Anthrax outbreak among Grevy's zebra (*Equus grevyi*) in Samburu, Kenya

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Abstract

An anthrax outbreak occurred in the Wamba area of southern Samburu, Kenya, between December 2005 and March 2006. The outbreak affected equids including the endangered Grevy's zebras (*Equus grevyi*), plain zebras (*Equis Burchelli*) and donkeys (*Equus asinus*). Most of the deaths were localized in Nkaroni area just west of Wamba town. The diagnosis of anthrax was rapidly confirmed by bacteriological methods. The relevant government departments, including the Kenya Wildlife Service and Veterinary Department, and other stakeholders were promptly informed. Fifty-three Grevy's zebra and 26 plains zebras died from anthrax. An equal number (eighteen) of adult male and female Grevy's zebras succumbed to the disease. The outbreak affected immature and mature individuals equally. The dead plain zebras included fifteen adult females, two adult males and nine immature individuals. The Veterinary Department responded by vaccinating livestock while Kenya Wildlife Service vaccinated 620 Grevy's zebras within southern Samburu. Examination of sites at which carcasses of animals which succumbed to the disease were burnt, revealed that unsupervised burning did not eliminate anthrax spores in 42% of the cases (n = 14). There is an urgent need to incorporate strategic wildlife disease monitoring in the struggle to save Grevy's zebras and other endangered species.

Key words: anthrax outbreak, *Equus grevyi*, vaccination control

Résumé

Une épidémie d'anthrax a touché la région de Wamba, dans le sud du pays samburu, au Kenya, entre décembre 2005 et mars 2006. La maladie a touché les équidés, y compris les zèbres de Grévy (*Equus grevyi*) qui sont en danger, les zèbres de Burchell (*Equus burchelli*) et les ânes (*Equus asinus*). La plupart des morts ont été rapportées du côté de Nkaroni, juste à l'ouest de la ville de Wamba. Le diagnostic fut rapidement confirmé par des analyses bactériologiques. Les départements gouvernementaux concernés, y compris le *Kenya Wildlife Service* et le Service vétérinaire, et les autres partenaires ont vite été informés. Cinquante-trois (53) zèbres de Grévy et 26 zèbres de Burchell sont morts de cette maladie. Le même nombre de mâles et de femelles adultes (18) sont morts chez les zèbres de Grévy. L'épidémie a touché de la même façon les jeunes et les adultes. Chez les zèbres de Burchell, on compte 15 femelles adultes, deux mâles adultes et neuf jeunes. Le Service vétérinaire a réagi en vaccinant le bétail tandis que le *Kenya Wildlife Service* vaccinait 620 zèbres de Grévy dans le sud-Samburu. L'examen des sites où l'on avait brûlé les carcasses des animaux infectés a montré que l'incinération non contrôlée n'avait pas éliminé les spores d'anthrax dans 42% des cas (n = 14). Il faut d'urgence intégrer un monitoring stratégique des maladies de la faune sauvage dans la lutte pour sauver le zèbre de Grévy et les autres espèces menacées.

Introduction

During the past century the endangered Grevy's zebras (*Equus grevyi*) have experienced rapid decline in numbers and range (Williams, 2002; Nelson & Williams, 2003). This equid, whose historical range extended from Danakil desert in Djibouti and Eritrea to central Ethiopia and southwards to Northern Kenya up to Somalia, is currently restricted to northern Kenya and a few areas in southern Ethiopia. In 1977, their Kenyan population was estimated at about 13,700 (Williams, 2002) whereas the Ethiopian

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Alledeghi plains and Chew Bhir area were home to about 600 and 1500 individuals respectively. Grevy's zebras were also abundant in Yabello sanctuary and surrounding areas in Ethiopia. Their current population is estimated at 1600–2000 in Kenya (Williams & Low, 2004) and 110 in Ethiopia (Williams, Nelson & Kebede, 2003) suggesting about 2100 individuals in the wild. Recent surveys indicate that the Ethiopian population have declined further because of hunting and competition with livestock (A. Bekele, personal communication). This rapid decline in Grevy's zebra populations has been attributed to competition for resources with expanding human livestock populations and the resultant habitat degradation (Williams, 2002; Williams & Low, 2004). Other important factors thought to be limiting Grevy's zebra population include reduced access to water resources. The risk of diseases to Grevy's zebra populations is yet to be documented.

Disease outbreaks can cause sudden and unexpected local declines in abundance of endangered animals (Woodroe & Ginsberg, 1999; Cleaveland *et al.*, 2002). For example, an anthrax outbreak led to local decline among wild dogs in Luangwa valley, Zambia (Turnbull *et al.*, 1991). Laurenson *et al.* (1998) found that canid diseases were a significant threat to the future existence of the endangered Ethiopian wolf. Despite these lessons, the problem of disease outbreaks has been lowly ranked as a threat to Grevy's zebra conservation (Williams & Low, 2004). In their extensive review of disease concerns of wild equids, Radcliffe & Osofsky (2002) only found scanty information on diseases affecting Grevy's zebras and no report of anthrax. In this paper we document a recent anthrax outbreak that occurred in Wamba area of southern Samburu, Kenya. Specifically, we report the number and age/sex classes of Grevy's zebras and plains zebras which died during the outbreak and document prevalence of anthrax spores after carcasses were disposed of by burning.

Study area

The anthrax outbreak occurred in Wamba division, Samburu district of the Rift Valley Province of Kenya (Fig. 1). It is north of the Samburu National Reserve around the foothills of Matthew Ranges at about 37°E and 1°N. This is a hot and dry area with low, erratic rainfall (Pratt & Gwynne, 1971). Mean annual rainfall is 375 mm whereas the mean minimum and maximum temperatures are 24 and 33°C respectively. The area is rich in wildlife species including elephants, Grevy's zebras, plain zebras, reticulated giraffes, gazelles, gerenuks, dik diks, Beisa oryx, bat eared fox, wild dogs, lions, hyenas, cheetahs, pancake tortoises, buffaloes, shrews, rodents, and various birds including the Somali ostrich, vulturine and helmeted guinea fowls. Land is owned communally by Samburu pastoralists. Livestock reared in the area include cattle, goats, sheep, donkeys and camels. This area is very important for Grevy's zebra conservation. For example, from December 2002 to June 2006, a monthly mean of 294 (±37 SE) (range = 863–18) Grevy's zebras were encountered in Wamba area accounting for about 15% of the total wild Grevy's zebra population.

Methods

While conducting routine Grevy's zebra census in early December 2005, eight carcases of Grevy's zebras were encountered in Nkaroni area, Wamba Division of Samburu District, Kenya. In addition, several donkeys had died. The carcasses showed pathological signs that included discharge of dark tarry watery blood, which did not coagulate, from the natural body orifices (anus, vulva, nostrils, mouth, eyes and ears), rapid bloating and incomplete rigor mortis. These signs suggested anthrax (Merchant & Packer, 1983), therefore the carcasses were not opened (Radcliffe & Osofsky, 2002). In collaboration with a team from the Kenya Wildlife Service's (KWS) veterinary department, blood samples were collected from the carcasses of Grevy's zebra and a donkey. Samples were also collected from a sickly Grevy's zebra. In addition, blood smears were fixed with 1% mercuric chloride in the field for fast bacteriological examination in the laboratory. These samples were analysed at the KWS and Institute of Primate Research (IPR) laboratories using World Health Organization (WHO)-recommended Standard Operating Procedures (SOP) for microscopic examination of anthrax (WHO, 2003). In both laboratories, blood smears were stained using polychrome methylene blue stain and observed under a microscope. The results of these tests were passed on to KWS and Veterinary Department for action. Confirmatory tests went on at the IPR laboratories where pure cultures were prepared by culturing *Bacillus anthracis* in nutrient broth, sheep blood agar and PLET (a medium consisting of a heart infusion agar with polymyxin, lysozyme, ethylenediamine tetraacetic acid and thallous acetate) (WHO, 2003). Bacteriological smears were then prepared on sterile microscope slides. The colonies were

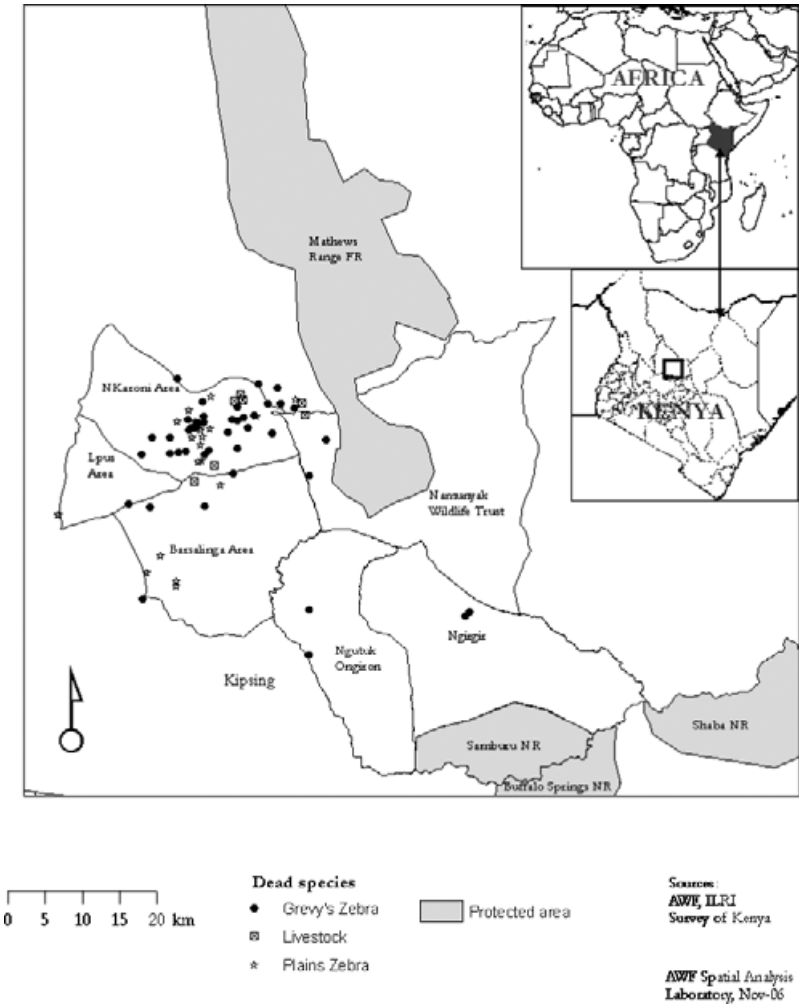

Fig 1 Areas where Grevy's zebras, livestock and plain zebras died from anthrax in southern Samburu (November 2005 to March 2006)

emulsified in normal saline, air dried then fixed with 40 g l^{-1} potassium permanganate for 10–15 min. One smear was stained by Gram stain, another one with methylene blue and then examined for characteristic microscopic appearance of *B. anthracis* (Gram-positive bacilli) or presence of amorphous capsule by methylene blue.

During the anthrax outbreak, we searched for Grevy's zebra, plain zebra or livestock carcasses in the areas known to be utilized by Grevy's zebras in Wamba area. Local community scouts also collected data on location and the age and sex of each carcass of wild and domestic animals showing clinical signs of anthrax. This information was available at Namunyak Wildlife Trust headquarters. We used Global Positioning System (GPS) receivers to locate the reported carcasses in order to confirm the scout's reports. The carcasses were disposed by burning.

In January 2006, ashes from fourteen burnt Grevy's zebra carcasses were collected and put into sterile tubes and double bagged. At each of the burning sites, soil samples within a radius of 3 m from the burnt carcass were collected and put into sterile tubes and double bagged. Water samples were collected from two temporary pans and two more from dry river bed channels. These were the only sources of water in Nkaroni area where most of the anthrax deaths occurred. We followed WHO (2003) SOP during sample collection, transportation, processing, isolation, identification and confirmation of *B. anthracis*. At the laboratory, all samples were processed in a Class II biosafety cabinet (Model 425-400; Ramsey, MN, USA) and all

disposable materials including pipettes, gloves, face masks were decontaminated using 10% sodium hypochlorite and then incinerated. As water samples were muddy they were treated as soil (WHO, 2003).

Data on the population of Grevy's zebras in Wamba area were available from our long-term Grevy's zebra monitoring in the area (Muoria, unpublished data) and were collected as follows. In December 2002, local field guides and herdsmen helped to identify the areas utilized by Grevy's zebras. Based on this information and our own observations, census routes were designed to cover all the potential areas used by Grevy's zebra in each study site. The census routes (transects) were surveyed every month from slow moving vehicle(s). Due to the limited number of roads and tracks in the area, most of the survey work was done by driving off-road. For each group of Grevy's zebras encountered, the following were recorded: their position using GPS, number and group composition, habitat and other large grazers within 400 m of the Grevy's zebras (Muoria *et al.*, 2005). Data obtained were used to (i) estimate the population size, (ii) population structure and (iii) distribution of Grevy's zebras in the area. Age-sex classes recorded were as in Decker & Ginsberg (1990). Each Grevy's zebra counted was photographed using digital cameras. As Grevy's zebras stripe pattern is unique for each individual, the photographs were used to develop a database of identified individuals and as a way of avoiding double counting. Single-factor ANOVA followed by Tukey test (Sokal & Rohlf, 1995) was used to test whether the number of Grevy's zebras sighted during the months of January, February and March of 2003, 2004, 2005 and 2006 differed significantly. Comparing the number of Grevy's zebras in the study areas during those months across years enables us to investigate the impact of the outbreak on the population size and structure.

Results

A total of 53 Grevy zebra carcasses were encountered during the anthrax outbreak in Wamba area from November 2005 to March 2006. This comprised eighteen adult male, eighteen adult female and twelve juvenile and infant carcasses. We could not establish the sex and age categories of five carcasses. Twenty-six plain zebras (fifteen adult females, two adult males and nine immature individuals) and at least five donkeys and two camels also died during the outbreak. Most of the deaths took place in Nkaroni area west of Wamba town and in the neighbouring community areas (Fig. 1). No deaths were reported in the national reserves (Buffalo Springs, Samburu and Shaba). Community scouts monitoring Grevy's zebras in Isiolo District did not report any deaths in areas adjacent to the reserves. However, unconfirmed reports indicate that more Grevy's zebras died in Kipsing area south of Wamba (Fig. 1). *Bacillus anthracis* spores were isolated in six of the ash samples (n = 14) but not in the soil (n = 14) and water samples (n = 4).

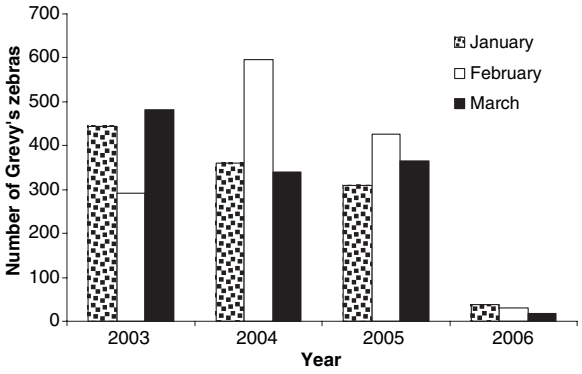

Fig 2 The number of Grevy's zebras sighted in Wamba area during the months of January, February and March 2003, 2004, 2005 and 2006

Figure 2 shows the number of Grevy's zebras sighted in Wamba area from January 2003 to March 2003 compared with the same months in the years 2004, 2005 and 2006. The number of Grevy's zebras sighted in the area differed significantly between years ($F = 12.41$, $P = 0.002$; d.f. $= 11$). The number of individuals sighted in 2003, 2004 and 2005 was statistically the same but that in 2006 was significantly lower than that in other years (2003: $Q = 7.057$, $P < 0.05$; 2004: $Q = 7.542$, $P < 0.05$ and 2005: $Q = 6.322$, $P < 0.05$).

Discussion

At least 53 Grevy's zebras died from anthrax infection during the outbreak. It was possible to miss other mortality cases as animals could have died in thickets, scavenged on death or missed out during the monitoring as the area is vast. Following our 3-year monitoring programme, Grevy's zebra numbers were lower in the region during the outbreak compared with similar periods in 2003, 2004 and 2005 (Fig. 2). This may be explained by severity of drought in the area (Fig. 3), which was widespread throughout the country. Grevy's zebras, and most

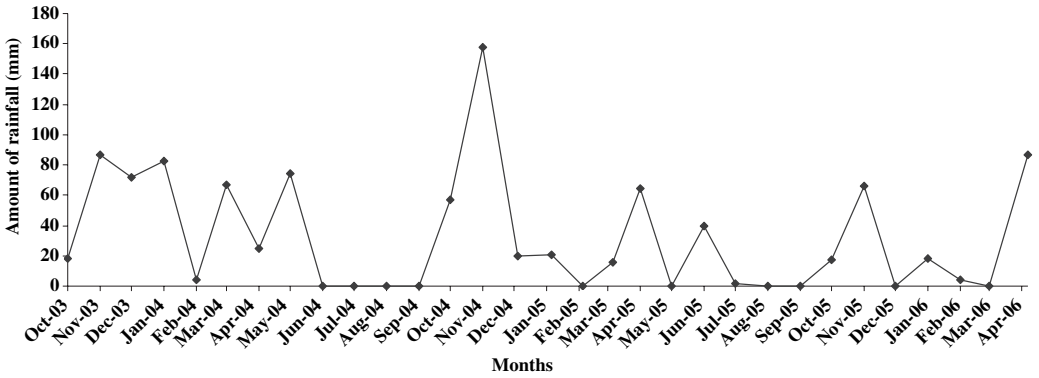

Fig 3 Monthly rainfall total in Nkaroni area from October 2003 to April 2006

livestock, usually move away from Wamba area during the dry months (P.K. Muoria, personal observation). Thus it is not possible to derive the impact of the anthrax outbreak on the Grevy's zebra population from these data.

Here, we give a breakdown of how the disease affected the population structure based on available data. Immature individuals (up to 3 years old) constituted 33% of the dead Grevy's zebras (n = 12). Census data collected in the area from January 2003 to June 2006 (P.K. Muoria, unpublished data) indicate that the immature individuals constitute 32% of the population. Thus the outbreak seems not to have affected Grevy's zebra's population structure as it affected both mature and immature individuals equally. An equal number of male and females died, although females outnumber males in Nkaroni area. This is probably because the outbreak took place during a drought when most Grevy's zebras emigrate from the area leaving some territorial males which continue guarding their territories. It is noteworthy that among adult plains zebras, anthrax killed more female than male.

Anthrax outbreaks in other areas have been attributed to adverse climatic conditions (Dragon & Rennie, 1995; Friedlander, 1997). For example, an outbreak in Texas was attributed to the ingestion of contaminated soil and grass in the drought-stricken area (Young, 1975). Grevy's zebras, plain zebras and donkeys could have become infected by ingesting the resistant spores while grazing on contaminated land (Dragon & Rennie, 1995; Dixon *et al.*, 1999; Radclie & Osofsky, 2002). The total amount of rainfall experienced in the study area in 2004 (total rainfall = 486 mm, monthly mean = 40.5 mm; coefficient of variation = 120, Fig. 3) was far much higher than that of 2005 (total rainfall = 225 mm, monthly mean = 18.8 mm; coefficient of variation = 132). This trend continued into 2006 (Fig. 3). Consequently, the amount of surface water and the quality and quantity of food available to Grevy's zebras during the anthrax outbreak had greatly declined. The dry conditions could have promoted trauma in the oral cavity of grazing equids, thus increasing the chances of acquiring anthrax causing *Bacillus* spores (Friedlander, 1997).

Control of anthrax involves breaking the cycle of infection which can only be achieved through the correct disposal of anthrax carcasses, correct disinfection and decontamination, disposal of contaminated materials and vaccination of exposed susceptible animals (Turner *et al.*, 1999a,b). In the Samburu case, the first deaths were recorded in early December 2005. The cause of the death was immediately diagnosed to be anthrax. The results were relayed to the relevant authorities including the Veterinary Department, Public Health Department and KWS for action. The Veterinary Department responded by vaccinating livestock in Samburu landscape (Manyimbe, Low & Chege, 2006) and imposing quarantine on livestock movement to and from Wamba area. KWS in collaboration with the local conservation organizations and local government authorities vaccinated 620 Grevy's zebras against anthrax in Wamba area, Samburu and Buffalo Springs reserves, and also in Lewa Wildlife Conservancy (Manyimbe *et al.*, 2006). The local community members assisted by stakeholders responded to the anthrax outbreak by burning all the carcasses of animals suspected to have died from the disease. Although vaccination of livestock and Grevy's zebras was carried out, there was no evidence that it was responsible for controlling the outbreak.

One important lesson from the outbreak is how unprepared conservationists are in the event of a disease outbreak in an endangered animal species. Grevy's zebras

had not been vaccinated against anthrax before. This forced KWS veterinarians to conduct trials in order to ascertain that the vaccine would have no adverse effects on the Grevy's zebras (Manyimbe *et al.*, 2006) because such data were lacking. This led to waste of valuable time. Because mass vaccination of threatened hosts is the most logical way to protect them against anthrax (Cleaveland *et al.*, 2002), it would be important to develop safe and effective protocols for vaccinating endangered animals to avoid wasting precious time when trying to contain an outbreak.

This anthrax outbreak also demonstrated the importance of monitoring an endangered species. The fact that we were there when deaths started occurring, confirming the cause immediately. Early detection of the outbreak helped to prevent the spread of this deadly pathogen among wild animals, humans and livestock (the mainstay of the local community). Importantly, we held meetings with local communities to let them know of the outbreak and to stay away from carcasses. There is an urgent need to incorporate disease surveillance to the ongoing Grevy's zebra monitoring activities. A multidisciplinary team of researchers, local communities and other landowners, veterinarians and other wildlife disease experts should be involved in such surveillance and preparedness.

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