BMC Research Notes

Short Report

Characterization of some Brucella species from Zimbabwe by biochemical profiling and AMOS-PCR

Gift Matope¹, Evison Bhebhe¹, John Bwalya Muma*², Eystein Skjerve³ and Berit Djønne⁴

Address: ¹Department of Paraclinical Veterinary Studies, University of Zimbabwe, PO Box MP 167, Mount Pleasant, Harare, Zimbabwe, ²Department of Disease Control, University of Zambia, School of Veterinary Medicine, PO Box 32397, Lusaka, Zambia, ³Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, PO Box 8146, Dep, 0033 Oslo, Norway and ⁴National Veterinary Institute, PO Box 8156 Dep, N-0033 Oslo, Norway

Email: Gift Matope - gmatope@vet.uz.ac.zw; Evison Bhebhe - ebhebhe1961@yahoo.com; John Bwalya Muma* - jbwalya@lycos.com; Eystein Skjerve - eystein.skjerve@veths.no; Berit Djønne - berit.djonne@vetinst.no

* Corresponding author

Abstract

Background: Bovine brucellosis caused by Brucella abortus is endemic in most large commercial and smallholder cattle farms of Zimbabwe, while brucellosis in other domestic animals is rare. The diagnosis of brucellosis is mainly accomplished using serological tests. However, some Brucella spp. have been isolated from clinical cases in the field and kept in culture collection but their biochemical profiles were not documented. We report biochemical profiling and AMOS-PCR characterization of some of these field isolates of Brucella originating from both commercial and smallholder cattle farming sectors of Zimbabwe.

Findings: Fourteen isolates of Brucella from culture collection were typed using biochemical profiles, agglutination by monospecific antisera, susceptibility to Brucella-specific bacteriophages and by AMOS-PCR that amplifies species-specific IS711. The results of the biochemical profiles for B. abortus biovar 1 (11 isolates) and biovar 2 (2 isolates) were consistent with those of reference strains. A single isolate from a goat originating from a smallholder mixed animal farm was identified as B. melitensis biovar 1. The AMOS-PCR produced DNA products of sizes 498 bp and 731 bp for B. abortus (biovar 1 and 2) and B. melitensis biovar 1, respectively.

Conclusion: We concluded that the biochemical profiles and AMOS-PCR characterization were consistent with their respective species and biovars. B. abortus biovar 1 is likely to be the predominant cause of brucellosis in both commercial and smallholder cattle farms in Zimbabwe.

Background

The Brucellae are small Gram-negative coccobacilli bacteria affecting both animals and humans [1,2]. There are nine recognized species; B. abortus, B. melitensis, B. suis, B. canis, B. ovis, B. neotomae, B. ceti, B. pinnipedialis and B. microti [3-5]. On the basis of phenotypic profiles, some Brucella spp. are further divided into biovars [3].

Studies of the genome of Brucella spp. have demonstrated the existence of more than 70% homology [6] and based
on DNA-DNA hybridisation, a single species, *B. melitensis* was once proposed, with the other species being biovars [7]. However, the traditional classification into nine different species is still used. This has been further supported by the recent discovery of *B. ceti* and *B. pinnipedialis* from marine mammals [5], or *B. microti* from a common vole *Microtus arvalis* [4]. The genomic similarity makes the differentiation of *Brucella* spp. difficult, and often a study of biological and physiological characteristics is required [3].

In Zimbabwe, only *B. abortus* and *B. melitensis* have been reported to cause animal brucellosis. *B. melitensis* infection was confirmed in a flock of cattle, believed to have been translocated from Mozambique [8,9]. Brucellosis in wildlife has been demonstrated by serology [10] and in one instance *B. abortus* was isolated from a Cape buffalo (*Syncerus caffer*) [11]. Bovine brucellosis is a problem in some commercial dairy cattle farms, while others have eradicated [12]. Previous studies showed higher seroprevalence of between 10-53% in commercial herds in different regions of the country compared to 0-16% in communal (smallholder) cattle [9,12]. The disease continued to be closely monitored by the use of the milk ring test (MRT), serological surveys and bacteriological investigations [12-14]. Consequently, from 1988 to 2006, *Brucella* spp. isolates have been collected from infected herds from different parts of the country and kept in our laboratories. Although some these isolates have been identified to the species level, the details of their biochemical profiles and biovars have not been documented. The aim of this study was to characterize all *Brucella* field isolates in our culture collection that originated from both commercial and smallholder cattle farms of Zimbabwe using biochemical profiles and polymerase chain reaction (AMOS-PCR). The PCR assay is based on the repetitive genetic element, the insertion sequence 711 (IS711), that is unique to *Brucella* spp. For most *Brucella* spp., multiple copies of the IS711 occur at a unique species or biovar-specific chromosomal locus [15].

**Materials and methods**

The details of the identity and origin of all the *Brucella* spp. isolates, including the reference strains, used in this study are listed in Table 1. Prior to use, all strains from culture collection were stored either as lyophilized or in a -80 °C deep freezer. Lyophilized isolates were re-constituted and cultured in tryptone soya broth (TSB) (Oxoid) and subsequently sub-cultured onto Farrell's medium (Oxoid) and assessed for purity on bovine blood agar (Oxoid). All plates were incubated at 37 °C under 10% CO₂. Isolates were then inoculated into TSB with 5% glycerol, frozen and exported to Norway for further characterization.

For the observation of colonial morphology, *Brucella* spp. isolates were cultured on Mueller-Hinton agar (Oxoid) and single colonies were examined using a low power stereoscopic microscope illuminated by obliquely reflected light as described [3]. The tests for production of urease, catalase, oxidase, H₂S and indole; sensitivity to dyes (thionin and basic fuchsin) were carried out as described by Alton and co-workers [3]. Further tests for CO₂ requirement, sensitivity to dyes, lysis by bacteriophages (Tbilisi, Tb; Berkeley, Bk; Firenze, Fi; Izatnagar, Iz; R/C) and agglutination by A, M and R monospecific antisera were carried out at the Central Veterinary Laboratory, UK, using the procedures described [3].

The *Brucella* spp. isolates were grown on Farrell's agar (Oxoid) and incubated for 48 hours at 37 °C under 10% CO₂. To yield DNA, a few colonies from a pure culture were harvested and suspended in 200 μl of sterile distilled water in Ependorf tubes. A homogeneous suspension was made by stirring with the inoculation loop. Bacterial cells were inactivated by heating the tubes at 100 °C for 10 minutes on a heating block (Grant Instruments, UK). To separate the DNA, killed bacterial cells were centrifuged at 15, 700 × g for 10 minutes. The supernatant containing crude DNA template was pipetted into new sterile Ependorf tubes and the sediment discarded. The concentration of the extracted crude DNA was measured using a ND-1000 V3.0 spectrophotometer (NanoDrop® Technologies Inc., USA). The DNA was stored at -20 °C until further tests were carried out.

The AMOS-PCR (*B. abortus*, *B. melitensis*, *B. ovis* and *B. suis*-Polymerase Chain Reaction) was done as described previously [15] but with minor modifications of the assay environment. Briefly, PCR assay reaction mixture consisted of the following: 1 × PCR buffer (Applied Biosystems), 3 mM MgCl₂, 200 μM (each) of the four deoxynucleotide triphosphates (dNTPs) (Finnzymes Oy, Espoo, Finland), and the 5 sets of primers (0.2 μM each) of *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis* and IS711-specific primer. One and half units (1.5 U) of AmpliTaq Gold® DNA polymerase (Applied Biosystems) per 45 μl reaction mixture was added and then dispensed into MicroAmp vials (Applied Biosystems). A total of 5 μl DNA template of killed bacteria (estimated at 200 ng/ml) was added per 45 μl reaction mixture. The PCR was performed with a PTC-200 Peltier Thermocycler (Roche Molecular Systems Inc, Almelda, USA). Amplification was performed for 35 cycles, each cycle comprised of denaturation at 95 °C for 1 minute and 15 seconds, annealing at 60 °C for 2 minutes, and extension at 72 °C for 2 minutes. The PCR products were incubated for a further 5 minutes at 72 °C to allow elongation of products before storage at 4 °C. The PCR products were separated by electrophoresis using 1.5% agarose gel (w/v) (BDH Electron®) at 100 V for 1.5 hours. Gels were stained.

For the recent discovery of *B. a*
with ethidium bromide and photographed using a gene snap camera (Syngene Pvt Ltd, UK).

Results
All 14 Brucella spp. isolates characterized in this study (Table 1) yielded the following results that are typical of the genus; Gram-negative coccobacilli, non-motile, positive for modified Ziehl-Neelsen staining, oxidase and catalase production, and negative for indole production (Table 2). Their growth on Mueller-Hinton agar produced colonies that were convex, with entire edges and a smooth shiny consistency. The morphological, growth characteristics and biochemical profiles of the field isolates were similar to their respective reference Brucella species and biovars (data not shown). Isolates belonging to the same biovars showed consistently similar results, except for their CO₂-dependence for growth (Table 3). Regardless of the biovar type, seven of the 13 B. abortus isolates were CO₂-independent, while the remaining six strains were CO₂-dependent. The B. abortus isolates were lysed by phages Tb, Fi, Bk₂, Iz1 and resistant to R/C. Only one isolate was lysed by the R/C phage. The single B. melitensis isolate was resistant to all phages but showed partial lysis to Bk₂ (Table 3). B. abortus isolates were agglutinated by A-antiserum and B. melitensis by the M-antiserum, but all were not agglutinated by the R-antiserum (data not shown). Eleven and two of the 13 B. abortus isolates were identified to be biovars 1 and 2, respectively (Table 3).

The Brucella isolates were detected by the AMOS-PCR and produced predicted amplicons of sizes 498 bp and 731 bp for B. abortus and B. melitensis, respectively. Similar DNA products were produced for the reference B. abortus biovar 1 and B. melitensis biovar 1, respectively.

Discussion
This paper provides the first detailed biochemical profiling and AMOS-PCR characterization of some Brucella spp. isolates from Zimbabwe. The phage sensitivity patterns of all the Brucella spp. isolates were consistent with what has

Table 1: Brucella isolates used in the study and the details of their geographical regions of origin.

| Field Brucella spp. | Reference number (Year isolated) | Specimen of origin | Farm name (type) and geographical region of origin |
|---------------------|---------------------------------|--------------------|--------------------------------------------------|
| B. abortus          | B1-2-2676 (1994)               | Aborted foetus     | Mazowe (S), MC                                   |
| B. abortus          | B4-11-438 (1998)               | Aborted foetus     | Mhuri (S), MD                                    |
| B. abortus          | B5(1999)                       | Hygroma            | Chinamhora (S), MSE                              |
| B. abortus          | B6-304 (1997)                  | Aborted foetus     | Pilosoff (C) MN                                  |
| B. abortus          | B7-307 (1997)                  | Aborted foetus     | Chikurubi Prisons (C) MSE                        |
| B. abortus          | B8-2160 (1996)                 | Aborted foetus     | Greyling (C) MW                                  |
| B. abortus          | B9-2260 (1996)                 | Aborted foetus     | Hensman (C) MW                                   |
| B. melitensis       | B10-6419 (1988)                | Aborted foetus (goat) | Muzarabani (S)MC                                 |
| B. abortus          | B12-555(?)                     | Aborted foetus     | (C), NE                                          |
| B. abortus          | B14-(2005)                     | Milk               | Mulanjeni (S), MD                                |
| B. abortus          | B15-H-56(?)                    | Aborted foetus     | NE (C), NE                                       |
| B. abortus          | B16-494-64 (?)+                | Aborted foetus     | NE (C), NE                                       |
| B. abortus          | B20- (2006)                    | Milk               | Lulaka (S), MD                                   |
| B. abortus          | B21-93-35 (?)                   | Aborted foetus     | NE (C), NE                                       |

Reference Brucella spp.

| B. abortus 1 | 544 | - | NVI |
| B. abortus 2 | 86/8/59 | - | NVI |
| B. abortus 3 | Tulya | - | NVI |
| B. abortus 4 | 292 | - | NVI |
| B. melitensis 1 | 16M | - | NVI |
| B. melitensis 3 | Ether | - | NVI |
| B. suis 1 | 1330 | - | NVI |
| B. suis 4 | 40 | - | NVI |
| B. canis | RM-6-66 | - | NVI |
| B. ovis | 63/290 | - | NVI |
| B. neotomae | 5K-33 | - | NVI |

(?) = Year of isolation not established, (S) = smallholder farm, (C) = Commercial farm, NE = not established, MC = Mashonaland Central province, MSE = Mashonaland East province, MD = Midlands province, MW = Mashonaland West province, MN = Matabeleland North province NVI = National Veterinary Institute, Norway

aObtained from the Central Veterinary Laboratory
bObtained from the University of Zimbabwe
been reported [16]. However, a single isolate of *B. abortus* (B15) was lysed by R/C phage and this susceptibility could be indicative of the presence of phage attachment sites which are present in the non-smooth phases of *Brucella* [16]. The *B. melitensis* (B10) also showed an atypical reaction because it was not lysed by the Iz1 phage which normally lyses smooth strains of this species. However, the examination of single colonies on microscopy by Henry illumination [3] showed no sign of dissociation and none of the isolates were agglutinated by the R-monospecific antiserum which is an indicator of dissociation.

### Table 2: Basic biochemical and metabolic profiles of field *Brucella* spp. from Zimbabwe.

| Biochemical properties | Growth on TSA in the presence of dyes |
|------------------------|---------------------------------------|
| reference no. | Cat | Oxi | Ure | Mot | Ind | MZN | T<sub>20</sub> | T<sub>40</sub> | BF<sub>20</sub> |
| B1 | + | + | + | - | - | + | - | - | + |
| B4 | + | + | + | - | - | + | - | - | + |
| B5 | + | + | + | - | - | + | - | - | + |
| B6 | + | + | + | - | - | + | - | - | + |
| B7 | + | + | + | - | - | + | - | - | + |
| B8 | + | + | + | - | - | + | - | - | + |
| B9 | + | + | + | - | - | + | - | - | + |
| B10 | + | + | + | - | - | + | - | - | + |
| B12 | + | + | + | - | - | + | - | - | + |
| B14 | + | + | + | - | - | + | - | - | + |
| B15 | + | + | + | - | - | + | - | - | + |
| B16 | + | + | + | - | - | + | - | - | + |
| B17 | + | + | + | - | - | + | - | - | + |
| B18 | + | + | + | - | - | + | - | - | + |

Cat, Catalase; Oxi, Oxidase, Ure; Urea hydrolysis; Mot, Motility test (+ = motile, - = non-motile); Ind, Indole production; MZN, Modified Ziehl Neelsen stain; TSA, Tryptone Soya agar; T<sub>20</sub>, 20 μl/ml thionin; T<sub>40</sub>, 40 μl/ml thionin; BF<sub>20</sub>, 20 μl/ml basic fuchsin; + = positive test result; - = negative test result.

Urea hydrolysis = All isolates positive within 2 hours of culture.

### Table 3: Summary of phenotypic characteristics of the field *Brucella* spp. from Zimbabwe

| Isolate No. | CO₂ Dependent | H₂S | BF | TH | A | M | Tb | BK₂ | Fi | Iz1 | R/C | Size of DNA detected | Interpretation |
|-------------|---------------|-----|----|----|---|---|----|-----|----|-----|-----|---------------------|---------------|
| B1, B6, B7, B14, B16, B21 | - | + | + | - | + | - | CL | CL | CL | CL | NL | 498 bp | *B. abortus* 1 |
| B15 | - | + | - | - | + | - | CL | CL | CL | CL | 498 bp | *B. abortus* 2 |
| B10 | - | - | + | - | + | + | NL | PL | NL | NL | 731 bp | *B. melitensis* 1 |
| B4, B5, B8, B9, B12 | + | + | + | - | + | - | CL | CL | CL | CL | 498 bp | *B. abortus* 1 |
| B20 | + | + | - | - | + | - | CL | CL | CL | CL | 498 bp | *B. abortus* 2 |

* All tests carried out by the reference laboratory (VLA), Weybridge, UK.
Isolate No. = Isolate identification.
DNA: Test by the AMOS PCR.
BF = Basic fuchsin at 20 μl/ml (1/50,000 w/v)
TH = Thionin at 20 μl/ml (1/50,000 w/v)
Phages: Tb = Tbilisi, BK₂ = Berkeley type 2, Fi = Firenze, Iz1 = Izatnagar, R/C = phage lytic for non-smooth species of *Brucella*
CL = Confluent Lysis
PL = Partial lysis
NL = No lysis
RTD = Routine test dilution
+ = positive (yes)
- = negative (no)
bp = base pairs
showed the predominance of A-specific and M-specific epitopes in our *B. abortus* and the *B. melitensis* isolate, respectively. All smooth strains of *Brucella* may possess either the A, M or both A and M antigenic epitopes on the O chains of the lipopolysaccharides [16].

Although phage typing is used primarily for identification at the nomen species level, some *Brucella* strains, especially *B. melitensis* may show deviation from the standard pattern of susceptibility to Bk$_{2}$, Iz1 and Wb phages [16]. The use of phage typing as a means of differentiating *Brucella* spp. has become less discriminatory as a typing tool because of the discovery of new strains with atypical sensitivity patterns [17].

The growth characteristics and the biochemical profiles of the field *Brucella* spp. isolates (Table 2) were similar to those of the reference strains used in this study. In addition, the results were consistent with what is reported for *Brucella* spp. and biovars [2,3,16]. However, the requirement for CO$_{2}$ for growth was at variance with reports from literature [3]. Although most strains of *B. abortus* biovars 1-4 require CO$_{2}$ for primary isolation, this attribute is quickly lost on repeated subcultures and such isolates will adapt to growing in atmospheres without CO$_{2}$ [3,16].

The use of the AMOS-PCR results were consistent with those reported elsewhere [15]. These results confirmed the identity of the *Brucella* spp. that was obtained using biochemical profiles. The IS711 analysis using AMOS-PCR can identify only three *B. abortus* biovars, 1, 2 and 4; all three biovars of *B. melitensis*; biovar 1 of *B. suis* and *B. ovis*, but the individual biovars within a species are not differentiated [15]. Therefore, further DNA fingerprinting methods such as the variable number of tandem repeat analysis (VNTR) [15] could be used to investigate the molecular epidemiology of these *Brucella* isolates.

Although the *B. abortus* used in this study originated from five of the eight geographical provinces of Zimbabwe (Table 1), it is difficult to conclude on the spatial distribution due to the limited number of isolates used. These isolates could possibly be restricted to one or a few geographical regions of Zimbabwe from where they have spread through movement of infected cattle. A study of more isolates is required to determine the spatial distribution of *B. abortus* in Zimbabwe. However, the predominance of *B. abortus* biovar 1 over biovar 2 suggested that it is the major cause of bovine brucellosis in both commercial and smallholder cattle farms. Another study which used fewer *Brucella* isolates from commercial dairy farms reported similar findings [12]. Although *B. abortus* biovar 2 was also detected and originating from both the commercial and smallholder cattle farms, its distribution could be limited to a few isolated areas. Elsewhere in South Africa biovar 1 had been reported to account for about 90% while biovar 2 contributed 10% of all the *B. abortus* isolates [18]. South Africa, to a large extent, shares similar geographic, climatic and livestock husbandry systems with Zimbabwe. While it is difficult to explain the reasons for the distribution of these *B. abortus* biovars in the cattle farming sectors, this could largely be influenced by movement of infected cattle between farms. Some farms often purchase cattle from other farms for the purpose of improving the genetics of their herds [9] or in the case of smallholder farms, to restock their herds which are continuously lost due to infectious diseases and lack of adequate grazing, especially during the drought seasons.

Despite the relatively few isolates studied, our results suggested that *B. abortus* biovar 3 and indeed other biovars may be rare in Zimbabwe, but this requires further study. *B. abortus* biovar 3 has been infrequently reported in South Africa, East and North Africa, while there seems to be no reports of isolation of the other biovars [2,18]. Worldwide, in countries where bovine brucellosis is endemic, *B. abortus* biovar 1 is predominant and *B. abortus* biovar 2 occurs less frequently while the other biovars are rare [2,18].

**Conclusion**

We concluded that the biochemical profiles and AMOS-PCR characterization were consistent with their respective species and biovars. *B. abortus* biovar 1 is likely to be the predominant cause of brucellosis in both commercial and smallholder cattle farms in Zimbabwe. Further studies are required that will apply DNA-fingerprinting to study distribution patterns of *B. abortus* biovars in Zimbabwe.

**Abbreviations**

AMOS-PCR: *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis*; Polymerase Chain reaction; bp: base pairs; DNA: deoxyribonucleic acid.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

GM: Principal investigator, conceived the study, and participated in the design of the proposal, collection of *Brucella* isolates and culture, phenotypic and molecular characterization of isolates, analysis of results and drafting the manuscript. EB: Supervision of *Brucella* isolate collection, participated in phenotypic and molecular characterization of isolates, result interpretation and helped in drafting of the manuscript. JB: Participated in the design of the study, molecular characterization of isolates, result interpretation and drafting of the manuscript. ES: Participated in the design, acquisition of funds and general coordination and helped to draft manuscript. BD: Participated
in phenotypic and molecular characterization of isolates, result interpretation, supervision of laboratory work and helped in drafting of the manuscript. All authors have read and approved the final manuscript.

Acknowledgements
This study was fully sponsored by the Norwegian Council for Higher Education and Development (NUFU), project vote: pro/2002/06. We are grateful to the members of staff at the National Veterinary Institute, Oslo, for their technical assistance and in particular Dr. Arve Lund for his material support and encouragement. Our sincere appreciation goes to Ms L. Perret of Central Veterinary Laboratory, Weybridge, the UK, for further typing of the Brucella isolates.

References
1. WHO/FAO: Brucellosis in humans and animals, World Health Organization and Food and Agricultural Organization, 20 Avenue Appia, 1211 Geneva, Switzerland. 2006:1-66.
2. Quinn PJ, Carter ME, Markey B, Carter GR: Clinical Veterinary Microbiology, Mosby International Limited, Edinburgh. 1999:261-267.
3. Alton G, Jones LM, Angus RD, Verger JM: Techniques for the brucellosis laboratory, Institut National de la Recherche Agronomique, Paris, France. 1988.
4. Scholtz HC, Hubalek Z, Sedláček I, Verger JM, Cloeckaert A, Letesson JJ, Godfroid J: Pheno
typic and molecular characterization of Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. Int J Syst Evol Microbiol 2008, 58:375-382.
5. Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A: Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. Int J Syst Evol Microbiol 2007, 57:2688-2693.
6. Clavareau C, Welmans V, Walravens K, Tryland M, Verger JM, Grayon M, Cloeckaert A, Letesson JJ, Godfroid J: Phenotypic and molecular characterization of a Brucella strain isolated from a minke whale (Balaenoptera acutorostrata). Vet Microbiol 1998, 144:3267-3273.
7. Verger JM, Grimont F, Grimont PAD, Grayson M: Brucella, a monospecific genus as shown by deoxyribonucleic acid hybridization. Int J Syst Bacteriol 1985, 35:292-295.
8. Madzima WM: Zimbabwe: Bovine brucellosis and brucellosis of small ruminants: Diagnosis, control and vaccination. In Technical series Paris, France: Office International des Epizooties: 1987.
9. Madsen M: The current status of brucellosis in Zimbabwe. Zimbabwe Veterinary Journal 1989, 20:133-145.
10. Madsen M, Anderson EC: Serologic survey of Zimbabwean wildlife for brucellosis. J Zoo Wildlife Med 1995, 26(2):240-245.
11. Condy JB, Vickers DB: Brucellosis in Rhodesian wildlife. Journal of the South African Veterinary Association 1972, 43:175-179.
12. Mohan K, Makaya PV, Muvavarirwa P, Matope G, Mhembere E, Pawandiwa A: Brucellosis surveillance and control in Zimbabwe: bacteriological and serological investigation in dairy herds. Onderstepoort Journal of Veterinary Research 1996, 63:47-51.
13. Manley FH: Brucellosis in Rhodesia. A report to the Director of Veterinary Services. Department of Veterinary Services, Harare, Zimbabwe: 1969.
14. Swanepoel R, Blackburn NK, Lander KP: The occurrence, diagnosis and control of Brucellosis in cattle in Rhodesia. Rhodesia (Zimbabwe) Veterinary Journal 1976, 7:24-31.
15. Bricker BJ, Halling SM: Differentiation of Brucella abortus bv. 1, 2, and 4, Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR. J ClinMicrobiol 1994, 32:2660-2666.
16. Garnery GM, Bell JA, Lilburn T: Family III, Brucellaceae Breed, Murray and Smith 1957, 394 AL. In Bergey's Manual of Systematic Bacteriology Volume II. 2nd edition. Edited by: Brenner DJ, Krieg NR, Staley JT. Springer Science and Business Media, Inc., New York, NY 2001, 13: 2005-370-392.
17. Jahans KL, Foster G, Broughton ES: The characterisation of Brucella strains isolated from marine mammals. Vet Microbiol 1997, 57:373-382.

BMC Research Notes 2009, 2:261  http://www.biomedcentral.com/1756-0500/2/261

Publish with BioMed Central and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."
Sir Paul Nurse, Cancer Research UK

Your research papers will be:
• available free of charge to the entire biomedical community
• peer reviewed and published immediately upon acceptance
• cited in PubMed and archived on PubMed Central
• yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

BioMedcentral