Protocol for Sampling Important Wildlife Diseases in the Kavango Zambezi Transfrontier Conservation Area

C. Foggin
2021

SAMPLING PROTOCOL FOR IMPORTANT WILDLIFE DISEASES IN THE KAVANGO-ZAMBEZI TFCA

Summarized, Practical Field Technique, with emphasis on Elephant

INTRODUCTION

This Protocol is intended as a Field Guide and Checklist for Field Veterinarians, and their Assistants, who are faced with wildlife mortality in the KAZA region, and who may be called out, at short notice, to post-mortem (PM) and sample carcasses in remote areas, under difficult circumstances. It is not meant to be a document which deals with all possible scenarios and diseases, or to replace previously published, more comprehensive works on wildlife PM technique.

This Protocol does not deal with targeted, disease-surveillance sampling; only diagnostic, or *ad hoc* surveillance sampling.

Because some of the diseases that may be encountered are potentially fatal zoonoses, adequate precautions are advised when undertaking such PMs, especially where there is no prior background information. However, it is also difficult to use restrictive Personal Protective Equipment (PPE) under field conditions, especially when it is very hot, or there are other field impediments. Perceived risk is weighed against PPE impediment.

A few points to consider:

1. The most important zoonotic conditions in KAZA are Anthrax and Rabies. Rift Valley fever has not been a major problem, though should occur. Wildlife TB is presently uncommon in KAZA, apart from Mongoose TB (possibly not zoonotic)
2. Knowledge of the historical presence of these diseases, or ongoing outbreaks in surrounding areas especially in domestic animals, is important
3. Per-acute diseases such as Anthrax or Pasteurellosis may initially be difficult to differentiate in the field from acute natural, or malicious, poisoning. Where multiple species are dead in close proximity (sometimes including invertebrates), malicious poisoning is likely.
4. Minimal sampling should be undertaken when serious zoonotic disease is suspected:
   - Anthrax
     - Only collect blood smear, blood / superficial tissue – DO NOT OPEN THE CARCASS
   - Rabies (with appropriate PPE)
     - Remove head and take, in leak-proof bag(s), to bio-secure facility where brain can be removed
     - Or open cranium and remove brain in the field with appropriate PPE (by rabies-vaccinated personnel)
     - Or disarticulate the head and dissect out a piece of brain-stem, via the foramen magnum with appropriate PPE (by rabies-vaccinated personnel)
   - TB and Rift Valley fever
     - Full PM can (and probably will) be done, taking much care, and with appropriate PPE, including face mask
5. Rule of thumb… apply as much PPE as can be comfortably accommodated under the prevailing conditions, unless a known, serious zoonotic condition is suspected. **Gloves and overalls are mandatory for all field PMs.**

PRIOR TO DEPLOYMENT (See Check-List: Appendix 1)

PM equipment – not all essential for all post-mortems

- Knives – flaying and de-boning (multiple required for elephant and other mega-herbivores)
- Sharpening steel + files (or coarse oil-stone / grinding stone)
- Axe and / or meat-cleaver
- Saw(s) – rip-saw or bow-saw (or both) – for cutting ribs etc; rib-cutters useful, but not essential
- Pruning shears / Secateurs - for cutting ribs etc in animals up to ± 20 kg
- Meat hooks (multiple, sharpened, and long-handled – looped handles - for rope attachment)
- Scalpel and multiple blades
- Forceps (rat-tooth and smooth, at least 18cm)
- Scissors (curved and straight, at least 22cm)
- Plastic organ containers (eg. buckets or 'baby-baths')
- Cutting board – panels of skin, with subcutis uppermost, can serve the purpose
- Nylon ropes (eg. 3 x 10m, ≥ 10mm diameter)

- PVC canvas – 4 to 8m^2 is preferable
- Knapsack sprayer (battery-operated is preferable)
- 20Ltr water container (full)
- Winch (vehicle-operated is preferable)
- Tape-measure, ≥ 3m
- Appropriate haversacks to carry loose equipment, if no road or helicopter access
- Refuse bags (tough plastic, and multiple)
- Metal detector (often unavailable)
- Wheelbarrow
- Shovel
- 5 Ltrs of diesel
- Matches (and firelighters)

PPE, plus other personal and bio-hazard equipment

- Overalls
- Face masks
- Goggles and / or face-shield
- Gloves – heavy-duty gardening, latex/nitrile and shoulder length; appropriate sizes
- Gum-boots
- Plastic apron
- Wide-brimmed hats, umbrella and sunscreen (especially for fair-skinned personnel)
- Sweat bands
- Household bleach (1 to 2Ltr)
- Hand-operated sprayer – 1 to 2Ltr (for disinfecting surfaces with bleach)
- Plastic bottles of drinking water (preferably frozen at the time of departure)
- Waterproof bandaging material and antiseptic ointment
- Disinfectant soap and scrubbing brush
- Disposable, Hand-towel roll

Sampling and recording equipment

- Blood slides (recently washed in alcohol and dried-polished, or washed in field with ≥ 90% alcohol)
- Slide-box and/or toilet paper to wrap the prepared slides
- 10% formalin in normal (0.9%) saline – preferably buffered; in leakproof (preferably plastic) containers ≥ 250ml
- 70% alcohol with 5% glycerine – 200ml (for parasites)
- Plastic sampling tubs (wide-mouth; eg. washed/rinsed 1Ltr yoghurt tubs)
- Plastic funnel
- Sterile / near-sterile plastic bottles (eg. recently-emptied 500ml mineral-water bottles – usually sterile)
- Sterile sample tubes – eg. 50ml Falcon, 15ml tissue, 10ml red-top, 5ml EDTA purple-top, 2ml cryotubes
- Insulation tape (for sealing tops)
- 10 or 20ml syringes and 14 to 16gu needles
- Bacterial swabs and agar tubes (sterile and preferably charcoal agar)
- Sample plastic bags – Biohazard or Tamper-proof and / or standard Zip-lock
- Waterproof pens, plus labels / masking-tape
- Digital camera (tough, disinfectable, with good macro facility; smart phones less desirable)
- GPS
- Clip-board and recording sheets / PM forms
- Chain-of-Custody forms (for forensic cases)
- Cyanide field-test kit – Na-picrate soln., filter-paper strips, 100ml bottle with airtight lid
- Polystyrene container and frozen ice-packs (for samples that must be immediately chilled)

Equipment taken to the site is dependent on accessibility, species / size of animal and manpower available; prioritize, if limited.

IN THE FIELD – PM PROCEDURE

Attention to background details

- Previous, or ongoing, mortality in immediate, or adjacent, areas – what was diagnosed before
- Multiple or single species involved in previous deaths

- Seasonal, climatic, or other environmental changes – especially sudden, significant variation
- Ecological changes – population dynamics / population pressures
- Unusual human or wildlife activity, especially if a result of climatic factors

Initial assessment of the carcass and surrounds

- Single or multiple carcasses (& species) – the presence of multiple carcasses frequently indicates poisoning (natural or malicious), or gunshot poaching
- All carcasses to be numbered, photographed otherwise undisturbed, and GPS'd
- Proximity to water – what kind of water (flowing, stagnant - large or small body; dead insects or birds)
- Proximity to natural salt-licks / mineralised soil (Cyanide, or other poisons may be mixed with salt in these)
- Proximity to human activity, crops
- Presence of unusual objects, unnatural fruits, toxic plants; exotic fruit, such as oranges, or natural fruits, such as melons, are used for placing poisons
- Attitude of the carcass:
  - elephant dying in sternal condition have died very suddenly
  - elephant lying laterally, with rigidly-straight legs may be anthrax-infected
  - ruminants with marked opisthotonos, and rigidly-straight legs, may be anthrax-infected
- Evidence of struggling ('paddling' scrapes on ground), or fighting, prior to death; elephant in poor body condition that are unable to rise, especially on soft substrate, may make very deep gouges in the soil
- Degree of scavenging; by what species; presence of dead scavengers (pesticide / sometimes cyanide)

Superficial examination

- If possible, both sides of the carcass must be examined; may be impossible in mega-herbivores, without adequate equipment and manpower
- If gunshot poaching likely, and if metal-detector available, scan the whole subcutaneous area because bullets often come to rest just under the skin
- Stage of decomposition (see **Appendix 2**) – Stage 3, and beyond, carcasses usually yield little information except for testing for residual toxins and anthrax; or bullets. Sometimes, the intestinal contents of Stage III to V carcasses may give information on intestinal function (impaction, sand ingestion)
- Body Condition Score (see **Appendix 3**)
- Age of animal – done on body size for the species, physical measurement (occiput/tail-base, shoulder, protruding tusk or girth of chest/forefoot – elephant), dentition (see **Appendix 3**)
- External parasites – tick loads are often greater in weakened / thin animals, though many ticks will usually detach after death
- Wounds – size, area covered, state of healing, obvious fractures or dislocations, bullet holes
- Skin discoloration – haemorrhages visible in pale skinned areas (eg. anthrax),
- Blood or fluids from orifices – fresh, un-clotted blood = anthrax; blood-tinged fluid in late stage 2 and stage 3 carcasses are not necessarily indicative of anything, except decomposition; many dead blow-flies and other insects may point to pesticide or cyanide poisoning
- Mucosae – conjunctivae, oral, vulva, rectal or prepuce (cyanosis, congestion, pallor, haemorrhages); scavenging vultures target these first

Opening the carcass

- Collect blood smears (see **Appendix 4**, as well as **Appendix 5** -specimens for Anthrax); additional notes:
  - Peripheral blood smears are best for haemo-parasites, but often only feasible in Stg 1 and early Stg II carcasses
  - Deep blood smears from extremities are best for Stg II and Stg III carcasses – deep cut on the caudal aspect just above the foot / coronet; a knife-stab at this site in an elephant will usually yield blood
  - Make smears in shade / under cover when hot-sunny, or wet, conditions prevail
  - Flies can destroy unprotected blood smears by feeding on them, therefore protect / wrap them
- When Anthrax or Rabies suspected, do restricted / minimal PM merely to obtain necessary samples (See Introduction, above, and **Appendix 6**)
- Whenever possible lie carcass on right side and **open on left**. This gives initial access to the spleen, stomach, left kidney and edge of left lobe of the liver. Reptiles and birds should be positioned in dorsal recumbency and opened ventrally

- When right-side opening is the only option, the intestinal tract may have to be completely eviscerated before good access to stomach and spleen are possible
- Skinning – total flaying is preferable for comprehensive subcutaneous assessment but, in the field, is usually only feasible in animals up to ± 200 kg
    - May be necessary in gunshot cases as exiting bullets may be found just under the skin
- Accessing body cavities:
    - In most species it is possible to remove the upper (left) front and rear legs by reflecting them through the shoulder muscles and hip joint respectively
    - Cut all muscles away from the rib-cage, exposing bones
    - Remove (left) abdominal muscles and peritoneum from ventral midline to lumbar spine, exposing abdominal viscera
    - Sever diaphragm at attachment to upper ribs
    - Remove (upper) rib-cage by sawing / axe-ing ribs at sternum and spine; one-by-one, if necessary
- Neck and head
    - Animals <± 500kg: usually possible to remove trachea (+ oesophagus) and lungs together; can include tongue by freeing tissues from inner aspect of mandibles
    - Brain accessed by severing spine at occipital joint, and sawing head in half from nose to occiput after cutting away skin and muscles; leaving 2 halves of brain
    - Larger animals - easier to use axe to access the brain (in the hands of a skilled operator); see Introduction above for rabies sampling
- Elephant:
    - Pull (top) front leg forward (by vehicle, winch, or only ropes - use 'truckers knot' round tree)
    - Or remove it entirely; may then be best to skin and de-bone first, depending on available equipment and manpower, and if full access to thorax required; cut tree-forks are useful to prop up leg while skinning / deboning
    - Pull rear leg backward, similarly (or remove entirely, as above)
    - Make dermal cut-lines and remove, by peeling back, the abdominal skin panel, in one or more pieces, from ventral to dorsal midline
    - Remove abdominal muscles – it may be helpful to leave the peritoneum holding abdominal organs in place
    - Remove ribs as above – axe needed to free them at sternal and spinous attachments; Note: elephant lungs are adherent to rib-cage and this attachment has to be cut free as each rib is removed, allowing the lungs to remain in the thoracic cavity

Removal and gross examination of organs

- General observations:
    - general colour of organs (pale, jaundiced, purple/cyanotic)
    - amount of fat
    - consistency and degree of clotting of blood
- Check body cavities for inflammation, haemorrhages, fluids (quantity, colour and turbidity) and adhesions
- **Potentially bio-hazardous cases** - organs may simply be sampled in situ once carcass is opened; eg. left kidney, spleen, stomach contents, left lobe of liver, left lung and heart muscle
- Abdominal cavity: suggested order of removal:
    - Spleen – size, swelling (rounded edges / bulging cut surface), surface haemorrhages (note: these can be normal in elephant) + other surface discolorations; colour / consistency of cut surface
    - Upper (left) kidney – peri-renal fat, discolorations (colour, distribution, size)
    - Adrenal – size, swelling, haemorrhages
    - Gastrointestinal tract:
        - Stomach – if possible, for potential poisoning cases, take a sample of stomach contents (see below, under sampling), **before removal from the abdomen**; cut through oesophagus at diaphragm, and remove / pull out of abdominal cavity; often best to tie off stomach with braided nylon cord / string at both ends (cardia and pyloris) before severing and removing as a separate unit
        - An elephant stomach, or rumen, too heavy to manhandle can be partially emptied first; a wheelbarrow or PVC canvas used as a 'sledge' can assist in the process; re-suture if necessary
        - free duodenum from common bile duct, loosen small intestine from mesentery while extracting from abdomen, in ruminants or simple-stomach species of mammals the large intestine is extracted with the small intestine

  - Large intestines of hindgut-fermenting mega-herbivores is handled as with the stomach
  - Spread intestines out, preferably on a non-sandy surface, if in the field – PVC canvas can be used as clean surface
  - Apart from taking toxicological samples, pathological assessment of the g.i.t. is best left to the end of the PM procedure (see below)
- Liver - cut free from diaphragm and remove – check swelling, parasites, local or general discolouration;
- Gall-bladder (absent in hindgut-fermenters & common duiker) – check filling, colour / consistency of bile; bile stones in elephant occur in the major bile ducts in the absence of a gall bladder
- Right kidney – in many cases this is removed with the liver; same examination as left
- Bladder – check amount, colour (eg blood) of urine
- Uterus – check for pregnancy; measure crown/rump length of foetus
- Testes – caudally adjacent to kidneys in elephant, otherwise external, though deep in the preputial fold in rhino; check size, consistency and inflammation

- Thoracic cavity – in animals < ± 500kg – trachea, lungs, oesophagus and heart are removed together; elephant heart may weigh 25kg; thus the heart, and each lung, must be removed separately, with the aid of meat hooks; pericardial sac is opened first, in situ, in elephant
  - Thoracic cavity – check adhesions (normal in elephant), fluid (amount and consistency)
  - Pericardium & Heart – check fluid/pus in pericardial sac; haemorrhages on or inside muscle; amount of coronary fat or "watery fat"; pale areas on cut surface; check heart valves for swelling or adherent inflammation
  - Trachea and major bronchi – check fluid, forth, blood, ingesta (commonly aspirated in dying ruminants)
  - Lungs – check if collapsed or not; heavy/fluid or blood-filled; areas of consolidation; froth in trachea; worms in trachea or bronchi
- Head (see above for access to organs)
  - Tongue and gingivae – check ulcers, abscesses
  - Teeth – check abnormal wear; missing teeth
  - Nasal cavity – check parasites (insect larvae), inflammation
  - Lymph nodes and salivary glands – check inflammation / abscessation
  - Brain and meninges – check congestion / haemorrhage / oedema, abscesses
- Musculo-skeletal
  - Open obviously swollen joints – check excess synovial fluid or pus
  - Make cuts into major muscle blocks, especially swollen regions – check haemorrhage or necrosis
- Spinal chord
  - Open if there were indications of posterior paralysis / paresis
  - Use axe or meat-cleaver to chop along spine, above the spinal cord Or
  - Using a saw make double cuts through the spine approx. 8cm apart and 'tease' the spinal cord out of the spinal canal of the cut section Or
  - Remove head, and then excise a small section of spinal cord from exposed foramen magnum and spinal canal
  - Check for compression, abscesses, traumatic injuries

Sampling procedures

A. General Considerations

1. Four diagnostic scenarios; range of specimens can depend on this:
   i. Confirm an obvious field diagnosis made on gross pathological findings (few targeted specimens)
   ii. Confirm a fairly obvious field, **or suspected**, diagnosis, but also to look for other conditions for surveillance purposes (multiple specimens)
   iii. No idea of the cause of death, but good quality, fresh samples available (multiple samples)
   iv. No idea, and samples of poor quality anyway (few targeted specimens)

   While it may seem best to collect in the field, and then send off, as many samples as possible regardless of any initial diagnosis, excess samples can overwhelm diagnostic or storage resources, and then not be properly optimized. Targeted sampling in the field, or rationalization of multiple samples during subsequent, laboratory pre-processing, can work out more satisfactory.

2. Targeted, good quality and well-preserved samples obviously yield best results
3. Coolest-possible site in the field and during transport to base, should be used for samples which are not in specific preservatives. Wet newspaper or even wet vegetation, wrapped around properly-sealed containers, is useful if no other cooling method is available

B. List of potential samples

See Appendix 6. For List of some potential diseases / Conditions and samples

1. Cytology
   - Blood smear – as described above; first sample to be taken
   - Impression or scrape-smears
     - Spleen
     - Lymph node
     - Stomach
     - Duodenum / jejeunum
     - Brain and / or meninges
     - Any purulent lesion
2. Histopathology– in 10% formalin / normal saline; size ± 4 x 2 x 2cm, or smaller (approx. half size of thumb); 'neat' edges; rinse off excess blood; include any lesions + adjacent 'normal'
   - Spleen
   - Liver
   - Kidney - include cortex and medulla
   - Adrenal – ditto
   - Lung
   - Heart
   - Skeletal muscle (diaphragm, thigh, and / or shoulder)
   - Lymph node – peripheral and visceral (especially if 'abnormal')
   - Gastrointestinal tract – stomach/rumen, duodenum, jejeunum, caecum
   - Brain – from any animal >20kg, section longitudinally; from any animal >100kg, section longitudinally and transversely – the latter multiple times depending on size; place in 'tubs' or plastic bags with 10% F/S (not in narrow-mouthed bottles)
   - Spinal cord – cranial and caudal, if possible
   - Any other tissue that appears abnormal
3. Bacteriology – use charcoal-agar swabs, or at least swabs with an agar-based medium; chill
   - Heart blood
   - Brain
   - Liver / spleen often less rewarding
   - Periphery of purulent lesions, especially if multiple
   - Small intestinal fluid - ≥ 2ml; where Clostridial septicaemia suspected
4. PCR / virology – 'finger-tip' sized samples; chill or freeze
   - Blood (1 to 2ml)
   - Spleen
   - Liver
   - Kidney
   - Lung
   - Brain
   - FMD vesicle fluid or epithelium
   - Other organs or tissues which show inflammation

5. Toxicology – chill or, preferably, freeze
    - Stomach fluid – not less than 100ml if fluid, or 500g if dry
        - if contents fairly dry, then squeeze handfuls, allowing fluid to run through a funnel into a container (eg Falcon tube, bottle or tub) Or
        - scoop out fluid with a wide-mouthed tub, and decant into a tube/bottle
    - Liver – 50 to 100g (chilled / frozen)
    - Kidney – if liver unavailable (as above)
    - Blood / serum - ≥ 5ml (if botulism or pesticide suspected)
6. Special samples for Specific Diseases – see under list in **Appendix 6**.
7. Forensic Samples & Containers
    - Crime Scene Investigations Unit should be present (Police of Parks Authority)
    - Use Tamper-Proof bags for whole samples or to contain (multiple) tubes of sample material
    - Intact, leak-proof plastic bags can be used; sealed with tape, and with signatures over seal + bag
    - Chain-of-Custody Form must be filled in on site (see below)

Recording

- GPS coordinates of each carcass recorded on PM Report (and Crime Scene Report, if relevant)
- Label all samples - including date, PM ID (species & carcass ID, if relevant), tissue of origin if not obvious
- Detailed labelling for forensic samples; includes the following:
    - Date and time collected
    - Unique Carcass identification (for that Date)
    - Unique Sample Identification (for that Date) Number
    - Person collecting
    - Sample details (Species, organ / substance)
    - Each Sample Number to be identified and correspond in Crime Scene Report
    - Chain of Custody Form to be filled in (**Appendix 7**); must accompany forensic samples
- Photographs to include
    - The scene prior to interference; including different aspects of the carcass(es)
    - Steps of opening the carcass
    - Organs in situ
    - Individual organs; including macro / close-up of lesions
    - Sampling / samples
    - Clean-up operations

  Photos should be taken to allow a viewer, who was not involved, to orientate; ie. a single close-up photo of part of an organ may be meaningless to someone who was not present when the photo was taken.
- PM Report to be completed and signed – see **Appendix 8** for suggested format

IN THE FIELD – CLEANING UP

Routine carcass disposal

- Many carcasses can be left for scavengers to deal with in an appropriate, conservation area; though not in a human frequented zone
- Carcasses potentially harmful are those likely to be infectious or toxic to other animals or humans (who may also scavenge the carcasses)
    - Burn, or bury, on site, if possible; includes intestinal contents that may be scattered
    - Cover unburned carcasses with Caustic Soda or Quicklime; even if they are buried; includes contaminated soil
- If impossible to burn / bury the carcasses, consider:
    - Covering them in diesel…. and lighting it, if unlikely to cause a wild bush-fire
    - Completely cover with plastic or branches (possibly after covering with diesel)
    - Guard carcasses until late Stage 3 decomposition

Cleaning up Poison / poison spill

- Poisoned carcass
  - Collect all traces of poison and burn completely on site (stand up-wind!)
  - Or take away in plastic bags and burn in a proper incinerator
  - Must include spilled intestinal contents
- Poison spill
  - Remove and bury (or burn) contaminated top-soil
  - Flood the rest of the area, where poisons were lying, with water and / or
  - Use chemical de-contamination – eg. Caustic Soda or Quicklime
- Decontamination for cyanide
  - Cyanide usually disappears quite rapidly, especially if wet / dissolved; half-life of cyanide in solution is approx. 2 hours
  - Cyanide 'Cakelets' (20g blocks of NaCN, used for processing gold ore) should be removed from the scene and neutralized elsewhere
  - Chemicals you can use for cyanide are:
    - Swimming Pool Acid (Hydrochloric acid)
    - Swimming Pool Chlorine (Calcium hypochlorite or "*hth*")
    - Use these wet, ie add water after you have put the chemical down
    - Beware of highly toxic HCN gas being released
    - If nothing else available, flood the area and put guards there for 1 to 2 days
- For poison-contaminated water bodies / pans
  - try to drain them and keep them dry thereafter
  - and / or guard for ± 5 days

References:

Lane, E.P., Foggin, C.M. & Kock, N. (2012). Necropsy techniques. In Chemical and Physical restraint of Wild Animals, 2nd Edition, (M.D. Kock &R. Burroughs eds.). International Wildlife Veterinary Services (Africa). ISBN 978-062052162-8; pgs. 339-349

Penrith, M-L and Thomson, G (2012). Analysis of the Status of Transboundary Animal Diseases and Their Control in the SADC Region During the Period 2005-2011, Focusing on the Five Countries that Contribute Land to the Kavango Zambezi (KAZA) Transfrontier Conservation Area (TFCA). Technical Report to the Wildlife Conservation Society's AHEAD Program. 72 pp. http://www.wcs-ahead.org/workinggrps_kaza.html.

Woodford, M.H., Keet, D.F., Bengis, R.G. (2000). Post-mortem procedures for wildlife veterinarians and field biologists. M.H.Woodford, ed.) Published by OIE, Care for the Wild and Veterinary Specialist Group/Species Survival Commission. pp 55. ISBN92-9044-491-6

Appendix 1.

FIELD POST MORTEM : CHECK-LIST

PM equipment

- Knives – flaying and de-boning ☐
- Sharpening steel + files / oil-stone ☐
- Axe ☐
- Meat-cleaver ☐
- Saw(s) – rip-saw or bow-saw ☐
- Secateurs ☐
- Meat hooks ☐
- Scalpel and multiple blades ☐
- Forceps (rat-tooth and smooth) ☐
- Scissors (curved and straight) ☐
- Plastic organ containers ☐
- Cutting board ☐
- Nylon ropes ☐
- PVC canvas ☐
- Knapsack sprayer ☐
- 20Ltr water container ☐
- Winch ☐
- Tape-measure, ≥ 3m ☐
- Haversacks ☐
- Refuse bags ☐
- Metal detector ☐
- Wheelbarrow ☐
- Shovel ☐
- Matches (and firelighters) ☐
- 5 Ltrs of diesel ☐

PPE + personal / bio-hazard equipment

- Overalls ☐
- Face masks ☐
- Goggles and / or face-shield ☐
- Gloves – heavy, latex/nitrile, shoulder ☐
- Gum-boots ☐
- Plastic apron ☐
- Wide-brimmed hats ☐
- Umbrella ☐
- Sunscreen ☐
- Sweat bands ☐
- Household bleach (1 to 2Ltr) ☐
- Hand-sprayer – 1 to 2Ltr (with bleach) ☐
- Frozen drinking water ☐
- Bandages + antiseptic ointment ☐
- Disinfectant soap / scrubbing brush ☐
- Roll of Paper Towel ☐

Sampling and recording equipment

- Blood slides ☐
- Alcohol for washing slides ☐
- Slide-box / toilet paper ☐
- 10% formal-saline containers ≥ 250ml ☐
- 70% alcohol with 5% glycerine ☐
- Plastic sampling (yoghurt) tubs ☐
- Plastic funnel ☐
- Sterile plastic bottles ☐
- Falcon tubes (50ml) ☐
- Tissue tubes (15ml) ☐
- Red-top tubes (10ml) ☐
- EDTA tubes (5ml) ☐
- Cryo-tubes (2 + 5ml) ☐
- Insulation tape ☐
- Syringes (10 or 20ml) ☐
- Needles (14 or 16gu) ☐
- Bacterial swabs + (charcoal) agar ☐
- Sample bags (Tamper-proof, Zip-lock, Biohazard) ☐
- Waterproof pens ☐
- Labels / masking-tape ☐
- Camera ☐
- GPS ☐
- Clip-board / PM forms ☐
- Chain-of-Custody forms ☐
- Cyanide field-test kit (Na picrate etc.) ☐

Additional items

USAID
FROM THE AMERICAN PEOPLE

Appendix 2. STAGES OF DECOMPOSITION OF A CARCASS

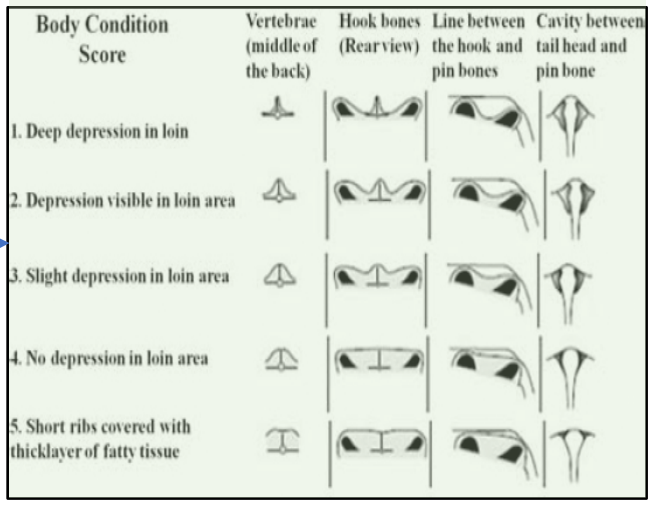
STAGE	TIME	CHANGES	INSECTS
1	0 to 6 hrs	"Normal" Rigor (stiffness) starting	Blowfly eggs
2	6 to 48 hrs	Bloating (abdomen) Starting to smell	Blowfly larvae Larvae growing
3	2 to 10 days	Very bloated (gas under skin) Very strong smell (due to ammonia) Becomes spongy Deflates Becomes liquid	 Blowfly larvae full size (1 cm) Larvae disappear (burrow) Beetles and other insects
4	10 to 21 days	Drying out Skin detaches from bones Skin becomes hard	No blowfly larvae
5	3 weeks to months	Skin decreases (contracts / breaks) Carcass still has 'shape' Skin finally disappears	Horn moths
6	Months to years	Bones only – slightly moist to DRY	
Affected by: size of animal, disease, scavengers, ambient temperature, rain			

Appendix 3.

Body Condition Score & Ageing

Body Condition Score (BCS)
How much are the bones sticking out
- Hip bones
- Back-bone
- Ribs

Scored 1 to 5
1 = very thin (emaciated)
2 = thin
3 = normal condition
4 = good condition (fat)
5 = very fat (obese)

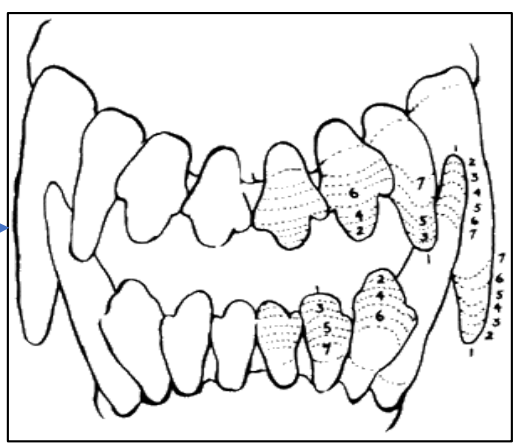


Ageing of Animal

On size and breeding
Baby (Jj) - born recently
Juvenile (J) - still with mother
Sub-adult (Sa) - not with mother, still small
Young adult (Ay) - nearly full size: not breeding
Adult (A) - breeding
Old (Aa) - near the end of its life

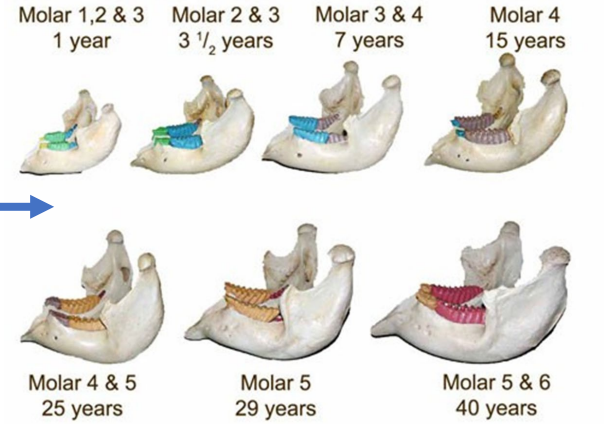
On teeth
- Milk teeth
- Changing to adult teeth (milk teeth and adult teeth)
- All adult teeth
- Adult teeth very worn

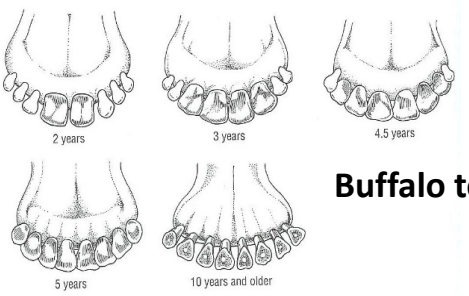
Lion teeth	
2 – 4 wks	I_{1-4}, and c out
6 – 8 mths	I_{1-2} out
12 – 14 mths	C out & growing
2 yrs	C fully out
3 – 4 yrs	I_{1-4} all out; C just worn
5 – 9 yrs	I_{1-4} and C worn as in diagram
10 - 14 yrs	I_{1-4} and C very worn / broken



Elephant teeth ageing - also on tusk length: tusks emerge at ± 30 months of age

Molar	Molar Eruption	Molar Loss
I	birth (5 lamellae)	2 years
II	birth (7 lamellae)	6 years
III	1 yr (10 lamellae)	13-15 years
IV	6 yrs (10 lamellae)	28 years
V	18 yrs (12 lamellae)	43 years
VI	30 yrs (13 lamellae)	65+ years





Buffalo teeth ageing

Appendix 4. The making of blood smears

Except when prepared for the examination of microfiliaria or trypanosomes, blood smears should be thin. One should be able to read newsprint through them. In the above instances, it is of some advantage to place the thick and thin smear on the same slide. In all cases, the smears should be dried quickly, labelled with a diamond pencil or grease pencil and wrapped (in tissue / toilet paper) or boxed to prevent damage by insects. Thin smears may be fixed by dipping in methyl alcohol but thick smears should not be fixed. Blood smears are best made from venous blood collected from a live animal but useful smears can be made from blood collected from an animal which has recently died usually by cutting off an ear. Peripheral capillary blood may be desirable for making smears for the detection of microfilariae, which are often cyclical in their appearance in the peripheral blood (Fig. 5).

Thick blood smears can be mad by putting a drop of blood on the slide and spreading it in a circular fashion with the corner of another glass slide, or even a dry, clean match-stick. The "spread" drop should be opaque and about 1cm in diameter.

Thin blood smears are made as follows: A clean slide (preferably washed in alcohol and dried in air) is placed on a horizontal surface and put a drop of fresh blood (this can be blood in anti-coagulant) near one end. A second slide, which may have its corners cut off, held at an acute angle, is placed on the horizontal slide and is drawn into the drop of blood. When the blood has spread evenly almost across the end of the spreader slide, the spreader should be pushed quickly along the length of the horizontal slide. An even pressure should be maintained on the spreader slide while making the smear. The smear is dried by waving in the air. Care should be taken to avoid exposure to flies.

Notes specifically for Anthrax

For smears made from suspect Anthrax cases, which may be quite rotten, the following procedure may be done.

- disposable gloves should be worn, if available
- using a new scalpel blade, cut into the back of a foot just above the hoof (coronet in a hoofed animal)
- as blood appears, collect it on the tip of the scalpel blade and transfer it to the slide (as in the diagram)
- spread the smear as shown and follow the rest of the procedures

If the carcass is unopened, formation of the resistant anthrax spores should be minimal from this procedure, though the smear should nevertheless undergo minimal handling. The gloves and scalpel blade can be disposed of, together with the carcass (by burning or burying).

Carnivores such as lions or leopards may develop more localized anthrax. If there is obvious swelling of the head or neck, make a tissue smear from fluid / blood under the skin, using the same procedure as for the blood smear.

If there is no blood, take a small piece of flesh / skin and put it into the bottle provided. If there is no flesh (very old carcass), take some soil from under the and put into the bottle

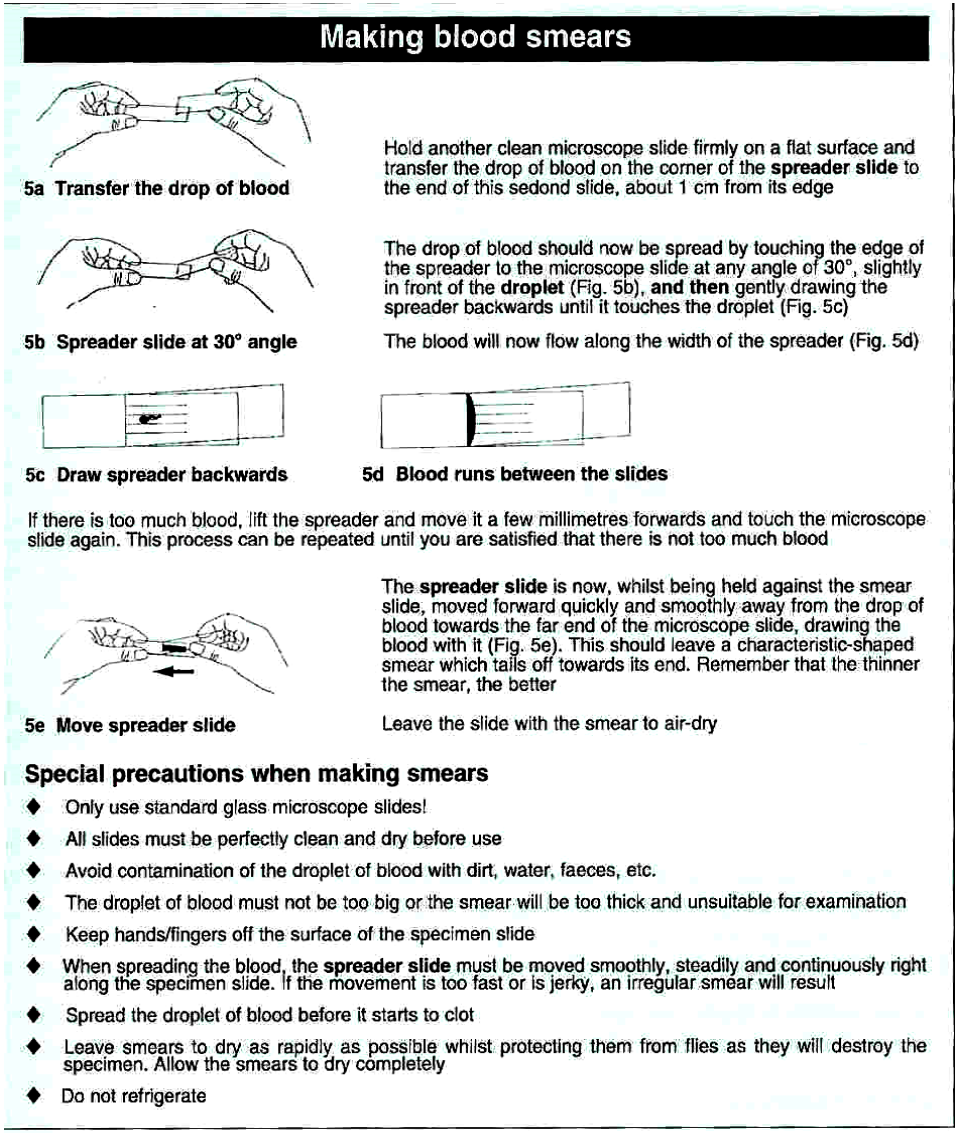


Fig. 5
Making blood smears
Courtesy: Onderstepoort Veterinary Institute, Pretoria, South Africa

Appendix 5.

General information **ANTHRAX : COLLECTION OF SAMPLES FOR DIAGNOSIS**

- Be careful because people can get Anthrax
- While collecting samples for Anthrax you might get SKIN ANTHRAX if you cut yourself or have an open would
- Skin Anthrax can be easily treated with Antibiotics

Provided some simple precautions are followed, the process of collecting samples should be almost risk-free.

- The Anthrax bacteria inside the carcass dies within a few days of the animal dying
- If the carcass is not opened then very little contamination of the area will occur
- Only if the carcass is opened and spread around will the Anthrax SPORES form
- These Spores are very resistant to heat, disinfectants and dessication (drying) and can last many years
- Therefore when collecting Anthrax samples do it with the least disturbance to the carcass

Precautions

- If possible, wear some protective clothing, preferably overalls and gumboots
- Avoid getting these covered in blood or carcass material
- After use wash overalls separate from other laundry with lots of detergent and rinsing
- Boots can be washed then wet with strong household bleach or 5% formalin
- WEAR GLOVES – it is possible to carefully collect samples without gloves, but not recommended
- Skin wounds should be covered with waterproof plaster, if possible; but rather have someone else collect the samples if you have open wounds
- After sampling wash your hands with lots of soap and water. You can use some household bleach as well
- Knives and other re-usable instruments should be disinfected with 5% formalin or strong bleach before washing with soap and water
- All other waste material (Gloves, Scalpel Blades etc should be burned or buried

Three types of samples can be collected, as indicated below:

1. Blood smears

- Blood Smears are best made from Stage 1 and 2 decomposition carcasses, and possibly early stage 3
- The “Anthrax Smear Kit” has 3 smear glasses(Glass Slides) wrapped in toilet paper, plus a scalpel blade, plus label on which to write details
- How to make a blood smear is described in the attached sheet on “How to Make Blood Smears”
- Once made, the smears are allowed to dry in the air, the re-wrapped in the toilet paper (separately – do NOT put slides together, touching) in the piece of toilet paper
- The form is completed and attached.

2. Tissue samples

- Can be taken from any carcass that still has 'wet' or soft tissue on it
- This can be 'Cultured' in a bacteriology laboratory to check for Anthrax.
- Cut a small piece of tissue, about 10mm x 10mm x 10mm is cut off the carcass with a scalpel
- Forceps can be used to told the piece, but if not available you can use a gloved hand or two pieces of stick
- Tissue should be collected from muscles, or thin skin (best is from penis or vulva, tongue, eyelid or nostril), or virtually any organ if the carcass has been opened)
- The tissue sample is placed in a bottle / tube with a secure lid, without contaminating the outside
- The bottle in turn should be tied in a plastic bag
- This should be kept chilled if possible
- The label should be completed with the same details supplied as for blood smears.

3. Soil samples (collected when there is no 'Wet Carcass Material' available)

- Soil is collected from the around a dried carcasses
- Collect an amount of about one or two teaspoons of soil
- This should be taken from just below the surface where "leakage" of blood from the carcass may have occurred before decomposition – even though the soil may be collected long afterwards
- This would be from under the mouth / nose, or vulva / rectum
- If the carcass was opened early (by scavengers) then the whole area is likely to be contaminated with spores, and any soil from underneath may be useful
- The soil can be collected with a piece of disposable metal or bark + stick etc., and should be put on a sheet of paper and then "funneled" into a bottle with a tight-fitting lid without contaminating the outside
- The same details, as for blood smears, should be provided.

After you have collected the samples

- Items such as scalpel blades, gloves sticks, paper etc should be put in the fire, or buried
- Other actions such as washing of implements, clothing etc has been described above.

Submission of samples

- Samples should be taken to the Veterinary Dept Offices

Appendix 6.

Wildlife Diseases & Conditions, possibly Significant in KAZA (adapted from Penrith and Thomson, 2012)

Disease	Impact (High, Med, Low)	Species & Explanation	Critical samples & Preservative	Laboratory Tests
Anthrax	**H**	Multiple; high mortality in some species; Zoonosis; Livestock infection	Blood smear (AD); swab (Ch); tissue (Ch)	Cytology; PCR; Bact culture
Pasteurellosis (Haemorrhagic septicaemia)	**H**	Elephant mortality; also buffalo; Livestock disease (not via wildlife); Zoonosis (low potential)	Organs, incl. brain (FS); swab (Ch); Blood / tissues (Ch/Fr)	Histo; PCR; Bact culture
Tuberculosis	**H**	**Bovine TB**: Multiple spp; Zoonosis; Livestock disease; control impossible; population effect in some spp	Lesion smear (AD); lesion (Ch); Lesion, l.node, organs (FS); serum (post-Rx tuberculin)	Cytology; Bact culture/PCR; Histo; IFNy serology
	H	**Human TB**: Elephant (especially domestic), primates (others ?); Zoonosis; possible 'escape' into wild elephant population	Lesion smear (AD); lesion (Ch); Lesion, l.node, organs (FS); serum (post-Rx tuberculin); serum (Fr)	Cytology; Bact culture/PCR; Histo; IFNy serology; DPP (lateral flow)
	M	**Mongoose TB**: Banded mongoose (Zoonosis / others?); long-term effect on population unknown	Lesion smear (AD); lesion (Ch); Lesion, l.node, organs (FS)	Cytology; Bact culture/PCR; Histo
Brucellosis (Br abortus and melitensis)	**M**	Buffalo (hygroma and abortion); antelope; Zoonosis;	Joint fluid (Ch); Serum (Ch); Foetus - placenta, lung (Ch & FS), abomasal fluid (Ch)	Culture; PCR; Histo
Clostridial septicaemia / cellulitis / myositis	**M**	Elephant mortality; also buffalo; using contaminated pans; Livestock disease (not via wildlife)	Tissue smear (AD); swab (Ch)	PCR; FAT; Bact culture
Botulism	**L**	Multiple; Rabies / paralysis DD	Serum; stomach / intest. contents (Ch/Fr)	PCR; biological
Clostridial perfringens enterotoxaemia	**L**	Rhino, (Elephant & others ?); usually under intensive conditions or sudden diet change	Small intestine contents (Ch/Fr); Intestinal smear (AD)	PCR; biological; Cytology
Dermatophilosis (Senkobo disease)	**L**	Occasional wildlife (confused with mange); Livestock disease	Skin scraping (AD); Skin lesions (Ch); Skin lesions (FS)	Cytology; Bact culture; Histo
Contagious Bovine Pleuro-Pneumonia (CBPP)	**Nil**	No known disease in wildlife; Livestock disease	Preparedness for diagnosis	
Avian Influenza H1, H5 and H7 (HPAI)	**H**	Waterbirds; ostriches (only domestic?); Disease of domestic poultry	Cloacal/tracheal swabs in virus medium, lung/spleen/kidney (Ch); Organs (FS)	PCR; virus isol.; Histo
Foot-and-Mouth disease (FMD)	**H**	Antelope - lameness (sometimes mortality from coronitis or myocarditis): Buffalo carriers; Livestock disease of economic importance	Heart (FS); Lesion & tonsil (Ch →VM);	Histo; virus isolation & PCR
Rabies	**H**	All, especially social carnivores; Zoonosis; Livestock disease	Brain and/or spinal chord (GS & FS + Ch)	IFAT; PCR; Histo (other encephalitides)
Canine distemper (CDV)	**M**	Lions, Wild dogs, (other carnivores ?); Disease of domestic dogs	Blood (Ch); Brain & lung (Ch); Organs incl. brain (FS); For Rapid Antigen test - ocular secretion, serum, urine	PCR; Histo; Rapid Antigen Test Kit
Malignant catarrhal fever (MCF) - Alcelaphine	**M**	Buffalo and antelope (very rare); Livestock disease (only reason for medium impact)	Blood & spleen (Ch); Organs (FS)	PCR; Histo
Newcastle disease (NDV)	**M**	Wild birds, incl.doves (Pigeon paramyxovirus); Disease of domestic poultry	Choanal swabs in virus medium (Ch); lung, spleen, kidney, brain (Ch); Lung, spleen, kidney, brain (FS)	PCR; virus isol.; Histo
Papillomatosis & sarcoid	**M**	Giraffe (cancer & mortality); zebra (cancer ?); lion & leopard	Lesion (FS & Ch)	Histo; PCR
Rift Valley Fever (RVF)	**M**	Buffalo (abortion) & antelope; Zoonosis; Livestock disease	Organs (FS); tissues (Ch); Blood (Ch)	Histo; virus isolation & PCR

USAID
FROM THE AMERICAN PEOPLE

Disease	Impact (High, Med, Low)	Species & Explanation	Critical samples & Preservative	Laboratory Tests
Encephalitis-myo-carditis virus	**L**	Elephant (esp. Ad ♂ mortality; associated with rodent 'explosions'	Organs esp. heart (FS); blood & heart (Ch)	Histo; PCR
Endothelio-tropic herpes virus (EEHV)	**L**	Elephant; wart-sarcoma in young animals; fatal in zoo Asian elephant	Tongue / Lesion (FS); Blood (Ch)	Histo; PCR
Peste de Petit Ruminants (PPR)	**L**	No wildlife disease, though possible wildlife reservoir; Livestock disease	Blood, L.node, spleen, lung (Ch); Organs (FS)	PCR; virus isol.; Histo
Middelburg virus	**L**	Rhino; Rabies / paralysis DD	Brain / spinal chord (FS & Ch)	Histo; PCR
Shuni virus	**L**	Rhino (mortality & paralysis DD)	Brain / spinal chord (FS & Ch)	Histo; PCR
West Nile virus	**L**	Crocodile (+ rhino ?); Mortality; Zoonosis	Brain / spinal chord (FS & Ch)	Histo; PCR
Theileriosis	**H**	Buffalo and antelope (naïve animals); Livestock disease	Blood / spleen / l.node smear (AD); Spleen (Ch); Organs (FS)	Cytology; PCR; Histo
Babesiosis	**L**	Elephant, Giraffe (fatal), some antelope, small carnivores (high parasitaemia); some transmission to livestock	Blood smear (AD); Spleen & blood (Ch/Fr); Organs (FS)	Cytology; PCR; Histo
Trypanasomiasis	**M**	White rhino; naïve / introduced wildlife; Zoonosis; Livestock disease; Tsetse fly absent in most of KAZA, but could return	Blood smear (AD); blood (Ch); brain + organs (FS); CSF (Ch)	Cytology; PCR; Histo
Epizootic Ulcerative Syndrome in fish (EUS)	**M**	Fish in some KAZA river systems	Lesions (Ch); Lesions (FS)	Culture; PCR; Histo
Poisoning - Pesticide (carbamate and organo-phosphate) (forensic case)	**H**	Elephant, Rhino, carnivores threatening livestock, scavengers esp. vultures	Stomach fluid, Liver, Blood (Ch/Fr); Bait (ch)	HPLC & MS (Chemistry Lab); Cholinesterase
Poisoning - Cyanide (forensic case)	**H**	Elephant and Rhino; non-target scavengers, esp. vultures	Stomach fluid (Ch/Fr); Muscle (Fr)	Na picrate field test (qualitative); MS (quantitative)
Poisoning - Cyanotoxin	**M**	Elephant and many other spp	Stomach fluid (Ch/Fr); Liver, Brain (Fr); Pan water and sediment	HPLC & MS (Specialized Chemistry Lab); Dipstick tests (Microcystin & Anatoxin a)
Poisoning - Plant	**L**	All herbivores; (Dichapetalum, Cyanogenetic plants eg Acacia, and many others	Stomach contents, esp. intact leaves & fruit (AD); Stomach fluid (Ch/Fr)	Plant ID; Biological test; Cyanide field test
Parasites - External (Ticks, mange etc.)	**M**	All spp (some more susceptible), esp. very young, old & nutritionally-stressed individuals	Gross path. assessment; skin scraping (for mange); skin (FS); parasites (Alc)	Parasite ID; Histo
Parasites - Internal (Helminths, Oestrid larvae, Pentastomes)	**M**	All spp, esp weaner, old & nutritionally-stressed individuals	Gross path. assessment; parasites (Alc); Organs (FS); Faeces (Ch/2%FS)	Parasite ID; Histo; faecal egg count
Poverty	**M**	All spp (giraffe & kudu - tannin-related?); age, spp density, quality/quantity forage-related and climate-related; competition for resources vs humans & livestock; final outcome often trapped in mud	Gross pathological assessment, incl. teeth wear and stomach / rumen contents; Secondary parasite overload **Samples:** Organs (FS); Parasites (Alc); Stomach fluid (Ch/Fr)	Histo; tannin analysis
Unnatural Trauma (Forensic cases)	**M**	All spp: includes RTA and gunshot poaching	Gross path assessment; **Samples:** bullets and cartridge cases	Balllistics Lab.
Natural trauma	**L**	All spp. Mostly Ad or SAd ♂, hierarchial aggression	Gross pathological assessment; **Note:** # mandible may be missed (rhino)	

Preservatives:

FS = 10% formalin in 0.9% saline (unless otherwise stated0
Ch = Chill (2 to 6°C)
Fr = Freeze (≤ 10°C)
AD = Air dry and wrap in tissue / toilet paper)

Alc = 70% ethanol (with 5% glycerol added)
GS = 50% sterilized, buffered saline
VM = Lab-prepared virus medium (buffer with antimicrobials)

USAID
FROM THE AMERICAN PEOPLE

Appendix 7.

	CHAIN OF CUSTODY RECORD	CASE ID NO:

BRIEF DESCRIPTION OF CASE:

DATE AND TIME OF SEIZURE:	LOCALITY OF SEIZURE (LOCSTAT if known):	PERSON SEIZING EVIDENCE:

SOURCE OF EVIDENCE/ PROPERTY (Person taken from, or Person received from, or Found at above locality):

DESCRIPTION OF EVIDENCE/ PROPERTY (include Seizure Tag Numbers and any serial numbers):

ITEM NO.	DESCRIPTION (NOTE - Underline below last item):	SEAL NUMBER:

CUSTODY RECORD OF ITEMS:

ITEM Nos.	RELEASED			RECEIVED		PURPOSE
	Date & Time	Person	Carrier	Date & Time	Person	
		Name			Name	
		Signture			Signature	
	Date & Time	Person	Carrier	Date & Time	Person	
		Name			Name	
		Signture			Signature	
	Date & Time	Person	Carrier	Date & Time	Person	
		Name			Name	
		Signture			Signature	
	Date & Time	Person	Carrier	Date & Time	Person	
		Name			Name	
		Signture			Signature	

USAID
FROM THE AMERICAN PEOPLE

Appendix 8. POST MORTEM RECORD REF NO _____

SPECIES _____ **GPS/AREA** _____ **DATE** _____

AGE _____**SEX**_____ **LACTATION**_____ **TIME OF DEATH / STG. DECOMP** _____

BACKGROUND

HISTORY LEADING TO DEATH

POSITION FOUND / DISTURBANCE, SITE

EXTERNAL OBSERVATIONS: injuries - loss of hair - bloating - blood from orifices - eye conjunctiva - anaemia - jaundice - cyanosis - diarrhoea

ECTOPARASITES type and location (samples taken)

BODY CONDITION SCORE (1 TO 5 - 1 = emaciated, 5 = obese)_____ **BLOOD SMEAR Y/N**_____

SUBCUTANEOUS DETAILS: amount of fat- bruising - congestion - other discoloration

LYMPH NODES – location- swelling (sampled)

ABDOMINAL CAVITY AND ORGANS (PTO for gastro-intestinal tract)

CAVITY - clear fluid - pus - adhesions

SPLEEN - normal - swelling - haemorrhage

LIVER - normal - swelling - local discoloration – parasites **GALL BLADDER** – filling, colour, bile stones

KIDNEY - normal - fat deposits - soft – discoloured **URETERS** - swollen

ADRENAL - normal - swelling

BLADDER - normal - urine dark (coffee) - red - cloudy

SEX ORGANS: ♀ **-** Gonads / Uterus **(**active, pregnant / foetus development). ♂ **Penis, Testes, Acc Organs** (size – swelling – consistency)

USAID
FROM THE AMERICAN PEOPLE

CHEST CAVITY AND ORGANS

CAVITY - clear fluid - pus – adhesions; **MEDIASTINUM & AORTA** – fat – discolouration: **THYMUS** – size - active

LUNGS - collapsed or not - heavy/fluid or blood filled - areas of consolidation - froth in trachea; **BRONCHIAL LYMPH NODE** - inflammation

HEART SAC - Fluid - clear/pus **HEART** – Fat – haemorrhage – discolouration – heart valves - arteries

GASTRO- INTESTINAL TRACT. Contents - amount - wet - dry - ulcers – parasites – discolouration of mucosa

OESOPHAGUS & STOMACH or **RUMEN** (rumen, reticulum, omasum, abomasum)

SMALL INTESTINE - contents - blood -mucus – ulcers **MESENTERY & LYMPH NODES** – fat - swelling

LARGE INTESTINE -(caecum, colon, rectum) – contents -

INTERNAL PARASITES - LOCATION & ABUNDANCE

HEAD

ORAL CAVITY / TONGUE / TEETH - eruption and wear

BRAIN - abscess - parasites - blood congestion - fluid

NASAL CAVITY – parasites - inflammation

LYMPH NODES & SALIVARY GLANDS – swelling – abscess

SKELETAL - MUSCLE AND BONES

LARGE MUSCLES - bruising/ blood congestion - scarring – calcification – gas formation

BONES – deformities - fractures - old breaks – arthritis - marrow

SUMMARY & PRELIMINARY DIAGNOSIS (DDs); ADDITIONAL NOTES

SPECIMENS

SIGNATURE:_____ **NAME:**_____