An outbreak of anthrax has been confirmed in Chama district in Zambia affecting close to 80 persons. A previous outbreak in the same area was confirmed in 2011 with 521 humans affected and 6 human and over 80 hippos dead. To understand the disease situation and provide technical support the Ministry of Health in collaboration with the World Health Organization (WHO) in Zambia and Center for Zoonoses control University of Zambia investigated the outbreak in various villages. The index case, a 22-year-old male presented at Pondo rural health centre with eschar invariably accompanied by oedema on the cheek with onset 22nd September 2016. More patients mostly below 20 years of age from five RHICs were seen thereafter with varied lesions papules, vesicles and eschars and treated with ciprofloxacin. Most patients were associated with eating hippo meat. Various interventions were put in place to control the outbreak including case detection, case management, contact tracing and community awareness. Field investigations observed dead carcasses of Hippo and Buffalo. *Bacillus anthracis* was isolated from humans, buffalo, hippo and the environment confirming the outbreak link to Anthrax infection suggesting the need to strengthen surveillance, diagnosis, community sensitization and treatment of affected persons for effective disease control. The rapid response by the Ministry of Health, WHO Zambia, Center for Zoonosis Control, and ZAWA necessitated by the availability of financial resources provided by MOH and WHO Zambia contributed significantly to the timely containment of the outbreak and avoidance of any fatalities.
Introduction
Anthrax is a zoonotic disease with its natural reservoirs being hoofed animals that are known to carry the Bacillus anthracis. Most commonly it is found in grazing herbivores such as cattle, sheep, goats, camels, horses, and pigs [1], but has also been associated with transmission from wildlife to humans by various modes, notably buffalo and hippos in Zambia [2,3]. It is a serious bacterial infection caused by Bacillus anthracis that occurs primarily in animals. Cattle, sheep, horses, mules, and some wild animals are highly susceptible [4]. Humans become infected when the spores of Bacillus anthracis enter the body by contact with animals infected with Bacillus anthracis or from contact with contaminated animal products, insect bites, ingestion, or inhalation [5].

The developed world has no or lower incidence of anthrax infection, meanwhile it continues to be a problem of public health concern in the developing world and countries that do not have veterinary public health programs that routinely vaccinate animals against anthrax [6]. Continued outbreaks in areas with previous outbreaks may occur because anthrax spores survive for decades, even under adverse conditions, contaminating the soil [7]. Anthrax infections in humans occur in three forms including cutaneous, gastrointestinal and pulmonary depending on route of exposure, with up to 95 % being cutaneous [8, 9].

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Number of samples</th>
<th>Number of positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound swabs (Human)</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Hippopotamus</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Buffalo</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Lagoon water</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Soil from pasture/</td>
<td>35</td>
<td>3</td>
</tr>
</tbody>
</table>

Cutaneous infections initially appear as painless, itchy papules on the face, neck, forearms or hands and ulcerate within 7 to 10 days. These subsequently form a black painless eschar. The patient may also incur localised swelling, usually of the face and neck, with painful swollen lymph nodes and systematic symptoms. Literature reveals no human to human spread, no racial, sexual or age association with the disease, although commonly among young and middle ages as it is often related to industrial exposures, way of life and livestock farming [6]. A well managed outbreak with appropriate therapy will reduce and even exclude fatalities.
Zambia has had outbreaks of anthrax in human and animal populations with previous studies showing that clostridia infections and anthrax outbreaks are higher in the Western Province and Luangwa valley than the rest of the country [10-12]. Confirmed outbreaks affecting cattle in Western province from 1989-1995, in which 1,626 suspected cases of anthrax were identified, 51 cases were confirmed with *Bacillus anthracis* infection. During the 1990 outbreak alone, 220 cases of human anthrax cases and 248 human cases during 1991-1998 were confirmed with 19.1% and 7.7% case fatality rates, respectively [13].

Recurrence of anthrax outbreaks have been linked to various ecological factors such as cycles of heavy rainfall followed by periods of dry weather, high evaporation potential of flood water, the presence of calcareous soils and ambient temperatures above 15.5 degrees [14-16]. The Anthrax spores are very resistant, remaining dormant and viable in nature for >100 years [2]. Chama district recorded an outbreak of anthrax in August 2011 that saw over 80 hippopotamuses die after showing signs of infection with *Bacillus anthracis*, followed by 521 suspected human cases resulting in six human community deaths associated with *Bacillus anthracis* infection and about 80 hippopotamus deaths [3, 17]. Five years later, Muchinga Provincial Medical Office received a report of a suspected anthrax outbreak on 27th September 2016 in the southern part of Chama district, recording up-to 79 human cases and 25 animals including hippos and buffalos by end of October 2016. A quick response team consisting of surveillance officers, epidemiologists, clinicians, laboratory scientists, veterinary staff and health promotion staff from the Ministry of Health, the World Health Organization in Zambia, Center for Zoonoses control University of Zambia, the Ministry of Fisheries and Livestock (MFL) as well as National Parks and Wildlife (NPW) investigated and provided technical support in managing the outbreak.

**Methods**

A descriptive cross sectional study of a recent outbreak in Chama was undertaken. The outbreak response team reviewed case records at the local facilities, undertook field visitations to affected areas and conducted interviews with health providers, NPW staff at the camps, village scouts, individual clients, Community Based Volunteers (CBVs),
community leaders, school teachers/pupils and community members and visited the risk areas, that is, Baghdad lagoon on the Luangwa river (GPS coordinates S 1161221, E03268300 and elevation 617), NPW camps and the affected villages in the RHCs catchment areas.

Local health officials in the affected area collected specimens from affected patients which, included swabs from vesicular lesions and blood. The specimens were collected by swabbing of cutaneous lesions from suspected patients. A total of 12 swabs and blood specimens were submitted for anthrax detection and confirmation.

In case of animals, sections of tissues and bones from carcasses were obtained from hippo and buffalo, while soil samples from hippo and buffalo grave sites and soil on land where hippos graze from were obtained. Water samples from the lagoon where hippos were struggling to survive were also collected. The collected samples were carefully transported to the laboratory for analysis.

Cuttings of flesh from the tongue, buccal mucosa and neck area of a Hippo and buffalo, as well as soil samples from hippo grave sites, soil on the land where the hippos died and water samples from Baghdad lagoon were tested for anthrax.

Study area
Chama district is one of the 7 districts in Muchinga Province in the north-eastern part of Zambia. It is a Game Management Area (GMA) and it experiences a lot of human-animal conflicts. Most agricultural activities are undertaken on the plateau while in the valley they heavily depend on wildlife. Chama South, the area affected by the outbreak is predominantly a game reserve with some game camps and lodges for professional hunters and tourists. Cases were recorded in Chigoma, Chikwa, Lundu, Kapichilasenga and Pondo Rural Health Centres (RHCs).

Case definition of Anthrax A suspected case was defined as person with acute onset characterized by several clinical forms that are:

Cutaneous form-any person with skin lesion evolving over 1 to 6 days from a papular through a vesicular stage to a black eschar invariably accompanied by oedema that may be mild to extensive;
Gastro intestinal-any person with abdominal distress characterised by nausea, vomiting, anorexia and followed by fever;
Pulmonary (inhalation anthrax)-any person with a brief prodrome resembling acute viral respiratory illness, followed by rapid onset of hypoxia, dyspnoea and high temperature with x ray evidence of meditational widening.

A confirmed case of anthrax in human can be defined as clinically compatible case of cutaneous, inhalational or gastrointestinal illness that is laboratory confirmed by isolation of Bacillus anthracis from an affected tissue or site [18].

Public Health response

The response to the outbreak included case detection, case management, contact tracing, community sensitizations and stakeholders’ involvement. All cases meeting the case definition were referred to the rural health centres for management as outpatients except for one who was treated as an inpatient for four days because of his presentation with abdominal pains, difficulties in breathing, extensive swelling of the face and head. The patient was discharged in a stable condition. A line list of cases was maintained and updated accordingly. Active cases were treated with oral ciprofloxacin of varied dosages according to age twice daily for 7 days: below 4 years 125mg; 4 to <15 years 250mg ; ≥ 15 years 500mg. Contacts traced were given a prophylactic stat dosage according to age as above.

Communication on the outbreak was strengthened at all levels and regular briefing reports were made at different levels and appropriate times. The Minister of Health issued a press statement about the outbreak that contributed to raising public awareness of the outbreak and response interventions that were in place. Community sensitization was conducted through the community radio, Zambia News and Information Services public address system, community and school meetings, door to door visitations by community health workers and meetings with different stakeholders and community leaders. Standard messages were developed on the types of anthrax, causes, signs and symptoms, risk factors, prevention, treatment and the importance of early care seeking and were disseminated widely in the community. Another key message given to the community was about the importance of reporting deaths of domestic and wild
animals and to avoid getting into contact or eating such animals.

Decontamination using lime was conducted on animal grave sites both on the river banks and other grave site on the land and the bush. All the dead buffaloes and hippos remains were burned and buried on their grave sites. A Mobile Bio-safety level 3 laboratory for quick confirmation of cases both in human and animal populations was put in place.

**Laboratory investigations**

A biosafety level 3 laboratory was deployed on site for analysis of samples from both human and animal cases. The samples were analysed for *B. anthracis* according to the World Health Organization (WHO) guidelines and involved culture, isolation and confirmation of the isolates by polymerase chain reaction (WHO, 2008).

The swabs and blood from suspected patients were directly inoculated on Blood agar (Himedia Laboratories Ltd., Mumbai, India) containing 5% sheep blood. The samples from animals and soil were decontaminated by subjecting them to heating in normal saline at 75°C for 5 minutes. The fresh samples of tissues were inoculated directly on Blood agar.

The confirmed isolates of *Bacillus anthracis* were then subjected to antimicrobial susceptibility tests to define the profile of antimicrobial sensitivity, using standard antimicrobial discs on Mueller-Hinton agar (Difco; Becton, Dickinson and Co, Franklin Lakes, NJ, USA) followed by an E-test to determine the minimum inhibitory concentration (CLSI, 2008). The antibiotics tested with the disc diffusion method were penicillin, chloramphenicol, cotrimoxazole, erythromycin, doxycycline, tetracycline,

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**Table 2 Anthrax cases by age groups, health facility and exposure**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48 (61)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (39)</td>
</tr>
<tr>
<td><strong>Age group (years):</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>14 (18)</td>
</tr>
<tr>
<td>5 – 14</td>
<td>22 (28)</td>
</tr>
<tr>
<td>15 – 24</td>
<td>16 (20)</td>
</tr>
<tr>
<td>25 – 34</td>
<td>10 (13)</td>
</tr>
<tr>
<td>35 – 44</td>
<td>9 (11)</td>
</tr>
<tr>
<td>45+</td>
<td>8 (10)</td>
</tr>
<tr>
<td><strong>Health Facility</strong></td>
<td></td>
</tr>
<tr>
<td>Chikwa</td>
<td>41 (52)</td>
</tr>
<tr>
<td>Pondo</td>
<td>24 (30)</td>
</tr>
<tr>
<td>Chigoma</td>
<td>11 (14)</td>
</tr>
<tr>
<td>Kapichilasenga</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Lundu</td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>Exposure Type</strong></td>
<td></td>
</tr>
<tr>
<td>Patient ate Hippo meat</td>
<td>76 (96.1)</td>
</tr>
<tr>
<td>Patient ate fish from Baghdad Lagoon</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Patient in contact with raw hippo meat</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Patient ate Buffalo Meat</td>
<td>1 (1.3)</td>
</tr>
</tbody>
</table>
streptomycin and gentamicin (Himedia Laboratories Ltd., Mumbai, India). Others were ciprofloxacin, amoxicillin, ampicillin and vancomycin (Oxoid Ltd., Basingstoke, UK). The MIC was determined for chloramphenicol, doxycycline, tetracycline, cotrimoxazole (Oxoid), ciprofloxacin, penicillin and erythromycin (Himedia Laboratories Ltd., Mumbai, India).

Results
In this outbreak, 79 human cases and 25 carcasses (18 hippos and 7 buffalos) were identified. Of these human cases and animals, 12 human specimens and all animal samples were tested for *Bacillus anthracis*. Furthermore, lagoon water samples, soil samples from animal grave sites and land/pasture where animals graze from were also tested for *Bacillus anthracis*.

The tested hippo samples were negative while buffalo samples (2 buffalos) were positive for *Bacillus anthracis*. The water samples were negative while some soil samples from the hippo and buffalo grave site and pasture were positive (Table 1). Antimicrobial sensitivity patterns on the cultured 6 *B. anthracis* isolates indicated sensitivity to a range of drugs used. Intermediate sensitivity was observed with cotrimoxazole and erythromycin, while resistance with vancomycin was noted. The minimum inhibitory concentration of antimicrobial agents was observed at various points: chloramphenicol (8 µg/mL); doxycycline (0.5 µg/mL); tetracycline (1 µg/mL); ciprofloxacin (0.5 µg/mL); cotrimoxazole (16 µg/mL); penicillin (0.12 µg/mL); and erythromycin (4 µg/mL). Of the human cases of clinically confirmed anthrax identified the majority 41 (52%) were from...
Chikwa RHC followed by 24 (30%) from Pondo RHC. Of the total anthrax cases 48 (61%) were males. Overall, most cases (28%) were from the 5 – 14 age group (p<0.01). These results are shown in Table 2. Analysis of age group in each sex group revealed that amongst the males, most of the cases were aged between the 15-24 age-group (33%) followed by the 5 -14 (29%) whereas for females, it was highest among the less than 5 years and 5 – 14 age-group at 26% in both groups followed by those above 45 years (23%), as shown in Figure 1. Analysis of differences between sexes in age groups showed a significant difference (p<0.001) between the sexes only in the 15 – 24 years age group. There was no significant difference (p=0.525) between children (<15 years) and adults (≥15years).

Clinical investigations noted the following signs and symptoms: eschar, rash like lesions among others. One patient had an allergic reaction features (figure 2 & 3). All cases except two were associated with consumption of dead hippo and/or buffalo meat. Of the two who had not eaten, one had eaten fish from the Baghdad lagoon where anthrax was confirmed and the other participated in butchering the carcasses.

The outbreak was contained within one month of onset between 19th September and 20th October 2016 (Figure 4). There were no fatalities.

Figure 3 Epi-curve on anthrax outbreak in Chama, 2016
Discussion

An outbreak of cutaneous anthrax associated with *Bacillus anthracis* occurred in Chama district affecting almost a total of 80 children and adults with no significant difference between age groups and about 25 animals. In this case another animal, the buffalo has come into the transmission pattern of anthrax. There were no positive results from hippopotamus. This could have been due to the decomposed carcasses. *Bacillus anthracis* is easily overrun by anaerobic bacteria upon death. This outbreak was less than that in the 2011 outbreak in the same district which affected about 520 humans with 6 deaths and 80 hippopotamus. Although there was no significant difference between age groups, it was noted on further analysis that in the 15 - 25 age group there was a significant difference between males and females, with males more likely to be infected than females. This could be attributed to the fact that the males of this age group were the ones handling the carcasses to dismember them for meat.

All human cases identified were associated with eating either one or both of hippo and buffalo meat obtained from carcasses in the outbreak area. Literature documents an association of anthrax infection with eating meat from infected carcasses or drinking contaminated water, through the skin by contact with infected material or by insect bites, and through the lungs by inhaling spores has been documented [19]. Some parts of Zambia are endemic of anthrax as evidenced by continued outbreaks impacting negatively on the economy of the livestock industry and public health generally. Social and economic determinants include poor food security resulting from draught in Chama area have been noted as contributing factors in these outbreaks. Despite knowing the consequences of eating infected meat, people prefer to get the disease than die from starvation. Similar determinants are described in the outbreaks in Western province [10]. This gap between knowledge and behavior became a threat to the communication effort that was mounted and required more interactive communication methods within the community to emphasise the dangers of anthrax and the importance of prevention and to promote community ownership and local solutions.

The Anthrax outbreak in Chama was fueled by human behaviour, particularly that of handling dead animals. These risky behaviours have been well documented in similar outbreaks of anthrax where the need to educate communities was emphasized [20, 21, 22]. Although the people in Chama were
aware about anthrax from previous outbreaks, it was very critical to sensitise the community and reinforce the knowledge on the causes, risk factors, signs and symptoms and to promote early care seeking. Early reporting of deaths of animals and wildlife by community members can also prevent the spread of anthrax. Equally, effective communication during an outbreak is important particularly using credible sources and ensuring the use of channels which can reach all the target audiences. The practice of communicating at different levels on a regular basis helped to build and to maintain trust of the community in line with WHO recommendations [24].

Another anthrax outbreak occurred in the same area in 2011. Chama district is located within a wildlife sanctuary, where normal anthrax intervention strategies cannot be applied. It has been documented that while livestock anthrax is generally on the decline in many parts of the world, it remains enzootic in many national parks, for example, in southern Africa and North America. This scenario represents a persistent risk for surrounding livestock and public health [19]. The timely containment and lack of any case fatalities may be attributed to the rapid response by the Ministry of Health with the support of the WHO country office in Zambia that provided financial resources (USD$7,176) and technical support (NPO/National Surveillance Officer and NPO/Health Promotion), National Parks and Wildlife (NPW) and the University of Zambia (UNZA) School of Veterinary Medicine Laboratory and Centre for Veterinary Research Institute (CVRI) who provided laboratory confirmation. A paper by Siamudaala et al. (2006) on the ecology and epidemiology of anthrax in some parts of Zambia indicated that challenges of anthrax control are complex and comprise of socio-political, economic, environmental and cultural factors. They also site inadequate funding, lack of innovative disease control strategies and lack of cooperation from stakeholders as the major constraints to the control of the disease in Zambia [10].

Having confirmed a clear picture of anthrax, the response included as part of the disease containment and prevention measures the following activities: confiscation and destruction of hippo meat and carcasses; active surveillance and contact training; treatment of cases and prophylaxis management of contacts; and community sensitisation including discouraging the community from eating dead animals. As a preventive measure for another outbreak in
the same area, formalin and lime were applied to the soils where animas died from (animal graves) and where animals were being cut and shared by the people.

In order to ensure prevention and control for anthrax, enhanced surveillance which would include mechanisms for disease detection, confirmation of diagnosis, reporting, collation of data and feedback of the data to the source, must be employed [20].

Conflict of interest Statement
We declare that we have no conflict of interest.

Acknowledgements
The authors wish to thank the patients and care givers for being cooperative. The authors also acknowledge the efforts by the health staff (Health centre, district level, provincial level) and the Provincial Medical Officer put into the investigation and control of the outbreak

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