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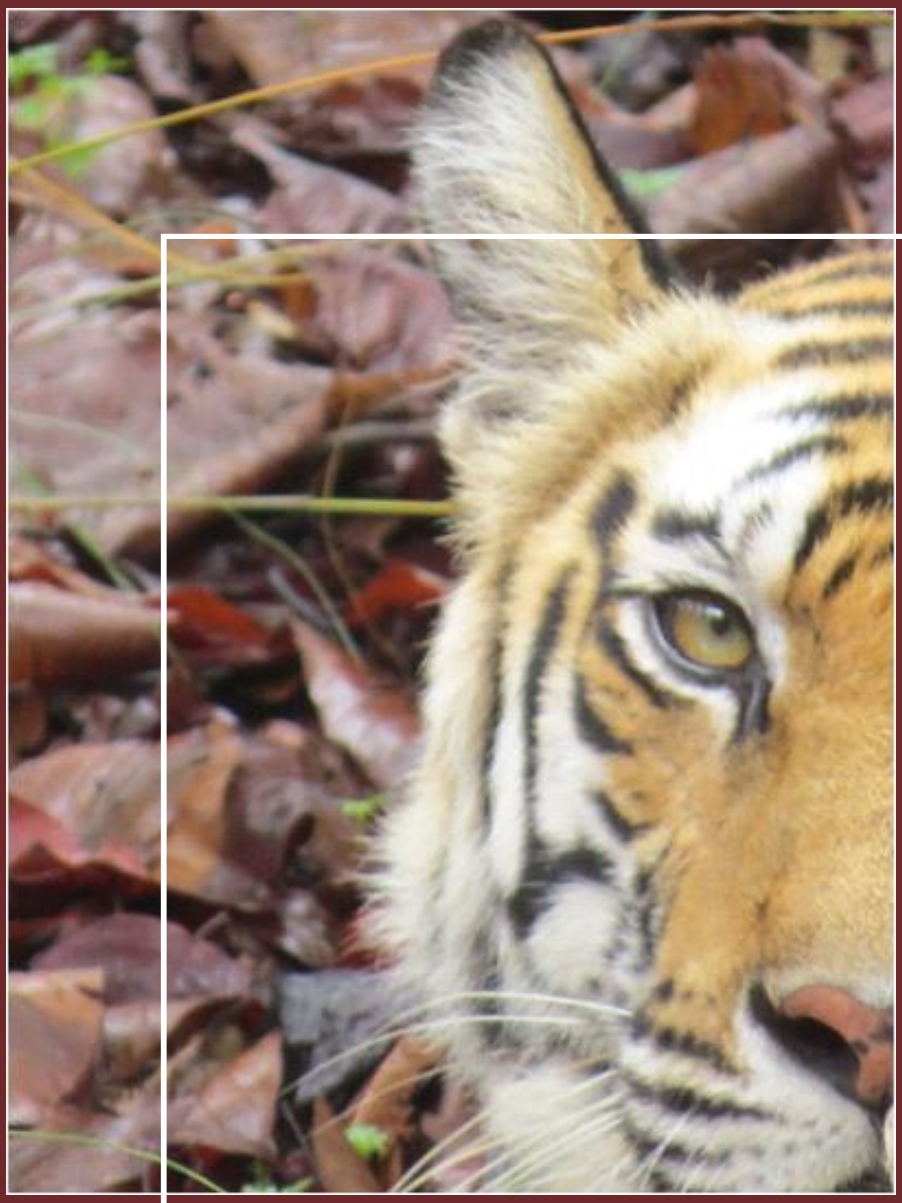

PATTERNS OF MORTALITY IN FREE RANGING TIGERS

(Technical Report 2016)

Partners:
Wildlife Institute of India
National Tiger Conservation Authority

PATTERNS OF MORTALITY IN FREE RANGING TIGERS

Technical Report 2016

[A Component of ongoing WII-NTCA Collaborative Project on "Strengthening Veterinary Interventions in Tiger Reserves"]

WILDLIFE INSTITUTE OF INDIA

Submitted to National Tiger Conservation Authority, New Delhi

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

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Front and Back Cover Photographs: Parag Nigam

Layout & Design:

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Contributors / Citation: Nigam, P., Muliya, S.K., Srivastav, A., Malik, P.K., Shrivastava, A.B. and Mathur, V.C. (2016). Patterns of Mortality in Free Ranging Tigers. Technical Report. Wildlife Institute of India – National Tiger Conservation Authority, pp.

Annexure 1

NECROPSY PROTOCOL (*PANTHERA TIGRIS TIGRIS)*

Necropsies are performed to get further insight into the cause of death and include a careful examination of the carcass both externally and internally. Necropsies generate a series of gross observations that help in establishing a diagnosis. Subsequent investigations, such as histopathology, microbiology or forensic analysis are then guided to rule out various potential causes until an etiology is established. Regardless of whether it is an anthropogenic cause or natural mortality event, by consistently conducting necropsies, trends in population health can also be monitored.

Except for tigers that are routinely monitored, carcasses are generally recovered in conditions that pose a challenge for conducting proper post-mortem examination. This is due to the fact that the environmental conditions (high ambient temperatures) result in rapid putrefaction and majority of the times the carcasses are partially/ completely scavenged before being detected. Laboratory examinations thus play key role for the eventual diagnosis in such cases. The laboratory results clearly depend on how well the necropsy is performed in field and how carefully the samples are collected, labeled, stored and transported to the laboratory.

This present protocol is designed to assist the wildlife professionals in carrying out all the procedures to support establishment of cause of death.

PRE-NECROPSY PROCEDURES

Prior to initiating any procedure, it is important to have proper planning and preparedness for conduct of PM procedures. It is imperative to have basic logistic support in terms of equipment and professionals/expertise to carry out such procedures.

Necropsy equipment:

Minimum recommended equipment to be carried in field necropsy kit are as follows:

Sn. no	Equipment	Minimal quantity
	Documentation equipment	
1.	Hand held GPS	1
2.	Digital camera	1
3.	Measuring tape	1
4.	15 cm - Plastic ruler (to be used as scale while photo-documenting lesions)	2
5.	Vernier calipers	1
6.	Portable weighing balance	1
7.	Metal detector	1
8.	Necropsy data sheets	10
9.	Clip board	1
10.	Permanent Marker	5
11.	Pencil	5
12.	Necropsy procedure and protocols	

Sn. no	Equipment	Minimal quantity
	Necropsy equipment	
1.	Sharp high quality necropsy knife	6
2.	Curved knife for skinning	1
3.	Knife sharpening stone or steel	1
4.	scalpel handles (of different sizes)	2 each
5.	Box of scalpel blades (based on handle size)	2 boxed each
6.	Small plain forceps	3
7.	Rat-tooth forceps	3
8.	Small dissecting Scissors	2
9.	Large dissecting scissors	2
10.	Artery Forceps	3
11.	Hack saw or bone saw	1
12.	Chisel and hammer	1 each
13.	Alcohol lamp or gas burner (to sterilize equipments on site)	1
	Sample collection	
1.	Disposable syringes (5 ml & 10 ml)	1 box each
2.	20G needles	1 box
3.	Ziplock bags	100 nos
4.	Large white plastic cutting boards (for cutting and photographing tissues)	2
5.	Vacutainer (EDTA Coated)	1 Box
6.	Vacutainer (Plain/ clot activator coated)	1 box
7.	Cryo vials	100 nos
8.	Sterile swabs	100 nos
9.	Glass slide	1 box
10.	Slide container	2
11.	Plastic screw cap/ tight fitting wide mouth containers (for tissue samples)	50 nos
12.	Aluminium foil	2 rolls
13.	Labels/ labeling tape	2 sheets
	Preservatives	
1.	10% buffered formalin	
2.	Absolute alcohol	
3.	70% ethyl alcohol + 2% glycerine	
4.	Viral transfer media and RNA Later	
5.	Silica crystals	
6.	Saturated salt solution	
7.	Insulated cooler box with ice blocks	

Personal safety: Personal safety should be exercised while carrying out a Post mortem examination. Personal protective equipment (PPE) should include:

1. Overalls with a washable rubber apron
2. Rubber boots
3. Face mask and goggles to cover eyes, and
4. Double gloves (rubber or plastic gloves)
5. Disinfectants (70% ethanol, quaternary ammonium compound / biguanide)

Important Considerations during Necropsy Examination (Sinha, 2010)

- Post mortem procedure is a specialist job and should be left to such person(s) who are suitably qualified for the same and have some previous experience and expertise of working with wildlife.
- Every detail of the procedure must be dutifully recorded. It is appropriate not to trust these to memory, to be committed to writing at some later stage. Recording the observation on a voice recorder or a note pad will be of great help.

NECROPSY PROCEDURES

Brief history: Accounts of health status of the animal including illnesses, recent treatments, laboratory findings, wounds, etc. should be noted in due consultation with protected area authorities. The area around the carcass should be thoroughly examined for evidences such as but not limited to presence of other tiger, signs of trauma, poisoning or anthropogenic activity, etc., to assist in establishing probable cause of death.

Assessing body condition (i) good and (ii) emasciated) © A.B. Shrivastav

Necropsy site: Efforts should be made to transport the entire carcass to the areas designated in each tiger reserve or to nearby veterinary institution for conduct of necropsy. In case this is not possible (due to remoteness of area), efforts should be made to ensure that the team carrying out the necropsy reaches the site at the earliest and conducts all procedures with utmost caution including proper biological sampling and carcass disposal.

A committee consisting of veterinarian of the reserve, a veterinarian from animal husbandry department and a trained pathologist from a recognized veterinary college should be constituted to carry out the procedure. Additionally, the team should consist of one or two trained veterinary assistants to support proper conduct of procedures.

Collection of preliminary information: Since necropsy gives a unique opportunity to gather ecological and health data, it is worthwhile to collect all possible information. The information collected should

include information on location including GPS co-ordinates and related data; time and date of necropsy examination; estimated time and date of occurrence of event; environmental conditions in the area; brief description of habitat and information for animal identification. The information for animal identification should include: age class (Juvenile, ≤ 1 Year; Sub adult 1 – 3 years; Adult 3 – 10 years or Geriatric ≥ 10 years), sex (male, female or unidentified), UTID: National Unique Tiger IDs assigned by NTCA and the Local ID (if any).

Ageing tigers: Since it is practically impossible to determine the exact age of tigers (unless they are being monitored from birth), it is advisable to document the age class. Apart from classifying animals into juvenile / cub group (based on obvious body size); the dentition and wear of teeth can be an indicator for classification into other age groups.

Tigers, similar to other felids; have fewer teeth (30) compared with canids and ursids (42) with bigger canines and shorter jaws (Kitchener et al, 2010). Teeth of tigers are exceptionally stout, with long and slightly curved canines (Mazák. 1981). The number and diversity of cheek teeth is reduced by loss of post-carnassial molars and the most anterior premolars, leaving a dentition that is highly specialized for carnivory (Kitchener et al, 2010). **The basic dental formula of felids is (I 3/3; C 1/1: PM 3/2; M 1/1) X 2 = 30 (**Mazák. 1981).

The teeth are subjected to unpredictably high load from struggling prey, which over time leads to wear and tear (Van Valkenburgh, 1988). As the age advances, tooth wearing is manifested by change in color from whitish to pale yellow and subsequently darker yellow; show retraction of gum line with gradual change in angulation of the jaws and considerable wear including missing or at times the broken tooth. In general, animals can be classified into following groups as described below.

Cubs: Tiger cubs are born toothless and their whitish deciduous (temporary) teeth come in within a week or two after birth. At about six to twelve months of age, these deciduous teeth fall out and are replaced by stronger adult teeth.

Sub adults (1 – 3 years): The permanent teeth are partially/ fully erupted, sharp, and mostly white. Only a small amount of wear can be observed on the incisors, canines, and premolars.

Adults (3 – 10 years): there is obvious wear on canines, incisors, and pre-molars. Canines are often yellowish in color with variable state of chipping in distal longitudinal ridge.

Geriatric animals (≥ 10 years), canines are often partially or completely broken showing varying degree of wear and damage.

Alternatively, gum-line recession however can be used more reliably in wildlife studies as an indicator of age for tigers. Gum-line recession is defined as the distance (mm) from the cementum - enamel junction to the gingiva (i.e., gum-line) on the left and right upper canines (Spinage, 1973). It is the exposure in the roots of the teeth caused by a loss of gum tissue and/or retraction of the gingival margin from the crown of the teeth as age advances.

(Fàbregas and Garcés-Narro, 2014)

Age (in months) = 20.47 + 12.60 X Mean gum – line recession

Based on results, animal can be then grouped into one of the four age classes, *viz.,* Juvenile, ≤ 1 Year; Sub adult 1 – 3 years; Adult 3 – 10 years or Geriatric ≥ 10 years.

Determination of gender: If the carcass is not detected early enough, putrification and scavenging might lead to difficulty in identification of the sex of tiger. Pugmark analysis suggesting the concept of square and rectangular frame in male and female respectively has served as a cost-effective, reliable, and accurate tool for monitoring sex ratios of free ranging tigers (Sharma et al., 2003). Thus, if a clear pugmark is available on a flat surface near the carcass, it could be used to identify the sex of dead tiger. However, more accurate method would be forensics based examinations, wherein PCR amplification using a set of primers would help determine the sex of the tiger reliably (De candia et al., 2016).

Thus, in cases where sex of the tiger is uncertain, tissue samples should be collected and stored in 90% ethanol. The tissue should be completely immersed in ethanol and transported to designated forensic laboratories within 5-7 days.

Recording vital morphometric measurements: Vital morphometric measurements such as body size and size of body structures are intimately related to life history and ecology of species (Temerin et al. 1984). Analysis of accumulated morphological data over period can aid in various ecological studies, including age estimation, gender identification and identifying dietary strategies (Athereya and Belsare 2008; Heymann et al. 2012). Since immobilizing tigers solely for the purpose for obtaining such data is not feasible, opportunities such as necropsy examination should be full utilized to collect such data. The minimum measurements to be documented in each carcass should include canine lengths (upper and lower), inter-canine width, gumline recession, length and breadth of forefoot paw (right and left), a body weight, height and length. Since body measurements are accurate only if taken from fresh carcass, bloated and decomposed carcass should always be excluded.

External Examinations: Actual necropsy procedure begins with thorough external examination, that can support and lead to some key findings. These are briefly discussed below.

1. Necropsy interval: Estimation of time of death (necropsy interval (NI)) is crucial as its influence the appropriateness of the conduct of necropsy procedures. As many factors operate soon after death, post mortality changes vary to a great extent in each individual. Rather than using one particular parameter, combination of parameters may be considered to allow estimation of necropsy interval with reasonable accuracy (Oates et al., 1984).

Evidence for estimating NI may come from “Carcassial evidence” i.e. evidences that are present in the body such as rigor mortis, state of decomposition, presence of insects (forensic entomology) etc. Additional information from environmental and associated evidences (that are present in the vicinity of the body) and anamnestic evidence (in monitored animals, evidences based on the animals movements, last known sighting and location etc) may help in assessing the NI.

2. Rigor mortis: Rigor mortis is one of the recognizable signs of post mortem changes, its onset and duration is governed by temperature (lower ambient temperatures accelerates the onset of rigor and prolong its duration, whereas the opposite is found in warmer temperatures), metabolic state of the body (vigorous activity immediately prior to death) and prior disease if any. Even though time of appearance and disappearance of rigor mortis cannot be generalized, it can be still used to establish an estimate range. Typically, the stages observed over time lapse based on Oates and co-workers (1984) are as follows:

	Stage	Time lapse since mortality event
1.	Carcass is fresh and rigor mortis is absent (flaccid state)	> 1 hrs
2.	Rigor setting in	1 to 6 hrs
3.	Rigor complete	6 - 24 hrs
4.	Rigor passing off	12 - 36 hrs

3. Changes in the eye: Following death the eye luminosity, color of pupil, and its constriction can form basis for estimating NI. At death, the cornea and eye fluids are transparent and the surface of the eye is

tight and smooth. The transparency and luminosity decrease as the intraocular fluids become cloudy. A basis to estimate NI in White tailed deer using the technique described by Gill and O'Meara (1965) may be used to estimate the NI with suitable modifications taking due considerations of the environmental factors, anatomical peculiarities and metabolic rate of the animal.

Hours	Changes in eyes
0.5	Eye lens and fluids fully transparent Light reflected from within the eye appears brilliant luminous green Pupil size however depends upon the prevailing light intensity at time of death. Pupil fully dilated shortly after death as the muscles are relaxed .
0.5 - 6	Lens and fluids remain transparent Luminosity and color may decrease slightly Pupil fully diluted and is almost round (as rigor progresses the pupil gradually flattens).
6 - 10	Colour changes toward gray Luminosity fades Pupil width may decrease to about one half original diameters.
12 -18	Colour fades to dull gray Luminosity fades away Pupil may narrow to one-third or less of original diameter.
30 and above	Color and pupil diameter remain the same. Hazy blue color appears over brown colored iris after about 48 hours. Normally on the third to sixth day after death, the eyeball partially collapsed

Additionally, changes in the skin (color and elasticity), mucus membranes (color), level of putrefaction, bloating and discharges from natural orifices can form basis in establishing NI.

4. State of decomposition: Decomposition occurs due to enzyme breakdown of tissues (autolysis), and bacterial activity (putrefaction). The process is known to be affected by ambient temperature, weather, size of the body, body composition, age and health of the animal (Brown, 2009). In general, carcasses can be assigned to following stages, based on state of decomposition.

Stage of carcass	Changes observed
Fresh	• Characterized as the beginning of decomposition immediately following death. • There are relatively few changes occurring during the fresh stage and the odor associated with the remains will still be the natural smell of the body (Parsons, 2009). • The fresh stage continues until the first signs of bloating begin, which is highly variable depending on the external environmental temperatures and conditions.
Early decomposition	• Early decomposition is marked by beginning of bloating in abdomen region and the skin begins to take on a green discoloration. • During the bloating period, the body begins to purge decompositional fluids and blood is released from the natural orifices (Terneny, 1997). • Bloating can occur rapidly in warm temperatures and last 2-5 days, however, it is extremely temperature dependent (Parsons, 2009).
Advanced decomposition	• Advanced decomposition starts with end of bloating phase; the tissues will sag and the abdominal cavity will have a sunken appearance. • Skin will take on a “wet” appearance where the liquefaction and disintegration of tissue begins (Payne, 1965). • The abdominal cavity will remain moist while other areas of the body such as the extremities will exhibit mummification or partial skeletonization depending upon external environmental conditions (Megyesi, 2005).

Stage of carcass	Changes observed
	• The odor of decay during this phase is strong and putrid and can be detected over long distances (Parsons, 2009).
Skeletonization	• Early phases of this stage are identified when the majority of soft tissue has decomposed or when mummified tissue begins to break down to reveal bone (Megyesi, 2005). • Odor is minimal and takes on a musty or moldy smell. • It can take place as quickly as two weeks in hot and humid environments but takes much longer to reach in areas characterized by cold and dry climates (Parsons, 2009).

Additional information: The condition of the heart may also be used as an indicator of the time of death and degree of postmortem change. The presence of unclotted blood in the left ventricle usually means that death was recent and rigor mortis has not yet set in. A disintegrating clot and dark haemolysed blood in the left ventricle is seen when an animal has been dead for 24 hours or more. This indicates that rigor mortis has occurred and that extensive post-mortem changes can be expected.

Fresh Carcass

Early Decomposition

Advanced Decomposition

Skeletonization

5. Forensic entomology: Forensic entomology intends to establish the time of death or NI by precisely showing how long the carcass has actually been exposed in the environment. Identification of the different species of insects and stages there off (e.g. eggs, larvae, pupae, maggots, flies and beetles) which are attracted to carcass are generally used for this purpose. Furthermore, insects may serve as important alternative species for toxicological analysis in cases where human samples are not available for this purpose (Verma and Paul, 2013; Bhat et al., 2011; Jadav & Sate, 2015)

The two most important species in determining NI are flies (Diptera) and beetles (Coleoptera). Diptera are the first insects to be attracted to and to colonize decomposing remains. It is the Dipteran larvae (maggots), which are responsible for much of the removal of decaying tissue. Following this initial stage and after the remains have dried out, most of the other insects, notably the beetles become involved in the process. The diversity and occurrence of insects on a carcass have been effectively documented by Jadav and Sate, (2015) and the details are provided below.

Insect species	Families	Sequence of occurrence on carcass
Sarcophaga lineatocollis Macquart	Sarcophagidae	Within 6 hours, fresh stage
Stomoxy scalcitrans Linnaeus	Muscidae	Within 6 hours, fresh stage
Chrysomya rufifacies Macquart	Calliphoridae	After 24 hours, bloated stage
Lucilia sericata Meigen	Calliphoridae	After 24 hours, bloated stage
Calliphora vicina Robineau- Desvoidy	Calliphoridae	After 24 hours, bloated stage
Necrophila rufithorax Selys	Silphidae	After 2-3 days
Dermestes maculatus (De Geer)	Dermestidae	At decay and dry stage
Necrobia rufipes (Fabricius)	Cleridae	After 2-3 days
Creophilus sp.	Staphilinidae	After 3-4 days
Hister sp.	Histeridae	After 3-4 days
Saprinus sp.	Histeridae	After 3-4 days

The growth rate of the maggots can be affected by changes in the temperature, geographic location, exposure to sun or shade, time of day and season, humidity, and rain (Hogon, 1999). As it is unlikely that a qualified forensic entomologist will actually be present on site for collection and documentation activities, it is therefore essential that the field veterinarians are well versed with various aspects of entomological documentation and collection procedures provided by Byrd et al., (2001).

6. Other relevant information: Additional information including nutritional status, presence of external injuries or marks should also be collected. All mucous membranes, including oral, nasal, conjunctiva, anus, vulva or prepucial mucus membranes should be thoroughly examined for any discoloration, discharges and presence of any abnormalities.

7. Classifying wounds: Wounds are known to act as either contributing factor or sometimes even primary cause of mortality in wild animals. A detailed inspection of the wound can lead to conclusive diagnosis in wide variety of cases, such as death due to gunshot, electrocution or natural mortality due to wound sustained from other tiger/ carnivore or prey species. A preliminary step in this process is to differentiate ante-mortem injuries from postmortem injuries. Major differences between the two are provided below.

Ante – mortem wounds	Post – mortem wounds
Evidence of copious hemorrhage	Hemorrhage minimal or absent

Wound edges gape due to normal elasticity and appear swollen and everted.	Edges do not gape due to loss of elasticity and appear in a closely approximated state.
Evidence of blood spurting	Spurting of blood does not occur
Firmly clotted blood	Blood is either clotted or liquefied
Tissue response might be observed if wound is old	Absence of tissue response

To aid in differential diagnosis, wounds should also be further classified based on their aetiology, location and type of injury, the details of which are provided below.

i. Bruise

External bruising is less common however skin and internal bruising from severe blunt force / trauma have frequently been encountered. These may result from vehicular collissions or when animal has been struck by blunt articles).

ii. Abrasions

These wounds result from friction applied approximately parallel to the skin's external surface. These are characterized by removal of variable amounts of the epidermis, dermis and hypodermis. Abrasions are typically superficial, trivial injuries, which bleed minimally and heal quickly leaving no scar.

iii. Punctur wound

A puncture wound has a depth greater than its length on the skin surface. Based on thickness and penetration, they are classified as either perforation wound (having both an entrance and an exit) or a penetrating wound (that has only an entrance).

iv. Incised wound

An incised wound is a breach of the skin resulting from contact with a clean and sharp edge objects. Penetration of full thickness of the skin is usual, but shallow, partial-thickness skin cuts can also occur.

v. Gunshot wound

A gunshot wound is a penetrating wound where the projectile passes through the skin. The wound has an entrance and exit, with later being larger and more irregular than entry. The exit wound may be absent if the bullet gets trapped inside the body.

vi. Degloving wound

A degloving wound is a type of avulsion injury in which an extensive section of skin is completely torn off the underlying tissue. Degloving injuries typically occur when an animal is pushed or dragged by a moving

vehicle or when an animal is trapped in snare/jaw trap, tries to free it selves by haphazardly pulling its trapped leg.

vii. Burns

Burn injuries may result from electric current passing through the body. Very often, a blister or burn, similar to the shape of the conductor (wire or fence) is seen at the contact site, confirming the diagnosis.

Internal examination

Though there is no single protocol applicable in all situations (as carcasses are usually found in varied state of decomposition and may be even incomplete due to scavenging), efforts should be made to use the opportunity to carry out as comprehensive post mortem examination as possible. It should include detailed examination of each organ system with proper recording of information as per the format provided. The format provided in the report has been adapted from the NTCA's SOP and includes minor additions that further support better information retrieval. Due emphasis has been given on proper sampling, preservation and transport. The format duly modified from existing format (NTCA) for recording of information during the post mortem examination is provided below:

RECORD OF NECROPSY EXAMINATION

Species: ***Panthera tigris tigris*** **Date:**

Common name: Bengal Tiger

A. LOCATION & EVENT INFORMATION

Protected Area/ Nearest Protected Area:		
If Inside Protected Area		**If Outside PA Protected Area**
Latitude: Longitude:		Latitude: Longitude:
Compartment:		Village:
Beat:		Tehsil:
Range:		District:

Date and time of Necropsy	Date: Time:	Estimated date and time of mortality	Date: Time:	Basis of estimation
Time of carcass acquisition by officials:		Time of carcass disposal:		
Ambient temperature:		Weather:		
Area/ Habitat description:				

B. ANIMAL IDENTIFICATION INFORMATION

Age group:	Juvenile (≤ 1 Year) ☐	Sub adult (1 – 3 years) ☐	
	Adult (3 – 10 years) ☐	Geriatric (≥ 10 years) ☐	
Sex:	Male ☐	Female ☐	Uncertain ☐
UTID*:	Local ID:		Unidentified ☐

*Permanent National ID as per "protocols for the establishment of a national repository of camera trap photogrphs of tigers".

C. CLINICAL AND PATHOLOGICAL OBSERVATIONS

I. Brief History

1. **Medical history before death (if any):**

2. **Relevant observations around carcass:**

II. Vital Measurements

1. Right fore paw dimensions:	Length X Width ……cm X……cm		
2. Left fore paw dimension:	Length X Width ……cm X……cm		
3. Canine dimensions:	Upper Right …….cm	Upper Left ……cm	Missing Broken UR ☐ ☐ UL ☐ ☐ LR ☐ ☐ LL ☐ ☐
	Lower Right …….cm	Lower Left ……cm	
Gum Line recession:	Upper Right …… mm	Upper Left ……..mm	
4. Inter canine width:	Upper ……..……..cm	Lower ……..……..cm	
MorphometricMeasurements (Columns below to be filled only if carcass in fresh (Not bloated/putrified)			
5. Body Weight	……..kg		
6. Body measurements	Body length (Nose tip to base of tail) …….cm	Tail length…….cm	
	Chest girth …….cm	Neck Girth……..cm	
	Shoulder height….cm	Hind leg ……cm	

III. External Observation

Rigor mortis:	Absent ☐	Setting in ☐	Complete ☐	Passing off ☐
Carcass condition:	Fresh ☐	Refrigriated ☐	Decomposed ☐	Incomplete ☐
State of Decomposition:	Fresh ☐	Bloated ☐	Putrified ☐	Skeletonised ☐
Physical condition:	Normal ☐	Obese ☐	Lean ☐	Emaciated ☐
External wounds:	Absent ☐	Ante-mortem ☐	Postmortem ☐	
Wounds (if present**):**	Bruice ☐	Abbresion ☐	Puncture ☐	Incision ☐
	Gunshot ☐	Blunt force ☐	Degloving ☐	Burns ☐
Wound Healing status (if present**):**	Fresh ☐	Infected ☐	Healing ☐	Healed ☐
Fractures:				
Detailed description of injuries (if present):				
Result of metal detector scanning:				
External orifices:		**Mucous membrane:**		

IV. Internal Examination

System	Appearance, color and observations
A. SUBCUTANEOUS TISSUE	
B. BODY CAVITIES 1. Position of visceral organs 2. Peritoneal Cavity 3. Pleural cavity and Pleura 4. If fluid present in the Peritoneum or thorax: Color, transparency and amount	
C. RESPIRATORY SYSTEM 1. Larynx 2. Trachea 3. Lung parenchyma 4. Diaphragm 5. Lymph nodes	
D. CIRCULATORY & LYMPHATIC SYSTEM 1. Pericardial SAC 2. Heart Muscle 3. Heart Chambers a. Right Atrium b. Left Atrium c. Right Ventricle d. Left Ventricle 4. Blood Vessels 5. Spleen 6. Lymph nodes	
E. HEPATIC SYSTEM 1. Liver 2. Liver Parenchyma 3. Gall Bladder	
F. DIGESTIVE SYSTEM	

1. Pharynx 2. Esophagus			
3. Stomach	Cardiac zone		
	Fundus		
	Pylorus		
	Contents		
4. Small intestine	Duodenum		
	Jejunum		
	Ileum		
	Contents		
5. Large intestine	Ceacum		
	Colon		
	Rectum		
	Contents		
G. UROGENITAL			
1.Urinary Bladder			
2.Kidneys		Left	Right
Capsule:			
Cortex:			
Medulla:			
3. REPRODUCTIVE ORGANS	Testes ☐		
	Ovary ☐		
	Penis ☐		
	Uterus ☐		
H. LYMPH GLANDS			
I. HEAD 1. Buccal Cavity 2. Nasal Orifices 3. Tongue 4. Submandibular/ Maxillary lymph nodes			
I. NERVOUS SYSTEM 1. Brain 2. Spinal cord			
J. SKELETAL SYSTEM			

V. Record sheet for parasites

Body Region	Parasite Type	Yes / No	Location	Description
Subcutaneous tissue/ Muscles	1. Worms 2. Cysts 3. Other			
Body cavities	1. Cysts 2. Worms			
Respiratory system	1.Lungworm 2.Cysts 3.Other			
Liver and Gall bladder	1.Flukes 2.Tape worms 3.Round worms 4.Cysts			
Heart and blood vessels	1. Worms 2. Blood flukes 3.Cysts			
Digestive system	1.Tapeworms 2. Round worms 3. Cysts			
Urogenital organs	1. Worms 2.Others			
External parasites	1. Tick 2. Flea 3. Lice 4. Mites			

VI. SUMMARY

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PROVISIONAL DIAGNOSIS: .....

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PLACE:
DATE :

SIGNATURE:
NAME :
DESIGNATION:
ADDRESS:

PLACE:
DATE :

SIGNATURE:
NAME :
DESIGNATION:
ADDRESS:

VII. Checklist for specimen collection for laboratory Analysis

S. No	Examination required	Preservative used	Specimen	Designated laboratory

PLACE:

DATE :

SIGNATURE:

NAME :

DESIGNATION:

ADDRESS

VIII. Photographs

Whole carcass as observed before necropsy	
Left lateral view	
Right Lateral View	

Oral cavity (Close up view)	
Gross lesion of major visceral organs (Heart, lungs, liver, spleen, GI tract, kidney, brain etc (including lymph nodes)	

Photographic records: Photographs serve as a backup to the written descriptions on the necropsy record. They also allow pathologists to assess gross lesions/observations in relation to samples sent for analyses. It is worthwhile to take at least one full body photograph of both the left and right flank of the carcass prior to initiating PM as it would aid in identification of the dead tiger based on stripe pattern. Additionally, photograph all the specific lesions, (the entire organ, with reference to its anatomical location) as well as a closer shot of the particular lesion (preferably on the cutting board, with a scale to depict details of the abnormality). These need to be part of the PM report.

Sample collection: Determination of the cause of death in wild animals is often difficult and requires laboratory support. Proper sampling is crucial and integral part of the any mortality investigation. All the specimens must be collected with utmost precaution, preserved and packed in suitable conditions, appropriately labelled, and transported to the designated laboratory by the fastest route possible.

As the quality of samples collected diminishes with the progression of the decomposition and creating greater uncertainty with results, it is important to prioritize sample collection, based on the condition of carcass at the time of sample collection.

- Samples from fresh carcass can be used for histology, cytology, microbiology (culture and PCR), virology (tissue), parasitology, toxicology, and genetics.
- Samples from moderately decomposed carcass (early decomposition) can be used for histology (limited value), microbiology (PCR), virology (PCR), parasitology, toxicology, and genetics.
- Samples from carcass with advanced decomposition can be used for histology (limited/ absent) virology (PCR), and genetics.
- Mummified/Skeletal Remains can be used for toxicology (limited), and genetics.

Diagnostic Activity	Type of Specimen	Preservation Method	Type of Container	Comments
Histopathology	Tissues and lesions	10% buffered formalin	Wide mouthed, Leak-proof glass or plastic	Sections no more than ¼ inch thick. Ratio of 10:1 formalin to tissue. Storage at room temperature.
Haematology	Whole blood in anticoagulant	Refrigeration	Glass or plastic tubes	Gently rotate tubes to mix blood with anticoagulant. Generally short storage
Serology	Blood and Serum	Refrigeration or freezing of serum/ portion of blood or in merthiolate	Clean, dry glass or plastic vials	Handle gently to avoid rupture of red cells. Transfer serum to separate container before freezing.
Virology	Organs, tissue, lesions or body fluids	50% glycerol saline or Phosphate Buffer saline (PBS)/Hank's balanced salt solution	Sterile plastic/glass containers	Care to avoid contamination is critical. Appropriate sampling varies with different diseases
Bacteriology	Whole blood, Organs, tissue, lesions, or body fluids, swabs	Usually refrigeration/on ice	Sterile plastic/glass containers	Care to avoid contamination is critical. Appropriate sampling varies with different diseases
Mycology	Hair sample & skin scraping Tissues/Deep skin scrap	At room temperature Usually refrigeration or freezing	Sterile plastic/glass containers	Care to avoid contamination is critical. Appropriate sampling varies with different diseases
Parasitology	Worms	5% formalin	Glass or plastic container	Storage at room temperature
	External parasites	70% alcohol or 5% formalin	Glass or plastic container	Storage at room temperature
	Blood parasites	Air dried blood films or whole blood in anticoagulant	Glass slides or tubes	Blood slides stored at room temperature. Tubes of whole blood refrigerated
Toxicology	Organs, fat, blood and ingesta or suspected contaminated foods	Refrigeration or freezing, Methanol, saturated salt solution	Clean glass, plastic, or metal foil	Accurate records are critical. Appropriate sampling varies with suspected toxin.

Diagnostic Activity	Type of Specimen	Preservation Method	Type of Container	Comments
	larvae, maggots, pupa and other entomological samples (In highly putrefied carcasses)	Refrigeration or freezing, Methanol, saturated salt solution	Clean glass, plastic containers	Accurate records are critical. Appropriate sampling varies with suspected toxin.
Forensic entomology	larvae, maggots, pupa and other entomological samples	80% ethyl alcohol	Clean, airtight glass or plastic containers	Associated habitat, photographic and metrological data should accompany samples
Ballistics	Wound swab (collected with cotton tip applicator))	Cotton tip applicator should be moistened with isopropyl alcohol or 5% nitric acid before collection.	Airtight zip lock pouches.	Accurate records and photo documentation is critical.
	Projectiles (Shrapnel, bullet fragments)	Without any preservative		
Genetics	Tissue, bone, hair samples.	90 % ethanol or Silica gel	Airtight containers	Storage at room temperature

All specimens should be clearly numbered and labeled. All relevant information should accompany the specimens and recorded appropriately in designated section of the Necropsy Record form.

Carcass Disposal: After collection of all the required samples, the carcass should be immediately disposed as per "Standard operating procedure for disposing the tiger/ leopard carcass/body parts" vide advisories of the Ministry of Environment & Forests/ Project Tiger/ NTCA on the subject (Advisory No: 1-60/89-WL I dated 04-11-1994 from the Addl. IGF (wildlife) Ministry of Environment and Forests). Available at http://projecttiger.nic.in/writereaddata/cms/sop_carcass-disposa25feb2013.pdf.

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Annexure 2

Necropsy Observations in Poisoning Cases

In a case of suspected poisoning, specimens should be collected in duplicates and placed in sealed containers, with suitable preservatives (as elaborated in ANNEXURE 1) taking due consideration of legalities. Further, detailed necropsy examination should be conducted and pathological observations made should be recorded accordingly. Photographs of the affected animals and the surroundings should be taken for future reference. In all legal cases, an accurately documented chain of custody of specimens and a thorough record of the investigation and related analyses and other diagnostic methods and results must be maintained (Cheeran, 2007).

Observations made during some commonly used poisons are as follows.

ORGANOCHLORINE COMPOUNDS

Organochlorine Insecticides (also known as chlorinated hydrocarbons) are mainly characterized by, presence of carbon (organo-), chlorine and hydrogen and sometimes oxygen atoms including a number of C-Cl bonds.These are contact insecticides and ectoparasiticides, which act on central nervous system, producing hyperactivity and tremor and occasionally convulsions.

Examples: DDT (*Dichloro Diphenyl Trichloroethane*), HCH (*hexachlorocyclohexane*), aldrin, dieldrin, endosulphan, etc.

Necropsy findings: Cyanosis**;** blood tinged froth in the respiratory system**;** pulmonary oedema**;** congestion in the lungs, liver and kidney**;** and dead maggots/ flies in and around fresh carcass (Cheeran, 2007).

Material required for laboratory analysis: Gut contents, fat, kidney, liver, brain and suspected poisoned prey carcass (if any) should be sent for laboratory analysis.

Preservative: Freezing is the preferred way of preservation. Alternatively, 50% ethanol (1 ml/g/tissue) or Saturated salt solution (specimen should be completely submerged) can be used incase freezing facilities are not available

ORGANOPHOSPHORUS COMPOUNDS

Organophosphorous insecticides (often refer to as organo phosphates) are neutral ester or amide derivatives of phosphorous acids carrying a phosphoryl (P-O) or thiophosphoryl (P-S) group. They are commonly used as insecticides, acaricides, soil nematicides, fungicides and in a variety of similar products and act by inhibiting cholinesterase activity, causing acute muscarinic manifestations.

Examples: Chlorfenvinphos, chlorpyriphos, coumaphos, demeton, diazinon, dichlorvos, dimethoate, ecothiophate, fenthion, malathion, methyl parathion, Schradan, andtrichlorfon.

Necropsy findings: Brick red in coloured visible mucus membrane; pulmonary oedema, congestion, cyanosis, frothy discharges in trachea and bronchial trees; haemorrhages and oedema of the heart, bowel as well as other organs; cerebral oedema; and necrotic patches on the skeletal muscles. The

intestinal tract will be usually dilated and fluid filled, with partially digested or undigested meat (Kalaivanan et al., 2011; Cheeran, 2007; Allwin et al., 2015).

Material required for laboratory analysis: Gut contents, visceral organs and suspected poisoned prey carcass (if any) should be sent for laboratory analysis.

Preservative: Freezing is the preferred way of preservation. Alternatively, 50% ethanol (1 ml/g/tissue) or Saturated salt solution (specimen should be completely submerged) can be used in case freezing facilities are not available

CARBAMATE COMPOUNDS

Carbamate are anticholinesterase insecticide and are synthetic derivative of physostigmine, also known as eserine, which is a principle alkaloid of plant, *Physostigma benenosum*, calabar bean. These are are commonly used to control or destroy insects, arachnids and nematodes.

Examples: Carbaryl, Methomyl, Propoxur, Aldicarb.

Necropsy findings: Generalized congestion and cyanosis, symptoms similar to organophosphorus poisoning (Cheeran, 2007; Venkataramanan et al., 2008).

Material required for laboratory analysis: Gut contents, visceral organs and suspected poisoned prey carcass (if any) should be sent for laboratory analysis.

Preservative: Freezing is the preferred way of preservation. Alternatively, 50% ethanol (1 ml/g/tissue) or Saturated salt solution (specimen should be completely submerged) can be used incase freezing facilities are not available

PHOSPHORUS

Yellow phosphorus is used as a common ingredient in rodenticides and is easily available in the market. It is a protoplasmic poison, strong irritant, corrosive and has a necrotising effect on the stomach mucosa.

Necropsy findings: The gastric contents will have typical garlic odour and could be phosphorescent; signs of severe gastroenteritis, fatty liver, multiple hemorrhages and black tarry blood that does not clot; signs of severe hepatitis, evidence of jaundice and renal damage could be present (Cheeran, 2007).

Material required for laboratory analysis: Gut contents, visceral organs and suspected poisoned prey carcass (if any) should be sent for laboratory analysis.

Preservative: Freezing is the preferred way of preservation. Alternatively, 50% ethanol (1 ml/g/tissue) or Saturated salt solution (specimen should be completely submerged) can be used incase freezing facilities are not available

ZINC PHOSPHIDE

Zinc phosphide is a common ingredient used in rodenticides. Upon ingestion, it reacts with dilute acids in the gastrointestinal tract, producing phosphine which enters the blood stream to cause toxicity.

Necropsy findings: The gastric contents will have a characteristic “dead fish” or acetylene odour due to emission of phosphine gas; hemorrhagic gastroenteritis, generalized visceral congestion and pulmonary oedema may also be present (Cheeran, 2007).

Material required for laboratory analysis: Gut contents, visceral organs and suspected poisoned prey carcass (if any) should be sent for laboratory analysis.

Preservative: Freezing is the preferred way of preservation. Alternatively, 50% ethanol (1 ml/g/tissue) or Saturated salt solution (specimen should be completely submerged) can be used incase freezing facilities are not available

STRYCHNINE

Strychnine is an alkaloid obtained from the seeds of the tree *Strychnos nuxvomica* (or seeds of a climbing shrub *Strychnos ignati*). It inhibits competitively and reversibly the inhibitory neurotransmitter glycine in the spinal cord and the medulla and causes death by asphyxia combined with acidosis.

Necropsy findings: Rigor mortis sets in fast due to muscular contractions; cyanosis and asphyxia, subcutaneous or intramuscular hemorrhages may be seen (Cheeran, 2007).

Material required for laboratory analysis: Gut contents, visceral organs and suspected poisoned prey carcass (if any) should be sent for laboratory analysis.

Preservative: Freezing is the preferred way of preservation. Alternatively, 50% ethanol (1 ml/g/tissue) or Saturated salt solution (specimen should be completely submerged) can be used incase freezing facilities are not available

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