Anthrax in Wildlife in the Luangwa Valley, Zambia

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ing strength of the bones of the birds between the two percheries. These observations suggest that there may be subtle differences between the design of the two percheries which allow the Gleadthorpe perchery birds to experience more accidents during flying and on landing. In this respect the differences between the overall heights of the perchery frames and in the distances between the perches and landing stages alongside the nest boxes may be important. The shorter the flight distance the less likely there is to be an accident on landing.

The experiments produced two other unexpected findings. First, in spite of their longer period of confined activity, end of lay battery hens (68 weeks old) which had been cage-reared as pullets for the first 18 weeks had stronger wing bones than battery hens which had been reared on deep litter for the first 18 weeks. In particular, the strength of the humerus at end of lay was 24 per cent greater in the cage-reared birds, and this difference was sufficient to cause a substantial difference in the extent to which the humerus was broken during depopulation. In one of the flocks the tibia was also significantly stronger in the cage-reared birds.

The second unexpected finding was that during moulting bone strength declined slightly but then improved during the second laying period; in the case of the tibia there was a 16 per cent increase in strength during this period.

The rearing of pullets on deep litter and the housing of hens in a perchery system are often thought to have advantages in terms of the welfare of laying hens. In contrast, forced moulting is sometimes considered to impose a stress on the birds. The results of these experiments suggest, first, that the presumed welfare advantages of the first two husbandry practices are offset to some extent by their effects on bone strength and the incidence of broken bones either during or at the end of lay and, secondly, that the effects of forced moulting are not wholly negative. It is clear that comprehensive information is essential when assessing the overall welfare benefits of particular methods of keeping livestock.

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Anthrax in wildlife in the Luangwa Valley, Zambia

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An abnormally high mortality among hippos *(Hippopotamus amphibius)* in the Luangwa River valley between June and November 1987 and estimated to number more than 4000 deaths was attributed to anthrax. Several other species, particularly Cape buffalo (*Syncerus caffer*) and elephant (*Loxodonta africana*), appear to have been affected. A smaller outbreak of anthrax in hippos occurred between August and September 1988, approximately 100 km up-river. A field study was arranged in August 1989 to assess the extent of environmental contamination by *Bacillus anthracis* and the risks to people in the area, to study possible methods of control and to equip local laboratory staff for continued monitoring of the disease. The study confirmed the enzootic status of the region. The characteristics of the outbreaks of anthrax in 1987 and 1988, and the results of the field study are described.

ANTHRAX, once one of the major scourges of man and his livestock throughout the world, has been so well controlled over the past half-century by the vaccination of livestock and improvements in animal husbandry, factory hygiene and public health measures that it has become an almost forgotten disease in the western world. However, in African wildlife, which cannot easily be vaccinated and in which the other aspects of control are not relevant, the disease remains a major cause of uncontrolled mortality in herbivores (Turnbull 1990).

The North and South Luangwa National Parks in Zambia are situated along the Luangwa River and comprise areas of 4636 and 9050 km^2, respectively, 700 to 800 km north east of Lusaka. They have their origins in a game reserve proclaimed in 1904, predominantly to protect giraffe. They were gazetted under their existing names as game reserves in 1938 and made into national parks in 1972.

The possibility that anthrax was a cause of mortality among the wildlife in the Luangwa Valley national parks does not seem to have been acknowledged before 1987, when a major epizootic in hippos was attributed to anthrax. Records from pre-independence (1964) days of cases of anthrax in the wildlife of that area would probably be difficult to obtain but discussions with local residents have provided strong anecdotal evidence that it had been recognised in those days.

This paper summarises the events which occurred during the 1987 epizootic, the situation in 1988 and the results of a field study and related work in 1989 to 1990.

The 1987 outbreak

Abnormal mortality in *Hippopotamus amphibius* began in mid-June 1987. A census of the hippo population in a selected 23 km section of the river, begun at the end of June, indicated that the epizootic reached its peak in early August and abated after the onset of the November rains. A census of the hippo populations in eight sections (14 to 31 km lengths) of the river totalling 167 km in June, July and November and a comparison of these with 1986 census figures indicated an average population loss of 21 per cent (5·7 to 55·5 per cent), or approximately 1420 hippos. Extrapolating this rate of loss over the total pop-

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ulation of 18,000 to 20,000 hippos living along the approximately 300 km of affected river, together with its innumerable lagoons and tributaries, suggested a total potential loss of more than 4000 hippos.

The first attempts to confirm that the epizootic was due to anthrax were made between July 20 and July 30, 1987. The results of an examination of blood smears from hippo 9, a young mature female, were interpreted as confirming the diagnosis. A post mortem examination on July 20 revealed a possible frothy nasal discharge and, internally, cardiac necrosis, a mildly enlarged spleen, and dark and non-clotting blood. On July 23 hippo 10, which had been found in respiratory distress and shot, was examined. A nasal discharge, dark non-clotting blood, an enlarged spleen and an enlarged haemorrhagic mesenteric lymph node were recorded. Tissue fluid taken from the ear of hippo 11 on July 30 was cultured on blood agar within 48 hours of collection.

In the Regional Veterinary Diagnostic Laboratory, Chipata large Gram-positive encapsulated bacilli were found in blood smears from hippos 9 and 10; in hippo 10 they were in chains. Bacteriological cultures of specimens from hippos 9, 10 and 11 yielded Gram-positive, sporulating, encapsulating, non-motile bacilli with a colonial morphology like that of *Bacillus anthracis*. The isolates were, however, reported to be penicillin-resistant.

A mouse injected with 0·3 ml of heart blood from hippo 10 did not develop anthrax. A second mouse injected with tissue fluid (0·5 ml) from the ear of hippo 11 died after 16 hours. Dark non-clotting blood and an enlarged spleen was reported in this mouse but no bacteriological cultures or blood smears were examined. It was concluded that the hippos had died of anthrax and this diagnosis was supported by tests carried out by Professor K. Shimuzu, at that time at the School of Veterinary Medicine, Lusaka.

By September, although *H amphibius* was the species continuing to exhibit the greatest abnormal mortality, it had become clear that other species were also probably succumbing to anthrax. In the small (30 to 50 km^2) areas of Mfuwe and Nsefu near the Chinzombo Research Station at the edge of the South Luangwa National Park, the following numbers of carcases were recorded in September to November 1987: 101 hippopotamus (*H amphibius*), 60 Cape buffalo (*Syncerus caffer*), 20 elephant (*Loxodonta africana*), 12 puku (*Kobus bardoni*), five kudu (*Tragelaphus strepsiceros*), five wild dog (*Lyceon pictus*), two baboon (*Papio ursinus*), two giraffe (*Giraffa camelopardalis thornicroftii*), two leopard (*Panthera paridus*) and one each of eland (*Taurotragus oryx*), hyaena (*Crocuta crocuta*), impala (*Aepyceros melampus*), porcupine (*Hystrix africae-australis*), waterbuck (*Kobus ellipsiprymnus*) and zebra (*Equus burchelli*). Blood smears were obtained and recorded by Professor Shimuzu as positive for anthrax from five buffalo, four elephant, four puku, four wild dog, three waterbuck, two hippo, two giraffe and one kudu.

Five 10 ml water samples collected from the river and pools where hippo had died were also sent to Professor Shimuzu. *B anthracis* was isolated directly from water sample 5.

After two- to fivefold concentration of these samples by centrifugation followed by heating at 80°C for 30 minutes, 0·2 to 0·3 ml were injected into mice intraperitoneally (two mice per sample) or subcutaneously (two mice per sample). An isolate from a Cape buffalo that had died of anthrax in August 1987 was also injected into four mice as a control. Of the water sample tests, one of the four mice injected with the heated concentrate of water sample 5 died. *B anthracis* was identified microscopically and by nutrient agar culture in the spleen and liver. All four control mice died, one, one day and the others two days after inoculation.

1988 to 1989 and the involvement of the Centre for Applied Microbiology and Research (CAMR)

May to October constitute the dry season in Zambia; the 1987 epizootic which had begun and remained centred in the Nsefu region died out with the onset of the November rains. No abnormal mortalities became apparent in the Nsefu district during the dry season of 1988 but in August of that year information reached Chinzombo of abnormal mortality in hippo in the Musalangu game management area more than 100 km north of Nsefu.

Information obtained by wildlife research staff sent to investigate the deaths indicated that they had begun in June, in the vicinity of the Lufila/Luangwa confluence, and had progressed up river to Chama. A count of hippo carcases or remains and live animals in three stretches of the Luangwa river in the Chama district showed a dead:live ratio of 28:187 in August and September 1988. This represents a minimum mortality rate because most dead hippos would have died in deep water and, after consumption by crocodiles and other scavengers, their remains would have rapidly disappeared. However, a major outbreak of the type seen in 1987 did not occur.

At the end of August 1988, the senior author was asked to examine some specimens to confirm that anthrax was the cause of the deaths. In the light of some ambivalent results in the mouse tests described, the apparent resistance of the organism to penicillin and the absence of any cases in people who had handled the hippo carcases, together with the economic losses and the dangers to other precious species and to human health, it was considered important to obtain the fullest possible understanding of anthrax in the Luangwa valley.

Accordingly, the appropriate import licence was obtained and the following specimens were received: blood films from two hippos (hippo A 17.10.88 and hippo 10.02.89) and two zebras (zebra C 10.2.89 and zebra 13.2.89), blood papers (strips of filter paper dipped in the blood and dried) from two hippos (hippo A 17.10.88 and hippo 3.12.88) and the two zebras, ear clippings from hippo B 22.10.88 and zebra C 10.2.89 and subcutaneous tissue from the forefoot of hippo B 22.10.88.

Hippo A 17.10.88 was estimated to have died three to four days before the specimens were collected and the remains of hippo B 22.10.88 were estimated to have been more than a month old when the specimens were collected. No details were supplied about the other carcases.

B anthracis was unequivocally isolated from the subcutaneous forefoot tissue of hippo B 22.10.88 and from the ear clipping from zebra C 10.2.89. The blood film from zebra C was also M'Fadyean positive. A single colony of *B anthracis* was found on a culture of the blood paper specimen from hippo 3.12.88. In view of the fact that 48-hour soakings of this paper injected into a mouse failed to induce anthrax, this finding must be regarded as equivocal. However, the failure to isolate *B anthracis* from the blood paper specimen from zebra C 10.2.89 demonstrated that the blood paper method of taking specimens is not a good one.

The blood film from zebra 13.2.89 was also M'Fadyean positive and it was thought that there were capsulated cells on the blood film of hippo A 17.10.88. However, numerous putrefactive organisms made it difficult to read the blood film from hippo A. The blood papers from both these animals were negative and attempts to culture *B anthracis* from the smears also failed.

August 1989 field study and related work

Specimens

The new awareness of anthrax in the Luangwa Valley, and the anxieties engendered, resulted in an investigation at the site designed to confirm the continued existence of the disease in the area, to assess the extent of environmental contamination by *B anthracis* and the risks to local villagers (who eat meat from animals found dead) and tourists, to study methods of control and to equip local laboratory staff so that they could continue to monitor the disease. Accordingly, a laboratory was established at the Chinzombo Research Station on the eastern edge of the South Luangwa National Park and intensive studies

were carried out from August 3 to August 18, 1989.

Only three carcases were seen within 24 to 48 hours of death. One was the only freshly dead hippo carcase observed but it was inaccessible and therefore not examined; the second was a zebra, EB 16.8.89, which had clearly been a kill, presumed by lion, and which was negative for *B anthracis*; the third was an elephant, LA 18.8.89, which was found after the laboratory had been closed and fumigated. This elephant showed the haemorrhagic signs typical of anthrax, but cultures made hastily and transported to the Regional Veterinary Diagnostic Laboratory, at Chipata were negative.

However, information received from patrolling National Parks and Wildlife Service staff, tour operators and researchers about animals that had been seen dying or freshly dead one week to one month before the field study were followed up and specimens were collected from their remains and from the soil or mud where the head and tail were believed to have been. Droppings of vultures and faeces of hyaenas that were likely to have fed on the carcases were also collected.

The remains of the animals usually consisted of the skull and other scattered bones. Pieces of soft tissue left uneaten by scavengers at the base of tusks or horns, and between the eyes, the marrow of bones (particularly if the remains of blood were visible) and the fine bones from the nostrils were taken when possible. These samples and soil samples were collected into sterile bags using, where possible, pre-sterilised tools.

The skulls and occasionally other bones of hippos that had died earlier in the year or in the 1987 outbreak were also examined. In this case the skull and bones were collected in a sheet, transported to Chinzombo Field Station and small sections cut or chipped off, then crushed with a hammer before examination.

In addition, a number of specimens had been collected in advance of the field study by the resident wildlife biologist and kept refrigerated pending the establishment of the laboratory at the Chinzombo Research Station.

Finally, a number of soil, mud and water samples were collected from lagoons where hippos had died in 1987, and from sites at the edges of these lagoons where carcases were recalled as having lain.

In all, 32 specimens from 18 animals and 24 specimens of soil, mud, water, hyaena faeces and vulture droppings were examined for the presence of *B anthracis* (Table 1). Because the supply of media for on-site bacteriology was limited, the specimens of soil, mud, faeces and droppings were mailed for full examination at CAMR, Porton Down, England.

TABLE 1: Results of culture of specimens collected between June 14 and August 11, 1989

Specimen	Number positive/ number tested	Interval after death (days)	Comment
Cape buffalo	1/1	30	Nearby hyaena faeces positive
Elephant	2/4	1 - 6	Terminal blood levels of *B anthracis* seem to vary
Impala	2/3	0	
Kudu	1/1	8	
Wildebeest	1/1	0	
Eland	0/1	8	Only scapula left; blood on underside swabbed. Nearby vulture droppings negative
Hippo	0/5	>100	Specimens from two hippos sent to CAMR in May, 1989 were positive
Warthog	0/1	> 15	Vulture droppings from nearby log were positive
Zebra	0/1	0	A kill
Soil *	1/8	-	Positive from head and tail region of elephant LA1 3.8.89. 180,000 spores/g under head
Mud *	0/5	-	
Water *	0/2	-	
Hyaena faeces	1/5	-	Associated with positive Cape buffalo
Vulture droppings	1/4	-	Associated with negative warthog

* Collected from sites of known or suspected anthrax carcases

Examination of specimens

When possible, blood or tissue fluid smears were prepared, alcohol-fixed and stained with polychrome methylene blue (British Drug Houses) for microscopic examination for the typical capsulated bacilli.

Other specimens were suspended in sterile water (approximately 1:1 w/v) and the suspensions plated with a standard bacteriological loop on blood agar before and after being heated at 62·5°C for 15 minutes. The unheated and heated suspensions (0·25 ml) were also spread on polymyxin-lysozyme-EDTA-thallous acetate (PLET) plates.

The blood agar plates were examined for *B anthracis* after 24 hours at 37°C and the PLET plates after 48 hours.

Suspect colonies were subcultured to test for gamma phage and penicillin sensitivities and those still thought to be *B anthracis* were confirmed by inoculation into horse blood and examination for capsule production after incubation for six to 18 hours at 37°C.

Results of examinations of specimens. — A summary of the results of the search for *B anthracis* in the specimens is given in Table 1.

Experiments on fate of B anthracis *in water*

The cycle of *B anthracis* outside its vertebrate host has long been a topic of debate. The bacterium depends, to by far the greatest extent, on multiplication within the host to maintain its endemic status in an area. There is much theory but few data on whether subsidiary cycles of germination, multiplication and resporulation occur in the environment and help to maintain the population of *B anthracis*.

In an attempt to determine the fate of *B anthracis* in the water around a hippo that has died of anthrax and to simulate the shedding of vegetative forms from the dying animal within the sanguineous oronasal or anal exudates, inocula of vegetative cells were suspended in 2 ml of laked horse blood and added to 50 ml of water from a lagoon where many hippo had died in the 1987 epizootic.

The inocula of vegetative cells were prepared by spreading 0·25 ml of an overnight culture of each of two strains (the Sterne vaccine strain and the on-site impala isolate 2IB/89) on blood agar plates which were incubated at 35°C for four-and-a-half hours, and then washing the growth off with phosphate buffered saline (PBS) at 5°C. The suspensions in PBS were kept refrigerated until they were used. Before the experiment they were diluted and added to laked horse blood to give an estimated *B anthracis* vegetative cell count of 5×10^6/ml. This dilution was intended to give a starting count of 2×10^5/ml in the inoculated water samples (pre-filtered through 0·45 µm low protein-binding Schleicher and Schuell membrane filters) which were then incubated at 35°C. At the times shown in Fig 1, 1·5 ml samples were withdrawn for estimations of the viable cell and spore numbers by the drop count method (International Commission on Microbiological Specifications for Foods 1978) before and after heating at 62·5°C for 15 minutes.

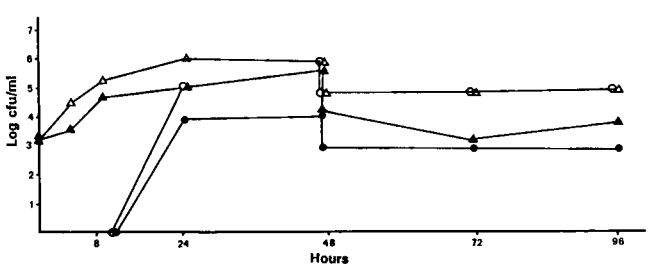

FIG 1: Fate of *Bacillus anthracis* vegetative cells (▲Δ) and spores (●○) in water samples from a lagoon in which numerous hippos had died in the 1987 epizootic. Inocula were added as 8×10^4 Sterne vaccine strain (▲●) or 7×10^4 local strain 21B/89 (Δ○) vegetative cells in 2 ml laked horse blood to 50 ml of water sample

Results of experiments on B anthracis *in water.* — The initial counts in the inoculated water samples were lower than intended, just over 10^3/ml (Fig 1). However, the results demonstrate the initial multiplication that occurred during the first 24 hours, with sporulation being 87 per cent and 77 per cent complete after 24 hours in strain 2IB and the Sterne strain, respectively, and 100 per cent and 74 per cent complete after 48 hours.

To simulate the dilution effect of the water on the *B anthracis* in shed blood, after 48 hours, 0·5 ml samples of the suspensions were added to 9·5 ml of freshly collected lagoon water. Fig 1 shows that the local isolate 21B/89 did not germinate, and the numbers of spores remained steady. There were obviously some vegetative cells in the Sterne strain suspension (the total counts were 0·5 to 1 log higher than the counts in the suspension after heating) reflecting the known reluctance of the Sterne strain to sporulate compared with normal wild-type strains, but again there was no evidence of multiplication.

The pH of the lagoon water was 7·90 before filtration and 7·84 after filtration. In all, 14 water samples were tested; their pHs ranged from 6·81 to 8·33, the highest value being recorded in the water from the Luangwa River.

Serology in human beings

Meat is a commodity that the villagers of the Luangwa River Valley generally cannot afford; as a result dead animals are taken advantage of. There is little doubt that meat from hippos that died in the 1987 outbreak was consumed by many of the villagers, but there were no indications that anyone had contracted anthrax. Nevertheless, to obtain data on the exposure of people to anthrax, blood was collected from 24 volunteers in seven of the villages along the Luangwa River. The sera were examined by the enzyme immunoassay of Turnbull and others (1986, 1988) for antibodies to the *B anthracis* protective antigen.

Results of serology in humans. — Positive results were obtained from 12 of the 24 sera tested; the titres ranged from 1:16 to 1:128 and the samples came from people who were fairly evenly distributed among the seven villages. The positive control was a vaccine with a titre of 1:1024.

Discussion

Diagnosis in the 1987 epizootic

The frothy nasal discharges, enlarged spleens and dark non-clotting blood observed in all the hippos and the 'respiratory distress' of hippo 10 are signs typical of anthrax. The large, Gram-positive, encapsulating square-ended bacilli which were found (sometimes in chains) in the blood and tissue fluid smears were highly suggestive of *B anthracis*. The colonial morphology and absence of motility were further indications that they were correctly identified.

However, the failure of culture positive blood from hippo 10 to kill a mouse, the death 16 hours after injection of the mouse inoculated with tissue fluid from hippo 11 and the report of penicillin resistance all provide evidence against the diagnosis. Anthrax is known to kill mice between 40 and 70 hours after injection; it is possible that the mouse which died at 16 hours did so from a clostridial infection acquired from soil or water contaminants in the ear tissue fluid. A Gram stain is not adequate for identifying the capsule of *B anthracis* and it appears that the results obtained by using a methylene blue stain at Chipata were equivocal. None of the isolates made before, during or after the field study exhibited penicillin resistance.

Isolates from the 1987 examinations were not retained (at least in Chipata) and so could not be re-examined.

It is difficult to explain why only one of the four mice injected with the concentrate of water sample 5 died. However, mice have an LD50 of 50 to 100 spores and low spore numbers could account for this low mortality. Heating at 80°C for 30 minutes is a severe treatment and *B anthracis* spores might be expected not to survive. Furthermore, the mouse which died within a day of inoculation did so earlier than would have been expected if its death had been due to anthrax.

The results of the examinations and tests are strongly suggestive of anthrax but there were anomalies which remain unexplained.

When so many hippos were dying, it is puzzling that there should have been any problem in establishing whether anthrax was the cause of death. Several factors may have been involved. First, access to the dying or dead animals was made difficult by the presence of other hippos that might have attacked a boat, and crocodiles that might have attacked a wader; secondly, the hippo's extremely tough skin makes it difficult to collect good specimens and, thirdly, *B anthracis* is rapidly destroyed in carcases by putrefactive processes which are encouraged by the warm conditions.

1989 field study

Although the 1989 field study took place at the same time of year as when many hippos were dying in 1987 and 1988, there was no outbreak of disease in hippos at the time of the field study. As a result the nine positive isolations of *B anthracis* were significant in confirming the endemic status of the area. Without the specific search and testing for anthrax these cases would have gone unrecognised and it is therefore possible that similar sporadic cases may occur every dry season, if not throughout the year, and it is unlikely that they are confined to the small section of the Luangwa National Park in which the field study took place.

The failure to isolate *B anthracis* from soil, mud and water samples which were not associated with a recent anthrax carcase was not surprising. Experience in the Etosha National Park, whose endemic status is now well defined (Turnbull and others 1989), has shown that the bacterium can only rarely be found in such samples. Of 373 samples examined in that park since 1985, only 27 (7.2 per cent) have yielded *B anthracis* (P. Lindeque, unpublished results). To some extent, this result may be due to the poor sensitivity of the detection systems but it does indicate that the soils, waters and muds of the Luangwa Valley are not 'loaded' with *B anthracis*.

The original position of the head of elephant LA1 3.8.89 (Table 1) was recalled from memory by a scout; it was therefore remarkable that 180,000 *B anthracis* spores/g were found in the soil collected from the site. It is the highest natural count the authors have encountered. The highest count recorded in the Etosha National Park is 25,000/g (P. Lindeque, unpublished results).

The isolation of *B anthracis* from carnivore faeces and vulture droppings has been reported before (Anon 1978, Turnbull and others 1989). The involvement of scavengers and predators in the spread of anthrax among wildlife has long been suspected (Pienaar 1967, Ebedes 1976, de Vos 1990). It is not possible to to definitely associate a positive sample of faeces or droppings with a particular carcase because the time taken by the ingested bacteria to pass through the gastrointestinal tract of the carnivore must be taken into account.

In the Etosha National Park, a geographical association between the regions of highest incidence of anthrax and artificial water holes or gravel pits had led to the belief that these water holes played a major role in the incidence of the disease (Ebedes 1976). However, experiments provided no evidence that *B anthracis* would multiply in water from either type of water hole in the absence of added nutrients; on the contrary, vegetative forms appeared to die off rapidly while the number of spores remained constant (Turnbull and others 1989). Blood was not added in those experiments although 4 per cent brain-heart infusion broth (final concentration) was added, this being the minimum needed to induce the germination of spores. The

4 per cent of blood added in the Luangwa tests bore only an arbitrary relationship to the blood levels around the nostrils or anus of an animal dying in the water, but there is probably considerably more nutrient in 4 per cent blood than 4 per cent brain-heart infusion broth. Further comparisons between the two studies cannot be made, unless the experiments are repeated with water samples from Etosha.

The nature of the bloody exudate from animals dying of anthrax has not, to the authors' knowledge, been analysed. When the blood is shed, the counts of *B anthracis* may already have reached the top of the exponential curve and the available nutrients may already have been used up. Ideally the experiments should be repeated at a range of blood concentrations and with different numbers of *B anthracis*, and it would be useful to know the range of blood concentrations and *B anthracis* counts that occur naturally. However, it may be concluded that in some circumstances, in still water, *B anthracis* may multiply around the site where an animal sheds the bacteria within a bloody exudate.

With the exception of one unexplained positive serum sample among 110 samples taken from blood donors (Kleine-Albers and others 1990), the authors believe that this is the first report of positive serological results in unvaccinated people who have not suffered clinical anthrax. The low titres, as compared with the vaccinated control, were in keeping with the low titres observed in sera from people with the disease (Turnbull and others 1988).

An inhibition enzyme immunoassay was used, which appears to be highly specific for antibodies to the protective antigen component of anthrax toxin. The results therefore suggest that the antibodies to anthrax were acquired naturally by these individuals. It cannot be proved that the antibodies were acquired as a result of consuming hippo meat in 1987; meat from other animals which died of anthrax may have been handled and, or, consumed and the antibodies may have resulted from a subclinical infection derived from this. In this case, the antibodies could have provided a level of protective immunity to these people and might have accounted for the absence of cases in 1987. The authors have observed that once antibody titres have been established in people who have been vaccinated, they persist for many years (Turnbull and others 1986). However, the ready availability in 1987 of hippo meat which is presumed to have been extensively contaminated with *B anthracis*, suggests that a high proportion of the serological positives in the community did develop their antibodies at that time.

After the initial diagnosis of anthrax as the cause of the epizootic in the hippos in 1987, a task force was set up on the instructions of the Director of Veterinary and Tsetse Control Services, Ministry of Agriculture, Zambia. Staff from the Ministry of Health joined this force later. In addition to monitoring deaths, diagnosing the causes of disease and observing any spread of the epizootic in the affected regions of the Luangwa Valley, the task force produced an information sheet entitled 'How to avoid contracting anthrax while in the infected area'. Among the recommendations were: (a) avoid going close to dead animals; (b) hunters are advised not to open up animals found dead; (c) avoid handling game meat, hides and trophies from animals suspected of having died of anthrax; (d) do not carry game meat, hides or trophies from animals which have died from unknown causes; (e) do not eat game meat from animals which have died from unknown causes; (f) avoid drinking unboiled surface water; it must be boiled for at least 30 minutes.

The task force was also responsible for the correct disposal of carcases and 'trophies', primarily hippo teeth and elephant tusks. Where possible, carcases were burnt under a pile of mopane logs dampened beforehand with diesel fuel. However, the 55 carcases successfully disposed of in this manner represented only a small fraction of the number of animals which died. 'Trophies' were collected by staff wearing rubber gloves, overalls and boots and wrapped in bed sheets for transport to the Chinzombo Research Station where the package was left in a chlorine bath for 48 hours. The trucks used for their transport were disinfected with 10 per cent formalin.

Attempts were made to vaccinate elephant and giraffe by dart gun, but they proved ineffective and it was realised that other means of applying the vaccine would be necessary.

After the field study, a recommendation was made that an organisation should be set up to continue to monitor the disease in the valley and that a more comprehensive successor to the information sheet should be drawn up.

The legislation in most countries, including Zambia, which requires the incineration or burial of anthrax carcases was formulated for domestic animals and is generally inappropriate or impractical for wildlife, owing to the lack of available personnel or resources and the fact that many carcases remain unobserved. However, carcases lying near human dwelling places, tourist lodges or tourist routes should be disposed of for aesthetic reasons and to safeguard public health. One measure suggested for decontaminating the environment of the site of an anthrax carcase was to spray a 10 per cent solution of formalin with a garden watering can over the area enclosed by a line drawn approximately 1·5 metres from the carcase. It was recognised that this was only a limited step because it could be done only after scavengers had disposed of the carcase.

The final recommendation was that a contingency plan be established for the emergency vaccination of a core of valuable species in the event of a major outbreak of anthrax and that research should begin as soon as possible on the best method of applying long term prophylactic cover.

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Correction

Otitis media in the dog The correct address of the authors of two papers on otitis media in the dog (*VR*, March 30, p293 and April 6, p319) should have been printed as the School of Veterinary Science, Langford House, Langford, Bristol BS18 7DU. Dr Little's current address is the Department of Veterinary Medicine, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 12H.