UNIVERSITÀ DEGLI STUDI DI PADOVA
Department of Comparative Biomedicine and Food Science

First Cycle Degree (B.Sc.) in
Animal Care

Postmortem examination procedures on field and causes of death in Costa Rica wildlife

Supervisor
Prof. Cinzia Centelleghe
Co-supervisor
Dott. Luca Spadotto

Submitted by
Gaia Lorenzin
Student n.
2003474

ACADEMIC YEAR 2023/2024

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Summary

Postmortem examination procedures are all the medical inspections performed on animals after death. They involve looking at the animal as a whole, as well as looking at each individual organ and body system to determine the cause, the mechanism and the manner of death.

There are some important differences between necropsies on field and the procedures done in the necropsy room; the main aspects of both processes, and the protocols usually utilized for these operations, will be analysed and discussed. It is fundamental to highlight the comparison between the two procedures to increase the knowledge about this topic and to improve the techniques adopted during these methodologies.

Following the discussion, it is inevitable not to mention the crucial role that postmortem investigations have, in the sphere of animal conservation, especially in this historical period, visibly in crisis due mainly to human impact on the environment and to its inhabitants, on a global scale.
Within the context of this topic, the essay will conclude, after traversing the Central part of the American continent, in Costa Rica, where I underwent my traineeship experience. Here, I had the opportunity to witness some necropsies and focus on the prevailing causes of death of local wildlife.

1. Introduction

The term necropsy refers to the postmortem examination of an organism through a dissection, typically conducted to ascertain the cause of death or ill health, underlie abnormalities or to study the anatomical structure and pathological conditions present at the time of death.

It involves a meticulous medical inspection of tissues, organs, and the entire systems of the body; often, it is performed by skilled professionals like pathologists or veterinary surgeons, to gain insights into the biological processes and factors contributing to the demise of the individual.[1]

Usually, it is advised to perform the necropsy in the shortest time, within the first hours following the death of the animals, before the decomposition process begins. Unfortunately, this is not always possible: the solutions adopted in this case are refrigerating or freezing the carcass. In this way, it can be preserved and the autolysis together with its consequences, such as morphological tissue changes, can be slowed down or avoided. For this reason, it is important to find a balance in this delicate situation: it is indeed better to perform the examination as soon as possible but if freezing may lead to a more analytical examination and better results obtained, this should be taken into consideration.[1] However, freezing can cause ice crystal formation within tissues, leading to cellular damage and disruption of tissue architecture. This can compromise the quality of histological samples and make it more difficult to identify microscopic abnormalities; freezing can introduce artifacts in tissues, making it challenging to distinguish between pathological changes and changes caused by freezing; it may be more difficult to sample frozen tissues compared to fresh ones. Obtaining representative tissue samples for analysis may be more challenging, leading to potential sampling bias.[2]

Necropsy encompasses a wide range of features essential to be aware of, in order to conduct a correct examination of the organism. Firstly, when available, consult the history of the animal; later, if there is the possibility, conduct the necropsy with a sufficient lighting and then examine the animal in a precise order, following a methodical approach: starting cranially to caudally, system by system.[2]

It must be taken into consideration that clinical signs and clinical pathologic findings are often not definitively explained until necropsy is performed [3], therefore it is inadvisable to make a diagnosis before having followed the entire examination protocol.

There are mainly two places where a necropsy can be carried out, according especially to the size of the animal and if transport is possible: on field, the exact place where the animal is found

lifeless, or into the necropsy room, a clinic, or a designated area of a structure. A critical point is that laboratories, where necropsies are accomplished, should be established in a separate room, which can be easily cleaned and with adequate supplies and correct structures ready to be used during the examination, such as draining floors and, air exchange systems.[4]

Some differences arise when it comes to comparing the two locations: primarily, the risk of spread of infection both to wild and domestic animals due to the opening of the carcass in the field; for example, it is known that ectoparasite and endoparasite burdens are more prominent in animals living in the wild than in the ones kept in captivity. [2] Moreover, it's important to note that conducting necropsies in the field requires specific skills, due to the variability of the anatomy among different species, therefore collaboration between professionals with complementary expertise is often beneficial, especially because in the field not always a specialist pathologist is present, and for this reason, a veterinary clinician, a member of the field staff or a biologist should be able to go through the entire examination if the situation requires it.[5]

The postmortem examination should not be underestimated: despite the cause of death being established, conducting this examination remains essential, as it yields a wealth of information across multiple dimensions and facilitates the collection of materials for subsequent analyses and comprehension.

2. Necropsy

Necropsy is a specialized medical procedure involving a comprehensive examination of a carcass through dissection. Dissection can be defined as a medical or biological procedure involving the disassembly of the body of a deceased animal according to specific protocols. This process is conducted to study the anatomical structure of the animal and/or to assess and sample specific organs and tissues for further examination and analysis.[4]

It is also important to emphasize the difference between the postmortem examination and the postmortem investigation; while the first one is actually the physical examination of a dead individual or animal's body, the second one, apart from the inspection of the body, refers also to the collection and analysis of additional information related to the death. This may include reviewing medical records, analysing toxicology reports, conducting forensic examinations, and consulting experts. A post-mortem investigation aims to gather comprehensive evidence to accurately determine the cause and circumstances of death. Thus, while a post-mortem examination specifically refers to the physical examination of a deceased body, a post-mortem investigation involves a more comprehensive inquiry that includes examining the body as well as gathering and analysing additional evidence to determine the cause and circumstances of death. Speaking of which, different types of cause of death can be analysed: the immediate cause of death relates to the ultimate condition or disease leading to death; on the other hand, the underlying cause of death, indicates the disease or injury that instigated the sequence of pathological events ultimately resulting in death. Moreover, there could be other notable conditions that might have played a role in the individual's death but did not directly cause it, named contributing factors.[5]

It may be helpful to gain an understanding of the circumstances surrounding the occurrence of death, by attempting to answer just six questions:

- *How did this animal die?*
- *Why did this animal die?*
- *How long did it take this animal to die?*
- *When did this animal die?*
- *Where did this animal die?*
- *Who was involved, intentionally or inadvertently, in its death?* [1]

By going into more detail, some of the situations that may arise in the context of performing a necropsy can now be analysed. Prior to conducting a postmortem examination on an animal, it is essential to consider two significant factors: the presence of zoonotic diseases and reportable and infectious illnesses.[2]

In the first case, different species may carry diseases that can be transmitted to humans, for instance *rabies* or *echinococcosis* in carnivores or *psittacosis* in birds can result in severe and potentially fatal human illnesses.[2]

It is also to take into consideration that diseases like *tuberculosis* or *foot-and-mouth* disease can transmit to other animals via environmental contamination during necropsy procedures. Individuals conducting necropsies on wild animals should be knowledgeable about the characteristic lesions of these diseases and exercise additional precautions during the decontamination of necropsy sites.[2]

Another aspect to take into account is autolysis, a natural biological process occurring as enzymes within the cells become activated, leading to the breakdown of cellular structures and the release of their contents. Autolysis typically results in the degradation of tissues. This can have an impact on the quality and integrity of the carcass to different degrees. For all these reasons maintaining the animal's body at the correct refrigerated temperature is crucial for obtaining meaningful necropsy results. [5] However, histopathology can help differentiate between actual lesions and post-mortem changes.

2.1 Necropsy in veterinary facilities and Necropsy on field: the comparison

All types of necropsies need to follow standardized protocols and guidelines to ensure thoroughness and accuracy in their observations and interpretations.[3]

The various steps of a necropsy protocol are presented below, which are generally applicable to both on field and laboratory settings; however, differences and similarities will be analysed.

The first step is gathering background history and identifying the species under examination; as it was previously said, the anamnesis of the individual or everything possible to collect about it, are essential: the photographs of the place where the animal was found, the temperature of the environment and its characteristics, whether it was in an urbanised area with houses and buildings or near natural elements such as rivers or lakes.[2]

Then, understanding the surroundings in which these animals are found is crucial: document any recent weather conditions that may have contributed to animal deaths, such as droughts, floods, electrical storms, etc. is very important;[3] it provides valuable clues to help interpret potential injuries and diagnose the cause of death at an early stage. This could also be facilitated by considering key indicators crucial for assessing the time of death:

- Cooling of the carcass
- Rigor mortis
- Desiccation
- Discoloration of skin and/or internal organs
- Maggot and other invertebrate activity
- Degree of post-mortem change

Additionally, it is vital to gather information about the characteristics of the areas within the animal's territory, including whether they encompass urbanized or industrialized regions. [6]

In the field, carcasses may need to be protected from scavengers and humans' illegal activities, until they can be examined. Wildlife carcasses are often found in an advanced decomposition state or as partial remains. The rate of decomposition is influenced by external factors such as wrapping method, burial depth, ambient temperature, soil composition and the presence of water; on the other hand, the factors intrinsic to the carcass are characterized by the size and weight of the animal, where larger and heavier carcasses typically decompose more slowly than smaller ones due to the increased mass and volume of tissues that need to break down or still carcasses with higher fat content may decompose more slowly than those with lower fat content due to differences in tissue composition and microbial activity. Also, the internal body

temperature at the time of death can impact the rate of decomposition. Higher temperatures can promote microbial growth and enzymatic activity, speeding up decomposition.[7]

Before starting the necropsy protocol, photographs should be taken of the carcass where it was found. These photos, with an appropriate scale bar, should capture the front, back, and a view including the extremities for the assessment of any additional findings. Any other distinguishing features such as ear tags, tattoos or brands should also be documented at this stage. It is advisable to ensure that the photographs are clean and uncluttered.[8]

In the second place, if the examination is performed on field, some important features need to be considered: although the optimal approach involves transporting sick or recently dead animals to a pathology laboratory for necropsy under the supervision of trained veterinarians, in many cases, transportation is not feasible, mainly due to the size of the animal [3]. Furthermore, what on field will not be possible to perform, are complementary tests such as radiography, computed tomography (CT), ultrasound, microbiological, parasitological, genetic, virologic and toxicological examination able to give further information. Nevertheless, samples can be made for these latter.

The primary action for the individuals performing the necropsy is to wear personal protective equipment such as a face mask, protective gloves, caps, foot covers, and protecting clothing, such as disposable aprons. Where disposable equipment is not available, clothing should be easy to wash and disinfect.[6]

In Table 1 can be seen the tools utilized throughout the examination.[1]

Species	**Essential**	**Useful additions**	**Special precautions**
All (vertebrate and invertebrate) species	Post-mortem table; Instruments for the dissection; Incinerator, macerator or other means of disposal; Refrigerator, freezer; Balance/scales and rulers for the measurements (a single person should perform all	Protective hood or flow cabinet; X-ray machine	Adequate drainage, disinfection and ventilation

	measurements using consistent equipment to minimise individual variation. Any asymmetry should be verified by measurement, taking into account potential observer bias. [1]) Disinfectant solutions; Steriliser or autoclave; Bottles and fixatives		
Mammals Birds Reptiles Amphibians Fish	As above; Instruments should include bone forceps and saw for examination of the central nervous system (CNS); The corpse should be kept moist during the examination of amphibians; Long forceps and scissors for toxic spines in fish	Vacuum cleaner or similar to remove hair and dander and feathers; container for head of venomous species in reptiles	As above
Invertebrates	As above (all); Micro instruments (e.g. ophthalmic forceps, dissecting microscope or magnifying loop) are	As above	As above

	often needed for a mini necropsy; Some aquatic species need to be kept damp or even immersed in saline water (to prevent desiccation or collapse) during examination		

Table 1. Main tools used during examination. ("Wildlife Biodiversity Conservation", chapter 10)

Regarding the equipment, a dissection kit must be available; It should include essential cutting tools such as knives, scalpels, scissors, hatchets, and bone saws. These tools can be adjusted according to the size of the animal and to the external surroundings. Moreover, forceps and tweezers are also present: they are tools which assist during cutting. It is imperative that this equipment is properly labelled as belonging to the necropsy service and is never repurposed for other uses. Additionally, thorough cleaning and disinfection of the equipment should be performed after each use. It's vital to have tools to record the information collected, including a notepad, audio recorder and a camera.[7]

Moreover, microscopy in the field is becoming increasingly practical with the development of expensive instruments that can be used in conjunction with a mobile phone.[1]

Once these steps have been completed, the next action would be to perform the necropsy. This is the pivotal point of the protocol: there are different species, and each can be treated and managed differently depending on the anatomy involved.

In the case of a carnivore, depending on the size, canids and small cats are done in dorsal decubitus for example, while others are placed on the left side, leaving the right side of the carcass open. On the other hand, all birds, reptiles and primates are placed on their backs.[8]

The first step is performing an external examination; it consists of an assessment of bleedings, external fluids, and leakages. In addition, nutritional and decomposition state are determined, as well as the evaluation of skin annexes. In Figure 1 there is an example of an external examination in a bird of prey.

Figure 1. External exam of a juvenile bald eagle "Haliaeetus leucocephalus".
https://cwhl.vet.cornell.edu/article/whats-necropsy-science-behind-valuable-diagnostic-tool

After that, before opening the body cavities, the skinning and the assessment of the subcutis are performed. Once the body cavities are opened, it is important to evaluate and ensure the proper positioning of all organs to check for any displacements before proceeding with organ removal. During this stage, in a very fresh carcass, a sterile blood sample can be extracted from the heart, preferably from the right atrium, for culture purposes. Subsequently, additional blood samples can be collected to obtain serum for serological tests.[9]

Once the overall condition of the animal has been documented, individual organs can be systematically removed, examined, and sampled. Any unusual findings should be described in detail; photographs of these abnormal observations offer the most comprehensive documentation for record-keeping purposes.[8]

Irregularities in organs or tissues are referred to as "lesions". They must be described in detail according to a precise outline:

- Numbers and distribution

- Location/orientation
- Raised, flat or depressed
- On surface only, or in deeper tissue
- Shape
- Size (cm and mm)
- Colour
- Consistency
- Odour

The colour of the lesions may be important, as colour changes have traditionally been used as an indicator of pathological changes to assist in the diagnosis of lesions. It is advisable to standardise descriptions by using a colour key.

Other phenomena that can lead to colour changes are hypostasis, also known as *livor mortis* or "lividity," it refers to the gravitational pooling of blood in specific areas of the body, particularly the lower regions such as the limbs and feet, after death. This phenomenon leads to colour changes and can sometimes be misinterpreted as bruising. Understanding hypostasis may be pertinent in determining the circumstances surrounding the animal's death.[5]

Colour changes are also indicative of bruises. They serve as significant indicators during post-mortem examinations.[1]

Based on necropsy reports, a logical order for opening and dissecting the body can and should be followed as it was said also previously. Figure 2 shows an example of a form for a post-mortem examination:

Owner's Name			Date of Necropsy
Animal's Name			Date of Death
Species			Euthanasia Method
Age	Sex		Body Weight at Necropsy
Band/Microchip #	Tattoo?		

Organ(s)	Normal	Abnormal	Description
Skin/Feathers			
Beak/Oral Cavity/Tongue			
Eyes/Ears/Conjunctivae			
Sinuses/Choana/Nasal Cavity			
Skeletal muscle/Bones/Joints			
Liver/Gall Bladder, if present			
Spleen			
Thyroids/Parathyroids			
Trachea/Lungs/Airsacs			
Kidneys/Adrenals			
Testes/Ovary/Oviduct			
Crop/Esophagus			
Proventriculus/Ventriculus			
Duodenum/Pancreas			
Jejunum/Ileum/Ceca, if present			
Colon/Cloaca			
Bursa/Thymus			
Brain/Meninges			
Spinal Cord/Vertebrae/Nerves			
Bone/bone marrow			
Middle & inner ear			
Heart/Great Vessels			
Gut contents wet mount results:			
Gut contents dried smear results:			
Organ impression smear results:			
Tissues in formalin:			
Tissues frozen:			

Figure 2. Example of a necropsy sheet based on a gross necropsy procedure on birds. ("Clinical Avian Medicine", chapter 26)

There are some important differences depending on the species studied:

- Carnivores: typically, they have well-developed musculature and specialized dentition for meat consumption. During necropsies, special attention may be given to the teeth, jaws, and digestive tract to assess any abnormalities related to diet or oral health; they have retractable claws and specialized paw pads for gripping and manoeuvring. Examination of the claws and pads may reveal signs of wear, injury, or infection.[8]
- Ungulates: Many of them, such as cattle, deer, and sheep, are ruminants with a complex, multi-chambered stomach designed for fermentation of plant material. The digestive tract may be carefully examined, paying close attention to the stomach compartments (rumen, reticulum, omasum, and abomasum), as well as the intestines, for signs of disease,

impaction, or foreign bodies. They have hooves adapted for support and locomotion; examination of them may reveal signs of wear, injury, or hoof diseases such as *laminitis.*[8]

- Birds: soak or spray the carcass in water containing a disinfectant to dampen the feathers as first step; next, pull back the skin to reveal the keel and breast muscles, ribs, and muscles covering the lower celomic cavity. Evaluate the level of body fat beneath the skin and within the body cavity and the amount of musculature over the keel. Following the same steps of the general outline, arriving in the gastrointestinal tract, the major difference with other species will be the removal of the proventriculus and ventriculus, the gizzard and intestines, including the cloaca and bursa of Fabricius.[3]
- Reptiles: the procedure is similar to that for birds, except for a few species' peculiarities within this class of vertebrates: The kidneys of lizards are situated deeper within the pelvic region; to remove the plastron in turtles, a saw is used at the junction with the carapace.[9]

Another important step in the necropsy protocol is samples collection. Each container, tube, slide and bag must be labelled with a marker. For added security, a second label may be placed in a plastic bag and attached to the container. For formalin-fixed tissues, a paper label with the animal identification written in pencil may be immersed in formalin with the tissues.[6]
The following information should appear on the labels:

- Date and Geographic location (Park name or nearest town, country)
- Species
- Sex and approximate age
- Tissue Identification (this is not necessary for formalin-fixed tissue samples)
- Animal ID (if available)

If collecting is done on field, there's the problem of transporting. Samples for culture should be refrigerated (for parasitology or bacterial cultures) or frozen (for toxicology or virology). Freezing at -70°C is preferable to -20°C (standard freezers). It is advisable to send frozen and fixed samples separately. If they must be shipped together, ensure that fixed tissues are protected from freezing by wrapping them in newspaper. In addition, take precautions because formalin is toxic and carcinogenic, as well as to avoid spillage of formalin, as fixation of frozen specimens makes it impossible to culture for bacteria or viruses and may alter cells on blood smears or cytology slides.

In addition, all specimens should be handled with care and unfixed specimens should be placed in leak-proof containers to prevent hazardous infectious materials from escaping during transport.[3] Some field techniques, reported in Table 2, can be used to improvise because there is not a fully equipped facility at disposal: [1]

Requirement	**The ideal situation**	**Possible improvisation**
Fixation of tissues for histological examination	Formalin or commercial alcohol	Alcoholic drinks, including wine and strong beer
Handling of contaminated or infected material	Rubber gloves and forceps	Plastic or paper bags Wooden spatulae
Sample collection	Laboratory containers	Re-used film pots

Table 2. Field techniques for improvisation. ("Wildlife Biodiversity Conservation", chapter 10)

Approaching the last stages, sanitizing the facilities is the next step.

It is recommended that disinfectant solutions are made readily available for post-necropsy cleanup, especially in cases where zoonotic diseases are suspected. However, it is important to exercise caution and ensure that these solutions and their fumes do not come into contact with the tissues being collected. This is because such contact could lead to cell lysis and the destruction of essential microorganisms required for culture. [6]

The necessary steps to be performed are the correct disposal of the carcass, along with all tissues and any blood-soaked dirt, depending on the sanitary risk; the same for contaminated paper or plastic materials. Instruments should be cleaned from all blood and residual tissues using soap and water, followed by disinfection. Necropsy boots and aprons should be cleaned, and any contaminated clothing should be thoroughly washed. Additionally, the external surfaces of containers holding samples should be washed.[6]

In conclusion, comparing records, evaluating laboratory results, and compiling a comprehensive report represent the final stages of the process. This means collating all the information to produce a final report, which must include the data analysed starting from the beginning with the history of the animal until the end of the examination.[2]

Overall, determining the cause of death in non-domestic animals, whether kept under human care (UHC) or in their natural habitat, poses significant challenges due to several factors:

- Lack of prior history of the animal.
- Absence of observations prior to death.

- Sudden death without/ with limited evident gross and microscopic lesions.
- Limited familiarity with the normal anatomy of wild animals among examiners.

In wild animals the cause of death may be unknown, whereas in UHC animals the apparent cause of disease during life may mask the true cause of death. This underlines the importance of taking appropriate samples and keeping accurate written and photographic records. [9]

3. Necropsy: A relevant role in the sphere of conservation

It is very difficult to determine the cause of death or understand what happened to the deceased animal without a post-mortem examination. This is the reason why necropsy is so important: it is performed to assess the possible cause of death. A post-mortem examination may uncover a disease capable of decimating an entire population of wild animals and it helps to improve the understanding of the disease itself.

Even here, there is the possibility to differentiate the scenario between two cases: captive facilities and natural habitat. For instance, as soon as the origin of a problem is identified, veterinarians in captive facilities can utilize vaccination to prevent the illness from spreading further. In zoos, the demise of a single animal due to an unidentified illness could signify a potential threat that could endanger all animals of that species and possibly animals of different species within the facility; conversely, in the wild, it is not this immediate, indeed, it is crucial to identify potential diseases that could wipe out an isolated population of animals. Even the loss of a single animal from that species significantly diminishes the available gene pool of animals.

For these reasons and more, the welfare and conservation of animals is an increasing concern, as well as the increment in the number of cases of wildlife crimes. Necropsy, as the definitive diagnostic tool, plays a key role in the assessment of evidence in cases of suspected wildlife crime.[10]

3.1 An increasingly growing aspect: Forensics

Within this topic, there are a few aspects that need to be taken into account. Primarily, before proceeding with the postmortem examination on the animal's body, it is essential to verify whether there is an official authorization to do so from the appropriate authority. This is of utmost importance. If the animal is insured, it is necessary to obtain a letter of request from the competent authority of the relevant insurance company. In the case of a private uninsured animal, the veterinarian must obtain permission from the regulatory authority. If, due to unavoidable circumstances, the veterinarian is unable to attend the necropsy, it is advisable to ask the police or the injured party to contact another veterinarian.[2] In the case of a wild animal found dead, the authority to conduct the procedure typically lies with relevant wildlife management or conservation authorities. It is essential to obtain permission or authorization from the appropriate wildlife management authority before proceeding with the necropsy.

In addition, the examination will have medical and legal significance if it is carried out carefully and accurately, ensuring that the chain of custody is maintained. This is essential for the identification and prosecution of those responsible [7]. But what is the chain of custody? The chain of evidence, or chain of custody, typically begins with the law enforcement officer who first encounters the animal(s) in the field. It is their duty to maintain custody of the animal until it reaches the laboratory. At this point, responsibility passes to the pathologist who will perform the necropsy. Specimens for forensic examination should never be left unattended at a laboratory. They must always be received by a responsible person to maintain the chain of custody. For this reason, at the time of submission, each specimen should be clearly identified with securely attached labels or tags.[10]

Nevertheless, it is essential to begin from the outset: the collection of the animal. The carcass should be collected by the competent authorities, accurately identified, and placed in a sealed bag. The bag should be marked with the identification of the animal, the keeper responsible for the collection, the date, and the location. In the next step, the representatives of the authorities are responsible for preparing the accident report and for collecting as much data and as many samples as possible at the scene of the accident. The animal should then be transported to a necropsy laboratory, where it should be refrigerated or frozen to slow down the process of autolysis.[7]

The identification of the species, sex, and age is crucial information, along with details of the animal's ante-mortem history, environmental conditions, territory, previous health status, behaviours, and any events that may have contributed to the cause of death. Based on the collected information, the veterinarian can attempt to establish the chronological sequence of events and ascertain the potential cause of death, based on observed lesions. Sampling is also crucial, such as obtaining samples for toxicology examination when poisoning is suspected. These samples should be properly identified and collected in duplicate. Photographs should be captured, preferably in unalterable formats that are resistant to manipulation through computer programs. Each photo should be accompanied by a small paper indicating the case identification, animal details, date, pathologist's name, and the specific organ being photographed. Guidelines for lesion measurement should also be included. The veterinarian, preferably with expertise in pathology, should systematically conduct the necropsy to gather comprehensive information. All observations should be meticulously recorded in the report, including samples collected, and whether or not they exhibit lesions (for toxicological or histological examination). Following the

delivery of the report, it is the duty of the pathologist to retain duplicate copies of the report, photographs, and samples until the judgment is made or until the legally stipulated duration (in some cases, mandatory storage of data for five years).[4]

In conclusion, necropsies have a central role in the field of conservation and protection not only of animals, to maintain their species, but also of public health: they may reveal diseases or contaminants that pose a risk to community health, such as zoonotic diseases or environmental toxins. Likewise, results from necropsies help to understand disease dynamics, population health, and ecosystem health. Thanks to this information policy making, wildlife management and conservation efforts can be informed. Finally, providing animal welfare through postmortem examination thanks to the revelation of cases of animal abuse, neglection or inadequate care, leads to actions to improve animal welfare standards, that denounce those who go against and review the laws aimed to preserve these creatures. In this way, this creates a history, a precedent so that these cases do not go unnoticed and, above all, if they happen again in the future, they will no longer be the only ones, but they will have similar episodes behind them that will defend their vision.[10]

3.2 Passive monitoring for the detection of diseases

Disease is only one of many elements that affect the sustainability of wild populations. In a well-balanced ecosystem, the majority of populations will endure with minimal levels of disease or experience occasional outbreaks. Nonetheless, human activities are causing wildlife populations to become increasingly dense through habitat encroachment, thereby heightening the likelihood of catastrophic epidemics within these populations, as well as the increment of the transmission of diseases between wild and domestic species. To assess disease risks to a population, it's essential to identify the causes of morbidity and mortality within that population. Risk assessment also entails understanding the natural history of infectious diseases in that environment, including the past occurrences of epidemics.[11]

Numerous wildlife disease epidemics impacting valuable wild animals' resources or livestock have remained undetected due to the lack of appropriate sampling for diagnostic testing from animals that died during the outbreak. However, with the collection of appropriate samples and the maintenance of written and photographic records, the cause of an epidemic can be determined in most instances, as it has been shown above.[12]

Normally, in veterinary public health, active and passive surveillance depends mainly on the definitive diagnosis of specific diseases by laboratory tests for identified pathogens.

Nevertheless, when a previously unknown or unexpected pathogen emerges in a population, this method may require additional time and may result in delays in the identification and detection of pathogens.[13]

For these reasons, syndromic surveillance systems (SYS) were developed [11], to improve conventional passive surveillance systems, and therefore, they are currently gaining prominence in veterinary medicine. By analysing and interpreting pre-diagnostic health-related data, veterinary surveillance systems assist in the early detection of disease threats; different data sources have been assessed for their suitability in veterinary surveillance systems globally: prominent among them, in the case of animals used for food production, data derived from post-mortem meat inspection and examinations of fallen livestock. Necropsies conducted by diagnostic laboratories on fallen livestock or diseased animals could offer supplementary insights into animal health that may be beneficial for surveillance systems. As a matter of fact, postmortem examinations play a crucial role in the investigation of disease outbreaks. Farmers may request veterinarians to investigate a sudden rise in morbidity or mortality due to an unknown or unrecognized disease. If animals have died suspiciously or were euthanized, veterinarians may conduct a field necropsy or arrange for the carcasses to be transported to a diagnostic laboratory, where a more comprehensive post-mortem examination and diagnostic assessment can be carried out.[11]

Necropsy reports could potentially be a valuable source of information for the identification of unexpected health events that occur within a population; as a result, syndromes covering a wide spectrum of diseases could be identified and tracked in a surveillance system based on pathological findings from necropsies. In sight of this, post-mortem examination of wild species plays a key role in zoonosis assessment, facilitating the collection of material for additional examination. It is estimated that over 70% of current zoonotic diseases originate from wild animals.[12] The rise in these diseases can be attributed to several factors, including over-exploitation of forests, expansion of agricultural land, illegal wildlife trade, ecotourism in remote areas, the wild meat market, and other contributing factors. This anthropogenic pressure on wildlife is resulting in an imbalance of ecosystems, leading to significant losses in terms of animal and human health.[11]

3.3 Passive epidemiological surveillance present also in Costa Rica

As a continuation of the topic relating to passive monitoring, the same approach can also be seen in Costa Rica, the main protagonist of this essay. Equatorial regions are among the areas of greatest natural biodiversity, with a concomitant high diversity of pathogens and thus a high potential for disease emergence.[10] In addition, this risk has increased significantly due to human-induced stress associated with overexploitation of natural resources and intensified land-use changes, which increase the likelihood of contact between wildlife, domestic animals and humans.

Thanks to the action of the World Organization for Animal Health (WOAH) and the World Health Organization (WHO) there was the creation of preventive strategies aimed to face the risk of epidemic events. All of this was possible by heightening initiatives to establish mechanisms for the early detection of pathogens, giving priority to those of zoonotic and nature conservation importance, through Wildlife Health Monitoring Programs. [14]

The main feature and advantage of this approach, as previously noted, is its ability to identify the causes of mortality within a particular species by analysing their pathological profiles through necropsy examinations. When used on a long-term basis, this method has been shown to provide essential information for decision-making and the formulation of policies, regulations, and strategies with a focus on disease prevention, even in cases where sampling is biased, or geographical coverage is incomplete.[15]

In Central America, some national programs using wildlife as sentinels to monitor specific diseases are well established and operating effectively; although, one problem remains: there are still no programs to monitor the general health of animals in the wild. This is a clear indication of the urgency of setting up this type of project involving wildlife; Costa Rica, for instance, has the infrastructure and maintains adequate surveillance programmes to detect and control zoonotic diseases in livestock, but without any implication of wild animals. And here, in Costa Rica, numerous pathogens, including zoonotic parasites and directly transmitted viruses, have been detected in natural habitats. The main obstacles are significant financial and logistical constraints in monitoring the health and spread of pathogens in wildlife due to limited resources present in nations like Costa Rica. Apart from these impediments, it was demonstrated that is achievable to modify the existing infrastructure to implement this programme. In addition, this adaptation has facilitated the analysis of wildlife carcasses, allowing the detection of zoonotic pathogens.[14]

4. Costa Rica: main causes threatening the ecosystem

Diseases and epidemics are just one of the major causes affecting the trend of wildlife species.

Global environmental transformation represents a challenge for everyone, especially for those who are in contact with nature such as indigenous populations and wildlife.

The disappearance of species and the decline of biodiversity worldwide is an increasingly frightening common scenario today, posing a significant threat to wildlife in particular.

Human-induced and ecological changes threats human and animal health, and the list that follows describes only some of the most important threats:

- Trauma related to human activity: such as impact with vehicles, buildings or other man-made structures, like powerlines or fencing, causing electrocution;
- Captivity: meaning animals kept under human care, seized by rangers or law enforcement agencies as a result of poaching or the illicit pet trade; animals discovered in inappropriate locations by accident and/or left orphaned due to unnecessary human interference; this latter is also linked to animals dependent on their parents for survival, either abandoned or orphaned due to the death of their parents;
- Deforestation with the consequent fragmentation of the territory and habitat loss;
- Disease, as described in the previous chapter, which may be of an infectious, parasitic, metabolic, or nutritional nature;
- unknown causes.[16]

The most widespread causes leading to the decline of wildlife will be analysed with a focus on Central America, and more specifically the country of Costa Rica.

4.1 Personal experience of traineeship in Costa Rica

I had the opportunity to experience some of these realities during my traineeship in Costa Rica, more precisely in the *"Wild Sun Rescue Centre*", near the Capo Blanco Natural Reserve, in Cabuya on the southern tip of the Nicoya Peninsula. This facility is just one of many rehabilitation centers built in the country, dedicated to rescue, rehabilitation, and release of local wildlife back to the wild.

As said in the previous paragraph, local animals face many threats here in this land, due mainly to human action and for this reason, the centre accepts everyday sick, injured and orphaned creatures.

Necropsies are often performed in the center. The scenarios that occur more frequently are especially electrocuted primates like howler monkeys *Alouatta palliata*, the most diffused species in the peninsula.

Figures 3 and 4, for example, show severe burns on a juvenile specimen. In the first picture is possible to observe the dorsal part of the thoracic region almost entirely burnt, while in the second one, the detail of the distal part of the forelimb is shown.

Figures 5 and 6 illustrate another young howler monkey been electrocuted who arrived at *Wild Sun Rescue Center*: the specimen has quite different types of lesions compare with the previous case: this time the burns are distributed throughout the face and on the left forelimb up to the three fingers.

Most individuals arrived agonizing or already dead because of the electric shocks. Often, babies become orphaned because in the early months of life, they stay on the back of the mother, travelling across the trees; therefore, they will inevitably be affected by the accident. However, orphaned babies usually survive because their injuries are cushioned by their mother's body, and after a period of rehabilitation, they are released back into the wild. *Figures 7 and 8* show an instance of these cases arrived at *Wild Sun Rescue Center*: in the first picture, the adult female howler monkey together with her young baby are represented after the electrocution; while in the second picture, there is the young baby who manage to survive.

In *Figure 9 and 10* other similar electrocution lesions are shown. In this case, the primate species belonged to the New World Monkey is the black-tufted marmosets, *"Callithrix penicillate"*.

Figure 3. The lesion is yellow reddish with quite distinct margins, a depressed outline and an ulcerated surface; the shape follows the anatomical structure, while the texture is compact, and the distribution is focally extensive. ("Journal of Medical Primatology", Vol. 50, Issue 5).

Figure 4. This lesion is yellow reddish with distinct irregular margins and a depressed outline. It has an ulcerated surface and a linear shape following the anatomical structure making the phalanges visible. It is focally extensive distributed. ("Journal of Medical Primatology", Vol. 50, Issue 5).

Figure 5. The left forelimb almost completely burnt presents yellow brownish lesion with distinct and irregular margins; the outline is depressed with eroded surface and linear shape following the forelimb structure. The texture is compact with a focally extensive distribution. ("Wild Sun Rescue Center" archive).

Figure 6. The lesion is black with defined and irregular margins, a depressed outline and an eroded surface; the shape follows the anatomical structure of the face with a compact texture and focally extensive distribution. ("Wild Sun Rescue Center" archive).

Figure 7. Adult female howler monkey together with her young baby after the electrocution. ("Wild Sun Rescue Center" archive)

Figure 8. The orphaned young baby howler monkey who survived. Photos taken from Wild Sun Rescue Center. ("Wild Sun Rescue Center" archive)

*Figure 8. Electrocuted black-tufted marmosets. **a** Lower limb with carbonization of tissues and deep ulcer on the skin. **b** Torso with dark reddish parallel electrical wiring marks in the subcutaneous and muscle tissues. ("Primates", Vol. 61).*

*Figure 9. Electrocuted black-tufted marmosets. **a**. Lower limb with electrothermal injury on the plantar surface and ulcer formation on the skin; **b.** Upper limb with severe electric shock injury with exposure of the underlying tissues and fracture of phalanges. ("Primates", Vol. 61).*

However, they are not the only species: mammals, like jaguars, sloth, and kinkajous (animals closely related to racoons) are target of these threats. *Figures 11 and 12* illustrate an electrocuted adult kinkajou, “Potos flavus” arrived at Wild Sun Rescue Center.

Furthermore, these species are also victims of car accidents, dramatically frequent; this occurred particularly in areas where roads intersect with natural habitats. As urbanization and infrastructure development expand, roads increasingly fragment wildlife habitats, leading to higher rates of vehicle-wildlife collisions. This can result in injury or death for wildlife as well as damage to vehicles and potential risks to human safety.

Figures 12 and 13 show a young female howler monkey arrived at Wild Sun Rescue Center after been hit by a car.

Figure 9 and 10. Young female kinkajou “Potos flavus” suffered from electrocution. On the left, the forelimbs and the face are quite affected by burns with whitish, quite distinct margins and a depressed outline; it has a multifocal distribution. On the right, the trajectory of the electric shock from the forelimbs to the hind ones can be observed. Photos taken from Wild Sun Rescue Center. (“Wild Sun Rescue Center” archive).


Figure 11 and 12. Young female howler monkey hit by a car. The lesion is red withish, with distinct irregular margins; it has a nodular/ irregular shape, a lifted outline with an ulcerated surface and a multifocal distribution. Photos taken from Wild Sun Rescue Center.
("Wild Sun Rescue Center" archive).

Thanks to this experience, I was able to observe the reality of this country at close quarters and learn how to make a difference to help what is now the survival of these species in the midst of the ever-increasing human actions that are taking over.

4.2 Deforestation

Despite covering just 0.03 per cent of the earth's surface, Costa Rica is home to almost 6 per cent of the world's biodiversity. The total amount of species presents in this territory amount to roughly 500,000 different species including mammals, birds, reptiles, amphibians, fish, invertebrates, and plants. [17] This is evidence of the remarkable density of biodiversity within a compact nation.

Much of this biodiversity thrives in its forests, and they play a crucial role in supporting biodiversity. The tropical rainforests provide habitat for countless species of plants, animals, fungi, and microorganisms; moreover, forests also contribute to biodiversity by regulating local climates, supporting nutrient cycles, and offering protection against natural disasters, as well as serving as carbon sinks, helping to mitigate climate change by absorbing carbon dioxide from the atmosphere. For all these reasons, protecting and conserving forests is essential for maintaining biodiversity on both local and global scales. Unfortunately, once again, human activities are the

protagonist of the degradation of forests in Costa Rica. Presently, Costa Rica's forests harbor 179 species listed on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species. Among these, 70 species are labelled as "*Vulnerable*," while 76 are designated as "*Endangered*." The remaining 33 species fall under the classification of "*Critically Endangered*," indicating an extremely precarious state of existence, that warrants urgent conservation attention.[17] Costa Rica is experiencing a rapid loss of its rich and diverse wildlife, a trend that unfortunately appears irreversible.

One of the worst consequences of deforestation is certainly the high levels of habitat fragmentation resulting in a reduction of the total habitat area, which is leading to a reduced connectivity within living spaces. This is where the problem for wildlife arises: species that are unable to pass through the "non-habitat" area will be restricted to the habitat fragments. Over time, these fragments become too small and too far apart to support viable populations. Animals need large territories to secure adequate vital supplies, especially in regions with fluctuating environmental conditions. In general terms, to ensure that there is enough food, water and shelter for the entire population, larger animals need more territory.[18]

For example, the case of the Central American squirrel monkey, "*Saimiri*",[17] highlights the effects of forest fragmentation. This animal natural geographic range, primarily found in the Pacific lowlands of southern Costa Rica and northern Panama, became split into two separate regions. What caused this ecologic disturbance was the uncontrolled the deforestation in certain areas of the habitat of this species, and it is mainly related to urban sprawl, livestock farming, and farming practices, such as banana plantations, sugar cane, and rice farms. The forest patches forming the newly fragmented habitats are smaller and exhibit more irregular shapes compared to their original configurations. Therefore, the population is restricted to areas that are inadequate for sustaining their numbers. The survival and reproduction of the Central American squirrel monkey has been greatly affected by the lack of resources such as food and shelter within these fragments and now this species finds itself listed as "*Endangered*" on the IUCN Red List with a steadily declining population.[17]

While it's accurate that species like the Central American squirrel monkey aren't exclusive to Costa Rica, the decline in their numbers within the country corresponds to a global decrease in their overall populations. This inhibits their capacity to recover from endangerment and to restore their populations to their original levels.

This case is just one of many examples of wildlife being at risk from human activity. First of all, the awareness of these situations must be clear; and then, reforestation and habitat restoration projects may be a beneficial initiative, aimed at replanting native vegetation in fragmented areas to help reconnect habitats and provide corridors for wildlife movement; moreover, implementing zoning regulations and land-use policies that prioritize conservation and minimize habitat fragmentation can help maintain ecological connectivity, as well as all the activities related to the education and awareness of public about the importance of biodiversity and the impacts of habitat fragmentation which can lead to greater support for conservation efforts.[13]

By implementing a combination of these strategies, Costa Rica can work towards mitigating the effects of habitat fragmentation and conserving its rich biodiversity.

4.3 Climate change

In Central America, the effects of climate change are expected to be widespread in the coming years. In particular, tropical rainforests are especially vulnerable to fluctuations in climate. In Costa Rica, temperature and rainfall have a significant impact on forest ecosystems, given that various forest types exhibit distinct ranges of temperature and precipitation.

A peculiar example is now presented: amphibians are the central figures of this case: frogs, toads, and salamanders are highly vulnerable to environmental changes due to their affinity for water and their permeable, absorbent skin. They often experience the initial effects of environmental changes.[19]

In Costa Rica, the increase in warmer and wetter conditions has led to a reduction in rainforest leaf litter; this layer of decomposing leaves releases nutrients into the soil which are then absorbed by plants, contributing to the overall fertility of the rainforest ecosystem. Additionally, the leaf litter provides habitat and food for many species. With the advent of these conditions and the decrease in the rainforest leaf litter, more than 30 species of amphibians and reptiles, are marked by a drastic decline, in fact, a recent study performed in a protected old-growth lowland rainforest, states that the populations of these species have dropped by 75% in 35 years. [19] This substantial decline is concerning for several reasons: mainly because this study was performed at “La Selva Biological Station” an exceptional neotropical research center located in the province of Heredia, this facility is located in a protected area, and this implies that habitat destruction is likely to be minimum to non-existent. The cause of these species’ decline is still enigmatic and contested but many hypotheses are proposed. The infectious disease named chytridiomycosis,

caused by the chytrid fungi "*Batrachochytrium dendrobatidis"* was one of the leading causes that needed attention; however, this species cannot withstand high temperatures and thrives, it grows exclusively in temperate regions or mountainous areas, not comparable with the condition of "La Selva". Moreover, the fungus appear in the population through swift declines within months, whereas the declines observed over decades. It is also known that, reptiles do not share amphibians' susceptibility to chytridiomycosis, because of the anatomical difference of the skin, so the chrytridiomycosis hypotheses lost its relevance. Climate change was investigated as main cause of these species decline, and it was noted that in the last 35 years, "La Selva" has encountered increased precipitation and higher temperatures. There has been a rise of one degree C° in temperatures, which has resulted in slowed growth of local trees and a decrease in the volume of shed leaves, leading to a reduction in levels of leaf litter in the forest. In this way, both amphibians and reptiles were deprived of their habitats. At this point, also within this protected area, habitat destruction is occurring unnoticed. The concept of climate change explains another peculiar discovery: the populations of amphibians and reptiles had not diminished in nearby abandoned cacao plantations. This is attributed to the fact that cacao trees shed their leaves consistently throughout the year, ensuring a continuous provision of new leaf litter.[19]

4.4 Electrocution

As described before, uncontrolled construction and escalating human development in rural areas have introduced the problem of inadequately designed electric infrastructure; together with inadequate urban planning, represent a substantial menace to the biodiversity of the country. More specifically, electrocution poses a significant threat to wildlife in Costa Rica; the lethal consequences of contact with electrical infrastructure have raised concerns among conservationists and policy-makers alike. In this paragraph, the situation of the howler monkeys' species *Alouatta palliata* living in the region of Guanacaste, will be analysed. The rapid loss of habitat compels animals to utilize power lines as pathways across the region.[12] Regrettably, the true impact on wildlife remains uncertain, but certainly the number of accidents that occurred far exceeds the hundreds.[20]

When electricity flows through a body, it can cause injury through various energy transduction mechanisms. However, there are many variables contributing to the injury process that are not measurable or calculable. The route of the current through the body, from the point of entry to the point of exit, determines the number of organs affected and, consequently, the nature and

severity of the injury. Accurate determination of the electrical pathway is critical for both acute management and long-term prognosis. The longer the animal is in contact with electricity, the more energy can cause electrothermal heating of tissues, increasing the degree of tissue destruction at skin contact points and in internal organs.[21] External injuries are primarily electrothermal burns and typically occur in high-voltage accidents. There was observed that almost one hundred howler monkeys were victims of electrocution; unfortunately, many of them perish on their journey to a rescue canter or upon their arrival. In these situations, the veterinarian's first step is usually to carry out a physical examination and check the symptoms; often the burns are critical, and many do not survive the next few days or weeks despite treatment. Blood values can serve as a valuable tool to gain a deeper insight into the overall condition of the patients and to assess the extent of the damage inflicted by the electricity. In addition to burns, muscle and kidney damage and impaired glucose metabolism have been reported.[22]

This situation is becoming increasingly common in Costa Rica and demands a significant investment of time, effort, care, and financial resources to offer these patients a second chance at survival. Addressing this problem requires a prevention strategy from government agencies and electricity companies, in collaboration with wildlife rescue and rehabilitation centers, to effectively mitigate this growing issue. The government should establish the obligation to carry out environmental impact studies regarding electricity distribution to protect wild species, creating subterranean, insulated, or semi-insulated cables together with the establishment of biological corridors, especially in -highly dense wildlife-populated areas; in this way, animals will be allowed to move between different areas, promoting genetic diversity and reducing isolation, but most of all, increasing the survival of the wildlife so valuable for the ecosystem.[20]

5. Conclusion

To conclude, the aim of this work has been to highlight the purpose of necropsies in relation to the conservation of wild animals.

What emerges from all these observations is that postmortem examinations are very useful for multiple reasons: first of all, necropsies can reveal the cause of death helping mitigate threats to wildlife populations. Then, monitoring health trends by examining multiple necropsy reports over time; accordingly, it is possible to identify trends in wildlife health and disease prevalence. This information can update conservation strategies, as passive monitoring, and management decisions to protect vulnerable species. Furthermore, necropsies can help detect and monitor the emergence of new diseases or pathogens in wildlife populations. Early detection of disease outbreaks can facilitate rapid response efforts to prevent further spread. Eventually, findings derived from postmortem examinations, can contribute valuable data to scientific research on wildlife health and conservation. This information can help prioritize research and conservation efforts, guiding resource allocation and management strategies to maximize conservation outcomes.

Shifting the focus to the main causes of wildlife mortality in Costa Rica, there is an opportunity to deal with such threats requiring coordinated efforts between government agencies, conservation organizations, local communities, and international partners. Conservation initiatives focused on habitat protection, sustainable land use practices, biodiversity monitoring, and public awareness are essential for safeguarding Costa Rica's precious ecosystems for future generations.

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