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Human Brucellosis: A Case Series Over 12 Months From A Rural Hospital In Western Maharashtra, India.

Vaibhav Vitthalrao Rajhans¹, Shahariar Bahman Roushani²,
Deepika Shivaji Bhalerao³, Anagha Subhash Vaidya⁴, and
Anita Balakrishnan Nair^{5*}.

¹Associate Professor, Microbiology, Dr. Balasaheb Vikhe Patil Rural Medical College, Loni, Maharashtra, India.

²Professor & HOD, Microbiology, Dr. Balasaheb Vikhe Patil Rural Medical College, Loni, Maharashtra, India.

³Professor, Microbiology, Dr. Balasaheb Vikhe Patil Rural Medical College, Loni, Maharashtra, India.

⁴Professor, Microbiology, Dr. Balasaheb Vikhe Patil Rural Medical College, Loni, Maharashtra, India.

⁵Assistant Professor, Microbiology, Dr. Balasaheb Vikhe Patil Rural Medical College, Loni, Maharashtra, India.

ABSTRACT

Human brucellosis is very common in developing countries like India, more so in the rural population. We encountered 8 cases of brucellosis diagnosed by blood culture & Vitek 2. Blood cultures were done by automated blood culture system BacT/ALERT 3D 120 by Biomerieux; and bacterial identifications were done by Vitek 2 Compact system by Biomerieux. *Brucella melitensis* was isolated from the blood cultures of 8 patients. On provisional diagnosis, no patients were clinically suspected to be having brucella infection. Most of the patients presented with complaints of body aches, predominantly backache, which is a very common presentation of brucellosis. 7 out of 8 patients were successfully treated with antibiotics. Animal exposure which is very common in rural parts seems to be the main factor for the occurrence of this zoonotic disease in humans. There feels a dire need for proper guidelines on antibiotic susceptibility testing of brucella, which is not available either in CLSI or EUCAST. Clinicians and microbiologists have to work hand in hand to rapidly diagnose and treat this condition to minimize morbidity & mortality.

Keywords: bacteraemia, BacT/ALERT, blood culture, *Brucella melitensis*, brucellosis, Vitek, zoonosis.

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**Corresponding author*

INTRODUCTION

Humans can get brucellosis through various routes like consumption of un-boiled or non-pasteurised milk or infected dairy products, eating infected meat, contact with infected livestock, infections of the breaches in the skin surface, conjunctival inoculation and aerosol inhalation [1, 2].

Human brucellosis is known to be one of the major bacterial zoonotic diseases and it is reported from all over the world. Globally, around 5 lakh new cases are reported every year, and the annual incidence varies from as low as 2 cases to more than 500 cases per 10 lakhs from populations of different geographical areas [2]. Although so many possible modes of transmission of brucellosis are there, fortunately, there is no evidence of man-to-man transmission of this disease [3]. Despite being one of the major zoonosis and an occupational hazard; clinicians miss many cases of brucellosis because it is not considered a common disease [4]. However, literature says the otherwise. Several publications suggest that human brucellosis can be a fairly common condition and this holds for developing countries like India [5]. We hereby reported 8 cases of human brucellosis diagnosed by an automated blood culture system and automated bacterial identification system at a tertiary care hospital in a rural setup of western Maharashtra, over 12 months.

MATERIALS AND METHODS

One of the important ways to limit the hospital-level and community-level transmission of infections is early detection. Thanks to the recent advances in technology which can provide rapid, accurate tests for early detection of infectious diseases in humans as well as animals [6].

As with all other routine blood cultures samples, blood cultures from all 8 patients of brucellosis were processed in BacT/ALERT 3D 120 (Biomérieux) automated blood culture system. The BacT/ALERT system blood culture bottle incubation was set to the maximum of 5 days, meaning, bottles loaded in BacT/ALERT system were flagged negative if there was no change of colour in the bottom disc even after 5 days of incubation. The consent of the patients &/or family was taken as per the hospital protocol.

If blood culture bottles were flagged positively in BacT/ALERT, subcultures were done on Blood agar and MacConkey agar. The culture media plates were incubated at 37°C overnight. After obtaining growths on the culture media plates, the growth was subjected to Gram staining & other routine biochemical tests like catalase test, oxidase test, and motility test.

Tiny moist grey-coloured non-haemolytic colonies on Blood agar, showing very short gram-negative coccobacilli which are nonmotile and giving positive oxidase reaction; along with scanty or no growth on MacConkey agar were presumptively identified as *Brucella*. The urease test was done as an additional identification test [7]. The pure growth of the same was then subjected to automated identification in the Vitek-2 compact (Biomérieux) system, using the GN ID cards.

Case Wise Presentations

Case 1: 40-year-old male presented to this hospital with neck pain and generalised body ache. Provisionally was diagnosed as having infective spondylitis (C4 - C5) and blood culture and other laboratory investigations were done. He was positive for HBsAg & HBeAg, was having pan-leucocytosis & the ESR was raised. After diagnosis of brucellosis, he was treated with cephalosporins to which he responded well.

Case 2: 85 years male was admitted with complaints of fever with disorientation. Given the serious condition, he was clinically diagnosed with a case of Pneumonia with septicaemia along with ischaemic heart disease (IHD) and was treated in cardiac ICU. For laboratory investigations, Blood cultures and other samples were sent. Haemoglobin was low, LDH was raised & there was neutrophilic leucocytosis. After the diagnosis of brucellosis, he was treated with multiple antibiotics. But as some other major comorbidities were present, the patient could not be saved.

Case 3: 48 years old female presented with fever & chills along with body ache. After a full clinical examination, she was admitted as a case of anaemia, hepatic haemangioma and cholelithiasis. Blood culture and other routine laboratory investigations were done. Iron deficiency anaemia was detected.

Brucella was detected in blood culture in 2 different samples taken 3 days apart. After the diagnosis of brucellosis, she was treated with cephalosporins and she recovered.

Case 4: 56-year-old male came to the hospital with complaints of fever with chills and body ache. He was a known alcoholic. The provisional diagnosis was Pyrexia of unknown origin (PUO). Blood culture showed brucellosis. Multiple antibiotics were administered and he recovered well.

Case 5: 17 years female presented with walking difficulty. There was pain in the right hip which increased on walking. Fever was also present. She was provisionally diagnosed as having septic hip & blood culture and other laboratory investigations were done. Serum urea was found to be marginally increased (46 mg/dl). After diagnosing with brucellosis, she was treated with cephalosporins and good recovery was seen in the patient.

Case 6: A 40-year-old female patient was admitted for backache. Lumbar spondylitis was provisionally diagnosed and laboratory investigations including blood culture were done. Haemoglobin was low (8 mg/dl) & blood culture showed *Brucella* bacteraemia. She was treated with multiple antibiotics and she responded well.

Case 7: A 2-year-old girl was admitted to the paediatric ward for weakness. She was clinically diagnosed as having Congestive cardiac failure. Haemoglobin was 6 mg/dl, and blood culture showed growth of *Brucella*. She responded well to the conventional treatment & was discharged after full recovery.

Case 8: 8-year-old female child was brought to the hospital with complaints of fever, weakness and body ache. The provisional diagnosis was Pyrexia of unknown origin (PUO). Blood culture & other laboratory investigations were done. After the diagnosis of brucellosis, she was treated with cephalosporins to which she showed good response.

RESULTS

All 8 isolates from the blood cultures of the 8 patients were identified as *Brucella melitensis* on Vitek 2 compact. The antibiograms were not given by the Vitek-2 compact system to any of the isolates; as there are no standard guidelines available for antibiotic susceptibility testing against brucella isolates.

For additional confirmation and cross-checking of the Vitek results, randomly selected isolates of *Brucella melitensis* were sent to **Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh**. The sent isolates were confirmed as *Brucella melitensis* by the said referral centre.

Age distribution: Out of 8 cases, only 3 cases were on the younger side (2 years, 8 years & 17 years). All other cases were middle age and above. (2 cases 40 years, 1 case 48 years, 1 case 56 years & 1 case 85 years)

Sex distribution: There were 3 male & 5 female cases of brucellosis.

Symptomatic presentation: Out of 8 cases, 6 cases presented with body ache, mainly in the joints, backache or pain while walking. In 1 case, an 85-year-old male presented with complaints of fever with disorientation, & the 2-year-old female patient was brought to the hospital with complaints of weakness.

DISCUSSION

According to the available literature, incubation of 3 days is sufficient for the detection of routine bacteria and yeast when using blood culture bottles of BacT/ALERT by Biomerieux [8]. According to our observation, all the blood cultures which were identified as brucella were flagged positively after a minimum of 3 days of incubation. This is certainly in contrast with routine bloodstream infections as blood cultures are flagged positive well before 3 days of incubation for pathogens other than brucella, in BacT/ALERT. However, after subculturing from BacT/ALERT bottles, the culture media plate incubation didn't take more time. Growth of brucella on enriched culture medium such as blood agar, was seen after single overnight incubation, as with any other routine bacteria.

Brucellosis can be said to be one of the major debilitating illnesses, causing severe human disease and high economic losses. Number of cases is on the higher side in the western parts of Asia, some Middle Eastern countries & some countries from southern Europe and Latin America. In India, brucellosis is a significantly increasing health issue in animals & human populations because around 80% of the population lives in rural areas. Owing to the different occupations on rural sides, humans do have close contact with domestic as well as wild animal populations. Ours is principally an agrarian country. Animal husbandry is the 2nd largest occupation in rural parts of India. The prevalence of brucellosis has been widely reported in different states of India such as Punjab, Orissa, Andhra Pradesh, Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Goa. It can be said that the human population is at a greater risk of contracting zoonotic diseases such as brucellosis [2].

Serologically, brucellosis can be diagnosed presumptively by demonstrating rising antibody titres to *Brucella* antigens, yet, bacterial culture of blood, bone marrow, or tissue culture remains the gold standard. Because of the suboptimal recovery rate of brucellae even from blood, it is generally advised to try isolating brucellae from culture of bone marrow aspirates, liver tissue or lymph nodes to improve the recovery rate of the organism. This method is more useful because *Brucella* organisms survive the intracellular killing by phagocytes and polymorphonuclear white blood cells and can localize in the reticuloendothelial system [1].

Brucella bacteria have a longer doubling time of two to four hours, as compared to other human bacterial pathogens. Through the conventional methods, incubation of blood cultures for 30 days instead of the routine just 7 days period and performance of blind subcultures are advised to increase the chances of recovery of these fastidious organisms because brucellae present in blood culture broths are many times without any visible evidence and take much longer before attaining detectable numbers [1].

In older literature, incubation of blood culture for up to 35 days is suggested even by Castaneda's method. Blood cultures of brucella even by automated systems need more time, the reason for which lies in the metabolism of the bacteria. The slow release of CO₂ by members of the genus doesn't reach the threshold or detectable levels as it does for other common bacteria detected in the blood [1]. This perhaps explains the longer incubation in BacT/ALERT system required for brucella, observed in our cases.

Barua A et al (2016) mention that among all *Brucella* species, *Brucella melitensis* is the predominant species responsible for human brucellosis, mainly in south-east Asian countries including India [9]. This is consistent with the findings in our study.

As described by DP Patil *et al* (2016), although the affliction of various organs by *Brucella* infection is known, almost all *Brucella*-positive cases in their study presented with a history of fever, followed by fever with chills and rigors, low backache, scrotal pain and swelling. According to their study, night sweats are said to be a characteristic feature of brucellosis. Large or medium-sized joint infections and infections of sacroiliac joints or the spine is the most common localised complication of brucellosis. The other common complications include orthopaedic complications such as spondylitis, synovitis and arthritis [2]. Spondylitis and arthritis were observed in our brucellosis cases as well.

Chahota R *et al* (2015) mention that the most common clinical features of human brucellosis are undulant fever, sweating, arthralgias, myalgias, lymphadenopathy and hepatosplenomegaly. According to their study too, arthritis is one of the common complications[10].

Arthritis or backache or pain while walking was a predominant clinical feature as seen in our study also. Fever was reported in 5 out of the 8 cases as one of the main symptoms.

According to Mantur BG *et al* (2010), human brucellosis most commonly presents with fever as its primary symptom. A few other studies mention sweating with bad odour to be pathognomic. We were not able to verify this finding in our patients. Mantur BG *et al* (2010) also mention that the most frequent complications of brucellosis are bone and joint involvement and occur in up to 40% of the cases. Three distinct forms of arthritis are seen; peripheral arthritis, sacroiliitis and spondylitis. Out of these three forms, peripheral arthritis is the most common. Their study also says that some cases may present with only joint pain or only low back ache [5]. This observation is also seen in our study.

Adequate epidemiologically relevant information such as animal contact from patients must be gathered which will be helpful for the control of this public health problem. An accurate diagnosis followed by recommended anti-Brucella therapy is curative [2]. All the patients in our study came from rural populations, all were either directly or indirectly exposed to the livestock present in villages.

For a useful antibiotic treatment, the doses should start on time, broad-spectrum antibiotics with at least one drug having a good penetration into cells should be given and it also should have prolonged blood levels. According to the World Health Organization, the recommended treatment for acute brucellosis in adults is as follows-

- Rifampicin 600 to 900 mg twice daily for a minimum of six weeks
- Doxycycline 100 mg twice daily for a minimum of six weeks.

Childhood brucellosis can be effectively treated with a combination of the same two drugs in the following dosage

- Rifampicin 10 mg/kg/day orally for six weeks
- Doxycycline 4 mg/kg/day orally for six weeks

Brucellosis in pregnancy responds well to rifampicin with or without cotrimoxazole [5].

According to Mantur BG et al (2001), brucellosis patients showed good response to combination therapy of tetracycline & streptomycin [11].

Outcomes

Out of the 8 cases, 7 cases responded to the treatment very well, were treated in general wards and were discharged eventually after recovery. Only one patient, who was the oldest (85 years) among the 8; and also had a history of ischaemic heart disease, needed intensive care (ICU), but couldn't be recovered and succumbed to the illness.

Limitations of the study

We did not perform any antibiotic susceptibility testing, neither conventional nor automated, for the brucella isolates. There are no standard guidelines available for the same. For the diagnosis of brucellosis, we relied only on blood culture. Bone marrow culture or any other tissue culture was not done. No serological tests for brucellosis were done.

CONCLUSION

Brucellosis is still observed in rural populations because of people's close association with livestock. Automated blood culture and automated identification systems certainly help in the early and certain identification of brucella bacteraemia.

The guidelines for antibiotic testing for brucella in-vitro need to be prepared so that exact and specific antimicrobial therapy can be instituted in brucellosis patients as soon as possible and with fair confidence.

Control of brucellosis in domestic livestock by mass vaccination of animals can be said to be a good way to prevent human brucellosis. This becomes more crucial because of the lack of human vaccines and lack of effective control measures. That's why it becomes necessary for doctors and other healthcare workers to take protective measures for themselves and others in turn. Occupational occurrence of brucellosis can be reduced by the use of protective or barrier clothing while handling animal stillbirths & products of animal conception and the use of personal protective equipments (PPEs) while processing the microbiological cultures in the lab. As a general measure in the beginning and at a household level, consuming only properly pasteurised dairy products will prevent infection in the community.[5]

For early diagnosis & effective treatment of brucellosis, sound coordination between clinicians and microbiologists is important.

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