admission, respectively, whereas physical examinations were normal. The time lapse between the appearance of the index case and the secondary cases in each family could not be established because the symptoms, osteoarticular disorders and severe hepatic sepsis, confused the diagnosis, so that this infection was not suspected at first.

Bacteriology

By conventional bacteriological methods *Brucella* was isolated from the blood of eight out of nine patients (three from members of family A and five from family B) and all strains were identified as *B. melitensis* biovar 1 (Alton *et al.*, 1988; Corbel & Banai, 2005; Osterman & Moriyón, 2006). In Argentina the population of nearly 4 million goats is concentrated mainly in the provinces located in the north-west of the country. Recent surveys revealed a 0.5–0.8% prevalence of brucellosis, and *B. melitensis* biovar 1 was isolated from infected goats (Samartino, 2002). The use of Rev.1 vaccine to control goat infection was authorized at the end of 2006 (SENASA, 2006). Although the true incidence of human infection is unknown, the isolation of strains from humans reflects its presence in the animal population.

Molecular typing

Brucella species are characterized by a high level of nucleotide similarity, although they vary widely in host tropisms, microbial and disease phenotypes, and pathogenicity. For many years the development of molecular typing tools was hampered by this lack of diversity, but gradual progress has been made in identifying useful tools (Whatmore, 2009). Multilocus VNTR analysis has proven useful to discriminate very limited genomic diversity within genetically conserved bacterial species. When applied to isolates obtained in this study VNTR analysis confirmed the epidemiological link between isolates within each of the two familial clusters. Isolates from the two family groups comprised two very distinct genetic clusters with only very minor differences apparent within family groups (Fig. 1) and genotypic profiles were shown to be very distinct from those of reference *B. melitensis* isolates.

These findings highlight the potential value of VNTR-based typing when applied to *Brucella* to confirm epidemiological linkages and to allow traceback to identify sources of infection. This is particularly valuable for an organism where until recently epidemiological linkage was reliant on only very crude tools such as biotyping. Unfortunately, in the cases described here, there were no longer samples of cheese available that would allow categorical linkage of the outbreaks with the suspected source of infection. However, the clear discrimination between the two groups of *B. melitensis* biovar 1 isolates from distinct families, and from *B. melitensis* reference strains, highlights how such matching would be possible given the appropriate samples. These findings are consistent with recent studies that have shown substantial heterogeneity in VNTR profiles even within restricted areas of endemicity (e.g. Marianelli *et al.*, 2007; Smits *et al.*, 2009; Kang *et al.*, 2009) and have demonstrated the value of VNTR in the recognition of human outbreaks that relate to a common source (Valdezate *et al.*, 2007; Al Dahouk *et al.*, 2007; Kattar *et al.*, 2008) or to confirm relapse (Al Dahouk *et al.*, 2005).

Measuring genetic diversity within a population is of particular importance because it may reflect differences in

Table 1. Clinical findings, epidemiological data and strains isolated from families A and B

Family/ case	Age (years)	Filiation	Occupation	Clinical finding	Duration of symptoms (months)	Isolation	Strain
A/1	26	Uncle	Van driver	Fever, arthralgias	2	<i>B. melitensis</i> 1	F6/09-3
A/2	6	Index case		Arthralgias of the hip and knee, fever	2	<i>B. melitensis</i> 1	F6/09-8
A/3	65	Grandfather	Retired	Fever, arthralgias, sweating	2	<i>B. melitensis</i> 1	F6/09-7
A/4	29	Uncle	Carpenter	Arthralgias, right hip and knee pain	3		
B/5	40	Mother	Housewife	Fever, vomiting, abdominal pain	1	<i>B. melitensis</i> 1	F6/09-2
B/6	19	Sister	Student	Fever, nausea, asthenia, mild arthralgias	1	<i>B. melitensis</i> 1	F6/09-6
B/7	18	Index case	Student	Fever, severe hepatic insufficiency	2	<i>B. melitensis</i> 1	F6/09-1
B/8	16	Brother	Student	Fever, nausea, mild arthralgias	1	<i>B. melitensis</i> 1	F6/09-4
B/9	14	Brother	Student	Fever, asthenia, mild arthralgias	1	<i>B. melitensis</i> 1	F6/09-5

Fig. 1. Relationships between isolates from the two outbreaks as determined by VNTR analysis (Whatmore *et al.*, 2006). Profiles obtained from the three *B. melitensis* biovar reference strains and vaccine strain Rev.1 are included for comparison. In the case of mixed allele signals, which were apparent in four cases (Hoofprint 1, F6/09-5, minor peak=9; Hoofprint 1, F6/09-3 minor peak=8; Hoofprint 5, F6/09-8, minor peak=7; Hoofprint 7, F6/09-4, minor peak=4), the dominant allele is presented here.

virulence or other important phenotypes, such as antibiotic susceptibility. In the outbreaks in our study, although the clinical manifestation varied, fever characterized most patients in both families, but arthralgias and osteoarticular complaints were mainly present in family A while hepatic disorders, malaise and abdominal pain characterized family B. It is possible that the similar clinical findings in members of the same family indicate that differential virulence of different bacterial clones, as indicated by the VNTR data, could have influenced the course of the disease. Brucellosis may often trigger an exacerbation of existing underlying conditions in certain target organs (Akritidis & Pappas, 2001) and it could be argued in these cases that different clinical findings might be due to familial genetic backgrounds or are simply coincidental rather than reflecting variations in the virulence of *B. melitensis* strains. It is thought that host susceptibility to brucellosis is linked to host genetics (Bravo *et al.*, 2003, 2007, 2008; Budak *et al.*, 2007). However, differences in the pathogenic processes associated with different *Brucella* isolates have been reported. For example, Troy *et al.* (2005) reported a high rate of bone and joint infections in patients infected with *B. abortus*, while patients infected with *B. melitensis* had a higher rate of hepatosplenomegaly complications. More recently intriguing evidence has been provided that susceptibility to specific *B. melitensis* multilocus VNTR analysis genotypes in Peru may be age related and that specific genotypes may be more frequently isolated from patients presenting with specific clinical conditions such as splenomegaly and hepatomegaly (Nöckler *et al.*, 2009).

Conclusion

In this report we highlight several important aspects concerning recognition and reporting of persons with

brucellosis due to goat cheese consumption and recommendations concerning treatment and follow up. The hypothesis that there may be species-specific or clone-specific differences in the virulence of the genus *Brucella* should be investigated. Forthcoming genome sequencing projects, which with the benefit of technological advances will enable full characterization of large numbers of isolates, will be useful in examining such hypotheses. The risk of foodborne transmission of this zoonotic disease and its prevention should be considered.

ACKNOWLEDGEMENTS

We are very grateful to Sandra M. Ayala for helpful assistance with the typing of the isolates and to Stephen Shankster for technical assistance.

REFERENCES

- Akritidis, N. & Pappas, G. (2001). Ascites caused by brucellosis: a report of two cases. *Scand J Gastroenterol* **36**, 110–112.
- Al Dahouk, S., Hagen, R. M., Nöckler, K., Tomaso, H., Wittig, M., Scholz, H. C., Vergnaud, G. & Neubauer, H. (2005). Failure of a short-term antibiotic therapy for human brucellosis using ciprofloxacin. A study on *in vitro* susceptibility of *Brucella* strains. *Chemotherapy* **51**, 352–356.
- Al Dahouk, S., Flèche, P. L., Nöckler, K., Jacques, I., Grayon, M., Scholtz, H. C., Tomaso, H., Vergnaud, G. & Neubauer, H. (2007). Evaluation of *Brucella* MLVA typing for humans brucellosis. *J Microbiol Methods* **69**, 137–145.
- Alton, G. G., Jones, L. M., Angus, R. D. & Verger, J. M. (1988). Bacteriological and serological methods. In *Techniques for the Brucellosis Laboratory*, pp. 13–136. Paris: Institut National de la Recherche Agronomique.
- Ariza, J., Bosilkovski, M., Cascio, A., Colmenero, J. D., Corbel, M. J., Falagas, M. E., Memish, Z. A., Roushan, M. R. H., Rubinstein, E. &

- other authors (2007).** Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. *PLoS Med* **4**, e317.
- Bravo, M. J., Colmenero, J. D., Alonso, A. & Caballero, A. (2003).** HLA-B*39 allele confers susceptibility to osteoarticular complications in human brucellosis. *J Rheumatol* **30**, 1051–1053.
- Bravo, M. J., Colmenero, J. D., Martín, J., Alonso, A. & Caballero, A. (2007).** Polymorphism of the transmembrane region of the MICA gene and human brucellosis. *Tissue Antigens* **69**, 358–360.
- Bravo, M. J., Colmenero, J. D., Queipo-Ortuño, M. I., Alonso, A. & Caballero, A. (2008).** TGF- β 1 and IL-6 gene polymorphism in Spanish brucellosis patients. *Cytokine* **44**, 18–21.
- Bricker, B. J., Ewalt, D. R. & Halling, S. M. (2003).** *Brucella* “HOOF Prints”: strain typing by multilocus analysis of variable number tandem repeats (VNTRs). *BMC Microbiol* **3**, 15.
- Budak, F., Göral, G., Heper, Y., Yilmaz, E., Aymak, F., Baçstürk, B., Töre, O., Ener, B. & Oral, H. B. (2007).** IL-10 and IL-6 gene polymorphisms as potential host susceptibility factors in brucellosis. *Cytokine* **38**, 32–36.
- Castell Monsalve, J., Rullan, J. V., Peiro Callizo, E. F. & Nieto Sandoval Alcolea, A. (1996).** Epidemic outbreak of 81 cases of brucellosis following the consumption of fresh cheese without pasteurisation. *Rev Esp Salud Publica* **70**, 303–311 (in Spanish).
- CDC (1997).** Case definitions for infectious conditions under public health surveillance. *MMWR Recomm Rep* **46**, 1–55.
- Corbel, M. J. (2006).** *Brucellosis in Humans and Animals*, WHO/CDS/EPR/ 2006–7. Geneva: World Health Organization.
- Corbel, M. J. & Banai, M. (2005).** Genus I. *Brucella* Meyer and Shaw 1920, 173AL. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 370–386. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Elberg, S.S. (1981).** *A Guide to the Diagnosis, Treatment, and Prevention of Human Brucellosis*, VPH/81.31. Geneva: World Health Organization.
- Kang, S. I., Her, M., Heo, E. J., Nam, H. M., Jung, S. C. & Cho, D. (2009).** Molecular typing for epidemiological evaluation of *Brucella abortus* and *Brucella canis* isolated in Korea. *J Microbiol Methods* **78**, 144–149.
- Kattar, M. M., Jaafar, R. F., Araj, G. F., Le Fleche, P., Matar, G. M., Abi Rached, R., Khlife, S. & Vergnaud, G. (2008).** Evaluation of a multilocus variable number tandem repeat analysis scheme for typing human *Brucella* isolates in an endemic country. *J Clin Microbiol* **46**, 3935–3940.
- Lucero, N. E. & Bolpe, E. (1998).** Buffered plate antigen test as a screening test for the diagnosis of human brucellosis. *J Clin Microbiol* **36**, 1425–1427.
- Lucero, N. E., Foglia, L., Ayala, S. M., Gall, D. & Nielsen, K. (1999).** Competitive enzyme immunoassay for diagnosis of human brucellosis. *J Clin Microbiol* **37**, 3245–3248.
- Marianelli, C., Graziani, C., Santangelo, C., Xibilia, M. T., Imbriani, A., Amato, R., Neri, D., Cuccia, M., Rinnone, S. & other authors (2007).** Molecular epidemiological and antibiotic susceptibility characterization of *Brucella* isolates from humans in Sicily, Italy. *J Clin Microbiol* **45**, 2923–2928.
- Mendez Martinez, C., Paez Jimenez, A., Cortes Blanco, M., Salmoral Chamizo, E., Mohedano Mohedano, E., Plata, C., Varo Baena, A. & Martinez Navarro, F. (2003).** Brucellosis outbreak due to unpasteurised raw goat cheese in Andalucía (Spain), January – March 2002. *Euro Surveill* **8**, 164–168.
- Nöckler, K., Maves, R., Cepeda, D., Draeger, A., Mayer-Scholl, A., Chacaltana, J., Castañeda, M., Espinosa, B., Castillo, R. & other authors (2009).** Molecular epidemiology of *Brucella* genotypes in patients at a major hospital in Central Peru. *J Clin Microbiol* **47**, 3147–3155.
- Osterman, B. & Moriyón, I. (2006).** International Committee on Systematics of Prokaryotes. Subcommittee on the Taxonomy of *Brucella*. Minutes of the meeting, 17 September 2003, Pamplona, Spain. *Int J Syst Evol Microbiol* **56**, 1173–1175.
- Pappas, G., Akritidis, N., Bosilkovsky, M. & Tsianos, E. (2005).** Brucellosis. *N Engl J Med* **352**, 2325–2336.
- Samartino, L. E. (2002).** Brucellosis in Argentina. *Vet Microbiol* **90**, 71–80.
- SENASA (2006).** Se autoriza la importación, producción y el uso de la vacuna Rev. 1, en las condiciones que determinen la Dirección Nacional de Sanidad Animal y la Dirección de Laboratorios y Control Técnico. Resolución 216/2006. Buenos Aires: Servicio Nacional de Sanidad y Calidad Agroalimentaria. (<http://www.senasa.gov.ar/contenido.php?to=n&in=1029&io=4627>)
- Smits, H. L., Espinosa, B., Castillo, R., Hall, E., Guillen, A., Zevalleta, M., Gilman, R. H., Melendez, P., Guerra, C. & other authors (2009).** MLVA genotyping of human *Brucella* isolates from Peru. *Trans R Soc Trop Med Hyg* **103**, 399–402.
- Troy, S. B., Rickman, L. S. & Davis, C. E. (2005).** Brucellosis in San Diego: epidemiology and species related differences in acute clinical presentations. *Medicine* **84**, 174–187.
- Valdezate, S., Cervera, I., Hernandez, P., Navarro, A. & Saez Nieto, J. A. (2007).** Characterization of human outbreaks of brucellosis and sporadic cases by the use of hyper-variable octameric oligonucleotide fingerprint (HOOF) variable number tandem repeats. *Clin Microbiol Infect* **13**, 887–892.
- Whatmore, A. M. (2009).** Current understanding of the genetic diversity of *Brucella*, an expanding genus of zoonotic pathogens. *Infect Genet Evol* **9**, 1168–1184.
- Whatmore, A. M., Shankster, S., Perret, L. L., Murphy, T. J., Brew, S. D., Thirlwall, R. E., Cutler, S. J. & Macmillan, A. P. (2006).** Identification and characterization of variable number tandem repeat markers for typing of *Brucella* spp. *J Clin Microbiol* **44**, 1982–1993.
- Yagupsky, P. & Baron, E. J. (2005).** Laboratory exposure to brucellae and implications for bioterrorism. *Emerg Infect Dis* **11**, 1180–1185.