OPEN ACCESS
Scholars Bulletin
Abbreviated Key Title: Sch Bull
ISSN 2412-9771 (Print) |ISSN 2412-897X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Subject Category: Zoology

A Review on Laboratory Investigation of Anthrax as Causative Infectious Disease and Future Perspectives

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DOI: 10.36348/sb.2021.v07i07.006 | **Received:** 11.06.2021 | **Accepted:** 14.07.2021 | **Published:** 20.07.2021

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Abstract

Anthrax is a zoonotic disease that is associated with herbivores and other domestic animals including humans. The causative agent of this disease is bacterium *Bacillus anthracis*. It is Gram positive, non-motile, anaerobic, spore forming bacterium living in soil. It can transmit in animals by entering spores in their bodies through skin, lungs and gastrointestinal tract. This can be passed into humans by improper handling and contact with infected animals. Symptoms of this disease are different in animals and humans according to the type of anthrax. It is diagnosed by different methods including differential and laboratory methods (PCR). It can be treated by vaccination and therapies that are described. Gastrointestinal anthrax occurs only after eating infected, undercooked meat. This review helpful to understand the anthrax disease, diagnosis and possible treatment.

Keywords: *Bacillus anthracis*, anthrax, transmission of anthrax, pathology of anthrax, cutaneous anthrax, gastrointestinal anthrax, pulmonary anthrax.

INTRODUCTION

The world anthrax is derived from Greek world "anthrakis" which means "coal black coal skin". Anthrax is a zonotic disease caused by a bacterium called Bacillus Anthracis. It is a rare but serious disease of herbivores and other animals including human also [1, 2]. It mostly affects the livestock, wild game and then transfer to human beings so called as zonotic disease. It may cause the death of the animal if leads to serious conditions. It is most common in the countries which have poor agricultural system i.e. United State, South America, Central America, Southern and Eastern Europe, Africa, Ciribben and Middle East. Animals are affected to it when they ingest or breathe the spores of bacteria previously mentioned from polluted soil, plants or water. When spores enter in the human body they divide here and multiplied and spread in the whole body, produce toxic substances and cause serious disease anthrax. People may also gain the spores through cut on the skin [3, 4].

History and ecology

Due to its rapid spread it has great attention in recent years in understanding how it causes the disease. Due to its agricultural importance this bacterium has becomes the subject of many scientists. Today it is taken as biological weapon and cause the immediate death. It was also described in early literature, books of Romans and Hindus [1-3]. It is written in the Egyptian book Genesis (1419) BC that the possible loss of sheep, goat, horses and donkeys may be the anthrax. In the 19th century the research made on the anthrax and a lot of medicines were made for the treatment. In 1850 Pierre Rayer firstly described the filifor bodies called as small rod like structuresin the blood of thesheep that has dieddue to thisdisease [4]. This observation leads to the information that the cause of the anthrax are the living organisms that multiply in the body, transfer to blood stream and cause the death due to poisoning. The culture of the bacteria was not present that time [5].

This observations leads to Robert Kotch who was successful for describing the Germ Plasm Theory of disease and explain three postulates. He applied these postulates in the Germony during the Wollstein. He isolates the bacteria from the skin of sheep and prepares the culture. He then used this culture by growing these bacteria in the aqueous humor of the OX's eye and again injects these bacteria in the healthy sheep. He then performed same experiment by taking bacteria from rod cells of the ox eye. He studied that underperformed favorable conditions these bacteria reduce spores especially in the absence of oxygen but again form the spores when conditions are favorable. When any animal dies the blood goes to the soil, the bacteria formed in the soil, convert into spores when oxygen is available. Thus he discovered the technique named as vaccine. Thus the first vaccine was made against anthrax [6-8].

Types of Anthrax

There are four different types of anthrax depending upon from which part of body they enter in the body.

1. Cutaneous anthrax

Also called skin anthrax contributes to 95% of the anthrax disease. It occurs when spores of the bacteria enters to the body by the skin cut or by the detritions of skin such as touching the defile wool, mask, leather and hair products of infected living organisms [6, 7]. Skin infections occur as annoying skin crash looks similar to the insect biting and within 1-2 days it develops the vesicle which has diameter of about ½ inch with black area. Mostly those areas of the body are affected which are exposed such as arm, hands, face, neck and foot. It can be cure by proper treatment but if it lefts untreated then it may cause serious conditions. 20% of the untreated cases died due to anthrax [8].

2. Gastrointestinal anthrax

This occurs when animal eats the bareness or uncooked food. Spores enter to body by eating and mostly affects the upper gastrointestinal system i.e. throat, esophagus, stomach and intestine. This may also cause the oral esophageal ulcer and enlargement of lymph nodes. Infection occurs within 1-7 days. More than 50% died due to untreated anthrax. However, by applying the proper treatment 60% patients can survive [9].

3. Inhalation anthrax

This occurs when person breath in such places where anthrax spores are found. These spores mostly occur in the mils of wool, tanneries, and slaughterhouses [10]. The spores may also enter in the body by breathing while dealing the contaminated animals or products. Its symptoms are flue like i.e. fever, cough and muscle aches. It firstly causes the infection in lymph nodes and in chest and then spread in rest parts of the body and hence causes serious problems of breath. It is most serious form of anthrax occurs within 2 months. Only 10-15% patients survive by treatment. However, by proper and complete treatment 555 patients may survive. It is fetal if lefts untreated [11-13].

4. Injection anthrax

It was firstly introduced in North America while people dealing with the Heroin injections. Its symptoms are similar to the cutaneous anthrax. Its infection is deep under skin and on the muscles especially where drug is injected. It spread to body fast so due to which it is very easy to recognize it and to treat it. Many times infection occurs on body due to other reasons also. Therefore, an infection does not, means that the person is infected by anthrax [14].

Morphology of Bacillus anthracis

Bacillus anthracis is arod shaped gram positive (gives pink color when staining) bacterium. It is aerobic (grow in the presence of oxygen) or facultative anaerobic (alsogrow without oxygenbutgrow best in the presence of oxygen. It forms the endospores which are highly resistive in the environment. Its size is 4µm in length and 1µm in width. The spores are of 1µm size and grow at 37℃. spores germinate when they enter in the environment which is rich in amino acids, nucleosides and glucose. Under microscope this bacterium appears like chain of cells. These chains appear two to few cells in length when smear (blood smear, tissue smear) is prepared. When smear is prepared in vitro it forms long chains just like string. In the presence of oxygen it form capsule around its cell which are resistive. In the absence of oxygen it releases its spores and remains dormant [15-17].

Modes of Transmission

Spores of this canenter the body through1) skin lesion or cutaneous anthrax, 2) lungs or pulmonary anthrax and 3) gastrointestinal route orgastrointestinal anthrax andthen they germinate, producing a vegetative form.Anthrax is a concern of public health also in many countries where agriculture is the main source of income [18].

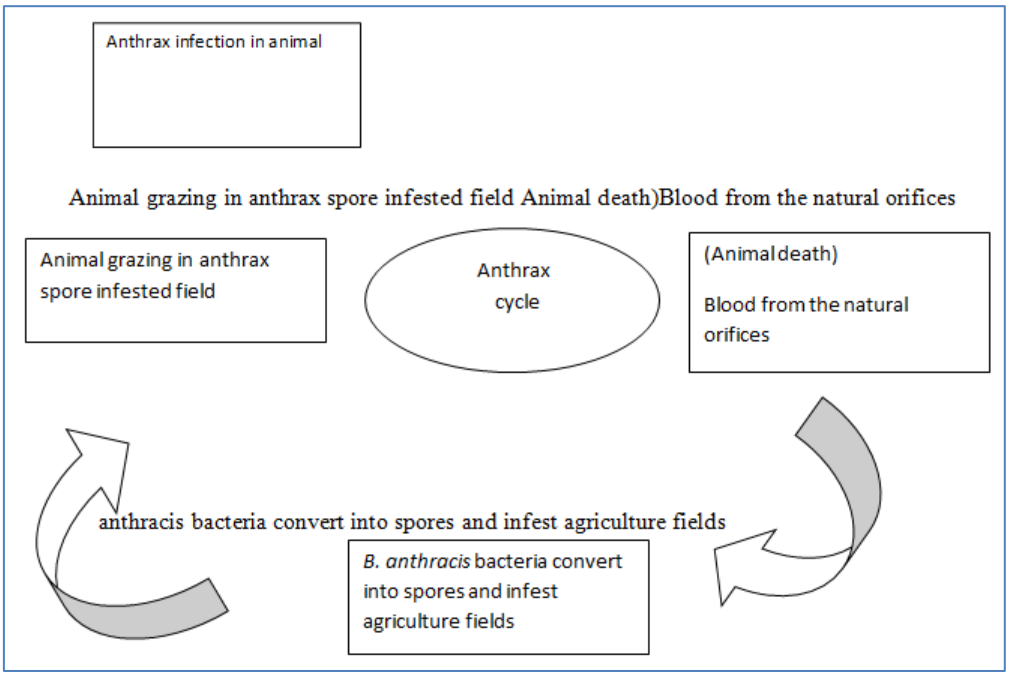

Fig-1: Shows the different steps involved in Life cycle of Anthrax

It mostly causes the infection in the wall of the terminal ileum and caeccum. The orophyranx, stomach and upper ileum may also affected by anthrax. It is found in two forms abdomen and oro esophageal anthrax. The symptoms of the abdominal anthrax include the nausea, vomiting, fear of getting fat and fever. The acute abdominal pain, diarrhea and haematemesis also occur followed by the death. The symptoms of the oro esophageal anthrax include the sore throat, dysphagia, fever, edema and enlargement of lymph nodes. It also leads to high mortality rate [15-19].

Table-1: Shows the specific antibiotic and dosage of drugs against anthrax

Recommended anti-toxin organization regimens for Bacillus anthraics			**References**
Antibiotic	**Dosage for adults**	**Dosage for children**	**[2]**
Penicillin V	5oo mg orally, 4 times per day	25-50 mg/kg/day orally in4 divided doses	**[6]**
Penicillin G, procaine	0.6-1.2 mU IM EVERY 12-24H	2500-50000 U/kg/day IM	**[9]**
Penicillin G, sodium or potassium	4 Mu intravenously every 4-6 hours	300000-40000 u/kg/day in divided doses every 4-6 hours	**[11]**
Ampicillin	1-2 g intravenously every 4-6 hours	5-200 mg/kg/day intravenously divided every 4-6 hours	**[15]**
Amoxicillin	500 mg orally every 8 hours	Weight> 20 kg 500 mg orally every 8 hours Weight<20 kg 40 mg/kg orally in 3 doses every 8 hours	**[19]**
Ciprofloxacin	500 mg orally every 12 hours or 400 mg intravenously every 8 hours	Not generally recommended for children in emergency 10-15 mg/kg twice daily not to exceed1 g/day	**[20]**
Clarithromycin	500 mg PO or IV every 8 hour	Not recommended for children	**[7]**
Clindamycin	150-300 mg orally every 8 hours or 600-900 mg IV every 6-8 hours	8-25 mg/kg/day every 60-8 hours orally IV 15-40 mg/kg/day in 3-4 divided doses	**[2]**
Doxycycline	100 mg every 8 hours	Not recommended For less then 8 years In emergency less then or equal 2.2 mg/kg twice daily then 8 year and weight. Then 45 kg 100 mg twice daily	**[7]**
Erythromycin	500 mg orally every 6hours	30-50 mg/kg/day in 4 divided doses	**[9]**
Rifampicin	o.6-1.2 g daily IV PO in 2-4 divided doses	10-20 mg/kg/day 12-24 hours	**[11]**
Streptomycin	1 g/day IM	20-40 mg/kg/day	**[12]**
Tetracycline	500 mg orally every 6 hours	Not approved	**[21]**
Vancomycin	1 g IV very 12 hours	40 mg/kg/day in 2 or 4 divided doses every 6-12 hours	**[27]**

Diagnosis

There are different methods for the diagnosis of anthrax.

1. Differentia Diagnosis

Diagnosis of anthrax was made on the base of clinical finding. Enzyme- linked immunosorbent and PCR are use to diagnosis of anthrax but available at national reference laboratories. Microbiology also help to identify the initial diagnosis of anthrax. Most microbiologist use blood culture which give growth within 6-24 hours. If symptoms of cutaneous anthrax present then gram staining and culture of vesicular fluid confirm the disease [17].

Nearby or state wellbeing offices, clinic disease transmission experts, and the neighborhood or state wellbeing research center ought to be told quickly when Bacillus anthracis is suspected. Rules are accessible from the CDC for clinical and research facility finding, example taking care of, sterilization of gear, and postexposure prophylaxis [11].

2. Laboratory Diagnosis

Diagnosis in hospital and laboratories is depend upon the direct Gram's-stained smear of a skin lesion, blood showing encapsulated, broad, cerebrospinal fluid, gram-positive bacilli. It is also diagnosis on the base of growth on the sheep's blood and used agar culture. Different types of agar used but growth dose not appeared on MacConkey agar. Confirmatory tests are performed in B level laboratories. In these laboratories growth of virulent strains visualized on the nutrient agar in the presence of India-ink staining. Further conformation tests are performed by the exposure of gamma phage or direct fluorescence-antibody [19-22].

ELISA technique also uses to test the presence of B. anthracis. Performing such measures on serum tests got from patients with known and associated cases with Bacillus anthracis amid the intense and improving stages would be of an incentive in approving these serologic tests and potentially in affirming the determination in cases in which coordinate culture has not yielded the life form. Serologic trial of contacts would likely not be of assistance in settling on choices about somebody with late introduction, in perspective of the way that a serum test acquired amid the recovering stage half a month later would be important for serologic analysis. Nonetheless, such tests may be of epidemiologic incentive for the later analysis of Nasal-swab culture use to detect the presence of inhalation B. anthracis [22-25].

3. PCR

PCR is also use to identify the presence of virulence genes and B.anthracis present or not.

Treatment

Treatment of anthrax based on the historical information about animal. Now it is established that timely antibiotic therapy give good results for the treatment of animals and humans. In clinical laboratories broth microdilution method was used with staphylococcal breakpoints for the treatment of Bacillus Anthrax. Historical 65 isolates was testing. These isolates links with 2001 outbreak which were sensitive to meropenem, quinolones, tetracycline, chloramphenicol, imipenem, , rifampin , clindamycin, and the aminoglycosides, vancomycin. The use of pencilline for the treatment of anthrax is complicated. Cases of B. anthrax sensitive to pencelline which available easily .Ciprofloxacin is also studied in primates quinolones not studied in primate cases. Bacteremic patient often treated with many drugs in the begninig. In multi drug treatment ciprofloxacin or doxycycline along with one or more agents given to organism for inhalation anthrax. For the treatment of cutaneous disease are also ciprofloxacin or doxycycline given to organism. Penicillin such as amoxicillin or amoxacillin/clavulanic acid are given during testing the disease. Inhalational and cutaneous anthrax being treated with 60 days of antibiotics. But zoonotic cutaneous anthrax is treated 7 to 10 day with antibiotics[26-29].

Pre-exposure prophylaxis

In Pre-exposure prophylaxis vaccination is available. Filter vaccines are licensed but available to military persons. A major drawback of these vaccines are initial series of injections and d annual booster immunizations [30].

Post-exposure prophylaxis

Post-exposure prophylaxis for anthrax is use for high risk exposure. Ciprofloxacin or doxycycline full dose use for 60–100 days. Data showed that exposure of spores in human for 24 days after release from livestock and in monkeys for 100 days [31].

Treatment in humans

Penicillin G is as yet the medication of decision in the treatment of normally happening Bacillus anthracis in many parts of the world [1, 7]. In gentle uncomplicated instances of cutaneous Bacillus anthracis, the treatment for the most part prescribed is intramuscular procaine penicillin, 500 to 600 mg each 12– 24 hours for 3– 7 days. Intravenous anti-microbial treatment is not prescribed in gentle cases. On the off chance that the patient rejects intramuscular infusion, penicillin Vor on the other hand amoxicillin (500 mg orally at regular intervals for 3– 7 days) are worthy choices. Cutaneous sores normally turned out to be sterile inside the initial 24 hours of such regimens and the going with oedema for the most part dies down inside 24 to 48 hours at the same time, albeit early treatment will restrict the measure of the sore, it won't

modify the developmental stages it must experience [9, 14, 19].

Treatment duration

Continuation of treatment for 7– 14 days or longer has turned out to be standard, however often it is not valued that the injuries, or other poison related harm, will keep on advancing through their cycles of improvement and goals in any case of the end of the Stainting B.anthracis. For direction, it is recommended that the term of anti-toxin treatment in uncomplicated cutaneous Bacillus anthracis be 3– 7 days at the same time, in the nonappearance of clinical involvement with short-course anti-microbial treatment in foundational Bacillus anthracis, treatment in instances of fundamental Bacillu anthracis ought to be proceeded for 10– 14 days[22, 29].

CONCLUSION

Penicillin G is as yet the medication of decision in the treatment of normally happening Bacillus anthracis in many parts of the world. Intemperate anti-microbial treatment might be inefficient and counterproductive, perhaps offering ascend to antagonistic reactions. Strong treatment turns out to be more vital after the initial couple of days. Gastrointestinal anthrax occurs only after eating infected, undercooked meat. This review helpful to understand the anthrax disease, diagnosis and possible treatment.

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