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Verbal Autopsy and Outbreak Investigation of Anthrax in Livestock and Wildlife at the Virunga National Park Interface Area, Democratic Republic of the Congo

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Abstract

Anthrax is a worldwide environmentally transmitted fatal zoonotic disease with socioeconomic and public health impacts. It frequently occurs among livestock and wildlife in North Kivu province, Democratic Republic of the Congo. However, due to a poor animal health control system, it is poorly understood and managed and often underreported. Therefore, to understand, document, and report the epidemiology of anthrax in this province, a cross-sectional survey was conducted in 2015 involving arbitrarily selected 120 farmers to access their knowledge on clinical manifestations, transmission, prevention, attitudes, and practices concerning anthrax. Subsequently, a field investigation was conducted in 2021 during an anthrax outbreak to investigate the disease trend and routine practices. We established that most farmers know and can identify anthrax; they greatly fear and try to prevent it despite some unhealthy practices associated with handling and eating anthrax-contaminated meat. Extensive transhumance livestock management systems, anthropogenic activities, and the lack of a surveillance program have primarily contributed to anthrax outbreaks in North Kivu province. The presence of anthrax in this region was confirmed by detecting *Bacillus anthracis*, the etiological agent, during an outbreak investigation. Hence, the need for collaborative efforts for continued surveillance effectively manage anthrax outbreaks to reduce this serious threat to health and livelihood in this area.

Keywords: Anthrax, livestock, wildlife, occurrences, North Kivu province

Introduction

The exponential growth of the world population requires investing in food self-sufficiency, including the intake of meat proteins [1]. Arable land is needed, but across the Earth, only a third of the soil is suitable for agriculture [2]. However, in the Democratic Republic of the Congo (DRC), the eastern regions are endowed with arable soils and a favorable climate for livestock farming. Agriculture and pastoralism are the main income-generating activities in the eastern region, contributing to improved livelihood and the socio-economy of the area. Unfortunately, these regions have been under terror from armed groups whose presence and activities have profoundly affected agricultural activities for several decades. These armed groups indiscriminately raid and slaughter domestic animals from farmers but, as a countermeasure, constantly move their livestock. Farmers move into protected areas such as the Virunga national park and the surrounding interface areas. This scenario increases animal densities, over-utilization of common pool resources by livestock and

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wildlife, and increases opportunities for infectious disease transmission. Since farmers move with their livestock, there is an increased risk of emergence, re-emergence, and dissemination of infectious diseases, particularly zoonosis, such as anthrax.

Anthrax, also known as woolsorter's disease, murrain, or spleen blood, is a multi-specie zoonotic disease caused by a spore-forming aero-anaerobic bacterium: *Bacillus anthracis* (*B. anthracis*). [3, 4]. Except for Antarctica, anthrax is present and endemic on all continents and presents a socioeconomic and public health concern [5, 6]. However, the global distribution of *B. anthracis* is poorly understood; many countries, especially Africa, possess inadequate surveillance systems, even within known endemic regions [7]. This has resulted in under-reporting of the disease and, in some cases, neglected; thus, anthrax endemism and outbreak intensity has been characterized at extremely coarse scales. In animals and humans, the bacterium is often found in the vegetative form, but on exposure to air, it forms highly resistant spores that can remain viable for many years in some soils and therefore serves as a source of infection to grazing herbivores [8-11]. Thus, the ability of *B. anthracis* to form long-lasting, highly resistant spores is central to the persistence of anthrax in an area.

Once herbivores consume the spores, they germinate, changing to the growing and dividing vegetative form, which multiplies rapidly and causes the disease. *B. anthracis* virulence is due to the production of a tripartite toxin composed of a protective antigen, lethal factor, and edema factor [12, 13]. The clinical manifestations vary depending on the location of the occurrence, the time of year, the susceptibility of the infected animal species, and the form of the disease [14, 15]. It manifests as fatal febrile sepsis with or without hemorrhagic lymphadenitis [14, 16]. Peracute septicemic forms are brutal, sometimes asymptomatic, and suddenly fatal [3]. On the lesional level, sticky, incoagulable, and blackish blood can flow from the body's natural orifices [14]. Bulky and muddy spleen (spleen blood) and hemorrhages from the bladder are also observed [3, 16]. Human anthrax cases are secondary to outbreaks in animals through contact or consumption of anthrax carcasses. There are three types of human anthrax infection: cutaneous, gastrointestinal, and pulmonary. The most common cutaneous form is acquired through contact, the gastrointestinal form through ingestion of infected meat, and the pulmonary form through inhalation of *B. anthracis* spores [13, 17]. "Hunger for meat," being the primary driver of consumption of anthrax carcasses, contributed to environmental contamination with anthrax spores through the dressing of anthrax carcasses, a major source of primary infection for livestock and wildlife. These anthropogenic activities and the lack of knowledge of anthrax strongly intimate their contribution to anthrax recurrence and maintenance in endemic areas [18].

Notwithstanding this context, anthrax can be controlled if animal species involved in its maintenance and transmission, anthropogenic factors, spatiotemporal distribution, and its impact on the human or animal population and the household economy are known. Unfortunately, due to poor animal health control and the absence of an effective surveillance system for animal diseases in the DRC, this zoonosis is not monitored, and the parameters contributing to its maintenance and dissemination are scarce or not documented. Furthermore, epizootic reports and alerts from park wardens and breeders are often under-reported and masked due to limited human resource, making this country's data on anthrax unavailable. Therefore, this survey was designed to assess the knowledge regarding symptoms, transmission, and prevention of anthrax among farmers, to describe attitudes, and determine the risk and health-seeking practices in the event of anthrax. Simultaneously, the clinical investigation provides some evidence to understand, document, and report the epidemiology of this disease in the Kisongwere and Lulimbi groups and generate essential information for implementing appropriate measures for the control of anthrax in this environment.

Farmers in the North Kivu province frequently report morbidity and mortality suspecting Anthrax in domestic animals. Wild animal deaths are also sometimes reported with possible Anthrax signs. However, none of these alerts are investigated since the area is not secure because the decades-long war is still ongoing. Taking advantage of a given opportunity, a survey was conducted in 2015 that led to the gathering of information on the epidemiology of this disease in the area using verbal autopsy whereby farmers were interviewed on their knowledge, attitude, and practices related to Anthrax risk transmission and control. In 2021, alerts were reported by park guards on the mortality of buffaloes and hippopotamuses in the Virunga national park with suspected Anthrax signs. Despite the insecurity of the area, a team was sent to the field to conduct investigations on these cases. The present report underlines the findings of the 2015 survey and the 2021 investigations on Anthrax occurrence in this area.

Materials and Methods

Study environment

These two activities were conducted in the North Kivu province in the Democratic Republic of the Congo (DRC) (Figure 1). The study was done in some localities (Kyavinyonge Muko, Makungwe Muvula, Kisunga Vwino) of the Kasongwere group and Lulimbi city. These agglomerations are located not far from the Virunga national park in Beni, North Kivu Province of the DRC. The primary source of livelihood in this area is agriculture, including livestock rearing (cattle, goat, sheep and pig) and crop

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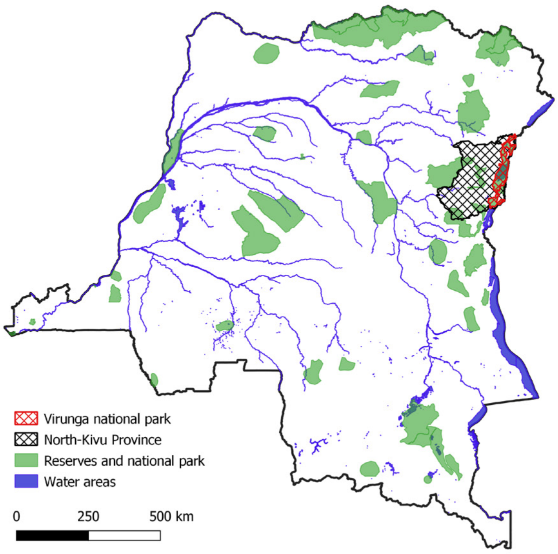

Figure 1: Map of the DRC showing the national parks and nature reserve areas. The studied area in North Kivu province, Virunga national park, is also shown.

production. Other economic activities are fishing and crafts. Kasongwere enjoys the mountain climatic influences of the Rwenzori massif, Mount Tchabirimu, the Semuliki plain, and Lake Edward. Livestock is reared on a transhumance grazing system, sharing pastures and watering points with a wide range of wild animals, including buffaloes (*Syncerus caffer caffer*) and hippopotamuses (*Hippopotamus amphibius)* in the Virunga national park and the surrounding game management areas. The investigation on anthrax alerts that were reported in hippopotamuses and buffaloes by Virunga national park guards took place in Lulimbi, a city located near the Ishasha river, in the savannah plain, in the eastern part of the Virunga national park.

Study Design

For verbal autopsy, we conducted a cross-sectional study in 2015 using a pre-tested semi-structured questionnaire partially adapted from other studies to assess the local people's knowledge, attitudes, and practices about anthrax. Informed consent was obtained from 120 farmers from randomly chosen localities prior to each interview [19]. Additionally, in 2021, a field investigation was carried out in response to the alerts from park rangers reporting an active suspected anthrax outbreak with mortalities in hippos and buffaloes in Lulimbi, on the eastern part of Virunga national park (Figure 1). Unclotted blood samples were collected from exudates from all carcasses for laboratory confirmation. Blood smears were made, stained with polychrome methylene blue, and then observed under a Nikon ECLIPSE E2000 microscope (Nikon USA, Melville, NY).

Data management and Analysis

The data from the questionnaire survey was entered into an Excel database (Microsoft Office 2016). The analysis consisted of enumerating the variables and their respective responses. Then, statistical calculations were used to assess the proportion of each element studied concerning what was expected.

$$[Proportion = (\frac{observed\ frequency}{sample\ size}) \times 100\,]$$

Results

The questionnaire study evaluated a total of 120 livestock farmers from 3 localities (40 farmers per locality) in the group of Kisongwere who consented and joined the survey. These represented livestock farmers study areas that had had a history of the disease (Kyavinyonge Muko, Makungwe Muvula) and those without (Kisunga Vwino and some of its bordered agglomerations), which ensured unbiased evaluation of the levels of awareness about anthrax.

It was noted that in the Kisongwere group, free-range (37%) and extensive management systems (63%) (n=120) are the most common modes of exploitation of domestic animals in these localities. In the free-ranging system, animals did not receive any form of food supplements but had to forage in the nearby grasslands unguided. While in the extensive system, animals were taken daily to the pasture by a herder, then brought back to a kraal by nightfall. In addition, some farmers (42%) in the extensive system practiced a transhumance grazing system, in which cattle were constantly moved in search of pasture into the park and only returned home during the rainy season when vegetation in the neighborhood was sufficient and nutritious.

This study also shows that livestock farmers in the Kisongwere group and park wardens know the signs of anthrax and can quickly identify sick animals or those that had died from anthrax. They mentioned high fevers (87%), the oozing of dark and non-coagulated blood through the natural orifices (oral, nasal, auricular, and anal) (13%), generalized congestion of the corpse (31%) with edema and effusions of blood in the serosa (82%), an enlarged, purplish and softened or muddy spleen (7%), rapid putrefaction (deterioration) of the carcass (40%) and bloating of the belly (67%).

It was also noted that the mortality due to anthrax is expressed differently in the localities studied, Kyavinyonge Muko (58,3%) being the most affected. It was followed by Makungwe Muvula (26.7%) and then Kisunga Vwino (15%).

As for the seasonality of the occurrence of mortalities attributed to anthrax, this survey shows that in the Kisongwere group, these mortalities were recorded throughout the year (12.5%) and were particularly amplified during the rainy season (69.2%) than in the dry season (18.3%). According to these breeders, pigs (30.8%) were most affected, followed by cattle (25.8%), dogs (18.3%), goats (14.2%), and other animals approximately (10.8%).

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The carcasses of animals that died of anthrax are more buried than incinerated. For animals still alive, preventive measures include isolating suspected patients and avoiding grazing animals in the park. For humans, prevention consists of smoking the meat before consuming the flesh of a suspected animal (Table 1).

Table 1: Proportion of respondents with knowledge about anthrax-affected animal's, symptoms and necropsy signs, mortality, attitudes and, perceived risk for anthrax

Variable	Category	Frequency	Percentage
Affected animals	Pig	37	30.8
	Cattle	31	25.8
	Dog	22	18.3
	Goat	17	14.2
	Other animals	13	10.8
Sign of anthrax	**Sick animals***		
	High fever	87	72.5
	Oozing of dark and non-coagulated blood	13	10.8
	Rapid putrefaction (deterioration) of the carcass	40	33.3
	Bloating of the belly	67	55.8
	Dead animals*		
	Generalized congestion of the carcass	31	25.8
	Edema and effusions of blood in the serosa	82	68.3
	Enlarged, purplish, and softened or muddy spleen	7	5.8
Mortality	**Animal number/year***		
	1-25	104	86.7
	26-50	7	5.8
	51-75	5	4.2
	>76	4	3.3
	Seasonality*		
	Rainy season	83	69.2
	Dry season	22	18.3
	Rainy and Dry seasons	15	12.5
	Studied area*		
	Kyavinyonge Muko	70	58.3
	Makungwe Muvula	32	26.7
	Kisunga Vwino	18	15
Carcass treatment	**Measure***		
	Burial	68	56.7
	Incineration	35	29.2
	Meat consumption after smoking	17	14.2
Prevention	**Measures***		
	Do not graze animals in the park	83	69.2
	Isolate any suspected anthrax animal	37	30.8
Consequences of anthrax	Animal mortality	88	73.3
	Poverty	15	12.5
	Human death	17	14.2

*Specify the concerned category

Concerning the effects of anthrax within the community, herders identified the mortality of their animals (88%), contamination to humans and its consequences (17%), as well as poverty (15%) resulting indirectly from all these effects.

After receiving alerts about the anthrax epizootic in wildlife in Lulimbi during April, the rainiest month in North Kivu province, active surveillance was conducted. The park rangers, park authorities, and surrounding communities were asked to monitor and report cases of clinical illness and any mortality in domestic or wild animals and animal movements restriction. Dead animals were enumerated retro, and prospectively, clinically ill animals were followed up, and some blood smears were made. From the observation of sick animals, it was noted that the hippopotamuses clinically affected cried out plaintively, presented skin redness, difficulty, and respiratory distress, and then died. Oozing from the natural orifices of blackish and non-coagulated liquid blood was observed on the carcasses. For the dead animals, 16 hippopotamus corpses were counted at the mouth of the Ishasha River and Lake Edward (Figure 2), apart from those found in inaccessible sites where the remains were surrounded and protected by congeners. Only three buffalo carcasses were observed; the rest were not accessible. Some hippopotamus carcasses in lakes or swampy places exuded directly into the water. The smears prepared and stained with polychrome methylene blue made it possible to highlight *B. anthracis* (Figure 3) under the microscope.

a
b
c

Figure 2: Corpses of anthrax dead animals, hippos (a, b) and buffalo (c) floating in the Ishasha River at Lulimbi, Virunga national park (Photos taken by Eddy Syaluha)

a
b

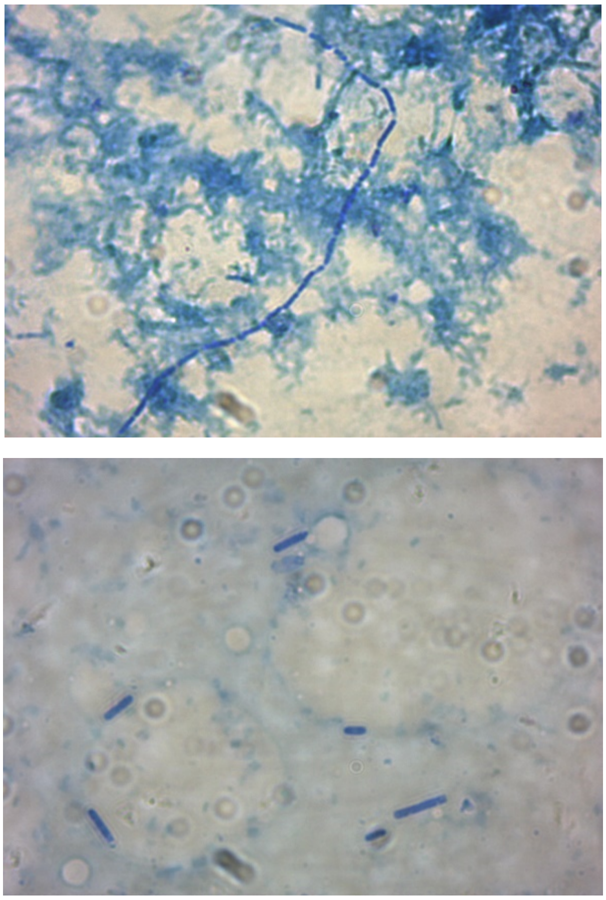

Figure 3: Results of microscopic analyses: blood smears from blood collected in digital pad of hippos (a, b) dead from anthrax stained with polychrome methylene blue and showing rods of *Bacillus anthracis* (Photos taken by Eddy Syaluha)

Discussion

Anthrax is caused by bacteria transmitted to humans through contact with infected animals/contaminated products. Given the ability of *B. anthracis* to sporulate and the conditions required for its culture, a clinical approach can be used, as is the case in this survey, to understand the epidemiology of this disease [20]. Clinical signs and postmortem lesions, including those pathognomonic for anthrax, which breeders mentioned, would reflect the clinical picture of this disease, suggesting that they were experienced and observed animals/ their carcasses during epizootics [3]. This would indicate the presence of this disease in the studied areas and the danger it represents to the rural population. Studies have shown that the highest risk for contracting and developing human anthrax is by handling carcasses of animals that die from anthrax [21, 22].

Although the clinical course of anthrax is often rapid and asymptomatic, most of the farmers interviewed in this study could identify it at postmortem. This validates the presumption of the presence of anthrax in this part of the

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DRC, and probably, at the proportions noted in the different localities studied. The observed difference in the case reports between localities could be influenced by the presence of hunters or poachers, animal densities, and levels of environmental contamination. Hunters/poachers sell meat for wild animals and livestock in the cities. It is not uncommon for the meat sold to include that which is obtained from *B. anthracis* contaminated carcasses since it is opportunistically and conveniently collected) and results in the gastrointestinal form of anthrax outbreaks in cities [23]. Anthrax carcass handling and meat distribution contribute to environmental contamination and the dispersal of *B. anthracis* spores to new areas. The spores in the environment serve as a future source of infection as they are grazed together with pastures causing persistent anthrax outbreaks. In addition, due to limited pastures around human settlements in the study area, herders make arrangements with the game wardens to enter the park for their animals to access pastures and water. This would expose domestic animals to graze or drink around unidentified, and undelimited anthrax grave fields since not all wildlife death sites are known or disinfected [7, 24]. Thus, wildlife has been viewed as an obstacle to controlling anthrax outbreaks [4].

In endemic areas, the likelihood of anthrax outbreaks was cyclic and linked to ecological factors, breeding behaviors, and activities of susceptible animal hosts [25-27]. In this study, there was also a variation of infection and mortality rates throughout the year stratified by season, suggesting the influence of environmental conditions, temperature, and humidity. More cases, as well as the investigated outbreak, were recorded in the rain than in the dry season, and could be explained by the increased activity of earthworms, including those containing *B. anthracis* spores, and by runoffs which bring the spores to the soil surface and on grass, thus exposing them to be ingested by animals [28-30]. This finding strongly intimates that there are a lot of anthrax-cursed fields in the study area and underreporting of wildlife anthrax cases. Previous studies also noted variations in mortality due to anthrax depending on the environment and the season, with seasonal peaks in the rainy season [15, 25, 27]. This survey also shows that multiple species were affected, with the highest mortality rate recorded among pigs, ahead of cattle and dogs in the Kasongwere group. This is an unusual finding; literature has shown that anthrax is rare in pigs and carnivores given that they are less susceptible compared to herbivores [8, 31, 32]. This could reflect the importance of eating habits for the pig in the Kasongwere group. Pigs were kept on the free-range management system, feeding from kitchen residues to scavenging in anthrax-cursed fields, possibly for fresh carcass remains and earthworms, which are abundant in the soils around the grave sites [10]. established that animals are fatally attracted to anthrax carcass sites because of localized carcass-mediated nutrient pulses, which improved soil and vegetation, and that *B. anthracis* was found on grasses up to 2 years after death. "This attraction may enhance transmission rates, suggesting that hosts are limited in their ability to trade off nutrient intake with parasite avoidance". However, the number of animals killed annually during anthrax epizootics may have been underestimated. In the north Kivu province, the lack of a strong animal diseases surveillance program, the inaccessibility to certain areas where animals die, and the non-existence of an elaborate program to control this disease mean that all mortalities were not exhaustively documented. Considering the rapid progression of the disease and premonitory signs that may go unnoticed, a definitive diagnosis is difficult to establish; thus, the cases of the disease are underestimated [3, 33]. Nonetheless, all the farmers and park wardens interviewed are aware of symptoms of anthrax, postmortem lesions, as well as its public health implication, 57% of carcasses are buried, and 29% are incinerated by burning. Burying without additional chemical treatment or burning as a mode of disposal is not sufficient to prevent the formation of cursed fields, especially when it is not well conducted. To bury anthrax carcasses, it is recommended to dig a hole of at least two meters deep, block the orifices, and sprinkle a large quantity of quicklime [3]. Seemingly, the graves are not dug deep, and the 14% of the carcasses which are dressed for consumption encourage scavenging by pigs and dogs. In addition, dressing of *B. anthracis-infected* carcasses contaminate the environment with spores, promoting the recurrence of anthrax outbreaks in this area.

The consequences of anthrax within the community could not be evaluated due to the underestimation of these mortalities, but its socioeconomic impacts are evident. Poverty being the main driver of anthrax carcass consumption, the scarcity of grazing lands around the communities, thus taking the animals into the national park, and the general lack of a systematic disease control program have primarily contributed to outbreaks at the Virunga human-livestock wildlife interface area. The *B. anthracis* viable spores are resistant to harsh environmental conditions, and their dissemination can lead to a new risky environment or exposure and subsequent anthrax outbreaks [34]. This study found some carcasses in lakes or swampy places exuded directly into the running water. Therefore, the probable dissemination of spores that can form from anthrax carcasses can also lead to new outbreaks or pose a public health concern. This survey has revealed evidence of anthrax in multiple species in the Virunga national park and the surrounding interface areas. The outbreaks peak during the rainy season and mortalities are highest in pigs. Complimentary ecological parameters, a free-ranging livestock management system, anthropogenic activities, and the lack of a surveillance program have primarily contributed to the outbreaks. Nevertheless, we believe that the control of anthrax through vaccination and appropriate disposal of anthrax carcasses will significantly mitigate its socioeconomic

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impact and improve poverty levels. Therefore, there is a need to establish veterinary infrastructure and extension services for anthrax control and other livestock diseases and zoonosis in this park and its surrounding areas.

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Authors Contributions

S M. D, L P. B and, M J *contributed to the study conception and design. Material preparation, data collection and analysis were performed by* S M. D, L P. B, M J, B M. É, N F and S K. E. *The first draft of the manuscript was written by* S M. D, L P. B and, M J, S K. E, B M. É. A*ll authors including* K K. H, B M. T; M L; A D. H and; M S *commented and* revised critically *previous versions* with important intellectual content*. All authors read and approved the final manuscript for publication.*

Ethics Approval

This study was approved by the Ministry of Fisheries and Livestock, Central Veterinary Laboratory of Kinshasa authorities in charge of research.

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