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Anthrax bio-surveillance of livestock in Arua District, Uganda, 2017–2018

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ABSTRACT

Anthrax, caused by *Bacillus anthracis*, is a widespread zoonotic disease with many human cases, especially in developing countries. Even with its global distribution, anthrax is a neglected disease with scarce information about its actual impact on the community level. Due to the ecological dynamics of anthrax transmission at the wildlife-livestock interface, the Sub-Saharan Africa region becomes a high-risk zone for maintaining and acquiring the disease. In this regard, some subregions of Uganda are endemic to anthrax with regular seasonal trends. However, there is scarce data about anthrax outbreaks in Uganda. Here, we confirmed the presence of *B. anthracis* in several livestock samples after a suspected anthrax outbreak among livestock and humans in Arua District. Additionally, we explored the potential risk factors of anthrax through a survey within the community *kraals*. We provide evidence that the most affected livestock species during the Arua outbreak were cattle (86%) compared to the rest of the livestock species present in the area. Moreover, the farmers' education level and the presence of people's anthrax cases were the most critical factors determining the disease's knowledge and awareness. Consequently, the lack of understanding of the ecology of anthrax may contribute to the spread of the infection between livestock and humans, and it is critical to reducing the presence and persistence of the *B. anthracis* spores in the environment. Finally, we discuss the increasingly recognized necessity to strengthen global capacity using a One Health approach to prevent, detect, control, and respond to public threats in Uganda.

1. Introduction

Anthrax is a zoonotic disease caused by the Gram-positive bacterium *Bacillus anthracis* rods, a soil-transmitted pathogen found on all continents and reported in several islands like Haiti, the Philippines, and Indonesia (Carlson et al., 2018). Globally, an estimated 20,000 to 100, 000 human anthrax cases occur annually, particularly in developing countries with poor rural settings (Swartz, 2001). Naturally, the enzootic cycle of anthrax is characterized by a combination of long-term spore persistence in soil and an obligate-lethal transmission route of ingestion, primary in herbivorous mammals (Carlson et al., 2018). *B. anthracis* spores are known to survive for decades in alkaline soil rich in calcium ions which are believed to facilitate sporulation hence driving landscape-level patterns of spore persistence (Carlson et al., 2018; Hugh-Jones and Blackburn, 2009). Both livestock and wild herbivores are exposed to *B. anthracis* spores from the soil while grazing. They become infected and usually return spores to the soil when dead and decomposed (Turner et al., 2014).

Anthrax is a neglected disease, and its global distribution is still poorly characterized (Carlson et al., 2018). It has been shown that wildlife epizootics may lead to downstream infections in both humans and livestock (Mukarati et al., 2020). Sub-Saharan Africa is a high-risk

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zone because wildlife and livestock frequently share grazing fields in National Parks (Mwakapeje et al., 2018). One particular example is the Queen Elisabeth National Park in the Western Region of Uganda where hundreds of hippopotamus scammed to deadly anthrax in 2004–2005 (Driciru et al., 2018). The sub-Saharan Africa has an estimated population of 16.2 million poor livestock keepers (Carlson et al., 2019), who stand at high risk of acquiring the disease by occupational exposure. Human clinical presentations of anthrax, case-fatality rates, and mortality are a function of the exposure pathway determined by the ecological dynamics at the wildlife–livestock interface (Alexander et al., 2012). In some regions, such as the West Nile sub-region in north-western Uganda, anthrax is hyperendemic, and cases follow regular seasonal trends (Ntono et al., 2021).

In Uganda, anthrax outbreaks occur sporadically and continuously from different subregions of the country (Coffin et al., 2015). As a result, the disease has been ranked as one of the most critical zoonosis alongside Ebola, brucellosis, African sleeping sickness, plague, and rabies, which are prioritized zoonotic diseases selected to be controlled and maintained at minimum levels (Sentumbwe et al., 2018). Therefore, control of anthrax requires a One Health approach involving both human and animal health stakeholders for cross-sectoral integration and coordination of activities to prevent, detect, and respond to endemic anthrax in Uganda.

Anthrax still remains a challenge to many farming production systems in Uganda (Vudriko et al., 2021). Opening infected carcasses by community members in all anthrax hot spot areas and leakage of internal discharges in the grazing ground occurs, putting at risk the health of a large population of ruminants and humans in the region. Such activities contribute to spore transformation, transmission as well as environmental contamination. Uganda faces a scarcity of statistics about bovine data associated with anthrax outbreaks compared to other countries like Zambia, in which a total of 1216 bovine cases were reported in the Zambezi flood plain between 1999 and 2007 (Munang'andu et al., 2012). The lack of information associated with anthrax outbreaks is attributed to farmers who do not report suspected cases to the veterinary department in Uganda, making it more difficult to understand the magnitude, impact, and control of the disease's spread (personal communication).

This study aims to identify and confirm the presence of anthrax in biological samples and determine case fatalities among livestock after a suspected outbreak of anthrax in Arua between 2017 and 2018, a northwestern district of Uganda located in the West Nile sub-region. We also aim to establish the potential risk factors of anthrax that affect the cattle population in Arua District at the community level. Understanding the risk factors has important implications for preventing, detecting, controlling, and responding to future anthrax outbreaks in the region, meeting the requirements of the International Health Regulations (World Health Organization, 2008) and Global Health Security Agenda (Wolicki et al., 2016).

2. Materials and methods

2.1. Study area

Arua District (Fig. 1) is located in the West Nile sub-region of northwest Uganda. In 2020, the population of the Arua District was estimated at 750,000 habitants (Citypopulation.de, n.d.). It is bordered by other districts of Uganda (Yumbe, Moyo, Maracha, Koboko, and Adjumani) and the Democratic Republic of the Congo (DRC) to the west. Because of its localization in a corner of Uganda that borders DRC and South Sudan, the most significant amount of local community activity results from cross-border trade. Agriculture is the mainstay of the Arua's

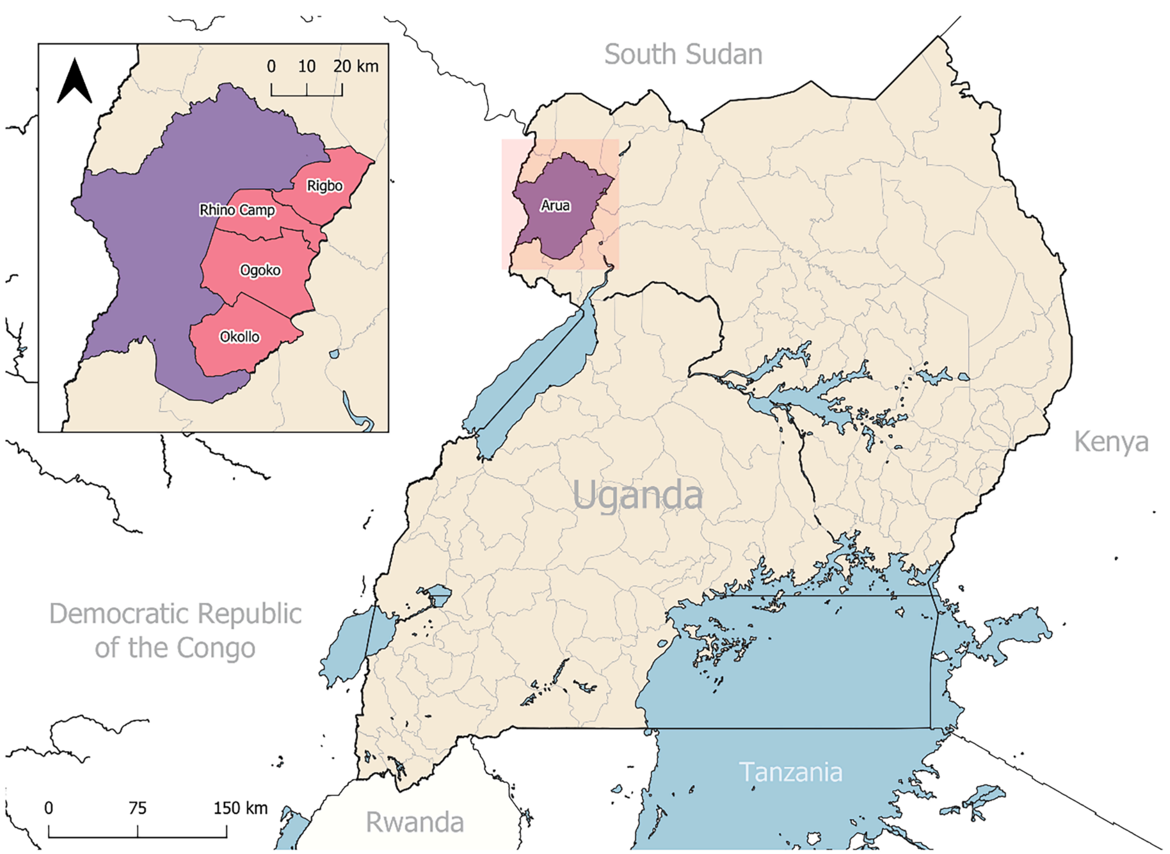

Fig. 1. Location of Arua District (purple) in Uganda and detail of hot spots Sub-Counties (pink) of anthrax outbreaks in Arua District in 2018 (Rigbo, Rhino Camp, Ogoko, and Okollo).

District economy, with approximately 117,000 head of local Zebu cattle. Rigbo, Rhino Camp, Ogoko, and Okollo Sub-Counties were the centres of anthrax outbreaks reported in 2018 (Fig. 1).

2.2. Data collection from Arua District

To better understand the status of anthrax outbreaks and the dynamics among domestic animals in Arua District, data was collected from community herds of livestock (*kraal*) through surveys in the Arua Sub-Counties with elevated mortality reports of suspected anthrax outbreaks in livestock between 2017 and 2018 (Rigbo, Rhino Camp, Ogoko, and Okollo Sub-Counties). Data sources were collected from 57 community herds of livestock from responsible *kraal* leaders. The questionary was designed and previously pretested before its usage. Additionally, the district veterinary officer and the Sub-County chief were interviewed about the anthrax situation.

Data collected comprised both qualitative and quantitative variables of demographic information of respondents (name, age, sex, and size of their family), localization (Sub-County, parish, and village), knowledge about anthrax disease, education received (none, elementary, secondary, or higher education), ownership of companion animals, and herd size. The data frame also recorded the species affected, the number of domestic animals and livestock suspected to be infected with anthrax during the outbreaks, and clinical signs, if any (loss of appetite, fever, recumbency, external bleeding, sudden death, blotting, and *rigor mortis*). Information about human activities that facilitate disease dispersion was also recorded (i.e., management of the dead carcasses, opening and skinning the dead animals in the field to consume and sell the meat, carcass disposal and soil treatment methods, transportation of animal products with dripping blood discharges to trading centers for sell, and transport means used to carry meat to the selling points).

Interviews with the Veterinary Department included records about vaccination status, if any, species affected, physical control actions (i.e., quarantine and other events which contributed to the persistence or control of the disease in the area), management or disposal of carcasses, availability of personal protective equipment, biosecurity measures if any, capacity to the regional veterinary laboratory to handle the diagnosis of anthrax samples, the level of community reporting of suspected cases, number of reported dead animals that succumbed to suspected anthrax, and availability of chemicals to disinfect carcasses and burial sites as well as the availability of emergency fuel for burning dead animals.

2.3. Anthrax standard case definitions in livestock

During the study, outbreaks were still occurring in which animals were sick, and others died suddenly in the *kraals* and the field. Therefore, the team decided to collect biological specimens, observe several cases' conditionality, and develop clinical descriptions for the target population as described below.

A suspected case of herbivore anthrax was defined as any case of the sudden death of animals with/without bleeding from natural orifices such as anus, eyes, and ears (unclotted dark tarry blood), absence of or incomplete *rigor mortis*, and rapid bloating of carcasses. In pigs and carnivores, the main symptoms suspected of anthrax were local edema and swelling of the face and neck.

A confirmed animal anthrax case of required laboratory detection of *B. anthracis* either in smears, rapid screening test, or bacterial isolation. As a criterion for laboratory diagnosis confirmation, we used internationally recognized standard diagnostic techniques. The interpretation for anthrax diagnosis in animals is reported in the Manual of Standards for Diagnostic Tests and Vaccine for Terrestrial Animals of the Office International des Épizooties (World Organization for Animal Health, 2021). The samples were subjected to the Active Anthrax Detect (AAD) rapid test lateral flow immunoassay, which has been previously tested in animal suspected anthrax tissue samples in Namibia with a specificity of 82% and sensitivity of 98%, showing better sensitivity than the gold standard culture, Laboratory Response Network real-time PCR, or immunohistochemistry (Kolton et al., 2019). However, additional testing (microscopy) was performed to confirm the cases.

2.4. Collection of biological specimens from the Arua District

In the Arua District, twenty-four (24) biological samples from suspected anthrax cases were collected from community herds of cattle during the outbreak seasons. Samples comprised of fixed blood smears, pooled unclotted blood, smoked meat, fresh meat, bovine skin scrapings, ear notch swabs, human skin lesions, and environmentally contaminated material. Human samples from Arua health centres were submitted to the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) to test and confirm zoonotic anthrax in the affected districts under the One Health arrangement. The AAD rapid test lateral flow immunoassay and microbiological methods such as Gram staining and polychrome methylene blue staining (M'Fadyean stain) were used to detect and confirm *B. anthracis*.

2.5. Active Anthrax Detect rapid test

Detection of *B. anthracis* in animal tissues was performed using a simple, rapid, and field-deployable kit provided by InBios to purposely screen and respond to the outbreak faster and reduce the burden of livestock disease. The AAD is a screening assay designed to identify the *B. anthracis* capsular polypeptide (polyglutamic acid) antigen (Gates-Hollingsworth et al., 2022, 2015; Kolton et al., 2019). Samples were tested following the manufacturer's instructions. Positive (the wild strain of encapsulated *B. anthracis*) and negative quality controls (*B. subtilis*) were run alongside the test samples based on immunochromatographic principles. Test results were read after 15 min. Each cartridge developed test and control lines. For a positive result, both lines appeared. For a negative result, only the control line was visible. Samples that did not show any of the lines were repeated. Samples were further confirmed using Gram and M'Fydean stains.

2.6. Gram staining method

The samples were tested following a previously validated protocol for the Gram staining method. Both thick and thin smears were prepared on labelled clean glass slides and allowed to dry by air and on a heating block for 2 min. With the addition of crystal violet stain for 1 min, then flooded with mordant iodine for 1 min. Followed by decolorizing the sample with 70% acetone-alcohol, the tissue was stained with dilute carbol-fuchsin as a counterstain. All excess stains were washed off with clean tap water, dried, and examined at room temperature under magnification $\times$ 100 with a light microscope using immersion oil. For quality control, known internal quality controls strains of wild *B. anthracis* and gram-negative *Escherichia coli* were examined alongside samples.

2.7. The M'Fadyean stain

Samples were also assessed by the M'Faydean staining method (M'Fadyean, 1903). Briefly, thin and thick smears were prepared using coverslips on a clean glass slide. The specimens were dried at room temperature and fixed with potassium permanganate (40 g/L) solution for 10 min. Then, the smears were covered with Loffler's polychrome methylene blue for 1 min, and the excess stain was removed using tap water. The slides were wiped with soft tissue and allowed to dry at room temperature under a biosafety cabinet. Samples were examined with a light microscope using immersion oil at $\times$ 100 magnification. For quality control, known prepared positive control smears of a wild strain of *B. anthracis* and negative controls (*B. subtilis* Sterne strain) were examined.

2.8. Statistical methods and data analyses

Kraals' survey data were analyzed using the *survey* package (Lumely, 2020) in R Core Team, (2019). Descriptive statistics for continuous and categorical variables were determined. Additionally, subpopulation analyses were performed, associations between variables were determined using the Wald test, and multiple linear regression models were conducted, including several predictors to the variable "knowledge of anthrax" and "anthrax outbreaks in people". Statistical significance was set at $p < 0.05$. Data were expressed as mean ± standard deviation unless otherwise stated.

3. Results

3.1. Arua District kraal surveys findings

Data from *kraals* surveys were obtained in 6 villages (Agera, Ndara, Owodromati, Pasumu, Payawe, and Tumawele) located in the Rigbo, Rhino Camp, Ogoko, and Okollo Sub-Counties of the Arua District. Some community members concealed information for fear of restriction from trade and consumption of their beef. All the responsible *kraal* leaders (57 individuals) were males with an average of 34.6 ± 12.1 years (minimum 19 years, maximum 70 years).

We recorded 6781 cattle, with a herd average number of 119 ± 71.5 animals. Additionally, the number of sick and dead goats, sheep, pigs, and dogs were recorded. A total of 339 animals were reported sick with clinical signs compatible with anthrax, with a case fatality rate of 66.4% (225/339). Clinical signs compatible with anthrax include sudden death (49%), *rigor mortis* (44%), fever (44%), external bleeding (39%), loss of appetite (37%), blotting (32%), and recumbency (26%). More than a quarter of the dead animals were abandoned in the bush (31.1% - 70/225) without any disinfection or proper elimination method of the carcasses. Of the total animals reported dead, 86.2% were cattle (194/225), 8% were goats (18/225), and 5.8% were sheep (13/225). Mortality in cattle was 2.9% (194/6781). Pigs and dogs were not affected by the anthrax outbreak. However, 40 people were affected: 20 people in Payawe, 12 people in Ndara, 5 people in Owodromati, 2 people in Agera, 1 person in Tamuwele, and 0 people in Pasumu.

A quarter of the responsible *kraal* leaders knew about anthrax (24.6% - 14/57), whereas 75.4% lacked knowledge of the disease (43/57) (Fig. 2). More than one third of the responsible *kraal* leaders were illiterates (38.6% - 22/57), 21.1% received primary education (12/57), 36.8% received secondary education (21/57), and 3.5% received higher education (2/57). The statistical analysis showed that the knowledge about anthrax disease was associated with the education level ($p<$ 0.001). Almost the totality of the responsible *kraal* leaders within the population knowing about anthrax received secondary education (92.9% - 13/14), representing the 61.9% of the total population that received secondary education (13/21). The remaining 7.1% of the people knowing about anthrax received primary education (1/14), representing the 8.3% of the total population that received primary education (1/12). Receiving secondary education was statistically significant to knowing about anthrax disease ($p < 0.001$). Additionally, higher knowledge about anthrax disease was found in the Payawe and Ndara villages (located in the Rhino Camp and Ogoko Sub-Counties, respectively; $p < 0.05$) and, interestingly enough, were the villages in which more cases of affected people were reported (20 and 12 people, respectively; $p < 0.01$).

The practice of eating dead animals is widely spread in these communities-89.7% of the communities consumed the meat of dead animals (51/57). More than half of the dead carcasses were opened for meat consumption (57.3% - 129/225), and 12.9% were skinned (29/225). The source of infection in people was contact with livestock (50%) and contact with contaminated meat or animal products in local markets (50%). Villagers transported contaminated meat to the local markets shipping beef on their backs (21.7%) and motorbikes (78.3%) without any protective clothing. Type of transportation means of contaminated meat was not associated with anthrax outbreaks in people ($p > 0.05$).

3.2. Arua District Veterinary Department interviews

Arua District Veterinary Department is faced with numerous challenges attributed to a lack of resources to contain the spread of the disease in the region. The district lacked information, education, and communication materials for educating communities about the signs and risks of disease and preventative measures. Records from the Department revealed numerous challenges surrounding the outbreak in 2017–2018. Vaccines were not available to prevent and protect community livestock from spreading anthrax in the district. Additionally, quarantine and law enforcement procedures to limit the movement of livestock in and out of the district were weak. From the community, carcasses were opened for consumption. During the outbreak, the district technical response team lacked personal protective equipment to handle carcasses, appropriate chemicals such as soda ash and fuel to burn the carcasses. The wrong depth of the pit dug to bury carcasses (about less than 6 feet), and no biosecurity measures taken to contain the spread of spores enabled scavengers like dogs to disturb the burial sites in the search for beef.

The community's level of reporting diseased animals was very poor, and the data provided was not significant enough for proper planning. It is believed that more animals died due to suspected anthrax, but reports at the center show that only 125 cattle, 9 sheep, and 16 goats died of suspected anthrax. In addition, because of logistical challenges in the Veterinary Department, no real-time surveillance was conducted to establish the scope of infection and respond timely. The regional veterinary laboratory in Arua lacked laboratory reagents to test, detect, and

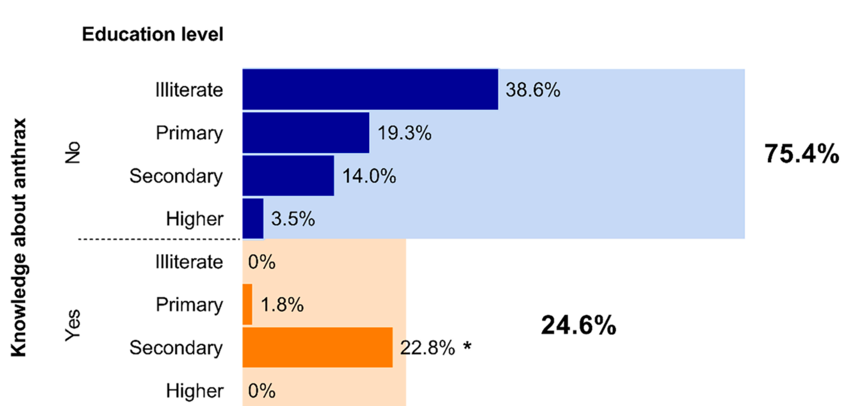

Fig. 2. Knowledge about anthrax associated with the education level of responsible *kraal* leaders. Asterisk indicates the education level statistically significant to knowing about anthrax disease.

confirm anthrax in real-time. Samples were collected and shipped to NADDEC and Uganda Virus Research Institute for a conclusive laboratory diagnosis. In Pawor Sub-County, Odupi, and Rhino Camp 65, 30, and 29 cattle were reported dead, respectively.

3.3. Tested samples

From the 24 samples submitted to NADDEC during the outbreak (Table 1), 19 samples tested positive (79.2%) on Gram and polychrome methylene blue stains. Positive samples demonstrated Gram-positive dark blue rods with a capsule on gram stain and polychrome methylene blue-stained large blue rods surrounded by red amorphous capsules characteristic of *B. anthracis* and numerous chains of non-sporulated rods. Under the light microscope, the observed positive smears demonstrated an uneven distribution of square-ended Gram-positive rods ranging from low to high intensity (+ to +++) of sporulated and non-sporulated Gram-positive rods. For the ADD rapid test (Table 1), 12 samples tested positive (50%).

4. Discussion

In domestic animals and people, anthrax is particularly common in parts of Asia, the Middle East, and Africa, where control measures in the livestock sector remain inadequate (Antonation et al., 2016). Anthrax is a notifiable disease in Uganda, and it is a disease of public health and veterinary importance (Republic of Uganda - Ministry of Health, 2021). However, Uganda has a national surveillance plan for anthrax developed in 2018 with the support of the Food and Agricultural Organization of the United Nations - Uganda purposely to control anthrax in the country (Food and Agriculture Organization of the United Nations, 2018).

The disease remains endemic with high incidences of anthrax in livestock and is one of the listed priority zoonotic diseases of public health and veterinary importance in Uganda (Vudriko et al., 2021). Despite annual anthrax outbreaks in animals, there is a weak surveillance system in the animal sector, which has led to underreporting and failure to detect early cases for immediate intervention. Therefore, a reliable and sensitive diagnostic method is of higher value to reduce the chance of zoonotic transmission and understand the burden diffusion and prevalence in time. In 2019, due to the lack of a rapid and reliable diagnostic method and other factors, the community herds surveyed in the Arua District reported 339 livestock infected with anthrax, 225 animals were reported dead, and 40 people were affected by *B. anthracis*.

Keeping livestock for livelihood in agricultural communities is a common practice in Arua District. However, animal husbandry practices in this community do not consider preventing animal diseases like anthrax. In Arua District, animals graze extensively, and others spend 24 h in the field, where they may frequently contact contaminated soil and decomposing livestock carcasses. Additionally, political and war pressures in the Republic of South Sudan triggered the movement of people with their livestock to settle in Rhino Camp, in Arua District. This may have contributed to the unquantified risk of introducing animal diseases in Uganda. Moreover, no measures were put in place to screen the foreign livestock for possible epidemics, which may increase the risk of animal diseases, including anthrax outbreaks. Similarly, the risk of disease transmission between the indigenous and foreign livestock is likely to occur because all livestock share one drinking water point: river Nile banks and grazing fields.

Table 1
Summary of human and animal tested samples using three diagnostic methods during the suspected anthrax outbreak in Arua District (2018).

Sample Type	Tested samples	Positive cases		
		GS	PMB	AAD
Bovine fixed blood smears	9	6	6	0
Bovine pooled unclotted blood	3	3	3	3
Smoked meat bovine	1	1	1	1
Fresh meat bovine	2	0	0	0
Bovine skin swabs	1	1	1	1
Bovine ear notch swabs	5	5	5	4
Environmental samples (blood on grass)	2	2	2	2
Human cutaneous swab	1	1	1	1
Total	**24**	**19 (79.2%)**	**19 (79.2%)**	**12 (50%)**

GS: Gram staining; PMB: polychrome methylene blue stain; AAD: Active Anthrax Detect rapid test.

Particularly, cattle were mentioned and linked to cutaneous anthrax human infections in the district. The 69 human cases reported with cutaneous anthrax in Olujobo health center were linked to livestock farming and behavioral risks of shipping beef on their backs and motorbikes to trading centers without the protection of their bodies using any protective clothing, except using their bare hands. The susceptibility to anthrax infection varies depending on the host species, where cattle are known to be the most devastated species, followed by sheep and goats (Fasanella et al., 2010). A similar situation was noticed in Arua District between 2017 and 2018. According to the district veterinary officer and the community surveys, more heads of cattle died of anthrax than goats and sheep.

Cattle tend to pull pasture directly from the ground with their roots, unlike sheep or goats, which browse on shrubs on off the ground level (Hornitzky and Muller, 2010). Therefore, cattle are most likely to ingest high doses of the encapsulated bacteria from potentially contaminated soil compared to browsing ruminants. Humans have a moderate susceptibility to the infection, while pigs and carnivores are more resistant to the deadly spores (Epp et al., 2010). According to these results, our survey data showed that pigs and dogs were not reported to be sick or dead due to anthrax outbreaks. However, dogs may act as instrumental in scavenging on the carcasses and spreading the infectious agents mechanically to clean areas.

The most accepted philosophy for browsing animal infection in Africa is that blowflies feeding off the carcasses move to rest on the leaves of nearby trees and shrubs and deposit anthrax spores on these leaves (De Vos, 1994). Browsing animals become infected after feeding on contaminated leaves. Additionally, biting flies are suspected of transmitting anthrax amongst wild animals and playing important roles in the wild epidemics (Hugh-Jones, 2016), making it difficult to halt the infection in the susceptible population if flies are heavily involved in the spread of spores. In Arua District, a similar scenario of decomposing bodies of livestock on the scene was heavily surrounded by active flies, making it difficult to halt the spread of spores to the clean environments. Furthermore, seasonal variations of anthrax transmission and unreported livestock deaths in the district may also contribute to the persistence of the outbreaks (Ashkenazi-Hoffnung et al., 2009). A discussion with a local area leader in Pasumu village revealed that many sudden deaths of animals in the region occurred but were not reported to the veterinary authorities. Animals fell sick, lost appetite, failed to respond to treatment, blood started oozing out of the nostrils, and finally died. In Ndara village, 13 animals were lost in December 2018, and one of the handlers of beef, a 17-year-old boy, developed skin lesions 4 days after carrying meat on his back.

Due to ignorance of the risk and custom, some community members exhumed dead animals and admitted that the common method of carcasses disposal was consumption since it is taboo to bury animals. As a risk factor, the digging process may expose the anthrax bacterium, which can further cause a spread of the contaminated soil extensively by blowing wind and lead to fresh outbreaks in the area (Ravenel, 2008). Due to a lack of proper disposal practices and resources, other cattle keepers decided to abandon dead animals in the bush. These carcass disposal methods were contrary to appropriate burning procedures, followed by disinfection of the site, which are better and more efficient mechanisms to reduce the chances of spore survival and further outbreaks in the affected areas (Mwakapeje et al., 2017).

The community's lack of awareness and knowledge is a risk factor that contributes to the spread of diseases. Our data identified that educational status and previous contact with the disease are associated with the knowledge of anthrax. This could be because education impacts on information access and capacity to comprehend health messaging. Furthermore, the previous contact with the disease may increase awareness of avoiding contracting anthrax. Therefore, education is the cornerstone to expanding the knowledge of anthrax among the farmers' population and preventing its spread. These findings are supported by a study conducted in Ethiopia, in which education background and knowledge of anthrax were associated with better anthrax prevention practices (Mesfin et al., 2021). Other factors include sex (females had better anthrax prevention practice than males), time spent arriving at the nearby clinic, and attitude towards anthrax prevention (Mesfin et al., 2021).

In the wildlife sector, sporadic anthrax outbreaks occurred in and around Queen Elizabeth National Park, where anthrax collectively killed over 500 wildlife, particularly hippopotamus and small herbivores and 400 domestic animals (Coffin et al., 2015). Comprehensive studies in the tropical rainforest of Africa revealed that *B. anthracis* is accountable for widespread incidences and persistent mortality among domestic herbivores and wild mammals for 3 decades with significant losses (Hoffmann et al., 2017). With the presence of Ajai Game Reserve in the Arua District and zero control of environmental contamination, there is a likelihood of anthrax spilling over to the wildlife herbivores in the future. In this case, grazing animals may acquire the disease by ingesting spores when grazing over sites where previous victims died and deposited the spores in the environment. For instance, licking of bones (pica or osteophagia) in search for minerals like calcium from animals that died of anthrax may result in cases or outbreaks in the wildlife (Lafferty and Chapman, 2014).

Failure of livestock vaccination programs in enzootic districts is a risk factor for livestock and wildlife herbivores (Rao et al., 2019). Vaccination of susceptible animals is one of the options to confer protective immunity and preserve animal health (Rao et al., 2019). From 2015–2019, no livestock vaccination was conducted as a predisposing factor, which may also have contributed to the growing number of anthrax fatalities among cattle. Due to poverty, livestock owners could not afford to buy their own vaccines because of a high cost of a single dose, which ranges from US$8 to US$19, which is another risk factor that paves the way for continuous anthrax outbreaks in the region unless government or donor organizations step in to support the district.

Only in 2020, the Food and Agricultural Organization of United Nations - Uganda, supported the district of Arua with 30,000 doses of anthrax vaccine (personal communication). Livestock, including cattle, goats, sheep, and pigs, were vaccinated against anthrax and black quarter. The vaccination targeted animals in high-risk areas where refugees and host communities reside, including Omugo, Odupi, Invepi, and Uriama, all in Terego County. Due to high population livestock density, many animals missed out on the vaccination due to inadequate quantity of the vaccine doses provided. Another category of farmers ignored taking their animals for the vaccination exercise. According to the Veterinary District officer, the vaccine served only 50% of the targeted livestock population, leaving another 50% of the population without protective immunity against future anthrax outbreaks.

Due to quarantine and trade barriers associated with livestock diseases, including anthrax, animal keepers were afraid to disclose households with infected livestock. Therefore, prompt identification and early response interventions were affected. Secondly, concrete data to establish the actual burden of the disease was next to impossible. Strengthening regional level laboratory capacity for anthrax is critical for the early identification of an outbreak. There is a need to strengthen laboratory surveillance at the regional level and timely detection of anthrax. Quick and reliable disease detection and confirmation methods are essential to correctly identify *B. anthracis* and understand its real diffusion and prevalence (Zasada, 2020), particularly in remote environments in which regular anthrax cases occur. In this regard, real-time polymerase chain reaction is a preferred method for detecting *B. anthracis* in animal tissue and blood samples (Avberšek et al., 2021; Berg et al., 2006). Awareness in the community is vital for the locals to understand forms of anthrax, species affected, impact and disease presentation. The importance of reporting to the Veterinary Departments is paramount in controlling the spread of the disease and reducing human infections as well as livestock mortalities.

5. Conclusions

This study identified and confirmed the presence of anthrax in biological samples after a suspected outbreak in Arua District. The cattle population was more affected by anthrax than any other reared species included in the study, such as goats and sheep. Lack of awareness among cattle owners and community concerning the nature, the transmission of anthrax from animals to humans, scarcity of the livestock vaccine, social norms, cultures, environmental factors, and poverty were the key drivers that may have contributed to the spread of anthrax in livestock, as noted in this study. The grazing system where cattle are not controlled and monitored may easily promote the transmission and maintenance of anthrax outbreak cycles in the Arua District's grazing field and other Uganda districts.

Ethics approval

The field and laboratory outbreak investigations were conducted in accordance with the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and World Oragnisation for Animal Health (WOAH) requirements for responding to and managing Animal disease outbreaks in Uganda. The primary intent of the investigation was public health control, as did not require ethics committee approval or written consent of the farmers.

CRediT authorship contribution statement

Michael Omodo: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **Jaume Gardela:** Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alice Namatovu:** Supervision. **Rose Ademun Okurut:** Supervision. **Martin Esau:** Investigation. **Merab Acham:** Investigation. **Maria Flavia Nakanjako:** Investigation. **Mugezi Israel:** Investigation. **Emmauel Isingoma:** Investigation. **Mwanja Moses:** Investigation. **Lumu Paul:** Investigation. **Ben Ssenkeera:** Investigation. **Stella A. Atim:** Investigation. **Doreen N. Gonahasa:** Investigation, Writing – review & editing. **Musa Sekamatte:** Investigation, Writing – review & editing. **Meriadeg Ar Gouilh:** Writing – review & editing. **Jean Paul Gonzalez:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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