Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Department of Food Safety and Infection Biology

Philosophiae Doctor (PhD)
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Use of a One Health Approach for Understanding the Epidemiology and Management of Anthrax Outbreaks in the Human-Livestock-Wildlife and Environmental Health Interface Areas of Northern Tanzania

Bruk av en Én Helse tilnærming for forståelse av epidemiologiske forhold og håndtering av miltbrann hos mennesker og dyr i grensesnittområder i Nord-Tanzania

Elibariki Reuben Mwakapeje

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Dedication

To

My family for their patience, support, love and care

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Acknowledgement

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PhD on time. Thank you God and help me with your wisdom and guidance in my future endeavors to serve your purpose for the rest of my life.

List of acronyms and abbreviations

AUC	Area Under the Curve
AHIS	Animal Health Information System
BSL	Biosafety Levels
B.C	Before Christ
CI	Confidence Interval
DC	District Council
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Sorbent Assay
GPS	Global Position System
GHSA	Global Health Security Agenda
GCLA	Government's Chemist Laboratory Agency
HMIS	Health Management Information System
IDSR	Integrated Diseases Surveillance and Response
IHR	International Health Regulation
IPC	Infection Prevention and Control
JEE	Joint External Evaluation
KCRI	Kilimanjaro Clinical Research Institute
KEMRI	Kenya Medical Research Institute
KINAPA	Kilimanjaro National Park
LCA	Latent Class Analysis
LGA	Local Government Authority
MoHCDGEC	Ministry of Health Community Development Gender Elderly and Children
MLFD	Ministry of Livestock and Fisheries Development
MNRT	Ministry of Natural Resources and Tourism
NIMR	National Institute for Medical Research
OR	Odds Ratio
OH	One Health
NCA	Ngorongoro Conservation Area
NCAA	Ngorongoro Conservation Area Authority
NHLQATC	National Health Laboratory Quality Assurance and Training Centre
PHEIC	Public Health Events of International Concern
PoE	Point of Entry
PORALG	President's Office Regional Administration and Local Government
QGIS	Quantum Global Information System
ROC	Receiver Operating Characteristics
TAWIRI	Tanzania Wildlife Research Institute
TALIRI	Tanzania Livestock Research Institute
TFDA	Tanzania Food and Drugs Authority
TVLA	Tanzania Veterinary Laboratory Agency
VIC	Veterinary Investigation Centre
WHO	World Health Organization

Summary

Zoonotic diseases are infections, which are transmissible between animals and humans. In both low and middle-income countries (LMIC), animals (livestock or wildlife) and humans often live close together. In LMIC, people are especially dependent on livestock and poultry for food, clothing, fertilizer, draught power, workforce, and as an important financial security. This interaction between humans, livestock and wildlife can easily result in an interspecies transmission of zoonotic diseases.

The overall aim of this study was to identify the drivers of infection and options for prevention and control of anthrax outbreaks in the humans-livestock-wildlife and environmental health interface areas of Northern Tanzania. Specifically the study intended: (i) to identify hotspot areas of anthrax outbreaks in the human-livestock-wildlife interface areas of northern Tanzania; (ii) to assess the efficiency of the existing structures for response to anthrax outbreaks using a One Health approach; (iii) to determine the risk factors associated with frequent anthrax outbreaks in the hotspot areas of northern Tanzania and (iv) to determine the influence of climatic and environmental factors for the spatial distribution of *Bacillus anthracis* spores in Tanzania and use this information in disease control and prevention.

The reported incidence rate of human anthrax over 2013-2016 was much higher in Arusha region (7.88/100,000) followed by Kilimanjaro region (6.64/100,000) than any other regions of the Tanzania Mainland, and these regions were identified as hotspots for anthrax outbreaks in the country. The records from selected health facilities showed that there were 187 human anthrax cases (57%) in Kilimanjaro and 143 (43%) in Arusha region in the period of 2006-2016. The majority (86.1%) of all human anthrax cases reviewed at the selected health facilities were of the cutaneous form, and most of the patients (65.2%) were male.

From 2006 to 2016, TVLA received 161 anthrax suspect specimens from different livestock and wildlife species for laboratory analysis. Most of the submitted specimens came from cattle (66%). A total of 103 specimens (64%) tested positive for *B. anthracis*, and 68 (66%) of the positive specimens came from cattle, followed by 18 goats (17%). During the same period, a total of 57 wildlife specimens obtained from the active surveillance done in the Serengeti ecosystem were tested for anthrax at TAWIRI laboratory based at Serengeti National Park. Of

these 18 (32 %) were positive for anthrax of which most of them (67%) came from African buffalo.

In another survey on the anthrax outbreak, done in Monduli district, a total of 131 carcasses of wildlife were counted, the majority (83%) being wildebeest. Other carcasses of wild animals included Grant gazelles (16%) and rabbits (0.8%). Out of 21 humans suspected anthrax cases, the majority were under 5 years of age (43%) followed by the age group of 6 -15 years (33%). Methylene blue staining and PCR techniques were used to confirm the existence of the anthrax outbreak in Selela ward. Local officials in Monduli district further reported that livestock carcasses (10 cattle, 26 goats, and three sheep) were either been consumed or hidden by owners.

In the case-control study, cases were recruited from 7 districts (Hai, Meru, Monduli, Moshi DC, Ngorongoro, Rombo and Siha. The study showed that male participants constituted the majority (59.3.%) compared to female (40.7%). The age range was 1–80 years with a median age of 32 years. A total of 83 (70.3%) of the study subjects had no formal education. In the group that included the youngest individuals, the exposure status was strongly linked to anthrax transmission (OR=25) in the younger group and a bit lower in the older group (OR=3.2).

In the ecological niche modeling study for prediction of the potential geographic distribution of *Bacillus anthracis* spores in Tanzania, the soil types demonstrated a high percentage contribution (56.5%) to persistence and environmental suitability for *B. anthracis* spores followed by soil pH (23.7%). The isothermally also added some explanatory power. The soil types were solonetz, fluvisols, and lithosols. The risk maps indicated that regions with high and very high risks for anthrax outbreaks were Arusha and Kilimanjaro from the northern part of the country, while other regions like Mara, Manyara, Simiyu, and Singida had few patches of high and very high-risk areas. Regions like Dodoma, Mwanza, Dar es Salaam, Lindi, Mbeya, Rukwa, Katavi, and Kigoma were predicted to have a medium risk in a few locations and the rest of the regions in the country had low risks for geographic suitability of *B. anthracis* spores persistence. It is therefore envisaged that implementing targeted control measures based on the disease risk mapping is more cost-effective due to reduced cost for carcass disposal, laboratory reagents and cost for outbreak management in general. A targeted livestock vaccination and intensified human and animal disease surveillance can be established by focusing more closely on the predicted high and very high-risk districts.

A One Health approach is required for responding to anthrax outbreaks as this disease can affect humans, livestock and wildlife during a single outbreak in the hotspot areas. The *B. anthracis* spores can stay in the soil for a long time. Emphasis should be given to effective communication, coordination and collaboration among all the involved sectors. A One Health approach, which has been established in Arusha region specifically in Selela ward, in Monduli district is one of the best practices emanated from this study and should be used as a model to extrapolate the same approach to the rest of the regions in the country.

Sammendrag

Zoonotiske sykdommer er infeksjoner, som er overførbare mellom dyr og mennesker. I lav- og mellominntektsland lever ofte dyr og mennesker tett sammen. I slike land er folk avhengige av husdyr til mat, klær, gjødsel og trekkdyr, og husdyra er også en viktig del av deres økonomiske sikkerhet. Dette samspillet mellom mennesker, husdyr og vilt kan lett resultere i overføring av zoonotiske sykdommer.

Det overordnede målet med denne studien var å identifisere drivere for infeksjon og beskrive muligheter for forebygging og bekjempelse av miltbrannsutbrudd i grensesnittet mellom mennesker, husdyr og vilt i Nord-Tanzania. De spesifikke målene var å (i) identifisere hotspot-områder av miltbrannsutbrudd i Nord-Tanzania, (ii) vurdere effektiviteten av eksisterende strukturer for respons på utbrudd ved hjelp av en Én helse tilnærming (iii) finne risikofaktorer knyttet til hyppige miltbrannsutbrudd i hotspot-områdene i Nord-Tanzania og (iv) identifisere klimatiske og miljømessige faktorer som bestemmer forekomst av sporer av Bacillus *anthracis* i Tanzania og i lys av dette foreslå passende kontrolltiltak.

Den rapporterte insidensen av miltbrann hos mennesker i 2013-16 var mye høyere i Arusha-regionen (7,88/ 100,000) etterfulgt av Kilimanjaro-regionen (6,64 /100,000) enn noen andre regioner på Tanzanias fastland, og disse regionene ble identifisert som hotspots for miltbrann. Data fra utvalgte helsestasjoner viste at det var 187 miltbranntilfeller hos mennesker (57%) i Kilimanjaro og 143 (43%) i Arusha-regionen i perioden 2006-2016. Flertallet (86,1%) av disse var hudformen av miltbrann, og de fleste pasientene (65,2%) var menn.

I perioden 2006 til 2016, mottok det tanzanianske veterinærlaboratoriet 161 prøver fra forskjellige dyre- og dyrearter, mistenkt for miltbrann. De fleste innleverte prøver kom fra storfe (66%). Totalt ble 103 prøver (64%) testet positive for *B. anthracis*, og 66% av de positive prøvene kom fra storfe, etterfulgt av geiter (17%). I samme periode ble totalt 57 viltprøver fra overvåking i Serengeti-økosystemet testet for miltbrann. Av disse var 32% positive for miltbrann, de fleste kom fra afrikansk bøffel (67%).

Under et utbrudd i Monduli-distriktet, ble 131 dyreprøver undersøkt, og flertallet (83%) var gnu. Andre kadaver av ville dyr var Grant-gaselle (16%) og kaniner (0.8%). Av 21 mistenkte tilfelle hos mennesker, var flertallet under 5 år (42,9%) etterfulgt av aldersgruppen 6-15 år (33,3%). Metylenblåttfarging og PCR-teknikker ble brukt til å bekrefte forekomst av miltbrannsutbrudd i

Selela Ward. Lokale myndigheter rapporterte også at husdyr (10 storfe, 26 geiter og tre sauer) enten ble konsumert eller skjult av eiere.

I en oppfølgende kasus-kontroll-studie ble miltbrannstilfelle og kontroller rekruttert fra Hai, Meru, Monduli, Moshi DC Ngorongoro, Rombo og Siha. Blant studiedeltakere var det flere mannlige deltakere (59,3%) sammenlignet med kvinner (40,7%). Alder varierte mellom 1 og 80 år med en median på 32 år. De fleste (70,3%) av pasientene hadde ingen formell utdanning. Under analysene av studien viset det seg at yngre (1-20 år) ble rekruttert hyppig blant pasientene (44,1%), mens bare fire kontroller (6,8%) var fra denne gruppen. Med hjelp av en såkalt latent-klasse analyse kunne vi påvise at utdanning var knyttet til miltbrann som prediktor for eksponering, men ikke direkte knyttet til miltbrannoverføring I den yngste gruppen var eksponeringsstatus sterkt knyttet til miltbrannoverføring (Odds Ratio=25,0). I den eldre gruppen var koblingen til eksponering også høy (OR=3,2).

I den avsluttende økologiske nisjemodelleringsstudien viste jordtyper en høy forklaringsgrad (56,5%) etterfulgt av pH (23,7%) Flere jordtyper ble identifisert som de viktige (solonetz, fluvisoler og litosoler). Risikokartene indikerte at regioner med høy og svært høy risiko for miltbrannsutbrudd var Arusha og Kilimanjaro fra den nordlige delen av landet, mens andre regioner som Mara, Manyara, Simiyu og Singida hadde noen mindre områder med høy og svært høy risiko. Regioner som Dodoma, Mwanza, Dar es Salaam, Lindi, Mbeya, Rukwa, Katavi og Kigoma ble vurdert å ha en middels risiko på enkelte steder og resten av regionene i landet hadde lav risiko for geografisk overlevelse av *B. anthracis* sporer

Målrettede kontrolltiltak basert på kartlegging av sykdomsrisiko er mer kostnadseffektivt på grunn av redusert kostnad for destruksjon av dyr, kostnader til laboratorieanalyser og generelt redusert kostnad for utbruddshåndtering. En målrettet husdyrvaksinasjon og intensivert overvåking av mennesker og dyr gjøres best ved å fokusere på høyrisikodistriktene. En Én helse tilnærming er nødvendig for å håndtere miltbrannsutbrudd da denne sykdommen rammer mennesker, husdyr og vilt, samtidig som *B. anthracis* sporer kan holde seg i jorden i lang tid. Det bør legges vekt på effektiv kommunikasjon, koordinering og samarbeid mellom alle involverte sektorer. Den Én Helse-tilnærmingen som er etablert i Arusha-regionen, er et hovedprodukt fra denne studien, og bør benyttes som en modell for hele Tanzania.

List of Papers

Paper I

Elibariki Reuben Mwakapeje, Sol Høgset, Robert Fyumagwa, Hezron E. Nonga, Robinson H. Mdegela, and Eystein Skjerve: **Anthrax outbreaks in the humans -livestock and wildlife interface areas of Northern Tanzania: a retrospective record review 2006-2016.**

BMC-Public Health (2018) 18:106. DOI: 10.1186/s12889-017-5007-z

Paper II

Elibariki R. Mwakapeje, Justine A. Assenga, John S. Kunda, Ernest E. Mjingo, Zachariah E. Makondo, Hezron E. Nonga, Robinson H. Mdegela and Eystein Skjerve: **Prevention, detection, and response to anthrax outbreak in Northern Tanzania using One Health approach: A case study of Selela ward in Monduli district.**

International Journal of One Health. DOI: 10.14202/IJOH.2017.66-76

Paper III

Elibariki R. Mwakapeje, Sol Høgset, Adis Softic, Janneth Mghamba, Hezron E. Nonga, Robinson H. Mdegela, and Eystein Skjerve: **Risk factors for transmission of cutaneous anthrax in the hotspot areas of Northern Tanzania. Unmatched case-control study.**

The Journal Royal Society Open Science. DOI: 10.1098/rsos. 180479

Paper IV

Elibariki R. Mwakapeje, Sood Ndimuligo, Gladys Mosomtai, Samuel Ayebare, Luke Nyakarahuka, Hezron E. Nonga, Robinson H. Mdegela and Eystein Skjerve**: Ecological niche modeling as a tool for prediction of the geographic distribution of *Bacillus anthracis* spores in Tanzania.**

Submitted to the International Journal of Infectious Diseases. IJID-D-18-00513

Introduction

Like many other developing countries, Tanzania is affected by frequent anthrax outbreaks without clearly knowing any reason for their repeated occurrences. This thesis tries to fill some of the knowledge gaps and give answers to some of the speculated hypotheses linked to the understanding of the epidemiology and management of anthrax outbreaks in animals (livestock and wildlife) and humans in a Tanzanian context.

Tanzania

The United Republic of Tanzania (URT) is located in East Africa between 3°S and 12°S and 26°E and 41°E. It has a coastline (800 km) of the Indian Ocean to the East, and borders eight countries namely, Kenya, Uganda, Rwanda, Burundi, the Democratic Republic of Congo, Zambia, Malawi, and Mozambique. The total area including Zanzibar is 945,087 km^2, of which 883,087 km^2 is land and 62,000 km^2 is water.

The country is divided into 31 Regions (26 in the mainland, 3 in Unguja and 2 in Pemba), with seven administrative zones in Tanzania Mainland (**Figure 1)**. In total, there are 169 districts, also known as local government authorities (LGAs). In Tanzania, the climatic condition varies with geographical zones: tropical on the coast where it is hot and humid (March-May), semi-temperate in the mountains with short rains (November-December) and long rains (February-

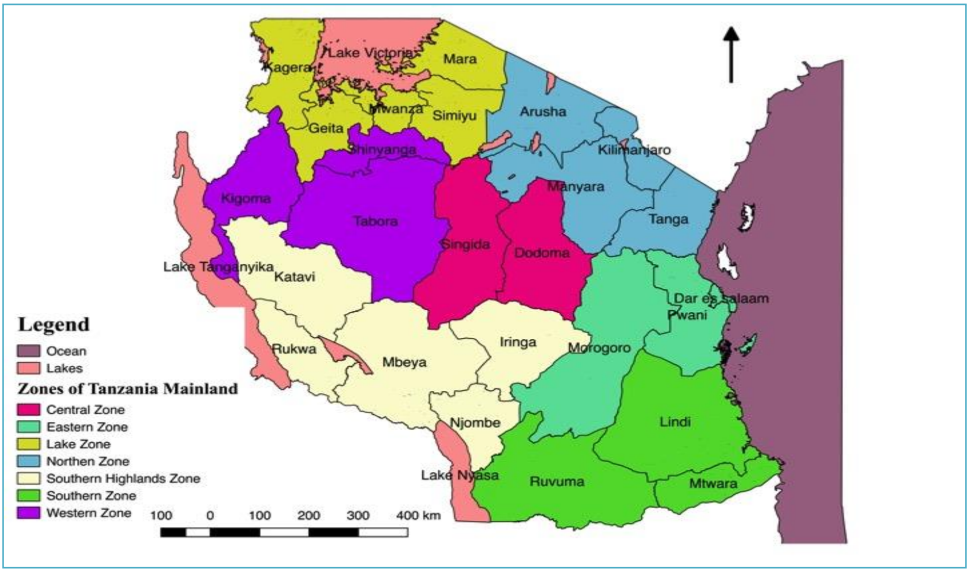

Figure. 1: Map of Tanzania showing the distribution of administrative zones of Tanzania Mainland including the northern zone where Arusha and Kilimanjaro regions are located (**Drawn in QGIS Software**)

May); while it is drier in the plateau regions with considerable seasonal variations in temperature. Rainfall is well distributed throughout the year, reaching its peak during the period of March through May. The diverse climate attracts a wide range of vectors of veterinary and public health importance.

Demography

The population of Tanzania consists of more than 120 ethnic groups (Lawson et al. 2014). According to the National Bureau of Statistics (NBS), the 2018 population projection estimates the total population to be 54.2 million (male, 26.5 million and female, 27.7 million). More than 44.8% of the population is under 15 years, 52% between 15 and 64 while only 3.2% are over the age of 64 years (http://www.nbs.go.tz). The population density varies from $12/km^2$ in less populated regions, such as Katavi, to $3,133/km^2$ in highly populated regions, such as Dar es Salaam. The overall population growth rate is estimated to be 2.9% per year with the urbanization growth rate standing at 4.77% per year (Tanzania Bureau of Statistics 2010). Agriculture supports the livelihoods of 82% of the population, 70% of which is rural (Annual Agricultural Sample Survey Report 2016).

Agriculture, livestock, and wildlife

Tanzania is a developing country, with most of the population still depending on agriculture and livestock. In the context of this thesis, the large population of livestock and wildlife constitutes a unique setting for a complex interaction between humans, livestock and wildlife producing the pattern of zoonotic infections found, with anthrax being one of the important diseases within this interface.

Livestock

Livestock farming is one of the major agricultural activities in the country. The livestock industry plays an important role in Tanzanian's socio-economic development and contributes towards household food and nutritional security. Tanzania's animal populations include 30.5 million cattle, 18.8 million goats, 5.3 million sheep, 1.9 million pigs, 38.2 million local chickens and 36.6 million-hybrid chickens (Ministry of Livestock and Fisheries 2018). More than 99% of these livestock are kept under traditional production systems, owned and managed by resource-poor mixed pastoralists or agro-pastoralists who operate under the traditional husbandry system with little or no access to good and reliable animal husbandry practices and reliable veterinary services (Annual Agricultural Sample Survey Report 2016).

Livestock production is regarded as the cultural heritage of many resource-poor rural communities in Africa. It serves as a major source of livelihood and a pathway out of poverty for many rural farmers in Sub-Saharan Africa. Apart from providing a rich source of animal protein, many livestock are kept as “bank on hooves”. These animals are sold to earn hard cash necessary to settle important family problems. In addition, livestock are also used to accomplish many cultural and traditional religious practices such as marriage dowry price, naming ceremonies, tribal rituals and religious sacrifices (Tanzania Ministry of Agriculture 2012).

Wildlife

In Tanzania, approximately 233,300 km^2 (28%) of the total land area is set aside in protected areas for wildlife conservation, including 16 national parks, 38 game reserves, 43 game controlled areas, Ngorongoro Conservation Area (NCA), and Selous–Nyassa Wildlife Corridor (Ministry of Natural Resources and Tourism 2018). The various types of protected areas are interwoven with agro-pastoral and pastoral community lands, some of which also support substantial biodiversity. Tanzania contains 20% of Africa’s large mammal population, found across its national parks, game reserves, conservation areas, and marine parks.

In many protected areas, including major national parks (Ruaha & Serengeti), there is an increased interaction between livestock, wildlife, and humans forming a complex ecosystem with a potential for disease transmission (Holmern et al. 2004). The Ngorongoro Conservation Area (NCA) is a multiple land use area where livestock and wildlife are legally allowed to live together and in the game controlled areas, limited human activities like livestock keeping and temporary shelters are allowed (Mangesho et al. 2017).

Zoonotic diseases of Tanzania

Zoonotic diseases are diseases and infections, which are naturally transmissible between vertebrate animals to humans. Approximately 61% of human infectious diseases are zoonotic (Han et al. 2013), but public health practitioners rarely consider the implication of these types of infections in humans.

Animals (livestock and wildlife) and humans often live close together in the less and middle-income countries (LMIC), and although at a different pattern, also in the developed countries. In the LMIC, people are especially dependent on poultry and livestock for food, clothing, fertilizer, draught power, workforce, and animals also represent an important part of financial security. In some cases, wildlife is also used as a source of bush-meat and other uses such as tourism attractions (Martin, Caro, and Mulder 2012). The animals and their products create disease risks for the pastoral and agro-pastoral communities in the LMIC, who mostly depend on animals for their livelihood. Pets and companion animals can pose a similar risk to humans in the developed countries.

The frequent interaction among humans, livestock and wildlife can easily result in interspecies transmission of zoonotic diseases (Gadaga et al. 2016). The increasing human population and the increased demand for land, food, and use of natural resources are the root causes of increased transmission of zoonotic diseases. Zoonotic diseases are of great importance at the interface among humans, livestock and wildlife, especially in self-identified populations that primarily rely on raising livestock on 'natural' pasture, otherwise referred to as "pastoral communities" (Macgregor, Waldman, and Macgregor 2017). It is also a response to the national livestock policy, which advocates for increased livestock production to meet the increasing demand for meat and milk as a country is moving towards the industrialized economy (Zadoks, R; and Crump 2017).

Although many zoonotic diseases have been reported, few of them have been properly described and confirmed in Tanzania. Important zoonotic diseases include viral diseases (Rift Valley Fever and rabies), bacterial diseases (anthrax**,** brucellosis, salmonellosis, leptospirosis), parasitic diseases (giardiasis, hydatidosis, sleeping sickness, cysticercosis/taeniasis, cryptosporidiosis etc.), and fungal (dermatophytoses, sporotrichosis) (OHCEA report 2011). However, Tanzania has prioritized a list of zoonotic diseases including rabies, Rift Valley Fever, zoonotic influenza virus, anthrax, trypanosomiasis, brucellosis, and other viral hemorrhagic fevers (Department of Health and Human Services USA 2017)

Anthrax and *Bacillus anthracis*

Anthrax is a potentially fatal disease of humans and animals (herbivores in particular) caused by the Gram-positive, rod-shaped and spore-forming *Bacillus anthracis*, a bacterium to which all warm-blooded animals are susceptible. *Bacillus anthracis* endospores are resistant to heat, ultraviolet light, gamma radiation, and many disinfectants.

Bacillus anthracis is similar to other genetically related, but phenotypically different bacteria among the broader group called *Bacillus cereus* group. *Bacillus cereus, B. thuringiensis,* and *B. anthracis* are soil-borne pathogens which are similar in their ability to sporulate but *B. anthracis* can be differentiated from other members of the *B. cereus* group in the microscope by its squared end and encapsulated bacilli, and lack of motility, lack of hemolysis on blood agar and its sensitivity to penicillin (Spencer 2003).

Anthrax outbreaks are increasingly becoming a threat to humans, livestock and wildlife in Arusha and Kilimanjaro regions of Northern Tanzania. Anthrax outbreaks have been causing massive deaths to animals leading to continued economic losses to the communities residing in the livestock-wildlife interface areas of Arusha and Kilimanjaro regions. The disease also causes a lot of sufferings in humans, which may be associated with deaths especially the vulnerable groups in the community like children, women and the elderly. Human sufferings from anthrax experience a reduced time for production due to illness, linked to a direct and indirect socio-economic impact in these communities. The massive deaths of wildlife affect the tourism industry, which is one of the dependable sources of income in Tanzania.

***Bacillus anthracis* as a bioterror agent**

Besides its importance as an infectious disease, *B. anthracis* is also one of the major agents linked to biological warfare and terrorism. Following the accidental Sverdlovsk outbreak near Moscow in 1979, many individuals suffered gastrointestinal, cutaneous, and inhalational anthrax attributed to secret biological weapons activity nearby the military compound (Howard and Borry 2012; Riedel 2005). In 1993, an aerosol containing *B. anthracis* was released from a building in Kameido, Tokyo. No deaths were reported, but many neighborhood residents complained of unpleasant smells, loss of appetite, nausea, and vomiting. Before and after the September 11, 2001 incident, anthrax spores in powder form were sent to Senators Patrick

Leahy, Tom Daschle, and NBC news anchor Tom Brokaw, and the New York Post. Some employees who handled the letter contracted inhalational anthrax or the skin form of anthrax; a number of them became sick and a few died (Howard and Borry 2012). A total of 10 confirmed cases of inhalational anthrax were due to intentional release of *Bacillus anthracis* in the United States (J. A. Jernigan et al. 2001; D. B. Jernigan et al. 2002).

Historical perspective

*A*nthrax is a classic disease, also described in the Bible in the book of Exodus (chapters 7 to 9) as the 5th (death of livestock) and 6th (boils) plagues, which had inflicted the Egyptians and had shown typical symptoms of anthrax (Sternbach 2003). The first scientific description of the *B. anthracis* goes back to 1876 when Robert Koch discovered *B. anthracis* spores through his experiment of inoculating a bacterium into a number of mice, which later on developed a disease, which was named as anthrax in a later stage. Robert Koch also used *B. anthracis* to develop a postulate of transmission of infectious diseases (J. Chu 2009).

Anthrax originated from the Greek word, *anthracites*, meaning coal, referring to the black eschar, coal-like lesions mainly seen on the human cases of cutaneous anthrax (Sternbach 2003). In 1881, Louis Pasteur vaccinated two different herds of cattle with the virulent *B. anthracis* strain; the first herd was vaccinated with his vaccine while the control group was not. When all the vaccinated animals survived and the others died, he further proved his hypothesis that *B. anthracis* causes anthrax (Sternbach 2003).

At the beginning of the 1900s, a number of human inhalational cases of anthrax were reported in the United States among the workers of the textile and tanning industries who processed goat's hair, skin, and wool (Riedel 2005). The largest anthrax outbreak recorded in history was that from 1979 to 1985, which occurred in Chikubo and Ngandu villages of Murewa district in Zimbabwe affecting 10,000 human cases with cutaneous anthrax (Mwenye, Siziya, and Peterson 1996).

Clinical features of anthrax in animals

Anthrax is an infectious disease of livestock and wildlife herbivorous, but also occasionally affects humans and scavengers/carnivores. The incubation period for anthrax infection in susceptible animals under normal conditions ranges from 1 to 14 days and the acute form of the disease takes 36 to 72 hours in which the animal can present with behavior changes followed by sudden death (Bagamian et al. 2013).

In herbivores, the disease is often peracute, acute or subacute and mostly fatal. The common clinical presentations of anthrax in herbivores are septicaemia, generalized oedema and sudden death with/without bleeding from natural orifices and subcutaneous haemorrhages. Because of the disturbances in haemopoietic system, the carcasses from the anthrax cases normally ooze non-clotting dark blood from the natural orifices.

The peracute form of anthrax occurs in herbivores and it is characterized by sudden death without premonitory signs and symptoms, although there may be fever, dyspnea, muscle tremors, congestion of the mucosa and terminal convulsion in few animals. The acute form of the disease takes about 2 hours and it is characterized with severe depression and listlessness followed by death. Fever (42°C), anorexia, labored breathing, congested and hemorrhagic mucosae; raised heart rate, rumen stasis, and reduced milk production are common features. There may be non-clotting black blood oozing from the mouth, ear, nostril, anus, and vulva. Moreover, dysentery and edema of the tongue, sternum and bloodstained or yellow milk is produced. Pregnant animals may abort. Animals then collapse and die after terminal convulsion.

Post-mortem examination of carcasses suspected to have died from anthrax is not recommended because exposure of vegetative cells to open air triggers the formation of endospores which are resistant to stressful environmental conditions (Dixon et al. 1999).

Differential diagnosis of anthrax in animals should consider other causes of sudden death associated with oozing of non-clotting blood; including African horse sickness, botulism, peracute babesiosis, chemical poisoning (heavy metal and other poisons), and consumption of poisonous plants, snake-bite, lightning strike, or metabolic disorders like lactic acidosis, magnesium deficiency, and bloat. These differential diagnoses will vary depending on a species in question and geographical area (Rushton 2008).

Anthrax and its epidemiology in animals

A soil-borne, spore-forming, and Gram-positive bacterium, *B. anthracis* is the causative agent of anthrax (FAO-OIE-WHO 2008). The anthrax spores may remain viable in the soil for an extended period of time and can be dispersed by wind, predators, fertilizers, or effluents from factories processing contaminated animal products (Rushton 2008). Other studies have shown that lowland areas, which are prone to floods, are the hotspots for anthrax outbreaks. Sometimes, it is also associated with temperature, rains or drought, soil type, vegetation, host condition and population density (Hugh-Jones and Blackburn, 2009; Fasanella et al., 2013).

Ingesting feeds, water or soil contaminated with *B. anthracis* spores infects animals, and abrasions of the oral mucosa aid in the penetration of the bacteria. Upon ingestion, spores enter macrophages of a susceptible host and are transported to lymph nodes where they germinate into vegetative form (Akoachere et al. 2007), migrate into the bloodstream and release toxins which cause systemic effects (Koehler 2009).

Mechanical transmission by biting necrophagous flies has also been reported in different parts of the World (Turell and Knudson 1987). However, non-biting flies act as carriers for transmission of *B. anthracis* spores by providing a link between a carcass and its environment particularly to browsing animals such as kudu, *Tragelaphus stepsiceros* (Beyer 2018). Wildlife can act as carriers of anthrax which makes it difficult to control this disease in areas bordering national parks and game reserve areas as it is impossible to restrict movement of wild animals or implement vaccination programs effectively (Rushton 2008).

Anthrax is widespread and has been reported in different parts of the World including America, Asia, Europe and Africa (Lewerin et al. 2010). In Sub-Saharan Africa anthrax outbreaks were linked to high mortalities of animals during 1960-70s and since then, it has been continuously being reported in Sierra Leone, Ghana, Chad, Ivory Coast, Uganda, Nigeria, Botswana, Tanzania, Kenya, and Republic of South Africa (Rushton 2008). Effective vaccination programs in domestic animals have significantly minimized the incidences of this disease in most countries in recent years (Rushton 2008).

Anthrax is one of the major threats to animals and humans in the Western part of Zambia. In 2010, it affected 45 cattle and three humans (Munang'andu, Banda, Chikampa, et al. 2012). A total of 306 hippopotami died from a confirmed anthrax outbreak in the Queen Elizabeth National Part of Uganda in 2004, representing 11.6% of the total hippo population in the park (Wafula, Patrick, and Charles 2008). In 2004-2005, authorities in Uganda disposed of 500 carcasses of wildlife and 400 livestock due to anthrax (Coffin et al. 2015). Another outbreak affected 124 animals of different species: 81 cattle, 15 sheep, 9 goats, and 11 horses in Basilicata region and 8 deer of Pollino National Park in Italy (Chakraborty et al. 2012). In Bangladesh, a multi-sectoral team investigated 14 anthrax outbreaks and identified a total of 140 animal carcasses and 273 human cases of cutaneous anthrax in the recent years (Mondal and Yamage 2014; Chakraborty et al. 2012).

Clinical features of anthrax in humans

The three forms of anthrax in humans are cutaneous, gastrointestinal, and inhalational anthrax. The modes of transmission leading to different forms of anthrax in humans are shown in **Figure 2** and spores are central to the transmission cycle. However, the cutaneous form of anthrax accounts for 95% of all human anthrax cases worldwide. It is speculated that 10-20% of untreated cutaneous cases are expected to result in death, and less than 1% of treated cases are fatal (FAO-OIE-WHO 2008). In contrast, gastrointestinal tract and pulmonary cases are mostly fatal because more often they go unrecognized until at a late stage when it is not possible to provide an effective treatment. As a result, meningitis development is a possible complication of the three forms of anthrax and it has a case fatality rate (CFR) of 100% (FAO-OIE-WHO 2008).

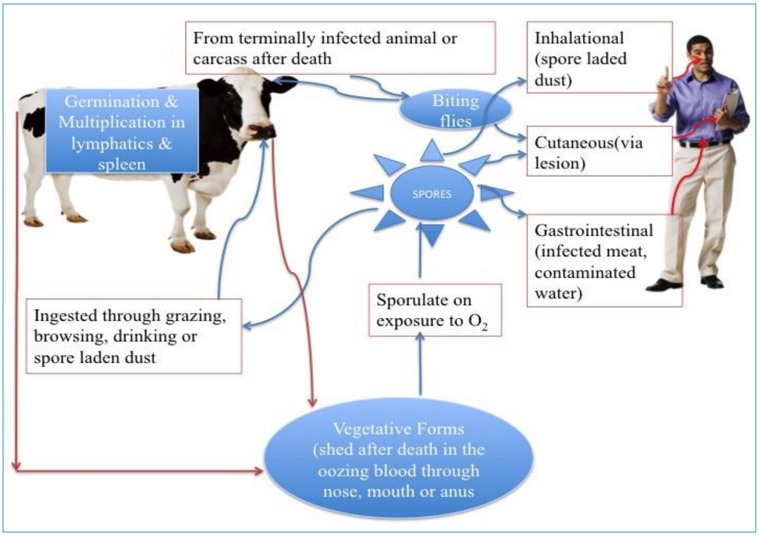

Figure 2: The cycle of anthrax transmission in humans and animals: **Adopted from FAO-OIE-WHO Guidelines 2008**

The incubation period for cutaneous anthrax ranges from 9 hours to 2 weeks, mostly 2 to 6 or 7 days with the following general scenarios: (i) day 0 → entry of the infective spores through a skin lesion (cut, abrasion, or insect bite), (ii) day 2-3 ➔ a small pimple appears, (iii) day 3-4 ➔ a ring of vesicles develops around a pimple and vesicular fluids may be exuded, (iv) day 5-7 ➔ the pimple ulcerates to form characteristics eschar, and at this stage topical swabs will not pick up *B. anthracis* and the fluid will probably be sterile if a suspected patient has been given antibiotics. Clinical symptoms may be extensively severe if the lesion is located in the face, neck or chest. Symptoms of anthrax in a more severe form of infection are high fever, toxemia, regional painful edema, shock, and eventually death (FAO-OIE-WHO 2008).

Two gastrointestinal forms may occur after ingestion of *B. anthracis* spores in contaminated food or water. (i) Intestinal anthrax, whose signs and symptoms are nausea, vomiting, fever, abdomen pain, hematemesis, bloody diarrhea, and massive ascites. If early treatment is not provided toxemia and shock may develop, followed by death. However, it is documented that mild unidentified cases may recover. The differential diagnoses include food poisoning, acute abdomen pain, and hemorrhagic gastroenteritis. (ii) Oropharyngeal anthrax whose clinical features include a sore throat, dysphagia, fever, localized lymphadenopathy in the neck and toxemia. Even when treatment is given the mortality rate goes up to 50%. The differential diagnoses are streptococcal pharyngitis, Vincent's angina, para-pharyngeal abscess, while deep tissue infection of the neck may be also considered (Doganay, Almaç, and Hanagasi 1986). In order to diagnose inhalational anthrax in a suspected patient, thorough history taking and assessment of the symptoms are required; these symptoms may include mild fever, fatigue and malaise, headache, muscle pain, chills, and fever. However early symptoms are non-specific and flu-like with mild upper respiratory tract discomfort may prevail (FAO-OIE-WHO 2008).

Epidemiology and transmission of anthrax in humans

Livestock and wildlife serve as potential sources of infections in humans. In humans, the infection occurs when *B. anthracis* penetrates through skin abrasions or mucous membranes when someone comes into contact with infected animals or animal products, or through inhalation of *B. anthracis* spores. Infection can also be acquired through consumption of raw or undercooked infected meat, milk, milk products and blood (Bengis and Frean 2014). Depending on the route of transmission, the disease in humans can occur as cutaneous, gastrointestinal, or as an inhalational (Lembo et al. 2011). It is estimated that a total of 2,000-20,000 human anthrax cases are being reported annually worldwide (Khomenko et al. 2013). China, for instance, experienced three large-scale anthrax outbreaks with 112,000 human cases from 1956 to 1997 (Chen et al. 2016).

Other studies have reported a total of 52 cases of cutaneous anthrax, and 24 cases of oral pharyngeal anthrax in humans after anthrax was found in water buffaloes in March-April 1982 in Chiang Mai, northern Thailand (Sirisanthana and Brown 2002). However, there is no documented human-to-human transmission of anthrax and laboratory-acquired anthrax is rare (Collins 1988). Anthrax in humans is classified into **non-industrial** and **industrial** anthrax.

Non-industrial anthrax occurs in pastoralists and agro-pastoralists, butcherers, knackers, veterinarians, and other groups of people who are directly dealing with animals resulting into contact with infected carcasses. This usually manifests itself as a cutaneous form and it mostly occurs on a seasonal basis in parallel with seasonal disease pattern in animals from which it is contracted. This form of anthrax is also manifested as gastrointestinal and inhalational anthrax depending on the route of transmission. Human consumption of raw or undercooked anthrax-infected carcasses results in gastrointestinal anthrax while the inhalation of contaminated air with *B. anthracis* spores leads to the inhalational anthrax.

Industrial anthrax affects those who are employed in industries, which are processing bones, hides, wool, and other animal products. Movement of animals and animal products can also introduce a disease to non-endemic areas (**Figure 3)**. This form of anthrax mostly occur as gastrointestinal tract anthrax acquired by consumption of contaminated meat and pulmonary (inhalational) anthrax acquired from breathing in contaminated air with anthrax spores, (FAO-OIE-WHO 2008).

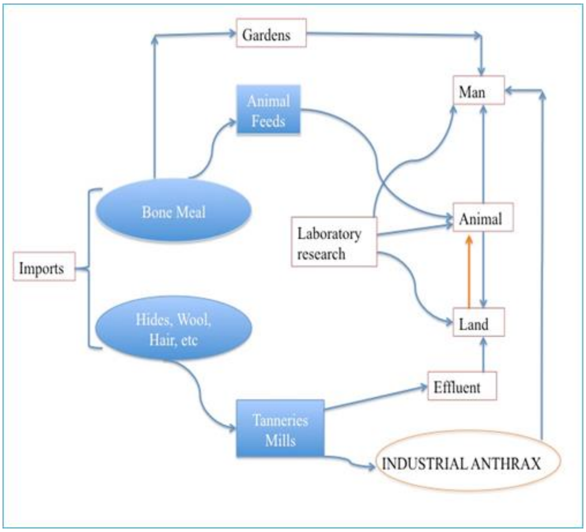

Figure 3: Importation of anthrax infection from endemic countries to other countries, and cycles within the importing country. **Adopted from FAO-OIE-WHO Guidelines 2008**

In the 18th century, anthrax epidemic affected almost half of the sheep population in Victorian England. This disease was termed as wool-sorters disease as it affected workers in the mill industry especially those ones who were frequently exposed to animal fibers which were contaminated with *B. anthracis* spores (Stefan 2005). Among the industrial workers who are at high risk for contracting the respiratory form of the disease are those involved in handling sacks of dried bones especially in the production of the bone meal. Early in the 20th Century, in the US, anthrax outbreak affected those who were handling materials woven from contaminated animal fibers and also this disease bears a high risk of importation to other countries through transportation of contaminated animal products (American Society for Microbiology 2008).

Anthrax outbreaks in Tanzania

The first anthrax outbreak in Tanzania was documented in the wildlife species in the national parks during 1962-1998 which caused deaths of 1,200 impalas, posing a great risk to humans and susceptible livestock (Mlengeya et al. 1998). Later on, sporadic human cases were being reported in different parts of the country. In 1985, a total of 239 human anthrax cases were reported in the Rukwa valley in southwest of Tanzania (Webber 1985), and between January 1984 to June 1988, a total of 11 human cases of cutaneous anthrax were admitted and treated at Mvumi Hospital in Dodoma region of central Tanzania after getting into contact with the infected animal carcasses (Yorston and Foster 1989). In 1985, hundreds of carcasses from different species of wildlife were laboratory confirmed to have died from anthrax in the Selous game reserve of southern Tanzania (Gainer 1987). Subsequently, in 1988 a big anthrax outbreaks in wildlife was reported in the Tarangire national park in which 142 impalas, 3 zebras, 4 wildebeests, and one giraffe were counted dead (Mbise, Nsengwa, and Mbasha 1991). Since then, different species of wildlife and livestock, and humans are frequently being affected by *B. anthracis* with varying disease pattern between years in terms of the size of outbreaks and species affected (Hampson et al. 2011).

Anthrax outbreaks are exacerbated by the increased proximity to wildlife-protected areas, human behavior of consuming raw or undercooked carcasses from sick or dead animals, as it is illustrated in **Figure 4**. Moreover, poor farming practices, and mismanaged cross-border movement of animals (livestock and wildlife) from one area to another as far as neighboring countries are concerned can also facilitate disease transmission.

Figure 4: Photographs (**own source**) showing the Maasai people at risk for anthrax infection by (**A**) skinning a carcass suspected to die from anthrax, (**B**) poor cooking condition on anthrax-infected carcass, (**C**) gangrene of a finger due to anthrax infection and (**D**) anthrax-related skin lesions

In Tanzania, studies have shown that anthrax outbreaks are regularly occurring in the country, for instance, in 1998, 2003, 2006, and 2009; many species were affected including livestock, humans, and wildlife.

Overall, seropositivity was found to be higher in carnivores from Serengeti National Park and Ngorongoro Crater by 90% and 57%, and significantly lower in herbivores by 46% and 14%, respectively (Hampson et al. 2011). The higher seroprevalence finding in carnivores (lions, hyena, etc.) than reported death rates, can be explained by the fact that wild carnivores are frequently being exposed to *B. anthracis* without apparent deaths which suggest for protective immune response presumably associated with more frequent exposure through scavenging infected prey, but domestic dogs may also be frequently exposed when they scavenge infected livestock and wildlife carcasses (Clegg et al. 2004; Down and Drive 1992; Lembo et al. 2011). In 2016, an anthrax outbreak was reported in Monduli district in Northern Tanzania in which 131 carcasses of wild animals were disposed of and 39 carcasses of domestic animals were consumed. Photographs of carcasses of different animal species are shown in **Figure 5.**

Figure 5: Photographs (**own source**) showing carcasses of wildlife and livestock that had died of anthrax infection whereby (**A)** elephant, (**B)** zebra, (**C)** cattle, and (**D)** goat. All the carcasses were confirmed to be anthrax positive and people consumed some of them.

Anthrax is a disease of public health importance and it forms part of the priority diseases in the National Integrated Diseases Surveillance and Response (IDSR) system. However, for anthrax, being transmitted between livestock and wildlife to humans, a joint surveillance system is required using a One Health approach through initiation of a joint anthrax reporting system and outbreak investigations apart from the existing challenges in both human and animal surveillance systems at different levels (Halliday et al. 2012), In Tanzania, some human anthrax outbreaks have been documented. Hospital records reviewed in 2011 showed that 7,538 cases were suspected for anthrax and 8 cases were confirmed as gastrointestinal anthrax with four deaths during 1999 -2006 (Lembo et al. 2011).

During a survey of anthrax infection for data collection of this thesis, there were reports of gastrointestinal anthrax at Pinyinyi dispensary in Ngorongoro district, where the reported cases presented with a stomach ache, bloody diarrhea and had a history of consuming a carcass from animals suspected to have died of anthrax. Human cases of respiratory anthrax were observed during the current study at Endulen Hospital in Ngorongoro district, Arusha region and Kilimanjaro Christian Medical Centre (KCMC) in Moshi Municipality, Kilimanjaro region of northern Tanzania. The probable means of transmission could be through inhalation of contaminated air/dust while cultivating their fields to grow crops or through exposure while dancing their local dramas using drums made from anthrax contaminated skins.

Recent reports indicate the occurrence of the anthrax outbreak in Rombo district of Northern Tanzania affecting four people with one death (Case Fatality Rate 25%) after acquiring infection from infected cattle in 2016 (Happiness Tesha 2016). Another recent outbreak occurred in Monduli district of Arusha region from Northern Tanzania, where 21 human cases were reported and a number of domestic and wildlife being affected.

In pastoral communities of northern Tanzania, mortality rates are high for individuals who get infected due to consumption of meat from infected dead animals. In wildlife-protected areas, the disease is associated with drought and outbreaks are often predictable (Hampson et al. 2011). The pastoral communities normally consume raw blood, raw milk and milk products, and raw or undercooked meat, which are all potential sources of *B. anthracis* infections (Crump et al. 2013).

During the current study, it was also found that there is a close temporal relationship between the occurrence of anthrax outbreaks in animals (livestock and wildlife) and in humans. This might be attributed by the existing interaction between the environment, livestock, wildlife, and humans. Scavengers like stray dogs consuming carcasses and poor disposal of carcasses can be some of the facilitating factors. Transmission of anthrax in places where the outbreaks have occurred being facilitated by sharing of water collection points between humans and animals, an increased interaction of wildlife and livestock during grazing and poor disposal of carcasses from animals died of anthrax as it is illustrated in **Figure 6**.

Figure 6: Photographs (**own source**) showing (**A**) a dog feeding on the abandoned carcass of a sheep, (**B**) unattended sheep oozing blood from its body openings as it was found in one of the study sites (**C**) animals and humans sharing a water source in one of the anthrax hotspot areas, and, (**D**) livestock and wildlife grazing together in Ngorongoro district, northern Tanzania

The ecological and epidemiological disease patterns of anthrax in northern Tanzania are not well understood, despite the frequent occurrence of anthrax outbreaks in humans, livestock and wildlife. Other studies reported that, areas with ambient temperature above 15.5^{o}C (Munang'andu, Banda, Siamudaala, et al. 2012), and cyclic rainfall pattern with high evaporation potential characterized by calcareous soil (Winsemius et al. 2006) tend to favor long-term survival of the *B. anthracis* spores in that environment leading to repeated anthrax outbreaks.

The endemicity of *B. anthracis* in northern Tanzania may be linked to the nature of the soil, which is calcium-rich alkaline types of soil (Deocampo, 2013), reported to provide favorable conditions for the long term survival of *B. anthracis* spores (Hugh-Jones and Blackburn 2009). This makes even the control measures of the disease to become more difficult.

Tanzania is among the few countries, which were evaluated for the local capacity to implement the pillars of the Global Health Security Agenda (GHSA), and it was found that the country has diverse ecosystems with open protected areas facilitating the human-livestock-wildlife interactions leading to high risk of transmission of zoonotic diseases. This situation is exacerbated by lack of knowledge of zoonotic diseases and existence of various socio-cultural norms including eating raw-meat, drinking raw-milk and milk products, and blood among pastoral communities, poor reporting of zoonotic diseases in human and animal surveillance systems, and poor linkages of animal and human laboratories on sharing information, experts and facilities for testing of specimen (JEE Report 2016).

Diagnosis of anthrax and methods for detection of *B. anthracis* in humans, animals and the environment

The confirmation of anthrax is done by laboratory testing (bacterial culture) of samples collected from suspected animals or humans. If the outbreak is in an animal species, consider the fact that, the fresher the carcass the better the chances of identifying the *B. anthracis* from the collected specimen. The specimen collection mechanisms should adhere to the following conditions:- (a) swabs should be collected aseptically from a peripheral vein or from the mouth, nose and anus of the carcass using appropriate gauge needles and a Vacutainer tube, (b) in case if there are fluids oozing from swellings on the carcass, swabs should be collected at the opening from where the fluid is exuding, (c) if there is blood discharge from the body orifices onto the soil, a soil sample should be collected and placed into a sterile falcon tube. However, opening the carcass of the anthrax suspected dead animals should be avoided, as it will facilitate the sporulation of *B. anthracis* in an aerobic environment.

The blood samples should be collected within 2 – 8 days post exposure and should be left to clot at room temperature overnight and subsequently centrifuged at 3000 rpm for 10 minutes to obtain clear serum which can be aliquoted in cryovials and stored at -20°C until serological analysis by ELISA is done (Dauphin et al. 2012). The ‘culture technique’ is a gold standard to confirm for the diagnosis of anthrax and the specimens should be collected prior to antimicrobial therapy. Moreover, culture plates should be examined for growth after 24 hours. Non-hemolytic, large, dense, grey-white irregular colonies with colony margin of “Medussa Head” or “curled-hair lock” appearance can be suspected to be *B. anthrancis.* The diagnostic polymerase chain reaction (PCR) can be used for confirmation of colonies. The PCR technique can be done as described by Turnbull (Turnbull TCB 1998) and the target should be the identification of pX01, pX02 plasmids using the appropriate primers.

The Giemsa stain technique can also be used to test for anthrax following preparation and fixing the blood smear on a microscope slide, the appearance consistent with the presence of *B. anthracis* should be observed. The management of anthrax specimens should be done in the laboratories with the well-established biosafety containment levels. However, the primary risk criteria used to define the four ascending levels of containment, referred to as biosafety levels 1 through 4, are infectivity, severity of disease, transmissibility, and the nature of the work being conducted (U.S. Department of Health and Human Service 2009).

Currently, in Tanzania, the Mbeya referral Hospital laboratory (from southwestern part of the country) has been upgraded to biosafety level (BSL-3), appropriate for agents with a known potential risk for aerosol transmission, for agents that may cause serious and potentially lethal infections that are indigenous or exotic in origin. The WHO has accredited this laboratory to perform diagnostic tests of highly infectious pathogens. The National Health Laboratory Quality Assurance and Training Centre (NHLQATC) and Tanzania Veterinary Laboratory Agency (TVLA) have also been accredited and have been given the ISO 15189 status of international standard. The two laboratories have demonstrated good biosafety measures in the country for conducting conventional microbiological methods such as testing for specimen of highly infectious diseases like Viral Hemorrhagic Fevers (VHF) such as Marburg, Ebola, Rift Valley Fever, Yellow Fever Chikungunya and others like anthrax. However, as a requirement for the implementation of IHR (2005) in the context of IDSR, the main challenge remain to be poor linkages between laboratory services and real-time surveillance through providing timely and reliable results for an informed decision on the management of epidemic-prone diseases (Phalkey et al. 2015).

Prevention and control measures

Anthrax is a neglected disease in most of the endemic countries, and its control mechanisms require a One Health approach by the proper inclusion of social realities and political mechanisms. It requires several immediate actions in order to avoid unnecessary morbidity and mortalities in human and animal population and also limit further spread of the infection through contaminated environment. The only control measures of anthrax in humans are through control or elimination of the disease in animals. These actions may include, but not limited to the following (Cunningham et al. 2017):

i. Provide anthrax vaccine to all susceptible livestock in the affected herd and surrounding areas
ii. Ensure restriction of animal and animal's products (bone meal, hides, and skins) movements from infected/endemic areas
iii. Provide treatment of infected humans by using the recommended antibiotics in order to stop any incubating infections
iv. Conduct an epidemiologic investigation to identify the source of infection, localize the outbreak and establish the magnitude of the problem
v. Avoid opening of the anthrax suspected carcass in order not to expose vegetative cells to oxygen which leads to sporulation and formation of endospores which is resistant to harsh environmental conditions
vi. Intensify surveillance and monitor areas surrounding the infected areas for detection of any existing additional cases

A proper waste management procedures should be observed especially in the laboratory and hospital environment after managing anthrax patients (CDC 2008). This should also go hand in hand with adhering to infection, prevention, and control (IPC) practices and ensuring that autoclave, incinerator, disposal pits, hot air oven, and reliable water supply are available in the laboratory.

One of the recommended control measures of anthrax outbreak is a safe disposal of carcasses through either burying or burning of carcasses depending on the physical condition (fresh or dry) of the carcass. Burying can only be considered when the carcasses are fresh and intact because the *B. anthracis* spores can remain virulent for many years under favorable soil condition and become a source of future outbreaks when exposed to the aerobic environment. The burial pit should be 6 feet deep and lime has to be poured on top of the pit and the surrounding environment in order to decontaminate the location. This method is tedious and labor intensive, as it requires manpower to excavate the burial pit and collection of carcasses into the pit followed by the application of formalin 10% on the burial site.

In contrast, incineration of the carcasses (fresh or dry) should be given high priority as one of the best and most effective control measures during anthrax outbreaks in which fire woods and petrol can be required. It tends to destroy completely the carcasses and *B. anthracis* spores and therefore it minimizes the chance of the spore's survival and prevents further spread of the disease. These options are demonstrated in **Figure 7.**

Figure 7: Photographs (**own source**) showing different options for disposal of wildlife carcass during response to anthrax outbreak in one of the hotspot districts of northern Tanzania whereby (A) making fire for burning of dry carcasses, (B) carcasses on top of fire, (C) collection of carcasses, and (D) burying carcasses followed by a spray of lime powder for decontamination of the burial site.

Furthermore, health education is paramount for control of disease spread during anthrax outbreaks. Important and targeted messages are aired to the affected communities through public address, leaflets, and use of the most respected local leaders like 'the Laigwanan' in the Maasai community. These leaders are more influential to the society so the messages given by them are highly trusted and comprehended by their community members. An example of a session of health education delivered during the current study is shown in **Figure 8**. The key messages provided include: (i) don't open the carcass of animals suspected to have died from anthrax because by doing so, you expose the spores into the aerobic environment and they can sporulate and cause more outbreaks, (ii) don't eat raw and/or undercooked meat from dead animals, (iii) take your animals for vaccination depending on the schedule given by your local veterinary expert and, (iv) when fall sick go to the neighboring health facility for medical attention.

Figure 8: Photograph (**own source**) showing a well-informed Laigwanan delivering health education to the Maasai community during anthrax outbreak in Monduli district, Northern Tanzania.

The Tanzanian surveillance systems

Human health surveillance system

Tanzania continues to face various public health threats, which require a timely response in order to avert death and disabilities. Having a robust surveillance system is a key element towards achieving this goal. The Health Management Information System (HMIS) is the main surveillance system in Tanzania, but the Integrated Diseases Surveillance and Response system (IDSR) is placed for surveillance of diseases, which require rapid response including epidemic-prone diseases (like anthrax), among others (Rumisha et al 2007). The IDSR system has started an electronic reporting system in more than 15 regions in Tanzania and it has immediate and weekly reporting schedules. In this surveillance system, a standard case definition for anthrax has been developed. At **Health Facility level**) a **Suspected anthrax** case is any person with acute onset of illness characterized by several clinical forms (i) Localized form, (ii) Systemic forms-Gastrointestinal, Pulmonary, Meningeal and a **Confirmed case**: Laboratory confirmed of *B. anthracis* from clinical specimen. At **Community level** any person with fever, difficulty in breathing, skin conditions or abdominal pain or altered consciousness, in a person with a history of contact with sick or dead animal.

According to IDSR, all health facilities, Points of Entry (PoE) and any other location (in conjunction with a nearby community) must report the total number of cases and deaths of the IDSR priority diseases observed in a given period (for example, monthly or weekly). This number of cases is analyzed and the results are used to monitor progress towards disease reduction targets, measure achievements of disease prevention activities in the district, and identify unforeseen outbreaks or problems so that early action can be taken. Immediate reporting is indicated when an epidemic-prone disease or other potential public health events of international concern (PHEIC) is suspected or is otherwise required under the International Health Regulations (World Health Organization 2005).

Tanzania is also implementing a community-based diseases surveillance system in which community health workers are engaged on diseases surveillance and response as far as rumors and public health events are concerned (Ministry of Health, Community Development, Gender, Elderly and Children 2017). Currently, community health workers are being chosen by the community members themselves and they are given a formal training for conducting community-based diseases surveillance in collaboration with a nearby health facility where they submit their weekly reports which are reported to higher levels through the existing reporting systems.

Animal health surveillance system

The animal health information system (AHIS) simply refers to a database for collection, storage, analysis, and reporting of animal diseases and their determinants with the objective being to (i) enable informed decision/s (ii) basic information which is unbiased and of known precision (iii) support implementation of disease control programs and (iv) meet international disease reporting obligations. Data sources are farms, laboratories, clinics, livestock markets, slaughter facilities and dip tanks. More than 80% of the disease information is based on clinical observations and 95% of the system is paper-based (outbreak investigation reports, surveillance reports, and treatment reports). The structure of animal health system is composed of community animal health service under the public sector, and animal health care centers and clinics under the private sector (OHCEA report 2011).

The animal health experts who are working with wildlife institutions in Tanzania like Tanzania Wildlife Research Institute (TAWIRI), Tanzania National Parks (TANAPA), and Ngorongoro Conservation Area Authority (NCAA) conduct the wildlife health services. The wildlife veterinary researchers and other scientists who are officially registered by TAWIRI to conduct various wildlife health research projects in Tanzania also provide information on a regular basis regarding the health status of the wild animals. In addition, the District Veterinary Officers offer health services to wildlife after being approved by the TAWIRI in the event where there is no wildlife Veterinarians. Laboratory analysis of samples is done at veterinary investigation centers (VICs), TAWIRI Wildlife Laboratories, Tanzania Veterinary Laboratory Agency (TVLA), and some other laboratories outside Tanzania. The reports on wildlife diseases reports are submitted through the Veterinary Section at TAWIRI and occurrence of any disease is reported to the Director of Veterinary Services (OHCEA report 2011).

In the Serengeti ecosystem of northern Tanzania, seroprevalence surveys have been reported in herbivorous species often hunted for bush meat (wildlife which are killed for human consumption) (Lembo et al. 2011). Spillover infections in wildlife can sustain the disease and become a source of spillback infection to humans and livestock (Thompson 2013). Spillover/spillback can be explained as an event of which a disease causative pathogen can move from one species (i.e. animals) into another species (i.e. humans) and vice – versa, resulting into an outbreak (Streicker and Pedersen 2012).

A One Health approach to control for zoonotic diseases

While the established systems are ambitious, their qualities are not too well documented, and this was one of the starting points for this thesis work. The poor surveillance system in the human health sector regardless of the electronic IDSR system has contributed to a failure to estimate the burden of anthrax in the country including in the hotspot areas of northern Tanzania. This is compounded by the lack of well-structured surveillance system in the animal sector.

Nevertheless, currently, there is no legal binding regulation for the sectors to have a mutual understanding for sharing surveillance information on a regular basis. To address these challenges a One Health Approach was recommended and Tanzania has established a One Health Coordination desk and developed a strategic plan for 2015-2020 (One Health Strategic Plan 2015). It has been recognized that the problem lies in a slow pace of changing the mindset of professionals from working continuously in their respective Ministries, professional organizations, and sectors. Various sub-committees were established to oversee different clusters which all aimed to come up with key intervention strategies for addressing various health-related issues in a "One Health Approach". Among the clusters is the "One Health Disease Surveillance, Prevention, and Control" which needs a well-structured collaboration between sectors responsible for humans, livestock, wildlife, and environmental health to ensure for prevention and control of zoonotic diseases (Thompson 2013) (**Figure 9)**. In this case, veterinary services are managed under the Ministry of Livestock and Fisheries development (MLFD); public health service is managed under the Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) at the national level and at sub-national (regional) level, by the President's Office

Regional and Local Administration (PORALG) monitors the implementation of all activities at district and lower levels, while the wildlife service is managed by the Ministry of Natural Resources and Tourism (MNRT) and the environmental affairs are under the Vice President's Office (One Health Strategic Plan 2015). Therefore, the coordination of all these Ministries is undertaken under the Prime Minister's Office, through the One Health coordination desk, which was officially launched in February 2018.

Figure 9: The One Health Triad showing the collaborative efforts to obtain optimal health for humans, livestock, wildlife, and the environment. **Credit**: R.C. Andrew Thompson, 2013

The affiliated academic and research institutions or agencies within each respective ministry provide technical support. Such agencies and institutions include Tanzania Veterinary Laboratory Agency (TVLA), Tanzania Livestock Research Institute (TALIRI), and National Institute for Medical Research (NIMR), Tanzania Food and Drug Authority (TFDA), Government Chemist Laboratory Agency (GCLA) for Public Health, Tanzania Wildlife Research Institute (TAWIRI), Tanzania National Parks Authority (TANAPA), Ngorongoro Conservation Area Authority (NCAA), Sokoine University of Agriculture (SUA), Muhimbili University of Health and Allied Sciences (MUHAS), and the Tanzania Wildlife Research Institution (TAWIRI) (OHCEA report 2011).

Anthrax outbreaks in humans, livestock, and wildlife species have been recurring in the game reserve and protected areas without anyone knowing clearly the reasons for their occurrences. This indicates that people from those areas probably are not aware of the disease and therefore they get exposed to potential sources of *B. anthracis* infection. However, despite the frequent occurrence of anthrax outbreaks in Tanzania, there is no comprehensive survey that reports on the magnitude and spatial distribution of the disease from the human, livestock, and wildlife health sectors (Zhang et al. 2016).

Cultural practices may influence the distribution of anthrax between men and women. For instance, men in the Maasai community are reported to be at a higher risk of acquiring anthrax than women (Odontsetseg N et al 2007). This may be attributed by the fact that men prefer to slaughter, handle and eat undercooked meat from the carcass of dead or slaughtered sick animals without being inspected by a designated livestock officer (Chen et al. 2016). More often they also eat undercooked or raw meat while grazing their animals in the wilderness, only bringing home any remaining meat and offal for the wives and children. Moreover, men are the decision makers of the family who also dictate whether women and children should go to the hospital when they fall sick. This has impacted on the health-seeking behavior of females creating a false representation in hospital registers regardless of the true disease status.

Effective clinical management of zoonotic diseases depends on various factors including the health-seeking behavior of individuals in a respective locality. For those who manage to attend the health facility seeking for medical care, it is a common practice for medical practitioners not to consider anthrax infection especially the gastrointestinal and respiratory forms of infection in their list of differential diagnoses. This leads to a continued misdiagnosis of anthrax ultimately causing false documentation of the burden of zoonotic disease in Northern Tanzania (John, Kazwala, and Mfinanga 2008).

In different aspects of food-borne diseases inspection in the Tanzanian food processing industries and/or local markets, risk assessment and setting appropriate control measures is paramount. In this aspect, there is limited consideration of anthrax among the potential food-borne diseases; which is transmitted through consumption of contaminated raw and/or undercooked meat from sick and dying cattle, sheep, goats, camels (Newell et al. 2010). This has led to lack of awareness of anthrax-related food-borne diseases in the food chain value of northern Tanzania and beyond, and therefore it is poorly reported and documented (Zadoks, R; and Crump 2017).

Knowledge Gaps

Anthrax and how it may spread has been known for more than a century, and we also understand some of the disease dynamics in a Tanzanian context. However, when the study was initiated, we observed a set of knowledge gaps of importance for prevention and control of anthrax in the country as follows:

1. While reports indicated a large difference between the frequencies of reported outbreaks in various parts of Tanzania, it was unclear how much this depended on the quality of the operating reporting systems.
2. The comparison of reported anthrax outbreaks in humans and animals had not been done, an indication of a lack of One Health approaches in disease prevention and control in the human-animal-environmental health interface areas of Tanzania.
3. While a known relationship between anthrax in humans, livestock, wildlife, and the environment has been reported in several places, a detailed assessment of this relationship in Tanzania had not been done.
4. The drivers of infection in the hot-spot areas were not known, a key to understanding how the disease can be prevented.
5. There was a lack of knowledge about the importance of ecological factors (humidity, temperature, vegetation, livestock density, and soil pH and soil types.) as drivers for anthrax outbreaks in Tanzania.
6. Reporting (through formal and informal channels) and surveillance systems for human and animal diseases have been operating, but there was a clear need for an improvement of the surveillance systems for zoonotic diseases in general, as they mostly depend on collaboration between professionals and authorities in human and animal health sectors as well as wildlife.

Aims and objectives

The overall aim of this study was to identify drivers of infection and describe options for prevention and control of anthrax outbreaks in the human-livestock-wildlife-environmental health interface areas of northern Tanzania within a One Health approach.

Specific objectives

1. To identify hotspot areas of anthrax outbreaks in the human-livestock-wildlife interface areas of northern Tanzania (**Paper I**)
2. To assess for the efficiency of the existing structures for response to anthrax outbreaks using a One Health approach (**Papers II**)
3. To determine the risk factors associated with frequent anthrax outbreaks in the hotspot areas of northern Tanzania (**Papers III**)
4. To determine the influence of climatic and environmental factors for the spatial distribution of *Bacillus anthracis* spores in Tanzania and propose for appropriate control and preventive measures (**Paper IV**)

Materials and methods

Study areas

In **paper I**, after compiling the national data for humans and livestock (for the whole of Tanzania), the focus of the study was on the identified hotspot areas of northern Tanzania (**Arusha** and **Kilimanjaro** regions) for more detailed studies of anthrax outbreaks in humans and livestock as well as wildlife. **Figure 10** shows a detailed map of these regions. For **Papers II & III**, the study areas were the identified hotspot districts for anthrax outbreaks in northern Tanzania which were also used to collect the occurrence data for predicting the geographic distribution and persistence of *B. anthracis* spores in the whole country (**Paper IV**).

The Arusha region lies on the Kenyan border, encompassing savannahs and part of the Great Rift Valley. Wildlife-protected areas include the Ngorongoro Conservation Area (NCA), which contains the massive Ngorongoro Crater, and Arusha National Park, which covers volcanic Mount Meru. There are also Manyara National Park, Tarangire National Park, Grumet Game Reserve, and Lake Natron game Reserve. In this region, the selected study districts were Meru, Ngorongoro, and Monduli district councils.

Kilimanjaro region is home to a part of Kilimanjaro National Park (KINAPA). The region is bordered to the north and east by Kenya, to the south by the Tanga region, to the southwest by the Manyara region, and to the west by the Arusha Region. The selected study districts in this region were Hai, Moshi rural, Rombo, and Siha district councils.

More detailed descriptions of materials and methods can be found in the publications from this study which are referred to in this thesis by the roman numerical number I – IV.

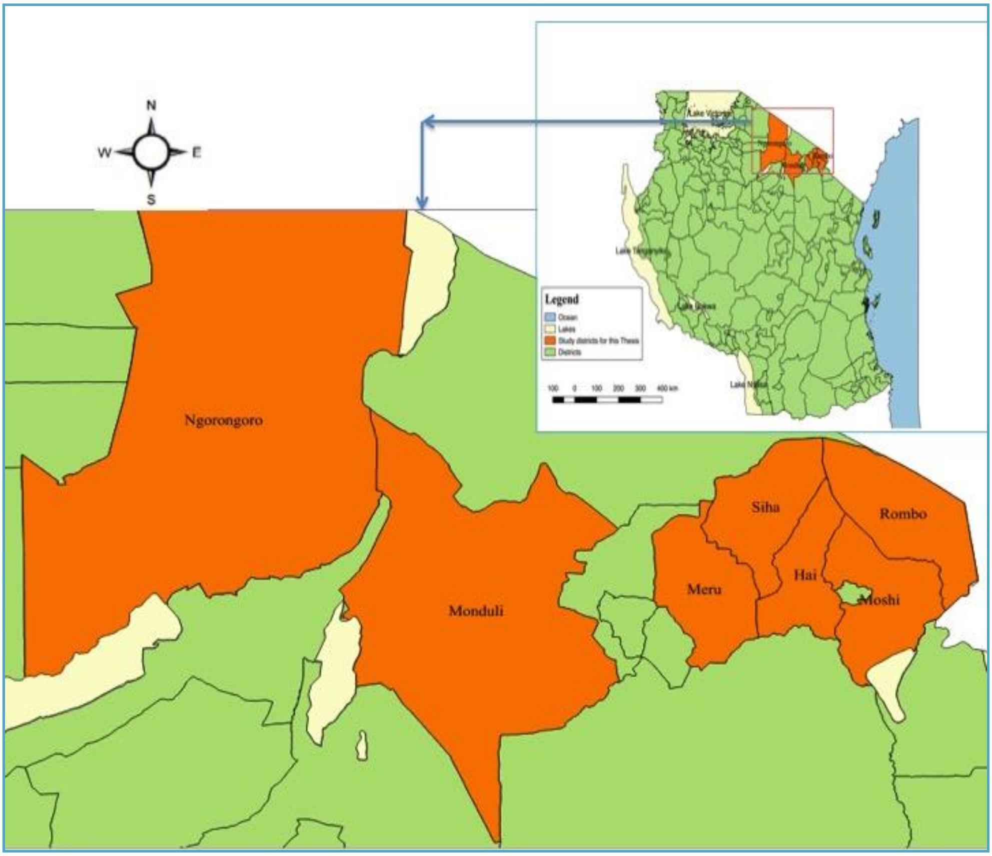

Figure 10: Map of Tanzania Mainland showing the study districts for this thesis in Arusha and Kilimanjaro regions of northern Tanzania (**Drawn in QGIS Software**)

Study population

After the initial study covering the whole of Tanzania, the study population consisted of different subsets of human and animal populations in the interface areas of the **Arusha** and **Kilimanjaro** regions. The initial data collection was done from September to November 2016 (**Papers I, II and III**), various administrative and fieldwork activities for this research are shown in **Figure 12**. Additional data were collected in February 2018 as a follow-up study, which led to the ecological niche modeling of *B. anthracis* spores in Tanzania (**Paper IV**).

Study design and implementation

The series of papers are illustrated in **Figure 11**. These studies assisted to develop evidence-based preventive and control strategy by involving public health, veterinary (livestock and wildlife) and environmental health sectors in the country on how they can work better together using a One Health approach.

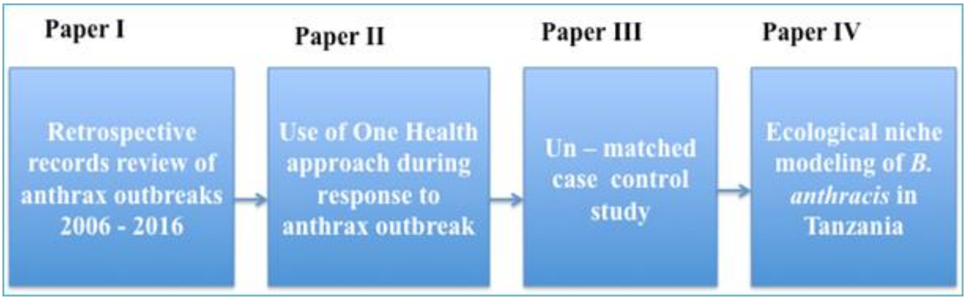

Figure 11: The research process for this thesis indicating a series of developed articles

For **Paper I**, the temporal trends and identified hot-spot areas of occurrences of anthrax outbreaks at the human-livestock and wildlife interface in a period of 10 years (2006-2016) were described. A retrospective review of reported anthrax outbreak records was conducted from the human, livestock, and wildlife surveillance systems of Tanzania from January 2013 to December 2016. This was followed by a more thorough examination of data from Arusha and Kilimanjaro regions for 2006 to 2016. During the follow-up in Arusha and Kilimanjaro regions, data from Health facilities, the Tanzania Wildlife Research Institute (TAWIRI), Serengeti National Park, Tanzania Veterinary Laboratory Agency (TVLA), District Veterinary Offices, Livestock Field Offices at the Ward and Village Levels and District Medical Offices were used as data sources. These institutions were used as sources of data for a comprehensive retrospective records review of anthrax outbreaks in the humans, livestock and wildlife interface areas of Northern Tanzania for a period of 2006 to 2016.

At the health facility and animal diagnostic centers**,** data reviews were carried out through the health management information systems (HMIS) for both inpatients and outpatient’s booklets at health facility level. Formal and informal meeting minutes, internal memos and official outbreak notification letters and raw data in the form of typed or handwritten reports, tables and spreadsheets from the district medical and veterinary offices were also captured solely for this purpose. The global positioning system (GPS) points were recorded at each level of record review. Moreover, animal anthrax data were reviewed from the laboratory units of TVLA in Arusha and TAWIRI in Serengeti National Park.

For **Paper II,** an anthrax outbreak investigation and response using a **One Health approach** in one of the identified hot-spot district was established. Using both qualitative and quantitative methods for livestock, wildlife, humans, and environmental health sources of the epidemiological pattern of the outbreak was described.

The obtained information on anthrax cases was used to trace these cases at a community level through their village leaders. The traced and found cases were asked verbally to consent for participation in this study and upon agreeing, other ethical related procedures were dealt with. Later on, a control was purposively selected in a neighborhood of a case.

The use of questionnaires and other sources of community information were implemented in an **unmatched case-control study** (**Paper III**) in order to establish a causal pathway for disease transmission in the identified hotspot areas. The minimum sample size for the case-control study was calculated using the online epidemiologic ***Epitools AusVet sample size calculator*** (http://epitools.ausvet.com.au/content.php?page=Sample Size) with the assumption that the frequency of exposure in controls was 20%, and the odds ratio was to be detected at 3.0, with 80% power and 95% confidence interval. With these assumptions, the minimum sample size of 61 cases and 61 controls was calculated. A total of 59 cases and 59 corresponding controls were subsequently recruited.

A case was defined as any person residing in the selected hotspot districts of Northern Tanzania who had ever developed skin lesions by itching of the affected area followed by papular lesion vesicular stage over 2–6 days, eventually developing into depressed black eschars, sometimes accompanied by mild or severe edema. A case was eligible for inclusion in the study if found recorded in the medical register at a randomly selected health facility in the hotspot districts of Arusha and Kilimanjaro regions during the past two weeks before the beginning of this study. The patient should have met the case definition for cutaneous anthrax (as defined above) and his/her name found registered in medical records and had resided in the hotspot districts of Arusha and Kilimanjaro regions for not less than six months before the time of recruitment.

A control was defined as any person who resided in a neighborhood with an eligible case and had not contracted cutaneous anthrax during the past six months. This study excluded anthrax suspected cases with a history of coming from other places apart from Arusha and Kilimanjaro regions in a period of one week before onset of signs and symptoms of anthrax. Children of ≤18 years old were included in the study, but their parents/ guardians were interviewed as a proxy on their behalf.

A semi-structured questionnaire was developed in English to be administered to both cases and the controls. The questionnaire included questions related to potential biological exposure to *B. anthracis* as well as information about demographic factors such as age, sex, occupation, and ethnic group, level of education, district/place of residence, and potential links to travel outside the village in the last two weeks before onset of the disease. The questionnaires were pre-tested, and necessary changes were made based on the identified ambiguities. The questionnaire was subsequently translated into Kiswahili, the national language spoken by most Tanzanians.

For **Paper IV**, findings from papers I, II and III were used as a background to establish an **ecological niche model** in order to better understand the epidemiology of anthrax in the hot-spot areas as they are affected by ecological factors linked to climate and soil variables. A database of 191 mixed cases of humans, wildlife and livestock was constructed from sporadic anthrax outbreaks, which occurred in different places of Northern Tanzania from October 2016 to March 2018. A total of 23 environmental covariates were obtained from open-access satellite-linked climatic and biophysical variables. The *Worldclim* database was used to download these variables representative for distinct temperature and precipitation measurements (www.bioclim.org). Various administrative and fieldwork activities for this research are shown in **Figure 12.**

Figure 12: Photographs showing events during a fieldwork for this thesis. (**A**): questionnaire administration, (**B**): courtesy call to the Kilimanjaro administration prior to the launch of a fieldwork, (**C**): records review at Kibosho hospital, (**D**): specimen collection from a wildebeest carcass during a response to anthrax outbreak in Selela ward, Monduli district – northern Tanzania.

Laboratory methods

The studies were based upon various diagnostic procedures in various human health, animal health and wildlife laboratories. The different papers describe the specific use of these techniques.

The **human specimens,** and especially skin lesion swabs were tested by Gram or Methylene blue staining techniques for anthrax in some of the selected health facilities. Other health facilities were managing suspected human anthrax cases clinically as they did not have any diagnostic capacity in place. However, health facilities not able to diagnose anthrax specimen collected from anthrax suspected cases and shifted them to TVLA in Arusha town for Giemsa staining to be able to appropriately manage the patients. With this approach, it takes about 3 days from the time specimens were collected until when they receive back the laboratory results, depending mainly on the distance of that health facility to Arusha town.

Moreover, all the anthrax sample remnants were immediately autoclaved at 121°C and then incinerated into ash in order to avoid disease transmission to humans, animals, and contamination to the environment. These procedures were done in a laboratory with biosafety level-2 Cabinet at TVLA in Arusha and Dar es Salaam, Kilimanjaro Clinical Research Institute (KCRI) and National Health Laboratory Quality Assurance and Training Centre (NHLQATC) in Dar es Salaam, Tanzania.

The collaboration of human and animal laboratories through sharing of equipment, reagents, consumables, and staff during testing for anthrax specimen from human, environment, and animals (livestock and wildlife) is a good indication of the implementation of One Health during response to public health threats in Tanzania. This was evident when I was allowed to process some of the animal specimens at KCRI and TVLA regardless that I have a background of Public Health. The TVLA, in Dar es Salaam is producing various animal vaccines including 34F Sterne for anthrax control, which is a surrogate for preventing the disease in humans if it is stored, transported and administered in a proper way.

From domestic animals, blood samples of 5 ml were collected from the jugular vein of well-restrained adult cattle; goats and sheep (within the households with reported human anthrax cases) separated into the plain and EDTA vacutainer tubes. The samples in the plain vacutainer were left to clot at a room temperature overnight and subsequently centrifuged at 3000 rpm for 10 minutes to obtain clear serum which was aliquot in cryovials and stored at -20°C until serological analysis by ELISA. Other blood samples in the EDTA vacutainers were stored in a refrigerator for molecular laboratory analysis.

In addition, real-time qPCR test was done as described by Turnbull (Turnbull TCB 1998). The target plasmids were pX01 which is a tripartite toxin complex for protective antigen (PA), edema Factor (EF) and lethal factor (LF) while pX02 is for a capsule, the relevant primers were used and interpretation of results was conducted according to the manufacturer's instructions. The animal (livestock and wildlife) specimens are routinely processed at the three diagnostic centers in northern Tanzania, i.e. TVLA in Arusha Township, KCRI in Moshi Township and TAWIRI Serengeti Centre in the Serengeti National Park.

Data management and analyses

For **Paper I&II**, the descriptive data analysis was done in an Excel spreadsheet before the dataset was exported into Stata software SE for Windows version 14 (Stata Corp, College Station, TX) for further analysis. The Quantum GIS (QGIS) (https://www.qgis.org/en/site/) software was used to create maps using geo-coordinate points collected at every eligible household and also for making the map of Tanzania to show the country's profile and locating the study areas. The measures of levels and variability in regard to anthrax infection and possible risk factors were determined and the results were reported with a 95% confidence interval.

For the case-control study in **Paper III**, considering the questionnaire used in this study, it was noticed that there were groups among our investigated population which were characterized by different patterns of behavior, caused by different preferences, which could lead to anthrax. Still, there was no single specific variable, describing such behavioral differences. Hence, a latent class analysis (LCA) method was used to analyze the data in Stata software SE for Windows version 15 (Stata Corp, College Station, TX). All variables linked to the expected exposures to anthrax were used to construct two Latent Classes using the generalized structural equation modeling (*gsem)* command in Stata. The binomial family and the logit link function defined the variables. The study subjects were classified with a probability of belonging to an **Exposed** class and the rest as **Not exposed** by using a posterior probability of greater than 0.5 as a cut-off/threshold between the two classes.

Before building the Maxent model in **Paper IV**, a pairwise Pearson correlation analysis for environmental variables was done using ENMTOOLs. This was done in order to ascertain whether the environmental variables and species presence data were spatially auto correlated. Only variables with a correlation < ±0.75 were retained for model fitting. After this procedure, the non-correlated environmental variables were chosen for the development of a species distribution model. The area under the curve (AUC) as a receiver operating characteristics (ROC) was employed to assess the predictive performance of the obtained 4 best model subset-using measures of specificity (absence of commission error). The ROC analysis is a threshold and sensitivity (absence of omission error). A receiver operating characteristics (ROC) of the curve is mostly generated to evaluate the accuracy of the performance of the model at all-important points. Evaluation of the important individual environmental predictors in the model development was done by a jackknife test. In the Maxent test, an area under the operating characteristics curve (AUC) of 0.93 was obtained indicating that a model had good ability to predict the presence of *B. anthracis* spores in the hotspot areas of Tanzania.

Ethical consideration

The National Institute for Medical Research (NIMR) in Tanzania approved this study (Research Permit Number NIMR/HQ/R.8a/Vol.IX/2286), and the authorities of TAWIRI, TVLA, and Ministries of Human health, Livestock, Kilimanjaro and Arusha regions together with authorities in the selected study districts. Altogether, granted permissions to review historical data, hospital and laboratory records. Verbal informed consent was sought from all human subjects before being involved with study activities. For underage (<18 years), parents or guardians consented on their behalf.

In the whole process of data collection, confidentiality of human data was strictly observed by not exposing details of medical records, by using unique identifiers instead of their real names and only the information required for the purpose of this study was reviewed. The dissemination of the research findings was done to authorities at various levels in order to inform for policy changes towards having a holistic One Health approach in the management of anthrax outbreaks in the country. Moreover, positive results obtained from human suspected cases were communicated back to relevant health facilities in order to assist in the further clinical management of those cases.

Main Results

Summary of individual papers

Paper I: Anthrax outbreaks at the human-livestock and wildlife interface areas of Northern Tanzania: a retrospective records review (2006-2016)

The objectives were (i) to identify the districts assumed to be an anthrax hotspots in Tanzania Mainland, (ii) to evaluate the efficiency of the anthrax reporting and response system and diagnostic capacity at national, regional and district levels, (iii) to describe the epidemiology of anthrax in the hotspot areas and (iv) to identify potential areas for further observational studies to better understand the complex epidemiology and ecology of anthrax.

In this study, it was found that the reported human anthrax incidence rate over 2013-16 per 100,000 population was much higher in Arusha region (7.88/100,000) followed by Kilimanjaro region (6.64/100,000) than any other regions of the Tanzania Mainland, and these regions were identified as hotspots for anthrax in the country. Records from selected health facilities showed that there were 187 human anthrax cases (57%) in Kilimanjaro and 143 (43%) in Arusha region for the period of 2006- 2016. The majority (86.1%) of all human anthrax cases reviewed at the selected health facilities were of the cutaneous form and a majority of these were males, (65.2%). Most of them were managed clinically due to lack of diagnostic capacity in these health facilities.

It was also found that there were human anthrax cases in record books since 2006 and beyond despite the disease not being included in the HMIS and IDSR reporting forms at that time. Overall, the Ngorongoro district reported more human anthrax cases, (80%) compared to other districts of Arusha and Kilimanjaro regions. The Endulen Hospital reported more human anthrax cases (52.2%) compared to other health facilities in Ngorongoro district in the period of review.

From 2006 to 2016, TVLA received a total of 161 specimens from different livestock and wildlife species, which were anthrax suspect for laboratory analysis. Most of the submitted specimens came from bovine (66%). A total of 103 specimens (64%) tested positive for *B. anthracis*, and 68 (66%) of the positive specimens came from bovines, followed by 18 caprines (17%). In the same period, a total of 57 wildlife specimens obtained from active surveillance done in the Serengeti ecosystem were tested for anthrax at TAWIRI laboratory located in the Serengeti National Park. Of these 18 (32 %) were positive for anthrax of which most of them came from African buffalo, *Syncerus caffer* (67%). Anthrax outbreaks have occurred across human, livestock and wildlife populations with peaks of outbreaks recorded in March and September through November and this corresponds to specific environmental and climatic condition.

Through records review, Arusha and Kilimanjaro regions were identified as the key hotspot areas for anthrax outbreaks in Tanzania Mainland affecting humans, livestock and wildlife due to exacerbated interactions between animals and humans residing in close proximity to protected areas i.e. National Parks and Game Reserves.

Paper II: Prevention, detection, and response to anthrax outbreak using One Health Approach: a case study of Selela Ward in Monduli District

The objectives of this paper were (i) to create and strengthen regional, district, and village multi-sectoral teams to manage the outbreak using a One Health approach, (ii) to determine the magnitude of anthrax outbreak in humans, livestock, and wild animals, and (iii) to sensitize the community on their involvement in the disease prevention and control, the team also intended to address challenges facing the community on prevention and control of anthrax, and other epidemics.

In this article, a survey on the anthrax outbreak was done in Monduli district where a total of 131 carcasses of wildlife were counted, the majority (83%) being wildebeest (*Connochaetes taurinus)*. Other carcasses of wild animals (16%) were for grant gazelle (*Nanger granti)* and 0.8% for the rabbits (*Oryctolagus cuniculus*). Out of 21 human suspected cases, the majority were under 5 years of age (42.9%) followed by the age group of 6 -15 years (33.3%). Methylene blue staining and real-time rtPCR techniques were used to confirm the existence of the anthrax outbreak in Selela Ward. Samples were amplified either with Pag (pXO1) or Cap (pXO2) that was run with control samples amplified with a standard curve CQ value average of 34.6. Local officials further reported that livestock carcasses (10 cattle, 26 goats, and three sheep) were either consumed or hidden by owners.

The human index case was reported on 5th November 2016 through the electronic-IDSR system, and this was preceded with the verbally reported deaths in livestock and wild animals in Selela village in Monduli district. A total of 21 human anthrax suspected cases were mostly (61.9%) from the villages of Selela ward. Other affected wards in different proportions were Esilalei (28.6%), Lepurko (4.8%), and Mswakini (4.8%). The number of cases started to decline after a national multi-sectoral team was deployed and conducted an intensive health education campaign for appropriate anthrax prevention and control in the affected areas in Selela ward.

The livestock kept by the Maasai in Monduli district were not vaccinated against anthrax. The Maasai spend most of their time taking care of their animals, and the grazing environment makes their domestic animals in close contact with wildlife. The local people were observed dressing dead livestock and wildlife for human consumption, and women and children mostly did the carcass dressing from dead animals. The dried hides and skins from such animals were used as bedding materials and also as ropes or donkey luggage pockets and wildebeest tail brush as swats for chasing out flies. These activities expose them to the risk of contracting zoonotic diseases including anthrax.

A total of eight affected households were visited, all were close to areas where wildebeest carcasses were found, and most of the members of the households admitted to having been consumed meat from the anthrax related carcasses. The heads of household reported grazing their livestock near places where some of the decomposing wildebeest were scattered on the ground. It was also reported that there were few livestock extension officers for providing extension and veterinary services to livestock and for meat inspection. This led to livestock keepers treating their animals themselves after buying antibiotics and other veterinary drugs from the street vendors. It was a common practice for the livestock keepers to administer veterinary drugs through wrong routes and also giving wrong doses. Livestock keepers requested a response team to investigate on the suspected poisonous grass called *endule* in the Maasai language, which was believed to cause livestock mortalities during the beginning of every rainy season.

Finally, a local multi-sectoral group of experts and local leaders was formed in Selela ward to ensure for early reporting of suspected anthrax cases and other epidemic-prone diseases in humans and animals from their areas, and also to trigger for a timely response and prevention of further spread of the disease.

The formed team composed of livestock field officers, Clinical Officer, Community Development Officer, Ward Health Officer, Community Health Worker, Wildlife Officer, Agriculture Extension Officer, Village Chairperson, Village Executive Officer, Ward Executive Officer, and Councilors. The terms of reference were developed to describe the roles and responsibilities of each team member.

A One Health multi-sectoral Team was also developed at Arusha regional level comprising members from Monduli district (Human, livestock, and wildlife Departments), TAWIRI, TANAPA, Tanzania Wildlife Authority, Nelson Mandela African Institute of Science and Technology, and Regional Secretariat (Veterinary Services, Human health, and Tourism Services). The developed team agreed to conduct joint meetings once per week and developed outbreak response action plan 2016-2018.

A multi-sectoral collaboration using a One Health approach is crucial for the effective management of anthrax outbreaks and other zoonotic diseases in the sense that, it fosters for cost-effective mechanisms during outbreak response. A One Health model team was formed in the Arusha region and Monduli district, with terms of reference (ToR) and schedules of conducting their meetings was put in place. This team is still available and is working on responding to various public health threats in the region through health education on how to avoid contracting the disease and conducting joint investigation of various forms of public health emergencies. Using a One Health model with joint fieldworks depending on the nature of the outbreak and sharing of information on a timely fashion is adhered to; this can also be extrapolated to other regions in the country.

Paper III: Risk factors for human cutaneous anthrax outbreaks in the hotspot districts of Northern Tanzania: unmatched case-control study

The objectives of this study were (i) to identify demographic and behavioral factors associated with human cutaneous anthrax outbreaks in the identified anthrax hotspot areas of northern Tanzania, and (ii) to understand the causal relations of risk factors for anthrax outbreaks and give advice for potential intervention strategies in the region.

The study found that cases were recruited from Hai (n=6, 10.2%), Meru (n=3, 5.1%), Monduli (n=20, 33.9%) Moshi DC (n=3, 5.1%) Ngorongoro (n=12, 20.3%), Rombo (n=7, 11.9 %) and Siha (n=8, 13.5 %). Among the study participants, there were more male participants (n=70, 59.3%) compared to female (n=48, 40.7%). The age range was 1–80 years with a median age of 32 years. A total of 83 (70.3%) of the study subjects had no formal education. During analysis, it was realized that younger cases (1-20 years) were more recruited, with 26 (44.1%) of the 59 cases, while only four controls, (6.8%) were from this group.

In the univariable logistic regression analysis, study subjects were initially grouped into four age quantiles and subjected to cross-tabulations with demographic characteristics (sex, education status, and occupation) and history of travel, biological factors (skinning/burying dead animal, contact with animals, contact with animal products, and type of sleeping materials). These are the factors which may predispose an individual to anthrax infection while other variables were source of animal feeds, knowledge on the animal diseases preventable with vaccine, disposal of animal carcasses, death of animals at home steady and keeping animals/dogs.

The results from the subsequent multivariable logistic model indicated that having primary school education was protective against getting anthrax infection. There was no statistical association between knowing any of the animal’s vaccine preventable diseases and anthrax. Increasing age, 21-30 years (OR = 0.07) and 31-40 years (OR = 0.08) appeared as protective against acquiring anthrax infection compared to the younger age group, 1-20 years.

The latent class analysis (LCA) model was used to shift the focus from simple associations in the multivariable analysis model to describing a potential causal pathway of the exposures and anthrax infection with age and education being used as primary variables, based upon the assumed causality. The level of education was not considered for the youngest age group (1-20 years). The LCA showed that (61.9 %) of the study subjects had a high probability of being classified as exposed, while (38.14%) of the study subjects were in the unexposed group. Since there was a strong age bias among cases, two final structural equation models; one with the youngest (1-20 years) group and one for the older group (>20 years) were established.

In the youngest group, exposure status was strongly linked to anthrax transmission (OR= 25.0, 95% CI = 1.5-410). In the older age group, the link to exposure was weaker (OR = 3.2, 95% CI = 1.28-8:00). The most distinct difference between the SEM model and the ordinary logistic model was that education was identified to be linked to the model as a predictor of exposure, but not directly to anthrax.

There is a strong connection between gender inequality, economic status, and level of education, keeping livestock, cultural practices and acquiring anthrax infection in a given community. This is because poor families cannot afford to take their children to school, which is linked to the fact that these children are spending most of their time for keeping livestock and/or engaging themselves in agricultural practices. All these activities are predisposing them to anthrax transmission through breathing polluted air, which is contaminated with anthrax spores, or getting into direct contact with infected animals or animal products.

Paper IV: Ecological niche modeling as a tool for prediction of the potential geographic distribution of *Bacillus anthracis* spores in Tanzania

In this study, the objectives were (i) to predict for the potential geographic distribution of *B. anthracis* spores in Tanzania and (ii) to produce the epidemiological and ecological evidence for effective management of anthrax outbreaks in Tanzania. In the Maxent model, an area under the operating characteristics curve (AUC) of 0.93 was obtained indicating that the model had an 'excellent' ability to predict for the persistence of the *B. anthracis* spores in the most risky areas of Tanzania Mainland. The Maxent test indicated that the difference between the AUC from model prediction and the AUC at random is statistically significant, showing that the model performs better than random prediction.

Out of the 23 environmental variables, variables identified as non-collinear included isothermally (BIO3), temperature seasonality (BIO7), moisture index arid quarter (MIAQ), potential evapotranspiration (PET), soil types and soil pH. The percentage contribution of each of these variables indicated that the soil types demonstrated a high percentage contribution (56.5%) followed by pH (23.7%) so the two (soil type and pH) variables in total contributed by 80.2%.
The Jackknife test of variables indicated that omitting any of those six variables affects the regularization gain; AUC and test gain in the model. It was found that the soil types were the most important contributing variables to persistence and environmental suitability for *B. anthracis* spores followed by soil pH. However, pH decreases the gain the most when removed from the model. By looking at the AUC of the Jackknife test, the most significant variables with scores of > 0.7 (above fair) were soil type, soil pH, BIO3, and BIO7. The response curve for soil pH shows that the probability of geographic suitability increases with the level of alkalinity (corresponding to high levels of calcium) in the soil.
Soil type, soil pH and isothermally were the most important variables, with soil types as the single most important variable, accounting for 56.5% to the model prediction with the following soil types identified as the most significant; solonetz, fluvisols, and lithosols.
The risk map indicated that regions with stable areas of high and very high risks were Arusha and Kilimanjaro from the northern part of the country, while other regions like Mara, Manyara, Simiyu, and Singida had few patches of high and very high-risk areas. Regions like Dodoma, Mwanza, Dar es Salaam, Lindi, Mbeya, Rukwa, Katavi, and Kigoma were predicted to have a medium risk in few locations and the rest of the regions in the country had low risks for geographic suitability of *B. anthracis* spores persistence.

This research has revealed that environmental factors like rainfall, temperature, and soil characteristics have a direct link to persistence and spatial distribution of *B. anthracis* spores in Tanzania. Based on this experience, it has been easy to prepare risk maps, which helped to predict for the potential risk areas, and a population at risk to experience continued anthrax outbreaks in some parts of the country.

Discussion

General discussion of results

This study aimed to assess for the benefits of using a One Health approach in order to understand the epidemiology and management of anthrax outbreaks in the human, livestock, wildlife and environmental health interface areas of Northern Tanzania. This overall aim was addressed through specific objectives which have been implemented in Papers I, II, III and IV. In the subsequent text key results, which have emerged from this work, are discussed paper-by-paper.

Paper I

This paper argues that the interaction between humans and animals through human looking for food (meat and milk) and use of animal skin as bedding materials facilitates the transmission of anthrax from animals to humans, in line with other findings (Mikolon et al. 2013; Molyneux et al. 2011). The disease starts from animals which acquire the disease during dry-hot climatic condition due to overgrazing on short contaminated grasses, nutritional stress and congregation of animals at some points which propagate the transmission of anthrax among the animals (M. Hugh-Jones and Blackburn 2009) and then spills over to humans. Identifying a seasonal pattern of a disease is very important because it helps the authorities to activate their preparedness capacities like stockpiling of livestock vaccines against anthrax, distribution of human prophylactic antibiotics to the communities at high risk and targeted health education campaign. All these should be implemented a few months before the expected periods for outbreaks. However, more emphasis should be given to the provision of livestock vaccination as a control measure of a disease in animals which will automatically control this disease in humans (Munang'andu, Banda, Siamudaala, et al. 2012).

Furthermore, anthrax outbreaks in animals have an economic implication in terms of losses through massive deaths of animals in the affected areas. The expenses of laboratory reagents and other consumables, and cost of carcass disposal through burial or incineration can be estimated, while other costs cannot be easily quantified, such as the cost of human illness, cost of absence from work due to illness and cost of not producing in the field due to anthrax related illness. Therefore, a tangible investment in the fight against this disease is needed in order to ensure for a better livelihood of the society (Mullins et al. 2015).

In this review, it was found that the diagnostic capacity was not only poor in the study areas but also in the entire country. The only available routine diagnostic technique performed at some health facilities were either Giemsa or methylene blue staining of fixed blood smears prepared from either suspected humans or animals. They also test skin lesion swabs collected from suspected humans with the same technique. A gross under-reporting of the electronic IDSR system was found in Arusha and Kilimanjaro regions, regardless of the fact that these two regions were among the first regions to use an electronic-reporting system in the country. Among the challenges identified were poor network connectivity of the mobile phone used for reporting, the existence of parallel reporting system between paper and electronic systems, and staff turnover, as those health workers who attended a training for electronic reporting were not staying permanently at the original health facility. Generally, many anthrax outbreaks are mismanaged especially in the rural areas where it is unlikely to have been adequately diagnosed nor reported on time to higher levels to guide for response decision.

The response to anthrax outbreaks which affects both livestock, wildlife, and humans, requires a multi-sectoral collaboration through a One Health approach and the focus should be on capacity building, research opportunities, surveillance systems and diagnostic capacities (Mbugi et al. 2012). The existing opportunity in Tanzania is the availability of the One Health Coordination Desk with a budgeted strategic plan for a period of 2015-2020 (One Health Strategic Plan 2015), a multi-sectoral national action plan for health Security (Mghamba et al. 2018), and a list of prioritized zoonotic diseases, anthrax being inclusive (Department of Health and Human Services USA 2017) which will help to start with as other milestones are being set.

Paper II

This paper revealed that the affected communities associated the massive death of animals to the fresh, lush pastures that follow long periods of drought season. This might be a reason for pastoralists to consume the anthrax-associated carcasses. Pastoralist might be aware of the risks associated with the consumption of raw milk, blood or undercooked meat (Chakraborty et al. 2012), but they still practice these risky behaviors especially in rural areas (Swai, Schoonman, and Daborn 2010). This is supported by other studies, which reported that the consumption of meat from dead animals could be attributed to poverty. To compensate the economic loss from a dead animals most of the community members eat, share or sell the meat from dead animals (Chikerema, Matope, and Pfukenyi 2013; Chakraborty et al. 2012).

As a control measure for anthrax, all the carcasses found lying on the ground were burnt. Effective burning destroyed carcasses so as to prevent further transmission of anthrax to other grazing and browsing animals. Burying was however tedious, time-consuming and required an extensive workforce for excavating the burial pit of 6 feet deep. A disinfectant (formalin 10%) was also required to be poured on top of the burial pit. In the described outbreak, all carcasses of wildlife irrespective of the species showed classical features of anthrax including exuding black and non-clotting blood in all body openings, illustrating that the occurrence of anthrax outbreak is dependent on the existence of the susceptible host (M. E. Hugh-Jones, Vos, and de Vos 2002).

During this outbreak, the health seeking behavior was very low, such that one family was found with eight suspected anthrax patients, and no one of them had a history of attending a health facility seeking for medical care. The victims were rather observed with smeared cow dung on the affected parts of the body as part of their traditional medicine practice. Health education campaign using various channels (television spot, radio spot, leaflets and billboards) was conducted and it was seen that people were reluctant to change their behavior. This is in line with what was reported in Zambia that people can still consume infected meat with anthrax due to lack of education, cultural belief, poverty and economic reasons (Sitali et al. 2017).

A well-functioning One Health approach is critical for understanding the role of ecology, epidemiology, climate change and socio-economic aspects of the infectious diseases transmission dynamics (Hitziger et al. 2018).

As a part of the response to this outbreak, a One Health team was established at regional, district and ward levels in order to ensure multi-sectoral collaboration at all levels. The established team was given terms of reference describing the roles and responsibilities of every participating partner. A composition of this team included experts from veterinary services, medical services, environmental Health, wildlife, chemical, sociology, tourism, and research institutions. A schedule for conducting their routine meetings was developed and they conduct meetings regularly even if there is no any outbreak reported in their respective working areas/sectors. The regional team is extrapolating this approach to other districts. Arusha region has been set as a model for the establishment of a well functioning One Health teams at the intermediate levels in the country.

Paper III

This paper confirmed that activities such as keeping livestock, skinning dead animals, coming into contact with animals or their products, manipulating carcasses, and touching while burying carcasses were linked to development of cutaneaous anthrax. In the analyses these variables were combined into a latent class (LC) as an exposure variable for disease occurrence. The use of a LC approach supported to identify the direct causal links between eduction, exposure and disease. These factors are essential as having animals at their households brings about the high chances of coming into direct and/or indirect contact with dead animals and/or animal products, which predisposes humans to acquire cutaneous anthrax (Islam et al. 2017).

In the pastoral Maasai community, animal skins are mostly used as bedding materials. The use of skin from infected animals would facilitate direct contact with *B. anthracis* spores if the animals had died of anthrax. Other studies have also reported that processing skin and hides for making sleeping materials facilitated anthrax transmission in the susceptible population in Zambia (Sitali et al. 2017).

Dressing of dead livestock and wildlife, and using the meat for human consumption was a risk factor for disease transmission. On some occasions during the anthrax outbreak, dead and decomposed animals were found lying on the grounds and being eaten by scavengers. This may be another pathway that may have contributed to an increased disease transmission to the unaffected animals and humans in the hotspot areas.

In a Zambian study, people who participated in skinning infected carcasses, consumption of contaminated meat, processing hides and skins for making sleeping materials were highly exposed to the infection (Munang'andu, Banda, Siamudaala, et al. 2012). Another study from Lake Rukwa valley in south-west Tanzania reported that manipulating infected carcasses and animal products was a potential risk for anthrax transmission in the community (Webber 1985).

Age and gender distribution can also facilitate disease transmission in a society due to the allocation of duties among the members of the community. In the Maasai community, men of older age (>20 years) were more often affected by cutaneous anthrax, perhaps because they are the ones responsible for taking care of the animals while grazing. In addition, they are also responsible for milking, slaughtering and skinning the carcasses.

Illiteracy was at high levels among the study subjects, and in anthrax cases in particular, and education level was found as a critical factor in explaining the set of exposure variables. Illiteracy is linked to poverty, and poor people opt to dress the carcasses from animals dying on their own and use them as meat, which exposes them to *B. anthracis* infection by contact in cases of infected animal products (Chikerema, Matope, and Pfukenyi 2013). The uneducated part of the community also face challenges in following or understanding critical messages through written materials (leaflets, billboards and magazines), which are provided during health education campaigns. Another study reported that poverty is centered in Sub-Saharan Africa where most people are illiterate, and the community is predisposed to many infectious zoonotic diseases due to their increased contacts with animals, yet with limited access to good health services for humans and animals (Grace et al. 2017).

The pastoral and agro-pastoral Maasai communities in Northern Tanzania depend on livestock for their livelihood. However, the animal husbandry systems do not take into account prevention of animal diseases like anthrax. Because of extensive grazing of livestock, they frequently come into contacts with wildlife which may have died from anthrax or the contaminated pastures, soil and water (De Garine-Wichatitsky et al. 2013). This also increases the risks of bringing the infection nearer to humans (Sitali et al. 2017). The pastoral and agro-pastoral communities practice an intimate contact with livestock in most of the cultural aspects and are combined as part of the unified social and ecological context (Cunningham, Scoones, and Wood 2017). Therefore, social factors and cultural practice dimensions influence the human and animal interactions that propagate the transmission of anthrax in this community. Similarly, researchers have to consider immutable beliefs and cultural practices, which exacerbate risks for transmission of zoonotic diseases in order to advocate for adequate individual behavior change (MacGregor and Waldman 2017).

Paper IV

There are a lot of competing priorities for the distribution of financial resources in the country, and the findings of this study provide important insights for allocating resources for anthrax prevention and control based on the predicted level of risks (very high, high, medium and low) at each district, which is an important administrative level for diseases prevention and control policy implementation in Tanzania.

In the current study, an ecological niche modeling technique was used to predict for the potential suitable habitat distribution of *B. anthracis* spores in Tanzania. It is the first study that presents potential risks distribution associated with occurrence of *B. anthracis* spores in Tanzania using climatic and abiotic factors such as soil type and soil pH. The Arusha and Kilimanjaro regions had a higher risk of *B. anthracis* spores habitat suitability in Tanzania. The observations in the current study are in line with what were previously reported in (paper I) where the Arusha region had a high incidence of 7.9 human anthrax cases per 100,000 population followed by Kilimanjaro (6.6) region.

In the predicted risk map, it was also observed streaks of predicted very high and high risks in Tanga, Coastal region, Manyara, and Singida regions. From the predicted risk regions, the corresponding districts predicted with very high and high risks are Arusha region (Ngorongoro, Monduli, Longido, Arusha rural and Meru), Kilimanjaro region (Hai, Siha, Moshi rural and Rombo), Mara region (Serengeti) Manyara region (Simanjiro, Hanang and Babati urban), Simiyu region (Bariadi and Itilima), Tanga region (Kilindi) and Singida region (Mkalama and Iramba), these regions are being arranged in the order of risk preference.

Some of the predicted districts with high risks such as Hanang, Simanjiro, Itilima, Serengeti, Bariadi, Kilindi, Mkalama and Iramba have not reported anthrax cases through the surveillance systems in recent years. This might be attributed to the poor human and animal surveillance systems leading to severe under-reporting hence misleading disease burden information (Gibbons et al. 2014). Monduli and Ngorongoro are among the districts with predicted high and very high risk for suitability of anthrax spores from Arusha region, corresponding with the recent frequent anthrax outbreaks in Monduli district. In the late 2016, a total of 130 wildlife carcasses, 39 livestock carcasses, and 21 human cases were confirmed to be infected with anthrax (Paper II). It is therefore envisaged that implementing targeted control measures based on the disease risk mapping is more cost-effective due to reduced cost for carcass disposal, the cost for laboratory reagents and reduced cost for outbreak management in general. It also helps to implement a targeted livestock vaccination and intensified human and animal disease surveillance by focusing more closely on the predicted high and very high risky districts using a One Health approach (Cleaveland et al. 2017; Baum et al. 2017).

This model demonstrated that the environmental suitability for the persistence of *B. anthracis* spores was highly influenced by the soil types, soil pH, isothermally, annual temperature range, moisture index arid quarter and potential evapotranspiration variables respectively. Environmental variables such as soil and climate favor and extend the survival of *B. anthracis* spores in the soil for a long period. This finding supports what was reported by other studies that anthrax outbreaks are exacerbated by warmer temperatures, moist soils and high organic matter content (humus) which favors the anthrax spore amplification (Dey, Hoffman, and Glomski 2012).

Soil types and soil pH were the most important variables for long-term persistence of anthrax spore in the identified high and very high-risk areas. This is supported by other studies showing that soil with high moisture, alkaline pH and humus are suitable for anthrax spores germination and sporulation outside a mammal host which are one of the critical variables that lead to the occurrence of anthrax outbreaks in animals with spillover to humans (Kreuder Johnson et al. 2015). Other studies have documented that soil pH above 6.1 (alkalinity) in a combination with calcium levels are important variables for the long-term survival of anthrax spores (Kracalik et al.

2017). This kind of soil is regarded as a natural reservoir for *B. anthracis* spores (Barro et al. 2016).

Methodological limitations

The major challenge during the implementation of this research was lack of a biosafety cabinet level three (BSL-3) laboratory for performing advanced laboratory techniques such as culture for *B. anthracis*. This would have helped to perform genotyping of *B. anthracis* for isolates obtained from humans, livestock, soil, and wildlife specimens and determine their genetic relatedness (similarities and differences) to guide for setting up effective preventive and control measures of anthrax outbreaks in the identified hotspot areas in the country.

Poor surveillance systems in both human and animal sectors, contributed to the failure of estimation of a true burden of anthrax in the country, as it was stated earlier. Therefore, the estimates obtained from the retrospective review may not be providing a true reflection of the disease status on the ground.

In paper III, a convenience sampling technique was used in the recruitment of anthrax patients through a record review performed at selected health facilities in the hotspot districts. This might have led to sampling bias, as follow-up of cases were so widely dispersed, affecting on their environmental risk factors attributable to the disease occurrence. However, this potential bias was controlled in the analysis stage by employing both latent class analysis and logistic regression techniques. Age was also found to be a confounder because children (<18 years) had more risk of acquiring infection because they spend more time grazing animals and coming into contact with infected animals and animal products. It was also found that most of the children were not in a formal education of which is a risk for infection because they cannot read the information materials provided to the community for disease prevention and control. However, a high response rate of about 97% obtained by recruiting 59 cases out of the intended 61 cases and selecting the control from the same community as the cases helped to minimize this kind of bias.

Misclassification bias (recall bias) might have been introduced due to a fact that the same questionnaire was used for cases and controls such that, control might not been able to recall their previous exposure. This might have led to underestimated exposure prevalence among the controls compared to cases causing the Odds Ratio to be artificially inflated (Austin et al. 1994)..

In the models behind the maps of paper IV, factors like livestock density, number of dry months, elevation and length of the longest dry season were highly correlated with the most significant variables. Apart from the identified most significant variables favoring the persistence of anthrax outbreaks in the areas with high and very high risks there were other factors, which contribute to anthrax outbreaks in Tanzania. However, it is still trusted that the predicted suitable environment for anthrax outbreaks are important regions and/or districts to be given more attention because they have been identified as hotspot areas for anthrax outbreaks in the previous studies.

Anthrax surveillance in a One Health Approach; improving the reporting systems

A One Health (OH) surveillance can be defined as a systematic collection, collation, validation, analysis, and interpretation of data collected on humans, animals and the environment and the obtained information disseminated to decision makers for more effective, evidence-based health interventions (Stärk et al. 2015). The routine collection of data is mostly based on the signs and symptoms (syndromic surveillance), a collection of health-related information, which may precede the formal diagnosis of a particular disease. This information can show a probability of change of a health status of a given population which can trigger for further investigation (Hoinville et al. 2013).

Syndromic surveillance can serve as an early warning system which involves a collection of health indicators in a defined population in order to increase the likelihood of early detection of the unexpected threats in the community (Hoinville et al. 2013). An effective OH surveillance system should capture both formal and informal information through various networks between livestock keepers and veterinary and medical sectors as a key for regular sharing of information (Hoinville et al. 2013).

Livestock can be used as a surveillance tool to predict human disease due to a fact that they share the same environment as humans. Once they show any signs and symptoms of zoonotic diseases such as anthrax then control measures can be mounted instantly. In Tanzania there is still a poor surveillance system in the animal sector leading to the under-reporting which might be due to inability of the surveillance system or unwillingness of experts to report or inability of the surveillance system to capture the reportable diseases or conditions (World Bank 2010). **Figure 13** illustrates various factors contributing to the under-reporting of zoonotic diseases in any surveillance system.

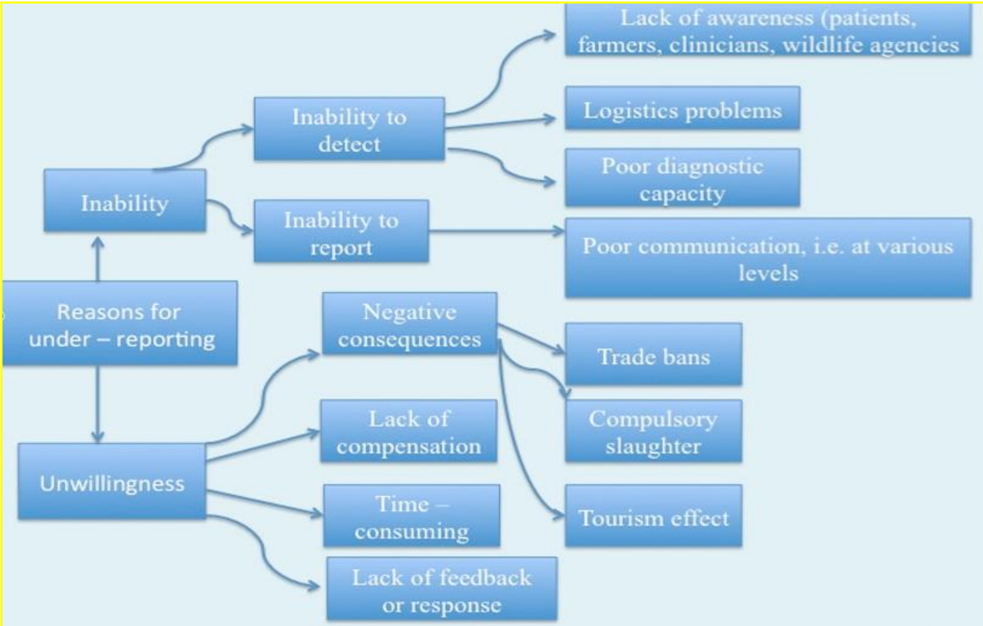

Figure 13: Possible reasons for under-reporting of zoonotic diseases. **Adopted from the World Bank 2010**

The following are some recommendations for improvement of the reporting systems using a One Health approach in Tanzania

i. Use of the existing mobile phone system (electronic IDSR), to include animal surveillance system and capture anthrax as well, in order to ensure the real-time syndromic surveillance and etiological confirmation of infectious diseases at the animal – human interface

ii. Create a standardized laboratory approach between the sectors for comparability of diagnostic tests between human (NHLQATC, KCRI & Regional Hospitals), animals (TVLA, KCRI &Zonal Veterinary Centers) and food safety diagnostic centers (TFDA)
iii. Establish a clear and timely data sharing mechanisms at the line Ministries through a Memorandum of Understanding (MoU). This should also regulate joint surveillance activities in a more holistic approach.
iv. Address the existing gaps between the laboratory and epidemiology units in both human and animal sectors
v. Community health workers (public health sector) and community animal health workers (CAHWs) should be engaged to deliver some services in selected areas where there is shortage of professionals to collect information (death of animals and/or humans without a clear cause of death, unknown illness affecting both humans and animals, vector/rodent infestations) which can be integrated into the disease reporting system.

Conclusions and recommendations

This study has been able to describe the reported incidence of anthrax in Tanzania, a process used to identify the two hotspot regions for anthrax transmission namely Arusha and Kilimanjaro regions both being located in Northern Tanzania, and both in close proximity to protected areas (National Parks and game Reserves). In these regions, there are extensive porous interface areas for humans, livestock and wildlife, and this study has identified those areas to be at high risk for contracting anthrax infection. This is due to an increased interaction between animals and humans especially the pastoral Maasai communities, with a high dependence on animals for their livelihood (meat, milk, bedding materials, workforce, and paying for dowry price). Moreover, risk factors for anthrax transmission have been identified and they can be categorized as demographic, biological, behavioral, environmental (soil types, soil pH, and soil nutrients) and climatic factors (seasonal rainfall, and temperature)

Vaccination of livestock against anthrax should be given a priority in order to ensure for an effective prevention and control of the disease in animals and ultimately control the disease in humans. Anthrax vaccine should be a public good under a public-private partnership scheme. Priority of intervention setting should be given depending on the output of the risk mapping and therefore more emphasis to be given in the predicted high-risk districts.

A One Health approach is required for responding to anthrax outbreaks as it affects humans, livestock and wildlife. It is also important to remember that the *B. anthracis* spores can stay in the soil for a long period of time, illustrating the long-term perspective of controlling a disease such as anthrax. Emphasis should be given to effective communication, coordination and collaboration among all the involved sectors.

It is recommended that, a One Health approach, which was established in Arusha region with its entire districts and in Selela ward of Monduli district of this region, as one of the best practices emanated from this study should be, used a model to extrapolate the same approach to the rest of the regions in the country.

Future research

In this thesis, the challenge of lack of advanced anthrax laboratory diagnostic tests for animals and humans at lower levels has been mentioned many times. Therefore, an operational research for adopting the use of the animal snap test for anthrax should be introduced to assess the feasibility of this test in the Tanzanian setting. This test needs a specimen from a dead animal (tissue or blood) and results can be obtained within a short time to guide decision-making on what precautions should be taken while handling the carcasses. Once this test is approved and established in Tanzania, it can be used as a rapid diagnostic test for anthrax in both animals and humans during outbreak response especially in the hard to reach areas. The issues of concern for this research can be acceptance, affordability, and accessibility of the relevant technology and the required gadgets in the Tanzanian settings.

In the strategy for prevention and control of anthrax in both humans and animals, livestock vaccination is documented to be a most efficient and effective disease control approach. The International Organization for Animal Health (OIE) is recommending livestock vaccination as a strategy for controlling anthrax in humans and animals (Khomenko et al. 2013). However, a systematic review and meta-analysis of this intervention should be done in order to inform policy makers for ensuring that livestock vaccination against anthrax is given more emphasis to ensure routine access of the vaccine to pastoralists by making it a public good.

This thesis has clearly elaborated on various cultural aspects, which propagates anthrax transmission in the Maasai pastoral communities. Anthropological studies are recommended in order to establish a more holistic approach to address a socio-cultural aspect that affects the health status of individuals in this community with a relevancy to anthrax transmission.

Molecular characterization of *B. anthracis* should be done in order to determine the distribution of various genotypes of the pathogen in the country, in humans, livestock, and wildlife. This genetic relationship can be studied further through ecological niche modeling by assessing the environmental suitability for the persistence of each strain and then acquire knowledge on the spatial and temporal distribution of various strains of *B. anthracis* in the country. This kind of study has been done in other countries like Ghana (Kracalik et al. 2017).

Anthrax outbreaks are bringing about increased number of deaths of both livestock and wildlife and it also affects human beings in different ways. The pastoral communities are affected economically by the loss of their animals, which are the most important assets for their livelihood. No studies have been conducted to quantify the economic loss due to death of animals following anthrax outbreaks in Tanzanian setting. This kind of study should be conducted in order to ascertain the economic implication in a wider aspect covering costs for disposal of carcasses, diagnostic consumables and surveillance related activities apart from the cost encountered at the family level during outbreaks.

Several studies have indicated that necrophagous flies have a role to play as a carrier for anthrax transmission. This is because they tend to regurgitate or defecate and contaminate the vegetation for browsers and transmit the infection easily (Blackburn et al. 2010). Hematophagous flies are biting flies and can act as mechanical vectors and can successfully carry *B. anthracis* between animals (Fasanella et al. 2010). Similar studies should be conducted in Tanzania during anthrax outbreaks in order to gain more knowledge about the role of vectors for anthrax transmission in the hotspot areas.

The impact of various research activities conducted by academic and research institutions in the endemic areas for zoonotic diseases is realized through increased awareness and knowledge about zoonotic diseases (Mangesho et al. 2017). This is on top of the Government's initiatives of providing health education to affected communities as part of responding to outbreaks. This is done through the use of leaflets, roadshow, and radio broadcasting. However, due to high level of illiteracy to the mostly affected Maasai communities and lack of more sustainable approach of health education campaign to these communities and knowledge update to health care providers from both human and animal sectors. A digital health intervention system should be introduced in order to improve the knowledge uptake and retention for prevention and control of anthrax using a One Health approach in the hotspot areas of northern Tanzania. Its assessment should be conducted through experimental studies in order to determine the opportunities and challenges for scaling up this kind of intervention in the country.

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Anthrax outbreaks in the humans - livestock and wildlife interface areas of Northern Tanzania: a retrospective record review 2006–2016

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Abstract

Background: Anthrax outbreaks in Tanzania have been reported from the human, livestock and wildlife sectors over several years, and is among the notifiable diseases. Despite frequent anthrax outbreaks, there is no comprehensive dataset indicating the magnitude and distribution of the disease in susceptible species. This study is a retrospective review of anthrax outbreaks from the human, livestock, and wildlife surveillance systems from 2006 to 2016. The objectives were to identify hotspot districts, describe anthrax epidemiology in the hotspot areas, evaluate the efficiency of the anthrax response systems and identify potential areas for further observational studies.

Methods: We prepared a spreadsheet template for a retrospective comprehensive record review at different surveillance levels in Tanzania. We captured data elements including demographic characteristics of different species, the name of health facility, and date of anthrax diagnosis. Also, we collected data on the date of specimen collection, species screened, type of laboratory test, laboratory results and the outcome recorded at the end of treatment in humans. After establishing the database, we produced maps in Quantum GIS software and transferred cleaned data to Stata software for supportive statistical analysis.

Results: Anthrax reported incidences over 4 years in humans were much higher in the Arusha region (7.88/100,000) followed by Kilimanjaro region (6.64/100,000) than other regions of Tanzania Mainland. The health facility based review from hotspot districts in parts of Arusha and Kilimanjaro regions from 2006 to 2016, identified 330 human anthrax cases from the selected health facilities in the two regions. Out of 161 livestock and 57 wildlife specimen tested, 103 and 18 respectively, were positive for anthrax.

Conclusion: This study revealed that there is gross under-reporting in the existing surveillance systems which is an obstacle for estimating a true burden of anthrax in the hotspot districts. Repeated occurrences of anthrax in livestock, wildlife and humans in the same locations at the same time calls for the need to strengthen links and promote inter–disciplinary and multi-sectoral collaboration to enhance prevention and control measures under a One Health approach.

Keywords: Anthrax outbreaks, Wildlife interface areas, Record review, Northern Tanzania

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Background

Anthrax is a zoonotic infectious disease caused by *Bacillus anthracis*, a spore-forming, Gram-positive bacterium [1]. The disease occurs in humans, and wild and domestic mammalian species, in particular, herbivores [2]. Anthrax cases in humans are classified into three forms according to the clinical features and transmission routes: the cutaneous form, accounting for about 95% of all reported human cases worldwide, the gastrointestinal form, and the pulmonary form [3]. There is no evidence of person-to-person transmission of *B. anthracis* [4], and humans normally acquire the disease from direct contact with anthrax-infected animals or anthrax-contaminated animal products [4, 5]. Both domestic and wild animals serve as potential sources of infections in humans [6, 7]. The clinical presentation of this disease in susceptible herbivores is usually characterised by septicaemia and sudden death with/without bleeding from natural orifices and subcutaneous haemorrhages. Other symptoms in livestock and some wild herbivores are fever, dyspnoea, agitation, convulsions followed by sudden death. In pigs, carnivores, and primates the main symptoms are local oedema and swelling of the face and neck. Failure of the blood to clot, the absence of rigor mortis and the presence of splenomegaly are the most significant necropsy findings [6].

Worldwide, anthrax occurs at a low incidence in developed countries but remains endemic in African and Asian regions [6]. The African experience also illustrates the classic One Health aspects of anthrax where humans, livestock, wildlife and environment are important part of the epidemiological pattern. Anthrax outbreaks in Tanzania have been reported in humans, livestock, and wildlife over several years, and areas mostly affected are those in the livestock-wildlife interface [7]. Anthrax outbreaks in hippos were reported in Ugalla Game Reserve in 2000 and 2001 as well as in Mtera dam in 2003 [4]. Several large outbreaks (> 500 deaths) have also been reported in cattle, goats, and sheep in the eastern part of the Serengeti National Park [7]. In September 2016, a total of 153 hippopotamus died in Kilombero River due to anthrax outbreak, and in early October 2016, an anthrax outbreak in livestock occurred in Ngorongoro district, where ten humans were infected, two of them died [8]. Anthrax outbreaks have been reported in the Serengeti ecosystem for many years, mostly with sporadic outbreaks in several endemic hotspot areas affecting humans, livestock and wildlife animals [9].

Anthrax incidence in a given locality is related to temperature, rains or drought, soil, vegetation, host condition and population density [10, 11]. The local weather condition of an area may directly or indirectly influence possibilities for animals to come into contact with *B. anthracis* spores. This may include grazing closer to the soil in dry periods when grasses are short or sparse, and movement of herds to protected areas for wildlife conservation when water becomes scarce. The general state of health of the hosts may also affect their level of resistance to infection [6].

The health personnel in the Ministries responsible for health of human, livestock and wildlife (Epidemiology Unit) and the Ministry of Regional Administration and Local Government are responsible for responding to disease outbreaks as soon as they get outbreak notification from lower levels. During the disease outbreak response, their role is to identify and characterize the outbreak etiologic agents, monitoring the progress of the outbreak and putting the effectiveness of control and preventive strategies in place [12]. From the national level, information of public health emergence or disease outbreak is required to be communicated to WHO within 24 h [13, 14]. For the human surveillance system, communication during surveillance, reporting, and the response is by telephones (mobile and landlines), internet, fax, radio (national and local stations), television, letters, technical Meetings (National task force) and workshops [15]. Laboratory diagnostic reports of anthrax from Tanzania Veterinary Laboratory Agency (TVLA) and Tanzania Wildlife Research Institute (TAWIRI) Serengeti are regularly shared by the Ministries responsible for livestock and wildlife respectively. These reports are crucial for setting up control measures of anthrax outbreaks in livestock and wildlife.

Regular analysis of diagnostic and surveillance data from livestock and wildlife are essential for efficient management of anthrax outbreaks in animals and protecting human population [16].

However, despite the frequent occurrence of anthrax outbreaks in Tanzania, there is no comprehensive analysis of data indicating the magnitude and spatial distribution of the disease from the human, livestock and wildlife health sectors. It is therefore important to coalesce and summarize the available information to assess more comprehensive epidemiological patterns of anthrax in Tanzania.

We conducted a retrospective review of reported anthrax outbreak records from the human, livestock, and wildlife surveillance systems of Tanzania from January 2013 to December 2016. This was followed by a more thorough examination of data from Arusha and Kilimanjaro regions for 2006 to 2016. The specific objectives were (i) to identify the districts assumed to be an anthrax hotspots in Arusha and Kilimanjaro regions, (ii) to evaluate the efficiency of the anthrax reporting and response system and diagnostic capacity at national, regional and district levels, (iii) to describe the epidemiology of anthrax in the hotspot areas and (iv) to identify potential areas for further observational studies to better understand the complex ecology of anthrax.

Methods

Study areas

A follow – up was done at national level involving the Ministries responsible for health of humans, livestock and wildlife using a structured checklist. During this follow up, all regions of the Tanzania Mainland were assessed for the described anthrax outbreaks during the period of 2013–2016. After compiling the National data for humans and livestock, we focused on the identified hotspot areas of **Arusha** and **Kilimanjaro** regions for more detailed studies of anthrax data for humans and livestock as well as wildlife.

Arusha region lies on the Kenyan border, encompassing savannahs and part of the Great Rift Valley. It has a total area of 37,576 km^2 with a human population of 1.7 million [17]. Wildlife conservation areas in this region include (a) the Ngorongoro Conservation Area, which contains the Ngorongoro Crater, (b) Arusha National Park, which covers volcanic Mount Meru, (c) Loliondo Game Controlled Area and (d) Lake Natron Game Controlled Area, which contains the active volcanic mountain - Oldoinyo Lengai. This region has seven districts which are Arusha City, Arusha rural, Meru, Ngorongoro, Karatu, Monduli and Longido districts. Based on the history of frequent anthrax outbreaks, a data review was purposively done in Ngorongoro, Meru and Monduli districts for the period of 2006 to 2016.

Kilimanjaro region is a home to the highest mountain in Africa, Mt. Kilimanjaro and Kilimanjaro National Park. It is bordered to the north and east by Kenya, to the south by Tanga region, to the southwest by Manyara region and the west by Arusha Region, and has a total area of 13,250 km2 with a population of approximately 1.6 million [17]. The region has seven districts: Hai, Moshi rural, Rombo, Mwanga, Siha, and Same districts, and Moshi Municipality. Hai, Siha, Moshi rural and Rombo districts were conveniently selected for a comprehensive retrospective data review for anthrax outbreaks in the period of 2006 to 2016.

The National Anthrax Surveillance Systems

Anthrax is among the notifiable diseases in humans in Tanzania and is therefore also included in the current human health electronic integrated diseases surveillance and response system (eIDSR) [14]. Under the described surveillance system, a registered mobile phone is used to report a human suspected anthrax case within 24 h after having met the standard case definition for anthrax at a reporting health facility. All health facilities, Points of Entry (PoE) and any other location (in conjunction with a nearby community) must report the total number of human cases and deaths seen in a given period.

Anthrax is also a notifiable disease in livestock and wildlife, and surveillance systems linked to farms, laboratories, clinics, livestock markets, slaughterhouses and dip tanks are among the data sources for animal health information system (AHIS). More than 80% of disease information obtained is based on clinical observations, and 95% of the surveillance system is paper based investigation, surveillance and treatment reports.

The animal health system is composed of community animal health service under the public sector, and animal health care centres and clinics under the private sector [15]. In wildlife, anthrax reports are submitted through the Veterinary Section at (TAWIRI), where the occurrence of any disease (outbreak, infectious, zoonotic, unknown) is reported to the Director of Veterinary Services. The laboratory personnel working within the wildlife health system include laboratory attendants, laboratory technicians and laboratory technologists [15].

All final human and animal anthrax reports are made available from the Ministries responsible for the health of humans, livestock, and wildlife.

Standard case definitions

In our follow-up we defined a human anthrax case as follows:

At the health facility level, a suspect human anthrax case was any person with acute onset of illness characterized by one of several clinical forms:

1) **Localized form**
   Cutaneous; skin lessions evolving from a papular through a vesicular stage, to a depressed black scar invariably accompanied by oedema that may be mild or extended.
2) **Systemic form**
   a) Gastrointestinal; any person with abdominal distress characterized by nausea, hematemesis, blood dirrhoea, vomiting, anorexia and followed by fever
   b) Pulmonary; anyone with an acute illness resembling a viral respiratory illness followed by hypoxia, dyspnea or acute respiratory distress with resulting cyanosis and shock.
   c) Meningeal; any person with acute illness revealing fever, convulsions, coma, or meningeal signs.

At the community level, a suspect anthrax was anyone with fever, difficulty in breathing, skin conditions or abdominal pain or altered consciousness, with a history of contact with sick or dead animal [14].

We defined a suspect anthrax case in a non-immunized livestock or wildlife animal as an acute disease characterised by septicaemia and/or sudden death with/without bleeding from natural orifices and/or could include subcutaneous hemorrhages. Other symptoms in cattle, horses, sheep and some wild herbivores are fever, dyspnoea, agitation, convulsions followed by sudden death. In pigs, carnivores, and non human primates symptoms could include local oedema, and/or swelling of the face and neck. Necropsy, if completed, could reveal failure of the blood to clot, the absence of rigor mortis, and/or the presence of splenomegaly [6].

Confirmed case: A suspect case with one of the following:

a. Culture and identification of *B. anthracis* from clinical specimens by the designated laboratory or demonstration of *B. anthracis* antigens in tissues by immunohistochemically staining.
b. A four-fold increase or change in antibodies to protective antigen between acute and/or paired convalescent sera
c. Evidence of *B. anthracis* DNA in blood, swab or tissue specimens collected from a normally sterile site or lesion of other affected tissue (skin, pulmonary, reticuloendothelial, or gastrointestinal).

National anthrax records review

Sources of data used in this review were the various Epidemiology sections of the Ministries responsible for human and livestock services.We examined nationally stored databases of each Ministry to retrieve the relevant surveillance data for the period of January 2013 to December 2016. The Ministry of Natural Resources and Tourism (MNRT) headquarters receives the active surveillance reports from TAWIRI which prepares reports after every wildlife related outbreak occurring in the protected areas of Tanzania. Therefore, we did a record review for 2006 to 2016 at the TAWIRI research centre located in the Serengeti National Park. We also compiled information about the population structure of humans and livestock based upon the statistics obtained from the National Bureau of Statisticts, Ministry of Finance [17]. The incidence risk (IR) was calculated by taking into account the number of new cases who got anthrax infection in a projected population from 2012 census per 100,000 population in each region of the Tanzania mainland for a period of 2013–2016. The time period experienced by members of the population during which events of anthrax outbreaks occurred was also considered (Table 1).

Follow-up in hotspot areas in Arusha and Kilimanjaro regions

For the follow-up in Arusha and Kilimanjaro regions, we used data obtained from the randomly selected health facilities of the identified hotspot districts, Tanzania Wildlife Research Institute (TAWIRI) Serengeti National Park, Tanzania Veterinary Laboratory Agency (TVLA), District Veterinary Offices, Livestock Field Offices at the Ward and Village Levels and District Medical Offices. We conducted a comprehensive retrospective review of anthrax outbreak records in the human, livestock and wildlife health sectors of Northern Tanzania for the period of 2006 to 2016.

Review at the health facility and animal diagnostic centres

Data reviews were carried out using the health management information systems (HMIS) booklets for both in–patients and out-patients at the health facility level. We also captured formal and informal meeting minutes, internal memos and official outbreak notification letters, and raw data in the form of typed or handwritten reports, tables and spreadsheets from the district medical and veterinary offices. Moreover, animal (livestock and wildlife) anthrax data were reviewed from the laboratory units of TVLA in Arusha, and TAWIRI in Serengeti National Park. We conducted this review in a period of two months, early October to late November 2016.

Data management and analyses

We compiled the national datasets for humans and livestock into separate Excel® sheets. Cross-tables were obtained using Pivot tables in Excel, supported with tables generated in the statistical software Stata (Stata14/ SE, StataCorp, College Station, TX). We also entered follow-up data from Arusha and Kilimanjaro into Excel®. We classified the recorded human anthrax cases according to the name of the region, district, village/area of residence, health facility, sex, age, and date of anthrax diagnosis, and the outcome of treatment. We also used Excel® to create a trend (with computer generated moving average) of anthrax outbreaks from hotspot districts for the period of 2006–2016. As a means of data quality control, we excluded cases without proper records (as listed above) in the database. For livestock and wildlife diagnostic laboratory data the spreadsheet captured the name of the region, district, date of specimen collection, nature of specimen submitted, animal species, kind of laboratory tests and test results. All of this information was reviewed from the registers at TVLA in Arusha and TAWIRI in Serengeti. However, we omitted from the database any suspect animal cases without clear information on the date of specimen submission to TVLA. We entered all data into an Excel® spreadsheet, after establishing the Excel® databases, we created a map to indicate the locations of human cases by using the Quantum Geographical Information System (QGIS) software (http://www.qgis.org/en/site/forusers/index.html). We cross-tabulated for age, sex, the location of human and animal cases, date of symptom onset, form of anthrax, final treatment outcome of human cases, and laboratory results across the species, over all seasons.

Results

Anthrax in humans

We found that the reported human anthrax incidence risk over 2013–16 per 100,000 population was much higher in Arusha region (7.88/100,000) followed by Kilimanjaro region (6.64/100,000) than any other regions of the Tanzania

Table 1 Spatial distribution of reported anthrax cases across various species in Tanzania Mainland, 2013 to 2016, based on the human and livestock National Surveillance Systems

Region	Estimated populations, 2012 Census (million)				Reported Cases and (Deaths) 2013–2016	Livestock deaths, 2013–2016			eIDSR (Human Cases) 2013–2016	Human (Incidence risk per 100,000 Pop)
	Human	Bovine	Caprine	Ovine	Human	Bovine	Caprine	Ovine		
Dodoma	2.08	1.5	1.0	0.26	0 (0)	23	39	87	0	0.00
Arusha	1.7	1.6	1.9	0.84	134 (8)	87	23	8	96	7.88
Kilimanjaro	1.64	0.65	0.69	0.25	109 (2)	17	35	26	38	6.64
Tanga	2.05	0.77	0.82	0.22	0 (0)	27	34	32	x	0.00
Morogoro	2.22	0.88	0.49	0.13	10 (0)	23	34	54	x	0.45
Pwani	1.10	0.54	0.19	0.04	0 (0)	7	32	32	x	0.00
Dar es Salaam	4.36	0.27	0.16	0.02	22 (6)	9	0	5	6	0.50
Lindi	0.86	0.26	0.10	0.01	0 (0)	7	8	9	x	0.00
Mtwara	1.27	0.17	0.23	0.02	14 (0)	28	4	12	x	1.10
Ruvuma	1.38	0.47	0.32	0.03	0 (0)	0	0	0	x	0.00
Iringa	0.94	0.66	0.20	0.04	0 (0)	0	0	0	x	0.00
Mbeya	2.71	1.45	0.56	0.08	2 (0)	16	2	0	x	0.07
Singida	1.37	1.37	0.83	0.29	6 (0)	12	31	21	0	0.43
Tabora	2.29	2.23	0.95	0.27	4 (0)	23	12	5	x	0.17
Rukwa	1.00	0.64	0.23	0.04	0 (0)	9	2	0	x	0.00
Kigoma	2.13	0.51	0.26	0.05	2 (0)	1	5	1	x	0.09
Shinyanga	1.53	1.30	0.62	0.20	0 (0)	21	13	4	x	0.00
Kagera	2.46	0.85	0.73	0.08	0 (0)	12	2	0	0	0.00
Mwanza	2.77	1.33	0.57	0.13	12 (0)	27	19	4	0	0.43
Mara	1.74	1.65	0.76	0.34	22 (8)	12	3	0	2	1.26
Manyara	1.43	1.81	1.54	0.58	8 (0)	26	13	4	1	0.55
Njombe	0.70	0.27	0.11	0.02	0 (0)	0	0	0	x	0.00
Katavi	0.56	0.36	0.18	0.03	0 (0)	12	3	0	x	0.00
Simiyu	1.58	1.60	0.93	0.39	0 (0)	2	0	0	x	0.00
Geita	1.74	0.82	0.43	0.05	0 (0)	0	0	0	0	0.00
Total	43.63	23.97	14.91	4.39	345 (24)	401	314	304	142	

Reported cases through the electronic system (eIDSR) and the reported human's anthrax Incidence risk over the period of 2013–16 is also given

Mainland (Table 1), identifying these regions as hotspots for anthrax. Records from selected health facilities showed that there were 187 human anthrax cases (57%) in Kilimanjaro and 143 (43%) in Arusha region for the period 2006–2016 (Table 2). Figure 1 indicates the spatial distribution and magnitude of anthrax cases in humans, while Fig. 2 shows the distribution of anthrax forms of human cases over the different health facilities. The majority (284/330, 86.1%) of all human anthrax cases reviewed at the selected health facilities were of the cutaneous form. A majority of reported human anthrax cases was in males, (215/330, 65.2%) compared with females. Figure 3 shows the trends of human anthrax cases in Arusha and Kilimanjaro regions for 2006–2016, illustrating an increasing trend with the highest number of 163/330 (49.4%) cases in 2016 showing 2 cases per moving average in Ngorongoro district in a time series of ten years.

Table 2 Distribution of human anthrax cases in study hotspot districts, Arusha and Kilimanjaro regions from 2006 to 2016

Regions	Districts	Number of Cases (% of Cases)
Arusha	Ngorongoro	115 (80)
	Meru	7 (5)
	Monduli	21 (15)
Total		143
Kilimanjaro	Moshi rural	71 (38)
	Hai	77 (41)
	Rombo	17 (9)
	Siha	22 (12)
Total		187

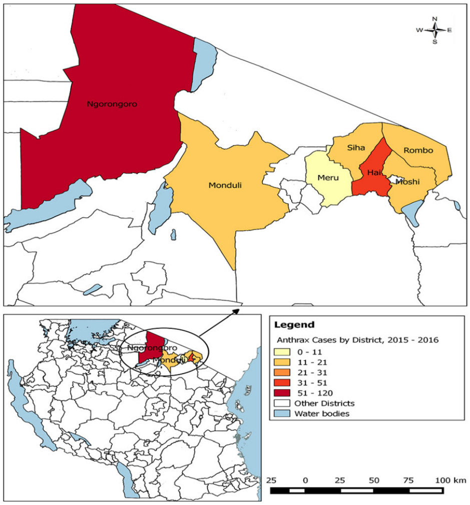

Fig. 1 Map indicating the magnitude of human anthrax cases in the hotspot districts of Arusha and Kilimanjaro regions for the period of 2015 - October 2016

We found that there were human anthrax cases in record books since 2006 and beyond despite the disease not being included in the HMIS and IDSR reporting forms at that time. Overall, the Ngorongoro district reported more human anthrax cases, 115 (80%) compared to other districts of Arusha and Kilimanjaro regions. The Endulen Hospital reported more human anthrax cases (60/115, 52.2%) compared to other health facilities in Ngorongoro district in the period of review.

We further found a gross under-reporting of the electronic surveillance system in Arusha and Kilimanjaro regions. For instance, Arusha region reported 96 anthrax human cases through the electronic surveillance system from all the districts compared to 134 human anthrax cases obtained from health facility's record review in hostspot districts only (Ngorongoro, Meru and Monduli) in the period of 2013 to 2016. Similarly, a total of 109 anthrax human cases were revealed following the health facility's record review from hotspot districts only of Kilimanjaro region (Hai, Moshi rural, Siha, and Rombo) compared to 38 human cases reported by the electronic system from all districts in the region during the same period.

Anthrax in livestock and wildlife

From 2006 to 2016, TVLA received a total of 161 specimens from different livestock and wildlife species for laboratory analysis (Table 3). Most of the submitted specimens came from bovine (106/161, 66%). A total of 103 specimens (64%) tested positive for *B. anthracis*, and 68 (66%) of the positive specimens came from bovines, followed by caprine (18/103, 17%). In the same period, a total of 57 wildlife specimens obtained from active surveillance done in the Serengeti ecosystem were tested for anthrax at TAWIRI Serengeti laboratory. Of these 18 (32%) were positive for anthrax of which most of them came from African buffalo (12/18, 67%), (Table 4). Anthrax outbreaks have occurred across human, livestock and wildlife populations with peaks of outbreaks in the months of March and September through November and this corresponds to specific environmental conditions (Fig. 4).

Generally, it was found that, the diagnostic capacity for anthrax in human, livestock and wildlife sectors was

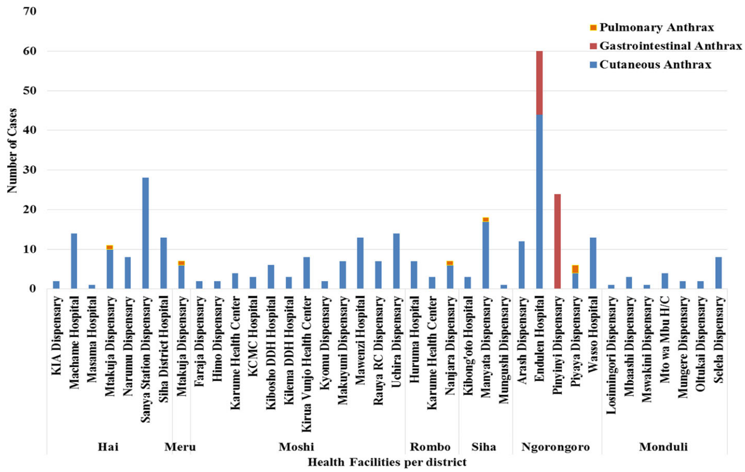

Fig. 2 Distribution of clinical forms of human anthrax cases per health facility in hotspot districts of Northern Tanzania, 2006 to 2016

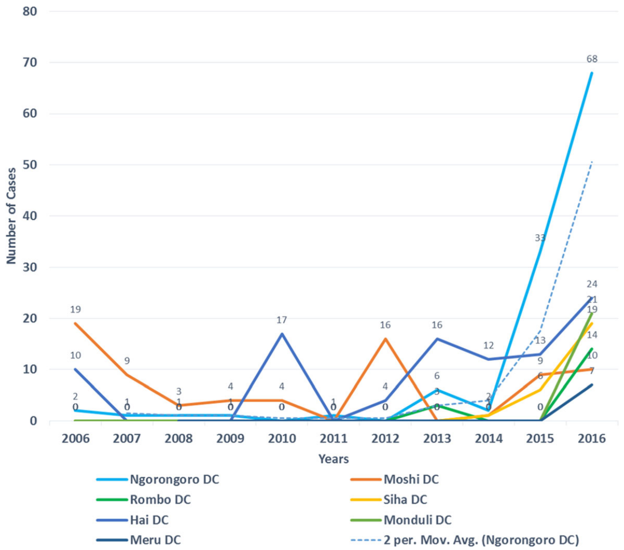

Fig. 3 The trend of human anthrax cases from the hotspot districts of Arusha and Kilimanjaro regions, 2006 to 2016

inefficient in the hotspot districts. This is because there were only two diagnostic centres for anthrax in Northern Tanzania, i.e. TVLA and TAWIRI Serengeti Centre for management of animal (livestock and wildlife) specimens. Human specimens especially skin lesion swabs were tested by Gram or Methylene blue staining techniques for anthrax in some of the selected health facilities. Other selected health facilities were managing anthrax cases clinically as they did not have any diagnostic capacity in place. No selected health facilities were found with advanced diagnostic capacity for anthrax like culture and PCR techniques.

Discussion

This study has revealed that there is a close temporal correlation between the occurrence of anthrax outbreaks in animals (livestock and wildlife) and humans in the Arusha/ Kilimanjaro ecosystems. It might be attributed to the ongoing interactions between humans and animals such as types of husbandry, humans looking for food (meat and milk) and other livelihood issues like the use of animal skin as bedding materials, a common practice in the pastoral community.

Table 3 Summary of livestock species tested for *B. anthracis* at TVLA – Arusha, 2006 to 2016

Species Screened	Samples tested	*B. anthracis* (Positive)	% Samples testing positive
Bovine	106	68	66.
Caprine	23	18	17
Ovine	8	7	7
Swine	5	3	3
Wildlife trophies[a]	19	7	7
Total	161	103	

[a]Wildlife trophies: for the purpose of this review, means a group of unique wild animals whose parts of their body like horns, skin and skull are used for decorations like Waterbuck and Topi.

We also, found more cases occurring in the dry season starting from September through November which might be a facilitating factor for anthrax transmission in animals and then into humans. The observed seasonal occurrences of anthrax show that climate-related factors (precipitation and ambient temperature) play a crucial role in triggering outbreaks, although there are variations between locations and therefore contributing factors are debated [9]. Some African countries, like this study have been reporting anthrax outbreaks at the end of dry seasons which indicates that over grazing, nutritional stress and congregation of animals at watering points might propagate the disease transmission [18, 19]. Also, animals tend to assemble themselves in certain places when there is pasture shortage, increasing chances of occurrences of anthrax [20].

Furthermore, water bodies may collect and accumulate spores in "storage areas" [21]. As water storage points are the last locations to hold water during dry seasons, these are the dangerous areas where animals tend to acquire the infection through drinking spore-contaminated water [22]. Our data demonstrated a seasonal pattern of anthrax outbreaks in northern Tanzania with peaks of outbreaks in humans, livestock and wildlife during March (start of long rain season) and September through November (end of dry

Table 4 Summary of wildlife species tested for *B. anthracis* at TAWIRI Serengeti laboratory in Serengeti National Park, 2006 to 2016

Species Screened	Samples tested	*B. anthracis* (Positive)[a]	% of Samples testing positive
African Buffalo	28	12	67
Elephant	2	2	11
Wildebeest	5	0	0.0
Black Rhino	1	1	6
Hippo	1	0	0.0
Giraffe	1	1	6
Horse	2	0	0.0
Zebra	11	1	6
Lion	1	0	0.0
Wildlife Trophies	5	1	6
Total	57	18	

[a]Using a microscopy test: Positive *B. anthracis* was obtained by staining a dry fixed blood smear with polychrome methylene blue. A typical morphology of the bacilli was observed to be gram positive, thick, long with square or truncated and swollen ends with characteristic 'bamboo stick' appearance

season) each year in the last decade. This shows a high potential for anthrax infection in the human-livestock and wildlife interface areas of northern Tanzania [12], representing critical information to decision makers that they will need to set up preventive measures.

These measures could include strategic vaccination of livestock against anthrax, distribution of human antibiotic prophylactics to hotspot areas, and health education to high risk communities a few months before the expected time of anthrax outbreaks. Nevertheless, effective anthrax control depends on ensuring that the disease is controlled in livestock through routine targeted vaccination which may automatically control the problem in humans [20]. Restriction of free movement of healthy livestock during outbreak periods and safe disposal of dead animals (in a pit of six feet deep added with 10% formalin poured on top of the carcasses). This should be followed by soil decontamination on the area where the carcass is laying and removal of bloody soil, so it can help to prevent further occurrences of the disease [23].

We further found that men were at higher risk of acquiring anthrax (65.2%) than women, which might be due to slaughtering and handling the meat from the carcass of dead or slaughtered sick animals without inspection by a designated livestock officer. This is more likely to be the route of exposure for many of the cutaneous anthrax cases we found in this review [24]. More often they also eat meat while grazing their animals in the wilderness, only bringing home any remaining meat and offal for the wives and children. Moreover, men are the decision makers of the family who also dictates whether women and children should go to the hospital when they fall sick. This might impact on the health seeking behaviour of women creating a false representation in hospital registers regardless of the true disease status. Effective clinical management of zoonotic diseases depends on various factors including health seeking behaviour of individuals [16].

Pastoralists handle sick animals before dying and dress the carcasses after death [25]. Also, extensive handling of meat at different stages of preparations with direct skin contact with anthrax infected materials. It is a risk

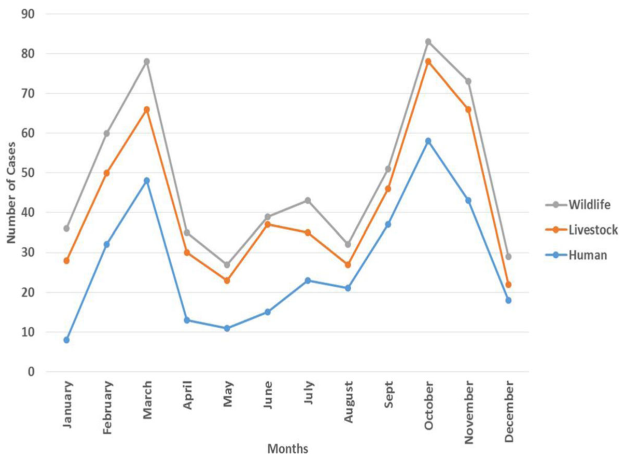

Fig. 4 Monthly distribution of anthrax cases from all 3 health sectors (human, livestock and wildlife) in selected regions of Northern Tanzania, 2006–2016

for contracting the infection and entry of *B. anthracis* into the human skin abrasions and can cause the cutaneous form of anthrax which we mostly found during this review. The cutaneous forms of anthrax are easily diagnosed clinically in health facilities and in laboratories by performing a Giemsa or Methylene blue staining on the discharges from lesions to detect the presence of *B. anthracis*. Other forms of anthrax like gastrointestinal and pulmonary are not as easily diagnosed at most of the existing health facilities in the anthrax hotspot districts in Tanzania.

High numbers of recorded human anthrax cases (Table 1) may partly be due to many patients that report at the specified health facility and good systems of recording patients in the health management information system (HMIS) outpatient & in-patient department booklets. In a few instances, some health facilities had no HMIS booklets which may account for no or a low number of reviewed human anthrax cases. For example, the Magaiduru dispensary in Ngorongoro district had no HMIS booklets to keep records of human anthrax cases regardless of some verbal information on the presence of human anthrax suspected cases in the village they serve. The same applies for IDSR reporting forms that in past years anthrax was not one of the IDSR priority diseases. Therefore some facilities did not bother to report the disease until 2013 when a revised National IDSR Guidelines included anthrax as one of the immediately reportable diseases. However, in Hai district they historically improvised a slot on the reporting forms for capturing anthrax cases in the infectious diseases weekly ending (IDWE) reporting forms which accounts for a high number of anthrax cases in Hai district compared to other hotspot districts of Kilimanjaro region. Overall, Arusha region has reported more anthrax human cases in a time series of the last ten years and Ngorongoro district having more anthrax human cases compared to other districts. This might be contributed by the pastoral communities living in close proximity with wildlife conservation areas and facilitating disease transmission between livestock and wildlife animals and then to humans.

Anthrax outbreaks cause substantial economic losses through livestock and wildlife losses, the cost of laboratory reagents and carcass disposal (burial or incineration). Therefore investment in the control of this disease is inevitable [26]. Response to these outbreaks requires joint collaborative efforts of the Ministries responsible for human health, livestock, and wildlife services. However, one of the biggest challenges in the control of zoonotic diseases is the current lack of joint approaches for responding to disease outbreaks. Therefore, there is a need for the creation of joint response action plans with combined technologies and infrastructures from both public health and veterinary professionals including sociologists, and ecologists to initiate approaches to contain zoonotic diseases [27]. Worldwide, a One Health approach is a call to action for the establishment of closer professional interactions, collaborations, capacity building and research opportunities across the science professionals and related disciplines to improve the health status of humans, livestock, wildlife, and the environment [28]. In Tanzania, one of the challenges for initiating the joint surveillance system under a One Health approach would be lack of compatible surveillance systems between the ministries responsible for human health, livestock and wildlife. Nonetheless, the right opportunity is the existence of a strong IDSR system within human health sector which can be improved and expanded to cover a harmonized list of priority zoonotic diseases in a 'One Health' approach.

In countries like Kenya the five diseases identified as top priority zoonotic diseases are anthrax, trypanosomiasis/HAT, rabies, brucellosis and Rift Valley Fever (RVF) in descending order [29]. This highlights the importance of prioritizing zoonotic diseases in Tanzania, as well as presenting opportunities to focus on diseases with the greatest local public health burden and not focus only on diseases that have greater global attention [29]. Most often, authorities start looking for the disease in livestock and take appropriate actions only after they report human cases and deaths in that particular area. When disease surveillance and control take this approach, humans essentially serve as a sentinel species (human illness and death) act as proxy indicators of disease prevention and control in livestock [12].

We also observed that there is poor anthrax diagnostic capacity, not only in the hotspot districts in northern Tanzania, but the entire country. The only routine diagnostic techniques performed at TVLA in Arusha and TAWIRI in Serengeti National Park are either Giemsa or Methyline blue staining of fixed blood smear from either humans or animals (livestock and wildlife). They also test swabs collected from skin lesions of human anthrax suspected cases by the same technique at some health facilities of the hotspot districts in Northern Tanzania. The Ministry of Livestock and Fisheries headquarters has a laboratory (Biosafety Level 2 Laboratory) with a capacity for diagnostic polymerase chain reaction (PCR) for anthrax, but is located in Dar es Salaam about 700 Km away from the hotspot areas. This hampers the diagnosis of other forms like pulmonary and gastrointestinal anthrax, which are currently managed clinically at respective health facilities and may be confused with so many other diseases with a potential of causing pneumonia and/or bloody diarrhoea within the hotspot areas. There is a concern for biosafety in clinical laboratories; the requirements vary in different countries. We consider Biosafety Cabinet level 2 appropriate for clinical laboratory analysis, while biosafety level 3 is more suitable for research related studies involving spore suspensions in liquids formulation or large-scale cultures [30]. We would therefore recommend an animal US snap test for anthrax, which needs a specimen from a dead animal (tissue or blood) and obtain results within a short time. Similar tests can be pursued for humans after having good response in the animal sector in terms of supportive political will, user acceptance and accessibility of the relevant technology and gadgets.

In most instances, many anthrax outbreaks are mismanaged, particularly in rural areas, where it is unlikely to have been adequately diagnosed, reported on time and forwarded to the central levels for rapid response. Underreporting of the IDSR priority diseases (including anthrax) through the electronic surveillance system was revealed in Arusha and Kilimanjaro regions. Therefore, anthrax cases detailed in this study include only those that were recorded, communicated and reviewed in the surveillance systems of Tanzania. It only provides an estimate of the magnitude of the disease which could be a significant under - estimate of the disease burden. Furthermore, the collected anthrax data would assist in future ecological niche modelling in order to map for areas where anthrax outbreaks are more likely to be occurring. This may be a tool for optimization of control measures and improving epidemiologic knowledge of this disease in Tanzania. The causality of various potential risk factors for anthrax transmission in the affected communities of northern Tanzania could also be tested with appropriately designed future observational studies.

Conclusion
The findings of this study are critical for consideration by respective authorities for setting up prevention and control measures of anthrax outbreaks in the human, livestock and wildlife sectors within Tanzania. There is a gross underreporting of anthrax cases in existing human and animal surveillance systems, which can be an obstacle for estimating the real burden of anthrax in the hotspot districts. We also noticed that people living in the marginalised communities like the Maasai remain at high risk of contracting anthrax infection given their ties to cultural practices of handling and consuming dead animals and their products. Moreover, repeated occurrences of anthrax in livestock, wildlife, and humans suggest for strengthening links and promoting inter–disciplinary and multi-sectoral collaboration to enhance the improved prevention and control measures for anthrax outbreaks in a One Health approach.

Abbreviations
AHIS: Animal Health Information System; eIDSR: electronic Integrated Diseases Surveillance and Response System; GIS: Geographical Information System; GPS: Global Positioning System; HAT: Human African Trypanosomiasis; HMIS: Health Management Information System; IDSR: Integrated Diseases Surveillance and Response; IHR: International Health Regulations; IPD: inpatient Department; MNRT: Ministry of Natural Resources and Tourism; NIMR: National Institute for Medical Research; OPD: Outpatient Department; PCR: Polymerase Chain Reaction; PoE: Point of Entry; QGIS: Quantum Geographical Information System; RVF: Rift Valley Fever; TAWIRI: Tanzania Wildlife Research Institute; TVLA: Tanzania Veterinary Laboratory Agency; WHO: World Health Organization

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Availability of data and materials
The dataset used for the current study will not be made publicly available in order to protect for participant's identity but can be obtained from the corresponding author on reasonable request.

Authors' contributions
ERM designed a study, conducted records review, analysed, interpreted data and drafted the manuscript. SH participated in the record review; RF provided expertise on wildlife laboratory data review; HEN participated in designing a study and supervised records review; RHM participated in designing a study and supervised records review and ES supervised the conception of the study, data analysis, interpretation and drafting the manuscript. All the authors have critically reviewed and commented on the manuscript.

Ethics approval and consent to participate
This study was approved by the National Institute for Medical Research (NIMR), Tanzania (Reference Number. NIMR/HQ/R.8aVol.IX/2286). Authorities of TAWIRI, TVLA, Ministries responsible for human health and livestock, the Kilimanjaro and Arusha regions together with districts in the study areas granted permission to review historical data, hospital and laboratory records. The permission was given solely for the purpose of completion of this study. Moreover, human data were given high confidentiality in order not to expose details of medical records of research subjects; we achieved this by using unique identifiers instead of real names of the eligible research subjects.
The research subjects record captured were only the information needed for this study and were properly coded and stored in the manner that we did not disclose the information to any other person [31].
Finally, we will disseminate the findings of this study to the responsible authorities and study subjects so that they adopt a proper way to set control measures of the anthrax outbreak in a more holistic approach.

Consent for publication
Not applicable.

Competing interests
The authors declare that there were no competing interests.

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Paper II

RESEARCH ARTICLE
Open Access

Prevention, detection, and response to anthrax outbreak in Northern Tanzania using one health approach: A case study of Selela ward in Monduli district

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Abstract

Background: Anthrax is an infectious fatal zoonotic disease caused by *Bacillus anthracis*. Anthrax outbreak was confirmed in samples of wild animals following rumors of the outbreak in wild animals, livestock, and humans in Selela ward, Monduli district of Northern Tanzania. Therefore, a multi-sectoral team was deployed for outbreak response in the affected areas.

Objectives: The aim of the response was to manage the outbreak in a One Health approach and specifically: (i) To determine the magnitude of anthrax outbreak in humans, livestock, and wild animals in Selela ward, (ii) to assess the outbreak local response capacity, (iii) to establish mechanisms for safe disposal of animal carcasses in the affected areas, and (iv) to mount effective control and preventive strategies using One Health approach in the affected areas.

Materials and Methods: This was a cross-sectional field survey using: (i) Active searching of suspected human cases at health facilities and community level, (ii) physical counting and disposal of wild animal carcasses in the affected area, (iii) collection of specimens from suspected human cases and animal carcasses for laboratory analysis, and (iv) meetings with local animal and human health staff, political, and traditional leaders at local levels. We analyzed data by STATA software, and a map was created using Quantum GIS software.

Results: A total of 21 humans were suspected, and most of them (62%) being from Selela ward. The outbreak caused deaths of 10 cattle, 26 goats, and three sheep, and 131 wild animal carcasses were discarded the majority of them being wildebeest (83%). Based on laboratory results, three blood smears tested positive for anthrax using Giemsa staining while two wildebeest samples tested positive and five human blood samples tested negative for anthrax using quantitative polymerase chain reaction techniques. Clinical forms of anthrax were also observed in humans and livestock which suggest that wild animals may contribute as reservoir of anthrax which can easily be transmitted to humans and livestock.

Conclusion: The rapid outbreak response by multi-sectoral teams using a One Health approach managed to contain the outbreak. The teams were composed of animal and human health experts from national to village levels to control the outbreak. The study testifies the importance of multi-sectoral collaboration using One Health approach in outbreak preparedness and response.

Keywords: anthrax outbreak, human – livestock and wild animal's interface, response, Tanzania.

Introduction

Anthrax is a zoonotic infectious disease caused by a Gram-positive, rod-shaped spore-forming bacterium called *Bacillus anthracis* [1]. The disease affects mainly herbivores, causing fatalities in the majority of infected cases [2]. Infection in human occurs when *B. anthracis* penetrates through skin abrasions or mucous membranes when there is a contact with infected anthrax carcasses or animal products, inhalation of spores, or consumption of undercooked infected carcass [3]. Three types of anthrax occur in humans depending on the route of transmission; these include cutaneous, gastrointestinal, and inhalational forms [1]. The inhalational form is acquired through inhaling anthrax spores, while the gastrointestinal form is more severe, acquired through consumption of raw or inadequately cooked products from infected animals. It may also represent a significant burden which is both poorly reported and misdiagnosed [4]. The soil is the primary reservoir of *B. anthracis* [5].

Herbivores are infected when they graze in an area where the soil or water sources have been contaminated by *B. anthracis* spores [3]. Anthrax outbreaks are often associated with low-lying areas with soil that has high moisture, calcium, prolonged periods of hot and dry weather, organic content, and alkaline pH [1]. Insects have been implicated in the spread of anthrax outbreaks, including both transmissions of a disease by biting or carrion flies spreading the spores onto vegetation which is then consumed by browsing animals [3,6,7]. Spores can persist in the soil under extreme weather and environmental conditions for an extended period [8].

The burden and economic impacts of anthrax in domestic animals are not fully understood [9]. However, epizootics occur each year, resulting in massive deaths of animals, and spill over to humans often occurs by direct contact with infected animals or their products such as meat, hides, bones, and other materials. Estimates show that a total of 2000-20,000 human anthrax cases are being reported annually worldwide [2]. Endemic hotspot areas for anthrax outbreaks exist in most parts of the world including Africa, Asia, United States, and Australia [10]. China, for instance, has experienced three large-scale anthrax outbreaks with 112,000 human cases from 1956 to 1997 [11]. Another outbreak affected 124 animals of different species: 81 cattle, 15 sheep, 9 goats, and 11 horses in Basilicata region and 8 deer of Pollino National Park in Italy [12,13]. In Bangladesh, a multi-sectoral team investigated 14 anthrax outbreaks and identified a total of 140 animal carcasses and 273 human cases of cutaneous anthrax in the recent years [14,15]. Other studies have reported a total of 52 cases of cutaneous anthrax, and 24 cases of oropharyngeal anthrax in humans after anthrax was found in water buffaloes in March-April 1982 in Chiang Mai, Northern Thailand [4].

Anthrax is epizootic throughout Africa, leading to considerable economic losses of livestock and wild animals, costs for laboratory testing and carcass disposal (burning or burial), and severe, sometimes fatal infection in humans. In Zimbabwe, during 1995-2005, a total of 282 outbreaks and 2978 animal cases (livestock and wildlife) were reported [16]. Anthrax is one of the major threats to animals and humans in the Western part of Zambia, in 2010, it affected 45 cattle and three humans [17]. A total of 306 hippopotami died from a confirmed anthrax outbreak in the Queen Elizabeth National Part of Uganda in 2004. It was representing 11.63% of the total hippo population in the park [18].

In Tanzania, studies show that anthrax outbreaks are frequently occurring in the country, for instance, in 1998, 2003, 2006, and 2009, many species were affected including livestock, humans, and wildlife. Overall, seropositivity was found higher in carnivores from Serengeti National Park and Ngorongoro Crater by 90% and 57%, respectively, and significantly lower in herbivores by 46% and 14%, respectively [19]. In humans, hospital records show that 7,538 cases were suspected for anthrax and 8 cases were confirmed for gastrointestinal form with four deaths (case fatality rate 50%) during 1999-2006 [5]. Recent reports indicate the occurrence of the anthrax outbreak in Rombo district of Northern Tanzania affecting four people leaving one died after acquiring infection from infected cattle in 2016 [20]. However, the available statistics on the magnitude of anthrax in the country might not be exhaustive, due to poor surveillance systems and diagnostic capacities in both human and animal sectors [21].

Anthrax has become a disease of public health and economic importance because of its increased incidences both in humans and animals and also impairing the livelihood of human beings [22]. It might be exacerbated by the increased proximity to wildlife protected areas, human behavior of consuming raw or undercooked carcasses from sick or dead animals, poor farming practices, and mismanaged cross-border movement of animals (livestock and wildlife) from one area to another as far as neighboring countries are concerned [23-25]. Control measures against anthrax outbreaks addressed in Tanzania include targeted routine livestock vaccination, intensified disease surveillance, multi-sectoral response to outbreaks, and health education to communities at risk [23].

The Government of Tanzania through the Prime Minister's Office has developed a National One Health Strategic Plan for the period 2015-2020. The plan has a clear focus on ensuring the implementation of human and animal health services by engaging various sectors to enhanced collaboration among livestock, wildlife, and human health sectors for prevention and control of zoonotic diseases [26]. These teams are currently centered at the national level, and plans are underway to replicate these teams at region, district, ward, and village levels countrywide.

Early November 2016 rumors circulated about massive deaths of animals and existence of suspect human anthrax cases in Selela ward, Monduli district of Northern Tanzania. The initial report showed that 80 wildebeests, 6 impala, and 28 cattle deaths were reported to the District Executive Officer. After preliminary laboratory investigation results, the District Commissioner announced the existence of anthrax outbreak in that area. The Monduli District Medical Officer (DMO) communicated the outbreak information to the Ministry of Health, Community Development, Gender, Elderly, and Children that there were two suspected cases of human anthrax in Mto wa Mbu and Mbaash Dispensaries. The human suspect cases were also reported through the electronic integrated diseases surveillance and response (e-IDSR) system.

The One Health Coordination Unit, under the Disaster Management Department of the Prime Minister's Office, formulated a multi-sectoral response team of experts from human, livestock, and wildlife sectors to the region, district, and the affected villages. This team constituted experts from the Prime Minister's Office (National One Health Coordination Unit), Ministry of Health, Community Development, Gender, Elderly and Children, Regional Medical Officer's office, Tanzania Wildlife Research Institute (TAWIRI), DMO, District Veterinary Officer (DVO), and the District Game Officer. The main aim of the response was (i) to create and strengthen regional, district, and village multi-sectoral teams to manage the outbreak, (ii) to determine the magnitude of anthrax outbreak in humans, livestock, and wild animals, and (iii) to sensitize the community on their involvement in the disease prevention and control, the team also intended to address challenges facing the community on prevention and control of anthrax, and other epidemics.

Materials and Methods

Ethical approval

The study was approved by the National Health Research Review Committee of the National Institute for Medical Research (NIMR), Tanzania (Reference Number. NIMR/HQ/R.8aVol.IX/2286). Verbal informed consent was sought from all human subjects before being involved with study activities. For underage, parents or guardians consented on their behalf. We observed confidentiality at all times during the study, names or personal identifications were not used nor disclosing personal details including laboratory results without prior permission. Moreover, during human blood sampling, pre-counseling was conducted, and all measures were taken to make sure minimal pain is inflicted to study participants. All confirmed cases were treated according to Tanzania Standard Treatment Guidelines for anthrax.

Study design

This study was designed as a cross-sectional survey employing both quantitative and qualitative methods in data collection.

Study area

We conducted this study in Monduli district of the Northern part of Tanzania. Monduli district is one of the districts of Arusha region with the majority of people keeping animals. The district forms a part of the Great East African Rift Valley, characterized by some isolated mountains in the flat and rolling plains. Steep escarpments extend along the Western border of the district with the highest point being 2900 m above sea level and the lowest near Lake Natron, 600 m above sea level. The district is located in the middle of one of Tanzania's most important world renowned wildlife and nature-based tourism regions. About 95% of the land area of the district is made up of game controlled areas where wildlife migrates to the wet season from the surrounding National Parks.

Administratively, the district is divided into three divisions (Manyara, Makuyuni, and Kisongo), 15 wards, and 48 villages. The district is part of the northern tourist circuit, surrounded by some of the world's most famous natural attractions. To reach those attractions, tourists must travel through Monduli district. They include the following, to the West of Monduli district (Serengeti National Park - with vast herds of wildlife, including the wildebeest migration, Tarangire National Park - with a high concentration of different species of animals, particularly elephants in this low intervention National Park. The park is well known for its tree-climbing pythons, Lake Manyara National Park with tree-climbing lions, groundwater forests, hot springs, and Ngorongoro crater and conservation area). The main economic activities of Monduli district are livestock keeping, agriculture production, and tourism. More than 90% of the district population is engaged in livestock keeping and agricultural activities. The major ethnic group of this district is the Maasai (whose main activity is livestock keeping), and they constitute about 40% of the entire population. The second ethnic group is the Waarusha who constitute about 20% of the population, and they practice livestock keeping and agricultural activities. The rest of the population who are not indigenous constitutes 40%, and their main activities are farming and trading [27].

Response to anthrax outbreak and field survey

A suspect case of human anthrax was defined as any person with an acute onset of illness characterized by several clinical forms including (i) localized form - skin lesions and (ii) systemic forms - gastrointestinal, pulmonary, and meningeal. A confirmed case was any suspect case with the above symptoms and laboratory confirmation of *B. anthracis* from a clinical specimen [28,29]. A suspected animal case of anthrax occurs when the animal suffers a sudden death accompanied with one of the following signs: Lack of rigor mortis (legs not stiff), blood oozing from the nose, mouth, and other natural body openings, subcutaneous swellings, rapid bloating, and dark non-clotting blood [30]. Additional symptoms in cattle, horses, sheep, and some wild herbivores include fever, dyspnea, agitation, and convulsions followed by death [31].

During field surveys, the following methods were used to find cases and collect data (i) active searching of suspect human anthrax case at health facilities and community level, (ii) species identification and physical counting of carcasses of wild animals in the affected areas, (iii) collection of specimens from suspected human cases and carcasses of wild animals for laboratory analysis, (iv) burying and burning of carcasses of wild animals followed by disinfection of the burial area using lime or 10% formalin, and (vi) meetings with local political and traditional leaders at the district headquarters and Selela ward, and also visiting households with reported anthrax human cases to observe the herd status and search for active human cases at community level. During the visits, sensitization on the mode of transmission, prevention, and control of anthrax was done. At the end of the survey, carcasses of dead wild animals were either buried in a pit of 6 ft and disinfected with 10% formalin or incinerated. The field work for this outbreak response was done during the 2nd and 3rd weeks of November 2016.

Laboratory diagnosis

The DVO initially collected specimen from three wildebeests which included the impression blood smears. The specimens were sent to the Tanzania Veterinary Laboratory Agency (TVLA), Northern zone in Arusha for laboratory analysis, where methylene blue staining technique diagnosed *B. anthracis*, the causative agent of anthrax. Additional tissue samples of wild animals (6 wildebeest, 2 grant gazelle, and one rabbit) and five human blood samples (5 ml each) were taken into an EDTA vacutainers from suspected cases. All samples were transported at a refrigeration temperature to the TVLA in Arusha for further laboratory analysis using polymerase chain reaction (PCR) techniques. We used the QIAamp Mini DNA Kit (Qiagen, Germany) for the DNA extraction following manufacturer's instructions. Homogenized tissues were mixed with QIAGEN Protease (proteinase K) and a lysis buffer proportionally, and the mixture was incubated at 56°C for 10 min. Afterward, proteins were precipitated by addition of 200 µl ethanol to the sample mixture by pulse vortexing for 15 s. The mixture was then centrifuged in the 1.5 ml microcentrifuge tube to remove drops from inside the lid. The lysate was passed through a QIAamp Mini spin column and added 500 µl buffer AW1 without wetting the rim and then centrifuged at 8000 rpm for 1 min. DNA was eluted using 50 µl of sterile water and stored at −20°C until a real-time quantitative PCR (qPCR) for *B. anthracis* was performed. The detection of *B. anthracis* was done by aliquoting 122 µl (24 reactions) of grade water in Eppendorf then Dynamo Color flash master mix, primer (R and F), probe 10 µM, and grade water were added. After vortexing, the master mix for 10 s and in each well of the PCR plate, 22.5 µl of the master mix, and 2.5 µl of DNA template and control sample were added into a PCR plate. The mixture was put into the PIKO - real-time qPCR machine which was connected to a computer with an installed software and the results were read according to a quick guide of PCR analysis procedure version 4 of 2016 with a CQ value range of 25-35. From each sample, we ran against anthrax (*B. anthracis*) virulence plasmid Pag (pOX1) and Cap (pOX2) as described by Fasanella *et al.* [32]. Control-positive DNA used was obtained from the Finnish Defense Forces Center for Bio-threat Detection (MIL - Con).

Data management and analyses

A checklist was prepared to capture quantitative data of human, suspect cases, and the variables collected were: The location of the cases, age, sex, date of disease onset, signs and symptoms, specimen taken, date of specimen collection, type of drugs given (if the patient presented to a health facility), and the outcome of treatment (died or recovered). The qualitative information was obtained by observation during house-to-house visits and while conducting meetings with local officials. A map to indicate locations of the human cases was drawn on the Quantum GIS software (http://www.qgis.org/en/site/forusers/index.html). Data were entered in the Microsoft Excel Spreadsheet and analyzed by producing pivot tables and graphs. Furthermore, the databases were transferred to STATA (SE/14 for Windows, StataCorp, and College Station, TX) for additional statistical analyses [33].

Findings dissemination strategy

We disseminated the findings of the study to responsible officials in Selela ward, Monduli district in Arusha region and at the national level through reports, and meetings and recommendations were made for further control and prevention of the outbreak.

Results

Laboratory investigation confirmed anthrax through methylene blue staining and real-time qPCR techniques. Other samples did not amplify either with Pag (pXO1) nor did Cap (pXO2) that was run with control samples amplified with a standard curve CQ value average of 34.59. As mentioned earlier, livestock carcasses (10 cattle, 26 goats, and three sheep) were not available for sampling as they were reported to have either been consumed or hidden by owners. A total of 131 carcasses of wild animals were counted, the majority (83%) being wildebeest. Other carcasses of wild animals (16%) were for grant gazelle and 0.8%

Table-1: Distribution of human cases and animal carcasses identified in Selela ward following anthrax outbreak, November 2016.

Species affected	Human cases and animal carcasses	Frequency (%)
Human cases, sex	Male	11 (52.38)
	Female	10 (47.62)
Human cases, age group	≤5	9 (42.85)
	6 - 15	7 (33.33)
	16 - 25	3 (14.28)
	≥26	2 (9.54)
Livestock carcasses	Cattle	10 (25.64)
	Goats	26 (66.67)
	Sheep	3 (7.69)
Wildlife carcasses	Wildebeest	109 (83.21)
	Grant gazelle	21 (16.03)
	Rabbit	1 (0.76)

for the rabbit. Out of 21 human, suspected cases, the majority were under 5 years of age (42.85%) followed by the age group of 6-15 years (33.33%) as shown in Table-1.

Five villages were visited, these included Selela, Mbaash (Selela ward), Mungere (Mto wa Mbu ward), and Oltukai (Lake Manyara ward) for active searching of anthrax suspected cases and dissemination of health education on prevention and control of anthrax outbreak. We observed that the Maasai communities were still engaging themselves in activities which are posing a risk for anthrax transmission. All the livestock kept by the Maasai in Monduli district were not vaccinated against anthrax. The Maasai spend most of their time taking care of their animals, and the grazing environment makes their animals become in contact with wild animals. Due to their intimate contact with livestock and their products, the Maasai are predisposed to different zoonotic diseases including anthrax. The local people were observed dressing dead domestic and wild animals for consumption, and this was mostly done by women and children. The dried hides and skins from such animals were used as bedding materials, ropes or donkey luggage pockets and wildebeest tail brush as swats for chasing flies.

In the meetings conducted with local people, anthrax was reported as being brought by bad spirits of Maasai ancestors. When anthrax outbreak occurs, they tend to tie a small piece of animal skin on the finger as a way of chasing out the bad spirit from the household which is perceived to protect human from acquiring anthrax. It was further observed that anthrax outbreak in livestock, wildlife animals, and humans occurred at the same time with overlapping dates in November 2016 (Figure-1). It is an indication that the existing interactions between animals and humans in different ways pose risks for anthrax transmission across the species.

A total of two suspected cases of human anthrax were reported from livestock keeping households identified during community-based case searching, and all had skin lesions suggestive of cutaneous anthrax. All patients were treated at Mto wa Mbu health centre, Selela, Mungere, Mswakini, Oltukai, and Simangori dispensaries and recovered. On history taking at health facilities where they were attended, it was found that all of the human anthrax patients had a history of coming into direct contact with carcasses (touching or butchering) of dead animals whose causes of death was not established.

The human index case was reported on 5th November 2016 (Figure-1) through the e-IDSR system, and this was preceded with the verbally reported deaths in livestock and wild animals in Selela Village. The 21 human anthrax suspected cases were mostly (61.9%) from the villages of Selela ward. Other affected wards in different proportions were Eslalei (28.6%), Lepurko (4.8%), and Mswakini (4.8%) (Figure-2). The incubation period of anthrax infection in humans is up to about 5-7 days depending on the microbial load. The number of cases started to decline after a national multi-sectoral team was deployed to the affected areas as it is illustrated in the epidemic curve of the human anthrax outbreak in Selela ward (Figure-3).

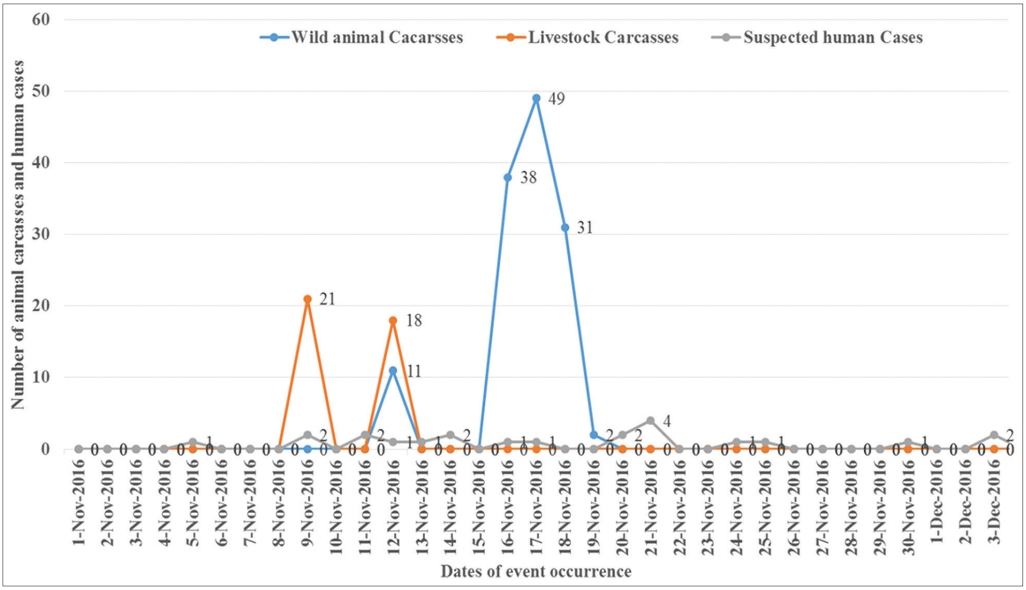

Figure-1: Trend of anthrax outbreak occurrences at the human, livestock, and wildlife interface in Selela ward in Monduli district, November 2016.

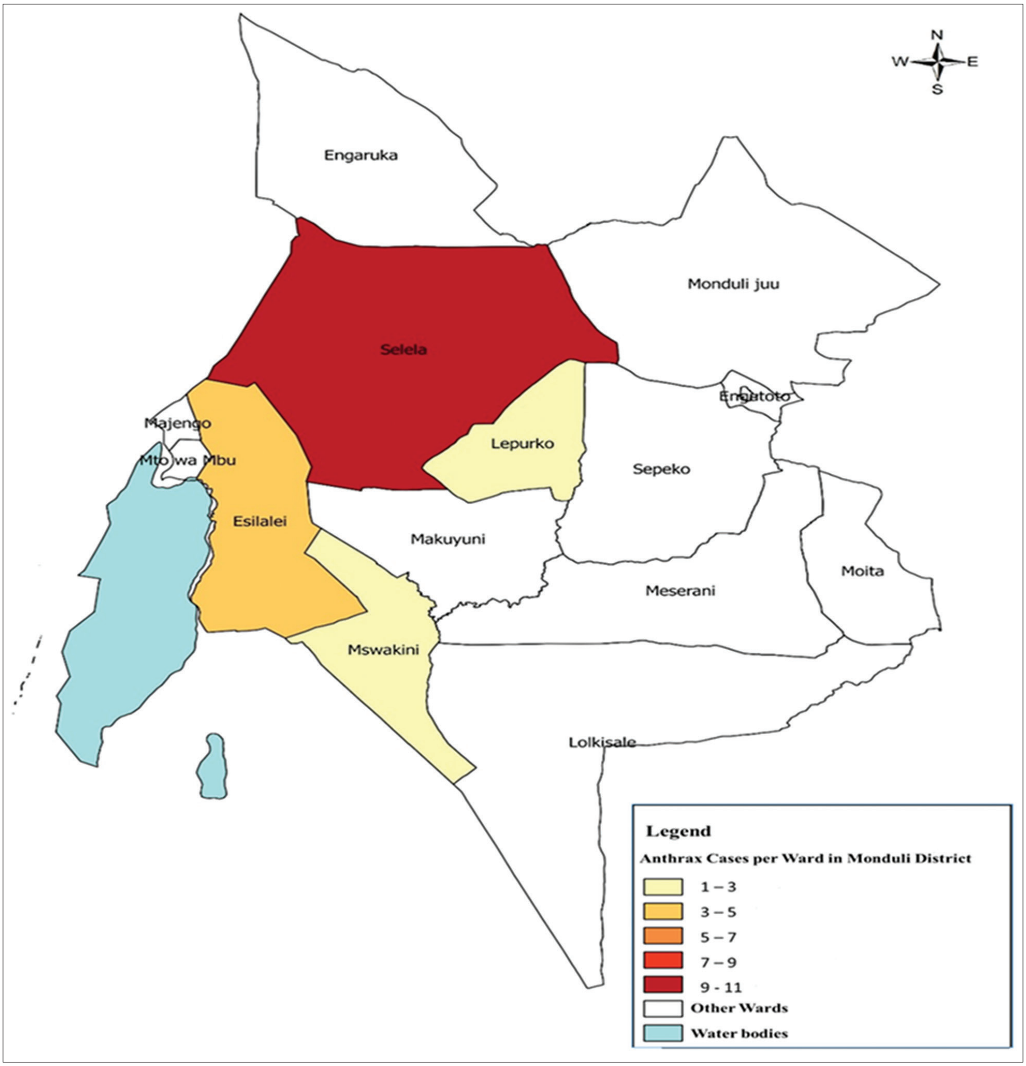

Figure-2: Map of Monduli district, showing the distribution of human anthrax cases in Selela ward, November 2016.

A total of eight affected households were visited, all were close to where wildebeest carcasses were found, and most of the members of the households admitted to having consumed meat from carcasses. The household herds reported grazing their livestock in proximity to places where some of the decomposing wildebeest were scattered on the ground. It was also reported that there were few livestock extension officers for providing extension and veterinary services to livestock and meat inspection. This led to livestock keepers treating their animals themselves. Livestock keepers requested to a response team to investigate on the suspected poisonous grass called *endule* in the Maasai language which is believed to cause livestock mortalities at the beginning of every rainy season.

The Laigwanan are the highly respected traditional leaders whose orders and directives are obeyed by the whole community within the locality. We educated and sensitized them on how to prevent transmission of anthrax and on early health-seeking behavior to a nearby health facility once any member of the family gets sick. They were also requested to ensure that sick and dead animals are immediately reported to the nearby livestock field officer and other authorities. The aim of using them was for easier dissemination of anthrax knowledge to the community because they are key people in the society. Finally, a local multi-sectoral group of experts and local leaders was formed in Selela ward to ensure early reporting of suspected anthrax cases and other epidemic-prone diseases in humans and animals.

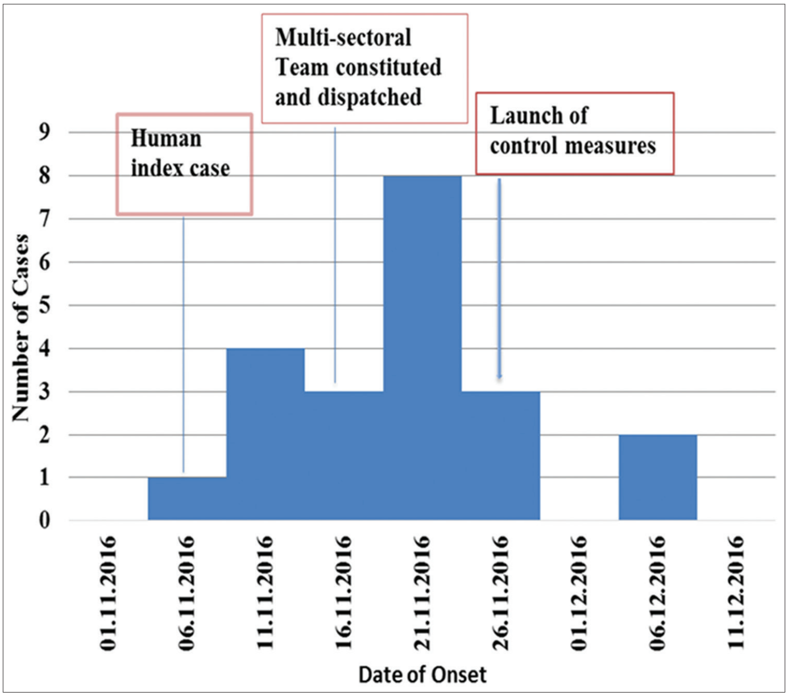

Figure-3: Epidemic Curve of human anthrax cases in Selela ward, Monduli district, November 2016.

The team consisted ofward livestock field officers, Clinical Officer, Community Development Officer, Ward Health Officer, Community Health Worker, Wildlife Officer, Agriculture Extension Officer, Village Chairperson, Village Executive Officer, Ward Executive Officer, and Councilors. The terms of reference were developed to describe the roles and responsibilities of each team member, and the official launching of the established team was proposed to be done in January 2017. A One Health Multi-sectoral Team was also developed at Arusha regional level comprising members from Monduli district (Human, livestock and wildlife Departments), TAWIRI, TANAPA, Tanzania Wildlife Authority, Nelson Mandela African Institute of Science and Technology, and Regional Secretariat (Veterinary Services, Human health, and Tourism Services). The developed team agreed to conduct joint meetings once per week and developed outbreak response action plan for the year 2016-2018.

Discussion

The findings on the carcasses such as body fluids including blood oozing from different natural orifices, excessive bloating, laboratory results of specimens taken from the carcasses, and the PCR assays confirmed that the animals died of anthrax. The skin lesions on human cases who attended health facilities after consuming carcasses also provided strong evidence to support the diagnosis of anthrax. The finding that human smear results were negative may be attributed to the effect of antibiotics taken after anthrax was suspected. Similar findings were observed in Chama district, Zambia, where the diagnosis of anthrax in human specimen was masked by antibiotic treatment initiated before blood sampling [34,35].

Clinical presentation in suspected humans coupled with a history of adequate exposure to infected animals and their products such as consumption of raw or undercooked meat and evidence of physical contact with infected carcasses are of great importance in initial diagnosis even before laboratory results. The advanced laboratory tests such as real-time PCR or serology (ELISA) serve as confirmatory tests, but most of the times, they are not readily available in typical field settings and also are not cost effective. The ideal laboratory test for anthrax should be sensitive, specific, and inexpensive [36]. Laboratory culture technique should be considered as a backup diagnostic procedure to support the conventional investigations, requiring standardized equipment, materials, and instructions at the levels of diagnosis [37].

As a control measure, burning of carcasses was the method of choice as scavengers had already opened most of the carcasses. This is because burying was, however, tedious, and costly as it required extensive workforce for excavating the burial pit of 6 ft deep. Burning also destroyed and killed spores that contaminated bushes and served as a preventive measure for disease transmission to other grazing and browsing animals. In the current outbreak, wildebeests were the most affected wild animals, followed by grant gazelle and rabbit. All carcasses of wild animals, irrespective of the species, showed classical features of anthrax including exuding blood in natural body openings. As anthrax outbreak is dependent on the existence of susceptible hosts [38], intensified surveillance using a One Health approach, vaccination of livestock, proper disposal, and liming of the disposal sites of livestock and wild animal carcasses are the most efficient approaches for prevention and control of future occurrences of outbreaks. These may also serve as ways to reduce transmission of anthrax to humans [2].

It was evident that health-seeking behavior among members of the village with anthrax outbreak requires urgent attention. The eight households visited had cases with active skin lesions suggestive of being anthrax infection, but none of the cases had a history of attending to the nearby health facility for medical attention. Some cases vividly showed signs and history of using traditional ways including smearing cow dung on skin lesions. Eating carcasses were observed to be one of the common practices in Selela ward besides some of the on-going awareness campaigns and health education interventions. We suggested different intervention methods including the use of the influential local leaders known as Laigwanan and political leaders. Some pastoralists in the Maasai community mostly consider the extent of decomposition of a dead animal which they want to consume rather than potential risks of zoonotic disease transmission [23].

Livestock keepers in Selela ward requested for the investigation of the toxic grass called *endule* in Maasai language as they believed it was the cause of animal deaths every year. We collected samples of the reported toxic grasses to the NIMR laboratory for investigation but the results were not conclusive. To support toxicological investigation determination of seeds or plant materials in the rumen or stomach content or feces on autopsy and postmortem lesions are required to rule out plant poisoning [39].

The teams discovered several unreported deaths of wild animals during the survey. The local community associated the deaths to the fresh, lush pastures that follow long periods of drought season. It might be the basis for them to consume all the livestock carcasses in addition to some wild animals which died close to their *bomas*. Pastoralists might be aware of the risks associated with consumption of raw milk, blood, or raw or undercooked meat, but they still practice these risky behaviors particularly in rural areas [24]. The Maasai community has a belief that, drinking raw blood is important for young boys who have just been circumcised, as they believe that, raw blood replenishes nutrients lost during the procedure. On the other hand, lack of appropriate health education, poverty, or economic reasons can facilitate anthrax transmission as community members may tend to consume raw or undercooked meat, milk, or blood from animals infected with anthrax [40].

In Selela ward, it was evident that anthrax outbreak occurred at the human/livestock/wild animal's interface and this was facilitated by the existing interactions between them. Shortage of experts (livestock field officers and clinical officers) in this ward was reported which also contributes to late reporting of suspected human and animal cases, and hence, delayed response to the outbreaks. In most occasions, many anthrax outbreaks, especially from peripheral areas, are unlikely to have been properly diagnosed and reported timely to the district level leading to delayed outbreak response.

As opposed to the animal health sector where disease reporting at the village level is still a problem, the human/public health sector has the e-IDSR system, that allows a health facility to report cases of prioritized diseases to the higher levels of action. When the first case of human anthrax presented to the Mbaash dispensary in Selela ward with skin lesions suggestive of anthrax, headache, and fever, the facility clinician immediately fed the information onto the e-IDSR system using his mobile phone. It enabled all the higher levels including the Ministry of Health to get notified of the outbreak occurrence, so appropriate interventions were employed.

In Chama district, Zambia, the IDSR system reported two suspected cutaneous human anthrax cases, and the next day, a multi-sectoral response team was constituted and deployed to respond [41]. The animal health surveillance system in Monduli district is not well structured, with lack of veterinary extension officers to report or record deaths of livestock in the village and to the higher levels. It illustrates the challenges facing the veterinary sector and the need to address some of the issues for an effective One Health approach.

The previous analysis of anthrax epidemiological data in the world indicates the following estimated ratios: (i) 1 human cutaneous anthrax case to 10 anthrax livestock carcasses; (ii) 1 incidence of enteric human anthrax to 30-60 anthrax-infected animals eaten; and (iii) in humans, 100-200 cutaneous cases for each enteric case that occurs [42]. The clinical appearance of cutaneous anthrax is similar to a malignant pustule surrounded by edema at the infection site [43].

Selela ward has a National Park nearby and the surrounding bushy areas with free movement of wild animals. Studies have indicated that wild animals as being the reservoir of many human infectious diseases including anthrax [44]. It is estimated that more than third of new, emerging, or re-emerging human infectious diseases since an early 21st century have been caused by pathogens originating from animals or products of animal origin [45]. Viruses, bacteria, and parasites have had their reservoirs in a host of animals such as those found in the wild, peri-domestic, and domestic [44].

The wide array of host species and the complex natural history of the pathogens concerned, pose big challenges for effective surveillance, prevention, and control of zoonotic diseases [1]. Several factors have been shown to facilitate the spillover of new diseases from livestock and wild animals into humans. These include environmental changes, population increase, microbiological adaptation to hosts and environment, and human practices and behavior [46]. Therefore, there is a need for various sector's collaboration during anthrax outbreak investigation and response including sharing the standards for livestock vaccination, meat inspection, and food hygiene in the country, East Africa Community (EAC) region and beyond.

Selela ward is a few kilometers from the border with Kenya, some livestock keepers cross the border to Kenya with their livestock, and there is also a free movement of wild animals across the Tanzania-Kenya border to Selela ward. The report of the EAC meeting is noted that Tanzania had developed country initiatives for cross-border diseases outbreak investigation and response. It was through sharing of information, surveillance data, laboratory confirmation and response initiatives in satellite laboratories, cross-border meetings, the establishment of cross-border diseases surveillance committees, and joint field simulations/investigations between Burundi, Rwanda, Kenya, Uganda, and Tanzania [47]. This approach can, therefore, be expanded to involve livestock and wildlife sectors using One Health approach in the EAC region.

About 95% of the members of the community of Selela ward are livestock keepers, and 5% are involved in crop production and business. The occurrence of anthrax in wild animals and the spillover to livestock and human is a wakeup call for a targeted: (i) Comprehensive multi-sectoral strategy involving routine vaccination of susceptible livestock (cattle, sheep, and goats) in anthrax hotspot areas using quality-assured and tested vaccines; (ii) enhanced surveillance system (with clear case definition) both in the public health and animal health sectors to ensure timely reporting and investigation of sudden death in livestock and wild animals; (iii) rapid disposal of dead livestock and wild animals, contaminated bedding materials and control of scavengers; (iv) extensive public awareness and compliance with general hygiene principles, including use of personal protective equipment by people who might be in contact with sickened or dead animals; (v) laws and regulation enforcement pertaining to anthrax control including quarantine of infected animals and animal products, and last but not least, enhanced communication and collaboration between countries to strengthen cross-border networks and strategies to curb zoonotic outbreaks.

Moreover, the next step for our project will be to map for a more detailed ecological niche modeling to better understand the epidemiologic knowledge of anthrax outbreaks. It will also assist to explore for a normalized difference vegetation index to get a better idea of how specific location might be associated with lives of grazing animals which are getting exposed to risks of disease transmission.

Limitation

The outbreak response did not test the statistical significance of the documented potential risk factors for anthrax transmission in Selela ward. Therefore, a qualitative anthropological study is recommended to measure the significance of the mentioned cultural-related practices that propagated disease transmission in the Maasai pastoralist communities living in the wildlife-livestock interface areas. The team did not find any livestock carcass, and hence, no sample was collected from livestock, it is possible that animals were consumed after they died. The intake of antibiotics before collection of blood samples from suspected cases compromised the confirmation of anthrax in humans.

The team had to use a translator to communicate with the Maasai as the majority of them did not speak Kiswahili which is the national language. Therefore, awareness of anthrax, health education, and other relevant outbreak information had to be translated to Maasai language. To some extent, this could not ascertain whether the right information was conveyed.

Conclusion

Anthrax outbreak was confirmed in wild animal samples taken from Selela ward, Monduli district, Arusha region in Northern Tanzania. The sudden death of animals with carcasses showing signs of anthrax was the first clear indication of the disease in animals. Clinical manifestation of cutaneous anthrax in human cases who consumed the meat from carcasses of dead domestic and wild animals during the outbreak cemented the diagnosis of an anthrax outbreak.

Although vaccination for livestock is considered to be among the most important interventional methods to prevent and control anthrax outbreaks in both humans and animals, no anthrax vaccination for livestock was observed during this outbreak response as in Tanzania vaccination is a private enterprise. Therefore, most livestock keepers do not consider it a cost-effective exercise, and hence, they either cannot afford to, or they opt not to vaccinate their animals. The authors would, therefore, recommend for anthrax vaccine to be a public good under a public-private partnership scheme. The study concludes that for an effective zoonotic diseases prevention and control, multi-sectoral coordination, communication, and collaboration using a One Health approach is paramount.

Authors' Contributions

ERM designed a study, conducted field survey, analyzed, interpreted data, and drafted the manuscript. JAA participated in the field survey; JSK participated in the field survey. EEM participated in the field survey. ZEM provided expertise on specimen laboratory analysis, HEN participated in designing a study and supervised drafting and writing of the manuscript; RHM participated in designing a study and supervised drafting and writing of the manuscript, and ES supervised the conception of the study, data analysis, interpretation, and drafting the manuscript. All authors revised and approved the final version of this manuscript.

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Competing Interests

The authors declare that there were no competing interests.

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Paper III

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Risk factors for human cutaneous anthrax outbreaks in the hotspot districts of Northern Tanzania: an unmatched case–control study

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Bacillus anthracis is an aerobic, Gram-positive and spore-forming bacterium, which causes anthrax in herbivores. Humans get infected after coming into contact with infected animals' products. An unmatched case–control study was conducted to identify the importance of demographic, biological and/or behavioural factors associated with human cutaneous anthrax outbreaks in the hotspot areas of Northern Tanzania. A semi-structured questionnaire was administered to both cases and controls. The age range of participants was 1–80 years with a median age of 32 years. In the younger group (1–20 years), the odds of being infected were 25 times higher in the exposed group compared to the unexposed group (OR = 25, 95% CI = 1.5–410). By contrast, the odds of exposure in the old group (≥20 years) were three times lower in the exposed group compared to the unexposed group (OR = 3.2, 95% CI = 1.28–8.00). Demographic characteristics, sleeping on animal's skins, contacting with infected carcasses through skinning and butchering, and not having formal education were linked to exposure for anthrax infection. Hence, a One Health approach is inevitable for the prevention and control of anthrax outbreaks in the hotspot areas of Northern Tanzania.

1. Introduction

Bacillus anthracis is an aerobic, gram-positive and spore-forming bacterium belonging to the family Bacillaceae [1]. The release of this bacterium (the causative agent for anthrax) from a dead infected host into the environment induces spore formation [2], enhancing the agent's ability to survive in the soil for a long time [3]. Despite being well controlled in developed countries, anthrax continues to have a devastating global effect on the poor and marginalized populations that depend on small-scale livestock farming in rural areas [4]. Anthrax is continuously ranked as a significant poverty-related neglected zoonotic disease, defined by the World Health Organization as a disease that 'perpetuate poverty by affecting not only people's health but also their livelihoods' [4,5]. Flooding, drought and biological vectors (birds, insects or scavengers) or areas of temporary stagnant water may exacerbate anthrax outbreaks [6]. The release of *B. anthracis* from an infected host into an aerobic environment with insufficient nutrients to sustain bacterial replication induces sporulation [7]. The *B. anthracis* spores are resistant to extreme conditions such as pH [8], heat, cold, desiccation and chemical agents, and may, in specific environments, survive up to 200 years [9]. Owing to the extended persistence of *B. anthracis* spores in the environment, regular epidemics may occur after a long time, such as a recent outbreak in Sweden after 27 years [10].

Anthrax affects all mammals, but wild and domesticated herbivorous dominate the numbers, as they are often infected through ingestion or inhalation of spores while grazing [11]. The susceptibility to infection differs depending on the host species [12], with cattle and sheep being the most vulnerable species followed by goats, dogs and horses [13]. Humans are considered to have a moderate susceptibility, while pigs and carnivores are more resistant [11]. Upon ingestion, spores enter macrophages of a susceptible host and are transported to lymph nodes where they germinate into vegetative form [14] and migrate into the bloodstream and release toxins which cause systemic effects [11].

Humans typically get infected with *B. anthracis* through oral, cutaneous and respiratory routes [15], and the infection could occur during direct contact when butchering, eating raw or undercooked meat, or handling products from infected animals [16]. Cutaneous anthrax is the most frequently diagnosed form of the disease in humans and occurs within 2–6 days after direct contact with anthrax spores [17]. It presents as a papular to a vesicular ulcer which forms a depressed black eschar which is accompanied by oedema [18].

The first anthrax outbreak in Tanzania was documented among the wildlife species in the national parks during 1962–1998, causing the death of 1200 impalas, and posed a great risk to humans and susceptible livestock [19]. Later on, sporadic human cases have been reported in different parts of the country. In 1985, a total of 239 human anthrax cases were reported in the Rukwa valley in southwest of Tanzania [20], and in 1988, a total of 11 human cases of cutaneous anthrax were admitted and treated at Mvumi Hospital in the Dodoma region of central Tanzania after patients came into contact with the infected animal carcasses [21].

In 1985, hundreds of different species of wildlife carcasses were laboratory-confirmed to have died from anthrax in the Selous game reserve [22], and in 1988, a big anthrax outbreak in wildlife was reported in the Tarangire national park in which 142 impalas, three zebras, four wildebeests and one giraffe were counted dead [23]. Since then, different species of wildlife and livestock and humans have frequently been affected by *B. anthracis*, with varying disease patterns between years in terms of the size of outbreaks and species affected [24].

Anthrax is a notifiable zoonotic disease in Tanzania, and it is a disease of public and animal health importance [16]. Despite the seriousness of anthrax outbreaks in animals, there is a poor surveillance system in the animal sector leading to under-reporting of reportable diseases [25], including anthrax.

Moreover, episodes of anthrax outbreaks are increasingly becoming a threat to humans, livestock and wildlife in Northern Tanzania, specifically in the Arusha and Kilimanjaro regions. For instance, in November 2016, anthrax outbreaks were reported in Monduli district, Arusha region in Northern Tanzania in which 131 carcasses of wild animals were disposed of and 39 carcasses of domestic animals were reported to be consumed [18]. In the Serengeti ecosystem of Northern Tanzania, serological reactions have been reported in herbivorous species often hunted for bushmeat that comes from wildlife which is smuggled in for human consumption [26]. Spillover infections in wildlife can sustain the disease and become a source of spill-back infection to humans and livestock [27].

Therefore, recurrent outbreaks of anthrax in Northern Tanzania are probably due to the extensive interactions of human, livestock and wildlife in the interface areas. Sporadic, non-fatal cutaneous anthrax lesions are common in individuals who handle infected meat or come in direct contact with infected animal materials [18]. Although it is well known that cutaneous anthrax is caused by skin contact with contaminated surfaces [28], during these outbreaks it was not clear which surfaces were the most important vehicle for transmitting *B. anthracis* to humans in specific geographical and cultural settings.

Other studies have reported that there is limited knowledge on the community's awareness of the role contributed by the interaction of animals and humans in the transmission of zoonotic diseases [29,30]. Our retrospective study of health facilities and animal diagnostic centres from 2006 to 2016 revealed a list of hotspot districts for anthrax outbreaks in Northern Tanzania, and that most reported human cases pertained to cutaneous anthrax infection [16]. Moreover, the Arusha region had a reported incidence of 7.9 human anthrax cases per 100 000 population followed by the Kilimanjaro region with 6.6 per 100 000 population [16].

During anthrax outbreaks, the multisectoral teams comprising experts from the ministries responsible for human, livestock and wildlife health were dispatched to the affected regions. In these affected areas, a team collaborated with the regional and district's multisectoral teams to contain the outbreaks by mounting preventive and control measures including intensified surveillance, community awareness, improved diagnostic capacity and livestock vaccination against anthrax in the affected areas [31]. In Tanzania, the coordination of response to disease outbreaks is under the One Health coordination desk within the Prime Minister's Office [32,33].

The current study was conducted to identify demographic and behavioural factors associated with cutaneous human anthrax outbreaks in the anthrax hotspot areas of Northern Tanzania. The study was conducted to better understand the causal relations and improve on potential intervention strategies in the region.

2. Material and methods

2.1. Study area

The areas for this study were the hotspot districts for anthrax in the Arusha and Kilimanjaro regions of Northern Tanzania. The eligible districts for the Arusha region were Ngorongoro, Monduli and Meru, while in the Kilimanjaro region the study was conducted in Siha, Hai, Rombo and Moshi rural districts. The health facilities involved in each district included Wasso DDH, Endulen Mission Hospital, Pinyinyi, Piyaya, Arash and Magaiduru Dispensaries in Ngorongoro District. Selela, Oltukai, Mto wa Mbu, Mungere, Mbaash and Mswakini Dispensaries were the studied health facilities in Monduli District, while Majengo Dispensary was in Arumeru District. Other health facilities in the study were Hai District Hospital, Sanya Station, KIA and Mtakuja Dispensaries in Hai District; Himo, Rauya RC Dispensaries in Moshi rural district, while Kibong'oto Hospital and Manyata Dispensary were in Siha District. Lastly, Huruma DDH, Nanjara and Karume HC were included in Rombo District. Figure 1 shows the wards where the health facilities and villages involved in the study are located. All the studied districts have a majority of residents practising both subsistence farming and animal husbandry. The study districts are also in the interface areas surrounded by different wildlife conservation areas in the northern circuit of Tanzania. There are soft/porous borders between wildlife conservation areas and human settlements, due to an increased interaction between wildlife and livestock during grazing and at water points [34] in Northern Tanzania. Humans are also posing a risk of zoonotic disease transmission through farming intensification in close proximity to conservation areas, leading to clearance of bushes (change of landscape) and hence destruction of the wildlife ecosystem, causing an increased rate of contact between disease pathogens and humans, livestock and wildlife [35,36]. The data collection for this study was done from 6 October to 5 December 2016.

2.2. Study design and sample size

This study was of a non-matched case–control design, with cases being retrieved from the local health facilities. For each case, a control was selected from a nearby randomly selected household within the same locality as the eligible case.

The minimum sample size was calculated using the Epitools AusVet sample size calculator (http://epitools.ausvet.com.au/content.php?page=SampleSize) with the assumption that the frequency of exposure in controls was 20%, and the odds ratio was to be detected at 3.0, with 80% power and a

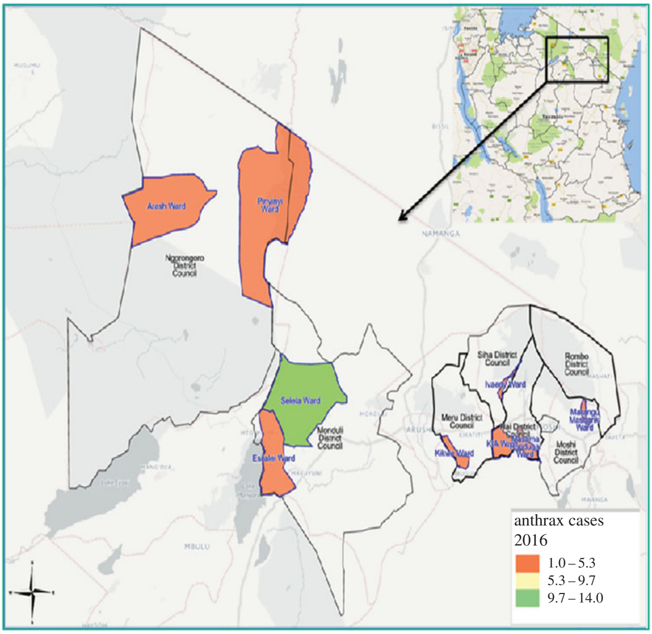

Figure 1. Spatial distribution of anthrax cases in the affected wards from hotspot districts, Northern Tanzania 2016.

95% confidence interval. With these assumptions, the minimum sample size of 61 cases and 61 controls was calculated. A total of 59 cases and 59 corresponding controls were subsequently recruited.

2.3. Inclusion and exclusion criteria

A case was defined as any person residing in the selected hotspot districts of Northern Tanzania who had ever developed skin lesions by itching of the affected area followed by papular lesions and thereafter a vesicular stage over 2–6 days, eventually developing into depressed black eschar sometimes accompanied by mild or severe oedema [1]. A case was eligible for inclusion in the study if records were found in the medical register at a randomly selected health facility in the hotspot districts of the Arusha and Kilimanjaro regions during the preceding two weeks. The patient should have met the case definition for cutaneous anthrax (as defined above) and his/her name found registered in medical records and had resided in the hotspot districts of the Arusha and Kilimanjaro regions for not less than six months before the time of recruitment. A control was defined as any person who resided in a neighbourhood with an eligible case and had not contracted cutaneous anthrax during the preceding six months. This study excluded anthrax suspected cases with a history of coming from other places apart from the Arusha and Kilimanjaro regions in a period of one week before the onset of signs and symptoms of anthrax. Children under 18 years old were included in the study, but their parents/guardians were interviewed as a proxy on their behalf.

2.4. Data collection

A semi-structured questionnaire was developed in English to be administered to both the cases and the controls. The questionnaire included questions related to potential biological exposure to *B. anthracis* as well as information about demographic factors such as age, sex, occupation, ethnic group, level of education, district/place of residence, and potential risks linked to travelling outside the village in the last two weeks before onset of the disease. The questionnaires were pretested, and necessary changes were made based on the identified ambiguities. The questionnaire was subsequently translated into Kiswahili, the national language spoken by almost every resident.

Before visiting the eligible households, a brief interview was conducted with the ward and village executive officials. Locally available public health officers, livestock extension officers and natural resources officers were also interviewed to document their views on the occurrences of the human and animal anthrax cases in their areas within a period of one month before the time of data collection.

In each household of an eligible human anthrax case, interviews were conducted using the questionnaire and in the event of an underage case (less than 18 years), the proxy (parent or guardians) was interviewed in the same household. After the case interview, the questionnaire was administered to the head of households near the cases, which served as the control.

2.5. Statistical methods and data analysis

The data obtained were entered into a Microsoft Excel® spreadsheet by allowing comparison for duplicate data entry errors, and data cleaning was done to ensure the quality of the information entered in the dataset. The cleaned dataset was then transferred into STATA (Stata15/SE for Windows, StataCorp, College Station, TX, USA) for statistical analysis.

Essential demographic, biological and other characteristics were described for cases and controls. The relationship between anthrax transmission and potential risk factors or other covariates was initially assessed using univariable logistic regression. As many cases were younger compared to the controls, the analysis was split into four age quantiles. Further recoding of all exposure factors as dichotomous (yes/no) variables was done. Candidate variables with $p < 0.25$ from the initial logistic models were subsequently assessed for collinearity in a cross-tabulation using a Goodman and Kruskal's gamma test. For highly correlated variables, only one of them was selected for further analyses. Variables were identified as confounders and included in the final model if including or excluding the variable altered the effect estimate for another variable by more than 10%.

The first statistical model was developed using a multivariable logistic regression, with a backward elimination strategy among candidate variables. The models were built based upon the Wald test and the likelihood test ($p < 0.05$). We finally used a Hosmer–Lemeshow test for the goodness of fit and the area under the curve of the receiver operating characteristics to assess the reliability of the final constructed model.

As many exposure variables were correlated, we were faced with the difficulty in establishing a realistic and stable statistical model. Considering the questionnaire used in this study, we noted that there were groups within our investigated population. Those groups were characterized by different patterns of behaviour, caused by disparate preferences, which could lead to anthrax infection. Still, we could not identify any specific variable which describes such behavioural dichotomy. Hence, we adjusted our statistical analyses using a latent class analysis (LCA) method. All variables linked to the expected exposures to anthrax were used to construct two latent classes using the generalized structural equation modelling (*gsem*) command in STATA. The binomial family and the logit link function defined the variables. The study subjects were classified with a probability of belonging to an *Exposed* class and the rest as *Not Exposed* by using a posterior probability of greater than 0.5 as a cut-off/threshold between the two classes. Based on a directed acyclical graph (DAG) model drawn in the DAGitty software [37], the final statistical model was established using a structural equation model (SEM). The SEM was also built on the *gsem* platform with a logit link function between the anthrax cases and the *Exposed* class. Initially, the primary model was built using the graphical interface in the *sembuilder*, before modifying the model in the *gsem* command syntax. Demographic factors such as age, sex, occupation and education as well as the history of travel were used as predictors for *Exposed* and were not linked directly to anthrax cases. As there was a strong age bias in the dataset due to the high number of young cases, separate SEM models for the first age quantile (less than 20 years) and older study subjects were established.

3. Results

3.1. Respondents' characteristics

Cases were recruited from Hai ($n = 6$, 10.2%), Meru ($n = 3$, 5.1%), Monduli ($n = 20$, 33.9%) Moshi DC ($n = 3$, 5.1%), Ngorongoro ($n = 12$, 20.3%), Rombo ($n = 7$, 11.9%) and Siha ($n = 8$, 13.5%). Figure 1 illustrates the relative density of cases recorded in each of the wards from the hotspot districts. The timeline for the cases recorded in the different districts is found in figure 2. Table 1 gives the main categories of the demographic and biological variables recorded. Among the study participants, there were more male ($n = 70$, 59.3%) than female participants ($n = 48$, 40.7%). The age range of participants was 1–80 years with a median age of 32 years. Figure 3 shows the distribution of age across cases and education groups. A total of 83 (70.3%) of the study subjects had no formal

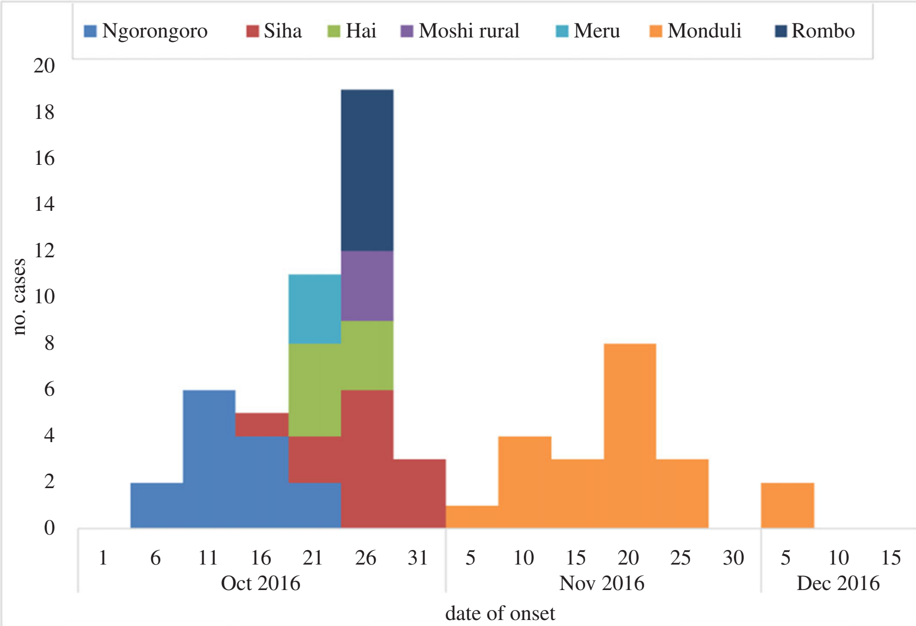

Figure 2. The cumulative epidemic curve for identified anthrax cases in the hotspot districts of Northern Tanzania in the period of October–December 2016.

education. During analysis, it was realized that younger cases (1–20 years) were more recruited, with 26 (44.1%) of the 59 cases, while only four controls (6.8%) were from this group.

3.2. Logistic regression analysis

In the univariable logistic regression analysis, study subjects were initially grouped into four age quantiles and subjected to cross-tabulations with demographic characteristics (sex, education status and occupation) and history of travel and biological factors (skinning/burying dead animal, contact with animals, contact with animal products and type of sleeping materials). These are the factors which may predispose an individual to anthrax infection; the other variables were source of animal feeds, knowledge on the animal diseases preventable with vaccine, disposal of animal carcasses, death of animals at home and keeping animals/dogs. Potential candidate variables ($p < 0.25$) are presented in table 1. The results from the subsequent multivariable logistic model are presented in table 2. These results indicate that having primary school education was protective against getting anthrax infection (OR = 0.02). There was no association between knowledge of disease prevention through vaccination or of anthrax as a disease. Increasing age, 21–30 years (OR = 0.07) and 31–40 years (OR = 0.08) were protective against acquiring anthrax infection compared with the younger group, 1–20 years. A worrying sign was that the biologically relevant variables such as skinning infected animals, touching the infected animals and their products, and sleeping on the infected animal's skin, which were linked to direct exposure to anthrax infection, disappeared from the multivariable model.

3.3. Latent class analyses

The LCA model was used to shift the focus from simple associations in the multivariable analysis model to describing a potential causal pathway of the exposures and anthrax infection with age and education being used as primary variables, based upon the assumed causal diagram shown in figure 4. The level of education was not considered for the youngest group (1–20 years), as they are not eligible for enrolment in primary education, which mostly starts at the age of 7 years or/and above. The LCA showed that 73/118 (61.9%) of the study subjects had a high probability of being classified as exposed, while 45/118 (38.14%) of the study subjects were in the unexposed group. As there was a strong age bias among

Table 1. Univariable logistic regression analysis of demographic, biological and other risk factors associated with anthrax transmission, Northern Tanzania 2016. Results are given as the OR with the corresponding *p*-values.

variable	variable description, *n* (%)	dataset, OR (*p*-value)	age quantiles (years)			
			1–20 OR (*p*-value)	21–30 OR (*p*-value)	31–40 OR (*p*-value)	≥41 OR (*p*-value)
demographic characteristics						
education	some education, 35 (30)	0.4 (0.02)[a]	6 (0.001)[a]	0.1 (0.05)[a]	0.4 (0.32)	2.1 (0.31)
	not educated, 83 (70)					
sex	male, 70 (59)	0.8 (0.70)	3.0 (0.06)[a]	0.5 (0.5)	1.3 (0.70)	0.9 (0.9)
	female, 48 (41)					
occupation	risky, 112 (95)	1.0 (1.00)	omitted	omitted	omitted	omitted
	not risky, 6 (5)					
biological factors						
skinning/burying	yes, 75 (64)	1.6 (0.18)[a]	3.3 (0.27)	0.7 (0.74)	0.6 (0.53)	4.2 (0.09)[a]
	no, 43 (36)					
contact with livestock	yes, 78 (66)	6.1 (0.00)[a]	2.5 (0.47)	3.8 (0.14)[a]	14.6 (0.01)[a]	3.1 (0.18)[a]
	no, 40 (34)					
contact with animal products	yes, 78 (66)	6.1 (0.00)[a]	12.0 (0.04)[a]	4.6 (0.09)[a]	4.4 (0.10)[a]	3.1 (0.18)[a]
	no, 40 (34)					
history of travel	yes, 9 (8)	1.2 (0.72)	omitted	omitted	omitted	0.4 (0.50)
	no, 109 (92)					
sleeping materials	mattress, 64 (54)	2.6 (0.01)[a]	5.5 (0.13)[a]	3.25 (0.15)[a]	1.3 (0.71)	0.5 (0.56)
	animal skins, 54 (46)					

(*Continued.*)

Table 1. (*Continued.*)

variable	variable description, n (%)	dataset, OR (p-value)	age quantiles (years)			
			1–20 OR (p-value)	21–30 OR (p-value)	31–40 OR (p-value)	≥41 OR (p-value)
other variables						
source of animal feeds	risky, 82 (69)	0.6 (0.02)[a]	0.3 (0.16)[a]	0.81 (0.66)	0.7 (0.4)	0.9 (0.8)
	not risky, 36 (31)					
knowing animal's vaccine preventable diseases	yes, 30 (25)	0.3 (0.04)[a]	0.7 (0.7)	omitted	0.27 (0.27)	1.3 (0.74)
	no, 88 (75)					
animal died at compound	yes, 65 (55)	13.16 (0.00)[a]	75.0 (0.01)[a]	14.8 (0.01)[a]	28.8 (0.00)[a]	1.5 (0.56)
	no, 53 (45)					
disposal of animal carcasses	consume, 64 (54)	14.37 (0.00)[a]	75.0 (0.01)[a]	14.8 (0.01)[a]	28.8 (0.00)[a]	2.13 (0.33)
	not applicable, 54 (46)					
source of meat	home slaughter, 71 (60)	1.9 (0.09)[a]	2.5 (0.4)	1.8 (0.40)	1.4 (0.6)	0.6 (0.5)
	from the butcher, 47 (40)					
keep animals	yes, 94 (80)	3.87 (0.01)[a]	8.3 (0.16)[a]	1.75 (0.54)	3.6 (0.26)	2.6 (0.31)
	no, 14 (20)					
keep dogs	yes, 73 (62)	3.5 (0.002)[a]	25 (0.02)[a]	3.8 (0.14)[a]	1.6 (0.52)	1.2 (0.8)
	no, 45 (38)					

[a]Candidate variables.

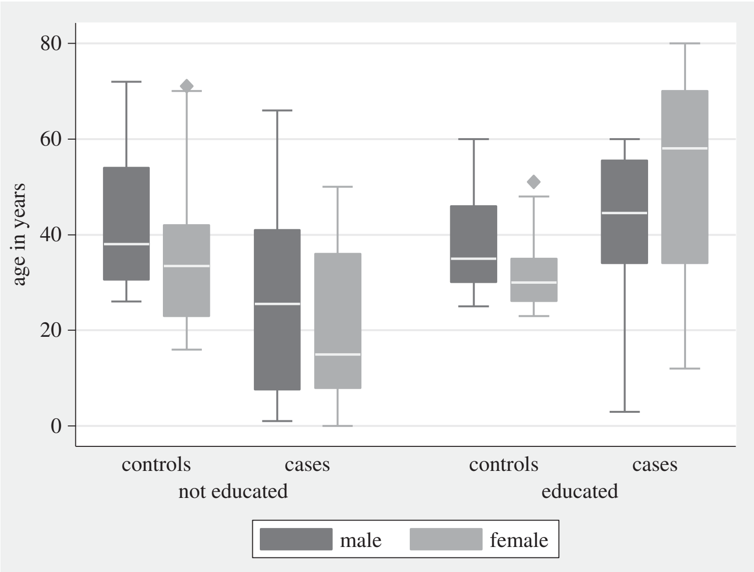

Figure 3. Demographic characteristics of study subjects in the hotspot districts of Northern Tanzania, 2016.

Table 2. Results from multivariable logistic regression analysis for potential predictors associated with anthrax transmission in Northern Tanzania, 2016. Results are given as the odds ratio (OR) with 95% CI and corresponding *p*-values.

variable description	OR (95% CI)	*p*-value
age 1–20 years	1.00 (–)	—
age 21–30 years	0.07 (0.011–0.47)	0.006
age 31–40 years	0.08 (0.016–0.43)	0.003
age $\geq$40 years	0.56 (0.13–2.43)	0.445
no formal education	1.00 (–)	—
have formal education	0.02 (0.0024–0.16)	$<$0.001
does not know animal diseases preventable by vaccines	1.00 (–)	—
know animal diseases preventable by vaccines	0.23 (0.055–1.02)	0.053

cases, two final SEMs: one with the youngest (1–20 years) group and one for the older group (greater than 20 years) were established. In the youngest group, exposure status was strongly linked to anthrax transmission (OR = 25.0, 95% CI = 1.5–410). In the older group, the link to exposure was smaller but still high (OR = 3.2, 95% CI = 1.28–8.00). The most distinct difference between the SEM model and the ordinary logistic model was that we were able to identify that education was linked to the model as a predictor of exposure, but not directly to anthrax infection (table 3).

4. Discussion

The occurrence of anthrax outbreaks in a particular location mostly depends on the existence of interacting factors, which include unique characteristics of the bacterium, environmentally related features, animal densities and human activities [9,38].

In this study, we found that some human activities predisposed people to risk factors for cutaneous anthrax infection. For the younger group (less than 20 years), we only found a strong relationship (OR = 25) between the set of exposures measured as a latent class representing many exposures and cutaneous anthrax. In the older group (age greater than 20 years), we still found a considerable risk as measured by an OR of 3.1 among the exposed. However, using the SEM, we could explain the probability of belonging

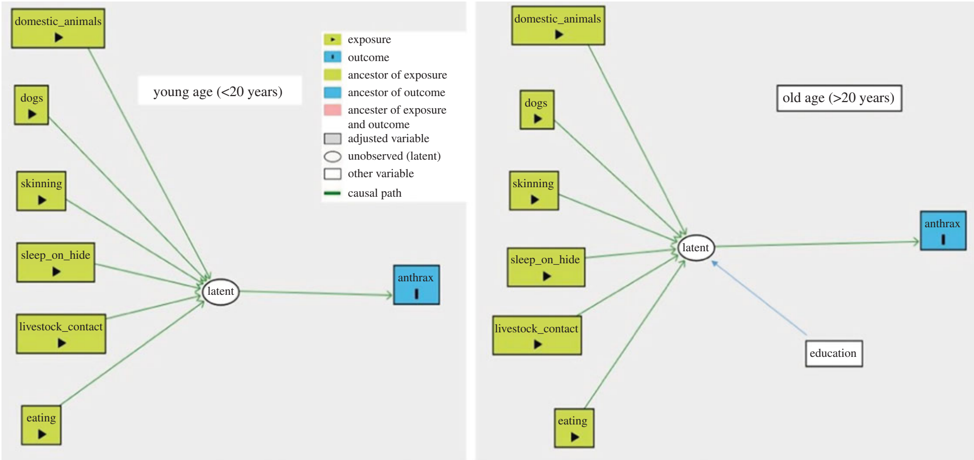

Figure 4. Direct acyclical graph (DAG) for the suggested causal pathway of anthrax transmission in the hotspot districts of Northern Tanzania, 2016.

to the *exposed* class by the education level. Thus, factors such as knowledge of animal diseases preventable by vaccines found in the ordinary multivariable model were found to be proxy variables better represented by the level of education. We applied a strict causal understanding in establishing the SEM, especially benefitting from using the latent class to represent a series of correlated exposure variables. The exposure variables discussed below all disappeared as risk factors using the standard multivariable approach. The neglect of biological plausibility in statistical analyses, during epidemiological studies such as this, is an inherent issue [39], which we sought to address in this study. Activities such as keeping livestock, skinning dead animals, coming into contact with animals or their products, touching carcasses and burying carcasses were in our analyses set as exposure variables for disease occurrence. The nature of livestock rearing within these households brings about a high chance of direct/indirect contact with contaminated animals or animal by-products, which makes these factors pivotal to a high predisposition of humans to acquiring cutaneous anthrax [17].

In the pastoral Maasai community, animal skins are mostly used as bedding materials. The use of skin from infected animals would facilitate direct contact with *B. anthracis* spores if the animals had died of anthrax. Other studies have also reported that processing skin and hides for making sleeping materials facilitated anthrax transmission in a susceptible population [40].

This study also found that dressing of dead livestock and using them as meat for human consumption was a risk factor for disease transmission. On some occasions, dead and decomposed animals were found lying on the grounds and being eaten by scavengers. This may be another pathway that may have contributed to an increased disease transmission to the unaffected animals and humans in the hotspot areas. Other studies report that burning, or burying followed by disinfection of the burial site, is an appropriate disposal mechanism for anthrax carcasses and limits further spread of the disease in the affected areas and beyond [18].

In a study done in Zambia, people who participated in skinning infected carcasses and processing meat and hides as well as skins for making sleeping materials were highly exposed to anthrax infection [41]. Another study from Lake Rukwa valley in southwest Tanzania reported that touching infected carcasses and animal products was a potential risk for anthrax transmission in the community [20].

Age and gender distribution can also facilitate disease transmission in a society due to the allocation of duties among the members of the Maasai community. In our study, we found that men of older age were more often affected by cutaneous anthrax, perhaps because they are the ones responsible for taking care of the animals while grazing. In addition, they are also dealing with milking, slaughtering and skinning the carcasses.

Illiteracy was at high levels among the study subjects, and in anthrax cases in particular, and education level was found to be a critical factor in explaining the set of exposure variables. Illiteracy is linked to poverty, and poor people opt to dress a carcass and use it as meat, which exposes them to *B. anthracis* infection by contact in cases of infected animal products [29]. The uneducated part of the

Table 3. SEM for predictor variables for anthrax transmission in the hotspot districts of Northern Tanzania, 2016. Results are given as the OR with 95% CI and the corresponding *p*-values.

variable description	age 1–20 years, OR (95% CI); *p*-value	age greater than 20 years, OR (95% CI); *p*-value
not exposed (LCA class)	OR = 1.00 (–)	1.00 (–)
exposed (LCA class)	25.0 (1.5–410); $p = 0.024$	3.2 (1.28–8.0); $p = 0.013$
no formal education	—	1.00 (–)
have formal education	—	0.23 (0.09–0.58); $p = 0.002$[a]

[a]Having formal education was a predictor for being exposed, not directly linked to skin anthrax.

community also has trouble following or understanding critical messages through written materials (leaflets, billboards and magazines), which are provided during health education campaigns. Another study reported that poverty is centred in sub-Saharan Africa where most people are illiterate, and the community is predisposed to many infectious zoonotic diseases due to their increased contacts with animals, yet with limited access to good health services for humans and animals [5].

Keeping livestock for the livelihood of pastoral and agro-pastoral Maasai communities is a common practice in Northern Tanzania. However, the animal husbandry systems do not take into account the prevention of animal diseases like anthrax. Because of extensive grazing of livestock, they frequently come into contact with carcasses of wild animals, which may have died from anthrax [42]; this increases the risk of human–pathogen interaction [40]. This is because the pastoral and agro-pastoral communities have intimate contact with livestock in their daily cultural practices as part of a unified social and ecological context [43]. Therefore, social factors and cultural practice dimensions influence the human and animal interactions that propagate the transmission of anthrax in this community [44]. Similarly, researchers have to consider immutable beliefs and cultural practices, which exacerbate risks for transmission of zoonotic diseases, in order to advocate for adequate individual behaviour change [44,45]

In the pastoral and agro-pastoral communities, it is a common practice to keep dogs for security purposes against wild animals and to assist in the herding of livestock in grazing areas. Previous reports found a high seroprevalence of anthrax in dogs owned by pastoralists during a large anthrax outbreak in livestock in the Ngorongoro Conservation area, Northern Tanzania [26].

5. Conclusion

Sleeping on animals' skins and contact with infected carcasses through skinning or butchering were linked to the *exposed* latent class, which proved a robust predictor of anthrax infection. For older participants (greater than 20 years), being exposed to the pathogen could be explained by the level of education; where a lack of a formal education was linked to higher risks of anthrax infection. Prevention and control strategies of anthrax in pastoral and agro-pastoral Maasai communities need a well-framed approach with a clear understanding of social mechanisms. Educational materials will need to breach the high levels of illiteracy and provide a socially relevant context.

Therefore, preventive and control measures of anthrax outbreaks such as livestock vaccination, safe carcass disposal (preferably incineration) of dead animals from anthrax, public awareness campaigns, stockpiling of antibiotics, identification of competent laboratories for human and animal anthrax diagnosis, and an intensified surveillance system for human and animal sectors should be implemented using the One Health approach in the hotspot districts of Northern Tanzania. The collaboration of anthropologists and veterinary, medical and public health professionals could bridge the education gap in this unique African environment.

Ethics. The permission to carry out this study was sought from and granted by the Tanzanian National Institute for Medical Research (NIMR) with reference number: NIMR/HQ/R.8aVol.IX/2286. Permissions were also sought from Arusha and Kilimanjaro region authorities and identified anthrax hotspot districts, namely Ngorongoro, Monduli, Meru, Siha, Hai, Rombo and Moshi rural in order to conduct this research in their areas. At the community level, local authorities were engaged, and they facilitated access to the eligible households for interviews. Participation of the eligible subjects in the study was on voluntary bases. Verbal consent was obtained from each of the selected participating cases and controls following explanation of the purpose and importance of the study by adhering to the rules and regulations of research in humans from NIMR. Confidentiality of the collected information was strictly observed, and preliminary findings of this research were disseminated to the responsible authorities at national, regional, district and village levels.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. E.R.M. designed the study, administered questionnaires, analysed and interpreted data, and drafted the manuscript. S.H. participated in the fieldwork. A.S. participated in the data analysis. J.M. participated in the design of the study. H.E.N. and R.H.M. participated in the design of the study and supervised this research. E.S. supervised the initial design of this research, analysis, interpretation of data and drafting of the manuscript. All the authors have constructively reviewed and commented on this article.

Competing interest. We declare we have no competing interests.

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Paper IV

Ecological niche modeling as a tool for prediction of the potential geographic distribution of *Bacillus anthracis* spores in Tanzania

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Abstract

Introduction: Anthrax is caused by the spore forming, Gram-positive bacterium *Bacillus anthracis*. The aim of the study was to predict the potential distribution of *B. anthracis* in Tanzania and produce epidemiological evidence for management of anthrax outbreaks in the country.

Materials and Methods: Maxent algorithm was used to predict risky areas for anthrax outbreaks based on the occurrence and environmental data in Arusha and Kilimanjaro regions and later transferring the model to predict the entire country. 70% of the specimens were used to train the model whereas 30% were used for model evaluation.

Results: Four regions of northern Tanzania are predicted to have high risk for anthrax outbreaks while the southern and western regions had low risk areas. Soil type (56.5%) , soil pH (23.7%) and isothermally (10.4%) were the most important variables to the model prediction and the most significant soil types were solonetz, fluvisols and lithosols.

Conclusion: A strong risk level across districts of Tanzania Mainland was identified in this study. A total of 18 districts are predicted to have very high risks for occurrence of anthrax outbreaks in Tanzania. These findings are important for policy makers to effectively mount targeted control measures of anthrax outbreaks in Tanzania.

Introduction

Bacillus anthracis, the causative agent of anthrax in wildlife, livestock and humans is a soil—borne, spore–forming and Gram-positive bacterium [1]. Upon entry into a susceptible host, the spores germinate into vegetative cells which replicate rapidly in the blood stream forming septicemia [2]. The septicemic infection leads to hemorrhage of hosts which results into blood oozing on the soil [3]. It is speculated that death of the susceptible host occurs due to a tripartite toxin produced by this bacterium [4]. The disease can occur as peracute, acute or chronic depending on the host susceptibility, immunity status of the host and size of the spore inoculum, however, the peracute form is the most common infection in herbivores, while scavengers such as dogs may be infected without showing symptoms [5]. In humans, the skin form is the most common [6].

Disease transmission pathways are a complex system that involves several agents of dispersion. In wildlife conservation areas such as Serengeti, Ngorongoro conservation area, Kilimanjaro, Arusha and Mkomazi National Parks, it is quite impossible to have rapid and proper disposal of anthrax-infected carcasses. Bloody vultures contaminate water bodies through bathing after opening up and feeding on anthrax contaminated carcasses [7]. It can also be dispersed by insects like necrophagous flies which play a crucial role in spreading anthrax [8]. Hugh-Jones and De Vos [9] reported that blow–flies which feed on fluids of the anthrax-contaminated carcass and deposit their feces or regurgitate liquids on leaves of vegetation nearby from a carcass, ready for transmitting a disease to a next animal, but biting/hemophagic flies are also considered to transmit anthrax among wild animals and livestock [10] and even to humans [11]. Vector Infection in human occurs when *B. anthracis* penetrates through skin abrasions or mucous membranes when he/she comes into contact with infected animal [12] or animal products, inhalation of spores, or consumption of raw or undercooked infected meat [13].

The disease burden and economic impact of anthrax in domestic animals is not yet fully documented [14]. However, it is estimated that 2000–20,000 human anthrax cases are being reported annually Worldwide [15] with more cases coming from Africa, Asia, United States and Australia [16]. For instance, China reported 112,000 human cases in the period of 1956 – 1997 [17], in 2004, the Pollino National Park in Italy reported animal deaths counting to

81 cattle, 15 sheep, 9 goats, 11 horses and 8 deer due to anthrax [18]. In Zambia, 521 human cases and 5 deaths (CFR, 0.95%) were reported in 2011 [19], Kenya had 53 deaths of Grevy's zebra in 2006 [20] and Uganda reported 500 deaths of wildlife and 400 domestic animals in 2004 – 2005 [21]. In 2016, Tanzania experienced a big anthrax outbreak in Monduli district, Arusha region affecting 21 humans, 109 wildebeest, 21 grant gazelle and one rabbit, 10 cattle, 26 goats and 3 sheep [22].

In conducive environmental conditions, the bacteria from drained blood form spores, which can remain dormant for extended period of time in the soil, possibly decades, until it affects a new susceptible host [23]. Studies on the environmental suitability for the persistency of spores shows that soil parameters such as alkalinity, calcium and high organic matter contents [2, 24], elevation, precipitation, temperature and vegetation biomass [25, 26] may support the extended survival of the *B. anthracis* spores in the environment. In our previous retrospective study, we established that recurrence of anthrax outbreaks in the human, livestock and wildlife interface areas of Northern Tanzania were highly correlated with cycles of short rainfall followed by dry – hot weather [27]. However, the spatial ecology and anthrax outbreaks pattern in the country are not well understood. Other studies reported that, areas with ambient temperature above 15.5ºC [28], and cyclic rainfall pattern with high evaporation potential characterized by calcareous soil [29] tend to favor long term survival of the *B anthracis* spores causing frequent anthrax outbreaks in such areas.

Ecological niche modeling is a tool for identifying geographic and ecologic areas suitable for species persistence based on the environmental variables of known occurrence sites [30]. During modeling of species distribution, presence only or both presence and absence data may be used. Using both presence and absence data has shown to improve the model performance in some cases [31]. While absence data is challenging to verify [32] and therefore a modeling technique with a presence data only [33] can be employed. However, the species can be absent from the suitable habitat for historical reasons or due to failure to disperse to those areas [34]. Various presence only modeling techniques to predict the geographic distribution of *B. anthracis* have been employed such as Maxent (Maximum entropy) [35] and GARP (genetic algorithm for the rule – set prediction) [36]. Comparing correlative models such as Bioclim, GARP and Maxent using the same input data, Maxent was found to give the best predictions [37]. In this study, therefore we used Maxent [38] to model for *B. anthracis* persistence and its spatial distribution using the presence-only data.

In the present study, we aimed to predict the potential geographic distribution of *B. anthracis* in Tanzania and produce epidemiological evidence for management of anthrax outbreaks in Tanzania. This information will provide a better ecologic and epidemiologic understanding of frequent anthrax outbreaks in the most risk areas. It will also help to zone the country based on risks and inform decision makers to effectively allocate resources for targeted preventive and control measures such as intensified surveillance, community awareness, improved diagnostic capacity and livestock vaccination against anthrax in the identified high risk areas [39].

Materials and Methods

Study areas

The study was conducted in Arusha and Kilimanjaro regions of northern Tanzania, where the occurrence data for anthrax outbreaks were collected for modeling the whole country. The Arusha region lies on the Kenyan border at latitude -3.36667 and longitude 36.683330 with elevation of 1, 415 m above sea level, the population size (2012 census) for this region was estimated to be 1,694,310 [40]. This region encompasses the savannahs and part of the Great Rift Valley. It has wildlife protected areas including the Ngorongoro Conservation Area (NCA), which contains the massive Ngorongoro Crater, and Arusha National Park, which covers volcanic Mount Meru. There are also Manyara National Park, Grumet Game Reserve, and Lake Natron game Reserve. In this region, the selected study districts were Meru, Ngorongoro and Monduli district councils.

Kilimanjaro region is located on the northern part of Tanzania Mainland, south of the equator at 2°25′ and 4°15′, longitudinally it lies between 36°25′30″ and 38°10′45″ east of Greenwich and the region has an elevation of 2400 m above sea level. The 2012 census estimated a population of approximately 1,640,087 with an average annual population growth of 1.8% (Tanzania 2012 Population and Housing Census).The region has three ecological zones such as lowland (1,500 m and below), highland (1,501 – 3,000 m) and forest (3,001 m and above) [41, 42] Kilimanjaro region is bordered to the north and east by Kenya, to the south by the Tanga region, to the southwest by the Manyara region, and to the west by the Arusha Region. The selected study districts in this region were Hai, Moshi rural, Rombo, and Siha district councils. Figure 1 illustrates both the studied area and the distribution of the occurrence data

Anthrax occurrence data

A database of 192 mixed cases of humans (68), wildlife (21), livestock (80) and environmental (23) samples, was constructed from sporadic anthrax outbreaks, which occurred in different places of *Arusha* and *Kilimanjaro* regions in northern Tanzania from October 2016 to March 2018. This information was used to map risks of anthrax outbreaks for the whole of Tanzania.

However, it is a standard practice that under normal circumstances during outbreaks, specimens for laboratory analysis were not taken from all suspected human cases, preferably a few were collected for confirmation of the existence of an outbreak. We omitted from the database all specimens with errors in geo coordinates and missing information. Therefore, a total of 108 (56.25%) specimens were maintained in a database out of which 44 (40.74%) tested positive for *B. anthracis*. The positive cases for anthrax were linked to the geo–coordinates (latitude/longitude) which were collected either at a residence of a human suspected case or at a burial point of an animal carcass (livestock or wildlife). If a suspected human case reported to have any livestock dead in a period of two weeks prior to data collection then a geo – coordinates were collected at a carcass disposal site using a hand held global positioning system machine. It should also be noted that, in some occasions repeated the same geo coordinates recorded outbreaks in different time periods; this has also contributed to less number of occurrence data in the final database compared to what was recorded initially.

The collected data were stored in a Microsoft Excel Spreadsheet followed by editing and removing records with geo – coordinate errors, not tested and negative laboratory results were removed from the database. A final version with geo coordinates and positive laboratory results were saved in a comma separated value (csv) format while environmental covariates were saved in ESRI ASCII format for further analysis in a Quantum GIS software. Figure 1 shows the map of Tanzania illustrating the distribution of the geographical position of the sampled areas with their laboratory results of the collect specimen following sporadic anthrax outbreaks in northern Tanzania. However, we used occurrence data from a small sampled area for model calibration and then predicted the habitat suitability of anthrax spores for the whole country.

Environmental covariates

A total of 21 climatic variables summarized in Table 1 were obtained from 1 km grid Africlim database consisting of two categories of data, (i) temperature variables that consist annual mean temperature (BIO1), mean diurnal range (BIO2), isothermally (BIO3), temperature seasonality (BIO4), maximum temperature of warmest months (BIO5), minimum temperature of coldest month (BIO6), temperature annual range (BIO7), mean temperature of wettest quarter (BIO8), mean temperature of driest quarter (BIO9), mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter (BIO11), and (ii) precipitation variables that consist of annual precipitation (BIO12), precipitation of wettest month (BIO13), precipitation of driest month (BIO14), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18), precipitation of coldest quarter (BIO19), Moisture index arid quarter (MIAQ) and Potential evapotranspiration (PET) obtained from http://doi.org/10.1111/aje.12180. The variables were obtained under the current scenario that comprise of monthly measurements obtained from weather stations around the world between 1950 to 2000 modeled under 4.5RCP (representative concentration pathways) scenario. Furthermore, 1 km grid soil type and soil pH data obtained from ISRIC African soil database were also included; soil pH was included as predictor variable because it has been shown that epidemics of anthrax are associated with an alkaline pH [2]. Soil type as a categorical variable was also included in the model, this is because the influence of soil type on the *B. anthracis* persistence is ecologically documented and it is speculated that there is significant relationship between the soil types and the extensive presence of anthrax outbreaks in certain areas [43].

Model development

A pairwise Pearson correlation analysis for environmental variables was done using an ENMTOOLs [44]. This was done in order to reduce multicollinearity of the environmental variables and only variables with a lower than (±0.75) were retained for model fitting. After this procedure, the non–correlated environmental variables were chosen for development of a species distribution model. The candidate variables identified included Isothermally (BIO3), Temperature seasonality (BIO7), Moisture index arid quarter (MIAQ) Potential evapotranspiration (PET), Soil types [45] and soil pH .

A Maxent model was fitted using 100 bootstrap runs with 70/30 partition percentage for the training/testing datasets. Default Maxent model parameter settings (auto features, convergence threshold of 0.00001, maximum number of background points = 10,000, regularization multiplier = 1) were used [46]. In order to train Maxent one fold was used to fit a model and the remaining folds were treated as independent data for evaluation of predictive ability of the model performance (testing). We created a masked file and used in the model in order to constrain the selection of background values and then evaluated the performance of the model. In each iteration, the contribution of every single variable to the general distribution was determined by the Jackknife statistical technique, which allowed us to identify variables which had the greatest influence on the probability of persistence of *B. anthracis* and spatial distribution in Tanzania.

Model evaluation

Several methods exist for evaluating model accuracy but the most common method involve the used of area under the curve (AUC) of receiver operating characteristics (ROC) [47]. A successful model has an AUC value close to 1.0, the higher the AUC, the better the model distinction of presence from absence of a species while models with no clear distinction have an AUC close to 0.5 [48]. Evaluation of the critical individual environmental predictors in the model development was done by a jackknife test and also response curves are used to show how each environmental variable affects the Maxent prediction and how the logistic prediction changes as each environmental variable is varied by keeping all other variables at their average sample value. Figure 2 is illustrating a summary of the model development process and methods used for obtaining significant variables for modeling.

Results

In our Maxent model, we obtained an area under the operating characteristics curve (AUC) of 0.93 indicating that a model had 'excellent' ability to predict the presence of *B. anthracis* spores in the most risk areas of Tanzania Mainland, as it is shown in Figure 3. The test indicates that the difference between the AUC from model prediction and the AUC at random is statistically significant showing that the model performs better than random prediction.

Out of the 23 environmental variables, the following variables were identified as non collinear, Isothermally (BIO3), Temperature seasonality (BIO7), Moisture index arid quarter (MIAQ), Potential evapotranspiration (PET), Soil types [45] and soil pH .Table 2 indicates the percentage contribution of each of these variables whereby the soil types have demonstrated a high percentage contribution (56.5%) followed by pH (23.7%) so the two (soil type and pH) variables in total contributed by 80.2% while the Jackknife test helped us to identify the most contributing variables in the persistence and geographic distribution of *B. anthracis* spores in Tanzania.

The Jackknife test of variables indicates that omitting any of those six variables affects the regularization gain, AUC and test gain in the model, as it was indicated in Figure 4, the soil types were the most important contributing variables to the model followed by pH among the retained variables in the model. However, pH decreases the gain the most when removed from the model. Therefore, by looking at the AUC of the Jackknife test, the most significant variables with scores of > 0.7 (above fair) are soil type, soil pH, BIO3 and BIO7 and response curves for these variables to the suitability for prediction of *B. anthracis* spores geographic distribution are shown in Figure 5.

The response curve for soil pH shows that the probability of geaographic suitability increases with the level of alkalinity (corresponding to high levels of calcium) in the soil.

The soil characteristics for the soil types that were identified as having the highest predictive power for B. anthracis spores survival is as shown in the response curve for soil types in Figure 5 (D) and Table 3 were Calcic Cambisols (2), Lithosols (9), Eutric Fluvisols (11), Eutric Histosols (16), and Orthic Solonetz (20). The Soil type, soil pH and isothermally were the most important variables, however, soil type was the single most important variable that accounted for 56.5% to the model prediction with the following soil types identified as the most significant; solonetz, fluvisols and lithosols.

Figure 6, shows a risk map indicating regions with very high, high, medium and low probability of environmental suitability for persistence and spatial distribution of B. anthracis spores in Tanzania. The regions with stable areas of high and very high risks were Arusha and Kilimanjaro from northern part of the country while other regions like Mara, Manyara, Simiyu and Singida had few patches of high and very high-risk areas. While regions like Dodoma, Mwanza, Dar es Salaam, Lindi, Mbeya, Rukwa, Katavi and Kigoma were predicted to have medium risk in few locations and the rest of the regions in the country had low risks for geographic suitability of B. anthracis spores persistence.

Discussion

Despite of the fact that, anthrax is a disease of both public and livestock importance in Tanzania, the risk mapping of the disease has not been used there previously. Consequently, there is no evidence – based allocation of resources for prevention and control of the disease bearing in mind that, there are a lot of competing priorities for the distribution of financial resources in the country. Therefore, the findings of our study provide important insights for spatially allocating and prioritizing resources for anthrax surveillance, prevention, and control based on the predicted level of risks (very high, high, medium, and low) within each district, which is an important administrative level for diseases prevention and control policy implementation in Tanzania.

In the current study, we used ecological niche modeling technique to predict potential suitable habitat distribution of anthrax spores in Tanzania. It is the first study that presents potential risks distribution associated with B. anthracis in Tanzania using climatic and abiotic factors such as soil type and soil pH. The Arusha and Kilimnjaro regions had higher risk (very high and high risks) of Bacillus anthracis spores habitat suitability as it was illustrated in the national prediction - risk map shown in Figure 6. The observations in the current study is in line with what was previously reported by Mwakapeje et al (2018), that in a restrospective study (2006 – 2016), Arusha region had 7.88 incidence of human anthrax cases per 100,000 population followed by Kilimanjaro region (6.64) [27] .

In the same predicted map, we observed streaks of predicted very high and high risks in Tanga, Coastal region, Manyara and Singida regions. From the predicted risky regions, the corresponding districts predicted with very high and high risks are indicated in Figure 7 which are Arusha region (Ngorongoro, Monduli, Longido, Arusha rural, and Meru), Kilimanjaro region (Hai, Siha, Moshi rural, and Rombo), Mara region (Serengeti) Manyara region (Simanjiro, Hanang, and Babati urban), Simiyu region (Bariadi, and Itilima), Tanga region (Kilindi) and Singida region (Mkalama, and Iramba), The ares within these regions are color-coded in the order of risk recognition. The ability to identify the risk associated with specific places and areas is extremely important in having an efficient disease surveillance and control program.

In fact, some of the predicted districts with high risks such as Hanang, Simanjiro, Itilima, Serengeti, Bariadi, Kilindi, Mkalama and Iramba have no reported anthrax cases through the surveillance systems. This might be attributed by the poor human and animal surveillance systems leading to severe under reporting hence misleading disease burden information [49]. Monduli and Ngorongoro are among the districts with predicted high and very risky for suitability of anthrax spores from Arusha region, this corresponds with the recent frequent anthrax outbreaks in Monduli district, for instance in the late 2016, a total of 130 wildlife carcasses, 39 livestock carcasses and 21 human cases were confirmed to have been infected by anthrax [22]. It is therefore envisaged that implementing targeted control measures based on the disease risk mapping is more cost-effective due to reduced cost for carcass disposal, cost for laboratory reagents and reduced cost for outbreak management in general. It also helps to implement a targeted livestock vaccination and intensified human and animal disease surveillance by focusing more closely on the predicted high and very high risky districts using One Health approach [50, 51].

Our model demonstrated that the environmental suitability for persistence of B. anthracis spores were highly influenced by the soil types, pH, BIO3, BIO7, MIAQ and PET variables respectively. Apart from soil type and soil pH, other variables are categorized into temperature (BIO3 – isothermally, and BIO7 – annual temperature range) and precipitation (MIAQ – moisture index arid quarter and PET – potential evapotranspiration) variables. Environmental variables such as soil and climate are postulated to favor and extend the survival of B. anthracis spores in the soil for a long period. This finding supports what was reported by other studies that anthrax outbreaks are exacerbated by warmer temperatures, moist soils and high organic matter content (humus) which favors the anthrax spore amplification [52]

In this study, we found that soil types and soil pH were the most significant variables for long term persistence of anthrax spore in the identified high and very high-risk areas. This is supported by other studies that, soil with high moisture, alkaline pH and humus are suitable for anthrax spores germination and sporulation outside a mammal host which are one of the critical variables that lead to the occurrence of anthrax outbreaks in animals with spillover to humans [53]. Other studies have documented that soil pH of more than 6.1 (alkalinity) in a combination with calcium levels are important variables for the long term survival of anthrax spores [54]. This kind of soil is regarded as a natural reservoir for Bacillus anthracis spores [36].

Study limitations

During model building, factors like livestock density, number of dry months, elevation and length of longest dry season were highly correlated with the identified most significant variables. Therefore, we are scientifically convinced that, apart from the identified most significant variables favoring the persistence of anthrax outbreaks in the areas with high and very high risks there are other factors, which contribute to anthrax related deaths in Tanzania, for example the Gainer – Kolonin hypothesis of hyperacute deaths involving latent infections, climate stress, and severe seasonal biting-fly activity in the absence of suitable soils [55]. However, we still trust that the identified suitable environment for anthrax outbreaks are important regions and/or districts to be given more attention because they have been identified as hotspot areas for anthrax outbreaks in the previous studies.

Conclusions

Our study modeled the occurrence data and environmental variables to create risk maps with categorized risks, which assisted to establish districts with high, very high, medium and low risks for anthrax outbreaks emergence in Tanzania. The results have identified northern Tanzania to have a higher probability of the occurrence of anthrax outbreaks more than other parts of the country. The identified most significant factors for anthrax persistence were soil types, soil pH, isothermality, mean temperature range, moisture index arid quarter and potential evapotranspiration.

The categorized risks are important and will help directing decision makers on resource allocation in a most cost–effective approach. In the identified high risk districts, they have to reduce mortalities in livestock and prevent the disease in humans through continued pre – outbreak targeted livestock vaccination, safe carcass disposal (preferably incineration) of dead animals from anthrax, public awareness campaign, provision of relevant diagnostics for livestock and human care facilities, and intensified human and animal surveillance systems. These activities if implemented effectively will help significantly to control for the existing devastating frequent anthrax outbreaks in the predicted high-risk areas in Tanzania.

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Conflict of interest

The authors declare no conflicts of interest.

Author's contributions

ERM – conceptualized the study, collected specimens, participated in he laboratory confirmation of anthrax, data analysis and interpretation of the results, drafted the article, addressed comments from the co – authors until the article was ready for submission to the peer review journal; SAN – participated on the conceptualization of the study, data analysis and interpretation of the results, reviewed and commented on the article; GM – participated on the conceptualization of the study, data analysis and interpretation of the results, reviewed and commented on the article; SA – participated on the conceptualization of the study, data analysis and interpretation of the results, reviewed and commented on the article; LN – participated on the conceptualization of the study, data analysis and interpretation of the results, reviewed and commented on the article; HEN – Supervised the design of the study, reviewed and commented on the article; RHM – Supervised the design of the study, reviewed and commented on the article and ES – Supervised the initial design of the study, data analysis and interpretation of the results, conceptualization of the study, drafting the article, reviewed and commented on the article.

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Table 1: Bioclimatic variables used for modeling by Maxent Software

Variable code	Variable description	Unit
	1. Temperature variables	
Bio1	Annual mean Temperature	^{o}C
Bio2	Mean daily temperature	^{o}C
Bio3	Isothermally (Bio2/Bio7) x 100	-
Bio4	Temperature seasonality (Standard deviation x 100)	^{o}C
Bio5	Maximum Temperature of warmest month	^{o}C
Bio6	Minimum Temperature of coldest month	^{o}C
Bio7	Temperature annual range (Bio5 – Bio6)	^{o}C
Bio8	Mean Temperature of wettest quarter	^{o}C
Bio9	Mean Temperature of driest quarter	^{o}C
Bio10	Mean Temperature of warmest quarter	^{o}C
Bio11	Mean Temperature of coldest quarter	^{o}C
Pet	Potential evapotranspiration	mm
	2. Precipitation variables	
Bio12	Annual precipitation	mm
Bio13	Precipitation of wettest month	mm
Bio14	Precipitation of driest month	mm
Bio15	Seasonal rainfall (Coefficient of variation)	mm
Bio16	Precipitation of wettest quarter	mm
Bio17	Precipitation of driest quarter	mm
Bio18	Precipitation of warmest quarter	mm
Bio19	Precipitation of coldest quarter	mm
mimq	Moisture index moist quarter	n/a
miaq	Moisture index arid quarter	n/a
dm	Number of dry months	months
llds	Length of longest dry season	months

Table 2. The percentage contribution and permutation importance of the variables used in Maxent modeling

Variable	Percent contribution	Permutation importance
Soiltype	56.5	37.4
Soil pH	23.7	20.6
bio3	10.4	26.8
miaq	5.2	9.4
pet	3.2	2.9
bio7	1.1	2.9
mask	0	0

Table 3. Summary of soil types with strong association to persistence and environmental suitability for B. anthracis spores in Tanzania

Soil code number	Soil type key	Soil type
20	So	Orthic Solonetz
11	Je	Eutric Fluvisols
9	I	Lithosols
2	Bk	Calcic Cambisols
16	Oe	Eutric Histosols

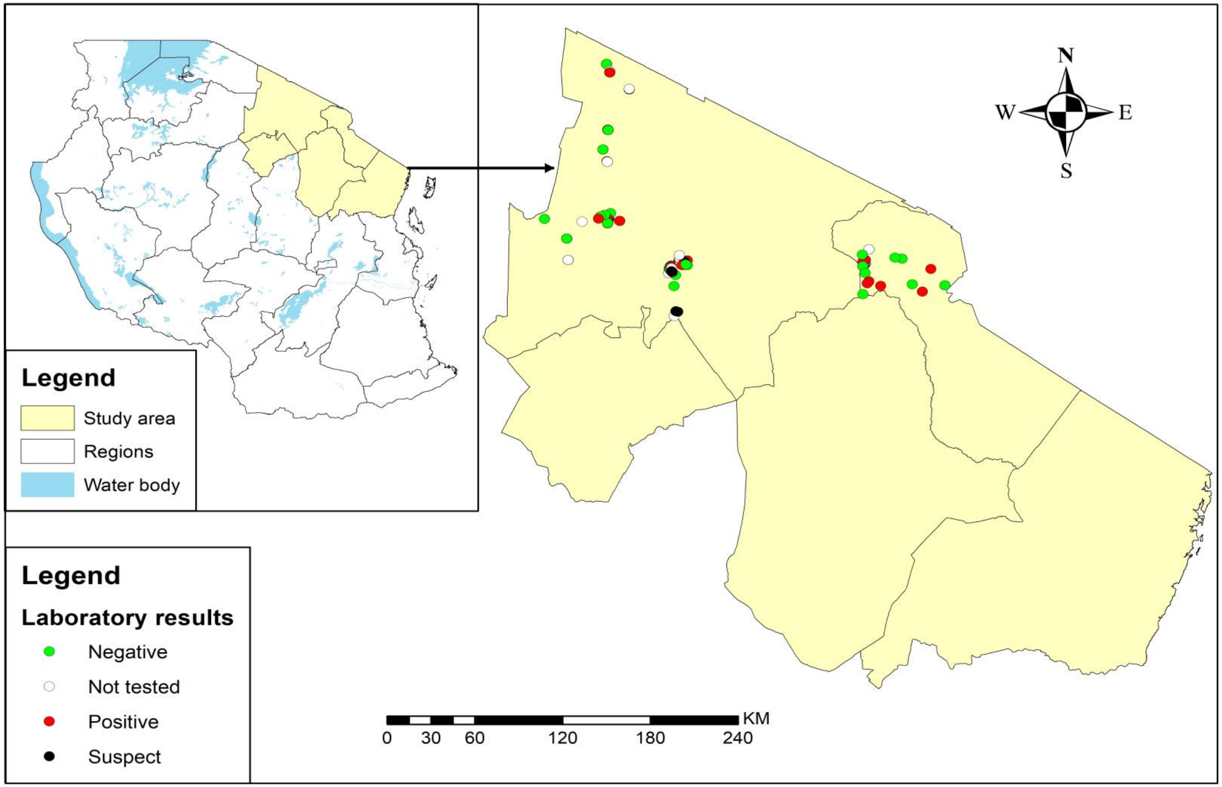

Figure 1: Map showing the study ares and distribution of the laboratory results from the sampled areas of northern Tanzania

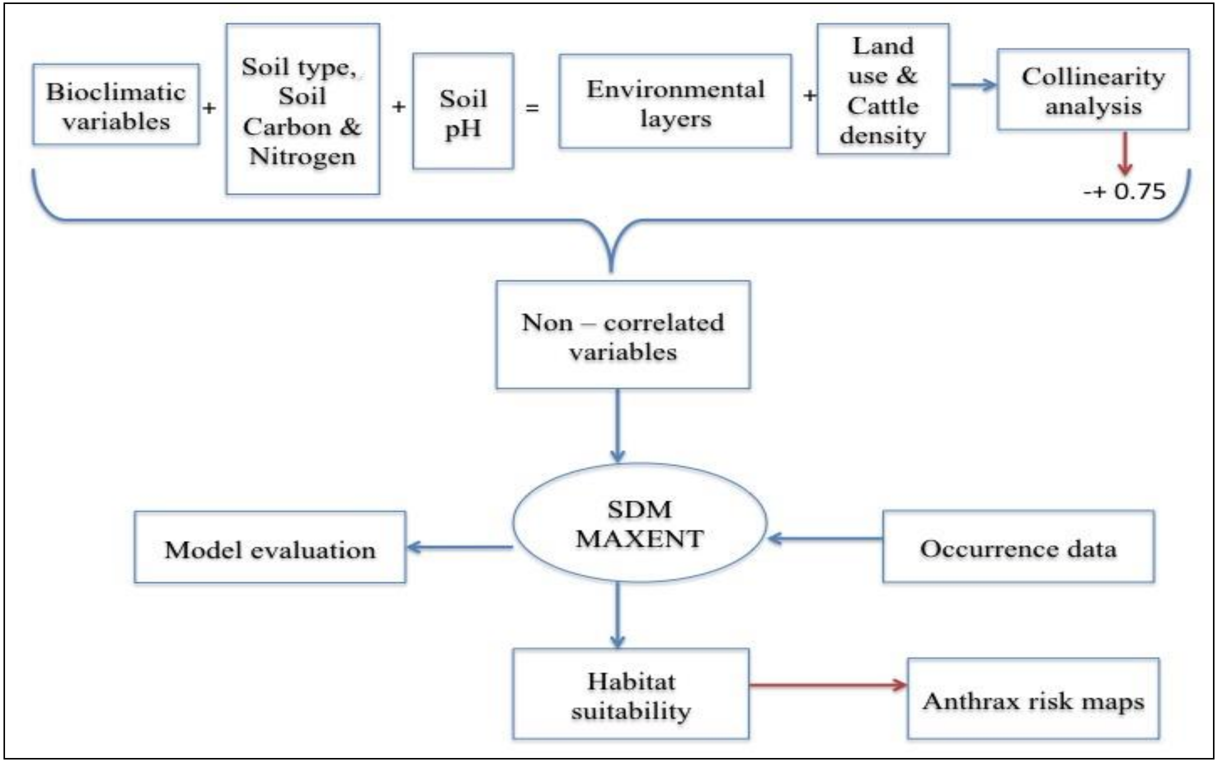

Figure 2: Flowchart indicating the model building process and included potential variables

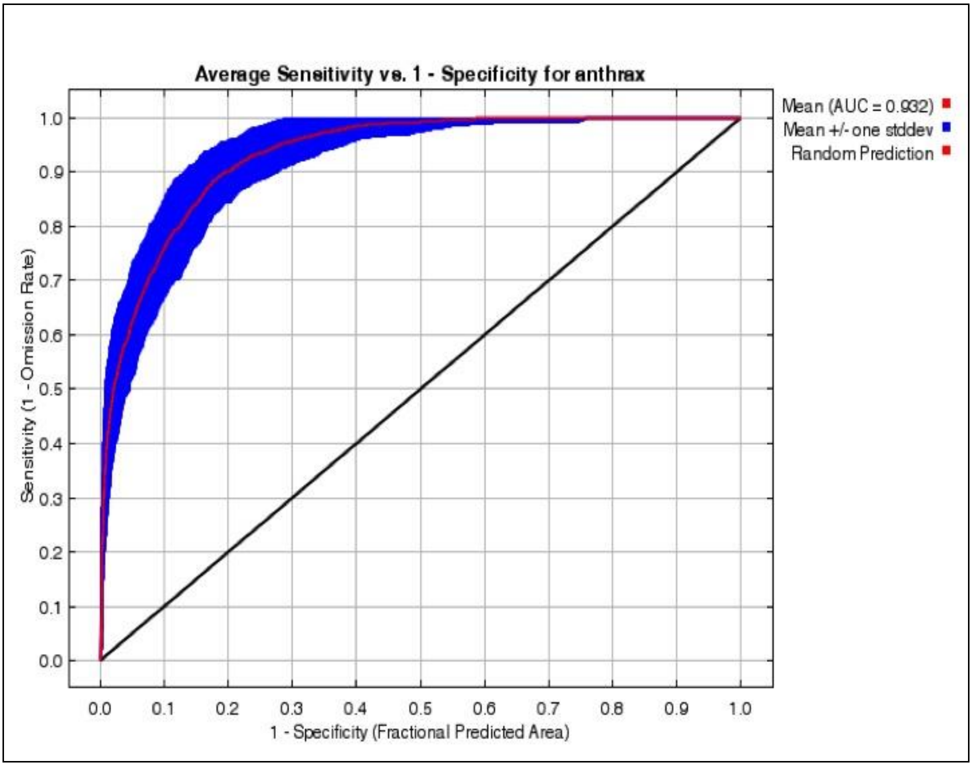

Figure 3: Average receiver operating characteristics (ROC) and related area under the curve (AUC) of the 100 bootstraps replicates

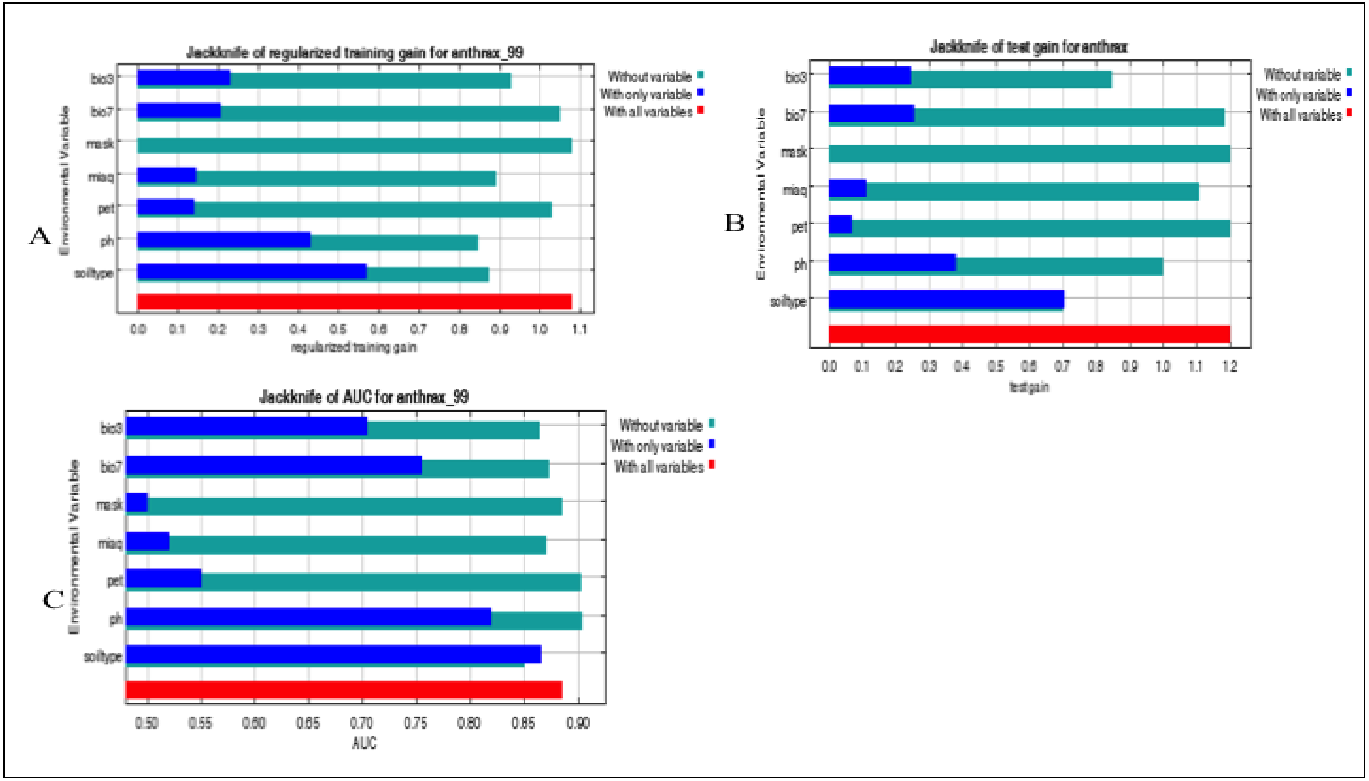

Figure 4: Jackknife tests of variable importance with (**A**) regularized training gain, (**B**) test gain, and (**C**) AUC. The light blue bars illustrate the model gain without variable inclusion while the solid blue bars show its gain with the variable only

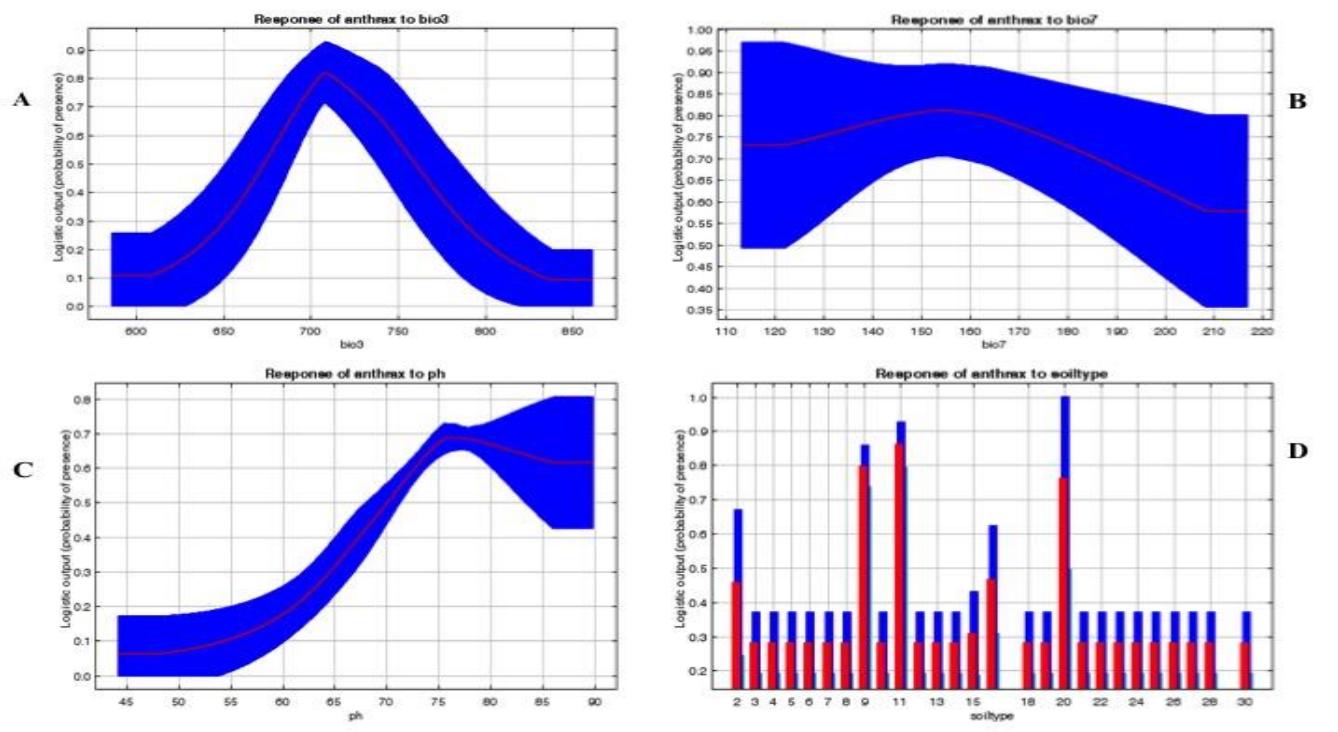

Figure 5: Response curves for the most significant variables in a Maxent Model for persistence of *B. anthracis* in Tanzania. Indicating **A** - BIO3 (Isothermal), **B** - BIO7 (Annual temperature range), **C** - Soil pH and **D** - Soils (soil type). The red lines indicate the mean values while blue ares denote 1 Std deviation limits resulted by cross – validation model runs

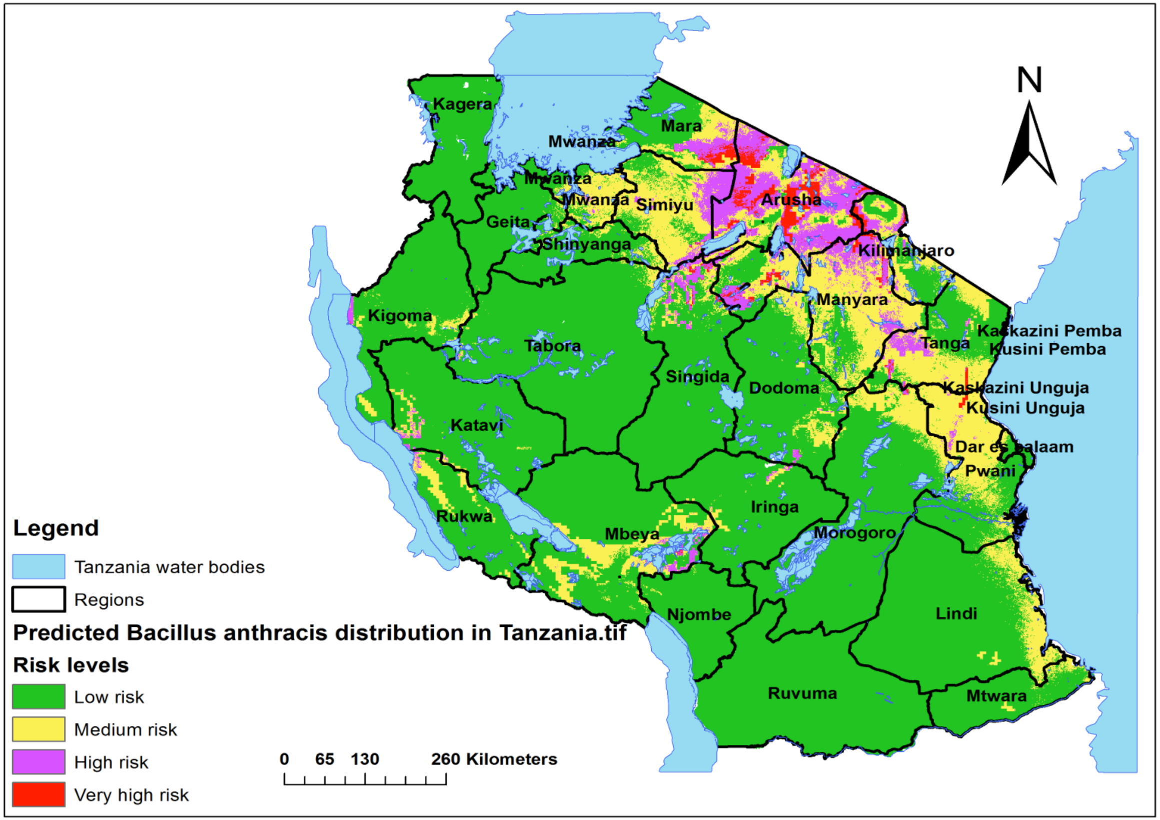

Figure 6: Risk map for the predicted environmental suitability of *B. anthracis* within regions in Tanzania Mainland.

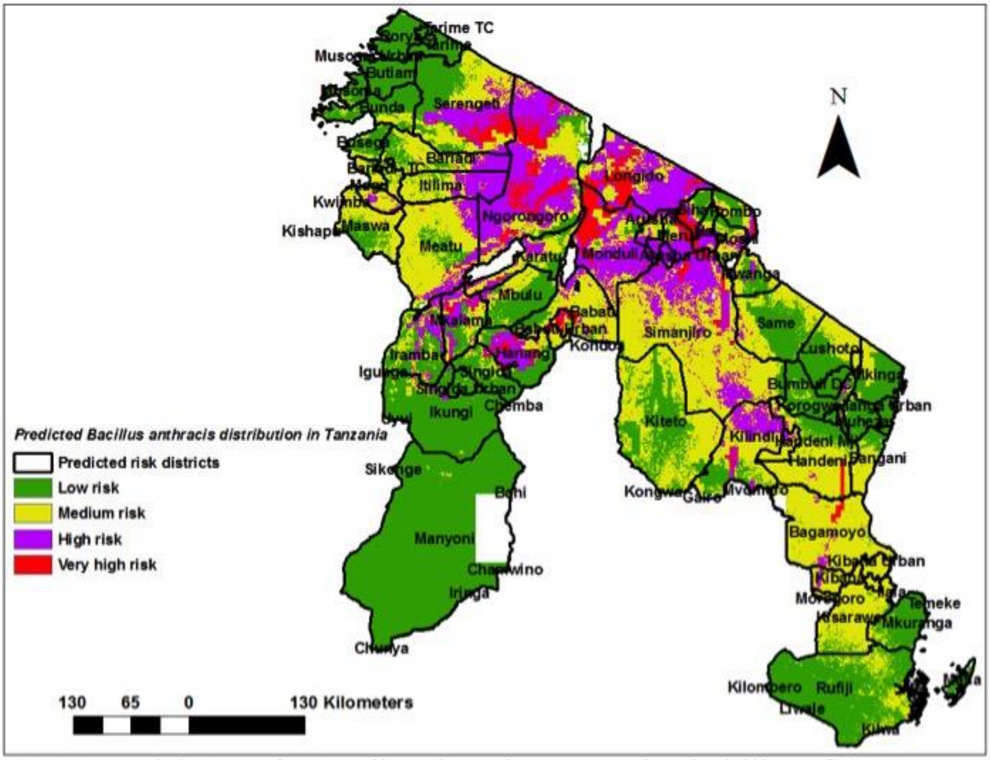

Figure 7. Risk map for predicted environmental suitability of *B. anthracis* at a district level from very high risk regions in Tanzania

Errata list

PhD candidate: Elibariki Reuben Mwakapeje
Thesis: Use of a One Health Approach for Understanding the Epidemiology and Management of Anthrax Outbreaks in the Human-Livestock-Wildlife and Environmental Health Interface Areas of Northern Tanzania
Date: 6th February 2019

Side	**Line**	**Original text**	**Corrected text**
Page 1	Para 2 (line 4)	The total area including Zanzibar is 947,303 km2, of which 886,040 km^2 is land and 62,050 km^2 is water.	The total area including Zanzibar is 945,087 km^2, of which 883,087 km2 is land and 62,000 km^2 is water.
Page 8	Para 4(line 4)	Sierra Leone, Ghana, Chad, Ivory Coast, Uganda, Nigeria, Botswana, Tanzania, Kenya, Republic of South Africa	Sierra Leone, Ghana, Chad, Ivory Coast, Uganda, Nigeria, Botswana, Tanzania, Kenya, **and** Republic of South Africa
Page 12	Para 2 (line 9)	…confirmed to have died from anthrax in the Selous game reserve of western Tanzania	…confirmed to have died from anthrax in the Selous game reserve of **southern** Tanzania
Page 14	Para 1 (line 4)	…in which 131carcasses of wild animals disposed of	…in which 131carcasses of wild animals **were** disposed of
Page 15	Last para (line 6)	…sharing of water collected points	…sharing of water **collection** points
Page 16	Last para (line 2)	…anthrax outbreaks in humans, livestock and wild animals	…anthrax outbreaks in humans, livestock and **wildlife**
Page 29	Para 1(line 3)	..detailed studies of anthrax outbreaks from humans and livestock as well as wildlife	..detailed studies of anthrax outbreaks **in** humans and livestock as well as wildlife
Page 33	Last para(line 3)	…in the hot-spot areas as it is affected by ecological factors	..in the hot-spot areas as **they are** affected by ecological factors
Page 36	Para 1(line 5)	…specimens are routinely processed at three diagnostic centers in northern Tanzania	..specimens are routinely processed at **the** three diagnostic centers in northern Tanzania
Page 48	Para 2(line 10)	… training for electronic reporting are not staying permanently at the original health facility	…training for electronic reporting were not staying permanently at the original health facility
Page 54	Para 1 (Line 1)	..the implementation of this research was a lack of a biosafety cabinet	..the implementation of this research was lack of a biosafety cabinet

Page 54	Para 3 (line 1)	..a convenience sampling technique was used in the retrospective anthrax patients	..a convenience sampling technique was used in the **recruitment of** anthrax patients
Page 54	Para 3 (Line 9)	..because they cannot **lead** the information	..because they cannot **read** the information
Page 58	Para 4(Line 5)	(tissue or blood) and results can be obtained	(tissue or blood) and **the** results can be obtained
Page 58	Para 4 (Line 7)	Once this test is approved and established in **the** Tanzania	Once this test is approved and established in Tanzania
Page 59	Para 3 (Line 4)	each strain and then peroxide knowledge on the	each strain and then **acquire** knowledge on the

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