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ANTHRAX: DEADLY BIOTERRORISM THREAT AND PREVENTION CHALLENGES IN INDONESIA

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ABSTRACT

Anthrax is a disease caused by the bacterium Bacillus anthracis. The disease can be a threat to bioterrorism because it causes a high mortality rate and is easy to spread. The characteristics of sport anthrax are resistance to extreme environmental conditions. Furthermore, the Vegetative form of anthrax bacteria can form biofilms. This characteristic causes the bacteria to become a potential biological weapon agent because it is persistent and resistant to some antimicrobial agents. Antibiotic therapy is still considered the main treatment choice for anthrax disease. Antibiotic recommendations vary depending on the location, severity of the disease, and level of antimicrobial sensitivity that should be tested through bacterial culture. Anthrax prevention and control procedures in Indonesia are still limited in monitoring the entry of animals from anthrax-free areas and administering vaccines to animals in endemic or enzootic areas. The high rate of death, illness, and economic burden due to anthrax outbreaks requires health workers to increase medical vigilance against clinical anthrax. Early detection efforts and policy-making to prevent the spread of anthrax, especially against the possibility of its use as bioterrorism, should be a concern.

KEYWORDS anthrax, bioterrorism, mitigation

INTRODUCTION

Bioterrorism is the deliberate use of biological agents against civilians, which can be bacteria, viruses, fungi, or toxins, with the aim of causing panic, mass casualties, or severe economic disruption (Ahmed & Kiani, 2024). Microorganisms have been used as weapons since prehistoric times (Alam et al., 2022). There were 37 bioterrorism attacks around the world from 1981 to 2018 (Ogunleye et al., 2024). Anthrax caused by B. anthracis bacteria is included in category A of priority pathogens for biological defense due to its easy-to-obtain properties, high mortality rate, and ease of spread (Doganay, Dinc, Kutmanova, & Baillie, 2023). In addition to being easy to obtain, Anthrax spores can also be easily made in the laboratory and have a long half-life in the wild (Williams et al., 2021).

The main paradigm of the B. anthracis life cycle has vegetative bacilli that sporulate after the death of the host, and the spores remain dormant until they meet the next host

(Wales & Mackintosh, 2023). The relative resistance of spores to environmental conditions such as drought, heat, rain, cold, radiation, and disinfectants is one of the reasons why these organisms have been explored as potential biological weapons agents (Maves & Berjohn, 2020). In addition, some vegetative forms of anthrax can easily form biofilms that keep them persistent in the soil and resistant to some antimicrobial agents. This makes anthrax difficult to eradicate (Bower, 2023; Pilo & Frey, 2018; Savransky, Ionin, & Reece, 2020).

The impact of anthrax attacks is estimated that if the spread of anthrax is carried as much as one kg for 10 million through the air, more than 100,000 people will die from the attack (Khairullah et al., 2024). This figure would increase up to seven times if the distribution of antibiotics was slower (SHENENI & AKOMOLAFE, 2023). In addition, anthrax also affects the economic system, where the process of decontamination and treatment requires a large cost estimated at one trillion rupiah (Caffes, Hendricks, Bradley, Twenhafel, & Simard, 2022). The high mortality rate, morbidity, and economic losses from anthrax outbreaks make the author interested in raising the topic of anthrax as a threat to bioterrorism (Olani, Dawo, & Lakew, 2020). This paper aims to increase awareness of the threat of bioterrorism, as well as to encourage early detection and policy-making to prevent the spread of anthrax (Aminu et al., 2020).

RESEARCH METHOD

This study employs a literature review methodology, collecting and analyzing data from various secondary sources such as scientific journals, government reports, and books. The approach aims to synthesize existing knowledge on anthrax as a bioterrorism threat and its mitigation efforts in Indonesia and globally.
he steps involved in the literature review include:

Data Collection

Relevant articles and reports were selected from reputable databases such as PubMed, Google Scholar, and government publications. Keywords used in the search included "Anthrax," "Bioterrorism," "Anthrax Mitigation," and "Indonesia."

Inclusion and Exclusion Criteria

Only articles published in the last ten years were included to ensure up-to-date information. Studies focusing on unrelated biological threats or non-zoonotic diseases were excluded.

Data Analysis

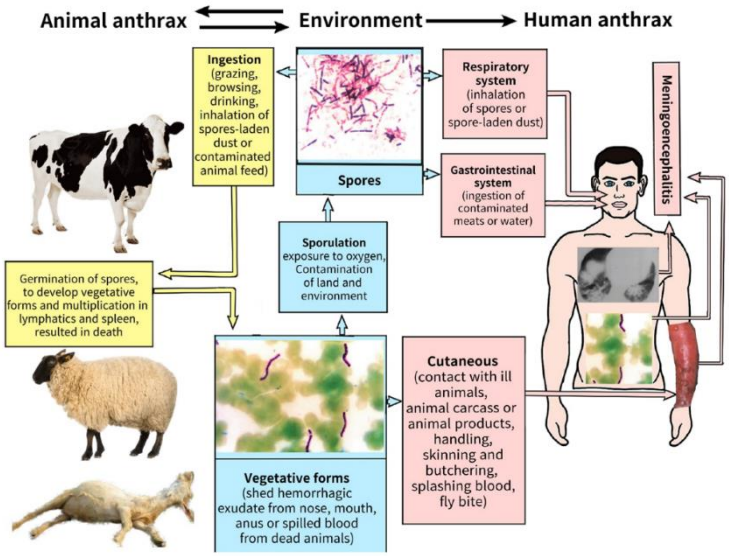
Thematic analysis was conducted to identify recurring themes related to anthrax pathogenesis, the role of Bacillus anthracis as a biological weapon, and mitigation strategies. Information from global and local contexts was compared to assess the current state of preparedness and challenges in Indonesia.

This methodology allows for a comprehensive understanding of the subject matter, drawing on existing research to identify gaps and provide recommendations for future policy and practice.

RESULT AND DISCUSSION

Anthrax is a zoonotic, contagious, and deadly disease that affects both herbivorous animals and humans. The causative agent of anthrax is the bacterium B. anthracis, which is in the form of a rod with gram-positive, non-motile, aerobic, or facultative anaerobic capsules and produces spores. There are two forms of bacteria, namely vegetative and spore. Spores begin to form from the carcasses of animals that have died infected with anthrax. After the body fluids from the carcass of the animal come out of the animal's body and are exposed to the environment, the bacteria will sporulate. The ambient temperature between 12 - 42°C and the presence of oxygen exposure facilitate sporulation, while conditions at < 12°C do not cause Bacillus sporulation.

Spores can survive in hostile environments such as being resistant to heat, cold, drying, chemical disinfection, pH, and radiation. This allows the spores to survive for a long time in the environment in a dormant state without replication for decades. Spores are more potent than the aggressive form of bacteria. Vegetative cells that cannot survive long (>72 hours) outside the host's body in the absence of sporulation. Vegetative is the result of the development of spores ingested by livestock so that the vegetative can only live and infect the host's body cells.



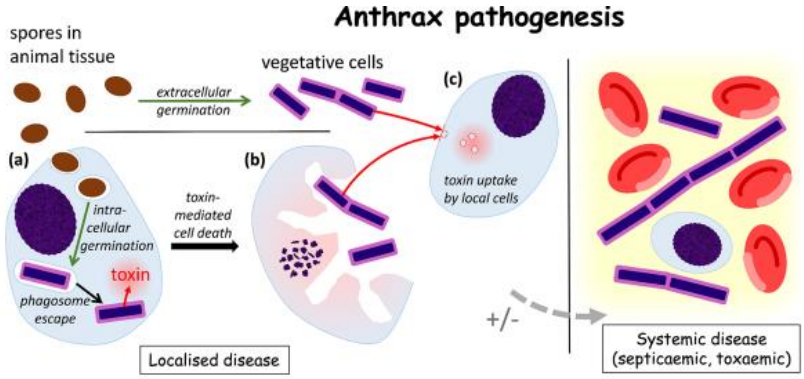
Picture 1. Life Cycle Bacillus Anthracis.

The life cycle of B. anthracis is where the soil is the main reservoir of the pathogen and is contaminated by spores released from the carcasses of infected animals. Animals that eat grass from land or carcass meat contaminated with spores become infected animals, giving rise to a new cycle of infection. In addition to ingestion, there are other major forms of infection routes, namely inhalation and skin (open wounds, intravenous injections, or insect bites).

Anthrax Pathogenesis

Mature spores that were previously outside the host are still in inactive or inert form and then enter the host's body through the infection route. The presence of incoming spores signals the non-specific immune system in the form of macrophage migration to the infected area. Macrophages will phagocytose the spores, and then macrophages go to the regional lymph nodes. Macrophages have an important role in the early stages of anthrax infection. The spores in macrophages undergo a germination process transformed

into a concurrent vegetative cell followed by activation of capsule genes and toxin complexes present in bacterial plasmids. In addition, spores can also germinate in extracellular tissues without going through phagocytosis, which often occurs on the skin index route.



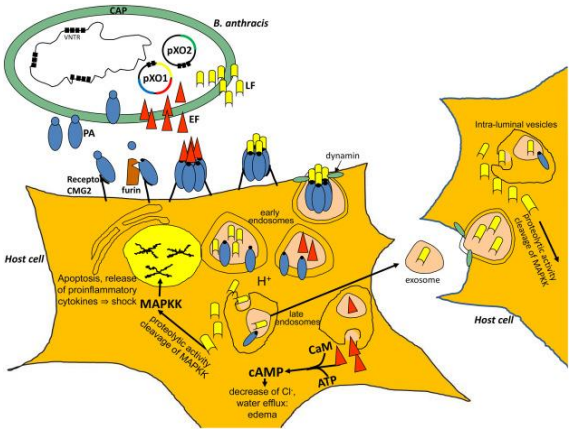
Picture 2. Pathogenesis of Antrax.

The main virulence factors resulting from B. anthracis vegetative cells are poly-γ-D-glutamic acid capsules and tripartite toxins. Gene activation of poly-γ-D-glutamic acid capsule genes is derived from the pXO2 code of plasmids, which have immunogenicity of decay so that it can provide resistance to phagocytosis and complement systems. This makes B. anthracis immune to the host's immune system, and the bacteria can escape from phagocytes and carry the toxin to the target cell. Tripartite toxin consists of a protective agent (PA), edema factor (EF), and lethal factor (LF) derived from the pXO1 code of the plasmid.

The process of toxin entering the cytoplasm of the target cell begins PA first binding to the Anthrax Toxin Receptor. Once the PA binds to the receptor, it will then be cleaved by the host-like furin to produce a PA that is shorter from 83kDa to 63kDA and assemble into a heptamer or octamer in a lipid membrane to bind to LF and EF. The LF: PA and EF: PA complexes enter the target cells through dynamin-clathrin-mediated endocytosis. After entering the cell, PA undergoes conformation triggered by the acidic environment (H+) in the endosome, forming pores to translate EF and LF to the cytoplasm, resulting in a toxic activity. LF, which is a metalloprotease, will inactivate MAPKK kinase through proteolytic cleavage. This results in excessive cytokine release, apoptosis, hypoxia, and finally, necrosis. In addition, LF stimulates the production of high levels of TNF-α and IL-1-β, leading to macrophage lysis, release of additional inflammatory mediators, multisystem organ failure, and death.

Then EF is adenyl cyclase, which converts ATP to cAMP and is about 100 times more active. An excessive increase in cAMP concentration leads to a decrease in chloride ions and the formation of massive extracellular edema that can end in hypovolemia shock. LF and EF target and inactivate immune cells such as macrophages and neutrophils in early infection conditions. This will block the immune system and cause acute infections with clinical symptoms such as fever, sore throat, diarrhea, and vomiting. Meanwhile, in advanced infection conditions, EF and LF form intraluminal vesicles so that they are protected from lysosomal enzymes. This causes toxins to be stored in the long term. The release of this toxin can occur at any time, even in the absence of B. anthracis bacteria. This is the basis that some patients can still experience systemic infections even though

they have succeeded in eradicating bacteria through antibiotics, especially in patients with conditions where treatment is given late.



Picture 3. Molecular Mechanism of Virulence Bacillus anthracite

Diagnosis And Management

The procedure for the diagnosis of anthrax consists of clinical manifestations, patient history, laboratory examination, and microbiological and radiological testing. A travel history, residence in an endemic area, work with animal contact, and exposure to sick or dead animals can indicate suspicion of anthrax infection. Suspect cases should be confirmed by the collection of appropriate samples of the patient's lesions or manifestations. Samples include skin swabs, blood, phlegm, feces, and cerebrospinal fluid.

Clinical manifestations can arise in one of three classic clinical forms, namely cutaneous, gastrointestinal, and inhalation. The severity of the infection depends on the patient's immunity, virulence, and the number of bacteria infected. Anthrax meningitis is a complication of one of the classic clinical forms. The majority of anthrax cases,95%, are skin lesions. These lesions can occur on the face, neck, hands, and arms. The incubation period is between 12 hours - and 7 days. Symptoms begin with itching and then appear in the stage of papules or macular erythematosus, which then turns brown with itemized rings and vesicles. The vesicle stage with the image of blisters erupting into hemorrhagic lesions, which continue to ulceration; the eschar stage will appear 2-6 days after the hemorrhagic vesicles mongering into black crust (croal-like), surrounded by significant non-pitting and item edema. This efflorescence is very typical in the clinical cortex of the skin.

Gastrointestinal (GI) anthrax has an incubation period of two – five days after exposure. Symptoms can start from oropharyngeal, such as sore throat, ulceration in the oral cavity, lymphadenopathy in the submandibular lymph nodes, and often swelling of the neck. Gastrointestinal symptoms are in the form of complaints of abdominal pain, anorexia, nausea, vomiting, diarrhea, and occasional hematemesis. This can be followed by the development of ascites, bloody diarrhea, toxemia, and toxic shock.

Clinical symptoms of inhaled anthrax appear 1-6 days after exposure, consisting of two stages, namely prodromal and acute. The prodromal stage lasts for 48 hours and consists of flu-like symptoms, namely unproductive cough, subfebrian fever, fatigue, and myalgia. A short period of improvement of 1-3 days may occur after prodromal symptoms

before worsening rapidly. The acute stage is in the form of severe dyspnea, stridor, high fever, and cyanosis. Massive lymphadenopathy, pleural effusion, and enlargement of the mediastinum can be seen at this stage. 10,12 Meningeal anthrax manifests as the onset of cerebral hemorrhage and finger edema. A typical pathology of bleeding due to anthrax is a subarachnoid hemorrhage that spreads on the cortical surface is classically called cardinal's cap.

Culture and isolation of bacteria with a medium to become the gold standard examination in identifying B. anthracis. After incubation in 18 – 24 hours, the bacteria grew on the blood agar with morphological characteristics of gray/white ground glass shape, flat or slightly increased, 2-5 mm in diameter, described as "tenacious" or "sticky," similar to petroleum jelly. In addition to culture, pathogen identification can be made by smearing gram methylene blue to see the vegetative form of gram-positive bacteria in blue and black surrounded by pink capsules. The use of PCR can also establish the diagnosis of Anthrax quickly, but the antibiotic sensitivity test for each individual is only obtained through bacterial culture. Aminu et al. in Northern Tanzania found that gram staining smear is the most effective and easiest to check in areas with limited resources.

Antibiotic therapy is still considered the main choice for the treatment of anthrax disease. However, the treatment approach of anthrax is different from other bacterial infections due to the unique pathogenicity of anthrax in the form of toxin production, antibiotic resistance problems and the high frequency of complications of meningitis events. Antibiotic prescribing recommendations vary depending on the location and severity of the disease. The recommended treatment regimen for uncomplicated cutaneous anthrax is doxycycline 2x100 mg or minocycline 1x200 mg, then 100 mg every 12 hours orally for 7-10 days or until clinical criteria are stable.

If there is a possibility of exposure to aerosols, patients should switch from treatment to a PEP regimen, while patients with clinical systemic cutaneous anthrax, inhalation, GI with or without meningitis are recommended a combination of two antimicrobials and Protein Synthesis Inhibitors (PSIs) or RNA synthesis inhibitors (RNAI). One of the recommended regimens is ciprofloxacin 3x400 mg IV + meropenem 3x2 gr IV + minocycline once 200 mg IV, then continued 2x100 mg IV with a treatment duration of 10 – 14 days. If there is a possibility of aerosols, the patient does not need to need antimicrobial drugs for PEP because the patient already has natural immunity.

Anthrax and The Threat of Bioterrorism

Anthrax disease has been feared for centuries due to the high mortality rate in animals and humans. The fifth item of the old covenant about the plague of Egypt is in the form of a plague of livestock, and the sixth is in the form of ulcers in humans. These symptoms are consistent with anthrax disease. Anthrax events also occurred in Europe in the 1600s and caused more than 60,000 deaths in humans and livestock. It was similar to the largest zoonotic outbreak in Zimbabwe's history in 1978-1980 caused by anthrax.

B. anthracis was first identified by a German researcher named Robert Koch in 1876. Anthrax became famous as a biological weapon until the formation of the Biological Weapons Convention signed by 180 countries in 1975, which prohibited the research, production, and use of anthrax as a biological weapon. After the ban, some continued to produce and distribute B. anthrax. The events in the Soviet Union in 1979 resulted in 77 cases of inhaled anthrax, 66 of which died, confirming the lethal nature of anthrax as a biological weapon. The most recent incident occurred in Washington DC, New York, and Florida in October 2001 when there were letters contaminated with

anthrax spores sent by U.S. mail to politicians and media offices. It infected at least 22 people and resulted in five deaths.

Bioterrorism or biological attack is the deliberate spread of viruses, bacteria, and other organisms that cause illness and death in humans, livestock, and food crops. One of the agents used for bioterrorism is 18 B. anthracis. Some of the reasons are that Anthrax spores are easy to find in nature, can be produced in laboratories, and can survive for a long time in a free environment. In addition, the size of the spores is so small that they cannot be seen, smelled, or felt and can be used without anyone knowing.

Spores can also be spread through goods, food, water and soil. LF and EF toxins inhibit the work of the innate immune response so that bacteria can circulate freely in lymphatic and systemic blood vessels, spreading throughout the host cell and producing large toxins. This condition supports the occurrence of sepsis as a complication of anthrax. It also causes death in patients in just a few days if not treated immediately. The highest mortality rate during 1940 – 2019 occurred in the primary meningitis anthrax infection route at 92%, 75% for inhaled anthrax, 72% for gastrointestinal anthrax, 33% for injectable anthrax, and 22% for cutaneous anthrax.

To prepare for public health and national security, the distribution of the most important pathogens for biodefense is essential. Pathogens are classified into three categories by the Centers for Disease Control and Prevention (CDC): Category A, Category B, and Category C. Category A pathogens are considered to have the highest risk and pose a significant hazard to public health and national security. These agents include organisms that can easily spread or be transmitted, resulting in high mortality rates, and have a significant impact on public health. Anthrax (Bacillus anthracis), botulism (Clostridium botulinum toxin), plague (Yersinia pestis), smallpox (variola major), and tularemia (Francisella tularensis) are some examples of category A pathogens.

Category B pathogens receive second priority. These agents are easily spread and have low morbidity and mortality rates. They need better CDC diagnostic capacity and more disease surveillance. Examples of category B pathogens include brucellosis (Brucella species), epsilon toxin of Clostridium perfringens, food safety threats such as Salmonella species and Escherichia coli O157:H7, and viral encephalitis caused by alphaviruses. New infectious diseases that can be engineered to be mass-disseminated in the future include category C pathogens, which are the third highest priority. Due to its high morbidity and mortality rates and significant health impacts, this pathogen is considered a new threat. Examples of Category C pathogens include the Nipah virus and Hantavirus. This division helps prioritize research and preparedness efforts to address the most significant threats of bioterrorism.

Disaster Mitigation for Anthrax

According to Law Number 24 of 2007, mitigation is an effort to reduce disaster risk, both through physical development and awareness and improvement of the ability to face disaster threats. Mitigation consists of two main patterns, namely (1) structural mitigation through the physical development of facilities and infrastructure with a technological approach and (2) non-structural mitigation which focuses on the formulation, decision-making and implementation of governance and increasing legislative capacity, insurance and so on. 21 Data in Indonesia has occurred several outbreaks starting from 1884 where there was a disease resembling Anthrax attacking buffalo cattle in Teluk Betung, Lampung. After that, the anthrax incident began to attack other areas. The latest data

recorded until 2017 was reported to have occurred in 22 provinces, and the last cases occurred in Gorontalo, South Sulawesi, and Yogyakarta.

The procedures for the prevention and control of anthrax are regulated in the technical instructions issued by the Directorate General of Livestock and Animal Health in 2018. Preventive measures for anthrax-free areas are based on strict regulations that supervise the entry of animals into the area. Meanwhile, in endemic/enzootic areas, preventive measures in the form of vaccination are recommended, followed by strict monitoring. Animals suspected of anthrax were injected with the antibiotic Penicilline 6,000 – 40,000 IU/kgBB IM or Oxytetracycline 1–2 grams/head IM for 3-4 consecutive days, SC or IV homologous serum at a dose of 50-150 ml, combined and followed by Anthravet vaccination of 10 million IU/ml/head after the next two weeks.

Efforts to control the disease where animals with anthrax must be isolated in a special place where there is a two-meter-deep hole to accommodate food scraps and feces from sick animals; if the animal dies or recovers or the hole has been filled up to 60 cm, then the hole is filled with fresh soil. Animals that are suspected of being sick within a period of 20 days do not show symptoms of illness; then they are released from isolation. Insulated enclosures that have been completed in use cannot be disinfected but must be burned. Likewise, animal carcasses that die from anthrax must be destroyed immediately by burning them in a hole or burying them at least two meters deep. Then for, the area must also be isolated. The disease is said to disappear if it passes 20 days since the death or recovery of the animal.

Several guidelines that recommend Anthrax preventive measures, namely the CDC, 2023 suggest prevention in a person who has been exposed in the form of post-exposure prophylaxis (PEP) by being given live antimicrobial drugs or live anti-toxin drugs if not available. The recommended antibiotic regimen (PEPAbx) is Docicicline 2x100 mg orally or ciprofloxacin 2x500 mg orally taken for 60 days for those exposed to aerosols. However, if PEPAbx is used in conjunction with vaccination (PEPVx), the antimicrobial is discontinued 42 days after the first dose or 14 days after the last dose of the vaccine, whichever is longer. If exposed to non-aerosols (skin or ingestion), PEPAbx is taken for seven days and the vaccine is not required. The PEPVx regimen uses a single anthrax antitoxin with the choice of BioThrax (Anthrax Vaccine Adsorbed, AVA) vaccine for Anthrasil (Anthrax Immune Globulin Intravenous, AIGIV), raxibacumab and obiltoxaximab.

The Advisory Committee on Immunization Practice (ACIP) and the United States Department of Defense (DOD) recommend conducting pre-exposure prophylaxis in high-risk populations such as defense workers who go to high-risk areas of exposure, laboratory workers, farmers, veterinarians and livestock workers who may be handling infected animals. The recommended vaccine for pre-exposure prophylaxis (PrEP) is AVA at a dose of 0.5 ml intramuscularly (IM at 0, 1, and 6 months with a booster at 6 and 12 months after. AVA can also be used as a PEP regimen with a dose of 0.5 ml administered subcutaneously (SC) at 0, 2, 4 weeks.

CONCLUSION

Anthrax poses a serious threat as a biological weapon due to its high mortality rate, ease of production, and resistance to extreme environmental conditions. The unique pathogenicity of Bacillus anthracis, particularly its ability to form long-lasting spores, makes it a primary concern for bioterrorism and public health preparedness. In Indonesia, limited resources for monitoring and controlling anthrax underscore the need for stronger

mitigation strategies, such as enhanced early detection, comprehensive vaccination programs in endemic areas, and the strengthening of policies and education related to bioterrorism prevention. Collaboration between government agencies, health organizations, and researchers is essential to minimize the impact of anthrax threats on public health and national security. Further research is needed to develop advanced diagnostic methods, explore innovative treatment options, and evaluate the effectiveness of existing mitigation measures.

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