



सत्यमेव जयते

Ministry of Health & Family Welfare
Government of India

A Comprehensive Learning Guide

Anthrax



**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
Directorate General of Health Services
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Government of India

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DIRECTOR GENERAL OF HEALTH SERVICES



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स्वास्थ्य एवं परिवार कल्याण मंत्रालय
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



Acknowledgments

The **Comprehensive Learning Guide** for Anthrax 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 development, drafting and finalizing this guide. Their collaboration has been pivotal in ensuring the quality and relevance of this resource.

Dr. Simmi Tiwari
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Preface

Anthrax derives its name from a Greek word “anthrakites”, meaning coal-like referring to the typical black eschar seen in cutaneous involvement of the disease. It is potentially a fatal and highly contagious zoonotic infection. Bacterium *Bacillus anthracis* (*B. anthracis*) being causative organism. Anthrax has a worldwide distribution. Herbivore animals are the natural host of the disease, and all warm-blooded animals are susceptible. Humans mostly contract the infection through animals.

The bacterial spores have extraordinary stability and remain viable in soil for hundreds of years. They are resistant to many of the disinfectants which demand extremely careful handling of samples or bacterial cultures and following stringent decontamination procedures. Anthrax remains a remarkably under-reported disease due to failure to suspect, detect and diagnose the disease in many parts of India.

The guide aims to equip learners with the knowledge and tools necessary to educate and train individuals involved in various aspects of Anthrax management. It provides a comprehensive overview of the disease, covering its epidemiology, clinical presentation, laboratory diagnosis, treatment options, and preventive measures.

The guide 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 Anthrax 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 Anthrax.

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

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AST	Aspartate Aminotransferase
CBC	Complete Blood Count
CDC	Centre for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Amendments
CRP	C-reactive Protein
CSF	Cerebrospinal Fluid
DSO	District Surveillance Officer
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ESR	Erythrocyte Sedimentation Rate
GLP	Good Laboratory Practice
HEPA	High-Efficiency Particulate Air
IHIP	Integrated Health Information Platform
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IDSP	Integrated Disease Surveillance Programme
IV	Intravenous
IVRI	Indian Veterinary Research Institute
LDH	Lactate Dehydrogenase
MCQ	Multiple Choice Questions
MLVA	Multiple Locus Variable Number Tandem Repeat Analysis
MCD	Municipal Corporation of Delhi
NCDC	National Centre for Disease Control
NIV	National Institute of Virology
PA	Protective Antigen
PCR	Polymerase Chain Reaction
PCMB	Polychrome Methylene Blue
PEP	Post-Exposure Prophylaxis
PPE	Personal Protective Equipment
RSV	Respiratory Syncytial Virus
SSO	State Surveillance Officer
US	United States
WBC	White Blood Cell
WOAH	World Organisation for Animal Health (formerly OIE)

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Introduction to Learning Guide for Anthrax

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. Despite being poorly documented, zoonotic diseases pose a significant public health problem in India. They equally impact animal, human, and environmental sectors, causing substantial damage to the nation's economy. To protect all sectors, it is essential for India 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 Anthrax, its outbreak potential, health emergencies, risk communication, diagnostic procedures, laboratory safety, surveillance, data reporting, and their applications.

The guide are based on national and international Learning Guidelines and serve as a comprehensive training resource for individuals involved in zoonotic disease diagnosis and surveillance activities. They provide a deeper insight into the understanding of Anthrax. 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' Learning Guide (FG). The FG 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 Anthrax training guide is 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 anthrax 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 Anthrax preparedness and response guide includes a comprehensive pre 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 anthrax 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 anthrax. The pre-training quiz will consist of MCQs that cover various aspects of anthrax, 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 Anthrax 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 anthrax. 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 anthrax-related concepts and procedures.

Course Syllabus

This guide is tailored to facilitate a deep understanding of anthrax management and its impact on both human and animal health. It covers essential topics such as anthrax epidemiology, clinical presentation, diagnostics, treatment, and preventive measures, providing participants with the knowledge to effectively respond to anthrax outbreaks.

Course Goals

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

- Equip participants with skills for anthrax 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:

- Well-versed with clinical presentation of anthrax.
- Apply diagnostic and treatment protocols for anthrax management.
- Implement preventive strategies and outbreak management tactics.

Participant Selection Criteria

Participants for this anthrax 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' learning 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 guide 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 Anthrax, its epidemiology, bioweapon potential, 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 anthrax, 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 setting ground rules	15 min
2.	Ice Breaker: Talk about yourself • Contribution to the management & control of Zoonoses • Expectations from the training	30 min
3.	Training objectives	15 min
4.	Pre-Test Quiz	15 min
5.	Session 1: Introduction to Anthrax	15 min
6.	Session 2: Epidemiology	30 min
7.	Session 3: Anthrax as a Bioweapon	15 min
8.	Session 4: Anthrax in Humans	20 min
9.	Session 5: Anthrax in Animals	15 min
10.	Session 6: Laboratory Diagnosis	20 min
11.	Session 7: Treatment, Prevention & Control	20 min
12.	Session 8: Outbreak Management	20 min
13.	Session 9: Surveillance and Risk Communication	15 min
14.	Post - Test Quiz	15 min
15.	Q/A session, review and discussion	15 min
16.	Closing remarks	5 min

Session-1

Introduction to Anthrax

Course No. 001	Session 1: Introduction to Anthrax
Duration	15 minutes
Learning Objective	• Describe disease overview • Understand causative agent and its characteristics
Pre-requisite knowledge/skill	• Basic understanding of microbiology, including bacterial and viral pathogens. • Familiarity with host-pathogen interactions and disease mechanisms. • Awareness of the clinical and public health significance of infectious diseases.
Competencies to be achieved	• Learners would gain knowledge of the basic characteristics and features of Anthrax • Understanding of the characteristics and classification of causative agent

INTRODUCTION

Anthrax is a **zoonotic** and **occupational** disease that primarily affects ruminant animals. It is a highly infectious disease caused by bacterium *Bacillus anthracis*. Humans become incidentally infected by handling infected animals, carcasses, meat, hides, or wool, as well as goods obtained from infected animals, such as drumheads.

Anthrax is called by many other names like **Wool sorters' disease Rag-picker's, disease, Knacker's disease, Hide-porter's disease (cutaneous anthrax), Malignant edema, Malignant carbuncle, Malignant pustule, Charbon disease, Milz brand disease, Splenic fever, Black Baine, and Siberian Plague**. It can be a fatal disease, if not diagnosed and treated on time.

In 2001, anthrax reemerged into US with 22 cases of confirmed inhalational. The disease in humans' manifests in 4 forms viz, i) Cutaneous ii) inhalational and iii) gastrointestinal and iv) Injectional. The most commonly reported form of anthrax in humans is **cutaneous anthrax** (95%–99%).

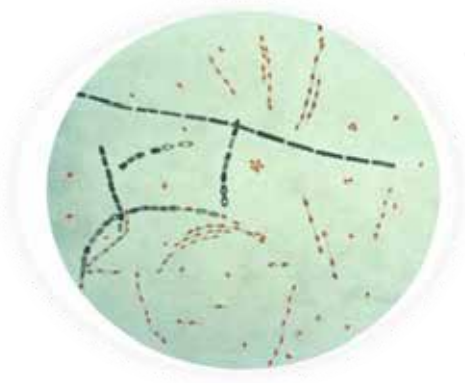
The zoonotic aspect of Anthrax emphasizes the importance of One Health approaches, recognizing the interconnections between human, animal, and environmental health. It also underscores the significance of effective surveillance and preventive measures to mitigate outbreaks.

The name **Anthrax** is derived from the Greek word “**anthrakites**,” which means “black,” and refers to the necrotic lesion found in cutaneous anthrax. The *Bacillus anthracis* is a tiny, encapsulated, spore-forming rod that is aerobic or facultatively anaerobic, gram-positive or gram-variable measuring about 3 to 5µm long and 1 to 1.2µm wide. Toxins produced by the bacterium are vital for clinical pathogenicity. It grows well on blood agar, forming huge, irregular-shaped colonies.

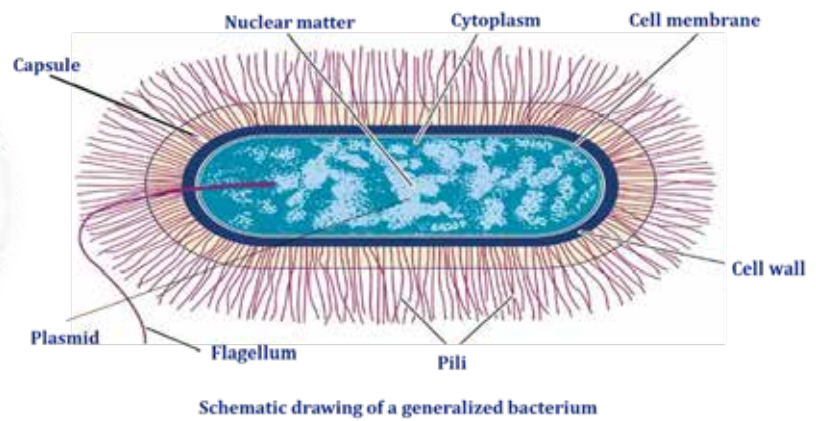
Bacillus anthracis spores are ecologically stable and may contaminate soil globally, resulting in herbivore illnesses while grazing. Spores can remain dormant and viable in the environment for decades.

Plasmids, **pX01 and pX02** encode the primary virulence factors of *B. anthracis*. The **pX01** plasmid is **184.5 kbp** in size and **codes for** three toxins that cause **bleeding, oedema, and necrosis**. They are composed of the 83 kDa lethal factor, the 89 kDa oedema factor (calmodulin dependent adenylate cyclase), and the 85 kDa protective antigen, which contains the 20 kDa host cell receptor. Exotoxins are binary in nature, with the protective antigen serving as the binding domain that allows the toxin to enter the host cell.

The **95.3 kbp** plasmid **pX02** encodes three genes (**cap B, cap C, and cap A**) involved in the production of the polyglutamyl capsule that inhibits host phagocytosis of the vegetative form of *B. anthracis*. Both plasmids are required for full virulence; the absence of one result in an attenuated strain.

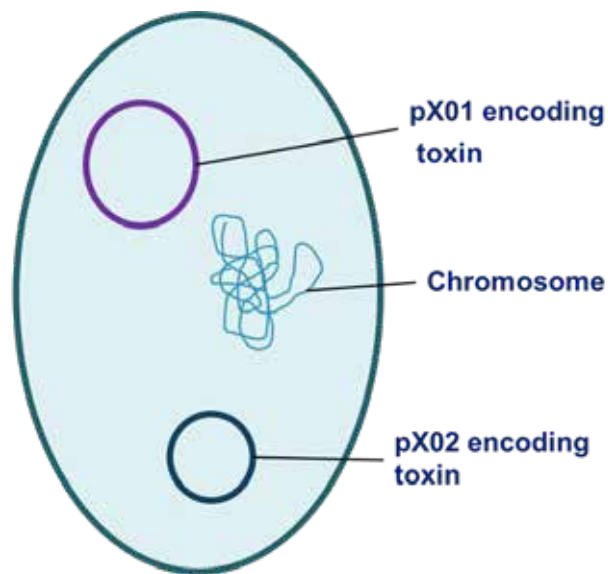


Source: <https://www.ncbi.nlm.nih.gov/books/NBK507773/>



Source: <https://www.britannica.com/science/bacillus-bacteria#ref282300>

Fig 1: Morphology of *Bacillus anthracis*



Source: <https://www.ncbi.nlm.nih.gov/books/NBK507773/>

Fig 2: Characteristics of *Bacillus anthracis*



Fig 3: Clinical presentations of Anthrax

(Image source:1. Stier DM, Mercer MP, Chin RL. Anthrax. In: Chin RL, Frazee BW, Coralic Z, eds. *Emergency Management of Infectious Diseases*. Cambridge University Press; 2018:480-489. 2. Outbreak of anthrax in adults and adolescents: a review of nine cases in a regional teaching hospital in East Hungary. Orlos, Z. et al. *Clinical Microbiology and Infection*, Volume 23, Issue 1, 52 – 54)

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The diagram shows a cigarette with the word "ANTHRAX" written on its orange filter. Various callout boxes are connected to different parts of the cigarette:

- Primarily a disease of herbivorous animals, although all mammals, including humans, and some avian species can contract it.** (Points to the cigarette tip)
- It is not a contagious disease.** (Points to the filter)
- Bleeding through natural orifices followed by sudden death is common.** (Points to the cigarette tip)
- Causative agent is the spore-forming, Gram-positive rod-shaped bacterium, *Bacillus anthracis*.** (Points to the cigarette tip)
- Worldwide distribution** (Points to the cigarette tip)
- Zoonotic disease** (Points to the cigarette tip)
- Mediated by exotoxins** (Points to the cigarette tip)
- Anthrax infected carcasses should never be opened.** (Points to the cigarette tip)
- Potential bioweapon** (Points to the cigarette tip)
- BSL - 4 agent** (Points to the cigarette tip)
- Characteristic lesion of Anthrax is a cigarette burnt appearance - Charbon (French-coal)** (Points to the cigarette tip)
- In India, Anthrax cases have been reported from Andhra Pradesh, Harkhand, Odisha, Tamil Nadu, and West Bengal.** (Points to the cigarette tip)

Center for the Study of the History of Mathematics

The image consists of two parts. On the left is a circular micrograph showing a rod-shaped bacterium with several flagella extending from one end. On the right is a schematic diagram of a generalized bacterium. The diagram is an oval shape with a thick outer layer labeled 'Capsule'. Inside this is a thinner layer labeled 'Cell wall', followed by a wavy line labeled 'Cell membrane'. The interior is filled with 'Cytoplasm' and contains a dense, blue, irregular mass labeled 'Nuclear matter'. At one end, there are several short, hair-like structures labeled 'Pili'. At the other end, there are several long, thin, whip-like structures labeled 'Flagellum'.

<https://www.ncbi.nlm.nih.gov/books/NBK507773>

Source: <https://www.britannica.com/science/bacillus-bacterial/ref282300>

Center for the Study of Mind, 301 NC, 30101

A diagram of a cell containing a chromosome and two plasmids. The chromosome is a large, blue, circular structure. The two plasmids are smaller, circular structures, one labeled 'pX01 encoding toxin' and the other 'pX02 encoding toxin'.

- <https://www.ncbi.nlm.nih.gov/books/NBK507773>

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[illegible]

Clinical presentations of Anthrax

- ❑ Infection in humans happen incidentally due to close contact with infected animal or animal product
- ❑ The disease in humans manifests in 4 forms viz, i) *Cutaneous* (most common 95-99%), ii) *inhalational* (5%) and iii) *gastrointestinal* iv) *Injectional*
- ❑ Bacterial spores remain viable for decades and can enter the body through skin abrasions, inhalation or ingestion

Image source: Slier DM, Mercer MP, Chin RL. Anthrax. In: Chin RL, Frazee BW, Coralic Z, eds. Emergency Management of Infectious Diseases. Cambridge University Press; 2018:440-489.
Image source: Outbreak of anthrax in adults and adolescents: a review of nine cases in a regional teaching hospital in East Hungary. Orsi, Z. et al. Clinical Microbiology and Infection, Volume 23, Issue 1, 52 - 54



Notes

Session-2

Epidemiology of Anthrax

Course No. 002	Session 2: Epidemiology of Anthrax
Duration	30 minutes
Learning Objective	• Understand history and epidemic potential of Anthrax • Understand geographical distribution of Anthrax
Pre-requisite knowledge/skill	• Basic knowledge of infectious disease epidemiology and outbreak patterns. • Familiarity with zoonotic diseases and their transmission dynamics. • Awareness of global and regional disease surveillance and reporting systems.
Competencies to be achieved	• Epidemic potential assessment of Anthrax • Analysis of seasonality and understanding its implications • Assessment of geographical distribution and prevalence of Anthrax disease

EPIDEMIOLOGY OF ANTHRAX

Global: Anthrax is common in agricultural regions of Central and South America, sub-Saharan Africa, central and south-western Asia, and southern and eastern Europe. Although outbreaks still occur in livestock and wild herbivores in the United States, Canada, and Western Europe, human anthrax in these areas is now rare. The most common type of anthrax in humans (95%-99%) is cutaneous anthrax. The global prevalence of the Livestock anthrax was 28%.

Certain regions, such as India and Bangladesh, experience particularly high levels of anthrax endemism. The reservoir is contaminated soil. Factors contributing to this endemism include environmental conditions conducive to spore survival and the presence of susceptible animal populations. The persistence of the disease in these areas underscores the importance of region-specific strategies for surveillance and intervention.

Handling infected animals, as well as butchering and consuming meat from those animals, has been linked to outbreaks of cutaneous and ingestion anthrax. The majority of these outbreaks occurred in endemic areas of Africa and Asia. A few cutaneous instances in travelers who had direct or indirect contact with animals, or their byproducts have been reported.

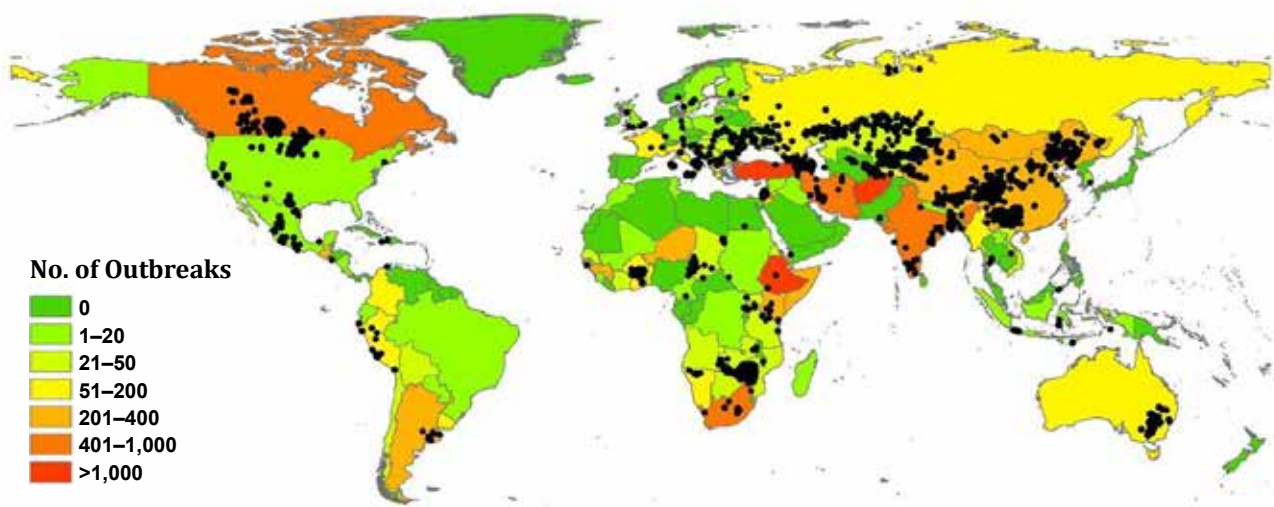


Fig 4: Global distribution of outbreaks by country and geographic locations of anthrax events

(Image source: Carlson et al; Nature Microbiology, 2019)

India: Most human anthrax outbreaks in India are reported from **eastern coastal regions**. Odisha was of special concern, with around **62 outbreaks** reported since 2009, with occurrences almost every year. Frequent outbreaks were reported from districts **Koraput, Sundergarh, Rayagada, and Deogarh**. **West Bengal, Andhra Pradesh, and Jharkhand** were also reported to be the states of concern. Several outbreaks of human anthrax were recorded from Tamil Nadu before 2000. Only two cases in 2009 and one in 2004 reported from **Tamil Nadu** state. According

to a time-trend analysis, the frequency of outbreaks surged between **2015 and 2017**. **Koraput** district in Odisha had **33 cases in 2019**, indicating continued transmission.

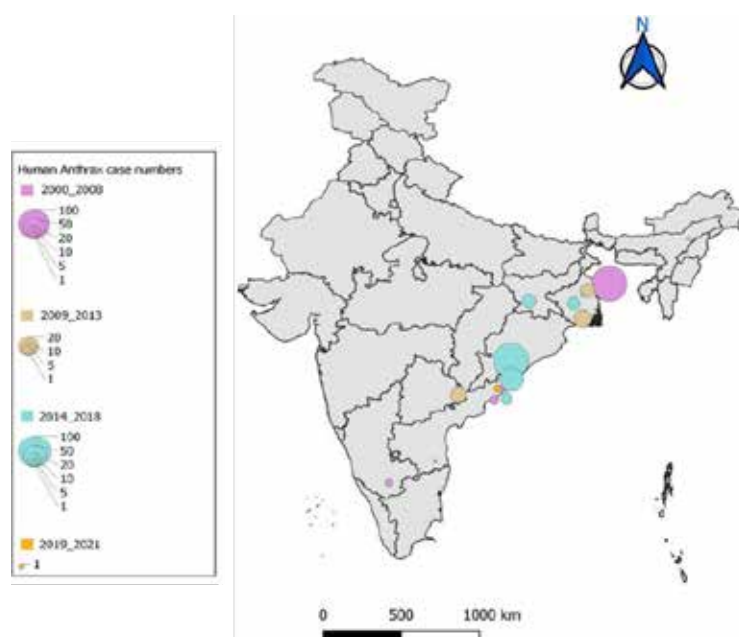


Fig 5: Human anthrax outbreaks reported in India during 2000–2021

(Image source: Jayaprakasam et al; One Health 2023)

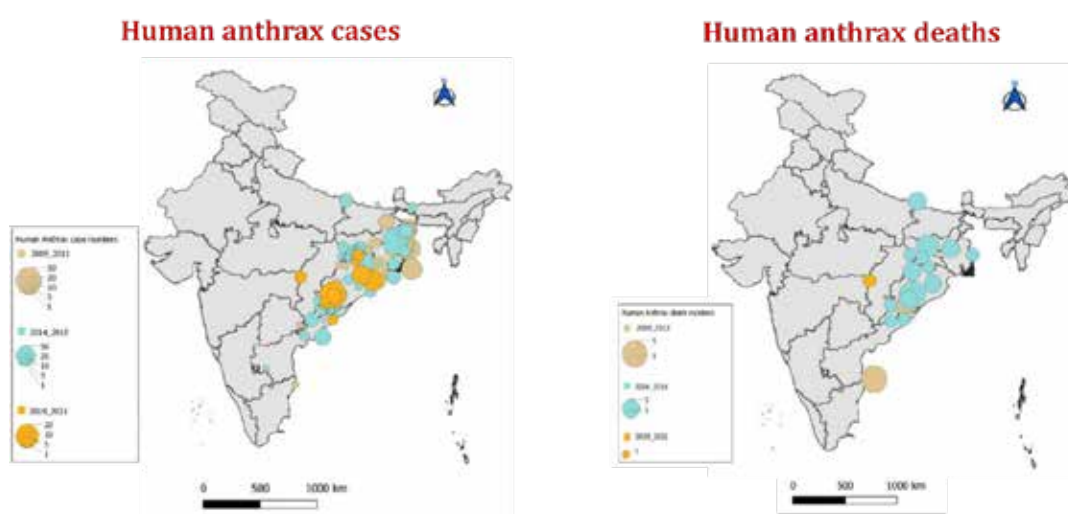


Fig 6: Spatiotemporal distribution, 2009–2021

(Image source: Jayaprakasam et al; One Health 2023)

Global Scenario

Common in agricultural regions of Sub-Saharan Africa, Central and South America, central and southwestern Asia, and southern and eastern Europe.

Outbreaks in livestock and wild herbivores common in Canada, the United States, and western Europe, while human anthrax outbreaks rare in these regions

Anthrax outbreaks worldwide mostly are due to cutaneous type (95%–99%)

Cases of cutaneous (n=4), ingestion (n=1), and inhalation (n=3) anthrax reported in people who have handled, played, or made drums from goatskin.

Some cases of cutaneous anthrax reported in travelers also

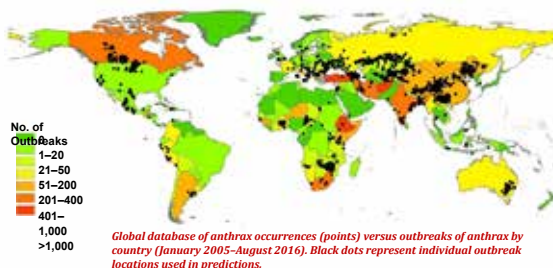


<https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/anthrax/diagnosis>

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ANTHRAX

Global distribution of outbreaks by country and geographic locations of anthrax events



Carlson et al; Nature Microbiology, 2019

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ANTHRAX

India

- Human anthrax outbreaks in India mostly from eastern coastal regions (**Odisha, Andhra Pradesh, West Bengal and Jharkhand**).
- Maximum number of cases from Odisha (n=439), West Bengal (n = 260), Jharkhand (n=137) and Andhra Pradesh (n = 81)
- 62 outbreaks reported since 2009 in Odisha with occurrence in almost every year
- Several outbreaks of human anthrax also recorded from Tamilnadu before 2000
- Sporadic cases reported from Assam and Karnataka as well
- 100% case fatality from gastrointestinal and inhalational types while case fatality ranges from 2% to 38% for cutaneous cases if not treated

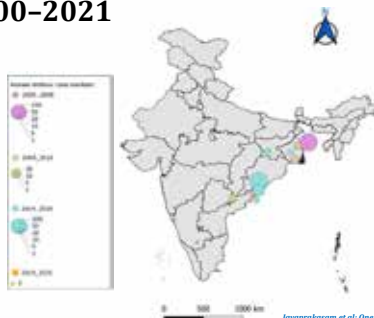


Jayaprakasham et al; One Health 2023

Centre for One Health, NCDC, MoHFW

ANTHRAX

Human anthrax outbreaks reported in India during 2000–2021



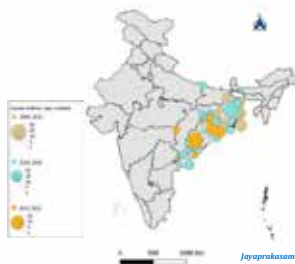
Jayaprakasham et al; One Health 2023

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ANTHRAX

Spatiotemporal distribution, 2009–2021

Human anthrax cases



Session-3

Anthrax in Humans

Course No. 003	Session 3: Anthrax in Humans
Duration	20 minutes
Learning Objective	• Describing transmission and Symptoms • Disease Progression • Identification of risk individuals • Case definitions
Pre-requisite knowledge/skill	• Basic understanding of infectious disease transmission routes and host susceptibility. • Familiarity with clinical signs and diagnostic criteria. • Knowledge about case definitions and risk factor assessment
Competencies to be achieved	• Explaining high risk/zoonotic individuals and the transmission route • Enumerate types of Anthrax disease in humans • Understanding and identifying symptoms • Classifying case in humans

CAUSATIVE AGENT

Bacillus anthracis, a spore-forming, Gram-positive bacterium that survives in spore form outside hosts and spreads through contact with infected animals or products. Transmission routes include cutaneous, inhalation, ingestion, and injection.

The lifecycle of *B. anthracis* involves two primary forms: the vegetative form and the spore form. In low-oxygen environments, such as within an infected host, the bacterium remains in its vegetative form. However, once exposed to oxygen outside the host, the vegetative cells begin to sporulate, forming highly resilient spores capable of surviving in the environment for extended periods.

ENVIRONMENT

Factors like temperature (8°C to 45°C), high humidity (>96%), pH (5-9), oxygen availability, and calcium-rich, alkaline soils favor spore persistence. Spores can survive for long periods in the environment, but the vegetative form quickly dies outside the host.

HOST

Primarily affects herbivores, with humans contracting it through contact with infected animals, products, or contaminated materials (e.g., wool, hides). Inhalation and ingestion also contribute to transmission.

CYCLE OF INFECTION

The infection cycle of anthrax is driven by the formation and uptake of spores. Within an infected host, the spores germinate into vegetative cells that multiply, ultimately killing the host. When the host dies, the vegetative cells are released into the environment, typically into the soil under the carcass, where they sporulate upon exposure to air. These spores can then remain viable for many decades, waiting to be taken up by a new host, thus continuing the cycle of infection. This uptake can occur at any time, from less than an hour to many decades later.

TRANSMISSION

Most human infections with *B. anthracis* result from handling *B. anthracis*-infected animals or their carcasses, meat, hides, or wool. Products derived from infected animals (e.g., drumheads, wool clothing) are additional documented sources of human infection.

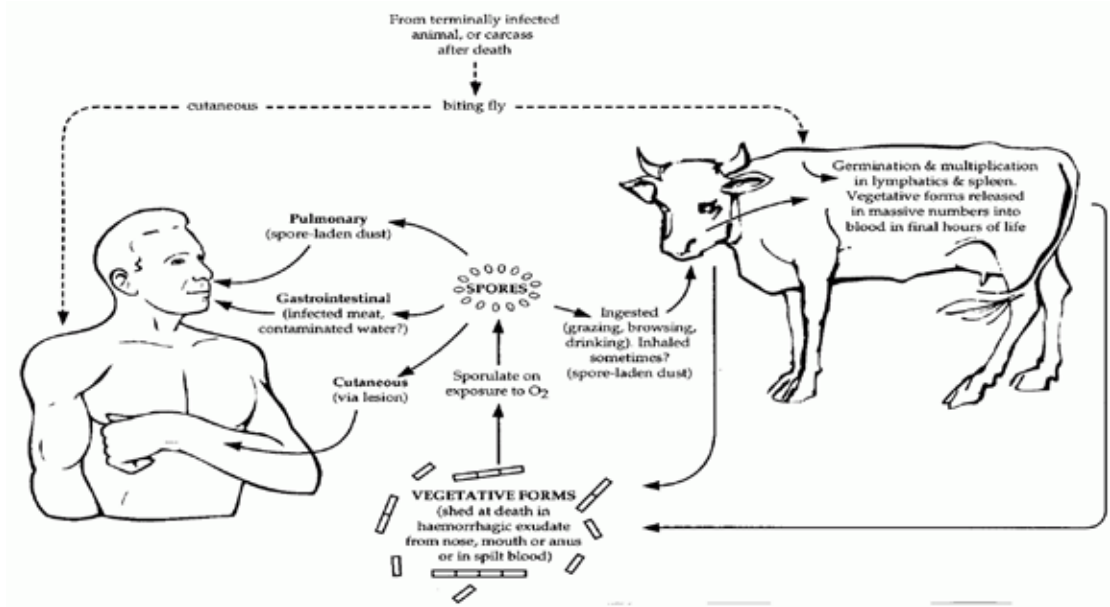


Fig 7: Primary routes of infection transmission

(Image Source: WHO)

Anthrax infection can occur via cutaneous, ingestion, injection, and inhalation routes. Spores introduced through the skin can result in cutaneous anthrax; breaks in the skin increase susceptibility. Eating meat from infected animals can result in ingestion (also called gastrointestinal) anthrax. Since 2000, injection transmission has been reported in cases of *B. anthracis* soft-tissue infections among intravenous heroin users in northern Europe. Aerosolized spores from contaminated hides or wool can cause inhalation anthrax. Anthrax in humans generally is not considered contagious; person-to-person transmission of cutaneous anthrax has been reported only rarely and only in instances of extremely close contact with an infected person (e.g., breastfeeding, dressing a wound, direct skin contact with the blood from a patient with anthrax).

Animals contract the disease by ingestion of bacterial spores, transmission usually happens from animals to humans but rarely from human-human or between animals. If ingested or inhaled by an animal, or after entering through cuts in the skin, they can germinate and cause disease.

CASE SCENARIO: SUSPECTED ANTHRAX

Patient Profile

- Age: 50 years
- Gender: Male
- Chief Complaints: High-grade fever, severe headache, fatigue, muscle pain, and a painless black raised sore on the hand.

Medical History: No significant past medical history reported.

Physical Examination

- General Appearance: Unwell
- Vital Signs: Temperature of 40°C (104°F)
- Cutaneous Findings: A 3 cm black, necrotic ulcer on the hand with surrounding edema.

History

- The patient is a farmer who recently handled animal carcasses and was involved in shearing and sorting wool.

Timely recognition, appropriate case definitions, and targeted interventions play a vital role in mitigating the impact of anthrax on human health.

IDSP CASE DEFINITION

- **Probable Case:** A person who has clinical illness* with an epidemiological link to confirmed or suspected animal cases (bleeding from natural orifices or bloated carcass) OR has exposure to contaminated animal products from these animals.
- **Clinical description:**
 - ◇ Cutaneous anthrax (most common after direct exposure): Skin lesion begins as a painless, pruritic papule on exposed parts (hands, feet and neck) which develops into a vesicle (usually 1-3 cm in diameter) and then a painless ulcer with a characteristic black necrotic (dying) area in the centre surrounded by erythema and edema. Systemic symptoms are mild and may include malaise and low-grade fever. There may be regional lymphangitis and lymphadenopathy.
 - ◇ Gastrointestinal anthrax: There are two clinical forms of intestinal anthrax - Symptoms include nausea, vomiting, fever, abdominal pain, hematemesis, bloody diarrhea and ascites.
 - ◇ Oropharyngeal anthrax – clinical features are sore throat, dysphagia, fever, lymphadenopathy in the neck and toxemia.
 - ◇ Pulmonary (inhalation): brief prodrome resembling acute respiratory illness, rapid onset of hypoxia, dyspnea and high temperature.]
- **Confirmed Case:** A presumptive case with: Isolation and identification of *B. anthracis* from relevant samples and identified by colony morphology, microscopy and biochemical tests Gamma phage lysis OR Validated PCR (Toxin and capsule genes) may be used for final confirmation (Validated 3PCR on direct clinical sample is also acceptable).

CLINICAL PRESENTATION

Anthrax manifests in four primary forms: cutaneous, ingestion, injection, and inhalation. Cutaneous anthrax results from direct contact with infected animals or their products, causing characteristic skin lesions. Ingestion of contaminated food leads to gastrointestinal anthrax, characterized by severe gastrointestinal symptoms. Injection anthrax occurs through the

injection of contaminated drugs, causing localized skin infections and potentially systemic disease. Inhalation anthrax, the most dangerous form, results from inhaling anthrax spores and can lead to severe respiratory illness and even death.

The symptoms of anthrax depend on the type of infection and can take anywhere from 1 day to more than 2 months to appear.

Cutaneous Anthrax

After an incubation period of 1 to 7 days, the infection begins with the appearance of a painless, itchy papule, usually within 2 to 3 days of exposure. This papule develops into a ring of vesicles surrounded by marked edema (swelling), and by days 5 to 7, it ulcerates, forming a characteristic round, regular-bordered lesion. As necrosis sets in, a black eschar (hardened scab) forms. The eschar gradually dries and sloughs off over the course of several weeks, beginning around day 10. The infection is often accompanied by localized swelling, regional lymph node enlargement, and sometimes systemic symptoms like malaise, headache, and fever. The head, neck, forearms, and hands are the most common areas affected.

The painless nature of the lesion and the formation of a black eschar are hallmark signs, distinguishing cutaneous anthrax from other infections. Without treatment, the mortality rate of cutaneous anthrax is approximately 20%, but with appropriate antibiotic therapy, it drops to less than 1%. Despite the severe appearance of the lesion, treatment is highly effective, and patients generally recover well with timely medical intervention.

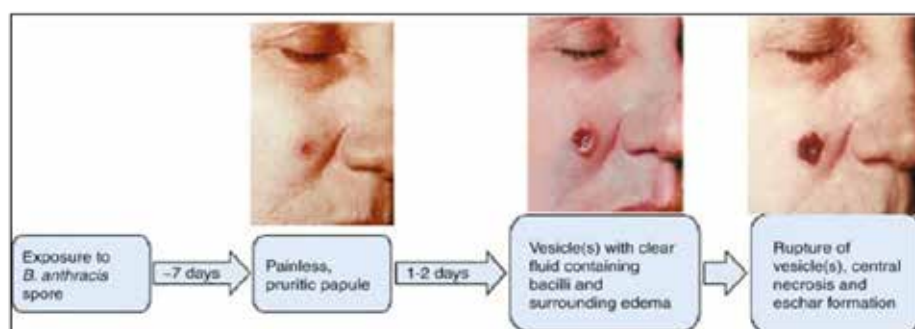


Fig 8: Progression of Cutaneous Anthrax

(Image source: https://doi.org/10.1007/978-3-319-75623-3_22)

Ingestion or Gastrointestinal Anthrax

Gastrointestinal anthrax occurs when a person consumes raw or undercooked meat from an animal infected with *Bacillus anthracis* spores. The infection can affect the upper gastrointestinal tract, stomach, and intestines. Symptoms typically develop within 1 to 7 days after ingestion, though incubation periods as long as 16 days have been reported. Clinically, gastrointestinal anthrax can present as either intestinal or the less common oropharyngeal form. Both forms of gastrointestinal anthrax are associated with fever and chills. Without treatment, more than half of those infected may die; with appropriate medical intervention, the case-fatality rate decreases to less than 40%.

Oropharyngeal Anthrax

Patients typically present with high fever, severe sore throat, ulcers in the posterior oropharynx, difficulty swallowing (dysphagia), and regional lymphadenopathy causing marked neck swelling. Early lesions in the oropharynx appear edematous and congested, progressing by the end of the first week to central necrosis and ulceration.

Intestinal Anthrax

Initial symptoms include nausea, anorexia, vomiting, and high fever. As the condition worsens, patients may experience severe abdominal pain, hematemesis (vomiting blood), bloody or melanic stools, and sometimes ascites. This condition may resemble an acute abdomen, with severe pain, distention, and tenderness. Involvement typically affects the terminal ileum and cecum, and without treatment, intestinal anthrax can lead to septicemia.

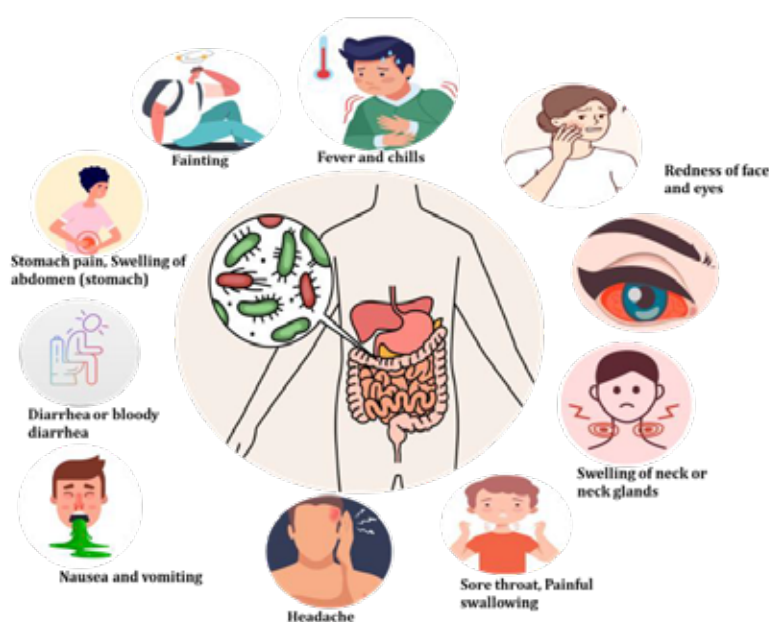


Fig 9: Symptoms of Ingestion or Gastrointestinal Anthrax

Injection Anthrax

Injection Anthrax occurs primarily in injection drug users and typically develops within 1 to 4 days of exposure to anthrax spores. It is associated with a high mortality rate, with more than 25% of confirmed cases resulting in death. Patients commonly present with severe soft-tissue infections, including swelling, erythema (redness), and extensive bruising at the injection site. Despite the significant swelling, the pain experienced is often less severe than expected. A distinguishing feature of injection anthrax is the appearance of small vesicles or papules at the injection site, which progress to painless ulcerative lesions, similar to cutaneous anthrax.

One of the challenges with injection anthrax is that it can be more difficult to diagnose compared to other forms of anthrax. The condition often progresses rapidly to systemic illness, with most patients becoming septic. This rapid progression and the initial presentation as a localized

soft tissue infection make it harder to identify and treat early, contributing to the high risk of severe complications and mortality.

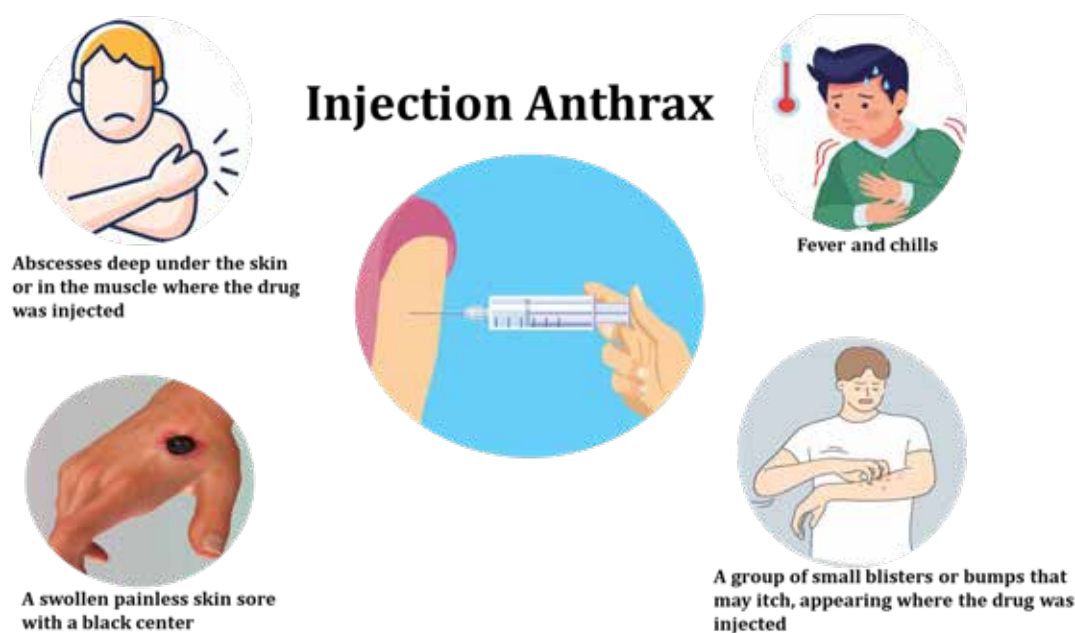


Fig 10: Symptoms of Injection Anthrax

Inhalation Anthrax

Inhalation anthrax occurs when a person inhales anthrax spores, commonly in environments such as wool mills, slaughterhouses, and tanneries where infected animals or contaminated products are present. The incubation period is typically within a week, though it can extend up to 2 months.

The illness begins with nonspecific symptoms (Prodromal Period) like fever, chills, fatigue, cough, shortness of breath, chest pain, and sometimes nausea or vomiting, making it difficult to differentiate from other respiratory illnesses like influenza, COVID-19, or pneumonia. As the disease progresses, symptoms such as severe shortness of breath, chest pain, altered mental status, headache, and nausea become more pronounced. In some cases, patients experience severe upper respiratory symptoms, though myalgias (muscle pain) are rare. Altered mental status or significant respiratory distress often brings patients to medical attention, marking the beginning of the fulminant phase.

During the second stage of illness, patients develop severe hemorrhagic lymphadenitis and mediastinitis, often leading to bacteremia, respiratory failure, and hemodynamic collapse. Meningitis occurs in up to 50% of cases, causing confusion, headache, and progression to coma. A chest X-ray may show a widened mediastinum due to lymphadenopathy, and pleural effusions or pulmonary infiltrates, which may be hemorrhagic, are also common. This is the deadliest form of anthrax, with a rapid course; symptoms can progress to death within 1 to 10 days. Without treatment, the survival rate is only 10-15%, but with aggressive medical intervention, the survival rate improves to around 55%.

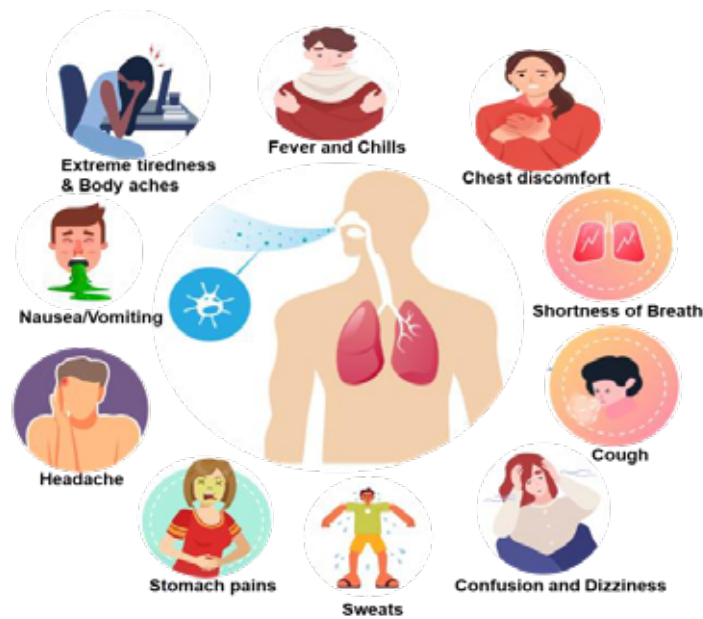


Fig 11: Symptoms of Inhalation Anthrax

DISEASE PROGRESSION

Cutaneous Anthrax

- **1-10 days post-exposure:** Development of a painless, pruritic papule.
- **1-2 weeks later:** Progression to a painless ulcer with a black, necrotic center and surrounding edema.
- **Healing:** Eschar formation and eventual healing.

Inhalation Anthrax

- **1-6 days post-exposure:** Initial flu-like symptoms (fever, chills, fatigue, chest discomfort).
- **1-2 weeks later:** Severe respiratory distress, mediastinitis, and sepsis.
- **Rapid progression:** Can lead to death within days if untreated.

Ingestion Anthrax

- **1-7 days post-exposure:** Abdominal pain, nausea, vomiting, and bloody diarrhea.
- **Progression:** Severe sepsis and potential death.

Injection Anthrax

- **1-4 days post-exposure:** Development of a painless ulcerative lesion at the injection site.
- **Potential for systemic infection:** Can progress to a more severe illness, similar to inhalation anthrax.

DIFFERENTIAL DIAGNOSIS

Key distinguishing features of anthrax compared to its differential diagnoses.

Table 1: Cutaneous Anthrax Differential Diagnosis

Diagnosis	Key Characteristics	Epidemiological Clue	Laboratory Tests	Elevated Biochemical Levels
Cutaneous Anthrax	Painless, pruritic papule progressing to a necrotic ulcer with a characteristic black eschar.	Exposure to livestock, animal hides, or contaminated soil in endemic areas.	Gram stain and culture of lesion; PCR for <i>Bacillus anthracis</i> DNA; serology; CBC may show leukocytosis.	CRP, ESR elevated; possible mild elevation of liver enzymes (ALT/AST) if systemic involvement.
Staphylococcal Skin Abscess	Painful, localized swelling with pus; no black eschar.	History of skin trauma, poor hygiene, or recent hospitalization.	Gram stain and culture of pus; CBC shows leukocytosis; ESR/CRP elevated.	CRP, ESR elevated; no significant liver or kidney function abnormalities unless sepsis develops.
Cat Scratch Disease	Papule or pustule at the scratch site, regional lymphadenopathy; no necrotic ulcer or eschar.	History of cat scratch or bite; common in children or young adults with cat exposure.	Serology for <i>Bartonella henselae</i> (IgM/IgG); PCR for <i>Bartonella</i> DNA; CBC shows mild leukocytosis or eosinophilia.	CRP, ESR elevated; ALT/AST mildly elevated if systemic symptoms occur.
Tularemia	Painful skin ulcer at the site of entry, fever, and lymphadenopathy; lacks characteristic eschar.	Exposure to rabbits, ticks, or deer flies; handling animal carcasses in endemic regions.	PCR for <i>Francisella tularensis</i> DNA; serology; culture of ulcer exudate or lymph node aspirate; CBC shows leukocytosis.	CRP, ESR elevated; ALT/AST elevated in systemic tularemia; possible elevated LDH and bilirubin .
Spider Bite	Local swelling and erythema; may ulcerate but lacks painless black eschar.	History of bite in an endemic area for venomous spiders (e.g., <i>Loxosceles</i> or <i>Latrodectus</i> species).	Clinical diagnosis; no specific tests unless secondary bacterial infection; CBC and wound culture if infected.	No specific biochemical elevations; secondary infections may cause elevated CRP, ESR or mild liver enzymes .
Ecthyma Gangrenosum	Associated with <i>Pseudomonas</i> infections; rapidly progressing gangrenous ulcers in immunocompromised patients.	Common in immunocompromised individuals (e.g., neutropenic patients, diabetes, or those with recent hospitalizations).	Culture of wound exudate or tissue biopsy for <i>Pseudomonas</i> ; blood cultures; CBC shows neutropenia; ESR/CRP elevated.	CRP, ESR elevated; may have elevated ALT/AST, creatinine if severe sepsis or renal involvement.

Key Highlights

1. **Cutaneous Anthrax:** Painless papule with black eschar; exposure to livestock or contaminated soil. Elevated CRP, ESR, mild liver enzyme increases in systemic cases.
2. **Staphylococcal Skin Abscess:** Painful swelling with pus, no eschar. Common with skin trauma or poor hygiene. Elevated CRP, ESR, leucocytosis; no significant liver/kidney issues unless sepsis develops.
3. **Cat Scratch Disease:** Papule and regional lymphadenopathy, no eschar. Linked to cat scratches/bites, especially in children. Mild ALT/AST, elevated CRP, ESR in systemic cases.
4. **Tularemia:** Painful ulcer with fever and lymphadenopathy, no eschar. Linked to rabbits/ticks, elevated CRP, ESR, ALT/AST in systemic cases.
5. **Spider Bite:** Local swelling and erythema, may ulcerate, no eschar. History of venomous bite. Elevated CRP, ESR if secondary infection occurs.
6. **Ecthyma Gangrenosum:** Rapid gangrenous ulcers, common in immunocompromised. Elevated CRP, ESR, ALT/AST, creatinine if severe.

Table 2: Inhalation Anthrax Differential Diagnosis

Diagnosis	Key Characteristics	Epidemiological Clue	Laboratory Tests	Elevated Biochemical Levels
Inhalation Anthrax	Rapid progression to severe respiratory distress and systemic illness; mediastinal widening on chest X-ray.	Exposure to contaminated animal products, hides, or soil in endemic areas.	Gram stain and culture of sputum or blood; PCR for <i>Bacillus anthracis</i> DNA; chest X-ray for mediastinal widening.	CRP, ESR elevated; possible elevated LDH, liver enzymes (ALT/AST) in systemic involvement.
Community-Acquired Pneumonia	Fever, cough, sputum production; lacks mediastinal widening and rapid systemic progression.	History of recent respiratory illness or exposure to pathogens in the community.	Chest X-ray or CT scan for consolidation; sputum culture or PCR for bacterial pathogens (e.g., <i>Streptococcus pneumoniae</i>).	CRP, ESR elevated; WBC count may be elevated depending on bacterial or viral etiology.
Influenza	Sudden onset of fever, fatigue, muscle aches, and cough; does not lead to mediastinal widening.	Recent exposure to infected individuals or seasonal outbreaks, often in winter months.	Rapid antigen test for influenza A/B; PCR for confirmation; chest X-ray shows no mediastinal widening.	CRP, ESR mildly elevated; WBC count typically normal or slightly elevated, ALT/AST elevated if complications occur.
Respiratory Syncytial Virus (RSV)	Common in children, presents with wheezing and bronchiolitis; lacks severe systemic features.	Recent exposure to infected children or in daycare settings, seasonal outbreaks.	Nasal swab for RSV antigen or PCR; chest X-ray may show bronchial obstruction or hyperinflation.	CRP, ESR mildly elevated; WBC count typically normal or slightly elevated.
Pneumonic Plague	Hemoptysis and high fever, with pulmonary consolidation; less likely to show mediastinal widening.	Exposure to infected rodents, fleas, or animals in endemic areas (e.g., <i>Yersinia pestis</i> in rodents).	Blood culture for <i>Yersinia pestis</i> ; PCR for <i>Yersinia</i> DNA; chest X-ray for consolidation but no mediastinal widening.	CRP, ESR elevated; WBC count elevated, often with a left shift; LDH and ALT/AST may be mildly elevated.
Tularemia	Pulmonary form shows patchy infiltrates on X-ray, fever, and pleuritis; lacks widened mediastinum.	Exposure to infected animals (rabbits, rodents), ticks, or contaminated water in endemic regions.	PCR for <i>Francisella tularensis</i> DNA; serology for <i>Francisella</i> antibodies; chest X-ray for pulmonary infiltrates.	CRP, ESR elevated; WBC count may be elevated; mild ALT/AST elevation if systemic or liver involvement.

Key Highlights:

1. **Inhalation Anthrax:** Rapid progression and mediastinal widening on chest X-ray, often with severe systemic illness.
2. **Community-Acquired Pneumonia:** Classic pneumonia with fever, cough, and sputum but without mediastinal widening.
3. **Influenza:** Abrupt onset with systemic symptoms like fever and muscle aches; chest X-ray typically shows no mediastinal widening.
4. **RSV:** Wheezing and bronchiolitis, common in children; does not present with systemic features or mediastinal widening.
5. **Pneumonic Plague:** Haemoptysis and high fever, with pulmonary consolidation; less often mediastinal widening.
6. **Tularemia:** Pulmonary form shows patchy infiltrates, often with fever and pleuritis, without mediastinal widening.

Table 3: Gastrointestinal Anthrax Differential Diagnosis

Diagnosis	Key Characteristics	Epidemiological Clue	Laboratory Tests	Elevated Biochemical Levels
Gastrointestinal Anthrax	Sudden onset of severe abdominal pain, bloody diarrhea, and vomiting.	Exposure to contaminated food or undercooked meat from infected animals (typically herbivores like cattle, sheep).	Stool culture or PCR for <i>Bacillus anthracis</i> DNA; blood culture for systemic infection; abdominal X-ray may show bowel edema.	CRP, ESR elevated; LDH, ALT/AST may be elevated due to systemic involvement; mild leukocytosis.
Ulceroglandular Tularemia	Local ulcer with painful lymphadenopathy; lacks severe abdominal pain and bloody diarrhea.	Exposure to rabbits, rodents, ticks, or contaminated water in endemic regions.	PCR for <i>Francisella tularensis</i> DNA; serology for antibodies; wound culture or biopsy for <i>Francisella tularensis</i> .	CRP, ESR elevated; WBC count may be elevated; mild ALT/AST if systemic involvement occurs.
Bubonic Plague	Painful, swollen lymph nodes (buboes), fever; no bloody diarrhea or severe abdominal pain.	Exposure to fleas from infected rodents or close contact with infected animals (e.g., <i>Yersinia pestis</i> in rodents).	Blood culture for <i>Yersinia pestis</i> ; PCR for <i>Yersinia</i> DNA; chest X-ray for lung involvement, if present.	CRP, ESR elevated; WBC count elevated with a left shift; LDH, ALT/AST mildly elevated in severe cases.

Key Highlights:

1. **Gastrointestinal Anthrax:** Severe abdominal symptoms, bloody diarrhoea, and vomiting with possible systemic infection. Elevated CRP, ESR, and liver enzymes in severe cases.
2. **Ulceroglandular Tularaemia:** Local ulcer with regional lymphadenopathy but no gastrointestinal involvement. Elevated CRP, ESR and mild ALT/AST in some cases.
3. **Bubonic Plague:** Characterized by painful buboes and fever; no abdominal symptoms or diarrhoea. Elevated WBC count, CRP, and ESR.

Table 4: Injection Anthrax Differential Diagnosis

Diagnosis	Key Characteristics	Epidemiological Clue	Laboratory Tests	Elevated Biochemical Levels
Injection Anthrax	Severe soft tissue infection with extensive edema, often without a black eschar; progresses rapidly to systemic illness.	History of injection of contaminated drugs (especially heroin); exposure to animal products in some cases.	Gram stain and culture of wound exudate; PCR for <i>Bacillus anthracis</i> DNA; blood cultures; chest X-ray if systemic spread.	CRP, ESR elevated; LDH, ALT/AST may be elevated if systemic illness develops; possible leukocytosis.
Necrotizing Fasciitis	Rapidly spreading tissue infection with severe pain, crepitus, and necrosis; typically caused by Group A <i>Streptococcus</i> or <i>Clostridium</i> species.	History of trauma, surgery, or immunocompromised state; can follow viral infections or insect bites.	Blood culture; Gram stain and culture of wound exudate; imaging to assess tissue involvement.	CRP, ESR elevated; WBC count elevated with a left shift; ALT/AST, LDH elevated in systemic cases.
Cellulitis	Localized skin infection with redness, swelling, and warmth; lacks rapid systemic progression and severe edema.	History of skin trauma or wound, especially in diabetics, immunocompromised, or elderly patients.	Gram stain and culture of wound exudate; CBC shows mild leukocytosis; skin biopsy if the diagnosis is unclear.	CRP, ESR mildly elevated; WBC count mildly elevated in bacterial infections.
Abscess	Localized collection of pus, tender and fluctuant; no rapid spread or systemic illness.	History of trauma or infection, often in areas with poor hygiene or immunocompromised individuals.	Culture of pus or fluid from abscess; CBC with mild leukocytosis if infected; ultrasound or CT to assess depth.	CRP, ESR mildly elevated; WBC count elevated in acute cases.
Gas Gangrene	Caused by <i>Clostridium perfringens</i> , presents with gas in tissues (crepitus), severe pain, and systemic toxicity.	History of trauma, surgery, or chronic disease (e.g., diabetes); often occurs in deep, contaminated wounds.	Gram stain and culture of wound tissue; PCR for <i>Clostridium</i> species; imaging for gas in tissues.	CRP, ESR elevated; WBC count elevated with a left shift; LDH, ALT/AST elevated in severe cases.

Key Highlights:

1. **Injection Anthrax:** Severe soft tissue infection with extensive oedema, possible systemic spread, **elevated CRP, ESR, and LDH.**
2. **Necrotizing Fasciitis:** Rapid tissue necrosis with crepitus and severe pain, caused by Group A Streptococcus or Clostridium, elevated **WBC count, CRP, and LDH.**
3. **Cellulitis:** Localized skin infection, mild **CRP** and **ESR** elevations; no systemic spread or severe oedema.
4. **Abscess:** Localized pus collection, mild biochemical changes (**CRP, WBC count**) if infected.
5. **Gas Gangrene:** Caused by Clostridium perfringens, characterized by gas in tissues (crepitus), severe pain, and systemic toxicity, with **elevated CRP, ESR, and LDH.**

COMPLICATIONS

Meningitis: It is a severe form of anthrax that can develop from any of the other three forms of the disease. It is characterized by hemorrhagic leptomeningitis, which involves intense inflammation and edema of the meninges. Symptoms include neck pain, headache, altered mental state, vomiting, and high-grade fever, followed by elevated cerebrospinal fluid (CSF) pressure, blood in the CSF, disorientation, loss of consciousness, and rapid death. Neurological signs may include cranial nerve palsies, myoclonus, fasciculations, decerebrate posturing, and papilledema.

The hemorrhagic component is a distinctive feature, rarely seen in other central nervous system infections. Prognosis is extremely poor, with survival being rare and contingent on early diagnosis and prompt treatment. In some cases, meningeal signs appear first due to early spore translocation to the meninges. Definitive diagnosis is achieved by identifying capsulated bacilli in the CSF or through culture.

Sepsis: It occurs when Bacillus anthracis spreads from a primary lesion (cutaneous, gastrointestinal, or pulmonary) into the bloodstream. It is characterized by high fever, toxemia, and shock, leading to rapid death. Definitive diagnosis is achieved by isolating B. anthracis from the primary lesion and blood cultures, or by detecting its toxin or DNA in these samples.

LABORATORY CRITERIA FOR DIAGNOSIS

Laboratory confirmation of anthrax can be achieved through one or more of the following methods:

- Isolation of Bacillus anthracis from clinical specimens such as blood, lesions, or discharges.
- Microscopic Examination: Demonstration of B. anthracis in stained smears from clinical specimens like blood, cerebrospinal fluid, pleural fluid, or stool.
- Serological Tests or Molecular Tests: Positive results from serological tests (ELISA) or molecular tests like PCR.

Veterinarians



Animal handlers



Handlers of drums made from contaminated goatskins



Butchers and abattoir workers



Agricultural workers



Livestock producers dealing with sick animals



Laboratory staff who handle Anthrax



Uncooked Meat eaters

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Case Profile	
Patient's age	50 years
Gender	Male
Medical conditions	- High grade fever, severe headache, fatigue, muscle pain from past few days. - Painless black raised sore on hand
Previous treatments	None reported
Medical Examination	On examination, he appears unwell with a temperature of 40°C (104°F). There is a black, necrotic ulcer on his hand, approximately 3 centimeters in diameter, with surrounding edema.
History	He works on a farm who recently handled animal carcasses and has been involved in the shearing and sorting of wool.



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- ✓ **Probable Case:** A person who has clinical illness* with an epidemiological link to confirmed or suspected animal cases (bleeding from natural orifices or bloated carcass) OR has exposure to contaminated animal products from these animals. Clinical description:

- **Cutaneous anthrax (most common after direct exposure):** Skin lesion begins as a painless, pruritic nodule on exposed parts (hands, feet and neck) which develops into a vesicle (usually 1-3 cm in diameter) and then a painless ulcer with a characteristic black necrotic (dying) area in the centre surrounded by erythema and edema. Systemic symptoms are mild and may include malaise and low-grade fever. There may be regional lymphangitis and lymphadenopathy.
- **Gastrointestinal anthrax:** There are two clinical forms of intestinal anthrax - Symptoms include nausea, vomiting, fever, abdominal pain, hematemesis, bloody diarrhea and ascites.
- **Oropharyngeal anthrax** - clinical features are sore throat, dysphagia, fever, lymphadenopathy in the neck and toxæmia.
- **Pulmonary (inhalation):** brief prodrome resembling acute respiratory illness, rapid onset of hypoxia, dyspnea and high temperature.

- ✓ **Confirmed Case:** A presumptive case with: Isolation and identification of *B. anthracis* from relevant samples and identified by colony morphology, microscopy and biochemical tests Gamma phage lysis OR Validated PCR (Toxin and capsule genes) may be used for final confirmation (Validated 3PCR on direct clinical sample is also acceptable)



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Anthrax has 4 main clinical presentations—cutaneous, ingestion, injection, and inhalation. Anthrax meningitis can complicate any of the 4 main clinical presentations

Cutaneous Anthrax



Direct contact with an infected animal

Ingestion Anthrax



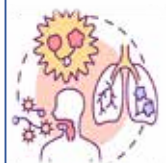
Ingestion of contaminated food

Injection Anthrax



**Contracted through
injecting contaminated
drugs.**

Inhalation Anthrax



Inhaling bacterial spores

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Cutaneous Anthrax



A painless skin sore (ulcer) with a black center small blisters or bumps (usually on face, neck, arms, or hand)



A group of small blisters or bumps that may itch

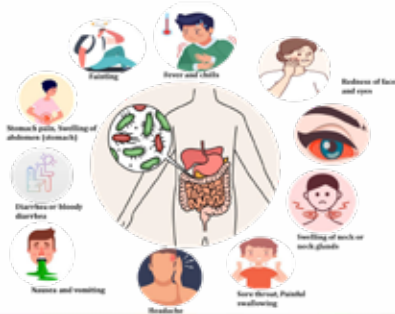


Progression of Cutaneous Anthrax

Centre for One Health, NCDC, MoHFW

ANTHRAX

Ingestion or Gastrointestinal Anthrax



Centre for One Health, NCDC, MoHFW

ANTHRAX

Injection Anthrax



Abscesses deep under the skin or in the muscle where the drug was injected



A swollen painless skin sore with a black center



Fever and chills



A group of small blisters or bumps that may itch, appearing where the drug was injected

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ANTHRAX

Inhalational Anthrax



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ANTHRAX

[illegible]

- Development of pruritic papular lesion 1-10 days post infection.
- Development of painless ulcer
- Progression
 - into necrotic and blackened center surrounding non-pitting edema
 - 1-2 weeks later eschar dries and lesion heals



- Fever, sweats, nausea, vomiting, malaise, chest pain and nonproductive cough after 1-6 days post infection
- Hemorrhagic lymphadenitis and mediastinitis, progression to bacteremia
- Dyspnea, and stridor from increasing lymphadenopathy, respiratory failure and hemodynamic collapse
- The time from onset of symptoms to death ranges from 1 to 10 days



- Fever, nausea, vomiting, and diarrhea-like symptoms appear 1–7 days after infection
- Bloody diarrhea, hematemesis, and massive ascites
- Untreated patients progress to septicemia
- Mortality ranges from 25% to 60%.



- Development of papules at the injection site. Symptoms usually appear 1-4 days post exposure
- Painless ulcerative lesion similar to cutaneous anthrax
- Difficult to recognize and may progress more rapidly to systemic illness than cutaneous anthrax

- **Laboratory confirmation by one or more of the following:**
- Isolation of *Bacillus anthracis* from a clinical specimen (e.g., blood, lesions, discharges).
- Demonstration of *B. anthracis* in a clinical specimen by microscopic examination of stained smears (vesicular fluid, blood, cerebrospinal fluid, pleural fluid, stools)
- Positive serology (ELISA), or molecular tests like PCR.



Session-4

Anthrax in Animals

Course No. 004	Session 4: Anthrax in Animals
Duration	20 minutes
Learning Objectives	• To understand host range and reservoirs for Anthrax in animals • Describe clinical signs and symptoms • Understand disease transmission in animals • Describe disease progression • Understand case definitions
Pre-requisite knowledge/skill	• Basic knowledge of veterinary infectious diseases and zoonotic reservoirs. • Familiarity with clinical signs and progression of diseases in domestic and wild animals • Understanding of disease transmission dynamics and case definition in animal health surveillance.
Competencies to be achieved	• Capability to explain the signs and symptoms in different animals • Comprehension disease progression • Understanding disease transmission • Defining case of Anthrax • Understanding Post-Mortem risks and lesions

Anthrax is a serious bacterial disease that primarily affects herbivores such as cattle, sheep, goats, and horses. Animals typically become infected by ingesting anthrax spores present in contaminated soil, water, or feed. Carnivores can also become infected by consuming infected carcasses. Environmental factors, like flooding, can expose animals to spores, triggering outbreaks. Once ingested, the spores germinate in the animal's body and release toxins, leading to a rapid onset of symptoms and often resulting in death within hours or days. The bacteria can also form spores, allowing it to survive in the environment for extended periods. The incubation period for anthrax in cattle can vary from 1 to 14 days. However, for trade purposes, the OIE (World Organisation for Animal Health) considers a 20-day incubation period. Anthrax can manifest in different forms in animals, including peracute, acute, subacute, and chronic forms. The peracute and acute forms often lead to sudden death with minimal clinical signs, while the subacute form may show signs of illness like fever, depression, and swollen lymph nodes. The chronic form is rare and characterized by localized lesions. Understanding the epidemiology, clinical signs, and transmission routes of anthrax in animals is crucial for effective prevention and control measures.

CLINICAL MANIFESTATIONS

Ruminant animals are often found dead without indication that they are ill. In the acute form, there may be high fever, muscle tremors, and difficulty breathing seen shortly before the animal collapses and dies. Un-clotted blood may exude from the body's natural orifices and the body may not stiffen after death. The subacute form may be accompanied by progressive fever, depression, inappetence, weakness, prostration, and death.

In horses and (on occasions) in ruminants there may be digestive upsets and colic, fever, depression and sometimes swelling. These symptoms may last for up to four days before death results.

In carnivores when the animal feeds on an infected source there may be an intestinal form of the disease with fever and cramps from which animals sometimes recover.

Anthrax primarily spreads from animals to humans, but rarely between humans or animals. Animals can contract anthrax through various routes: ingestion of contaminated water or food, consumption of infected animal carcasses by carnivores, and insect bites that transmit the bacteria from infected animal bodies. Additionally, direct contact with infected animals or their products can lead to skin infections in humans.

TRANSMISSION CYCLE IN ANIMALS

Animals become infected by ingesting anthrax spores present in contaminated soil, water, or feed. Once ingested, the spores germinate, leading to a rapid onset of symptoms and often resulting in death. The bacteria form spores, allowing it to survive in the environment for extended periods. This cycle of infection, death, and spore formation perpetuates the disease.

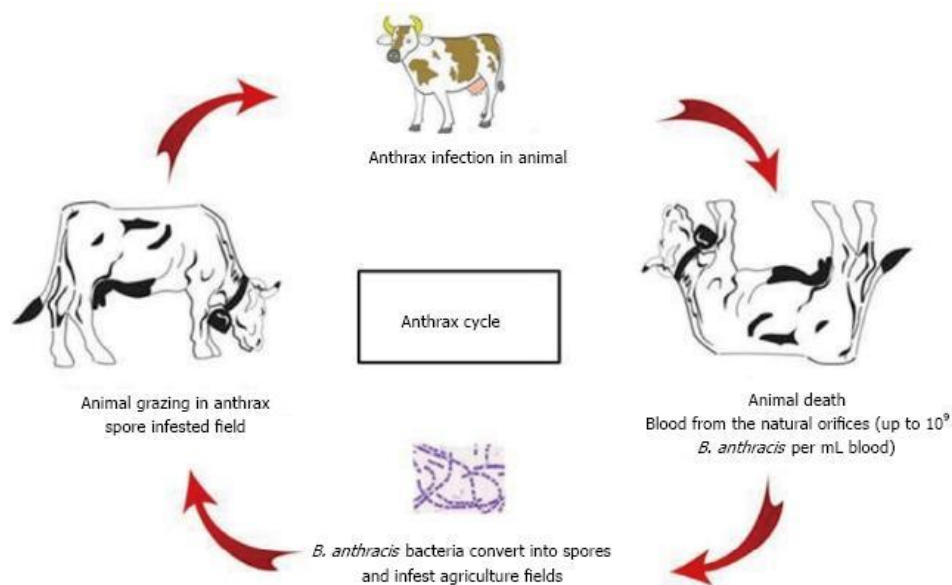


Fig 12: Transmission in Animals

(Image source: <https://rr-middleeast.woah.org/en/our-mission/one-health/anthrax/>)

RECOMMENDED CASE DEFINITION: ANIMALS

Suspected case: A case that is compatible with the clinical description. In enzootic areas a suspected case can be:

1. A case of sudden death
2. Un-clotted blood-stained discharges through mouth, nose or anus.

Confirmed case: A suspected case that is laboratory confirmed.

Anthrax in animals can manifest in different forms, each with its own progression and clinical signs. The peracute form is the most severe, leading to sudden death without any noticeable symptoms. In the acute form, animals may exhibit fever, weakness, loss of appetite, and swollen lymph nodes, often leading to death within a few days. The subacute form is characterized by a slower progression, with symptoms such as progressive fever, depression, weakness, and inappetence. The chronic form, which is rare, presents with enlarged lymph nodes as the primary sign. Understanding these different disease progressions is crucial for timely diagnosis and implementation of appropriate control measures.

Transmission Cycle in Animals

The diagram illustrates the transmission cycle of Anthrax in animals, showing a continuous loop of infection and shedding. At the top, a brown and white cow is labeled "Anthrax infection in animal". A red arrow points from the cow to a box in the center labeled "Anthrax cycle". From the box, a red arrow points to a cow lying on its side, labeled "Animal death. Microbes from the cultural infection (up to 10⁷ A. anthracis per ml, blood)". A red arrow points from the dead cow to a purple, multi-lobed structure labeled "A. anthracis bacteria convert into spores and infect agriculture fields". A red arrow points from the spores to a cow standing in a field, labeled "Animal grazing in anthracis spore infected field". A red arrow points from the grazing cow back to the infected animal at the top, completing the cycle.

Transmission Cycle in Animals

Anthrax infection in animal

Anthrax cycle

Animal death. Microbes from the cultural infection (up to 10^7 A. anthracis per ml, blood)

A. anthracis bacteria convert into spores and infect agriculture fields

Animal grazing in anthracis spore infected field

Control for One Health, NCDC, Mumbai

ANTHRAX

Suspected	Confirmed
A case that is compatible with the clinical description. In enzootic areas a suspected case can be: ✓ A case of sudden death ✓ Un-clotted blood stained discharges through mouth, nose or anus	A suspected case that is laboratory confirmed

Disease Progression in Animals

Per-acute

- Found dead condition, no ante mortem signs visible

Acute

- no ante mortem signs visible

Sub-acute

- progressive fever, depression, inappetence, weakness, prostration and death

Chronic

- Rare
- Enlarged lymph glands

Source: *For One Health, INDC, 2018/19*

ANTHRAX

[illegible]

Session-5

Laboratory Diagnosis

Course No. 005	Session 5: Laboratory Diagnosis
Duration	20 minutes
Learning Objectives	• Describe sample collection, transport and storage • Following Good laboratory practice • Familiarity with various presumptive and confirmatory techniques to identify the pathogen • Knowledge about referral laboratories in India
Pre-requisite knowledge/skill	• Basic knowledge of biosafety, aseptic techniques, and sample handling procedures. • Familiarity with standard microbiological diagnostic methods and lab workflows. • Awareness of the laboratory network for zoonotic diseases in India.
Competencies to be achieved	• Understanding materials to be needed for sampling • Describe PPE kit and triple packaging system • Explain various presumptive and confirmatory tests • Capability to describe referral laboratories in India

LABORATORY DIAGNOSIS

Since Anthrax is a highly virulent organism, biosafety level class II/III cabinets are mandatory. All samples must be treated as potentially biohazardous. Accurate diagnosis of anthrax requires meticulous sampling, appropriate PPE use, familiarity with identification techniques, and access to referral laboratories. Following recommended procedures ensures the safety of healthcare professionals, accurate diagnosis, and effective management of anthrax cases.

Collecting appropriate specimens is critical for accurate diagnosis. Skin lesions, blood, and tissue samples are common choices. Use sterile instruments, avoiding contamination. Transport specimens in sealed containers, preferably with a cold pack to preserve integrity. Store samples at appropriate temperatures to maintain viability for testing.

Proper PPE is essential to prevent exposure. Wear gloves, masks, gowns, and eye protection while handling potentially infected materials. Disinfect PPE after use and wash hands thoroughly. Adhering to PPE Learning Guidelines ensures safety for both laboratory workers and the environment.

Various techniques aid in identifying *Bacillus anthracis*. Presumptive tests include gram staining, showing characteristic gram-positive rods. Confirmatory methods involve culturing samples on specialized media, followed by biochemical and genetic analyses. Polymerase chain reaction (PCR) is often used to detect specific DNA sequences.

Several laboratories across India are equipped for anthrax testing. High-level Biosafety Level 3 (BSL-3) labs handle live anthrax cultures. The National Institute of Virology, Pune, and Indian Veterinary Research Institute, Uttar Pradesh, are key referral centers. State-level animal disease diagnostic laboratories also offer testing services.

SAMPLE COLLECTION

Specimen Collection- Human Anthrax

All samples must be treated as potentially biohazardous.

- **Cutaneous Anthrax:**
 - ◇ Collect vesicular exudate from lesions using a sterile swab at an early stage.
 - ◇ At a later stage, collect material from underneath the eschar by lifting it with sterile forceps.
- **Intestinal Anthrax:**
 - ◇ Collect a fecal specimen if the patient is not severely ill.
 - ◇ If the patient is severely ill, collect ascitic fluid (peritoneal fluid).

- **Pulmonary Anthrax:**
 - ◊ Collect sputum if the patient is not severely ill.
 - ◊ If the patient is severely ill, collect bronchial lavage.
- **Sample Processing:** Process samples in a BSLII type B2 bio-safety cabinet with Biosafety Level 2 practices. Use BSL3 standards when working with quantities of the organism, activities with high potential for aerosol production, or activities with antibiotic-resistant strains.

SAMPLE PROCESSING

Process samples in a BSLII type B2 bio-safety cabinet with Biosafety Level 2 practices. Use BSL3 standards when working with quantities of the organism, activities with high potential for aerosol production, or activities with antibiotic-resistant strains. Sample transportation

SAMPLE TRANSPORTATION

The proper packaging and transportation of biological samples, such as those containing anthrax, is crucial to prevent accidental exposure and contamination. The process involves multiple layers of containment to ensure safety.

Key Points for Safe Packaging and Transportation of Biological Samples:

Primary Containment:

- Place the sample in a sealed plastic bag with absorbent material.

Secondary Containment

- Place the sealed plastic bag inside a shipping canister lined with absorbent material.
- Label the canister as "Biohazardous Material."

Tertiary Containment

- Place the shipping canister in a polystyrene foam-lined box with dry ice.

Documentation

- Include an itemized list of contents in a separate sealed bag.
- Clearly label the package with sender's information and biohazard symbol.

Table 5: Specimen Types and Containers

Sample Type	Container Type	Volume Requirements
Aspirated Pus (exudate)	Universal disposable sterile container (wide-mouthed, screw-capped, leak-proof)	>0.5 ml
Swab from lesion	Sterile tube with two sterile swabs	Each swab should be insinuated beneath the scab and rotated 2-3 times
Fluids (bleb, ascitic, peritoneal, etc.)	Universal disposable sterile container (wide-mouthed, screw-capped, leak-proof)	>0.5 ml
CSF	Sterile Eppendorf tube (provided in the lumbar puncture set)	>0.5 ml
Blood	BacT/Alert blood culture bottles	Maximum volume: 10 ml
Sputum & Gastric Aspirate	Universal disposable sterile container (wide-mouthed, screw-capped, leak-proof)	>0.5 ml
Stool	Universal disposable sterile container (wide-mouthed, screw-capped, leak-proof)	>0.5 ml

Key points for Specimen Transport

- 1. Leak-Proof Containers:** Collect specimens in appropriate leak-proof, break-resistant (preferably screw-capped) containers and secure them.
- 2. Disinfection and Labeling:** Wipe the container with a disinfectant solution (sodium hypochlorite), label, and triple package.
- 3. Transport:** Place the sample container in a clear plastic cover with a biohazard label, seal, and immediately transport to the laboratory (hand carried).
- 4. PPE for Processing:** Wear gloves, N95 mask, apron, visor, and boot cover during sample processing and follow-up.

DIAGNOSTIC TESTS

Early and accurate diagnosis of anthrax is essential for effective treatment and outbreak prevention. The timing of sample collection depends on the clinical presentation of the disease. Diagnosis involves both presumptive and confirmatory methods. Presumptive diagnosis is based on clinical signs, epidemiological factors, and preliminary laboratory tests, including microscopy, biochemical tests, and serological tests. Confirmatory diagnosis relies on laboratory techniques such as bacterial culture, Gram staining, ELISA, PCR, and Multi-locus Variable-Number Tandem Repeat Analysis (MLVA). Combining these diagnostic approaches enables healthcare professionals to make timely and accurate decisions, facilitating effective treatment and prevention strategies.

SAMPLING WINDOW AND LABORATORY TESTS FOR ANTHRAX

Table 6: Sampling window and laboratory tests

Clinical Presentation	Optimal Sample Collection Time	Recommended Tests	Sample Type
Cutaneous Anthrax (Early Stage)	1-10 days post-exposure	Gram stain, culture, PCR, biochemical tests (e.g., catalase, oxidase, sugar fermentation)	Skin lesion swab or biopsy
Cutaneous Anthrax (Late Stage)	1-2 weeks post-exposure	Gram stain, culture, PCR, biochemical tests (e.g., catalase, oxidase, sugar fermentation)	Eschar swab or biopsy
Inhalation Anthrax (Early Stage)	1-6 days post-exposure	Blood culture, PCR, serological tests (e.g., ELISA)	Blood, sputum, nasopharyngeal swab
Inhalation Anthrax (Late Stage)	1-2 weeks post-exposure	Blood culture, PCR, serological tests (e.g., ELISA)	Blood, pleural fluid, lung tissue
Gastrointestinal Anthrax (Early Stage)	1-7 days post-exposure	Stool culture, PCR	Stool sample
Gastrointestinal Anthrax (Late Stage)	Sepsis	Blood culture, PCR, serological tests (e.g., ELISA)	Blood

PRESUMPTIVE DIAGNOSIS

Presumptive diagnosis of anthrax involves a combination of microscopy, biochemical tests, and serological tests. Microscopy techniques like Gram staining and special stains for spores help identify the characteristic morphology of *Bacillus anthracis*. Biochemical tests, such as nitrate reduction and sugar fermentation tests, further aid in identifying the organism. Serological tests, including ELISA and direct immunofluorescence, detect specific antibodies or antigens associated with anthrax infection.

Microscopic Tests

Preparation and Staining Procedures: smears to be prepared using clinical samples and subjected to:

- **Gram Stain:**
 - ◊ Gram-positive large thick rectangular bacilli
 - ◊ Size: 4x1µm
 - ◊ Box car shaped with truncated ends, giving a “bamboo-stick” appearance
 - ◊ Non-bulging oval central or sub-terminal spores
 - ◊ In tissue: single or short chains of bacilli without spores
 - ◊ In culture: long chains with spores

- **Methylene Blue Staining:** Bacteria stain deep blue, leukocytes stain blue against a light blue background, and the capsule of *B. anthracis* stains pink.
- **Polychrome Methylene Blue (PCMB) Staining:**
- **McFadyean's Reaction:** *B. anthracis* capsule appears as amorphous purple material surrounding blue bacilli.

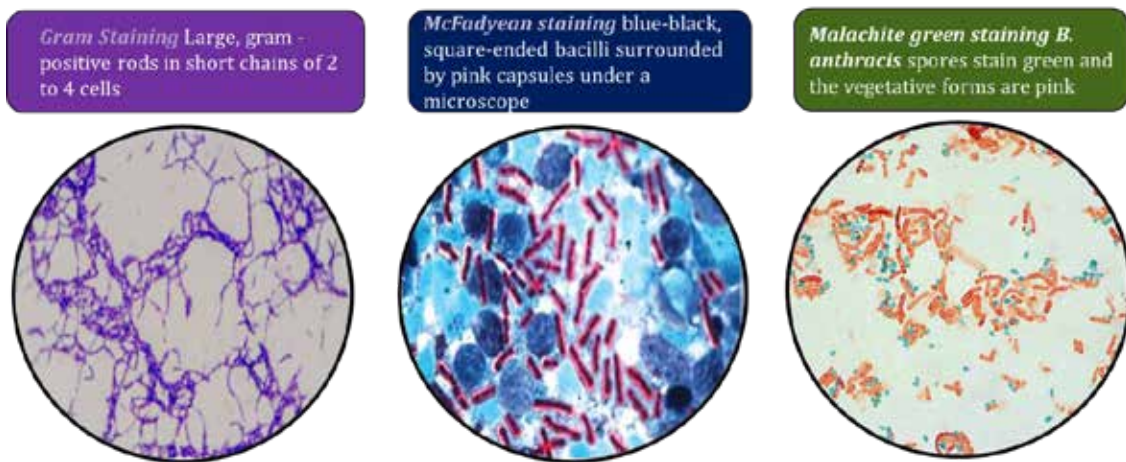


Fig 13: Microscopic identification of B. anthracis

Serology

- **Antibody Detection Antibodies in Convalescent Sera:** Can be detected by ELISA or immunodiffusion in gel methods.
- **Direct Immunofluorescence Test:** Detects capsular and cell wall polypeptide antigens using fluorescent-tagged monoclonal antibodies.

Detection of Anti-PA Antibodies

- **Purpose:** To detect anti-PA (protective antigen) antibodies, indicating exposure to *Bacillus anthracis*.
- **Principle:** Indirect ELISA; IgG antibody to protective antigen (anti-PA) binds to antigen on a micro-titre plate. The intensity of color generated after adding enzyme-linked conjugate (anti-species antibody) correlates with the amount of antibody present.
- **Interpretation:**
 - ◇ <0.9: No detectable antibody to PA IgG by ELISA.
 - ◇ 0.9-1.1: Borderline positive. Follow-up testing recommended if clinically indicated.
 - ◇ 1.1: Indicative of vaccination, current, or previous anthrax infection.

CONFIRMATORY DIAGNOSIS

Confirmatory diagnosis of anthrax is essential for accurate identification and appropriate treatment. Bacterial culture on blood agar, nutrient agar, and gelatin stab agar is a gold standard method. Molecular techniques like PCR and MLVA provide rapid and sensitive detection of the bacteria. Additionally, testing for susceptibility to penicillin G helps Learning Guide treatment strategies.

Culture

- **Nutrient Agar:**
 - ◇ Colonies are 2-3 mm in size, irregular, raised, dull greyish white with frosted glass (ground glass) appearance.
 - ◇ **Medusa Head Appearance:** Viewed under a low-power microscope, the edge of the colony appears as locks of matted hair, giving a medusa head appearance.
 - ◇ Colonies have a tacky consistency.
- **Blood Agar:** Non-hemolytic, dry white or greyish white, wrinkled, irregular, low convex colonies with tailing along the streak line.
- **Blood Agar with Penicillin:** Colonies appear as string pearls due to the action of penicillin.
- **Nutrient Broth:** Floccular deposit with clear or slightly turbid supernatant. Perform a hanging drop preparation from the overnight incubated nutrient broth. *B. anthracis* is non-motile.

Table 7: Differentiating Characteristics of *Bacillus* Species

Characteristics	<i>B. anthracis</i>	<i>B. cereus</i>	<i>B. mycoides</i>	<i>B. thuringiensis</i>	<i>B. megaterium</i>
Capsule ^a	+	-	-	-	+/-
In-vitro McFadyean test*	+	-	-	-	-
Parasporal crystals ^b	-	-	-	+	-
Motility ^c	-	+	-	+	+/-
Rhizoid spreading colonies	-	-	+	-	-
Haemolysis on blood agar	-	+	+/-	+	+/-
Egg-yolk lecithinase*	+/-	+	+/-	+/-	-
Growth on penicillin agar ^d	-	+	+	+	+
Lysis by McCloy's γ -phage*	+	-	-	-	-
Growth on Knisely's PLET agar*	+	+	-	-	-
Growth on 0.25% chloral-hydrate agar*	-	+	+	+/-	+
High virulence in mouse ^e	+	-	-	-	-

a Growth on serum agar or 0.7% NaHCO₃ nutrient agar incubated at 35°C in air plus 5-10% CO₂, mucoid growth, capsules seen in India ink wet film.

b Square or diamond-shaped inclusions by phase-contrast microscopy.

c By hanging drop or growth through semi-solid agar in a Craigie tube.

d Growth on nutrient agar with 5 mg/litre benzylpenicillin.

e Fatal septicaemia after intraperitoneal injection of 0.1 ml broth culture diluted to contain only 10-1000 spores.

* Refer to methods in Mackie & McCartney Practical medical Microbiology

List National Referral Laboratories for Anthrax Diagnosis in India

1. National Centre for Disease Control (NCDC), Delhi
2. Indian Veterinary Research Institute (IVRI), Izatnagar, UP
3. Defence Research and Development Establishment (DRDE), Gwalior, MP
4. ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru
5. Regional Medical Research Centre (ICMR), Port Blair (A&N Islands)
6. Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai
7. Christian Medical College (CMC), Vellore
8. BJ Medical College, Ahmedabad
9. National Institute of Epidemiology (NIE), Chennai
10. Government Medical College, Surat
11. Madurai Medical College, Madurai

Molecular Diagnosis

- Purpose: Detect DNA of *B. anthracis* directly from samples and cultures.
- Principle: Real-time PCR detects a segment of DNA unique to the pXO1 and pXO2 genes using a commercial assay kit.

GOOD LABORATORY PRINCIPLES (GLP)

Good Laboratory Practices (GLPs) are essential for ensuring the quality and reliability of laboratory results. These practices encompass various aspects, including regular training and revisions for laboratory personnel, timely vaccinations for lab staff, proper hand hygiene, safe storage of contaminated materials, and the prohibition of mouth pipetting and consumption of food or cosmetics within the laboratory. Additionally, maintaining accurate records of accidents and incidents is crucial for identifying potential hazards and implementing preventive measures. By adhering to these GLPs, laboratories can minimize risks, improve efficiency, and maintain the integrity of their research.



Sample type



Blood Specimen, Cerebrospinal fluid



Fecal specimen, peritoneal fluid (Gastrointestinal Anthrax)



Swabs from the lesion (Cutaneous Anthrax)



Sputum, Bronchial lavage (Inhalational Anthrax)

Note: Samples must be taken before the patient begins taking antibiotics for treatment

Image Source: <https://openres.ensjournal.com/content/5/1/00165-2018>

Sample collection

- ❑ Acute sample (blood) is collected ≤7 days after symptom onset
- ❑ Convalescent sample (blood) is collected 14-35 days after symptom onset



10 ml of blood sample is collected aseptically from a peripheral vein using appropriate needles and serum separator tube.

- The tube is allowed to stand in the upright position at room temperature for 30 minutes before centrifugation
- Centrifugation is carried out to separate the serum in a sterile vial

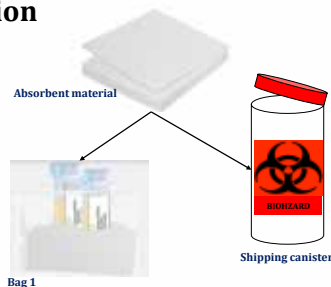


The tube is labeled and sealed properly, and stored at -20°C until tested or shipped to testing laboratory

<https://www.cdc.gov/anthrax>

Packaging and Transportation

1. Place absorbent material and the plastic vials holding the serum inside bag 1, and seal it
2. Place the sealed plastic bag into the shipping canister. Place a biohazard label on the outer surface of the shipping container. Line a plastic screw-top canister (shipping canister) with absorbent sheets. Use enough absorbent material to absorb the entire contents of the plastic vials, should there be a leak or spill.
3. Place dry ice into a polystyrene foam-lined box (the overpack). Place the shipping canister into the overpack



<https://www.cdc.gov/anthrax>

Packaging and Transportation

4. Place an itemized list of contents inside a second sealable plastic bag (bag 2). Seal bag 2 and place it inside the overpack.



Polystyrene foam-lined box with dry ice



Bag 2



Overpack

5. Label the package properly as "Biohazardous material" on the outside. Name, address & contact no. of person responsible for shipment should be clearly mentioned on the package. This person must be knowledgeable about the shipment and know how to remediate in case of spill or leakage.

Culture Methods

Blood Agar	• Colony size smaller, 2-4 mm in diameter • Colonies white or grey-white with matt appearance, • non-haemolytic and having curly tailing at the edges	
Nutrient Agar	• Colonies irregular, raised, dull, opaque and greyish-white with "frosted glass" (ground glass) appearance • Occasionally the colonies may have curled tailing at edges (Medusa head appearance)	
Gelatin Stab Agar	• The gelatin hydrolysis test detects the ability of bacteria to produce gelatinases • <i>B. anthracis</i> are positive for gelatin hydrolysis	
Bicarbonate Agar	Colonies of fully virulent isolates are mucoid in nature on this medium when incubated overnight under CO2 due to capsule formation.	

Control for Area Health, NCHC, Wafatim

ANTHRAX

Character	<i>B. anthracis</i>	<i>B. cereus</i>	<i>B. mycoides</i>	<i>B. thuringiensis</i>	<i>B. megaterium</i>
Capsule ^a	+	-	-	-	+/-
In-vitro McFadyean test ^a	+	-	-	-	-
Parasporal crystals ^b	-	-	-	+	-
Motility ^c	-	+	-	+	+/-
Rhizoid spreading colonies	-	-	+	-	-
Haemolysis on blood agar	-	+	+/-	+	+/-
Egg-yolk lecithinase ^a	+/-	+	+/-	+/-	-
Growth on penicillin agar ^d	-	+	+	+	+
Lysis by McCloy's y-phase ^a	+	-	-	-	-
Growth on Knisely's PLET agar ^a	+	+	-	-	-
Growth on 0.25% chloralhydrate agar ^a	-	+	+	+/-	+
High virulence in mouse ^e	+	-	-	-	-

Molecular Identification

- The invention of nucleic acid amplification has revolutionized the identification of microorganisms by enabling faster, easier, safer, and more accurate diagnosis as compared to traditional culture-based methods
- PCR/Real-time PCR with specific primers can be used for further confirmation
- Molecular typing (MLVA/AFLP) can be used for epidemiological studies to trace the source of infection



Center for Drug Use, Health, and Justice

ANTHRAX

Real Time-Polymerase Chain Reaction (RT-PCR)

- Targets genes located on anthrax virulence plasmids **pX01** and **pX02**.
- pX01 contains genes coding for anthrax toxin (protective antigen, edema factor, lethal factor) and pX02 contains genes coding for capsule

The diagram illustrates the qPCR workflow, a circular process with four main stages: **Setup**, **Run**, **Analyze**, and **qPCR workflow** (center).

- Setup** (top right) involves 'PCR Reagents' (green box), 'Primers' (green box), and 'PCR Master Mix' (green box).
- Run** (bottom right) shows 'Real Time PCR Instruments' (a thermal cycler machine).
- Analyze** (bottom left) features 'PCR Analysis Software' (a computer monitor displaying a graph).
- The central **qPCR workflow** is represented by a large blue circle connecting the stages.

 Additionally, a 'Primer PCR Assay' (green box) is shown near the Setup stage, and a 'PCR Reagents' (green box) is shown near the Run stage.

Center for Disease Control, NCHD, WorldNet

ANTHRAX

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Session-6

Treatment, Prevention & Control

Course No. 006	Session 6: Treatment, Prevention & Control
Duration	20 minutes
Learning Objectives	• Describe standard treatment options in humans and animals • Understand Learning Guidelines for effective anthrax control program • Describe preventive measures to protect exposed personnel and pets • Describe disinfection procedures • Describe vaccination protocols
Pre-requisite knowledge/skill	• Basic understanding of antimicrobial therapy and vaccination strategies in humans and animals. • Familiarity with infection control practices, including disinfection and personal protection. • Awareness of public health guidelines and frameworks for zoonotic disease control programs.
Competencies to be achieved	• Comprehension of available treatment options • Thorough understanding of preventive measures to adopt for both animals and at-risk individuals • Ability to describe various disinfection options • Knowledge about available vaccines for humans and animals

TREATMENT

Effective anthrax management requires understanding treatment options, implementing control programs, adopting preventive measures, and following disinfection protocols. Vaccination and collaborative efforts among veterinary and public health authorities are key to minimizing the impact of anthrax on both human and animal populations.

In humans, early administration of antibiotics like ciprofloxacin, doxycycline, or penicillin is crucial for successful treatment. Supportive care, including fluid replacement and respiratory support, may be necessary for severe cases. In animals, antibiotic therapy is also effective if initiated early. Vaccination of susceptible livestock is a preventive measure.

A comprehensive anthrax control program involves surveillance, early case detection, and rapid response. Close collaboration between veterinary and public health authorities is essential. Strategies include livestock vaccination in endemic areas, proper carcass disposal, and decontamination of affected environments.

POSTEXPOSURE PROPHYLAXIS (PEP) IN HUMANS

Antimicrobial Drugs: First-line Agents like Ciprofloxacin or doxycycline or amoxycillin

Dosage and Duration: Administered for 60 days to ensure complete eradication of *B. anthracis* spores.

Table 8: Patient-Specific Treatment Approaches for Anthrax

Condition	Treatment Type	Preferred Antimicrobial Drug(s)	Alternative Antimicrobial Drug(s)	Duration	Additional Notes
Nonpregnant Adults Aged ≥18 Years					
Cutaneous Anthrax (without meningitis)	PEP	Ciprofloxacin, Doxycycline	Levofloxacin, Moxifloxacin, Penicillin VK (if penicillin susceptible)	60 days (aerosol exposure), 7 days (non-aerosol exposure)	Use antitoxin if no antimicrobial drugs are available
	Treatment	Ciprofloxacin, Doxycycline	Levofloxacin, Moxifloxacin, Penicillin G (if penicillin susceptible)	7-10 days or until clinical stability	Transition to PEP regimen if aerosol exposure
Systemic Anthrax (with or without meningitis)	Empiric Treatment	Two bactericidal drugs (Ciprofloxacin, Meropenem, Imipenem/Cilastatin) + PSI (Doxycycline, Minocycline) or RNAI (Rifampin)	One bactericidal drug + PSI or RNAI, Two PSIs, One bactericidal drug	2 weeks or longer	Use antitoxin as adjunctive therapy; transition to oral medication based on improvement

Condition	Treatment Type	Preferred Antimicrobial Drug(s)	Alternative Antimicrobial Drug(s)	Duration	Additional Notes
Pregnant and Lactating Persons Aged ≥18 Years					
Cutaneous Anthrax (without meningitis)	PEP	Ciprofloxacin, Amoxicillin (if penicillin susceptible)	Levofloxacin, Moxifloxacin, Doxycycline	60 days (aerosol exposure), 7 days (non-aerosol exposure)	Use antitoxin if no antimicrobial drugs are available
	Treatment	Ciprofloxacin, Amoxicillin (if penicillin susceptible)	Levofloxacin, Moxifloxacin, Doxycycline	7-10 days or until clinical stability	Transition to PEP regimen if aerosol exposure
Systemic Anthrax (with or without meningitis)	Empiric Treatment	Two bactericidal drugs (Ciprofloxacin, Meropenem, Imipenem/Cilastatin) + PSI (Doxycycline) or RNAI (Rifampin)	One bactericidal drug + PSI or RNAI, Two PSIs, One bactericidal drug	2 weeks or longer	Use antitoxin as adjunctive therapy; transition to oral medication based on improvement
Children Aged ≥1 Month to <18 Years					
Cutaneous Anthrax (without meningitis)	PEP	Ciprofloxacin, Doxycycline	Levofloxacin, Moxifloxacin, Penicillin VK (if penicillin susceptible)	60 days (aerosol exposure), 7 days (non-aerosol exposure)	Use antitoxin if no antimicrobial drugs are available
	Treatment	Ciprofloxacin, Doxycycline	Levofloxacin, Moxifloxacin, Penicillin G (if penicillin susceptible)	7-10 days or until clinical stability	Transition to PEP regimen if aerosol exposure
Systemic Anthrax (with or without meningitis)	Empiric Treatment	Two bactericidal drugs (Ciprofloxacin, Meropenem, Imipenem/Cilastatin) + PSI (Doxycycline, Minocycline) or RNAI (Rifampin)	One bactericidal drug + PSI or RNAI, Two PSIs, One bactericidal drug	2 weeks or longer	Use antitoxin as adjunctive therapy; transition to oral medication based on improvement

RECOMMENDED ANTIBIOTICS FOR ANIMALS

Anthrax in animals is treated with antibiotics like penicillin and streptomycin. Anti-anthrax serum can be administered in severe cases. Vaccination is a crucial preventive measure, especially in regions with a high prevalence of the disease.

PREVENTION OF ANTHRAX IN HUMANS

Vaccination

The Anthrax vaccine, BioThrax, is recommended for high-risk individuals such as those handling animals or animal products, veterinarians, and laboratory staff. It can also be administered to people exposed to anthrax as part of a public health response. The vaccine is given in five doses to individuals aged 18-65, with annual boosters for those at continued high risk. **However, it is not recommended in general population**

INFECTION CONTROL PRACTICES

Hand Hygiene

Protocol: Wash hands thoroughly with soap and water, especially after potential exposure to spores. Avoid alcohol-based hand rubs.

Frequency: Before eating, after using the restroom, and after contact with potentially contaminated materials.

Personal Protective Equipment (PPE)

Types of PPE: Gloves, masks, protective clothing (e.g., gowns, aprons).

Usage: When handling materials or working in environments where *B. anthracis* spores may be present. Ensure proper fit and integrity of PPE.

Decontamination

Disinfectants: Use EPA-registered products effective against *B. anthracis*, such as sodium hypochlorite (bleach).

Procedures: Regular cleaning and disinfection of surfaces, equipment, and clothing, focusing on high-touch surfaces and areas where contamination is likely.

PREVENTION IN ANIMALS

Prevention of anthrax in animals involves several key measures. Proper disposal of infected carcasses, typically through incineration, is crucial to prevent the spread of spores. Decontamination of the affected area and items used during testing is also essential. In addition, timely treatment and vaccination of exposed animals can help control outbreaks. Early detection of outbreaks, quarantine of affected premises, and destruction of diseased animals and contaminated materials are vital to prevent further spread of the disease.

Protection of Pets

To protect pets from anthrax, it's important to keep them away from areas with dead animals, prevent digging in potentially contaminated soil, and seek prompt veterinary care if they show any signs of illness. Additionally, minimizing contact between pets and wild animals can help reduce the risk of exposure to anthrax spores. By following these preventive measures, pet owners can help protect their furry companions from this serious disease.

Livestock Vaccination

Anthrax vaccination is a crucial preventive measure for livestock. Cattle should receive a 1 ml subcutaneous injection, starting at 4 months of age and annually thereafter. Sheep and goats require a 0.5 ml subcutaneous injection, also starting at 3-6 months of age and annually. In areas with persistent exposure to infection, vaccination every 6 months is recommended. However, pregnant animals should not be vaccinated. By vaccinating livestock, we can significantly reduce the risk of anthrax outbreaks and protect both animal health and public health.

Wildlife Management

Strategy: Vaccination of wildlife populations in areas prone to anthrax outbreaks where feasible. Techniques include oral bait vaccines for wild herbivores.

Disposal of Anthrax Carcasses

Incineration: The preferred method for disposing of anthrax carcasses is to incinerate them at the site of death. Ensure complete combustion to ashes to prevent any spores from surviving. If incineration is not possible at the site, move the carcass to a suitable location, such as a quarry, for burning.

Burial: If incineration is not feasible, deep burial is an alternative. Carcasses should be buried at least 2 meters deep and covered with quicklime before backfilling to hasten decomposition and prevent scavenging.

Site Disinfection: After removing the carcass, disinfect the site with a 5% formaldehyde solution. Ensure thorough soaking of the contaminated area to kill any residual spores.

Handling Carcasses: Carcasses can be wrapped in double-thickness plastic to prevent spilling of body fluids during transport. Ensure that transportation to the incineration or burial site minimizes the risk of contamination along the route.

Control Measures

Quarantine: Quarantine herds or flocks with suspected or confirmed anthrax cases to prevent the spread to other animals until the risk of transmission has passed and affected animals have been treated.

Surveillance: strengthening of surveillance by immediate reporting of suspected cases to veterinary and public health authorities for quick response and regular health checks and surveillance of livestock and wildlife for signs of anthrax, particularly in endemic regions.

Public Awareness

Education Campaigns: Periodic sensitization of farmers, veterinarians, animal handlers, and the general public about the importance of vaccination, early detection, proper handling of suspected anthrax cases, and preventive measures.

PREVENTIVE AND CONTROL MEASURES FOLLOWING OUTBREAK

During an outbreak, isolation and treatment of infected individuals are prioritized. Quarantine measures may be implemented to prevent further exposure. Surveillance systems track cases and identify potential hotspots. Public health communication ensures accurate information dissemination and reduces panic.

Upon discovering an anthrax-infected animal carcass, it's crucial to isolate the area to prevent accidental contact. Avoid disturbing the carcass as anthrax spores can become airborne.

Before approaching the carcass, wear appropriate personal protective equipment (PPE), including gloves, masks, goggles, and protective clothing. This prevents direct contact and inhalation of potential spores. Open burial is discouraged as it can lead to soil contamination and potential exposure.

Do not bury the carcass without proper decontamination. Spray the carcass with a chlorine-based disinfectant to kill anthrax spores. Allow the disinfectant to sit for a recommended period, then repeat the process.

Place the carcass in a heavy-duty plastic bag and seal it securely. This prevents any leakage or further contamination.

Transport the sealed carcass to a designated disposal site. After disposing of the carcass, thoroughly decontaminate any equipment, vehicles, or clothing that came into contact with the carcass or the disposal site.

Continue monitoring the area for any signs of contamination or new cases. Report the disposal and take necessary steps to prevent further outbreaks.

An effective outbreak response involves swift action. Rapid diagnosis, treatment, and isolation of cases prevent further spread. Preparedness includes training healthcare and veterinary professionals, ensuring stockpiles of antibiotics, and establishing communication networks for real-time information exchange.

Proactive measures can reduce anthrax risks. Vaccinating livestock in endemic areas and promoting hygiene among handler's lower exposure. Monitoring animal health and promptly reporting unusual deaths aids early intervention. Public awareness campaigns educate communities about preventive measures.

Public health preparedness encompasses planning, resource allocation, and coordination to address anthrax outbreaks. Collaborative efforts between health departments, veterinary agencies, and emergency response teams ensure a unified response to protect both human and animal health.

- ✓ The key to rescuing the patient is a rapid diagnosis and initiation of treatment
- ✓ Antibiotics should be recommended basis the drug sensitivity test results. A combination of broadly active agents are advised for treatment.
- ✓ Antibiotics and antitoxins effective for treatment of Anthrax include:
 - **Antibiotics**- Ciprofloxacin, Doxycycline, Levofloxacin, Moxifloxacin, Penicillin G, Penicilline VK, Meropenem, Imipenem/Cilastatin, Rifampin, Minocycline.
 - **Antitoxins**- Intravenous anthrax immune globulin (human): Raxibacumab, and Obiltoxaximab monoclonal antibodies



Recommended Antibiotics for Anthrax Treatment

Nonpregnant Adults (≥18 Years)

- **Cutaneous Anthrax (without meningitis)**
- Preferred: Ciprofloxacin, Doxycycline
- Alternatives: Levofloxacin, Moxifloxacin, Penicillin VK (if susceptible)
- **Systemic Anthrax (with/without meningitis)**
- Preferred: Two bactericidal drugs (Ciprofloxacin, Meropenem, Imipenem/Cilastatin) + PSI (Doxycycline, Minocycline) or RNAI (Rifampin)
- Alternative: One bactericidal drug + PSI or RNAI, Two PSIs, One bactericidal drug

Pregnant & Lactating Persons (≥18 Years)

- **Cutaneous Anthrax (without meningitis)**
- Preferred: Ciprofloxacin, Amoxicillin (if penicillin susceptible)
- Alternatives: Levofloxacin, Moxifloxacin, Doxycycline
- **Systemic Anthrax (with/without meningitis)**
- Preferred: Two bactericidal drugs (Ciprofloxacin, Meropenem, Imipenem/Glasattin) + PSI (Doxycycline) or RNAI (Rifampin)
- Alternative: One bactericidal drug + PSI or RNAI, Two PSIs, One bactericidal drug

. Children (≥ 1 month to <18 Years)

- Similar regimen as nonpregnant adults, with dose adjustments

Prognosis

- The majority of cases of anthrax have been cutaneous and these usually resolve with or without treatment.
- If treated promptly with antibiotics, the mortality rate is less than 2%.
- Inhalational anthrax is life-threatening and has poor prognosis. Mortality rate 50%, even with adequate treatment, have been reported.



Treatment in Animals

- ❑ Penicillin 10000 units per kg body weight twice daily through parenteral route.
- ❑ Streptomycin @ 8-10g in divided doses through IM
- ❑ Anti- anthrax serum @100-200 cc IV along with a course of penicillin can be given
- ❑ Treatment has to be continued for 5-7 days
- ❑ As a routine measure, case of anthrax may be brought under the regimen of aqueous penicillin through IV route by use of procaine penicillin through IM route.



Vaccination in Humans



- Anthrax vaccination is recommended for high-risk professions (people who handle animals or animal products), veterinarians, and laboratory staff.
- The vaccine may also be recommended to exposed community members as a component of a public health response after a bioterrorism event
- **BioThrax®** is administered in five doses to people aged 18 to 65 who are at high risk of exposure, with a booster dosage administered each year for those who remain at high risk of exposure.
- **However, it is not recommended in General Population**

Infection Control Practices

Hand Hygiene

- Protocol: Wash hands thoroughly with soap and water, especially after potential exposure to spores. Avoid alcohol-based hand rubs.
- Frequency: Before eating, after using the restroom, and after contact with potentially contaminated materials.

Personal Protective Equipment (PPE)

- Types of PPE: Gloves, masks, protective clothing (e.g., gowns, aprons).
- Usage: When handling materials or working in environments where B. anthracis spores may be present. Ensure proper fit and integrity of PPE.

Decontamination

- Disinfectants: Use EPA-registered products effective against B. anthracis, such as sodium hypochlorite (bleach).
- Procedures: Regular cleaning and disinfection of surfaces, equipment, and clothing, focusing on high-touch surfaces and areas where contamination is likely.

Prevention in Animals

In the event of a case or outbreak occurring in livestock, following control measures are necessary:

- ✓ correct (incineration) disposal of the carcass(es),
- ✓ decontamination of the site(s) and of items used to test
- ✓ initiation of treatment and/or vaccination of other animals as appropriate.
- ✓ The carcass should not be opened, since exposure to oxygen will allow the bacteria to form spores.
- ✓ Early detection of outbreaks, quarantine of affected premises, destruction of diseased animals and fomites

Preventive measures for protection of pets



Keep pets away from areas where there might be carcasses of infected animals, such as dead wildlife or livestock.



Prevent pets from digging in areas that may be contaminated.



Prompt Veterinary care if they exhibit any symptoms of illness



Prevent exposure of pets to wild animals.

Vaccination in Animals

- ❑ **Cattle** – 1 mL- subcutaneous injection (age 4 months and above)
- ❑ **Sheep and Goats** – 0,5 mL -subcutaneous injection.
- ❑ Animals should first be vaccinated at 3 to 6 months of age and annually thereafter.
- ❑ In areas where animals are likely to be subjected to continued exposure to infection, vaccination every 6 months is advisable.
- ❑ Under normal conditions, annual revaccination will usually suffice
- ❑ Pregnant animals should not be vaccinated.



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Wildlife Management and Carcass Disposal

Vaccination of Wildlife:

- Oral bait vaccines for wild herbivores in anthrax-prone areas.

Carcass Disposal:

- **Incineration:** Preferred method for complete destruction of spores.
- **Burial:** Deep burial (at least 2 meters) with quicklime to accelerate decomposition.
- **Site Disinfection:** Thorough disinfection with 5% formaldehyde solution.
- **Carcass Handling:** Double-bagging to prevent spillage during transport.

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Control Measures for Anthrax

- **Quarantine:** Isolate infected animals to prevent disease spread.
- **Surveillance:** Implement regular health checks and surveillance, especially in endemic regions.
- **Public Awareness:** Educate farmers, veterinarians, and the public about prevention, early detection, and proper handling of suspected cases.

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ANTHRAX

Anthrax Outbreak Control and Prevention

Key Strategies

- **Isolation and Treatment:** Isolate infected individuals and initiate appropriate antibiotic therapy.
- **Quarantine:** Implement quarantine measures to prevent further spread.
- **Surveillance:** Monitor for new cases and identify potential hotspots.
- **Public Health Communication:** Disseminate accurate information to reduce panic.

Carcass Disposal

- **Isolation:** Isolate the area to prevent accidental contact.
- **PPE:** Wear appropriate protective gear before handling the carcass.
- **Disinfection:** Disinfect the carcass with chlorine-based solution.
- **Safe Disposal:** Seal the carcass in a heavy-duty bag and transport to a designated disposal site.
- **Decontamination:** Thoroughly clean and disinfect all equipment and surfaces.

Long-Term Prevention

- **Vaccination of Livestock:** Vaccinate animals in endemic areas.
- **Public Awareness:** Educate communities about prevention and early detection.
- **Preparedness:** Train healthcare and veterinary professionals, stockpile antibiotics, and establish communication networks.

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Session-7

Outbreak Investigation and Management

Course No. 007	Session 7: Outbreak investigation and management
Duration	15 minutes
Learning Objectives	• Describe outbreak management • Understand risk reduction measures • Understanding public health scenario • Describe outbreak response and preparedness • Describe measures to be taken in the event of an outbreak of anthrax
Pre-requisite knowledge/skill	• Basic knowledge of outbreak investigation, response strategies, and emergency preparedness. • Familiarity with risk assessment, mitigation, and communication in public health emergencies. • Understanding of coordination among different sectors during zoonotic outbreaks.
Competencies to be achieved	• Understanding potential sources of outbreak • Understanding course of action in an Anthrax outbreak • Explain measures to improve outbreak response and preparedness • Competency in effectively managing Anthrax outbreak

OUTBREAK INVESTIGATION

In India Human Anthrax cases are monitored under IDSP in the IHIP Portal.

Outbreak investigations for Anthrax are essential to control the spread of this disease. These investigations involve a systematic process of various steps and actions to understand and respond to outbreaks effectively:

- 1. Confirmation of the outbreak:** The first step is to confirm the existence of an outbreak by analyzing surveillance data. This involves identifying an increased number of Anthrax cases or a cluster of similar cases. Furthermore, laboratory testing is crucial to confirm the diagnosis of Anthrax in affected individuals.
- 2. Establishing a multidisciplinary team:** A multidisciplinary team is formed, which includes professionals from various fields, such as public health officials, epidemiologists, clinicians, veterinarians, laboratorians, and other experts. This team collaborates to investigate and respond to the outbreak comprehensively.
- 3. Case definition:** A clear case definition for Anthrax is developed. This definition should encompass both clinical criteria, such as symptoms and signs, and laboratory criteria, which involve confirmation through specific tests. Having a standardized case definition ensures consistency in identifying cases.
- 4. Case Identification & Reporting:** Mechanisms are implemented to identify and report all suspected and confirmed Anthrax cases. Collaboration with healthcare facilities and laboratories is crucial to ensure comprehensive case identification. Timely reporting is essential for swift action.
- 5. Data Collection:** Detailed information is collected from both confirmed and suspected cases. This includes demographics, clinical symptoms, exposure history, travel history, and potential risk factors. Various methods, such as interviews, medical records review, and laboratory records, are employed to gather this information.
- 6. Descriptive epidemiology:** The collected data is analyzed to describe the outbreak in terms of person, place, and time. This step involves identifying demographic characteristics, the geographical distribution of cases, and temporal trends. Descriptive epidemiology helps in understanding the scope and pattern of the outbreak.
- 7. Hypothesis generation:** Hypotheses are generated regarding the source of the outbreak, potential modes of transmission, and risk factors. These hypotheses are based on the analysis of collected data, knowledge of the disease, and existing literature.
- 8. Analytical epidemiology:** Analytical studies, such as case-control studies or cohort studies, are conducted to test the generated hypotheses and identify risk factors associated with the outbreak. This involves comparing the characteristics and exposures of cases with those of non-affected individuals to assess the association.
- 9. Environmental investigation:** The potential sources and modes of transmission are thoroughly investigated. This may involve inspecting farms, slaughterhouses, dairies, and other relevant sites to collect samples for testing. The investigation aims to determine if the outbreak is linked to contact with infected animals, consumption of contaminated food products, or exposure to contaminated environments.

- 10. Control measures:** Appropriate control measures are implemented to prevent further spread of Anthrax. These measures can include public health education, enhanced surveillance, contact tracing, treatment of cases, and actions to mitigate exposure to infected animals or contaminated products.
- 11. Communication and collaboration:** Effective communication with key stakeholders and collaboration with local and national agencies are crucial. Information sharing, coordination of efforts, and support for control measures are essential components of this step.
- 12. Evaluation:** After implementing control measures, the effectiveness of these measures is assessed. The situation is continuously monitored to ensure that new cases are promptly identified, treated, and reported, thereby preventing further transmission of Anthrax.

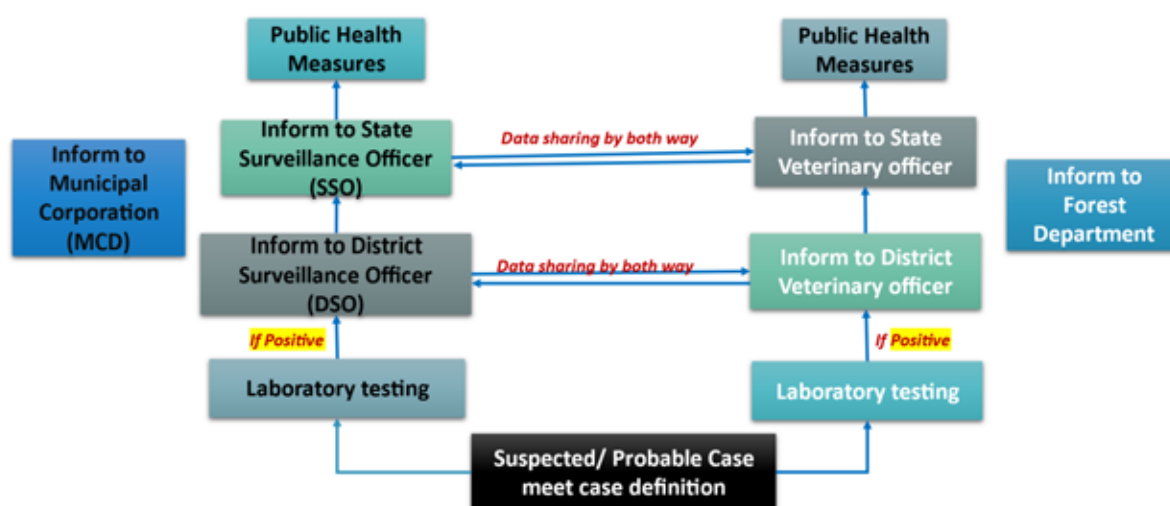


Fig 14: Data sharing by Human and Veterinary Department under "One Health Approach"

RISK REDUCTION MEASURES

Early detection of anthrax outbreaks is essential for timely intervention, which requires implementing effective surveillance systems and prompt reporting of suspected cases. Once an outbreak is confirmed, affected premises must be quarantined to prevent the spread of the disease. Diseased animals and contaminated materials should be destroyed to eliminate the source of infection. Strict sanitary procedures should be enforced in abattoirs and dairy factories to ensure the safety of animal products intended for human consumption.

DISINFECTION AND DECONTAMINATION MEASURES

Effective disinfection is crucial for controlling the spread of anthrax. Formaldehyde and glutaraldehyde are highly effective in destroying bacterial spores. For larger areas like stockyards and pens, a 10% NaOH or 5% formaldehyde solution is recommended. Household bleach, when diluted and adjusted to pH 7, can serve as a sporicidal agent. Gaseous sterilization with agents such as chlorine dioxide, vapor-phase hydrogen peroxide, or formaldehyde gas can also be employed under specific conditions. Additional methods like autoclaving at 121°C for at

least 30 minutes or gamma radiation are effective for eliminating anthrax spores. For personal decontamination, washing exposed skin with soap and hot water, followed by a disinfectant, is strongly recommended.

MEASURES TO BE TAKEN BY VETERINARIANS IN CASE OF A SUSPECTED OUTBREAK

In case of a suspected anthrax outbreak, veterinarians and other personnel should exercise extreme caution. Direct contact with potentially contaminated carcasses and soil should be avoided. Proper use of disposable personal protective equipment (PPE), such as gloves, masks, and gowns, is essential when handling suspected cases. Although the risk of respiratory infection is low, it is advisable for veterinarians and other personnel conducting postmortems, soil remediations, and carcass disposal to wear respirators with high-efficiency particulate air (HEPA) filters to minimize the risk of inhalation exposure.

Investigating an Anthrax Outbreak: Key Steps

➤ Confirmation of the outbreak	Analyze surveillance data for increased cases or clusters. Laboratory confirmation of Anthrax cases.
Multidisciplinary Team Formation	Assemble a team of experts from various fields..
Case Definition	Establish clear clinical and laboratory criteria for case identification.
1.Case Identification and Reporting	Implement mechanisms for timely reporting of suspected and confirmed cases.
Data Collection	Gather detailed information on demographics, clinical features, exposure history, and risk factors.
1.Descriptive Epidemiology	Analyze data to describe the outbreak in terms of person, place, and time.

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ANTHRAX

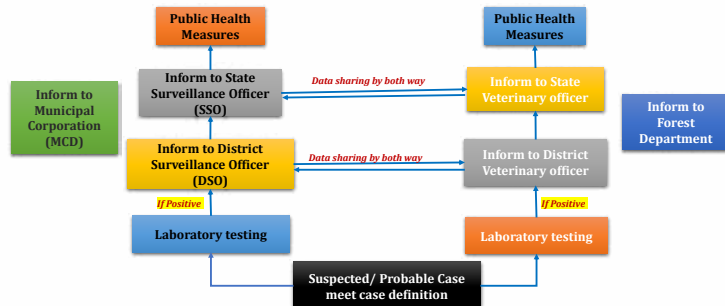
Investigating an Anthrax Outbreak: Continued Steps

➤ Hypothesis generation	Develop hypotheses about the source, mode of transmission, and risk factors.
Analytical epidemiology	Conduct case-control or cohort studies to identify risk factors.
Environmental investigation	Inspect farms, slaughterhouses, and dairies to identify potential sources. Collect samples for testing
Control measures	Implement public health education, surveillance, contact tracing, and treatment. Mitigate exposure to infected animals and contaminated products.
Communication and collaboration	Coordinate with local and national agencies. Share information and resources.
Evaluation	Monitor the effectiveness of control measures. Identify and address emerging issues.

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



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Sample anthrax case-report form

The form is divided into two main sections: Human and Veterinary. Each section contains a header for case identification and a table for detailed reporting. The human section includes fields for patient name, age, sex, and various clinical symptoms. The veterinary section includes fields for animal name, species, and various clinical signs. Both sections have a section for laboratory testing results.

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Outbreak Management

Proper investigation of the outbreak, confirmed through laboratory diagnosis, and source identification

Proper incineration of carcasses of infected cattle. Deep burial of ashes for prevention of body fluids spilling.

Timely vaccination of other animals in the affected herd and in neighboring premises

A buffer zone, 20-30 Km wide, is to be established around the infected area within which all cattle and exposed sheep are vaccinated and quarantined

Disinfection of contaminated site with 5% formaldehyde after disposal of carcasses

Quarantining affected area for at least 20 days after the last case or 6 weeks after vaccination

Milk collected from a cow, buffalo or goat showing signs of anthrax within 8 hours of milking is to be destroyed, along with any other milk that may have been mixed with the suspected milk.

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Outbreak Management continued...

People entering infected premises should wear protective clothing and footwear, which should be disinfected before leaving the premises

Persons who have handled infected animals or their carcasses should be vaccinated if their exposure is frequent.

People handling infected animals should either avoid any contact with others or should first change clothing, wash hands and take appropriate disinfection measures.

Movement of livestock from affected premises during an outbreak should be prevented.

Dust in industries handling wool or hides should be control.

Wool/hair from endemic areas should be washed and disinfected (e.g. with 10% formalin)

Vaccinate livestock in endemic areas

Avoid examination of suspected/infected carcasses

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Risk Reduction Measures

- Early detection of *outbreaks*
- Quarantine of affected premises
- Destruction of diseased animals and fomites
- Implementation of appropriate sanitary procedures at *abattoirs* and dairy factories will ensure the safety of products of animal origin intended for human consumption



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Disinfection

- The most effective disinfectants for destroying bacterial spores are formaldehyde and glutaraldehyde.
- A 10% NaOH or 5% formaldehyde solution can be used for stockyards, pens and equipment.
- Household bleach must be diluted with water and adjusted to pH 7 in order to prepare an efficient sporicidal agent.
- Gaseous sterilization can be done with agents such as chlorine dioxide, vapor-phase hydrogen peroxide, and formaldehyde gas, under specific conditions of humidity and temperature.
- Anthrax spores can also be eliminated by autoclaving at 121°C (250°F) for at least 30 minutes and exposing contaminated animal products to gamma radiation
- Exposed arms and hands can be washed with soap and hot water, and a disinfectant



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Measures to be taken by Veterinarians in case of a suspected outbreak

- Veterinarians and other personnel should avoid direct contact with potentially contaminated carcasses and soil
- Proper disposable personal protective equipment (PPE), should be used while handling suspected case
- Although the risk of respiratory infection is extremely small, veterinarians and other personnel conducting postmortems, soil remediations, and disposal of animal carcasses should wear respirators with a high-efficiency particulate air (HEPA) filter.

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Notes

Session-8

Surveillance and Risk Communication

Course No. 008	Session 8: Surveillance and Risk Communication
Duration	10 minutes
Learning Objectives	• Understand considerations of Anthrax surveillance in humans and animals • Describe risk communication and public awareness
Pre-requisite knowledge/skill	• Basic knowledge of surveillance systems for zoonotic diseases in both humans and animals. • Familiarity with principles of risk communication and public health messaging. • Understanding of community engagement strategies for disease awareness and prevention
Competencies to be achieved	• Comprehension of the various methods employed for surveillance and monitoring of cases • Description of strategies for risk communication and raising public awareness about AnthraxUnderstanding the importance of collaboration between healthcare providers and public health authorities in addressing Anthrax cases

ANTHRAX REPORTING

Anthrax surveillance requires close collaboration, data sharing, and effective communication among human and animal health sectors. Integrating surveillance efforts, educating the public, and implementing early warning systems contribute to enhanced preparedness and timely responses to anthrax threats. In India, Human Anthrax cases are reported in IDSP-IHIP portal.

The Integrated Disease Surveillance Programme (IDSP) was launched in 2004 to strengthen disease surveillance for epidemic-prone diseases in India. The primary objective of IDSP is to enable early detection of disease outbreaks, providing timely alerts or "early warning signals" to facilitate quick public health responses. The Integrated Health Information Platform (IHIP) was introduced in January 2022 as part of IDSP to further modernize and enhance the surveillance infrastructure. This platform streamlines disease data reporting and integrates health information for improved management and faster response to public health threats. The IDSP-IHIP Portal is primarily used for reporting human cases of various diseases. Healthcare providers and public health officials input data on suspected, probable, and confirmed cases into this system. The data is then analyzed to monitor disease trends, identify outbreaks, and implement control measures. IHIP Zoonoses Portal is specifically designed for reporting zoonotic diseases. This data is essential for early detection and prevention of zoonotic outbreaks.

The IDSP-IHIP platform includes several reporting forms that enable comprehensive surveillance across different health units. These include:

S Form: This form is filled by health workers to report data on suspected cases or syndromes. It is used for syndromic surveillance.

P Form: This form is filled by medical officers to report data on probable or clinically suspected cases of diseases. It is used for presumptive surveillance.

L Form: This form is designed to collect data on laboratory-confirmed cases of diseases. It is used for laboratory surveillance.

Prompt reporting of suspected cases is crucial for effective surveillance. Collecting accurate and detailed data about cases, including their location, demographics, and potential sources of exposure, helps in tracking patterns and responding swiftly.

Integrating surveillance efforts between human and animal health sectors is essential due to the zoonotic nature of anthrax. Collaborative data sharing enhances the understanding of disease dynamics and early identification of potential outbreaks.

RISK COMMUNICATION

Open and transparent communication is vital to manage anthrax risks effectively. Public awareness campaigns educate communities about anthrax, its transmission, and preventive measures. Clear risk communication helps mitigate panic and ensures adherence to recommended practices.

- Educate about sources of anthrax exposure, emphasizing avoiding contact with sick animals and their products.
- Promote proper hygiene practices after handling animals and during visits to outbreak-prone areas.
- Explain the importance of reporting unusual animal deaths and suspected cases promptly.
- Disseminate information about available vaccines for livestock in endemic areas.

Effective risk communication involves collaboration between public health authorities, veterinary agencies, local governments, and community leaders. Joint efforts enhance information dissemination and response coordination.

Developing early warning systems that integrate surveillance data, environmental factors, and outbreak history can provide insights into potential anthrax outbreaks. This allows for proactive responses and resource allocation.

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Session-9

Anthrax as a Bioweapon

Course No. 009	Session 9: Anthrax as a bioweapon
Duration	20 minutes
Learning Objectives	• Understanding bioterrorism • Describing the threat of an Anthrax attack • Preparing for an attack
Pre-requisite knowledge/skill	• Basic understanding of bioterrorism and biological warfare agents. • Familiarity with public health emergency preparedness and response frameworks. • Awareness of the clinical and environmental implications of deliberate anthrax exposure.
Competencies to be achieved	• Describing potential of Anthrax as a bioweapon • Understanding how Anthrax is used in bioterrorism • Explaining response and detection of an Anthrax attack

Anthrax can be acquired naturally or due to deliberate release which makes it a **potential weapon** for **bio-terrorism**. Anthrax spores can remain viable for decades. It can enter the body through skin abrasions, inhalation or ingestion and multiply to produce exotoxins. It can be released quietly without detection and can be concealed in powders, sprays, food, and water. An anthrax attack can occur through letters, food, water, or airborne release, posing a severe risk due to the ease of spreading the spores. Anthrax has been used as a weapon before, causing fatalities in the 2001 attack in the USA where spores were sent through postal letters. Inhalation anthrax is the most dangerous form, with potential for rapid fatalities if not treated immediately. Anthrax has been classified as a **Tier 1** biological agent, presenting significant risks for mass casualties and public safety threats. Therefore, attack detection and response is very essential. The possession, use, or transfer of *Bacillus anthracis* in the USA is regulated by the **CDC's Division** of Select Agents and Toxins.

BIOTERRORISM PREPAREDNESS: ANTHRAX AS A THREAT

Bioterrorism involves intentional release of biological agents to cause harm, fear, and societal disruption. Anthrax, due to its stability and virulence, is a prominent bioweapon candidate, posing grave threats to populations.

Anthrax's impact can be devastating. Aerosolized spores, when inhaled, cause severe respiratory disease with high mortality. Beyond immediate health consequences, an attack induces panic, disrupts societies, and has far-reaching economic implications.

Preparing for an Attack

- **Enhanced Surveillance:** Robust systems detecting unusual illness patterns aid rapid response and containment.
- **Public Health Readiness:** Equipped healthcare systems with stockpiles of antibiotics, vaccines, and treatment protocols to handle surges in patients.
- **Effective Communication:** Accurate and clear risk communication helps Learning Guide public actions, mitigate fear, and maintain order.
- **Research and Innovation:** Ongoing research for improved diagnostics, treatments, and vaccines strengthens response capabilities.
- **International Collaboration:** Bioterrorism is a global threat, necessitating information sharing, resource pooling, and coordinated efforts.

Anthrax has a history of being used as a bioweapon. In 2001, anthrax spores were mailed through the US postal system, infecting 22 people, including 12 mail handlers. Tragically, five of these individuals succumbed to the disease, highlighting the devastating potential of anthrax as a biological weapon.

The Attack

- One of the most likely agents used in a biological attack
- Anthrax spores are easily found in nature, can be produced in a lab,
- Spores last for a long time in the environment.
- The microscopic spores can be easily put into powders, sprays, food, and water which makes it a potential weapon for bioterrorism
- A **Tier 1** biological agent, presenting significant risks for mass casualties and public safety threats.



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Anthrax: A Bioterrorism Threat- Preparedness

- **Stability and Virulence:** Anthrax spores are highly stable and can easily be weaponized.
- **Devastating Impact:** Inhaled anthrax can cause severe respiratory disease with high mortality rates.
- **Societal Disruption:** Beyond health consequences, anthrax attacks can induce panic, disrupt societies, and impact economies.
- **Preparing for an Attack:**
 - Enhanced surveillance
 - Public health readiness
 - Effective communication
 - Research and innovation
 - International collaboration

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Fatalities Caused

- Anthrax was used as a bioweapon in the USA, in 2001.
- Powdered anthrax spores were put into letters and were mailed through the U.S. postal system
- Twenty-two people, including 12 mail handlers, got anthrax, and five of these 22 people died



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Disinfection

- The most effective disinfectants for destroying bacterial spores are formaldehyde and glutaraldehyde.
- A 10% NaOH or 5% formaldehyde solution can be used for stockyards, pens and equipment.
- Household bleach must be diluted with water and adjusted to pH 7 in order to prepare an efficient sporicidal agent.
- Gaseous sterilization can be done with agents such as chlorine dioxide, vapor-phase hydrogen peroxide, and formaldehyde gas, under specific conditions of humidity and temperature.
- Anthrax spores can also be eliminated by autoclaving at 121°C (250°F) for at least 30 minutes and exposing contaminated animal products to gamma radiation
- Exposed arms and hands can be washed with soap and hot water, and a disinfectant

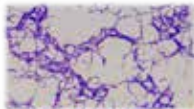


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ANNEXURES

Annexure- I (Gram Staining)

Purpose	Procedure	Interpretation
Gram stain is a staining procedure to visualize the gram stain positivity of bacteria present in clinical sample. Material required • Crystal violet (primary stain) • Gram's Iodine (mordant that fixes crystal violet to cell wall) • Decolorizer (e.g. ethanol) • Safranin (secondary stain) • Water	• Heat-fix the sample to the slide. • Add the primary stain (crystal violet) to the sample/slide and incubate for 1 minute. Rinse slide with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet. • Add Gram's iodine for 1 minute. • Rinse sample/slide with acetone or alcohol for ~3 seconds and rinse with water. • Add the secondary stain, safranin, to the slide and incubate for 1 minute. Wash with a gentle stream of water for a maximum of 5 seconds. • Examine the smear microscopically using the oil immersion objective (100x).	• Gram-positive thick, long, straight bacilli with square or truncated ends with parallel sides found usually single, in pairs or chains of 3 or 4 bacilli. • The chain is truncated with swollen ends gives a characteristic "bamboo stick" due to oval central or sub terminal spores . 

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Annexure- II (Mcfaydean reaction)		
Purpose	Procedure	Interpretation
Methylene blue is a simple direct stain used to reveal the morphology of bacteria and to visualize the presence of capsule.	• Make two thin smears from small drop of blood or swabs or tissue fluids • Air dry the smear • Fix the smear by dipping in absolute alcohol (95 - 100%) or methanol for 30 to 60 seconds • Air dry the smear slide • Cover the smear with Polychrome methylene blue and leave it for one minute • Rinse the smear gently with tap or distilled water into container containing hypochlorite solution • When dry, examine under the oil immersion lens.	The anthrax bacilli can be seen as purple amorphous material surrounding the blue-black bacilli.
Material required		
• Methylene blue • Absolute alcohol or methanol.		

Annexure- III (Malachite green stain)		
Purpose Malachite green stain is used to visualize the presence of spores.	Procedure • Dry the films and heat or alcohol fix. • Place the slide in a moist chamber (a petri dish with moistened filter paper will do). • Cover the film with 5% aqueous solution of malachite green, • Leave to act for 60 minutes. • Wash off stain with water using wash bottle (into hypochlorite solution). • Counterstain with 0.5% safranin or 0.05% basic carbol fuchsin for 30 seconds. • Wash again (into hypochlorite solution) and allow to dry. • When dry, examine under the oil immersion lens.	Interpretation Spores appear green and the vegetative bacilli red.

Annexure- IV (Nitrate reduction test)

Purpose	Procedure	Interpretation
Nitrate broth is used to determine the ability of an organism to reduce nitrate (NO_3^-) to nitrite (NO_2^-) using the enzyme nitrate reductase. Material required - Media: Nitrate Broth - Composition: Peptone 5 g/l, Meat extract 3 g/l, Potassium nitrate 1 g/l, Final pH 7.0 $\pm$ 0.2 at 25°C. Autoclave the media before use. - Sulfanilic acid (Reagent A) α-Naphthylamine (Reagent B)	- Inoculate the nitrate broths with clinical specimen/isolated bacteria. - Incubate the tubes at the optimal temperature 37°C for 24 hours. - Add 6-8 drops of nitrite reagent A and add the 6-8 drops of nitrite reagent B. - Observe for the reaction (color development) within a minute or less. - If no color develops add zinc powder. - Observe for at least 3 minutes for a red colour to develop after addition of zinc.	Positive Test: - Development of a cherry red coloration on addition of reagent A and B. - Absence of a red color development on adding Zn powder. Negative Test: - A development of red color on addition of Zn powder. 

Source: for Your Health, WDC, Mumbai

ANTHRAX

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NOTE TO FACILITATORS

This guide has been meticulously designed to enhance the understanding and expertise doctors, veterinarians, paramedical staff, and laboratory personnel in dealing with anthrax 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 Anthrax 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 Learning Guide participants through this comprehensive Anthrax 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 anthrax 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' Learning Guide (FG). Full set of the training guide: Facilitators' Learning 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

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 18 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 type of microorganism causes anthrax?

- a) Virus
- b) Bacteria
- c) Fungus
- d) Protozoa

2. Anthrax primarily affects which two main groups?

- a) Children and the elderly
- b) Healthcare workers and veterinarians
- c) Farmers and politicians
- d) Athletes and musicians

3. Which of the following is NOT a common route of anthrax transmission?

- a) Inhalation
- b) Ingestion
- c) Skin contact
- d) Sexual contact

4. Which region is NOT typically associated with the high prevalence of anthrax?

- a) Sub-Saharan Africa
- b) Asia
- c) Europe
- d) Middle East

5. Anthrax spores can be weaponized and dispersed through which means?

- a) Cooking
- b) Injection
- c) Inhalation
- d) Drinking water

6. Which of the following is a characteristic feature of anthrax as a bioweapon?

- a) Rapid treatment response
- b) High mortality rate
- c) Mild flu-like symptoms
- d) No potential for person-to-person transmission

7. What is the most common form of anthrax infection in humans?

- a) Inhalation anthrax
- b) Gastrointestinal anthrax
- c) Cutaneous anthrax
- d) Ocular anthrax

8. Which of the following is a symptom of inhalation anthrax?

- a) Skin ulcers
- b) Difficulty swallowing
- c) Joint pain
- d) Chest discomfort and fever

9. Which of the following is NOT a common way to contract cutaneous anthrax?

- a) Handling contaminated animal products
- b) Inhaling airborne spores
- c) Contact with infected animals
- d) Using contaminated items like wool or hair

10. Can anthrax be spread from person to person?

- a) Yes, easily
- b) Yes, but rarely
- c) No, never
- d) Only through sexual contact

11. Which diagnostic method is commonly used for detecting anthrax in clinical specimens?

- a) Blood test
- b) Urine culture
- c) Stool sample analysis
- d) Polymerase chain reaction (PCR)

12. What is a characteristic feature of anthrax bacteria under a microscope?

- a) Round shape
- b) Chain-like formation
- c) Spiral structure
- d) Cuboidal appearance

13. What is the first-line antibiotic treatment for anthrax?

- a) Penicillin
- b) Ciprofloxacin
- c) Azithromycin
- d) Doxycycline

14. Which of the following is a preventive measure against anthrax for individuals at high risk of exposure?

- a) Wearing a hazmat suit
- b) Avoiding all meat products
- c) Annual flu vaccination
- d) Frequent handwashing

15. In the event of an anthrax outbreak, what is the primary goal of public health authorities?

- a) Eradicate all infected animals
- b) Isolate and quarantine all affected individuals
- c) Administer mass vaccinations
- d) Implement early treatment to reduce morbidity and mortality

16. Which factor can contribute to the spread of anthrax during an outbreak?

- a) Warm and humid climate
- b) Widespread use of antibiotics
- c) Lack of suitable animal hosts
- d) High altitude areas

17. What is the purpose of anthrax surveillance?

- a) To track international travel patterns
- b) To monitor population growth
- c) To detect and respond to anthrax cases
- d) To study the genetic diversity of anthrax bacteria

18. Which of the following is an example of passive anthrax surveillance?

- a) Regular testing of livestock in high-risk areas
- b) Active case-finding through door-to-door surveys
- c) Monitoring social media for anthrax-related posts
- d) Analyzing climate data for potential outbreaks

ANSWER KEYS

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- a) To track international travel patterns
- b) To monitor population growth
- c) To detect and respond to anthrax cases**
- d) To study the genetic diversity of anthrax bacteria

18. Which of the following is an example of passive anthrax surveillance?

- a) Regular testing of livestock in high-risk areas**
- b) Active case-finding through door-to-door surveys
- c) Monitoring social media for anthrax-related posts
- d) Analyzing climate data for potential outbreaks

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