ISSN 2449-8955 European Journal of Biological Research *Review Article*

DOI: http://dx.doi.org/10.5281/zenodo.3773896

Laboratory diagnostic methods and reported outbreaks of anthrax in Ethiopia

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Received: 22 February 2020; **Revised submission:** 18 April 2020; **Accepted:** 25 April 2020

http://www.journals.tmkarpinski.com/index.php/ejbr

ABSTRACT: Anthrax is a zoonotic disease caused by *Bacillus anthracis*, a Gram-positive, non-motile, spore-forming bacterium. It is a globally distributed disease, having been reported from all continents that are populated heavily with animals and humans. The objectives were to review general laboratory diagnostic testing methods and reported outbreaks of anthrax in Ethiopia. Anthrax was second top zoonotic priority next to rabies and endemic in Ethiopia that may occur in May and June every year (Anthrax season) in several farming localities. Animal hosts acquire the disease through grazing, usually by ingestion or inhalation while there are three major routs of transmission: ingestion, inhalation and cutaneous. This review indicated that anthrax remains to be major public and animal health problem in Ethiopia. Although suspected cases of anthrax are reported from several districts, they are not well confirmed by laboratories. Prevention and control of anthrax in animals effectively reduces its impact on public health and the national economy. The control of anthrax outbreaks among domestic animals is primarily dependent on rapid identification and treatment of affected animals; enhanced surveillance for additional cases; implementation of control measures including quarantine, prophylaxis, vaccination and the proper disposal of dead animals with decontamination is critical.

Keywords: Anthrax; *Bacillus anthracis*; Diagnosis; Toxins; Zoonosis.

1. INTRODUCTION

Anthrax is naturally occurring diseases of warm-blooded animals, including humans. The disease is caused by *B. anthracis*, a gram-positive, non-motile, endospore-forming bacterium [1, 2]. The name of the bacterium is derived from “anthrakis”, the Greek word for coal, because anthrax in humans causes black, coal-like lesions on the skin at the site of inoculation [3]. World Health Organization Collaborating Center for Remote Sensing and Geographic Information Systems for Public Health (WHOCC) recorded anthrax outbreaks in animals are in nearly 200 countries by The World Anthrax Data Site. Country-of-origin, anthrax status, vaccination program, species affected, year of outbreak, number of outbreaks during the year, number of cases, number vaccinated and total livestock population were types of data recorded by The World Anthrax Data Site [4].

Anthrax is worldwide distributed disease, having been reported from all continents that are populated heavily with animals and humans. WHOCC [4] classified the status of anthrax as hyperendemic/epidemic, endemic, sporadic, probably free, free and unknown. The countries with hyperendemic/epidemic status are more frequent in Africa, although the status of Egypt is "Probably free". Examples of regions with unknown anthrax status are the polar extremes, the Arctic and the Antarctic [5].

Anthrax is currently recognized as a neglected zoonosis, causing a public health threat in many regions of the world, particularly in Africa [6]. The bacterium forms spores when exposed to oxygen and allowing it to remain viable in the environment for many years before coming into contact with a susceptible host and when exposed to a nutrient rich environment, such as the tissues or blood of an animal or human host [7, 8].

Molecular monomorphic characteristics of *B. anthracis* is challenging for differentiation by PCR. However, *VrrA*, gene containing variable-numbers of tandem repeats region (VNTR) involving five variants differing in the number of copies. Rapid PCR analysis can be used to distinguish the five variants and based on the number of VNTRs, it is possible to classify various strain and various withe the geographical origin of the isolates [9].

Moreover, the tripartite ministries, Ministry of Livestock and Fisheries (Currently Ministry of Agriculture), Ministry of Health and Ministry of Culture and Tourism (through the Ethiopian Wildlife Protection Authority), in collaboration with development partners established a national One Health Platform (OHP) with various technical working groups. Since the national OHP was established, various activities have been undertaken. One of the tasks was developing a list of priority zoonotic diseases as an entry for a multi-sectoral joint action to reduce and combat the impact of zoonotic diseases on public and animal health as well as on the national economy. The national zoonotic disease prioritization was conducted using a tool developed by CDC and resulted in 5 priority zoonotic diseases for inter sectoral collaboration of which anthrax was the second top priority next to rabies [10].

Animal anthrax is an endemic disease and seasonal in Ethiopia which occurs in May and June every year in different localities of the country. Several districts of the country are reporting suspected cases of anthrax outbreaks in animals, few of those are confirmed by laboratory [11] and research was not done yet to understand epidemiology of anthrax disease outbreak. To date, anthrax outbreak reports are based on history and clinical signs in both public and animal health sector. In addition to limitations in confirming anthrax, biosafety and biosecurity issues are also of major concern for culture and identification of *Bacillus anthracis* at the national and regional veterinary and public health laboratories.

Therefore, the main objectives of this review are: to review laboratory diagnostic testing methods for anthrax and to understand reported outbreaks of anthrax in Ethiopia.

2. BIOLOGY OF *BACILLUS ANTHRACIS*

Anthrax is disease caused by the spore forming bacteria *B. anthracis.* It is a Gram-positive, endospore forming, rod-shaped (Figure 1), non-motile bacterium that grows on nutrient media, typically sheep or horse blood, under aerobic or anaerobic conditions with an optimal temperature of 35-37^{o}C [1, 2]. Colonies show as irregular, raised, opaque white to grey when grown on blood agar. The colonies are about 2mm in diameter and tacky on teasing with a loop [12]. Colonies cultured on media containing bicarbonate and incubated in high carbon dioxide (5-20%) will produce capsule, causing a mucoid phenotype [12]. Under a microscope, the vegetative form of the bacteria appears as square-ended in chains of two or more with an elliptical spore in the middle of the cell [1].

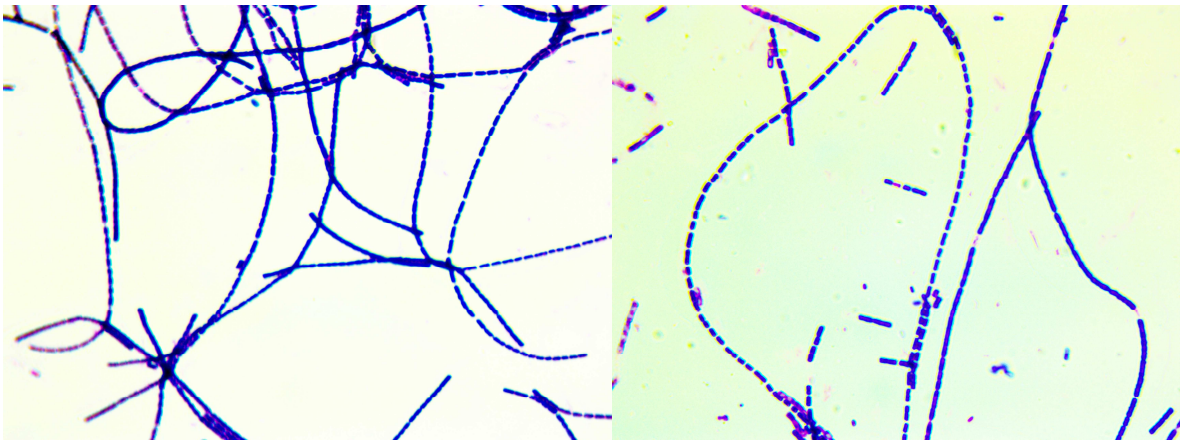

Figure 1. Gram staining shows Gram-positive rods in long chains, photo taken in May 2, 2019 during anthrax outbreak investigation at the National Animal Health Diagnosis and Investigation Center, Ethiopia.

Vegetative *B. anthracis* are poor to survive outside of a host and therefore produce endospores to survive long term in the environment [1, 13]. This characterizes *B. anthracis* as an obligate pathogen [12, 14, 15]. An endospore is the inactive form of a vegetative cell and formed in the presence of oxygen, towards the ends of exponential growth [16]. For *B. anthracis*, the spore is considered the infectious particle [17].

3. EPIDEMIOLOGY

3.1. Occurrences

Anthrax occurs on all the continents and commonly causes high mortality, primary herbivorous animals [12]. In domestic animals there is high fatality in cattle, sheep, goats, donkeys and pigs [15]. For wild animals, highest fatalities occur in zebra, antelope, bison, gazelles, impalas, elephants and hippopotami [2, 15]. Anthrax is the most common in agriculture region of central and South America, sub-Saharan Africa including Ethiopia, Central and southwestern Asia and southern Eastern Europe [18, 19].

Spores live on for decades in soil that rich in calcium, has a pH greater than 6.0, and when temperature is higher than 15.5^{o}C [20]. Anthrax disease is considered as a seasonal disease, because anthrax outbreaks follow a prolonged hot and dry period that is followed by heavy rainfall. It is thought that heavy rainfall can carry spores during runoff in clumps of organic matter to concentrate in standing pools or puddles. It is also thought that standing water can move spores upwards into the vegetation as it dries, leaving them in a better position to be ingested by grazing animals [20]. Spores are very resistant to heat, desiccation, cold, pH, chemicals and irradiation allowing them to survive in the environment for decades [1, 2].

3.2. Public Health Importance

Anthrax affects primarily herbivores animals. Humans usually become infected when butchering and eating of contaminated carcasses and come into contact with infected animals or their products and it is primarily an occupational hazard for handlers of processed hides, goat hair, bone products wool, infected wildlife and abattoir workers by contact with infected meat when contracted [21].

Anthrax spore most likely spread as an aerosol and can also be used as a bio-warfare or bio-terrorism agent, therefore; any new case can be assessed with this possibility in mind, particularly but not exclusively in cases of pulmonary anthrax [22].

3.3. Economic significance

Anthrax has significant economic impact by decreasing the efficiency which input or resources are converted into output or a product that means they reduced productivity. In most developing countries vaccination of susceptible animal in enzootic areas has decrease the prevalence of the disease to negligible proportions on national bases, but heavily losses may still occur in individual herds. Loss occurs due to mortality but also from withholding of milk in infected dairy herds and for a period following vaccination it also causes a great problem of death of animals, reducing animal products and complete condemnations of carcasses and by product as well as shutting down of abattoirs [23].

4. TRANSMISSIONS

4.1. Transmission in animals

The *B. anthracis* naturally remain viable in the soil, but its life cycle almost entirely takes place within the mammalian host [1]. Under the right conditions, spores can live on for years in the environment. Animal hosts get the disease through grazing, usually by ingestion or inhalation [1, 18]. Once enters in to the host, the spores germinate into the active form of the bacterium and begin to multiply rapidly inside the animal. The bacilli are then able to spread through the host system using toxin and capsule production as outlined above. Once the infection becomes systemic, the host dies from shock and hemorrhages blood and other bodily liquids. After death, the vegetative cells are exposed to air causing sporulation to occur and the spores return to the soil for the next host [1, 12, 13, 15, 18].

Animal meat, hides, hair, wool or bones may be transported long distance and spores can be carried with wind currents to new areas, especially during dry period. Insects are also thought to play a role in disease transmission, primary through blow flies. Animals that have died or are dying from anthrax provide the main source of infection for other animals through shedding of bacilli, which eventually become spores, into the environment [15].

4.2. Transmission in humans

In humans, anthrax transmitted in three major routes (ingestion, inhalation and cutaneous). The most common form of disease is cutaneous which accounts for 95% of human cases [18, 24]. This form is acquired through abrasions or open wounds on the skin that come into contact with either vegetative cells or spores. Cutaneous anthrax has a mortality rate of 5-20% for untreated cases [18]. Ingestion of anthrax develops after ingestion of spores or cells through contaminated meat. While uncommon, the mortality rate is thought to be much higher than cutaneous at 25-60%. The most fatal type of infection with anthrax is through inhalation which occurs from breathing in spores from environment [18].

Injection anthrax is becoming more common among intravenous (IV) drug user, which is characterized by cutaneous infection of soft tissue [25, 26]. This route presents differently than cutaneous infections, and has shown to be harder to treat, with infection leading to septic shock, meningitis and death in 34% patients despite treatment [18]. Biting insects are also thought to transmit disease, most prominently biting flies [2, 20]. Anthrax is not considered contagious, although in rare instances, human-to-human transmission can occur in the cutaneous form [19].

5. CLINICAL PROGRESSIONS

5.1. Clinical progression in animals

In animals, the incubation period can range from as little as 36-72 hours following the entry of bacteria or spore to 1-14 days [15]. The common incubation period in livestock is 3-7 days. According to the OIE international trade regulation the incubation period is considered to be 20 days. Clinical manifestations differ from species to species, presumably reflecting differences in susceptibility. Sudden death, bloody discharges from natural orifices (rectum, mouth, nostrils, etc.) rapid bloating of the carcass, absence or incomplete rigor mortis and the absence of clotting of the blood are the common characteristics of anthrax in susceptible animals. In more resistant species, local signs such as swellings of the oral and pharyngeal region are seen. In wildlife, sudden death is the invariable sign, often (but not always) oozing of blood from natural orifices, bloating, incomplete rigor mortis, dark blood and fail to clot [15].

Horses have hyperacute to acute disease with signs for 2-3 days before death. Frequently shows fever, severe colic, tremors, anorexia, depression, weakness, bloody diarrhea, and subcutaneous edema may be present on the neck, sternum, lower abdomen, and external genitalia. Death usually occurs within 2-3 days of onset. Sometimes, sick horses may live up to a week. Pigs are relatively resistant and infection is often subclinical. Clinical symptoms show localized swelling of pharynx, face and neck extending to chest and carnivores are more resistant and s typically have severe inflammatory edema of oropharynx and head, and acute gastroenteritis if clinically affected. Scavengers are relatively resistant [15].

5.2. Clinical progression in humans

Cutaneous anthrax presents in as little as 12 hours to as long as 19 days after initial infection [12], but is typically 1-12 days [18, 27]. A small, painless skin lesion with swelling usually appears on exposed regions of the body such as the face, neck, arms or hands [12, 18, 19]. The lesion eventually dries and forms a black center, called an eschar which sloughs in 2-3 weeks [12, 18]. During this period, fever can occur but is rare. It should be noted that toxin can be detected in the bloodstream even when systemic disease is not present [28].

Gastrointestinal of anthrax has an incubation period of about 2-5 days and can present in two different ways: Intestinal and oropharyngeal [12, 18]. During anthrax, vegetative cells cause ulcers or lesions throughout the small intestine [18]. Symptoms include nausea, vomiting, anorexia and fever [19]. In severe cases, abdominal pain, hematemesis, bloody diarrhea and septicemia can present. Oropharyngeal anthrax occurs when vegetative cells settle in the pharyngeal area and produce ulcers. Patients usually present with fever, neck swelling and sore throat [12, 19].

Inhalation of anthrax (also known as pulmonary) has the highest mortality and has historically been linked to industrial cases of disease, although bioterrorism has become the greatest concern in large-scale outbreaks [18, 24]. Inhalation cases take a biphasic clinical course and patients develop flu-like symptoms with fever, cough and myalgias after approximately four days [18].

6. PATHOGENESIS

The major virulence factors of *B. anthracis* are encoded on two virulence plasmids. The plasmids are circular, extrachromosomal, double-stranded DNA molecule [29]. Infection occurs after contact of spore through a break in the skin (cutaneous anthrax) or entry through mucosa (gastrointestinal anthrax). After ingestion by macrophages at the site of entry, within hours after uptake spores germinate and vegetative encapsulated bacilli proliferate, together with the production of capsule and toxins. The capsule plays an

important role in establishment of the infection while the toxins are more prominent in the final stages of infection [13].

6.1. Toxins

Genes encoding toxins (pX01) includes genes for both lethal toxin and edema toxin while pX02 includes genes for production and assembly of the capsule [1, 12, 13, 18]. The toxin complex is composed of three proteins: lethal factor (*LF*), edema factor (*EF)* and protective antigen (*PA*). *PA* is an intra member transporter that mediates cell binding and uptake of *LF* and *EF* [18]. Protective antigen (*PA*) in combination with *EF* and *LF* forms edema toxin and lethal toxin, respectively [1].

Edema toxin alters the production of cyclic-AMP (Adenosine monophosphate) which creates altered ions and water movements. This leads to the characteristic edema of anthrax. It is also thought to impair neutrophil function and prevent the inflammatory process [2]. Lethal toxin is an endopeptidese that disrupts signaling pathways and leads to the synthesis of cytokines that ultimately cause septic shock. It is also thought that lethal toxin may attack the endothelial cell linings of the capillary network that results in the necrosis of blood vessels. This leads to the characteristic hemorrhage from the nose, mouth and anus of infected hosts and the systematic release of bacilli into the environment, competing its life cycle [2].

6.2. Capsule

The capsule is a polymer that encases the bacterium. The capsule of *B. anthracis* is unique in that it is made from the protein, poly-glutamic acid, and not carbohydrate. It is formed under elevated CO_2 condition and in the presence of bicarbonate. The capsule allows virulent bacilli to grow unimpeded in the host for the initial stages of infection. It is theorized that the negative charge of the capsule inhibits host defense mechanisms, namely phagocytosis, allowing the bacteria to establish infection [30-32]. The plasmid pXO2 (95.3 kbp) is the smaller capsule that encodes three genes (*cap B, cap C, and cap A*) and involved in the synthesis of the poly-glutamyl capsule that inhibits host phagocytosis of the vegetative form of *B. anthracis* [31].

6.3. Spore and vegetative cell survival

Vegetative cells in unopened carcasses may survive for up to 1 to 2 weeks, but spores can persist for decades in a stable, dry environment. Spores are killed by autoclaving (121^{o}C/15 min) and dry heat (150^{o}C/60 min), but not by boiling (100^{o}C) for under 10 minutes. They are not highly susceptible to phenolic, alcoholic, oxidizing and chlorinating disinfectants, beta-propiolactone, and ethylene oxide are more useful. Heat fixation of smears does not kill spores [33].

7. DIAGNOSTICS

As various outbreaks are reported time to time from different areas, there is a great need of early diagnosis of the disease to save human and animal life. Besides, requirement of rapid and reliable detection, identification and diagnosis systems for anthrax has been emphasized by recent bioterrorism events. The early monitoring of the disease requires the detection of anthrax spores and infection both at environmental and clinical levels [34].

7.1. Culture and Gram staining

Bacterial culture and isolation are considered the gold standard and most important diagnostic tool for identification of *B. anthracis* and easy to grow on nutrient agar medium [35]. Specimen or culture can be

grown overnight on Sheep Blood agar at 35-37^{o}C. However, on sheep Blood agar (5%) and other routine culture media, almost all *Bacillus* species grow well [36]. After incubation for 18-24 hours, growth occurs on blood agar and shows the characteristic morphology of grey/white, flat colonies, 2-5 mm in diameter, flat or slightly raised, gray to white with a "ground glass" appearance and described as "tenacious" or "sticky" like petroleum jelly. After 18 hours of incubation on sheep blood agar (SBA) at 35°C, the slightly undulate margin may show curling, displaying a so-called "Medusa head" or described as comma-shaped protrusions [19]. Colonies should be observed for hemolysis (the rupture of red blood cells resulting in clearing of the medium) after incubation and a Gram stain should also be performed. *B. anthracis* will be negative for hemolysis and appear as purple rods after Gram stain [37].

Figure 2. Colony morphology of *B. anthracis* on Sheep Blood agar, photo taken in June 1, 2019 during anthrax outbreak investigation at the National Animal Health Diagnosis and Investigation Center, Ethiopia.

A selective media containing polymyxin-B, lysozyme, EDTA and thallous acetate (PLET media) can also be used for isolation of *B. anthracis* from contaminated and suspected samples [38]. It consists of heart infusion agar with polymyxin, lysozyme, ethylene diamine tetraacetic acid (EDTA) and thallous acetate (pH 7.35). Commercial readymade PLET agar base is also available to be used with Anthracis Selective Supplement (FD185) [39]. Another media (bicarbonate agar) is used to induce capsule formation for subsequent identification of *B. anthracis*. However, there is very little utility of these selective growth media because several closely related bacteria of *B. anthracis* like *B. cereus* and *B. subtilis* also grow well on these media.

7.2. Biochemical tests

Bacillus anthracis is highly susceptible to penicillin and *B. cereus* and the other spp are resistant. *B. anthracis* slowly produces an inverted fir tree type of gelatin liquefaction with side-shoots radiating from the stab line but *B. cereus*, *B. mycoides*, *B. thuringiensis* rapidly liquefy nutrient gelatin and non-motile (Table 1). *B. anthracis* characterized by various biochemical tests like catalase, oxidase, nitrate reduction, haemolysis, citrate utilization, urease [40].

Table 1. Differential characteristics of *B. anthracis* and *B. cereus.*

	B. anthracis	***B. cereus***
Hemolysis	-	+
Motility	-	+
Lysis by gamma phage	+	-
Capsule production	+	-
Penicillin susceptibility (10 unit disc)	S	R

+: positive reaction; -: negative reaction; S - Susceptible; R - Resistant; Quinn et al. [7].

7.3. Capsule production

Samples can also be tested for capsule production by incubation on certain types of agar followed by staining. Single colonies from Sheep Blood agar can be inoculated on to three different types of media. The first is Heart infusion broth (HIB) with 0.8% sodium bicarbonate. The broth should be incubated at 35-37^{o}c for 6-8 hours. Incubation can happen at an ambient atmosphere in a Co_2 enriched environment. The characteristic mucoid or smooth colony variant is correlated with capsule production ability of *B. anthracis* on capsule agar incubated in an atmosphere of 5% CO_2. The second medium is defibrinated horse blood. This should be incubated at 35-37^{o}C for 6-8 hours in an ambient atmosphere. The third medium is HIB supplemented with heat-inactivated horse serum and 0.8% sodium bicarbonate. This broth should be incubated at 35-37^{o}C for 6-8 hours in an ambient atmosphere or in a CO_2-enriched environment [15].

M'Fadyean (Polychrome methylene blue) stain is a simple stain containing methylene blue that is applied to fixed smears to visualize capsule. After staining, bacilli will appear dark blue with a narrow area around and between that is red/purple. A sample of culture should be smeared on a microscope slide and allowed to dry and then be heat-fixed. Stain is added to the smear and rinsed. The slide can be viewed at 40x or 100x with oil immersion [41]. M'Fadyean-stained blood smears examined at death will reveal large numbers of the capsulated bacilli which can also be isolated and confirmed bacteriologically [15].

7.4. Gamma-phage lysis

Gamma-phage is a bacterial virus that specifically lyses *B. anthracis* with 96% specificity. Most other strains of the *B. cereus* groups are not susceptible to lysis by Gamma-phage. A single colony is spread onto Sheep Blood agar plate, and small amount of gamma phage is aliquoted onto the first or second quadrant. If the test organism is susceptible to the phage, a clear zone of lysis will be present after overnight incubation at 35-37^{o}C. While gamma-phage lysis is a simple test to perform, proper propagation of active gamma-phage is essential. Unstable phage preparation can lead to false-negative results [15].

7.5. Serological tests

7.5.1. Enzyme-Linked Immunosorbent Assay (ELISA)

For serodiagnosis of cutaneous anthrax, an enzyme-linked immunosorbent assay was developed in India for determination of anti-PA IgGs with 99.4% specificity and 100% sensitivity [42]. A field-based qualitative visual ELISA for anti-PA IgG was also developed for serodiagnosis of anthrax [43]. Results of sensitivity and specificity of visual ELISA were found compatible with the results obtained from standard ELISA measuring OD values. Likewise, a quantitative ELISA was developed for measurement of the anti-PA IgG level in human serum samples [44]. The minimum detection limits and lower limits of quantification of

the assay for anti-PA IgG were 3.2 μg/mL and 4 μg/mL, respectively. The serum samples collected from the anthrax infected patients were found to have anti-PA IgG concentrations of 5.2 to 166 μg/mL [44].

7.5.2. Ascoli test

This test is to supply rapid retrospective evidence of anthrax infection in an animal. It was designed to detect *B. anthracis* antigens in the tissues of animals being utilized in animal by-products, and thereby to reveal when these products contained ingredients originating from animals that had died of anthrax [15]. This thermo-precipitation test is used if viable *B. anthracis* can no longer be demonstrated in tissues. About 2-3 g of homogenized materials in a little saline is briefly boiled and passed through filter paper. This filtrate is used as the antigen in a ring precipitation or gel diffusion test with known *B. anthracis* precipitating antiserum and the test is not suitable for detection of *B. anthracis* in environmental specimens [7].

7.6. MALDI-TOF mass spectrometry

Matrix Assisted Laser desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) can be utilized to detect lethal factor in serum samples of patients in the acute stage of illness. MALDI-TOF MS is a molecular technique used to separate protein or peptides of samples, seen as peaks on a spectrum. By analyzing serum samples mixed with a peptide that can be cleaved by lethal factor (LF), the presence of LF in the sample can be determined. If it is not present, a single protein peak will be seen after MALDI-TOF MS analysis while if LF is present, two distinct peaks will be seen as a result of the cleavage. This is only applicable to acute samples, as LF is circulating in the blood at that time [45].

7.7. Molecular diagnostics

Over recent years there have been several reports describing the use of molecular techniques for genotyping and distinguishing/ identification *B. anthracis* [46, 47], such as Multi Locus Sequence Typing (MLST) [48, 49], Multi Locus VNTR Analysis (MLVA) [50, 51], Single Nucleotide Repeat (SNR) Analysis [52], and real-time PCR Assays [49, 53-58]. The main targets to demonstrate virulence are the plasmids pXO1 and pXO2, encoding the toxin and capsule genes, respectively. Isolates lacking either one or both plasmids (pXO1_/ pXO2_) have been described. Only in conjunction with specific chromosomal markers insight into the backbone or genetic background can be gained to understand the pathogenic nature of *B. anthracis*. Due to lack of a specific chromosomal marker, differentiation of the pXO1_/pXO2_ form of *B. anthracis* from closely related *B. cereus* group species is difficult. In addition, naturally occurring as well as genetically modified *B. anthracis* strains cannot be characterized without ambiguity and differentiation of these strains from those B. cereus isolates that carry plasmids harbouring portions *of B. anthracis*-specific plasmids is a challenge [59, 60].

7.8. Differential diagnosis

Anthrax should be differentiated from other causes of sudden death such as: lightning strike and accidental electrocutions, pasteurellosis, piroplasmosis, blackleg, malignant oedema, food intoxications, botulism, peracute babesiosis, chemical poisoning (heavy metal and other poisoning), plant poisoning, snake bite, metabolic disorders (lactic acidosis), magnesium deficiency, bloat and others [61].

8. REPORTED OUTBREAKS OF ANTHRAX IN ETHIOPIA

Anthrax is an endemic disease which occurs in May and June every year (‘anthrax season’) in several

farming localities of the country, causing disease both in domestic and wild animals and humans. It is still a significant risk in most regions in Ethiopia and outbreaks frequently occur in humans and animals. In the country a retrospective record review from 2009-2013 showed that within five years a total of 26737 animal cases with 8523 animal deaths due to anthrax were reported [62]. This data showed that each year on average death of 1705 animals were recorded due to anthrax. Based on the report of Shiferaw [11] 26 cases of anthrax in animals were reported in Wabessa village of Dessie Zuria district, as a result death of 26 animals were recorded from the outbreak.

Moreover, retrospective study on the epidemiology of bovine anthrax in Elu Aba Bor Zone, South West Ethiopia showed that from the period of 2009-2016 within 8 years duration a total of 405 anthrax outbreaks with 1166 case and 739 deaths in cattle were registered (Table 2). This data revealed that each year 50 outbreaks of anthrax occurred in the area. Based on the report the hot dry season accounted for 29.6% of the outbreaks followed by the rainy and cold dry season (24.69%), and the post rainy season recorded the lowest proportion 20.99% [63].

Table 2. Animal and human anthrax cases and deaths reported in different parts of Ethiopia.

Year	Animal		Human		References
	Cases	Death	Cases	Death	
2018	NR*	NR*	38	1	[60]
2009-2016	1166	739	NR*	NR*	[56]
2010–2013	NR*	NR*	8	1	[59]
2009-2013	26737	8523	5197	86	[55]
2011-2012	NR*	NR*	3	0	[58]
2002	26	26	6	3	[12]
Total	27,929	9,288	5,250	91	

Note: NR* means not reported

In wildlife anthrax outbreak has been reported in southern parts of Mago national parks and spread to the northern part within two months beginning from September 1999. Moreover, another anthrax outbreak from September to October 2000 has also been reported in this park. According to the report in the first outbreak more than 1,600 wild animals were dead from 21 different species. Of all the species *Lesser kudu* was severely affected, which accounts for 95% of mortality and as a result more than 65% of *Lesser Kudu's* population within the park died [64].

Bahiru et al. [62] retrospective data showed that, a total of 5,197 human anthrax cases were reported from 2009 to 2013 with 86 human anthrax deaths (Case Fatality Rate: 1.7%) nationally. Human prevalence was found to be 1.3 per 100,000 populations per five years and this report revealed that each year on average 17 people death was recorded due to anthrax in the country. Based on the Shiferaw [11] report, 6 human cases and 3 human deaths were recorded due to anthrax in Dessie Zuria district of Amhara Regional state. As reported by Shiferaw et al. [65], a case series of 3 patients with periocular anthrax that were seen at Jimma University Specialized Hospital, Ethiopia from June 2011 to May 2012 and all the three patients were responded to intravenous antibiotics and the lesion resolved leaving scars which caused cicatricial ectropion.

Cutaneous anthrax cases were admitted to the rural General Hospital of Gambo, West Arsi Province of Ethiopia from 2010-2013 in eight patients (six female and two male, age range 1-56 years) as reported by Pérez-Tanoira et al. [66]. According to Pérez-Tanoira et al. [66] report, one patient suffered the loss of an

eyeball, and another died 12 hours after starting treatment. However, patients responded to treatment, and the lesions resolved, leaving eschars. Physicians working in rural areas of resource-poor settings should be trained in the clinical identification of cutaneous anthrax and early antibiotic treatment is recommended for decreasing morbidity and mortality. Moreover, Ethiopian weekly Epidemiological Bulletin of Ethiopian Public Health Institute reported 38 human cases and 1 death due to anthrax from different part of the country within one week in May 2018 [67].

9. TREATMENT AND PREVENTIONS

The control of anthrax outbreaks among domestic animals is primarily dependent on rapid identification and treatment of affected animals; enhanced surveillance for additional cases; implementation of control measures including quarantine, prophylaxis, and vaccination; prevention of animal access to suspected sources such as potentially contaminated feed or pastures; and appropriate disposal of infected carcasses and disinfection of affected premises.

The recommended procedure for treating animals showing clinical illness in which anthrax is thought to be the likely or possible cause is immediate intravenous administration of sodium benzylpenicillin as directed by the manufacturer's instructions (usually in the range 12 000–22 000 units per kg of body weight) followed 6-8 hours later by intramuscular injection of long-acting benzathine penicillin (manufacturers' instructions usually recommend a dose within the range of 6000–12 000 units per kg of body weight) or other appropriate preparation such as Clamoxyl® (15 mg/kg), a long-acting preparation of amoxicillin [15].

If long-acting preparations are unavailable, procaine penicillin (the dose recommended by manufacturers is usually 6000–12 000 units/kg) can be used for intramuscular injection, but should be administered again after 24 and 48 hours. Recommended doses of streptomycin to be administered together with penicillin intramuscularly are 5-10 mg per kg body weight in large animals and 25-100 mg per kg body weight in small animals [15].

Bacillus anthracis is susceptible to numerous antibiotics but treatment must begin early enough in infection to be successful. Penicillin and ciprofloxacin are considered the drug of choice although tetracycline, chloramphenicol, aminoglycosides, macrolides, imipenem, rifampicin and vancomycin can be used commonly in human [12, 13, 19]. Antibiotic treatment usually continues for 7-10 days although it can be extended in severe cases [12]. Along with antibiotics treatment, supportive care may be required as fluid drainage, blood pressure support and mechanical assistance with breathing [15].

Vaccination of host animals particularly cattle, sheep and horses, remains the gold standard for prevention of anthrax among both animals and people [2]. The veterinary anthrax vaccine is a live strain that contains the pXO1 plasmid but is missing the pXO2, meaning it is toxinogenic but non-encapsulated [1].

10. CONCLUSION AND RECOMMENDATIONS

Anthrax is an infectious disease caused by the bacteria *B. anthracis* and it affects both animals and humans. Generally, the disease causes a great problem by death of animals, prevent utilization of animal products and complete condemnation of carcasses and by-products as well as closure of abattoirs. The causative organism is sensitive to many antimicrobial agents. This review indicated that anthrax remains to be a major public and veterinary health problem in Ethiopia. Prevention and control of anthrax in animals effectively reduces its impact on public health and the national economy. A multi-sectoral collaborative approach and capacity building are essential for successful prevention and control of anthrax.

As the disease is endemic in Ethiopia and mostly under-reported, the following recommendations are forwarded:

- Strengthening diagnostic capacity of both veterinary and public health laboratories is very important for timely diagnosis, treatment, prevention, control and reporting of the disease.
- The regional laboratories should have been trained on biosafety, sample collection and diagnosis of anthrax suspected cases and investigation of any sudden or unexpected death in livestock.
- Active surveillance, proper animal immunization and awareness creation is important to curb the disease.
- Reported anthrax cases should be classified as suspected, probable and confirmed as per the WHO recommended case definition.
- Proper decontamination of the site where the dead animal was founds.

Authors' Contributions: Collected data and information from different publications and institutes: AO. Analyzed data, prepared the manuscript and reviewed the manuscript: AO, FD, ML. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare

Acknowledgment: The authors would like to acknowledge Ministry of Health and Ministry of Agriculture and National institutes under these Ministries for the provision of passive surveillance data about anthrax outbreak in country.

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