"CLINICAL PROFILE OF BRUCELLOSIS – A CROSS-SECTIONAL STUDY"

By

Dr. VIKRANT GHATNATTI

Dissertation submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D. MEDICINE

Under the Guidance of

Dr. A. J. DHUMALE MD
Associate Professor,

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAUM, KARNATAKA

MAY - 2009

KLE UNIVERSITY, BELGAUM,

KARNATAKA

DECLARATION

I hereby declare that this dissertation entitled **"CLINICAL PROFILE OF BRUCELLOSIS – A CROSS-SECTIONAL STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. A. J. DHUMALE.** $_{\text{MD}}$ Associate Professor, Department of Medicine, Jawaharlal Nehru Medical College, Nehru Nagar, Belgaum – 590 010.

Date:

Place: Belgaum (Dr. VIKRANT B GHATNATTI)

KLE UNIVERSITY, BELGAUM,

KARNATAKA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"CLINICAL PROFILE OF BRUCELLOSIS – A CROSS - SECTIONAL STUDY"** is a bonafide research work done by **Dr. VIKRANT GHATNATTI** in partial fulfillment of the requirement for the degree of **M.D. (GENERAL MEDICINE).**

Date :
Place:

Dr. A.J. DHUMALE.MD
Associate Professor,
Department of Medicine,
J. N. Medical College,
Nehru Nagar, Belgaum – 10

KLE UNIVERSITY, BELGAUM,

KARNATAKA

CERTIFICATE BY THE Co-GUIDE

This is to certify that the dissertation entitled **"CLINICAL PROFILE OF BRUCELLOSIS – A CROSS-SECTIONAL STUDY"** is a bonafide research work done by **Dr. VIKRANT GHATNATTI** in partial fulfillment of the requirement for the degree of **M.D. (GENERAL MEDICINE).**

Date:
Place

Dr. MAHANTESH NAGAMOTI
Professor,
Department of Microbiology,
J. N. Medical College,
Nehru Nagar, Belgaum – 10

KLE UNIVERSITY, BELGAUM,

KARNATAKA

ENDORSEMENT

This is to certify that the dissertation entitled **"CLINICAL PROFILE OF BRUCELLOSIS – A CROSS - SECTIONAL STUDY"** is a bonafide research work done by **Dr. VIKRANT GHATNATTI** under the guidance of **Dr. A. J. DHUMALE. MD**, Associate Professor, Department of Medicine, J. N. Medical College, Nehru Nagar, Belgaum – 590 010.

Dr. V. A. Kothiwale MD, Ph.D.
Professor and Head,
Department of Medicine,
J. N. Medical College,
Nehru Nagar, Belgaum – 10

Date:
Place: Belgaum

Dr. V. D. Patil MD,DCH
Principal,
J. N. Medical College,
Nehru Nagar, Belgaum – 10

Date:
Place: Belgaum

KLE UNIVERSITY, BELGAUM,

KARNATAKA

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the KLE University, Belgaum, Karnataka shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

Date :

(Dr. VIKRANT GHATNATTI)

Place : Belgaum

ACKNOWLEDGEMENT

First and foremost I would like to express my sincere gratitude and appreciation for my guide **Dr. A. J. Dhumale.** MD, Associate Professor, Department of Medicine, for his constant guidance, valuable suggestions, unparalleled encouragement and co-operation provided to me throughout the course of study. It has been a great pleasure and privilege to work under him. Without his immense professional insight and guidance I could have not completed this study.

I avail this opportunity to thank **Dr. V. A. Kothiwale** MD, Ph.D Professor and Head, Department of Medicine, for his keen interest, unparalleled encouragement and co-operation during this study.

I owe deep sense of gratitude and express my heartfelt thanks to my respected and beloved teacher **Dr. Vijay G. Somannavar** MD for his constant encouragement and support through out the course of my study.

I also would like to specially thank my co guide, **Dr. Mahantesh Nagamoti.** MD, Professor, Department of Microbiology who helped me conduct my thesis by providing the resources available in his laboratory.

I express my sincere gratitude to **Dr. V. D. Patil** MD, DCH Principal J. N. Medical College, Belgaum, **Dr. M. V. Jali** MD, Director and Chief Executive, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum for allowing me to utilize the facilities in above institution for the dissertation.

I take this opportunity to thank **Dr. H. B. Rajshekhar** MD, FCCP, **Dr. S. B. Kalagate** MD, **Dr. Rekha S. Patil** MD, **Dr.B.Srinivas** MD, **Dr. Neeta Deshpande** MD, **Dr. Prakash B** MD, **Dr. Arif M** MD, **Dr. Arathi Darshan** MD, **Dr. Naveen Angadi** MD, and **Dr .Mamta Patil** MD for their kind support.

I sincerely thank, **Dr. K. Ravishankar Naik** MD, DM (Neuro), **Dr Saroja A.O** MD, DM (Neuro), **Dr. Kutub Makandar** MD, DM (Neuro) and all other teachers of J. N. Medical College, Belgaum and all the technical staff at Clinical Laboratory, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum for their kind support and timely help during the study.

I sincerely thank all my post graduate colleagues for their help in the study. I am also thankful to my batch mates **Dr. Nitin Agarwal, Dr. Vishwanath Hesarur, Dr. Dhareppa Chougala, Dr. Manjunath Desai** and **Dr. Vivekanand** for their kind help rendered throughout the course of my study.

I express my sincere thanks to my friends **Dr. Tejus Chiranjeevi, , Dr. Shivakumar Hiremath, Dr. Adarsh E. S, Dr. Shailesh** and **Dr. Raghavendra Nayak** for their constant help, support, encouragement and cooperation in designing my dissertation.

This would have not been possible without the co-operation and understanding of my patients involved in the study. I also thank the authors of numerous publications whose knowledge have been freely utilized in the preparation.

With deep sense of gratitude and affection, I am very thankful to my parents **Prof Basalingappa Ghatnatti** and **Mrs. Vijaylaxmi Ghatnatti,** and my other family members who always have been a pillar of strength for me.

I thank my beloved brother **Lt. Harsha Ghatnatti,** my friends **Dr. Vishal Ratkal** and **Dr. Veeresh Manvi**, for their continuous support they have rendered, not only during course of this study, but in my life as well.

I would like to acknowledge the tireless and timely work done by **Miss. Veena** & **Mr. Deepak** of **Sai Xerox & D. T. P. Centre** for their excellent data processing and completion of this manuscript in a very short time.

Finally, I thank **Almighty** for all the blessings.

Date:
Place: Belgaum

Dr. VIKRANT GHATNATTI

LIST OF ABBREVIATIONS USED

ASLO	–	Anti streptolysin O
B. abortus	–	Brucella abortus
B. canis	–	Brucella canis
B. melitensis	–	Brucella melitensis
B. suis	–	Brucella suis
CDC	–	Centre for disease control
CNS	–	Central nervous system
CO_2	–	Carbon dioxide
CRP	–	C- Reactive protein
CSF	–	Cerbrospinal fluid
CT	–	Computerized Tomography
DM	–	Diabetes mellitus
DNA	–	Deoxyribose nucleic acid
DOA	–	Date of admission
DOD	–	Date of discharge
DOE	–	Date of expiry
ELISA	–	Enzyme linked immunosorbent assay
ESR	–	Erythrocyte Sedimentation Rate
FNAC	–	Fine needle aspiration cytology
HIV	–	Human immunodeficiency virus
HTN	–	Hypertension
IP	–	In patient
LPO	–	Lipopolysaccharide
MRI	–	Magnetic resonance imaging

No.	–	Number
PCR	–	Polymerase chain reaction
PS for MP	–	Peripheral smear for malarial parasite
PUO	–	Pyrexia of unknown origin
QBC for MP	–	Quantitative buffy coat for malarial parasite
RNA	–	Ribose nucleic acid
SAT	–	Standard agglutination test
TB	–	Tuberculosis
UTI	–	Urinary tract infection
VDRL	–	Venereal disease research laboratory.
2 ME	–	2 Mercapto ethanol

ABSTRACT

Background and objectives

Human brucellosis is an important but neglected disease in India. It is traditionally described as a disease of protean manifestations. The aim of this study was to assess the epidemiological, clinical and laboratory characteristics of brucellosis.

Methods

In this cross sectional study, all patients admitted with symptoms and signs suggestive of brucellosis were screened serologically for brucellosis by standard agglutination test. A total of 30 cases diagnosed as brucellosis were investigated in terms of spread of infection, age and sex distribution, clinical and laboratory characteristics and response to different treatment regimens.

Results

Our study revealed a prevalence of 0.61 percent in adults and 0.1 percent in children. Fever with drenching sweats remained one of the important symptoms of brucellosis. Other common symptoms were generalized weakness, anorexia, body ache, joint pain and headache. Amongst the signs, hepatomegaly and splenomegaly were more common where as lymphadenopathy was seen in only few cases. All patients responded to either of the drug regimens, namely rifampicin plus doxycycline or rifampicin plus streptomycin. Over all prognosis was good and none of the patients expired.

Conclusions and interpretation

It is concluded that brucellosis is a disease with protean manifestation with no single diagnostic symptom or sign. Brucellosis should be considered as a differential diagnosis in all cases of pyrexia of unknown origin, low backache, arthralgia, sciatica and in all cases of progressive weight loss.

Key words

Brucellosis ; diagnosis ;epidemiology ; prevention ; treatment

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

Brucellosis is a zoonosis widely distributed around the world. Half a million new cases are reported worldwide each year, but according to the World Health Organization, these numbers greatly underestimate the true incidence of human disease and brucellosis continues to be of great health significance and economic importance in many countries. Brucellosis has been present for millennia[1] and has managed to elude eradication, even in most developed countries.[2, 3] Gram-negative bacteria of the genus Brucella cause it and *Brucella melitensis* is the leading cause of brucellosis in humans.

It is transmitted directly or indirectly to humans from infected animals predominantly domesticated ruminants and swine. The illness is characterized by fever, sweats, weakness, malaise and weight loss often without localized findings. Brucellosis is also called as undulant fever, Malta fever or Mediterranean fever.

The interest in brucellosis has been increasing because of the growing phenomena of international tourism and migration in addition to the potential use of Brucella as a biological weapon.

Human brucellosis is an important but neglected disease in India. Only a few recent studies have addressed the prevalence and importance of human brucellosis as a human disease problem in India.

Human brucellosis is traditionally described as a disease of protean manifestations. Alertness of medical staff and high degree of suspicion is needed to recognize and diagnose the disease. Patients are often labeled pyrexia of unknown origin and subjected to various laboratory tests which do not include Brucella

serology. This is because of the general perception that brucellosis is only seldom encountered in this part of the world.

As the disease has a wide variety of clinical presentation, an attempt is made in this study to know the clinical presentation, diagnosis and complications of the disease.

OBJECTIVES

The objectives of the present study were to know the clinical features and complications of brucellosis.

REVIEW OF LITERATURE

HISTORICAL REVIEW

An unmistakable description of *Brucella melitensis* infection of goats is found in Genesis 31:38. A description of a chronic illness with undulating fever and relapses, found in the Hippocratic corpus, likely referred to ancient brucellosis. Prior to and especially during the 19th century, various febrile illnesses were described that were likely brucellosis. Local names were often attached to the term "fever" the predominant manifestation of brucellosis by exogenous military garrisons that may have been more vulnerable than the indigenous population to infection from endemic brucellosis. Hence, Constantinople fever as well as the fevers of Malta, Naples, Gibraltar, Crete, Crimea, Levant, Syria, and so forth. The undulating quality of fever caused the term malarial to be placed in some designations. Since febrile gastrointestinal manifestations may also be prominent, other names for the affliction included various terms designating typhoid.

In 1860, Marston provided the first modern clinical description of brucellosis, which he termed Mediterranean gastric remittent fever. The etiologic role of *Brucella melitensis* first was demonstrated in Malta in 1887 by Bruce and Carrauna-Secluna, who cultured and isolated this bacterium from the spleens of individuals who died from brucellosis. The original genus designation *(*Micrococcus*)* was subsequently changed in honor of David Bruce, a physician with the British army who carried out extensive studies of the organism. It must be mentioned in passing that Dr G. Carruana-Secluna played an important technical role in the success that was achieved

in culturing the organism, although it is said that Dr Bruce did not permit him to be listed as a coauthor.

Dr M. Louis Hughes, another colleague of Dr Bruce, was the first to isolate *B. melitensis* from brain, and it was he who provided the species name "melitensis." Hughes published a classic description of this illness in 1897, just prior to his death at age 32 in the Boer War. Hughes' term undulant fever became the most widely accepted clinical designation until "brucellosis" obtained currency, although Malta fever has also shown some staying power as a designation.

Hughes incorrectly concluded that the organism was to be found in the soil. Here again, Dr Carruana-Secluna played an important role together with Dr Themistocles Zammit. Their investigations (with the leading role accorded variously to one or the other) of goats at Chadwick Lakes established one of the most important principles of the epidemiology of infectious diseases, the zoonotic principle of the role of animals in transmission of disease to human. They demonstrated that more than half of Maltese goats were asymptomatically infected and that the organism could be transmitted to humans by the consumption of unpasteurized milk and milk products or by contact with infected goat urine.

Lennaire first isolated *B. melitensis* from spinal fluid in 1924.

Phage typing was discovered which was specific for *B. abortus* only but helped to distinguish *abortus*, *melitensis* and *suis* cultures. Later oxidative metabolic tests were discovered which helped in the speciation of Brucella and later still specific phases for all the six species of Brucella were discovered. Thus research on brucellosis still

continues and discoveries are made even today. A disease which was first discovered in a small island has been found to be of worldwide prevalence.

Brucellosis in India

In India around 1940 Polding made an all India survey of brucellosis among cattle, goats and sheep of organized farms.

From 1953 to 1973 Matur after an extensive study of the disease in cattle of organized farms and sheep of villages and patients with brucellosis established the epidemiology of brucellosis in India and concluded that almost all human infections were due to *Brucella melitensis* which was acquired from goats and sheep. He described 232 cases of human brucellosis.

Laxminarayan and Rammurthy from Chennai reported brucellosis in the patients of pyrexia of unknown origin and of those people who were exposed to brucellosis because of their profession.

The work on brucellosis in Belgaum has been going on since 1971 by Dr S. J Naglotimath.

Various workers like Shivarajan from Tanjavur, Koshi and Meyers from Vellore too have worked extensively on brucellosis. In 1985 a symposium was held at Udaipur. During the conference of Indian association of microbiologists and pathologists, various aspects of brucellosis were reviewed.

MORPHOLOGY OF BRUCELLA ORGANISMS

Brucellae are coccobacilli or short rods 0.5 to 0.7 micron X 0.6 to 1.5 micron in size arranged singly or in short chains. The bacteria are so small that they may be mistaken for cocci as done by Bruce who called them *Micrococcus melitensis*. They are non motile, non capsulated and non sporing. They are gram negative and non acid fast. The outer cell membrane closely resembles that of other gram-negative bacilli with a dominant lipopolysaccharide (LPS) component and three main groups of proteins. No Brucella species has been found to harbor plasmids naturally although they readily accept broad-host-range plasmids.

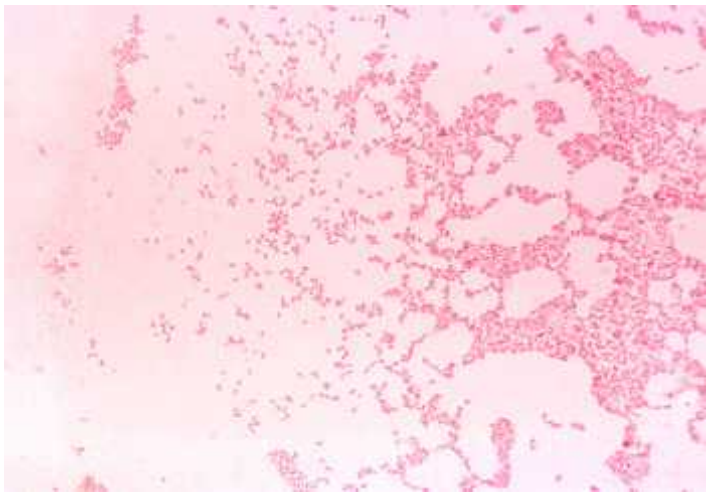


Figure No. 1: Gram-stain of Brucella showing the coccobacillary shape and Gram-negative staining. (Magnification unknown) *Source: Centers for Disease Control, Public Health Image Library #1937*

Cultural characteristics

Brucellae are strict aerobes and do not grow anaerobically. *B. abortus* is capnophilic, requiring 5 to 10% CO_2 for growth on primary isolation. The growth of *B. melitensis* is improved by CO_2 while *B. suis* is unaffected. The optimum temperature is 37^0 C and pH is 6.6 to 7.4. They may grow on simple media though growth is slow and scanty on primary isolation. Growth of Brucella is improved by addition of serum or liver extract. The media employed currently are serum dextrose agar, serum potato infusion agar, or tryptose agar. The addition of bacitracin, polymyxin or cycloheximide makes the media more selective.

In liquid media, growth is uniform, and a powdery or viscous deposit is formed in old cultures. On solid media, colonies are small, moist translucent and glistening.

Figure No. 2: Colony appearance: *Brucella* grows slowly, even on rich media to give pinpoint, translucent colonies with a smooth surface. *Source: CDC/ Courtesy of Larry Stauffer, Oregon State Public Health Laboratory, Public Health Image Library #1902*

Biochemical reaction

Brucellae are catalase positive, oxidase positive, (except for *Brucella neotomae* [*B.neotomae*] and *B.ovis* which are negative) and urease positive. Nitrates are reduced to nitirites by Brucellae. Citrate is not utilized and indole is not produced by Brucellae.

Resistance

Brucellae are destroyed by heat at 60^0C in 10 minutes and by one percent phenol in 15 minutes. They are killed by pasteurization. They may survive in soil and manure for several weeks. They remain viable for ten days in refrigerated milk.

Antigenic structure

The lipopolysaccharide (LPS) component of the outer cell membranes of brucellae is quite different— both structurally and functionally—from that of other Gram-negative organisms.[4,5] The lipid A portion of a *Brucella* organism LPS contains fatty acids 16 carbons long, and lacks the 14-carbon myristic acid typical of lipid A of Enterobacteriaceae. This unique structural feature may underlie the remarkably reduced pyrogenicity (less than 1/100th) of *Brucella* LPS, compared with the pyrogenicity of *Escherichia coli* LPS.[6, 7]

THE BRUCELLA GENOME

The complete sequencing of the *B. melitensis* genome was achieved in 2002.[8] The complete sequencing of *B. abortus* [9] and *B. suis* [10] has recently been accomplished as well. *B. melitensis* contains two circular replicons of 1.1 and 2.2 Mb, respectively, with a 57 percent GC content and no plasmids; 3197 open reading frames were sequenced, 2487 of which had an assigned function. *B. abortus* biovars 1 and 4 and *B. suis* biotype 1 are remarkably similar to *B. melitensis*. In contrast, *B. suis* biotypes 2 and 4 are composed of two replicons of 1.35 and 1.85 Mb, respectively, whereas *B. suis* biotype 3 is composed of a single circular replicon of 3.3 Mb.

EPIDEMIOLOGY

Considerable changes have taken place in the epidemiology of brucellosis during the past few decades with declining prevalence in some regions as the result of extension to increasing numbers of countries of sanitary or herd surveillance/culling practices, although contrary forces have negatively impacted distribution elsewhere. The negative forces have included political and socioeconomic forces that have fueled regional wars or reduced vigilance in control programs, as well as international travel of people and agricultural products that may carry the organisms far and wide.

Among the nations for which reliable data are available those with the highest current incidence in annual cases per million people (indicated in parentheses) are Syria (1603.4), Mongolia (605.9), Kyrgyzstan (362.2), Iraq (278.4), Turkey (262.2), Iran (238.6), Saudi Arabia (214.4), Tajikistan (211.9), Macedonia (148), Kazakhstan (115.8), Algeria (84.3), Albania (63.6), Azerbaijan (52.6), Turkmenistan (51.5), Lebanon (49.5), United Arab Emirates (41), Oman (35.6), Peru (34.9), Tunisia (34.5),

Kuwait (33.9), Armenia (31.3), Mexico (28.7), Georgia (27.6), Jordan (23.4), Greece (20.9), and Bosnia/Herzegovina (20.8). No data are available among other regions of importance for India, Pakistan, or Afghanistan where the disease is undoubtedly endemic.

Inspection of the list suggests that disease prevalence is adversely affected by poverty, famine, political unrest, and war and is understandably more common in regions where sheep, goats, and camels are abundant. Absence of careful supervision of abattoirs likely considerably increases risk of human brucellosis. Movement of herds of sheep and other animals in pursuit of grass may variably spread Middle Eastern cases of brucellosis from one nation to another.

The data for Syria, manifesting the highest international rate of human brucellosis cases (1603 cases per million population per year), are particularly alarming, and there is evidence that the number of cases have been nearly doubling each year. Turkey, with very high rates in the eastern portions of the country, also has worsening statistics, although the Turkish government has instituted a control project.

More than occasional cases are detected in countries surrounding the Mediterranean; India; China; other parts of Southwest, Central, and Southeast Asia; Africa; Central America; and South America (e.g., Peru). Northwest Iran and Northeast Turkey are areas of particularly high density of cases of brucellosis. Epidemiological factors of importance include consumption of unpasteurized dairy products obtained from cow, goat, and camel. Close occupational contact with animals (e.g., cows, goats, sheep, camels, pigs, hares, rabbits and reindeer) increases risk, as does the consumption of raw, poorly cleaned, or partly cooked meat from such

animals. Aerosol and hand-to-mouth transmission may occur in abattoirs or laboratories.

Brucellosis is much more common during summer than winter months, even in regions of the world where winters are comparatively mild. Worldwide, *B. abortus* accounts for the largest number of human and veterinary cases of brucellosis. Estimates of the veterinary toll alone in Latin America due to this organism range as high as $700,000,000. *B. suis* ranks second as a threat to public and veterinary health in the western hemisphere.

In the Mediterranean region and Middle East, the species of greatest importance is *B. melitensis.* In Malta, during the prime of Malta fever due to *B. melitensis,* peak incidence was in August, while the lowest incidence was in January and February. *B. melitensis* is harbored in sheep, goats, and camels. The brucellosis produced by this species is typically more severe than the brucellosis produced by other Brucella species.

Among the highest prevalence for human *B. melitensis* brucellosis is that of the Bedouins of Kuwait, who have more than 540 cases per 100,000. Seroprevalence for Brucella antibodies is one to seven percent in Turkey and Iraq, and 10% in Egypt. More than 40% of all cases of fever of unknown origin in Egypt are believed to be due to brucellosis.

Saudi Arabia and several adjacent countries experienced a very considerable rise in prevalence of human and animal brucellosis disease as the result of the investment of oil revenues into massive expansion of husbandry. The importation of

considerable numbers of untested and unvaccinated goats, sheep, and other animals outstripped efforts to monitor veterinary brucellosis. This husbandry was designed to provide food for Hajj pilgrims. As a result, 20% of Saudi Arabians have demonstrated seropositivity and two percent of the population are believed to have active disease. The incidence of new cases is highest during the Hajj; the increase is due to cases among pilgrims to Mecca.

Estimates of prevalence of human or animal brucellosis are not available for many countries of the world. Prevalence is likely high in many countries of Africa and Asia. In Nigeria, 55% of the population was found to be seropositive for *Brucella* species.

The economic importance of large-scale cattle and sheep husbandry in New Zealand and Australia has made brucellosis a particularly important consideration. The most recent estimate of incidence of human brucellosis in Australia is 0.9 cases per million people per year, while the rate in New Zealand is virtually nil. The last documented bovine case in New Zealand occurred in 1989, and both New Zealand and Australia were certified free from bovine brucellosis in 1992. For example, the eradication and ensuing freedom from *B. abortus* has been certified not only for Australia and New Zealand, but also for Austria, Canada, Denmark, Finland, Japan, Switzerland and various other countries.

Consumption of contaminated foods and occupational contact remain the major sources of infection. Examples of human-to-human transmission by tissue transplantation or sexual contact are occasionally reported but are insignificant.[22] Prevention of human brucellosis depends on the control of the disease in animals.

Although few recent outbreaks of disease caused by *B. suis* biovar 4 have been reported, foci of the infection persist in the Arctic regions of North America and Russia and constitute a potential hazard for the local population. *B. ovis* has not been demonstrated to cause overt disease in humans, although it is widespread in sheep. *B. canis* can cause disease in humans, although this is rare even in countries where the infection is common in dogs. Precise information on prevalence is lacking, but *B. canis* has been recorded in the United States, Mexico, Argentina, Spain, China, Japan, Tunisia, and other countries. The recent isolation of distinctive Brucella strains, tentatively named *Brucella maris*, from marine animals in the United Kingdom and the United States extends the ecologic range of the genus and, potentially, its scope as a zoonosis. A hitherto unreported incident of laboratory-acquired infection suggests that this type is pathogenic for humans. Infection could result from occupational contact with infected seals or cetaceans.

Brucella melitensis predominantly affects goats, sheep, hare, dog and camels. *Brucella abortus* affects the cattle like buffaloes, camels, deer, horses and dogs. *Brucella canis* infects mainly the dogs where as *Brucella ovis* mainly infects the rams. Of these six only four namely *Brucella abortus, melitensis, suis* and *canis* infect the humans.

Brucellosis in India

The true incidence of human brucellosis however, is unknown for most countries and no data is available for India. It has been estimated that the true incidence may be 25 times higher than the reported incidence due to misdiagnosis and underreporting. Several publications indicate that human brucellosis can be fairly common disease in India. A study by Mantur and coworkers[11] reported on 93 children with brucellosis who were identified by testing samples from children referred to the microbiology laboratory of BLDEA's Medical College in Bijapur during a period of 13 years. The seroprevalence was 1.6 percent by SAT (more than 1:160) and the diagnosis was confirmed by the isolation of *B. melitensis*. During the same period a total of 492 adult patients were diagnosed with brucellosis at the same hospital stressing the importance of childhood brucellosis. Importantly, it was noted that in only 15 cases brucellosis was suspected on first diagnosis and the remaining 78 cases were initially classified as pyrexia of unknown origin and rheumatic arthritis. [12-17]

Since many patients with brucellosis present with fever as the only manifestation, other groups have investigated the prevalence of brucellosis in patients diagnosed with pyrexia of unknown origin (PUO). Sen and coworkers identified 28 (6.8%) seropositive cases in a group of 414 patients with PUO and Kadri and coworkers identified 28 (0.8%) seropositive cases in a group of 3,532 patients with PUO.[18, 19]

BRUCELLA AS A BIOLOGICAL WEAPON

While Britain was focused on anthrax, brucellosis was the first microorganism that the United States chose to develop as a weapon; it even has the military code name US. The reasons for this included its low lethality, relative ease of manufacture, and its susceptibility to sunlight. The agent could be dispersed at night to affect those out in the open, that is, an army in the field without affecting civilians in houses. The bacterium would be killed shortly after sunrise, leaving civilians at low risk of infection, as it is not transmitted person-to-person. Field tests with live agents were conducted in the 1950's and it was shown that it could be effectively disseminated in four pound bombs.

PATHOGENIC FEATURES

The series of host-microbe interactions that takes place in humans differs in many crucial steps from the pathogenetic mechanisms first recognized in animal models.[20] Brucella is unusual in several ways. First, the bacterium does not bear classic virulence factors, such as exotoxins or endotoxins, and its lipopolysaccharide pathogenicity is not typical. Second, it exhibits a tendency to invade and persist in the human host through inhibition of programmed cell death.[21]

Brucella invades the mucosa, after which phagocytes ingest the organisms. In so-called nonprofessional phagocytes, internalization requires the expenditure of energy, and inhibitors of energy metabolism and receptor-mediated endocytosis can suppress this response.[22] Brucella has a two-component system called BvrS/BvrR, which codes for a histidine kinase sensor and controls the expression of molecular

determinants necessary for cell invasion.[23] After ingestion, the majority of Brucellae are rapidly eliminated by phagolysosome fusion. Of those bacteria, 15 to 30% survive[24] in gradually evolving Brucellae-containing compartments, in which rapid acidification takes place. How this unique environment is formed is incompletely understood, but it is responsible for limiting antibiotic action and explains the discrepancy between in vitro studies and in vivo events.[25] The induction of the vir B operon through a type IV secretion system (a system by which macromolecules are transferred) is of paramount importance during Brucella intracellular movement.[26] Replication of the bacterium takes place in the endoplasmic reticulum without affecting host-cell integrity. After replication, Brucellae are released with the help of hemolysins and induced cell necrosis.[27]

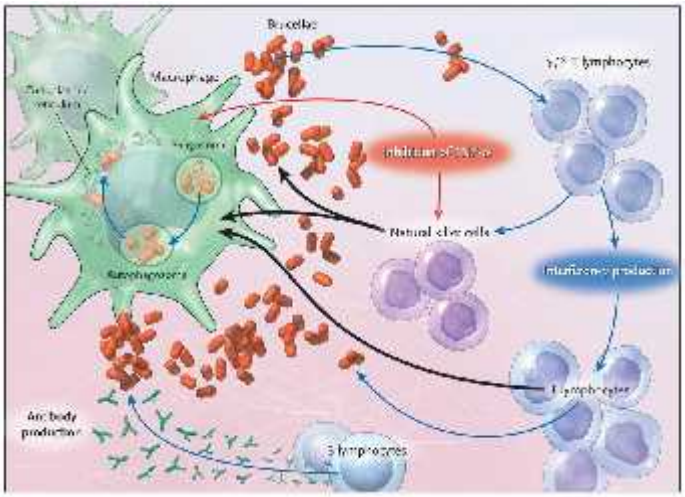


Figure No. 3: Schematic representation of major events in the pathogenesis of brucellosis and the host immune response. *Source: NEJM; Volume 352(22), 2 June 2005, pp 2325-233*6

Brucellae enter the macrophages, where the minority of the bacteria survive in specialized evolving compartments and multiply in the endoplasmic reticulum. The inhibition of tumor necrosis factor- alpha (TNF- α) by the bacteria disrupts the bactericidal effect of natural killer cells and macrophages. Interferon-γ production induces a bactericidal effect by natural killer cells and T lymphocytes directly and through macrophage induction. Antibody production by B lymphocytes is also induced but plays a minor role in the immune response. T lymphocytes include both helper and suppressor cells, depending on the stage of the disease.

THE HOST RESPONSE IN HUMANS

The host response in humans reflects unique features of Brucella. Smooth lipopolysaccharide does not activate the alternative complement pathway. Brucella is resistant to damage from polymorphonuclear cells owing to suppression of the myeloperoxidase-hydrogen peroxide-halide system and copper-zinc superoxide dismutase and the production of inhibitors of adenylate monophosphate and guanyl monophosphate. Impaired activity of natural killer cells and impaired macrophage generation of reactive oxygen intermediates and interferon regulatory factors have been documented.[28-30] CD4 lymphocytes play a limited role, acting either by facilitating clonal expansion of other cytolytic cells, as CD8, or by functioning as cytolytic effectors. An increase of γ/ δ CD4 and CD8 lymphocytes is characteristic in brucellosis, [31] as is the importance of a γ/ δ T-cell receptor.[32]

Studies using volunteers who have been vaccinated with the Rev 1 vaccine against *B. melitensis* have delineated the evolution of specific antibodies against Brucellae. Class M immunoglobulins against lipopolysaccharide appeared during the

first week of infection, followed by class G immunoglobulins as early as the second week. Both classes of immunoglobulin peaked during the fourth week, and the use of antibiotics was associated with a decline in both class M and class G titers. Class M titers persisted at levels that were higher than those of class G titers for more than six months, and both classes were present for almost a year. The appearance of class A immunoglobulins in conjunction with class G immunoglobulins for longer than six months was consistent with the presence of chronic disease. Antibody response in brucellosis, although extremely useful diagnostically, plays a limited part in the overall host response.

Interferon γ has a central role in the pathogenesis of brucellosis [33, 34] by activating macrophages, producing reactive oxygen species and nitrogen intermediates; by inducing apoptosis, enhancing cell differentiation and cytokine production; by converting immunoglobulin G to immunoglobulin G2a; and by increasing the expression of antigen-presenting molecules. That interferon γ has a central role in the evolution of brucellosis is highlighted by the effect of a genetic polymorphism in interferon γ (the +874A allele). Patients who are homozygous for the +847 allele may be relatively more susceptible to brucellosis and - in an interesting note - to tuberculosis.[35] Typically, serum interferon γ levels in patients with brucellosis are increased.[36, 37]

In contrast, the importance of tumor necrosis TNF-α in human brucellosis is the subject of debate. Although the induction of TNF-α was noted in murine models of brucellosis, the inhibition of TNF-α in human disease is an early, crucial step in infection. This inhibition may also be involved in the impaired activation and

cytotoxic function of natural killer cells owing to an active bacterial mechanism that involves outer-membrane protein 25, which has been identified as the down-regulator of TNF-α.[38] Serum levels of TNF-α were undetectable in patients with active brucellosis in one study,[37] but another study reported that serum levels were increased in a linear fashion with serum levels of interferon-γ and other inflammatory markers.[36] The role of interleukin-12, mainly as a regulator of interferon-γ production, has been extensively studied in animal models and humans.[37,39]

HUMAN DISEASE

Transmission of brucellosis to humans occurs through the consumption of infected, unpasteurized animal-milk products, through direct contact with infected animal parts (such as the placenta by inoculation through ruptures of skin and mucous membranes), and through the inhalation of infected aerosolized particles. Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories. One important epidemiologic step in containing brucellosis in the community is the screening of household members of infected persons.[40]

Consumption of unpasteurized dairy products - especially raw milk, soft cheese, butter, and ice cream - is the most common means of transmission. Hard cheese, yogurt, and sour milk are less hazardous, since both propionic and lactic acid fermentation takes place. Bacterial load in animal muscle tissues is low, but consumption of undercooked traditional delicacies such as liver and spleen has been implicated in human infection.

Airborne transmission of brucellosis has been studied in the context of using Brucella as a biologic weapon. In fact, *B. suis* was the first agent contemplated by the U.S. Army as a potential biologic weapon[41] and is still considered in that category. In a hypothetical attack scenario, it was estimated that release of an aerosolized form of Brucella under optimal circumstances for dispersion would cause 82,500 cases of brucellosis and 413 fatalities.[42] Cases of laboratory-acquired brucellosis are the perfect examples of airborne spreading of the disease.[43]

After entering the human body and being taken up by local tissue lymphocytes, Brucellae are transferred through regional lymph nodes into the circulation and are subsequently seeded throughout the body, with tropism for the reticuloendothelial system. The period of inoculation usually ranges from two to four weeks.

The classic categorization of brucellosis as acute, sub acute, or chronic is subjective and of limited clinical interest. Four species of Brucella can cause human disease: *B. melitensis, B. abortus, B. suis, and B. canis*. Disease from marine species has also emerged.[44] The vast majority of cases worldwide are attributed to *B. melitensis*. A recent study did not report any clinical differences between cases caused by *B. melitensis* and those caused by *B. abortus*.[45] Sufficient data on virulence and clinical presentation of biotypes of *B. melitensis* are lacking, although separate biotypes that predominate in various regions - for example type 2 in northwestern Greece, type 3 in Turkey, [46] and type 1 in Spain[47] - may account for variations in clinical presentation.

DETERMINANTS OF PATHOGENICITY OF BRUCELLA

The determinants of pathogenicity of Brucella have not been fully characterized, and the mechanisms underlying the manifestations of brucellosis are incompletely understood. The organism's survival strategy is centered on processes that permit survival within monocytic cells. The smooth Brucella LPS, which has an unusual O-chain and core-lipid composition, has relatively low endotoxin activity and plays a key role in pyrogenicity and in resistance to phagocytosis and serum killing in the nonimmune host. LPS is believed also to play a key role in suppressing phagosome-lysosome fusion and diverting the internalized bacteria into vacuoles located in endoplasmic reticulum, where intracellular replication takes place. Specific exotoxins have not been isolated, but a type IV secretion system (Vir B) that regulates intracellular survival and trafficking has been identified. In *B. abortus* this system can be activated extracellularly, but in *B. suis* it is activated (by low pH) only during intracellular growth. Brucellae then produce acid-stable proteins that facilitate the organisms' survival in phagosomes and may enhance their resistance to reactive oxygen intermediates. Virulent Brucellae are resistant to defensins and produce a Cu-Zn superoxide dismutase that increases their resistance to reactive oxygen intermediates.

PATHOLOGY OF BRUCELLOSIS

Between the three Brucella species there are distinctive differences in invasiveness and virulence which are reflected in the response of host's tissue and in the disease in guinea pigs were thoroughly investigated by Braude[48] who made comparative studies with many representative strains of each of the three species (*B.*

melitensis, B. suis and *B. abortus*). Both in animals and in man *B. melitensis* is most invasive and produces the most serious infections. *B. suis* is also very invasive and characteristically causes necrosis and suppuration in the tissues of the host. *B. abortus* is the least invasive of the species and causes a milder form of the disease.

The basic and characteristic tissue reaction for brucellosis involves mononuclear cells and the large phagocytes in tissues, with the formation of granulomas. These granulomas are comprised of epitheloid cells, giant cells of Langhans types, lymphocytes and plasma cells. Necrosis is usually slight or absent but abscess formation and even caseation may occur in association with these lesions. The granulomas eventually heal with fibrosis.

Human brucellosis tissue changes

In human brucellosis various tissue changes have been described by many workers[48]. Spink in 1949 observed liver changes in patients with brucellosis. They have described two types of lesions. In the first type they noted granuloma comprising of epitheloid cells and giant cells with a peripheral zone of lymphocytes. In the second type the lesions consisted of nodular inflammatory foci in the portal spaces with an infiltrate of plasma cells, lymphocytes and a few necrotic hepatic cells surrounded by mononuclear cells. Naglotimath[49] in 1979 observed similar non caseating granulomas in the liver biopsy of patients with brucellosis. In the same year Young had observed non caseating granulomas in infections with *B. abortus* and absence of granulomatous lesions with *B. melitensis.* In 1982 Cervantes from Spain reported non caseating granuloma in liver biopsies in patients with brucellosis.

In 1960 Perry described the changes in heart due to chronic brucellosis in human beings from autopsy studies. They noted the disease chiefly affects the myocardium and endocardium. The myocardial lesions were in the form of focal abscesses. Most of the times, the lesions were microscopic, perivascular, granulomatous or fibrotic. Brucella endocarditic was in the form of nodular deforming lesion in the aortic valves often having a tendency to calcify. Microscopically these lesions showed micro abscesses. The aortic valve showed the deposition of calcium.

Lymph node changes in brucellosis

These have been studied by Albertini in 1937 in patients suffering from brucellosis. He described the granulomatous form in the lymph nodes. These granulomas were formed by epitheloid cells and occasionally Langhans type of giant cells and plasma cells were seen. Rarely fibrinoid type of necrosis was seen in these granulomas. In exceptional cases caseation type of necrosis was present. This granulomatous reaction was most marked in the medullary region of the lymph node.

CLINICAL FEATURES

Human brucellosis is traditionally described as a disease of protean manifestations. However, fever is invariable and can be spiking and accompanied by rigors, if bacteremia is present, or may be relapsing, mild, or protracted. Constitutional symptoms are generally present. Physical examination is generally nonspecific, though lymphadenopathy, hepatomegaly, or splenomegaly is often present.

In sum, practically every organ and system of the human body can be affected in brucellosis - a fact that underscores the importance of including brucellosis in the differential diagnosis in areas of endemic disease, even if clinical features are not entirely compatible.

Clinical manifestations of acute brucellosis

In most instances, the manifestations of acute brucellosis consist of a characteristic fever and various constitutional signs and symptoms, but few localizing features.

The latency from infection to onset of symptoms of acute brucellosis is usually between 5 and 21 days, although occasionally the interval between infection and first symptoms is many months. The classic ensuing septicemic course is most likely to occur in regions of endemic disease and is usually due to *B. melitensis* infection.

- The severity of the illness ranges from mild to seriously ill. Mild cases may last for just a few days, while the acute phase of severe cases may persist for weeks to many months. In some cases this lingering illness consists of fever and malaise, which occur in most cases. In some cases, severe debilitation may occur.

- Common manifestations of acute brucellosis include fever (80-90% of cases), chills, anorexia, insomnia, joint pain (60-80% of cases), bone pain (40-60% of cases), myalgia (20-70% of cases), profuse night sweats (20-25% of cases), and irritability (common).

- The fever of acute brucellosis caused by *B. melitensis* usually lasts for 10 to 30 days, undulates irregularly, and is not associated with rash.
    - Some very severe cases are termed malariform brucellosis because the undulating fever spikes reach very high temperatures and are associated with chills, drenching sweats, and prostration from the very onset of illness.
    - The irregular undulation of fever spikes distinguishes malariform acute brucellosis from malaria, which produces quite regular fever spikes; the periodicity of malaria fever spikes (e.g., tertiary, quaternary) is determined by the type of malarial parasite that has infected the host.
    - Fever and other constitutional manifestations of acute brucellosis tend to be more severe and persistent in patients who attempt to remain active. Severity and duration typically are reduced by enforced bed rest.
- Some patients manifest focal abnormalities during acute brucellosis.
    - The most common focal manifestation of acute brucellosis is pain, usually localized to the lower spine, paraspinous muscles, or upper buttocks. In some cases, neuralgic pain is distributed along lumbosacral peripheral nerves, especially the sciatic. The region of the lumbosacral vertebrae may be tender to percussion, as may be the course of the sciatic nerve. Thus, these clinical features may closely resemble sciatica. The cost vertebral joints may be similarly afflicted.

- Occasionally, patients develop pain, tenderness, swelling of joints (often monoarticular, knees more than elbows) or bone ends.

- Skin ulcerations, purpura, erythema, or petechiae may be found, from which organisms may at times be cultured. Some of these changes, especially the purpura, arise as consequences of immune-mediated thrombocytopenia.

- Abdominal discomfort or pain may be associated with anorexia and weight loss. The pain may in some instances suggest an acute abdomen. In instances where there is right upper quadrant pain, hepatic abscess must be excluded, especially if associated jaundice is present.

- In some cases, tender enlargement of the spleen is discerned.

- Some patients develop constipation.

- In some instances, tender enlargement of the testicles due to epididymo-orchitis, resembling mumps orchitis, develops after the first few days of high fever and chills. Although it can be painfully persistent for a number of days, unlike mumps orchitis or brucellosis in sheep or goats, it seldom leads to sterility in humans.

- Urethritis or urinary tract infection may be found. Occasionally, the kidneys are involved, although the disease seldom results in renal failure.

- Unlike brucellosis of cattle, human acute brucellosis does not appear to carry any higher risk for abortion than any other form of bacteremic illness.

Sub acute brucellosis is distinguished from mild acute brucellosis by its more insidious onset, but this distinction is not always clear; hence, these two types of brucellosis exist on a continuum.

- Sub acute brucellosis does not have discrete onset of undulating fevers and does not produce marked constitutional symptoms.

- Low-grade fevers, aches and pains, and malaise are noted, but are relatively mild, resembling mild cases of influenza; their course persists for 10 to 13 days (in some cases many weeks, longer than is typical for influenza).

    - As with mild acute brucellosis, the sub acute form is most likely to be engendered by long-term, low-grade exposure to Brucella organisms, hence this form arises in some veterinarians or individuals with occupational exposure to herd animals.

    - As with mild acute brucellosis, *B. abortus* or *B. suis* infection is more likely than *B. melitensis* infections to cause sub acute brucellosis.

- Chronic brucellosis develops in the wake of some, but not all, sub acute cases.

- Brucella-related deafness is among the most common of the neurological consequences of sub acute brucellosis in regions of endemic disease.

- Some patients with findings suggestive of sub acute brucellosis are actually experiencing manifestations of a nonbrucellotic "chronic fatigue syndrome" or are manifesting psychologically induced complaints.

Clinical manifestations of chronic brucellosis

Chronic brucellosis develops in fewer than 15 percent of all patients who have had acute brucellosis. The risk for chronic brucellosis is reduced considerably if adequate treatment, including enforced rest, is provided for the acute phase of illness. Patients with chronic brucellosis are particularly likely to manifest anorexia and weight loss.

- Recurrence of fever after a fever-free interval is often the first sign of progression into the chronic phase of brucellosis.

- The interval between the acute and chronic phases of brucellosis varies from days to many months.

- Endocarditis, neurobrucellosis and other manifestations of chronic brucellosis are complications that often although not always are prevented by adequate treatment of acute brucellosis.

Endocarditis, neurobrucellosis and other forms of chronic brucellosis may in some instances develop without a known preceding bout of acute brucellosis.

- In such instances, the bout of acute brucellosis may have been very mild and mistaken for influenza or some other mild infectious illness, or the neurobrucellosis may develop as a complication of sub acute brucellosis.

- Failure to diagnose acute brucellosis prevents administration of adequate antibiotic treatment, hence missing the opportunity to prevent possible chronic brucellosis.

The onset of chronic brucellosis generally is announced by the reappearance of fever and constitutional symptoms (e.g., lethargy, irritability, fatigue).

- In many cases, the relapses consist solely of typical features of acute brucellosis, such as undulant fever, aches, sweats, and generalized weakness.

- Relapses resembling acute brucellosis have been known to continue, in the preantibiotic era, over intervals longer than 20 years. This was the case with the pioneering epidemiologist of brucellosis, Alice Evans, who contracted her illness in the laboratory.

- In such cases, the intervals between febrile relapses may entail sustained periods of easy fatigability, weakness, mental depression, headache, and other chronic aches and pains. The punctuating epochs of febrile relapse are markedly more debilitating than the intercurrent nonfebrile epochs.

In other cases, the relapsing illness includes additional abnormalities referable to specific organ systems.

- If these abnormalities are preponderantly or solely referable to a single organ system, the chronic brucellosis is referred to by some authorities as "focal." If many organ systems are involved, it is referred to as "diffuse."

- Organ systems that may be involved include cardiovascular, pulmonary, musculoskeletal, nervous systems (neurobrucellosis) and others which are described further under the section of complications.

COMPLICATIONS

1) Osteoarticular

Osteoarticular disease is universally the most common complication of brucellosis, and three distinct forms exist - peripheral arthritis, sacroiliitis, and spondylitis. Peripheral arthritis is the most common and is nonerosive, since it usually involves the knees, hips, [50] ankles, and wrists in the context of acute infection. Prosthetic joints can also be affected in peripheral arthritis. Brucellosis has also been proposed as a cause of reactive arthritis. A second form, characterized by sacroiliitis, is readily diagnosed, also usually in the context of acute brucellosis.[51] On the other hand, a third form of osteoarticular disease, spondylitis, remains notoriously difficult to treat and often seems to result in residual damage.[52] The lumbar spine is the usual site of involvement. Spondylitis can be easily diagnosed with plain radiography, in which the characteristic Pons sign (a step like erosion of the anterosuperior vertebral margin) can be identified, or with scintigraphy and magnetic resonance imaging. The latter imaging technique is popular and produces impressive scans but is costly and not always available. Osteoarticular complications are sometimes linked to a genetic predisposition, with recent data suggesting an association with HLA-B39.[53]

2) Neurobrucellosis

Neurobrucellosis is uncommon and neurologic manifestations of neurobrucellosis are diverse, and can affect any part of the central or peripheral nervous system, and clinical picture may be confused by the coexistence of two or more clinical syndromes in the same patient.[54] It is difficult to know how frequently the nervous system is affected, because of difficulties in diagnosis and variability in reporting such complications. The clinical neurological syndromes which may be caused by Brucella include acute toxic manifestations, meningitis, diffuse or localized encephalitis, myelitis , radiculitis, neuritis, multiple cerebral or cerebellar abscesses, ruptured mycotic aneurysm and subarachnoid hemorrhage, Guillain - Barre syndrome, cranial nerve palsies, hemiplegia, sciatica, myositis, and rhabdomyolysis. Papillitis, papilledema, retro bulbar neuritis, optic atrophy and ophtalmoplegia due to lesion in cranial nerve III, IV, VI may occur in Brucella meningoencephalitis. The most common neurologic manifestation is a sub acute or chronic meningoencephalitis. Many other CNS manifestations of neurobrucellosis have been reported: arachnoidits, cerebellar syndromes, ruptured basilar aneurysm, hemi parkinsonism, chorea, anterior poliomyelitis. Sometimes it may mimic brain tumor requiring neurosurgery. Acute toxic manifestations are seen during the acute phase of infection, and include headache, neck ache, backache, insomnia, depression and muscle weakness. Motor manifestations occur frequently and generally present in the form of paresis of variable intensity, with frequent gait disturbances. Sensory symptoms usually consist of paresthesias and occasionally gait apraxia. Involvement of the cranial nerves,

generally the sixth, seventh and eight is relatively frequent. The involvement of the eighth cranial nerve is very characteristic of the Brucellar meningitis.

3) Cardiovascular Complications of Brucellosis

The cardiac involvement is rarely seen in the course of the systemic brucellosis but is a life threatening complication of brucellosis, and the main cause of the mortality associated with this disease. Endocarditis is the most seen cardiac involvement. The reported incidence of endocarditis is 0.8 percent worldwide. The most common cause of mortality in the course of brucellosis is endocarditis.[55] Aortic valve and aortic root is the most commonly involved cardiac tissues, where vegetations and abscess formations are mostly seen.[56] Mitral and other valvular involvement are very rare in the course of the carditis associated with this disease. Mycotic aneurysms of ventricles, aorta and other arteries, [57] pericarditis and myocarditis have been reported in literature.

Endocarditis and vegetations may develop on damaged valves, prosthetic heart valves and especially normal valves, and there seems to be a high incidence of heart failure.[58]The atrial and ventricular septal defects may be involved by the disease.[59, 60] Pancarditis associated with systemic Brucellae infection is extremely rare and mortality is very high. Only one patient has been reported in literature so far.[61]

4) Hepatic complications of brucellosis

Hepatitis is common, usually manifesting as mild transaminasemia. Liver abscess and jaundice are rare.[62] Granulomas can be present in liver-biopsy specimens

in cases of both *B. melitensis* and *B. abortus*.[63] Ascites is often present, either as a temporary exacerbation of preexisting hepatic disease or as frank peritonitis.[64]

5) Genitourinary Complications of Brucellosis

The reproductive system is the second most common site of focal brucellosis. Brucellosis can present as epididymo-orchitis in men and is often difficult to differentiate from other local disease.[65]The effect of the local inflammation on subsequent testicular function has not been adequately studied. Brucellosis in pregnancy poses a substantial risk of spontaneous abortion.[66]

6) Pulmonary Complications of Brucellosis

Respiratory complications of brucellosis are considered rare. A recent multinational review of cases with respiratory complications indicated that approximately 16 percent of cases had pulmonary involvement that included lobar pneumonia and pleural effusions.[67]

7) Ocular Complications of Brucellosis

Diplopia or amaurosis is the result of occulomotor or optic nerve damage but disturbances of vision in brucellosis can also be due to direct involvement of the eye. Nummular keratitis, retinal thrombophlebitis and uveitis are rare complications during the acute or the chronic stage of the illness.

8) Cutaneous Complications of Brucellosis

Cutaneous lesions occur in about five percent of patients with brucellosis. The lesions that have been described include maculopapular rashes and erythema nodosum.[68, 69]

SPECIAL SITUATIONS

Childhood brucellosis generally exhibits a more benign course in terms of the rate and severity of complications and the response to treatment.

Although the relationship between brucellosis and T-cell-mediated immunity has been well described, brucellosis is not an opportunistic infection in patients who are infected with the human immunodeficiency virus (HIV) or who have AIDS, even in areas of endemic disease. Most patients with HIV infection and brucellosis have a benign clinical course in the early stages of HIV infection, according to the number of CD4+ T lymphocytes.

DIAGNOSIS OF BRUCELLOSIS

The development of a definitive diagnostic test for brucellosis remains an elusive target. Ever since the development of the first serologic test for brucellosis by Bruce more than a century ago, a definitive diagnostic technique has been actively pursued.

The absolute diagnosis of brucellosis requires isolation of the bacterium from blood or tissue samples. The sensitivity of blood culture varies, depending on individual laboratory practices and how actively the obtaining of cultures is pursued. The percentage of cases with positive cultures ranges from 15 to 70%.[70] Brucellae are cultured in standard biphasic (solid and liquid) mode or with the Castaneda bottle, which incorporates both solid and liquid mediums in the same container. Automated systems are also reliable in isolating Brucella.[71] Blood-culture sensitivity may be improved by a lysis-centrifugation technique.[72] Even with automated systems, subcultures should be performed for at least four weeks. Brucellae are small, gram-negative and oxidase- and urease-positive coccobacilli that resemble fine grains of sand. Catalase tests, which can have positive results for Brucella, should not be performed because the technique can cause the nebulization of particles. Species identification is performed on the basis of particular characteristics.

Bone marrow cultures are considered the gold standard for the diagnosis of brucellosis, since the relatively high concentration of Brucella in the reticuloendothelial system makes it easier to detect the organism. Furthermore, bacterial elimination from the bone marrow is equivalent to microbial eradication.[73] However, harvesting bone marrow for culture remains an invasive, painful technique, and results have not been universally reproducible.

There are two broad categories of serologic methods for diagnosing brucellosis: those based on antibody production against lipopolysaccharide and those based on antibody production against other bacterial antigens. Developed by Bruce, the serum agglutination test remains the most popular diagnostic tool for brucellosis.

Titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation. However, in areas of endemic disease, using a titer of 1:320 as diagnostic may be more specific. Seroconversion and evolution of the titers can also be used in diagnosis. Drawbacks of the serum agglutination test include the inability to diagnose *B. canis* infections; the appearance of cross-reactions of class M immunoglobulins with *Francisella tularensis, Escherichia coli O116 and O157, Salmonella urbana, Yersinia enterocolitica O:9, Vibrio cholerae, Xanthomonas maltophilia, and Afipia clevelandensis*; and the percentage of cases in which seroconversion does not occur. Lack of seroconversion can be attributed to the performance of tests early in the course of infection, the presence of blocking antibodies, or the so-called "prozone" phenomenon (i.e., the inhibition of agglutination at low dilutions due to an excess of antibodies or to nonspecific serum factors).[74] Some of these shortcomings can be overcome by modifications such as the addition of EDTA, 2-mercaptoethanol, or antihuman globulin. Other variations of agglutination tests[75] have not proven superior. A new dipstick test, however, offers a rapid and reliable diagnostic alternative in acute brucellosis.[76] The superiority of most of the other agglutination tests over the serum agglutination test has not been consistently proven. Serum agglutination tests have a major drawback in that they are not suitable for patient follow-up, since titers can remain high for a prolonged period.[77]

Indirect enzyme-linked immunosorbent assays (ELISAs) typically use cytoplasmic proteins as antigens. ELISA measures class M, G, and A immunoglobulins, which allows for a better interpretation of the clinical situation and

overcomes some of the shortcomings of the serum agglutination test. A comparison with the serum agglutination test yields higher sensitivity and specificity.[78] In patients with neurobrucellosis, ELISA offers significant diagnostic advantages over conventional agglutination methods.[79]

All told, antibody profiles do not have specific clinical correlations, and titers often remain high for a protracted period.[80] .The asymptomatic patient with an isolated positive titer of class G and A immunoglobulins, or A immunoglobulins only, has not been adequately studied. Variations of ELISA exist, such as competitive ELISA and sandwich ELISA, which may prove useful as a follow-up tool.

The development of a specific polymerase chain reaction (PCR) is a recent advance. PCR is fast, can be performed on any body tissue, and can yield positive results as soon as 10 days after inoculation. It was first developed for brucellosis in 1990, using a 635-bp fragment of *B. abortus* strain 19.[81] Subsequently, two major gene sequences have been used as targets: the 16S rRNA gene sequence,[82] which presents total genus-specific homology and has been satisfactory in clinical settings,[83] and the BCSP31 gene, which encodes an immunogenic protein of the external membrane of *B. abortus*[84] and has been extensively studied in clinical practice.[85] Cross-reactivity with ochrobactrum is noticed sporadically with both techniques. A comparison of the two techniques showed superiority of the 16S rRNA target in terms of sensitivity.[86]

Nested PCR has proved to have superior specificity and sensitivity, although it is more prone to contamination.[87] Real-time PCR is most likely the diagnostic tool of the future, offering the possibility of results in 30 minutes.[88-90] PCR ELISA is

another new promising variation.[91,92] Other variations of PCR exist, such as arbitrarily primed PCR, PCR with random amplification of polymorphic DNA, and a specific multiplex PCR that can concomitantly diagnose brucellosis, Q fever, plague, and anthrax and was developed for purposes of biowarfare defense.[93] Although PCR is very promising, standardization of extraction methods and set-up is lacking, and a better understanding of the clinical significance of the results is still needed.[94]

TREATMENT OF BRUCELLOSIS

Treatment of human brucellosis should involve antibiotics that can penetrate macrophages and can act in the acidic intracellular environment. There is a general need for combined treatment, since all monotherapies are characterized by unacceptably high relapse rates. Practitioners must weigh such questions as the optimal duration of treatment,[95] cost-effective and conveniently administered regimens, favorable pharmacokinetics and pharmacodynamics, and attention to local virulence factors.[96]

The general discrepancy between in vitro findings and in vivo observations precludes the study of resistance patterns of brucellosis or in vitro evaluation of the efficacy of individual antibiotics.

In 1986, the World Health Organization issued guidelines for the treatment of human brucellosis. The guidelines discuss two regimens, both using doxycycline for a period of six weeks, in combination with either streptomycin for two to three weeks or rifampicin for six weeks. Both combinations are the most popular treatments worldwide, although they are not used universally. The streptomycin-containing

regimen is slightly more efficacious in preventing relapse.[97] This may be related to the fact that rifampicin down-regulates serum doxycycline levels.[98] However, parenteral administration of streptomycin mandates either hospital admission or the existence of an adequate health care network - both of which are often absent in areas of endemic disease. On the other hand, the use of rifampicin in areas in which brucellosis is endemic, where tuberculosis is also usually endemic, raises concern about the development of community resistance to rifampicin.

Alternative drug combinations have been used, including other aminoglycosides (e.g., gentamicin and netilmicin).[99] Trimethoprim-sulfamethoxazole is a popular compound in many areas, usually used in triple regimens. Quinolones are an alternative. Various combinations that incorporate ciprofloxacin and ofloxacin have been tried clinically, yielding similar efficacy to that of the classic regimens.[100] Only in vitro observations exist for moxifloxacin and levofloxacin.[101]Although quinolones have been used and will continue to be used, the cost of this approach remains a major drawback. The action of macrolides is attenuated in the acidic phagolysosomal environment, and thus these agents are not useful in brucellosis.[102]

Most complications of brucellosis can be adequately treated with standard regimens. The protracted administration of triple regimens is used for neurobrucellosis. The addition of steroids in neurobrucellosis has not proved to be consistently beneficial.[103] A recent meta-analysis of the efficacy of various combinations for spondylitis advocated a duration of treatment of at least three months; the superiority of any particular regimen could not be proved.[104] Quinolones may prove cost-effective in spondylitis, according to preliminary results.[104]

Rifampicin is the mainstay of treatment in cases of brucellosis during pregnancy, in various combinations. Brucellosis in children is treated with combinations that are based on rifampicin and trimethoprim-sulfamethoxazole and with aminoglycosides.[105]

PROGNOSIS

In uncomplicated cases of acute brucellosis, fever, malaise, and many other manifestations improve rapidly with bed rest, while sustained physical activity may prolong or worsen the degree of illness.

Considerable improvement from the symptoms of the acute "toxic" phase of illness occurs in most cases within a few weeks, with or without treatment. In many cases this is followed by complete remission within two to six months. Recovery tends to be more rapid in individuals infected with *B. abortus* than in those infected with *B. melitensis* or *B. suis.*

Death seldom occurs as the consequence of acute brucellosis, although it has been reported. Fewer than one percent of patients die of brucellosis. When the outcome is fatal, death is usually a consequence of cardiac involvement; more rarely, it results from severe neurologic disease. Despite the low mortality rate, recovery from brucellosis is slow, and the illness can cause prolonged inactivity, with consequent domestic and economic losses.

Objective clinical and laboratory evidence for ongoing disease is demonstrable. Patients who do not have such evidence and who complain of occasional mild symptoms similar to those found in acute brucellosis are likely to

have psychoneurosis. This complication of acute brucellosis does not usually resolve with anti-brucellosis treatments, although such treatments may exert placebo effects for individual bouts. Psychiatric treatment may be indicated.

The likelihood of recurrence is greater in individuals who are not treated or who are inadequately treated for acute brucellosis. Recurrence is possible even in properly treated patients who have had acute brucellosis. Addition of oral rifampicin to oral tetracycline may reduce the recurrence risk for patients who are treated with that combined therapy for acute brucellosis. Chronic brucellosis may continue to trouble patients for as long as 25 years, but such cases are quite rare.

RELAPSE

Relapses, at a rate of about 10 percent, usually occur in the first year after infection, are often milder in severity than the initial disease, and can be treated with a repeated course of the usual antibiotic regimens. Most cases of relapse are caused by inadequate treatment.

PREVENTION

Prevention of human brucellosis focuses mainly on elimination of infection in hosts (i.e. goats, cows), along with hygiene, vaccine, and effecting heating of dairy products and related foods. The routine pasteurization of milk and milk products has been the single factor most responsible for the control of brucellosis. In many cases, human brucellosis can be an occupational hazard for veterinarians, abattoirs, farmers, and dairy workers. Because contact with infected materials can allow organisms to enter through skin lesions and gain access to the lymphatic system, hygienic

precautions are important. Vaccines developed to prevent this disease in humans have had limited efficacy and have been associated with serious medical reactions.[106, 107] Vaccines developed to prevent and control livestock infection are effective in reducing the incidence of human brucellosis.

Most veterinary vaccines focus on *B. abortus* and *B. melitensis*.[108]

There is little data on the role of routine screening for Brucella in populations at risk. Use of the SAT as a screening test is complicated by the uncertain significance of a low positive titer in an asymptomatic person. However, in certain populations, particularly the medically underserved, it may be reasonable to screen family or other community members of brucellosis patients, as they may share the same risk factors and if sick may not spontaneously present for diagnosis and treatment.[109]

VACCINATION OF ANIMALS

RB51 was approved for use in February 1996 and for use in the eradication program in March. It was the first new vaccine for brucellosis in 50 years and it is given only to calves four to 12 months old. This attenuated strain is less virulent in cattle so they will shed fewer organisms if they become infected. The biggest advantage to RB51 is the ability to protect via vaccination, but detect those infected with the wild type virus. This vaccine lacks the surface antigen, LPS-O, that induces an antibody response and therefore able to use it as a surveillance tool in serodiagnostic tests such as tube agglutination tests, the “card test”, milk ring, and complement fixation. Vaccination of cattle with RB51 does not induce antibody responses that cause positive reactions on diagnostic tests now used. This becomes a

problem when humans are accidentally inoculated through a needle stick. The advantage of testing seronegative in cattle after immunization makes diagnosis in human's post- exposure difficult because they too test seronegative. RB51 is considered infectious for humans and only accredited veterinarians should administer the vaccine.

VACCINATION IN HUMANS

Vaccines based on live attenuated Brucella strains, such as *B. abortus* strain 19BA or 104M, have been used in some countries to protect high-risk populations but have displayed only short-term efficacy and high reactogenicity. Subunit vaccines have been developed but are of uncertain value and cannot be recommended at present. Research in this area has been stimulated by interest in biodefense and may eventually yield new products, some of which may be based on the live attenuated WR 201 variant of *B. melitensis* strain 16M.

CONTROL OR ERADICATION

The World Health Organization "Mediterranean Zoonoses Control Project" has implemented surveillance, herd vaccination, and culling of infected animals using methods similar to those employed in the United States since 1945. This project has steadily eroded the prevalence of brucellosis in this region. Similar methods have been projected for other portions of the world, but implementation has been inadequate in many areas because of expense, warfare, lack of concern, and other reasons.

METHODOLOGY

We studied a total of 30 patients of brucellosis and observed for various clinical manifestations of patients with brucellosis presented to us and also tried to look for various laboratory parameters and complications of brucellosis.

Study design

One year cross sectional study.

Study period

The present study was conducted during January 2007 to December 2007.

Method of collection of data

Source of Data

Patients admitted in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum and fulfilling the inclusion criteria formed the material for the study.

Sample size

A sample size of 30 cases was calculated on the basis of 80 percent of the average number of similar cases admitted to KLES Dr. Prabhakar Kore Hospital Belgaum over a period of last three years.

Selection criteria

Inclusion Criteria

A clinically compatible case presenting with any of the following:

1 Fever of more than 10 days

2 Joint pains

3 Low backache

4 Body ache

5 Generalized weakness

Exclusion Criteria

Other diseases known to produce the symptoms in the present cases (malaria, UTI, upper respiratory tract infection, tuberculosis, enteric fever, syphilis, etc) were ruled out by all possible investigations.

Procedure

During the study period; all patients fulfilling the inclusion criteria were subjected to the serological tests and other tests if necessary were carried out to diagnose brucellosis. A diagnosis of brucellosis was made according to the CDC criteria.

Case classification: a clinically compatible case that is epidemiologically linked to a confirmed case or that has supportive serology (i.e. Brucella agglutination titer of greater than or equal to 160 in one or more specimens obtained after onset of symptoms).

Patients who are diagnosed to have brucellosis were examined according to the performa and other relevant investigations carried out after obtaining informed written consent. The ethical clearance had been obtained from the institutional committee authorized for the study.

The patients underwent the following investigations:-

1. Complete blood count
2. Urine routine
3. Serology (SAT, 2 ME, PS for MP, QBC for MP, VDRL, Widal, ASLO)
4. Blood culture
5. Chest X ray
6. Other relevant and special investigations were carried out as and when required.

RESULTS

The present study was conducted in KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum and the findings obtained are tabulated as below. During the study year from January 2007 to December 2007, **576** cases were screened for brucellosis and **30** cases of brucellosis were diagnosed. These 30 cases were studied for the following observations.

Out of these 30 cases, 27 cases were from the medical wards and 3 cases were from pediatric wards. Total number of admissions during this period was 4279 in medical wards and 2784 in pediatric wards.

Table No. 1: Prevalence of brucellosis in our hospital

YEAR	WARD	TOTAL ADMISSIONS	NO OF BRUCELLOSIS	%
January 07 – December 07	Medicine	4279	27	0.63
January 07 – December 07	Pediatrics	2784	03	0.1

The prevalence of brucellosis in medicine wards was 0.6% where as in pediatrics ward was 0.1 %.

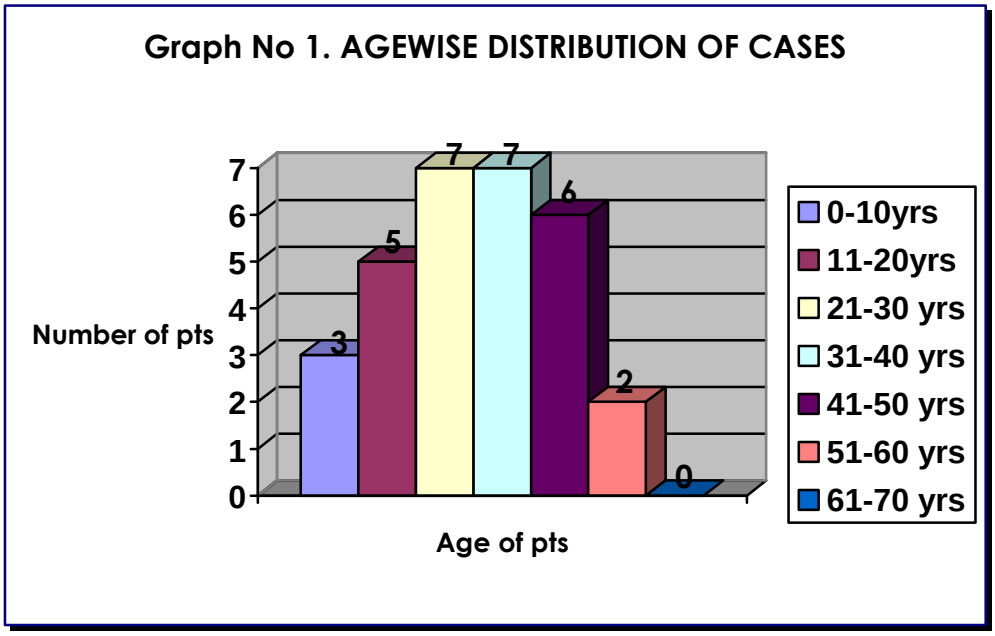
Table No. 2 Comparison of number of Enteric fever and Brucella cases

YEAR	NO OF ENTERIC FEVER CASES	NO OF BRUCELLA CASES	RATIO
January 07 – December 07	186	30	62:10

In the present study, it was found that for every 62 cases of enteric fever there were ten cases of brucellosis.

Table No. 3 Age distribution

Age (in yrs)	No of pts	Percentage
0 – 10	3	10%
11- 20	5	16.66%
21- 30	7	23.33%
31- 40	7	23.33%
41-50	6	20%
51-60	2	6.66%
61-70	0	—

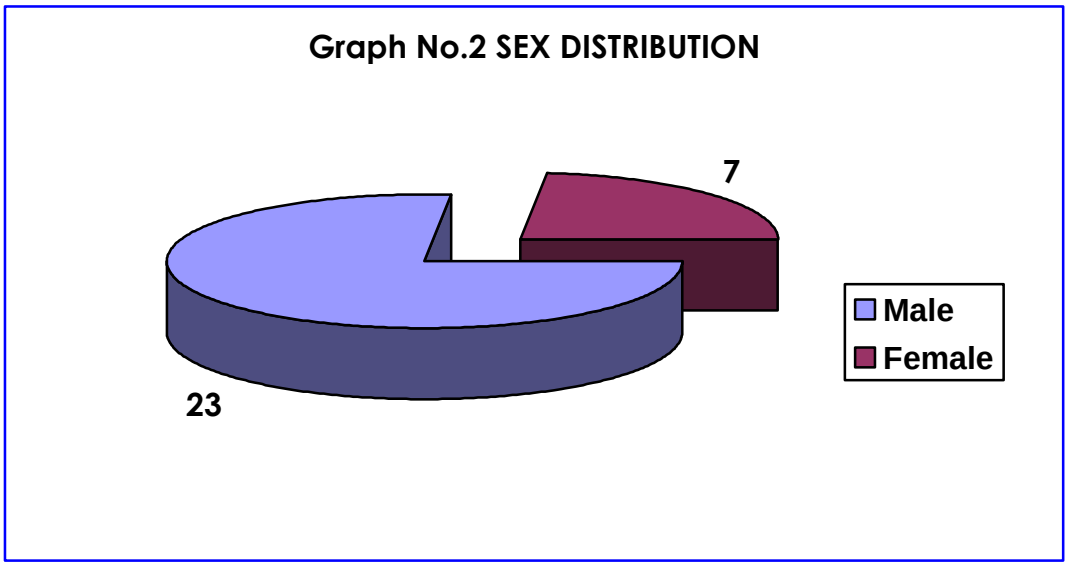


In the total of 30 patients, more number of cases i.e. seven cases each (23.33 %) were in the age groups of 41 to 50 years and 21 to 30 years. Out of 30 cases, six cases (20 %) were in the age group 41 to 50 years, five cases (16.66 %) in the age group 21 to 30 years, three cases (10%) in the age group zero to ten years and two

cases (6.66%) in the age group 51 to 60 years. We did not find any cases of brucellosis after the age of 60 years.

Table No. 4 Sex distribution

GENDER	No. of Pt's	%
Male	23	76.66
Female	7	23.33



We observed that males are more commonly affected with brucellosis than the females. In our study 23(76.66%) patients were males while 7(23.33%) were females, male to female ratio been 3:1.

Table No. 5 Occupational distribution

OCCUPATION	No. of Pt's
Farmer	13
Shepherd	2
Butcher	2
Veterinary	1
Others	12

In the present study, brucellosis was seen more commonly among farmers, shepherds and butchers.

Table No. 6 Locality of patients:

LOCALITY	No. of Pt's	%
Rural	26	86.66
Urban	4	13.33

We observed that the rural dwellers, 26 patients (86.66%) were more commonly affected than the urban dwellers, 4 patients (13.33%).

Table No. 7 Contact with Animals

H/O CONTACT WITH ANIMALS	No. of Pt's	%
Yes	27	90
No	03	10

In the present study, 27 patients had history of contact with animals, while 3 patients did not have history of contact with animals.

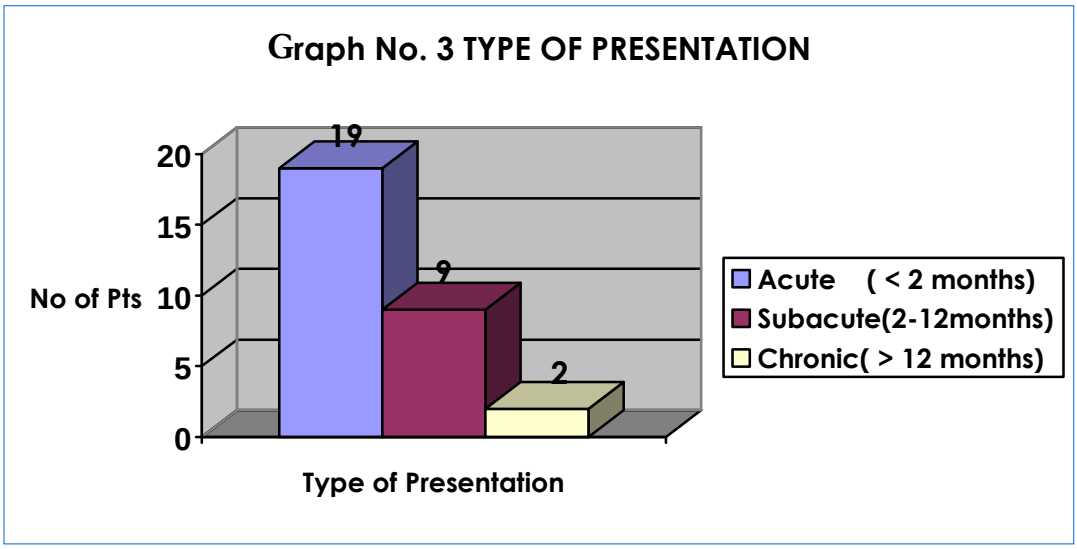
Table No. 8 Patients with history of raw milk consumption

H/O RAW MILK CONSUMPTION	No. of Pt's	%
Yes	09	30
No	21	70

In the present study, history of raw milk consumption was present in 9 patients whereas 23 patients did not have history of raw milk consumption.

Table No. 9 Type of presentation

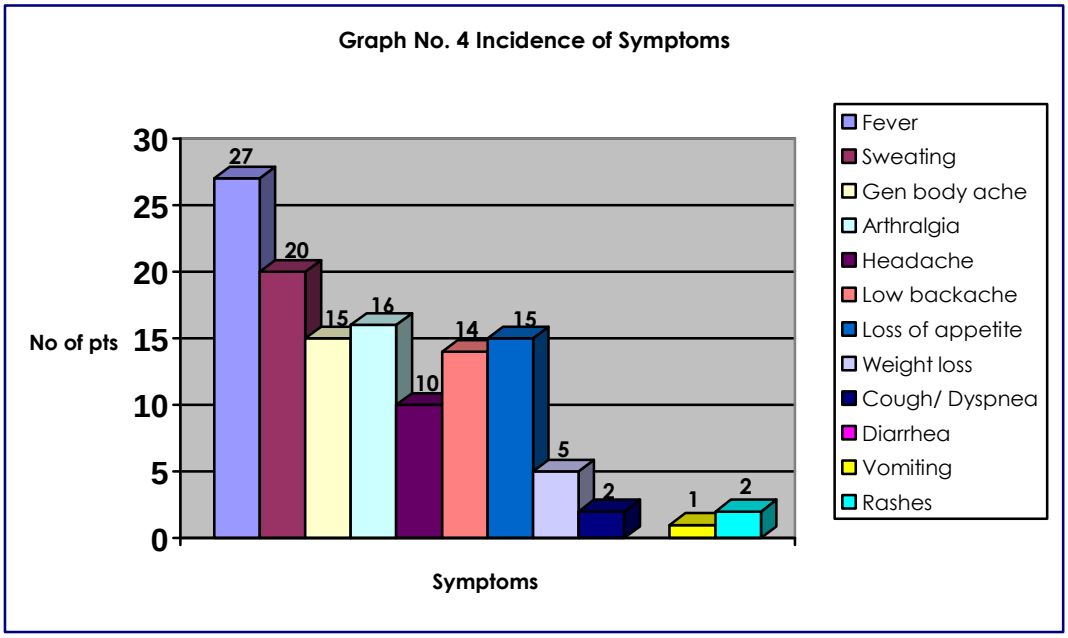
TYPE	No. of Pt's	%
Acute (< 2 months)	19	63.33
Sub acute(2-12months)	9	30
Chronic(> 12 months)	2	6.66



In the present study, acute presentation of brucellosis was seen in 19 patients (63.33%), sub acute in nine patients (30%) and chronic in two patients (6.66%).

Table No. 10 Incidence of Symptoms

SYMPTOMS	No. of Pt's	%
Fever	27	90
Sweating	20	66.66
Gen body ache	15	50
Arthralgia	16	53.33
Headache	10	33.33
Low backache	14	46.66
Loss of appetite	15	50
Weight loss	05	16.66
Cough/ Dyspnea	02	6.66
Diarrhea	--	--
Vomiting	01	3.33
Rashes	02	6.66



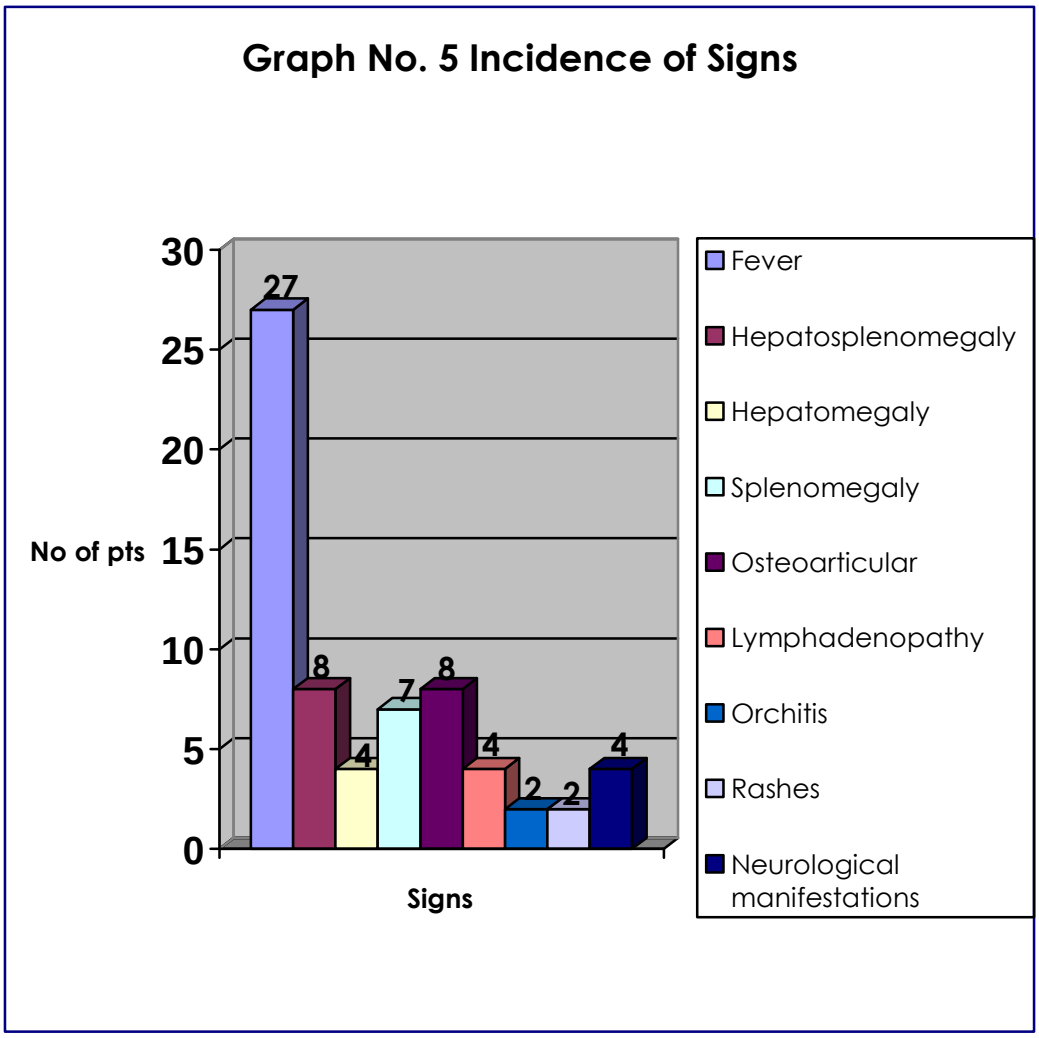
Out of the 30 patients, majority presented with fever- 27 patients (90%). Other common presentations were night sweats in 20 patients(66.66%), arthralgia in 16 patients(53.33%), generalized body ache and loss of appetite in 15 patients(50%),

low back ache in 14 patients(46.66%), headache in ten patients(33.33%) and weight loss in five patients(16.66%).

Other minor symptoms observed were cough/dyspnea in two patients (6.66%), rashes in two patients (6.66%) and vomiting in one patient (3.33%).

Table No. 11 Incidence of Signs

SIGNS	No. of Pt's	%
Fever	27	90
Hepatosplenomegaly	08	26.66
Hepatomegaly	04	13.33
Splenomegaly	07	23.33
Osteoarticular	08	26.66
Lymphadenopathy	04	13.33
Orchitis	02	6.66
Rashes	02	6.66
Neurological manifestations	04	13.33



In the present study, majority of the patient's .i.e. 27 patients (90%) had fever. Hepatosplenomegaly was seen in eight patients (26.6%), osteoarticular signs in eight patients (26.6%), splenomegaly in seven patients (23.33 %), hepatomegaly in four patients (13.33%), and lymphadenopathy in four patients (13.33%). Neurological manifestations, orchitis and rashes were rare manifestations. Ophthalmological signs were not seen in the present study.

LABORATORY PARAMETERS

Table no. 12 Total Leucocyte counts

TOTAL LEUCOCYTE COUNTS	No. of Pt's
Less than 4000cells/cmm	02
4000- 10,000 cells/cmm	25
More than 10000cells/cmm	03

The present study revealed that total leucocyte count is not much altered in brucellosis. Majority of the patients had counts in the normal range. Very few patients had counts above or below the normal.

Table no. 13 Erythrocyte sedimentation rate (ESR)

ESR	NO. OF PTS
0-20 mm/1st hour	04
20-40 mm/1st hour	12
More than 40 mm/1st hour	14

In the present study, it was found that ESR was between 20 to 40 mm at the end of first hour in 12 patients and more than 40 mm at the end of first hour in 14 patients. It was less than 20 mm at the end of first hour in only four patients.

Table no. 14 Agglutination titres

AGGLUTINATION TITRES	NO OF PTS
1:160	7
1:320	5
1:640	9
1:1280	5
1:2560	2
1:5120	1
1:10240	1

In the present study, 21 patients had titres in the range of 1:160 to 1:640 and titres of 1:1280 and above were seen in nine patients.

Table no.15 Titres according to the type of illness

Titre/ Type	1: 160	1: 320	1: 640	1: 1280	1:2560	1: 5120	1: 10240	P
Acute	2	5	5	3	2	1	1	0.636
Sub acute	4	0	3	2	0	0	0	
Chronic	1	0	1	0	0	0	0	

An attempt was made in this study to correlate the type of presentation with the standard agglutination titres. When we did a Pearson's correlation for the type of presentation and titres it showed negative correlation (r= - 0.307) which means that

titres were lower in chronic brucellosis than in acute brucellosis but this difference is not statistically significant (p=0.099). Chi square test was carried out to see if there is any significant difference of titres depending upon the type of presentation. The outcome revealed that there was no statistically significant difference (p=0.636) across the groups.

Table no.16 Blood culture

BLOOD CULTURE	NO. OF PT'S	%
Positive	11	36.66
Negative	19	63.33

Blood culture was done in all the patients. It was positive in 11(36.66%) cases and negative in 19 patients (66.66%).

Culture from other tissues /fluids

Bone marrow cultures were not done in any of the patients. CSF cultures which were done in two cases of chronic meningitis were negative.

Chest X Ray

Chest x ray did not show any signs specific for brucellosis in all the thirty patients. The X ray revealed cardiomegaly in one patient who had presented with infective endocarditis secondary to aortic regurgitation. Chest X ray was also normal in the only patient who had presented with cough.

Bone Marrow

Bone marrow study was done in one patient, although diagnosis of brucellosis was already established serologically. The bone marrow study showed a non specific granulomatous lesion.

Liver Biopsy

Similarly another patient diagnosed to have acute brucellosis on serology was subjected to liver biopsy and was found to have non caseating granuloma with kuppfer cell hyperplasia.

ECG

ECG was done in all the 30 patients. It was normal in 29 patients and only one patient, who was diagnosed to have aortic regurgitation with infective endocarditis showed evidence of left ventricular hypertrophy.

TREATMENT

All patients were treated with standard regimen of rifampicin plus doxycycline for six weeks or streptomycin for three weeks plus doxycycline for a period of six weeks. 14 patients were treated with rifampicin plus doxycycline. Nine patients were treated with streptomycin plus doxycycline. Six patients of neurobrucellosis which included two cases of chronic meningitis, three cases of radiculopathy and one case of myelopathy were treated with the standard regimen plus a third agent- cephalosporin for a duration of six months. One case of infective endocarditis was treated with rifampicin, doxycycline, gentamycin and a fourth agent ceftriaxone for a duration of six months.

COMPLICATIONS

Table no. 17 Complications

COMPLICATION	NO OF PT'S	%
Neurobrucellosis	6	20
Skeletal brucellosis	6	20
Infective endocarditis	1	3.33
Epididymo orchitis	1	3.33

We noted that, out of the 30 patients 14 patients suffered from various complications of brucellosis. Neurobrucellosis and skeletal brucellosis, each of which were seen in 6 patients (20%) were the most common complications. Among the six patients of neurobrucellosis, three patients had radiculopathy, two had chronic meningitis and one patient had myelopathy secondary to brucellosis. Among the six patients of skeletal brucellosis, four had sacroilitis, one had elbow arthritis and one patient had polyarthritis. Brucellar infective endocarditis was seen in one patient with rheumatic aortic regurgitation and one patient was diagnosed to have epididymo-orchitis.

PROGNOSIS

All patients recovered completely without any morbidity left behind. One patient with infective endocarditis who had persistent lesion despite medical therapy underwent successful surgical resection of the lesion.

DISCUSSION

576 cases of suspected brucellosis admitted in KLES Dr. Prabhakar Kore Hospital and Medical Research Centre from January 2007 to December 2007 were investigated clinically, serologically, bacteriologically and with other laboratory investigations to confirm the diagnosis of brucellosis. Out of the 576 cases, 30 cases were diagnosed as brucellosis according to the CDC criteria. Of the 30 cases 27 were in the adult patients and 3 cases were in the pediatric age group.

In the present study, prevalence of brucellosis in adult patients getting admitted to KLES Dr. Prabhakar Kore Hospital and Medical Research Centre was 0.61%. Prevalence in the present study was slightly less compared to the study of hospitalized patients at GMC, Srinagar[19] which quoted a prevalence of 0.8% and Mantur et al[110] reported a prevalence of 1.8% in patients hospitalized at BLDEA, Bijapur.

We observed cases of brucellosis through out the year although a small peak was observed in the months of June to August. Calving of animals usually occurs in April and May in this area, which could explain the peak incidence of brucellosis in this season in this study. The data clearly elucidate the endemicity of brucellosis in this area.

Karabay et a[111] have reported a prevalence of 1 percent in certain areas of Turkey, although another study from Turkey by Sumer et al[112] and one from Saudi Arabia by Al Sekait et al[113] have reported a higher prevalence of 3.2% and 4.5% respectively. The difference in endemicity may be due to the prevalent practices in the population and also the incidence of brucellosis in the community.

In this study more number of cases were seen in the age group of 11 to 50 years; which is in accordance with the study by Savas et al[114] of Turkey and Mantur et al[110] of Bijapur, India. This reflects the magnitude of the socioeconomic impact of brucellosis in this area, as it affects mainly the most productive group in the community.

In the present study males were more commonly affected than females. This is in accordance with the study by Mantur et al.[110] However in the study by Savas et al[114], females were more commonly affected than males.

This is probably due to the fact that outdoor activities and contact with animals is more in males than in females in our region.

In this study, most cases were from rural areas; indicating that brucellosis is still a disease of the rural population. This is in accordance with the study by Savas et al.[114]

We made an attempt of finding the source of infection in our study patients and found that 90 % of the patients had history of close contact with animals and 30% of the patients had history of raw milk consumption.

In the present study, acute and sub acute type of presentation was more commonly seen than chronic presentation; which is in accordance with the Savas et al[114] study.

In our study, symptoms like fever, sweating, generalized body ache, arthralgia, headache and low back ache were more commonly observed symptoms. Less commonly observed symptoms were cough, dyspnea and vomiting. This is almost similar to the study carried out by Savas et al[114] and Mantur et al.[110] Skin manifestations were more in our patients as compared to Mantur et al[110] and Savas et

al.[114] Patients presenting with eye manifestations and psychotic manifestations were not seen in this study which was seen by Buchanan et al and Lulu et al. However the incidence of the same in their study, was also much less.

In our study, signs like fever, hepatosplenomegaly, hepatomegaly and splenomegaly were common observations. In studies by Mantur et al[110] and Savas et al[114], sign of fever was seen with almost similar frequency but other findings like hepatosplenomegaly, hepatomegaly and splenomegaly were less common. Osteoarticular signs were almost similar in our study and the study by Mantur et al.[110] However it was more frequently seen in Savas et al[114] study. Lymphadenopathy in our study correlated with the study by Savas et al[114] but Mantur et al[110] observed it less frequently. Other signs like orchitis and neurological signs were slightly more in our study as compared to Mantur et al.[110]

Laboratory parameters like total leucocyte count and erythrocyte sedimentation rate were almost similar to the results observed by Savas et al.[114]
As reported elsewhere, [115] other than blood cultures and SAT, hematologic testing, such as white blood cell count and erythrocyte sedimentation have been of little value.

Standard agglutination titres were positive in all the patients. However the titres did not correlate with the type of presentation. This could probably be due to the different age, taking prior antibiotics and differing immune status of the patients. The yield of blood cultures in brucellosis ranges from 35% to 80.3%.[116, 117] We observed that blood cultures were positive in only 36.66% of our patients. This again could be due to fact that patients had received antibiotics effective against brucella organism prior to admission.

In our study neurobrucellosis was seen in 20% of the patients. Other studies have detected neurological involvement in 2% to 5% of the patients with brucellosis [118]. Meningitis is the most frequent CNS complication [119]. Musculoskeletal involvement is seen as the most frequent complication of brucellosis; however, its prevalence may vary from 0% to 70% [120].Skeletal brucellosis was seen in 20% of the patients in the present study. Endocarditis occurs in less than 2% of patients worldwide; however, in endemic areas, it may complicate 7%-10% of patients[121]. In a previous study of 530 patients with brucellosis, only 6 (1.5%) had endocarditis[47]. In the present study endocarditis was present in one patient (3.33%).The incidence of epididymo-orchitis

in brucellosis is estimated at 2%-20% [122]. Khan[123] investigated 100 patients with brucellosis in Saudi Arabia and found testicular involvement in 6%. In the present study, epididymoorchitis occurred in 3.33% of all patients with brucellosis.

In our study all the patients responded to both the drug regimens and no relapses were noted. However Savas et al[114] noted a relapse rate of five percent in their study.

No mortality was noted among the patients which in accordance with the other studies.

CONCLUSION

The present study was conducted in the KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with brucellosis and it was concluded as following;

1. Prevalence of brucellosis in admitted patients was 0.61 % in adults and 0.1 % in children.

2. Brucellosis was of acute type in 63%, sub acute in 30% and chronic in 6.6% of the patients.

3. Fever with drenching sweats remained one of the cardinal symptom of brucellosis. Other common symptoms were generalized weakness, anorexia, body ache, joint pain and headache.

4. Amongst the signs, hepatomegaly and splenomegaly were more common where as lymphadenopathy was seen in only few cases.

5. Total leucocyte counts were not much altered in majority of cases of brucellosis.

6. Brucella SAT was positive in all 30 cases, and there was no significant difference in SAT titres between acute, sub acute and chronic brucellosis.

7. Blood culture was positive in only 36.66% of the cases. Hence, it was not fruitful in the study.

8. All patients responded to either rifampicin plus doxycycline or rifampicin plus streptomycin regimen.

9. Over all prognosis was good and none of the patients expired.

10. Brucellosis should be considered as a differential diagnosis in all cases of pyrexia of unknown origin, low backache, arthralgia, sciatica and in all cases of progressive weight loss.

SUMMARY

The present study was conducted in KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 30 patients with brucellosis.

The objectives of the present study were to know the clinical features and complications of brucellosis.

All patients admitted with symptoms and signs suggestive of brucellosis were screened serologically for brucellosis by standard agglutination test. Patients who were diagnosed to have brucellosis were examined according to the performa and other relevant investigations including blood culture were carried out.

Our study revealed a prevalence of 0.61 percent in adults and 0.1 percent in children. Fever with drenching sweats remained one of the important symptoms of brucellosis. Other common symptoms were generalized weakness, anorexia, body ache, joint pain and headache. Amongst the signs, hepatomegaly and splenomegaly were more common where as lymphadenopathy was seen in only few cases. All patients responded to either of the drug regimens, namely rifampicin plus doxycycline or rifampicin plus streptomycin. Over all prognosis was good and none of the patients expired.

BIBLIOGRAPHY

1. Capasso L. Bacteria in two-millennia-old cheese, and related epizoonoses in Roman populations. J Infect 2002;45:122-7.

2. Corbel MJ. Brucellosis: an overview. Emerg Infect Dis 1997;3:213-21.

3. Young EJ. An overview of human brucellosis. Clin Infect Dis 1995; 21: 283-9.

4. Bundle DR, Cherwonogrodzky JW, Caroff M, Perry MB. The lipopolysaccharides of *Brucella abortus* and *B melitensis*. *Ann Inst Pasteur Microbiol* 1987; 138(1): 92–98.

5. Moreno E, Borowiak D, Mayer H. *Brucella* lipopolysaccharides and polysaccharides. *Ann Inst Pasteur Microbiol* 1987; 138(1): 102–105.

6. Goldstein J, Hoffman T, Frasch C. Lipopolysaccharide (LPS) from *Brucella abortus* is less toxic than that from *Escherichia coli,* suggesting the possible use of *B abortus* or LPS from *B abortus* as a carrier in vaccines. *Infect Immun* 1992; 60(4): 1385–1389.

7. Cherwonogrodzky JW, Perry MB, Bundle DR. Identification of the A and M antigens of *Brucella* as the Opolysaccharides of smooth lipopolysaccharides. *Can J Microbiol* 1987; 33(11): 979–981.

8. DelVecchio VG, Kapatral V, Redkar RJ. The genome sequence of the facultative intracellular pathogen Brucella melitensis. Proc Natl Acad Sci U S A 2002; 99: 443-8.

9. Sanchez DO, Zandomeni RO, Cravero S. Gene discovery through genomic sequencing of Brucella abortus. Infect Immun 2001; 69: 865-8.

10. Paulsen IT, Seshadri R, Nelson KE. The Brucella suis genome reveals fundamental similarities between animal and plant pathogens and symbionts. Proc Natl Acad Sci U S A 2002; 99: 13148-53.

11. Mantur BG, Akki AS, Mangalgi SS, Patil SV, Gobbur RH, Peerapur BV. Childhood brucellosis - a microbiological, epidemiological and clinical study. *J Trop Pediatr* 2004; *50*: 153-7

12. Banerjee TK, Pal AK, Das S. Neurobrucellosis presenting as acute meningoencephalitis. *Neurol India* 1999; *47*: 160.

13. Kochar DK, Agarwal N, Jain N, Sharma BV, Rastogi A, Meena CB. Clinical profile of neurobrucellosis - a report on 12 cases from Bikaner (north-west India). *J Assoc Physicians India* 2000; *48*: 376-80.

14. Kalla A, Chadda VS, Gauri LA, Gupta A, Jain S, Gupta BK, *et al*. Outbreak of polyarthritis with pyrexia in Western Rajasthan. *J Assoc Physicians India* 2001; *49*: 963-5.

15. Kadri MS, Tanvir M, Chowdhary ND, Hassan G, Gaash B. Pancytopenia in a brucellosis case investigated for pyrexia of unknown origin. *JK Practitioner* 2003;*10* : 222-3.

16. Kochar DK, Sharma BV, Gupta S, Jain R, Gauri LA, Srivastava T. Pulmonary manifestations in brucellosis: a report on seven cases from Bikaner (north-west India). *J Assoc Physicians India* 2003;*51* :33-6.

17. Chadda VS, Soni PK, Gupta A, Gupta BK, Chadda S, Nayak KC. Incidence of brucellosis in arthritis and chronic low backache pain in high risk group. *J Assoc Physicians India* 2004;*52* :338.

18. Sen MR, Shukla BN, Goyal RK. Seroprevalence of brucellosis in and around Varanasi. *J Commun Dis* 2002;*34* :226-7.

19. Kadri SM, Rukhsana A, Laharwal MA, Tanvir M. Seroprevalence of brucellosis in Kashmir (India) among patients with pyrexia of unknown origin. *J Indian Med Assoc* 2000;*98* :170-1

20. Dornand J, Gross A, Lafont V, Liautard J, Oliaro J, Liautard JP. The innate immune response against Brucella in humans. Vet Microbiol 2002; 90: 383-94.

21. Detilleux PG, Deyoe BL, Cheville NF. Effect of endocytic and metabolic inhibitors on the internalization and intracellular growth of Brucella abortus in Vero cells. Am J Vet Res 1991; 52: 1658-64.

22. Gross A, Terraza A, Ouahrani-Bettache S, Liautard JP, Dornand J. In vitro Brucella suis infection prevents the programmed cell death of human monocytic cells. Infect Immun 2000; 68: 342-51.

23. Guzman-Verri C, Manterola L, Sola-Landa A. The two-component system BvrR/BvrS essential for Brucella abortus virulence regulates the expression of outer membrane proteins with counterparts in members of the Rhizobiaceae. Proc Natl Acad Sci U S A 2002; 99: 12375-80.

24. Arenas GN, Staskevich AS, Aballay A, Mayorga LS. Intracellular trafficking of Brucella abortus in J774 macrophages. Infect Immun 2000; 68: 4255-63.

25. Pizarro-Cerda J, Moreno E, Sanguedolce V, Mege JL, Gorvel JP. Virulent Brucella abortus prevents lysosome fusion and is distributed within autophagosome-like compartments. Infect Immun 1998; 66: 2387-92.

26. Celli J, de Chastellier C, Franchini DM, Pizarro-Cerda J, Moreno E, Gorvel JP. Brucella evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum. J Exp Med 2003; 198: 545-56.

27. Gorvel JP, Moreno E. Brucella intracellular life: from invasion to intracellular replication. Vet Microbiol 2002; 90: 281-97.

28. Salmeron I, Rodriguez-Zapata M, Salmeron O, Manzano L, Vaquer S, Alvarez-Mon M. Impaired activity of natural killer cells in patients with acute brucellosis. Clin Infect Dis 1992; 15: 764-70.

29. Rodriguez-Zapata M, Reyes E, Sanchez L, Espinosa A, Solera J, Alvarez-Mon M. Defective reactive oxygen metabolite generation by macrophages from acute brucellosis patients. Infection 1997; 25: 187-8.

30. Ko J, Gendron-Fitzpatrick A, Splitter GA. Susceptibility of IFN regulatory factor-1 and IFN consensus sequence binding protein-deficient mice to brucellosis. J Immunol 2002; 168: 2433-40.

31. Bertotto A, Gerli R, Spinozzi F. Lymphocytes bearing the gamma delta T cell receptor in acute Brucella melitensis infection. Eur J Immunol 1993;23:1177-80.

32. Ottones F, Dornand J, Naroeni A, Liautard JP, Favero J. γ/ δ T cells impair intracellular multiplication of Brucella suis in autologous monocytes through

soluble factor release and contact-dependent cytotoxic effect. J Immunol 2000;165:7133-9.

33. Yingst S, Hoover DL. T cell immunity to brucellosis. Crit Rev Microbiol 2003; 29: 313-31.

34. Zhan Y, Cheers C. Endogenous gamma interferon mediates resistance to Brucella abortus infection. Infect Immun 1993; 61: 4899-901.

35. Bravo MJ, de Dios Colmenero J, Alonso A, Caballero A. Polymorphisms of the interferon gamma and interleukin 10 genes in human brucellosis. Eur J Immunogenet 2003; 30: 433-5.

36. Demirdag K, Ozden M, Kalkan A, Godekmerdan A, Sirri Kilic S. Serum cytokine levels in patients with acute brucellosis and their relation to the traditional inflammatory markers. FEMS Immunol Med Microbiol 2003; 39: 149-54.

37. Ahmed K, Al-Matrouk KA, Martinez G, Oishi K, Rotimi VO, Nagatake T. Increased serum levels of interferon-gamma and interleukin-12 during human brucellosis. Am J Trop Med Hyg 1999;61:425-7.

38. Jubier-Maurin V, Boigegrain RA, Cloeckaert A. Major outer membrane protein Omp25 of Brucella suis is involved in inhibition of tumor necrosis factor alpha production during infection of human macrophages. Infect Immun 2001;69:4823-30.

39. Zhan Y, Cheers C. Endogenous interleukin-12 is involved in resistance to Brucella abortus infection. Infect Immun 1995;63:1387-90.

40. Almuneef MA, Memish ZA, Balkhy HH. Importance of screening household members of acute brucellosis cases in endemic areas. Epidemiol Infect 2004; 132: 533-40.

41. Smart JK. History of chemical and biological warfare. In: Sidell FR, Takafuji ET, Franz DR, eds. Textbook of military medicine. Part I. Warfare, weaponry, and the casualty. Vol. 3. Medical aspects of chemical and biological warfare. Washington, D.C.: Government Printing Office, 1997:9-86.

42. Kaufmann AF, Meltzer MI, Schmid GP. The economic impact of a bioterrorist attack: are prevention and postattack intervention programs justifiable? Emerg Infect Dis 1997;3:83-94.

43. Ergonul O, Celikbas A, Tezeren D, Guvener E, Dokuzoguz B. Analysis of risk factors for laboratory-acquired brucella infections. J Hosp Infect 2004;56:223-7.

44. Sohn AH, Probert WS, Glaser CA. Human neurobrucellosis with intracerebral granuloma caused by a marine mammal Brucella spp. Emerg Infect Dis 2003;9:485-8.

45. Dokuzoguz B, Ergonul O, Baykam N. Characteristics of B. melitensis versus B. abortus bacteraemias. J Infect 2005;50:41-5.

46. Bodur H, Balaban N, Aksaray S. Biotypes and antimicrobial susceptibilities of Brucella isolates. Scand J Infect Dis 2003;35:337-8.

47. Colmenero JD, Reguera JM, Martos F, Sanchez-De-Mora D, Delgado M, Causse M . Complications associated with Brucella melitensis infection: a study of 530 cases. Medicine (Baltimore) 1997; 76:139.

48. Braude. Studies in the pathology and pathogenesis of experimental brucellosis. Ibid 1951; (89)37:122.

49. Naglotimath S, Darbar R, Joglekar : Granulomatous hepatitis in brucellosis, J. of I.M.A 1979; 1: 1-4

50. Bosilkovski M, Krteva L, Caparoska S, Dimzova M. Hip arthritis in brucellosis: a study of 33 cases in the Republic of Macedonia (FYROM). Int J Clin Pract 2004;58:1023-7.

51. Ariza J, Pujol M, Valverde J. Brucellar sacroiliitis: findings in 63 episodes and current relevance. Clin Infect Dis 1993;16:761-5.

52. Solera J, Lozano E, Martinez-Alfaro E, Espinosa A, Castillejos ML, Abad L. Brucellar spondylitis: review of 35 cases and literature survey. Clin Infect Dis 1999;29:1440-9.

53. Bravo MJ, Colmenero JD, Alonso A, Caballero A. HLA-B39 allele confers susceptibility to osteoarticular complications in human brucellosis. J Rheumatol 2003; 30:1051-3.

54. Shakir RA, Al-Din AS, Araj GF, Lulu AR, Mousa AR, Saadah MA. Clinical categories of neurobrucellosis: a report on 19 cases. Brain 1987;110:213-23.

55. Yavuz T, Ozaydýn M, Ulusan V, Ocal A, Ibrisim E, Kutsal A. A case of mitral stenosis complicated with seronegative brucella endocarditis. Jpn Heart J 2004;45:353-58.

56. Caldarera I, Albanese S, Piovaccari G. Brucella endocarditis: role of drug treatment associated with surgery. Cardiologia 1996;41:465-67.

57. Erbay AR, Turhan H, Dogan M, Erbasi S, Cagli K, Sabah I. Brucella endocarditis complicated with a mycotic aneurysm of the superior mesenteric artery: a case report. Int J Cardiol 2004;93:317-19.

58. Reguera JM, Alarcon A, Miralles F, Pachon J, Juarez C, Colmenero JD. Brucella endocarditis: clinical, diagnostic, and therapeutic approach. Eur J Clin Microbiol Infect Dis 2003;22:647-50.

59. Rahman A, Burma O, Felek S, Yekeler H. Atrial septal defect presenting with brucella endocarditis. Scand J Infect Dis 2001;33:776-77.

60. Kýzýltepe U, Uysalel A, Tutar E, Akalin H. Brucella melitensis endocarditis of ventricular septal defect patch: Successful surgical treatment without replacement. J Thorac Cardiovasc Surg 2003; 125: 196-97.

61. Chevalier P, Bonnefoy E, Kirkorian G, Touboul P. Brucella pancarditis with fatal outcome. Presse Med 1996; 25:628-30

62. Ariza J, Pigrau C, Canas C. Current understanding and management of chronic hepatosplenic suppurative brucellosis. Clin Infect Dis 2001;32:1024-33.

63. Cervantes F, Bruguera M, Carbonell J, Force L, Webb S. Liver disease in brucellosis: a clinical and pathological study of 40 cases. Postgrad Med J 1982;58:346-50.

64. Akritidis N, Pappas G. Ascites caused by brucellosis: a report of two cases. Scand J Gastroenterol 2001;36:110-2.

65. Navarro-Martinez A, Solera J, Corredoira J. Epididymoorchitis due to Brucella mellitensis: a retrospective study of 59 patients. Clin Infect Dis 2001;33:2017-22.

66. Khan MY, Mah MW, Memish ZA. Brucellosis in pregnant women. Clin Infect Dis 2001;32:1172-7.

67. Pappas G, Bosilkovski M, Akritidis N, Mastora M, Krteva L, Tsianos E. Brucellosis and the respiratory system. Clin Infect Dis 2003;37:e95-e99.

68. Mazokopakis E, Christias E, Kofteridis D. Acute brucellosis presenting with erythema nodosum. Eur J Epidemiol 2003; 18: 913-915.

69. Metin A, Akdeniz H, Buzgan T, Delice I. Cutaneous findings encountered in brucellosis and review of the literature. Int J Dermatol 2001; 40: 434-438

70. Memish Z, Mah MW, Al Mahmoud S, Al Shaalan M, Khan MY. Brucella bacteraemia: clinical and laboratory observations in 160 patients. J Infect 2000;40:59-63.

71. Bannatyne RM, Jackson MC, Memish Z. Rapid diagnosis of Brucella bacteremia by using the BACTEC 9240 system.J Clin Microbiol 1997;35:2673-4.

72. Yagupsky P. Detection of Brucellae in blood cultures. J Clin Microbiol 1999;37:3437-42.

73. Gotuzzo E, Carrillo C, Guerra J, Llosa L. An evaluation of diagnostic methods for brucellosis - the value of bone marrow culture. J Infect Dis 1986;153:122-5.

74. Young EJ. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. Rev Infect Dis 1991;13:359-72.

75. Araj GF. Human brucellosis: a classical infectious disease with persistent diagnostic challenges. Clin Lab Sci 1999;12:207-12.

76. Casao MA, Smits HL, Navarro E, Solera J. Clinical utility of a dipstick assay in patients with brucellosis: correlation with the period of evolution of the disease. Clin Microbiol Infect 2003;9:301-5.

77. Al Dahouk S, Tomaso H, Nockler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis - a review of the literature. Part II: serological tests for brucellosis. Clin Lab 2003;49:577-89.

78. Almuneef M, Memish ZA. Prevalence of Brucella antibodies after acute brucellosis. J Chemother 2003;15:148-51.

79. Araj GF, Lulu AR, Khateeb MI, Saadah MA, Shakir RA. ELISA versus routine tests in the diagnosis of patients with systemic and neurobrucellosis. APMIS 1988;96:171-6.

80. Ariza J, Pellicer T, Pallares R, Foz A, Gudiol F. Specific antibody profile in human brucellosis. Clin Infect Dis 1992;14:131-40.

81. Fekete A, Bantle JA, Halling SM, Sanborn MR. Preliminary development of a diagnostic test for Brucella using polymerase chain reaction. J Appl Bacteriol 1990;69:216-27.

82. Romero C, Gamazo C, Pardo M, Lopez-Goni I. Specific detection of Brucella DNA by PCR. J Clin Microbiol 1995;33:615-7.

83. Nimri LF. Diagnosis of recent and relapsed cases of human brucellosis by PCR assay. BMC Infect Dis 2003;3:5.

84. Baily GG, Krahn JB, Drasar BS, Stoker NG. Detection of Brucella melitensis and Brucella abortus by DNA amplification. J Trop Med Hyg 1992;95:271-5.

85. Morata P, Queipo-Ortuno MI, Reguera JM, Garcia-Ordonez MA, Pichardo C, Colmenero JD. Posttreatment follow-up of brucellosis by PCR assay. J Clin Microbiol 1999;37:4163-6.

86. Navarro E, Escribano J, Fernandez J, Solera J. Comparison of three different PCR methods for detection of Brucella spp in human blood samples. FEMS Immunol Med Microbiol 2002;34:147-51.

87. Matar GM, Khneisser IA, Abdelnoor AM. Rapid laboratory confirmation of human brucellosis by PCR analysis of a target sequence on the 31-kilodalton Brucella antigen DNA. J Clin Microbiol 1996;34:477-8.

88. Redkar R, Rose S, Bricker B, DelVecchio V. Real-time detection of Brucella abortus, Brucella melitensis and Brucella suis. Mol Cell Probes 2001;15:43-52.

89. Probert WS, Schrader KN, Khuong NY, Bystrom SL, Graves MH. Real-time multiplex PCR assay for detection of Brucella spp., B. abortus, and B. melitensis. J Clin Microbiol 2004;42:1290-3.

90. Queipo-Ortuno MI, Colmenero JD, Baeza G, Morata P. Comparison between LightCycler Real-Time Polymerase Chain Reaction (PCR) assay with serum and PCR-enzyme-linked immunosorbent assay with whole blood samples for the diagnosis of human brucellosis. Clin Infect Dis 2005;40:260-4.

91. Vrioni G, Gartzonika C, Kostoula A, Boboyianni C, Papadopoulou C, Levidiotou S. Application of a polymerase chain reaction enzyme immunoassay in peripheral whole blood and serum specimens for diagnosis of acute human brucellosis. Eur J Clin Microbiol Infect Dis 2004;23:194-9.

92. Morata P, Queipo-Ortuno MI, Reguera JM, Garcia-Ordonez MA, Cardenas A, Colmenero JD. Development and evaluation of a PCR-enzyme-linked immunosorbent assay for diagnosis of human brucellosis. J Clin Microbiol 2003;41:144-8.

93. McDonald R, Cao T, Borschel R. Multiplexing for the detection of multiple biowarfare agents shows promise in the field. Mil Med 2001;166:237-9.

94. Navarro E, Casao MA, Solera J. Diagnosis of human brucellosis using PCR. Expert Rev Mol Diagn 2004; 4:115-23.

95. Solera J, Geijo P, Largo J. A randomized, double-blind study to assess the optimal duration of doxycycline treatment for human brucellosis. Clin Infect Dis 2004;39:1776-82.

96. Pappas G, Akritidis N, Tsianos E. Effective treatments in the management of brucellosis. Expert Opin Pharmacother 2005;2:201-9.

97. Solera J, Martinez-Alfaro E, Saez L. Meta-analysis of the efficacy of rifampicin and doxycycline in the treatment of human brucellosis. Med Clin (Barc) 1994;102:731-8. (In Spanish.)

98. Colmenero JD, Fernandez-Gallardo LC, Agundez JA, Sedeno J, Benitez J, Valverde E. Possible implications of doxycycline-rifampin interaction for treatment of brucellosis. Antimicrob Agents Chemother 1994;38:2798-802.

99. Solera J, Martinez-Alfaro E, Espinosa A. Recognition and optimum treatment of brucellosis. Drugs 1997;53:245-56.

100. Karabay O, Sencan I, Kayas D, Sahin I. Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis: a randomized clinical trial [ISRCTN11871179]. BMC Infect Dis 2004;4:18.

101. Lopez-Merino A, Contreras-Rodriguez A, Migranas-Ortiz R. Susceptibility of Mexican brucella isolates to moxifloxacin, ciprofloxacin and other antimicrobials used in the treatment of human brucellosis. Scand J Infect Dis 2004;36:636-8.

102. Solera J, Beato JL, Martinez-Alfaro E, Segura JC, de Tomas E. Azithromycin and gentamicin therapy for the treatment of humans with brucellosis. Clin Infect Dis 2001;32:506-9.

103. McLean DR, Russell N, Khan MY. Neurobrucellosis: clinical and therapeutic features. Clin Infect Dis 1992;15:582-90.

104. Pappas G, Seitaridis S, Akritidis N, Tsianos E. Treatment of brucella spondylitis: lessons from an impossible meta-analysis and initial report of efficacy of a fluoroquinolone-containing regimen. Int J Antimicrob Agents 2004;24:502-7.

105. Lubani MM, Dudin KI, Sharda DC. A multicenter therapeutic study of 1100 children with brucellosis. Pediatr Infect Dis J 1989;8:75-8.

106. Corbel MJ. Vaccines against bacterial zoonoses.J Med Microbiol 1997;46:267–9.

107. Cieslak TJ, Christopher GW, Kortepeter MG. Immunization against potential biological warfare agents. Clin Infect Dis 2000;30:843–50.

108. Nicoletti P. Immune responses and vaccination. In: Madkour MM, editor. Brucellosis. London: Butterworths, 1989:263–9.

109. Abramson O, Rosenvasser Z, Block C. Detection and treatment of brucellosis by screening a population at risk. Pediatr Infect Dis J. 1991;10(6):434–438.

110. Mantur BG, Biradar MS, Bidri RC, Mulimani MS, Verappa K, Kariholu P, et al. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. Journal of Microbiology 2006; 55: 897-903.

111. Karabay O, Serin E, Tamer A, Gokdogan F, Alpteker H, Ozcan A & Gunduz H. Hepatitis B carriage and Brucella seroprevalence in urban and rural areas of Bolu province of Turkey: a prospective epidemiological study. Turk J Gastroenterol 2004; 15: 11–13.

112. Sumer H, Sumer Z, Alim A, Nur N & Ozdemir L. Seroprevalence of Brucella in an elderly population in Mid-Anatolia, Turkey. J Health Popul Nutr; 2003: 21, 158–161.

113. Al-Sekait MA. Seroepidemiological survey of brucellosis antibodies in Saudi Arabia. Ann Saudi Med 1999;19: 219–222.

114. Savas L, Onlen Y, Savas N, Yapar AF, Aydin M and Tugal O. Infect Dis Clin Pract 2007; 15: 83–88.

115. Young EJ. Brucella species. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Philadelphia: Churchill Livingstone; 2000:2386–2393.

116. Namıduru M, Gungor K, Dikensoy O. Epidemiological, clinical and laboratory features of brucellosis: a prospective evaluation of 120 adult patients. J Clin Pract. 2003;57:20–24

117. Al-Eissa YA, Kambal AM, Alrabeeah AA. Osteoarticular brucellosis in children. Ann Rheum Dis. 1990;49:896–900

118. Bellissima P, Turturici MA. Neurobrucellosis: clinical and therapeutic features. Infez Med 1998; 6: 25-30.

119. Gür A, Geyik MF, Dikici B, Nas K, Çevik R, Saraç J et al. Complications of brucellosis in different age groups: a study of 283 cases in southeastern Anatolia of Turkey. Yonsei Med J 2003; 44: 33-44.

120. Geyik MF, Gür A, Nas K, Çevik R, Saraç J, Dikici B et al. Musculoskeletal involvement in brucellosis in different age groups: a study of 195 cases. Swiss Med Wkly 2002; 132: 98–105.

121. Reguera JM, Alarcon A, Miralles F, Pachon J, Juarez C, Colmenero JD. Brucella endocarditis: clinical, diagnostic, and therapeutic approach. Eur J Clin Microbiol Infect Dis 2003; 22: 647-650.

122. Navarro-Martinez A, Solera J, Corredoira J, Beato JL, Martinez- Alfaro E, Atienzar M et al. Epididymoorchitis due to Brucella melitensis: a retrospective study of 59 patients. Clin Infect Dis 2001; 33: 2017-2022.

123. Khan MY. Brucellosis: observations on 100 patients. Ann Saudi Med 1986; 6: 519–523.

ANNEXURE I – CONSENT FORM

Objective and Purpose of the study

This is a study of clinical profile of Brucellosis. The principal investigator of the study is Dr. A.J. Dhumale and the co-investigator is Dr. Vikrant B Ghatnatti. This research is intended to study the clinical profile of Brucellosis and its complications and my co-operation will be of great help to the patients of Brucellosis in future.

Procedure

If I agree to be a part of the study I will be asked the relevant history and will be subjected to relevant clinical examination and biochemical investigations.

Risk and Benefit

The only risk and possible discomfort I might get while taking blood from my arm, it may cause swelling, pain, redness, bruising or infection (rarely happens) at the site where needle is inserted and headache following lumbar puncture if it is done.

Alternatives

Taking part in this study is voluntary. I may choose not to take part in this study, or if I decide to take part I can later change my mind and withdraw from the study. My decision will not change the present or future health care or other services that I receive. The study doctor or sponsor may stop my participation in this study any time. If I choose not to take part in the study I will receive standard treatment for patients with my condition.

Privacy and Confidentiality

All information collected about me during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify me in this research record.

Institutional / Sponsors Policy

Does not apply to this research.

Financial Incentives for Participation

I will not be charged any amount for the investigations subjected to me. I will not receive compensation or reimbursement for taking part in this study.

Authorization to Publish Results

Information from this study may be published but my identity will be confidential in any publication.

Consent Statement

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form, and have had all my questions answered.

Name of Study Participant or legally authorized representative:

Signature / Thumb Print

In case of the queries during study or in future I may contact following person

Principal investigator	: Dr. A.J.Dhumale.	Phone : 94805 37477
Co-Investigator	: Dr. Vikrant B Ghatnatti	Phone : 98444 45598

Name of Witness	:	Signature	:
Investigator Name	:	Signature	:
Date	:	Place	:

ANNEXURE II – PROFORMA

I. Patient Identification

Name: Age: Sex:

Occupation: Marital status: I.P Number:

D.O.A.: D.O.D.:

Address: Provisional diagnosis:

Final diagnosis**:**

I. Symptoms

Symptoms	Present	Absent	Duration
1. Fever with chills			
Sweating			
Rigors			
2. Joint pain			
Major			
Minor			
3. Low back ache			
4. Body ache			
5. Gen. weakness			
6. Anorexia			
7. Loss of weight			
8. Pain abdomen			

9. Vomiting
10. Diarrhea
11. Constipation
12. Headache
13. Visual disturbance
14. Tingling & numbness in both LL & UL
15. Insomnia
16. Cough.
17. Menstrual disturbance.
18. Genitourinary disturbance.
19. Others.

Occupational history:

Farmer |Butcher |Veterinary Surgeon |Dairy worker |

Agricultural |Engineer |Other.|

H|O Animal contact: Yes |No

Goat |Sheep |Cattle |Hog s.

H|O Consumption of raw milk: Present | Absent.

H|O Consumption of mild products: Present | Absent

II. PAST HISTORY:

H|O similar complaints in the past. (present | absent)

H|O TB|HT|DM

Others

III. FAMILY HISTORY:

H|O similar complaints in family (Present | Absent).

IV. General Physical Examination

Built :

Nourishment :

Pallor :

Icterus :

Lymph nodes :

Temperature :

Pulse :

Blood pressure :

Respiratory rate :

Skin eruptions :

V. Per Abdomen

VI. Respiratory system

VII. Cardiovascular system

VIII. Central nervous system

IX. Investigations

Hemoglobin :

Total leukocyte count :

Differential count :

ESR :

Random blood sugar :

Chest X-ray :

Others :

X. Serology

SAT titres :

2 ME titres :

XI. Blood culture :

XII. Other Investigations :

ANNEXURE III – PHOTOGRAPHS

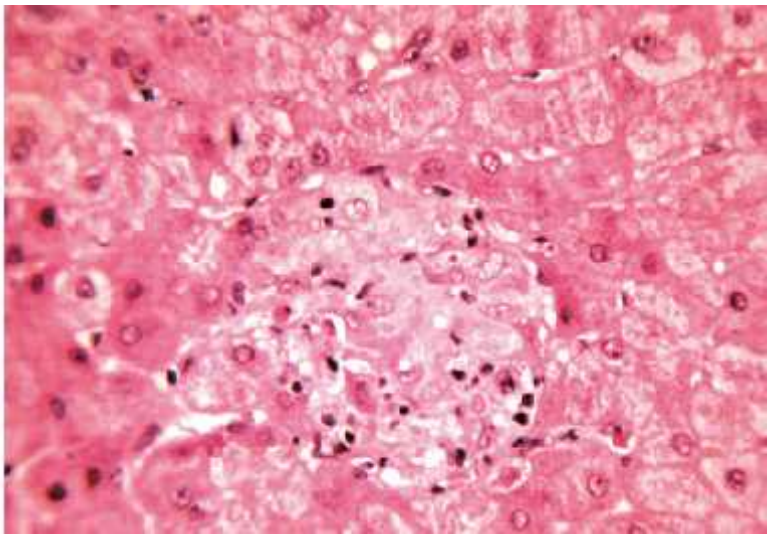


Figure No. 4: Liver biopsy specimen from a patient with brucellosis shows a non caseating granuloma

Figure No. 5: Photograph of David Bruce

ANNEXURE-IV

ANNEXURE IV – KEY TO MASTER CHART

+	-	Present
-	-	Absent
✓	-	Present
Arth	-	Arthralgia
Ac	-	Acute
B	-	Basophil
BC	-	Blood culture
BR	-	Butcher
BS	-	Businessman
Con	-	Contact with animals
Cons	-	Constitutional symptoms
CNS	-	Central nervous system
CSF	-	Cerebrospinal fluid
DC	-	Differential count
d	-	Days
E	-	Eosinophil
ESR	-	Erythrocyte Sedimentation Rate
F	-	Female
FA	-	Farmer
H	-	Hepatomegaly
HS	-	Hepatosplenomegaly
Hb	-	Hemoglobin

HW	-	House wife
IE	-	Infective endocarditis
IP. No.	-	In Patient Number
L	-	Lymphocytes
(L)	-	Left
Ln	-	Lymphadenopathy
LOA	-	Loss of appetite
M	-	Male
M.	-	Monocytes
mth	-	Months
N	-	Neutrophils
Orc	-	Orchitis
Ost	-	Osteoarticular
R	-	Rural
(R)	-	Right
S	-	Splenomegaly
SAT	-	Standard agglutination test
S. No.	-	Serial Number
SH	-	Shepherd
ST	-	Student
TLC	-	Total leucocyte count
U	-	Urban
VT	-	Veterinarian
Wt	-	Weight

S. No.	IP No.	Age	Sex	Occ	U/R	Con	Raw Milk	Diagnosis	Fever	Sweating	Arth	Headache	Back ache	Myalgia	coug/ dysp	Wt loss	LOA	Duration	HS	H	S	Ost	Ln	CNS	Orc	Hb	ESR	TLC	DC					Titre	BC	Special Inv.
																													N	L	M.	B	E			
1	174139	50	F	HW	R	+	-	Ac brucellosis	✓	✓				✓			✓	30 d		✓						10.5	24	5800	52	46	2	0	0	640	-	Liver biopsy
2	174934	32	M	FA	R	+	-	(L) sacroilitis	✓	✓	✓			✓				30 d				✓				13.5	12	7450	68	28	0	0	4	320	-	
3	179449	24	M	FA	R	+	-	L3 radiculopatly				✓	✓				✓	45 d								11.2	25	9850	77	20	0	0	3	640	+	
4	180250	45	F	HW	R	+	-	T12 transv myelopathy	✓				✓				✓	15 d						✓		10.5	28	6850	65	32	1	0	2	1280	+	
5	188036	31	M	FA	R	+	+	Polyarthitis			✓			✓				6 mth				✓				10.8	36	8900	60	35	1	0	4	1280	-	
6	188805	10	M	ST	R	+	+	(L) Elbow arthritis	✓	✓	✓			✓				6 mth	✓			✓				11.6	42	6600	52	39	8	0	1	160	+	
7	190296	20	M	FA	R	+	-	(L) Sacroilitis	✓		✓		✓	✓				7 d	✓			✓				12	20	####	71	27	0	0	2	160	-	
8	191437	17	M	SH	R	+	+	Chronic brucellosis	✓	✓						✓		18 mth			✓		✓	✓		11	38	7150	56	40	2	0	2	160	-	
9	205508	20	M	ST	R	+	-	(R) Sacioilitis	✓		✓	✓		✓				10 d		✓		✓				9	95	####	80	20	0	0	0	1280	-	
10	215477	32	M	FA	R	+	+	Br. Epididymoorchitis	✓	✓		✓		✓				1 mth					✓		✓	10	36	####	54	40	2	0	4	2560	-	
11	2207555	45	M	BS	U	-	+	C8 T1 radiculopathy										20 d			✓					14.1	12	7900	50	38	8	0	4	160	-	
12	222660	56	M	FA	R	+	-	Ac Brucellosis	✓	✓			✓					1 mth	✓							12	36	6500	40	54	2	0	4	640	+	
13	222896	28	M	FA	R	+	+	IE sec to brucellosis	✓	✓	✓	✓		✓	✓	✓	✓	12 mth			✓					8.5	41	8000	49	44	2	0	5	640	+	
14	223165	48	F	HW	R	+	+	Ac Brucellosis	✓	✓		✓					✓	15 d	✓							8.7	98	5500	38	54	8	0	0	2560	+	
15	227393	50	M	FA	R	+	-	Ac Brucellosis	✓	✓						✓	✓	2 mth			✓		✓			8	105	3900	62	34	0	0	4	640	-	

S. No.	IP No.	Age	Sex	Occ	U/R	Con	Raw Milk	Diagnosis	Fever	Sweating	Arth	Headache	Backache	Myalgia	coug/dysp	Wt loss	LOA	Duration	HS	H	S	Ost	Ln	CNS	Orc	Hb	ESR	TLC	DC					Titre	BC	Special Inv.
																													N	L	M.	B	E			
16	2308799	23	M	ST	R	+	-	Chronic Meningitis	✓	✓		✓	✓	✓			✓	7 mth			✓			✓		13	38	7800	65	30	0	0	5	160	-	CSF-SAT+
17	231826	21	M	BR	U	-	-	(R) sacroilitis	✓	✓	✓		✓	✓	✓	✓	✓	2 mth		✓		✓				13.9	18	8500	58	32	9	0	1	1280	+	
18	232781	50	M	FA	R	+	-	Ac Brucellosis	✓		✓	✓		✓				1 mth			✓		✓			12.7	38	3380	43	48	9	0	0	320	-	
19	257559	22	M	BR	U	-	-	Ac Brucellosis	✓	✓	✓		✓					15 d	✓							10	42	6800	50	40	8	0	2	320	-	
20	255425	50	M	VT	U	+	-	Ac Brucellosis	✓	✓				✓			✓	8 d								13.8	40	7660	53	38	7	0	2	160	-	rashes (+)
21	258291	35	F	FA	R	+	-	Ac Brucellosis	✓				✓				✓	10 d	✓							10	48	7850	62	34	1	0	3	640	-	
22	259956	38	M	FA	R	+	-	L5 S1 radiculopathy	✓	✓			✓			✓		2 mth			✓				✓	10.4	36	4300	45	43	8	0	4	640	-	
23	260772	22	M	SH	R	+	-	Chronic meningitis	✓	✓	✓		✓	✓			✓	2 mth						✓		8.7	40	7400	50	38	7	0	5	640	+	
24	260975	14	M	ST	R	+	-	Ac Brucellosis	✓	✓	✓	✓	✓				✓	10 d	✓							7.8	32	6500	35	60	1	0	4	320	-	
25	263402	8	F	ST	R	+	+	Brucella arthritis	✓		✓							10 d								7.7	46	8400	66	25	8	0	1	5120	+	
26	263495	8	F	ST	R	+	+	Ac Brucellosis	✓			✓					✓	20 d	✓							10	45	6600	42	58	0	0	0	1280	-	
27	269522	25	M	FA	R	+	-	Ac Brucellosis	✓	✓	✓		✓	✓			✓	5 d								7.4	75	4500	52	48	0	0	0	10,240	-	
28	271421	17	M	ST	R	+	-	Chronic Brucellosis	✓	✓	✓	✓	✓	✓				5 d				✓				10.3	48	6400	51	39	10	0	0	640	+	
29	273008	55	F	HW	R	+	-	Ac Brucellosis	✓	✓	✓		✓				✓	2 mth								12.5	15	8800	53	38	4	0	4	160	-	rashes (+)
30	274214	35	M	FA	R	+		Ac Brucellosis	✓	✓	✓						✓	1 mth		✓		✓				8	76	4400	56	39	2	0	3	320	+	