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Geographic distribution of cattle anthrax in Western Zambia

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Abstract

Cattle anthrax outbreaks have been reported in Western Province, Zambia since the 1970s. Although several efforts have been made to control these outbreaks, their persistent occurrence remains a major problem. To understand the current epidemiological situation, we analyzed the annual, seasonal, and geographical distributions of cattle anthrax in Zambia from 2000 to 2016. Results indicate a decrease in the annual number of reported cases and a change in geographical distribution of outbreaks. Anthrax vaccinations can influence the epidemiological situation of the Western Province; therefore, the seroprevalence of *Bacillus anthracis* protective antigen antibodies was determined in 1619 cattle. The overall seropositive rate was 24.9% (403/1619) and it varied among sampling areas, suggesting that anthrax vaccination programs must be reinforced in Zambia.

Key Words: anthrax, geographical distribution, Zambia

Anthrax, a zoonotic disease affecting wildlife, livestock, and humans, is caused by *Bacillus anthracis*[2]. Handling and consuming infected carcasses or their products is the main risk factor for anthrax infection in humans[4,5,7,10]. Lack of awareness, poor practices of animal husbandry, and inadequate vaccination of livestock are some factors contributing to the persistence of anthrax in endemic areas[3, 9].

In Zambia, anthrax outbreaks usually occur in May-January and peak toward the end of the dry season (between October and November)[11]. The species that are most frequently affected include cattle and goats. Cattle anthrax

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outbreaks invariably lead to the occurrence of the disease in humans[15]. The occurrence of cattle anthrax has usually been reported in remote rural communities of the Western Province of Zambia, particularly those along the Barotse Floodplain (Fig. S1). The Barotse Floodplain has an estimated area of $>5,000\,km^2$ extending from Lukulu in the north to Senanga in the south[17]. The onset and duration of inundation vary greatly, influencing various anthropogenic activities in the area as well as the time spent by cattle in the floodplain[20]. Flooding plays a significant role in agriculture as it is a source of grassland irrigation, which is essential for cattle in the traditional farming sector. Farmers in this area practice the transhumance type of farming, in which cattle spend most of the time in the floodplains but moving out of the floodplains when flooding peaks.

Regular cattle anthrax outbreaks in the Western Province of Zambia have been reported since the 1970s[1]. An average of 232 and 135 cattle anthrax cases were annually reported between 1989 and 1995, and between 1999 and 2007, respectively[11]. Throughout the years, the number of reported cattle anthrax cases has decreased; however, the frequency and persistence of such outbreaks are still not under control. Although livestock anthrax is the leading cause of anthrax infections in humans in endemic areas of several developing countries[19], the geographical distribution of cattle anthrax outbreaks and the situation on cattle anthrax vaccinations remains unclear. In Zambia, the lack of this information for managing and controlling cattle anthrax outbreaks has resulted in the underestimation of the impact of anthrax on veterinary medicine, public health, politics, and economics.

The anthrax toxin comprises the following proteins: edema factor, lethal factor and protective antigen (PA). PA an essential part of the anthrax toxin is responsible for cell surface receptor binding and translocating the edema factor and lethal factor into the cytosol[21]. Most currently used veterinary live spore anthrax vaccines are prepared from the Sterne strain of *B. anthracis*, and the conferred immunity is primarily attributed to PA[18]. The detection of anti-PA antibodies in the serum of vaccinated livestock can be useful for monitoring the extent and effectiveness of anthrax vaccination programs.

The objectives of this study were to determine the geographical distribution of cattle anthrax in the Western Province of Zambia using retrospective data on cattle anthrax outbreaks and assess the seroepidemiological situation in the anthrax outbreak areas.

In order to understand the current epidemiological situation of cattle anthrax outbreaks in the Western Province of Zambia, the annual, seasonal, and geographical distribution of cattle anthrax outbreaks from 2000 to 2016 were analyzed. The National Livestock Epidemiology and Information Centre, Ministry of Fisheries and Livestock, Lusaka, Zambia approved the use of national livestock surveillance data of anthrax in the Western Province of Zambia from 2013 to 2016. Data from 2008 to 2012 were obtained from annual reports on cattle anthrax outbreaks in Zambia deposited in the World Animal Health Information Database[13]. The number of cattle anthrax cases reported from 2000 to 2007 was obtained from the literature[11]. In addition, data were obtained from annual reports of the Provincial Veterinary Office in the Western Province of Zambia.

In the reports, field diagnosis of anthrax was done by district veterinarians. The diagnostic criteria were based on clinical signs and epidemiological history (outbreak location and location of previous outbreaks). Blood smears prepared from collected samples (blood swabs or ear from carcass) were subjected to Giemsa staining and microscopic examination. Field-diagnosed cases were termed "suspected anthrax cases" until confirmation by culture testing and bacterial isolation at the Central Veterinary Research Institute (CVRI), Ministry of Fisheries and Livestock, Lusaka, Zambia and the School of Veterinary Medicine, University of Zambia. The

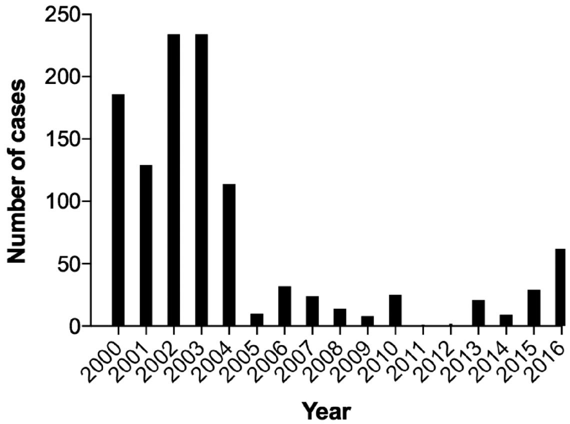

Fig. 1. Annual distribution of cattle anthrax cases from 2000 to 2016. Number of cattle anthrax cases reported from 2000 to 2007[11].

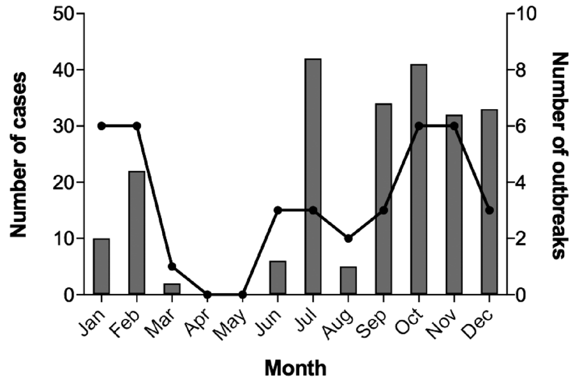

Fig. 2. Monthly distribution of cattle anthrax cases and outbreaks from 2006 to 2016. Bar graph: number of cattle anthrax cases; Line graph: number of cattle anthrax outbreaks. Descriptive statistical analyses and graphs were produced using GraphPad Prism, 7.0b (San Diego, CA).

culture and isolation of *B. anthracis* was performed as previously described by OIE[14]. For this study, cattle anthrax outbreak was defined as the occurrence of anthrax at least once and confirmed via culture testing. The case fatality rate was calculated by dividing the number of deaths in a herd by the total number of affected cattle in that herd. Additionally, population-at-risk was defined as the number of cattle exposed to the affected herd.

Firstly, we analyzed the general trend of the occurrence of cattle anthrax from 2000 to 2016. A total of 1,134 cattle anthrax cases were reported, with an annual mean of 66.71 [95% confidence interval (CI): 24.88–108.5] cases. Overall, a decreasing trend was observed for the number of cattle anthrax cases reported during this period, with the highest number being reported in 2002 and 2003 (n = 234 per year) and the lowest in 2011 (n = 1) (Fig. 1).

Secondly, we used the cumulative monthly outbreak data from 2006 to 2016 to analyze the seasonal distribution of cattle anthrax cases (monthly outbreak data from 2000 to 2005 were unavailable). From 2006 to 2016, 39 cattle anthrax outbreaks were recorded, with an annual mean of 3.25 (95% CI: 1.79–4.71). During the same period, 227 cattle anthrax cases were recorded, with an annual mean of 18.9 (95% CI: 8.3–29.5) (Table S1). The year 2008, 2010, and 2016 witnessed the highest number of cattle anthrax outbreaks. The frequency of these outbreaks was the highest in October, November, January, and February; the number of cattle anthrax cases peaked in July and October (Fig. 2). The frequency of cattle anthrax outbreaks and cases decreased from February to May. The majority of these outbreaks (58.9%) and cases (74.6%) were reported during the hot/dry season (May–November). Cattle anthrax cases reported between 2013 and 2016 were almost invariably fatal, with the case fatality rate ranging from 0% to 100% (Table 1).

The overall monthly distribution of cattle anthrax remained unaltered compared with that of livestock anthrax reported in the Western Province of Zambia from 1999–2007[11]. From 2008 to 2016, the frequency of outbreaks peaked equally during the hot/dry season and at the beginning of the rainy season (December–April). Since this study is based on the data collected from three different sources, there is a possibility that we over- or under-estimated the number of cases and outbreaks reported during the study period. Moreover, because the identification of cattle anthrax outbreaks is generally based on reports, missed or underestimated reports of the outbreaks may also underestimate the actual prevalence of anthrax in the field.

Table 1. Cattle anthrax cases and deaths from 2013–2016

Year	District	Herd size	Cases	Mortality	Case fatality rate %	Population at risk
2013	Kalabo	54	1	1	100	54
	Mongu	230	20	20	100	330
2014	Kalabo	81	1	1	100	1300
	Luampa	210	4	4	100	210
	Lukulu	50	3	3	100	100
	Nalolo	80	1	1	100	80
2015	Limulunga	378	10	9	90	2210
	Sioma	60	7	7	100	500
	Mwandi	ND	11	0	0	ND
	Senanga	ND	1	0	0	ND
2016	Kalabo	1600	40	25	63	5900
	Limulunga	ND	4	4	100	12000
	Nalolo	ND	6	6	100	12000
	Shangombo	ND	4	4	100	11000
	Sioma	ND	8	8	100	6000

ND: not determined

Thirdly, we analyzed the geographical distribution of cattle anthrax cases from 2000 to 2007 and 2012 to 2016. From 2008 to 2012, data on cattle anthrax outbreak locations were unavailable. From 2000 to 2007, >90% of cattle anthrax outbreaks were reported in areas along the Barotse Floodplain. The affected districts were Lukulu, Mongu, Senanga, Sesheke, Kaoma, and Kalabo. Mongu, followed by Senanga, had the highest rate of cattle anthrax outbreak recurrence. From 2013 to 2016, cattle anthrax outbreaks were reported in Lukulu, Mongu, Kalabo, Limulunga, Sioma, Nalolo, Shangombo, Luampa, and Mwandi. The Kalabo district had the highest number of cattle anthrax outbreak recurrences, followed by Sioma, Limulunga, and Nalolo (Table 1). Subsequently, the annual distribution of cattle anthrax cases from 2013 to 2016 was analyzed. In 2013, cattle anthrax outbreaks occurred in districts lying northwestern to the floodplain, and in 2014, 2015, and 2016, these outbreaks occurred in the northeastern, southern, and southwestern and northwestern parts, respectively (Fig. 3). Notably, districts that previously had no reports of outbreaks such as Shangombo reported cattle anthrax cases in 2015 and 2016. The majority of the affected districts were adjacent to districts that experienced such outbreaks in the previous years.

The finding regarding the highest number of cattle anthrax outbreak recurrences in the Mongu and Senanga districts in this study is consistent with that in previous studies[1,15]. Moreover, we found that Kalabo and Sioma districts were among those with the highest number of such outbreak occurrences from 2013 to 2016. The present analysis showed that cattle anthrax cases were also reported in rarely flooded areas, apart from the floodplain. Considering the incubation period of anthrax (approximately 3–5 days)[2], we hypothesize that cattle acquired the infection in the floodplain, resulting in subsequent death in the adjacent upland areas, establishing new foci of infection. In this study, mapping at the district level was performed because we were unable to obtain georeference data on the locations of cattle herds. Mapping at the herd or veterinary-camp level may be more useful in identifying high-risk areas and the actual geographical extent of cattle anthrax outbreak occurrence.

Fig. 3. Annual distribution of cattle anthrax cases from 2013 to 2016 in the Western Province of Zambia. Most of the newly affected districts were adjacent to districts with previously reported cattle anthrax outbreaks. Districts 1–16 are indicated in Fig. S1. Maps were produced using ArcGIS 10.4.1 (ESRI, CA, USA).

Because the pattern and extent of anthrax vaccination programs may influence the geographical distribution of outbreaks, we assessed the seroepidemiological situation in the Western Province of Zambia by measuring the seroprevalence of anti-PA antibodies in 1619 Barotse cattle. Cattle serum samples were collected from Mwandi, Limulunga, Mitete, Senanga, Mongu, Sesheke, and Mulobezi districts (Fig. 4). These samples were collected between December 2016 and February 2017 (9–10 months after the anthrax vaccination period) as part of the national surveillance program on vaccine-preventable diseases and were stored in the serum bank at the CVRI. We screened the serum samples for anti-PA antibodies using the *B. anthracis* PA-D1 in-house ELISA as described previously[16].

Of the 1619 cattle serum samples screened for anti-PA antibodies, 24.9% (95% CI: 22.7–27.0) were positive (Table 2). Seropositive rate was the highest in Mwandi (44.3%, 95% CI: 33.3–55.3), whereas the lowest rate was observed in Senanga (15.8%, 95% CI: 10.0–21.5). Seropositive rates significantly differed among the districts (Kruskal Wallis test, $P < 0.05$). We also found significant differences in the seropositive rates between veterinary camps in Sesheke and Mulobezi. Compared to the current seropositive rate of 24.9%, our previous study reported a seropositive rate of 8% (15/187) at 18 months after an anthrax outbreak and retrospective vaccination[16].

During the period between 2000 and 2016, we were only able to collect serum samples from December 2016. The results on the seropositive rate from the period between December 2016 and February 2017 suggest that the outbreak frequency during this period might be the result

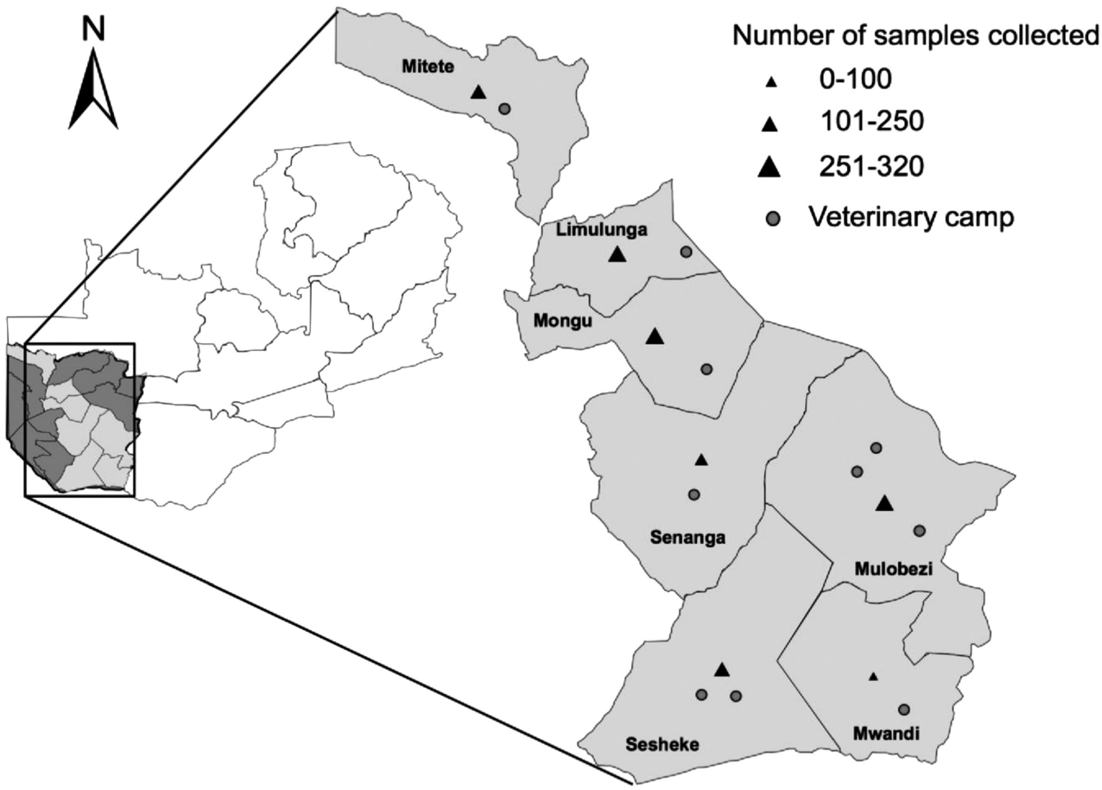

Fig. 4. Study area and number of samples collected in each district. Number of samples collected. Mulobezi, 295; Senanga, 154; Mongu, 320; Sesheke, 230; Mwandi, 79; Limulunga, 306; Mitete, 235.

of insufficient cattle anthrax vaccinations. Combined with the geographic distribution data showing that districts adjacent to the outbreak areas reported cattle anthrax cases in the subsequent years, it is possible that cattle anthrax vaccinations were mainly administered in the affected areas after an outbreak occurrence. Therefore, efforts must be made to strengthen and promote serosurveillance programs that will identify specific areas with poor vaccination practices and will educate livestock farmers on the benefits of anthrax vaccination in the Western Province.

In the Western Province of Zambia, anthrax vaccinations are provided for a part of the population-at-risk following an anthrax outbreak. In areas where vaccination coverage was >80%, there was a reduction in disease prevalence[1]. On the other hand, during the 2016 anthrax outbreak in the Kalabo district, 31.3% (1,846/5,900 animals) of the cattle population-at-risk were vaccinated[12]. These data are consistent with those of previous reports indicating inadequate anthrax vaccination of cattle in Zambia and in anthrax endemic areas elsewhere[6,8].

The transhumance type of farming and inadequate resources render the provision of veterinary services (i.e., herd health management, vaccination, and farmer education) challenging. Until 2012, the Western Province of Zambia was divided into seven districts, which may have had a negative impact on the detection and reporting of cattle anthrax cases. District veterinarians and veterinary assistants had to travel long distances (>100 km) to rural communities and the duration of time between case detection and intervention would vary from a day to a month[1]. Currently, the province comprises 16 administrative districts, increasing the ability to detect and report cattle anthrax cases. Considering these recent changes, the findings of this study may be useful in formulating and

Table 2. Seropositivity of cattle sera from districts sampled

District	Number of samples tested	Number of samples positive	Number of samples positive %	95% CI
Mwandi	79	35	44.3	33.3–55.3
Limulunga	306	76	24.8	19.9–29.6
Mitete	235	45	19.1	14.0–24.1
Senanga	154	24	15.6	10.0–21.5
Mongu	320	81	25.3	20.5–30.0
Sesheke				
Camp 1	75	10	13.3	5.6–20.9
Camp 2	155	42	27.1	20.8–34.9
Sub total	230	52	22.6	17.2–28.0
Mulobezi				
Camp 1	77	2	2.5	0.0–5.9
Camp 2	75	10	13.3	5.6–20.9
Camp 3	143	78	54.5	46.3–62.7
Sub total	295	90	30.5	25.2–35.7
Total	1619	403	24.9	22.7–27.0

implementing strategies for controlling anthrax in Zambia. Future studies focusing on the improvement of detection and reporting systems are warranted to determine high-risk areas and to optimize the distribution of resources.

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