



सत्यमेव जयते

Ministry of Health & Family Welfare
Government of India

A Comprehensive Learning Guide

Brucellosis



**NATIONAL CENTRE FOR DISEASE CONTROL
DIRECTORATE GENERAL OF HEALTH SERVICES**
Ministry of Health & Family Welfare, Government of India



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National Centre for Disease Control
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भारत सरकार
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स्वास्थ्य सेवा महानिदेशालय

Government of India
Ministry of Health & Family Welfare
Directorate General of Health Services



Message

Zoonotic diseases pose a significant and ongoing threat to biodiversity, human and public health and economic stability. Given India's diverse ecosystem, high population density and increasing human-animal action, the country remains particularly susceptible to zoonotic outbreaks. Past occurrences of plague, Nipah virus, avian influenza, leptospirosis, and Kyasanur Forest Disease emphasizes a critical need for robust surveillance, early detection, and a coordinated, intersectoral response.

Recognizing the importance of addressing zoonotic diseases as a public health priority, the Centre for One Health, under the National Centre for Disease Control (NCDC), is committed for strengthening surveillance, prevention, and response mechanisms through an integrated One Health approach. As part of this effort, the Centre for One Health has developed comprehensive learning guides on Priority Zoonotic Diseases. These modules provide scientifically validated, evidence-based guidance on the epidemiology, diagnosis, treatment, and prevention of key zoonotic diseases.

Strengthening disease surveillance, ensuring early diagnosis, and implementing timely interventions through a coordinated and multi-sectoral approach are critical to mitigating the risks associated with zoonotic diseases.

I am pleased to release these learning guides as a significant step in our ongoing efforts to combat zoonotic diseases. I urge all healthcare professionals, veterinary experts, and community leaders to actively utilize these resources to enhance their clinical index of suspicion for zoonosis and eventually contribute towards strengthening our health care system for responding timely and more efficiently to future outbreaks.

(Atul Goel)



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MESSAGE

It is my pleasure to know that the Centre for One Health at NCDC has prepared **Comprehensive Learning Guides** on 10 priority zoonotic diseases, which is now being shared with all concerned.

These resources become relevant in considering; India has encountered multiple zoonotic disease outbreaks in recent years, highlighting their persistent and evolving threat to public health. The re-emergence of **Nipah virus** in Kerala, **Kyasanur Forest Disease (KFD)** in Karnataka, and **avian influenza** across several states underscores the complex interconnections between human, animal, and environmental health. The rising cases of **leptospirosis** and **scrub typhus**, driven by climatic changes and ecological disruptions, further illustrate the unpredictable nature of zoonotic spillovers. With the additional challenges posed by **emerging coronaviruses** and **antimicrobial resistance (AMR)**, there is an urgent need for a **cohesive, evidence-based approach** to mitigate these risks effectively.

In recognition of this pressing need, the **National Centre for Disease Control (NCDC)** has intensified its efforts to fortify **zoonotic disease surveillance, outbreak preparedness, and intersectoral coordination**. As part of this initiative, the **Centre for One Health, NCDC**, has meticulously curated **Comprehensive Learning Guides** on priority zoonotic diseases. These guides, underpinned by the latest scientific insights and global best practices, serve as indispensable resources for **healthcare professionals, veterinarians, and epidemiologists**, equipping them with the knowledge to anticipate, detect, and mitigate zoonotic threats with precision.

To effectively curtail the impact of zoonotic diseases, a **harmonized One Health approach** is paramount—one that transcends disciplinary boundaries and fosters synergies across the human, animal, and environmental health sectors. I urge all stakeholders to **leverage these scholarly resources, enhance disease intelligence networks, and champion research-driven interventions**. By embracing a proactive and interdisciplinary ethos, we can **bolster pandemic preparedness, fortify national health security, and avert future spillover events**.

I sincerely **appreciate** my colleagues at the Centre for One Health, NCDC for their dedication to strengthening zoonotic disease control. Their efforts in enhancing surveillance, preparedness, and public health response are crucial in preventing and managing emerging health threats.

I am glad to see their commitment translating into evidence-based interventions and capacity-building initiatives that enhance zoonotic disease surveillance, outbreak preparedness, and overall health system resilience.

Through continuous innovation, collaboration, and unwavering dedication, we can fortify our health systems and safeguard our nation against zoonotic threats.

Wishing you all happy learning and skill enhancement wherever possible.

रंजन दास 19/3/25
Prof. (Dr.) Ranjan Das



Antibiotic resistance Containment Stewardship: Our Role, Our Responsibility
Judicious Use of Antibiotic: Key to Contain Antibiotic Resistance





भारत सरकार

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Message

Zoonotic diseases of public health importance continue to pose significant challenges to public health. These diseases, often driven by intricate human-animal-environment interactions, present substantial health risks, particularly to vulnerable populations, and demand a coordinated and robust response from the health sector.

In recognition of the pivotal role healthcare providers play in addressing these challenges, the **Centre for One Health** has developed a **Comprehensive Learning Guide for Priority Zoonotic Diseases**. This guide is designed to build capacity across the health sector, equipping professionals with the essential knowledge and skills needed to identify, manage, and prevent these diseases effectively. By enhancing surveillance systems, fostering rapid and accurate diagnostics, and implementing targeted prevention and control measures, healthcare providers can play a transformative role in mitigating the impact of zoonotic diseases.

The guide emphasizes the importance of building a resilient health workforce capable of responding to outbreaks, engaging communities, and collaborating across disciplines. Through these efforts, we aim to reduce the burden of zoonotic diseases, improve health outcomes, and contribute to the creation of a safer and healthier future.

I encourage all healthcare professionals to utilize this guide as a resource for improving their understanding and response to zoonotic diseases. Together, let us strengthen our health systems and work towards a future free from the threats posed by zoonotic diseases.

Dr. Simmi Tiwari
Joint Director and Head
Centre for One Health



Antibiotic Resistance Containment Stewardship: Our Role, Our Responsibility
Judicious Use of Antibiotic: Key to Contain Antibiotic Resistance



Acknowledgement

The **Comprehensive Learning Guide** for Brucellosis has been developed to address the significant public health challenges posed by zoonotic diseases, which have serious health, economic, and social implications, particularly for vulnerable populations. The priority zoonotic diseases necessitate robust surveillance, timely diagnosis, and effective prevention and control measures. This guide aims to strengthen the capacity of healthcare professionals by equipping them with the requisite knowledge and skills to mitigate these public health threats and improve health outcomes.

We acknowledge with deep appreciation the valuable contributions of the **subject matter experts** from leading medical institutions and research organizations, whose guidance and technical expertise has been critical in shaping this **Comprehensive Learning Guide**.

The Centre for One Health, NCDC, expresses its sincere gratitude to Ms. Punya Salila Srivastava, Secretary, Health and Family Welfare, Government of India; Ms. L.S. Changsan, Additional Secretary, Ministry of Health and Family Welfare, Government of India; Prof. (Dr.) Atul Goel, Director General of Health Services (DGHS), Government of India; Ms. Latha Ganapathy, Joint Secretary, Department of Health & Family Welfare, Government of India for their leadership, guidance, and support.

Lastly, we acknowledge the USAID-funded RISE project team, implemented by Jhpiego, for their technical support in drafting and finalizing this guide. Their collaboration has been pivotal in ensuring the quality and relevance of this resource.

Dr. Simmi Tiwari
Joint Director and Head
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National Centre for Disease Control
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Preface

Brucellosis is a highly contagious zoonotic disease with significant morbidity and potential for causing lifetime sterility. The training module aims to equip learners with the knowledge and tools necessary to educate and train individuals involved in various aspects of Brucellosis management. It provides a comprehensive overview of the disease, covering its epidemiology, clinical presentation, laboratory diagnosis, treatment availability, and preventive measures. The module is structured in a user-friendly manner, allowing learners to effectively communicate complex scientific concepts to their audience. It contains detailed explanations to enhance understanding and engagement. The content is supported by the latest scientific research and public health guidelines, ensuring the information presented is accurate and up to date.

It is our sincere hope that this module will serve as a valuable resource for learners and educators, enabling them to grasp essential knowledge and skills. By equipping professionals and individuals at risk with the necessary information, we can enhance our collective ability to detect, prevent, and respond to Brucellosis outbreaks, thereby safeguarding public health.

We would like to express our gratitude to all the experts, researchers, and contributors who have provided their invaluable insights and expertise in developing this scientific module. Their dedication and commitment to public health have been instrumental in creating a comprehensive resource that will contribute to the effective management of Brucellosis.

We hope you find this module informative, engaging, and ultimately, empowering. Together, let us work towards building a safer and healthier future by combating Brucellosis through knowledge and preparedness.

Abbreviations

ABSL	Animal Biosafety Level
BCG	Bacille Calmette-Guérin
BSL	Biosafety Level
CBC	Complete Blood Count
CO₂	Carbon Dioxide
CRP	C-Reactive Protein
DIVA	Differentiating Infected from Vaccinated Animals
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immuno-sorbent Assay
ESR	Erythrocyte Sedimentation Rate
FA	Fatty Acid
FMD	Foot and Mouth Disease
HCP	Health Care Provider
ICAR-NIVEDI	Indian Council of Agricultural Research - National Institute of Veterinary Epidemiology and Disease Informatics
IDSP	Integrated Disease Surveillance Program
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INAPH	Information Network for Animal Productivity and Health
LDH	Lactate Dehydrogenase
LPS	Lipopolysaccharide
MRT	Milk Ring Test
NADCP	National Animal Disease Control Programme
NAAT	Nucleic Acid Amplification Test
PCR	Polymerase Chain Reaction
PEP	Post-Exposure Prophylaxis
RB51	Rough Brucella abortus Strain 51
RBT	Rose Bengal Test
RNA	Ribonucleic Acid
SOP	Standard Operating Procedure

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Introduction to Learning Guide for Brucellosis

Historically, zoonotic diseases have had a significant impact on human evolution, particularly on cultures and societies that domesticated and raised animals for food and clothing. Zoonoses pose a frequent and feared risk to humanity, transcending natural boundaries and occurring worldwide. Their profound effect on the global economy and health is well recognized, ranging from the international movement of animals and the importation of diseases to bans on animal product imports and restrictions on other international trade practices. Thus, zoonoses are no longer confined to national borders, and global surveillance is necessary for effective control.

Controlling zoonoses remains a priority for international agencies, addressing the specific health and economic challenges of each region. They equally impact animal, human, and environmental sectors, causing substantial damage to the nation's economy. To protect all the sectors, it is essential to rapidly identify, diagnose, and manage these diseases. The One Health approach plays a crucial role in this endeavor, as we have learned valuable lessons from the COVID-19 pandemic regarding the need for strong surveillance and diagnostic preparedness to handle future pandemic situations.

To provide comprehensive training, the Center for One Health, NCDC has developed Learning Guide. These guides serve as both trainers' guides and self-directed learning resources. The training focuses on zoonoses and zoonotic diseases of public health importance in India (ZPHI) and emphasizes the One Health approach to responding to zoonotic diseases. By the end of the training, participants should have a solid understanding of the core principles of Brucellosis, its outbreak potential, health emergencies, risk communication, diagnostic procedures, laboratory safety, surveillance, data reporting, and their applications.

The guide is based on national and international guidelines and serve as a comprehensive training resource for individuals involved in zoonotic disease diagnosis and surveillance activities. The curriculum is designed to enable facilitators and learners to undergo step-by-step learning within a specified time frame, ensuring a thorough understanding of each module. The guide caters to various audiences, including medical professionals including doctors, veterinarians, paramedical staff and laboratory personnel who are expected to acquire knowledge and competencies beyond their regular field of work.

The guide consists of a Facilitators' Guide (FG) which provides instructional processes and supports educators in facilitating and assessing learning. Each module follows a standardized structure, including a session outline, detailed session plans, slide decks, and a wide range of references and resources for further reading. These resources offer comprehensive information on the respective topics.

The learning guide aims to provide a comprehensive education for medical professionals including doctors, veterinarians, paramedical staff and laboratory personnel. It helps them understand, identify, and differentiate between zoonotic diseases and equips them with the necessary knowledge and orientation for diagnostics procedures and surveillance systems.

COURSE DESCRIPTION

Course Design

This comprehensive Brucellosis guide tailored for medical professionals including doctors, veterinarians, paramedical staff and laboratory personnel. The course is designed to enhance their understanding and expertise in dealing with Brucellosis cases, spanning various aspects of prevention, diagnosis, and treatment. The training approach emphasizes practical application, aiming to equip participants with hands-on skills and competencies that can be directly applied in their professional roles.

Evaluation Approach

The evaluation approach for the Brucellosis preparedness and response guide includes a comprehensive pre-training and post-training quiz questionnaire designed to assess participants' knowledge and learning progression. The questionnaire utilizes multiple-choice questions (MCQs) to gauge participants' understanding of Brucellosis related concepts before and after the training. This approach allows for a clear measurement of knowledge enhancement and provides valuable insights into the effectiveness of the training program.

- ***Pre-Training Quiz:***

Before the training begins, participants will be administered a pre-training questionnaire. This questionnaire serves as a baseline assessment of participants' existing knowledge and awareness of Brucellosis. The pre-training quiz will consist of MCQs that cover various aspects of Brucellosis, including its etiology, modes of transmission, clinical manifestations, prevention measures, and response strategies. The purpose of the pre-training quiz is to establish a starting point and identify areas where participants may require more focused training.

- ***Post-Training Quiz:***

Upon completion of the Brucellosis preparedness and response training guide, participants will be given a post-training quiz questionnaire. This questionnaire will contain a similar set of MCQs as the pre-training quiz, covering the same topics related to Brucellosis. The aim of the post-training quiz is to evaluate participants' knowledge advancement and to measure the effectiveness of the training in enhancing their understanding of Brucellosis Virus-related concepts and procedures.

COURSE SYLLABUS

Course Goals

Foster an in-depth comprehension of Brucellosis and its diverse forms.

- Equip participants with skills for Brucellosis diagnosis, management, and prevention.
- Emphasize the importance of the One Health approach in addressing zoonotic diseases.
- Develop proficiency in risk communication, laboratory safety, and surveillance strategies.

Participant's Learning Objectives

By the course's conclusion, participants will be able to:

- Identify varied clinical manifestations of Brucellosis.
- Apply diagnostic and treatment protocols for Brucellosis management.
- Implement preventive strategies and outbreak management tactics.

Participant Selection Criteria

Participants for this Brucellosis response and preparedness training should be professionals from various fields involved in emergency response and public health:

- Emergency Responders: Including public health experts, medical personnel, and first responders.
- Laboratory and Biosafety Staff: Those experienced in handling biohazards.
- Government and Decision-Makers: Officials with authority in emergency coordination.
- Community Leaders and Experts: Community influencers, researchers, and mental health specialists.

Using the Resource Guide

All training activities in this course will be conducted in an interactive, participatory manner using the training guide appropriately as suggested in the course outline in the facilitators' guide. To accomplish this, the trainer will have to change roles throughout the course. For example, the trainer is a facilitator when conducting small group discussions and finally, when objectively assessing performance, the trainer serves as an evaluator.

Composition of participants

The training is intended for 15-20 participants hailing from various state and healthcare fraternities. There can be a single facilitator as an expert of the disease.

COURSE SCHEDULE

Morning Session

Introduce and welcome participants

- Discuss participant expectations and prior knowledge
- Outline the objectives of the course and learning outcomes

Review the course schedule and its components

- Provide an overview of the training materials.
- Conduct a pre-course knowledge assessment to gauge participants' baseline understanding.
- Distribute course materials, including handouts and resources.

Interactive presentations

- Take Sessions 1-4 and discuss in detail about basics of Brucellosis, its epidemiology, clinical disease and its significant features in humans and animals.
- Interactive discussion and Q&A session after the lecture

Afternoon Session

Interactive presentations

- Take Sessions 5-10 and discuss in detail about frequently asked questions, diagnosis of Brucellosis, its treatment, prevention & control, outbreak management and surveillance.

Debriefing and group discussions on simulation outcomes

- Interactive discussion and Q&A session after the lecture.

Review, Assessment, and Closing

- Comprehensive review of key concepts covered during the training.
- Interactive quiz to reinforce learning.
- Final assessment to measure participants' understanding of course material.
- Reflection session: Participants share insights and takeaways from the course.
- Closing remarks, thanking participants for their engagement.

COURSE OUTLINE

The course outline presented here follows a model structure for the training curriculum. It delineates the enabling objectives necessary to achieve the participant learning goals outlined in the course syllabus. The outline offers recommendations for suitable learning activities and essential resources and materials for each enabling objective. Depending on specific participant requirements, the trainer has the option to introduce supplementary practice activities, devise case studies, role-play scenarios, or tailor learning situations accordingly.

The course outline comprises four columns:

- **Duration:** This column specifies the estimated time allocated for each learning activity. This allocation ensures an equitable distribution of time across the diverse training components.
- **Learning Objectives:** This column lays out the enabling objectives and their alignment with corresponding learning activities. The objectives are sequenced to provide guidance on the training structure. The interplay of objectives and activities—ranging from icebreakers and interactive group tasks to practical exercises and breaks—constitutes the overall training progression.
- **Pre-requisite Knowledge/Skill:** This column addresses the foundational knowledge or skills that participants should possess prior to the session. It underscores the importance of existing competencies for effective engagement in the training.
- **Competencies to be Achieved:** The fourth column enumerates the competencies participants are expected to acquire by the conclusion of the session. It underscores the practical outcomes that participants will attain through engagement in the training.

AGENDA

S. No	Topic	Duration
1.	Welcome & Introduction of the training agenda and setting ground rules	15 mins
2.	Ice Breaker: Talk about yourself - Contribution to the management & control of zoonoses - Expectations from the training	30 mins
3.	Training objectives	15 mins
4.	Pre-Test Quiz	15 mins
5.	Session 1: Introduction to Brucellosis	15 mins
6.	Session 2: Epidemiology	20 mins
7.	Session 3: Brucellosis in Human	15 mins
8.	Session 4: Brucellosis in Animals	15 mins
9.	Session 5: Laboratory Diagnosis	45 mins
10.	Session 6: Management of Brucellosis	15 mins
11.	Session 7: Prevention and Control	15 mins
12.	Session 8: Outbreak Management	15 mins
13.	Session 9: Surveillance	10 mins
14.	Post - Test Quiz	20 mins
15.	Q/A session, review, and discussion	15 mins
16.	Closing remarks	05 mins

Session-1

Introduction to Brucellosis

Course No. 001	Session 1: Introduction to Brucellosis
Duration	15 minutes
Learning Objective	• Describe disease overview. • Understand causative agent and its characteristics. • Understand the disease transmission.
Pre-requisite knowledge/skill	• Basic understanding of Brucellosis and infectious disease concepts. • Familiarity with host-pathogen interactions and immune responses.
Competencies to be achieved	• Learners would gain knowledge of the basic characteristics and features of Brucellosis. • Understanding of the characteristics and causative agent.

BRUCELLOSIS: A CLOSER LOOK

Brucellosis is a highly contagious zoonotic disease associated with significant morbidity. The Centers for Disease Control and Prevention (CDC) estimates the global incidence of human brucellosis to be 1.6–2.1 million new cases annually, though this figure is likely an underestimate due to underdiagnosis and underreporting. The World Health Organization (WHO) has identified brucellosis as one of the top neglected zoonoses, underscoring its public health importance.

In India, brucellosis has been reported across all states, with prevalence rates ranging from 0.8% in Kashmir to as high as 26.66% in Ludhiana. The causative agents, *Brucella* spp., are small, nonmotile, non-spore forming, gram-negative coccobacilli that are facultative intracellular bacteria. Among these, *B. melitensis* is responsible for the most severe human disease, while *B. suis* is often linked to abscess formation.

The disease primarily affects individuals working with livestock or animal products, classifying it as a zoonotic occupational disease. Transmission occurs primarily through direct or indirect contact with infected animals or their products. Person-to-person transmission is rare. Human infection often results from consuming contaminated dairy products, particularly unpasteurized milk, or uncooked meat and offal. Additionally, exposure to animal urine, feces, vaginal secretions, or uterine excretions can lead to infection through abraded skin or the respiratory tract.

Infected individuals present with a wide range of symptoms, from debilitating nonspecific illness to localized involvement of various organs. This variability in clinical presentation can complicate diagnosis, as the disease in humans and cattle often mimics other conditions.

B. melitensis is the most common cause of human brucellosis and is closely associated with infections in sheep, goats, and camels. Other species such as *B. abortus*, *B. suis*, and *B. canis* can also infect humans. Two relatively new species, *B. ceti* and *B. pinnipedialis*, have been identified in marine mammals such as seals and dolphins, with occasional reports of human infections. The geographic distribution of *Brucella* spp. varies, with *B. abortus* being more widespread but typically causing less severe disease. The true incidence of brucellosis is believed to be 10–25 times higher than reported figures.

In India, approximately 80% of the population resides near domestic or wild animals, heightening the risk of zoonotic diseases like brucellosis. Rural populations show a higher prevalence of anti-*Brucella* antibodies, especially among individuals in livestock-related professions, such as farmers, veterinarians, slaughterhouse workers, and livestock keepers.

The *Brucella* genome typically contains two circular chromosomes of 2.1 Mb and 1.5 Mb, except in *B. suis* biovar 3, which has a single 3.1 Mb chromosome. Whole genome SNP analysis has revealed spatial clustering of *B. melitensis* isolates into five genotypes. Asian isolates predominantly cluster into genotype II, whereas European and American isolates fall into genotypes IV and V, respectively.

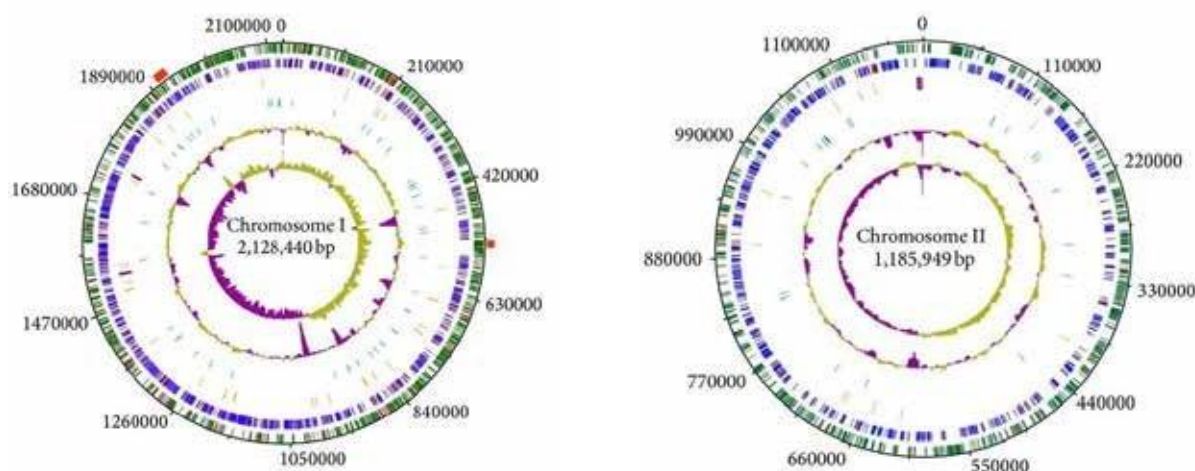


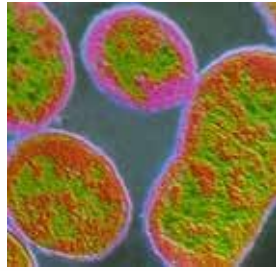
Fig 1: Genetic Characterization and Comparative Genome Analysis of *Brucella melitensis* Isolates from India.

(Image source: International Journal of Genomics, Volume: 2016, Issue: 1, First published: 25 July 2016, DOI: (10.1155/2016/3034756))

POINTS TO REMEMBER

- Brucellosis affects 1.6–2.1 million people annually worldwide, though the actual numbers are likely higher due to underreporting, and it is recognized as a top neglected zoonotic disease by WHO.
- In India, prevalence ranges from 0.8% in Kashmir to 26.66% in Ludhiana, with higher risk among livestock-related professionals such as farmers, veterinarians, and slaughterhouse workers.
- The disease is primarily transmitted through contact with infected animals or consumption of contaminated dairy products and meat, with person-to-person transmission being rare.
- Brucellosis is caused by *Brucella spp.*, with *B. melitensis* responsible for the most severe disease, presenting symptoms that range from nonspecific illness to organ-specific complications.
- *Brucella spp.* have a unique genomic structure, and studies reveal regional clustering, with Asian isolates of *B. melitensis* predominantly in genotype II.

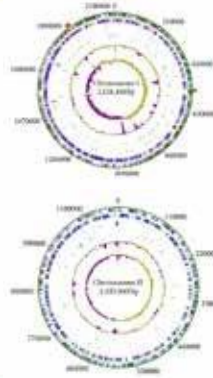
- It is a wide spread, neglected zoonotic disease caused by bacterium ***Brucella***, represented by 12 species of microorganisms.
- Serious impacts on human health affecting people of all ages and gender.
- Human brucellosis is predominantly caused by ***Brucella melitensis***
- Only 10 to 100 organisms are enough to cause infection making it a potential **agent of bioterrorism**.
- Occupational health hazard to livestock handlers, Farmers, Butchers, Hunters, Veterinarians and Laboratory Staff.
- Known by several names like **Mediterranean fever, Malta fever, gastric remittent fever, undulant fever, Gibraltar fever, Crimean fever, goat fever and Bang disease.**



- Brucellae are **gramnegative** coccobacilli measuring about 0.61.5µm length and 0.50.7µm width.
- They are **nonsporulating, nonmotile, facultative intracellular** pathogens.
- Require aerobic or microaerophilic conditions for growth.
- Genus includes four clinically relevant species: ***B. melitensis*** (acquired by humans from sheep, goats, and camels), ***B. suis*** (from swine), ***B. abortus*** (from cattle or buffalo), and ***B. canis*** (from dogs).
- The genomic structure of *Brucella* consists of **two chromosomes, with no plasmids**, making it unique in Bacteria.
- Slow multiplication at optimum temperature (37°C) and requires enriched medium for adequate growth.



- The genome contains two circular chromosomes of 2.1 Mb and 1.5 Mb except *B.suis* biovar 3, which has a single chromosome of 3.1 Mb.
- The whole genome SNPs revealed spatial clustering of *B. melitensis* isolates into five genotypes.
- All the Asian isolates of *B. melitensis* clustered into genotype II, whereas the isolates from Europe and America clustered into genotypes IV and V, respectively.



Agent	<i>Brucella</i> (Mainly <i>Brucella melitensis</i>)				
Reservoirs					
Transmission	Directly Contact with infected animals, abortions, excretions, skin wounds, ocular excretions and nasal discharges			Orally By ingestion of unpasteurized milk or milk product	
Contamination	Excretions pollutes the soil, pens, beds of straw, water from streams and wells			<i>Brucella</i> survives in environment for long period - in feces, up to 100 days - in milk, up to 300 days - in frozen environment for months	
Signs & Symptoms					
	In humans			In animals	
Prevention & Treatment			Antibiotics (Doxycycline 100 mg BID for 4 weeks or Doxycycline + rifampin 1gm Day 100 (4 weeks) and Omeprazole for Relapse)		
	Vaccination		Prevention of milk		

Session-2

Epidemiology of Brucellosis

Course No. 002	Session 2: Epidemiology of Brucellosis
Duration	20 minutes
Learning Objective	• Learn the history of brucellosis emergence. • Explain the geographical distribution of brucellosis globally.
Pre-requisite knowledge/skill	• Basic knowledge of infectious disease epidemiology and zoonoses. • Familiarity with modes of transmission and risk factors for zoonotic infections.
Competencies to be achieved	• Epidemic potential assessment of brucellosis • Analysis of seasonality and understanding its implications • Assessment of geographical distribution and prevalence of Brucellosis disease

Global Burden and Incidence

Brucellosis is a significant zoonotic disease caused by brucella species, affecting both humans and animals globally. The disease imposes a considerable burden on public health and the economy, particularly in developing regions. Its prevalence and distribution are highly variable, influenced by agricultural practices, socioeconomic factors, and the effectiveness of surveillance systems.

Expansion of Epidemic Territory

Over the past few decades, brucellosis has expanded its geographic reach dramatically. In 2006, the disease was reported in 53 countries, but this figure has since increased to at least 97, highlighting both the actual expansion of the disease and improvements in surveillance and diagnostic capacities. Newly affected areas include 18 countries in Europe, 12 in the Americas, nine in Asia, and four in Africa. These shifts reflect the role of globalization, increased livestock movement, and inadequate veterinary oversight, which facilitate the spread of the disease into previously unaffected regions.

Global Incidence and Prevalence

The global incidence of brucellosis varies significantly, ranging from 0.029 to 200.41 cases per 100,000 people annually. This variation highlights disparities in disease burden between regions, which are shaped by differences in surveillance quality, diagnostic capabilities, and public health infrastructure.

The Eastern Mediterranean region reports the highest incidence, with countries such as Syria, Turkey, Iraq, and Saudi Arabia being hotspots. Here, traditional practices such as consuming unpasteurized dairy products and close contact with livestock exacerbate the disease burden. Despite official statistics, the true incidence is likely much higher, as brucellosis often remains underreported due to misdiagnosis and limited laboratory access.

In Asia, the disease continues to spread, with many countries experiencing a significant increase in cases. Poor hygiene standards in animal husbandry and limited awareness about zoonotic diseases contribute to the high prevalence in countries such as China, India, and Central Asia. Urbanization and industrial scale farming are additional factors facilitating disease transmission.

In Europe, brucellosis shows a mixed trend. While the overall prevalence is declining in many parts of Western Europe due to effective control measures, the disease remains endemic in certain areas of Southeastern Europe, such as Bosnia and Herzegovina, North Macedonia, and Greece. In the Americas, the disease persists in rural and agricultural regions of Mexico, Peru,

Argentina, and Brazil, where livestock farming is a primary livelihood. Poor veterinary practices and the consumption of raw dairy products remain significant contributors to the prevalence in these areas.

Africa presents a unique challenge, as insufficient data and weak surveillance systems make it difficult to estimate the true burden of brucellosis. However, the disease is believed to be widespread in regions reliant on pastoral farming. The lack of diagnostic facilities and low public awareness further contribute to its underreporting and continued prevalence.

Oceania is largely free of brucellosis, with only sporadic cases reported in Australia and New Zealand. This success is attributed to stringent biosecurity measures and advanced livestock management practices that prevent the introduction and spread of brucella species.

Incidence of human brucellosis worldwide (2017-2019)

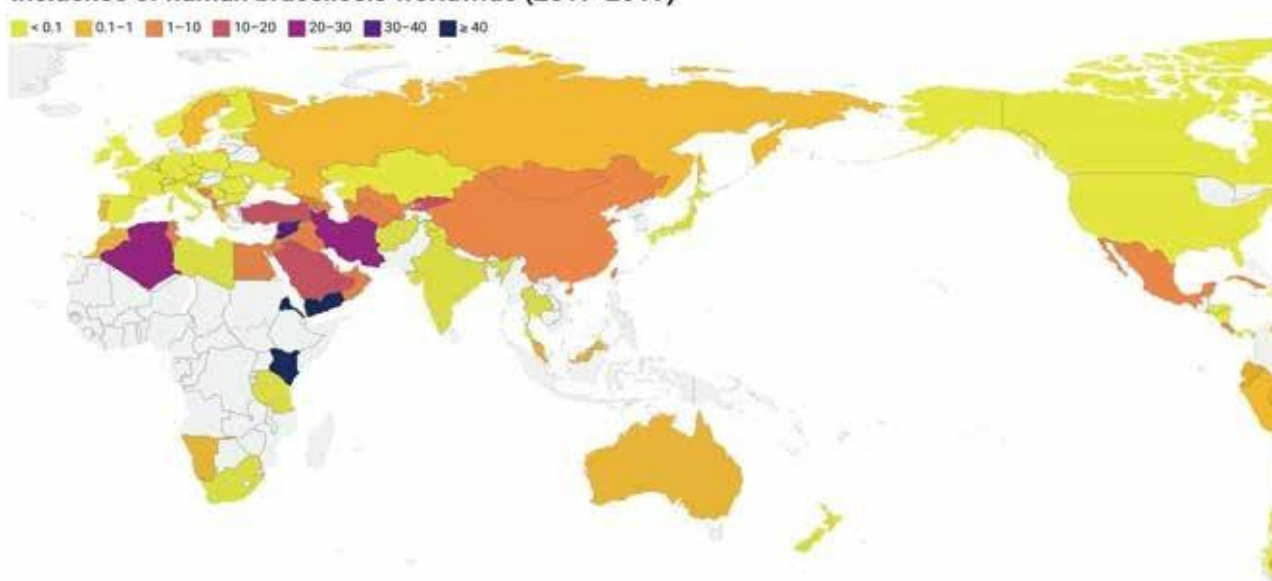


Fig 2: Incidence of human brucellosis worldwide (2017-19)

(Image source: Pappas, Georgios et al. "The new global map of human brucellosis." *The Lancet. Infectious diseases* 6 2 (2006): 919.)

Regional Trends

The burden of brucellosis demonstrates distinct regional trends shaped by cultural, environmental, and socioeconomic factors:

- 1. Eastern Mediterranean:** This region bears the highest documented burden of brucellosis globally. High incidence rates are attributed to unregulated livestock trade, traditional dietary practices involving unpasteurized dairy, and limited veterinary services.
- 2. Asia:** Cases are on the rise across many Asian countries, reflecting ongoing public health challenges. Increased demand for livestock products and poor disease control measures have exacerbated the problem, especially in densely populated regions.

3. **Europe:** Western Europe has seen success in reducing brucellosis through livestock vaccination programs and pasteurization campaigns. However, endemic foci persist in the Balkans and parts of Southeastern Europe, where socioeconomic constraints hinder eradication efforts.
4. **The Americas:** Endemic brucellosis in Latin America is tied to agricultural practices and economic reliance on livestock. Despite vaccination programs, challenges remain in regions with poor access to veterinary care and weak enforcement of food safety standards.
5. **Africa:** The true burden of brucellosis in Africa is challenging to determine due to insufficient surveillance data. However, it is likely underdiagnosed and endemic in many regions, particularly among nomadic and pastoralist communities.
6. **Oceania:** Rare cases of brucellosis have been reported in Australia and New Zealand, largely linked to importation risks. The region remains effectively free of the disease due to robust prevention strategies.

Burden and Impacts

Brucellosis continues to impose a heavy burden on public health, particularly in endemic regions. Its effects are not limited to acute illness; many cases lead to chronic conditions such as undulant fever, arthritis, and neurological complications. Moreover, the disease causes significant economic losses in livestock industries due to reduced productivity, abortions, and infertility. The burden is further compounded by the high costs associated with treatment and diagnostic delays, which are common in resource-poor settings.

EPIDEMIOLOGY OF HUMAN BRUCELLOSIS IN INDIA

Human brucellosis poses a significant public health challenge in India, where the close coexistence of humans and livestock creates an elevated risk of zoonotic infections. The disease is deeply intertwined with animal husbandry, which is a primary livelihood for many communities across the country. The prevalence of *Brucella* infection in animals, primarily *Brucella melitensis* and *Brucella abortus*, further contributes to the consistent reporting of human brucellosis cases nationwide.

Regional Distribution and Seroprevalence

Human brucellosis is prevalent throughout India, with wide variations in prevalence across states and risk groups. Punjab records the highest prevalence, with 26.6% of cases attributed to human brucellosis. Other regions with significant prevalence include Maharashtra (19.83%), Andhra Pradesh (11.51%), Gujarat and Belgaum (8.5%), and Karnataka (17-21.1% in specific districts like Gadag and Haveri). The northern state of Kashmir reports a lower prevalence (0.8%), while metropolitan cities like Delhi also report seropositivity rates of 0.9%, reflecting a wide geographical distribution.

Endemicity has been documented in states such as Odisha, Andhra Pradesh, Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, Uttarakhand, and Goa, emphasizing the widespread nature of the disease. Variations in prevalence are influenced by local livestock practices, access to veterinary care, and public health infrastructure.

High-Risk Groups

Several occupational groups are at a heightened risk of brucellosis due to their close association with livestock and animal products. These include:

- **Slaughterhouse workers:** Studies have reported seroprevalence rates of 17-34% in this group.
- **Veterinarians and para-veterinarians:** Prevalence rates range from 6.8% in Assam to 25% in New Delhi and 21% in Goa. Para-veterinarians and animal handlers are especially vulnerable, with prevalence rates reaching 16% compared to 5-6% in veterinarians and artificial insemination workers.
- **Dairy farmers and animal handlers:** These groups also show a high prevalence of brucellosis, reflecting the role of occupational exposure in disease transmission.

A notable study in Punjab revealed that 25.28% of veterinary para-clinical staff tested positive, followed by dairy workers (16.10%) and veterinarians (11.01%). The risk of infection is significantly associated with longer durations of exposure, emphasizing the occupational hazard for individuals engaged in animal care and livestock industries.

Clinical and Demographic Trends

Human brucellosis in India presents with varied clinical symptoms, often characterized by fever of unknown origin (FUO), spondyloarthropathy, and other systemic complications. Studies report prevalence rates of 0.8% to 6.8% among FUO patients. Intermittent fever (71.62%), spondyloarthropathy (52.70%), and epididymo-orchitis (12.16%) are among the most common symptoms. Chronic manifestations such as infertility and polyarthritis have also been observed, with studies noting fever and upper back pain as significant predictors of acute and chronic brucellosis, respectively.

The disease affects individuals across all age groups but is most commonly reported in children and young adults aged 21-40 years. In Karnataka, studies observed a seroprevalence of 6.75-8.90% in this age group, with children and young adults in rural areas being disproportionately affected. Regarding sex-based trends, female children show higher infection rates (14.3%) compared to males (10.9%). However, males overall are more frequently affected in adult populations, likely due to their occupational exposure.

SOURCES OF INFECTION

The primary hosts for *Brucella* bacteria include cattle, sheep, goats, swine, buffaloes, horses, and dogs. These animals can excrete the *Brucella* organism in various bodily fluids, such as urine, milk, placenta, uterine and vaginal discharges, and feces, making them potential sources of infection for humans.

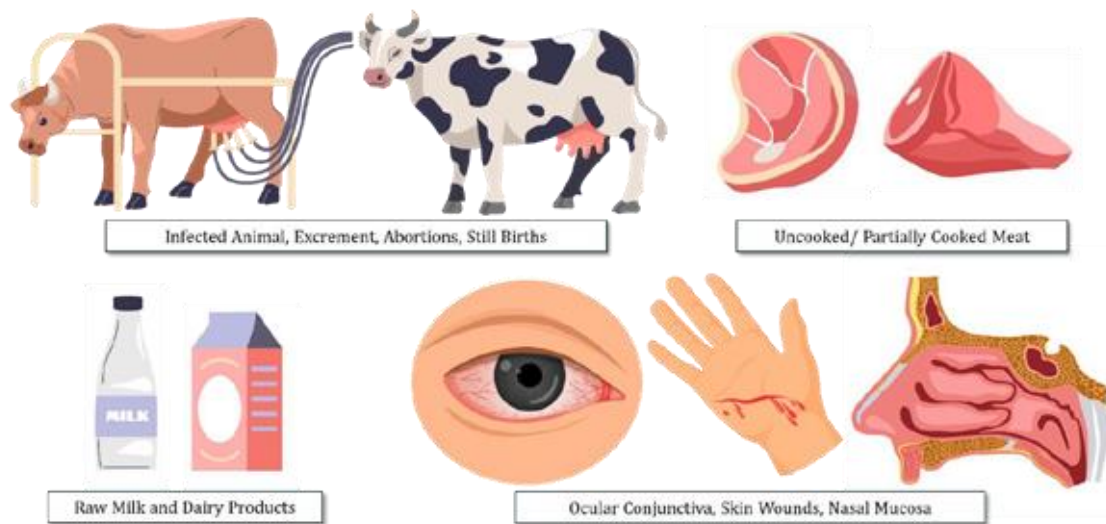


Fig 3: Sources of Infection

TRANSMISSION OF BRUCELLOSIS

One distinctive feature of *Brucella* is its ability to survive in damp environments and soil for prolonged periods, further increasing the risk of transmission to humans in agricultural and livestock-related settings. The mode of infection in humans occurs through three primary routes:

- 1. Direct Contact:** Infection can occur through contact with contaminated animals or their products, such as touching infected tissues, handling contaminated milk or meat, or coming into contact with animal secretions. The organism can enter the human body through abraded skin, mucous membranes, or the conjunctiva of the eye.
- 2. Contaminated Food and Water:** Consumption of contaminated dairy products, especially unpasteurized milk, or uncooked meat and offal can lead to infection. This route emphasizes the importance of proper food handling and hygiene.
- 3. Airborne Route:** *Brucella* bacteria can also be transmitted through aerosols or splashes, though this is a rare mode of transmission. Exposure to aerosols containing the pathogen can lead to respiratory tract infections, highlighting the need for safe practices in settings with infected animals.

In rare cases, *Brucella* has been reported to be transmitted through sexual contact or from lactating mothers to their breastfed infants.

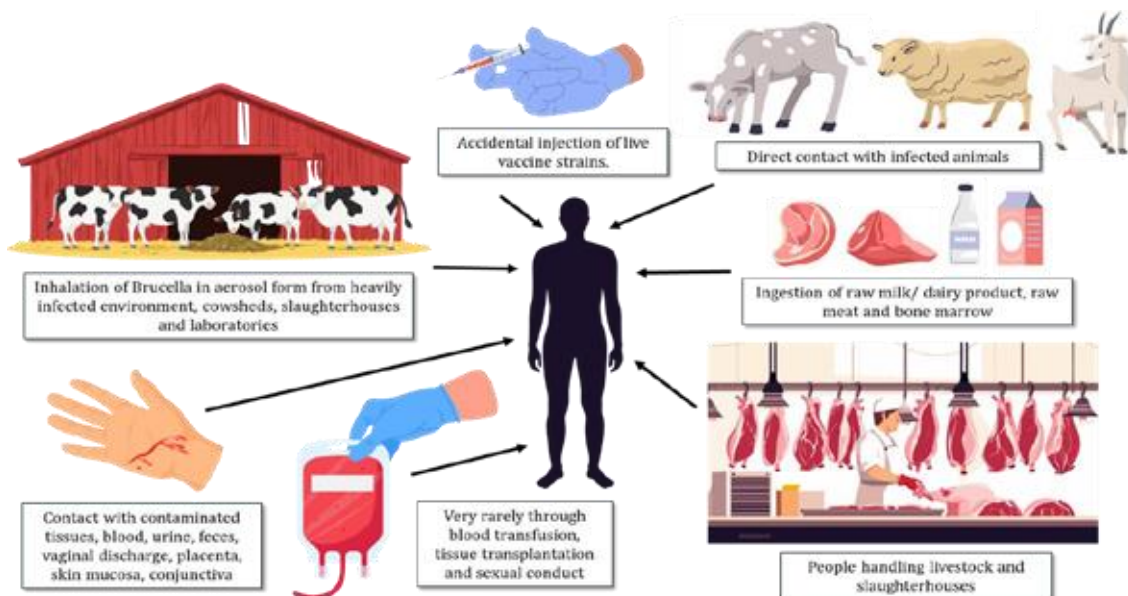


Fig 4: Transmission in Humans

RISK OF CONTRACTING BRUCELLOSIS

In the general population, the majority of brucellosis cases arise from consuming raw milk or its unpasteurized derivatives, such as fresh cheese. These infections are often linked to products derived from sheep and goats.

Brucellosis is also recognized as an occupational hazard for individuals in the livestock industry. Those who handle animals or come into contact with infected materials such as blood, placentas, fetuses, and uterine secretions face a heightened risk of exposure. This mode of transmission predominantly affects professionals like farmers, butchers, hunters, veterinarians, and laboratory workers.



Fig 5: People at Risk

POINTS TO REMEMBER

- Brucellosis has expanded to 97 countries from 53 in 2006, showing improved surveillance and actual spread.
- Global incidence ranges from 0.029 to 200.41 cases per 100,000, with the highest burden in the Eastern Mediterranean.
- Rising cases in Asia and Southeastern Europe contrast with declining prevalence in Western Europe due to effective control.
- Disease expansion is driven by globalization, livestock trade, and inadequate veterinary controls.
- In India, seroprevalence ranges from 0.8% in Kashmir to 26.66% in Punjab, with occupational exposure being significant.
- High-risk groups include veterinarians, slaughterhouse workers, and dairy farmers due to close contact with livestock.
- Transmission occurs via infected animals or products, contaminated environments, or aerosols, highlighting its zoonotic nature.

- **Wide Geographic Spread:**
 - Endemic in states like Punjab, Maharashtra, Andhra Pradesh, Gujarat, Rajasthan, Uttar Pradesh, Odisha, and Goa.
 - Prevalence:
 - Punjab: 26.6% (highest)
 - Maharashtra: 19.83%
 - Gujarat/Belgaum: 8.5%
 - Andhra Pradesh: 11.51%
 - Delhi: 0.9%

- **Abattoir Workers:** 17-34% prevalence.
- **Veterinary Professionals:**
 - 25% in New Delhi; 21% in Goa; 6.8% in Assam.
 - Para-veterinarians: 16% (higher risk than veterinarians).
- **Dairy Farmers and Animal Handlers:** Significant prevalence reported in Punjab and Karnataka.

A world map illustrating the distribution of the number of countries in each region that have signed the Convention on the Rights of the Child (CRC). The map is color-coded according to the number of signatories per region, with a legend at the top left showing the following categories: 1-4 (light yellow), 5-6 (yellow), 7-8 (orange), 9-10 (dark orange), 11-12 (red), 13-14 (dark red), 15-16 (purple), 17-18 (dark purple), 19-20 (black), 21-22 (dark grey), 23-24 (light grey), 25-26 (medium grey), 27-28 (dark grey), 29-30 (black), 31-32 (dark grey), 33-34 (light grey), 35-36 (medium grey), 37-38 (dark grey), 39-40 (black), 41-42 (dark grey), 43-44 (light grey), 45-46 (medium grey), 47-48 (dark grey), 49-50 (black), 51-52 (dark grey), 53-54 (light grey), 55-56 (medium grey), 57-58 (dark grey), 59-60 (black), 61-62 (dark grey), 63-64 (light grey), 65-66 (medium grey), 67-68 (dark grey), 69-70 (black), 71-72 (dark grey), 73-74 (light grey), 75-76 (medium grey), 77-78 (dark grey), 79-80 (black), 81-82 (dark grey), 83-84 (light grey), 85-86 (medium grey), 87-88 (dark grey), 89-90 (black), 91-92 (dark grey), 93-94 (light grey), 95-96 (medium grey), 97-98 (dark grey), 99-100 (black).

- Fever of Unknown Origin (FUO): 0.8% to 6.8%.
- Intermittent Fever: 71.62%.
- Spondyloarthropathy: 52.70%.
- Other: Infertility (8.10%), polyarthritis, and back pain.

- Most common in **21-40 years age group**.
- **Children:** Higher prevalence in females (14.3%) vs. males (10.9%).
- Males more affected in occupational groups.

- Rural areas report higher prevalence due to close livestock proximity.
- Punjab, Karnataka (districts like Gadag, Haveri), and Maharashtra are key hotspots.

- Significant association with livestock exposure ($p < 0.05$).

The infographic is divided into four quadrants, each with a title and an illustration of a source of E. coli infection:

- Top Left:** Titled "Infected Animal, Excrement, Abortions, Still Births". It shows a brown cow and a black and white cow. The brown cow is in a yellow metal frame, and the black and white cow is standing next to it.
- Top Right:** Titled "Uncooked/Partially Cooked Meat". It shows two pieces of raw, red meat.
- Bottom Left:** Titled "Raw Milk and Dairy Products". It shows a glass bottle of milk and a carton of milk.
- Bottom Right:** Titled "Ocular Conjunctiva, Skin Wounds, Nasal Mucosa". It shows a close-up of a human eye, a hand with a red scratch, and a cross-section of a nose showing the nasal cavity.

Session-3

Brucellosis in Human

Course No. 003	Session 3: Brucellosis in Human
Duration	15 minutes
Learning Objective	• Understanding the source of infection. • Understand the disease progression and clinical manifestation in humans.
Pre-requisite knowledge/skill	• Basic knowledge of zoonotic diseases and their reservoirs. • Familiarity with human immune response and pathogenesis of infections. • Ability to recognize general signs and symptoms of systemic infections.
Competencies to be achieved	• Knowledge of the source of infection. • Explaining the disease transmission and clinical sign and symptoms in human.

BRUCELLOSIS IN HUMANS

Disease Progression

When it manifests in humans, brucellosis presents a highly variable and often nonspecific clinical picture. This zoonotic disease can affect virtually any organ or body system, making its symptoms diverse and sometimes challenging to distinguish from other medical conditions. One key difficulty in diagnosing brucellosis is the absence of pathognomonic (distinctive) symptoms, leading to frequent confusion with other illnesses.

Diagnostic hints can be drawn from the patient's history, such as visiting an endemic region, handling diagnostic work in a microbiology lab, consuming unpasteurized dairy products like soft cheeses, interacting with animals, accidental exposure to veterinary *Brucella* vaccines, or, in endemic areas, the occurrence of similar illnesses among family members, reported in almost half of the cases.

The incubation period for brucellosis typically ranges from 1 to 4 weeks, although in some cases, it may extend to several months, depending on bacterial load, host immune status, and the species of *Brucella* involved. In the initial stages, patients often experience continuous low-grade bacteremia (the presence of bacteria in the bloodstream). This subtle and prolonged presence of the pathogen complicates early diagnosis, necessitating a high degree of suspicion, especially in regions where brucellosis is prevalent.

Clinical Presentation

Brucellosis presents a wide range of symptoms in affected individuals. The onset of symptoms can be abrupt or insidious, with the disease manifesting in acute, subacute, or chronic forms.

The early presentation is often non-specific. Patients typically report fever, which is often undulant (fluctuating), and is accompanied by malaise, headache, chills, night sweats (noted for their distinctive foul odor, likened to mold, or wet hay), anorexia, and weight loss. Fatigue is almost universally present and can be debilitating. As the disease progresses, other systemic symptoms, such as generalized weakness, myalgia, arthralgia, and migratory joint pains, may become prominent.

In chronic cases, symptoms may persist for years after the initial infection. Patients may describe cyclical fever episodes, ongoing joint pain, or backache, reflecting the disease's ability to persist intracellularly and evade immune defenses.

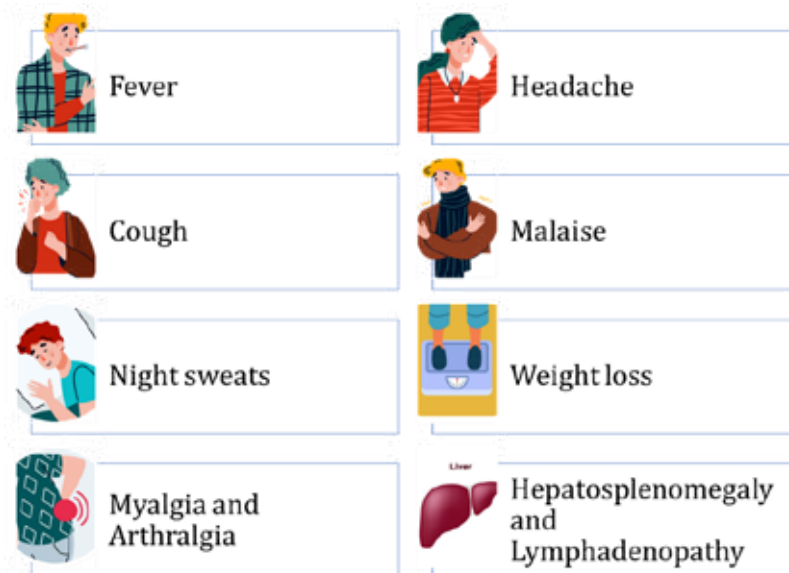


Fig 6: Clinical Manifestation in Humans

Patterns of Clinical Presentation

Brucellosis commonly presents in one of three patterns:

- 1. Febrile Illness Resembling Typhoid Fever:** This is characterized by fever, headache, and malaise. It is less severe than typhoid and may lack gastrointestinal symptoms.
- 2. Localized Disease:** Symptoms are specific to the organ system involved, such as joint pain, back pain, or genitourinary symptoms.
- 3. Relapsing or Chronic Disease:** Patients may have recurrent fever, night sweats, and arthralgia, with symptoms sometimes appearing years after the initial infection.

Organ Specific Manifestations

1. Musculoskeletal Involvement

The musculoskeletal system is the most commonly affected, with symptoms in 40–50% of cases:

- **Sacroiliitis:** A hallmark of brucellosis, sacroiliitis typically presents with severe unilateral low-back pain radiating to the hip or thigh. It often limits movement and may be confused with ankylosing spondylitis or tuberculosis.

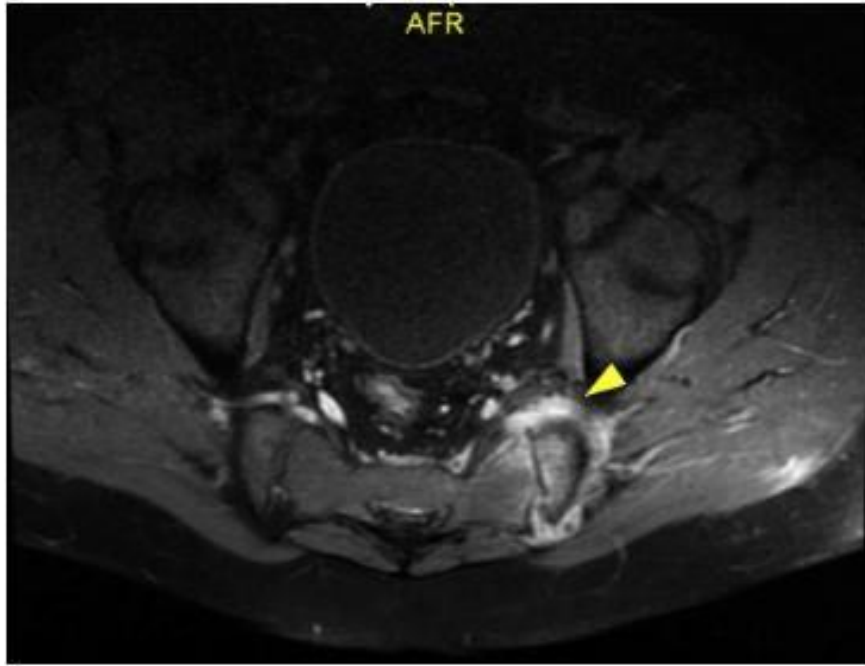


Fig 7: MRI of the lumbar spine with inflammatory changes in the left sacroiliac joint and in the adjacent fat

Image source: <http://www.scielo.org.co/pdf/rcrc/v29n2/0121-8123-rcrc-29-02-145.pdf>

- **Septic Arthritis:** Mono or polyarthritis frequently affects large joints like the hip, knee, or shoulder. Symptoms include swelling, warmth, and restricted range of motion.
- **Spondylitis and Spondylodiscitis:** These are common in chronic cases, especially involving the lumbar and low thoracic vertebrae. Imaging often shows anterior vertebral erosion, sclerosis, and osteophyte formation.
- **Osteomyelitis:** A serious complication that may lead to bone destruction if untreated.
- **Epidural Abscess:** Rare but can cause neurologic deficits requiring immediate intervention.

Septic arthritis in brucellosis progresses slowly, starting with small pericapsular erosions. In the vertebrae, anterior erosions of the superior end plate are typically the first features to become evident, with eventual involvement and sclerosis of the whole vertebra. Anterior osteophytes eventually develop, but vertebral destruction or impingement on the spinal cord is rare and usually suggests tuberculosis.

2. Neurological Manifestations (“Neurobrucellosis”)

Neurological involvement occurs in ~5% of cases, often presenting as:

- **Lymphocytic Meningoencephalitis:** Mimics tuberculosis or leptospirosis, with fever, headache, and neck stiffness.
- **Guillain-Barre Syndrome:** An autoimmune response linked to acute brucellosis.
- **Focal Neurological Deficits:** Resulting from brain abscesses or subarachnoid hemorrhage.
- **Myelitis and Increased Intracranial Pressure:** Manifest as papilledema, cranial nerve palsies, and ataxia.

3. Cardiovascular Manifestations

Cardiovascular involvement is rare but serious:

- **Endocarditis:** Occurs in 1–2% of cases and predominantly affects the aortic valve. It is the leading cause of mortality in brucellosis.
- **Pericarditis and Aortic Fistulas:** Rare complications that may present with chest pain, pericardial rub, or effusion.

4. Respiratory System

Respiratory symptoms occur in ~25% of cases:

- Common complaints include cough, dyspnea, and pleurisy.
- Radiographic findings are often normal, but rare complications like lung abscesses, granulomas, or effusions have been documented.

5. Hepatobiliary and Gastrointestinal Systems

- **Hepatosplenomegaly:** Found in 25–50% of cases, often accompanied by granulomatous hepatitis.
- **Hepatic Abscesses:** Though rare, they indicate severe disease progression.
- **Spontaneous Bacterial Peritonitis:** Occasionally reported as a complication.

6. Reproductive System

- **Males:** Epididymo-orchitis is observed in ~10% of cases. Symptoms include tender, swollen scrotum with overlying erythema.
- **Females:** Brucellosis is associated with miscarriage, but teratogenic effects are not reported.

7. Dermatological Manifestations

- Skin findings are uncommon but include maculopapular rashes, erythema nodosum, panniculitis, and abscesses.



Fig 8: Exanthemas / maculopapular rashes s. *Exanthema infectiosum*(EI)



Fig 9: Erythema nodosum (EN)/ Erythema nodosum-like (EN-like) lesions s. Erythema nodosum syndrome (left), Papules, Nodules /Papulo-nodular eruptions/ Urticaria-like (center and right)

8. Hematologic and Lymphatic Involvement

- Lymphadenopathy is noted in 10–20% of patients.
- Rare cases of immune-mediated thrombocytopenic purpura can result in bleeding diatheses.

9. Ocular Manifestations

- Ocular involvement includes uveitis, keratoconjunctivitis, optic neuritis, and iridocyclitis. Cataracts may occur in severe cases.

The clinical diversity of brucellosis makes it a challenging condition to diagnose and manage, requiring a high degree of clinical suspicion, particularly in endemic regions.

Complications of Brucellosis

While timely and appropriate treatment usually ensures a smooth recovery from brucellosis, complications can still arise in some cases, particularly when diagnosis and intervention are delayed beyond a month. The risk of more severe outcomes increases with prolonged untreated infection, and relapse is a possibility.

Brucellosis complications span a wide range of systems, reflecting the disease's complexity. Prompt and appropriate treatment of brucellosis generally ensures recovery, but complications can arise, especially if diagnosis is delayed beyond a month. Cardiovascular complications like endocarditis and mycotic aneurysms are severe yet rare. Genitourinary issues may include orchitis, pyelonephritis, and abortion.

The CNS may be affected, leading to meningitis, stroke, or optic neuropathy, while musculoskeletal problems such as arthritis, spondylitis, and osteomyelitis are common. Hematologic risks include disseminated intravascular coagulation. Gastrointestinal complications range from hepatitis to abscesses in critical organs. Early intervention is crucial to minimize these diverse and potentially serious outcomes.

Prognosis and Follow-up

Although recovery from brucellosis can be slow, the prognosis for most patients is generally excellent, with overall mortality remaining very low, likely below 2%. Symptoms typically resolve within 3-6 months, and physical activity often aids recovery more effectively than prolonged rest. However, relapse occurs in up to 30% of patients who are poorly compliant with treatment and can be either localized or systemic. Relapses respond well to extended courses of the original therapy, and clinical follow-up for up to two years is recommended to monitor recurrence.

Patients with underlying conditions, such as heart or lung disease, may experience more disabling effects, and the prognosis is particularly poor for those who develop congestive heart failure due to endocarditis. Indicators such as weight gain and general health are more reliable for assessing recovery than serology, as IgG antibody levels may persist in the diagnostic range for over two years post-treatment. Immunity is not durable, leaving patients vulnerable to reinfection after repeated exposures. Despite the low mortality rate, brucellosis can lead to prolonged inactivity, impacting domestic life and economic productivity. Advances in diagnostics, such as assays for *Brucella* antigens or DNA, may improve the identification and management of ongoing infections in the future.

Case Definition of Brucellosis

SUSPECTED CASE

A case that is compatible with the clinical description and is epidemiologically linked to a suspected/confirmed animal cases or contaminated animal products.

PROBABLE CASE

An illness characterized by acute or insidious onset of fever with any of the following -

Night sweats, arthralgia, headache, fatigue, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis, or focal organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly)

AND

Important risk factors to be kept in mind are

- Slaughterhouse workers
- Meatpacking plant employees
- Veterinarians
- Ingesting undercooked meat
- Consumption of unpasteurized/raw dairy products.
- Assisted animals giving birth

LABORATORY CONFIRMED CASE

A case compatible with the clinical description of brucellosis with at least one of the following:

1. High titre of IgM antibodies in ELISA (evaluated with locally determined cutoff) for single clinical sample*.
2. A fourfold rise in the SAT (total antibodies) between acute and convalescent phase serum specimens run parallel.
3. Seroconversion on ELISA in paired serology (demonstrating the conversion of IgM to IgG antibodies).

**A single serum sample showing high titres of IgM antibodies may indicate acute infection. These 13 tests are the preferred tests as ELISA are widely acceptable*

Other - Isolation and Validated PCR can be done in patients who have not received antibiotic and in early stage of diseases (preferably less than 7 days)

Differential Diagnosis

Brucellosis presents with a wide array of clinical symptoms that can mimic numerous other medical conditions, necessitating a meticulous approach to differential diagnosis. Key conditions to rule out include typhoid, malaria, tuberculosis (TB), lymphoma, dengue, leptospirosis, rheumatic diseases, Epstein-Barr virus infection, toxoplasmosis, meningitis, Mycoplasma pneumonia, cytomegalovirus infection, and HIV-associated illnesses.

The difficulty lies in the significant overlap of symptoms, such as fever, fatigue, and generalized muscle pain, with these other conditions. Accurate diagnosis is essential to initiate targeted treatment and avoid mismanagement. Clinicians must rely on a combination of patient history, clinical examination, and specialized laboratory tests to distinguish brucellosis from these other possibilities.

For instance, brucellosis associated fever is often characterized by an undulant pattern with relapses, which can help differentiate it from typhoid and malaria. Also, musculoskeletal symptoms like myalgia may also provide diagnostic clues.

Table 1: Differential diagnosis of Brucellosis

Disease	Epidemiological Clues	Key Clinical Differences	Laboratory Tests
Ankylosing Spondylitis (AS)	Common in younger males (teens to 40s); genetic predisposition (HLA-B27); no zoonotic exposure.	Insidious onset of lower back pain, worse at night, stiffness improves with activity. No fever, weight loss, or hepatosplenomegaly.	Hematological: Mild anemia, ESR and CRP elevated.
			Biochemical: Normal LFTs, renal function.
			Serology/Special Tests: HLA-B27 positive, X-ray or MRI showing sacroiliitis.
Undifferentiated Spondyloarthropathy	Similar to AS but less specific; no significant systemic or zoonotic risk factors.	Episodic joint pain and swelling, mainly affecting lower extremities; fever and systemic signs are uncommon.	Hematological: Mild leukocytosis, elevated ESR/CRP.
			Biochemical: Normal.
			Serology/Special Tests: Negative <i>Brucella</i> serology, imaging may show enthesitis.
Influenza	Seasonal outbreaks, close person-to-person transmission (droplet spread).	Acute onset of fever, myalgia, sore throat, and cough; rapid resolution within 5–7 days.	Hematological: Mild lymphopenia or lymphocytosis.
			Biochemical: Normal.
			Serology/Special Tests: Influenza PCR or rapid antigen test positive; negative <i>Brucella</i> serology.
Cryptococcosis	Exposure to bird droppings; commonly affects immunocompromised patients (e.g., HIV/AIDS, transplants).	Presents with pulmonary or CNS symptoms (e.g., headache, meningitis); no relapsing fever, arthralgia, or hepatosplenomegaly.	Hematological: Leukopenia or anemia in advanced disease.
			Biochemical: Normal unless disseminated disease.
			Serology/Special Tests: Cryptococcal antigen positive, CSF showing India ink positive yeasts.
Leptospirosis	Risk factors: exposure to floodwater, livestock, or contaminated water; endemic in tropical areas.	Conjunctival suffusion, jaundice, acute kidney injury (Weil's disease) in severe cases; myalgia in calves is prominent.	Hematological: Thrombocytopenia, mild leukocytosis.
			Biochemical: Elevated bilirubin, creatinine, CK.
			Serology/Special Tests: Microscopic agglutination test (MAT) or PCR positive for <i>Leptospira</i> .
Viral Hepatitis (A, B, C, E)	History of contaminated food/water (A/E) or high-risk behavior (B/C); endemic in some regions.	Prominent jaundice, nausea, anorexia; hepatomegaly; no relapsing fever or arthritis.	Hematological: Mild anemia or leukopenia.
			Biochemical: Markedly elevated ALT/AST (>10x normal), hyperbilirubinemia.
			Serology/Special Tests: Positive markers for hepatitis (e.g., HBsAg, anti-HCV antibodies).

Disease	Epidemiological Clues	Key Clinical Differences	Laboratory Tests
Malaria	Travel to endemic areas, mosquito exposure, seasonal outbreaks.	Intermittent or cyclical fever with chills, splenomegaly, fatigue; no arthralgia or lymphadenopathy.	Hematological: Anemia, thrombocytopenia.
			Biochemical: Normal to mild hyperbilirubinemia.
			Serology/Special Tests: Thick/thin smear or rapid diagnostic test (RDT) positive for <i>Plasmodium</i> .
Tuberculosis (TB)	Contact with TB cases, endemic in developing countries; risk factors include HIV, poor nutrition.	Gradual onset fever, night sweats, chronic cough, weight loss; extrapulmonary TB (e.g., lymphadenitis, meningitis) possible.	Hematological: Normocytic anemia, leukocytosis, elevated ESR.
			Biochemical: Normal unless organ specific damage (e.g., elevated protein in TB meningitis).
			Serology/Special Tests: AFB smear, GeneXpert, or culture positive for <i>Mycobacterium tuberculosis</i> .
Infectious Mononucleosis	Teenagers/young adults; spread through saliva (e.g., kissing disease).	Pharyngitis, posterior cervical lymphadenopathy, splenomegaly, fatigue; no relapsing fever.	Hematological: Atypical lymphocytes, mild leukocytosis.
			Biochemical: Mildly elevated transaminases.
			Serology/Special Tests: Positive Monospot test or EBV-specific antibodies (e.g., anti-VCA IgM).
Typhoid Fever	Contaminated food/water in endemic regions; poor sanitation.	Sustained high fever, abdominal pain, rose spots; hepatosplenomegaly, diarrhea or constipation.	Hematological: Anemia, leukopenia.
			Biochemical: Mild LFT elevation.
			Serology/Special Tests: Blood culture positive for <i>Salmonella typhi/paratyphi</i> .
Collagen Vascular Diseases (e.g., SLE)	Females of childbearing age; autoimmune predisposition.	Multi-system involvement (e.g., arthritis, malar rash, nephritis); fever may mimic infectious diseases.	Hematological: Hemolytic anemia, thrombocytopenia, leukopenia.
			Biochemical: Hypocomplementemia (low C3/C4).
			Serology/Special Tests: Positive ANA, anti-dsDNA, and anti-Smith antibodies.
Chronic Fatigue Syndrome	Often follows viral illness; no zoonotic or occupational risk.	Persistent fatigue without fever, arthralgia, or organomegaly.	Hematological: Normal CBC.
			Biochemical: Normal LFTs, renal function.
			Serology/Special Tests: Diagnosis of exclusion; no specific tests.

Disease	Epidemiological Clues	Key Clinical Differences	Laboratory Tests
Malignancy	History of cancer or associated risk factors; older adults.	Weight loss, localized masses, persistent fever in advanced stages; hepatosplenomegaly in hematologic malignancies.	Hematological: Pancytopenia or leukocytosis (depending on type).
			Biochemical: Elevated LDH, abnormal tumor markers.
			Serology/Special Tests: Imaging (e.g., CT, PET) or biopsy.
Osteomyelitis	Trauma, recent surgery, or sepsis history.	Localized bone pain, erythema, restricted movement, fever; no systemic lymphadenopathy or relapsing fever.	Hematological: Leukocytosis, elevated ESR/CRP.
			Biochemical: Normal unless associated with systemic illness.
			Serology/Special Tests: Positive culture from bone biopsy or imaging showing osteomyelitis (MRI preferred).

The table below gives the differentiating features between the osteological manifestations of brucellosis and tuberculosis.

Table 2: Radiology of the Spine: Differentiation of Brucellosis from Tuberculosis

Feature	Brucellosis	Tuberculosis
Site	Lumbar and other regions	Dorsolumbar
Vertebrae Involvement	Multiple or contiguous	Contiguous
Diskitis	Late	Early
Body Morphology	Intact until late	Morphology lost early
Canal Compression	Rare	Common
Epiphysitis	Anterosuperior (Pom's sign)	General: upper and lower disk regions, central, subperiosteal
Osteophyte Formation	Anterolateral ("parrot beak")	Unusual
Deformity	Wedging uncommon	Anterior wedge, gibbus
Recovery	Sclerosis, whole-body involvement	Variable
Paravertebral Abscess	Small, well-localized and discrete	Common
Psoas Abscess	Rare	More likely

Case Presentation

Presenting Complaint

A 6-year-old boy from New Delhi presented with **fever lasting 5 days**, accompanied by **dry cough, headache**, and **three episodes of vomiting** (non-bilious) over 1 day. He also experienced **two episodes of epistaxis and reduced appetite**.

HISTORY OF PRESENT ILLNESS

- Onset and Progression
 - Fever was **high-grade**, remittent, and not associated with chills.
 - No history of joint pain, abdominal pain, diarrhea, or skin rashes.
 - Developed vomiting and epistaxis a day before admission.

- **Travel/Exposure History**
 - Traveled to a hill station in northern India **2 months prior** to the onset.
 - Consumed **unpasteurized dairy products**, a known risk factor for brucellosis.
 - No history of animal exposure, insect bites, or handling of carcasses.
- **Family History** - No similar illness in household members.

Examination Findings:

- **General Condition** - Conscious, oriented, afebrile with **cool extremities**.
- **Vitals** -
 - Pulse: **144/min (low volume)**
 - BP: **96/50 mmHg**
 - Respiratory rate: **26/min**
 - Capillary refill time: **>3 seconds**

SYSTEMIC EXAMINATION

- **HEENT** - Single whitish exudate over the right tonsil. No congestion or pallor.
- **Abdomen** -
 - **Hepatomegaly**: Liver palpable 3 cm below costal margin.
 - **Splenomegaly**: Spleen palpable 1.5 cm below costal margin.
 - No tenderness, distension, or free fluid.
- **Lymphadenopathy** - Tender cervical and axillary lymph nodes (1-1.5 cm in size). No fluctuation or inguinal node enlargement.
- **Neurological** - Normal with no focal deficits, meningeal signs, or fundus abnormalities.

INVESTIGATIONS

- **Hematology**
 - Hemoglobin: **11.2 g/dl**
 - TLC: **3600/cu mm** (Neutrophils: 56%, Bands: 9%)
 - Platelets: **1.3×10^5 /cu mm**
- **Liver Function Tests** - Elevated ALT (**254 IU**) and AST (**196 IU**).
- **Brucella Serology**
 - **Agglutination test**: Positive for *B. abortus* (1:160).
 - **ELISA**: IgM positive for *B. abortus* in blood; CSF negative.
- **Blood Cultures** - Sterile after 5 days of incubation (modern automated methods).
- **Imaging**
 - **Abdominal Ultrasound and CT**: Hepatosplenomegaly with a hypodense lesion in the spleen, suggestive of a brucelloma.
 - **Thoracic CT**: Bilateral pleural effusion with no hilar lymphadenopathy.

DIAGNOSIS

Brucellosis, confirmed by positive serology (*B. abortus*) and imaging findings consistent with splenic involvement.

Management as per NCDC Guidelines

- Treated with a combination of
 - **Doxycycline (6 weeks)** and **rifampin (6 weeks)** for brucellosis as per WHO and NCDC protocols.
 - **Gentamicin (10 days)** for severe complications (splenic involvement).
- Supportive care
 - Intravenous fluids for dehydration.
 - Antipyretics and close monitoring for complications.

Outcome and Follow-Up

- Fever resolved within **48 hours** of initiating therapy.
- Abdominal symptoms and lymphadenopathy subsided within **72 hours**.
- Discharged after **2 weeks** with advice to complete oral antibiotic therapy at home.
- **Follow-up**
 - Regular serological and clinical monitoring every 3 months.
 - Post-treatment ultrasound showed resolution of splenic lesion.
 - Child remained asymptomatic with no relapse or residual hepatosplenomegaly during 6-month follow-up.

POINTS TO REMEMBER

- Brucellosis presents with highly variable, nonspecific symptoms such as fever (acute or insidious), night sweats, fatigue, myalgia, and weight loss. Focal symptoms like arthritis, spondylitis, and hepatosplenomegaly occur in many cases, complicating diagnosis.
- Severe cases may involve endocarditis (1% of cases), neurological complications (e.g., meningoencephalitis), or reproductive issues like epididymo-orchitis and fetal loss in pregnant women.
- Prognosis is excellent with low mortality (<2%), but relapses occur in up to 30% of poorly compliant patients. Recurrences can be systemic or localized and typically respond to extended therapy.
- Common sources of infection include unpasteurized dairy, undercooked meat, contact with infected animals, and occupational risks (e.g., veterinarians, slaughterhouse workers).
- Diagnosis requires serology (ELISA for IgM/IgG) or a fourfold rise in SAT titers in paired samples. PCR and culture are valuable in early, untreated cases.
- Brucellosis symptoms often mimic conditions like typhoid, malaria, tuberculosis, leptospirosis, and dengue, requiring careful clinical and laboratory evaluation.

- The clinical presentation of brucellosis in humans is variable and unspecific
- It can affect any organ and body system
- Symptoms are not pathognomonic, the disease is often confused with other medical conditions
- In the initial stages of brucellosis, patients experience a continuous lowgrade bacteraemia

Temperature increases to 40-41° C, then subsides gradually to normal or near normal with profuse sweating in the morning.

	Fever		Headache
	Cough		Malaise
	Night sweats		Weight loss
	Myalgia and Arthralgia		Hepatosplenomegaly and Lymphadenopathy

1. **Febrile Illness Resembling Typhoid Fever:** This is characterized by fever, headache, and malaise. It is less severe than typhoid and may lack gastrointestinal symptoms.
2. **Localized Disease:** Symptoms are specific to the organ system involved, such as joint pain, back pain, or genitourinary symptoms.
3. **Relapsing or Chronic Disease:** Patients may have recurrent fever, night sweats, and arthralgia, with symptoms sometimes appearing years after the initial infection.

- **Sacroiliitis** - A hallmark of brucellosis, sacroiliitis typically presents with severe unilateral low-back pain radiating to the hip or thigh. It often limits movement and may be confused with ankylosing spondylitis or tuberculosis.
- **Septic Arthritis** - Mono- or polyarthritis frequently affects large joints like the hip, knee, or shoulder. Symptoms include swelling, warmth, and restricted range of motion.
- **Spondylitis and Spondylodiscitis** - These are common in chronic cases, especially involving the lumbar and low thoracic vertebrae. Imaging often shows anterior vertebral erosion, sclerosis, and osteophyte formation.
- **Osteomyelitis** - A serious complication that may lead to bone destruction if untreated.
- **Epidural Abscess** - Rare but can cause neurologic deficits requiring immediate intervention.

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BRUCELLOSIS

Myelitis and Increased Intracranial Pressure - Manifest as papilledema, cranial nerve palsies, and ataxia.

BRUCELLOSIS

- Spontaneous Bacterial Peritonitis: Occasionally reported as a complication.

BRUCELLA

- Males - Epididymo-orchitis is observed in ~10% of cases. Symptoms include tender, swollen scrotum with overlying erythema.
- Females - Brucellosis is associated with miscarriage (similar to fetal loss in livestock) but teratogenic effects are not reported.

BRUCELLA

[illegible]

Dermatological manifestations

Skin findings are uncommon but include maculopapular rashes, erythema nodosum, panniculitis, and abscesses.



Erythema nodosum (EN)/ Erythema nodosum-like (EN-like) lesions. Erythema nodosum syndrome (left), Papules, Nodules /Papulo-nodular eruptions/ Urticaria-like (center and right)

Exanthemas / maculopapular rashes. Exanthema infectiosum (EI)



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BRUCELLOSIS

Systemic manifestations of brucellosis



Hematologic and Lymphatic Involvement

Lymphadenopathy is noted in 10–20% of patients. Rare cases of immune-mediated thrombocytopenic purpura can result in bleeding diatheses.



Ocular Manifestations

Ocular involvement includes uveitis, keratoconjunctivitis, optic neuritis, and iridocyclitis. Cataracts may occur in severe cases.

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BRUCELLOSIS

Prognosis and follow-up

- Recovery typically takes **3–6 months**, with physical activity aiding recovery better than bed rest.
- **Mortality is very low**, likely less than 2%.
- **Relapse occurs in up to 30%** of poorly compliant patients, often responding well to extended therapy.
- **Follow-up for 2 years** is recommended to monitor for recurrence, which can be localized or systemic.
- Poor outcomes are seen in cases of **heart failure due to endocarditis**.
- Patients with **heart or lung disease** may experience more severe effects.
- **Weight gain and general well-being** are better indicators of recovery than serology.
- **IgG antibodies** can remain elevated for over 2 years post-treatment.
- **Immunity is not long-lasting**, and reinfection can occur with repeated exposures.
- Future diagnostics using **antigen or DNA tests** may improve the detection of ongoing infections.

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BRUCELLOSIS

Case Classification

SUSPECTED CASE

A case that is compatible with the clinical description and is epidemiologically linked to a suspected/confirmed animal cases or contaminated animal products.

PROBABLE CASE

An illness characterized by acute or insidious onset of fever with any of the following:

Night sweats, arthralgia, headache, fatigue, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis, or focal organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly)

AND

Important risk factors to be kept in mind are

- Slaughterhouse workers
- Meatpacking plant employees
- Veterinarians
- Ingesting undercooked meat
- Consumption of unpasteurized/raw dairy products.
- Assisted animals giving birth

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BRUCELLOSIS

Notes

BRUCELLA

BRUCELLOSIS

RESCUE LOSS

BSEB/2011/10534

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Radiology of the Spine: Differentiation of Brucellosis from Tuberculosis

Feature	Brucellosis	Tuberculosis
Site	Lumbar and other regions	Dorsolumbar
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Osteophyte Formation	Anterolateral ("parrot beak")	Unusual
Deformity	Wedging uncommon	Anterior wedge, gibbus
Recovery	Sclerosis, whole-body involvement	Variable
Paravertebral Abscess	Small, well-localized and discrete	Common
Psoas Abscess	Rare	More likely

RECELLULOSIS

Case Scenario

Patient: 6 year old boy	Emergency Department Findings	Key History Finding	Diagnosis and Treatment	Lab Test	Result	Normal Range
Chief Complaint: Persistent high-grade fever (39–40°C) with chills and rigors for 3 weeks, occurring 3–4 times daily Previous Treatment: Managed at multiple hospitals with IV ceftriaxone, amikacin, and artemether; fever persisted despite treatment Past & Family History: Unremarkable	Vitals: Temperature 40°C, heart rate 130 bpm Physical Exam: No rash, lymphadenopathy, or hepatosplenomegaly	Exposure: Consumed unboiled goat milk for years and had direct contact with goats at home	Diagnosis: Suspected brucellosis, confirmed with elevated serum antibody titer (1:640; normal <1:80) Treatment Initiated: Rifampicin (10 mg/kg/day) and doxycycline (4 mg/kg/day)	Total White Blood Cell Count Neutrophils Lymphocytes Blood Culture Urine Culture Malaria Test ICT Malaria	4.5 × 10 ⁹ /L 45.6% 47.6% No growth No growth Negative Negative	510 × 10 ⁹ /L 4070% 2040%

Follow-up (2 weeks): Afebrile, no documented fever recurrence

RESCUE LOSS

Notes

[illegible]

Session-4

Brucellosis in Animals

Course No. 004	Session 4: Brucellosis in Animals
Duration	15 minutes
Learning Objective	• Understand the disease transmission and clinical manifestation. • Understand the treatment and prevention in animals
Pre-requisite knowledge/skill	• Familiarity with common zoonotic pathogens and transmission routes in animals. • Understanding of principles of disease prevention, vaccination, and antimicrobial use in veterinary practice.
Competencies to be achieved	• Enumerate Brucellosis transmission in animals. • Describing the clinical manifestation in animals, their treatment and prevention.

BRUCELLOSIS IN ANIMALS

Brucellosis is a highly contagious disease with substantial economic implications for livestock industries. The highest incidence of this disease is particularly evident in regions such as the Middle East, the Mediterranean area, sub-Saharan Africa, China, India, Peru, Mexico, and countries in Central and Southwest Asia. Brucellosis is not a uniform disease, as different types of *Brucella* bacteria infect different animal species, further complicating efforts to control and manage the disease. This zoonotic infection poses a significant threat to both animal and human health, making its surveillance and control crucial for the well-being of livestock and the people who depend on them.

Brucellosis in animals is primarily spread through various modes of contact with infected tissues and fluids. Direct contact with birthing tissues and fluids, including the placenta, aborted fetuses, fetal fluids, and vaginal discharges, poses a significant risk of transmission. In addition, ingestion of contaminated substances such as milk, blood, urine, and semen from infected animals can lead to the transmission of *Brucella* bacteria. Furthermore, the disease can be contracted through direct contact with mucous membranes, such as the eyes, nose, and mouth, or through breaks in the skin when handling infected animals. It is important to note that *Brucella* can also be transmitted indirectly via contaminated objects, known as fomites, which may include equipment, clothing, shoes, hay, feed, or water.

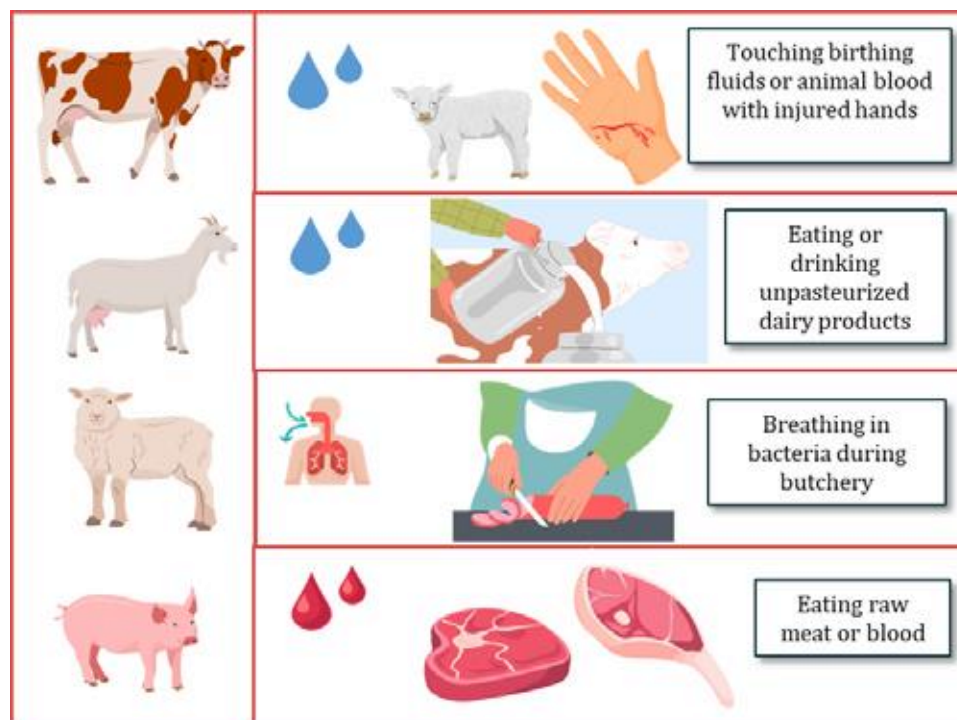


Fig 10: Transmission routes

Key clinical signs include infertility, recurrent abortions, stillbirths, and a progressive decline in milk production. Affected animals may also develop arthritis, mastitis, lameness, and skin lesions, compromising their overall health and farm productivity.



Fig 11: Clinical Manifestations in Animals

What makes brucellosis particularly challenging is that infected animals can shed bacteria into the environment, even if they show no signs of illness. Asymptomatic carriers can continue to excrete the pathogen for extended periods, posing a continuous risk to other animals within the herd. This underscores the importance of implementing stringent biosecurity measures to prevent the spread of brucellosis in livestock populations, thereby safeguarding both animal health and the livelihoods of those who depend on them.

Preventing and controlling brucellosis in animals requires a multi-pronged approach. This includes regular testing and surveillance, culling of infected animals, and vaccination in some cases to reduce the prevalence of the disease within herds. Strict hygiene and biosecurity practices, such as disinfecting equipment and maintaining quarantine measures, are also essential in preventing the transmission of *Brucella* between animals.

In regions with a high prevalence of brucellosis, public health efforts often extend to educating livestock owners and workers about the risks associated with the disease and the importance of personal protective measures. These comprehensive strategies aim not only to protect animal health but also to reduce the potential for zoonotic transmission, thereby safeguarding the well-being of both animals and humans in close contact with them.

Treatment of Brucellosis In Animals

The treatment of brucellosis in animals presents a formidable challenge due to the intracellular sequestration of the *Brucella* organisms within various tissues, including lymph nodes, the mammary gland, and reproductive organs. *Brucella* species are known to be facultative intracellular bacteria, capable of surviving and multiplying within the cells of the macrophage system, which is a key component of the immune response. Treatment failures in brucellosis are primarily attributed to the inability of antimicrobial drugs to effectively penetrate the cell membrane barrier where the bacteria reside. This unique intracellular survival mechanism, rather than the development of antimicrobial resistance, is the primary reason behind the

limited success of treatment in infected animals. As a result, prevention, through strategies such as vaccination and stringent biosecurity measures, remains a central focus in the control and management of brucellosis in animal populations.

Prevention and Control of Animal Brucellosis

Preventing and controlling brucellosis in animals is crucial not only for the well-being of livestock but also to reduce the incidence of infection in humans. Several key measures are integral to managing the disease:

Vaccinating Animals

Vaccination is a cornerstone in the efforts to control brucellosis in animals. Administering effective vaccines to livestock, particularly cattle, sheep, and goats, helps reduce the prevalence of the disease within herds. This strategy not only protects animal health but also minimizes the potential for zoonotic transmission to humans. Vaccination with *B. abortus* Strain 19 are employed, with the goal of achieving a vaccination coverage of over 90% within a span of 7 to 10 years, ultimately working toward the elimination of the disease.

Cleaning and Disinfecting

Proper cleaning and disinfection of areas that have been exposed to diseased animals, their urine, blood, milk, or discharges are vital in preventing the spread of the disease. Thorough disinfection protocols help reduce the environmental reservoir of *Brucella* bacteria, decreasing the risk of transmission to both animals and humans.

Isolation

Isolating healthy animals from infected ones is a key biosecurity measure to prevent the spread of brucellosis. This practice minimizes direct contact between susceptible and infected animals, limiting the potential for transmission and the development of new cases within a herd or flock.

Examination and Culling

In the case of sheep, regular examination of rams before the breeding season is essential. Culling of rams with genital abnormalities or signs of infection helps reduce the transmission of *Brucella* organisms during mating, thereby lowering the prevalence of brucellosis in sheep populations.



Fig 12: Prevention in Animals

National Animal Disease Control Programme (NADCP)

The **National Animal Disease Control Programme (NADCP)**, launched in September 2019 by the Hon'ble Prime Minister, is a flagship initiative to combat Foot and Mouth Disease (FMD) and brucellosis. It aims to vaccinate 100% of cattle, buffaloes, sheep, goats, and pigs against FMD, and all bovine female calves (4-8 months) against brucellosis. The programme has a budget of ₹13,343 crore for five years (2019-2024).

Objectives

The NADCP targets controlling FMD by 2025 through vaccination and achieving its eradication by 2030. It also focuses on intensive brucellosis management in animals, reducing its impact on human health. The programme is fully funded by the Central Government under its Central Sector Scheme.

Major Activities

- 1. Mass Vaccination:**
 - Regular vaccination of susceptible animals every six months.
 - Primary vaccination of bovine calves (4-5 months).
- 2. Preparatory Steps:**
 - Deworming prior to vaccination.
 - Awareness campaigns and training at village, state, and national levels.
- 3. Animal Identification and Records -**
 - Ear-tagging animals and registering them in the **INAPH system**.
 - Keeping vaccination records using Animal Health cards.
- 4. Monitoring and Surveillance -**
 - Sero-surveillance and sample testing (pre- and post-vaccination).
 - Investigating outbreaks and virus typing.
- 5. Logistics and Regulation -**
 - Ensuring proper storage with cold-chain equipment.
 - Quarantine and movement regulation during outbreaks.
- 6. Data and Evaluation -** Tracking progress through data analysis and impact assessment.
- 7. Incentives -** Vaccinators receive ₹3 per vaccination dose and ₹2 for ear-tagging and data entry.

Through its comprehensive measures, the NADCP aims to strengthen animal health, reduce economic losses, and mitigate zoonotic risks.

POINTS TO REMEMBER

- Brucellosis in animals is highly contagious, spreading through contact with infected tissues, fluids, or contaminated objects. Asymptomatic carriers can shed bacteria into the environment, maintaining a constant risk of transmission.
- Vaccination is crucial for controlling brucellosis in livestock. Effective vaccination programs aim for over 90% coverage within a 7 to 10 year span to significantly reduce disease prevalence.
- Prevention and control efforts include strict biosecurity measures like isolation of infected animals, cleaning and disinfection, regular examinations, and culling when necessary.
- The National Animal Disease Control Programme (NADCP) targets the eradication of brucellosis by 2030 through mass vaccination, surveillance, and robust record keeping systems, supported entirely by the Central Government.

- A contagious disease of livestock with significant economic impact.
- The highest incidence is observed in the **Middle East, Mediterranean region, SubSaharan Africa, China, India, Peru, and Mexico** and also countries in **central and southwest Asia**.
- Different *Brucella* types infects different animal species

- Direct contact with infected birthing tissues and fluids (e.g., placenta, aborted fetuses, fetal fluids, vaginal discharges)
- Ingestion of contaminated milk, blood, urine and semen found in infected animals
- Direct contact with mucous membranes (eyes, nose, mouth), or breaks in the skin of infected animals
- *Brucella* can also be transmitted by contaminated objects (fomites) such as, equipment, clothing, shoes, hay, feed or water
- Asymptomatic animals with no signs of illness can shed the bacteria into the environment for long periods of time, infecting other animals in the herd.



Infertility	Continuous reduction in milk yield	Arthritis in cows and pigs, mastitis and lameness in goats and oozing skin lesions in horses	Frequent abortions and still births

- Treatment is unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs.
- *Brucella* spp. are facultative intracellular bacteria that can survive and multiply within the cells of the macrophage system.
- Treatment failures are considered to be due not to the development of antimicrobial resistance but rather to the inability of the drug to penetrate the cell membrane barrier.

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The disease can be reduced by regular examination before breeding and culling of animals with abnormalities

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BRUCELLOSIS

National Animal Disease Control Programme (NADCP) is a flagship scheme launched by Hon'ble Prime Minister in September 2019 for control of Foot & Mouth Disease and Brucellosis by vaccinating 100% cattle, buffalo, sheep, goat and pig population for FMD and 100% bovine female calves of 4-8 months of age for brucellosis with the total outlay of Rs.13,343.00 crore for five years (2019-20 to 2023-24).



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RECELLULOSIS

Mass Vaccination of Bovine Calves



Publicity and Awareness Campaigns till Block Level



Strengthening of Laboratories and Remuneration for Mobilizations



Post Vaccination Sero-monitoring by Two Stage Sampling Schemes



Recording in Central Database with Unique ID Eartags

Consider the flow models in Fig. 10. Multiple

R52HFE1.1 OS/2

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Session-5

Laboratory Diagnosis

Course No. 005	Session 5: Laboratory Diagnosis
Duration	45 minutes
Learning Objectives	• Describe the key diagnostic methods used for brucellosis, highlighting advantages and limitations. • Identify the biosafety protocols required for handling <i>Brucella</i> specimens to minimize the risk of laboratory acquired infections.
Pre-requisite knowledge/skill	• Basic understanding of microbiological laboratory techniques and diagnostic principles. • Familiarity with serological and molecular diagnostic tools. • Awareness of laboratory biosafety levels and infection control practices.
Competencies to be achieved	• Ability to select and perform appropriate laboratory diagnostic tests (serological, culture, molecular) based on clinical presentation and resource availability. • Proficiency in recognizing <i>Brucella</i> colonies and interpreting results while ensuring biosafety during diagnostic procedures. • Capability to recommend diagnostic approaches and safety practices for effective management of suspected brucellosis cases in a laboratory setting.

LABORATORY DIAGNOSIS

Human brucellosis can affect any organ or body system, and its symptoms are non-specific, making it easily confused with other medical conditions. This lack of distinguishing features means that brucellosis may be misdiagnosed, leading to unnecessary treatments and potentially overlooking other serious illnesses, whether infectious or non-infectious. The treatment of brucellosis can be difficult, as it requires prolonged use of antimicrobial combinations that are not typically prescribed for other infections. Accurate diagnosis of brucellosis is critical, not only for effective patient care but also for public health. A correct diagnosis may point to exposure to infected animals, consumption of contaminated food (particularly dairy), lapses in laboratory safety, or even the deliberate release of the bacteria as a biological weapon.

In India, laboratory testing for brucellosis is conducted in a variety of settings, including public health laboratories, veterinary institutes, research facilities, and designated biosafety level-2 (BSL-2) or higher laboratories. The diagnostic approaches primarily involve direct methods to detect the organism and indirect methods to identify host immune responses. The following sections provide an overview of these methodologies and their application in the Indian context.

Evaluation of a Patient Suspected of Brucellosis

A comprehensive evaluation of a patient suspected of having brucellosis involves both clinical and laboratory investigations to confirm the diagnosis and assess disease severity. The key diagnostic steps are outlined below:

1. Complete Blood Count (CBC)

A CBC often reveals neutropenia and anemia in brucellosis patients. Thrombocytopenia may also occur, potentially due to hepatosplenomegaly or immune-mediated processes. In some cases, pancytopenia is observed, reflecting more extensive bone marrow involvement.

2. Inflammatory Markers

Patients typically exhibit elevated levels of inflammatory markers, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum lactate dehydrogenase (LDH), and alkaline phosphatase. These elevations correlate with the systemic inflammatory response.

3. Liver Enzymes

Hepatic involvement is common in brucellosis, with raised liver enzyme levels often indicating hepatomegaly and the extent of liver involvement. Monitoring these enzymes helps gauge disease severity.

4. Culture Techniques

Brucellosis can be diagnosed through blood or bone marrow cultures. Blood cultures may take over a week due to the slow growing nature of *Brucella*. Bone marrow cultures generally provide a higher diagnostic yield because the bacteria tend to localize in the reticuloendothelial system. Specimen handling should follow strict biohazard protocols due to the risk of aerosol transmission.

5. Serologic Testing

Serology is widely used in endemic regions. The standard agglutination test is common, with titers above 1:160 considered indicative of infection when paired with clinical symptoms. Higher titers, especially above 1:320, are more specific. Complementary tests like the Rose Bengal test and Enzyme Linked Immunosorbent Assay (ELISA) enhance diagnostic accuracy. Serial testing may be necessary if initial titers are low.

6. Polymerase Chain Reaction (PCR)

PCR provides a rapid and highly sensitive method to detect *Brucella* DNA in blood specimens. Targeting genes such as BCSP31 and the 16S-23S rRNA operon, PCR is particularly valuable for early diagnosis.

7. Imaging Studies

Radiological findings may include spondylodiscitis features, such as anterior vertebral erosions, disc space narrowing, and bone destruction (e.g., Pedro Pons sign). Hepatic imaging may reveal nonspecific hepatitis or granulomas.

8. Biopsy

In cases requiring definitive diagnosis, biopsies may be performed. Bone marrow aspiration and biopsy are helpful for identifying *Brucella* in challenging cases. Liver biopsies, especially in patients with suspected hepatic granulomas, can provide evidence of granulomatous hepatitis or hepatic microabscesses.

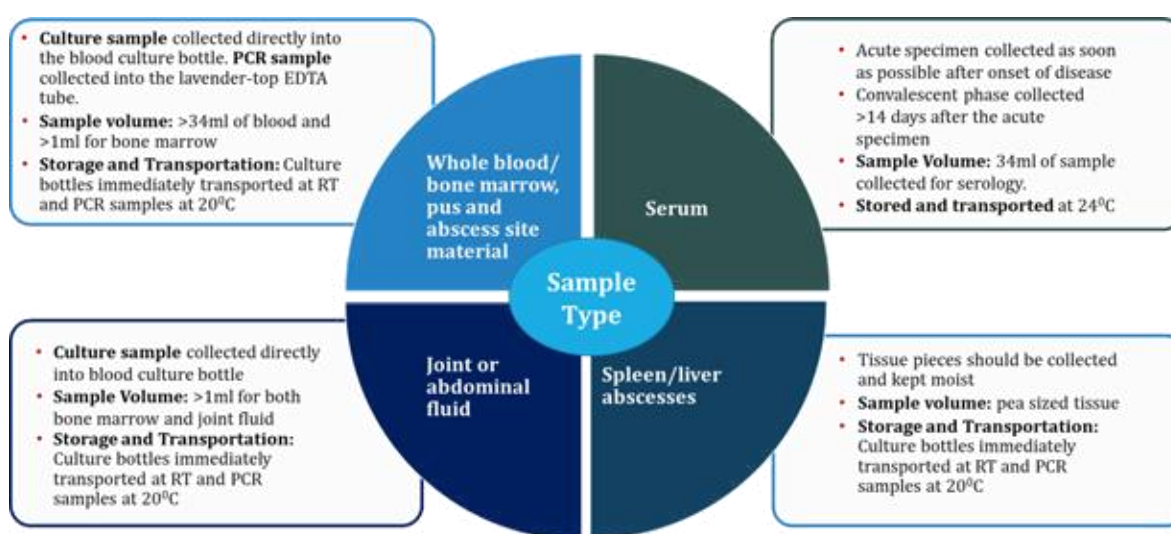


Fig 13: Preferred sample for laboratory tests

Sample Collection, Storage and Transport

Sample collection and transportation: Although Brucellae can be isolated from bone marrow, blood, cerebrospinal fluid, wound, pus, tissue, etc., can also be used depending on the clinical scenario. However, the specimens need to be obtained early in the disease for bacteriological culture. Samples should ideally be collected before initiating antimicrobial therapy to avoid reduced diagnostic sensitivity. It should be ensured that samples are appropriately stored and transported to prevent Brucella degradation, particularly for cultures.

Table 3: Sampling window depending upon the sample collected from the patient.

Time (Days/Weeks)	Symptoms	Samples	Diagnostic Tests
Day 1–7 (Acute Phase)	Fever, night sweats, fatigue, anorexia, muscle/joint pain.	Blood (Serum/Plasma)	Blood/Bone Marrow Culture - Gold standard for early detection. RT-PCR - Detects Brucella DNA.
Day 7–14 (Transition Phase)	Persistent fever, localized pain (e.g., arthritis, back pain).	Blood (Serum/Plasma)	IgM ELISA - Detects recent infection. SAT - Confirms diagnosis with specific titers.
Day 14–21 (Chronic/Recovery Phase)	Resolving or recurring symptoms; complications like arthritis, neurobrucellosis.	Blood (Serum/Plasma)	IgG ELISA - Indicates ongoing or past infection. Coombs Test - For low-titer antibodies in chronic stages.

Serum samples should be packaged in an airtight and leak proof container & kept at temperatures of 4-10°C. Ensure samples are not placed directly on the ice used for cooling the shipping container. For culture, samples should be transported at room temperature.

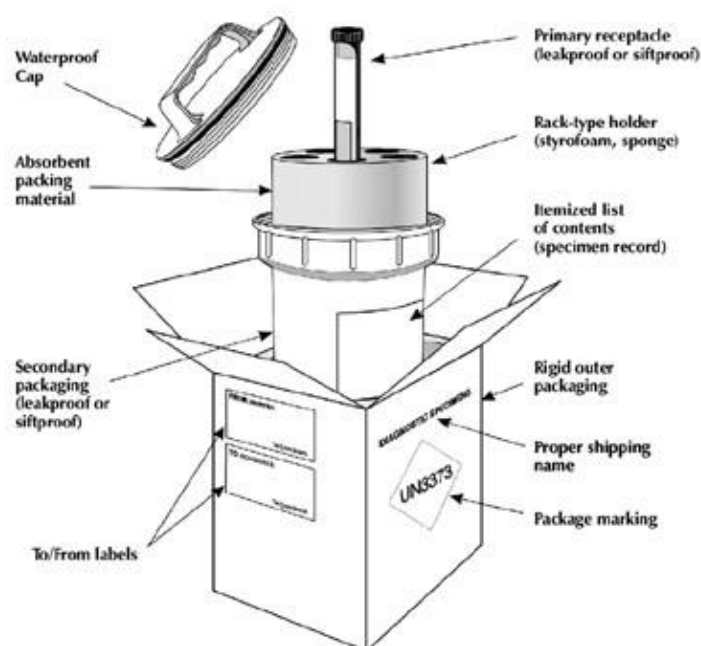


Fig 14: Packing of specimens for transport to laboratories

Table 4: Storage and Transport of different kinds of samples

Sample	Remarks	Volume	Storage & Transport
Whole blood or bone marrow, pus, abscesses material	- Considered the best specimen for culture. - Collect directly into an appropriate blood culture bottle. - Collect whole blood into lavender top EDTA collection tubes for PCR. ≤4 days post symptom onset for PCR based diagnosis.	- Collect > 3-4ml blood. - Collect > 1ml for bone marrow.	Culture: Transport bottles at room temperature as soon as possible to obtain the diagnosis PCR: Sample storage at -20°C. Transport at -20°C. Dry ice could be used.
Serum	- Collect at least 3-4 ml without anticoagulant for serologic diagnosis. - Acute specimen is collected as soon as possible after onset of disease. - Convalescent-phase should be collected >14 days after the acute specimen.		- Serological test: Sample storage: at 2 - 4°C. - Transport at 2 - 4°C.
Joint or abdominal fluid	Collect directly into an appropriate blood culture bottle.	- Collect > 1ml for bone marrow. - Collect > 1ml for joint fluid.	Culture: Transport bottles at room temperature as soon as possible to obtain the diagnosis PCR: Sample storage at -20°C. Transport at -20°C.

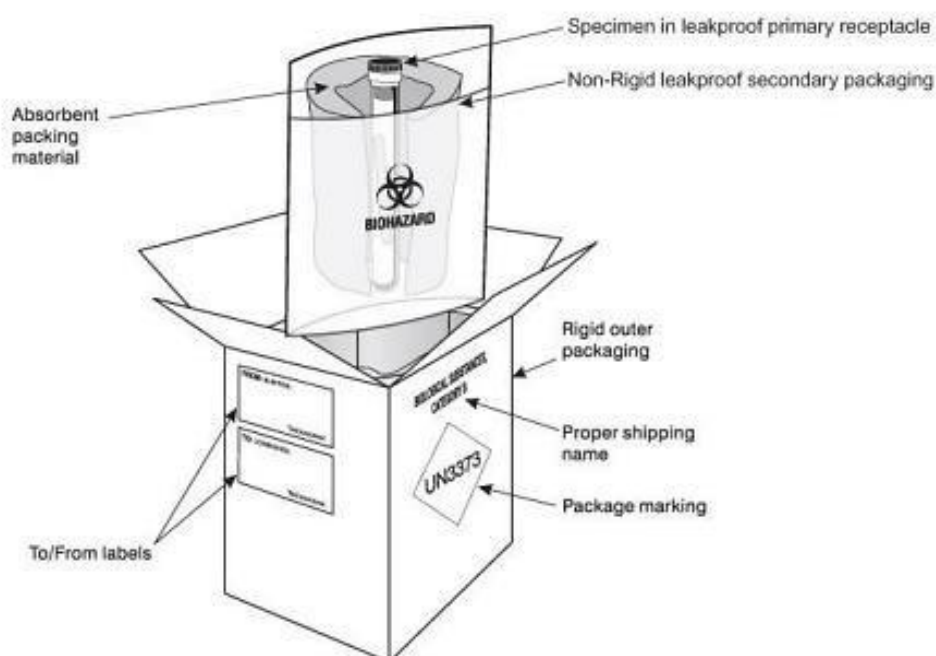


Fig 15: Basic triple packaging system

Safety Measures

Use of Class II Biological Safety Cabinet: All Brucella manipulations should be performed in a safety cabinet to prevent aerosol formation and exposure. For handling suspect cultures, animal necropsies, and conducting experimental studies with animals, it is advised to employ BSL-3 and ABSL-3 containment measures, equipment, and facilities. BSL-3 practices are also suitable for preliminary work on cultures or contaminated samples intended for automated identification systems. Similarly, BSL-3 containment protocols, equipment, and infrastructure are recommended for production related tasks. For initial inoculation of cultures derived from potentially infectious clinical samples, BSL-2 level practices, containment tools, and facilities are recommended. Activities with a high likelihood of producing infectious aerosols should utilize primary containment tools, such as biological safety cabinets, centrifuge safety cups, or sealed rotors.

Specimen Handling

Blood cultures – These are the most common source of Brucella. Although automated blood culture systems reduce risks, exposure increases during and after incubation when handling solid or liquid media.

Safety Cabinets – Positive blood culture vials should be processed in safety cabinets until Brucella is ruled out.

Avoid Open Bench Work – Small Gram-negative coccobacilli growing on blood and chocolate agar but not on MacConkey agar should not be handled on an open bench. A “rule-out-or-refer” approach should be used.

Discontinue Aerosol Generating Procedures – In high-risk regions, procedures such as the Isolator microbial tube method and antibiotic susceptibility testing on Brucella isolates have been discontinued to minimize exposure.

Regular Surveillance – Establish infection control policies that include routine blood cultures in safety cabinets and limit aerosol generating activities.

Laboratory Tests for Brucellosis

Diagnosing brucellosis in both humans and animals involves a range of methods that help healthcare providers and veterinarians confirm the presence of the disease. These diagnostic techniques includes -

Serological Tests

- Standard Agglutination Test
- Rose Bengal Test (RBT)
- ELISA (Enzyme Linked Immunosorbent Assay)

Bacterial Culture

- Culture on Agar Plates
- Culture by Castaneda Method
- Automated Culture

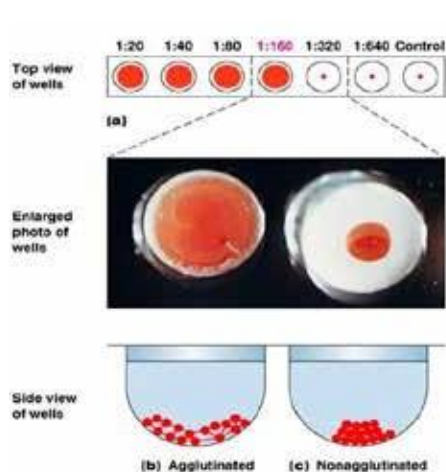
NAAT

- Polymerase Chain Reaction (PCR)
- Real-Time PCR

Serological Tests

A. Standard Agglutination Test

The Standard Agglutination Test is a valuable diagnostic tool used to determine the antibody titers present in a patient's serum. This test is specifically designed to detect antibodies directed against the O-polysaccharide (LPS) component of *Brucella*. It utilizes an antigen derived from a killed strain of *Brucella abortus* to facilitate the detection of antibodies targeting this critical LPS component. The Standard Agglutination Test is highly effective in diagnosing infections caused by several *Brucella* species, including *B. abortus*, *B. melitensis*, and *B. suis*, as it focuses on a shared antigenic element among these pathogens. However, it's essential to recognize that this particular test is not suitable for diagnosing infections stemming from *Brucella canis*, as this particular species lacks the O-polysaccharide (LPS) component. The Standard Agglutination Test plays a pivotal role in the accurate identification and differentiation of *Brucella* species, aiding in the prompt diagnosis and management of brucellosis in affected individuals or animal populations.



(a) Each well in this microtiter plate contains, from left to right, only half the concentration of serum that is contained in the preceding well. Each well contains the same concentration of particulate antigens, in this instance red blood cells.

(b) In a positive (agglutinated) reaction, sufficient antibodies are present in the serum to link the antigens together, forming a mat of antigen-antibody complexes on the bottom of the well.

(c) In a negative (non-agglutinated) reaction, not enough antibodies are present to cause the linking of antigens. The particulate antigens roll down the sloping sides of the well, forming a pellet at the bottom. In this example, the antibody titer is 160 because the well with a 1:160 concentration is the most dilute concentration that produces a positive reaction.

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Fig 16: Standard Agglutination Test

B. Rose Bengal Test

The Rose Bengal test (RBT) serves as a widely utilized tool in hospital settings for the swift diagnosis or exclusion of brucellar infections, particularly in patients presenting with symptoms of a febrile syndrome. This diagnostic test involves a card format and employs an 8% suspension of pure, smooth killed cells derived from *Brucella abortus* strain 99 that has been phenolized. The bacterial suspension is combined with Rose Bengal dye, buffered to maintain a specific pH of 3.65 ± 0.05 .

One noteworthy characteristic of the RBT is its ability to react with both IgG and IgM antibodies. Notably, it tends to detect IgG antibodies at an earlier stage, making it particularly useful for identifying subclinical infections. Moreover, it remains effective over an extended period during the disease, even in its chronic stage, which distinguishes it from conventional tube agglutination tests.

The results of the RBT are based on the presence or absence of visible agglutination, serving as an indicator for the presence or absence of antibodies in the tested samples. If agglutination is observed, it signifies an antibody anti-Brucella concentration equal to or greater than 25 IU/mL. The test is examined for agglutination in bright light. The last dilution showing agglutination is taken as positive. A titre is defined as the reciprocal of the highest dilution of serum, which produces a definite agglutination reaction.

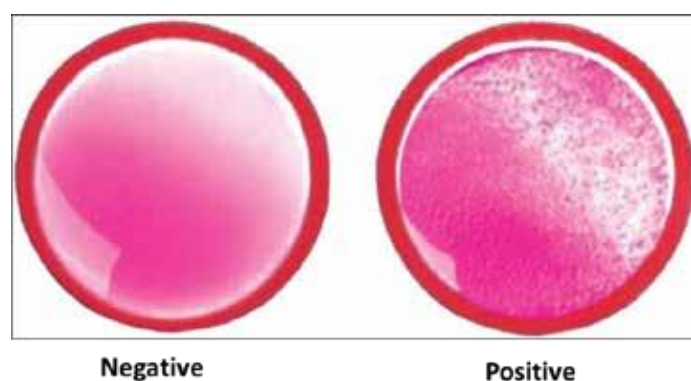


Fig 17: Rose Bengal Test

C. ELISA

ELISA stands out as a highly sensitive, rapid, and convenient method for definitively diagnosing brucellosis. This diagnostic approach boasts both high sensitivity and specificity, making it a valuable tool for routine laboratory testing when brucellosis is suspected in clinical practice.

One of the notable advantages of ELISA is its suitability for screening brucella specific IgG and IgM antibodies in patient sera. By detecting these antibodies, ELISA enables accurate identification and confirmation of brucella infections, thereby contributing to timely and effective patient management. Its sensitivity and speed make it a preferred choice for clinicians and laboratory professionals when diagnosing this infectious disease.

Culture

Blood culture remains a critical diagnostic method for confirming brucellosis, particularly in its early stages when serological tests may yield negative or inconclusive results. The isolation of *Brucella* from blood provides definitive evidence of infection, facilitates precise species identification, and enables genotyping to trace sources and assess antibiotic susceptibility. Despite challenges such as *Brucella*'s slow growth, low bacterial concentration in blood, and intermittent bacteremia in advanced cases, blood cultures continue to play a vital role in the diagnosis and management of the disease.

The slow-growing nature of *Brucella* and its ability to evade host immune responses make its detection in blood challenging. Prolonged incubation and specific laboratory techniques are often necessary. Automated systems may miss the bacterium if standard protocols are followed, as *Brucella* may require incubation beyond the usual 5–7 days.

Methods of Blood Culture

Different blood culture methods have evolved to enhance the detection of *Brucella* species:

1. Conventional Monophasic Method -

- This traditional approach involves inoculating blood into liquid media such as Tryptic Soy Broth (TSB) or Brain Heart Infusion (BHI).
- Bottles are incubated at 37°C and monitored for turbidity or growth signs.
- Subcultures are performed periodically on agar plates to detect slow-growing organisms.
- While cost-effective, sensitivity can be low unless extended incubation (up to 4 weeks) is employed.

2. Biphasic Methods -

- **Castañeda Method:** This inexpensive technique uses a biphasic medium comprising solid agar and liquid broth in a single container, reducing the need for repeated subcultures. The broth is periodically tilted to bathe the agar, encouraging bacterial growth. Although widely used in resource-limited settings, it requires extended incubation (up to 35 days) and may not distinguish *Brucella* from contaminants without further testing.
- **TUMS Medium:** An enhancement of the Castañeda method, this biphasic medium uses a urea agar base to leverage the urease activity of *Brucella* for faster identification.
- **Hemoline Medium:** A modified biphasic medium specifically designed for *Brucella* blood culture. This medium combines liquid enrichment with a solid surface for bacterial recovery and reduces contamination risks. It is faster than the Castañeda method in identifying *Brucella* but is less commonly available.

3. Lysis-Based Methods -

- These techniques improve detection by lysing host cells to release intracellular bacteria. The lysed samples are then plated on solid media.
- **Lysis-Centrifugation:** Highly effective in reducing detection time, this method is particularly useful in acute infections. Studies from India have shown its superiority, with a detection rate exceeding 90% and faster results (2–3 days) compared to traditional methods.

- **Isolator Tubes:** Commercially available lysis-centrifugation systems incorporate anticoagulants and detergents, streamlining the process. These tubes offer improved sensitivity and significantly reduced time to positivity.

4. Automated Systems -

Automated Blood Culture Systems for Brucella Detection

Automated blood culture systems represent a significant advancement in microbiological diagnostics. These systems streamline the detection of BRUCELLA species by automating incubation, monitoring, and alerting for bacterial growth, thereby reducing human error, labor, and time to detection. However, because BRUCELLA is a slow-growing and intracellular pathogen, specific considerations must be addressed to optimize the use of these systems for brucellosis diagnosis.

Common Automated Systems

1. BACTEC™ System (BD Diagnostics):

- Uses specific culture bottles such as the **BACTEC Myco/F Lytic vials**, designed for intracellular organisms like BRUCELLA.
- **Principle:** The system monitors the metabolic activity of microorganisms by detecting carbon dioxide production or oxygen consumption. These changes indicate bacterial growth.
- **Advantages:**
 - Continuous, automated monitoring every 10 minutes.
 - Faster detection compared to conventional methods (averaging 5–7 days for BRUCELLA versus 10–14 days manually).
 - Reduction in contamination risks due to closed system design.
- **Challenges:**
 - Requires extended incubation periods for slow-growing bacteria like BRUCELLA (up to 30 days).
 - Specific vials for lysis and cell processing may increase costs.

2. BacT/ALERT® System (bioMérieux):

- Utilizes colorimetric technology to monitor microbial growth in specialized culture bottles.
- Detects changes in pH or carbon dioxide production that result in color shifts within the culture medium.
- **Advantages:**
 - High sensitivity for a variety of pathogens, including BRUCELLA, with appropriate media (e.g., BacT/ALERT FA Plus for aerobic organisms).
 - User-friendly interface with automated alert notifications.
- **Challenges:**
 - Requires prolonged incubation for BRUCELLA, as early signals may not appear within routine observation periods.

3. VersaTREK® System (Thermo Fisher Scientific):

- Detects microbial growth by monitoring changes in gas pressure within the culture bottle.
- Specifically designed media, such as VersaTREK REDOX 1, enhance the recovery of slow-growing organisms.
- **Advantages:**
 - Simplified operation with automated incubation and detection.
 - Enhanced recovery rates for organisms, including BRUCELLA, in complex blood samples.
- **Challenges:**
 - Sensitivity is highly dependent on proper specimen collection and media choice.

Challenges in Blood Culture

- **Low Sensitivity:** The sensitivity of blood culture varies widely (10–90%) due to factors like low bacterial load, prior antibiotic use, or intermittent bacteremia.
- **Extended Incubation:** Routine 5–7 day protocols may not suffice for Brucella recovery, with some cultures requiring up to 4 weeks.
- **Safety Concerns:** Handling Brucella in laboratories necessitates stringent biosafety measures to prevent lab-acquired infections.

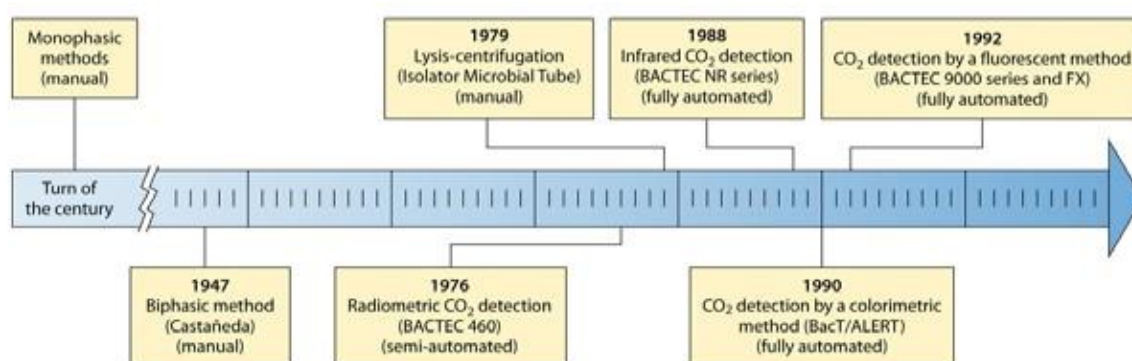


Fig 18 : Timeline for introduction of Brucella blood culture methods

Colonies will be subjected to Gram Staining and other biochemical tests. In Gram staining, Gram negative, coccobacillus will be seen. The modified ZN stain of blood culture bottles/ pus can also be done which shows acid-fast coccobacillus.

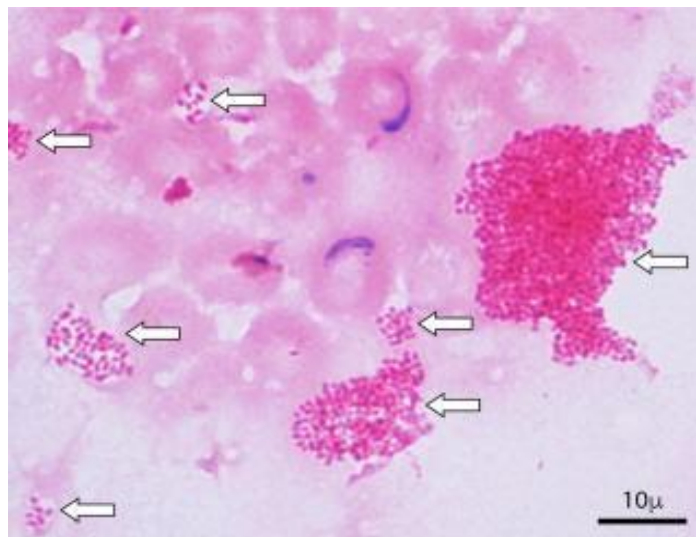


Fig 19: Gram stain of a positive aerobic Bactec blood culture vial showing *Brucella melitensis* microcolonies(arrows).

Table 5: Various Biochemical tests which need to be done following culture is listed below

Species	Growth on dye at routine test dilution			H2S production	Urease test (maximum time to positivity)
	Fuchsin	Thionine	Safranin		
<i>B. melitensis</i>	Yes	Yes	Yes	No	24 h
<i>B. abortus</i>	Yes ^b	No	Yes ^c	Yes	24 h
<i>B. suis</i>	No ^d	Yes	No	No	15 min
<i>B. canis</i>	Variable	Yes	No	No	15 min
<i>Marine species</i> ^e	Yes	Yes	Yes	No	90 min

aMany strains show lack of correlation with species.

bExcept biotype 2.

cExcept biotype 5.

dExcept biotype 3.

eB. pinnipedialis and B. ceti.

3. NAAT

A. Polymerase Chain Reaction

Compared to traditional diagnostic methods, PCR-based testing offers significant advantages, including increased speed, heightened sensitivity, specificity, reproducibility, and ease of execution.

In the context of diagnosing *Brucella* infections, a particular gene *bcsp31* is frequently targeted for DNA amplification. This gene encodes a 31-kDa antigen that is conserved among various *Brucella* species. Commercially available real-time PCR kits come pre-equipped with the necessary reagents and enzymes to specifically amplify the DNA of *Brucella* species. During the amplification process, the system emits fluorescence, which is subsequently measured by the real-time system's optical unit. This real-time PCR technology not only facilitates the efficient and accurate detection of *Brucella* DNA but also significantly reduces the time required for diagnostic results, making it a valuable tool in modern diagnostic procedures.

Genomic approaches for detecting brucellosis in humans and animals are accurate and fast, even in asymptomatic cases, but a positive result may not always indicate active infection. While nucleic acid amplification and serological tests are effective, culture remains the gold standard. Key gene targets for molecular diagnosis include bcs₃₁, which is reliable, though others like 16S rRNA and IS711 can face issues with cross-reactions or sequence variations. Various PCR methods, such as real-time PCR and LAMP, are used for species identification. Despite advancements, traditional methods like culture and serology remain essential, and reference labs play a crucial role in confirming species and tracking outbreaks.

Diagnostic Algorithm for Human Brucellosis

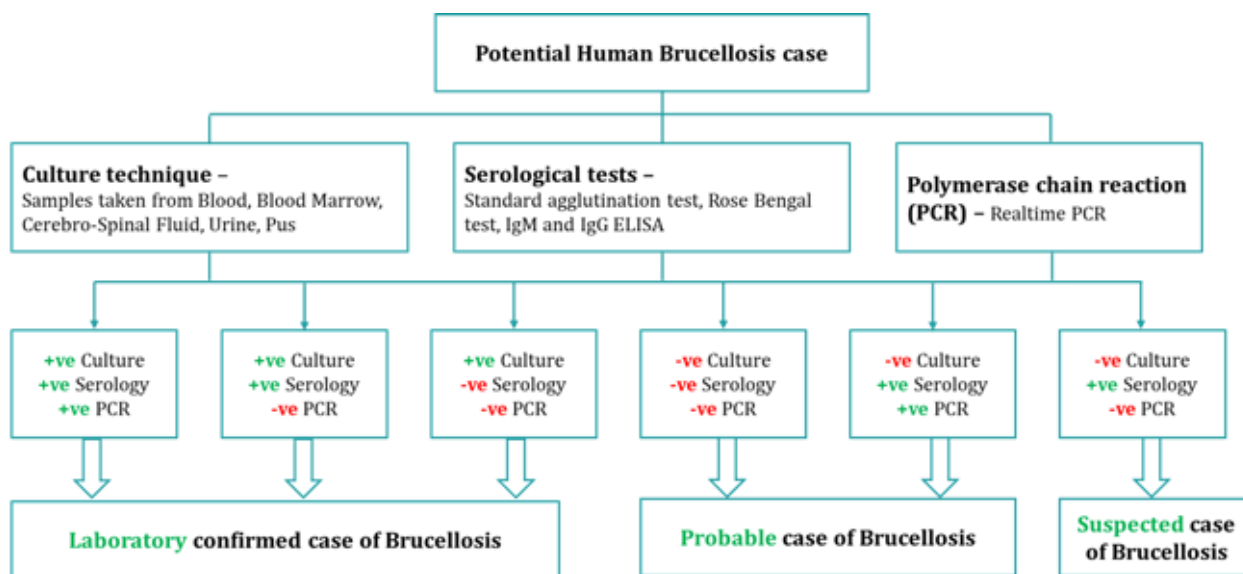


Fig 20: Diagnostic algorithm for Human Brucellosis

POINTS TO REMEMBER

- Common methods like the Standard Agglutination Test (SAT), Rose Bengal Test (RBT), and ELISA are widely used for detecting Brucella antibodies in humans and animals.
- Isolation of Brucella from blood is the gold standard for diagnosis, requiring prolonged incubation and specific media due to the bacteria's slow growth.
- Polymerase Chain Reaction (PCR) provides rapid and specific identification of Brucella DNA, making it highly valuable for early diagnosis.
- Handling Brucella requires strict adherence to biosafety levels (BSL-2 or BSL-3) to prevent laboratory acquired infections, especially during culture work or aerosol-generating procedures.

Notes

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Use of Class II Biological Safety Cabinet

BIOHAZARD



Biosafety Level 2

AUTHORIZED PERSONNEL ONLY

Containment Guidelines

- ### Handling Infectious Aerosols

Use primary containment tools like –

- 6 / A Comprehensive Learning Guide-Brucellosis

Specimen Handling Guidelines

Blood Cultures

- Primary source of Brucella.
- Use safety cabinets for processing positive vials.

Avoid Open Bench Work

Do not handle small Gram-negative coccobacilli (e.g., from blood/chocolate agar) on open benches.

- Employ a "rule-out-or-refer" strategy.

Minimizing Aerosol Exposure

- Discontinue high-risk procedures like the Isolator microbial tube method.
- Avoid direct antibiotic susceptibility testing on Brucella isolates.

Regular Surveillance

Implement infection control policies –

- Routine use of safety cabinets for blood cultures.
- Restrict aerosol-generating activities.

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BRUCELLOSIS

Methods for Diagnosis

SEROLOGICAL TESTS

- Agglutination test
- Rose Bengal Test (RBT)
- ELISA

BACTERIAL CULTURE

- Culture on Agar Plates (Blood Agar, Chocolate Agar, MacConkey Agar)
- Culture by Castaneda Method.
- Automated Culture

NAAT

- Polymerase chain reaction (PCR)/ Real Time PCR

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BRUCELLOSIS

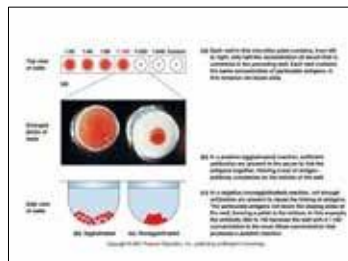
Standard Agglutination Test

Diagnosing *Brucella* spp (*B. melitensis*, *B. suis*, *B. abortus*) containing Opolysaccharide (LPS) component.

Antibody (Ab) content of serum is measured by adding a constant amount of *Brucella* antigen to serially diluted serum



High Ab titers above 80 units are considered clinically significant

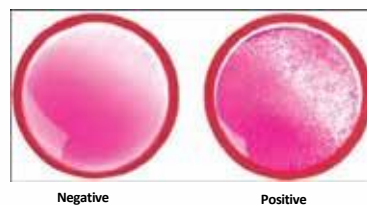


(Annexure -1)

BRUCELLOSIS

Rose Bengal Test (RBT)

- An agglutination test that uses a suspension of *Brucella abortus* in acidic buffer.
- Detects agglutinating and non-agglutinating antibodies
- Most popular method of diagnosis in hospital emergencies for being simple, rapid, cost effective and highly sensitive
- Can detect both IgG and IgM class of antibodies in the patient serum



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BRUCELLOSIS

Notes

- ELISA is a sensitive, fast, and convenient definitive method for diagnosing Brucellosis
- It shows high sensitivity and specificity and can be used as a routine lab test when Brucellosis is suspected in clinical practice.
- A best suitable method for screening of IgG and IgM *Brucella* specific antibodies in patient sera.



Image Source: <https://fos.cmb.ac.lk/blog/enzymelinkedimmunosorbentassayelisa/>

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BRUCELLOSIS

- Culture is regarded as the **"gold standard"** in the laboratory diagnosis of brucellosis due to its clinical and epidemiological importance.
- It isolates the etiologic agent from blood, bone marrow or other tissues and body fluids and allows defining the source of infection and the initial stage of the disease



Center for Data Mining, NICTD, shah@nctd.org

BRUCELOSIS

- Agar plates are used for growing bacteria.
- blood or body fluids are processed by Lysiscentrifugation (LC) technique to concentrate intracellular *Brucella* spp. in blood samples and increase the test sensitivity.
- The colonies are small, convex, smooth, translucent, slightly yellowish and opalescent, and may turn brown with age.

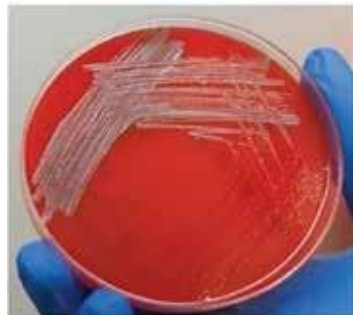


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BRUCELLOSIS

Blood culture vials are inoculated with patients' sample (Bone marrow, blood, CSF, pus), incubated at 35°C, and examined for bacterial growth



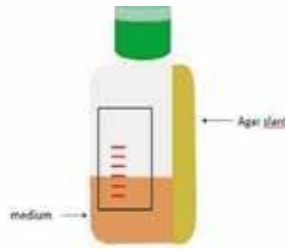
Image Source: <https://microbenotes.com/typesofculturemedia/>

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BRUCELLOSIS

[illegible]

- Castaneda Method:** a diphasic medium, one being agar (Trypticase soy agar) and another broth (Trypticase soy broth) is used for culturing *Brucella*. Subculturing is done without opening the bottle by tilting the bottle and allowing the broth to flow over agar at intervals of times. Agar surface is examined for colonies
- TUMS medium:** Castaneda flask variant method in which a solid urea agar base is used on the slant and brain heart infusion in the liquid phase. The principle behind this formulation is that all members of the genus *Brucella* exhibit positive urease activity that is revealed by the color change of a pH indicator, shortening the time required for identifying the isolate
- Hemoline medium:** Blood samples obtained from individuals with presumptive brucellosis are inoculated into flasks, incubated for 3 weeks, and subjected to terminal blind subcultures. Normally, the median time to detection is 5 days

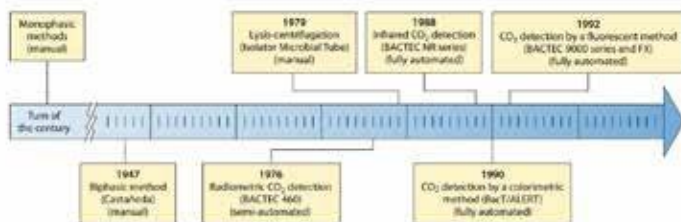


- Automated culture method determines the metabolic activity of growing bacteria by detecting either increase in the CO₂ concentration or decrease in O₂ level in the culture vials
- The time for diagnosis is shortened since changes in the gas composition can be detected considerably earlier than cloudiness of the medium becomes evident
- Some common Instrumentations: Bactec 9000 instruments, BacT/Alert system

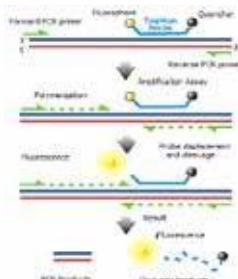


Timeline of CO₂ detection methods:

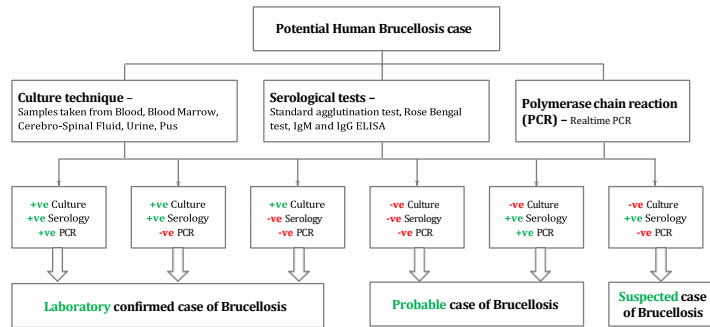
- 1947:** Bypass method (Canta-haba) (manual)
- 1972:** Lyso-centrifugation (Isolator Microbial Label) (manual)
- 1976:** Radiometric CO₂ detection (BACTEC 460) (semi-automated)
- 1988:** Infrared CO₂ detection (BACTEC 914 series) (fully automated)
- 1990:** CO₂ detection by a colorimetric method (Bac/ALERT) (fully automated)
- 1992:** CO₂ detection by a fluorescent method (BACTEC 9600 series and FR) (fully automated)



- Polymerase chain reaction (PCR) is an *in vitro* technology which amplifies nucleic acid fragments and aids in detection of an infecting organism.
- PCR based testing methods are faster and more sensitive, specific, reproducible and easy to perform as compared to the traditional methods for diagnosis
- Most frequently, the *bcsp31* gene, which encodes a 31kDa antigen conserved among *Brucella* spp. is used to amplify DNA fragments from bacteria
- Commercial ready to use real time PCR kits contains reagents and enzymes for the specific amplification of the *Brucella* spp. DNA
- Fluorescence is emitted and measured by the real time system's optical unit during amplification



Diagnostic algorithm



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BRUCELLOSIS

Notes

Session-6

Management of Brucellosis

Course No. 006	Session 6: Management of Brucellosis
Duration	15 minutes
Learning Objectives	• Understand the rationale behind using combination antibiotic therapy in the management of brucellosis. • Identify the appropriate treatment regimens for brucellosis based on patient age, pregnancy status, and disease severity. • Recognize the potential complications of brucellosis and the role of surgical intervention in managing these complications.
Pre-requisite knowledge/skill	• Basic knowledge of antimicrobial classes and their mechanisms of action. • Familiarity with principles of clinical pharmacology and patient specific treatment considerations. • Understanding of systemic infectious disease complications and indications for surgical management. • Knowledge of antibiotic classes and their indications.
Competencies to be achieved	• Develop the ability to choose an effective antibiotic regimen for brucellosis based on individual patient characteristics and clinical presentation. • Demonstrate proficiency in managing special populations, including pregnant women and children, by selecting appropriate treatment options. • Apply knowledge of symptomatic treatment to manage pain, fever, and complications associated with brucellosis.

MANAGEMENT OF BRUCELLOSIS

The primary objectives of brucellosis management are to rapidly control the disease, minimize the risk of complications, and prevent relapses. Early diagnosis, appropriate antimicrobial therapy, and, when necessary, surgical intervention, are critical to achieving these outcomes.

Antibiotic Therapy

The cornerstone of brucellosis treatment is a combination of antibiotics, as the bacteria reside intracellularly, necessitating prolonged and multidrug regimens. Monotherapy is avoided due to a high risk of relapse. Key treatment protocols include:

- **Standard Regimens:**
 - o **Doxycycline (100 mg twice daily for 6 weeks)** in combination with **streptomycin (1 g daily for 2 weeks)** is a widely used and effective treatment.
 - o An alternative combination pairs doxycycline (100 mg twice daily for 6 weeks) with **rifampin (15 mg/kg/day, typically 600–900 mg for 6 weeks)**. This regimen is often chosen for its oral administration convenience.
- **Alternative Therapies:**
 - o Streptomycin can be substituted with **gentamicin (5 mg/kg daily for 7–10 days)**, especially in settings where streptomycin is less accessible. However, no direct comparative studies exist to establish superiority between these two aminoglycosides.
- **Special Populations:**
 - o **Children:** Due to concerns about tooth discoloration and growth issues, doxycycline is avoided in children under 8 years of age. Instead, a combination of **trimethoprim sulfamethoxazole (co-trimoxazole)** with **rifampin or an aminoglycoside (streptomycin, gentamicin)** is recommended for 4–6 weeks.
 - o **Pregnant Women:** Rifampin is the preferred option during pregnancy to minimize fetal risks. Postpartum, co-trimoxazole may be added to enhance efficacy.
 - o **Complicated Cases:** For severe forms like spondylitis or sacroiliitis, a three-drug regimen of doxycycline, rifampin, and an aminoglycoside (such as gentamicin) is recommended for the initial 2–3 weeks. This is followed by an extended 6-week course of doxycycline and rifampin to ensure eradication.

Supportive Care

Symptomatic management plays a significant role in addressing discomfort and improving quality of life during treatment:

- **Antipyretics and Analgesics** are used to manage fever and alleviate pain.
- **Corticosteroids** may be administered in cases of severe complications such as brucella meningitis, although robust clinical evidence supporting their use is limited.

Surgical Interventions

Surgical management is reserved for complications arising from localized infections or structural damage:

- **Endocarditis:** This life-threatening complication may require valve replacement surgery to address damaged heart valves or repair associated fistulas.
- **Localized Abscesses:** Drainage of pyogenic joint effusions, paraspinal abscesses, or other collections may be necessary to control infection.
- **Spondylitis:** In advanced cases, surgical debridement and bone grafting are performed to manage spinal infections and restore structural integrity.

Treatment failure is rarely due to drug resistance but is commonly associated with poor adherence to therapy. Ensuring patients complete the full course of treatment is critical to prevent relapses and achieve long-term success.

POINTS TO REMEMBER

- Brucellosis is typically treated with a combination of doxycycline (100 mg twice daily for 6 weeks) and streptomycin (1 g daily for 2 weeks) or rifampin (15 mg/kg/day, 600-900 mg daily for 6 weeks).
- Monotherapy with doxycycline alone is not recommended due to a high relapse rate, with combination therapy being essential for effective treatment.
- For children under 8, doxycycline is avoided, and a regimen of trimethoprim-sulfamethoxazole with rifampin or an aminoglycoside is recommended for 4–6 weeks.
- Pregnant women are treated with rifampin during pregnancy, with co-trimoxazole added postpartum, to minimize risks to the fetus.
- In severe cases like spondylitis, a combination of doxycycline, rifampin, and an aminoglycoside is used initially for 2–3 weeks, followed by 6 weeks of doxycycline and rifampin.

Treatment of Brucellosis (Antibiotic Therapy)

Combination Therapy

- Essential due to intracellular nature of *Brucella* bacteria and to prevent relapses.
- Monotherapy is avoided due to high relapse rates.

Standard Regimens

- Doxycycline (100 mg twice daily for 6 weeks) + Streptomycin (1 g daily for 2 weeks).
- Alternative: Doxycycline (100 mg twice daily for 6 weeks) + Rifampin (15 mg/kg/day, 600–900 mg for 6 weeks).

Alternative Options

- Gentamicin (5 mg/kg daily for 7–10 days) can replace streptomycin if needed.

Complicated Cases (e.g., spondylitis/sacroiliitis)

- Initial 2–3 weeks: Doxycycline + Rifampin + Aminoglycoside (gentamicin).
- Followed by 6 weeks of Doxycycline + Rifampin.

Special Population

- **Children:** Trimethoprim-sulfamethoxazole (co-trimoxazole) + Rifampin or Aminoglycoside (4–6 weeks).
- **Pregnant Women:** Rifampin during pregnancy; co-trimoxazole added postpartum.

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BRUCELLOSIS

Notes

Supportive Care and Surgical Management



Supportive Care

Symptomatic Treatment -

- Antipyretics and analgesics for fever and pain.
- Corticosteroids for severe cases (e.g., meningitis).

Surgical Interventions

For complications such as:

- Endocarditis: Valve replacement and fistula repair.
- Spondylitis: Surgical debridement and bone grafting.
- Abscesses: Drainage of pyogenic joint or paraspinal abscesses.

Goals of Treatment

- Rapid disease control.
- Prevention of complications and relapses.

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BRUCELLOSIS

Session-7

Prevention and Control of Brucellosis

Course No. 007	Session 7: Prevention and Control of Brucellosis
Duration	15 minutes
Learning Objectives	• Understand the modes of transmission and risk factors associated with brucellosis in humans and animals. • Identify effective prevention strategies, including hygiene measures, vaccination, and safe handling of animal products. • Recognize the importance of community education and surveillance in controlling brucellosis.
Pre-requisite knowledge/skill	• Basic understanding of zoonotic disease transmission dynamics. • Familiarity with public health strategies and vaccination programs. • Awareness of health education, community engagement, and disease surveillance principles.
Competencies to be achieved	• Ability to implement preventive measures, including proper disposal of contaminated materials and personal hygiene practices. • Proficiency in educating communities about the risks of brucellosis and promoting safe consumption practices. • Capability to advocate for and manage livestock vaccination programs to minimize disease spread.

PREVENTION AND CONTROL OF BRUCELLOSIS

Brucellosis prevention involves a combination of meticulous hygiene, safe practices in animal handling, and community education. Whether in structured farms or migratory settings, proactive measures can minimize the risk of transmission.

Farm Sanitation

Effective sanitation on farms is crucial. Workers should wear protective gear, especially during high-risk activities such as assisting with births or handling animals that have recently aborted. Contaminated materials like aborted fetuses and placentas must be collected in sealed containers and disposed of through incineration or burial with lime at locations away from water sources. Disinfecting areas where infected animals have been, and sterilizing tools used in handling contaminated materials are also essential to control the spread. Regular cleaning of dung and safe disposal of liquid manure can further reduce risks.

Personal Hygiene and Occupational Safety

Individuals in high-risk occupations, such as veterinarians, farmers, and slaughter house workers, must adopt strict personal hygiene practices. Wearing protective clothing, such as gloves, masks, and goggles, helps prevent contact with infectious agents. Facilities should provide washing stations, and workers must disinfect themselves and their equipment thoroughly after handling animals or their by-products. Respiratory protection is critical in environments with airborne contaminants, such as slaughter-houses.

Nomadic and Migratory Livestock Management

In communities practicing nomadic livestock farming, strict hygiene is often challenging. However, education about brucellosis, its transmission, and its impact can significantly reduce risks. Teaching populations to avoid raw animal products, such as unpasteurized milk and undercooked meat, is vital. Preventing children from contact with animals during birthing or after abortion is particularly important in reducing infection rates.

Food Hygiene

Consuming pasteurized dairy products and thoroughly cooked meat can prevent brucellosis from spreading through contaminated food. Establishing food safety protocols, especially in areas with endemic brucellosis, is a foundational aspect of prevention.

Community and Worker Education

Awareness campaigns highlighting the risks and prevention strategies for brucellosis are indispensable. Workers in high-risk settings must receive ongoing training about safe practices and the importance of protective measures. Community education can address cultural practices that increase risk, promoting safer alternatives without disrupting livelihoods.

Vaccination

Vaccination is very important in the control of brucellosis, particularly among livestock. The ***B. abortus* S19 strain** has long been the standard for calfhood vaccination in India, offering lifelong protection against the disease. However, traditional vaccines come with challenges, including potential side effects like reduced milk production, abortions, and interference with diagnostic tests.

Recent advances have introduced a modified version of the **S19 strain**, addressing these limitations. This innovative vaccine is designed to be safer and more effective, showing improved immunogenicity and reduced side effects in buffaloes—a natural host for brucellosis. A key feature of this vaccine is its **DIVA (Differentiating Infected from Vaccinated Animals) capability**, which supports accurate disease surveillance without diagnostic interference. Furthermore, it requires a significantly reduced dose while maintaining high levels of protection.

POINTS TO REMEMBER

- Wear protective clothing and follow hygiene practices when handling animals, especially during births or abortions.
- Safely dispose of contaminated materials like placentas and aborted fetuses through incineration or deep burial with lime.
- Disinfect areas, tools, and equipment exposed to infected animals using approved disinfectants.
- Avoid consuming raw milk, undercooked meat, or animal products to minimize transmission.
- Educate communities, particularly in nomadic settings, about the risks and transmission of brucellosis.
- Vaccinate livestock using safe and effective vaccines, such as modified *B. abortus* S19 strains, to reduce infection.

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BRUCELLOSIS

BIODEGRADATION



Session-8

Outbreak Management

Course No. 008	Session 8: Outbreak Management
Duration	15 minutes
Learning Objectives	• Explain the outbreak management strategy for Brucellosis. • Describe the One health approach to prevent and control Brucellosis
Pre-requisite knowledge/skill	• Basic knowledge of outbreak investigation and response strategies. • Understanding of intersectoral collaboration in zoonotic disease control. • Familiarity with the One Health concept integrating human, animal, and environmental health.
Competencies to be achieved	• Describing outbreak management strategy for Brucellosis • Describing the One health approach to prevent and control Brucellosis

STEPS FOR OUTBREAK INVESTIGATION IN BRUCELLOSIS

1. Confirmation of the Outbreak

- Review available surveillance data to determine if there is an unusual increase in cases or clustering of brucellosis.
- Confirm cases using laboratory tests such as serological methods (e.g., Rose Bengal Test, ELISA) or molecular diagnostic tools (e.g., PCR) to verify the presence of *Brucella* species in affected individuals or animals.

2. Establishing a Multidisciplinary Team

- Assemble a team that includes public health officials, epidemiologists, veterinarians, clinicians, laboratorians, and other specialists as needed.
- This team collaborates to investigate the outbreak comprehensively, ensuring both human and animal health aspects are addressed.

3. Case Definition

- Develop a clear and standardized case definition that includes clinical signs (e.g., fever, joint pain, abortions in animals) and laboratory confirmed evidence of *Brucella* infection.
- This definition ensures consistent identification and classification of cases during the investigation.

4. Case Identification & Reporting

- Collaborate with healthcare providers, veterinary services, and laboratories to identify and report all suspected and confirmed cases.
- Ensure mechanisms for rapid reporting to facilitate timely interventions and control measures.

5. Data Collection

- Collect detailed information on confirmed and suspected cases, including demographics, clinical symptoms, occupational exposure, travel history, contact with animals, and consumption of unpasteurized dairy products.
- Use interviews, medical record reviews, and laboratory data to compile comprehensive case histories.

6. Descriptive Epidemiology

- Analyze the data to describe the outbreak in terms of person, place, and time.
- Create epidemic curves to visualize the progression of the outbreak, map geographic distributions of cases, and identify affected populations or high-risk zones.

7. Hypothesis Generation

- Based on the initial analysis, propose hypotheses regarding the outbreak's source, transmission routes, and associated risk factors.
- Hypotheses may include exposure to infected animals, consumption of contaminated milk or milk products, or occupational risks in farming or meat processing.

8. Analytical Epidemiology

- Conduct studies such as case-control or cohort studies to test the hypotheses.
- Compare affected individuals (cases) with non-affected individuals (controls) to identify significant risk factors and quantify their associations with disease occurrence.

9. Environmental Investigation

- Conduct inspections of relevant sites, such as farms, slaughterhouses, dairies, and other facilities.
- Collect and test environmental samples, including animal carcasses, birthing materials, milk, and waste products, to identify the source of infection and potential transmission pathways.

10. Control Measures

- Implement immediate actions to prevent further transmission, such as isolating infected animals, restricting animal movement, and vaccinating susceptible livestock.
- Promote public health education, including the importance of consuming pasteurized milk and maintaining good occupational hygiene.
- Ensure proper disposal of animal carcasses, waste, and birthing materials to minimize environmental contamination.

11. Communication and Collaboration

- Share findings and collaborate with key stakeholders, including public health authorities, veterinary services, community leaders, and international agencies if needed.
- Provide clear communication to the affected community on the risks and preventive measures.

12. Evaluation

- Assess the impact and effectiveness of the implemented control measures.
- Continuously monitor the situation to detect new cases and ensure the outbreak is fully contained.
- Use lessons learned to refine surveillance systems and improve preparedness for future outbreaks.

One Health Approach

To date there is no “One Health” surveillance and control system for endemic (i.e., enzootic) diseases like brucellosis, particularly but not exclusively in developing countries.

The flowchart illustrates the data-sharing framework between the human and veterinary departments under the “One Health Approach” to effectively manage zoonotic diseases. It demonstrates the bidirectional communication of data between public health and veterinary systems. When a suspected or probable case meets the case definition, human and animal samples are subjected to laboratory testing. Positive human cases are reported to the District Surveillance Officer (DSO), who communicates with the State Surveillance Officer (SSO) and ensures public health measures are implemented. Similarly, positive animal cases are reported to the District Veterinary Officer, who informs the State Veterinary Officer and oversees veterinary interventions. Both streams ensure data sharing between human and veterinary sectors, enabling coordinated action. Additionally, relevant authorities like the Municipal Corporation and Forest Department are notified as required, emphasizing an integrated response.

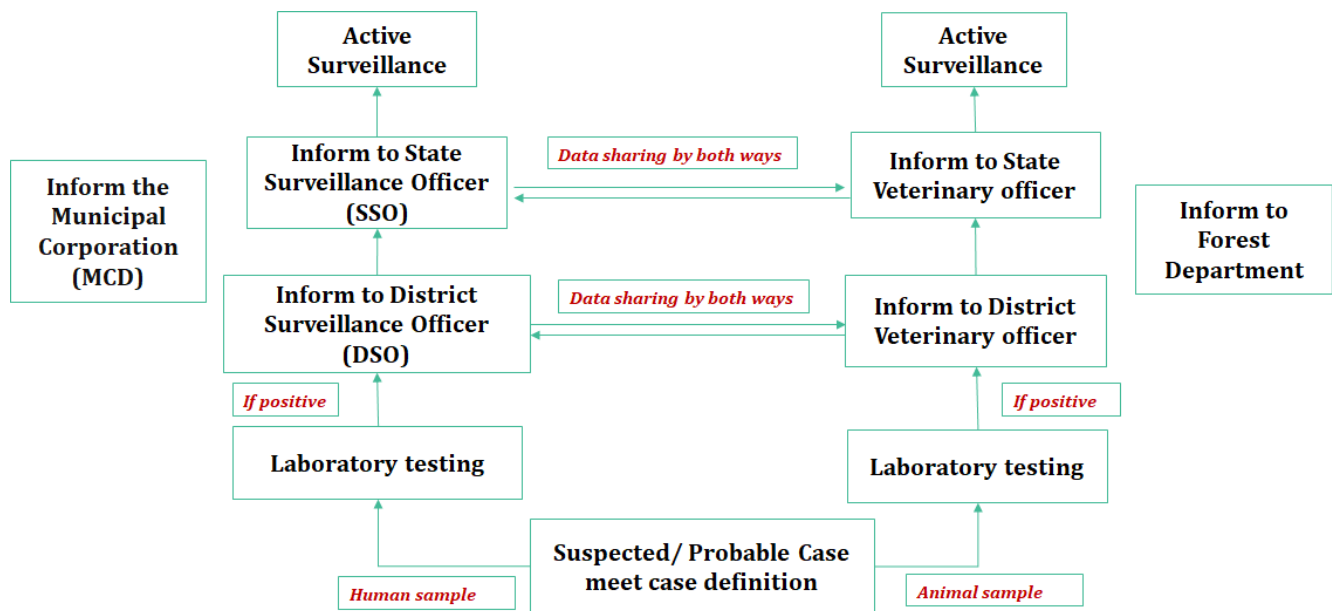


Fig 21: Data-sharing framework between the human and veterinary departments under the “One Health Approach

Bioterrorism

Brucellosis as a bioterrorism agent presents unique challenges, primarily targeting economic stability and environmental health rather than causing high fatalities. A deliberate release in regions reliant on livestock could lead to significant losses in animal productivity, long-term economic disruption, and environmental contamination. This contamination might also trigger secondary airborne outbreaks, compounding public health risks.

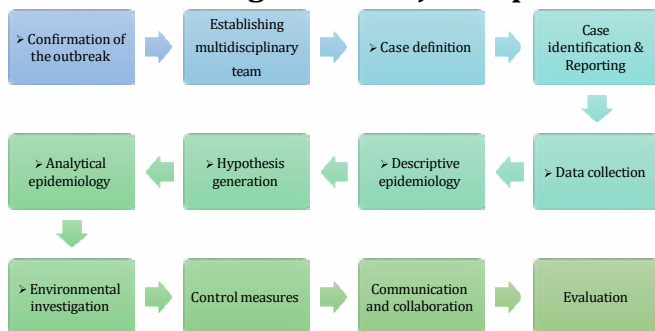
Attack scenarios vary widely depending on local conditions. In endemic areas with greater public awareness, the impact might be limited, as seen in projections where an attack on a city of 100,000 led to just two fatalities and minimal disruption. However, in less prepared regions, the consequences could be more profound.

While not highly lethal, brucellosis as a weapon could destabilize agricultural economies, create sustained health challenges, and disrupt societal functioning, making it a potent tool for prolonged destabilization.

POINTS TO REMEMBER

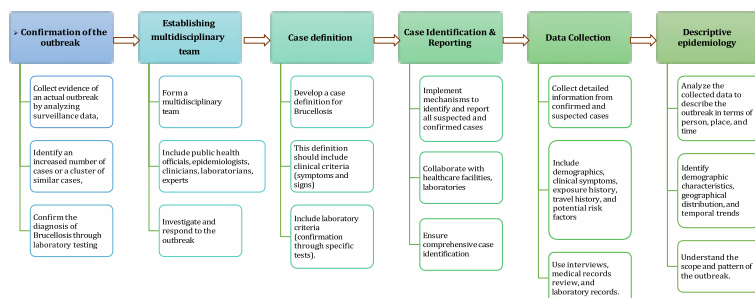
- Outbreak investigations for brucellosis involve collaboration between public health and veterinary sectors, integrating case identification, laboratory confirmation, and control measures under the One Health framework.
- Bidirectional data sharing between human health authorities (DSO, SSO) and veterinary officers ensures timely identification and response to zoonotic cases, supported by laboratory testing of both human and animal samples.
- Effective communication with local authorities, such as the Municipal Corporation and Forest Department, enhances the implementation of control measures and ensures comprehensive management of zoonotic outbreaks.

Outbreak Investigation – Major steps



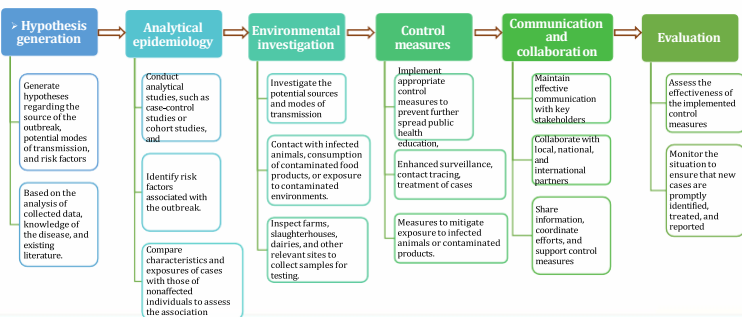
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Outbreak Investigation in detail



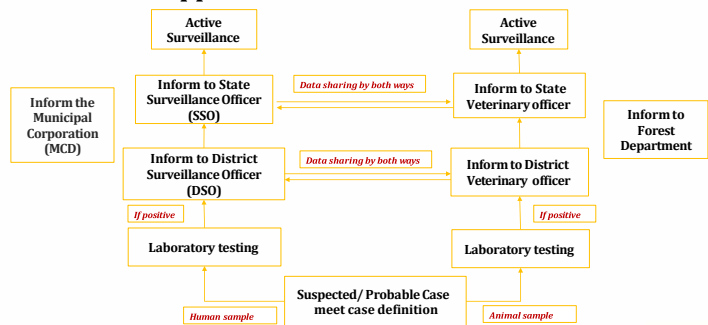
RECELLULOSIS

Outbreak Investigation



Researcher's Note

Data sharing by Human and Veterinary Department under “One Health Approach”



BSEB/2011/10534

Notes

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Brucellosis as bioterrorism agent

Economic and Environmental Impact

- Targets livestock-dependent regions, causing significant economic disruption.
- Leads to long-term environmental contamination.
- Secondary airborne outbreaks compound public health risks.

Attack Scenarios

- Minimal impact in prepared, endemic regions (e.g., projections show two fatalities in a city of 100,000).
- Severe consequences in less-prepared areas with inadequate awareness and infrastructure.

Key Risks

- Not highly lethal but capable of destabilizing agricultural economies.
- Sustains health challenges and disrupts societal functioning.
- A potent tool for prolonged destabilization.

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Notes

Session-9

Surveillance

Course No. 009	Session 9: Surveillance
Duration	10 minutes
Learning Objectives	• Explain the transmission, prevalence, and impact of brucellosis. • Describe surveillance tools and methods used in livestock and humans. • Advocate for the One Health approach in brucellosis control.
Pre-requisite knowledge/skill	• Basic understanding of zoonotic disease epidemiology and public health impact. • Familiarity with disease surveillance systems in both human and animal health sectors. • Awareness of the One Health framework and its relevance to zoonoses control.
Competencies to be achieved	• Analyze surveillance data to guide interventions. • Apply diagnostic tools effectively for brucellosis monitoring. • Collaborate across sectors for integrated disease management.

SURVEILLANCE OF BRUCELLOSIS

Brucellosis is a zoonotic disease with significant economic and public health implications, affecting a wide range of domestic animals, marine animals, and humans. In animals, it is primarily characterized by reproductive disorders, including abortion, which can severely impact livestock productivity. India, where approximately 80 million rural households are engaged in milk production—largely by small and marginal farmers—faces a heightened risk of brucellosis transmission due to the close association between humans and livestock. Recognizing the dual burden of this disease on animal and human health, the Government of India has implemented the National Animal Disease Control Programme (NADCP) for brucellosis, emphasizing regular sero-surveillance and monitoring to mitigate its impact.

Surveillance in Livestock

Surveillance in bovine populations focuses on identifying cases of brucellosis, estimating infection prevalence, and monitoring progress towards disease control goals. These efforts provide actionable data for policymakers and stakeholders to evaluate program outcomes and ensure adherence to regulatory standards. For example, ICAR-NIVEDI (Indian Council of Agricultural Research - National Institute of Veterinary Epidemiology and Disease Informatics), in collaboration with the Department of Animal Husbandry and Veterinary Services in Karnataka, undertook extensive monitoring using the Milk Ring Test (MRT). Between July 2012 and March 2013, 64,818 bulk milk samples from 30 districts were screened, highlighting the disease's spread in dairy populations.

In addition to milk testing, large-scale serosurveys have been conducted to estimate the seroprevalence of brucellosis in bovines. For instance, 12,054 serum samples comprising 9,236 from cattle and 2,818 from buffaloes were analyzed across 15 Indian states using protein G indirect ELISA. The findings revealed a true prevalence of 8.3% in cattle and 3.6% in buffaloes, underscoring the importance of targeted interventions in these populations.

Surveillance in Small Ruminants

Brucellosis in small ruminants, predominantly caused by *Brucella melitensis*, is another area of concern. A nationwide sero-screening conducted during 2017–2018 employed indirect ELISA to assess the seroprevalence and geographic distribution of the disease in small ruminants. The study found an apparent prevalence of 7.45% and a true prevalence of 3.79%. These results offer crucial insights into the disease dynamics within small ruminant populations, aiding the design of region-specific control strategies.

Human Surveillance

While robust surveillance mechanisms exist for livestock, systematic national-level brucellosis surveillance in humans is currently non-existent. This is because Brucellosis is not included as a priority communicable disease under the Integrated Disease Surveillance Program [IDSP].

Limited studies targeting high-risk populations have provided valuable insights. For example, ICAR-NIVEDI collaborated with the National Institute of Mental Health and Neurosciences (NIMHANS), medical colleges, and the Department of Animal Husbandry to screen human samples suspected of brucellosis. One study in Karnataka screened 1,050 samples from occupationally exposed individuals, such as veterinarians and dairy workers, reporting a seropositivity rate of 7.04%. These findings highlighted the need for active human surveillance, particularly in high-risk groups, to estimate the true burden of brucellosis and guide public health interventions.

Inter-Sectoral Collaboration

Surveillance efforts in brucellosis demonstrate the value of inter-sectoral collaboration between veterinary and human health sectors. The One Health approach, involving partnerships between organizations such as ICAR-NIVEDI, NIMHANS, and government departments, has been instrumental in identifying at-risk populations, improving diagnostic capacities, and fostering integrated disease control measures.

POINTS TO REMEMBER

- Brucellosis significantly impacts livestock productivity and human health in India, particularly among rural households engaged in milk production, necessitating robust control measures like the National Animal Disease Control Programme (NADCP).
- Surveillance in bovines involves tools like the Milk Ring Test (MRT) and serosurveys, revealing notable seroprevalence rates of brucellosis in cattle (8.3%) and buffaloes (3.6%), aiding targeted interventions.
- Nationwide sero-surveillance in small ruminants showed a true prevalence of 3.79%, emphasizing the importance of region-specific control strategies for *Brucella melitensis*.
- Systematic human brucellosis surveillance is lacking in India, though targeted studies in high-risk populations (e.g., veterinarians and dairy workers) have identified a seropositivity rate of 7.04%.
- Inter-sectoral collaboration through a One Health approach has been critical in improving diagnostics, understanding disease dynamics, and integrating veterinary and public health efforts for brucellosis control.

Brucellosis Surveillance in Humans

Current Gaps -

- No national-level human surveillance.
- Limited studies target **high-risk populations** (veterinarians, dairy workers).

Key Findings -

- Karnataka study: **7.04% seropositivity** among 1,050 high-risk individuals.

Inter-Sectoral Collaboration -

- Partnerships: ICAR-NIVEDI, NIMHANS, medical colleges, and government agencies.
- Focus on **diagnostic improvements** and **integrated One Health approach**.

Way Forward -

- Expand **human surveillance programs** alongside livestock monitoring.
- Strengthen **One Health initiatives** for sustainable control of brucellosis.

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Notes

Brucellosis Surveillance in Livestock

National Animal Disease Control Programme (NADCP) launched for brucellosis.

Focus: **Sero-surveillance** and **sero-monitoring** in animal populations.

Examples of Animal surveillance

ICAR-NIVEDI & Karnataka collaboration -

- **64,818 bulk milk samples** screened (Milk Ring Test, 2012–2013).
- Seroprevalence study: **8.3% in cattle** and **3.6% in buffaloes** (15 states, ELISA).

Nationwide screening in small ruminants (2017–2018) -

- True prevalence: **3.79%** (*Brucella melitensis*).



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Annexures

Post Exposure Prophylaxis for Laboratory Personnel

ANNEXURE – 1: BRUCELLA LABORATORY RISK ASSESSMENT AND POST-EXPOSURE PROPHYLAXIS (PEP): MINIMAL RISK

Specimen handling	Exposure scenario	PEP	Follow-up/monitoring
Routine clinical specimen (e.g., blood, serum, cerebrospinal fluid)	Person who manipulated a routine clinical specimen in a certified Class II biosafety cabinet, with appropriate personal protective equipment (PPE) (i.e., gloves, gown, eye protection).	None	N/A
	Everyone present in the lab while someone manipulated a routine clinical specimen in a certified Class II biosafety cabinet, or on an open bench where manipulation did not involve occurrence of aerosol generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes).		May consider symptom watch for following scenarios: Person who manipulated a routine clinical specimen on an open bench with or without appropriate PPE, or in a certified Class II biosafety cabinet without appropriate PPE.
Enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Person who manipulated enriched material or reproductive clinical specimen in a certified Class II biosafety cabinet, with appropriate PPE.		Person present in the lab while someone manipulated a routine clinical specimen on an open bench, resulting in aerosol generating events.
	Everyone present in the lab while someone manipulated enriched material or reproductive clinical specimen in a certified Class II biosafety cabinet.		

ANNEXURE – 2: BRUCELLA LABORATORY RISK ASSESSMENT AND POST-EXPOSURE PROPHYLAXIS (PEP): LOW RISK

Specimen handling	Exposure scenario	PEP	Follow-up/ monitoring
Enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Everyone present in the lab at a distance of > 5 feet from someone manipulating enriched material or reproductive clinical specimen, on an open bench, with no occurrence of aerosol-generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/ splashes).	May consider if immunocompromised or pregnant. Discuss with health care provider (HCP). Note: RB51 is resistant to rifampin in vitro, and therefore this drug should not be used for PEP or treatment courses.	Regular symptom watch (e.g., weekly) and daily self-fever checks through 24 weeks after last known exposure. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks after last known exposure. Note: no serological monitoring currently available for RB51 and B. canis exposures in humans.

ANNEXURE – 3: BRUCELLA LABORATORY RISK ASSESSMENT AND POST-EXPOSURE PROPHYLAXIS (PEP): HIGH RISK

Specimen handling	Exposure scenario	PEP	Follow-up/ monitoring
Routine clinical specimen (e.g., blood, serum, cerebrospinal fluid)	Person who manipulated a routine clinical specimen, resulting in contact with broken skin or mucous membranes, regardless of working in a certified Class II biosafety cabinet, with or without appropriate personal protective equipment (PPE) (i.e., gloves, gown, eye protection).	Doxycycline 100mg twice daily, and rifampin 600 mg once daily, for three weeks. For patients with contraindications to doxycycline or rifampin, consider TMP-SMZ in addition to another appropriate antimicrobial. Two antimicrobials effective against Brucella should be given. Pregnant women should consult their obstetrician. Note: RB51 is resistant to rifampin in vitro, and therefore this drug should not be used for PEP or treatment courses.	Regular symptom watch (e.g., weekly) and daily self-fever checks through 24 weeks after last known exposure. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks after last known exposure. Note: no serological monitoring currently available for RB51 and B. canis exposures in humans.
	Person who manipulated or is ≤ 5 feet from someone manipulating enriched material or reproductive clinical specimen, outside of a certified Class II biosafety cabinet.		
Enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Person who manipulated enriched material or reproductive clinical specimen, within a certified Class II biosafety cabinet, without appropriate personal protective equipment (PPE).		
	Everyone present when aerosol-generating events occurred (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes) with manipulation of enriched material or reproductive clinical specimen on an open bench.		

Additional slides for Laboratory Personnel

Notes

Annexure I (Oxidase test)

Principle

- The oxidase test is a biochemical reaction that assays for the presence of **cytochrome oxidase**.
- In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride reagent becomes an oxidized a dark purple coloured product.

Material Required

- Filter paper (moist) impregnated with the substrate (1% *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride)
- Wooden sticks or platinum wire loop.

Procedure

Take a moist filter paper with the substrate (1% *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride).



Pick a small portion of a bacterial colony from the agar surface using a platinum wire or wooden stick and rub the sample on the filter paper.



Observe inoculated area of paper or disk for a colour change to deep blue or purple within 10 seconds.

Result Interpretation

Positive: Development of a dark purple colour within 10 seconds.
Negative: No colour development

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Annexure II (Urease test)

Principle

- Detects the alkaline fermentation of urea with the resultant production of ammonia by microorganisms.
- Urea medium, whether a broth or agar, contains urea and the phenol red as a pH indicator. *Brucella* bacteria produce the urease enzyme, which catalyse the splitting of urea to release two molecules of ammonia and carbon dioxide which turns the medium alkaline & the indicator from orangeyellow colour to bright pink.

Material Required

- Urea agar base (commercially available)
- Urea solution (commercially available)
- Sterile glass tubes
- Inoculating loop

Procedure

Media Preparation: As per manufacturer recommendation

A loopful of a wellisolated colony is inoculated on the agar slants, and the butt shouldn't be stabbed.



The tubes are then incubated with loosened caps at 35 to 37°C.



The tubes are observed for the development of pink colour for as long as 7 days.

Result Interpretation

Positive: Development of intense magenta to bright pink colour in 15 min to 24 hours.
Negative: No colour change.

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Annexure III (Motility test)

Principle

- Motile bacteria use flagella for movement. Motility by the bacterium is demonstrated in a semisolid agar medium.
- Motility is indicated by the character of the growth in the butt of this tube.
- Motile organisms will produce a general clouding of the medium or a fuzzy stab line. Nonmotile organisms will give a sharply delineated stab line.

Material Required

- Media: Motility agar medium.
- Sterile straight needle
- BOD incubator

Procedure

With a sterile straight needle, touch a colony of a young culture growing on agar medium.



Single stab down the centre of the tube to about half the depth of the medium.



Incubate at 35-37°C and examine daily for up to 7 days.

Result Interpretation

Motility Test positive: Diffuse growth outward away from the stab line or turbidity of the media
Test negative or nonmotile: A clear tube (the same as the uninoculated media) with growth only along the line of inoculation.

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Annexure IV (Standard tube agglutination test)

Principle

- This test helps to assess the titre of the antibodies in the serum.
- Detect antibodies against Opolysaccharide (LPS) component of *Brucella*.
- Antigen used Killed strain of *B. abortus*.
- Used for diagnosis of *B. abortus*, *B. melitensis*, *B. suis*.
- Not useful for *B. canis* as it does not contain Opolysaccharide (LPS) component.

Material Required

- Brucella abortus* plain antigen
- Test serum suspected for brucellosis
- Known brucella positive and negative serum
- Wasserman's tubes
- Rack for placing Wasserman's tubes
- Normal saline
- Syringe (2mL) and Tuberculin syringes with long needles
- Water bath

Procedure

Place 10 numbers of Wasserman's tube in agglutination rack

Add 0.8mL of normal saline to the first tube, and 0.5mL of normal saline to the other remaining 9 tubes, using 2mL syringe.

Add 0.2 mL of serum to be tested in the first tube, using tuberculin syringe.

Mix the saline and serum gently without formation of air bubbles. The titre in the first tube will be 1:5.

Transfer 0.5mL of this mixture to the 2nd tube and mix with 0.5mL of saline in this tube which results in 1:10 titre, and then transfer 0.5mL of the mixture to the 3rd tube.

Continue the serial twofold dilutions up to the 8th tube (up to the dilution of 1:640). After mixing serum and saline in the 8th tube, discard 0.5mL of mixture.

Add 0.5mL of brucella plain antigen to each tube, using a separate 2mL syringe.

Keep positive and negative control: Positive control in the 9th tube with 0.5mL of saline and negative control in the 10th tube with 0.5mL of bacterial suspension (antigen).

Keep the tubes with the rack in a water bath at 56°C for 30 minutes or 37°C overnight.

Observe the tubes for agglutination.

0.5 ml transferred from tube to tube

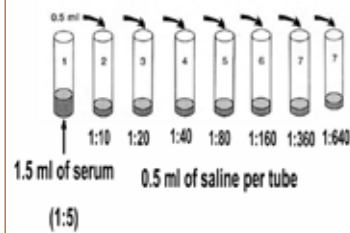
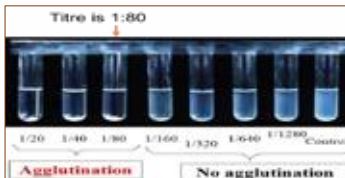


Figure: Serial dilution of test serum for Standard tube agglutination test

Interpretation of result

- A titre is defined as the reciprocal of the highest dilution of serum, which produces a definite agglutination reaction (100%).
- 100% agglutination: Agglutination at the bottom and clear supernatant. 50% agglutination: Agglutination at the bottom, but supernatant is not clear.



Notes

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NOTE TO FACILITATORS

This guide has been meticulously designed to enhance the understanding and expertise of doctors, veterinarians, paramedical staff, and laboratory personnel in dealing with Brucellosis cases. Your role is crucial in ensuring the success of this training, so please follow these instructions carefully -

Familiarize Yourself with the Content

Thoroughly review all the materials provided in the Brucellosis training guide. This includes presentations, handouts, case studies and assessment tools.

Tailor the Training to Your Audience

Understand the professional backgrounds of your participants (doctors, veterinarians, paramedics, and lab personnel). Tailor your examples and discussions to make the training relevant to their specific roles.

Set Clear Objectives

Begin each session by clearly stating the objectives participants are expected to achieve by the end. This will give them a sense of direction and purpose throughout the training.

Create an Interactive Learning Environment

Encourage active participation and open discussions. Use real-world scenarios to prompt critical thinking and problem-solving.

Use Multimodal Techniques

Utilize a mix of teaching methods, such as presentations, group discussions and case-based learning. This will cater to different learning styles and keep participants engaged.

Encourage Questions

Welcome questions and create a safe environment for participants to seek clarifications. This fosters deeper understanding and engagement.

Provide Regular Feedback

Throughout the training, provide constructive feedback on participants' performance. This will help them identify areas for improvement and enhance their skills.

Summarize and Review

At the end of each module, summarize key points and takeaways. Conduct a brief review to reinforce learning before moving on to the next topic.

Assessment and Certification

Develop an assessment mechanism, such as quizzes, practical assessments, or case presentations. Issue certificates of completion to participants who meet the training requirements.

Adapt to Participants' Pace

Gauge the participants' pace of learning. Adjust the training speed accordingly, ensuring that everyone can follow and grasp the content.

Facilitate Networking

Create opportunities for participants to interact and network with each other. This can lead to valuable knowledge exchange and collaboration.

Remember, your role as a trainer or facilitator is to guide participants through this comprehensive Brucellosis training with expertise, enthusiasm, and a dedication to enhancing their professional skills. Your efforts will contribute to building a more capable and knowledgeable medical workforce prepared to handle Brucellosis cases effectively.

Course Outline

It is imperative to underscore that the course outline harmonizes with the course schedule. Consequently, any modifications or adaptations made to the course schedule should be duly reflected in this course outline. This synergy between the schedule and the outline ensures consistency and alignment between training plans and their actual implementation.

Note: Make copies of the schedule, one for each participant and include in the training material bag.

TIME	TOPICS/ACTIVITIES	TRAINING/LEARNING METHODS	RESOURCES/MATERIALS
20 min	Welcome participants Facilitate introductions of participants Explore participants' expectations for the course	Open course with a word of welcome by organizers, lead trainers, etc. Facilitate the introductions of all participants and trainers. Explore participants' expectations for the course. Allow participants to freely explore their expectations. In the next session on review of the course objectives, address which expectations can be met and which cannot be met.	Prepare welcome sign, Name badges
20 min	Review course objectives and schedule Review components of the training guide	Review the course goals and objectives; the course design and expected outcomes; Review the course schedule, including starting and ending times and times for breaks and lunch; Review the materials to be used in the course and ensure that participants understand the use of course materials.	Copies of course schedule, one for each participant from Facilitators' Guide (FG). Full set of the training guide: Facilitators' Guide (FG) Questionnaire
20 min	Assess participants' pre-course knowledge	Distribute the Pre-course Knowledge Assessment sheet to each participant. Ask them to answer each question. Allow 15 minutes for assessment. Immediately review the correct responses—Have participants grade the papers while the trainer reviews the correct answers with them and collect the papers after reviewing all the answers.	Pre-Training Quiz Sheet for each participant
4-5 hours	Presentation/Discussion	Use the PowerPoint slides to present information	Power Point slides on the sessions planned as per agenda

20 min	Assess participants' post training knowledge	Distribute the Post Training Knowledge Assessment sheet to each participant. Ask them to answer each question. Allow 15 minutes for the Post Training Knowledge Assessment.	Copies of Post Training Knowledge Assessment Sheets (one for each participant)
15 min	Course Evaluation	Ask questions of the participants and engage them in the presentation of the information.	
15 min	Closing	Closing remarks by training organizers. Distribute certificates to participants.	Certificate for each participant

PRE/POST TRAINING QUIZ

Instructions for the trainer:

- The trainer/instructor will administer the test before the training and collect the response.
- The same test will be given to the trainee after the completion of the training.
- The difference in scores will be recorded for training evaluation purpose.

Trainee Name: _____

Date: _____

Designation: _____

Test taken before the training (yes/no): _____

Test taken after the training (yes/no): _____

Instructions for the trainee:

- The test quiz carries 30 questions.
- Attempt all the questions.
- Put a tick mark on the correct option or fill the blanks with True/ False.
- Please check whether all questions are answered before submitting.

1. What is the most common *Brucella* species causing severe human brucellosis?
 - a) *B. abortus*
 - b) *B. melitensis*
 - c) *B. suis*
 - d) *B. canis*
2. What type of disease is brucellosis classified as?
 - a) Vector-borne
 - b) Zoonotic
 - c) Nosocomial
 - d) Opportunistic
3. Which occupation has the highest risk of contracting brucellosis?
 - a) Software engineers
 - b) Veterinarians and slaughterhouse workers
 - c) Teachers
 - d) Lawyers

4. What is the estimated annual global incidence of human brucellosis according to the CDC?
 - a) 100,000-500,000 cases
 - b) 1.6–2.1 million cases
 - c) 5–10 million cases
 - d) 500,000–1 million cases
5. Which geographic region reports the highest incidence of brucellosis?
 - a) Eastern Mediterranean
 - b) Western Europe
 - c) Oceania
 - d) North America
6. A patient presents with fever, night sweats, weight loss, and hepatosplenomegaly. He consumed unpasteurized milk during a recent rural visit. What is the most likely diagnosis?
 - a) Tuberculosis
 - b) Typhoid fever
 - c) Brucellosis
 - d) Dengue fever
7. A 6-year-old boy develops fever and dry cough after consuming unpasteurized dairy. He also has hepatosplenomegaly and cervical lymphadenopathy. Which diagnostic test would be most specific to confirm brucellosis?
 - a) Blood culture
 - b) ELISA for Brucella IgM and IgG
 - c) PCR for Brucella DNA
 - d) Standard Agglutination Test
8. A veterinarian presents with joint pain and undulating fever. Blood cultures identify *B. melitensis*. What is the best treatment plan?
 - a) Amoxicillin for 7 days
 - b) Doxycycline and rifampin for 6 weeks
 - c) Ciprofloxacin and metronidazole for 2 weeks
 - d) Single-dose gentamicin
9. A farmer who was exposed to infected cattle presents with fever, back pain, and positive serology for brucellosis. Imaging reveals osteomyelitis of the lumbar vertebrae. Which complication does this represent?
 - a) Neurological brucellosis
 - b) Musculoskeletal brucellosis
 - c) Genitourinary brucellosis
 - d) Cardiovascular brucellosis

10. A laboratory technician develops fever after working with Brucella cultures without adequate protection. What biosafety protocol breach is most likely responsible?
 - a) Open handling of culture bottles
 - b) Improper disposal of media
 - c) Lack of vaccination
 - d) Working in a BSL-1 laboratory
11. Which Indian state reports the highest prevalence of human brucellosis?
 - a) Kashmir
 - b) Punjab
 - c) Maharashtra
 - d) Karnataka
12. What is a common source of brucellosis in the general population?
 - a) Mosquito bites
 - b) Aerosol transmission
 - c) Consumption of unpasteurized dairy products
 - d) Contact with contaminated water
13. Which occupational group in India shows the highest seroprevalence for brucellosis?
 - a) Dairy farmers
 - b) Paraveterinarians
 - c) Slaughterhouse workers
 - d) Artificial insemination workers
14. What is the primary mode of transmission of brucellosis in humans?
 - a) Direct contact with infected animals or their products
 - b) Airborne spread in public spaces
 - c) Contaminated food and water
 - d) Person-to-person transmission
15. Which high-risk group in India shows a seroprevalence of up to 25%?
 - a) Artificial insemination workers
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16. Which diagnostic method is considered the gold standard for brucellosis?
 - a) ELISA
 - b) Blood culture
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17. What is the most sensitive sample for detecting Brucella in early-stage infection?
- a) Serum
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21. Which measure is central to preventing brucellosis in livestock?
- a) Mass culling
 - b) Vaccination
 - c) Antibiotic treatment of herds
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22. Which vaccine is used in India for bovine brucellosis?
- a) BCG vaccine
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 - c) B. melitensis Rev 1
 - d) FMD vaccine
23. What is the target age for vaccinating female bovine calves under the NADCP?
- a) 2-4 months
 - b) 4-8 months
 - c) 6-12 months
 - d) 1 year

24. What is a critical biosafety measure in laboratories handling *Brucella*?
- a) Conducting all procedures in open areas
 - b) Use of Class II biological safety cabinets
 - c) Avoiding refrigeration of samples
 - d) Disposing samples in general waste bins
25. How long does the NADCP aim to achieve 90% vaccination coverage for brucellosis?
- a) 2 years
 - b) 5 years
 - c) 7-10 years
 - d) 12-15 years
26. A patient treated for brucellosis 4 months ago presents with fever and joint pain. Which diagnostic step should be performed?
- a) Repeat PCR test
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27. A 30-year-old male with brucellosis reports worsening back pain. Imaging shows osteomyelitis. What is the recommended management?
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28. In a brucellosis outbreak investigation, several cases were traced to a specific dairy farm. What should be the first public health action?
- a) Conduct serologic testing of livestock
 - b) Quarantine the infected workers
 - c) Administer mass antibiotics to the local population
 - d) Close the farm indefinitely
29. A pregnant woman with suspected brucellosis tests positive for IgM antibodies but has normal imaging. What is the appropriate intervention?
- a) Immediate termination of pregnancy
 - b) Antibiotic therapy with rifampin and trimethoprim
 - c) Close monitoring without antibiotics
 - d) PCR to confirm diagnosis
30. A 50-year-old veterinarian develops fever after handling aborted fetuses. Blood culture grows *B. abortus*. Which public health measure is most important?
- a) Vaccinate his household contacts
 - b) Isolate the veterinarian
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ANSWER KEY

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