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Recent Understanding of the Epidemiology of Animal and Human Anthrax in Ethiopia with Emphasis on Diagnosis, Control and Prevention Interventions-Review

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Abstract: Anthrax is a soil borne bacterial disease caused by Bacillus *anthracis,* facultative anaerobic bacterium about 1 by 9 µm in size. Anthrax is virtually a disease of all warm-blooded animals, including humans. The organism form spores that help persisting in the environments for long period of time. The capsule and the toxin complex are the two known virulence factors of *B. anthracis*. In animals and humans, anthrax is particularly common in parts of Africa, Asia and the Middle East where control measures in animals are inadequate. The most important sources of infection for anthrax are sick animals that expel the organism together with feces, urine and different discharges. Outbreaks of the disease is usually associated with flooding or soil disturbance that lead to exposing the spore to the ground. Sudden death, bleeding of non-clotting blood from orifices, subcutaneous hemorrhage, without prior symptoms or following a brief period of fever and disorientation are among clinical signs of anthrax. The most important factor for transmission of anthrax is the carcass of dead animals as it contains large amount of B. *anthracis* organism. Diagnosis of anthrax is based on the case history, epidemiology of the disease, clinical signs and laboratory examination. Anthrax can be treated by commonly available antibiotics. Rapid identification and treatment of affected animals and humans, vaccination, quarantine, disinfection of premises and disposal of infected materials are recommended control and prevention measures of anthrax.

Key words: Anthrax · Transmission · Diagnosis · Control · Prevention · Animal · Human

INTRODUCTION

Anthrax is a soil borne bacterial disease caused by *Bacillus anthracis*. *Bacillus anthracis* is a spore forming, Gram-positive, rod-shaped and facultative anaerobic bacterium about 1 by 9 µm in size [1].

Anthrax is virtually a disease of all warm-blooded animals, including humans. The name 'anthrax' is derived from the Greekword,' anthrakos', meaning coal, referring to the characteristic eschar in the human cutaneous form of the disease. It has different names in different areas and named as splenic fever, wool sorter disease, Siberian ulcer, Charbon, Milzbrand [2].

Anthrax is found all over the world on all continents except Antarctica. But it is common in parts of Africa, Asia and the Middle East where control measures in animals are inadequate [3]. Soil contaminated by sick animals that expel the organism together with feces, urine and different discharges are important source of the infection [4].

The most important factor for transmission of anthrax is the carcass of dead animals as it contains large amount of *B. anthracis* organism [5]. The disease occurs in all vertebrates but is most common in cattle and sheep and less frequently in goats and horses. Humans occupy an intermediate position between this group and pigs, dogs and cats are relatively resistant [6]. The capsule and the toxin complex are the two known virulence factors of *B. anthraces* organism [7].

Diagnosis of anthrax is based on the case history, epidemiology of the disease, clinical signs and laboratory examination [8]. The disease can be treated by commonly available antibiotics [9].

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Rapid identification and treatment of affected animals and humans, vaccination, quarantine, disinfection of premises and disposal of infected materials are commonly recommended control and prevention measures of anthrax [2].

Although both the human and animal anthrax is endemic in Ethiopia, very few articles are available on the epidemiology, diagnosis and control and prevention of the disease [10]. Therefore, the objectives of this review were:

- To highlight the epidemiology of anthrax with emphasis on Ethiopia
- To highlight the status of the diseases in Ethiopia and
- To review the current diagnosis and control and prevention interventions of the disease.

Epidemiology

Occurrence and Distribution: WHO has estimated that there are between 2,000 and 20,000 human cases of anthrax per year. The actual number of anthrax cases worldwide is difficult to determine owing to poor reporting [11]. Anthrax outbreaks in animals are more prominent and common than humans [12]. According to Inter African Bureau for Animal Resources [13], 2013 in 2011, 21 member states reported animal anthrax outbreaks to AU-IBAR recording a total of 629 outbreaks, 5655 cases and 1735 deaths. The highest number of outbreaks were reported by Ethiopia (452), followed by Somalia (44) and South Africa (25). The highest number of deaths was also recorded by Ethiopia (1102), followed by Zimbabwe (119), Guinea Bissau (109) and Cote d'Ivoire (103).

The disease is found all over the world on all continents except Antarctica. The disease probably originated in sub Saharan Africa and has spread to have a worldwide distribution. There are endemic areas with more frequent outbreaks, other areas are subject to sporadic outbreaks. This area in tropics and sub tropics where there is a continuous epidemic (epizootics) of anthrax is called incubator area of B. *anthracis* [14].

In domesticated animals and people, anthrax is particularly common in parts of Africa, Asia and the Middle East where control measures in animals are inadequate. It also occurs in South and Central America. This disease is infrequently reported in North America and Europe. In Europe, it is mostly seen in the south, while cases in North America currently occur in limited foci in western and Midwestern U.S. states and in parts of Canada [3].

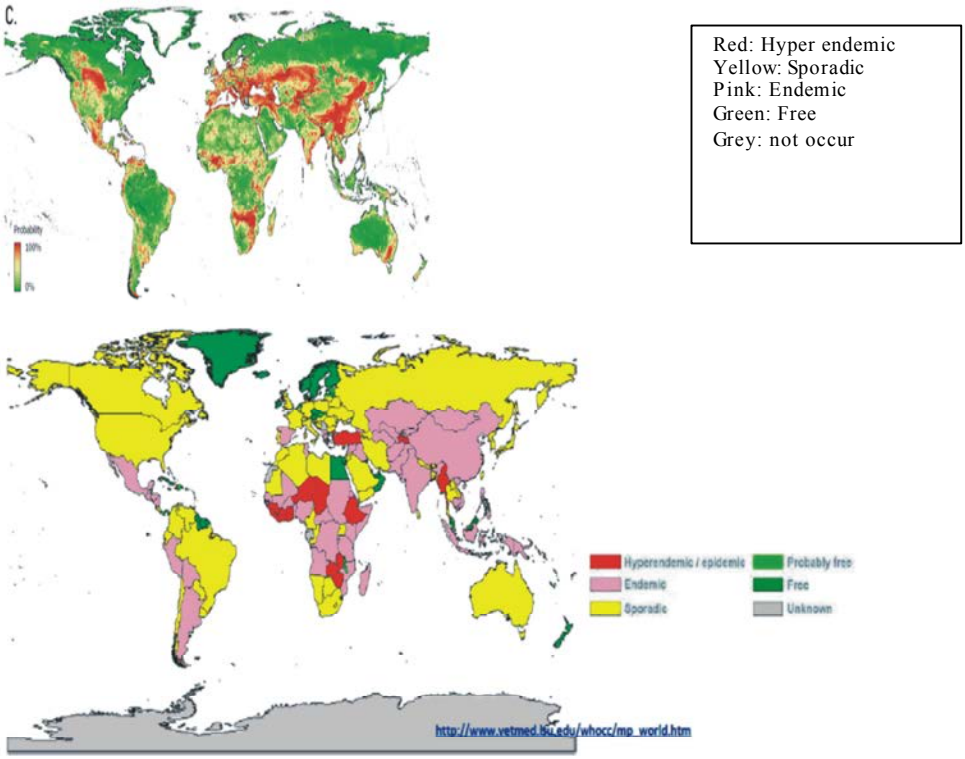


Fig. 1: Global environmental suitability for *Bacillus anthracis* (left) and temporal occurence of anthrax in the world (right). Source: [15].

Fig. 2: Contact with infected animal during shearing(left) and animal feeding contaminated pasture(right). Source: [16].

Source of Infection: Sources of infection for anthrax are sick animals that expel the organism together with feces, urine and different discharges. The organism may also be expel from sick animals together with different exudates (through necrotized carbuncle) and together with black tarry blood through natural orifices. The organism can also expel from sick animal with haematophages (as mechanical vectors) [4]. Contaminated soil with spores of B. *anthraces* is common source of infection for animals. That is why anthrax is one of soil borne diseases of animals [1]. The major sources of human anthrax infection are direct or indirect contact with infected animals, or occupational exposure to infected or contaminated animal products [2].

Mechanism of Transmission: The most dangerous factor for transmission of anthrax is the carcass of dead animals as it contains large amount of *B. anthracis* organism. *B. anthracis* can infect animals directly from the soil or fodder grown on infected soil, contaminated bone meal or protein concentrates, infected excreta, blood, or other discharges from infected animals [1]. Biting flies, mosquitoes, ticks and other insects have often been found to harbor anthrax organisms (mechanical vectors). The most important disseminator of *B. anthracis* is scavengers like vulture birds, street dogs and different wild carnivores [9].

The disease typically does not spread from animal to animal or from person to person. If spores are ingested or inhaled by an animal, or on entering through cuts in the skin, they can germinate and cause disease [17]. Carnivores and humans are mostly infected by eating meat from an infected animal. Person-to-person transmission is rare and only reported for cutaneous anthrax, where direct contact with skin lesions is required for infection [18].

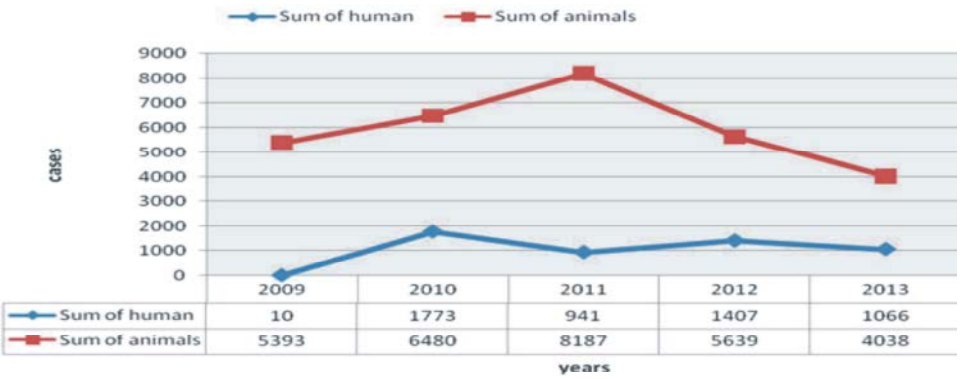
Risk Factors

Host Factor: The disease occurs in all vertebrates but is most common in cattle and sheep and less frequently in goats and horses. Humans occupy an intermediate position between this group and pigs, dogs and cats are relatively resistant [1]. In farm animal the disease is almost invariably fatal, except in pig and even in this species the case fatality rate is high [18]. The disease has also been reported in water buffalo, camels and South American camelids. Outbreaks have been reported in mink and wild species in zoos, as well as in free-living wildlife. Birds appear to be highly resistant, although a few clinical cases have been seen. Species that were affected included ostriches, poultry, eagles and pigeons [13].

Agent Factor: The capsule and the toxin complex are the two known virulence factors of *B. anthracis*. The poly. *D. glutamic* acid capsule is presumed to act by protecting the bacterium from phagocytosis.

The toxin complex, which consists of three synergistically acting proteins, Protective Antigen, Lethal Factor and Oedema Factor is produced during the log phase of growth of *B. anthracis*. These three components act synergistically to produce the toxic effect seen in bacillus *anthraces* [1]. The other pathogen factors of the disease are forming spores. When material containing anthrax bacilli is exposed to the air, spores are formed that protract the infectivity of the environment for very long periods, the spores are resistant to most external influences including the salting of hides, normal environmental temperature and standard disinfectant [19].

Environmental Factor: There is little dispute that anthrax is a seasonal disease; its incidence in any one place is related to temperature, rains or drought; however, examination of the literature shows that the conditions



	2009	2010	2011	2012	2013
Sum of human	10	1773	941	1407	1066
Sum of animals	5393	6480	8187	5639	4038

Fig. 4: Human and animal anthrax cases by year (2009-2013). Source: [10].

which predispose to outbreaks differ widely from location to location [20]. Climate probably acts directly or indirectly by influencing the way in which an animal comes into contact with the spores (for example, grazing closer to the soil in dry periods when grass is short or sparse, or movement of herds to restricted sites when water becomes scarce), or by affecting the general state of health of the hosts and thereby affecting their level of resistance to infection [14]. In tropical and sub-tropical climate with high annual rain falls, the infection persists in the soil, so that frequent, serious outbreak of anthrax are commonly encountered. Animal anthrax is an endemic disease in Ethiopia which occurs in May and June every year (anthrax season) in several farming localities of the country [21]. In temperate, cool climate only sporadic out break drive from the soil borne infection. Temperature, humidity, water activity, pH, oxygen availability, sunlight and the presence of certain cations, particularly Mn++ and Ca, are all influencing factors. Temperatures between about 8 and 45°C, pH between 5 and 9, a relative humidity greater than 96% are among the optimum condition for the survival of the organism [22].

Status of the Disease in Ethiopia: Although anthrax is endemic in most species of domestic animals and also cases have been commonly reported in humans in Ethiopia, very few studies are officially confirmed [10]. In last year, from January to June 2018, Ethiopia has reported 40 outbreaks in different species of animals with 1222 cases [23]. According to the Federal Democratic Republic of Ethiopia, Ministry of Health surveillance data, In the Ethiopian fiscal year 2003, a total of 1,096 suspected human anthrax cases and 16 deaths with a Case Fatality Rate (CFR) of 1.5% were reported from four regions (Tigray, Amhara, Oromia and SNNPR). A total of 5197 and 26737 cases and 86 and 8523 deaths of human and animal anthrax respectively were documented in the years 2009-2013 nationally [10].

According to Seboxa and Golden [24] twenty-seven patients with cutaneous anthrax were identified over a three-year period at Gondar College of Medical Sciences in North Central Ethiopia. According to Menghistu *et al.* [25], a total of 15 anthrax cases were reported in different animal species in South Tigray, North Wollo and Ab'ala (Afar), Ethiopia areas from 2012-2016. Out of total recorded cases, 11 cases were observed in bovine and 2 cases each were reported in goats and camel. Shiferaw [26] has reported a total of 82 anthrax cases in different animal species (13 cattle, 17 donkeys and 52 goats) in Wabessa village in Dessie zuria district of Ethiopia in the year 2002.

According to Mebratu *et al.* [27] retrospective data results from in and around Tanqua-Abergelle district, Northern Ethiopia have indicated that a total of 504 anthrax cases were registered in cattle in the veterinary clinics and 2,680 human anthrax cases were recorded in the human hospital/clinics from the year 2008 to 2012.

Agro-ecologically, 72.0 and 65.2% of the cattle and human anthrax cases, respectively, were originated from lowland areas of the district. Seasonally speaking, the disease was observed to commonly occur in the spring (52%) than the rest seasons indicating the significance of the hot environment in the spring and lowland area for the formation of *Bacillus* spores and its proliferation [27].

In Elu Ababor, Western Ethiopia, During the year 2009-2016, retrospective data results revealed that a total of 405(0.028%), 1166(.083%) and 739(.05%) anthrax outbreak, cases and deaths in cattle were respectively registered in the Veterinary Epidemiology department at zone level and veterinary clinics in each Woreda. 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 (20.99%) [21].

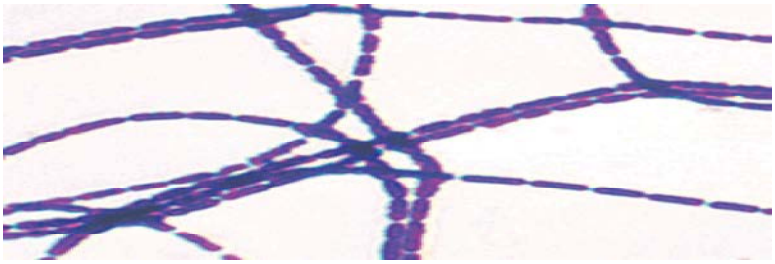


Fig. 5: Gram-positive rods, square-ended, chains of Bacillus *anthraces* on Gram staining. Source: [28].

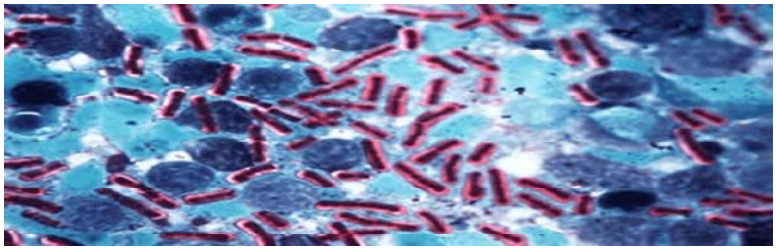


Fig. 6: B. *anthraces* in polychrome methylene blue staining. Source: [30].

Diagnosis: Tentative diagnosis of anthrax is based on the case history, epidemiology of the disease, clinical signs and necropsy findings (this should be done with precautions). While laboratory diagnosis gives the confirmatory diagnose of the disease. In epidemiological diagnose of the disease, species of animals that are affected in the area, sudden death of animals in pasture in endemic areas after agricultural activities and flooding should be considered. In clinical signs, per acute and acute nature of the disease in most cases with septicemia and/or carbuncle and angina on affected animals should be considered [8].

In animals, rapid diagnostic methods require testing the carcass while it is still fresh. Once the carcass is putrefied or scavenged, culture is required to isolate *B. anthraces*. Samples should be collected without opening the carcass. Specimens can include blood, tissue, exudates, other fluids and nasal turbinates [1].

In humans Specimens can include blood, skin lesion exudates, pleural or ascitic fluid, cerebrospinal fluid, or stool. Specimens should always be collected prior to antibiotic therapy. Culture and Gram stains will likely be negative if specimens are collected after antibiotic therapy has been initiated, regardless of the form of disease. The likelihood that antigen or molecular testing methods will be positively decreased with the length of antibiotic treatment prior to sample collection [28]. All samples (except sera for serology) must be collected prior to the initiation of antibiotics [29]. Available laboratory diagnostic methods for Anthrax are briefly explained below. Zoonotic importance of the disease must be recognized when dealing with the organism and pro [1].

Gram Staining: Gram staining of Bacillus *anthraces* reveals Gram-positive rods, square-ended, in pairs or short chains. As shown in figure below.

However, a Gram's stain may not reveal capsule and could result in false negative diagnosis if the carcass is not fresh (after 24 hours of death) or putrified. In addition, it may not be possible to find bacilli in smears or to isolate *B. anthracis* from animals that were treated with antibiotics before death [28].

Polychrome Methylene Blue Stain (McFadden Stain): This is the ideal method for demonstration of the capsule of Bacillus *anthraces* from the sample. The capsule looks pink amorphous material surrounding the blue-black bacilli and rods are in pairs or short chains, sometimes as single rods. A positive and negative control should be included with every test [30].

Direct Antigen Detection: Ascoli precipitin test (thermostable antigen test) is the very old test dating from 1911**.** It 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. Over the years, it has been one of the most valuable tools for controlling

anthrax in most European countries. The test is not suitable for detection of *B. anthracis* in environmental specimens; numerous other *Bacillus* species can be expected to occur in these [28].

Currently immunochromatographic method become widely available as the replacements for **t**he ascoli test. Updated immunochromatographic device described by Levine, Tang and Pei [30] utilizes a monoclonal capture antibody to the anthrax protective antigen (PA) bound to a nitrocellulose membrane and a second monoclonal antibody specific for a different epitope of PA bound to colloidal gold particles which become visible when they accumulate at the capture sites.

The assay can detect as little as 25 ng/ml of PA and is performed in a few minutes without the need for special reagents.

It therefore lends itself to direct diagnosis of cases of anthrax by detection of PA in the blood or body fluids, or to retrospective analysis of extracts from the types of sample of animal origin for which the Ascoli test was designed. As such, it has excellent specificity and sensitivity, but it has not become commercially available [2].

Serology and Delayed Type Hypersensitivity Testing: Effective serological enzyme immunoassays for confirmation of the diagnosis of anthrax have been designed and proved to be useful diagnostic, epidemiological and research aids. Currently accepted as the best serological procedure is the ELISA in microtitre plates coated with the Protective Antigen (PA) and Lethal Factor (LF) components of the anthrax toxin. The toxin antigens appear to be truly specific for *B. anthracis*. PA and LF are available commercially but are costly. This tends to mean that anthrax serology is currently confined to a few specialist laboratories [2].

The Anthrax in T delayed type hypersensitivity test is also available in reference laboratories and it involves intradermal injection of 0.1 ml of AnthraxinT. A positive test is defined as erythema of ≥ 8 mm with induration persisting for 48 hours [28].

Culture: Culturing on nutrient agar is the gold standard diagnostic method for identification of bacillus anthracis. *Bacillus anthracis* grows readily on most types of nutrient agar, however, 5-7% horse or sheep blood agar is the diagnostic medium of choice. Blood is the primary clinical material to examine. Swabs of blood, other body fluids or swabs taken from incisions in tissues or organs can be spread over blood agar plates. After overnight incubation at 37°C, *B. anthracis* colonies are grey-white to white, 0.3-0.5 cm in diameter, non-hemolytic, with a ground-glass surface and very tacky when teased with an inoculating loop. Tailing and prominent wisps of growth trailing back toward the parent colony, all in the same direction, are sometimes seen [31].

Confirmation of *B. anthracis* should be accomplished by the demonstration of a capsulated, spore-forming, Gram-positive rod in blood culture. Absence of motility is an additional test that can be done.

Anthrax-specific phages were first isolated in the 1950s and the specifically named gamma phage was first reported in 1955 and quickly became the standard diagnostic phage for anthrax. Gamma phage belongs to a family of closely related anthrax phages [28].

Two tests for confirming the identity of *B. anthracis* are gamma phage lysis and penicillin susceptibility. The typical procedure for these tests is to plate a lawn of suspect *B. anthracis* on a blood or nutrient agar plate and place a 10-15 µl drop of the phage suspension on one side of the lawn and a 10-unit penicillin disk to the other side. Allow the drop of phage suspension to soak into the agar before incubating the plate at 37°C. A control culture, e.g. the Sterne vaccine or the NCTC strain 10340, should be tested at the same time as the suspect culture to demonstrate the expected reaction for gamma phage lysis and penicillin susceptibility [32]. If the suspect culture is *B. anthracis*, the area under the phage will be devoid of bacterial growth, because of lysis and a clear zone will be seen around the penicillin disk indicating antibiotic susceptibility. Note that some field isolates of *B. anthracis* may be phage resistant or penicillin resistant. As the performance of the gamma phage lysis assay may be affected by the density of bacterial inoculum, [33] recommend streaking the suspect culture on the agar plate over several quadrants instead of using a lawn format and inoculating a drop of gamma phage on the first and second quadrants on the plate. If antibiotic or phage resistant *B. anthracis* is suspected then polymerase chain reaction (PCR) diagnostic methods may be applied.

Molecular Method (PCR): PCR is becoming more widely available means of confirming the presence of the virulence factor (capsule and toxin) genes. For routine purposes, primers to one of the toxin genes (usually the Protective Antigen gene) and to one of the enzymes mediating capsule formation are adequate [34]. Confirmation of virulence can be carried out using the PCR. Real-time PCR assays have been developed for enhanced speed, sensitivity and specificity of detection of pXO1, pXO2 and chromosomal genes of *Bacillus anthraces* [35].

Treatment: Bacillus anthraces are susceptible to penicillin, chloramphenicol, streptomycin, tetracycline and erythromycin. Treatment should continue for at least five days. However, in acute anthrax, antimicrobial treatment is often useless. Treatment initiated 24 hours after infection with any antibiotics protected the animals during treatment, but many of the animals died of anthrax after treatment was stopped, the antibiotics conferring degrees of protection ranging from 10-90 percent. Animals whose treatment was delayed beyond 24 hours post-infection developed varying degrees of bacteremia and toxemia [6].

Control and Prevention

Rapid Identification and Treatment of Affected Animals and Humans: In enzootic countries or areas, whether or not animal vaccination is carried out, all suspected sudden deaths in animals should be investigated as possible cases of anthrax [28]. Flocks or herds in direct or indirect contact with positive human cases should be investigated, as the human infection will have derived from animal cases and any infected herds or flocks should be identified. Prophylactic administration of a single dose of long acting tetracycline or penicillin is a much commoner tactic to susceptible animals. For outbreak of the disease in humans; Rapid identification of animal source of outbreak, persons exposed to source, Outpatient treatment of uncomplicated cutaneous cases, Provision of antibiotics and supportive care for systemically ill patients should be considered [6].

Vaccination: In livestock, anthrax can be prevented largely by vaccination of all grazing animals in the endemic area and implementation of control measures during epizootics [6]. Vaccination should be done 2-4 weeks before the season when outbreaks may be expected [1]. In Ethiopia anthrax vaccine is being produced by National veterinary Institute [35].

Quarantine: Prohibition of movement of milk and meat from the farm during the quarantine period should prevent the entry of the infection into the human food chain [6]. Quarantine should be kept as soon as possible and it need not be longer than 21 days after vaccination of the affected herd has been carried out. Restricting access to suspected sources (feed or pastures) and if flies are suspected of being important vectors, therefore, fly control should be considered [28].

Appropriate Carcass Disposal: Disposal of infected materials is important and hygienic and it is one of the biggest single factors in the prevention of spread of the disease. Infected carcasses should not be opened but immediately burned or buried, together with bedding and soil contaminated by discharges. If this cannot be done immediately, a liberal application of 5% formaldehyde on the carcass and its immediate surroundings will discourage scavengers [2].

Disinfection of Affected Premises and Materials: Disinfection of premises can be carried out immediately before spore formation can occur, ordinary disinfectants or heat (60°C (140°F) for a few minutes are sufficient to kill vegetative forms. However, when spore formation occurs (i.e. within a few hours of exposure to the air), disinfection is almost impossible by ordinary means. So that strong disinfectants such as 5% Lysol require being in contact with spores for at least 2 days. Strong solutions of 8-10 % of formalin or 5-10 % of sodium hydroxide are probably most effective [2]. Prevention of Bacillus anthraces exposure through annual products imported from other areas requires disinfection of such material as hair, wool and skin by gamma radiation [28].

Case Surveillance: Surveillance is also the preventive measures for early case detection and prevention of outbreaks in animals and humans. The process comprises of routine clinical and laboratory surveillance. Disease reporting also plays a vital role in controlling. Anthrax is reportable disease in both the animals and humans. The veterinarians and health professionals who encounter or suspect anthrax should follow and report the disease according to guidelines for disease reporting [28]. According to Gemeda *et al*. [36] study in Gomma district of Jimma zone, Ethiopia, although majority of health care providers heard about anthrax, significant number of them do not know that anthrax attack human and animals. This implies that health care providers in the district can easily miss anthrax cases that can in turn contribute to a number of needless human and animal deaths and delayed outbreak detection and allow the disease to persist in the population.

CONCLUSIONS AND RECOMMENDATIONS

Anthrax is a soil born disease which affects many species of animals including human and endemic in both developing and developed countries of the world. Anthrax is a serious zoonotic disease that can affect most mammals and particularly important in herbivores. The organism form spores that help to persist in the environments for long period of time, outbreaks are

usually associated with flooding or soil disturbance. Infection gains entrance to the body by Ingestion of contaminated food or water, Inhalation and through the skin (macro and micro wounds). Diagnosis of the disease is done on the basis of clinical history, ante-mortem findings, post-mortem findings and laboratory examination of clinical specimens. Vaccination, surveillance, disinfection of premises, prophylaxis and proper carcass disposal are important prevention and control strategy against the disease. Although the disease is endemic and causing series effect in animal and human population in Ethiopia, this review revealed that few studies are available on general understanding of the diseases. Therefore, based on the above conclusion the following recommendations are forwarded:

- Efforts should be made by both governmental and non-governmental bodies to mitigate the effects of the disease on human and animal population.
- A regular and strategic vaccination should be given throughout the country.
- Researches should be done in line with the epidemiological situation, rapid diagnosis and prevention and control methods of the disease as general.

Abbreviations:

AU	African Union
AU-IBAH	Interafrican Bureau for Animal Resources
ELISA	Enzyme-linked Immunosorbent Assay
EPHI	Ethiopian Public Health Institute
MoA	Ministry of Agriculture
NVI	National Veterinary Institutions
OIE	Organization International Epizootic
PCR	Polymerase Chain Reaction
SNNPR	Southern Nation and Nationalities People Region
UK	United Kingdom
WHO	World Health Organization

REFERENCES

1. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2007. A textbook of the diseases of cattle, horses, sheep, pigs and goats. Veterinary Medicine, 10: 2045-2050.
2. Turnbull, P.C.B., M.E. Hugh-Jones and O. Cosivi, 1999. World Health Organization activities on anthrax surveillance and control. Journal of Applied Microbiology, 87(2): 318-320.
3. Aulinger, B.A., M.H. Roehrl, J.J. Mekalanos, R.J. Collier and J.Y. Wang, 2005. Combining anthrax vaccine and therapy: a dominant-negative inhibitor of anthrax toxin is also a potent and safe immunogen for vaccines. Infection and Immunity, 73(6): 3408-3414.
4. Carter, G.R. and J. D. Wise, 2004. Essentials of Veterinary Bacteriology and Mycology, 6thed. IowaStatePress, Ames, IA, pp: 179-182.
5. Hirsh, D.C. and Y.C. Zee, 2003. Veterinary microbiology. USA: Black well Science, pp: 246-249.
6. Misgie, F., A. Atnaf and K. Surafel, 2015. A review on anthrax and its public health and economic importance. Acad. J. Anim. Dis, 4(3): 196-204.
7. Shiloach, J., S.H. Leppla, D.M. Ramirez, R. Schneerson, J.B. Robbins, S.D. Hsu and M.J. Rosovitz, 2010. US Department of Health and Human Services (HHS) Methods for preparing Bacillus anthracis protective antigen for use in vaccines. U.S. Patent, 7: 763,451.
8. Berg, T., H. Suddes, G. Morrice and M. Hornitzky, 2006. Comparison of PCR, culture and microscopy of blood smears for the diagnosis of anthrax in sheep and cattle. Letters in Applied Microbiology, 43(2): 181-186.
9. Hirsh, D.C., Y.C. Zee and A. de Souza Coutinho, 2003. Veterinary microbiology. USA: Black well Science, pp: 148-180.
10. Bahiru, G., A. Bekele, B. Seraw, L. Boulanger and A. Ali, 2016. Human and animal anthrax in Ethiopia: A retrospective record review 2009-2013. Ethiopian Veterinary Journal, 20(2): 76-85.
11. Stern, E.J., K.B. Uhde, S.V. Shadomy and N. Messonnier, 2008. Conference report on public health and clinical guidelines for anthrax. Emerging Infectious Diseases, 14(4): 10-30.
12. Goel, A.K., 2015. Anthrax: a disease of biowarfare and public health importance. World Journal of Clinical Cases: WJCC, 3(1): 20.
13. AU-IBAR, 2011. Panafrican animal health yearbook 2011.
14. Blackburn, J.K., K.M. McNyset, A. Curtis and M.E. Hugh-Jones, 2007. Modeling the geographic distribution of Bacillus anthracis, the causative agent of anthrax disease, for the contiguous United States using predictive ecologic niche modeling. The American Journal of Tropical Medicine and Hygiene, 77(6): 1103-1110.
15. Carlson, C.J., I.T. Kracalik, N. Ross, K.A. Alexander, M.E. Hugh-Jones, M. Fegan and M. Bagirova, 2019. The global distribution of Bacillus anthracis and associated anthrax risk to humans, livestock and wildlife.

16. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID). (2015): Anthrax. Basic information; Available online: Basic Information% 20% 20 Anthrax% 20%20 CDC.html.
17. Turner, W.C., K.L. Kausrud, W. Beyer, W.R. Easterday, Z.R. Barandongo, E. Blaschke, C.C. Cloete, J. Lazak, M.N. Van Ert, H.H. Ganz and P.C. Turnbull, 2016. Lethal exposure: An integrated approach to pathogen transmission via environmental reservoirs. Scientific Reports, 6(1): 1-13.
18. Read, T.D., S.N. Peterson, N. Tourasse, L.W. Baillie, I.T. Paulsen, K.E. Nelson, H. Tettelin, D.E. Fouts, J.A. Eisen, S.R. Gill and E.K. Holtzapple, 2003. The genome sequence of Bacillus anthracis Ames and comparison to closely related bacteria. Nature, 423(6935): 81-86.
19. Moir, A. and D.A. Smith, 1990. The genetics of bacterial spore germination. Annual review of Microbiology, 44(1): 531-553.
20. Hugh-Jones, M. and V. De vos, 2002. Anthrax and wildlife. Rev. Sci. Tech., 21(3): 359-383.
21. Esthete, T., G. Chali, T. Wakshum, S. Tafa, M. Husen and Deneke, 2017. Retrospective Study on the Epidemiology of Bovine Anthrax in Elubabur Zone, South West Ethiopia, Global Veterinaria, 19(4): 590-595.
22. Hugh-Jones, M. and J. Blackburn, 2009. The ecology of Bacillus anthracis. Molecular Aspects of Medicine, 30(6): 356-367.
23. OIE, 2018. Anthrax, Animal health in the World, Information on aquatic and terrestrial animal diseases: Available online: Anthrax%20 OIE-World Organisation for Animal Health.html Pediatrics, 2009; pp: 211-214.
24. Seboxa, T. and J. Golden, 1989. Anthrax in Ethiopia, Trop. Geoger Med., 41: 108-112.
25. Menghistu, H.T., K.T. Hailu, N.A. Shumye and Y.T. Redda, 2018. Mapping the epidemiological distribution and incidence of major zoonotic diseases in South Tigray, North Wollo and Ab'ala (Afar), Ethiopia. PloS one, 13(12): 0209974.
26. Shiferaw, G., 2004. Anthrax in Wabessa village in the Dessie Zuria district of Ethiopia. Rev. sci. tech. Off. int. Epiz, 23(3): 951-956.
27. Mebratu, A.T., Z. Hailu and Y.H. Weldearegay, 2015. A retrospective survey and assessment of farmer's indigenous knowledge on anthrax in and around Tanqua-Abergelle District, Northern Ethiopia. Academic Journal of Animal Diseases, 4(1): 10-16.
28. World Health Organization, 2008. Anthrax in humans and animals, 4th edition, WHO press, Geneva, Switzerland.
29. Martin, G.J. and A.M. Friedlander, 2010. Bacillus anthracis (anthrax). Mandell, Douglas and Bennett's principles and practice of infectious diseases. 7th ed. Philadelphia: Churchill Livingstone, pp: 2715-2725.
30. Levine, S.M., Y.W. Tang and Z. Pei, 2005. Recent advances in the rapid detection of Bacillus anthracis. Reviews in Medical Microbiology, 16(4): 125-133.
31. Shadomy, S.V. and T.L. Smith, 2008. Anthrax. Journal of the American Veterinary Medical Association, 233(1): 63-72.
32. Schofield, D., N.J. Sharp and C. Westwater, 2012. Phage-based platforms for the clinical detection of human bacterial pathogens. Bacteriophage, 2(2): 105-121.
33. Abshire, T.G., J.E. Brown and J.W. Ezzell, 2005. Production and validation of the use of gamma phage for identification of Bacillus anthracis. Journal of Clinical Microbiology, 43(9): 4780-4788.
34. Iqbal, S.S., M.W. Mayo, J.G. Bruno, B.V. Bronk, C.A. Batt and J.P. Chambers, 2000. A review of molecular recognition technologies for detection of biological threat agents. Biosensors and Bioelectronics, 15(11-12): 549-578.
35. National veterinary institute, 2016. Product catalogue, user manual for anthrax vaccine, document no: nvi-qms-qf- 64(2).
36. Gemeda, D.H., A.G. Sime, K.W. Hajito, B.D. Gelalacha, W. Tafese and T.T. Gebrehiwot, 2016. Health Care Providers' Knowledge and Practice Gap towards Joint Zoonotic Disease Surveillance System: Challenges and Opportunities, Gomma District, Southwest Ethiopia. BioMed Research International, pp: 3942672.