DOI: 10.7860/JCDR/2020/46011.14270

Original Article

Microbiology Section

Brucellosis, Unravelling an Enigma: Eight Years of Experience from a Tertiary Care Hospital in Central Kerala, India

M ARDRA[1], CHITHRA VALSAN[2], KA SATHIAVATHY[3]

ABSTRACT

Introduction: Various emerging and re-emerging infectious diseases have made the existence of mankind in this world a great challenge. In the midst of these havocs, some important diseases has undermined in the dark. Brucellosis is an endemic zoonotic disease in most of the developing world and it has far-reaching and deleterious effects on humans and animals alike. In humans, brucellosis shows a variety of non-specific clinical signs. To recognise and diagnose this neglected but debilitating disease, the awareness and alertness of medical personnel has to be enhanced.

Aim: To determine the prevalence of brucellosis in Tertiary Care Hospital.

Materials and Methods: A retrospective study was conducted from June 2011 to May 2019, all culture proven cases of human brucellosis admitted in a Tertiary Care Hospital in central Kerala, India were reviewed. Demographic data, clinical presentations, laboratory parameters, treatment and outcomes of the same were analysed in Microsoft excel sheets as percentages.

Results: Of the 12 culture proven *Brucella* cases, 11 presented as Pyrexia of Unknown Origin (PUO) cases and one was a soft tissue infection. Eleven patients had history of either consumption of unpasteurised milk products or had contact with animals before the symptoms developed. A 75% cases were imported cases from middle-east countries. In one case the route of entry was by close personal contact probably sexual transmission. All the patients complained of fever and malaise (100%), while low backache and arthralgia was noted in 83%. Most common clinical and laboratory findings associated with brucellosis were hepatosplenomegaly (41.7%) and anaemia (66.6%). Oral doxycycline for six weeks combined with either aminoglycoside or rifampicin was used for treatment. There was no death or relapses noted.

Conclusion: This study emphasises the close collaboration of an alert clinician and an experienced microbiologist to correctly diagnose and treat an infection with multiple presentations as brucellosis, in endemic areas also.

Keywords: *Brucella melitensis*, Imported brucellosis, Pyrexia of unknown origin

INTRODUCTION

Brucellosis is a zoonotic disease which is caused by genus *Brucella* and widely observed around the world. Among the various species of the genus *Brucella, B. melitensis* has the highest virulence and causes the most severe disease. They are transmitted to humans by contact with infected secretions, inhalation of contaminated aerosols or ingestion of unpasteurised dairy products. It is an important human disease in many parts of the world especially in the Middle East. Five of the 10 countries with the highest incidence for human brucellosis are in this area [1]. This highlights the importance of eliciting a positive history by physicians dealing with PUO and travel medicine. In human beings, brucellosis infection can cause serious dysfunction and debilitation. It is rarely fatal, but it is reported that approximately 2% of the untreated patients die of brucellosis [2]. This chronic and persistent disease is capable of affecting any organ system with granulomatous change [1]. Clinical diagnosis of brucellosis is often challenging as it presents with non-specific signs and symptoms. Blood culture is the gold standard in the laboratory diagnosis of brucellosis, but this method is successful in only 40-70% of the cases [3]. *Brucella* spp. can also be isolated from various specimens like pus, tissue, Cerebrospinal Fluid (CSF), and pleural/joint/ascitic fluid [4]. In the absence of culture facilities, the diagnosis of brucellosis traditionally relies on serological testing with agglutination tests and Enzyme Linked Immunosorbent Assay (ELISA). ELISA which measures IgG, IgM, and IgA, is a standardised assay for brucellosis [3]. In acute brucellosis, specific IgM antibodies dominate and in relapsing and chronic cases specific IgG antibodies are present in the serum of patients. The specificity of ELISA, however, seems to be less than the agglutination tests [3]. Molecular confirmation by Polymerase Chain Reaction (PCR) utilising different gene targets has become the most common approach for confirmation of human and animal isolates [5].

The World Health Organisation (WHO) suggests only a limited number of antibiotics with good intracellular access and confirmed clinical effectiveness for the treatment of brucellosis [6]. The present study evaluated eight years' experience with brucellosis in a tertiary care teaching hospital in Kerala, India focusing on the clinical findings, occupational history, laboratory parameters, treatment and outcome of the patients.

MATERIALS AND METHODS

A retrospective observational study was conducted in Jubilee Mission Medical College, Thrissur, a Tertiary Care Teaching Hospital in central Kerala, India including all patients with a positive culture for *Brucella melitensis* during a period of eight years (1/6/2011 to 31/5/2019). Identification of these isolates was done by Vitek 2 compact system (BioMerieux, India Ltd.,). The clinical records of culture proven brucellosis cases were analysed six monthly every year from medical records library for collecting data regarding the demographic characteristics such as age, gender, residency, exposure history, clinical spectrum of infection, antibiotic therapy and outcome of the patients. The follow-up was done with the help of the contact details (telephone numbers) available in the clinical records of the patient after discharge from the hospital. The mean follow-up period was of six months.

Definitions used

Relapse: Reappearance of the symptoms after initial improvement followed by treatment within a month after the end of the prescribed regimen [7].

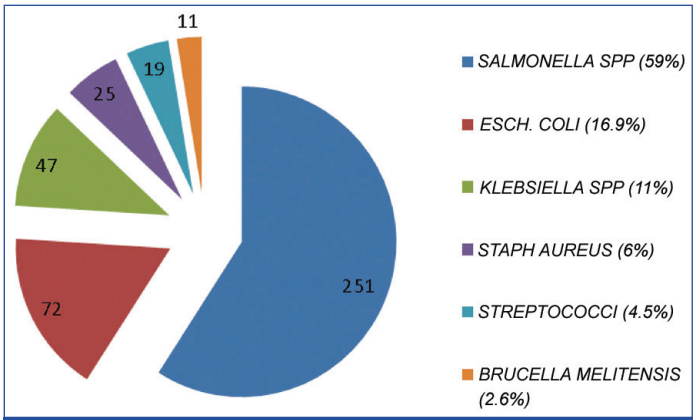
Cure: No recurrence of symptoms and signs during the six-month follow-up period [7].

STATISTICAL ANALYSIS

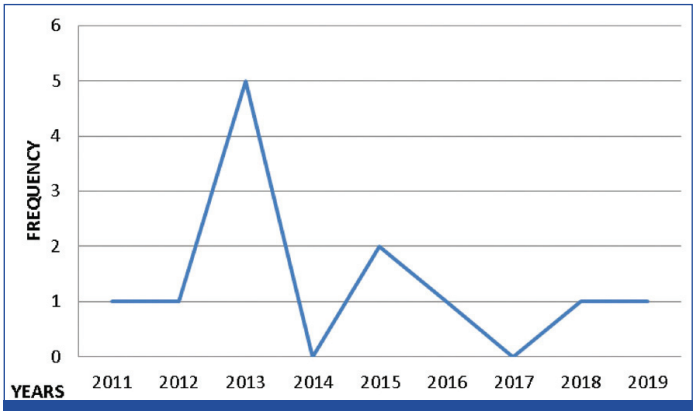
After coding, data was entered and analysed in Microsoft Excel version 10 and percentages were calculated for different parameters.

RESULTS

Twelve cases of *Brucella melitensis* were reported from the Department of Microbiology, Jubilee Mission Medical College, Thrissur, Kerala, India during this eight year period. Eleven of them were PUO cases (from non-repetitive blood samples) and one a soft tissue infection case (breast abscess). A total of 52880 blood samples were processed in Microbiology Department over a span of eight years and among which 5182 (9.8%) blood cultures were from patients suspecting PUO. Blood culture positivity rate of the laboratory was 8.2% (425). The organisms isolated from blood culture are detailed in [Table/Fig-1]. The annual distribution of *Brucella* cases are summarised in [Table/Fig-2].



[Table/Fig-1]: Details of blood culture isolates.



[Table/Fig-2]: Annual distribution of *Brucella* cases.

Demographic and exposure details of these 12 positive cases were collected. Distribution of cases based on districts of residence, Middle-east countries associated and departments in which patients got admitted is shown in [Table/Fig-3]. Presumed types of exposure of all the patients to the agent as per the history were outlined in [Table/Fig-4]. Out of 12 patients, nine (75%) were males and three were (25%) females with an age ranging from 25-57 years (mean age 37 years).

The severity of symptoms varied from mild illness to severe painful localised disease. Most of the cases (11/12) presented to the hospital after several weeks of mild non-specific complaints. Only one case presented as a localised painful lesion in the soft tissue. The illness duration prior to diagnosis ranged from seven to

	Frequency	Percentage
Sex distribution		
Male	9	75
Female	3	25
District wise case distribution		
Palakkad	6	50
Thrissur	5	41.7
Malappuram	1	8.3
Distribution of imported cases (n=9)		
Saudi Arabia	6	66.7
Sharjah	1	11.1
Oman	1	11.1
Abudubai	1	11.1
Admitted medical specialities		
Medicine	10	83.34
Surgery	1	8.3
Orthopaedics	1	8.3

[Table/Fig-3]: Demographic characteristics.

Type of exposure	Frequency	Percentage
Consumption of unpasteurised milk products or contact with animals	11	91.7
Other (close personal contact)	1	8.3

[Table/Fig-4]: Mode of transmission of *Brucella* spp among cases.

120 days. All the patients gave history of fever and malaise. History of all culture proven *Brucella* spp patients were thoroughly taken with an emphasis on type of exposure they probably had to get the infection. Various clinical presentations and laboratory parameters of the 12 cases are summarised in [Table/Fig-5].

	Number of patients	Percentage
Symptoms		
Fever	12	100
Malaise	12	100
Lower back pain and arthralgia	10	83
Gastrointestinal symptoms like vomiting, diarrhoea	4	33
Evening rise of temperature, weight loss, headache	3	25
Clinical findings		
Hepatosplenomegaly	5	41.7
Lymphadenopathy	4	33.3
Splenomegaly alone	3	25
Osteo-articular involvement	1	8.3
Breast abscess	1	8.3
Endocarditis	1	8.3
Deranged laboratory parameters		
Anaemia	8	66.6
Elevated erythrocyte sedimentation rate	7	58.3
Elevated liver enzymes	7	58.3
Thrombocytopenia	5	41.7
Leucopenia	1	8.3

[Table/Fig-5]: Clinical presentations and laboratory parameters of the brucellosis cases.

Blood samples of all the 11 PUO cases were indicated positive by automated blood culture system (BacT/Alert, BioMerieux India Ltd.,) in a mean detection time of three days. Blood and pus aspirate samples, on subculture yielded small greyish white non-haemolytic oxidase positive, urease positive, Gram negative cocco bacilli which were identified as *Brucella melitensis* by Vitek 2 compact system.

All the cases received doxycycline for minimum of six weeks. The treatment strategy followed is explained in [Table/Fig-6]. Two

cases were lost to follow-up (including one endocarditis case and the other case of PUO) for which complete treatment history could not be traced. There was no death or relapses noted during six month follow-up.

Antibiotics	Frequency (N=10)	Percentage
Doxycycline 100 mg BD for 6 weeks plus Gentamycin 120 mg IV OD for 10 days	5	41.7
Doxycycline 100 mg BD for 6 weeks plus Streptomycin 1 g IM OD for 2 weeks	3	25
Doxycycline 100 mg BD for 6 weeks plus Rifampicin 600 mg/Day for 6 weeks	2	16.6

[Table/Fig-6]: Details of brucellosis treatment regimens followed.
(n=2 lost to follow up)

DISCUSSION

Brucellosis is a zoonotic infection worldwide with more than 500,000 new cases annually with prevalence rate in some countries exceeds 10 cases per 100,000 population [1]. *Brucella* is classified as a Class B Bioterrorist agent [8] and its recent re-emergence had led to a renewed scientific interest in human brucellosis. *B. melitensis* is prevalent in Southeast Asian countries including India and mostly responsible for human brucellosis [5]. In many developing countries, brucellosis is endemic but remains under-diagnosed and under-reported because of its masquerading capability. Whether ingestion of contaminated dairy products or contact with infected animals has occurred is to be specially looked for. Detailed history taking from the patients is crucial especially in urban and non-endemic areas, and in cases of imported *Brucella*, in which travellers acquire the disease abroad and become ill in non-endemic settings [8]. In humans, the symptoms presents in majority of brucellosis cases are weight loss, undulant fever and night sweats. In endemic areas, it is one of the important causes of PUO and the reason for fever of prolonged duration [9]. Brucellosis results in mild chronic disease so that the diagnosis of the disease is difficult. In this study majority of cases (11/12) were diagnosed as PUOs. Some uncommon presentations of brucellosis include cutaneous and soft tissue lesions. In the present study, also there was a case of brucellosis with soft tissue infection presenting as breast abscess. An extremely rare manifestation of brucellosis in humans is Breast involvement but in animals it is common [10]. Based on a case series of brucellosis by Andriopoulos P et al, the reported prevalence of human brucellosis presenting as breast abscess is 0.7% [7].

The Middle-east countries are regarded as endemic for brucellosis. Analysing the demographic and occupational data in this study, it was found that 75% of the cases had a direct association with the Middle East countries in the form of occupational exposure or by ingestion of unpasteurised dairy products from there. A literature review of 505 imported cases of brucellosis including immigrants and foreign travellers illustrated that most imported cases were associated with travel and/or consumption of unpasteurised dairy products in or from endemic countries [11]. *Brucella* may enter the body through the gastrointestinal tract, the lungs or respiratory mucosal layers and spread through the blood and the lymphatic system to any other organ. Fresh milk and dairy products prepared from unpasteurised milk such as soft cheese, yoghurts and ice-creams may contain high amounts of the bacteria and consumption of these is an important cause of human brucellosis [12]. In the present study also, 91.7% of the brucellosis cases had given a history of consumption of unpasteurised milk in the form of fresh milk or milk shakes. In another study by Andriopoulos P et al., 84% of *Brucella* cases were by food borne transmission [7]. Thus, the main mode of brucellosis transmission is presumed to be through gastrointestinal route [13]. In this study, there was one case of human to human transmission of brucellosis probably sexually transmitted, where spouse of a brucellosis patient developed breast abscess. She presented with clinical features of breast abscess two months after the onset of her husband's disease. The chance of the lady acquiring disease from her husband is high as the appearance of disease falls in the incubation period. In cases of brucellosis in humans, genitourinary complications such as prostatitis and epididymo-orchitis are seen and seeding of these organs could perhaps involve in the disease transmission from person to person which is extremely rare [14]. The mean age of the patients with brucellosis in this study was 37 years (ranging from 25-57 years) with a male preponderance (75%). Although brucellosis affects all age groups, in this study, young adults were more commonly found to be affected. The clinical expression of brucellosis, including the frequency and type of signs, symptoms and complications are influence by the age distribution of the study population [15]. A similar observation has been made by Patil DP et al., where the mean age of the patients was 31 years and 72.2% were males [16]. Consistent with this result, the majority of studies had a male preponderance [13]. Sex distribution of patients with brucellosis varies widely- There are studies showing either equal distribution [17] or, even predominance of female patients [18].

According to the duration of symptoms, cases of brucellosis are classified arbitrarily as "acute" (less than eight weeks), "sub-acute" (from eight to 52 weeks), and chronic (more than 52 weeks). Acute brucellosis may progress to a more persistent disease with localised infections or a non-specific syndrome sometimes referred to as 'chronic fatigue syndrome' [16]. Complications observed can be very diverse including osteo-articular, gastrointestinal, hepato-biliary, respiratory or genitourinary [12]. In this study, acute and sub-acute type of presentations was commonly seen than the chronic type. The median illness duration prior to diagnosis was 47 days (range from seven to 120 days). Brucellosis duration prior to diagnosis is an important parameter, because it is directly correlated with the complication rate and unfavourable outcome [15]. Human brucellosis is considered as one of the "great imitators" because of its wide spectrum of clinical manifestations. The clinical features of brucellosis depend on the stage of the disease as well as the organs and systems involved [8]. In this study, fever and malaise (100%) were the predominant symptoms reported by the patients followed by low backache and arthralgia (83%) which was similar to that reported in previous literature [8,12]. *Brucella* spp after entering the gastrointestinal tract (most common route) localises the reticulo-endothelial system. According to Patil DP et al., the liver being the largest organ of reticulo-endothelial system is frequently affected resulting in hepatomegaly which is present in 32-63% of cases. The incidence of splenomegaly ranges from 29 and 56.6% of the cases. Anorexia, vomiting, diarrhea and constipation are other non-specific gastrointestinal manifestations of brucellosis [16]. In the present study hepatosplenomegaly (41.7%) was the most common clinical finding seen in the patients followed by lymphadenopathy (33.3%) and osteo-articular involvement (lumbosacral region) (8.3%). According to Andriopoulos P et al., splenomegaly (51%), osteo-articular involvement (42%), lymphadenopathy (31%) and hepatomegaly (25%) constituted the common clinical findings [7].

Mild haematologic abnormalities such as anaemia and leukopenia are associated with *Brucella* infection commonly [7]. In a case series by Dilek I et al., the incidence of anaemia, leucopenia and thrombocytopenia has been reported as 44 to 74%, 7.7% to 68% and 5% to 13.7%, respectively [19]. In this study, 66.6% patients had anaemia, 41.7% had thrombocytopenia, 8.3% had leucopenia, 58.3% had elevated liver enzymes and elevated Erythrocyte Sedimentation Rate (ESR). High incidence of anaemia and elevated ESR were also reported by Liu J and Zhao X and also by Sathyanarayanan V et al., [20,21]. The gold standard in diagnosis of brucellosis is identification of isolates from culture [22]. More culture positivity is with acute brucellosis than chronic or localised forms of disease [7]. Brucella is a slow growing bacterium and it may take weeks of incubation in blood culture bottles to get growth. The use of automated blood culture systems has shortened the time needed for detection. In this

study, blood samples were incubated in automated machine BacT/ALERT and the bottles were flagged positive in a mean detection time of three days. One study reported a mean detection time of 51.2±8.2 hours using BacT/ALERT standard aerobic bottles [23] where as another group showed the mean detection time between 1.8 and 3.7 days (mean: 2.5 days) [24]. The identification was done by Vitek 2 compact system in present study.

According to WHO, oral doxycycline for six weeks combined with gentamycin or streptomycin during the first two to three weeks of therapy is effective for human brucellosis [6]. Five out of 12 cases (41.7%) were treated successfully with doxycycline in combination with gentamycin, three cases (25%) received doxycycline in combination with streptomycin and two cases (16.6%) were treated with doxycycline in combination with rifampicin. Follow-up details of two cases were lost (including one endocarditis case and the other case of PUO) for which complete treatment history could not be traced. There was no death or relapse noted during the six months follow-up. *Brucella* endocarditis involving aortic valve is a rare but most serious complication of human brucellosis. Though it was observed in less than two percentage of the brucellosis cases, it is the main cause responsible for up to 80% of infection-related deaths in brucellosis. This condition is conventionally managed by a combined medical and surgical approach. Surgical intervention includes valve replacement with adequate debridement [25]. Brucellosis, remains as a diagnostic puzzle in both endemic and non-endemic regions due to unusual presentations and non-specific symptoms. It is frequently overlooked, misdiagnosed, or diagnosed incidentally; therefore, physicians must be aware of such a treacherous illness and consider brucellosis in their differential diagnosis of febrile diseases with peculiar musculoskeletal or other focal findings.

Limitation(s)

It was primarily associated with challenges inherent to retrospective review of clinical and epidemiological data. As a result, information on the treatment and follow-up were missing for some cases. Health education regarding preventive aspects of the disease including vaccinating the animal reservoirs, consumption of pasteurised milk and milk products, avoiding occupational risk exposures, also could not be emphasised. Brucellosis, remains a diagnostic puzzle in both endemic and non-endemic regions due to unusual presentations and non-specific symptoms. It is frequently overlooked, misdiagnosed, or diagnosed incidentally; therefore, physicians must be aware of such a treacherous illness and consider brucellosis in their differential diagnosis of febrile diseases with peculiar musculoskeletal or other focal findings.

CONCLUSION(S)

Brucellosis is an infection with multiple presentations, and whether in an endemic region or not, a thorough history of exposure, travel and clinical suspicion are required for early diagnosis of brucellosis and to facilitate prompt therapy that helps in timely management of this infectious disease with a successful outcome.

REFERENCES

[1] Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis. 2006;6(2):91-99.
[2] Madkour MM, Madkoure. Brucellosis. New York: Springer Verlag; 2001.
[3] Christopher S, Umapathy BL, Ravikumar KL. Brucellosis: Review on the recent trends in pathogenicity and laboratory diagnosis. J Lab Physicians. 2010;2(2):55-60.
[4] Mantur BG, Biradar MS, Bidri C, Mallana S, Verrappa K, Kariholu P, et al. Protean clinical manifestation & diagnostic challenges of human brucellosis in adults: 16 yrs experience in an endemic area. J Med Microbiol. 2006;55(7):897-903.
[5] Bamaiyi PH, Hassan L, Khairani-Bejo S, Zainal Abidin M. Update on Brucellosis in Malaysia and Southeast Asia. Malaysian J Vet Res. 2014;5(1):71-82.
[6] Corbel MJ. Brucellosis in Humans and Animals. Geneva: World Health Organization in Collaboration with the Food and Agriculture Organization of the United Nations and World Organization for Animal Health 2006. Pp.36-38.
[7] Andriopoulos P, Tsironi M, Deftereos S, Aessopos A, Assimakopoulos G. Acute brucellosis: Presentation, diagnosis, and treatment of 144 cases. Int J Infect Dis. 2007;11(1):52-57.
[8] Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. Lancet Infect Dis. 2007;7(12):775-86.
[9] Pathak AD, Dubal ZB, Doijad S, Raorane A, Rodrigues S, Naik R, et al. Human brucellosis among pyrexia of unknown origin cases and occupationally exposed individuals in Goa Region, India. Emerg Health Threats J. 2014;7:23846.
[10] Al Abdely HM, Halim MA, Amin TM. Breast abscess caused by *Brucella melitensis*. J Infect. 1996;33(3):219-20.
[11] Norman FF, Monge-Maillo B, Chamorro-Tojeiro S, Pérez-Molina JA, López-Vélez R. Imported brucellosis: A case series and literature review. Travel Med Infect Dis. 2016;14(3):182-99.
[12] Smits HL, Kadri SM. Brucellosis in India: A deceptive infectious disease. Indian J Med Res. 2005;122(5):375-84.
[13] Akhvlediani T, Bautista CT, Garuchava N, Sanodze L, Kokaia N, Malania L, et al. Epidemiological and clinical features of brucellosis in the country of Georgia. PLoS One. 2017;12:e0170376.
[14] Vigeant P, Mendelson J, Miller MA. Human to human transmission of *Brucella melitensis*. Can J Infect Dis. 1995;6(3):153-55.
[15] Bosilkovski M, Krteva L, Dimzova M, Vidinic I, Sopova Z, Spasovska K. Human brucellosis in Macedonia- 10 years of clinical experience in endemic region. Croat Med J. 2010;51(4):327-36.
[16] Patil DP, Ajantha GS, Shubhada C, Jain PA, Kalabhavi A, Shetty PC, et al. Trend of human brucellosis over a decade at tertiary care centre in North Karnataka. Indian J Med Microbiol. 2016;34(4):427-32.
[17] Shehabi A, Shakir K, el-Khateeb M, Qubain H, Fararjeh N, Shamat AR. Diagnosis and treatment of 106 cases of human brucellosis. J Infect. 1990;20(1):05-10.
[18] Demiroglu YZ, Turunc T, Aliskan H, Colakoglu S, Arslan H. Brucellosis: Retrospective evaluation of the clinical, laboratory and epidemiological features of 151 cases. Mikrobiyol Bul. 2007;41(4):517-27.
[19] Dilek I, Durmus A, Karahocagil MK, Akdeniz H, Karsen H, Baran AI, et al. Hematological complications in 787 cases of acute brucellosis in Eastern Turkey. Turk J Med Sci. 2008;38(5):421-24.
[20] Liu J, Zhao X. Clinical features and serum profile of inflammatory biomarkers in patients with brucellosis. J Infect Dev Ctries. 2017;11(11):840-46.
[21] Sathyanarayanan V, Razak A, Saravu K, Ananthakrishna SB, Prabhu M, Vandana KE. Clinical profile of brucellosis from a tertiary care center in southern India. Asian Pacific Journal of Tropical Medicine. 2011;4(5):397-400.
[22] Barua A, Kumar A, Thavaselvam D, Mangalgi S, Prakash A, Tiwari S, et al. Isolation & characterization of *Brucella melitensis* isolated from patients suspected for human brucellosis in India. Indian J Med Res. 2016;143(5):652-58.
[23] Sümerkan B, Gökahmetoglu S, Esel D. *Brucella* detection in blood: Comparison of the BacT/Alert standard aerobic bottle, BacT/Alert FAN aerobic bottle and BacT/Alert enhanced FAN aerobic bottle in simulated blood culture. Clin Microbiol Infect. 2001;7(7):369-93.
[24] Baysallar M, Aydogan H, Kilic A, Kucukkaraaslan A, Senses Z, Doganci L. Evaluation of the BacT/ALERT and BACTEC 9240 automated blood culture systems for growth time of *Brucella* species in a Turkish tertiary hospital. Med Sci Monit. 2006;12(7):235-38.
[25] Sasmazel A, Baysal A, Fedakar A. Treatment of *Brucella* endocarditis: 15 years of clinical and surgical experience. Ann Thorac Surg. 2010;89(5):1432-36.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India.
2. Professor, Department of Microbiology, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India.
3. Professor and Head, Department of Microbiology, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. M Ardra,
Assistant Professor, Department of Microbiology, Jubilee Mission Medical College, Thrissur-680005, Kerala, India.
E-mail: mardra85@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 26, 2020
- Manual Googling: Oct 07, 2020
- iThenticate Software: Nov 12, 2020 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 20, 2020**
Date of Peer Review: **Aug 25, 2020**
Date of Acceptance: **Oct 07, 2020**
Date of Publishing: **Dec 15, 2020**