Application of Polymerase Chain Reaction to Detect *Burkholderia Pseudomallei* and *Brucella* Species in Buffy Coat from Patients with Febrile Illness Among Rural and Peri-urban Population

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ABSTRACT

Context: *Melioidosis* and *Brucellosis* are important endemic infections among people in India, especially in rural settings. Conventional detection techniques have several limitations. Only a few studies exist on the prevalence of *Melioidosis* and *Brucellosis* in rural area especially in India. **Aim:** We sought to evaluate detection of *Burkholderia pseudomallei* and *Brucella spp.* among patients presenting febrile illness. **Material and Methods:** Previously described polymerase chain reaction **(**PCR) assays for both pathogens were evaluated with Deoxyribonucleic acid extracts of buffy coat samples collected from 301 patients recruited prospectively. Data was not amenable to statistical analysis. **Results:** The PCR showed specific amplification and no non-specific amplification with heterologous Gram-negative bacilli. The lower limit of detection of the assay for *B. pseudomallei* was determined to be 1 colony-forming unit /mL and for *Brucella* it was 1.95 x 10^3 plasmids per microliter. Blood culture in automated blood culture system was negative for all the samples. This prospective study carried out in southern India for the first time. PCR for *Brucella* was positive in 1% of the patient samples whereas 0.3% was positive for *B. pseudomallei*. **Conclusion:** The finding of *Brucella* and *Burkholderia* infections in our populations leads us to suggest that tests for *Brucella* and *B. pseudomallei* should also form part of a diagnostic platform for patients with Pyrexia of unknown origin in tropical developing countries.

Key words: 16-23S rRNA, *Brucella*, buffy coat, *Burkholderia pseudomallei*, *omp2*

INTRODUCTION

Pyrexia of unknown origin (PUO) is a major cause of morbidity and mortality in developing countries especially India. Many individuals present with undifferentiated fever which is categorized as PUO pending specific investigation for tuberculosis, enteric fever, *Brucellosis*, viral fevers and *Melioidosis* (the great imitator of tuberculosis).[1,2] This problem has to be addressed by improving the comprehensive diagnosis of infectious etiology of PUO in countries like India. Furthermore, there is no report of the proportionate role of *Brucellosis* and *Melioidosis* in cases of PUO especially from South India.[3]

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Burkholderia pseudomallei are the causative agents of *Melioidosis*, a fatal septicaemic infection in humans which can at times become chronic. The chronic infection shows many features common to tuberculosis. The organism is ubiquitous in nature and exists in soil and water. It causes infection when ingested or inhaled, or by inoculation through skin abrasions and wounds. This is considered to be an important organism causing undiagnosed fever.[4,5] *Melioidosis* is reported to be prevalent among people involved in rice cultivation and raising of farm animals especially in Southeast Asia, where it is as common as enteric fever in India.[6] *Brucellosis* in humans could be caused by any of the four main species *viz*, *B. abortus*, *B. melitensis*, *B. suis* and *B. canis*.[7] This is a severe zoonotic disease presenting as acute or chronic infection in humans, and manifesting as a septicaemic febrile illness or localized infection of bone, tissue, or organ systems.[8,9] In India, where cattle rearing is common, *B. abortus* and *B. melitensis* is known to cause life threatening illnesses.[10] It is transmitted by the ingestion

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of raw or unpasteurized milk, and other dairy products, by direct contact with infected animal tissues, or by accidental ingestion, and inhalation.

India is reported to be endemic for both pathogens as *Melioidosis* is increasingly reported from several parts of the country,[3] where *Brucellosis* is also well known to be present. Culture based identification is the gold standard for diagnosis for both these organisms. However, most often *Melioidosis* or *Brucellosis* may go undiagnosed in cases of septicemia being misinterpreted as non-fermenting Gram negative bacteria (*B. pseudomallei*) or use of inadequate blood culture media (*Brucella*). In India, optimal blood, bone-marrow culture facilities for such reticulo-endothelial pathogens are not widely available. The standard agglutination test (SAT) could be used with and without 2-mercaptoethanol treatment of the serum. The four-fold fall in titer between untreated and 2-mercaptoethanol treated serum helps to establish acute infection. The SAT titer is greater than 160 in chronic *Brucellosis*. The SAT is convenient to perform but has lower sensitivity compared to bone marrow culture. The bone marrow culture results are usually available after 3 to 4 weeks, and is not performed in many laboratories.[11] The standard agglutination test (SAT) for *Brucella* is used despite its lower sensitivity and specificity. In the case of *Melioidosis*, the serological assays have not gained wide acceptance. More and more laboratories in India are introducing polymerase chain reaction (PCR) for disease diagnosis. This impression is gained by oral survey of microbiology consultants from different parts of India (personal communication Prof. UC Chaturvedi, Lucknow). Hence, the present study was carried out to evaluate PCR based detection of *Melioidosis* and *Brucellosis* in a tertiary care hospital located in a rural area of Vellore district. The gene targets for PCR were 16S-23S rRNA spacer region for *B. pseudomallei* and *omp*2 gene for *Brucella* species, and two independent non-nested PCRs were used in this study.

B. pseudomallei and Brucella species are reported to infect monocytes,[6,12,13] and can be detected from peripheral blood.[14] We report here the evaluation of the PCR on buffy coat (White blood cells, WBC) DNA extracts of the blood sample collected from patients with PUO. In the study, blood culture was carried out prospectively in an automated commercial blood culture system as a standard method for comparison.

MATERIALS AND METHODS

Specimens

A total of 301 samples were collected from patients during the period of Nov 2008 to Jun 2009 attending a tertiary care hospital in rural area of Vellore district, Tamilnadu. Patients who came to hospital or who were admitted to the medical wards and gave a history of an acute/chronic undifferentiated febrile illness (temperature of 101°F) of 5 to 15 days or more duration, and who gave consent for blood collection were recruited as study subjects. The consent to participate in the study was obtained from each patient and a clinical questionnaire was duly filled by the examining physician and later used for analysis. Human immunodeficiency virus (HIV) status was not routinely established in the patients. The exclusion criteria were immuno-compromised patients other than HIV infected individuals, with hematological malignancy, autoimmune disorders, and patients on immunosuppressive drugs and with an obvious focus of infection such as urinary tract infections, lower respiratory tract infections, bacterial meningitis and abscesses. These conditions were excluded to enhance screening of primarily PUO cases without evidence of a definitive focus of infection or non-infectious inflammatory causes of fever.

Fever was recorded for every patient and the temperature ranged from 99 to 106°F. Duration of fever was between 3 and 90 days, among them 28 (8.3%) had intermittent fever and others had continuous fever. Only 2 (0.66%) of 301 patients gave a history of fever longer than 15 days of fever duration. The majority of patients (84.4%) had fever of 100-105°F, only two (0.66%) had hyperpyrexia (more than 105°F).

In our study subjects, males were 185, and females were 170 in number. The patients from rural community were 240 (67.6%) and from peri-urban community were 115 (32.4%). The age of the patients recruited in the study ranged from 2 to 81 years, among which two were less than 5 years; 16 were between age 5 to 15 years and 283 were more than 15 years of age. On analysis of the occupation/vocation and animal rearing habits of the patient volunteers, it was found that, 1 among 301 worked as a butcher. Five patient volunteers reared animals in their homes, and 2 of 301 had a habit of drinking unpasteurized cow's milk. A limitation of this observation was lack of information on individuals regarding rice paddy cultivation.

Sample collection

Venous blood samples (15 mL) were collected; 5 mL for routine blood culture and 5 mL for *M. tuberculosis* culture. The other 5 mL was collected in a sterile falcon tube containing Ethylenediaminetetraacetic acid (EDTA) for buffy coat preparation.

Blood culture processing was in a completely automated machine-BacT/Alert, according to manufacturer's instructions (3D, 120, Biomérieux, NC, USA), and if growth occurred, the sample was plated on MacConkey agar and blood agar. The organisms were identified by appropriate biochemical tests. All blood cultures were done in real time and results communicated to the treating physician as they were ready. The antibiogram of the causative agent was performed as per the recommendations of The Clinical and Laboratory Standards Institute (CLSI), USA.

Deoxyribonucleic acid preparation for *Burkholderia pseudomallei* and *Brucella abortus*

A DNA extracted from *B. pseudomallei* strains (NCTC 13178), was kindly provided by Dr. N. Ketheesan (JCU, Australia). For *Brucella* species, DNA was extracted from killed *B. abortus* (Indian Veterinary Preventive Medicine Institute, Ranipet, Tamilnadu). These DNA acted as templates for standardizing the respective PCR assays as positive control.

Buffy coat preparation and deoxyribonucleic acid extraction

Buffy coat was prepared from the third fraction of blood as indicated above. DNA was extracted in batches using QiaAmp blood mini kit (Qiagen GmBH, Hilden, Germany) as per the manufacturer's instructions. The extracted DNA was stored at -20°C and used for PCR assay.

Polymerase chain reaction testing for *Burkholderia pseudomallei* and *Brucella* species

Following extraction, PCR assays for the detection of *B. pseudomallei* and *Brucella* spp. were performed from the DNA samples. The target gene for *B. pseudomallei* was 16S-23S rRNA spacer region coding gene (species specific) and that for *Brucella* was *omp*2 gene coding for an outer membrane protein (genus specific). Primers used are listed in table 1. Primers were commercially synthesized and obtained from Metabion, GmBh, Germany. All the PCR reagents including Hotstar Taq polymerase were procured from Qiagen (Hilden, Germany). Negative controls were included in every assay replacing the template with nuclease free water (Qiagen GmBH, Hilden, Germany).

Table 1: Primer sequence used in the study

Organism	Target region		Primer sequence	Reference
B. pseudomallei	16S-23S rRNA	Fwd	5'-CGATGATCGTTGGCGCTT	Merritt *et al.*[15]
		Rev	5'-CGTTGTGCCGTATTCCAAT	
Brucella	*omp2*	Fwd	5'-TGGAGGTCAGAAATGAAC	Mitka *et al.*[16]
		Rev	5'-GAGTGCGAAACGAGCGC	

Conditions for PCR testing for *B. pseudomallei* (30 cycles) were 95°C for 15 minutes, 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 45 seconds, 72°C for 10 minutes (after last cycle) for final extension. And that for *Brucella* were 95°C for 15 minutes, 94°C for 40 seconds, 50°C for 1 minutes, 72°C for 1 minutes, and 72°C for 10 minutes (after 30th cycle). All precautions were taken for PCR testing like flow through, disposable plastic ware, and gloves, filter blocked tips and dedicated micropipettes. The PCR was carried out in Eppendorf thermal cycler (Mastercycler® personal 5332, Hamburg, Germany).

Analysis of amplification products

An aliquot of 5 µL amplicon was analyzed by gel electrophoresis in 2% agarose (Sigma, MO, USA) prepared in Tris-Borate-EDTA buffer containing 0.5µg/mL of ethidium bromide (Sigma, MO, USA). The gels were examined in a gel documentation system (Genei, Bangalore, India) for respective amplification products.

Establishment of lower limit of detection of *Burkholderia pseudomallei* by colony count

To establish the sensitivity (lower limit of detection) of PCR, the standard colony count method by surface streaking was carried out using unit volume per dilution. A typical biochemically and serologically characterized strain of *B. pseudomallei* was kindly provided by Dr. Mary V Jesudason (Pondicherry Institute of Medical Sciences, Pondicherry). Serial logarithmic dilutions of the culture suspension were plated on MacConkey agar to obtain the CFU units/mL. DNA was extracted using QiaAmp blood mini kit (Qiagen GmBH, Hilden, Germany) from a suspension of bacteria that contained 1000 CFU/mL. The DNA was diluted serially to facilitate testing of the equivalent of 100, 10, 1, 0.1, 0.01, and 0.001 CFU/mL in 5 µl input for the PCR mix. The sensitivity of the PCR assay was established as the least concentration of input DNA positive in at least two replicates of triplicate tested at each concentration. Adequate positive controls and negative controls have been used in this study as shown below. No external quality control was carried out as this was an assay development study. Furthermore, no centre could be identified within the country where these assays were done routinely.

Establishment of lower limit of detection of *Brucella* species by plasmid cloning

PCR products were produced with cycling conditions specific to *Brucella* primers with final extension of 10 minutes at 72°C. PCR products were checked by agarose

gel electrophoresis for single, discrete band. TOPO TA cloning kit (Invitrogen, CA, USA) was used to clone the PCR product as per manufacturer's instructions. Copy number of the cloned plasmids was calculated using the formula: weight of PCR fragment (in grams per mL)/(660 g per mol × the number of base pairs of the PCR fragment) × (6.023×10^{23})=the number of genome copies per microliter. The concentration of the plasmid was determined by measuring the optical density at 260 nm with a spectrophotometer (μQuant, Biotek instruments, Inc, VT, USA).

The probability of detecting *Brucella* species in a suspension of known concentration in the presence of defined DNA copy numbers was determined essentially as described previously.[17] The cloned plasmids were serially diluted 10-fold in TE buffer (pH 8.0) within the concentration range of 10^0 to 10^9 plasmid copies/μl. The dilutions were stored at -20°C until use. The approximate number of plasmid copies/μl of DNA suspension was determined by PCR using appropriate negative controls. Amplification shown in the highest dilution (least concentration) in at least two replicates of the triplicates tested at each dilution was taken as lower limit of detection as plasmid copies per microliter.

Specificity testing with heterologous organisms

Specificity of the PCR assays were established by screening DNA extracts of heterologous organisms such as *E. coli, Proteus mirabilis, Pseudomonas* spp. and *Enterobacter* spp. The *B. pseudomallei* and *Brucella* PCR did not show any heterologous amplification.

RESULTS

The PCR showed specific amplification of 16-23S spacer region (302 bp) of *B. pseudomallei* genome and for *Brucella* amplification of the *omp*2 region of the genome (282 bp) with the control strains and did not show non-specific amplification with heterologous Gram-negative bacilli. In experiments for determination of the lower limit of detection, the assay for *B. pseudomallei* was able to detect down to less than 1 CFU/mL and in the case of *Brucella* it was less than 2000 plasmid copies per microliter. Figures 1 and 2 show the gel analysis of the control strains in the study. Of the 301 blood cultures, 16 grew heterologous bacteria. None of the samples grew *B. pseudomallei* and *Brucella* in culture. Blood culture data is shown in Table 2. In PCR, 3 of 301 samples (1%) were positive for *Brucella* and 1 of 301 samples (0.3%) was positive for *B. pseudomallei*. Table 3 shows the PCR findings, duration and type of fever. The three *Brucella* positive individuals were from the rural

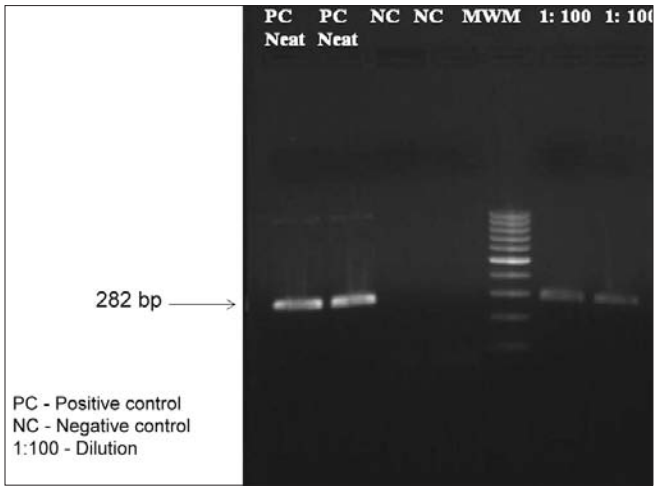

Figure 1: Gel analysis picture showing detection of *omp2* gene of *Brucella* species

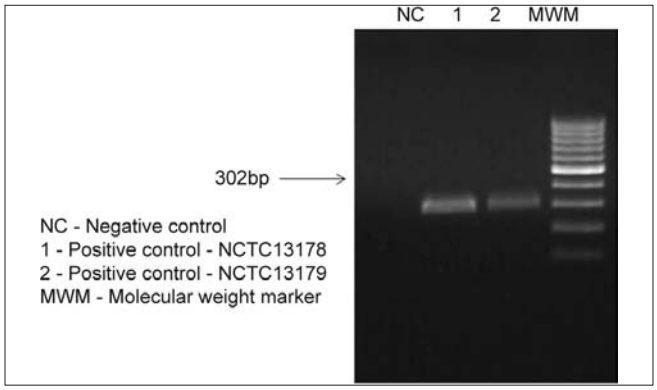

Figure 2: Gel analysis picture showing polymerase chain reaction for 16S-23S spacer region gene detection of *Burkholderia pseudomallei*

Table 2: Heterologous organisms grown in automated blood culture (BacT/Alert 3D)

Organism grown	Number of isolates
Staphylococcus species	4
Klebsiella	1
Proteus mirabilis	1
Pseudomonas species	3
Salmonella typhi	6
Non fermenting gram negative bacilli	1
No growth	285

None were positive for *M. tuberculosis*, *B. pseudomallei* or *Brucella species*

Table 3: Polymerase chain reaction findings in 301 cases of pyrexia of unknown origin

Study number	Polymerase chain reaction status	Blood culture status	Duration of fever	Type
56	*Brucella* and *S. typhi**	No growth	10	Intermittent
147	*B. pseudomallei*	No growth	8	Continuous
263	*Brucella spp.*	No growth	6	Continuous
264	*Brucella spp.*	No growth	6	Continuous

*Sample from study No. patient 56 grew *S. typhi* in blood and was buffy coat DNA extract positive by PCR for *Brucella* and *S. typhi* (PCR details not shown)

community. Two were female and 1 was a male. Among the *Brucella* positive individuals, one had intermittent fever and the other two had continuous fever. The lone case of *B. pseudomallei* positive individual was from the rural area. In all, 4 individuals positive for the two pathogens were from rural areas. No occupational risk factor was established in the study for the PCR positive individuals. The accuracy indices (sensitivity and specificity) were not calculated for the PCR assays, blood culture (gold standard) was negative for *Brucella* and *B. pseudomallei*.

DISCUSSION

In our study of 301 PUO cases, prevalence of *Brucellosis* was 1% and that of *Melioidosis* was 0.3% in patients. Both *Brucellosis* and *Melioidosis* positive patients were from rural community. No information was available on the antibiotic regimen prescribed and this is a limitation of this study. However, the patients who were positive for these agents were successfully treated as revealed by a home follow-up visit by a field worker. This study was hospital based and cross-sectional in nature wherein a sample of blood was collected and clinical information was obtained at the point of the first contact of the physician. The laboratory analysis included four organisms including *Mycobacterium tuberculosis*. The search for *M. tuberculosis* was included to assess its role as an agent presenting primarily with febrile illness in rural and peri-urban individuals. It must be stressed that for the study patients having fever of less than 15 days duration, were included. Though it is known that patients with tuberculosis would have prolonged fevers, the objective of including the search for *M. tuberculosis* was to investigate whether this organism would masquerade as a febrile illness without localizing symptoms. Furthermore, since the area has about 0.8% HIV seropositive status, it was important to obtain information on *M. tuberculosis* as a cause of febrile illness in this community. Prevalence of human *Brucellosis* has been reported in several parts of India. The disease is often ignored and misdiagnosed in the country.[11] The authors stated that the prevalence of human *Brucellosis* in India is underestimated and found that the situation is alarming. It is more closely associated with livestock systems and dairy products. In our study, although the patients positive for *Brucella* did not have contact with animals or a habit of drinking unpasteurized milk, about 1.7% of the study population had either been rearing animals at home or had a habit of drinking unpasteurized milk, and all of them were from rural population. Nimri and Batchoun[18] identified *Brucella* to be an important etiological agent in community-acquired bacteraemia. The prevalence of this pathogen was higher in rural population due to contact with infected animals, habits of drinking unpasteurized milk and consuming home-made soft cheese. This report did not have information on specific antibiotic therapy and treatment follow-up.

Ammari[19] identified *Brucellosis* apart from tuberculosis and typhoid fever to be a major cause of PUO. In the neighboring state of Karnataka, Mantur *et al.,*[13,20] based on a 16 years retrospective study period indicated that majority of cases are undiagnosed and untreated. Also, serology was found to be of poor value. In their study, a substantial number of patients (84.2%) presented with fever, and with fever alone in almost half of the cases. In a surveillance analysis by Mudaliar *et al.,*[21] among animal handlers in Pune, 5.3% were positive for *Brucella* antibodies. This included veterinary doctors who had 14.6% seropositivity among them. In Kerala, the seropositivity was 1.6% including veterinary students and general population.[22] Serodiagnosis seems to be complicated because of reported cross-reactivity with several gram negative bacilli such as *E. coli*, *Salmonella* and *Vibrio cholerae*.[13]

In a study by Demirtürk *et al.*,[23] among *Brucellosis* positive patients, only 17% were positive in blood culture. The most frequent symptom and clinical sign was fever. In a study by Shaheen *et al.*,[24] he could recover only 4 cases as positive for blood culture, out of the 21 (20%) *Brucella* positive patients. Blood culture is the gold standard for the isolation of *Brucella* as the treatment requires specific and prolonged antibiotics. Culture broths preferably Castaneda's medium have to be incubated for at least 45 days. A PCR assay targeting *omp*2 gene was developed for identification of human and animal strains.[25] However, it has not been evaluated on clinical samples. Our study, addressed these lacunae. In our study, no *Brucella* was positive by blood culture even in the automated system. This may be attributed to the use of the prior empirical antibiotic treatment taken by the patient themselves. Nevertheless, PCR proved to be a robust and sensitive method to detect *Brucella* from patients' buffy coat samples as this is a reticulo-endothelial parasite. One patient was co-infected with *Salmonella typhi*. This is in accordance with a previous report by Parker *et al.*,[26] who reported the occurrence of concomitant infections with pathogens such as *S. typhi* and *Brucella* in acute febrile illnesses.

Melioidosis is found to be endemic in Australia (Northern Australia) and Southeast Asia and sporadic cases were reported in many parts of our country.[3] The saprophyte can survive for years in hostile conditions in the soil which could act as a natural reservoir. The association between surface water and *Melioidosis* is attributed to the strong association with monsoonal rains and

occupational and recreational exposure to surface water and mud. This is particularly so with flooding of rice paddies and planting at the commencement of the monsoonal season. *B. pseudomallei* appear to be able to survive and multiply within professional phagocytes, including those of the macrophage/monocyte lineage. Hence, our study among rural patients is important and is the first prospective study on the role of *B. pseudomallei* in PUO cases primarily from rural patients in India. Though we found only 1 of 301 PUO cases to be positive for *B. pseudomallei*, it still indicates that unless improved screening of PUO cases especially for macrophage-tropic pathogens is done, there would be morbidity and mortality due to treatable infectious conditions. Among the various gene target evaluated for *B. pseudomallei*, the flagellin gene (*fli*C) was found to be useful in experimental infection.[27] Subsequently, in a study reported from Brazil by Merritt *et al*.,[15] using hemi-nested PCR targeting the 16S-23S rRNA intragenic spacer region, the authors found the assay to have high sensitivity and specificity. The second round of PCR did not improve the detection rate over the first round PCR alone. Therefore, we omitted the second round of the PCR and used a non-nested PCR format.

We had used PCR for detection of *S. typhi* and *M. tuberculosis*[28,29] apart from PCR for *Brucella* and *B. pseudomallei* in buffy coat samples of PUO cases. In all, 28 (9.3%) of 301 PUO cases had any of the 4 reticulo-endothelial pathogens detectable. *S. typhi* was seen in 14 (4.65%) cases and none of them were positive for *M. tuberculosis* either in culture or by nPCR. Hence, it may be suggested that tests for *Brucella* and *B. pseudomallei* should also form part of a diagnostic platform for patients with PUO. A convenient way for detecting multiple pathogens to establish the infectious etiology of PUO would be the development and evaluation of multiplex real time PCR or multiplex PCR followed by DNA microarray.

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REFERENCES

1. Jung A, Singh MM, Jajoo U. Unexplained fever-analysis of 233 cases in a referral hospital. Indian J Med Sci 1999;53:535-44.
2. Kejariwal D, Sarkar N, Chakraborti SK, Agarwal V, Roy S. Pyrexia of unknown origin: A prospective study of 100 cases. J Postgrad Med 2001;47:104-7.
3. Chugh TD. Emerging and re-emerging bacterial diseases in India. J Biosci 2008;33:549-55.
4. John TJ. *Melioidosis*, the mimicker of maladies. Indian J Med Res 2004;119:vi-viii.
5. Inglis TJJ, Rolim DB, Sousa Ade Q. *Melioidosis* in the Americas. Am J Trop Med Hyg 2006;75:947-54.
6. Cheng AC, Currie BJ. *Melioidosis*: Epidemiology, pathophysiology, and management. Clin Microbiol Rev 2005;18:383-416.
7. Sauret JM, Vilissova N. Human *Brucellosis*. J Am Board Fam Pract 2002;15:401-6.
8. Porbagher A, Pourbagher MA, Savas L, Turunc T, Demiroglu YZ, Erol I, *et al*. Epidemiologic, clinical, and imaging findings in *Brucellosis* patients with osteoarticular involvement. AJR Am J Roentgenol 2006;187:873-80.
9. Turunç T, Demiroglu YZ, Aliskan H, Colakoglu S, Timurkaynak F, Ozdemir N, *et al. Brucellosis* in cases of end-stage renal disease. Nephrol Dial Transplant 2008;23:2344-9.
10. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of *Brucellosis* in India. Vet Microbiol 2002;90:183-95.
11. Smits HL, Kadri SM. *Brucellosis* in India: A deceptive infectious disease. Indian J Med Res 2005;122:375-84.
12. Pearson GR, Freeman BA, Hines WD. Thin-section electron micrographs of monocytes infected with *Brucella suis*. J Bacteriol 1963;86:1123-5.
13. Mantur BG, Amarnath SK, Shinde RS. Review of clinical and laboratory features of human *Brucellosis*. Indian J Med Microbiol 2007;25:188-202.
14. Pongcharoen S, Ritvirool PN, Sanguansermsri D, Chanchan P, Jienmongkol P, Butkhamchot P, *et al*. Reduced interleukin-17 expression of *Burkholderia pseudomallei*-infected peripheral blood mononuclear cells of diabetic patients. Asian Pac J Allergy Immunol 2008;26:63-9.
15. Merritt A, Inglis TJJ, Chidlow G, Harnett G. PCR-based identification of *Burkholderia pseudomallei*. Rev Inst Med Trop Sao Paulo 2006;48:239-44.
16. Mitka S, Anetakis C, Souliou E, Diza E, Kansouzidou A. Evaluation of different PCR assays for early detection of acute and relapsing *Brucellosis* in humans in comparison with conventional methods. J Clin Microbiol 2007;45:1211-8.
17. Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of *Salmonella* PCR: Towards an international standard. Appl Environ Microbiol 2003;69:290-6.
18. Nimri LF, Batchoun R. Community-acquired bacteraemia in a rural area: Predominant bacterial species and antibiotic resistance. J Med Microbiol 2004;53:1045-9.
19. Ammari F. Fever of unknown origin in North Jordan. Trop Doct 2006;36:251-3.
20. Mantur BG, Biradar MS, Bidri RC, Mulimani MS, Veerappa, Kariholu P, *et al*. Protean clinical manifestations and diagnostic challenges of human *Brucellosis* in adults: 16 year's experience in an endemic area. J Med Microbiol 2006;55:897-903.
21. Mudaliar S, Bhore A, Pandit D. Detection of antibodies to *Brucella abortus* in animal handlers. Indian J Med Sci 2003;57:181-6.
22. Ajay Kumar VJ, Nanu E. Sero-positivity of *Brucellosis* in human beings. Indian J Public Health 2005;49:22-4.
23. Demirtürk N, Demirdal T, Erben N, Demir S, Asci Z, Kilit TP, *et al. Brucellosis*: A retrospective evaluation of 99 cases and review of *Brucellosis* treatment. Trop Doct 2008;38:59-62.
24. Shaheen HI, Mohamady HI, Ghabour S, Weiner M, Nakhlla I, Armstrong A. ASTMH 56th annual meeting abstract book: Abstract 905. Am J Trop Med Hyg 2007;77:258-344.
25. Bardenstein S, Mandelboim M, Ficht TA, Baum M, Banai M. Identification of the *Brucella melitensis* vaccine strain rev 1 in animals and humans in Israel by PCR analysis of the *Pst*I site polymorphism of its omp2 gene. J Clin Microbiol 2002;40:1475-80.
26. Parker TM, Murray CK, Richards AL, Samir A, Ismail T, Fadeel MA, *et al*. Concurrent infections in acute febrile illness patient in Egypt. Am J Trop Med Hyg 2007;77:390-2.

27. Hagen RM, Gauthier YP, Sprague LD, Vidal DR, Zysk G, Finki EJ, *et al*. Strategies for PCR based identification of *Burkholderia pseudomallei* DNA in paraffin wax embedded tissues. Mol Pathol 2002;55:398-400.
28. Nandagopal B, Sankar S, Lingesan K, Appu KC, Padmini B, Sridharan G, *et al*. Prevalence of *Salmonella typhi* among patients with febrile illness in rural and peri-urban population of Vellore district as determined by a nested PCR targeting the flagellin gene. Mol Diagn Ther 2010;14:107-12.
29. Nandagopal B, Sankar S, Lingesan K, Appu KC, Sridharan G, Gopinathan AK. Evaluation of a nested PCR targeting *IS6110* of *Mycobacterium tuberculosis* for detection of the organism in the leukocyte fraction of blood samples. Indian J Med Microbiol 2010;28:227-32.

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