

Original Research Article

Clinical profile of Brucellosis from a Tertiary care Hospital in South India

Running Title : Clinical spectrum of Brucellosis.....

Abstract:

Background :Brucellosis is an under reported,emerging zoonotic disease with worldwide distribution, which is particularly endemic in many countries of the Mediterranean basin. We reviewed thirteen cases of culture proven Brucellosis and their clinical manifestations admitted at our Institute.

Materials and Methods: Medical records of thirteen cases of Brucellosis were reviewed between Jan 2013 to 2017 June (a period of 3years 6months) mographic data ,clinical manifestations ,clinical complications and outcome of these cases were analysed

Results:The age range of patients was between 26-57 years with a female preponderance (7females and 6 males).All the patients had history of occupational exposure to cattle and they were hailing from rural areas(13 /13).In 8 patients with Pyrexia of unknown origin (PUO) fever was the main presenting symptom and the duration of fever was for more than 3 months with associated joint pains,headache and vomitings.Five patients had features suggestive of Infective Endocarditis (IE) with vegetations on Aortic valve. 13/13 cases were positive for Brucella culture.5/13 cases positive for Brucella antibodies were also positive by culture.8/13 cases were negative for Brucella antibodies.

Conclusion: Early diagnosis of brucellosis facilitates prompt therapy that helps in timely management of this Infectious disease with a successful outcome.

Key words: Brucella endocarditis, Aortic valve,*Brucella melitensis*,zoonotic,

Background: Brucellosis is a worldwide zoonotic infection which is prevalent in western parts of Asia,Middle Eastern,Southern European,and Latin American countries.[1].Humans are infected by close contact with an animal,or by the consumption of meat or dairy products infected by Brucella.The global burden of human brucellosis remains to be more than 500,000 infections per year.[2] The three most frequent agents of human brucellosis are *B .melitensis*, *B. abortus*, *B. suis* and less frequently *B. canis*. [3]
The most common causative agents of Brucellosis in India are *B. melitensis*, and *B. abortus*.

B. melitensis is the most virulent strain and currently accounts for most recorded cases globally with cattle emerging as an important reservoir. [4]

The clinical spectrum of human brucellosis ranges from inapparent, asymptomatic infection to severe disease with life-threatening complications. Symptoms of brucellosis are usually non-specific and include fever, sweating, malaise, weight loss, fatigue, headaches, arthralgia and myalgia. Osteoarticular manifestations are the most frequent focal complications, often affecting the spine and sacroiliac joints followed by pneumonia, meningitis and endocarditis. Usually Brucellosis patients present with pyrexia associated with backache and joint pains and a high degree of early clinical suspicion about the disease and adequate management is essential for the diagnosis. [5] The incidence rate of brucellosis in patients of Pyrexia of unknown origin is 132.4 per 100,000 cases. [6]

Cardiac involvement comprises an important complication of brucellosis and is responsible for up to 80% of *Brucella*-related deaths, [7] although *Brucella* endocarditis itself occurs in only 1–2% of all cases. [1] In *Brucella* endocarditis, aortic valve involvement is frequently observed and endocarditis is the most common cause of death [8,9]

We present 13 case series of Brucellosis to create awareness regarding the disease in specific clinical situation, particularly in the persons belonging to high risk category for Brucellosis and to look into various clinical manifestations and discuss them along with a review of the published literature on brucellosis. We reviewed eight cases of Brucellosis which presented as Pyrexia of unknown origin (PUO) with fever, joint pains as the major clinical manifestation and 5 cases of Brucellar Endocarditis.

Objective: To review the demographic, clinical complications, laboratory findings and outcome of Brucellosis in our case series.

Materials and Methods : Medical records of thirteen cases of culture proven Brucellosis and their clinical manifestations were reviewed, admitted at our Institute between Jan 2013-2017 (a period of 4 years 6 months). Brucellosis was diagnosed by the presence of antibodies against brucella > 1:160 by the standard tube agglutination test and/or by isolation of brucella from blood cultures in addition to clinical symptoms consistent with brucellosis. From each patient 2 sets of blood cultures were collected in 24 hours, 10ml each in SN and FN plus aerobic blood culture bottles and incubated in BacT/AlerT 3D continuous monitoring blood culture system (CMBCS). *Brucella melitensis* was isolated from 2 sets of Blood cultures within 3 days by using BacT/AlerT SN and FN plus aerobic bottles. Isolates were identified by Vitek 2 system using ID GN panel. Serological diagnosis of brucellosis was done by standard tube agglutination test.

Microbiological workup: *Brucella melitensis* was isolated from 2 sets of Blood cultures within 3 days (average) from each of these patients by using BacT/Alert FA Plus and Standard aerobic bottles (*bioMerieux,France*).Blood cultures flagged positive at an average time of 1.8-2.8 days.Positive Blood cultures broth and aortic valve tissue was subcultured on blood agar and chrome agar and incubated in a 5% carbondioxide generating pouch at 37°C for 48hours.Grey white pin point moist non haemolytic colonies were observed on both media after 48 hours.(Figure:1) Grams stain of colonies showed Gram negative coccobacilli. (Figure:2) The isolates were identified by Vitek 2 system (*bioMerieux,France*) using ID GN panel. Standard tube agglutination was done for all the cases for detection of *Brucella melitensis* antibodies

Results:The age distribution of our cases were between 26-57 years with a female preponderance (6males and 7females).Majority were in the age group of 51-60 years (Table:1)All our patients (13/13) had history of occupational exposure to cattle and they were hailing from rural areas.Eight cases presented as PUO and 5 cases presented as Brucellar Endocarditis.(Table:2) In PUO and Brucellar Endocarditis cases the duration of fever varied (Table:3)Fever and chills with rigors were the main presenting symptoms in our study followed by other symptoms (Table 4).We observed the laboratory characteristics of these patients as described in Table.Trans oesophageal echocardiography (TEE) was done in 6/13 cases where vegetations were observed seen in 5/13cases.Surgical procedure aortic valve replacement (AVR) was done for two patients.

13/13 cases were positive for Brucella culture. 5/13 cases positive for Brucella antibodies were also positive by culture.8/13 cases were negative for Brucella antibodies.(Table:5) Serological titres of Brucella antibodies ranged from 1:160 to 1:1280 (Table:6)

In 2 cases with Infective Endocarditis *Brucella melitensis* was also isolated from aortic valve tissue and was treated successfully with doxycycline in combination with Gentamicin for 6-8 weeks.All our PUO cases were treated with doxycycline in combination with Streptomycin for 6-8 weeks with a successful outcome. There was no mortality recorded in our case series

Discussion: Brucellosis is one of the most common zoonotic infections globally distributed. This disease was fully elucidated by Sir David Bruce,Hughes,and Zammit working in Malta. [10].It is a facultative intracellular,oxidase positive,urease positive, Gram negative coccobacilli. [11]

Brucella is an emerging potential biothreat pathogen with public health importance. As a biosafety level three organism, suspected Brucella spp. should be handled with personal protective equipment (PPE) that includes protective laboratory clothing, gloves, face and eye protection and respiratory protection.Brucellosis is a bacterial disease transmitted to humans

by consumption of infected, unpasteurised animal milk or through direct contact with infected animals, particularly aborted fetuses.[12]

The pathogenic species of Brucellosis are *B.melitensis*,*B.abortus* and *B.suis*.*B.suis* is rarely found in 5% cases.In all our cases *B.melitensis* was isolated from culture which remains a gold standard test.[3]

This disease is more common in rural areas.All our patients were also hailing from rural areas similar to Mohan et al study[13].Brucellosis is a protean disease with multisystemic involvement.Misdiagnosis and delay in treatment leads to complications like bone and joint involvement,endocarditis, meningitis, pneumonia and epididymo-orchitis.[14]

Female preponderance in our study was probably because of direct contact with animals while handling them for milk which was found to be significantly most important pre-disposing factor.

All the cases presented as PUO to our tertiary care centre had varied history of fever for prolonged duration.The commonest presenting symptom in our cases was fever followed by chills and rigors. similar to Mohan et al [14] Definitive diagnosis of Brucellosis is arrived at by the clinical picture of patients, repeated blood cultures and serological tests.In our study all the PUO cases were confirmed by blood cultures and also by standard tube agglutination test.

Serological diagnosis of brucellosis was based on demonstrating antibodies for *B.melitensis* in significantly high titer ($\geq 1:160$) against brucella antigen by standard tube agglutination test (SAT) SAT remains the most popular and yet used worldwide diagnostic tool. SAT measures the total quantity of agglutinating antibodies (IgM and IgG) SAT titres of 1:160 or greater are considered diagnostic in conjunction with a compatible clinical presentation.[14]Avneet Kaur et al .[6] reported that 6% cases were positive for Brucella Isolates by ELISA out of 100 isolates whereas none of the blood cultures were positive.In our study 38.46% of our cases were positive by standard agglutination test. 

Cardiovascular complications of Brucellosis include endocarditis, myocarditis, pericarditis, aortic root abscess, thrombophlebitis with pulmonary aneurysm and pulmonary embolism.

Brucella endocarditis constitutes a rare but severe complication of brucellosis and most cases described in the literature are single reports or small series.It is observed in less than 2% of brucellosis cases,responsible for up to 80% of deaths.[15]It may occur on previously normal valves and most commonly affected is the aortic valve in 75% of the cases as similar in our cases.Diagnosis of brucella endocarditis can be made with history, clinical findings, echocardiographic images confirming the diagnosis of infective endocarditis, isolation of the agent from tissue samples, positive agglutination test. Increased diagnostic and therapeutic vigilance is required for timely and efficient treatment of *Brucella* endocarditis.

In our study Brucella endocarditis was confirmed by TEE, by blood cultures, after surgery from excised aortic valve tissue and also by serological testing for Brucella antibodies .

Despite adequate medical therapy, Brucella endocarditis generally requires urgent or emergency cardiac surgery with the replacement of the affected valve in combination with conservative management for a large period of time, are deemed necessary for the successful treatment usually, because of the inability to control the infectious process with progression to a medically uncontrollable congestive cardiac failure. On the other hand, the microbe causes tissue destruction, with a tendency for progressive ulceration and significant risk of embolism.[9]

However, in a recent report Cohen et al. described one case and found another 12 in the literature, which were cured by antibiotic therapy alone.[16] Fifteen well documented patients were cured by medical treatment alone in another study.[17]. We treated 3 cases of Brucella endocarditis with conservative management alone.

But in the majority of cases surgery is indicated by failure of adequate conservative therapy for a reasonable period of time in the presence of an haemodynamically significant valve lesion.

2 of our cases presented with severe aortic regurgitation (AR), large vegetation at aortic leaflet, mild tricuspid regurgitation (TR) suggesting Infective endocarditis of aortic valve. Surgery was planned and tab augmentin 1.2mg IV 12th hrly was started prophylactically the same day before surgery was done. Urgent aortic valve replacement was done with 23mm SJM non rotatable valve for congenital rheumatic heart disease (CRHD), sev AR, post IE

Jacobs et al recommended a combination of antibiotic administration with valve replacement as the most effective therapy.[18] , the antibiotic schemes have varied from single to triple therapy, but most authors believe that single antibiotic therapy must be avoided because the development of resistance has been frequently demonstrated in this situation. Generally, rifampicin and/or co-trimoxazole must be added to the classic doxycycline in dosages usually higher than those recommended for other infections [18].

The optimal treatment regimen for brucellosis is doxycycline combination with aminoglycoside. Aminoglycoside is to be administered for the first seven to 14 days and doxycycline-rifampicin continued for six to eight weeks. brucellosis is an intracellular infection treatment should be administered for six weeks or longer as 30 days or less results in an increased relapse rate, even with the more potent doxycycline-streptomycin regimen.

All eight cases of PUO were treated with Tab doxycycline 100mg BD for 6 weeks in combination with Streptomycin 750mg IM for 5 days in a week for 3 weeks.

No treatment recommendations exist specific to brucella endocarditis. The treatment recommended by WHO in acute brucellosis in adults is rifampicin 600 mg and doxycycline 100 mg for a minimum of 6 weeks. Gentamicin 3 mg/kg in 3 divided doses can be added in cases of endocarditis for 2 weeks.[19] 

All five cases of Brucella .Endocarditis was treated successfully with Tab doxycycline P.O (per oral) 100 mg BD for 6 weeks in combination with Gentamicin 80mg IV 8th hourly for 3 weeks and all were discharged under stable condition with a successful outcome.

These five patients were followed-up at regular intervals for the next 6 months. For the two cases which have undergone aortic valve replacement the postoperative antibiotic therapy was continued for a period of 6 months. No relapses occurred during the six month follow-up period of the patient.

The literature shows considerable variation in the duration of the antibiotic therapy after surgery, varying from 2 weeks to more than 1 year. However, there is some consensus about the need to prolong the treatment for at least 3 months post-surgery. [20] Even in our cases emergency surgery was done without any complications. The postoperative antibiotic therapy was continued for a period of 6 months . 

Overall mortality due to brucellosis is low (<1%), but endocarditis is responsible for the majority of deaths (80%) related to this disease.[21]  gestive heart failure is responsible for the majority of deaths in Brucella endocarditis.[22] Our patients were treated successfully and there was no mortality documented in our cases.

Conclusion:  Brucellosis poses a great diagnostic challenge to clinicians due to its uncommon presentations, lack of awareness, and low index of clinical suspicion often it remains under-diagnosed and undiagnosed. Establishing a history of travel to endemic countries and also exposure history to animals and/or their products is crucial in order to help and make reach clinicians towards an early clinical diagnosis. Using automated blood culture systems with improvised culture medium promotes growth and increases the rate of isolation of these fastidious organisms. Early diagnosis facilitates prompt therapy that helps in timely management of this Infectious disease with a successful outcome

Consent: It is not Applicable

Ethical Approval : It is not applicable

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Table:1 Age distribution of Brucellosis patients

S.No	Age	No of Patients
1	0-20	Nil
2	21-30	1
3	31-40	4
4	41-50	4
5	51-60	5
6	61-70	1

Table 2: Distribution of Brucellosis cases

Distribution of cases	Number (n=13)	Percentage (%)
Pyrexia of unknown origin(PUO)	8	61.5
Infective Endocarditis	5	38.4

Table:3 Duration of fever in PUO & Endocarditis cases

Fever duration in PUO cases (n=8)	Fever duration in IE cases(n=5)
4 months (n=1)	2 months(n=3)
1 month (n=3)	6months (n=1)
10 months (n=1)	20 days (n=1)
2 months (n=1)	
25 days (n=1)	
5months (n=1)	

Table 4: Clinical manifestations of Brucellosis

Clinical manifestations	Number n =(13)	Percentage (%)
Fever	13	100
Chills &rigors	7	53.8
Joint pains	2	15.3
Sudden onset of breathlessness(SOB)	3	23.07
Cough	4	30.76
Chest pain	3	23.07
Vomiting	2	15.3
Headcahe	5	38.4
Backache	2	15.3
Splenomegaly	1	7.69
Hepatomegaly	1	7.69
Lymphadenopathy	1	7.69

Table:5 Culture positivity of Brucella culture & serology

Only culture positive	13/13 (100%)
Culture Positive & Serology positive	5/13 (38.46%)
Serology negative	8/13 (61.5%)

Table 6: Serological titres of Brucella antibodies

Serological Titre	Number
1:160	1
1:320	2
1:640	1
1:160	1

Figure:1 Colony morphology of *B.melitensis* on Blood agar and Chrome agar plates with grey white pin point shiny moist non haemolytic colonies.

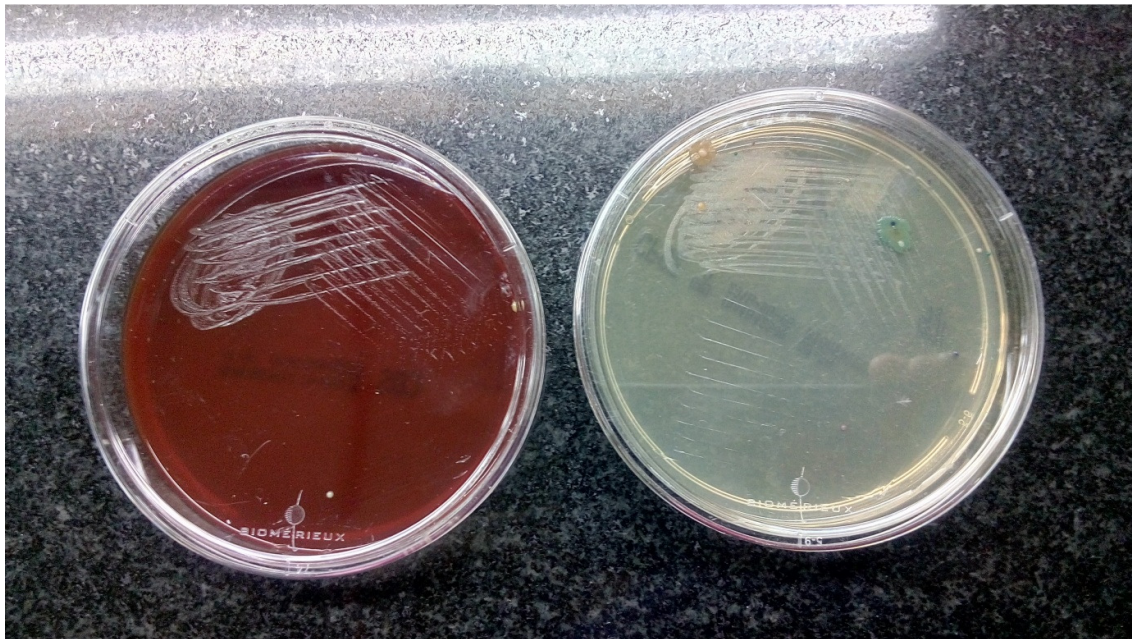


Figure 2: Grams stain of colonies showing Gram negative coccobacilli

