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INVESTIGATIONS INTO THE DEATH OF LARGE FELIDS IN THE WILD: THERE IS MORE TO IT THAN NECROPSY

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ABSTRACT

Large felids including the tiger (*Panthera tigris*) and leopard (*P. pardus*) are grouped in schedule I of the Wildlife Protection Act, 1972. The population trends of both these animals are decreasing globally. While natural death among these animals may be due to old age, territorial (intraspecific) fights, trauma and injuries caused during hunting (interspecies fight) and diseases, poisoning, (both primary and secondary), as well as poaching, make up for the most common causes of unnatural deaths in wild carnivores. The exact causes of death of many of the large felid that have happened in India are reported to be still under scrutiny. Hence, it is mandatory that every single event of death among these animals is thoroughly investigated, not only to understand the cause, but also to rule out other conflict related reasons. An understanding of the mandatory regulatory provisions as well as skill in necropsy procedures is required for the veterinarian to exactly diagnose the cause of death and prepare a report that can be used to prosecute offenders in the court of law. While most veterinarians have the requisite knowledge on basic skills in necropsy techniques, he or she might not be fully aware of the additional details, including safety precautions, adherence to relevant regulations and forensic investigations that have to be followed in case of wild animals. This paper outlines the general guidelines (apart from necropsy skills) that may be followed to perform 'forensic necropsy' of a schedule I animal like a tiger or leopard.

INTRODUCTION

Tiger (*Panthera tigris*), the largest living felid on earth, along with the leopard (*P. pardus*) are grouped in schedule I

of the Wildlife Protection Act, 1972. Globally, tigers are waging a grim battle against extinction, with their numbers and distribution range being less than one tenth of what was in the past and some of their subspecies getting extinct in the wild during the past decades (Dalinsky, 2020). Leopards have lost more than 70 per cent of their original, historic range. Of the nine recognized subspecies of leopards, some like *P. pardus orientalis* make up only 2 per cent of the population (Jacobson *et al*., 2016). Increasing urbanization has led to frequent conflicts with humans in almost every state of India. A staggering 1110 tigers and 4381 leopards have been reportedly poached in India during the 22 year period from 1994 to 2016 (Report, 2017). A total of 826 tiger deaths have been documented in India since 2012 to 2020 (Report, 2020) and among the 313 tiger deaths since 2017, the exact causes of about 19 per cent of the deaths are not known and are still 'under scrutiny'. It is mandatory that every single event of death among these animals is thoroughly investigated, not only to understand the cause but also to rule out other conflict related reasons. Poisoning, (both primary and secondary), as well as poaching, make up for the most common causes of unnatural deaths in wild carnivores. Natural death in wild tigers could be attributed to old age, territorial (intraspecific) fights, trauma and injuries caused during hunting (interspecies fights) and disease. A systematic approach is essential to accurately diagnose the cause of death. The veterinarian is therefore expected to perform a 'forensic necropsy' which not only involves necropsy but also an accurate forensic reconstruction of the events that could have preceded the death, based on scientific analysis of the evidences present, and submit the report in the prescribed proforma to enable this task. Most veterinarians are skilled in the basic techniques of necropsy. However, unlike the necropsy of a domestic animal, there are additional details to which attention needs to be paid, while conducting a forensic necropsy of wild animals under such special circumstances. This paper outlines the general guidelines on the additional details that are required to be followed while performing necropsy of a schedule I animal like a tiger or leopard.

GENERAL GUIDELINES FOR CONDUCTING FORENSIC NECROPSY ON A LARGE FELID

National Tiger Conservation Authority Guidelines:

The National Tiger Conservation Authority (NTCA) mandates that the Standard Operating Protocol (SOP) drawn out to deal with cases of death of a tiger (Report, 2010) is followed strictly

in every investigation of tiger death. The document draws out, in an exhaustive manner, the details pertaining not only to the constitution of the investigation team, format of post-mortem report, etc. but also the other relevant provisions like the list of equipment needed for necropsy and the basic format of specimen sheet. It would serve the veterinarians well to make themselves conversant with the contents of the document. While the onus of complying with the various provisions of the SOP lies with the forest authorities who work with the veterinarians during such an investigation, it is also the bounden duty of the veterinarian to have a thorough understanding of the same, to ensure that the necropsy is approached in a systematic manner and the cause of death is identified with certainty.

Preliminary precautions and documentation of data before necropsy:

1. Upon being intimated of the death of a large felid, ensure that the entry of unauthorized personnel to the immediate vicinity of the carcass (Scene of Crime, SoC) is restricted. This prevents destruction and/or tampering of evidences.

2. Make sure that required tools, equipment, instruments and reagents are adequately stacked in the necropsy kit (Report, 2010). Personal protective equipment (PPE) including disposable aprons, goggles, masks and gloves are mandatory. Unlike in domestic animals, wildlife necropsy can be tedious. As they are usually conducted in remote locations, it might not be possible to bring in materials to the spot as and when required.

3. Ensure personal safety - Tigers guarding a kill can be possessive and will sometimes attack people who venture near. Similarly, necropsies of tiger cubs (and elephant calves) have to be conducted after ensuring that there is no threat from the mothers, which are protective and persistently tend to stay at the spot.

4. Take note of the region (core area, buffer zone or human habitation), terrain (slope, valley, undulating etc.), vegetation (grassland, forests, deciduous, scrub etc.), topography (near a stream, road, etc.) and the existing weather conditions. Ensure the SoC and the carcass are photographed both from close and long range, from various angles.

5. Make arrangements for scanning the perimeter of the carcass (for up to 300-500 m radius) for the presence of other (prey) carcasses. If such other carcasses are present, secure the same also for necropsy. Look for evidence of baiting

(empty pesticide covers, tins, presence of granules over the prey carcass, etc.) near that carcass. If present, collect and preserve them for toxicological analysis (Venkataramanan *et al*., 2008) and also photographically document the same. Evidences of human activity (cigarette butts, shoe marks, tire marks of vehicle, hasty attempts to remove electric poles, etc.) in the vicinity, if present, must also be documented and materials preserved.

6. If the carcass is found near human habitations, enquire from locals if there had been previous sightings of the animal and collect any other information like recent kills, vocalizations and any other pertinent information.

7. Record the indirect evidences of the presence of other animals (pug marks, hoof marks, dung, etc.) in the vicinity of the carcass and photo-document them with a size indicator like a pen (Fig. 1).

8. Conduct the necropsy during day, preferably at the same site where the carcass was originally found. If immediate necropsy is not possible, arrange to preserve the animal in a refrigerator or deep freezer for the night and conduct the necropsy the next day. Document peculiar appearances if any (like carcass half-submerged in water, hanging on tree branches or power lines etc.), damage to vegetation around the carcass (as evidence of struggle before death) and scratch marks on the ground that could indicate fighting prior to death.

Necropsy:

1. Conduct a thorough forensic necropsy (Brownlie and Munro, 2016). Systematic and thorough post-mortem with gentle handling of tissues will unearth evidences even in putrefied carcasses and skeletal remains. Photograph all salient lesions using bright pointers. Documenting the findings 'on-the-go' in sheets of paper or as audio recordings will help prepare a thorough final report without missing out anything important. Maintain a time-stamp of all activities from when the site was reached, when necropsy began and ended, and when/how the carcass was disposed, as well as any other significant event(s) in between.

a. Ascertain the sex and age of the dead animal. Tentative age can be assessed by the body size and the dentition (Fig. 2). Juveniles of large felines under one year usually have pinkish 'delicate' teeth with a median projection in the canines. Young adults usually have the full component of robust dentures, which are off-white. As they get older, loss of teeth starting from the incisors may be

Fig. 1. A feline pug mark documented without a size indicator (a) can be variably interpreted as that of a tiger (b) or a cat (c) depending upon the perspective of the observer.

Fig. 2. Assessing the tentative age by dentition. The temporary teeth of a under-a-year juvenile (a) are pinkish-white. The canines are slender and have a median projection (arrow). A sub-adult (2-3 years) shows a full set of permanent, robust 'off-white' teeth (b), while older animals (8-10 years) may show loss of both incisors and canines (c), with the latter being longer and yellowish. Animals more than 12 years old usually have most of their dentures worn out (d) with almost complete loss of incisors.

noticed. With age, the teeth also become longer and yellowish with prominent longitudinal grooves. Very old animals usually have hardly any teeth left. A more accurate assessment of age can be made using the data from body size, body characteristics, stage of teeth eruption, extent of wear and colouration, intensity

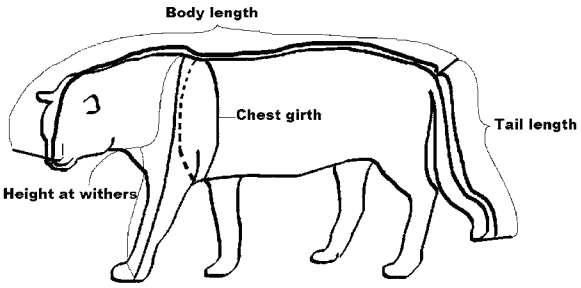

Fig. 3. The various measurements (in centimetres) to be recorded from carcasses of large felids

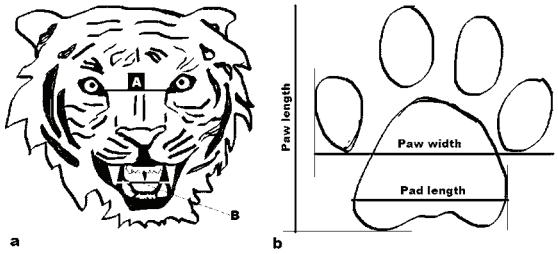

Fig. 4. Additional measurements (a) including the interocular distance (A) and inter-canine distance (B) as well as measurements of the paws (b) including paw length, paw width and pad length can be recorded.

of nose pigmentation and level of gum-line recession (Fàbregas and Garcés-Narro, 2014; Jhala and Sadhu, 2017)

b. Take accurate measurements of the animal, including body length (nose tip to base of tail), tail length, height at withers and chest girth (Fig. 3), preferably in centimetres. Other measurements that can be useful in generating data about the animal are inter-ocular distance, inter-upper canine distance, paw dimensions or paw impressions taken on a graph sheet (Fig. 4). If possible, weigh the animal or try to estimate the approximate weight.

c. Assess the degree of rigor mortis present.

Fig. 5. The presence or absence of 'trophy items' including all nails (a), external genitalia (b) and canine teeth (c) must be recorded.

Fig. 6. Injuries on leopard carcasses. Numerous deep bite marks (a) on the back of the head of an old leopard killed in a territorial fight and (b) injury caused by the tusk of a wild boar. Note the jagged edges of the wound.

Fig. 7. Extensive contusions on the right abdominal region of a leopard (a), possibly resulting from attack by an Indian gaur. Gaur hoof marks and dung were noticed in the region. Haemorrhage, contusions and subcutaneous tissue damage (b) in the neck and throat regions of a leopard caused by the defensive attack of a wild boar.

Note the extent of maggot infestation of the carcass, if any. Measure the size of the largest maggot present and collect the different types of maggots in 10 per cent formalin to identify the species and stage. The data thus generated, along with the information on putrefactive changes (discoloration, bloat, sloughing of skin, mutilation by predators, etc.) will enable to arrive at a tentative time of death (Sharma *et al*., 2015).

Fig. 8. Thick, flaky mesenteric fat depots (arrows) in a healthy animal (a) and thin strands of mesenteric fat in a comparatively weaker animal (b).

Fig. 9. Poisoning of a leopard. The stomach contents show the presence of hair from a calf which was predated by the animal. Subsequent retaliatory poisoning, by baiting of the carcass with phorates, lead to the death of the leopard.

Fig. 10. Carcass of a tiger in a shallow water body (a). Toxicological analysis confirmed death due to poisoning. Presence of mud and dirt (arrows) in the oesophagus of a leopard carcass found floating in a pond (b).

d. Record the status of the 'trophy items' which are items of commercial value (Fig. 5). The presence (or absence) of the canine teeth and all claws should be documented and when present, their representative lengths measured. Ensure

that the external genitalia in males are intact and record the findings. While absence of such trophy items in the carcass would strongly point to poaching as the cause of death, it is also possible that they were removed secondary to unrelated death.

e. Thoroughly examine the skin and natural orifices for the presence of injuries. If present, document them with their measurements and location. Deep bite marks at the back of the head (Fig. 6a), with or without scratch marks on the body and belly region could indicate death resulting from fights with other felids (same or different species). Injuries with 'jagged edges' without smooth outline (Fig. 6b) can be caused by tusks of wild boars, vehicle parts during road-hit and hard objects during a fall. Look for evidence of fracture of limbs, damages to paws from snares (wires) or leghold traps, damages to any other body part due to constricting by snares, signs of electrocution and gun-shot wounds (see section 'h'). The presence and extent of ectoparasite infestation must be recorded and ectoparasites collected for identification.

f. Flay the animal and examine the subcutaneous tissues for the presence of injuries/contusions. If present, document their shape, measurements, extent (mild, sever) and location. Subcutaneous contusions caused by blunt force trauma are sometimes the only lesions noticed in felids killed due to defensive attacks of large prey animals like the gaur (Fig. 7).

g. Record the status of the fat depots in the carcass (Fig. 8). Note the nature (excellent, fair, and poor) and consistency (thick, flaky, gelatinous) of the fat in the subcutaneous, epicardial, perirenal and mesenteric depots. An animal with excellent fat depots is not likely to have died due to starvation or chronic infectious causes.

h. Systematically examine each organ for the presence of lesions and parasites. In forest buffer areas and human habitations, pay special attention to conflict-related causes like poisoning (stomach contents-see below, haemorrhages in most visceral organs, etc.) and electrocution (presence of source of electricity in the vicinity, singeing at the point of contact and exit, shrivelled heart with empty chambers, etc.). Look out for evidence of death due to injuries caused by leghold-traps (wounds in the limbs) and gun-shot wounds (entry and sometimes exit wounds, presence of bullets in tissues). If required, employ imaging techniques and metal detectors for identifying foreign bodies like bullets in the carcasses. All organs

must be examined for the presence of endoparasites (Kalaivanan *et al*., 2015) or lesions (like nodules) caused by them. Parasites found in the organs must be carefully picked out and placed in 70 per cent alcohol or 10 per cent formalin for identification. Special attention should be paid for detecting lesions of some of the common infectious diseases of domestic carnivores like canine distemper, rabies, leptospirosis, feline panleukopenia, etc.) and collect samples to rule them out.

i. Examine the stomach and record the volume and nature of the contents. An empty stomach virtually rules out acute death due to poisoning. If contents are present in the stomach, try to identify whether it is from natural prey or domestic livestock by examining the hairs (Fig. 9), hooves, etc. Ascertain if the contents have any peculiar odour (phorates have a unique odour while carbofuran is odourless). Irrespective of the other lesions present, mandatorily collect about 250 g of contents each from stomach and intestines separately, and place them in plastic containers of 500 ml capacity. Similarly, collect ~250 g of liver and kidney separately. The above materials, preserved in saturated sodium chloride solution, should be despatched for toxicological analysis to a competent laboratory, along with a sample of the preservative alone, all sealed in leak and tamper-proof conditions by a special messenger following mandatory protocols (Dinis-Oliveira *et al*., 2016). If prey carcass is found in the vicinity of the carnivore carcass, include muscle samples (and other materials like pesticide covers, empty bottles) from them too. If the carcass is in an extreme stage of putrefaction, collect long bones, the soil underneath the carcass along with the putrefaction fluids, as well as the maggots, if any, present at the time of necropsy and process as above for toxicological analysis. Samples for toxicological analysis can also be sent frozen (in solid CO_2), without any preservative. Toxicological analysis can conclusively identify primary (Venkataramanan *et al*., 2008) or secondary (Kalaivanan *et al*., 2011) poisoning of felids.

j. Collect specimens for routine laboratory analysis. This would include (but not limited to) representative samples of all vital organs (the area with lesions) in 10 per cent formalin for histopathology, heart and peripheral blood smears and all organ impressions on clean glass slides, sterile swabs from heart blood and samples from other lesions as required, and also pieces of lymphoid (and other) organs in ice for viral

isolation. If possible, collect clots of blood from heart for serum separation. Prepare a clear and exhaustive inventory of the samples collected. It serves to keep ready containers identified with indelible (freezer-proof) labels for different samples in advance, so that no samples are missed.

2. Large cats do not normally die due to drowning (Fig. 10a). Carcasses found floating in water bodies should be thoroughly examined for predisposing causes (like infectious diseases, primary or secondary poisoning and injury sustained during the act of predation) that could have incapacitated them. Poisoned animals often stagger to water bodies to quench their thirst and could stumble into water and drown. In such carcasses, the presence of water, often along with the vegetation and mud, found in the oesophagus (Fig. 10b), respiratory tract and lungs, supported by appropriate histopathological findings should be able to establish death due to drowning. Such carcasses also have water in their stomachs, as any animal that drowns will usually swallow water. Similar attention (to rule out poisoning) should also be paid for suspected road-kills and cases where the evidence and lesions point to death due to a trauma caused by fall from heights.
3. In carcasses of adult females, the mammary glands should be checked for enlargement and extrusion of milk from teats, as these indicate a nursing mother. If the female proves to be a nursing mother, efforts should be made for the rescue and rehabilitation of the orphaned cubs.

Disposal of carcass:

After satisfactory completion of necropsy procedures, the entire carcass must be safely incinerated according to NTCA guidelines after ensuring that the valuable body parts (including the skin, canine teeth, claws and male genitalia) are mandatorily included in the pyre and completely burnt. The whole sequence of action must be photo documented. The PPE and other items like masks must also be destroyed by incineration.

Data management:

Data management must be competent and fool-proof. Data related to history, case definition, necropsy findings, samples collected, storage, despatch details, tests done, laboratory results and photographs taken must be properly organised, documented and retained as both soft and hard copies indefinitely and archived. Remember that most such cases are listed for hearing in the courts after many years and it would be impossible to recollect the

chain of events or trace the documents at that time, if not properly stored. Ensure that the 'chain of custody' is properly maintained by documenting from whom the carcass was 'received' and to whom the samples, body parts and other material collected from the carcass were handed over, by proper attestation.

CONCLUSION

The guidelines above are not exhaustive but provide a basic framework, based on experience, for ensuring that the obvious is not missed. Tentative diagnosis is arrived at based on the preliminary investigations with differentials prioritised and necropsy findings. A post-mortem examination report is no way complete till the results of laboratory investigations are incorporated in the necropsy report. Therefore, it is mandatory to update the provisional diagnosis made based on the gross pathological lesions and circumstantial evidences. Communication of results to relevant authorities is important at the end. In wild felid necropsy and investigation, determining the time of death, whether alterations occurred before or after death of the animal, describing what is seen and putting it on paper without trying to memorise, complete photo documentation from carcass presentation to sampling and despatch, documentation of evidences in crime scene, diagnosis and differential diagnosis, laboratory investigations and documentation are to be followed systematically.

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