

**ANTHRAX VACCINATION TRIAL IN GREVY'S ZEBRA  
AT LEWA WILDLIFE CONSERVANCY**

**STATUS REPORT**

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## EXECUTIVE SUMMARY

Grevy's zebra is an endangered species and a near-endemic to northern Kenya with less than 2,200 animals remaining globally. An outbreak of anthrax in northern Kenya that began in December 2005 appears to be disproportionately affecting equines and in particular Grevy's zebra. To date, 30 Grevy's zebra have been confirmed dead and it is estimated that at least 50% of all cases are reported therefore a relatively accurate guess-estimate of total deaths would be 60.

Although the situation is being closely monitored and livestock vaccination programmes are being undertaken in the affected areas, consensus among wildlife and anthrax epidemiology experts is that a broader vaccination programme is recommended whereby the most important Grevy's zebra populations are vaccinated against the disease.

Although free ranging wildlife has been vaccinated against anthrax with reported success, similar vaccinations in a new and especially endangered species, such as Grevy's zebra, cannot be done without proper precaution: the safety and efficacy of the vaccine to the particular species must be ascertained before it is used on a wide scale. The Kenya Wildlife Service has therefore undertaken a vaccination trial on a sample group of Grevy's zebra at the Lewa Wildlife Conservancy.

Adverse reactions from vaccinations occur within the first 1-4 days after vaccination. Four days into the trial and preliminary results show that no adverse reactions have taken place and only minor reactions were observed at the injection site, which are common with many vaccinations and do not pose a threat to the animal.

This report recommends that if the trial animals continue to show normal behaviour one week after vaccination, then a broader vaccination programme should be undertaken. Based on their importance in a global context, the target populations would be Lewa Wildlife Conservancy, Samburu, Buffalo Springs and Shaba National Reserves, and the Wamba population.

This report also recommends that the trial animals be re-vaccinated six weeks after the initial vaccination in order to determine whether a booster vaccination is required to induce protective levels of antibodies. These results will help to inform the protection strategy in the event of future outbreaks.

## **BACKGROUND**

### **Status of Grevy's zebra**

In recent history, Grevy's zebra (*Equus grevyi*), a near-endemic to Kenya, has undergone one of the most substantial reductions of range of any African mammal. In addition, there have been significant declines in the numbers of Grevy's zebra. Towards the end of the 1970s, the total wild population of Grevy's zebra was estimated to be approximately 15,000 animals; present day estimates are between 1,700 and 2,100 animals (Williams & Low, 2004). This represents an 86 – 89% decline in numbers over the past 25 years. The decline in numbers is continuing over the greater majority of their range, primarily as a result of killing and competition for critical resources.

### **Anthrax in wildlife**

The onset of anthrax epidemics has been related to temperature, drought or flood, and the conditions that trigger outbreaks vary widely between locations. For example, anthrax outbreaks in Etosha and Kalahari National Parks tend to occur after the major rains, whereas outbreaks in the Kruger National Park occur before the major rains (Hugh-Jones & de Vos, 2002). The outbreak in northern Kenya has been attributed to the extreme drought conditions prevailing at this time and it is unlikely that this cycle will break until the onset of the long rains in April (Richard Kock, *pers. comm.*)

Anthrax affects a wide range of mammal species, with certain wildlife species tending to be over- or under-represented in different outbreaks. Browsers are thought to be more susceptible than grazers (de Vos, 1994), however this is not always the case. And even within the same geographic area, different species appear to be disproportionately affected in different epidemics. For example, outbreaks in the central Serengeti predominantly affected impala in 1998 (Mlengya, et al., 1998) and in 2003 but mainly zebra in 2001 (TANAPA, 2001).

### **Status of the current anthrax outbreak in northern Kenya**

To date, 30 Grevy's zebra deaths have been recorded in northern Kenya (Figure 1), the majority of which showed clinical signs of anthrax after death (Figures 2-4). Three blood samples (two from Grevy's zebra and one from livestock) confirmed anthrax (Figure 5). A further four samples have been collected and will be analysed by the Kenya Wildlife Service in conjunction with Dr Paul Capstick, an expert in the diagnosis of anthrax who has also been responsible for manufacturing the vaccine at Coopers Kenya Ltd.

The spatio-temporal distribution of cases (Figure 1) showed that the Wamba area has been most affected by this epidemic. A team consisting of personnel from the Northern Rangelands Trust and Namunyak Wildlife Conservation Trust is operational on the ground and has been monitoring the outbreak closely. This team has responded to all reports of deaths in Grevy's zebra, other wildlife and livestock and follows up each report, recording clinical signs of death, GPS location and age and sex of animal. Blood samples are also taken and the carcass is then burnt using diesel and firewood (Figure 6).

### **Livestock vaccinations**

In response to the outbreak, the Samburu District Veterinary Officer, Dr Thuo, requested financial assistance to launch a livestock vaccination programme in order to prevent the disease from spreading. The Northern Rangelands Trust provided support for the programme and Dr Thuo has vaccinated 5,000 head of livestock around the Wamba area. He is continuing the programme this week and plans to vaccinate a further 7,000 animals. Together with the ground

team, he has burnt all fresh carcasses and is creating awareness among the communities on how to identify anthrax in their livestock and ensure that they do not eat the infected carcass.

### **Protection of the Lewa Grevy's zebra population**

The Grevy's zebra population at Lewa is probably the most important in the global context as it holds over 25% of the world's population and these animals are free from the threats they typically face in pastoral land. It is therefore considered a priority among conservationists to protect the Lewa Grevy's zebra population. As such, the management has closed the wildlife corridor in the northern boundary to prevent potentially infected animals coming in from the north. Lewa has also undertaken a livestock vaccination programme in surrounding communities in order to create a protective buffer around the Conservancy.

### **PROBLEM STATEMENT**

Recent epidemics of anthrax in wildlife have proved that the disease has the potential to wipe out endangered species. Control of the disease in domestic livestock is mainly by hygienic disposal of carcasses, vaccination, and control of animal movements-measures that prove difficult to apply in free ranging wildlife. Although hygienic disposal of carcasses is considered the single most effective way of preventing spread of the disease in domestic livestock, its value in wildlife is sharply limited due to inability to find new deaths in time, before carrion eaters dismember them. Carcass disposal is therefore not only capital intensive but also relatively ineffective in stopping the progression of anthrax epidemics in wildlife. Control of movement of free ranging wildlife is impractical during epidemics and would require fencing, which is not only expensive but is also incompatible with landscape conservation paradigms. Endangered species on the other hand are so sensitive to disease epidemics that any sound, direct intervention should be applied to protect them from extinction. Although free ranging wildlife have been vaccinated against anthrax with reported success, similar vaccination in a new and especially endangered species, such as Grevy's zebra, cannot be done without proper precaution: the safety and efficacy of the vaccine to the particular species must be ascertained before it is used on a wide scale.

### **Objectives of the trial**

In this trial, we sought to determine whether the Blanthrax® vaccine (Welcome Coopers) was safe and efficacious in Grevy's zebras, for controlling a current epidemic in the northern rangelands of Kenya (Wamba area), as well as similar outbreaks in future. We aimed at answering the following questions:

1. Does the vaccine have adverse effects on vaccinated Grevy's zebras?
2. Might the vaccine have adverse effects on unvaccinated Grevy's zebras that come into contact with the vaccinated ones?
3. Will booster vaccination(s) be required in order to induce protective levels of antibodies?

### **Experimental design**

Eight Grevy's zebras were captured from the wild, physically examined, ear-tagged, bled for laboratory tests, and assigned randomly to two experimental groups and one control group. The animals were transported and kept in four spacious, suitably designed holding pens (Figure 7). One of the experimental groups was comprised of four animals that were inoculated with Blanthrax® vaccine into the right rump muscle; the other was comprised of two non-vaccinated animals kept with vaccinated ones in the same pen. The control group was comprised of two

unvaccinated animals kept in a pen separate from the vaccinated experimental animals. The ages and sexes of animals in various groups were as indicated in table 1 below.

**Table 1: Age and sex distribution of Grevy's zebras in various groups of the anthrax vaccination trial in Lewa Wildlife Conservancy**

Animal Id	Group	Age*	Sex
1	Control	7-9 yrs	Female
2	Control	9-11 yrs	Female
3	Vaccinated	3 yrs	Female
4	Vaccinated	3-4 yrs	Female
5	In-contact	5-6 yrs	Female
6	Vaccinated	2.5 yrs	Male
7	Vaccinated	4 yrs	Male
8	In-contact	5-6 yrs	Female

\* Age was determined using *Tooth Development and Age Determination in the Plains Zebra* by Hans and Ute Klingel, 1996, Der Zoologische Garten (NF), Band 33, Heft 1/3

### Materials and methods

Eight Grevy's zebras were chemically immobilized using a combination of 5.5mg Etorphine Hydrochloride (M99®) and 60 mg Xylazine Hydrochloride, delivered using the Daninject® system, from vehicle position. They were physically examined, ear-tagged with identification numbers 1-8, treated for ticks using an acaricide cream and pictures of their teeth taken for aging purpose. Individuals were randomly assigned to various groups using a ruffle to minimise bias. Four of the zebras were vaccinated with 2ml of Blanthrax® vaccine into the right rump muscle (Figure 8). Blood was drawn from the jugular vein (Figure 9) using 20 ml syringe and 19-gauge needle and kept in three plain tubes and two tubes coated with ethylene diamine tetraacetate (EDTA). The blood was transported in a cool box. Blood drawn in the evening in EDTA tubes was stored in the fridge at 4 °C and analysed the following morning, while that drawn in the morning was analysed the same day, for complete blood cell counts. Blood in plain tubes was kept for about 6 hours for the clot to retract and then centrifuged at 15,000 rpm for 10 minutes to separate the serum. The serum was drawn using syringe and needle into cryovials, labelled with an indelible marker pen. It was stored at -20°C to await shipment for antibody analysis. An aliquot of the serum from each animal was analysed for organ function tests within three days of sampling.

Chemically immobilized animals were blindfolded using a wet towel, while in standing narcosis, and physically restrained. They were lifted using a stretcher into a specially modified canter truck (Figure 10). They were revived, after loading into the truck, using 15 mg of Diprenorphine hydrochloride (M5050®) given intravenously. The animals were transported into the holding pens about 30 to 40 minutes drive from the capture sites. They were offloaded at an offloading ramp into the holding pens (Figure 11).

The animals were provided with lucerne, hay and water *ad lib*. A professional animal keeper from Saint Louis Zoo observed them daily, with consistent intensity. The observer was kept unaware of the vaccination status of the animals, in order to control observer bias. Animal behaviour parameters (such as feeding, walking, defecating etc) were recorded for each animal at three-minute intervals, a total of nine hours of observation per day. The right rumps of all animals were examined for injection-site reactions on an hourly basis. Observation was designed to go on for two weeks. After two weeks the animals would be immobilized and bled

for antibody tests. The four vaccinated animals would be fitted with collars, released back to the conservancy and monitored closely for two months. After two months, they would be revaccinated by injection dart and immobilised two weeks later to bleed them again for antibody tests.

## PRELIMINARY RESULTS

### Observations in Grevy's zebra during the anthrax vaccination trial

Observations made on the eight Grevy's zebras for a period 2-4 days revealed no adverse reaction associated with vaccination, in both the vaccinated and in-contact animals. Two of the vaccinated zebras, a male and a female, showed an extremely mild injection site reaction comprising of a spot of blood and missing hair around the injection site. These resolved rapidly, within two days, without causing any swelling, soreness or infection. The reactions are summarized in table 2 below.

**Table 2. Observations of behaviour, systemic reaction and injection site reaction made in eight Grevy's zebras in two experimental and one control group in anthrax vaccination trial, on days 2-4 after parenteral inoculation with Blanthrax® vaccine.**

<b>Animal Identity</b>	<b>Group</b>	<b>Days after challenge</b>	<b>Systemic reaction</b>	<b>Injection site reaction</b>	<b>Behaviour reaction</b>
Zebra 1	Control	0-5	None	None	Normal
Zebra 2	Control	0-5	None	None	Normal
Zebra 3	Vaccinated	0-4	None	None	Normal
Zebra 4	Vaccinated	0-4	None	A spot of blood and missing hair, resolving by day 3	Normal
Zebra 5	In-contact	0-4	None	None	Normal
Zebra 6	Vaccinated	0-2	None	None	Normal
Zebra 7	Vaccinated	0-2	None	Transient spot of blood and missing hair.	Normal
Zebra 8	In-contact	0-2	None	None	Normal

## **Recommendations**

During the process of monitoring the outbreak and gathering information, several experts on anthrax have contributed to the recommendations outlined below. These experts are the following:

Dr Peter Turnbull – author of the WHO Anthrax Manual and a world authority on anthrax

Dr Roy Bengis – wildlife veterinarian at Kruger National Park, South Africa

Dr Richard Kock – wildlife veterinarian at Zoological Society of London

Dr Steve Osofsky – author of the health section on zebras under the IUCN series

Dr Andrew Dobson – studied anthrax in the Serengeti, Princeton University

Dr Patricia Moehlman – Chair of the Equid Specialist Group

The recommendations address the Grevy's zebra populations in the north and on Lewa separately:

- 1. Lewa Wildlife Conservancy**
  - Vaccinate the Grevy's zebra population
  - Vaccinate most susceptible other wildlife species (buffalo and kudu)
  - Continue livestock vaccination programme until buffer area secured
  
- 2. Northern populations**
  - Vaccinate the Grevy's zebra in the national reserves of Samburu, Buffalo Springs and Shaba
  - Vaccinate the Grevy's zebra around Wamba area
  - Continue intensive monitoring until outbreak ends
  
- 3. Continue Grevy's zebra vaccination trial to inform future outbreaks**
  - Re-vaccinate trial animals in six weeks via injection dart (no immobilisation)
  - Measure anti-body response two weeks later

APPENDIX I: FIGURES 1-11

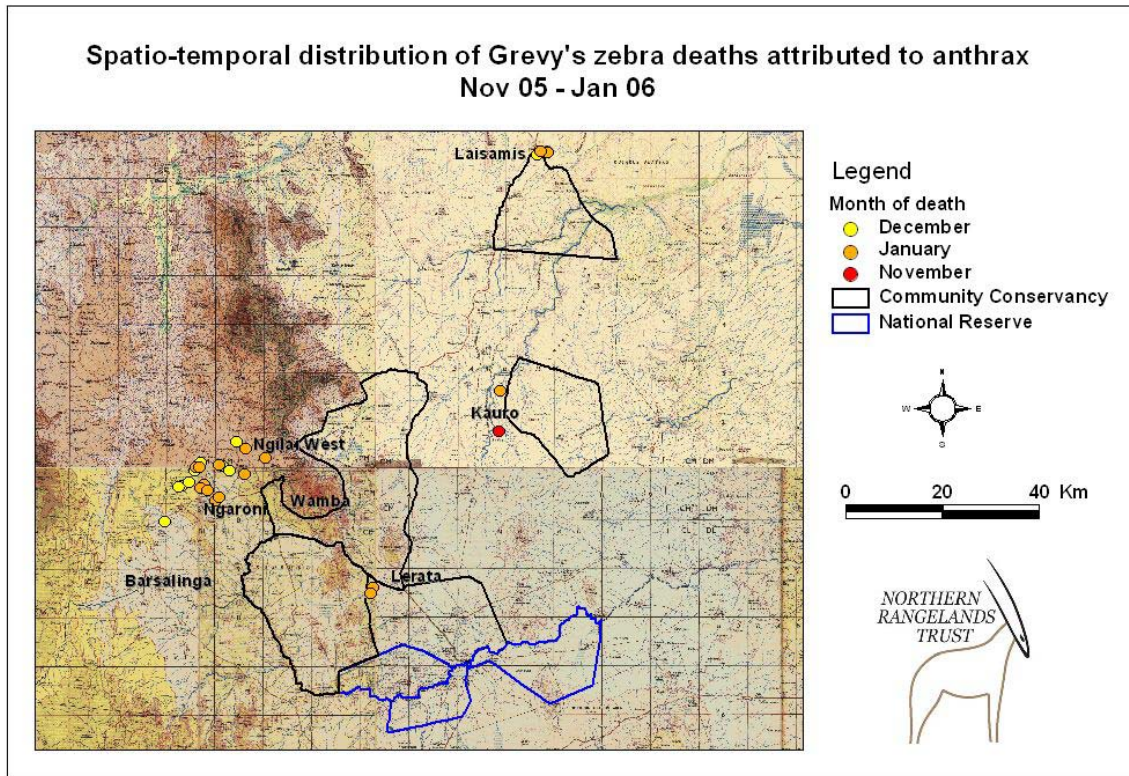


Figure 1: Distribution of Grevy's zebra deaths



Figure 2-4: Grevy's zebra carcasses (2 and 4 clearly show the clinical signs of anthrax)







**Figure 5: Results of a blood sample taken from a Grevy's zebra foal showing *Bacillus anthracis***



**Figure 6: Burning of carcasses**



**Figure 7: Holding pens**



**Figure 8: Delivering the Blanthrax® vaccine**



**Figure 9: Drawing blood**



**Figure 10: Loading the Grevy's zebra onto the canter**



**Figure 11: Off-loading the Grevy's zebra into the holding pens**