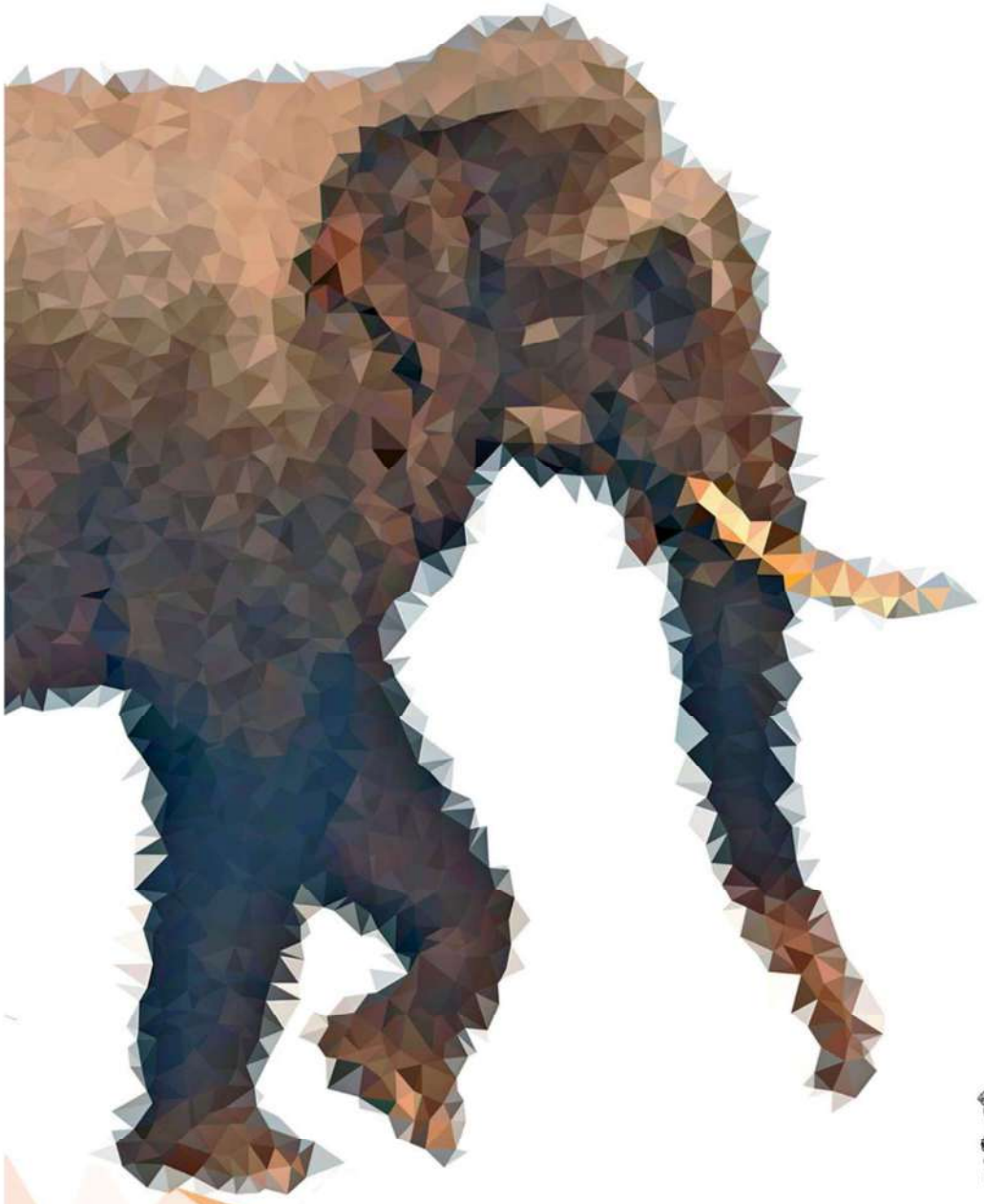
NECROPSY AND CARCASS DISPOSAL OF ASIAN ELEPHANT

RECOMMENDED OPERATING PROCEDURE



Project Elephant Division
Ministry of Environment, Forest and Climate Change
Government of India
2023

NECROPSY AND CARCASS DISPOSAL OF ASIAN ELEPHANT

RECOMMENDED OPERATING PROCEDURE

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Ministry of Environment, Forest & Climate Change

PROJECT ELEPHANT GOVT. OF INDIA

Project Elephant Division
Ministry of Environment, Forest and Climate Change
Government of India
2023

ISBN: 81-85496-82-5

Photo Credits (Cover and Section breaks): *Jayjit Das, Ritesh Vishwakarma, Devvrat Singh, Cheryl Laite, Parag Nigam*

Printer: Saraswati Press, Green Park, Niranjanpur, Dehradun, UK.

Suggested Citation: PE-MoEF&CC- WII (2023). Necropsy and Carcass Disposal of Asian Elephant: Recommended Operating Procedure. Published by Project Elephant Division, Ministry of Environment Forests and Climate Change, Government of India and Wildlife Institute of India. Pp.120

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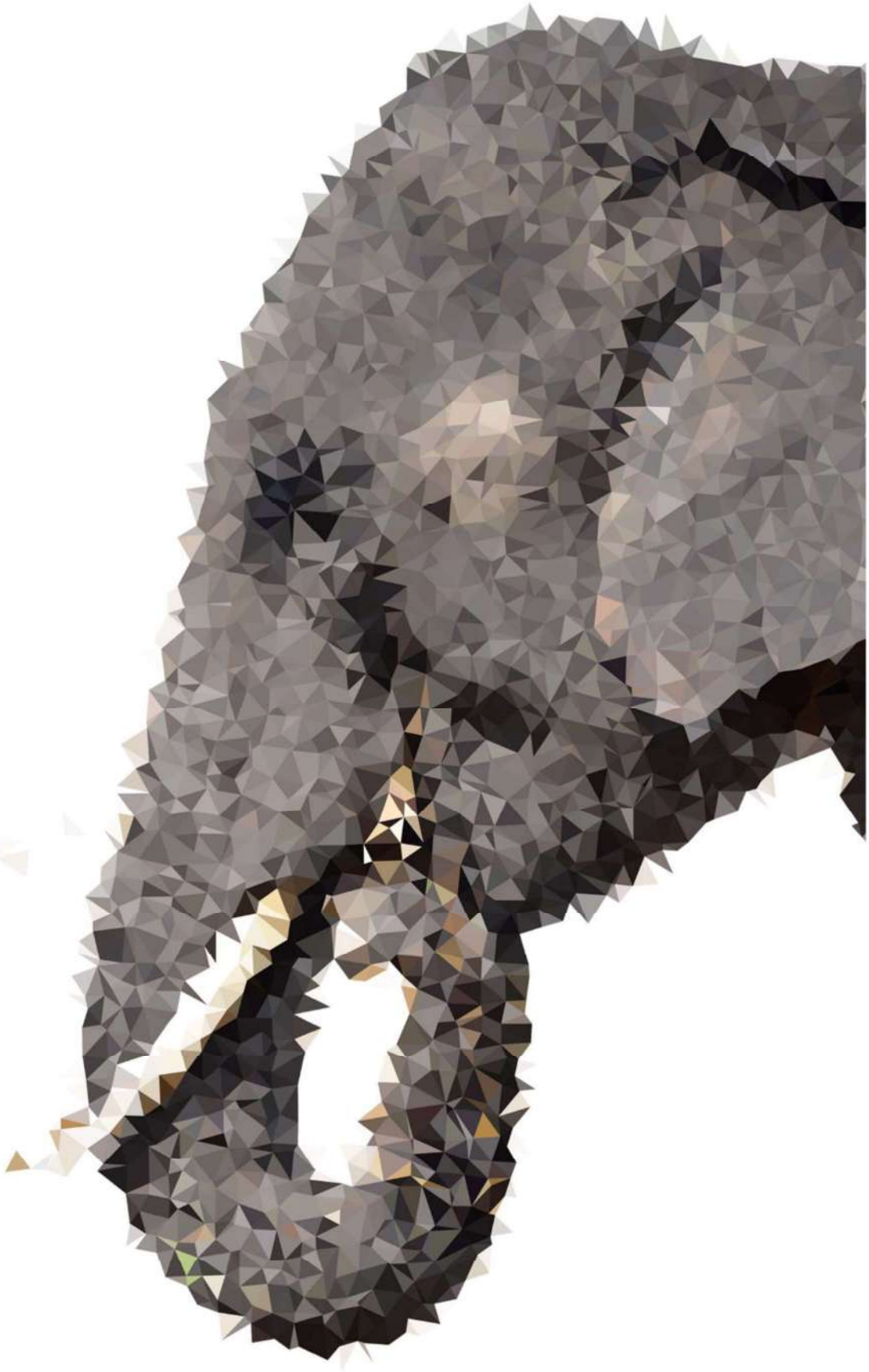

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GOVERNMENT OF INDIA

स्वच्छ भारत
एक कदम स्वच्छता की ओर

भूपेन्द्र यादव
BHUPENDER YADAV

आज़ादी का अमृत महोत्सव

FOREWORD

Elephants enjoy the highest legal protection in India and they are revered as cultural icons. They are recognized as flagships in biodiversity conservation. Strong legislations, favourable public opinion, and enforcement have subjugated ivory poaching, which, outside our country remains a major conservation threats for elephants. Detailed necropsy investigation and appropriate carcass disposal are integral processes to detect any emerging challenges. A thorough necropsy examination can also provide crucial insights on natural population cycles, emergence of infectious diseases, and response of elephant populations to environmental conditions. Thus, the importance of conducting a systematic necropsy cannot be undermined. However, being the largest land mammal with anatomical peculiarities, conducting the necropsy and disposing the carcasses is challenging especially in areas with limited veterinary capacities. Even in areas with sufficient infrastructure, lack of relevant resource material for reference and use in the field continues to be a major gap.

I am glad that the Project Elephant Division and the Elephant Cell at Wildlife Institute of India have made earnest attempts to fill this gap by bringing out a comprehensive 'Recommended Operating Procedures' to effectively conduct elephant necropsy and dispose the carcasses safely. The effort is very timely and I appreciate the efforts from the authors in putting this document together. I congratulate the Project Elephant Division of the MoEFCC for steering this activity.

Date: 28 **.03.2023**

(Bhupender Yadav)

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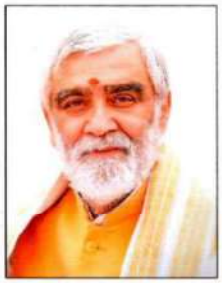
एक कदम स्वच्छता की ओर



FOREWORD

In India, elephants and people share a unique and long-lasting bond, where elephants are revered as a symbol of prosperity. They are cultural icons and omnipresent in the art, literature, mythology and folklore of the nation. India holds a special place for elephants, and they are declared as the country's National Heritage Animal. The cultural reverence of elephants is just not rhetoric, but also translates into on-ground conservation action. India holds the largest population of Asian elephants in the world. More than 60% of the wild populations occur in the country. More importantly, the elephant population in India has remained highly stable and even increased in a few landscapes. We have very strong legislation and political will to conserve elephants. In fact, nature conservation and compassion towards other forms of life has been hardwired in the country.

India has also been advancing aspects of scientific management in management of elephant landscapes and addressing human–elephant conflict in the frontier areas. The MoEFCC has been steering several activities in collaboration with the State Forest Departments and the associated Institutions with an objective of improving Institutional capacities in elephant management. As part of these ongoing efforts, the Project Elephant and the Elephant Cell at Wildlife Institute of India have come up with a very important manual that elaborates the necropsy process and carcass disposal therein pertaining to elephants. As elephants are massive animals, safely carrying out necropsy to properly investigate the cause of mortality requires training, skills and theoretic understanding. This comprehensive manual would serve as an important tool and guiding material for the frontline force including that of the veterinarians, forest officials and the support staff. I congratulate the authors of the manual and the Project Elephant for their dedicated efforts in bringing out this important manual.

(Ashwini Kumar Choubey)

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MINISTRY OF ENVIRONMENT, FOREST
& CLIMATE CHANGE

FOREWORD

Being a global leader in conservation of iconic large mammals like the Asian elephants, India has always embraced science and technological advancements in wildlife management – The country is using the best of science available to estimate populations of tigers, elephants, and other endangered animals. Modern technologies like the GPS-satellite telemetry tracking is being increasingly used to monitor wildlife populations. Forensic science has also improved significantly enabling fast and effective detection of wildlife-related crimes. In addition to these scientific advancements concerning live animals, the importance of understanding aspects of wildlife mortality cannot be underestimated. Thus, impetus is being placed on systematic mortality investigation of wild animals so that preventive and corrective action, as appropriate, may promptly be taken in case of rare and endangered animals. For long-living species like elephants, understanding patterns of mortality would be crucial for informed management.

Despite the importance, carrying out necropsy on elephants requires high levels of technical knowledge that is not readily available in all the regions that elephants occur in India. I am glad that Project Elephant and the Elephant Cell functioning under it have taken note of this lacuna and have come up with this comprehensive ready-reference manual that is intended to help the field practitioners and frontline staff of the Forest Department. This indeed is a much-awaited important contribution of the Project Elephant towards scientific management of elephants in India. I congratulate the contributors for their steadfast efforts and contributions. I am hopeful that the field personnel would make best use of the 'Recommended Operating Procedures'.

[Leena Nandan]

New Delhi, the 24th March, 2023

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CLIMATE CHANGE

FOREWORD

Understanding the demography of a free ranging, highly mobile species like elephants is often challenging. However, this knowledge about demographic patterns can contribute information related to population trends of wild animals and prove valuable for management interventions; like habitat improvement. While there are many intensive approaches to understand elephant demography, well maintained mortality and necropsy records can provide crucial details on the age class of elephants dying due to various reasons. Besides obtaining information of species biology, detailed field necropsy can also provide information on emerging infectious diseases, their severity and appropriate management responses. In spite of the benefits of carrying out a detailed field necropsy, the obvious limitations cannot be overlooked. Formidable size and specialized anatomy of elephants combined with a need for skilled personnel are major field limitations. It is encouraging that the Project Elephant has recognized these limitations and along with the Elephant Cell at the Wildlife Institute of India has come up with the "Recommended Operating Procedures" on carrying out carcass investigation, and its safe disposal.

I understand that the manual is a result of painstaking efforts of the Committee constituted by the Project Elephant and the individual authors who have made significant contributions. I appreciate the efforts of the Project Elephant and the Elephant Cell at Wildlife Institute of India for this timely contribution towards implementing best practices in the field while conducting elephant necropsy and augmenting the technical knowledge base.

[Chandra Prakash Goyal]
24.03.2023

New Delhi, the 24th March, 2023

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GOVERNMENT OF INDIA
MINISTRY OF ENVIRONMENT, FOREST AND CLIMATE CHANGE

FOREWORD

When it comes to inclusive conservation strategies for endangered wildlife, India is at the forefront. Being a rapidly growing economy, India continues to harbour the largest global populations of tigers, elephants, rhinos and many others. Nature worship and attaching a high survival value to every life form has always been an integral part of this country's philosophy. For example, a deceased elephant or a tiger elicits a lot of empathy in people and it is not uncommon to see local people worshipping these animals with garlands and lamps. Mortality is a natural process for every living organism on the planet. However, causes of the mortality, not excluding the threats of emerging infectious diseases in wild animals can potentially put entire populations at risk and this remains a source of concern in wildlife management. There has been a long-felt need to have reliable reference materials that are easy to use in the field.

This attempt at creating the "Recommended Operating Procedures" for Asian Elephant necropsy and carcass disposal is indeed commendable. I congratulate the Project Elephant Division of the MoEF&CC and the Elephant Cell at Wildlife Institute of India for this comprehensive document. I sincerely congratulate the authors; the committee constituted by the Project Elephant who put in time and effort to see this document to fruition.

[Dr. Satya Prakash Yadav]

New Delhi, the 24th March, 2023

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Azadi Ka Amrit Mahotsav

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Virendra Rambahal Tiwari, IFS **Director**

FOREWORD

As a country, India has been doing remarkably well in every sphere of biodiversity conservation. We have the largest and the most stable wild populations of elephants and tigers anywhere in the world. We have also recovered critically endangered species like one-horned Rhinos from the brink of extinction, which currently is thriving in many Protected Areas of North and the North East. While the rest of the world is suffering from species extinctions, the only mammal that became extinct in India after Independence was the Cheetah. This ecological mistake was also rectified with the reintroduction of Cheetah in Kuno a few months ago. Thus, as a Nation, India has exhibited its deep-rooted commitment and political will to conserve biodiversity. The country is also not far behind in adopting the most sound and effective principles of scientific wildlife management. Scientific research and wider knowledge dissemination aimed at effective management of wildlife habitats and populations remain the forte of Wildlife Institute of India.

It is noteworthy that the Project Elephant Division of the MoEFCC and the Elephant Cell at WII had come up with a very important field document that elaborates the fundamentals of conducting systematic necropsy of elephant carcasses, disease investigation techniques, and effective and safe ways of carcass disposal. The learning that deceased elephants possibly provide through systematic necropsy procedures could be vital for effectively managing emerging diseases in the living elephants. More importantly, details like age-specific mortality of wild elephants for a variety of environmental conditions can provide useful insights for adaptive management. Thus, it is extremely crucial to improve wildlife necropsy techniques in the country.

I am glad that this comprehensive document fills in the major gap that existed in carrying out proper necropsy of elephants and safe carcass disposal therein. I wholeheartedly congratulate the Project Elephant for this important contribution and also appreciate the efforts of the Elephant Cell at WII for their involvement. I hope that the field personnel would make best use of this ready-reference manual and also provide suggestions, as appropriate.

Dated: 27th March 2023

(Virendra Rambahal Tiwari)

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AND CLIMATE CHANGE

FOREWORD

India holds the largest and one of the most stable populations of the endangered Asian elephants. The Wildlife (Protection) Act, 1972 accords highest legal protection to elephants irrespective of whether the elephant is wild or captive. To further the cause of elephant management and conservation in India, Project Elephant is making steadfast efforts in enhancing the institutional and technical capacities of different facets of routine field management. In this regard, the Project Elephant Division and its associate Institutions have come up with many easy-to-use high-quality reference materials that are readily available to the field personnel. One of the Project Elephant's overarching aims is improving the technical know-how of field professionals is also being addressed. In continuation with these efforts, the Project Elephant has come up with this important "Recommended Operating Procedure" document on conducting elephant necropsy and safely disposing the carcasses targeting the field veterinarians and forest officials engaged in managing elephant populations. The aim of this manual is to improve the standards of conducting elephant necropsy and sample collection techniques so that critical scientific information related to elephant demography, emerging diseases of local and national importance, and habitat-specific variations in death rates can be obtained. The manual is the culmination of hard work of the committee and the contributing authors to whom I express my sincere gratitude and appreciation. I also hope that the field practitioners make best use of the manual, streamline necropsy and related data collection processes.

[Ramesh Kumar Pandey]

New Delhi, the 24th March, 2023

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आज़ादी का अमृत महोत्सव

PREFACE

In Africa, during the 1960s and 70s, basic insights into elephant ecology, hitherto unknown, were obtained from dead elephants that were controversially culled for population management. In India, elephants are revered both in life and after death and it is not uncommon to see villagers grieving and offering last rites to dead elephants. The relationship between elephants and people in India are deep-seated and hard to fathom. Nevertheless, there is no denial that a careful post-mortem examination of the dead elephants can provide crucial insights to managing elephants that are in the wild and under human care. Elephants are vulnerable to a wide range of diseases, disorders, illnesses, conflict situations, accidents etc., many of which can have potential negative feedback on their health and well-being. Even in well-protected habitats, stable elephant populations can have an average natural mortality at the rate of 1 to 2% per annum. Thus, it is important to develop robust necropsy and carcass disposal protocol. The latter becomes very important to prevent episodes of epidemics for elephants that could surface from time to time, and also to ensure personnel safety of frontline workers that engage in such operations.

Despite its recognized importance, conducting a systematic post-mortem of elephants, and safe disposal of carcass therein are seldom easy and hampered by inadequate elephant-specific training to the veterinary professionals. There are only limited practitioners with hands-on experience on aspects of elephant-specific veterinary procedures. Consequently, there is a long-felt need to enhance the capacity of veterinary professionals working in elephant areas through training as well as making relevant resource materials readily available. To fill this lacuna, the Project Elephant and the Elephant Cell at Wildlife Institute of India embarked on an ambitious attempt to come up with a document on "Recommended Operating Procedure for Necropsy and Carcass Disposal of Asian Elephants".

This document is essentially a sequel to the much acclaimed "A Handbook for Veterinarians, Biologists & Elephant Managers on Techniques & Procedure for Post-Mortem of Elephants" authored by Dr. Jacob Cheeran and published by Project Elephant and the Central Zoo Authority in 2003. The current effort is an attempt to revisit the issue in detail with the aim of inclusion of newer techniques and advances in the field. The document provides detailed information on the procedures and techniques required for conducting an elephant necropsy, including anatomical considerations, sample collection, and diagnostic testing, carcass disposal methods and biosecurity.

The manual is arranged section-wise to ensure easy reference. The first section deals with aspects of elephant anatomy. The second section elaborates on the procedures to be followed immediately after the elephant dies. The third section elaborately deals with the necropsy procedure, while the fourth section details the sample collection techniques. Post necropsy procedures that are important, but often ignored, have been comprehensively dealt with in the fifth section. The sixth section provides a comprehensive account of major diseases for elephants and also about commonly encountered pathological lesions. In addition to these technical sessions there are also annexures of data forms that will be useful for the field personnel.

This information is presented in a lucid and concise manner, with the aim of providing guidance and support to those who are involved in this critical work. It outlines the complexity and importance of elephant necropsy with emphasis on following the proper procedures for sample collection and diagnostic testing, we can gain valuable insights into the health and well-being of that individual animal, as well as the overall health of elephant populations.

Documents such as this are often a product of collaboration and generous knowledge sharing by many frontline practitioners. This document is not an exception. We would like to express our gratitude to the experts who contributed to this document, as well as to the many individuals who have supported this important work. We hope that this document on 'Necropsy and Carcass disposal of Asian Elephant-Recommended Operating Procedures' will serve as a valuable resource for all those who are involved in the care and conservation of elephants, and that it will help to advance our understanding of these magnificent animals.

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Special thanks to

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Dedicated to the legends who contributed to Elephant Conservation

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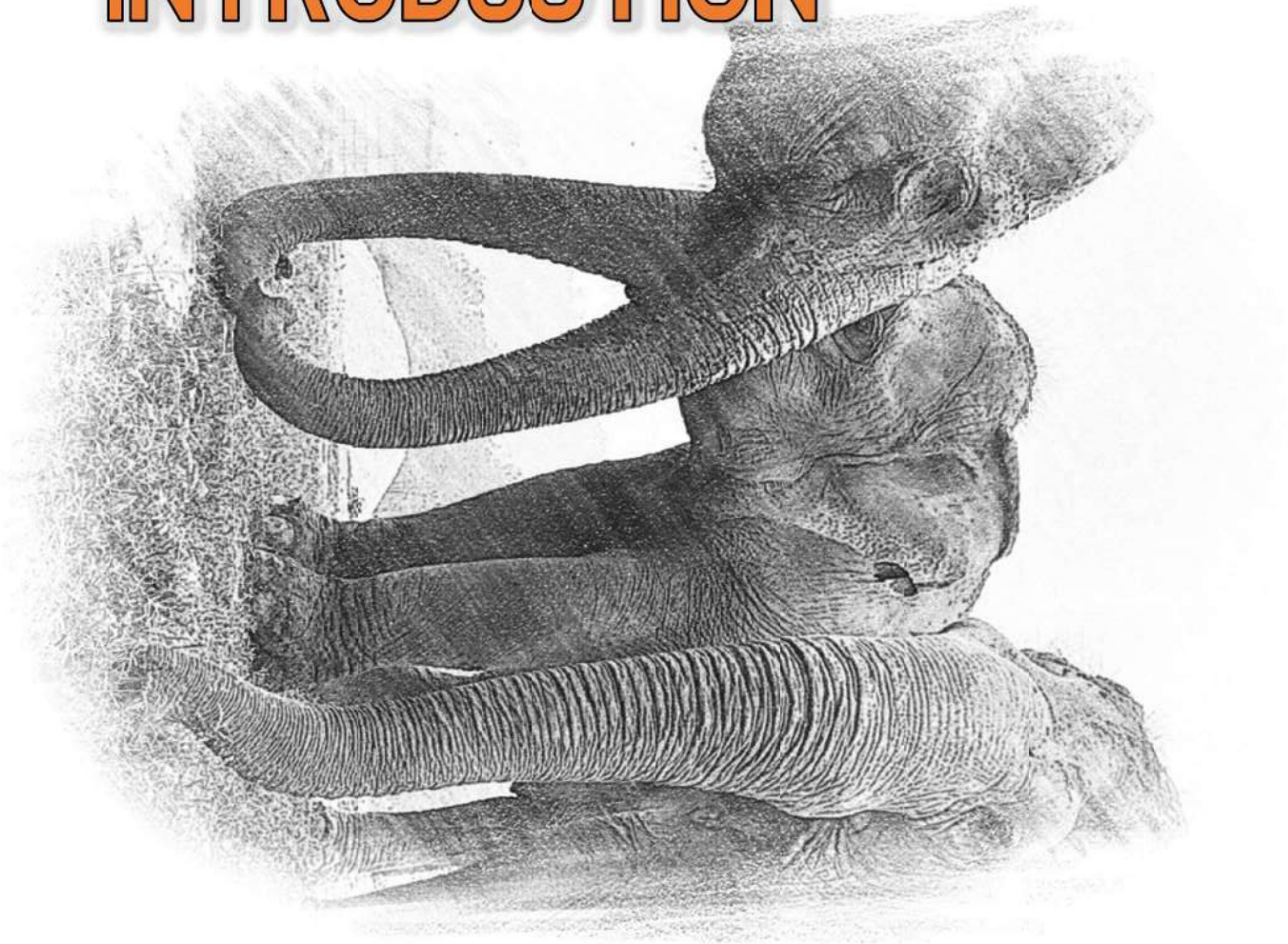
INTRODUCTION



INTRODUCTION

Across the globe, the health and wellbeing of wildlife are being increasingly compromised by incremental human population growth and the concomitant threats like habitat fragmentation, degradation and destruction. Consequently, the interface between people and wildlife are increasing unprecedentedly with potential consequences of wild animals interacting with humans and the livestock. Further to this, the ongoing global climate change combined with globalization is causing the spread of diseases into new areas where they may act as novel pathogens and for which the native fauna may be immunologically naïve. Additionally, the exchange of pathogens at the domestic-wildlife interface areas has potential negative consequences for both. Consequently, the study of wildlife diseases thus assumes a significant role in the ongoing biodiversity conservation efforts.

Understanding diseases in wildlife is a task that is easier said than done as there are gaps in information regarding aspects of anatomy and physiology for a large number of species. Further studies on disease dynamics in free-ranging wildlife are challenging, as it involves capture and marking of the animals in the large numbers. There are also limitations with respect to institutional capacities, availability of infrastructure and the skillsets required to undertake such operations. Thus, post-mortem examination (necropsy) of dead animals is often the only detailed source of information on various biological aspects and diseases for most species.

A necropsy is the examination of an animal after its death. The purpose of a necropsy is typically to determine the cause of death, or extent of disease. This involves a careful process of dissection, observation, interpretation, and documentation that are an aid to arriving at logical conclusion for identifying the cause of death and other associated diseases in the carcass.

For a necropsy to serve its purpose in the mortality investigation process, a thorough understanding of the species biology, behaviour, physiology, anatomy, pathology, nutrition including aspects of habitat where they reside are essential. This information though crucial is often lacking for most animal species and can be obtained by collation and analysis of data of necropsies conducted for the individual species over a period of time. The necropsy of a wild animal thus besides identifying the cause of death of the individual also aids in developing knowledge about the species. The key areas of knowledge that a detailed necropsy provides are listed below:

Diagnostic Value of Necropsy

Limited know-how, inherent limitations to examine and observe wild animals at close quarters, and difficulty in collecting relevant biological samples for diagnostics remain a challenge in most free-range situations when addressing interventions in wildlife health management. A detailed necropsy can provide a means to compare the accuracy of interpretation of clinical signs and ante-mortem diagnostic tests. Collation and analysis of data from necropsies conducted over a period of time helps increase the accuracy of diagnosis for the practitioner.

Contributions to Knowledge

A necropsy examination contributes to the body of scientific knowledge by increasing our understanding of anatomy, physiology and the ecological interactions of the individual in health and disease. It is an important tool in developing the skills of veterinary students and professionals in addressing health issues of wildlife as conventional veterinary education curriculum focusses primarily on domesticated species. The knowledge gained from necropsies can aid decision making and planning interventions for effective conservation of the species by policy makers and managers alike thereby serving to improve the welfare status of both free-ranging and captive wildlife.

Legal Functions

Increasing technological advancements have an important role in economic development of the human society. On the flip side; however, technological advancements are also resulting in increased crime against wildlife. Combating such crimes would require robust necropsy procedures and forensic investigation therein. Additionally, standard operating procedures adopted for the conservation of certain key species make it mandatory to carry out detailed necropsies for identification of cause of death. It is therefore critical to adopt standardised procedures and reporting processes while carrying necropsies.

Disease Surveillance and Monitoring

The continued loss of habitats for wild species and increased movement of pathogens between regions has contributed to disease threats emerging as a cause of species declines. Controlling the spread of diseases; however, is a knowledge driven process that can be obtained through continued surveillance and monitoring. Disease surveillance can be either active or passive. Active disease surveillance involves a systematic collection of information through

analysis of biological samples collected as a planned effort. The challenges in collection of such information for free-range populations often precludes active surveillance and monitoring.

The option remaining for managers is to use passive surveillance that involves collation and analysis of chance information i.e. an unplanned effort where all available information is collected from field observations, examinations, treatments and necropsies. The necropsy data can help in determining disease risks in a population by providing insights into causes of morbidities and mortalities in the population being examined. The information collected indicating primary cause of death and the differential diagnoses related to associated diseases can provide information to public health and regulatory authorities. This is beneficial for assessing the status of a disease, guide treatment and plan and appropriately implement pre-emptive strategies for managing disease spread.

Challenges in Necropsy of Wild Animals

A detailed and systematic necropsy provides insight into the cause of death of the animal and involves a thorough investigation of the carcass both externally and internally. It generates a series of gross observations that support identifying the cause of death. Subsequent laboratory investigations (histopathology, microbiology or forensic analysis to identify potential cause until an aetiology is established) are essential for a precise diagnosis. Additionally, a forensic assessment is valuable in collecting evidence that can lead to apprehension and prosecution of the offender(s) in vetero-legal cases.

A comprehensive necropsy examination conducted at the earliest, ideally before autolytic changes set-in will provide the valuable information. Carcasses of wild animals are however, seldom found in suitable condition for necropsy examination and may yield limited information. Often mortality events may occur in remote forested locations with poor accessibility. Autolytic changes set-in soon after death and it is often challenging to distinguish them from the ante-mortem changes that resulted in the death of the animal. Putrefactive changes are hastened by high ambient temperatures and humidity and can make interpretation of lesions difficult. Scavenger activity may often leave behind very little for a detailed necropsy. A detailed necropsy should be attempted with collection of appropriate biological samples for subsequent laboratory examination.

As previously indicated, regardless of the cause, consistently conducting necropsies can provide critical information related to health of populations that can be used for further management. Hence, it is necessary to standardize

the necropsy procedure for arriving at a conclusive diagnosis regarding the cause of death and add to the body of knowledge.

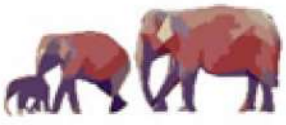
Need for the Document

Asian elephants are charismatic, threatened mega-herbivores found across most of the country as both free-ranging and captive populations. Being a global leader in conservation of iconic large mammals like the Asian elephants, India has always embraced science and technological advancements in wildlife management. A vast body of knowledge on the ecology, behaviour, anatomy and physiology of the species has been accrued over time. Nevertheless, the aspects of systematic mortality investigation remain an area that require major improvements. In particular, as elephants are long-lived, understanding the patterns of mortality would be crucial for informed management. Despite the importance, carrying out necropsy on elephants requires high levels of technical knowledge that is not readily available in all the regions that elephants occur in India.

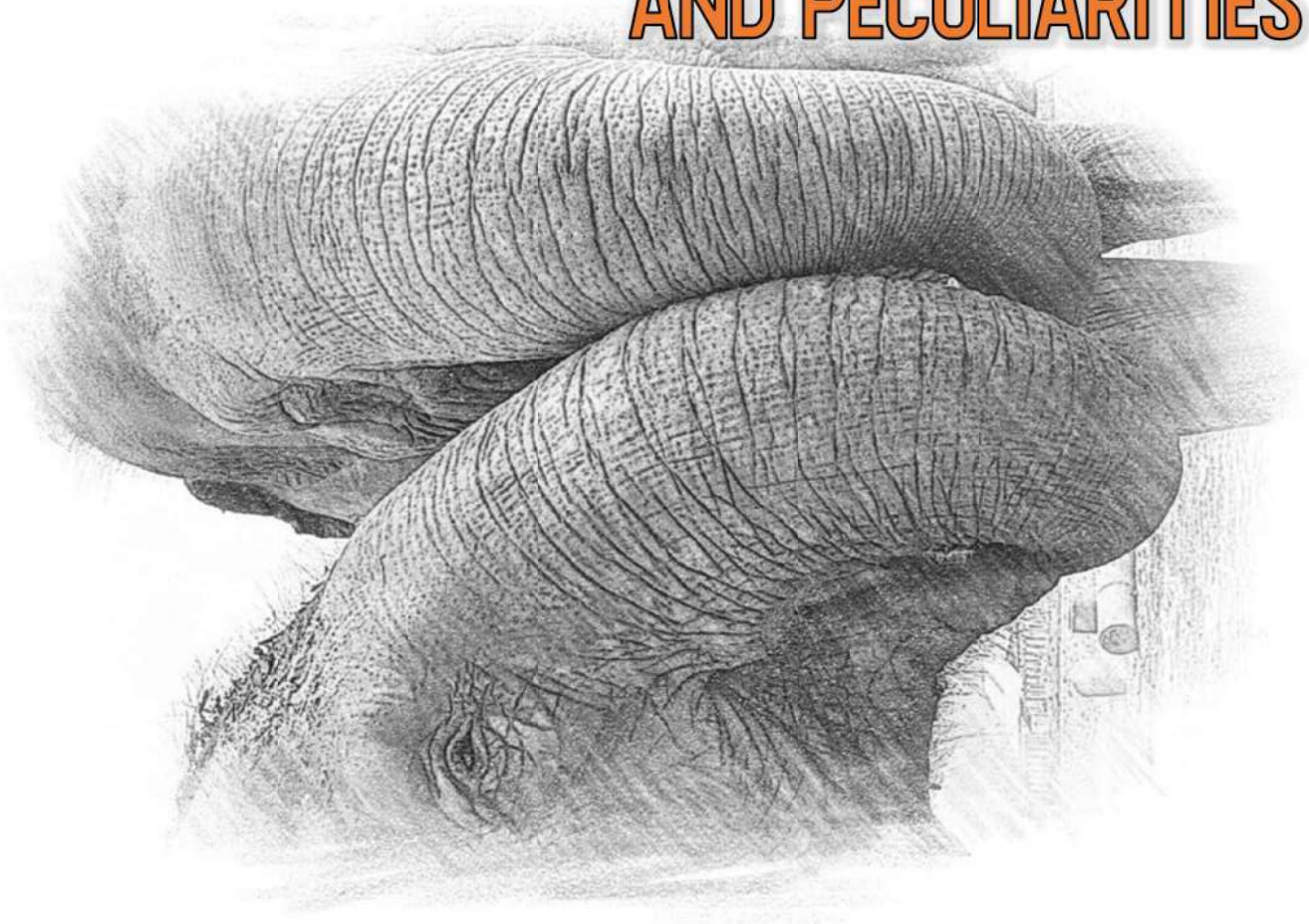
Recognizing this lacuna, the Project Elephant and the Elephant Cell at the Wildlife Institute of India have come up with this comprehensive ready-reference document that is intended to help the field practitioners and frontline staff of the Forest Department.

The document is intended to serve as a ready-reckoner in the field to better understand the species prior to conducting a systematic necropsy. The document is essentially a sequel to the much acclaimed "A Handbook for Veterinarians, Biologists & Elephant Managers on Techniques & Procedure for Post-mortem of Elephants" authored by Dr. Jacob V. Cheeran, an elephant veterinarian of repute. This manual was published by the Project Elephant and the Central Zoo Authority during the year 2003, and provided standardized protocols for undertaking mortality investigations of the species in the country.

In the current effort, the PE Division and the Elephant Cell at the Wildlife Institute of India collaborated with subject matter experts for the review of the earlier document for identifying gaps and preparing a revised, updated document. It is hoped that the protocols/ procedures documented here are used by field veterinarians in carrying out necropsies of elephants that are able to conclusively identify the cause of death and associated morbidities in the cases being examined by them.



SECTION I
ANATOMICAL ATTRIBUTES AND PECULIARITIES



ELEPHANT: ANATOMICAL ATTRIBUTES AND PECULIARITIES

A systematic detailed necropsy would provide lead for confirmatory diagnosis however, the information on history, symptoms, necropsy findings and result of the laboratory examination are critical for making the report meaningful. The foundation for carrying out the post mortem would however require a detailed knowledge of the anatomical features specific to the species.

Elephants are the largest land mammal that have evolved differently as compared to other species. Carrying out necropsy would essentially require necessary knowledge, skills and expertise relevant to the species. Therefore prior to carrying out necropsy, a basic understanding of anatomy and peculiarities therein is essential. These are essentially required so that the person carrying out the necropsy is well verse with the basics of elephant anatomy and physiology so that the observations made during necropsy can be interpreted in a meaningful manner. It is pertinent to also have an understanding of the anatomical peculiarities of elephants that differ from other species. These have been provided in Table 1.

Table 1: Organ-wise description and peculiarities

Organ System	Description
General description	Elephants are large bodied animals that fall under Proboscidea with adult bulls reaching 9-10 ft. at shoulders and weighing 4000 – 5000 kg whereas cow reach 7-8 ft. at shoulder and weigh 2500 – 3700 kg.
Musculo-skeletal	Asian elephant has elliptically dome back from shoulder to tail. Total number of bones in the body are 228 (unossified elements are not counted) [**Vertebrae:** Cervical-7, Thoracic-19-20, Lumbar-3-5, Sacral-3-5, Caudal 24-34; **Ribs:** 38 (19 pairs) (Sternal 6, asternal 9, floating 4)] Bones of fore limb and hind limb are arranged in a vertical fashion without angulation and rest on fibro-elastic fatty pad. Muscle tissue has greater fibrous content as compared to other animals. Have five toes on the front feet and four in the rear though it may vary in individuals. Skin is pliable and varies in thickness across body. Color of skin is dark greyish black with light to heavy de-pigmentation over the head, trunk and ears. Depigmentation may increase as age advances.

Organ System	Description
	Tusks are bulky and prominent in males and may be small and oddly shaped in *Makhnas* (tuskless males) similar to cow elephants and are known as 'tushes'. The alveolar process in the skull houses 1/3rd of the total length of tusk and grows continuously throughout life to accommodate the growth of the tusk.
Respiratory system	Proboscis is made of two nasal tubes separated by the septum nasi and the tip has a prehensile finger. Besides acting as an organ for respiration, the functions are manifold. Parietal pleura is thick and adherent to visceral pleura thereby obliterating the pleural cavity making the animal very sensitive to pressure on the chest cavity. It is also closely adherent to the thoracic wall. Left lung is small and extends from 3rd to 16th rib whereas right lung is larger and extend from 2nd to 16th rib.
Cardiovascular system	Heart is situated in the middle mediastinum extending from the 1st to the 5th rib with the apex resting on the 4th inter-chondral space. It is large, ovoid and longer on the left side. Apex of heart is bifid with *incisura cordis* separating the two apices of the ventricles whereas it is single in neonate formed by left ventricle.
Haemopoietic system	Only few lymph nodes (Tracheo bronchial, sub mandibular, tonsillar, mesenteric, axillary, inguinal) are present. Hemal nodes are dark brown and intercalated in the course of blood vessels and outnumber the lymph nodes. Spleen is dark, elongated organ, wide in the middle and elongated at either ends and placed vertically to the left abdominal wall. The caudal border has is serrated. .
Digestive system	Elephants have proboscis that acts as prehensile organ. The upper lip merges with the lower face of the proboscis and the lower lip is elongated with a pointed tip. Pharynx is a funnel shaped musculo-membranous sac (distensible pharyngeal pouch) common to both digestive and respiratory system. The pharyngeal pouch terminates in a sphincter and lies superior to larynx. The soft palate divides the cavity into a dorsal large nasopharynx and a ventral small oeso-pharynx. Tonsils are absent. The oesophagus forms a deep diverticulum or receptacle (glosso-epiglottic space) behind the root of the tongue. It is thick walled musculo-membranous tube that is dorsoventrally compressed. The peritoneum lining the abdominal cavity is thick and is reflected covers the viscera. The greater omentum is one such reflection and is thin, lace like and devoid of fat. It is extensive and attached to greater curvature of the stomach and the origin of the duodenum. Stomach is simple, elongated, muscular membranous sac placed vertically behind the left part of the diaphragm and the liver. Small intestine has longitudinal and transverse folds in honeycomb pattern
Hepatic system	Diaphragmatic face of liver is strongly convex and bulging and the visceral face is deeply concave. Liver is divided by deep umbilical fissure into larger extensive right and smaller left lobe. Gall bladder is

Organ System	Description
	absent; however, bile duct is large and sacculated. Pancreas are dark brown, lobulated and situated in the mesoduodenum.
Urogenital	Kidneys are multi-pyramidal multi-lobed (7-9 lobes), covered by a dense layer of connective tissue and fat. Kidneys show distinct surface lobulation. The left kidney is placed ventral to the vertebral ends of the last four ribs. The dorsal face is convex while the ventral face is flat. The right kidney is larger than the left and is situated under the vertebral ends of the last three ribs. Urinary bladder is small and pyriform and lies at the pelvic inlet. It is covered partially by peritoneum and can hold 6-18 ltr. of urine. Testicles are intra-abdominal and situated on each side medial to the cordial pole of the kidney. Ovaries are oval and flat on the sides, situated in the abdomen, ventral to the iliac crest and posterior pole of the kidneys.
Nervous system	Brain smaller in size as compared to size of head and has large cortical cortex.
Other peculiarities	Temporal gland lies under the skin on both side of the head between the eye and ear canal. Varied degree of temporal discharge may be seen in males as well as estrus females. There are two pectoral mammary glands. Each gland has a small conical teat. It is placed between the front legs with multiple openings. Perineal bulge is prominent in males whereas perineum is concave in females

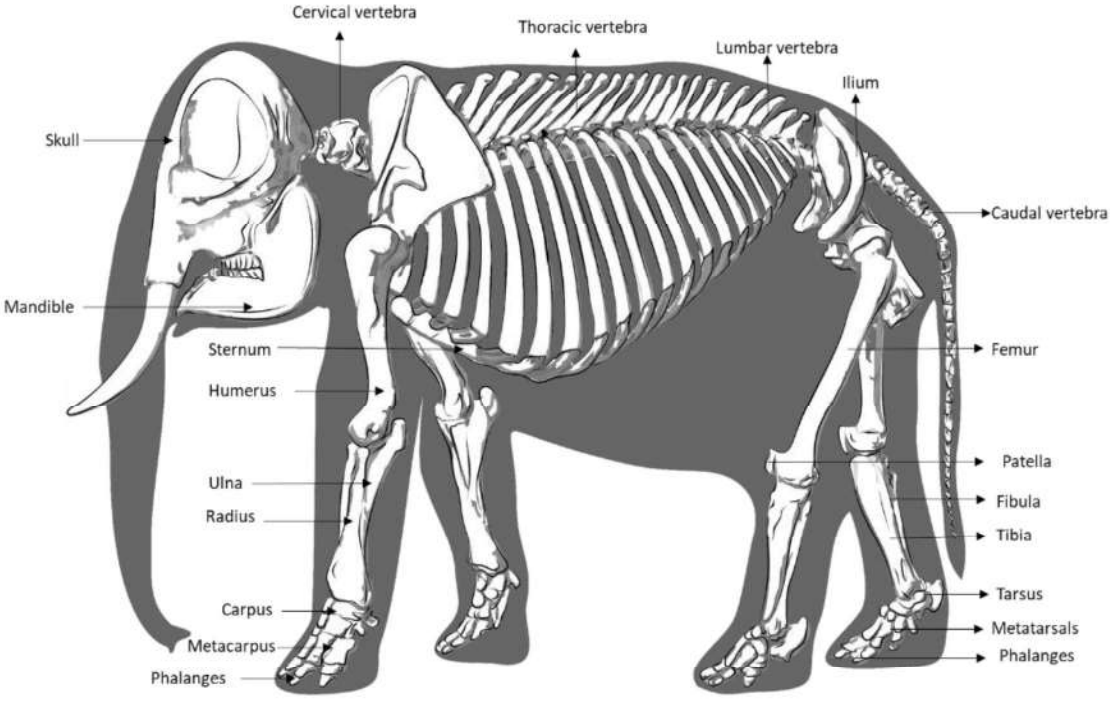


Fig 1. Mounted Skeleton of Asian Elephant

Dentition & ageing

Asian elephants have six set of molars (grinding teeth) (*Dental formula: Incisors-1/0, Canine-0/0, Premolars-3/3, Molars-3/3*) during the life span that appear at a specific age and get replaced as the age advances. Each molar consists of complex ridges or lamellae of dentine covered with enamel (marked as arrow in Plate 1) that are cemented together. The number of lamellae are specific to each molar set. A basic criterion for estimating age of animal based on the eruption and replacement of molar is provided in **Table 2.**

Table 2: Age estimation in Asian elephants based on molars eruption and replacement

Molar set	No. of lamellae	Eruption	Replacement
1	4	4 months	2 – 2 1/2 years
2	8	6 months	6 years
3	12	3 years	9 years
4	12 (wide)	6 years	25 years
5	16	20 years	50 – 60 years
6	24	40 years	60+ years

Plate 1: Mandibular and maxillary molar showing lamellae forming basis for age estimation

Identification of sex

The presence of tusks and perineal swelling in males can be used for identification of sex. Additionally, status of *mammae* and head characteristics can be used as supplementary information to establish sex. Teats in adult males (tusker and makhanas) look like a small protrusion from skin however these are conical with mammary gland tissue underneath in non-lactating females and prominent in lactating female. If the carcass is not detected early enough, putrefaction and scavenging might at times lead to difficulty in identification of the sex. An accurate method would be the forensics-based examinations, wherein PCR amplification using a set of primers helps determine the sex of the elephant. Tissue samples including bone marrow are excellent samples and can be stored in 90% ethanol. The tissue should be completely immersed in ethanol and transported to designated forensic laboratories within 5-7 days (Ahlering et al., 2011).

There might be instances wherein only the skeletal remains of the carcass are found in free ranging situations. Sex of the animal in such cases can be identified based on the dimensions of the skull.

Table 3: Characteristic of male and female skull

	Male Skull	Female Skull
FRONTAL VIEW		
Parieto-occipital crest (Marked A, Fig. 2)	Concave or distinctly depressed on the median plane of the dorsal boarder	Rounded and somehow convex on the dorso-median region
Frontal bone (Marked B, Fig. 2)	Narrower forehead compared to premaxilla width, "pinched" above nares	Wider forehead
External nares (Marked C, Fig. 2)	Well above orbit and placed higher as compared to female with the lateral edge sloping down	'Dumb bell' shape
Incisive fossa (Marked D, Fig. 2)	Deep and narrow and 'Scooped out' below inferior nares border	Inferior border of nares slopes gently into fossa
E. Tusk alveoli (Marked E, Fig. 2)	Larger and stouter, Incisive alveoli are larger than females though may be reduced in *makhnas*	Tusk often absent with reduced tusk alveoli

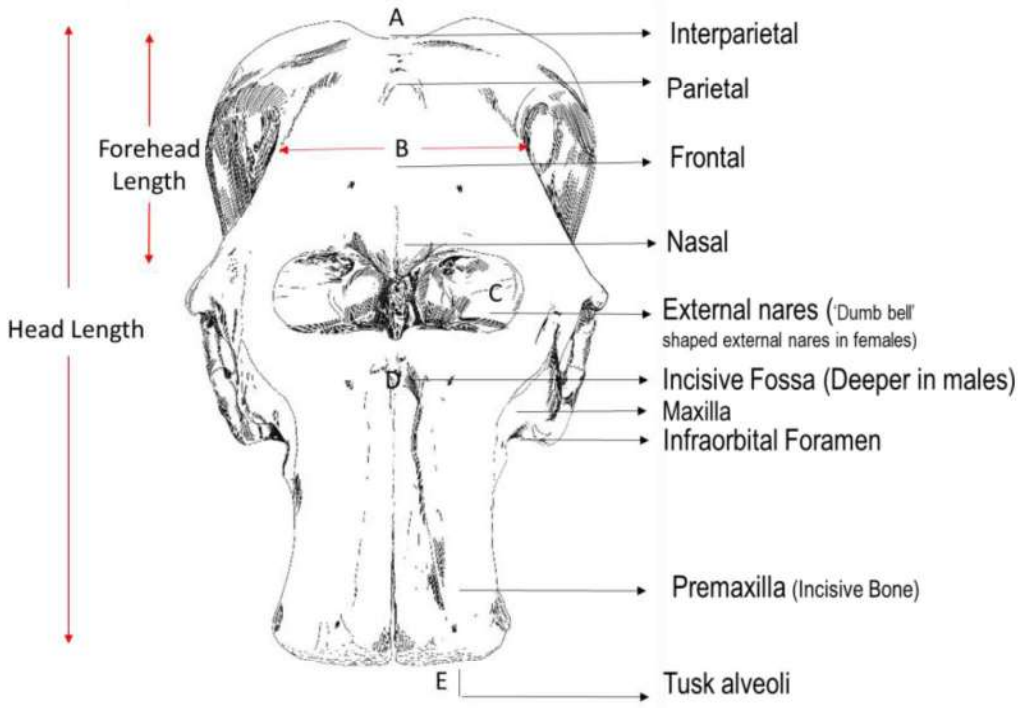


Fig 2. Frontal view of the cranium of Asian elephants (Female)

	Male Skull	Female Skull
OTHER MEASUREMENTS		
Ratio of Occipital length (Fig.4) and Forehead length (Fig.2) ratio	Greater than female and around 1.5-1.65	Almost equal
Ration of forehead length and head length (Fig.2)	Lesser than half of head length	Greater than half of head length

	Male Skull	Female Skull
LATERAL VIEW		
Frontal (Marked F, Fig. 3)	Slightly concave forehead	Rounded forehead
Temporal line (postero-medial wall of the temporal) (Marked G, Fig. 3)	Present with robust muscle attachment	Smooth and not so pronounced
Premaxillaries (Marked H, Fig. 3)	Large	Smaller
Parieto-occipital region (Marked I, Fig. 3)	Large, swollen bosses	Rounded

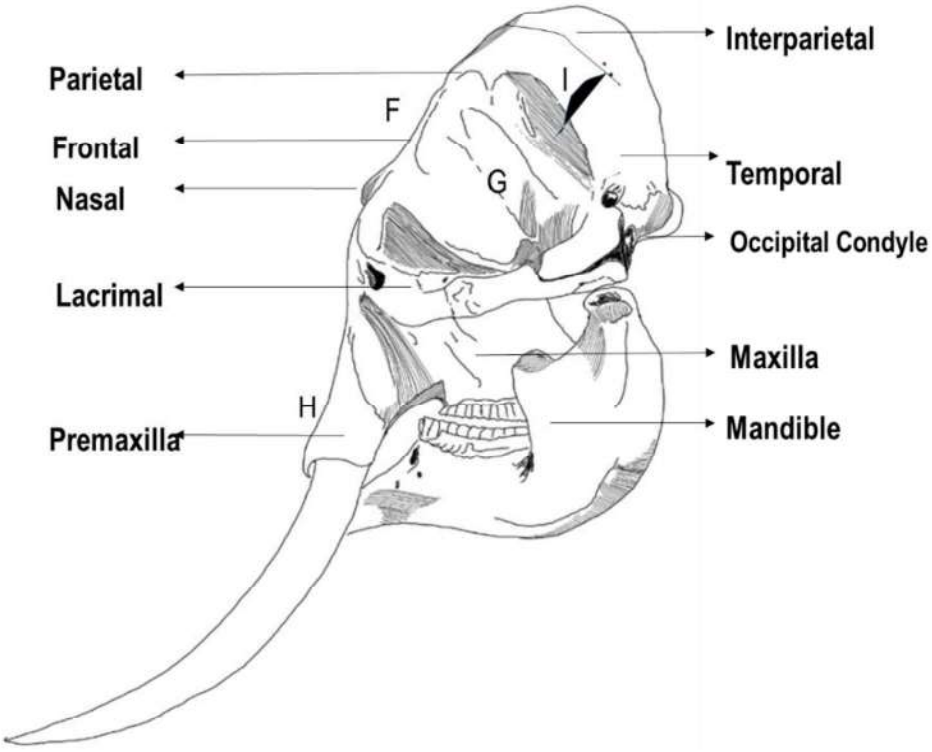


Fig 3. Lateral view of the cranium of Asian elephants (Male)

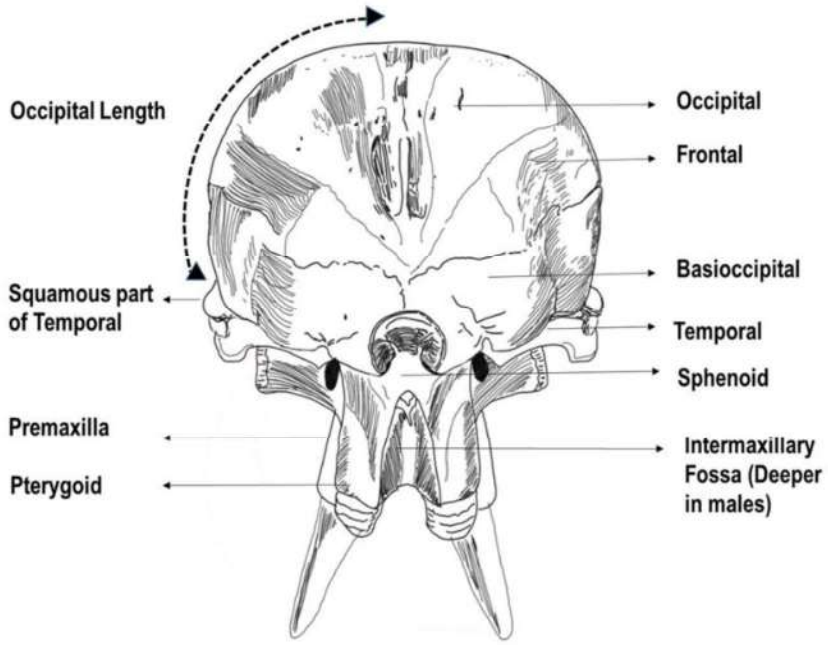


Fig 4. Posterior view of the cranium of Asian elephants (Male)

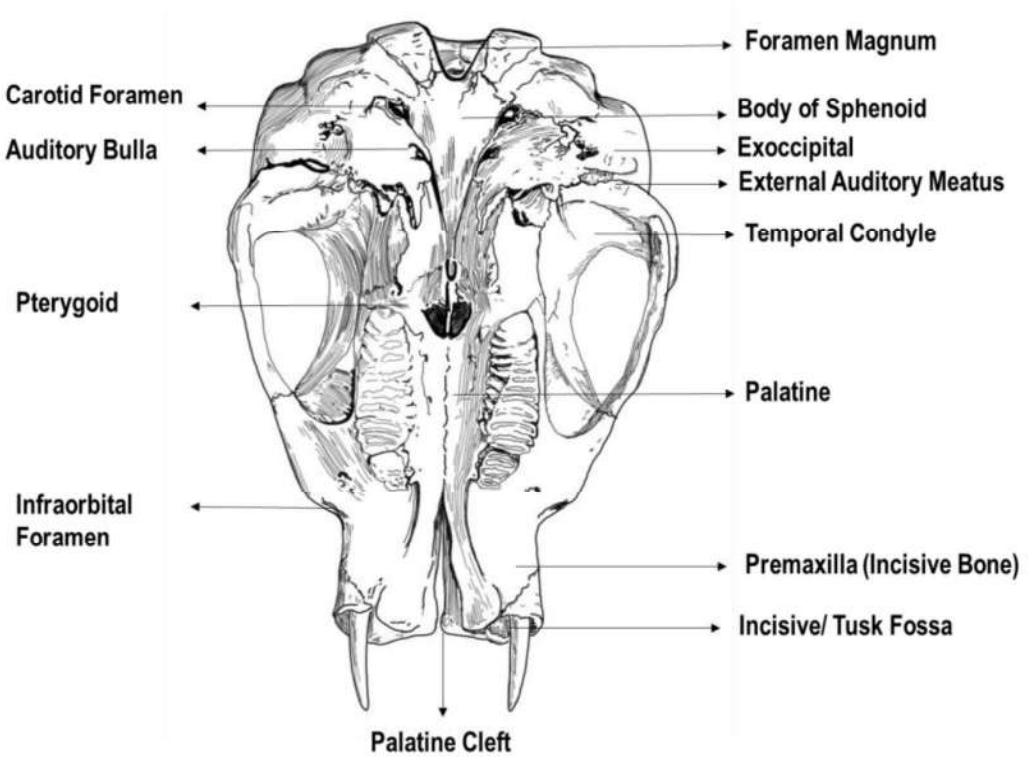
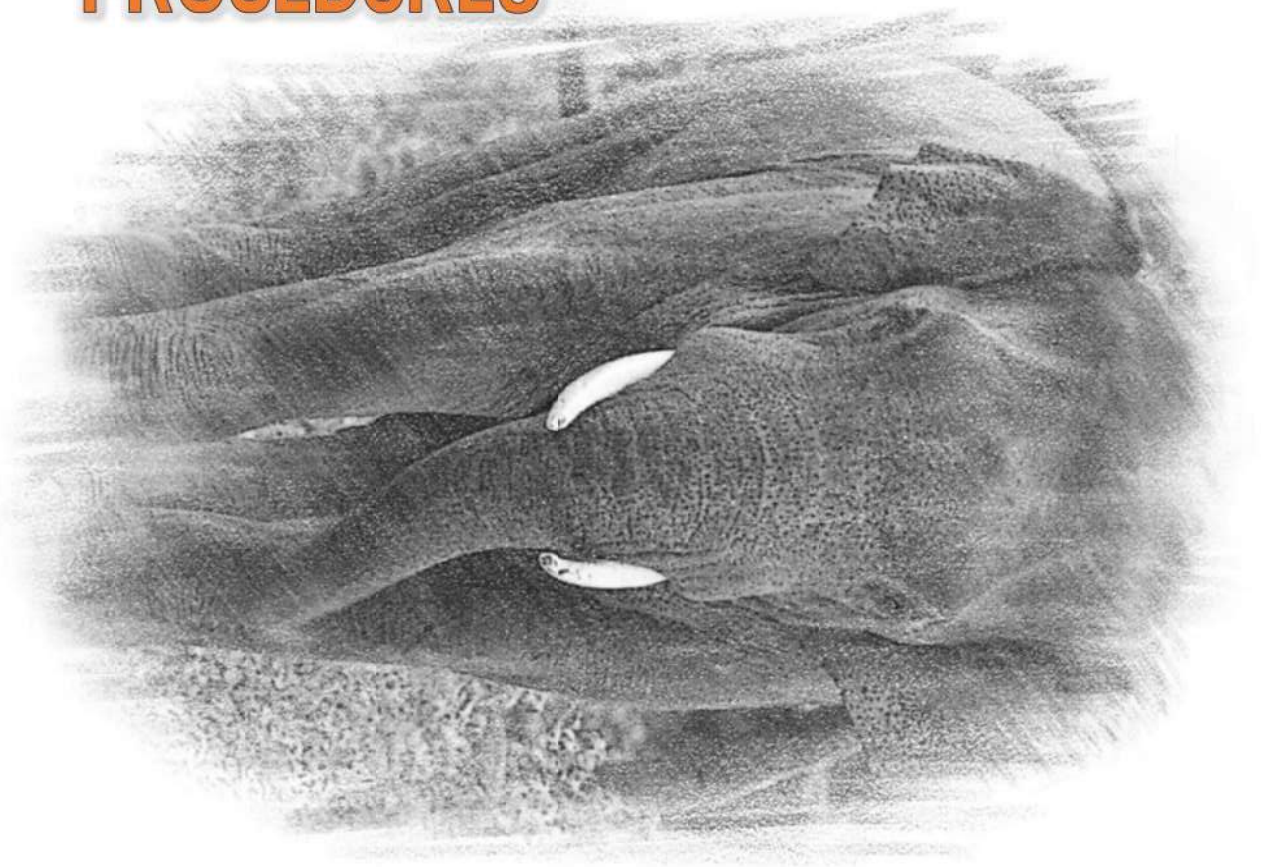


Fig 5. Inferior view of the cranium of Asian elephants (Female)

	Male Skull	Female Skull
MANDIBLE		
Lip bone (median projection from the anterior end of the mandibular symphysis)	Elongated, dropped downwards and somehow thick and broad	Thin, short and pointed

SECTION II
RECOMMENDED OPERATING PROCEDURES



RECOMMENDED OPERATING PROCEDURES TO BE FOLLOWED IN CASE OF ELEPHANT DEATH

Title: Necropsy and Carcass Disposal of Asian Elephant

Subject: Elephant death/Carcass disposal

Reference: A subcommittee under Captive Elephant Healthcare and Welfare Committee of the Project Elephant Division, Ministry of Environment, Forests & Climate Change, Govt. of India was constituted to revisit the document on 'Post Mortem of elephants' prepared by Dr. Jacob Cheeran and to develop a fresh Document on "Post Mortem of Elephants including Procedures for Carcass Disposal" vide OM No. -9/2014-PE dated 20th May 2022 **(Annexure 1).**

Purpose of ROP: The objective behind preparation of this "Recommended Operating Procedure" (ROP) is to ensure that the necropsy of elephants and disposal of carcass is carried out in a uniform manner across the country following established scientific protocols and procedures to ensure that the causative factors of elephant death are ascertained and taken to logical conclusion in the interest of elephant conservation. The ROP is also aimed at safe handling and investigation of elephant death in India.

Short summary: The ROP attempts to provide basic minimum steps required at the field level in carrying out elephant mortality investigation through systematic and scientific necropsy examination besides proper disposal of elephant carcass(es) to prevent spread of infection to human and other animals. It provides basic minimum steps that are required to be taken at the field level (Protected Area (PA) or elsewhere) for dealing with incidents of elephant mortality where the carcass is available.

Scope of the ROP: The ROP shall be useful to the field staff, veterinarians and field managers of all forest field formations (Elephant Reserve, Tiger Reserve, Protected Area, Reserve Forest, Revenue land or elsewhere).

Administrative responsibility in implementation of ROP: Necropsy should be carried out under supervision of the Field Director in the case of a

Elephant Reserve/ Tiger Reserve or the concerned Protected Area manager for a Protected Area (National Park / Wildlife Sanctuary). In the case of other areas (Revenue land/Conservation Reserve/Community Reserve/village/township), the Wildlife Warden, as per the Wildlife (Protection) Act, 1972, or Divisional Forest Officer/ Deputy Conservator of Forests (under whose jurisdiction the area falls), would be responsible for getting the necropsy. The overall responsibility at the State level would rest with the Chief Wildlife Warden of the concerned State.

Essentials for carrying out necropsy examination

1. **Constitution of team to carry out necropsy and oversee assessment and disposal of elephant carcasses:** Necropsy of an elephant is a laborious process and requires a team approach. It should be carried out by a pre-constituted team that is geared with all necessary equipment and logistics to carry out the necropsy. The team should comprise of following personnel who would carry out and supervise necropsy besides ensuring proper carcass disposal.

Table 4: Composition of team carrying out necropsy [PE Div. MoEF&CC, GoI, Letter No. 14-2/2019-PE (Part –I) dated 30th August 2022 **(Annexure II)**]

Necropsy Team	Constitution
Team Leader	Field Director of the Elephant Reserve/ Tiger Reserve/Officer of equivalent rank or in case of exigency an Authorized Officer not below the rank of Deputy Conservator of Forests under whose jurisdiction the area falls.
Three Veterinary Officers	Veterinary Officer of a Protected Area (Elephant Reserve, Tiger Reserve, National Park, Wildlife Sanctuary, Elephant Reserve) or Field Veterinary Officer in whose jurisdiction the area falls. Veterinary Officer from the State Animal Husbandry Department (District Veterinary Officer or his representative, who should necessarily be a veterinarian) preferably with experience of working in wildlife. An expert from a veterinary institution/ college preferably a pathologist may be co-opted, if required.
Representatives of Civil Society	Two representatives from civil society organization/ Non-Governmental Organization/ local Panchayati Raj Institution preferably nominated by Chief Wildlife Warden of the state
Additional	A representative of the NTCA if the incident pertains to a Tiger Reserve One or two trained veterinary field assistants/ Assistant Field Veterinary Officer may be co-opted to support proper conduct of procedures

2. Preparing for a Necropsy

The first action to be taken once a mortality is reported is to secure/ cordon the area and restrict movement to only those that are essential. This is required so that the evidence/s are not tampered with prior to arrival of the investigating team / team responsible for carrying out necropsy. The information should be transmitted at the earliest by the field officer to the competent authority seeking permission for carrying out the necropsy since elephants fall under Schedule-I of the Wild Life (Protection) Act, 1972. Efforts should be made to ensure that the team responsible for carrying out the necropsy reaches the site at the earliest and conducts all procedures with utmost caution.

State laws may also place certain restrictions on the necropsy of an animal that has died due to communicable disease and this should be considered before initiating the necropsy. These permissions should be sought without delay so that the necropsy can be carried out in a timely manner. Delay can result in putrefactive changes in the carcass that may impede proper diagnosis.

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

History: It is important that complete history of the case including observation records (clinical signs) prior to death (if possible), probable time of death or time when first encountered, position at the time of death, condition of carcass, wounds and discharges if any, its location, nearby water bodies, vicinity of human habitation, are obtained before attempting necropsy in free ranging situations.

In case of captive animal, information on case history, illness, treatment provided, management interventions, previous ailments and laboratory findings are essential while carrying out necropsy and may be sought from concerned authorities. These will help at arriving at a tentative diagnosis and can also provide a lead while finalizing the cause of death.

Steps to be taken prior to carrying out Necropsy:

1. The area around the carcass should be thoroughly examined for evidences such as but not limited to presence of other animals, signs of straining or trauma, poisoning or anthropogenic activity etc. to assist in establishing probable cause of death.

2. GPS co-ordinates; 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 (Age class, sex, unique ID in case of captive elephants) should be collected.

3. The necropsy should be always carried out in day light in an unhurried anner taking due care to record as many observations as possible.

4. Necropsy examination should preferably be conducted on-site since moving the carcass (involves hoisting, pulling, dragging of animal), can result in displacement of organs, post-mortem injuries and can confuse the veterinary professional while interpreting the observations. The chances of spread of infection cannot be ruled out. In case it is not possible to carry out the necropsy on site, every effort should be made to see that the carcass is handled in a manner that it does not lead to change of orientation of internal organs and biosafety protocols are followed. It is also advisable that following necropsy, the disposal of carcass should also be carried out at the same location so that the chances of pathogen/ disease spread are minimized. This would also help in containment of the disease though it would require adequate disinfection procedures; discussed in the subsequent section.

5. As far as possible, the gross lesions encountered during the necropsy are to be photographed properly to ensure proper documentation. Every detail of the procedure must be dutifully recorded or, preferably, dictated during the progress of the necropsy. It is recommended to not trust these to memory or to writing at some later stage. Recording the observation on a voice recorder or a note pad will be of great help.

6. Additional information including nutritional status, presence of external injuries or marks should also be collected. All mucous membranes, including oral, nasal, conjunctival and rectal should be thoroughly examined for any discoloration, discharges and presence of any abnormalities.

7. If the carcass is completely putrefied (with liquefaction of organs), partial necropsy can still be conducted. Considerable information such as signs of physical injuries, toxins and poisons, if any, time of death based on instar in the larval stages of flies, certain infectious diseases (from bone marrow of long bones) can provide leads to a conclusion.

8. Cases completely excluded from necropsies:

b. If the carcass is not presented as whole body but in parts thus not allowing accurate identification of the animal.
c. If the carcass is suspected to have died of **Anthrax**. In such cases, all-natural orifices should be plugged to avoid discharge of fluids and prevent chance of disease spread. Biological sampling as described in SOP (Project Elephant, GoI, 2019) may be carried out.
d. If the necessary authorization or permission for conducting necropsy from competent authority is not available, necropsy should not be performed, especially in cases with vetero-legal/ insurance implications.

3. Pre-requisites to Vetero-legal cases

Following points should be kept in mind prior to conducting necropsy of a vetero-legal case.

1. The necropsy should be conducted by written request from the police, the Magistrate or forest official and the contents of the request letter should be read carefully before examination.
2. There should be no unnecessary delay in conducting the necropsy.
3. No unauthorized person should be allowed to be present at the time of necropsy.
4. The particulars of identification marks of carcass furnished in the request memo should be checked and tallied with the carcass. Additional particulars of identification of carcass may be noted in the report.
5. The details of lesions observed by the veterinarian should be carefully recorded in the necropsy report on the spot at the time of examination.
6. The entire necropsy must be video recorded and adequately photographed for complete documentation of the procedures taking due account of the WCCB guidelines.
7. Metal detector must be used to look for bullets and other clues. If the carcass needs to be opened to retrieve any bullet or other evidence, the investigation team should take help of the necropsy team.
8. The appropriate morbid materials must be collected, preserved in appropriate preservative, and dispatched for desired laboratory investigations. The details of specimens collected, sample of preservative used, sample of seal used, copy of post-mortem report must be sent along with requisition letter for toxicological and other required investigation.
9. The report should be sent to the concerned agency only by registered post or sealed confidential envelope. A copy of report sent must always be filed separately for future reference at the time of evidence in court.

4. Risk assessment and safety precautions while conducting necropsy

Proper necropsy hygiene is two-fold process. In the first place, it is the duty of the veterinarian to protect himself/herself and his/her staff from the transmissible disease. Secondly the post- mortem room/ place should not serve as a source of infection for other living beings. Conducting necropsy 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. It is important to take all safety precautions while conducting post mortem. There are number of zoonotic diseases that can be easily transmitted to human if the procedures are not carried out in proper manner. A proper understanding of the probable diseases that can be transmitted while handling animals, safety measures and precautions required besides appropriate disinfection protocols and procedures is essential. Risk assessment prior to conduct of necropsy should consider following:

1. All necropsies should be approached with 'universal precautions' and all cases should be treated as high risk cases with possible risk of zoonotic disease exposure. The team conducting necropsy should be adequately protected against infectious diseases (e.g. Rabies, Tetanus etc.) and should take adequate protective measures while conducting necropsies.
2. Necropsies of carcasses suspected to have died of Tuberculosis, Rabies etc. should be conducted with utmost precaution and limited to interventions necessary for confirming a disease. Handling of such carcasses should take due considerations of biosafety and biosecurity precautions.

Major safety precautions that need to be taken while conducting necropsy:

1. In order to reduce the risk of disease spread, necropsy should be performed by trained personnel.
2. Unauthorized and non-essential persons should not be present in the necropsy facility/ area at the time of necropsy.
3. Personnel involved in or present at necropsies must wear personal protective equipment (PPE) e.g. overalls, lab coat, scrubs, disposable gloves and protective eye glasses/goggles or a full-face shield. Cut-proof gloves must be used when opening the body cavities and when working on high-risk cases.
4. Necropsy equipment should be used with great precaution to avoid injury or cut hazards. In general, hand tools are preferred over power tools due to lesser risk of injury and aerosol generation. Use of power tools should be reserved for cases with no alternatives. Additional precautions for respiratory protection such as face masks/ shields must be employed if

power equipment is used to protect from aerosol generated by such equipment.

5. Eating, drinking, grooming or other activities should be avoided during necropsy to avoid exposure to potential pathogens.
6. It is recommended that personnel involved in necropsy and handling carcass are duly protected against common diseases of zoonotic importance.
7. The site of necropsy/carcass disposal should be adequately disinfected subsequent to completion of the procedures. Details of various disinfectants are provided in **Annexure VII**.
8. Due care should be taken by all while conducting necropsy. Personal protective clothing (face masks, over-all, boots, protective gears for eyes) besides disinfection and sanitization essentials would help in minimizing the risk of disease to handlers especially for diseases that are contagious or are air borne.
9. Great care must be taken to ensure that all the specimens are collected, labelled, stored and transported safely and there is no risk of escape of infective materials during transportation.

5. **Necropsy equipment:** Minimum recommended equipment to be carried in a field necropsy kit are provided as A**nnexure III.**

Plate 2: Instruments of post-mortem box (© Karikalan, M.)

6. Necropsy procedure: external/gross examination

A thorough inspection of the carcass and the surroundings is important before starting the necropsy. Though assessing the overall body condition of the

carcass is a good measure of assessing overall health of the animal; it can only be done in fresh carcasses. The carcasses are usually encountered in a bloated condition thereby misrepresenting the actual condition of animal at the time of death. Due care should therefore be taken while interpreting these observations. Additionally, information on the position of carcass at the time of death, discharges from natural orifices, exudates, wounds, presence of rigor mortis and other signs of post-mortem changes must be recorded before initiating the necropsy. Following information may be collected during external examination:

1) Examination of the skin for swellings, exudates, injuries, wounds, eruptions etc.
2) Examination of temporal glands for any signs of discharge (indicative of *musth* in bull and oestrus in cow elephants).
3) Examination for evidence of inflicted injuries from gunshot, poaching and injury by mahouts.
4) Visible mucous membranes for pallor, icterus, cyanosis, congestion etc.
5) Examination of the extremities for any lesions or burn injuries.
6) Examination of natural orifices for oozing of blood or any discharges, lactation, prolapse, dental anomalies etc. Such signs are indicative of straining prior to death and may be encountered in variety of conditions and not just limited to Anthrax, acute poisoning, digestive disorders etc.

7. Estimating the Time of Death (TOD)

It is important to estimate time of death especially in the vetero-legal cases. Though some information is available to assess time of death in domestic animals, limited information is available for wildlife. Experienced pathologists may be able to estimate this interval with reasonable accuracy by observing the degree of post-mortem change in the organs. There are number of factors that influence rate of decomposition including ambient temperature, relative humidity, micro-environment, precipitation/rain/dew, cause of death, size of the animal and subcutaneous fat deposition etc. The changes become advanced with the passage of time. Following major factors may influence the rapidity of decomposition:

i. Environmental temperature – Elevated temperature especially during summers increases the rate of enzymatic and bacterial activity and hastens decomposition of carcass.
ii. Size of animal – Post-mortem changes occurs early in large size animals.
iii. Nutritional state of animal – Heat loss is slower in fat animal and can result in rapid decomposition.
iv. Cause of death- Rigor mortis is absent in cases of Tetanus and Anthrax though other post-mortem changes would be rapid. On the other hand, autolytic changes are rapid in cases of electrocution.

It is important to understand the normal autolytic changes that occurs soon after death from those changes that result in death and are briefly discussed below:

8. Autolysis/ Post-Mortem Changes

It includes the breakdown of tissues by the body's own enzymes characterized by uniform destruction of cells without any inflammatory reaction. Variation in autolytic changes in tissues may occur based on proteolytic enzymes in different tissues and due to invasion by "protein splitting anaerobic saprophytic organisms". Autolytic changes occur relatively quickly in liver, pancreas and kidneys as compared to muscle tissue. The protein splitting anaerobic saprophytic organisms results in the formation of gas and variety of foul-smelling substances which includes ammonia, hydrogen sulphide, indol, skatol and putrescent amines-like "*putrisciene* and *cadaverine*". The tissue turns black or dark-green as a result of the formation of iron sulphide (FeS) from broken down haemoglobin. The common putrefactive organisms are those belonging to *Clostridium* spp that normally reside in the gastrointestinal track and therefore, the autolysis occurs earliest in the abdominal cavity. Different stages in autolysis is provided below:

Algor mortis: Algor mortis is the gradual cooling of the body after death.

Rigor mortis: It is one of the most striking recognizable features of the post-mortem change, characterized by the stiffening of all muscles after death and relates to the contraction of muscle fibres as ATP decreases. ATP may be re-synthesized from glycogen and that's why delayed rigor is found in well-nourished/fed animal with high muscle glycogen. Rigor occurs more quickly in poorly nourished animals. Rigor mortis classically begins in two to six hours of death and passes in one or two days as the carcass putrefies. It begins in the cardiac muscle first and then progresses to the skeletal muscle of head, neck, throat and extremities. It gets enhanced by high temperature and increased metabolic activity before death. It is delayed in starvation, cold and cachexia. Rigor mortis disappears in the same order it appears and remains for 20-30 hours after death. It can therefore help in determining the time after death of the animal. This is of relevance in vetero-legal cases.

Livor mortis (Hypostatic congestion): It is the accumulation of blood in the ventral portion of organ and the carcass due to the influence of gravity. Hypostatic congestion indicates the side of the animal, which was ventral at the time of death. This is used in vetero- legal cases to indicate the position of the body at the time of death and if the carcass was moved after death. It

is most evident in the lungs and the skin as dark red coloration or in the kidneys as a black zone of coloration of the cortex on one side, which has been called "*pseudomelanosis*".

Post-mortem decomposition: It involves discoloration, softening, distension and displacement of tissues due to saprophytic organisms.

Post-mortem emphysema: It occurs due to accumulation of gas in the tissue mainly in gastro-intestinal tract (GIT) as a result of bacterial fermentation.

Discoloration of organs and tissues: It results from the break-down of haemoglobin and the action of bacterial hydrogen sulphide (H_2S) on haemoglobin resulting in Iron sulphide (FeS) [(Hb (Fe)+H_2S (Bacterial)= FeS (Iron sulphide) +H_2]. The shades of blue, green and black seen in the carcass are due to the above-mentioned reaction or changes.

Softening of tissue or organs: This is caused by "autolysis" with assistance from saprophytic bacteria or perhaps the normal flora of the tissue. This may be easily observed in liver and kidneys. The pancreas is very sensitive because of its enzymatic contents and softens rapidly which is accentuated by handling.

Distension: This occurs largely because of fermentation with gas production in the digestive tract. The gas distends all parts from the stomach downwards and extreme pressures built up in some organs may lead to rupture or the abdominal muscle may be torn from which GIT organs may protrude out. Large areas of the liver may become pale because if the blood has been pushed-out, or silhouette of the loops of the intestine may be imprinted on the surface of the liver. Gas bubbles are often present in liver and kidneys, usually in pale areas of autolysis and putrefaction, which are not uniformly spread through the tissues. Post-mortem abdominal distension with pushing of blood from the venous system in the abdomen makes the viscera pale at the same time the hind limb muscles, lungs and neck region may be quite congested. The mucosa of the stomach may peel off in large patches easily because of autolysis.

Rupture of organ and tissues: This occurs when gas produced causes progressive distortion of the organs and body structure until it bursts. Usually occurs in stomach, intestine, diaphragm, and ventral abdominal wall.

Displacement of organs: Occurs when the carcass is rolled or moved. Intestine is most commonly displaced and it must be differentiated from ante-mortem malposition of the viscera. Displaced viscera will show no passive hyperaemia.

Decomposition state

Based on the above-mentioned complexities, the estimation of time of death may be done to fall into one of the following periods namely fresh, less than 6 hours, between 12- 24 hours or more than 36 hrs, several days; weeks; months etc. Changes in the carcass after death can aid in tentatively predicting the time of death of an animal. These have been presented in Table 5

Table 5: Stage of carcass and changes observed (Adapted from Parsons, 2009, Megyesi, 2005).

Stage of carcass	Changes observed
Fresh	There are relatively few changes occurring during the fresh stage (algor mortis, rigor mortis) and the odor associated with the remains will still be the natural smell of the body. The fresh stage continues until the first signs of bloating begin, which is highly variable depending on the external environmental conditions.
Early decomposition	Early decomposition is marked by beginning of bloat of the abdomen. During the bloating period, the body begins to purge decomposition fluids and blood may ooze from the natural orifices. Bloating can occur rapidly in warm temperatures and last 2-5 days, however, it is extremely temperature dependent. Internal organs show post-mortem discolouration, gas formation, softening and liquefaction.
Advanced decomposition	Advanced decomposition starts at the 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. 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. The odor of decay during this phase is strong and putrid and can be detected over long distances.
Skeletonization	Early phases of this stage are identified when majority of the soft tissue has decomposed or when mummified tissue begins to break down to reveal bone. 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.

Plate 3: (a) Fresh Carcass in good body condition (Abdominal bloat barely discernible) (© Nitin Gupta), (b) Early decomposition as manifested by bloating of carcass. Note the clumps of fly eggs at anal region and in between the hind legs. (© Rajeshkumar, K.)

Plate 4: (a) Advanced decomposition of carcass. Most soft tissue decomposed leaving hard layer of skin over body cavity (© Apurba Chakraborty). (b) Advanced decomposition of carcass showing skeletonization. (© Nandakumar, S.)

Plate 5: (a) Advanced decomposition of carcass showing skeletonization. (© Karikalan, M.) **(b)** Bones exposed, skin consumed by keratinophagous beetles and skeletonization (© Rajeshkumar, K.)

Plate 6: Bones exposed, skin consumed by keratinophagous beetles and skeletonization (© Rajeshkumar, K.)

Forensic entomology

Forensic entomology has emerged as an important area that can assist in establishing the time of death, however, it needs adequate knowledge and expertise in the relevant field for its effective use. Forensic entomology intends to establish the time of death or Necropsy Interval (NI) based on the arthropod evidences on the carcass and stage of decomposition. Understanding of time and sequence of colonization of insects and their life cycle forms basis for establishing NI. Different species of arthropod colonize on the carcass at different stages of decay and may even prefer different tissue types. The method of estimation of time of colonization is based on the premise that different insect species are attracted to different stages of decomposition, and each wave of colonizers feeds upon the resource for a generation. The act of feeding fundamentally changes the resource, thereby rendering it unusable to species within the current wave yet attractive to other species in subsequent waves. The blending waves of insects can span weeks or months, making this method most useful for time of colonization estimations that may span many weeks or months. Insect colonization and decomposition of carcass is temperature dependent hence these factors should be considered while interpreting the results. Collection of these insects and the life forms and subsequent examination helps in determining the time of death. Identification of the different species of insects and their life-stages (e.g. eggs, larvae, pupae, maggots, flies and beetles) which are attracted to carcass are generally used for this purpose. Though morphological identification of

blow flies is complicated owing to phenotypic similarities amongst sub-species, molecular characterization of forensically important blowflies is precise, reliable and rapid for species identification of all developmental stages.

Table 6: Rough estimates of type of life forms that may be encountered during necropsy

Type of life form	Possible time
Only eggs laying in clumps over the body surface	8 to 10 hrs
Egg laying and hatching	Day 1
First stage larvae: hatching to first moult	Day 2
Second stage larvae: First moult to second moult	Day 3
Third stage larvae: Second moult to pre-pupa	Day 4-5
Pre-pupa to pupa	Day 5-6

The growth rate of the maggots however, can be affected by changes in the temperature, geographic location, exposure to sun or shade, time of day and season, humidity, and rain. 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 procedure. Furthermore, insects may serve as important alternative species for toxicological analysis in cases where tissue samples are not available for this purpose. The diversity and occurrence of insects on a carcass have been effectively documented and the details are provided below:

Table 7: Diversity and occurrence of insects on a carcass (Byrd et al., 2009; Byrd and Castner, 2009, Verma and Paul, 2013; Jadav & Sate, 2015).

Sequence of occurrence on carcass	Insect species
Within 6 hours, fresh stage	*Sarcophaga lineatocollis* Macquart, *Stomoxys calcitrans* Linnaeus
After 24 hours, bloated stage	*Chrysomya rufifacies* Macquart, *Lucilia sericata* Meigen, *Calliphora vicina* Robineau- Desvoidy
After 2-3 days	*Necrophila rufithorax* Selys, *Necrobia rufipes* (Fabricius)
After 3-4 days	*Creophilus* sp., *Hister* sp., *Saprinus* sp.
At decay and dry stage	*Dermestes maculatus* (De Geer)

For the groups of flies discussed here, the larval instar can be determined by observing the number of spiracular slits (the larva breathes from these slits located at the posterior end, that can be kept out of liquefied food; one slit

that is extremely difficult to see and a lack of an anterior spiracle = first instar, two slits = second instar, three slits = third instar). The last larval stage is followed by a transitional pupal stage where the insect body drastically changes form until the adult stage is formed. The adult stage in insects is the only stage to possess wings. Though limited efforts have been made thus far for using entomological tools in wildlife forensics, they have a potential to provide considerable information on the time of death and possibility of poisoning, including from decomposed carcasses. With the growing access to information on the entomological decomposition of carcasses, this field can form an important tool during wildlife crime investigation.

Plate 7: (a,b,c) Decaying elephant carcass with larvae of carrion beetle (*Diamesus osculans*) (© Rajeshkumar, K.)

9. Classifying wounds

Wounds are known to act either as 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 infighting. A preliminary step in this process is to differentiate ante-mortem injuries from any post-mortem damage. Major differences between the two are provided below (Spraker and Davies, 1994):

Table 8: Differentiating ante-mortem and post-mortem wounds

Ante-mortem wounds	Post-mortem wounds
Evidence of copious haemorrhage	Haemorrhage 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/ Puckered edges	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 are provided below.

Projectile wounds: Wounds in elephants characterized by circumscribed abrasions of the skin are typical of entrance wounds of projectiles from firearms. Exit wounds lack circumscribing abrasion of the skin. If there is an exit wound, deformation or fragmentation of a projectile as it passes through bone and soft tissue, diminishes the value of the wound shape and size in identifying the projectile. Wound paths that lead to a bone strike may provide evidence of the direction of the projectile and therefore the location of the entrance wound. Fragments of lead or bone sheared off in the bone strike will be present along the wound channel as the projectile continues its' path after the strike. In the field metal detectors and portable radiographs (X-rays) are extremely useful.

Non-projectile-induced wounds

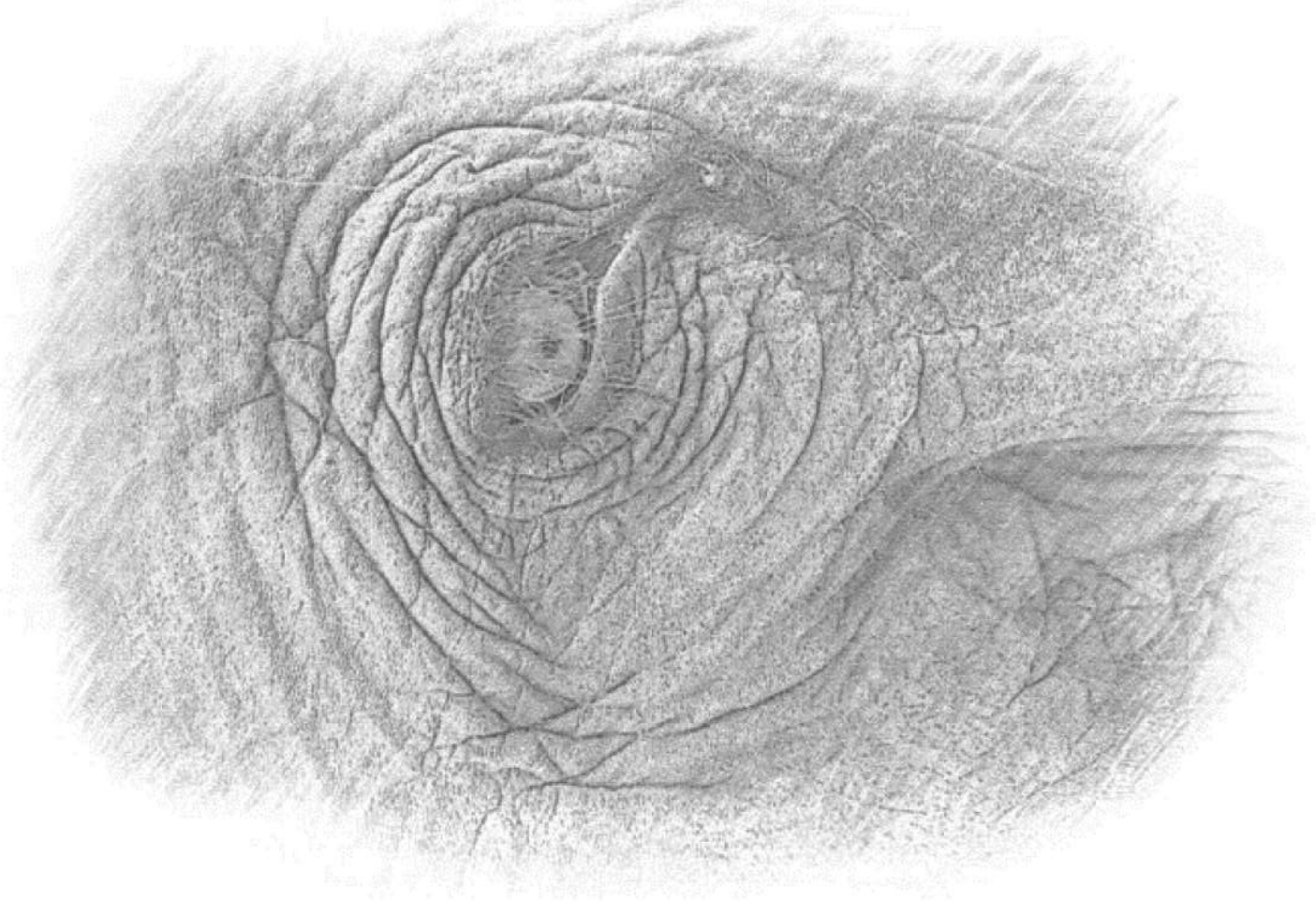
Wounds, superficially resembling bullet or knife wounds, occasionally are puncture wounds from tusk/s. Parallel lacerations characterize wounds from claws. Blunt trauma from an automobile strike to a protuberance of the hip or head of a bone may crush the overlying skin causing the bone to break, which superficially may appear to be a circular wound typical of a pellet. The most obvious distinction of these non-projectile wounds is the short depth of the wound channels.

Plate 8: Multiple Gore injuries on head including neck due to infighting. (© Manoharan, N.S.)

Plate 9: Fatal Tusk injuries on abdomen and neck due to infighting (© Karikalan, M.)

Plate 10 (a) Train hit injuries in elephant as evidenced by dragging marks and sloughing of skin (© Rajeshkumar, K.) (b) Burn Injury scar (Ante-mortem) on forehead (© Madhulal Valliyate)

SECTION III
NECROPSY PROCEDURE



Necropsy procedure: Internal Examination

Deskinning and subsequent examination: As a rule, skin should be examined on both the sides and subsequently deskinning should be done. Colour, condition of the fascia, amount of fat (normal or gelatinized),

Plate 11: (a) Initial incision for deskinning the carcass (© Karikalan, M.) **(b)** Flapping of skin to check the subcutaneous changes (© Rajeshkumar, K.)

hematoma (size and location), blood vessels and condition of blood (unclotted or clotted, color of blood – bright red, pale watery or chocolate) should be appropriately recorded.

Next the skin must be dissected out and the skin and subcutaneous tissue along the vertebral column lifted up. An incision should be made in the middle of the dissected skin, a rope passed through and tied. The dissected structures can be flapped by pulling the rope. The structures must be separated and removed by making deep incisions at the margins of the ribs at the cranial and abdominal ends of the thoracic wall and then connecting them with a deep ventral mid- line incision. The vertebral articulation must be cut and the thoracic cavity exposed by pulling the ribs along with the muscles as above and severing it from the costo-chondral articulations of the sternum.

Plate 12: Gross observations of visceral organs (*in-situ*) following deskinning and removal of ribs (© Rajeshkumar, K.)

Forelimbs: Forelimbs must be disarticulated and the segments removed after cutting through the shoulder joint, elbow joint, the radio-ulnar and carpal joints (A small axe can be used for cutting and a crow- bar for lifting the parts and severing the joints). The limbs should be tied with a rope and the segments pulled out.

Hind limbs: The segments should be removed (as described) above, after cutting through the hip joint, stifle joint, tibio- fibular and tarsal joints.

Muscles should be examined for their colour (pale or deep red or yellowish tinge), texture (normal, juicy, crepitating or necro-purulent sinuses/tract particularly in large muscle of thigh or limbs).

Opening of carcass: A superficial incision is made in the *linea alba,* taking due care to not puncture the viscera. In cases of excessively distended abdominal wall due to ante-mortem bloat or post-mortem accumulation of gases etc., a small puncture on the left abdominal wall with the point of knife can be made to allow the escape of gas and relax the wall. The incision is then extended from the opening in the *linea alba,* guided by two fingers, up to the xiphoid cartilage anteriorly and pubic symphysis posteriorly. The abdominal wall should be reflected along the costal arch on either side and in female along the border of the pubic bones as well. The abdominal cavity should be checked for fluid accumulation, colour and consistency. This becomes significant since cases of impaction leading to dilatation, gangrene and rupture of intestine at the region of colon resulting in peritonitis are quite common especially in captive animals. The omentum should be slipped off and the gastrointestinal tract can then be pulled out identifying each part of the intestine. While doing so, the mesentery should be examined since any case of torsion or intussusception will show gangrenous changes in the affected part of the intestine along with the mesentery.

The stomach should be removed separately. The spleen can then be seen attached to the greater curvature of the stomach. Inflammatory conditions may vary from congestion to haemorrhages. Ulcerative changes are also frequently encountered in young animals especially in cases of salmonellosis, colibacillosis, clostridial infections, Herpes etc.

The liver and spleen should be removed separately after dissecting their attachments. Liver should be examined thoroughly for the presence of trematode parasites (*Fasciola jacksoni*) and associated changes/ lesions. The kidneys, bladder and the reproductive organs are removed by separating their attachment. The position, shape and colour of liver, kidney and spleen should be noted. The consistency of the cut surface of the organs could be examined in detail for the presence of any gross lesions.

The organs of the thoracic cavity should be removed after examination of the cavity for any abnormal contents. The thoracic cavity can be cut open in case of young elephants or in case of adult animals, the organs can be accessed through the abdominal cavity by piercing the diaphragm.

Lungs along with the associated lymph nodes should be examined for nodular, congestive or suppurative changes. Lesions of pulmonary tuberculosis need to

be ruled out especially in captive elephants and necessary safety precautions while handling the carcass and tissues should be taken as this is a zoonotic disease.

The cardiac lesions for pericarditis, myocarditis, hydro-pericardium are also frequently encountered either as sequel to other chronic conditions or as a condition affecting the heart primarily. Haemorrhagic lesions in the epicardium are often seen in case of EEHV or in septicaemic conditions.

Dissection of head is best completed after separating it from the body. The ears should be dissected and removed. The trunk should be cut and removed at the level of the lower lip. The head can be disarticulated while cutting through the atlanto-occipital joints and separated from the body. The tusks should be dissected out. Several cuts will be required to sever the bones and to reach the base of the tusk. A good portion of the cranium must be cut to reach the brain (Large knives, long axe, chain saw and chisels can be used for cutting). Three connecting deep cuts should be made in the margins of the triangle formed at the base of the skull using an axe. The bony plates can be removed by lifting them with a crowbar and exposing the brain. The brain should be dissected out after severing the attachment.

Plate 13: Exposing of the brain (with intact meninges) after cutting through the skull (© Rajeshkumar, K.)

Examination of organs: All parts of an organ should be examined thoroughly. Emphasis should be given to differentiate lesions from post-mortem changes. After opening the carcass system-wise, individual organ should be examined for actual location and orientation; relative size and shape, presence of abnormal fluid in the cavities and nature of contents in the hollow organs. All lesions should be thoroughly examined and changes described and recorded. Representative samples for different tests that are to be sent to the reference laboratories should be collected while examining the organs. Proper documentation with photographic evidences should be part of the procedure. A measuring scale should be placed along the side of the dissected organ so that proper size of the organ can be noted. The methods for sample collection, preservation and dispatch for laboratory examination are described in the subsequent section.

After thorough examination of the carcass and prior to disposal of the carcass the preliminary observations should be recorded as per the format. If examination of some organ is not done or omitted while writing, it can be detected at this stage and necessary observations can be recorded. Once the details have been written out, procedures for carcass disposal should be initiated.

Tusk recovery, disposal including depositing tusk

In the case of bulls with tusks and cow elephants with tushes, it is necessary to dissect out the tusk prior to disposal of carcass. It would however require several cuts to severe the bones to reach the base of the tusk. Large knives, long axe, chain saw and chisels can be used for cutting.

In the case of suspected/confirmed Anthrax no part of the tusk/tushes must be collected or stored.

SECTION IV
BIOLOGICAL SAMPLING

Biological sampling

The conclusive cause of death in wild animals during necropsy based on gross examination of the organs alone is often difficult and thus laboratory support becomes crucial. Laboratory examination depends on the quality of biological and environmental samples collected, the method of preservation used and transport. Proper sampling is thus a crucial and integral part of the any mortality investigation and provides supporting information that helps in achieving a diagnosis of the disease and/or cause of death. Tissues with lesions collected from carcasses, parasites, ingested food, feces, or environmental samples should be sent to the laboratory.

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

1. Samples from fresh carcass can be used for histopathology, microbiology (culture and PCR), parasitology, toxicology, and genetics.
2. Samples from moderately decomposed carcass (early decomposition) can be used for histopathology (limited value), microbiology (PCR) (for select diseases), parasitology including forensic entomology, toxicology, and genetics.
3. Samples (bone marrow from long bones) from carcass with advanced decomposition can be used for microbiology (PCR) (for select diseases), forensic entomology, toxicology (select poisons) and genetic studies.
4. Mummified/Skeletal remains can be used for microbiology (PCR) (for select diseases), toxicology (limited), and genetics.

The details of the national institutes/regional centre/ laboratories and centres providing laboratory support are provided in **Annexure VIII**.

Table 9: Sample collection essentials

Sr. No.	Item		Quantity	Use
BASIC EQUIPMENT				
1.	Large tray/containers	PVC	05	Keeping organs

Sr. No.	Item	Quantity	Use
2.	Specimen jar/ Container (Wide mouth) 500 ml, 200 ml and 300 ml:	5 each	Sample preservation
3.	Sterile sample container (50 ml)	20 cups	Sample collection
4.	Screw capped tubes (10 ml)	20 tubes	Blood/ Urine
5.	Weigh scale (50 kgs)	01	Weigh organs
6.	Measuring tape (30 m)	01	External morphometry
7.	Permanent markers (Water and alcohol proof)	5	Labelling of samples
8.	Ice box/ wide mouth thermos flask	2	Sample dispatch
9.	Frozen ice packs	10	Sample preservation
10.	Painting brush	01	Picking up small parasites
11.	Magnifying glass	01	
SAMPLING SUPPLIES			
12.	Culture swabs	100	Bacteriological sample collection
13.	Glass slides (grease free)	1 pack	Blood smear and impression smear preparation
14.	Plastic Zip lock bags (10x8 inch and 6x4 inches)	100	Freezing tissue
15.	Aluminium foil (roll)	01	Freezing tissue
16.	Parafilm roll	02	Sealing of sample containers
17.	Bio Hazard bags	1 box	Disposal of biohazard
18.	EDTA Vials	1 box	Blood collection
19.	CLOT Activators	1 box	Serum collection
20.	Cryo tags (Cryo babies)	1 box	Sample marking
21.	Disposable sterile syringe and needle (2.5, 10 and 20 ml)	5 each	Collection of biological fluids
22.	Twin/thread roll	01	Tying off hollow organs
23.	Filter paper	01pkt	
24.	Rubber band	01 pkt	
25.	Absorbent cotton roll	01	
26.	Containers for sample collection; cylindrical plastic tubes	10-15	
27.	Viral transport media	100 ml	
CHEMICALS			
28.	10% neutral buffered formalin	5 litres	Fixing tissue

Sr. No.	Item	Quantity	Use
29.	2.5% chilled Glutaraldehyde	100 ml	Fixing tissue (for electron microscopy)
30.	70% ethyl alcohol + 2% glycerine	450 ml	Sample preservation
31.	Silica gel		Sample preservation
32.	Common salt	1 kg	Preservation of samples (Toxicological and genetic studies)
33.	Viral transfer media and RNA Later	20 tubes	
DOCUMENTATION ESSENTIALS			
34.	Ruler (30 cms)	01	To be used as scale while photo-documenting lesions
35.	Vernier callipers	01	Measurement of small specimens
36.	Measuring tape	02	Measurements
37.	Clip board	01	
38.	Water proof Marker	02	
39.	Pencils & Sharpner	02	
40.	Labels/ labeling tape	02 sheet	
41.	Microchip Reader	01	
MISCELLANEOUS			
42.	Coveralls, boots, gloves, caps, masks, protective eye and head gear, face shields (waterproof disposable suits are ideal)	10	
43.	Biohazard bags (red bags)		
44.	Alcohol lamp or gas burner (to sterilize equipment on site)	02	
45.	Match box/lighter	01	
46.	Sealing stamp and Lac Sealing wax		
47.	Soap and Nail brush	1	
48.	Hand sanitizer	01	Personal hygiene
49.	Towel	2	
50.	Field based diagnostic kits (select diseases)	5-10	

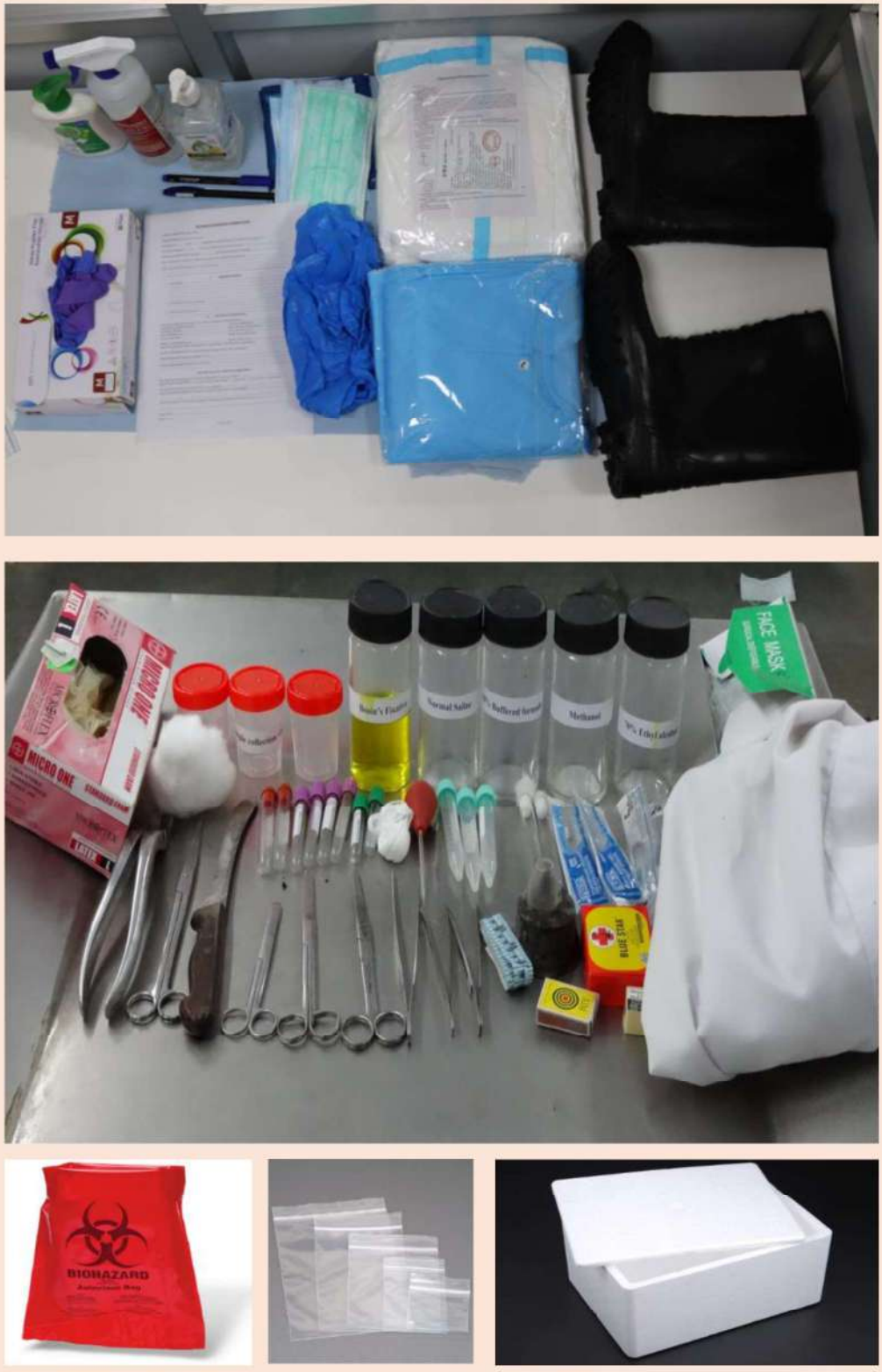


Plate 14: Sample collection consumables (© Karikalan, M.)

Microbiological examination

Heart blood and smear: During necropsy, in fresh carcasses, heart blood can be collected aseptically. A few blood smears can be prepared and fixed in methanol before packaging for laboratory examination.

Impression smear: The tissue piece from organ where lesions are seen should be cut by a sharp knife. The cut surface should be gently pressed with filter paper (for 2-3 occasions) and impressions should be taken on a clean slide. Generally, 2-3 impressions should be taken on a slide followed by fixing the same in ethanol or on a flame.

Swab: Swabs to be collected from the tissues showing any pathological lesions including those from nasal, pharyngeal, conjunctival, rectal, vaginal and prepucial areas. Swabs from abscess should be carefully collected by touching the pus membrane and not the pus as majority of times the pus is sterile and does not contain any live bacteria. All the samples should be sent to the laboratory aseptically under refrigerated condition by placing containers on ice packs. Sample collected for bacteriological examination should not be frozen. A variety of transport medias are available and can be used for transporting the samples. Select commercially available bacterial and viral transport media are provided below:

1. **For bacterial isolation**; nutrient broth, charcoal or Stuart transport medium without charcoal, Cary Blair transport medium, thioglycollate medium (anaerobic organisms).
2. **For viral isolation**, glycerol saline (50%) (Prepared by mixing equal amounts of glycerine and saline (0.85% NaCl), phosphate buffer saline (pH 7.2- 7.4) or cell culture media containing antibiotic and anti-mycotics.

Tissue samples: Various specimens including spleen, lymph node, liver, lung, heart, kidney, brain and loops of GIT have been used for bacterial and viral isolation. As discussed in the previous section, commercially available transport media may be used for transport of tissues. Cut piece of organs having lesions should be collected in sterile containers and transported on ice. One set of tissues should also be preserved in 10% neutral buffered formalin solution for histopathological examination and samples should be collected in wide mouthed container. The quantity of preservative should be in the ratio of 1 part of tissue: 9 parts of preservative. Tissue sample of 0.5 -1cm thickness from multiple areas of single organ having normal tissue and tissue with pathological lesions should be collected and sent to laboratory.

Intestinal loop: These are collected particularly in young animals with lesions indicative of haemorrhagic enteritis. A small loop of intestine by tagging both the ends by a thread can be collected and transported on ice.

Parasitological examination

The intestinal contents can be preserved by refrigeration for 48 to72 hrs. Equal volume of 10% formalin or formal saline added to contents acts as a preservative / fixative. Other samples like blood smears should be fixed in absolute methanol, skin scrapings in 10% KOH, endo-parasites (worms) in 10% formalin or 70% ethanol and ecto-parasites in 70% alcohol (for routine parasitological examination). The samples should be dispatched to the laboratory in containers with a tight lid. For trematodes and cestodes, the parasites should be placed in between two clean glass slides. The slides should be gently pressed so as to flatten the parasite. The glass slide should then be tied by a thread or rubber bands and fixed in 5% formalin solution and dispatched to laboratory.

Toxicological examination

Toxicological investigations are an important part of forensic examination especially in cases where death due to direct or indirect poisoning is suspected. Toxicological examination becomes more important in cases where intact carcass is not available and remains like skin, skeleton and decomposed organic matter are present. It requires knowledge about toxins/poisons, the effect of toxic substance on the animal, the animal's physiology and the potential for wider exposure. Such investigations require proper recording of information (environmental attributes, forensic evidences etc.) besides a detailed necropsy, biological sampling and laboratory examination.

Most commonly used compounds include organophosphates, organochlorine, synthetic pyrethroids and carbamates though variety of other compounds have also been used for poisoning. Type of samples to be collected from dead animals for toxicological examinations include liver, stomach, intestinal content, kidney, blood and fat samples. Other samples include feed, fodder, plant and water. The samples should be sent along with the tissue samples. If the carcass has already been buried and no visceral tissues are available, the remnant bones and bone ash should be collected for analysis. In case of putrefied materials, bone marrow preferably from long bones, synovial fluid, aqueous humor, hair, feed/food, soil and water should be used for analysis. Wet soil/ exudate under the carcass in case of purified carcass provides good led in deaths suspected for poisoning.

While collecting samples, the tissues should not be washed as this may lead to dilution of the toxic material. In addition to these samples, tissues from visceral organs viz. liver, lung, kidney, stomach, intestine, heart, spleen and

brain should be collected in 10% formol-saline for histopathological examination.

Common pesticides/ Heavy metal/ other toxicants reported in elephant mortality

1. Insecticides:

a. Organochlorine compounds (DDT, aldrin, dieldrin, endrin, chlordane, heptachlor, mirex, toxaphene, hexachlorobenzene)
b. Organophosphate esters (Malathion, parathion, chlorpyrifos, etc.)
c. Carbamates (Carbaryal, propoxure, baygon, etc.)
d. Pyrethroids (Cypermethrine, deltamethrine, permethrine, etc.)
e. Neonicotinoids (Imidacloprid etc.)

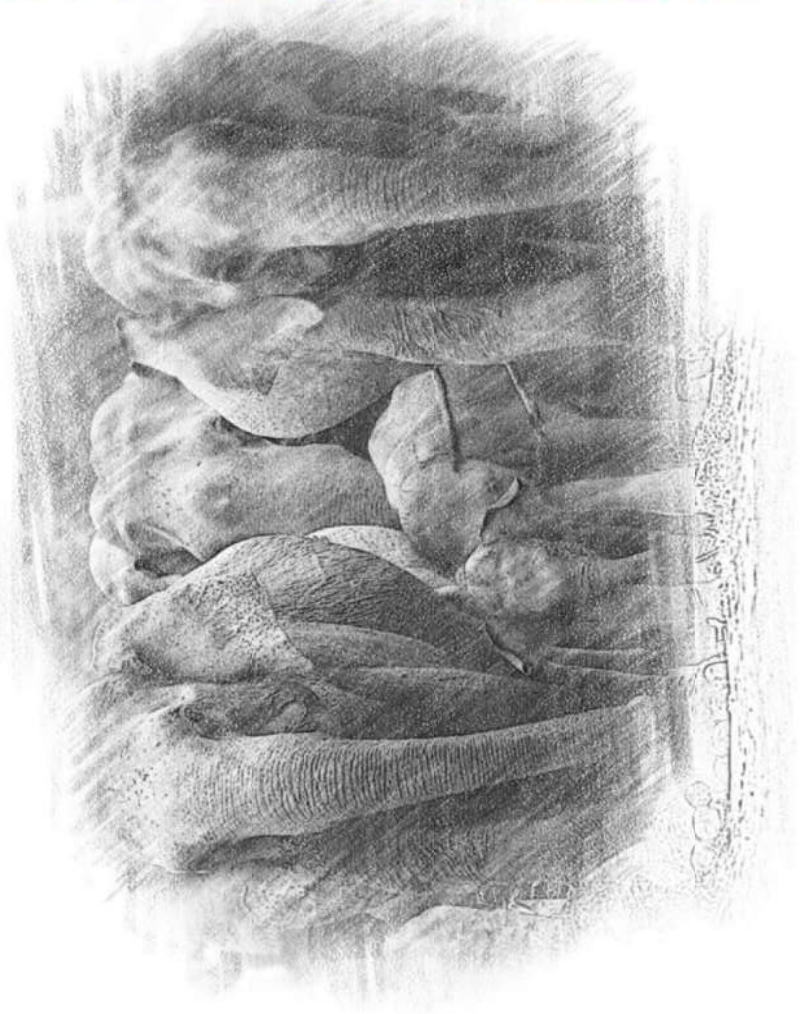
2. Fungicides: (Mancozeb, triadimefon, propiconazole, etc.)
3. Herbicides: (Chlorophenyl-acids; Paraquat, bipyridyl compounds or quaternary ammonium herbicides etc.)
4. Heavy metals: Cadmium, Arsenic, Lead, Mercury, Copper,
5. Others: Sodium chloride, nitrate/nitrite, urea, ammonia, hydrocyanic acid

Major toxicants, important lesions encountered and relevant biological sampling are provided as **Annexure IV**
Sampling essentials based on type of laboratory examination is provided as **Annexure V**

Other sampling essentials

- Samples should be free of any external contamination (dust, hair, earth etc.).
- Each sample should be kept in an independent container (wide mouth, leak proof sample container sealed in plastic bag with Ziploc closure), duly labelled with the case reference and nature of the sample. The set of samples of a particular case should be packed in a larger bag and labeled.
- If any preservative is in fact added to the sample, the type and amount should be specified in the accompanying report and a sample thereof shall be sent to the laboratory. Preservative shall be sent in a separate container and duly labeled, so that there is no possibility of confusion with a sample.
- All samples taken from the case should be placed in larger packaging and sealed in such a way as to identify tampering with the sample thereafter. This packaging shall bear references related to the case and accompanying reports.
- Samples will be sent to the laboratory in Polystyrene (thermocol) boxes or the like and suitably packed with refrigerating elements to ensure that the sample does not arrive thawed out. The inside should also be padded against impact and accidental opening of the containers during transport. Samples should ideally be sent at the start of the week to prevent them from thawing out during the weekend.

SECTION V
POST - NECROPSY PROCEDURE



POST-NECROPSY PROCEDURE

Waste and carcass disposal procedures: Upon completion of the necropsy, disposal of carcass and necropsy waste, sharps and infectious materials should be done in a manner that minimizes environmental contamination and exposure of the personnel or domestic or wild animals to infectious, toxins or other disease agents. It is pertinent to consider that any and all relevant regulations and protocols for carcass and medical waste disposal and the disposal methods should be decided after the relevant factors and risks are assessed.

Site of carcass disposal: For adult elephant carcasses, due to limitations resulting from size of a carcass(es), its location, field infrastructure and logistics, ability to safely move a carcass for disposal within a reasonable timeframe the disposal should be ideally carried out at the site itself. For captive elephants that have died in the city / town/ village, efforts should be made to lift the carcass into a heavy load truck using a JCB/ heavy machinery and then taken to select/ designated site for detailed necropsy and disposal. Special effort should be made to disinfect the site, vehicle and necropsy site once the carcass had been moved/ disposed to prevent spread of possible infection.

Carcass disposal methods: There are several methods for disposing off items used during necropsies and the wild animal carcasses. These include cremation either on a pyre or in an incinerator depending on the size of the carcass; burial in excavated pit or leaving the carcass for natural decay at the site of death and necropsy. Various options for carcass disposal are provided below

A. Trench Burial

Trench burial is the oldest disposal method and basically involves the excavation of a trench into the earth depending upon the size of the elephant carcass, placing of carcass and other materials in the trench, and covering the materials (backfilling) with excavated earth. However, it requires a careful selection of the burial site. Factors that may be considered while selecting the site for burial are provided below

1. **Proximity to human habitation:** The site should be away from towns, dwellings and major roads to reduce the risk of undesirable exposure of the public to dust and odour.
2. **Site accessibility, terrain selection and characteristics:** The site should be accessible to trucks and earthmoving equipment, allowing them

to enter easily and be effectively disinfected. The site should preferably not be on a slope and should allow for digging of a 5-metre deep pit with heavy equipment. Soil at the site should be stable enough to withstand the weight of machinery used to construct and fill the pit. It should be deep enough to avoid scavengers from opening up the grave. The top end of the buried carcass should be 6 ft below the ground.

Plate 15: Trench Burial of carcass (©Nitin Gupta)

3. **Proximity to drinking water supply:** When selecting a burial site, it is important to ensure that there are no nearby underground water sources or areas that may flood to reduce the chance of water contamination. It is preferable that the site is away from water catchment area. The seasonal maximum groundwater level at the site should preferably be at least 2 mts below the base of the burial pits, as far as possible. The site should also be away from any watercourses, lakes, ponds to reduce the likelihood of contamination of water systems by leachate and runoff. It includes natural or dammed fresh water, aquaculture ponds, sewage treatment ponds, reservoirs, water tanks etc. Surface runoff should be prevented from entering the pit by the construction of diversion banks. Similar banks should be constructed to prevent any liquids escaping from the burial site.
4. **Soil characteristics:** The site should preferably have soil of low permeability. Unlined burial is usually used when soil types or local geology can control the risk of leachate leakage, whereas lined burial is used when there are risks of leakage of leachate into subsoil or the water table.

Leachate production: Leachate is the liquid that is released during the decomposition. Leachate can potentially contaminate surface water and groundwater supplies however can be managed with appropriate planning for drainage and treatment at the time of pit construction. Lining or partial lining of pits may help to control the generation, release and degradation of leachate. It may allow use of sites where subsoil structure or depth of the groundwater is not known. Methods to mitigate leachate issues include using clay from excavations or nearby sources to put in place a compacted and channelled clay base, use of high-density polyethylene (HDPE) liners, and placement of absorbent layers of wood chips or hay.

Burial procedure: Site should be bigger than the size of the carcass allowing all parts of the carcass to be buried completely. The carcass can be pushed into the trench or placed in the trench using appropriate machinery and subsequently covered with sufficient quantity of lime/bleaching powder/ other disinfectants before covering it with earth. A layer of lime (approximately 5 cm) should be put both at the bottom of the burial pit and over the carcass before filling in soil. Following burial, the site should be fenced, protected and monitored for any breach.

Lime (calcium oxide)/ common salt can be used in the burial pits to increase the rate of decomposition of carcasses. The disinfectant properties of lime are due to its ability to raise the pH. A pH above 10 will disrupt bacterial cell walls and hydrolyse viral genome nucleotides. Unfortunately, this counteracts the acidification of carcasses that occurs naturally as part of the decomposition process and destroys many disease organisms. Hence, excess quantity of lime to burial pits is not recommended.

B. Thermal Treatment

Thermal treatment of the carcass through open burning or fixed facility incineration is another option for disposal of carcass. Open-air burning involves the burning of carcasses in an open setting, using combustible materials (dry wood) whereas incineration involves the combustion of organic materials in contained and controlled chambers, that are typically fuelled by gas or electricity. Though incineration is considered as an efficient and safe method of disposing of carcass (es), the major applicability of this method is for small sized captive animals and it has limited use for adult elephants. Though burning is practiced for disposal of elephant carcass in the field, it is

a highly resource-intensive method with regard to labour, fuel and other inputs.

An important consideration while burning the carcass (es) is to ensure sufficient airflow around the pyre for efficient combustion. Trenching underneath the pyre can facilitate easy air flow and provide draft that would help in efficient burning. The pyre design and the quality of the solid fuel used would determine the efficiency of combustion. The pyre should be constructed in a manner so as to maximise the airflow from wind. Pyres can be constructed in a rectangular shape, with the long edge at 90 degrees to the direction of the prevailing wind.

Burning can be done by placing dry wood/ iron rods over a trench over which the logs can be placed. The logs should be placed in a crisscross manner with adequate gap to facilitate air flow. The carcass can be lifted using JCB or heavy machinery and placed on the pyre. More wood and dry grass can be placed around the carcass before setting it on fire. Adequate fuel should be available for effective and complete burning of the carcass and the necropsy left overs. For disposal of carcasses by incineration, it should be ensured that incineration is complete and monitored till the whole carcass is reduced to ashes. Personnel on site must ensure that all possible controls are implemented to reduce the risk of fire spread including adequate clear area, supervision and presence of firefighting capability etc. The remaining ashes should be disposed of by burial on-site after the fire dies out.

Carcasses and tissues from necropsy of animals with known or suspected zoonotic agents must be incinerated. Carcass in all Anthrax/ suspected Anthrax cases should be burnt completely and under no circumstance be buried (Project Elephant. GoI (2019).

C. Leave *In-situ*

This method involves leaving the animal that has died of natural causes or accidents *in-situ*, and relies on changes in temperature and pH to reduce survival of the disease agent. Use of this method however, may only be possible in isolated areas following a detailed risk assessment. The risk assessment should include consideration of the potential for disease spread by scavenging species, and the potential for introduction of pathogens into wild or feral populations. In cases of confirmed natural death or confirmed mortality due to non-infectious/ non-malicious causes, the Chief Wildlife Warden of the state may be approached to permit natural decay at the site of death.

Other considerations:

1. Disposal of the necropsy waste, sharps and infectious materials should be carried out in a manner that minimizes the risk of environmental contamination and exposure of the necropsy team or domestic or wild animals to disease agents. They need to be appropriately disposed of.
2. It is appropriate to have a designated disinfection area to allow disinfection of vehicles, personnel and equipment leaving the burial site. Resources such as disinfectant, spray units and protective clothing would be additionally required. All personnel for this function will need to be trained in biosecurity.
3. It is important to consider health and safety issues while carrying out necropsy. The safety of the personnel involved in the operation is of paramount importance and every effort should be oriented towards minimizing exposure to pathogens through appropriate means. Besides the risks involved with manual handling associated with loading of carcass and handling of combustible materials should be adequately addressed.
4. The entire post-mortem process and elephant carcass disposal should be video- graphed and supplemented with still photographs and report prepared.

Cleaning and disinfection of necropsy area and tools

1. Necropsy areas and tools must be cleaned and disinfected at the end of necropsy using suitable disinfectants. The best choices for disinfectants are agents that have broad antimicrobial properties e.g. 0.5% sodium hypochlorite, 70% alcohol, borax etc. These disinfectants are effective against most common pathogens.
2. In cases of non-disposable instruments; blood and residual tissues on the instruments should be removed by washing with detergent solution followed by chemical disinfection or autoclaving. After thorough washing, the instruments should be rinsed with water and wetted thoroughly with 1:10 bleach solution or appropriate commercial disinfectants. Since bleach corrodes stainless steel and aluminum devices, it should be washed off after disinfection by thorough rinsing with water.
3. Disposable items, paper products, aprons, sponges and similar items should be placed into a biohazard container or bag and disposed-off appropriately.
4. Work surfaces and floors (in captive situations) should be washed with a detergent solution followed by disinfection with suitable disinfectant. Use of a cleaning agent such as trisodium phosphate or sodium carbonate dissolved in hot water facilitates cleaning of surfaces. Disinfectants recommended for general use on surfaces free of organic matter are 10% bleach, sodium or calcium hypochlorite, iodine, phenol, and quaternary

ammonium compounds or combinations e.g. quaternary ammonia and glutaraldehyde to enhance efficacy.

5. The selection of the best disinfectant for a particular situation as well as the contact time and concentration required for a particular pathogen is variable and should be carefully considered. The chosen disinfectant must be approved for the purpose and used in accordance with label directions including application for recommended contact time. Varity of disinfectants can be used for carrying out necessary procedures. These are summarized in **Annexure VII.**
6. High-pressure washing should be avoided until after the disinfectant has remained in contact with surfaces for the prescribed contact time.
7. The carcass should be disposed of appropriately. The blood and residual tissues over the instruments and tools should be removed with soap and water and subsequently disinfected. Disposal of carcass and the body parts should be done in the presence of the competent authority.

Standard format of necropsy report

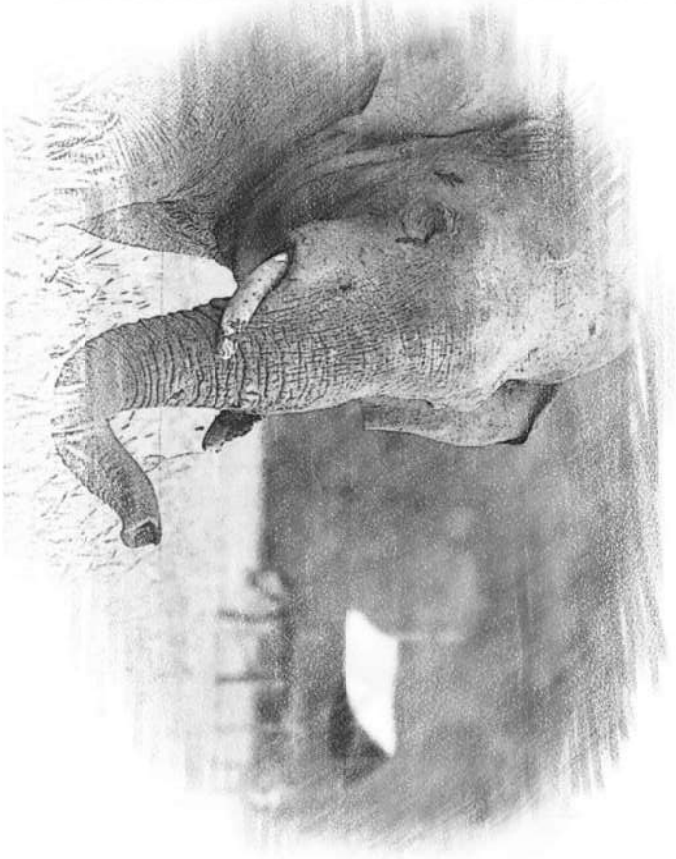
Recording of necropsy findings in a systematic, detailed and accurate manner is as important as the conduct of a thorough and systematic necropsy examination. Employing a standard format of necropsy report is extremely useful in accurate, detailed and systematic recording of necropsy findings. Use of standard format eliminates possibility of variability in reporting and omission/ missing of important data. A standard format of necropsy report is enclosed as per **Annexure VI.**

Conclusions

Necropsy examination has been accurately described as 'passing of knowledge from the dead to the living' as it enables clinicians not only in finding or confirming possible cause of death in animals but also in instituting timely and appropriate disease prevention and control measures.

Veterinarians must have the adequate knowledge of necropsy technique and related aspects such as biosafety, samples collection and preservation and writing of an informative necropsy report.

SECTION VI
MAJOR DISEASES SYMPTOMS & PATHOLOGICAL FINDINGS



Major diseases, symptoms and pathological findings during Necropsy

DISEASES	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Haemorrhagic septicaemia (HS) or Pasteurellosis caused by ***Pasteurella multocida*** serotypes (B, C & E) and *Mannheimia* (*Pasteurella haemolytica*) (All age groups)	Acute infectious disease is characterised by high fever, profuse salivation and severe dyspnoea. High fever, anorexia, listlessness, frequent yawning warm painful, oedematous swelling in throat region (head, neck, brisket) are characteristic and occur in nearly all cases. Swellings of various sizes in the region between jaws, neck, shoulder, anal region under the abdomen, groin and between hind legs and associated lameness may be encountered. Respiratory distress, and convulsions precede death by suffocation (resulting from to hypoxaemia and toxaemia.).	Widespread hemorrhages, oedema, and hyperemia, consistent with severe sepsis. Swelling of the head, neck, and brisket occurs in nearly all cases. Tongue dark and the lining of the mouth is coated with thick saliva. The palate may be dark and with mulberry spots. Sub-serosal petechial hemorrhages may occur throughout the body Thoracic and abdominal cavities often contain blood-tinged fluid Bronchi and bronchioles filled with "frothy mucus plug", heart may show varied degrees of haemorrhage over the endocardium with edematous atrial wall, liver congested and hard, spleen haemorrhagic, edematous thickening of stomach and intestine with haemorrhage in the mucosal wall Scattered petechiae may be visible in the tissues and lymph nodes, particularly the pharyngeal and cervical nodes; these nodes are often swollen and hemorrhagic Pneumonia or gastroenteritis occasionally occurs, but usually is not extensive There are no microscopic features that are specific for hemorrhagic septicaemia – all lesions are consistent with severe endotoxic shock and massive capillary damage	**Culture and isolation:** Heart blood in EDTA or heparinized vail, nasal and endocardial swabs, tissues (spleen) are good samples. Bone marrow from long bones (femur/humerus) can be collected from putrefied carcasses. Other visceral organs (lung, liver, kidney, heart) may also be collected for histopathological examination. **Molecular diagnosis:** Direct field samples such as nasal swab, spleen, bone marrow, and heart blood. **Blood smear**: Heart blood (characteristic bipolar Gram-negative coccobacillus are indicative). Samples should be collected and transported on ice

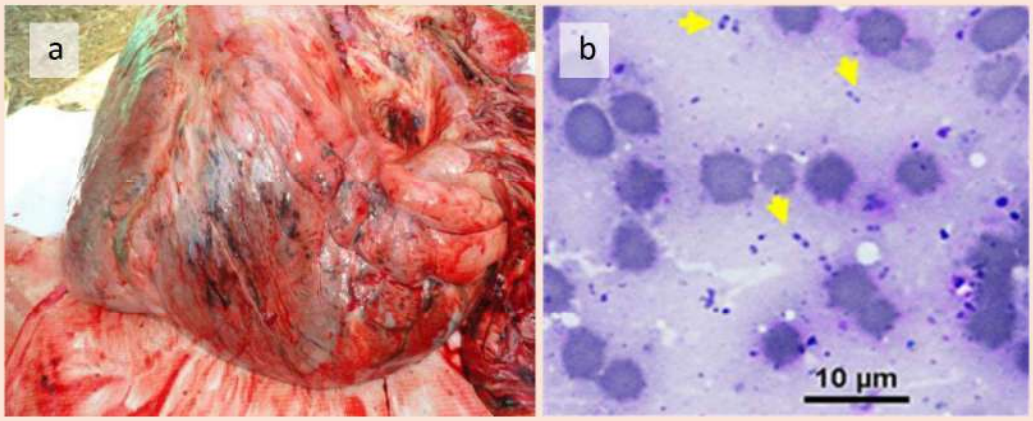


Plate 16: (a)Diffuse haemorrhages (Paint brush) in epicardium of elephant died of Pasteurellosis (© Nandakumar, S.); (b) Heart blood smears showing bipolar organisms, Giemsa staining X1000 (© Karikalan, M.)

DISEASE	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Tuberculosis	In captive animals- reluctance towards strenuous activity/ work with rapid exhaustion & breathlessness, chronic weight loss, progressive weakness, cough & discharge from trunk	Emaciated carcass, purulent exudates and caseated lesions in the lungs (multifocal to coalescing pale tan-to-white firm nodules (granulomas) effacing much of the lung parenchyma with areas of white chalky mineralization). Enlarged liver, enlarged mesenteric lymph nodes. Surface nodules containing caseated yellowish-white material besides serosanguinous fluid in the pericardial sac and focal areas of necrosis in the renal cortices	Impression smears of tubercle on visceral organs and lymph nodes (mesenteric). Ziehl Neilsen staining may reveal acid fast bacilli. Tissue samples in 10% formalin for histopathological examination Tissue samples in absolute alcohol for molecular studies (PCR amplification of the targeted bacterial genome of *Mycobacterium sp.*, gel documentation of the amplified products, and sequencing)

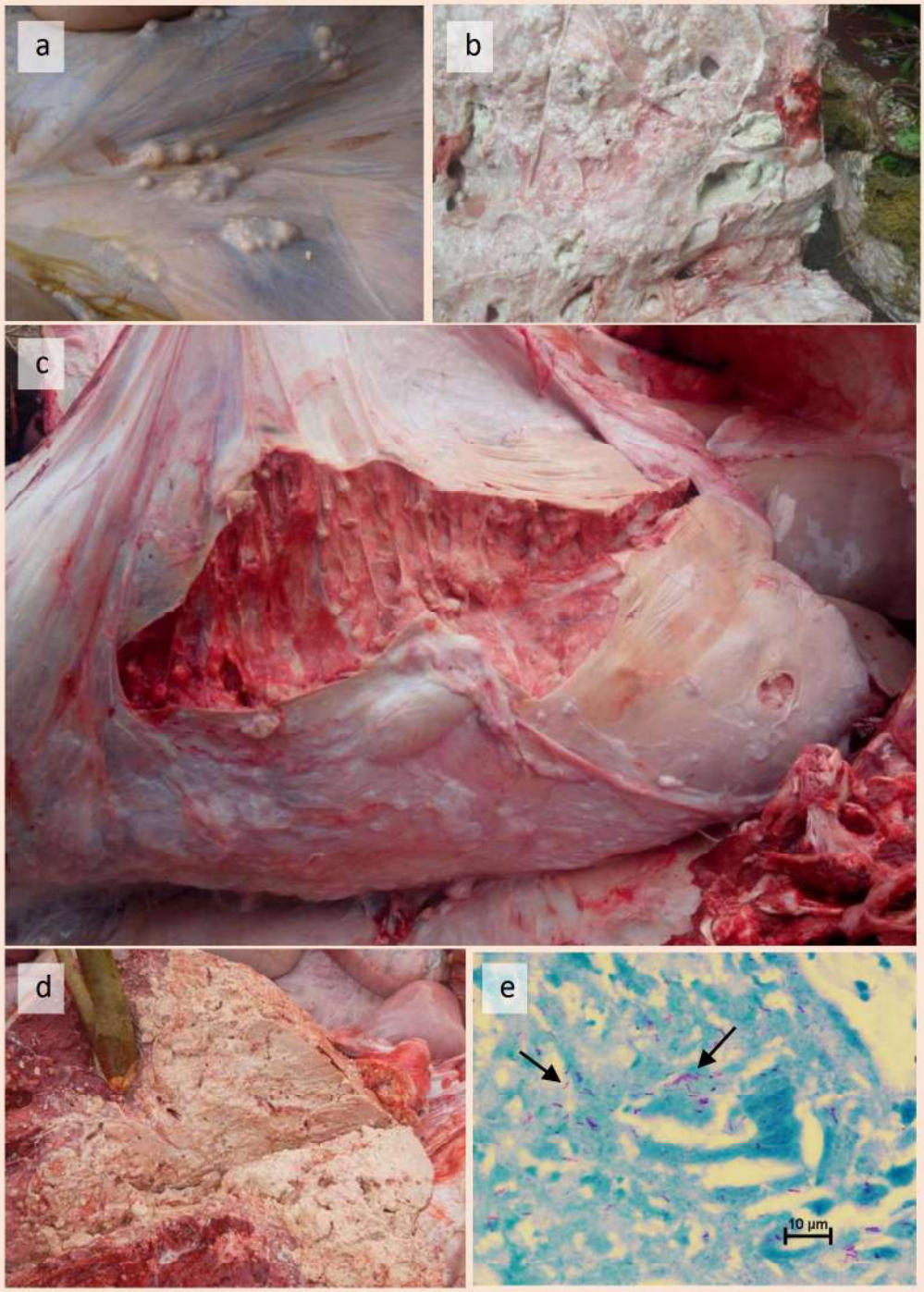


Plate 17: (a) Miliary tuberculosis with tiny caseous nodules; (b) Suppurative tuberculous pneumonia (© Nandakumar, S.); (c) Lung parenchyma cut surface showing miliary caseous nodules; (d) Large caseous mass in lung parenchyma; (e) Photomicrograph showing acid-fast bacilli in a tuberculous lung. (Zeil Neilsen Stain X 1000) (© Karikalan, M.)

DISEASE	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Anthrax (All age groups)	High fever, difficulty in breathing, "off-feed" swelling in body parts (thorax, shoulder, base of the tail, anal flap, belly), trunk resting, minimal movements followed by sudden death with bleeding from natural orifices	Carcass decompose rapidly with excess gas formation, Other signs include bleeding from natural orifices (ears, mouth, eyes, genitals, rectum, trunk). Blood is dark, tarry coloured and does not clot. Rigor mortis may be absent or incomplete.	Bloody discharges from natural orifices in sterile/ EDTA tubes and ear pinna piece under cold chain Blood Smears stained polychrome methylene blue/ Lieshman's stain

NOTE: No post-mortem should be carried out in deaths suspected for Anthrax and the procedures referred in SOP on Anthrax (Project Elephant. GoI, 2019) may be followed.

Plate 18: Bloated carcass showing tarry coloured blood oozing from natural orifices (suspected for Anthrax) (© Rajeshkumar, K.)

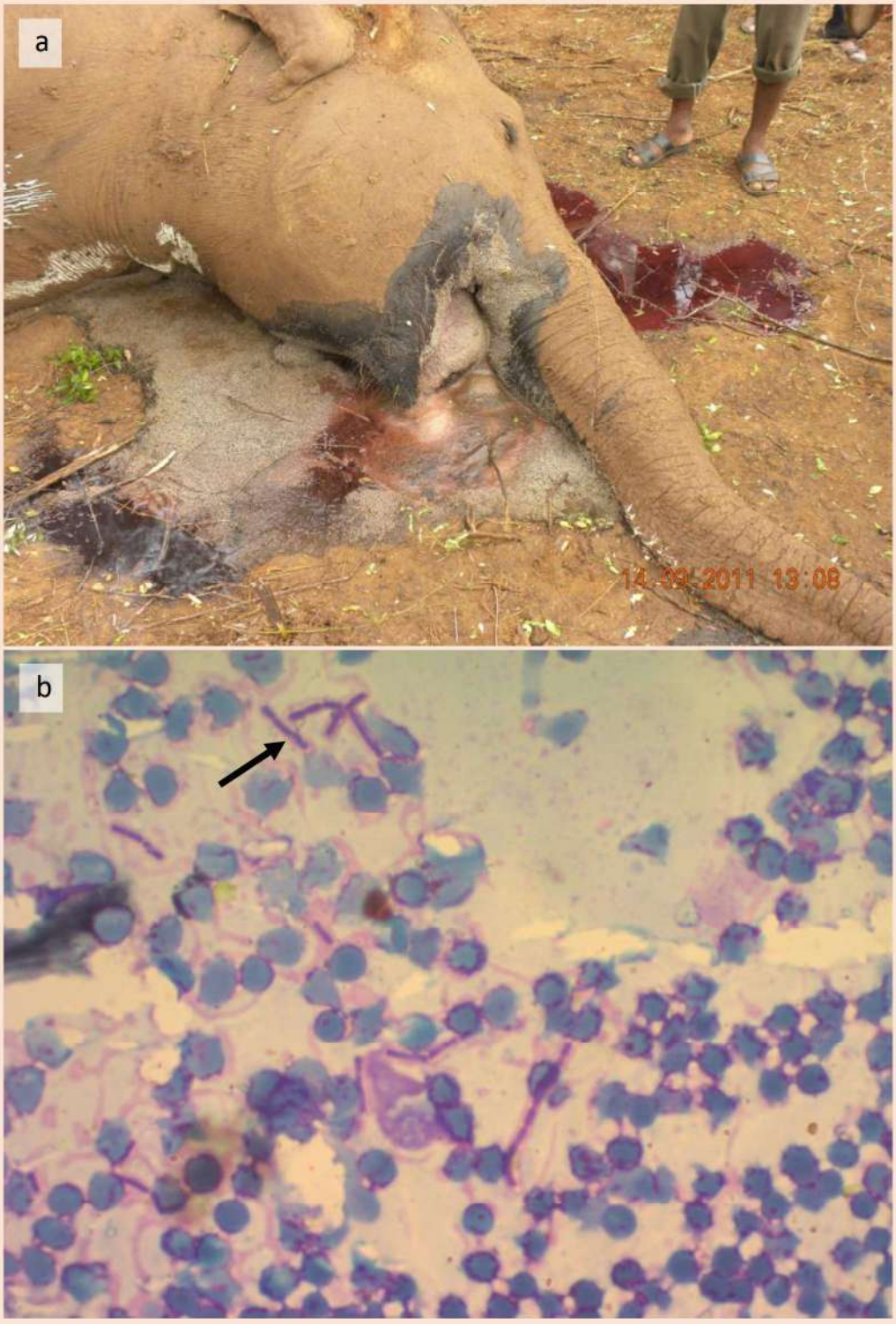
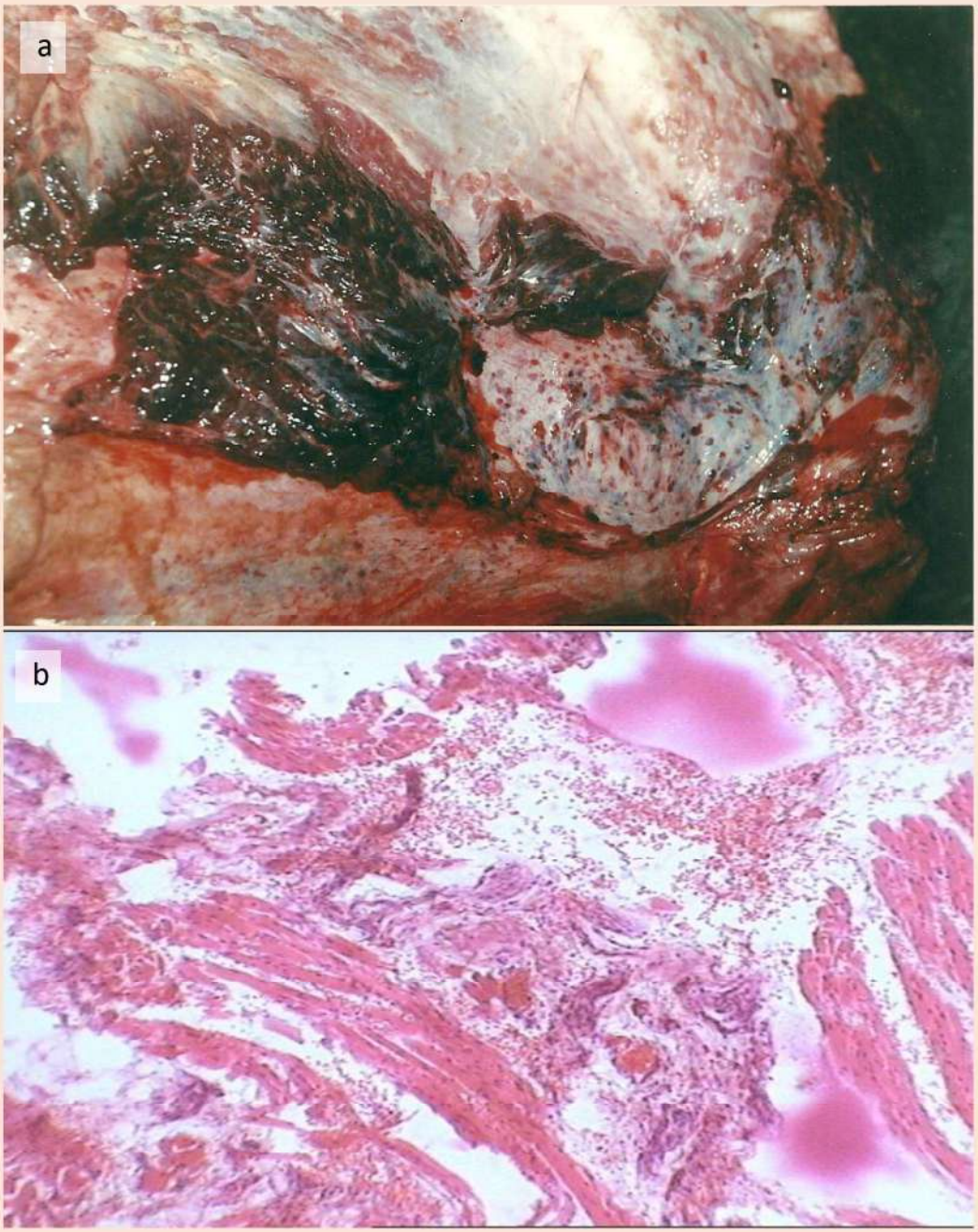


Plate 19: (a)Pooling of blood around the carcass in the dry soil (© Rajeshkumar, K) (b) Blood smear stained with Polychrome methylene blue showing characteristic *Bacillus anthracis* bacteria X 1000(© Karikalan, M.)

DISEASE	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Tetanus *Clostridium tetani* **(All age groups)**	Lock jaw, fever, breath will be noticeably hot to feel, red eyes; Buccal mucosa and trunk will be dark red, leg muscle hardening, tail stiff, periodic spasm, difficulty in walking. Muscle spasms, tetany is common	No characteristic lesions Blood may be black or tarry Rigor mortis sets in immediately after death No evidence of septicemia, encephalitis or meningitis encountered.	Pieces of visceral organs preserved in 10% formalin, for histopathological examination Blood smear, pus from wound for culture under anaerobic media
Clostridium perfringens type A beta2 toxin	Acute condition, Sudden off feed, weakness, anorexia, pyrexia and prolonged episodes of watery followed by bloody diarrhoea.	Diffused haemorrhagic and necrotic enteritis, lesions and edema in both small and large intestines. Discrete areas of necrosis on the walls of the caecum and colon.	
Clostridium perfringens type A alpha toxin	Anorexia, listless, pyrexia, lameness, stiff gait, oedematous swelling on neck, forelegs and back, Swellings pit on pressure	Myonecrosis as evidenced by diffuse haemorrhage with reddish to bluish discoloration of the muscles of the neck, tongue, back region and limb. Crepitation on palpation. Sero-sanguineous fluid from the emphysematous tissues on incision. Other septicaemic lesions in gastrointestinal tract, liver, spleen, kidneys, lymph nodes (mesenteric) and heart	Impression smear from the affected tissue for Gram's staining Tissue samples and Aspirated fluids for culture in anaerobic media Affected muscle tissue and visceral tissues in 10% formalin for histopathology
Clostridium septicum (Malignant oedema)	Edematous swelling around a wound that rapidly spreads in the subcutaneous tissue surrounding the	Edematous subcutaneous cellulitis.	

DISEASE	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
	wound. There is no gas formation Fever, rapid pulse, anorexia, depression, and weakness and sudden death has been reported		
Enterotoxemia Type C & D *(Clostridium perfringens type C & D)*	Severe diarrhoea, gas colic, prostration in Enterotoxemia Type C and sudden death, convulsions, circling posterior paralysis, minimal diarrhoea in Enterotoxemia Type D	Hemorrhages of the mucosa of intestine which is distended with gas and fluids. Hemorrhages on serosa of intestine, epicardium, and endocardium. No gas.	Use selective media to culture salmonella from feces (MacConkey agar, brilliant green agar, or selenite F broth) or anaerobic media for Clostridial species. Samples from tissue at necropsy may be plated directly onto blood agar.
C. diffcile	Altered behaviour, anorexia and listlessness Acute fatal enteritis	Fibrino-necrotic enterocolitis with considerable thickening of the ilium with mucosa covered with a yellowish fibrinous pseudo-membrane with multiple longitudinal ulcerations.	Samples of intestine, intestinal content, lung, heart, liver, kidney and spleen were collected aseptically for bacterial culture



Platc 20: (a) Hacmorrhagcs and nccrosis of muscle with cdcma in thc thigh rcgion in Clostridial infection in elephant calf. (b) Clostridial myonecrosis due to *Clostridium perfringens* type A. Affected muscles showing diffuse haemorrhages, intramuscular spaces with focal necrosis and empty spaces indicating the presence of gas bubbles (© Apurba Chakraborty)

Plate 21: Collibacillosis in a Calf: (a) Necrotic large intestinal epithelium showing haemorrhage with muddy ingesta (b) Extensive edema and thickening of the intestinal wall (c) Necrotic nodular ulcerative lesions in the large intestine in new-born calf. (*E. coli* rough strain, O37, O18) (© Apurba Chakraborty).

DISEASE	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Salmonellosis/ Colibacillosis (*Escherchia coli*) **/ Staphylococcus (**Staphylococcus spp**)/ Lactose fermenting coccobacilli /**	Most of the deaths of young and newly received calves are associated with gastrointestinal problems, including infections, indigestion, intolerance and diarrhoea.	Severe gastroenteritis, haemorrhagic gastroenteritis in Salmonellosis/ Colibacillosis	Blood and faeces for culture. Additionally, spleen, liver, and bone marrow are good tissues from which to obtain cultures. For invasive strains of *E. coli,* culture of the organism from what should be sterile sites such as joints, bone marrow, spleen, or blood is definitive.

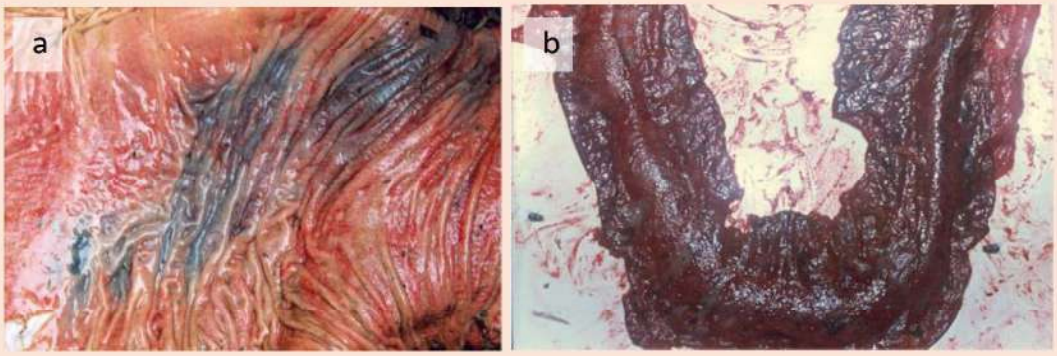


Plate 22: Salmonellosis: (a) Linear haemorrhagic gastritis (b) Severe haemorrhagic enteritis (small intestine) with massive destruction and sloughing of epithelium at places (© Apurba Chakraborty)

DISEASE	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Elephant Endotheliotropic Herpesvirus (EEHV)	**Primarily a disease of young animals** Silent infection Lethargy and Anorexia, pyrexia Edema of head, trunk and front leg, cyanosis of the tongue, mouth ulcers, mild gastroenteritis, lameness, nervous signs Acute mortalities in young elephants	Petechial and ecchymotic hemorrhage throughout internal organs and tissues, hemorrhage and edema of submucosa and sub-serosa of the gastrointestinal tract, ulcerations of the oral cavity, larynx, and large intestine, lingual cyanosis, hepatomegaly with hepatic sinusoidal expansion, erosive and ulcerative dermatitis of the skin, hepatomegaly Severe petechial hemorrhages on the epicardial and endocardial surface of the heart with accumulations of sero-sanguinous fluid in the pericardium Intranuclear inclusion bodies in endothelial cell lining of visceral organs, particularly liver sinusoid and spleen histopathologically	Blood, ocular swab and faecal swabs collected in viral transport media or Phosphate buffer saline and transported under cold chain. Trunk wash (Sharma et al., 2021) and processed pellets in anti-DNase or anti-RNase solution, Blood in EDTA/Heparinized vials and serum for serological tests Tissue (liver, heart, kidney, tongue, trachea, lung, trunk mucosa, brain, mammary gland, lymph node, urinary bladder, spleen, pancreas and intestine) in viral transport media under cold chain Samples from all organs that exhibit haemorrhagic lesions should be collected in 10% neutral buffered saline for histopathology and in absolute molecular grade alcohol (ethanol) for PCR Analysis

Plate 23: (a) Subcutaneous odema of head and jaw region attributed to EEHV Disease (b) Cyanosis of tongue (c) Mesenteric vasculitis (© Avadh B Shrivastav); (d) Diffusely congested brain with haemorrhages, (e) Ecchymotic to diffusive haemorrhages on epicardium in elephant calf (f) Acute haemorrhagic disease with severe endocardial haemorrhage (© Karikalan, M.)

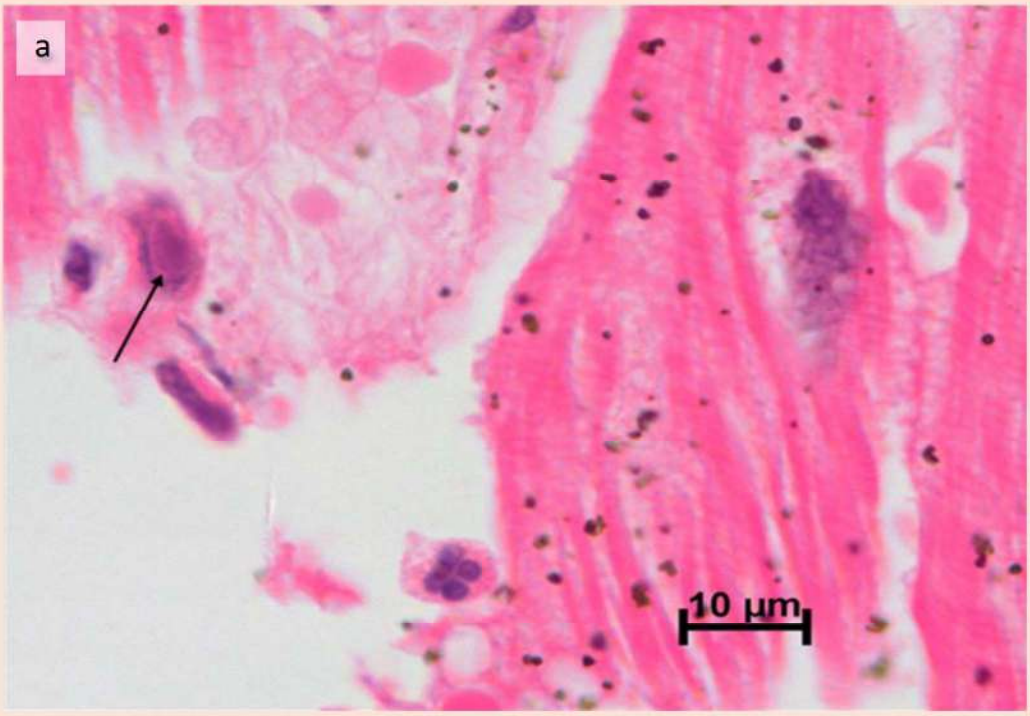


Plate 24: Intranuclear inclusion bodies in cardiac blood vessels (arrow) due to EEHV infection H&E 100X (© Karikalan, M.)

DISEASE	SYMPTOMS IN LIVE ANIMALS	PATHOLOGICAL FINDINGS	SAMPLING
Rabies	Aggressive and restless, difficulty in bearing weight on the hind limb, decreased urination and defecation, , temporal discharge, drooling of saliva from mouth and death in 5—9 days	The brain and meninges congested and more vascular than normal. Histopathological findings include non-suppurative encephalomyelitis, perivascular cuffing, formation of small glial nodules (Babes' nodules), neuronal degeneration and presence of Negri bodies (intra cytoplasmic eosinophilic inclusions) in the neurons.	Brain (cerebrum, hippo campus, cerebellum) in ice or 50% glycerol saline or impression smears from brain fixed in chilled acetone for Fluorescent antibody test (FAT), RT-PCR

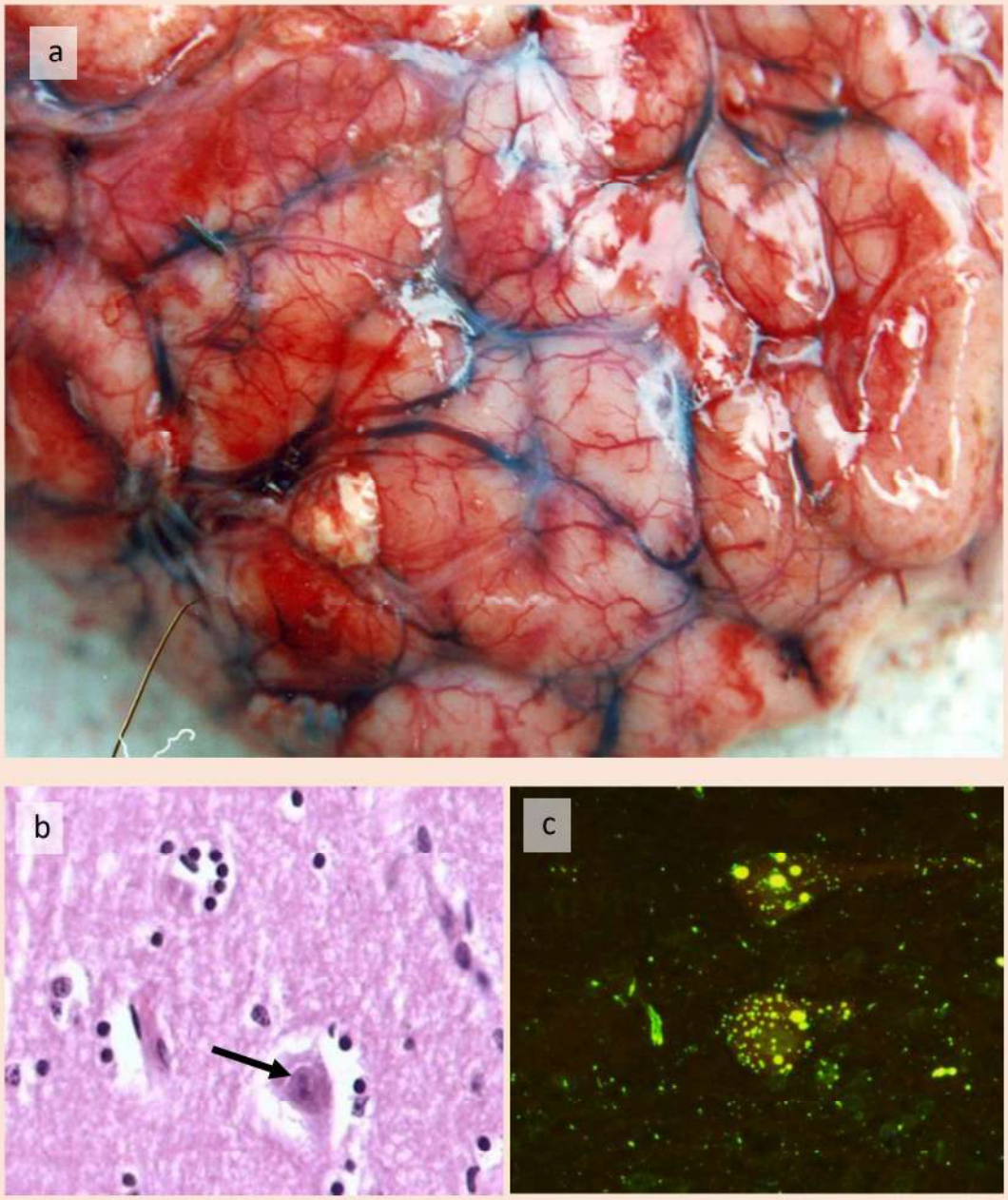


Plate 25: (a) Wide spread congested meninges and brain capillaries in Rabies infection (© Apurba Chakraborty); (b) Brain showing negri bodies in the cytoplasm of neurons (arrow) in the cerebrum with satellitosis. H&E X 400 (c) Impression smear of brain with dusty apple green fluorescence positive for rabies virus. FAT X 400 (© Karikalan, M.)

Major Endoparasites encountered in Asian elephant

Nematode (**Roundworm**)	*Strongylosis* ***Murshidia murshida, M. falcifera, M. indica*** **(Large intestine and caecum);** ***Decrucia additictia*** **(Large intestine and caecum);** ***Quilonia renniei, Q. travencra, Q. simhai, Q. guptai*** **(caecum);** ***Amira pileate*** **(Large intestine and caecum);** ***Choniangium epistemum, C. magnostomum*** **(caecum);** ***Equinurbia sipunculiformis*** **(caecum);** ***Bunostomum foliatum*** **(Stomach hook worm); &** ***Bathmostomum saneri*** **(Intestinal hook worm);** ***Leiperenia galebi*** **(intestine)** ***Mammomonogamus indicus, Grammocephalus veredastus, G. hybridatus*** **(Bile duct)** *Strongyloidosis* ***Strongyloides elephantis*** **(Small intestine)** *Parabronemiasis* ***Parabronema indicum*** **&** ***Parabronema smithi*** **(Stomah worm)** ***(*****small tumours and ulcers on the submucosa and muscular layer of stomach)** *Ascarids* ***Toxocara elephantis*** ***Balascaris lonchopetra*** **(bile duct)** *Eye worm* ***Thelazia sp.*** **(Eye)** *Filalrial Worm larvae* ***Indofilaria pattanhiramani*** **&** ***I. elephantis*** **(Cutaneous haemorrhagic nodules underneath abdomen, outer side of hind limbs);** ***Stephanofilaria srivastavi, S. assamensis*** **(sore on back)** *Blood worms* ***Onchocerca armillata*** **(Aorta)**
Trematode **(Fluke)**	*Fasciola jacksoni* (Bile duct) *Pfenderius papillatus* (Large intestine), *P. birmanicus, P. heterocaeca; Pseudodiscus collinsi* & *P. hawkesii*; (Large intestine), *Gastrodiscus secundus (Caecum);* *Bivitellobilherzia nairi* (Portal vessels)
Cestode **(Tapeworm)**	*Anoplocephala manubriata* (Intestine)
Flies/ Myiasis	*Cobboldia elephantis* (Asian elephant stomach bot fly): Gastric myiasis (larvae) *Haematomyzus elephantis* (Biting flies) *Elephantoloemus indicus (*Asian elephant skin maggot)
Ticks & Fleas	*Boophilus annulatus, Haemophysalis spinigera, Rhiphicephalus haemophysaloides* and *Ornithodorus savignyi; Vermipsylla sp.*(Flea)
Louse	*Haematomyzus elephantis*

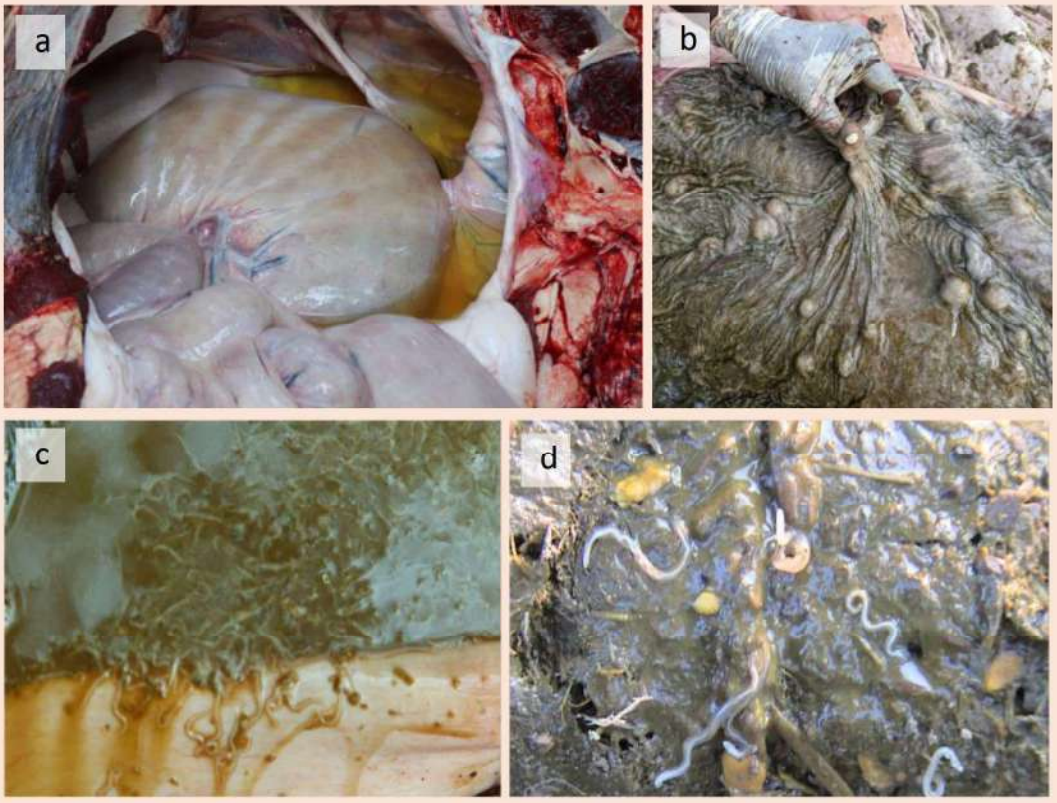


Plate 26: (a) Presence of clear transparent fluid (transudate) in the abdominal cavity (hypoprotenimia due to severe parasitic infestation) (© Karikalan, M.); (b) Verminous nodules of Amphistome (*Gastrodiscus secundus*) in cecum of elephant, (© Rajesh Kumar); (c) Large numbers of strongyles (*Mursidia mursidia, Choniagium epistomum* and other strongyle) in intestine of an elephant (d) Strongyle parasite on the faecal ball (© Apurba Chakraborty)

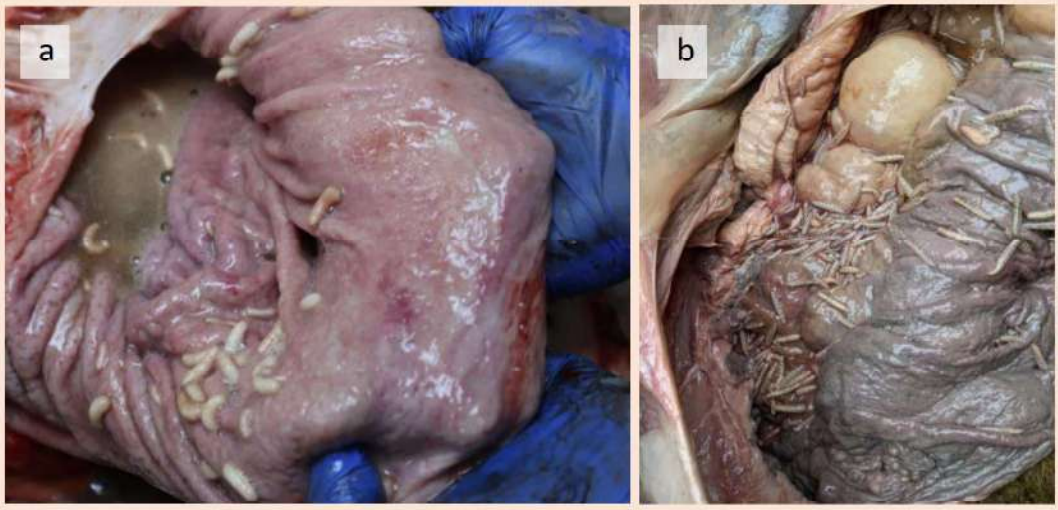


Plate 27: Stomach and abdominal cavity infested with dipteran larvae of *Cobboldia elephantis* (a)(© Karikalan, M.); (b) (© Rajeshkumar, K.)

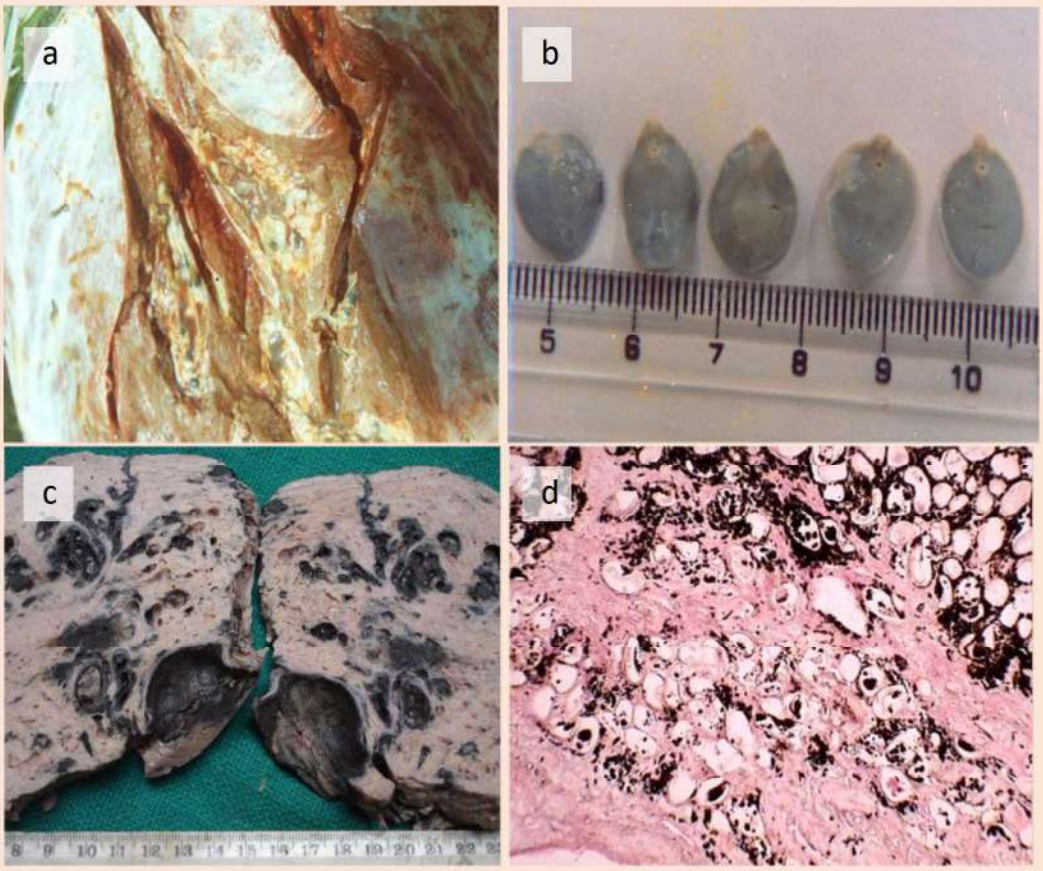


Plate 28: (a) *Fasciola jacksoni* in the bile ducts of an adult elephant (b) *F. jacksoni* recovered from the bile duct of elephant. The first and third specimens are placed dorsally (c) Formalin fixed cut section of liver showing massive destruction of parenchyma with bile ducts filled with necrotic materials giving a clay pipe appearance (d) Photomicrograph of liver showing fibrosis of parenchyma and tapping of large numbers of F. *jacksoni* eggs with the necrotic debris of bile ducts. H&E.X 100. (© Apurba Chakraborty)

Plate 29: Amphistome (*Pfenderius papillatus and Pseudodiscus collinensis*) in the large intestine of an elephant. Note edema of the intestines) (© Apurba Chakraborty)

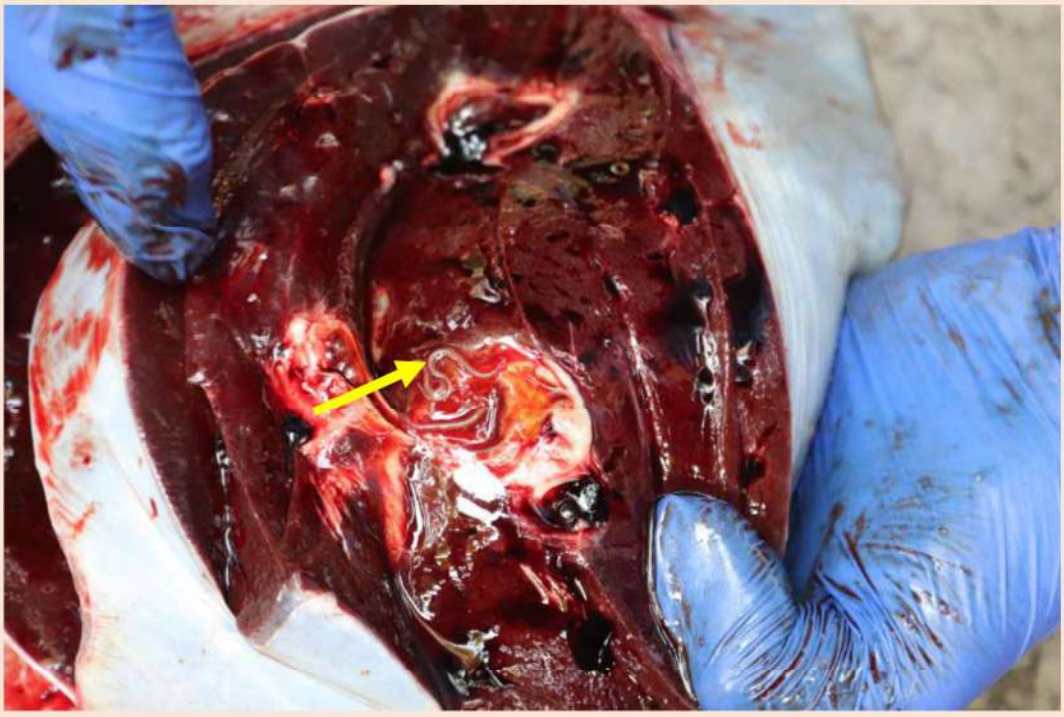


Plate 30: Hook worm (*Gramocephalus sp*) in bile duct (© Karikalan, M.)

Plate 31: (a) Skin lesion on the head from where *Stephanofilaria sp.* could be recovered (b) Photomicrograph from the skin showing cross section of *Stephanofiraial sp.* and infiltration of mononuclear cells H&E X 100. (© Apurba Chakraborty); (c) Ectoparasite infestation (*Amblyoma sp.*) (© Manoharan, N.S.)

Plate 32: Mycotic dermatitis in the abdominal region of an elephant caused by *Trichophyton sp.* (© Apurba Chakraborty)

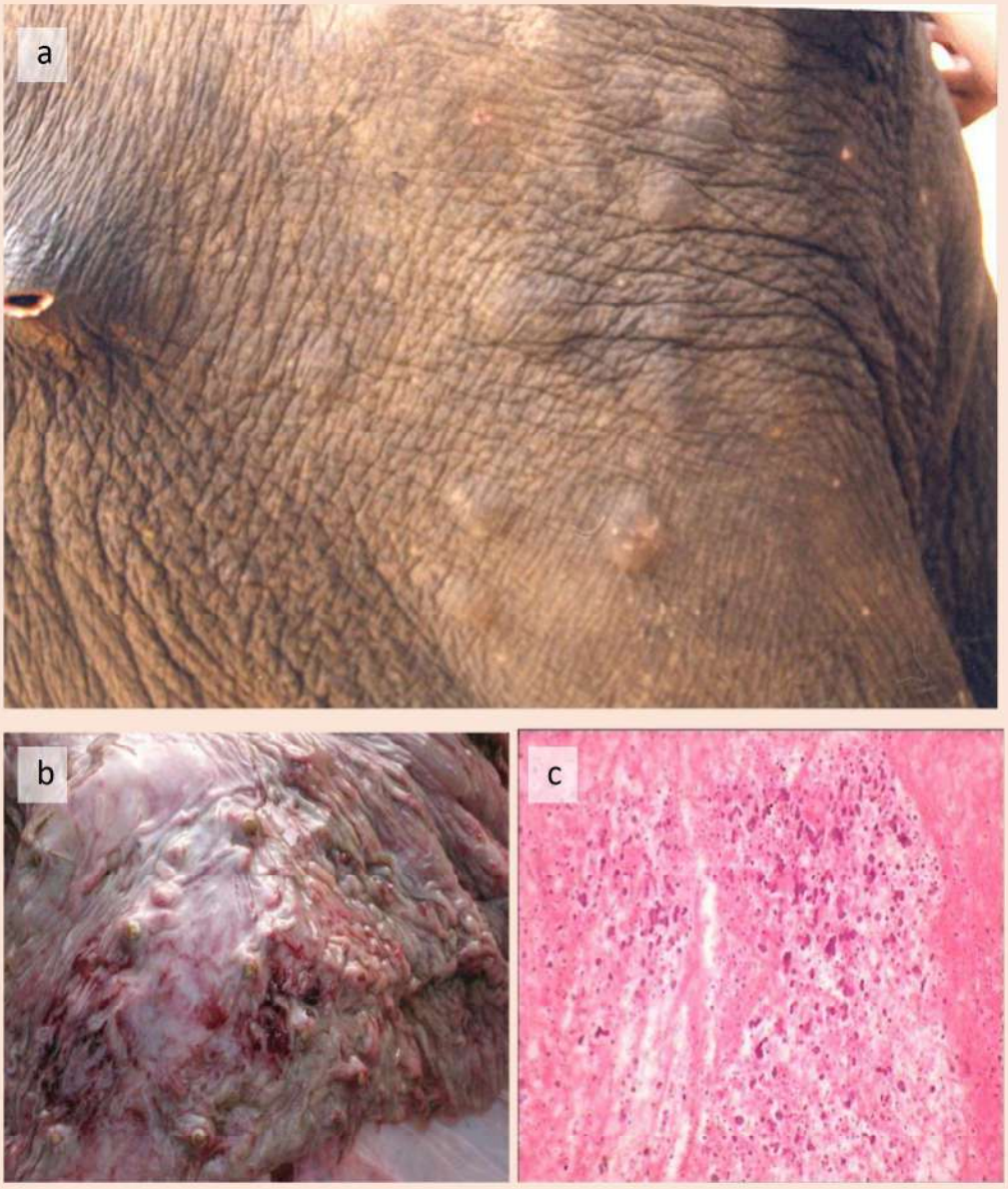


Plate 33: (a) Nodular circumscribed skin lesion of an elephant caused by an unidentified dipterous larva of non-biting fly. Dipterous larvae similar to *Cobbaldia elephantis* (though smaller in size) were observed on cut opening the nodules. (b) Nodular necrotic lesions measuring about 2-4 mm in diameter on the intestinal mucosa indicative of Candidiasis (© Abhijit Bhawal); (c) Photomicrograph of the nodule (figure b) showed presence of dimorphic fungi *Candida sp*. H&E X 100. (© Apurba Chakraborty).

CONDITION	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Electrocution (All age groups)	Electrocution gives a burnt appearance to the skin especially on the trunk and at times on the jaws where point of contact is obvious. (Noticeable burnt mark of the point of contact and exit of electric current can be appreciated)	Rigor mortis develops and passes quickly in electrocution cases. Electrical burn marks on the trunk and legs of the elephant are obvious while hemorrhage and congestion are encountered in the lungs, heart and omentum with considerable endocardial hemorrhage. Hardening of brain meninges may be noticed. Cooked appearance of the muscles is also evident in cases of electrocution The heart chambers contain black dried-up blood masses in charred carcasses. The mass is fragile and gives powdered feel, with appearance and consistency similar to that of charcoal powder.	Routine samples

Note: All cases of electrocution would invite provisions of the Wildlife Protection Act and should be handled as vetero-legal case.

Plate 34: (a) Focal areas of charred skin (© Rajesh Kumar), (b) Burn marks (© Sandeep Agrawal)

Plate 35: (a) Focal areas of charred skin (© Karikalan, M.); **(b)** Subcutaneous haemorrhage and congestion below the charred burnt area (© Jayjit Das)

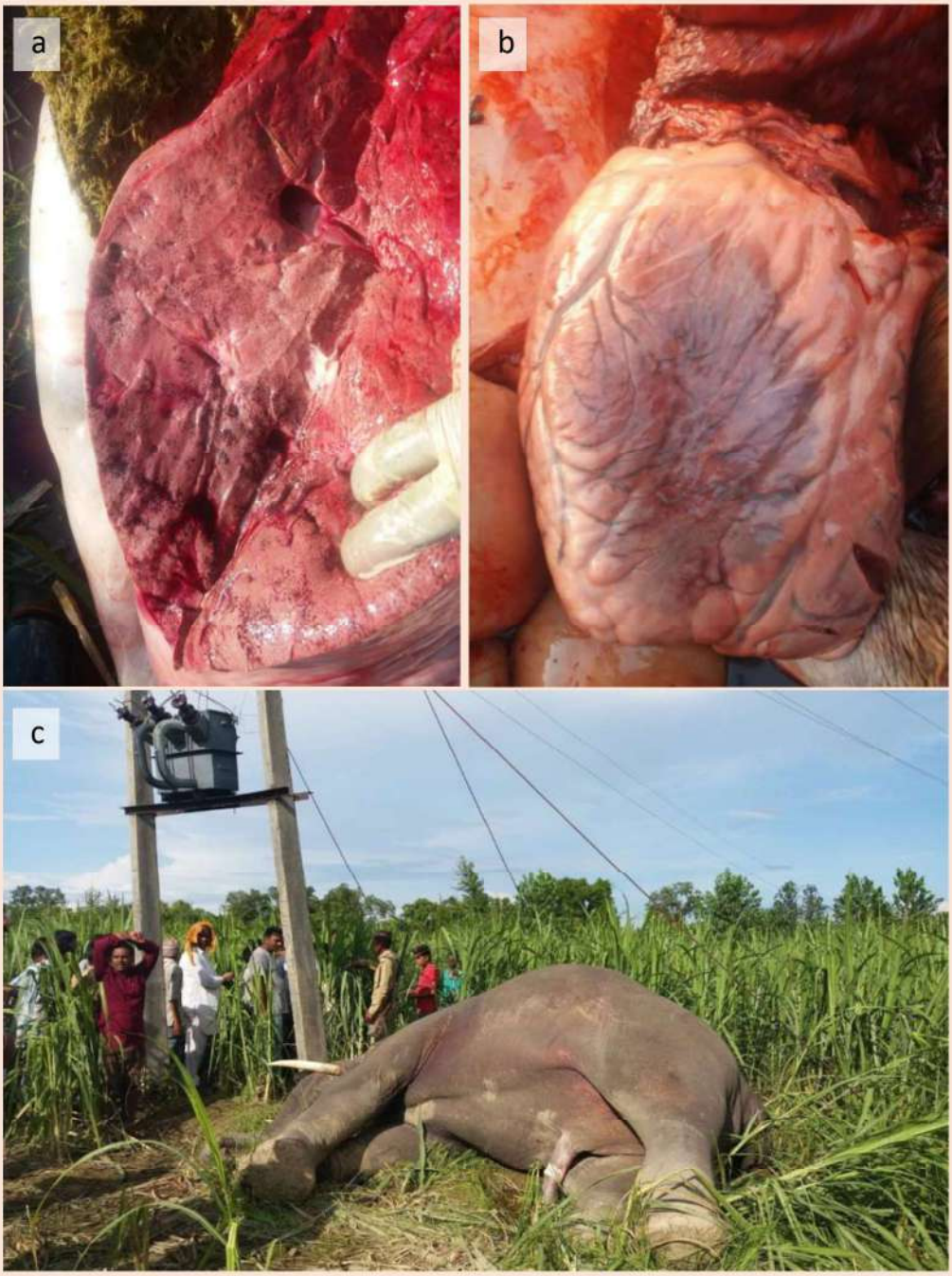
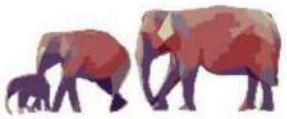


Plate 36: (a) Cut surface of liver showing multifocal areas of parenchymal haemorrhage (b) Patchy congested areas on epicardium, (c) Carcass of elephant died due to electrocution (Circumstantial evidences) (© Karikalan, M.)

CONDITION	SYMPTOMS	PATHOLOGICAL FINDINGS	SAMPLING
Poisoning (All age groups)	Hyper reactivity, excitement, discordant movements, arched back, ataxia, seizures, excitement and salivation, head pressing. Symptoms vary based on chemical/ toxicant used	Lesions are more commonly seen simultaneously with changes consistent with gastroenteritis, fatty liver, necrosis of liver, lesions on renal cortex, and pulmonary edema. Post-mortem lesions may vary based on chemical/toxicant. Yellowish coloration of the skin: possible phosphorus poisoning. Cherry coloured upper palate: indicative of cyanide or carbon monoxide poisoning Red colour: indicative of nitrates or nitrite poisoning Corrosive lesions on the skin, mouth and lips: possibly due to poisoning with strong acids or alkalis	In cases where the animal is still alive, the blood, urine, faeces, nearest water and feed source materials may be collected for analysis During post-mortem for toxicological examination stomach content, piece of liver, and loop of intestine and the viscera of the animal must also be collected and preserved for histological and toxicological examination. (Refer Cheeran, 2007)



Other Findings During Necropsy

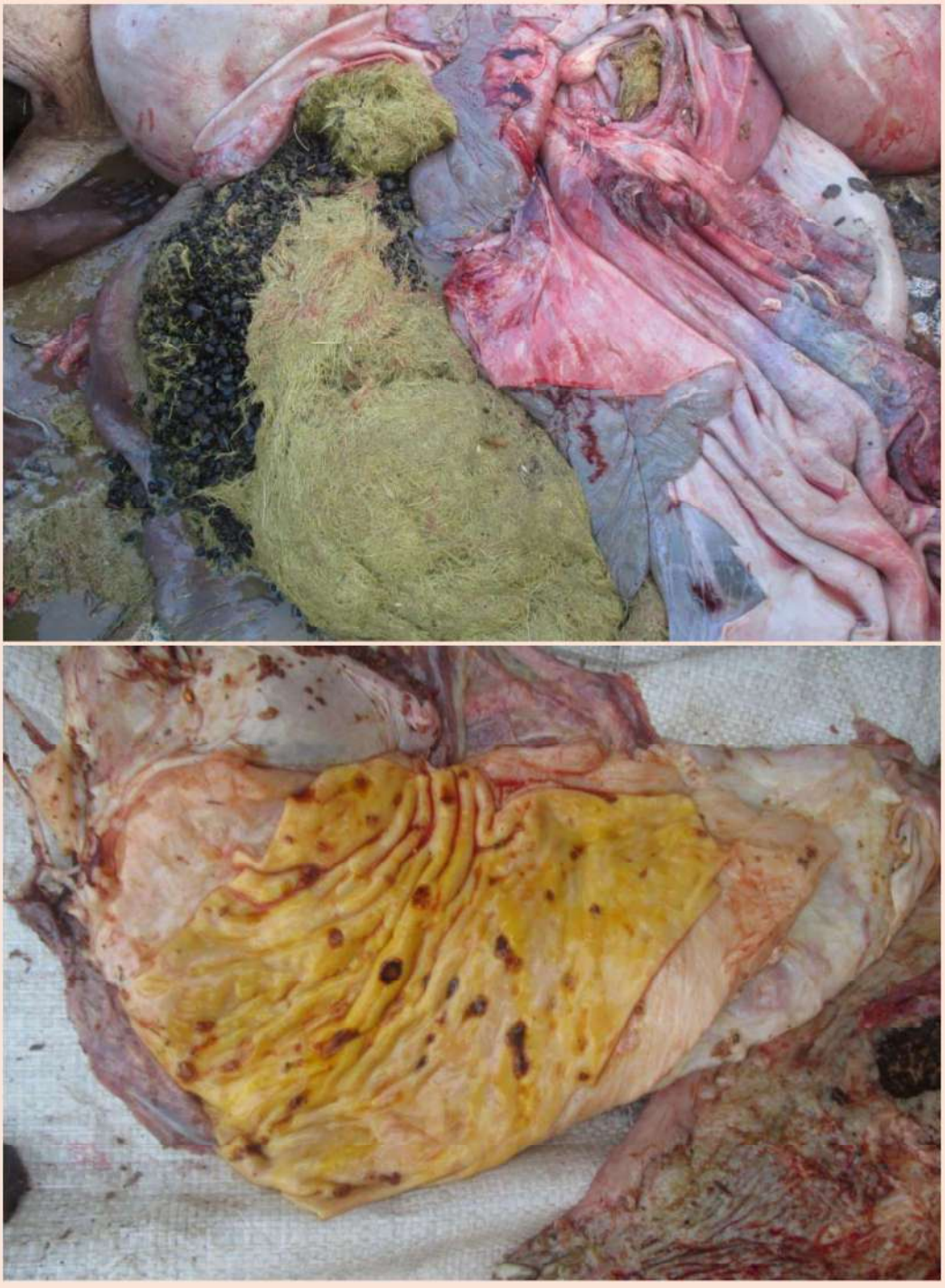


Plate 37: (a) Stomach containing screening gravel along with digested food (b) Gastric ulcer due to prolonged oral medication (Uremic condition) (© Nandakumar, S.)

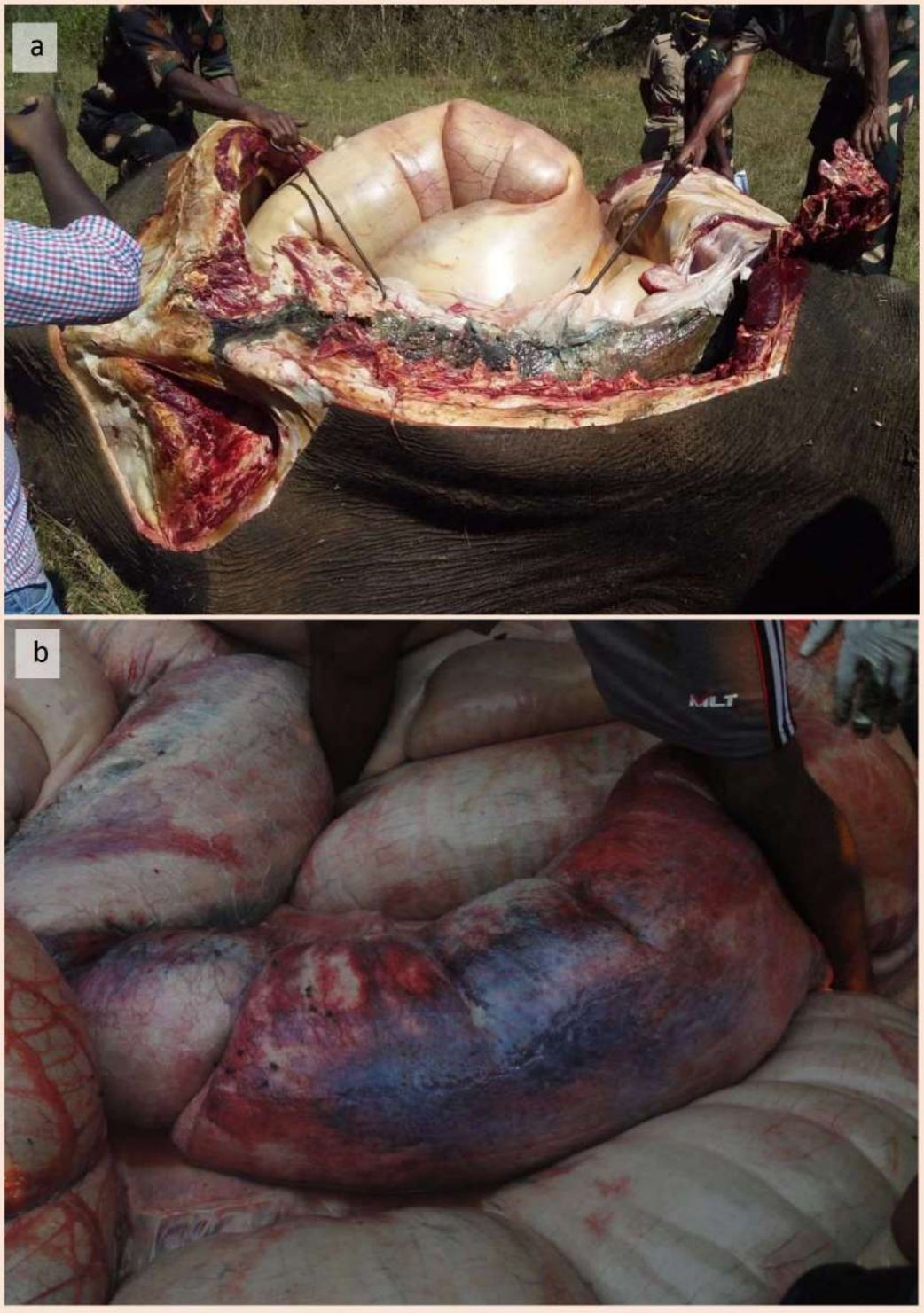


Plate 38: (a) Fistula extending from back on the ventral aspect of vertebral column (© Rajeshkumar, K.); (b) Congested bowel loop due to colonic impaction (© Nandakumar, S.)

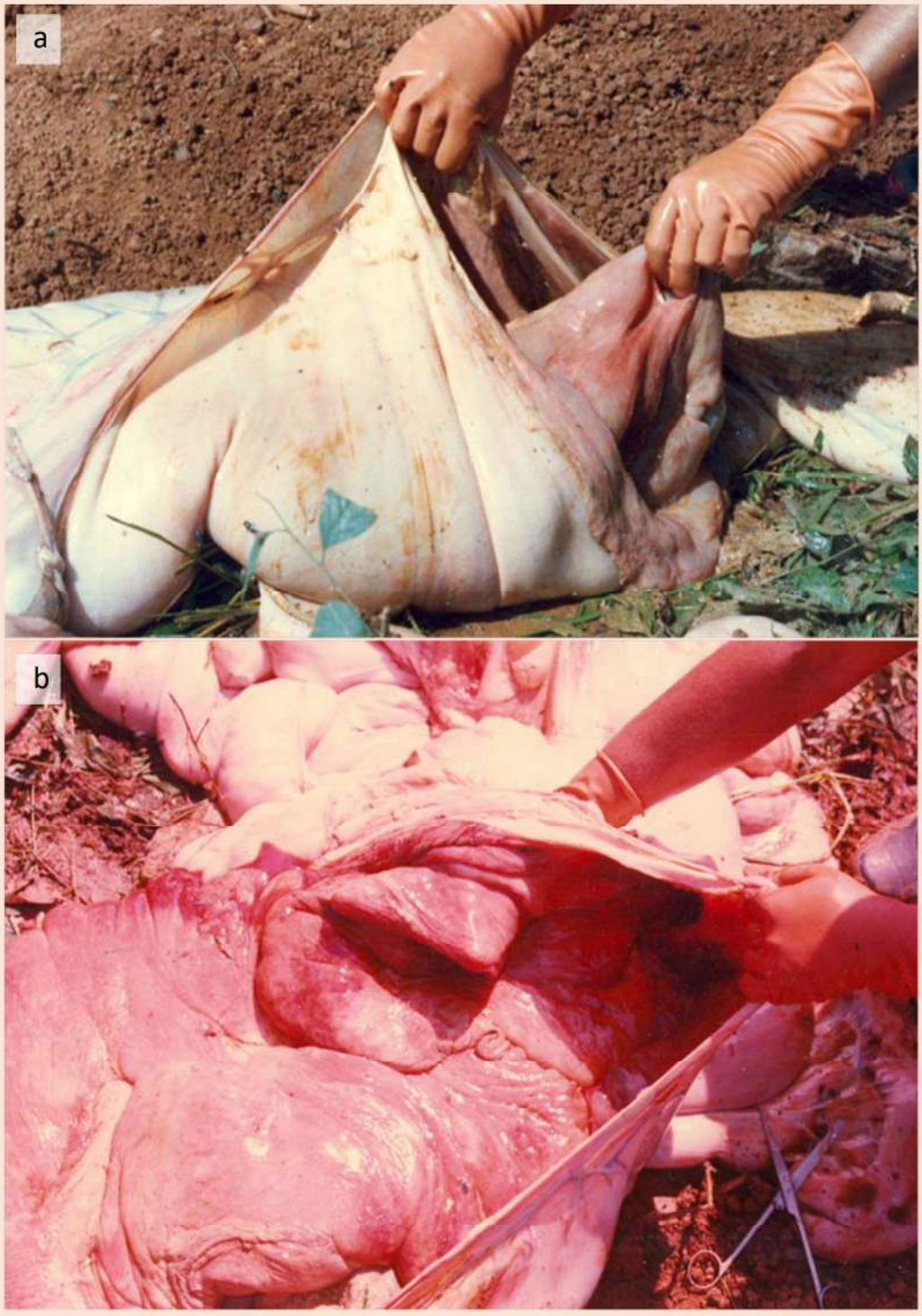


Plate 39: (a & b) Case of intussusception in an elephant (Telescopic invagination of jejunum into ilium) (© Apurba Chakraborty)

Plate 41: (a) Elephant calf carcass (b) Umbilical abscess in calf (c) Congestion in meninges, (d) in epicardium and (e) intestinal mucosa indicating septicaemic changes (© Rajeshkumar, K.)

Plate 42 Chronic deep-seated abscess on the back of an elephant (© Apurba Chakraborty)

Plate 43: Jaw abscess observed during necropsy resulting in death due to septicaemia (© Karikalan, M.)

Plate 44: Photo-sensitisation and associated dermatitis recorded in captive elephant carcass (© Nandakumar, S.)

Commonly encountered and misinterpreted autolytic changes

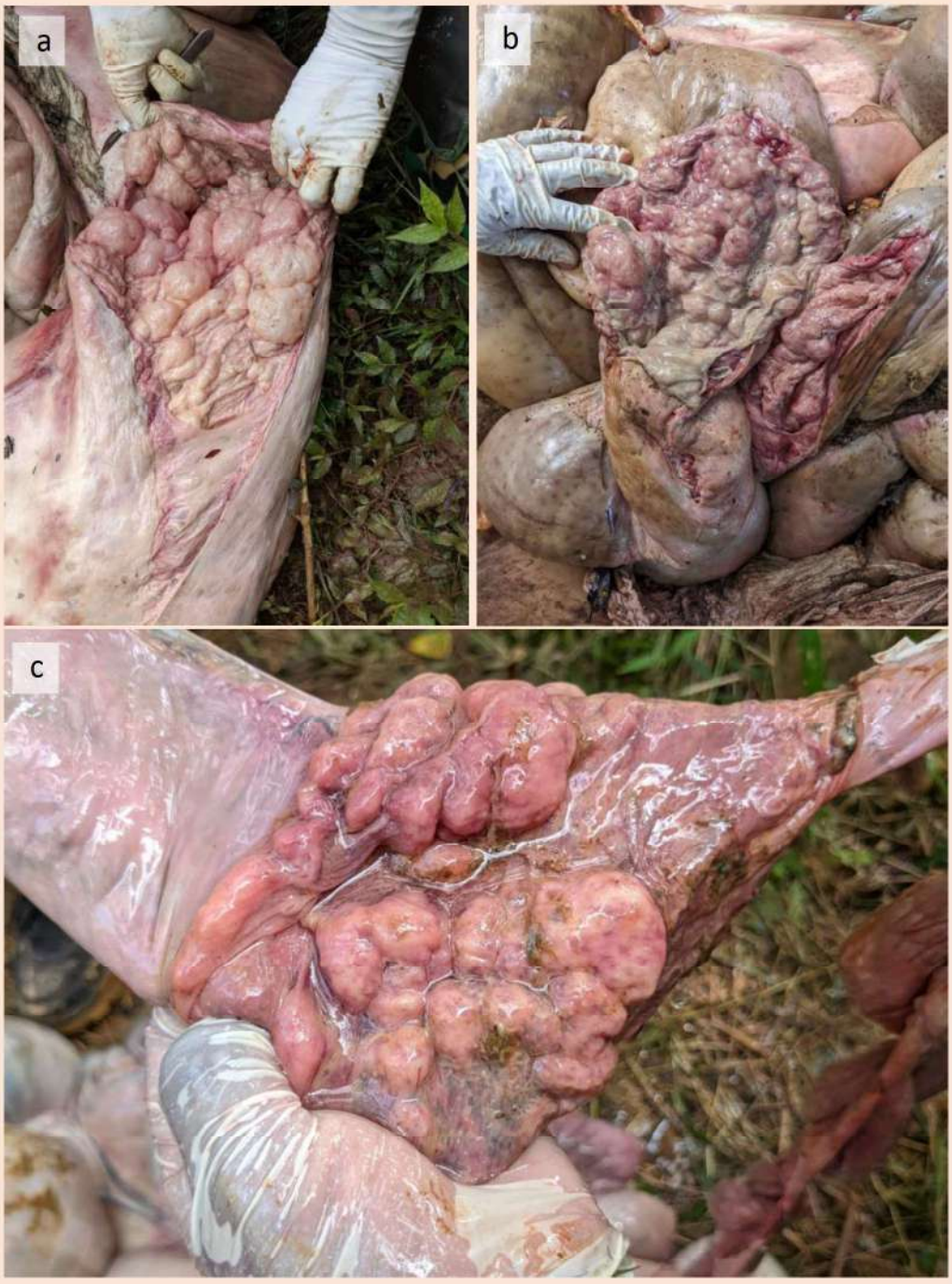


Plate 45: (a,b,c) Discoloured mucosa of the GIT with gas formation due to PM decomposition (© Rajeshkumar, K.)

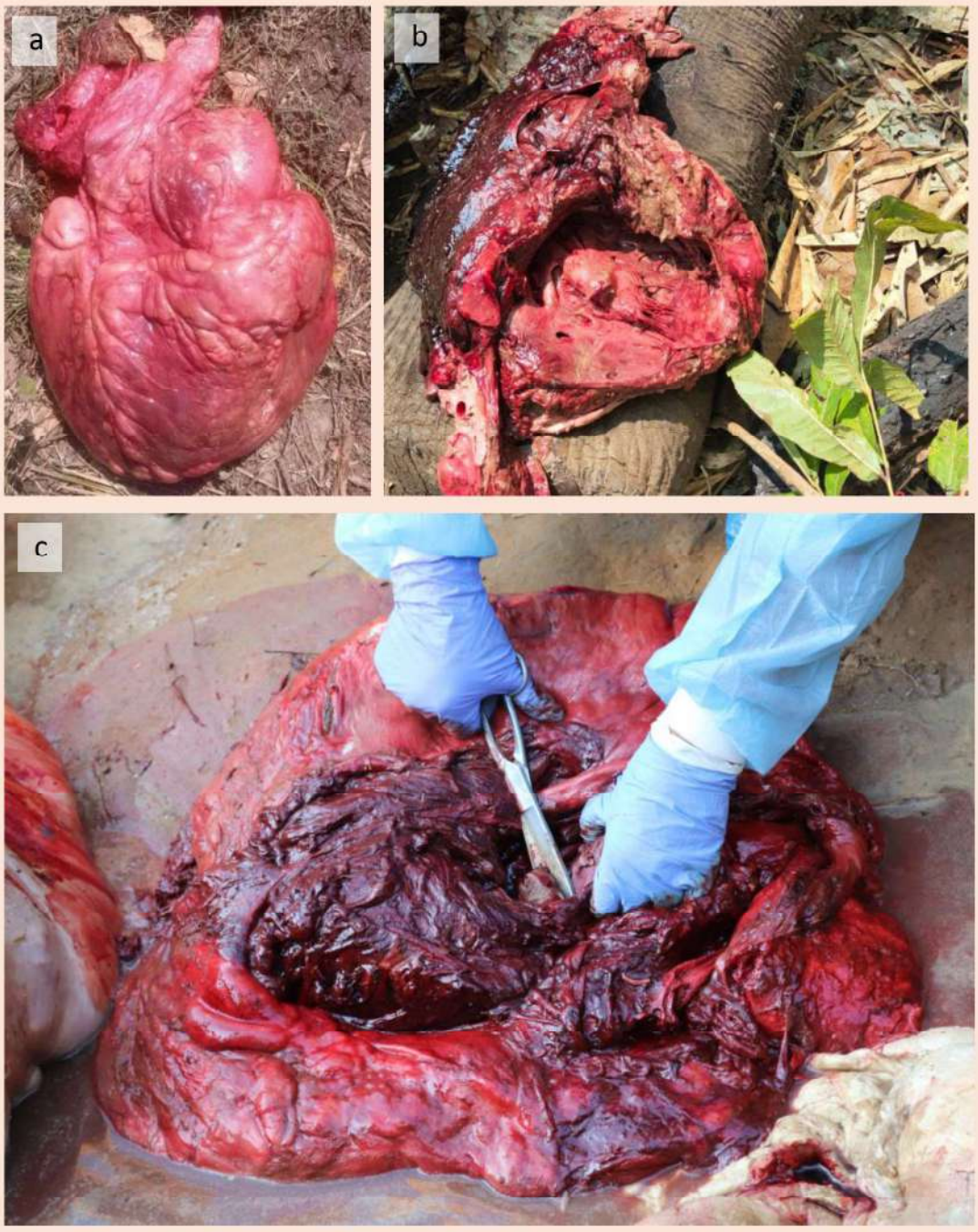


Plate 46: Autolytic Changes (a) Haemoglobin imbibition in pericardium (b&c) Haemoglobin imbibition in endocardium and myocardium after opening heart chambers (© Karikalan, M.)

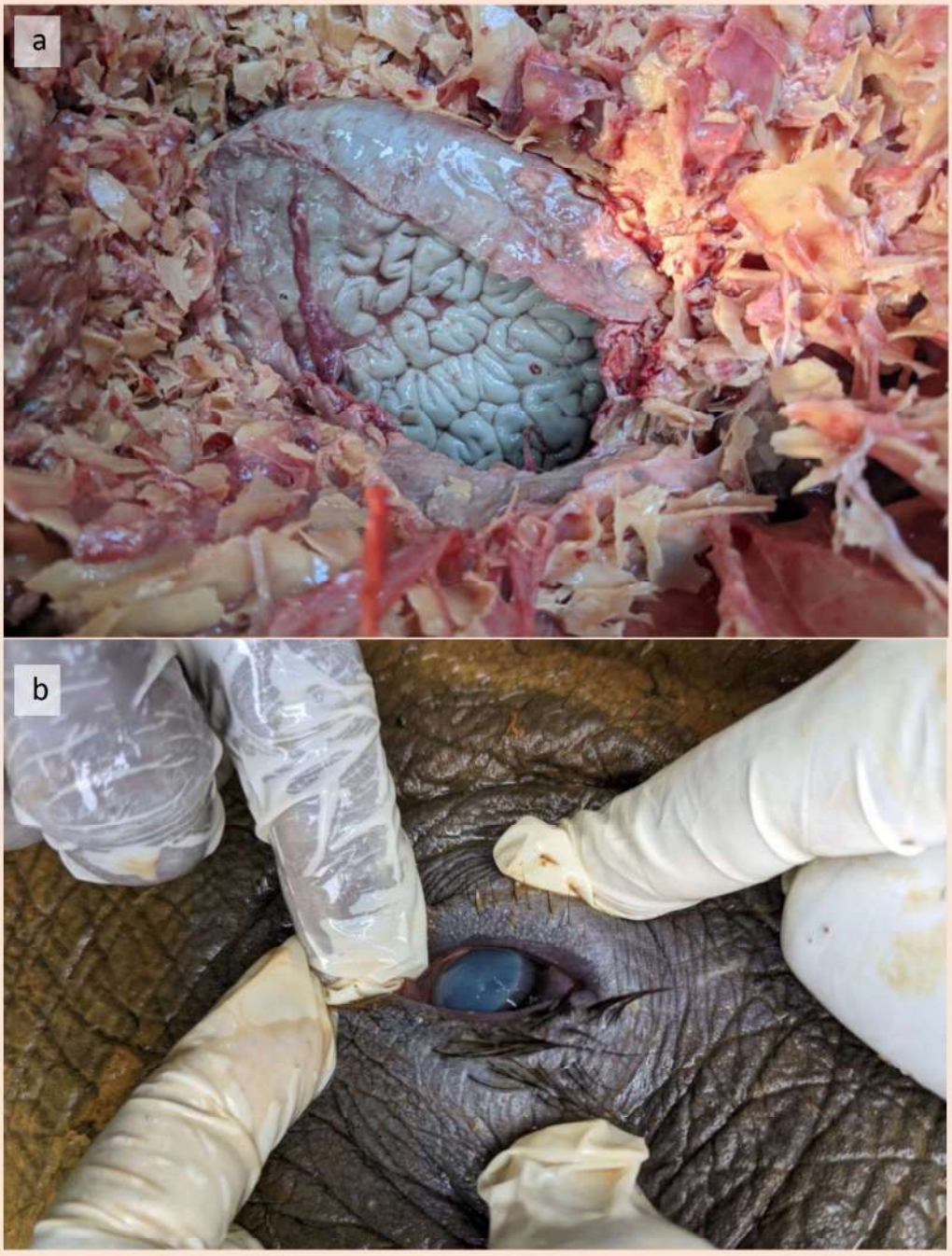
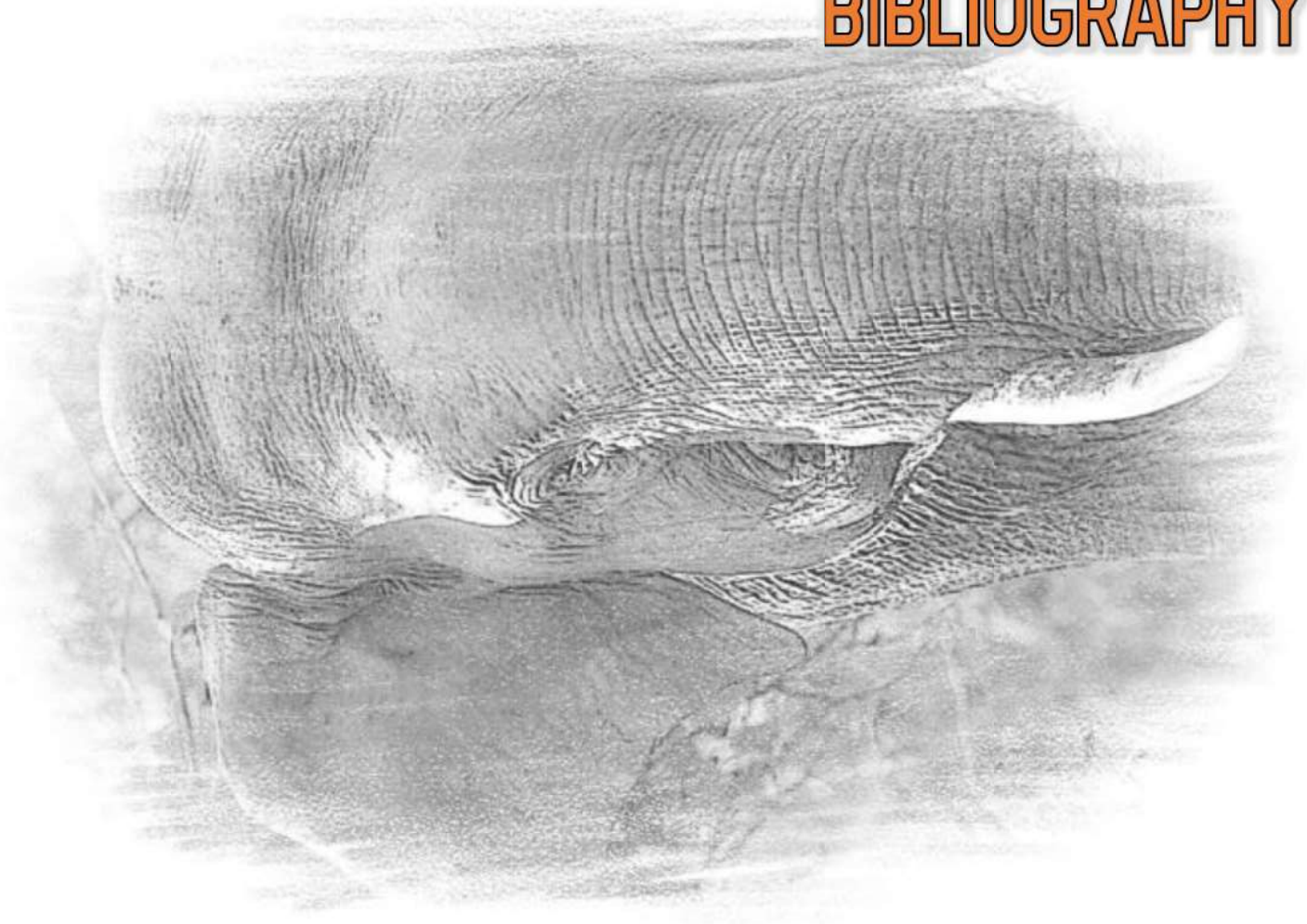


Plate 47: Autolytic Changes (a) Brain showing soft mushy mass with post-mortem discolouration, (b) Opaque eye due to absorption of aqueous humour (sign of decomposition) (© Rajeshkumar, K.)

SECTION VII
BIBLIOGRAPHY



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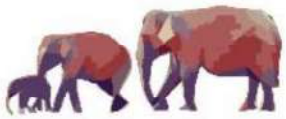
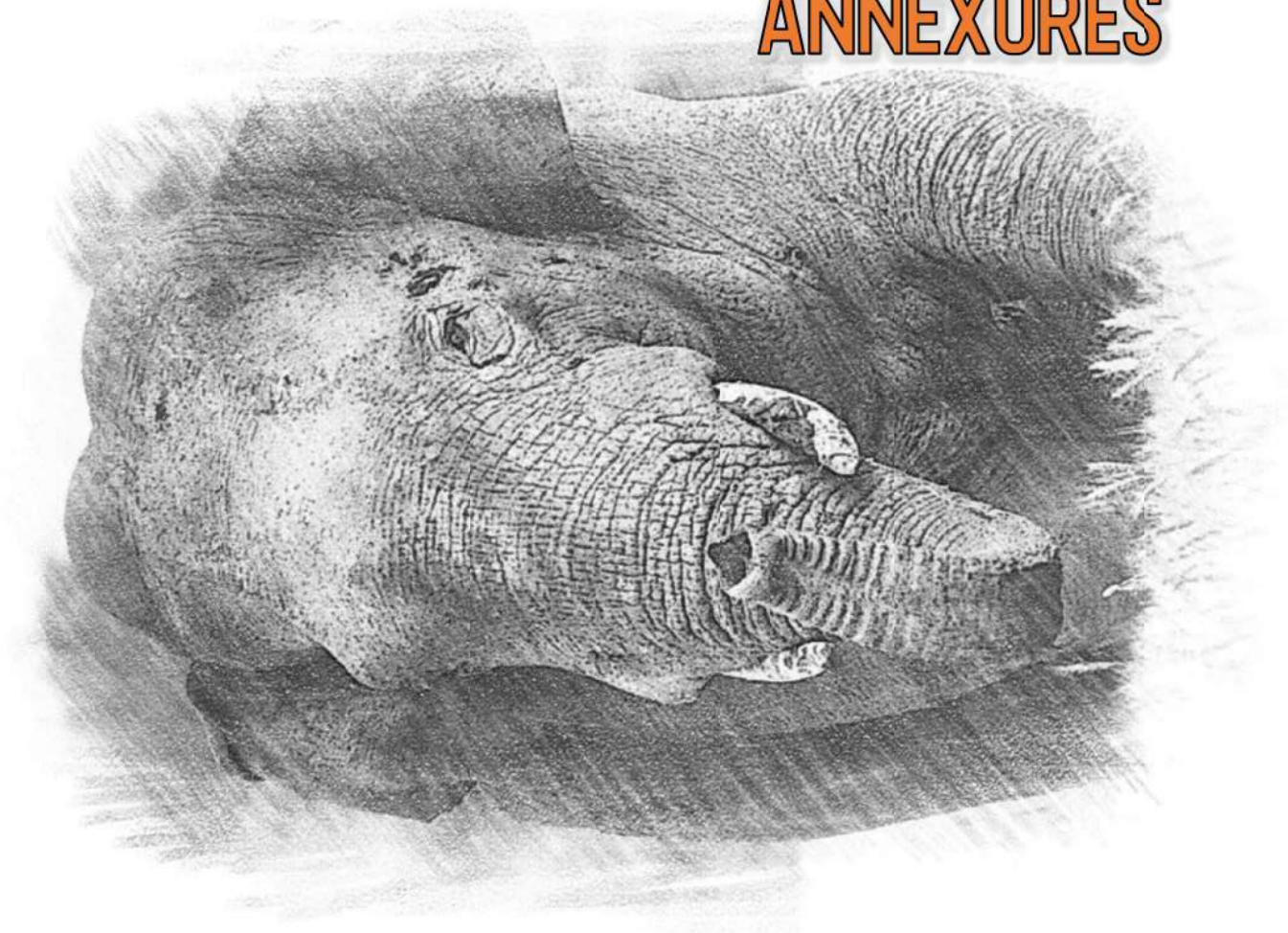


SECTION VIII
ANNEXURES



Annexure I

F. No. 2-9/2014-PE

Government of India/ भारत सरकार

Ministry of Environment, Forests & Climate Change/ पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय

(Project Elephant Division/हाथी परियोजना प्रभाग)

****

6th Floor, Vayu Wing,
Indira Paryavaran Bhawan,
Jor Bagh Road, Aliganj,
New Delhi-110003

Dated 20th May, 2022

OFFICE MEMORANDUM

Sub: Constitution of a sub committee under Captive Elephant Healthcare and Welfare Committee to revisit the document on Post Mortem of elephants prepared by Dr. Jacob Cheeran and to develop a fresh Document on "Post Mortem of Elephants including Procedures for Carcass Disposal"- reg

A sub committee under Captive Elephant Healthcare and Welfare Committee to revisit the document on Post Mortem of elephants prepared by Dr. Jacob Cheeran and to develop a fresh Document on "Post Mortem of Elephants including Procedures for Carcass Disposal", is constituted as under:

Sl. No.	Name and Designation	Member
1	Shri Ramesh Kumar Pandey, IGF & Director (PE)	Chairman
2	Dr. Avadh Bihari Shrivastav, Former Director, School of Wildlife Forensic and Health, NDVASU	Member
3	Dr. Apurba Chakraborty , Ex. Director of Research (Vety), Assam Agricultural University	Member
4	Dr. Vaibhav C. Mathur, IFS, Field Director, Manas Tiger Reserve, Assam	Member
5	Dr. Karikalan M. Scientist, Centre for Wildlife Conservation Management & Disease Surveillance, IVRI	Member
6	Dr. Nandakumar, S. Disease Investigating Officer, State Institute for Animal Diseases (Kerala)	Member
7	Dr. Gowri Mallapur, Veterinary Consultant, Central Zoo Authority	Member
8	Dr. Parag Nigam, Scientist F' Head, Dept. of Wildlife Health Management, Wildlife Institute of India,	Member Convenor

2. The Committee shall have the following Terms of References:
   - The Committee will review the Handbook of Techniques and Procedure for Post-Mortem of Elephants which was jointly published by project Elephant Division and CZA in 2003.
   - The Committee will develop a fresh Document on "Post Mortem of Elephants including Procedures for Carcass Disposal.
   - This will be a sub committee under the Captive Elephant Healthcare and Welfare Committee which is already in place.

3. TA/DA to Non-Official Members is to be paid by the Ministry through RTGS after submission of original bills of Airlines, Taxi etc. As per the Government of India Instructions non official members will have to book the ticket thorough Government approved agents.

4. The term of the Committee shall be for 30 days from the date of issue of this Office Memorandum, subject to modifications, if any, in the composition and functions of the committee, with the approval of competent authority.

5. This issues with the approval of the Inspector General of Forests & Director, Project Elephant, MoEF&CC.

(Dr. K. Muthamizh Selvan)
Scientist 'D' (Project Elephant)
Email id: km.selvan@gov.in
Telephone No. 011-24695067

Distribution:

- All the members of the Committee.

Annexure II

F. No. 14-2/2019-PE (Part-1)
Government of India/ भारत सरकार
Ministry of Environment, Forests & Climate Change/ पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
(Project Elephant Division/हाथी परियोजना प्रभाग)
****

6th Floor, Vayu Wing,
Indira Paryavaran Bhawan,
Jor Bagh Road, Aliganj,
New Delhi-110003

Dated 30th August, 2022

OFFICE MEMORANDUM

Sub: Postmortem of elephants by panel of 3 veterinarians - reg.

Reference is invited to the 1st meeting of Captive Elephant Health Care and Welfare Committee (CEHWC) held on 13th August 2022 at Periyar Tiger Reserve, Kerala, wherein it has been decided that postmortem of elephants should be done by a panel of 3 veterinarians in presence of two representatives of civil society (working in the field of wildlife conservation)nominated by the CWLW of the State. Therefore, the States/UTs may taken needful action accordingly for implementation of the decision.

(Dr. K. Muthamizh Selvan)
Scientist 'E' (Project Elephant)
Email id: km.selvan@gov.in
Telephone No. 011-24695067

To,

The Principal Chief Conservator of Forests (WL) &
Chief Wildlife Warden,
All States.

Copy to :

1. PPS to Director General of Forest & Special Secretary, MoEF&CC.
2. PS to Additional Director General of Forest (NTCA), MoEF&CC.
3. PS to Additional Director General of Forest (Wildlife), MoEF&CC.
4. PS to Inspector General of Forests (Project Elephant), MoEF&CC.

Annexure III

Table: Equipment and essential supplies for carrying out elephant necropsy

Sr.no	Item	Quantity
1.	Large animal necropsy knife straight (10 & 6 inch), curved (8 & 4 inch) & Knife sharpener	02 each
2.	Bone chopper	01
3.	Bone saw (Battery operated) with blades	01
4.	Post-mortem hammer (3 inches)	01
5.	Large axe (4-5 inches) with handle	01
6.	Shovel	01
7.	Chisel 0.5 & 1 inch	01 each
8.	Hacksaw handle	01
9.	Hacksaw blade	10
10.	Manual/ battery operated bone drill	01
11.	B.P. handle (no 24 and 28)	02 each
12.	B.P. Blades for 24 and 28	100
13.	Mayo scissors 23 cm straight, 21 cm curved, 6 inches straight	02 each
14.	Tissue forceps (7 inches & 3 inches)	02 each
15.	Rat toothed forceps (7 inches & 4 inches)	02
16.	Cutting board	04
17.	Tool kit box	02
18.	Apron (disposable full sleeve)	05
19.	Gum boots	04 pairs
20.	Post-mortem gloves and surgical gloves	01 box each
21.	Heavy straps rolls, Chains (30 m) & Thick ropes (30 m) for manoeuvring carcass	02 each
22.	Jute ropes / heavy straps/ chains	01 roll each
23.	Plastic/ Tarpaulin sheet 6 X 8 ft & 10 X 12 ft for Carcass placement	01 piece
24.	Metal detector	01
25.	Digital camera with close up lenses	01
26.	Green cloth (4x4) Use during photographic evidence collection	01
27.	GPS	01
28.	Hoist/crane/small tractor	01
29.	Mobile Refrigerator	01

Annexure IV

Table: Major toxicants, important lesions encountered and relevant biological sampling

Toxicants/ Poison	Important lesions	Important samples	Type of toxicity
Organophosphates, Carbamates Organochlorines	Pulmonary edema, blood tinged frothy exudates in the trachea Congestion and haemorrhages in heart, lung, meninges and brain	Gastric contents, liver, brain, kidney and body fat	**Acute toxicity**
Anticoagulant rodenticides	Pulmonary edema, gas in the stomach (irritant nature), congestion and haemorrhges in other visceral organs	Gastric contents, liver and kidney	
Bipyridyl herbicides and other other pesticides	Signs of gastrointestinal tract and kidney damage	Gastric contents, liver and kidney	
Lead	Gastroenteritis, congestion and haemorrhges in brain and meninges, Anaemic changes	Gastric contents, liver, kidney and bone	**Chronic toxicity**
Arsenic	Gastroenteritis, congestion and haemorrhges in intestine and brain	Gastric contents, liver, kidney, hairs and nails	
Mercury	Gastroenteritis (ulcers in GIT), congestion and haemorrhges in intestine and brain	Gastric contents, liver, kidney, brain, hairs and nails	
Cadmium	Gastroenteritis	Gastric contents, liver and kidney	
Nitrates	Cyanotic mucous membrane dark brown colour blood, hemorrhages in all the	Gastric contents, liver, CSF and eye ball	**Acute toxicity**

	body parts		
Cyanide	Dilated pupils, Frothy mouth, Cyanotic mucous membrane Bright red colour blood, hematuria, hemorrhages in all the body parts	Gastric contents, liver and muscle	
Sodium chloride	Congestion and haemorrhages in the brain and meninges, Gastroenteritis	Gastric contents, brain and liver	
Mycotoxins Aflatoxin, Ochratoxin	Severely enlarged liver and kidneys with congestion and haemorrhages Nodules in the liver Gastroenteritis	Gastric contents, liver, kidney and brain	**Acute/chronic toxicity**

Quantity of specimens to be collected for toxicological examination (in duplicate)

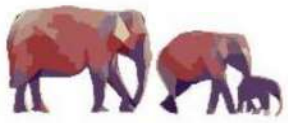
Sr. No.	Specimen	Quantity
1.	Clotted Blood (Heart)	10-30 gm
2.	Urine	100 ml or all available
3.	Pieces of Liver, Kidney, spleen & lung (collected separately)	Minimum 500g250 g
4.	Kidney	100 -250gm
5.	Brain	One-half brain
6.	Visceral fat	100 g
7.	Stomach contents/ Duodenal content	500- 1000 g
8.	Feed, and fodder	0.5 to 1 Kg
9.	Water	0.5 to 1 Lit
10.	Nearby vegetation suspected to have been consumed / found in stomach	Representative portion including root/ stomach content

Annexure V

Sampling essentials based on type of laboratory examination

Sr. No.	Type of lab exam	Specific sampling
1.	Bacteriological	Blood and exudates should be collected aseptically with sterile Pasteur pipettes/ syringes and put in sterile tubes or vials without any preservative and transported over ice. Pieces of affected organs with lesions should be collected in sterile vials and transported under cold chain. Appropriate sampling varies with different diseases
2.	Virological	Blood and exudates should be collected aseptically without any preservative and transported on ice. In some viral infections, samples may be collected in vials/ vacutainer containing EDTA. Pieces of affected organs should be collected under aseptic conditions and transported either on ice or in 50% buffered glycerol saline or appropriate viral transport media. Appropriate sampling varies with different diseases
3.	Mycological	Hair sample & skin scraping (superficial myotic infection) can be sent at room temperature in sterile plastic containers whereas deep skin scrap should be sent under refrigeration. For systemic mycotic infection, the affected tissues should be collected aseptically for fungal culture and tissue for histopathological examination. Appropriate sampling varies with different diseases.
4.	Parasitological	For identification of parasites and helminth ova, 10% formalin is the preferred transport medium to preserve the integrity of the ova. Nematode parasites should be preserved in 70% alcohol. For flukes and cestodes, the parasites should be pressed between two slides so as to flatten the parasite, tied with rubber band/clip and preserved in 10% formalin. For coccidial oocysts, 2.5% Potassium dichromate solution is the preferred transport medium. External parasites may be sent in 70% alcohol or 5% formalin. Air dried methanol fixed blood smears at room temperature or whole blood in anticoagulant may be sent under refrigeration for examination of blood parasites
5.	Histopathological	For routine histopathological examination, tissue pieces (including those with lesions and normal tissue) should be collected in 10% formal saline in wide mouthed leak proof bottles. Tissues should be no more than 0.5-1 cm thick and the quantity of formalin should be in the ratio of 10:1 formalin to tissue. It would be appropriate to take tissue samples from multiple places in single organ. Storage can be done at room temperature.
6.	Toxicological	For chemical analysis, fresh tissues and fluids (tissue, fat, blood and ingesta or suspected contaminated foods) should be dispatched on ice without adding any preservatives. If required, 90% ethanol/ methanol @ 1ml per gram of sample or saturated salt solution may be used to preserve the sample based on the recommendations of the specific laboratory.

Sr. No.	Type of lab exam	Specific sampling
		Larvae, maggots, pupa and other entomological samples from highly putrefied carcasses may be sent either under refrigeration (quantitative), saturated salt solution (qualitative), or alcohol based on laboratory protocol. Accurate records are critical and should accompany samples. Appropriate sampling varies with suspected toxin, hence clear instructions provided by laboratory for specific examination should be followed.
7.	Forensic entomological examination	Larvae, maggots, pupa and other entomological samples may be transported in 80% ethyl alcohol in clear, airtight glass or plastic containers. Associated habitat, photographic and metrological data should accompany samples
8.	Ballistics	Cotton tip applicator should be moistened with isopropyl alcohol or 5% nitric acid before sample collection from bullet wound and stored in airtight zip lock pouches. Projectiles (Shrapnel, bullet fragments etc.) should be collected without any preservative and sent to designated laboratory. Appropriate sampling varies with different laboratories and due consultation should be done prior to sending samples.
9.	Genetic	Tissue, skin and muscle, bone, hair samples should be collected in 90 % ethanol or Silica gel in Airtight containers Storage at room temperature



Annexure VI

RECORD OF NECROPSY EXAMINATION

PART A

Necropsy No ……..
Species: *Elephas maximus* **Common name: Asian Elephant**

A. LOCATION & EVENT INFORMATION

Protected Area/ Nearest Protected Area			**Zoological Park/ Temple/ Captive facility/ Rescue Centre/ Private ownership**
If Inside Protected Area	**If Outside PA Protected Area**		Details:
GPS Coordinates:	GPS Coordinates:		
Compartment:	Village:		Location:
Beat:	Tehsil:		
Range:	District:		
Multiple Animal Death?	Yes	No	Number of animals (If yes)

Carcass recovery date		Carcass Recovery Time		
Date and time of Necropsy	Date: Time:	Estimated date and time of mortality	Date: Time:	Basis of estimation
Carcass disposal: (Time/ Date)		Mode of Disposal (Burned to ashes/Trench Burial/ Left *In-situ*)		
Ambient temperature °C:		Weather:		
Area/ Habitat description (topography, water source etc.):				

Manner of Death	Predation	Disease	Human interaction (Poaching, Gunshot, Snared, Poisoned, Vehicular trauma, Problem animal control, Others)	Accident	Cannot be determined

B. ANIMAL IDENTIFICATION INFORMATION

Animal ID	(Name/Number/ID)					
Age group	Calf (0-1 y)	☐	Juvenile (1-5 y)	☐	Sub adult (5 – 15 y)	☐
	Adult (15 – 60 years)	☐	Geriatric (≥ 60 years)			☐
Sex:	Male	☐	Female ☐		Uncertain	☐
Microchip No.:			Local ID/ Name:		Unidentified	☐

C. CLINICAL AND PATHOLOGICAL OBSERVATIONS

I. Brief History

History of case before death (if any):

Observations around carcass:

Environment observations (Signs of struggle, signs of weather events such as lightening or flood, paddling, other gear/debris/ evidence found near animal, or evidence of supporting poisoning)

Carcass observations: (Clinical signs, presence of wounds, broken bones, external parasites, structural alterations caused by human to head/ appendages or body)

II. External Observation

Nutritional Condition (Subcutaneous fat/ Muscle mass)		Good	Fair	Poor
Rigor mortis:	Absent ☐	Setting in ☐	Complete ☐	Passing off ☐
Carcass condition:	Fresh ☐	Putrefied ☐	Advanced Putrefaction ☐	Incomplete ☐
State of Decomposition:	Fresh ☐	Moderate decomposition ☐	Severe decomposition ☐	Skeletonised ☐
Physical condition:	Normal ☐	Obese ☐	Lean ☐	Emaciated ☐
External wounds:	Absent ☐	Ante-mortem ☐	Post-mortem ☐	
Wounds (if present):	Location			
	Bruise ☐	Abbresion ☐	Puncture ☐	Incision ☐
	Gunshot ☐	Blunt force ☐	Degloving ☐	Burn/ singeing ☐
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:**	

Any other observations:

III. Internal Examination

System		Appearance, colour and observations
SUBCUTANEOUS TISSUE		
BODY CAVITIES **Position of visceral organs** **Peritoneal Cavity** **Pleural cavity and Pleura** **If fluid present in the Peritoneum or thorax:** **Color, transparency and amount**		
RESPIRATORY SYSTEM **Larynx** **Trachea** **Lung parenchyma** (Appearance, colour & consistency) **Diaphragm** **Lymph nodes** (NAD/enlarged/inflamed)		
CIRCULATORY & LYMPHATIC SYSTEM **Pericardial Sac** **Heart Muscle** (Epicardium, myocardium, endocardium) **Heart Chambers** (Right atrium, left atrium, right ventricle left ventricle) **Blood Vessels** **Spleen (Appearance, size, colour)** **Lymph nodes**		
HEPATIC SYSTEM **Liver (Appearance)** **Liver Parenchyma and Bile duct**		
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	

UROGENITAL			
1. Urinary Bladder			
2. Kidneys		**Left**	**Right**
Capsule:			
Cortex:			
Medulla:			
3. REPRODUCTIVE ORGANS	**Testes/ Penis/Glands**		
	Ovary/ Uterus / Vagina		
LYMPH GLANDS			
I. NERVOUS SYSTEM **Brain** **Spinal cord** **Meninges**			
J. SKELETAL SYSTEM			
Head (Buccal & Nasal Cavities/ Tongue/ Cheeks			

IV. Record sheet for parasites

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

V. Summary of Major Findings

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.....

.....

VI. Checklist for Specimen Collection for Laboratory Analysis

S. No	Specimen	Preservative used	Examination required	Designated laboratory

NB: Sample ID to be put as per sample register/Case ID and mentioned in the letter of communication to the laboratory.

PROVISIONAL DIAGNOSIS:

.....

.....

Place:

Date:

Necropsy performed by:

Signature..... Name..... Designation..... Signature..... Name..... Designation.....	Signature..... Name..... Designation..... Signature..... Name..... Designation.....

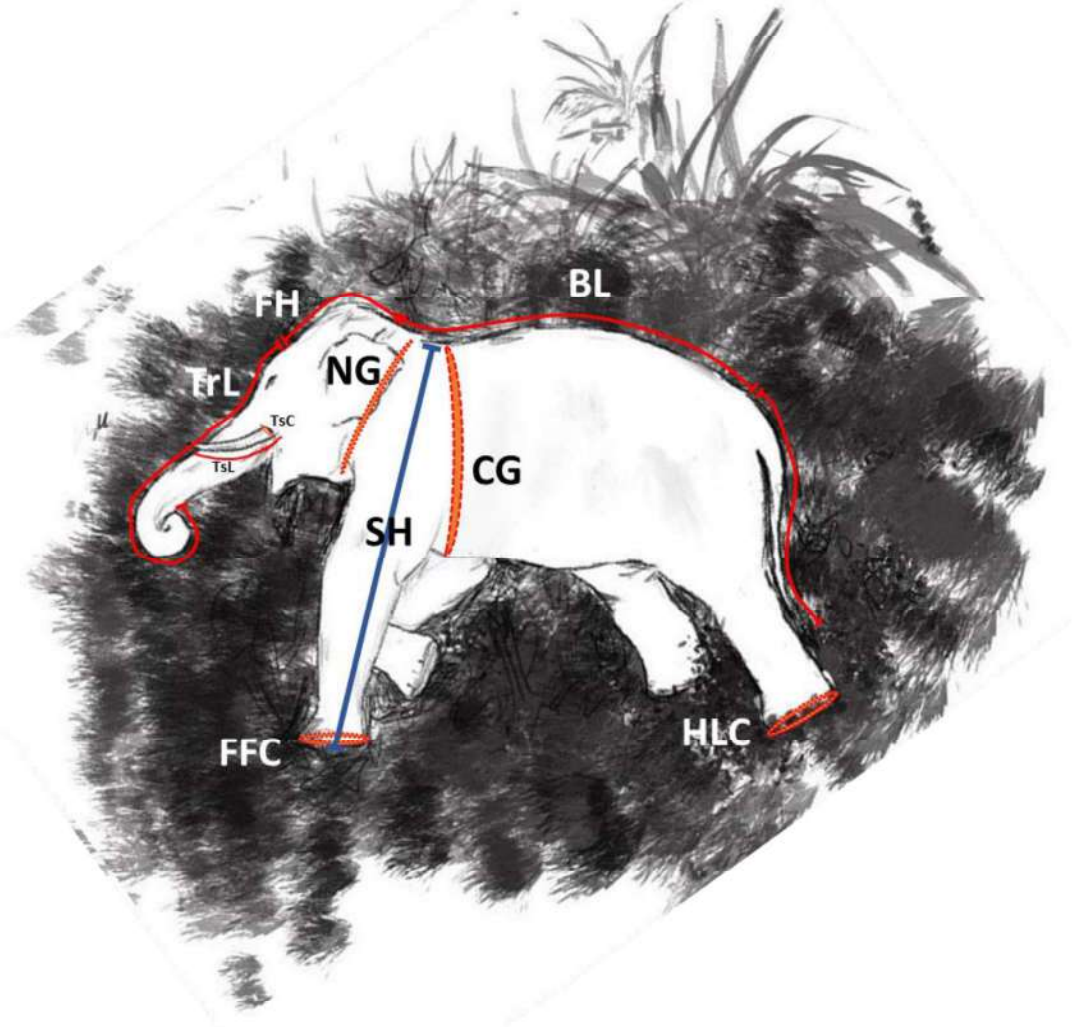
VII. Photographs

Whole carcass as observed before necropsy	
Left lateral view	
Right Lateral View	
Gross lesion of major visceral organs (Heart, lungs, liver, spleen, GI tract, kidney, brain etc (including lymph nodes)	

VIII. Vital Measurements
(Columns below to be filled only if carcass is fresh (Not bloated/putrified)

TrL: Trunk tip to forehead bump	**FH: Forehead (Forehead bump to base of occiput)**	**BL: Body Length (Base of occiput to base of tail)**
SH: Shoulder Height	CG: Chest girth	NG: Neck girth
TL: Tail Length	Tail characteristic: (Full/ broken/ kinked)	Tail tip:

FFC: Front Foot Circumference (Right……...Left……)	Toe Nails (Front foot) (Right……...Left……)
HLC: Hind Limb Circumference (Right……...Left……)	Toe Nails (Hind Limb) (Right……...Left……)
TsL: Tusk length (Right……...Left……)	TsC: Tusk circumference at base (Right……...Left……)



PART B

FINAL DIAGNOSIS REPORT

Serial no: File Name-Number/PA NAME/Range name/Camp name/serial no/date (For office use)

Name of PA: Range: Camp/Beat:
Sex: Approximate age:

Date of necropsy:
Date of dispatch of post mortem samples to respective laboratories:
Date of receipt of laboratory reports from respective laboratories:

Laboratory reports summary:

1. Bacteriology:
2. Virology:
3. Parasitology:
4. Histopathology:
5. Mycology:
6. Toxicology:
7. Others:

Final diagnosis/cause of death:

Veterinarian's name:
Designation & address:

Signature: Date:

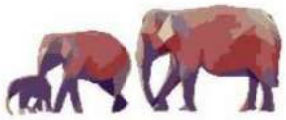
Memo No: xxxxxx (Office use)

Copies of the final diagnosis report to:

1. The PCCF (WL),….
2. The FRO,……
3. Office copy,…

Suggested format for maintaining PM records in official register and digital database

Sr. No.	PM Serial no.	Date of PM	Range/ Beat/ Camp	Provisional diagnosis	Sample collected (Each sample shall be recorded in separate row)	Sample sent to lab (Each reference shall be recorded in separate row)	Report retrieved from Lab (Each reference shall be recorded in separate row)	Confirmatory diagnosis reference (To be interpreted and compiled by veterinarian doing the PM)	Summary of confirmatory diagnosis by the veterinarian	PM entry done by (Name & signature

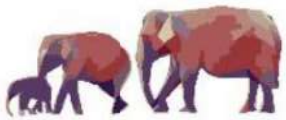


Annexure VII

Disinfectants

Category	Alcohols	Alkalis	Aldehydes	Halogens (Chlorine)	Halogens (Iodine)	Peroxygen compounds	Phenols	Quaternary Ammonium Compounds
Common Active Ingredient	Ethanol, Isopropanol	Calcium hydroxide, Sodium carbonate, Calcium oxide	Formaldehyde, Glutaraldehyde, Ortho-phthalaldehyde	Sodium hypochlorite (bleach), Calcium hypochlorite, Chlorine dioxide	Povidone-iodine	Hydrogen peroxide/ accelerated HP, Peracetic acid, Potassium peroxymonosulfate	Ortho-phenylphenol, Orthobenzylpara-chlorophenol	Benzalkonium chloride, Alkyldimethyl ammonium chloride
Mechanism of Action	Precipitates proteins and denatures lipids	Alters pH through hydroxyl ions; fat saponification	Denatures proteins; alkylates nucleic acids	Denatures proteins	Denatures proteins	Denatures proteins and lipids	Denatures proteins; disrupts cell wall	Denatures proteins; binds phospholipids of cell membrane
Characteristics	Fast acting and rapid evaporation, does not leave residue. Can swell or harden rubber and plastic	Slow acting, affected by pH, action best at high temperatures, corrosive to metals, can cause severe skin burns and irritation	Slow acting, pungent odour, noncorrosive and affected by pH and temperature. Can cause irritation of skin/ mucous membrane. Should be used in well ventilated areas	Fast acting affected by pH, requires frequent application, inactivated by UV radiation, corrodes metals, rubber and fabrics, Can cause mucous membrane irritation	Stable, affected by pH, requires frequent application, corrosive, stains clothes and treated surfaces	Fast acting, may damage some metals like lead, brass, copper, zinc. Powdered form may cause mucous membrane irritation, low toxicity at low concentration, Environment friendly	Can leave residual film on surfaces, can damage rubber, plastic; non-corrosive, stable in storage, Can cause irritation to skin and eyes	Stable in storage, best at neutral or alkaline pH, effective at high temperature, high concentrations is corrosive to metals, May cause irritation to skin, eyes, and respiratory tract

Category	Alcohols	Alkalis	Aldehydes	Halogens (Chlorine)	Halogens (Iodine)	Peroxygen compounds	Phenols	Quaternary Ammonium Compounds
Bactericidal	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Virucidal	Yes	Yes	±	Yes	Yes	Yes	Yes	Yes
Fungicidal	±	Yes	Yes	Yes	Yes	±	Yes	Yes
Tuberculocidal	Yes	±	Yes	Yes	Yes	±	Yes	No
Sporicidal	No	Yes	Yes	Yes	±	Yes	No	Yes
Factors affecting efficacy	Inactivated by organic matter	Variable	Inactivated by organic matter, hard water, soaps and detergents	Rapidly inactivated by organic matter	Rapidly inactivated by organic matter	Effective in presence of organic matter, hard water, soaps, and detergents	Effective in presence of organic matter, hard water, soaps, and detergents	Inactivated by organic matter, hard water, soaps and anionic detergents



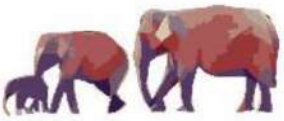
Annexure VIII

NATIONAL INSTITUTES/REGIONAL CENTRES FOR LABORATORY EXAMINATION

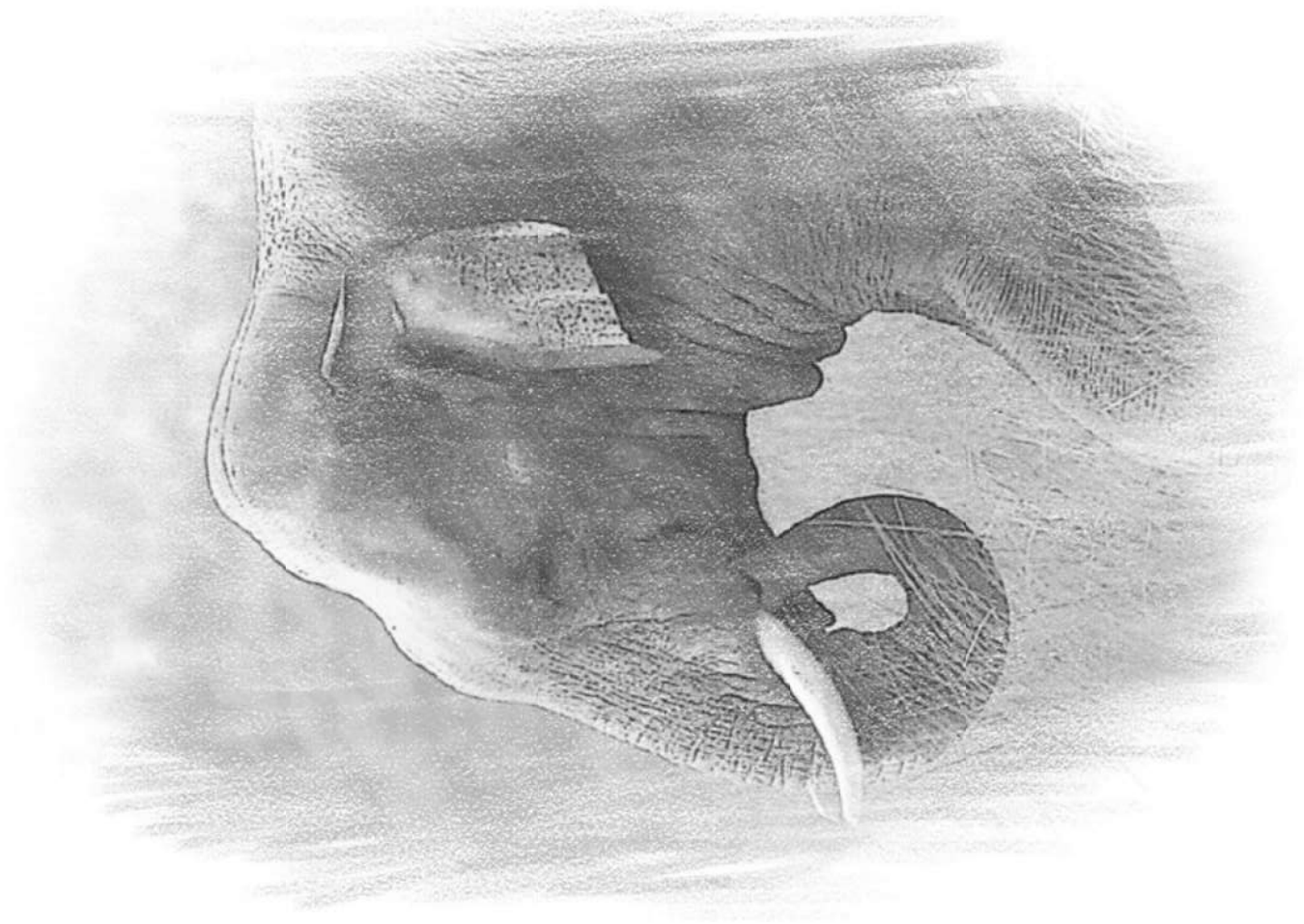
Sr. No.	Name of Institution	Sr. No.	Name of Institution
	NATIONAL INSTITUTES		
1	Director, National Referral Centre on Wildlife Healthcare) **OR** In-charge, Centre for Wildlife, Conservation, Management and Disease Surveillance ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh Email ids: directorivri@gmail.com, cwlincharge@gmail.com. Phone: +91 0581-2300096	2	Director National Institute of High Security Animal Diseases Indian Council of Agricultural Research, Anand Nagar, Bhopal- 462 022 Madhya Pradesh Email id: director.nihsad@icar.gov.in Phone: +91 755 2759204 (For exotic viral diseases)
3	ICAR- Directorate of Foot and Mouth Disease, International Centre of Foot and Mouth Disease Arugul, Jatni, District- Khorda, Bhubaneswar-752050, Odisha Email: Director.dfmd@icar.gov.in, Phone: +91-674-2601101 (O) Or Director, Project Directorate on Foot and Mouth Disease, Indian Veterinary Research Institute Campus, Mukhteshwar, 263138, Nainital, Uttrakhand Phone: +91-5942-286004 (For FMD work)	4	Director CSIR-Indian Institute of Toxicology Research Vishvigyan Bhawan, 31, Mahatma Gandhi Marg Lucknow - 226 001, Uttar Pradesh. Email: director@iitrindia.org Phone (Direct): +91-522-2613357, 2621856, 2628227 (For toxicological work)
5	Director Wildlife Institute of India P.O. Box 18, Chandrabani, Dehradun 248001 Uttarakhand Email: dwii@wii.gov.in Phone: +91-135-2646111-115 (For forensic related work)	6	Director Centre for Cellular & Molecular Biology, Habsiguda, Uppal Road, Hyderabad-500 007, Telangana Email: director@ccmb.res.in Phone: +91 40 27160222-31, 27160232-41, (For forensic related work)
	REGIONAL CENTRES/LABORATORIES (DADF)/SELECT INSTITUTIONS/CENTRES		
7	Joint Director Southern Regional Disease Diagnostic Laboratory, Biologicals, KVAFSU, Hebbal Bangalore-560024, Karnataka Email: info@iahvb.com Phone: 080 2341 1502	8	Joint Director Regional Disease Diagnostic Laboratory Animal Health Institute, Ladowali Road, Jallandhar-144001, Punjab Email: sddlnz@yahoo.com, nrddl2001@gmail.com Phone: 0181-2242335
9	Joint Director Regional Disease Diagnostic Laboratory (Disease Investigation Section), Department of Animal Husbandry, Govt. of Maharashtra, Aundh Pune-411007, Maharashtra Email ids: Jcahdis@hotmail.com dis.pune7@gmail.com Phone: 020-25692135	10	Deputy Director Animal Health Centre, North Eastern Regional Disease Diagnostic Laboratory, Animal Husbandry & Veterinary Department, Khanapara, Guwahati-781022, Assam Email: nerddlguwahati@gmail.com Phone: 0361-2334177

Sr. No.	Name of Institution	Sr. No.	Name of Institution
11	Joint Director Regional Disease Diagnostic Laboratory Institute of Animal Health & Veterinary Biological (IAH&VB), 37, Belgachia Road, Govt. of West Bengal Kolkata-700037, West Bengal Email: erddl_kolkata@yahoo.co.in Phone: 033-25328033	12	Director School of Wildlife Forensic & Health, South Civil Lines Jabalpur-482001, Madhya Pradesh Email: directorswfh@gmail.com Phone:0761-2627150 FAX: 0761-2627150
13	Coordinator Centre for Wildlife Health, College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, (OUAT) Bhubaneswar-751003, Odisha Tele-Fax: 0674-2397146	14	Joint Director Centre for Wildlife Studies, State Institute for Animal Diseases (SIAD), Palode, Trivanathapuram-695563, Kerala Email: cdio.ahd@kerala.gov.in Phone: 0471 2840252
15	Professor and Head Central University Laboratory, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Milk Colony, Chennai-600051, Tamil Nadu E-mail: culcahs@tanuvas.org.in Phone: +91-44-25551581	16	Special Officer Kerala Veterinary & Animal Science University, Centre for Wildlife Studies Pookode, Lakkidi PO Wayanad-673576 Kerala E-mail: chandy@kvasu.ac.in Phone: 8304073367
17	Principle Investigator Advanced Animal Disease Diagnosis and Management Consortium (ADMaC) College of Veterinary Science, AAU Khanapara, Guwahati, Assam, India Pin: 781022 Email:aadsmckhanapara.gmail.com Phone:+91 94 3555 8788	18	ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Indian Council of Agricultural Research, Post Box No. 6450, Ramagondanahalli, Yelahanka, Bengaluru-560064, India Email: sureshkp97 @gmail.com Phone:+91 80 2309 3110 / +91 80 2309 3111

Note: Samples may be also be sent to State Veterinary Colleges/ Laboratories/Institutions for routine work and also for specific diseases based on the facilities available. In case of ballistic investigations, samples may be sent to State Forensic Laboratories/ Central Forensic Laboratories.



CONTRIBUTORS



Contributor

Parag Nigam *holds a doctrate in Wildlfie Sciences and heads the Dept. of Wildlife Health Management at the Wildlife Institute of India and is the Nodal Officer of Elephant Cell of the Project Elephant Division, Ministry of Environment, Forest & Climate Change, Governement of India. He is responsible for developing and executing capacity development programs for wildlife professionals both nationally and internationally. He has played a key role in large carnivore and mega herbivore conservation . He steered the first ever tiger reintroduction program (Sariska Tiger Reserve) carried out as a collaborative initiative between the Rajasthan Forest Department, the National Tiger Conservation Authority and WII. He played a crucial role in the Gaur Reintroduction program in Bandhavgarh Tiger Reserve that was a collaborative initiative between Madhya Pradesh Forest Department and WII. He contributes to various wildlife ecological research projects through his inputs on animal capture and radio collaring and has worked across different landscapes. He is an invited member of the IUCN SSC- Wildlife Health Group.*

Apurba Chakraborty *, an eminent Veterinary Pathologist completed his graduation in Veterinary Science from College of Veterinary Science, Assam Agricultural University. He perused higher education in Veterinary Pathology leading to Master degree and PhD degree from the same university. He joined the Department of Veterinary Pathology, College of Veterinary Science, AAU in 1978 and worked in various capacities. He joined as Director of Research (Vety.) in Assam Agricultural University in 2008 and retired from active service after completing two terms as Director of Research in 2018. His major area of research interest has been diseases of wild animals and made notable contributions in wildlife disease investigations and diagnosis which includes findings of rare and emerging infections, rare parasites and mycotic diseases. He is known for his expertise in histopathology and scanning electron microscopic interpretations. Dr. Chakraborty has published 164 research papers in journals of national and international repute, 3 book chapter and one book on "Coloured Atlas of Diseases and Disorders of Wildlife". Work of Dr. Chkraborty has been recognized by various national and international agencies. He is Fellow of the National Academy of Veterinary Sciences and Fellow of Indian Association of Veterinary Pathologists.*

Karikalan M. *holds doctorate in Veterinary Pathology from the ICAR-IVRI, Izatnagar, Deemed University, Bareilly. He has been working as a Scientist (Veterinary pathology) at the Centre for Wildlife Conservation Management & Disease Surveillance, Indian Veterinary Research Institute, Izatnagar since 2015. He is involved in conducting post-mortem, histopathological and molecular diagnosis of diseases among wild animals. He is presently steering the DST-SERB funded project entitled "Patho-epidemiology of elephant endotheliotropic herpes virus among Asian Elephants" and aims to develop diagnostic kits against the disease. He has been providing technical services to various state wildlife departments, Central Zoo Authority and Project Elephant, MoEF & CC, Govt. Of India. He published more than 70 reasearch papers in National and International journals, 2 book chapters and 2 books related to wildlife diseases. He is member of Association of India Zoo and Wildlife Veterinarians and Indian Association of Veterinary Pathologists.*

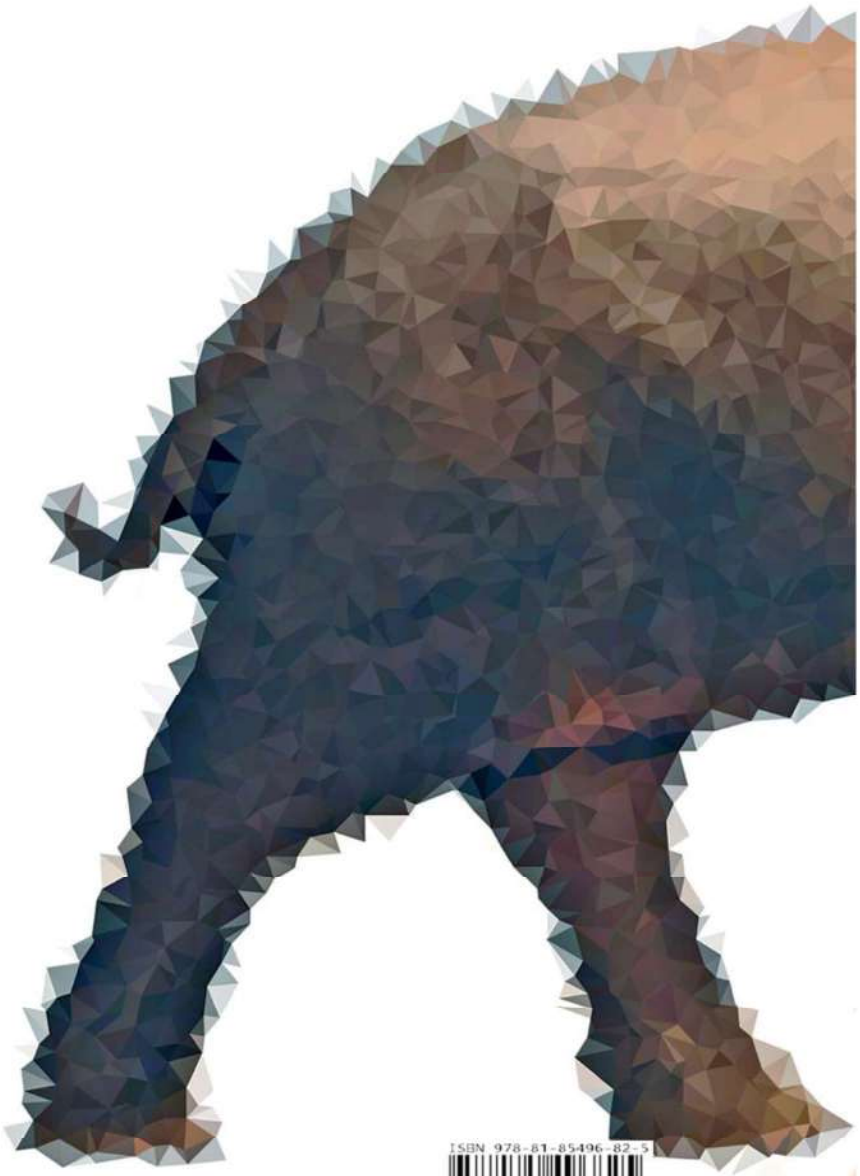
Avadh B Shrivastav *is former Director of School of Wildlife Forensics and Health, Nanaji Deshmukh Veterinary Science University, Jabalpur. He has led the foundation of wildlife health management practices in Madhya Pradesh and Central India. The Centre is providing technical guidance on various aspects of wildlife health and forensic to various state wildlife agencies. He is also actively involved in research projects as the lead investigator at state and national level. He is known for his expertise in wildlife pathology and has been confreed the prestigious Amrita Vishnoi Award for his contributions in wildflife health. He is presently the member of the Madhya Pradesh State Wildlife Advisory Board.*

Gowri Mallapur *is an experienced veterinarian and a passionate herpetologist with an unquantifiable eagerness to push boundaries. She holds Masters in Veterinary Sciences (Parasitology) from the Bombay Veterinary College and a Diploma in Sustainable Development and Natural History Management from the Ecological Society, Pune. She has worked as Director/ Veterinarian at The Madras Crocodile Bank Trust/ Centre for Herpetology and its associated Projects and subsequently as the Subject Matter Specialist (Veterinary Health Management) on a project entitled Biodiversity Conservation and Ganga Rejuvenation under the aegis of the Wildlife Institute of India and the National Mission for Clean Ganga. She is an invited member of the IUCN SSC- Crocodile Specialist Group. Dr. Mallapur currently works as the Veterinary Consultant with the Central Zoo Authority, in New Delhi, India.*

Nandakumar S, *Assistant Director with Dept of Animal Husbandry, Govt of Kerala is a Veterinary Pathologist with special interest in Wildlife Disease Investigations and diagnosis. He is presently in charge of the Centre for Wildlife Sciences at State Institute for Animal Diseases, Kerala. He has more than 2 decades of experience in field autopsies, veterinary forensics and histopathology. He has acted as Chairman of Committee for strengthening of elephant squads in the state and a member of various committees of Department of Animal Husbandry, Forests & Wildlife and Museum& Zoos for studying various management and disease control aspects of captive and free ranging wildlife. He is a keen proponent of One health and PGD holder in Wild Animal Disease Management.*

Vaibhav C. Mathur, *holds Masters in Epidemiology from the Indian Veterinary Research Institute and joined the Indian Forest Service in 2006. Borne on the Assam cadre, his keen interest in wildlife saw him complete the post graduate diploma in Wildlife Management form the Wildlife Institute of India in 2010. He was subsequently posted as Director of the Dibru Saikhowa Biosphere Reserve, wherein he managed two protected areas. During this stint, he successfully completed the Protected Area Tactical Enforcement Conservation Training at the Khao-Yai National Park in Thailand under the aegis of ASEAN-WEN. He moved to the National Tiger Conservation Authority in 2014, wherein he was part of several policy initiatives in the tiger conservation and wildlife sector. Importantly he handled data analytics in respect of tiger mortality across the country which is presented before the Parliament of India of every year. On his return to the state, he served as Joint Director at the Kaziranga Tiger Reserve and is currently posted as Field Director, Manas Tiger Reserve*

Rajeshkumar K. *holds a Bachelor's degree in Veterinary Science from Madras Veterinary College and subsequently joined the Tamilnadu Animal Husbandry Department in the year 2010. He served as Veterinary Officer in the Nilgiris Biosphere, Coimbatore and is presently working as the Forest veterinarian in Mudumalai Tiger Reserve. Throughout his professional career, he has worked towards ensuring health of livestock and wild animal. He headed the team for immunization of domestic cattle in forest fringe areas of The Nilgiris biosphere and served as full time veterinarian of Temple elephants rejuvenation camp of Tamil Nadu government for three years. He is presently serving as full time veterinarian of forest elephant camp at MTR where he plays a key role in providing veterinary services aimed at treating wild animals, rescue and rehabilitation of animals in distress, radio-collaring and translocation programs and carrying out necropsy.*



Ministry of Environment, Forest & Climate Change

हाथी परियोजना
भारत सरकार
PROJECT ELEPHANT
GOVT. OF INDIA

ISBN 978-81-85496-82-5

Project Elephant Division
Ministry of Environment, Forest and Climate Change
Government of India
2023