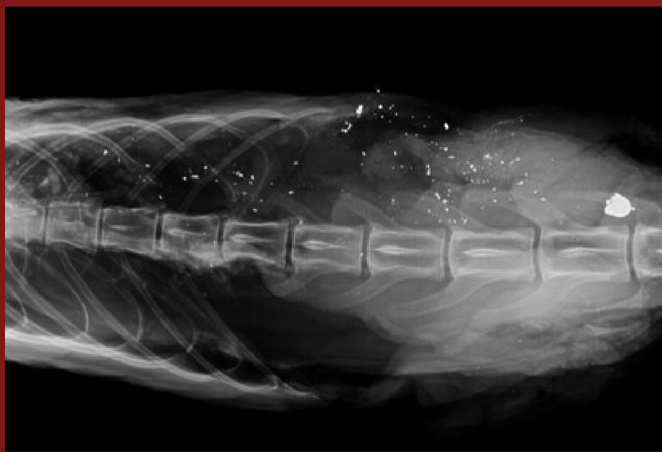
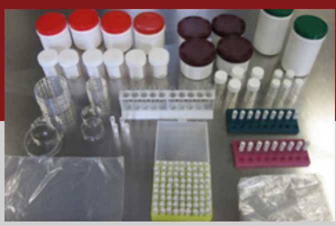
HANDBOOK

STANDARD OPERATING PROCEDURES FOR FORENSIC INVESTIGATIONS OF SUSPECTED ILLEGAL KILLING OF WILDLIFE





Christoph Beiglböck
Chris Walzer

Basics, techniques and methods for investigators

Practical recommendations for fighting the illegal killing of wildlife in the Alpine region and beyond

vetmeduni vienna

Interreg Alpine Space
ALPBIONET2030
EUROPEAN REGIONAL DEVELOPMENT FUND
EUROPEAN UNION

Handbook on Standard Operating Procedures (SOP) in Forensic Investigations of Suspected Illegal Killing of Wildlife

Basics, techniques and methods available for investigators and practical recommendations to fight the illegal killing of wildlife in the Alpine Area

Christoph Beiglböck

Chris Walzer

1st Edition

Impressum

Publisher: Research Institute of Wildlife Ecology (FIWI) of the University of Veterinary Medicine Vienna, Austria /Alpbionet 2030 project

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1st Edition December 2019

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ISBN: 978-3-200-06798-1

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Aknowledgments:
The authors are grateful to the numerous people who have contributed their input and criticism to the manuscript of this handbook, especially our fellow collegues at the the Research Institute of Wildlife Ecology of the University of Veterinary Medicine Vienna, Austria, the Environmental Crime Unit at the Criminal Intelligence Service of the Republic of Austria, and the numerous NGOs that provided their input, namely WWF and Birdlife Austria.

CONTENTS

PREFACE

Contrary to prevailing assumptions, illegal killing of endangered wildlife is not a problem of developing countries alone, it does occur in Europe and in the Alpine Region as well.

The Alpine Region located in the heart of Europe constitutes a refugium for many species of wildlife. However, it is also a partially densely populated area and is heavily used for recreational purposes as well as a main artery in continental traffic, thereby limiting exchange of animal populations due to lack of appropriate corridors.

Simultaneously, due to the manifold usage of the landscape, conflicting interests arise between the various stakeholders, like landowners and –users, and, therefore, illegal killings of some highly endangered species of animals have occurred in the past. Especially large predators like bears, wolves, lynx and birds of prey are at risk. These incidences counteract the many conservation-efforts and -programs by regional authorities and organizations to establish viable populations of these species.

Illegal killings of wildlife are not easy to detect, confirm and address, and the activities to prosecute this type of crimes are often flawed due to the lack of a systematic and comprehensive approach to them.

This handbook was compiled in course of the EU-funded Alpine Space Program called "ALPBIONET2030 - Integrative Alpine wildlife and habitat management for the next generation". It aims at assembling harmonized standard operating procedures for forensic methods in (suspected) wildlife crime cases. It is addressed to all authorities, persons, organisations etc. dealing with wildlife crime cases and spans the whole process from the detection of a dead animal to the prosecution in court.

We hope that our "Handbook on Standard Operating Procedures (SOP) in Forensic Investigations of Suspected Illegal Killing of Wildlife" will help in counter fighting illegal killings in the Alpine Area by presenting the basic facts and information as well as practical recommendations for the complete forensic process and investigations.

Vienna, December 2019 Christoph Beiglböck & Chris Walzer

1 INTRODUCTION

1.1 General

Wildlife crimes occur in many forms:

- Illegal keeping/trading of wildlife
- Killing of wildlife - under unlawful conditions
  - by inappropriate means or methods
- Damaging health of wildlife

Especially the illegal killing of wildlife and trafficking of the products is big business: "With a value estimated up to $23 billion, illegal wildlife trafficking is the fourth most lucrative global crime after drugs, humans and arms" according to the World Economic Forum.

Persecution and prosecution of alleged perpetrators is often unsuccessful or come to nothing because of the many pitfalls that may ensue. These include:

- Varying legislations, standardisations and responsibilities
- Lack of knowledge of legal authorities regarding essential biological aspects
- Prosecutors unaware of the legal aspects of wildlife crimes and their aspects in conservation efforts
- Improper CSI
- Improper handling, storage and transport of samples
- No/inexact necropsy
- Investigators and laboratories not familiar with wildlife crime incidents, the methods used for killing and the respective legal and methodical requirements
- Not initiating ancillary investigation techniques available
- Vague testimony

Many of the pitfalls mentioned above can be met by using standardized methods and procedures as well as by a deeper understanding of the possible problems associated with forensic investigations in suspected wildlife crimes. These investigations can be challenging, but, as with other types of crimes, the most important action is gathering evidence through thorough crime scene investigations and exhaustive and conclusive ancillary examinations. Distinguishing between an illegal killing and any accidental or natural cause of death is the paramount objective in a suspected wildlife crime case!

Our handbook exclusively deals with wildlife found dead under suspicious circumstances, and focuses on (highly) endangered mammals and birds of the alpine area that are known to be under threat of illegal persecution. It will NOT deal with other wildlife crimes such as illegal wildlife trade, forest crimes etc. The "target species" of this handbook are the Eurasian brown bear *(Ursus arctos arctos)*, the Eurasian lynx *(Lynx lynx)* and the Eurasian wolf (*Canis lupus lupus*) regarding mammals, and birds of prey in general. However, the information and recommendations in this handbook can easily be applied to incidents where other species of wild mammals and birds are involved.

For more information on the diagnosis of suspected kills of livestock and other animals by (large) carnivores, the readers should refer to the handbook "A Fieldguide for Investigating Damages Caused by Carnivores" that was compiled in course of the EU-funded "Life Dinalp Bear" project. This guide can be downloaded via this link: https://dinalpbear.eu/a-fieldguide-for-investigating-damages-caused-by-carnivores/

1.2 How to use this handbook

This handbook shall guide all those involved in suspected wildlife crime cases (legal authorities, veterinarians, biologists, stakeholders, NGOs,...) in choosing and interpreting the procedures and methods available via basic information on them, as well as providing "how-to" recommendations as "best-practise" models.

Since this handbook is available online in distinct chapters, all users are invited to compile their own handbook based on their specific needs. Some of the lists and files in the annex are intended to be completed and amended by the users to fulfil their specific commitments to wildlife crime cases and to support them at any level of the forensic and legal approach.

We greatly appreciate any feedback, comments, suggestions and other input by the users of this handbook. Based on these amendments, we will update the handbook on a regular basis, so checking the website occasionally may be worthwhile!

Any comment regarding the handbook should be sent to the following e-mail address:

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2 BASICS IN WILDLIFE FORENSICS

2.1 What is Wildlife Forensics?

The term wildlife "traditionally refers to undomesticated animal species, but has come to include all plants, fungi, and other organisms that grow or live wild in an area without being introduced by humans (Usher, 1986)". Forensics means the application of science to criminal investigations whilst collecting, preserving, analysing and reporting on evidence.
Although the use of science to support human forensic investigations dates back to as long as the 13th century, the application to veterinary forensics and especially wildlife forensic cases is a rather recent achievement.

2.2 Persons/Organisations involved in suspected wildlife crime cases

Many persons and /or organisations may be involved in suspected wildlife crime cases and their primary interests and intentions or tasks may vary.

2.2.1 Law enforcement officers/investigators

In most cases, these will be initiating the investigations at the crime site and filing the cases to be presented to the prosecutors. While having experience in criminal investigations in general, they may lack the biological aspects and background knowledge pertaining wildlife.

2.2.2 Prosecutors

Prosecutors will lead and decide on the steps necessary in pre-trial criminal investigations. By gathering and reviewing all the relevant information, they will decide whether to press a charge or drop the case. Further, they will decide whether the legal authorities will accept the costs for any investigations in a specific case. It may therefore be important to assure the prosecutor of the necessity of ancillary investigations to successfully resolve a crime case.

As with law enforcement officers, they may lack the biological aspects associated with suspected wildlife crimes.

2.2.3 Scientists at laboratories (veterinarians, biologists, toxicologists etc.)

These are the experts in their respective fields during a wildlife crime investigation. Their primary task is to report on their findings during the investigation process. It is of paramount importance that they stay absolutely objective and unbiased when reporting and witnessing on a case.

2.2.4 Veterinary authorities

The national veterinary authorities are government services that are responsible for the surveillance and control of (especially zoonotic) animal diseases, food production safety and animal welfare. However, as far as illegal killing of wildlife is concerned, the authorities may show reluctance to tackle the topic, referring this special issue to hunting and/or nature conservation authorities. Nevertheless, a good working relationship with the respective veterinary officers should be sought and the passing of relevant information in a suspected case of illegal killing should be discussed in advance.

2.2.5 Stakeholders

Various stakeholders may be involved in suspected wildlife crimes, e.g. hunting agencies/state wildlife management agencies, National park authorities and various NGOs with interest in wildlife conservation.

The type of involvement may range from constituting the official authority responsible for wildlife affairs to scientific attention regarding the respective species, often coupled with substantial financial investments in reintroduction- and/or monitoring programs of the affected species.

2.2.6 Media/public

Suspected wildlife crimes often generate high media attention and are perceived highly emotional among the public, and any statements and reports may spread very rapidly in the social media nowadays. This may put an extra pressure on the investigating authorities, and stakeholders may feel obliged to take advantage of the situation for their own interests, e.g. gathering additional donations for their work. However, pre-trial publicity, e.g. public statements like press releases and social media posts from people or organisations involved that only draw on conjectures ahead of the final results of the pending investigation process may seriously hamper the investigation process. As a worst case, this may render some of the investigational work unqualified for admission in court.

Consequently, it is important to have a strict “chain of reporting” of who passes what information when to whom. Ideally, a media representative of the primary investigating- or prosecuting-authority trained in handling press relations should coordinate public affairs and other personnel and organisations involved should defer media inquiries to him/her.

As a best practise, clarification on handling of all media affairs should be achieved by mutual agreement of all persons and organisations concerned in a very early stage of the investigation process in a suspected wildlife crime case.

2.3 Detecting Dead Wildlife

Detecting dead wildlife can be very tricky and depends on many circumstances, e.g. size of animal, area, environmental conditions, time of year and many others. Therefore, it is very often only by chance that dead animals are detected, an exception being GPS collared animals (e.g. in wildlife studies or reintroduction programs) providing position data at fixed intervals.

An experiment by Wobeser & Wobeser (1992) highlighted the difficulties associated with detecting dead animals in the field in an experiment. They placed a total of 250 dead chicks in a one ha area over a 5-day period (50 chicks/day) and 10 % of the area were searched daily for 10 days. Only two intact chicks that had been in place for longer than 24 hours were recovered while only three times a scavenger was observed preying on a chick. This clearly shows that, especially in smaller animals, detection of most cases (regardless of cause of death) is often unlikely. The main reasons being removal of carcass by scavengers, advanced decay and removal of illegally killed wildlife by the perpetrator.

In our experience, the willingness of, e.g., hunters to report suspicious deaths of wildlife increases with mutual reliance of involved authorities and their professional attitude.

If a wildlife crime is suspected but no carcass has been spotted or wildlife crimes happen regularly in a certain region, a number of actions may be proposed to help detecting dead wildlife:

- Raising public awareness (via press releases and social media activities by authorities or NGOs) is useful when wildlife crimes happen on a regular basis in a distinct area.

Locals that use the area for outdoor activities may be of help when asked to watch for any suspicious dead wildlife.

- A passive or active targeted surveillance program may be implemented with members of stakeholders (NGOs, local hunting association) searching defined areas on a regular basis.
- Search dogs are especially useful and several countries (e.g. Spain, Hungary, Czech and Slovak Republic) now have dogs specially trained for detecting poisoned birds of prey. The dogs may be privately owned and trained at the expense of NGOs or dedicated police dogs.

3 CAUSES OF DEATH IN WILDLIFE

Death of wildlife can be the result of many different causes, illegal human activities being only a very small proportion of it fortunately. It is the basic task of wildlife forensic investigations to rule out natural causes of death and possibly confirm illegal killing. Basically, death of wildlife can be attributed to natural and non-natural, human induced, causes. These can be refined into subcategories:

- **Natural**
  - Disease
  - Starvation/decrepitude
  - Predation
  - Intra-/interspecific fights
  - Ingestion of toxic plants
  - Natural Disasters: Avalanches, landslides, flooding, thunderstorms, forest fires, lightning strikes, etc.
  - ….

- **Non-natural**
  - Human induced „legal“ causes
    - Legal hunting
    - Collisions with vehicles, trains, aircraft, windmills, power lines
    - Electrocution
  - Human induced “illegal” causes
    - Illegal Shooting/Trapping
    - Poisoning (incl. secondary Poisoning)

While some of the causes are more or less apparent (e.g. a bird stuck into the fuselage after colliding with an airplane), many others are by far less obvious.

3.1 Natural causes

3.1.1 Disease

Diseases caused by infectious agents as a cause of death in wildlife are far more widespread than most expect. The outbreak of a certain disease and subsequent death may be limited to a single animal or to a few cases in a confined area, but may also develop to an epidemic, affecting animal populations in large regions.

When occurring as epidemics, diseases may play a significant role in the population ecology of the affected species and may have detrimental effects on it. These episodes do occur in Europe and the Alpine Region as well, examples being e.g. scabies (a skin disease caused by mites) in alpine chamois (*Rupicapra rupicapra*), infectious keratoconjunctivitis (an ocular disease caused by bacteria) in alpine chamois and alpine ibex (*Capra ibex*), tuberculosis in red deer (*Cervus elaphus*) in some parts of the Alpine Region, the reoccurring of the viral disease avian influenza in many parts of Europe every now and then, the present outbreak of African swine fever in wild boars (*Sus scrofa*) in parts of Europe, rabies in various carnivores that fortunately was eradicated in central Europe just recently, and others.

Research and knowledge on wildlife disease has dramatically increased in the last decades, partly due to concerns over possible spill-overs to domestic animals (which has occurred) and even humans (so called zoonotic diseases). Experts and dedicated institutions dealing with wildlife diseases are found in many countries nowadays. However, only few countries have long-term surveillance programs on diseases in wildlife and hence knowledge on the occurrence of distinct diseases still remains not comprehensive.

Non-infectious diseases (e.g. cancer, bone fractures) do occur in wildlife as well, but seem to play a minor role as causes of death compared to infectious ones.

Due to possible debilitation and weakness of the diseased animals, some of them (depending on species) will be prayed on by predators before they die of the disease. Nevertheless, chances are high that a wild animal found dead has died of a specific or non-specific disease. As with other causes of death in wildlife, this can only be confirmed or ruled out by a thorough necropsy of the carcass at dedicated institutions and ancillary investigations.

3.1.2 Starvation/decrepitude

Starvation and emaciation of wild animals is commonly encountered under certain circumstances. The reasons encompass unfavourable environmental conditions (e.g. snow cover), species overpopulation, lack of food, (chronic) disease, inability of food intake because of disease or injury amongst others. During necropsy, starvation is easily recognisable by lack of fat tissue.

Decrepitude may be hard to define in wildlife since the ultimate cause of death in this status may be chronic disease or starvation due to inability of food intake.

3.1.3 Predation

Predation by carnivorous animals is a common natural process. Although the "target" species of this handbook (large carnivores, birds of prey) are less likely to be preyed upon, predation should always be considered as a possible cause of death when investigating a carcass.

3.1.4 Intra-/interspecific fights

The reasons for intra- and interspecific fights are manifold, including fighting over territories, partners and food sources. The majority of these fights are non-lethal, but deaths may occur. Signs for fights may be found at the site the animal was found, e.g. disturbed soil or plants nearby, hairs or feathers and bloodstains in the vicinity etc.

3.1.5 Ingestion of toxic plants

Studies have shown that, in herbivorous or omnivorous animals, ingestion of toxic plants and subsequent disease and even death is more widespread than previously thought. Although animals may have some kind of a natural avoidance of toxic plants, this does not rule out their ingestion, tentatively due to impairment of the animals' senses or due to the plant being "unknown", e.g. invasive alien plants.

3.1.6 Natural Disasters

Many types of natural disasters like avalanches, landslides, flooding, thunderstorms, forest fires and lightning strikes may directly or indirectly (e.g. habitat loss) affect and kill wildlife. While some of these disasters may be obvious as causes of death, others may be more inconspicuous. For example, thunderstorms are known to have caused mass mortality events in birds due to the accompanying high winds and disorientation of the animals. Therefore, the recent environmental and weather conditions at the site one or more carcass was found should always be recorded. Further, inspection and documentation of the topographic characteristics of the site should be completed to rule out some of the natural disasters (e.g. avalanches and landslides will not occur in the plains).

3.2 Non-natural causes

3.2.1 Human induced „legal“ causes

3.2.1.1 Legal hunting

Legal hunting of designated game species is common in the Alpine Region both for wildlife management as well as for recreational purposes. Although different hunting systems and hence hunting laws exist in the alpine countries, counties and regions, they all have in common a clear description of which species are huntable at what specific time of year and in which manner. All large carnivores and most(?) raptors are protected year round in the alpine countries, thus shooting them is invariably illicit.

3.2.1.2 Collisions

The rapid growth of transport infrastructure like roads and railroads, negatively affect wildlife both in indirect ways - loss of connectivity, landscape degradation and fragmentation-, as well as directly via increased animal mortality by collisions.

Further, other infrastructure developments like wind farms and power lines that are built in or through wildlife habitats may endanger especially bird populations directly by increasing the threat of collisions, entanglement and electrocution.

3.2.1.2.1 Collisions with vehicles, trains and aircraft

Wildlife-vehicle collisions are a very common, and probably underrated, cause of death in wildlife. For example, the numbers of game-animals killed on Austrian roads are as high as 100.000 individuals per year. Most of these accidents occur on secondary and tertiary roads since most of the highways and motorways are fenced in the Alpine Region, thus making these roads impermeable for most ground dwelling animals. However, bears, lynxes and wolves with their notable abilities to dig and/or jump may nevertheless enter these sorts of roads. Further, there is a risk for raptors (and of course other birds) being hit by vehicles (especially trucks) although this seems to happen only rarely.

Deaths due to collisions with trains do occur, as happened for example in Grisons/Switzerland in 2012 and 2016 when brown bears M13 and M32 collided with local trains, with the latter bear succumbing to its injuries.

Some aspects have to be obeyed in forensic investigations when vehicle collisions are suspected. Not all animals die on the spot and stricken animals may even survive if they received only slight injuries (e.g. in “near-misses”). Especially larger animals involved in vehicle collisions may cover some distance from the road until they succumb to their injuries, e.g. internal bleedings from ruptured organs or blood vessels may last for a while until the animal dies from the subsequent loss of blood. Consequently, victims of vehicle collisions may be found distant from nearby roads.

Although there have been reports of ground-dwelling animals colliding with airplanes at airfields, cases of bird-strike are of much higher significance. While most of the cases with large passenger planes occur during the take-off and landing sequences (i.e. they happen rather close to an airport), military jets practising low-level flights and smaller sporting airplanes may also be involved.
Though rather unlikely to be the cause of death in a raptor (or other birds) found dead, bird strike should at least be considered under special circumstances. For example, Vienna International Airport is located at closest proximity to Donau-Auen National Park harbouring several highly protected raptors.

3.2.1.2.2 Collisions with man-made structures

Ground-dwelling animals may collide with and/or get entangled in (poorly perceivable wire-) fences that may cause significant injuries and even death.

In smaller birds, collision with windows is the most significant anthropogenic cause of mortality while larger birds are especially endangered by collisions with windmills and entanglement in powerlines. The latter may also be the cause of electrocution.

3.2.1.3 <u>Electrocution</u>

Especially larger birds are at risk of electrocution when using power lines and -poles as resting places. Electrocution occurs when the circuit between two different live-phase wires or between a live-phase wire and the ground-wire (or the insulators or poles) is completed by a bird. Normally this happens while spreading the wings during take-off from the resting place thereby completing the circuit with the wings and/or feet. While dry feathers provide some resistance, wet feathers have considerable less resistance, putting birds at higher risk in wet environmental conditions.

3.2.2 Human induced “illegal” causes

Illegal killing of wildlife occurs for many reasons, e.g. because wildlife is seen as a competitor over resources (hunting, agriculture, livestock) or because of intentional animal abuse.

For the most part, killing occurs by means of snaring/trapping, shooting or poisoning.

3.2.2.1 <u>Snaring/Trapping</u>

Snaring or trapping of certain species of (game) animals is legal in some but not all countries. If not set and placed correctly however, protected species may unintentionally get caught. Further, snares and traps may intentionally be used to catch/kill endangered wildlife.

3.2.2.1.1 Snares

Snares are simple devices to catch (small) mammals through anchored cables. They trap an animal around its neck, body or feet. Animals caught in snares alive are at risk of starvation and dehydration or hyper-/hypothermia if trapped for a longer period. They may struggle vigorously leading to progressively tightening of the wire and thereby cutting deep into the tissue with typical lesions that can easily be detected at necropsy. Sometimes, animals caught alive are killed by shooting, thus radiography should always be applied in such cases.

Figure 1: A typical snare to catch certain species of wildlife (Source: Wikimedia Commons).

3.2.2.1.2 Traps

Different types and sizes of traps exist, ranging from cage traps to catch animals alive to body-gripping traps (e.g. conibear traps; spring-traps) that are designed to quickly kill an animal.

Figure 2: Some types of traps (from left to right): Cage trap for trapping animals alive, conibear trap and spring trap, both designed for fatal trapping (Source: Wikimedia Commons).

As with snares, animals caught in cage traps are at risk of starvation and dehydration or hyper-/hypothermia if trapped for a longer period and sometimes the trapped animals are killed by shooting.

Body gripping traps kill the target animals (e.g. foxes, martens) quickly by tightly shutting jaws around the body, especially the neck, of an animal. However, non-target animals are at high risk by this type of traps, even if they are used legally. Especially if placed incorrectly (or intentionally), many species of birds of prey will get lured to the trap by the bait that is normally placed into the trap. When triggering the release mechanism, the jaws of the closing trap may instantaneously kill the bird or it will close around its feet (or sometimes other body parts) leading to injuries that range from rather superficial wounds to severe fractures and even amputation of the leg. The degree of the injuries is highly dependent on type and size of trap.

3.2.2.2 <u>Shooting</u>

In contrast to illegal shooting of wildlife, most shooting incidents in humans (homicide, suicide) involve handguns and the majority of the human forensic literature focusses on handgun shooting characteristics. Therefore, the respective literature should not be simply extrapolated to shooting incidents in wildlife. The interpretation of shooting wounds by the veterinary pathologist requires some knowledge of firearms and the ammunition used in wildlife crimes. This chapter shall present the respective background knowledge in more detail.

3.2.2.2.1 Types of firearms and ammunition

3.2.2.2.1.1 <u>Firearms</u>

There are several types of firearms that are used in wildlife crimes, mostly rifles and shotguns. To a lesser extent, handguns and air- or gas- powered guns may be used, although they produce significantly less energy and hence are deadly only in small animals and at short distances.

Most firearms except shotguns have rifled barrels, meaning that spiral grooves have been cut into the bore of the barrel. The purpose of this is to force the bullet into a longitudinal spin for better stabilisation and accuracy during flight. Rifling typically leaves tool marks on a fired bullet that may be characteristic for an individual weapon. The tool marks may be investigated in a special forensic laboratory and serve as important evidence and thus bullets recovered from a carcass should be handled with great care and kept as evidence (see chapter “Veterinary pathological Investigations”).

3.2.2.2.1.1.1 Handguns

Handguns are small firearms designed to be held and fired single-handed (although using both hands will increase accuracy). They are considered low-velocity weapons since the cartridges of handguns are shorter and hence carry fewer amounts of propellants (gunpowder) producing less energy than rifle ammunition. The muzzle velocity (i.e. the speed at which a bullet leaves the barrel) of handguns typically ranges from 200 m/s to under 500m/s, depending on calibre, barrel-length, and amount and burn rate of the propellant.

Due to the short length of the barrel of handguns and the relatively low velocity and thus energy of the bullets, handguns are much less precise and accurate than rifles. They are used only at short distances in hunting (e.g. finishing shots; self-defence purposes against attacking wild boars). For the same reasons, bullets fired from handguns are more likely to be retained in the carcass of an animal compared to bullets from rifles.

The two main types of handguns are revolvers and pistols.

Revolvers comprise a rotating magazine holding 5 to 10 cartridges. They are either single action or double action types. While in the former the hammer must be cocked manually each time a shot is to be fired (thus causing a rather slow shooting cadence), double-action revolvers fire a shoot each time the trigger is pulled. The cartridges are not ejected automatically after firing in revolvers.

Pistols are mostly semi-automatic firearms, meaning that they automatically extract and eject the cartridge and load the next bullet into the chamber once being fired, thus ensuring a high shooting cadence (a bullet is fired each time the trigger is pulled without the need of manual reloading in between). In pistols, the cartridges are stored in a removable magazine (typically holding between 10 and 20 cartridges) in the handgrip of the weapon.

Fig. 3: The two types of handguns: Revolver (left) and Pistol (right) (Source: Wikimedia Commons).

3.2.2.2.1.1.2 Rifles

Firearms that are designed to fire projectiles from the shoulder are called rifles. They have a rifled barrel and are high velocity weapons with muzzle velocities ranging from app. 600 m/s to more than 1000 m/s. Rifles are much more accurate (for hunting purposes up to 300m; more for military purposes) and more precise than handguns and thus are used for shooting at longer distances. The types of rifles comprise single shot rifles (no installed magazine, they have to be loaded manually after firing), bolt action rifles (loading is done by moving a handle back and forward, thereby extracting the used cartridge and loading a knew one) and semiautomatic rifles (each pull of the trigger automatically ejects, reloads and readies the weapon). Full automatic rifles ("machine guns" that fire as long as the trigger is pulled) are legal only for military purposes in Europe.

Figure 4: A typical hunting rifle. The lower picture depicts the riffling inside the barrel that leaves individual marks on the bullets once they are fired (Source: Wikimedia Commons).

3.2.2.2.1.1.3 Shotguns

Shotguns are firearms designed to fire dispersing shotgun pellets/buckshot or solid slugs at relatively short distances (in hunting typically < 50m) and at low velocities (<400m/s). They have a smooth bore with no rifling and are fired from the shoulder. Normally, they comprise two barrels mounted either side-by-side or stacked that fire consecutively by pulling the trigger twice or by activating two autonomous triggers. Shotguns have no magazine and must be loaded manually after firing. Therefore, two shots can be fired in very short cadence, whilst reloading for additional shots will take some seconds. Shotguns are used either for hunting small animals like birds and rabbits (using shotgun pellets) or, when using slugs, for finishing shots of wild boars at close distances.

Figure 5: A typical shotgun used for hunting purposes with two barrels mounted side-by-side and the respective ammunition (Source: Wikimedia Commons).

3.2.2.2.1.1.4 Air- or gas-powered weapons

These weapons use compressed air or gas (via built-in devices in the rifle) to propel a projectile at low velocity (<300m/s) and thus the ammunition comprises no cartridge containing a propellant but only the projectile itself. They are only seldom used in hunting since lethal wounds can only be inflicted at short distances in small animals. Their main purpose is recreational shooting.

3.2.2.2.1.2 Ammunition

3.2.2.2.1.2.1 Ammunition for handguns and rifles

The ammunition for handguns and rifles share the same basic principles. Each type of ammunition is identified by the calibre (i.e. the diameter of the bullet either in mm or inch) and the length of the cartridge.

They consist of a metal cartridge-casing that contains the propellant (gunpowder), the bullet (projectile) located at the tip of the cartridge and some kind of primer that ignites the propellant once the trigger is pulled. The primer is either located at the centre of the cartridge base ("centrefire weapons") or in a rim at the cartridge base ("rimfire weapons", usually only in small calibre-rifles).

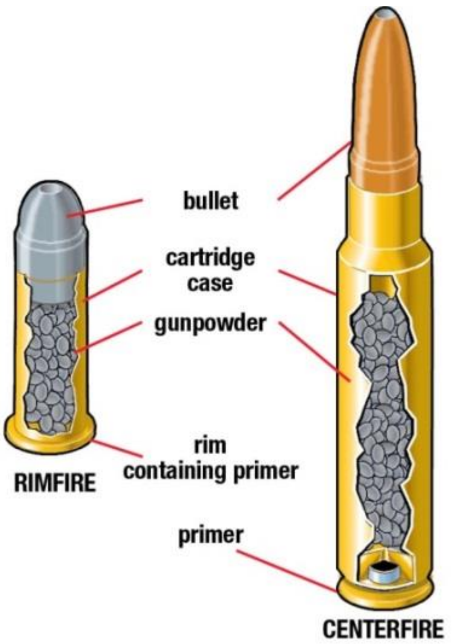

Figure 6: Rimfire ammunition (left) and centrefire ammunition (right) with their main components (Source: Wikimedia Commons).

The cartridge is ejected manually or automatically from the weapon after firing. Both the extraction process as well as the firing pin and other parts of the weapon may leave characteristic marks on the cartridge that may be used to identify the firing weapon. Therefore, searching the suspected crime site for cartridge(s) is paramount!

The bullets, i.e. the part that leaves the nozzle of the weapon, come in many different designs, especially in rifle ammunition for hunting purposes (the design of ammunition for pistols and revolver is less complex).

The aim of the different designs is to attain stable flight, high precision, to transfer as much energy as possible to the different animal species shot at and, as required, to create an exit wound.
Thus, the classification of the different bullet designs is complex. Technically, they used to be classified into solid bullets (made of one material, usually lead or hardened brass) and jacketed bullets (a core made of lead, steel or other metals and a [semi or full] jacket of other metals, mainly copper, zinc, nickel, aluminium or an alloy of any of them).
However, modern day rifle bullets used in hunting often cannot be assigned to a single category mentioned above. The designs encompass bullets made of different metals wielded together in varying shapes, bullets with soft or hollow points etc.

Figure 7: Cross sections of some bullets used for hunting purposes depicting the different designs regarding material, shape and jacketing (Source: Wikimedia Commons).

For the veterinary forensic examiner, classification according to the different behaviour of the bullets in the body is of importance to assess tissue damage:

- Stable shape bullets: These projectiles maintain their general shape in the target and retain their mass. They are mostly solid or fully metal-jacketed projectiles and are mostly used for hunting very large animals because they penetrate deep into the body.

- Deforming bullets: They deform their shape in the target (e.g. “mushrooming”, see later) and loose only a small percentage of their mass, thereby ensuring high energy-transfer to the tissue.

Figure 8: The shape of a deforming bullet before (left) and after hitting a body (right). Note the typical “mushroom shape” that ensures high energy-transfer in the body (Source: Internet).

- Frangible bullets: This design is intended to completely disintegrate in the target, again ensuring high energy-transfer.

Figure 9: Fragments of a disintegrated frangible bullet that is found along the wound path in the carcass (Source and © FIWI)

3.2.2.2.1.2.2 Ammunition for shotguns

3.2.2.2.1.2.2.1 Shotgun shells

These consist of a (mostly) plastic casing and, beginning from the bottom, a primer, propellant, wadding and shot. The shot vary in size, ranging from pellets with a diameter of <2mm (often referred to as "birdshot") to larger pellets (<9 mm, often referred to as "buckshot"). Pellets used to be composed solely of lead, but due to environmental concerns, today are increasingly made of other metals as steel or tungsten.

Labelling of the shotgun ammunition may vary between countries. In Europe, the most common terminology to describe the shells is a non-metric calibre specification, the most common being 12, 16 and 20 (with a respective bore diameter of app. 18,5, 16,8 and 15,7 mm), the length of the shell (in mm) and the size of pellets (in mm).

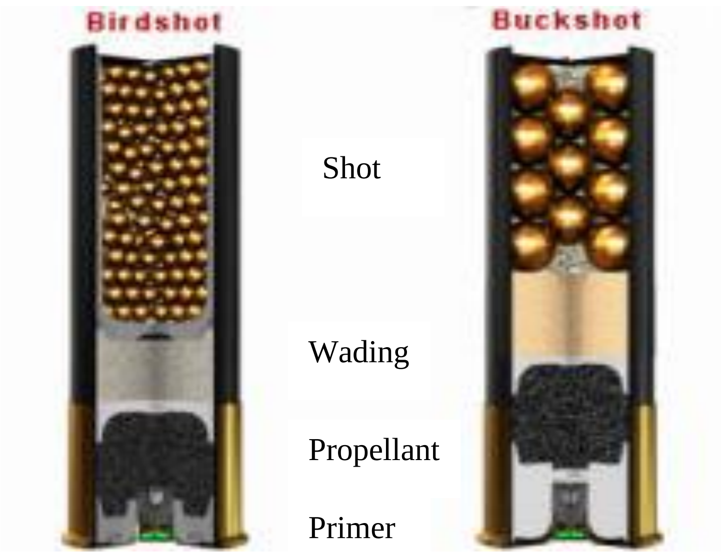

Figure 10: Cross section of two shotgun shells and their main components loaded with small pellets ("birdshot") and, respectively, large pellets ("buckshot") (Source: Wikimedia Commons).

3.2.2.2.1.2.2.2 Slugs

Shotgun shells may also carry slugs, in German often referred to as "Brenneke". These are commonly made of solid lead. They have a weight typically between 20g and 30g and rapidly loose velocity when fired and thus are also used only at short distances (<50m).

Figure 11: Cross section of a shotgun shell containing a slug (at left) (Source: Wikimedia Commons).

3.2.2.2.1.2.3 Ammunition for air- or gas-powered weapons

These come in various shapes, the most common type being the diabolo-style pellet that has a “wasp-waist”. They have only limited wounding capacity due to their small weight and rather low velocity. Nevertheless, when fired at closer distance on a small animal, they may inflict serious wounds.

Figure 12: Various shapes of projectiles for air- or gas-powered weapons (Source: Wikimedia Commons).

As a summary, table one illustrates the various physical characteristics of different types of weapons and the ammunition used.

		Sample Calibre	Main use in hunting	App. mass (g)	App. velocity at muzzle (m/s)	App. energy (J)	Viable shooting distance (m)
Handguns	Pistol	9 mm	Finishing shots/self defence	7	350	490	<10
	Revolver	.38	Finishing shots/self defence	9	220	230	<10
Rifles	"Rimfire"	.222	Very small game (e.g. fox)	6	1000	1500	<100
	"Centrefire" Standard calibre	7 mm	Small game (e.g. roe deer)	11	900	4000	<200
	"Centrefire " Large calibre	9.3 mm	Large game (e.g. wild boar)	16	800	5000	<200
Shotguns	Pellets (in total)	12	Birds/very small game	35	400	2800	<40
	Slugs	12	Large game on short distance (Self-defence)	32	400	2400	<40
Air-/Gas-powered	"diabolo"	5.5 mm	Recreational shooting/very small game	1	170	16	<30

Table 1: Characteristics of various types of weapons with some sample ammunitions.

3.2.2.2.2 Wound ballistics and wounding capacity

3.2.2.2.2.1 *Gunshots*

The wounding capacity of a gunshot projectile is mainly dependant on the energy it transfers to the tissue and its behaviour in the body (called wound ballistics).

Tissue damage occurs through:

- Direct laceration of tissues and complete organs that are directly hit by the bullet and its fragments on their trajectory through the body. Fluid-filled organs like the heart may completely rupture due to the non-compressibility of fluids.

- The cavitation effect.
  Deforming- or frangible-bullets begin to deform or to fragment as soon as they enter the body, thereby transferring much of their energy to the tissue. A pulsating, temporary wound cavity develops along the pathway due to the forces the rapidly decelerating bullet exerts on the surrounding tissue. This compression and contraction process causes damage in tissues that are not in the direct path of the bullet or its fragments.
  Stable shape bullets also cause a temporary wound cavity. However, this type of bullet penetrates deeper into the body before they begin to tumble due to the rapid deceleration. This tumbling process also creates a temporary wound cavity; however, the latter may be absent when a thin part of the body is hit with the bullet travelling straight through.
  When the pulsating of the wound cavity subsides (less than <0.1 sec.), a permanent cavity is formed being much smaller than the temporary one.
  This cavitation effect is especially seen with rifle bullets used for hunting and is far less pronounced in handgun bullets due to their lower energy.

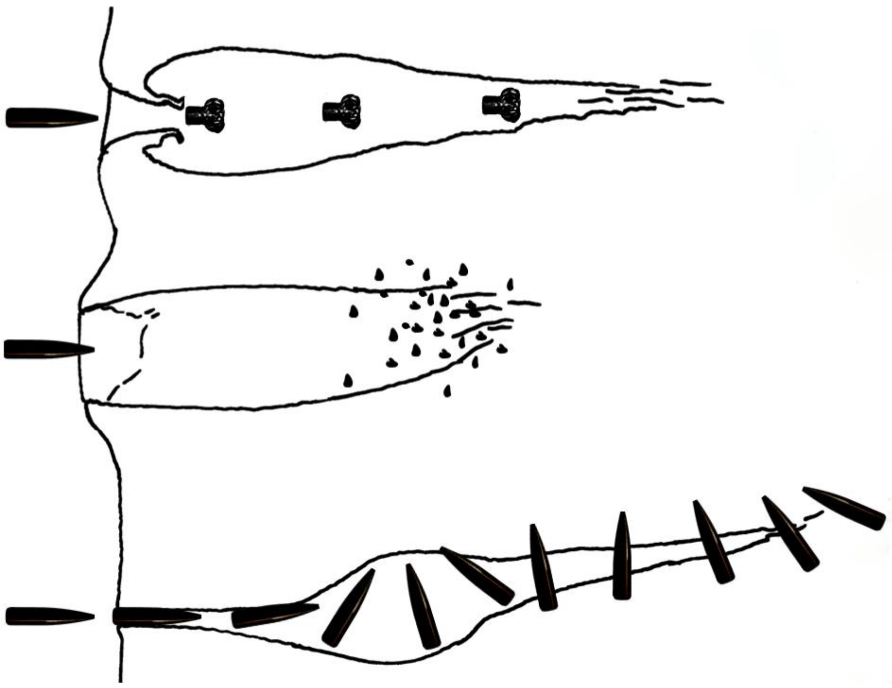

Figure 13: Illustration of the temporary wound cavities formed by different projectiles. From top to bottom: Deforming bullet, frangible bullet, stable shape bullet (drawing after Kneubuehl et al., 2011)

- Shock waves

Shock waves from the impacting bullet will travel through the whole body via blood vessels, nerves and other tissue and may seriously impair body function, even causing a "neuronal shock".

3.2.2.2.2.2 *Shotgun pellets*

The shot charge from shotguns radiates from the muzzle in a cone-like distribution ("Shot pattern"). The wounding capacity of shotgun shots is highly dependent on the firing distance.

At close range (<5 m) it will have destructive effects due to high energy transfer to the tissue, but as the distance gets longer, the pellets not only disperse but loose their energy very quickly. Thus, pellets will not even penetrate a thicker hide at longer ranges (>50m). When entering the body, pellets from a shotgun rapidly loose their energy and only seldom deform or disintegrate (unless they hit a bone), and they produce no temporary wound cavity.

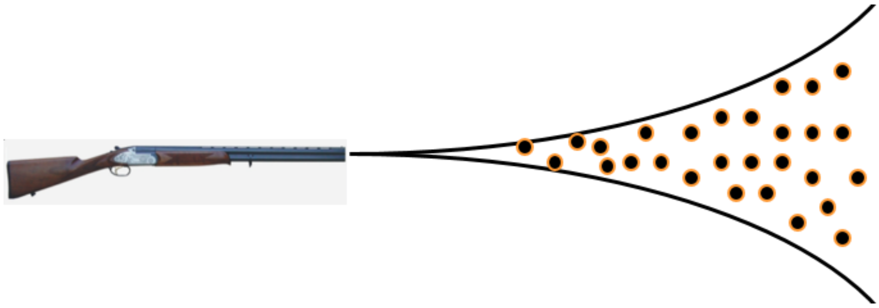

Figure 14: Scheme of a shotgun pattern after firing (Source and © FIWI).

Surprisingly, the exact mechanism of killing is still not fully understood and is mostly referred to as a neuronal shock due to the body being hit by multiple pellets on various locations of the body.

3.2.2.2.2.3 *<u>Shotgun slugs</u>*

As with shotgun pellets, slugs fired at close range from a shotgun have devastating effects due to their shear mass. At farther distances, however, they rapidly decelerate thus loosing energy. Since they are mostly made of lead, they very often deform and disintegrate in the body but produce no temporary wound cavity. When hitting a body with sufficient energy, the wounds will resemble a (massive) blunt trauma.

3.2.2.3 <u>Poisoning</u>

3.2.2.3.1 General

Poisoning of wildlife is still a matter of concern in some parts of Europe and a threat for several species of endangered species, especially raptors. Several national and international projects address this topic, e.g. the EU-funded “PannonEagle LIFE” – project in Hungary, Austria, Czech Republic, Slovakia and Serbia.

Not all poisoning events are intentional or malicious, however. Poisoning may occur, e.g., by the improper use of otherwise legal rodenticides, with raptors dying after feeding on the poisoned rodents (“secondary poisoning”). Another issue of non-intentional, secondary poisoning is the uptake of dead animals or their bowels that were shot with lead-containing ammunition. Lead is a chronic toxic substance that may cause disease and death in predators that feed on these animals or their remains containing lead particles.

Malicious poisoning mostly aims directly at “unwanted” raptors and carnivores by dispersing different types of baits (e.g. carcasses, meat, eggs) prepared with illegal, highly toxic substances like carbofuran. This kind of wildlife crime does not only threaten the targeted animals but also poses a risk for companion animals and humans that come into contact with the prepared baits.

While chronic lead poisoning and other unintentional secondary poisoning events are typically not very conspicuous, the findings at a crime scene of a malicious poisoning incident are often suspicious. These may include (but are not confirmative of intentional poisoning):

- Many victims found on one site or in its vicinity
- Baits (pieces of meat, whole animals, eggs, etc.) near the carcass
- Unusually coloured items/soil
- Dead insects on or near carcass
- Vomitus
- Ante mortem spasms
- Disturbed grass/soil (due to mortal distress of victim)

Care must be taken when processing the crime site and all participants should don their PPE.

Confirmation of poisoning in wildlife can only be achieved by necropsy of the carcass and subsequent toxicological investigation since findings at necropsy are seldom typical for a certain toxic substance. Lesions found at necropsy that may raise suspicion of intentional or unintentional poisoning:

- Poor body condition
- Generalized anaemia
- Ulcerations of gastrointestinal tract
- Abnormal content in gastrointestinal tract (e.g. discoloration)
- Bloody content in gastrointestinal tract
- Non-clotted blood in body cavities without obvious injuries
- Pulmonary edema
- Enlargement of organs
- Rapid onset of rigor mortis
- Birds: Broken feathers on wingtips due to dragging of wings, calluses on skin of legs due to immobility
- Histopathology: Inclusion bodies in cell-nuclei of kidney (indicative of lead intoxication)

3.2.2.3.2 Potential poisons causing poisoning in wildlife

3.2.2.3.2.1 Insecticides

3.2.2.3.2.1.1 Anticholinesterases/Organophosphates/Carbamates e.g.Carbofuran

Carbofuran is a very potent carbamate insecticide that was widely used in agriculture until its ban in the EU in 2008. It is still in use in several countries worldwide, posing severe threats to wildlife and also human health.

Carbofuran is highly toxic especially in birds, and therefore often used for intentional poisoning of raptors and other wildlife. Carbofuran comes in a variety of forms like granules, powders and dust and is often stained with a distinctive purple colour.

Figure 15: Stomach contents of a carbofuran poisoned animal with typical purple discolouration (Source and © FIWI).

The substance is neurotoxic, interacting with enzymes in the nervous system. The toxicity is dependent on many factors, including species, age and sex. Many birds die directly after ingestion of carbofuran with food still in the beak or are rendered flightless exhibiting spasms. Mammals show salivation, lacrimation and muscle tremor. In domestic pets, atropine can be used as an antidote.

3.2.2.3.2.2 Rodenticides

3.2.2.3.2.2.1 Anticoagulants

Oral anticoagulants are widely used rodenticides for pest control means. They come in different forms (grains, pellets, blocks, meat baits etc.) and encompass different compounds. While the first generation of anticoagulants like warfarin needed continuous baiting and many rodent species developed resistance to the substances, today's second generation rodenticides (brodifacoum, bromadiolone, indandione) have a longer half-life and a single baiting is sufficient. Note that these substances are not colour coded and visual identification of a particular anticoagulant therefore not possible.

The underlying mechanism of anticoagulants is their antagonistic property against Vitamin K. As the latter is an important factor in blood clotting, anticoagulant rodenticides lead to internal and external bleeding after three to five days.

During necropsy, haemorrhages from mouth, nose and other openings may be present, as well as sometimes massive generalized internal haemorrhages into body cavities or subcutaneous or muscular haematomas. Most tissues will exhibit mild to severe pallor.

3.2.2.3.2.2.2 Strychnine

Although forbidden for use as a rodenticide in many countries, Strychnine has been used for intentional oral poisoning albeit its bitter taste. Since inhibiting the release of certain neurotransmitters in the spinal cord, ingestion of strychnine rapidly leads to muscle spasms and convulsions, fatal respiratory arrest may ensue.

Usually, no specific lesions are found during necropsy.

3.2.2.3.2.2.3 Zinc/Aluminium phosphide

Known as "Giftweizen" in German speaking countries, phosphides are converted to phosphine gas after oral intake by the acid environment in the stomach. Phosphine gas interferes with cellular functions thereby damaging cells, especially in the heart, liver, kidney and brain.

A garlicky smell may be noted during necropsy that should be performed in a well ventilated room since inhalation of the gas is toxic to humans. Lesions found during necropsy are mostly non- specific and include pulmonary edema and hyperaemia of the gastrointestinal tract. Histological lesions may include interstitial edema, congestion and cellular degeneration.

Tissues for toxicological analysis must be taken as soon as possible and stored in airtight and shatter-proof containers as phosphine gas is very volatile.

3.2.2.3.2.3 *Lead*

Lead is a toxic heavy metal that unfortunately is still widely used in ammunition. Intoxication of wildlife with lead may occur in various ways:

- Non-lethal gunshot residues in the body of an animal that was shot at: May lead to protracted resorption of the lead and chronic lead intoxication depending on the tissue where residues are located.
- Ingestion of spent lead shots or lost lead sinkers for fishing purposes by geese, ducks and swans: Lead particles will be grinded in gizzard and resorbed leading to protracted lead intoxication.
- Ingestion of shot animals or their bowels with lead-ammunition particles in the carcass by raptors or scavengers: High pH value in their stomach will dissolve lead leading to resorption and subsequent intoxication.

Pathological findings in lead intoxication include broken feathers on wingtips due to dragging of wings; calluses on skin of legs due to immobility; anaemia; poor body condition, greenish faeces; diarrhoea and inclusion bodies in cell-nuclei of kidney seen in histopathology.

As with other poisons, however, these findings are not confirmative and subsequent toxicological analyses are essential.

4 BASICS IN WILDLIFE CRIME INVESTIGATIONS

4.1 General Recommendations

As mentioned before, wildlife crime investigations can easily become complex because of the many stakeholders, public authorities and scientific personnel (and their sometimes varying approaches and responsibilities) involved. It is therefore of great importance to establish working relationships with all institutions and authorities that may be involved.

4.1.1 What NOT to do

Just a reminder of some things that must NOT be done:

- "Just pick up" the carcass at the crime site
- No crime scene investigation
- No search of surroundings
- Wearing no PPE during recovery of carcass
- Freezing the carcass
- Improper or inaccurate labelling
- Improper transport and storage
- No necropsy
- No ancillary testing as necessary
- Imprecise or inaccurate reports
- Lack of communication between authorities, laboratories and stakeholders involved
- Dissemination of preliminary conjectures to public and/or press

4.1.2 Choosing a laboratory in wildlife forensic investigations

Seeking and establishing potential collaborations of the investigation unit with respective forensic laboratories (regardless of specialization) should be accomplished well ahead of a forensic investigation.

Any appointed forensic laboratories must meet several criteria. These include experience in forensic analyses, protocols for the respective investigations, packaging and transportation.

The methods used by a laboratory in a forensic investigation must be approved, standardised, scientifically sound and reproducible in order to stand in court. Further, the laboratory should be aware of the scheme of the chain of custody. Last but not least, the approximate costs of the respective analyses should be known in advance and prior approval of coverage of the cost by the lead investigator and/or prosecutor should be obtained.

In any case, the respective laboratory should be contacted in advance of a specific investigation to assure correct packaging and transportation and appropriate sample size needed for proper and diagnostically conclusive analyses.

The “first responding” laboratory must be a distinct veterinary pathology laboratory that also should have the possibility for radiography.

Further details on the respective laboratories and how to find them are given in the section “Laboratory Investigations in Suspected Wildlife Crime Cases”.

4.2 Personal Protective Equipment (PPE)

When handling a carcass or a piece of bait, personal protective equipment must be worn all time since a zoonotic disease (one that may spill over from animals to humans) cannot be ruled out! Further, during the process of decomposition, toxin-producing bacteria may be involved that pose risks for the handler of the carcass. Further still, some of the substances that are used for poisoning of wildlife are extremely toxic to humans as well.

While the technicians in laboratories will be trained in the handling of potentially hazardous material and the usage of the respective PPE, other personnel involved in wildlife crime cases (e.g. police officers at crime site) may be unaware of it.

The minimum equipment that should be donned during handling and recovery of the carcass include:

- Disposable latex gloves (in some cases double gloving to prevent cross contamination of samples)
- (Disposable) coverall
- Respirator mask, especially when working in a dusty environment to prevent inhalation of potentially contaminated dust

Figure 16: Disposable latex gloves, (disposable) coveralls and respirator mask are essential parts of the PPE when working with dead animals (Source: Internet)

Further, rubber-boots (or coverboots), an impermeable (rubber) apron and protective glasses (or shield) may be donned, especially when handling larger carcasses.

All non-disposable PPE equipment must be thoroughly cleaned and disinfected carefully after its use.

All disposable PPE must be put in a tight sealing bag after its use and disposed of properly at an appropriate waste collection facility.

5 CRIME SCENE INVESTIGATIONS (CSI) IN SUSPECTED WILDLIFE CRIME CASES

A thorough and exhaustive crime scene investigation (CSI) is of crucial importance in any case of suspected illegal killing of wildlife. Failure to do so may result in information and evidence being lost or overlooked and may hamper not only the investigation itself but also the outcome in court.

In general, the crime scene investigation in a suspected wildlife crime must follow the basic principles and rules as conducted in other types of crimes in context of securing, preserving, searching and documenting a crime site. Many methods and procedures applied when processing crimes involving humans can be utilized. Wildlife crimes, however, may pose a certain challenge to the investigators involved, and the tasks necessary at the site may not be as obvious as in other cases.

This chapter is not intended to teach already trained police investigators but shall provide information on the standards and procedures for those unfamiliar with CSI and point out some of the distinct characteristics of wildlife crime investigations.

5.1 Responding to a suspected wildlife crime scene

Prior to any investigation, the crime scene has to be secured by the first responder in an effective manner to prevent any destruction, contamination or otherwise disturbance of the initial scene. Onlookers, media and even responders not familiar with the, sometimes-distinct, requirements necessary to process a wildlife crime site should be prohibited from entering the scene using barrier tapes, emergency vehicles or uniformed police officers. It is important that the size of the “protected” crime scene is chosen to an extent that information and evidence in the vicinity of the scene is not destroyed or overlooked (e.g. cartridges in an illegal shooting, tyre tacks etc.). When responding to a suspected wildlife crime site, especially with multiple deaths, always bear in mind that you may be dealing with the outbreak of a notifiable disease (e.g. avian influenza) and additional legal requirements (e.g. veterinary and sanitary laws) have to be complied with. In any suspicious case, the veterinary state officer has to be notified.

All crime sites involving dead animals may pose a hazard to the health of the investigators involved, thus each team-member processing the crime scene must don PPE before entering the scene (for more information see the respective chapter). Under certain circumstances, changing of PPE (or double-gloving for example) during the investigation process will further reduce the risk of contamination/cross-contamination.

As mentioned before, crimes involving wildlife may be unfamiliar terrain for criminal investigators (e.g. the species of animal involved; especially in raptors, correct identification of the species may be difficult). Understanding animal systematics and biology, animal behaviour, animal diseases and knowledge of, e.g., local hunting regulations will aid in the investigations. Therefore, advice should be sought from specialists that preferably are called to the scene and help in processing the crime site. Once again, it is immensely helpful to establish contacts and good working relationships with dedicated specialists ahead of the occurrence of a wildlife crime. Experts that may be contacted to assist in crime scene processing include:

- Dedicated forensic veterinarians/pathologists
- Official veterinary officers
- Veterinarians/biologists with some expertise in wildlife crime investigations
- Specially trained police officers (in Austria e.g. “UKO - Umweltkundige Organe”)
- Staff members from NGOs (hunting agencies, wildlife conservation agencies etc.)

After forming the investigation team, a strict methodical working plan (including searching for, documenting, collecting and preserving potential information and evidence) should be established that clearly defines the tasks for each team member. Care should be given to the schedule of the tasks involved, as certain conditions, e.g. adverse weather that may destroy evidence like blood, warrant adaption of a routine sequence of actions.

5.2 Documenting a suspected wildlife crime scene

As soon as getting notified of a potential wildlife crime, documentation of the circumstances and the subsequent crime scene is paramount. Documentation must include:

- Written notes/oral recording on mobiles
- Photograph/videography records
- (Graphic sketches)

5.2.1 Notes

Written notes may be taken in various manners, starting from free-hand notes that are later incorporated in a concise record, to filing a pre-printed crime scene investigation worksheet. Under all circumstances, the basic information must include:

- Agency in charge of investigations
- Individual case number
- Leading officer of investigations with contact details
- Date and time notified
- Name and contact information of person making notification
- Address and location (preferably GPS coordinates) of crime scene
- Nearby structures that may be of importance (wind mills, power lines, roads, etc.)
- Weather conditions (temperature, precipitation etc.)
- Date and starting time of CSI
- List of experts plus contact information aiding in crime scene processing
- List of evidence and samples collected with information on their subsequent storage, transportation and recipient
- Completion time of CSI
- List of individuals interviewed plus contact information

5.2.2 Photography/Videography

The use of photography, and sometimes videography, is of great value for subsequent investigators (e.g. veterinary pathologists) dealing with the respective crimes, especially if they were not involved in or present at the primary CSI.

The use of DSLR-cameras or compact cameras with high resolution is much preferable to the use of smartphone cameras. Spare batteries and memory cards, a flash unit, a tripod as well as photo cards, rulers/scales and evidence placards should always be part of the basic equipment when documenting the crime scene. Pay attention to have date and time stamp correctly set in the menu of the camera in use!

For long-term storage, the photos must be transferred to a computer or an external HDD/SSD. Copies of the images may be processed to enhance their quality, however, the original image-files with the original names must be retained and no original image must be deleted.

The sequence of images taken should always be overview => mid-range => close up.

Overview images should provide information on the scene and its surroundings, the location of it and the relationship to other locations and structures (panoramic view). Including significant landmarks such as distinct buildings, natural structures such as distinct trees or other will greatly aid subsequent investigators in getting an overall picture of the scene and the place and surroundings a suspected wildlife crime has occurred.

Mid-range images will provide a closer look at the items of interest while still giving information on their spatial relationship to the crime site.

Close-up images shall be taken from any object of interest, preferably both with evidence placards and measuring devices (ruler, tape). To reduce distortion, take images at a 90° angle. Especially, all suspicious alterations of the carcass, e.g. discoloration, blood etc. must be documented.

For videography, the same principles as mentioned above apply. Using a tripod will greatly enhance the quality of the recordings. Audio should be turned off to prevent any unwanted or unnecessary communication to be recorded.

5.3 Searching a wildlife crime scene

A thorough and systematic search in an established pattern (clockwise/anticlockwise; grid pattern; spiral pattern) must be conducted at the crime site to recover and collect any possible item that may be of interest for the investigations. Possible evidence to be looked for includes:

- Other carcasses in the vicinity
- Bones/Hairs/Feathers
- Baits
- Vomit
- Faeces that may be indicative of longer immobility
- Spent ammunition/cartridges
- Cigarette butts and other items near the carcass with possible genetic evidence of the possible perpetrator
- Tyre tracks/foot imprints
- Signs of animal-fighting at the site (e.g. excessive amount of hairs/feathers at site, disturbed/discoloured soil)

Especially in suspected wildlife poisoning cases, the search must cover a relatively large area, since in our experience, multiple poisonings are common, e.g. if numerous individuals feed on a poisoned bait. A number of these animals may initially survive poison uptake and capable of moving away from the baiting site but subsequently succumb to the poison in the near vicinity. It is therefore recommended that a search radius of at least 250m around the initial crime site of a suspected poisoning event is undertaken. Further, in cases of suspected illegal shootings, an extensive search as far as the possible shooting distance may reveal evidence such as spent ammunition cartridges that may yield important evidence such as distinctive marks that later may be assigned to a distinct weapon by forensic laboratories.

5.4 Collection of evidence at a suspected wildlife crime site

Again, it cannot be overemphasized that, when collecting biological evidence, all handlers must don their PPE (personal protective equipment) to avoid any health hazards that might ensue in a suspected wildlife crime, e.g. poisons and pathogens (especially zoonotic agents). Further, all items must be seized in a manner that prevents destruction, degradation or cross-contamination. Regarding the latter, multiple changing of the gloves is therefore recommended, for example in between handling different carcasses at a multiple death crime site.

If the collecting team is in doubt about the appropriate method for sampling, they should consult with the respective laboratory.

Before collecting any material, an appropriate technique to collect fingerprints and DNA samples the possible perpetrator might have left (e.g. swabs) must be applied.

5.4.1 Labelling

To prevent any confusion during transport and the subsequent investigations, correct labelling the items that are retrieved at the crime site is of crucial importance. All packages must be sealed (e.g. adhesive tape) and it is recommended that the collector write initials, date and time over the seal. In order to identify the contents, a unique identifier, for example case number plus a sequential number, must be inscribed at the packaging at a prominent place with a permanent marker. Avoid, e.g., placing the identifier solely on the lid of a container since once the lid is opened, the contents of the container is not identifiable. It is recommended to photograph the final package with seal and labelling visible.

If the package has to be reopened, avoid, whenever possible, destroying the original seal and open the package from another location. Upon resealing, the seal should be labelled in the same manner as mentioned above.

After finishing collection and labelling of the recovered evidence, a complete list of evidence must be made. This list must include unique identifiers, brief description of the item, date and time of collection and the collectors ID.

5.4.2 Packaging, transportation and storage

All evidence has to be transported and stored appropriately in terms of safety, continuity and standard operating procedures of crime investigations. While any non-biological item like spent ammunition cartridges normally require no special procedures and considerations, carcasses and other biological samples from a wildlife crime must be given special attention to avoid degradation or even destruction of them during transport and storage and to minimize any possible hazard to the environment.

Biological samples from a wildlife crime site normally comprise the complete animal-carcasses and sometimes samples of bait. All further sampling for ancillary investigations is ideally done at the "first-responding" pathological lab with the staff aware of the individual requirements of sample-processing, -storage and -transport for the investigation in question.

Carcasses/baits must be packaged in impermeable and tight sealing bags (e.g. body bags; see below) to prevent any contamination and exposure of any personnel during storage and transport. It may be useful to cover the head of the animal with a plastic bag that is fixed around the neck to prevent any contamination of the rest of the body with fluids/ingesta leaking from the mouth and nose during transport.

Bones/teeth may be placed in cardboard boxes if completely dry. The items should be secured against movement during transport and the package must of course be sealed.

Hairs/feathers should be collected with tweezers and placed in paper envelops that are subsequently sealed.

If suspected toxins are found at the crime site, it is best to consult an appropriate laboratory for mode of collection. Normally, tight sealing plastic containers will be appropriate. The same does apply to soil samples.

Insects for entomological investigations may be collected directly at the crime site. However, an investigator with knowledge of this type of investigation should be tasked.

Preferably, transporting the carcass to the pathological laboratory is done immediately after the crime scene investigation has finished. To avoid any contamination of the environment and to minimize degradation, the carcass should be packaged as follows:

- Original tight sealed (body)-bag with unambiguous ID labelling (Source: Internet)

- Rigid and tight sealing outer container (metal preferable to hard plastic) with Biohazard Warning Sign according to UN 3373 and "Forensic Evidence" sign as well as emergency contact details of receiving laboratory/person/agency (Source and © FIWI).

- Absorbing material (e.g. absorbent cotton) between outer container and body bag to prevent any spillage if bag is leaking, and to prevent movement of bag in outer container.

- Cool packs/other cooling devices placed in container as necessary.

Whenever possible, transport should be done by the authorities. In cases where this is not feasible, e.g. large carcasses, courier services that have both the capabilities and procedures to adhere to the required criminal investigation protocol/procedures and the handling of biological samples may be commissioned with the transport. Private persons (e.g. members of NGOs) should never be assigned to transporting or storing any evidence since this may lead to concerns regarding the chain of custody (see below) and hence the admissibility of the respective item in court.

When immediate transportation to the lab is not possible (e.g. out of business hours of the lab), the CARCASS has to be stored cooled/refrigerated but NEVER FROZEN since this may lead to some ancillary investigations being unfeasible. Finding an adequate facility for storage of an animal-carcass in a criminal investigation may be challenging, though. Possible facilities may include cooling facilities for hunted game that are maintained by local hunters or cooling facilities at local hospitals or veterinary clinics. To complicate things, evidence should preferably be stored in a locked area with limited personnel access. However, this may be accomplished by using metal lockboxes to store the carcass in a non-restricted area under acceptable conditions.

Recommended storage conditions for samples for ancillary testings are described in the respective chapter in the section “Laboratory Investigations in Suspected Wildlife Crime Cases”.

As with other issues in wildlife crime investigations, it is advisable to check for storage options in advance.

5.5 Chain of custody

This term refers to the continuous documentation of the custody, transport, transfer, analysis and final deposition of any item of evidence. Failure to adhere to this process may lead to evidence not being accepted in court.

Each item of evidence should have its own chain of custody form that is sent along during transfer of the item. The form should include the following information:

- Description of item
- Date/Time/Names/Signature of individual and agency transferring and receiving item
- Purpose for transfer
- Analysis performed
- Any alteration of the item

A sample of a chain of custody form is found in the annex.

5.6 People to be interviewed in suspected wildlife crime cases

Wildlife crime is mostly a phenomenon of rural settings with the local community often being more aware of things happening in their surroundings than in urban centres. Thus, to maximise the investigation efforts in suspected wildlife crime cases, interviews of various persons and stakeholders can be extremely useful. Persons that should be interviewed include:

- Local hunters/local wildlife authorities: Often have a profound knowledge of the location and the local wildlife
  Ask for mounted wildlife cameras in the vicinity – they may yield useful material!
- Landowner of crime site
- Residents close to crime site
- Locals regularly using area for recreational purposes (running, cycling, dog-walking)

6 LABORATORY INVESTIGATIONS IN SUSPECTED WILDLIFE CRIME CASES

6.1 Veterinary pathological investigation

6.1.1 General

Veterinary pathology investigations are the pivotal part in all circumstances that involve conspicuous dead wildlife. The pathologist may determine not only the cause of death, including manner (e.g. blunt trauma) and reason (e.g. vehicle collision), but also define any cause of ill health and the underlying pathology. Further, forensic pathologists are experts able to assess and evaluate the necessity of ancillary investigations (e.g. toxicological, microbiological etc.) as a result of the pathological findings. Therefore, a full and thorough pathologic investigation at a designated laboratory is of paramount importance in suspected illegal killings! Remember that carcasses are more than dead bodies; they are packages full of information and future prospects!

The pathological investigation consists both of the macroscopic examination of the carcass and the tissues (= necropsy), and the microscopic examination on the cellular level of all relevant organs/tissues (=histological investigation):

External examination

- Scanning for and documenting any individual marking of the carcass (collar, microchips, rings in birds)
- Body condition, skin, pelage/plumage, body orifices, signs of injuries/predation

Internal examination of organs and body cavities

- Inspection of internal body cavities
- Dissection and assessment of inner organs: Size, weight, shape, colour, texture…

Sampling for additional investigations

Histological Examination

- Rule out/confirm disease

- Ageing of tissue damage (e.g. wounds) and inflammations (acute, subacute, chronic, etc.)

- Exact analysis of swellings (tumour, old trauma, etc.)

- Specific histopathological findings (e.g. lead intoxication)

6.1.2 Some considerations for on-site necropsies

In certain situations, an on-site necropsy may be deemed necessary. These circumstances include:

- Size of the animal
- Terrain making transport of carcass difficult
- Environmental conditions
- Time of year
- State of the carcass
- Available personnel

However, every effort should be made to transport the carcass to a pathological facility that act as the first responding lab. On-site necropsies can be very challenging and chances are high that important evidence is lost, e.g. due to lack of radiography in the field, contamination of samples due to unhygienic environment, lack of proper sampling tools/containers and many more.

6.1.3 Special questions during necropsy in wildlife forensic cases

6.1.3.1 <u>Species determination</u>

While the determination of distinct species is straightforward in (large) mammals like wolf, bear and lynx, the correct species-identification of birds of prey by morphological characteristics (size, coloration of plumage etc.) can be challenging. This is especially true for young individuals aged three years or less in certain species, e.g. harriers. Although there are many field guides available for easy identification of birds and other animals, the examiner may refer to biologists, especially ornithologists, if in doubt. If no working relation has yet been established with these experts, any museum of natural history or the biology department of a university may assist in finding one.

If no correct species determination is possible by morphological characteristics, e.g. the carcass being highly decomposed, a genetic investigation of any tissue of the carcass will provide the respective information. For sampling details and further information on genetic investigations, see the particular chapter in this book.

6.1.3.2 Age Determination

In a few cases, the correct determination of the age of an individual victim may be straightforward if the individual "is known". This may be the case if the individual was released in course of a reintroduction program or if it had been captured and marked (radio collar, microchip, ringing in birds etc.) during a scientific study. Normally, the age of the individual is determined in course of these projects/studies and thus contacting the respective project leaders is helpful.

In all other cases, age determination can be complex and advice from (zoological) specialists may be sought. As with species identification, various morphological characteristics are used depending on species: Size and weight of the individual; coloration of pelage/plumage; ossification of various bones and bone sutures (e.g. skull); ossification of various chondral tissues; dimension of bony structures; dentition, eruption and wear of teeth (in mammals).

In large carnivores, the dental characteristics, especially of the incisors, show consistent alterations with age and thus allow assigning an individual at least to distinct age categories, in lynx e.g. < 1 year, 1–2 years, 3–6 years, 7–9 years, 10–13 years, ≥ 14 years.

Very exact results of age determination in wolves, bear and lynx may be obtained by microscopic analysis of the so-called cementum annuli rings of the incisors, canines or premolar teeth (annually formed rings in the cementum layer of these teeth), but this technique is performed only by few specialised laboratories.

With the exception of some species of birds of prey (e.g. harriers), whose plumage show distinct coloration up to an age of approximately three years, age determination in birds is often restricted to simply classifying them as juvenile or adult since no distinct morphological characteristics allow for a more exact age classification. Remember that genetic analysis will not aid in age determination.

6.1.3.3 Determination of Post-mortem Interval

Right after death, many simultaneous chemical and physical processes commence in a carcass, leading to decomposition and, in most cases if undisturbed, final skeletisation. Decomposition comprises the autolysis process (decay of carcass by own body enzymes) and, sometimes, the putrification process (decay by bacteria). Under certain circumstances (dry climate with low humidity) mummification, literally the drying of the carcass, may occur.

Based on the sequence of processes, the pathologist may be able to determine at least a rough estimate of the time elapsed since the death of the individual during necropsy. Features that occur after death include:

- livor mortis: settling of blood in a carcass in a gravity dependant pattern
- rigor mortis: rigidity and subsequent relaxation of muscles after death
- algor mortis: cooling of body after death
- discoloration: (greenish) colour due to degradation of haemoglobin
- bloating: abdominal swelling due to (bacterial) gas discharge, mainly in herbivores
- sloughing of skin/hairs
- later stages of decay

It must be noted however, that all these processes are highly variable depending on many intrinsic and extrinsic variables, including species and size of animal, ante-mortem activity, cause of death (possible diseases, poisons leading to convulsions, e.g. carbofuran), environmental conditions (especially ambient temperature and -moisture, exposure to sun and rain) and many others. In large mammals, e.g. a bear in winter fur and with large fat depots, cooling is considerably slower than in emaciated birds. In human forensic sciences, post-mortem cooling models (e.g. Henßge’s nomogram) have been established for the first few hours after death, but there is only minimal data on distinct species of animals and human data cannot be simply extrapolated to individual animal species. Further, scavenger activity after death and mummification can be misinterpreted as stages of decomposition. Thus, determination of post-mortem interval based solely on the characteristics mentioned above is scientifically not well established and no practical field application is available. Caution is needed when interpreting the findings! Nevertheless, careful assessment and documentation of the post-mortem changes seen in a carcass must be performed by the pathologist.

It has recently been found that the intraocular pressure may be a good indicator for short (< 12 hours) post-mortem intervals and this method may hold future potential if validated in various species of animals. Other methods, e.g. variations in various body fluids characteristics, have not proven successfully in humans, let alone animal species.

Carcasses in wildlife forensic cases are often found at later stages of the post-mortem period, rendering the above-mentioned methods (lividity, cooling, rigidity etc.) useless. In such cases, entomological investigations of the insects and different stages of larvae that colonize a carcass after death may provide fruitful information on the minimum time (i.e. larvae need a certain time to develop even under "best" conditions) elapsed since death.

For more information on this technique, the reader may refer to the chapter on entomological investigations in this book.

6.1.4 Special findings in pathological investigations

6.1.4.1 Pathological findings due to Electrocutions

Electrocution is almost exclusively found in birds shortcutting the current while perching on and, especially, while lifting off from electrical wires. Electrical fences used to fence off a pasture, e.g., will not inflict any damage to ground dwelling animals due to their low intensity of current.

Electrical current usually travels the shortest route through the body from the contact point to the point of exit. In the body, the current flows along the least resistant tissues, especially nerves, blood vessels and wet tissues. Death usually occurs due to acute heart fibrillation and cardiopulmonary arrest or brain damage.

Pathological findings may range from extreme thermal burns to very subtle, almost non-detectable local marks, especially when the tips of feathers were the sole contact points.

Traumatic amputation of wings, legs or digits may occur through severe muscular contractions due to the current agitating the nervous system.

During necropsy, carefully inspect the wings and wrists for charred feathers. However, these may be difficult to distinguish from dirt and the use of a dissecting microscope is recommended. Further, charred feathers and skin may be detected by using an alternate light source at 530 to 570nm under a red filter. Inspect the feet for any discolorations. Further, check the complete skin of the carcass for entrance and exit holes of the current. These holes may exhibit only subtle charring.

Internal damage may comprise severe thermal injuries when the current entered the body for a prolonged period (e.g. when the bird was entangled after death). Muscles may exhibit a cooked appearance and discoloration and ruptures of viscera may occur. However, in many cases, internal damage is almost non-existent when the electrocution was very quick.

Histopathology may show coagulation necrosis and intraepidermal separation in the skin.

Figure 19: Extensively charred feathers in an electrocution victim (Source and © Ildiko Szabo/Beaty Biodiversity Museum)

6.1.4.2 <u>Pathological findings due to collisions with vehicles or other man-made structures (windmills, power lines etc.)</u>

Remember that an animal may not die instantly at the site of a vehicle collisions and hence is not necessarily found on or close to a road. Thus, finding an animal in a field or forest does not rule out a vehicle collision!

Many kinds of mammals and birds may collide with vehicles or trains. In small species, a frontal collision will result in multiple and severe, mostly blunt force, traumatic lesions, including bone-fractures, luxations, laceration and ruptures of inner organs and tissues. Multiple bleeding in various tissues will be prominent. Injuries to body areas that are remote from the direct impact area may be present due to projection of the animal, coup-contrecoupe effects due to the rapid acceleration/deceleration process in collisions etc.. Multiple abrasions of the skin and dirt embedded in the fur are often found in collision-victims.

Large collision-victims with massive muscles and/or thick layers of fat like bears may show only subtle pathological changes like bleedings in the hide and musculature when hit not frontally but in a peripheral region. External examination may reveal no obvious wounds, but check the exterior for vehicle paint chips. Nevertheless, because of the forces involved in collisions, death can occur due to rupture of inner organs and subsequent internal bleeding.

Flying birds may also be killed by larger or fast travelling vehicles by the downdraft created by them, i.e. no direct hit.

In windmill victims (birds), lacerations of the lungs may be a prominent feature.

6.1.4.3 <u>Pathological findings in snaring/trapping victims</u>

Remember that the traps and snares removed from a carcass are forensic evidence and may yield fingerprints and DNA of the perpetrator. Careful handling with gloves and appropriate evidence management should thus be ensured.

As a general rule, animals found dead in traps and snares should always be radiographed since sometimes the victims were entangled alive and killed by shooting.

The intensity of injuries due to illegal snaring/trapping largely depends on the species of animals involved and the devices used.

In smaller animals, jaw traps will have devastating effects with complete crushing and tear-off of feet, neck, wings and other body acra. In large animals, roundish, superficial to deep and sharp to blunt force traumata around the limbs are very common, as well as fractures of the extremities or the parts of the body where the jaws of the trap locked.

Injuries due to entanglement in (wire-) snares vary depending on the type of ligature, body location, length and amount of constriction etc. If skin and blood vessels are constricted, tissue swelling and edema with subsequent necrosis of the body parts distal from the ligature are found, mostly with a sharp margin to the healthy tissue. Subcutaneous haemorrhage, lacerations, abrasions and hair loss may be present due to struggling of the victim. Histologic samples of the wound and culture samples of the affected areas may give time estimates for the time the ligature was in place, based on infection and the evaluation of the granulation tissue.

If the animals are found in or near the trap, it is usually easy to attribute the lesions found at necropsy to the respective device. However, if the animal was killed by or in a trap and subsequently disposed of (i.e. no history of trapping exists), it may be complex to confirm illegal trapping.

Animals that were caught alive in cage traps may die of starvation/dehydration or hypo-/hyperthermia with the respective lesions found at necropsy, albeit giving no hint on illegal trapping.

6.1.4.4 Pathological findings in firearm victims

6.1.4.4.1 Handling of suspected firearm victims

Handling of the carcass should be reduced to a minimum both at the crime site and during external examination to avoid the loss of any evidence, e.g. a bullet fragment that is located near a wound falling out. After removal from the body bag used for transportation, the bag should be examined closely for the presence of any evidence that has exited the body during transportation.

6.1.4.4.2 Necropsy of suspected firearm victims

As a rule, all suspected firearm-victims must be radiographed BEFORE necropsy! (see respective chapter).

The usage of rapid lead test kits to detect lead residues of bullets in tissues is possible; however, due to the availability of lead-free hunting-ammunition for many types of weapons today, caution should be exerted when interpreting the results of these test kits, especially when they are negative.

Finding bullet parts or shots in a carcass does not necessarily mean that this relates to the death of the animal! Animals may suffer non-fatal shooting injuries (especially pellets) that do not interfere with any vital function. Especially when shotgun pellets are found in the hide or muscles, it is of paramount importance to determine if these are freshly inflicted wounds or if they stem from an older shooting event, or even if the animal was shot at after death. Look for any signs of bleeding, wound healing processes like the formation of fibrous tissue etc.! Further, despite various attempts by forensic experiments, determination of the exact shooting range is almost impossible. Especially with shotguns, due to the dispersal of the pellets after firing, the pattern, the amount and hence the density of the pellets in a carcass have been used to derive the probable shooting distance. However, since almost all shotguns comprise two barrels (that are normally both loaded during hunting), it cannot be excluded that an animal has been shot at twice or even more times in a single shooting incident. Thus, the pattern of the pellets in the carcass is no practical use when determining the shooting distance!

When shooting is suspected in wildlife crime cases, it can generally be assumed that the victims were shot at larger distances. Thus, evidence like soot marks (also called stippling or staining, i.e. stains left on the hair coat/skin from the ignition process of a bullet when fired at very close range) or searing of the skin or hairs/feathers from the superheated gases that leave the muzzle, will normally be absent.

6.1.4.4.2.1 <u>Gunshot wounds</u>

In gunshot victims, the determination of entrance and exit wounds, and consequently the trajectory the bullet has travelled through the body, is of highest importance. This enables re-creating the crime scene, e.g. the relative position of the victim and the shooter to each other. This is crucial under several circumstances, e.g. a suspected shooter claiming he had shot in self-defence.

Typically, the shape of gunshot wounds will remain relatively stable during decomposition.

The entrance wound of a gunshot is more or less round with a reddish to dark (depending on age of the wound) abrasion ring and sharp margins. The diameter is approximately the size of the bullet; however, it is not possible to determine the exact calibre by measuring the wound-diameter since both the flexibility and tension of the skin may have an influence on the shape. Blood and tissue particles may be found around the entrance wound that either have been expelled during the process of the formation and collapse of the temporary wound cavity or they may have leaked from the wound after death. In thick furred animals, the entrance wound may be difficult to find and a complete skinning of the carcass may be necessary.

The entrance wound may be more stellar when the skin over a superficial bone is hit with subsequent rupture of the bone. Further, a ricocheting bullet (i.e. a tumbling bullet that e.g. had hit a branch before entering the corpse) will produce an entrance wound with irregular appearance.

Figure20: Entrance gunshot wound in a red deer with some blood and small amount of tissue expelled due to pressure (Source and © FIWI).

Exit wound are typically much larger and irregular in diameter and shape and have jagged margins. Tissue fragments (e.g. bone) are usually found in the wound.

Figure 21: Exit gunshot wound in a young red deer with various tissues expelled (Source and © FIWI).

In rare cases, when the whole bullet (or its fragments) is retained in the body, an exit wound can be absent. This may happen when low energy firearms like handguns, rimfire rifles and gas-powered rifles have been used. Furthermore, slugs fired from shotguns will most likely be retained in the corpse of larger animals due to their low energy.

By determining the entrance and exit wounds, it is possible to reconstruct the trajectory of the projectile through the body. This is of importance since it may give hints on the orientation of the shooter and the animal, the distance of the shooter, and the ammunition used.

The easiest way to visualize the trajectory is to place a rod (preferably made of wood or plastic; not metallic) through the entrance and exit wounds. This should be done with care in order not to cause any artificial canal or to manipulate the canal. Therefore, placing a rod in the canal should be done after thorough exploration and documentation of the wound canal.

Once placed, photographs from various angles should be taken to exactly visualize the trajectory path.

Bear in mind that, under certain circumstances, e.g. when a superficial bone is hit by the bullet right after entering the body, the bullet or bigger parts of it may be deflected. Thus, the trajectory of the bullet in the corpse and the actual flight path of the bullet before hitting the body may not correspond exactly in a straight line.

In cases of multiple shooting, determining the exact trajectory of each shot and the sequence may be difficult and extra attention should be given to come to conclusive results.

Beside the determination of entrance and exit wounds and the trajectory, the necropsy should focus on the degree and severity of tissue damage that has been inflicted by the bullet. Injuries may be found distant from the trajectory path of the main part of the bullet when deforming bullets or fragmenting bullets were used due to the temporary wound cavity (see chapter "Wound ballistics and wounding capacity")

Larger bullet residues (app. > 3 mm) and pellets that appeared in the radiographs should carefully be searched for and removed from the carcass. Take care not to inflict any artificial marks on the fragments during this process, use plastic forceps, gloved fingers etc. since each weapon may leave distinctive marks on the bullet that may be used for further forensic investigations! After removal, carefully rinse the fragments with water to remove any tissue residues, than dry them to avoid possible oxidation. Store the fragments in a soft packaging and a rigid and appropriately labelled (plastic) container.

Carefully record all findings of the pathological investigation in an exhaustive written protocol and document them with multiple angle/distance photographs. To facilitate the description of superficial wounds (e.g. entrance and exit wounds) with reference to ballistic evidence, use schematic multiple-angle diagrams of the carcass.

Figure 22: Fragments from a deforming bullet retrieved from a carcass. Note the main fragment (bottom of the projectile) on the upper left (Source and © FIWI).

6.1.4.4.2.2 Shotgun wounds

When fired at contact or close distance, shotguns are extremely destructive. However, this scenario can more or less be ruled out in wildlife crime cases. At longer distances, the wounding capacity of shotguns is relatively low and therefore lethal only in small game like hares and birds.

The pellets do not form any temporary wound cavity but inflict small wounds resembling blunt trauma. The pellets themselves only seldom inflict damage to vital inner organs. Usually, they are found in the periphery of the body, e.g. subcutaneously or in the outer muscular layer without having penetrated the abdominal or thoracic cavity. However, exposed bones without a dense covering muscular layer such as the skull or distal extremities may fracture by the impact of the pellets.

6.1.5 Disposal of carcass

In many cases, gross pathological investigations will not yield unambiguous results whether illegal killing is evident or not. Commonly, this can only be achieved by the ancillary investigations and the interpretations of all the results.

Until a conclusive statement can be made, the carcass must be regarded as an important item of evidence and must not be disposed of. Permission to do so must be sought with the leading investigator of the legal authorities or the leading prosecutor, respectively. Bear in mind that long-term storage of the remains of the carcass under appropriate conditions (cooled/frozen) may be a legal requirement during legal investigations and a pending trial in court. This may soon pose a problem when larger wildlife is involved and suitable facilities should be identified before any wildlife crime investigations. Further, the costs for long-term storage should be discussed with the appropriate authorities in advance.

6.2 Imaging Techniques

6.2.1 General

The importance of the use of imaging techniques in suspected wildlife crimes cannot be overemphasised. Non-invasive imaging techniques are extremely useful examination tools in wildlife forensic cases to document various traumata, metal foreign objects, fractures, etc.

Several non-invasive imaging techniques are available that may yield important evidence during the investigation process in suspected wildlife crime cases. Due to its rather easy practicability and high information value, radiography is still the “gold standard” in imaging techniques. While radiography is the most commonly used method, other techniques may also be considered like computed tomography (CT), magnetic resonance (MR) and, to a lesser extent, ultrasonography. Remember that the determination if a shot was fatal or not is not possible by radiography alone, and statements on the shooting distance is pure speculation.

All these techniques and systems require appropriately qualified personnel, both to operate as well as to interpret the images. Thus, an expert report is required to describe and define the forensic significance of the findings for each imaging study, regardless of the method used.

Generally, all imaging techniques must be employed before necropsy to ensure that organs and tissues are in their correct anatomical position and any object of possible interest (e.g. bullets) is situated at its original location in the carcass. Images of single organs or body regions obtained after necropsy are of limited value. Further, pre-necropsy imaging may guide investigators to areas of interest that otherwise may have gone undetected, e.g. “chronic” foreign objects deep in muscles without acute reaction of the surrounding tissue.

The following parts will briefly describe the advantages and disadvantages of each method. Methods that can be used only in live animals (Scintigraphy, Positron-emission tomography [PET]) are not dealed with in this handbook.

6.2.2 Methods

6.2.2.1 Radiography

Forensic radiography (i.e. the use of x-rays) is an important and widely used complementary examination tool in forensic examinations. Various abnormalities and thus evidences may be documented with this method, the most significant being:

- Foreign objects (projectiles, projectile parts/-abrasion, etc.)
  Metal residues from projectiles made of steel or lead show high opacity in radiographs and even small fragments of them can be detected by radiography. Copper, which is often used as a jacketing material for bullets is less opaque and may be more difficult to detect. Take care not to confuse dirt on the skin of soiled carcasses or grit in the stomach of birds with bullet fragments. To complicate things further, a radiograph may also show metal residues in the stomach of birds of prey that have scavenged on hunted animals or their remains, thereby ingesting bullet remains.
  Always remember that finding metal residues in a corpse does NOT necessarily mean that the animal died due to shooting since, especially with shotgun-pellets, these may be retained in the body after a non-lethal shooting. Only a thorough necropsy will reveal the ultimate cause and manner of death!

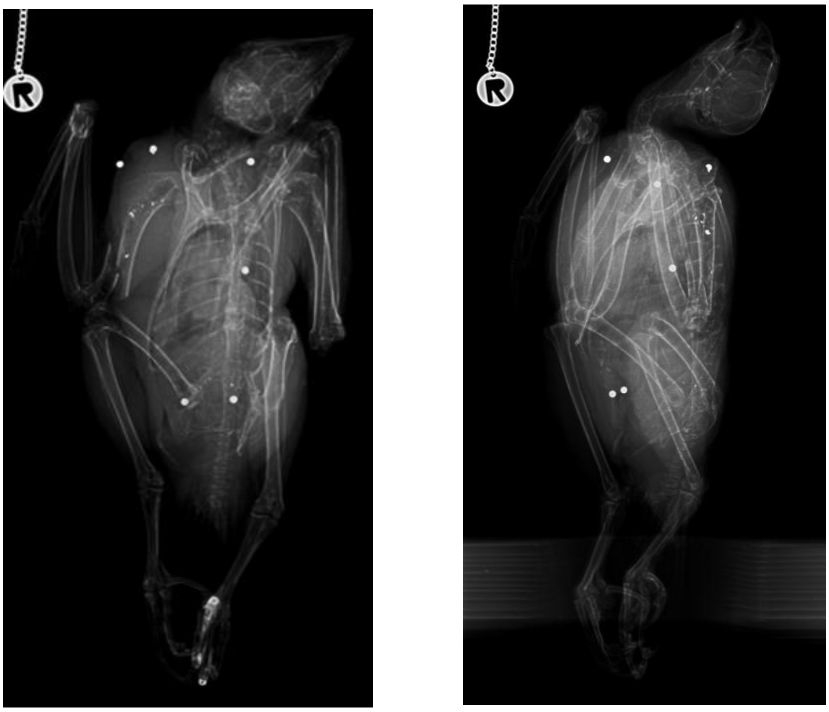

Figure 23: Corresponding radiographic images from two aspects of a peregrine falcon (*Falco peregrinus*) displaying multiple lead shots and bone fractures (correct labelling

is removed due to privacy protection). Note that the “R” marks the right side of the carcass in an image taken vertically (left figure) and indicates that the carcass rested on its right side in an image taken laterally (right figure) (Source and © FIWI).

- Traumata, especially skeletal injuries (fractures, dislocations etc.)
  Commonly, fractures and other skeletal injuries can easily be detected by radiography. However, it may be difficult to distinguish between ante-mortem and post-mortem lesions on a radiographic image. Therefore, a thorough necropsy must be done to assess possible accompanying findings like bleedings in surrounding tissues, swelling, and signs of heeling etc. that only occur in ante-mortem injuries.

- Measurement of bone density, age determination
  Bone density may give hints on the health - and the nutritional status - of an animal. Assessment of the epiphyseal plate or - line in long bones may help to determine the approximate age of an animal.

To a lesser extent, soft tissue injuries and accumulation of liquids and/or gaseous substances may be identified with radiography. However, these findings may be attributed to post mortem decomposition of the body and may be much easier to find and interpret through necropsy of the carcass.

Today, portable radiography systems are available and, although not entirely easy to transport in the field due to their weight and the amount of equipment necessary (e.g. protective clothing, see below!), radiography may be performed in the field when transportation of the carcass to designed laboratories is not possible – provided that power connection is available in the field.

Radiography uses ionizing radiation for imaging and thus precautions must be taken to minimize exposure of the staff. This can easily be achieved when working in premises designated for x-ray examinations (separation of examination room and operating/control room with lead-reinforced wall). However, when working in the field, protective clothing (lead gowns and gloves) must be donned by all personal and strict instructions by the operator of the radiography system must be given and obeyed (keeping distance etc.). Only a very experienced operator should use radiography equipment in the field!

To avoid contamination of the imaging device and to avoid loss of evidence during handling (e.g. bullets or bullet parts), carcasses should be radiographed in a plastic bag.

As a rule, the entire carcass should be radiographed and a minimum of two orthogonal images (views from two aspects with 90° deviation, e.g. laterally and vertically) must be taken to adequately localize and identify a certain structure. Whole body radiography of larger animals can increase image numbers quickly, since each region of the body (skull, thorax, abdomen, limbs etc.) must be displayed properly to avoid geometric distortion, confusion of anatomical structures etc.. Each image must be properly stamped with individual carcass label, acquisition date and left/right-side labelling. Although images may be edited to highlight details or enhance image quality (e.g. resizing, adjusting white balance, sharpening, etc.), a set of all images of the case, even those without obvious relevance, and without any alterations ("photoshopping") must be kept and handed to the court upon request.

6.2.2.2 Computed Tomography (CT)

While "ordinary" radiography is limited to two-dimensional imaging, CT provides three-dimensional, cross section-images of the body via computer processed multi-angle X-ray measurements. Despite the advantage of full body, three-dimensional images with high contrast, this technique is seldom used in wildlife forensic cases, mostly due to the adhering costs of each individual scan and the availability of the system. Additionally, foreign metal objects, like projectiles in the body, may produce artefacts on the images that may impede determination of the object's exact location in the carcass.

6.2.2.3 Magnetic Resonance (MR)

For MR-Imaging, the same advantages and limitations as for CT apply. While producing very high contrast images through magnetic fields and non-ionizing radio waves, the costs and duration of a MR-study and the limited availability of the system avert its application in most wildlife forensic cases.

Further, the (suspected) presence of ferromagnetic steel parts (as used in a variety of projectiles) are a contraindication for MR, as they may move in the body due to the strong magnetic field applied in this imaging technique.

6.2.2.4 Ultrasonography

Ultrasonography, as the name suggests, makes use of high frequency sound waves and their reflection of body-tissues to acquire images (called sonograms), mostly of soft tissues like internal organs, blood vessels, tendons etc. (ultrasound doesn't penetrate bones). Advantages of this technique are its radiation-free mode of action and most systems are easily portable.

Although widely used in live animal veterinary and human medicine, it has many limitations of usage in dead animals. Post mortem changes like gas accumulation in various cavities and tissues, as well as the progressive cooling of the body adversely affect imaging quality, imaging depth and thus the informative value of the sonograms of deceased animals.

6.3 Toxicological Investigations

6.3.1 General & Methods

These analyses aim at the detection of potentially toxic substances (pesticides, heavy metals, pharmaceuticals etc.) if poisoning is suspected in a wildlife crime case. They must be performed by a dedicated toxicological laboratory. Different methods are available including high-performance liquid chromatography (HPLC), atomic absorption spectroscopy (AAS) and gas chromatography-mass spectrometry (GC-MS). The laboratory will select the appropriate method depending on the preliminary report.

6.3.2 Sampling

Normally, samples for toxicology will be taken during necropsy, with the exception of baiting material at the crime site. It is often best to consult the respective laboratory for information on type of sample, size of sample and transport conditions, since laboratories often have their specific protocols and requirements for the collection, packaging and shipping of the material.

A small sample size will limit the number of tests performed if the type of toxin is unknown and multiple tests are required. On the other hand, multiple testing can result in high costs, so efforts should be made throughout necropsy to narrow down the substances in question. Therefore, all relevant information regarding the findings of the necropsy, the findings of ancillary tests, the findings at the crime site and other circumstances of the forensic investigations should be submitted to the analytical laboratory.

Sample materials for toxicological investigations can include organs, body fluids and other items.

- Blood/serum: good for pharmaceuticals, some heavy metals
- Liver: good for heavy metals, pesticides, pharmaceuticals
- Kidney: good for heavy metals, pharmaceuticals
- Brain: only in very fresh cases good for measuring enzyme activity in organophosphates-poisoning, e.g. carbofuran
- Lung: good for inhaled toxins

- Spleen
- Hair/feathers: May give time frame of intoxication
- Stomach contents/crop contents in birds/vomitus: good for recent ingestion of toxins, especially important if highly toxic substances that lead to acute death are suspected
- Urine: good for pharmaceuticals
- Baits: if illegal baiting is suspected
- Insects from carcass: Useful if carcass is very degraded; may indicate only presence or absence of toxin; toxins can be recovered from larvae, pupae and even pupal cases
- Plants/Water/Soil

All material for toxicological analyses must be stored and shipped in tight sealing containers. Generally, organ material, stomach contents and baits for toxicological analyses should be stored and shipped frozen at -20°C, while any fluid should be stored and shipped cooled at +4°C. However, it is again best to consult the respective laboratory on packaging and shipping conditions and advise the staff of the shipment in advance.

Thorough labelling and strictly adhering to the chain of custody is required as well as appropriate PPE since many toxins may be hazardous to humans

6.4 Bacteriological, Virological & Fungal Investigations

6.4.1 General

A large number of diseases in wildlife are caused by bacteria, viruses and fungi. While bacteria are "living" single-celled organisms that exhibit their own metabolism, viruses depend on living host cells of other organisms for reproduction. They comprise no cellular structure and consist solely of a protein capsule that surrounds the genetic material.

6.4.2 Sampling and Methods

Usually, suspicion of a bacterial, viral and fungal disease in wildlife is raised by the findings during necropsy and samples for the subsequent bacteriological and virological investigations will be taken during the necropsy by the veterinary pathologist.

Sampling material for bacteriological investigations include any affected tissue, blood and other body fluids or relevant swab samples. Organ samples and fluid samples should be stored and shipped cooled at app. +4° C (NOT frozen) in tight sealing and shatterproof containers. Since different types of swabs are available for bacteriological samples, swab samples should be stored and shipped according to the instructions by the manufacturer and/or the laboratory.

The appropriate method for the detection and identification of bacteria in a sample will be chosen by the laboratory. Methods include microscopic investigation (almost obsolete nowadays, except for some special staining methods), culture techniques on different growth media (e.g. agar plates) with subsequent characterisation of growth patterns and morphology, and genetic investigations (especially PCR) aiming at the identification of special genome-sequences of the bacteria.

Samples for virological investigations include mainly tissue samples. As with samples for bacteriological investigations, organs or organ parts should be stored and shipped cooled at app. +4° C in tight sealing and shatterproof containers, but virological samples may be frozen if stored for a longer period.

Methods for detection and identification of viruses include electron microscopy and genetic investigations (PCR).

All methods mentioned above aim at identifying the pathogen itself ("antigen") and thus are referred to as "direct" methods. Another possibility is to screen the blood or an organ for the presence of antibodies against a specific pathogen ("indirect" methods). However, the detection of antibodies is not necessarily a proof of acute disease since antibodies can persist for long periods after an infection. Many techniques for detecting antibodies are available (ELISA, gel diffusion precipitation…) and blood and tissue samples for these examinations should be treated as mentioned above.

6.5 Parasitological Investigations

6.5.1 General

The term “parasite” refers to various species of organisms that live in or on animals. They encompass such diverse genera as protozoa (single-celled organisms, e.g. coccidia), arthropods (insects, mites, ticks, fleas) and worms (roundworms, flatworms, tapeworms). Parasites can be subdivided into so called ectoparasites, i.e. parasites living on hairs, feathers or the skin of an animal, and endoparasites that live in an organ or a body-cavity of its host. The size of parasites ranges from several micrometres in single-celled coccidia to several meters in tapeworms. Most parasites “feed” on organ tissues, fluids or the ingesta of their respective hosts, thereby reducing the fitness of the infected animal or causing direct damage to it. Further, ticks e.g., may transmit various viral and bacterial diseases.

In wildlife, parasites are ubiquitous and almost 100% of wildlife harbour endo- and/or ectoparasites, the most common being worms found in the gastrointestinal tract, the liver and the lungs. While a low infestation with parasites normally do not have severe detrimental effects in the host, high infestation rates may have adverse effects on the health of the affected animals, causing direct damage to an organ, weakening, debilitating or emaciating the host and rendering it more vulnerable to other pathogens. Because of the high prevalence of parasites in wildlife, all investigations into the cause of death of a wild animal should routinely include parasitological examinations.

6.5.2 Methods

The most common technique used for the detection of endoparasites is analysis of the animals’ faeces. All endoparasites that colonize the gastrointestinal tract, the liver or the lungs shed their eggs via faeces during their reproductive cycle. These eggs can be detected and determined by microscopic inspection or by genetic analysis.

Large endoparasites may also be detected during necropsy, especially liver parasites (e.g. liver flukes) and large intestinal parasites.

Parasites of the blood can be detected by microscopic inspection of stained blood smears or by genetic analysis of blood samples.

Most ectoparasites (e.g. mites, ticks, fleas) can be detected by macroscopic inspection of the pelage or plumage of the animal during necropsy, or by microscopic inspection of the skin or a skin scrapping.
Dedicated laboratories for veterinary parasitological investigations will perform all the respective analyses.

6.5.3 Sampling

Usually, the appropriate samples for parasitological investigations will be taken during necropsy by veterinarians. Faecal samples should be stored and shipped cooled at app. +4° C (NOT frozen) in tight sealing and shatter-proof containers. Similarly, blood samples for analyses of blood parasites should be stored and shipped cooled in dedicated blood vials. If a whole organ (e.g. liver, lung) shall be analysed, the whole organ or the conspicuous part should also be stored and shipped refrigerated in tight sealing shatter-proof containers.

6.6 Genetic Investigations

6.6.1 General

Since the late 1980s, analysis of the DNA (short for deoxyribonucleic acid), the molecules that carry genetic information, has been used in human forensics. In almost any cell of an animal, two types of DNA are found: The linear shaped nuclear DNA located in the nucleus of a cell, and the circular shaped mitochondrial DNA located in the cell's mitochondria (organelles that produce energy in the cell). An important exception of DNA carrying cells are mature red blood cells of mammals.

For forensic purposes, the mitochondrial DNA is often better suited since the linear shaped nuclear DNA is highly susceptible to degradation in decomposing tissue. Further, a single cell may contain hundreds of copies of the mitochondrial DNA compared to only 2 copies of the nuclear DNA. While the nuclear DNA is passed by both parents and thus making it likely to undergo recombination, the mitochondrial DNA is inherited solely by the maternal line, making it more persistent. In any forensic case however, the respective genetic lab will decide which DNA to use as this also depends on the question that the forensic genetic investigation addresses.

6.6.2 Questions that can be addressed with genetic analyses

6.6.2.1 <u>Species Identification</u>

Blood stains, traces of saliva, faeces, feathers and hairs (all materials that normally cannot be clearly assigned to a unique species aside of hairs and feathers), often play an important role in wildlife crime cases. Further, since organic tissues may decompose to a degree that makes it impossible to identify the species it derives from by morphological characteristics, genetic investigations may help to identify the distinct species. Nowadays, publically-accessible genetic reference databases exist for most species and subspecies (e.g. GenBank, Barcode of Life) making it possible for examiners to compare the DNA profile of a sample in question with the unique profiles of species in the databases.

6.6.2.2 Region/Population of origin of an individual

Genetic analyses may reveal the origin of animal or animal parts, respectively. To accomplish this, a reference database is needed to compare the DNA profile of a sample with the known distinct DNA profile of animals in distinct regions and/or populations. In Europe for example, wolves can be can be allocated to the population and the region they originally came from since the distinct DNA profiles of the nine different populations are known.

6.6.2.3 Individual

For assigning, e.g., a hair sample to a certain individual or identifying an animal found dead individually, reference DNA profiles of the specific individual are needed. For this purpose, it is recommended to take samples for genetic analysis of all individuals that are handled during conservation projects, e.g. while individuals are raised in captivity for releasing or animals are captured for marking.

6.6.2.4 Matching samples

Genetic analyses can be used to determine if two or more samples are from one individual, e.g. if blood stains on a car stem from a recovered carcass, if antlers match with a certain individual and other issues in question.

6.6.2.5 Sex determination

Since male and female individuals carry distinct DNA sequences (for example the XX chromosomes in female mammals versus XY chromosomes in male mammals), determining the sex of the individual is straight forward by genetic analysis.

6.6.2.6 Parentage/Relatedness

If samples or a DNA profile of all individuals in question are available, parentage and/or relatedness of the individuals can be determined by genetic analysis.

6.6.3 Questions that cannot be addressed with genetic investigations

- Age of an individual
- Whether sample came from living or dead animal
- Size, colour or markings of an individual
- …

6.6.4 Sampling

When sampling biological material for genetic analysis in a wildlife crime case, the same procedures and protocols must be obeyed as with other samples regarding chain of custody, labelling, transport and storage. During sampling, all collection material and tools (swabs, containers, knives, scissors etc.) must be free of “external” DNA from the collector or other possible sources of contamination. The usage of sealed and disposable tools is recommended. In general, sampling should be done by qualified and trained personnel. Personal Protective Equipment (PPE) must be worn, and gloves should be changed between sampling of different materials and/or samples to avoid cross-contamination.

Storage and transport are crucial to avoid any degradation and contamination of the material and different materials require distinct conditions. If in doubt, it is always best to contact the laboratory performing the genetic analysis for the relevant information.

Nowadays, a wide array of commercial kits is available for sampling of genetic material. Using these kits in wildlife crime cases is highly recommended, as this reduces the risk of improper collection and handling of samples. All instructions of the respective manufacturer regarding the usage, storage and mode of shipment must be followed.

Soft body tissue: Any tissue of a carcass can be collected for genetic analysis (organs, muscles etc.). Usually, a small amount (5 gram) is sufficient except for samples of highly degraded remains. Place tissues in an airtight, shatter-proof container and store them at -20°C. Samples should be shipped via an express mail service in a cooler with ice packs.

Bones /Teeth/Antlers/Eggshells: These materials should not be cleaned or bleached before processing. Large molar teeth or large bones (humerus, femur) are best suited for DNA extraction. Storage and shipping of these materials can be done at room temperature.

Hair/Feathers: A number of hairs and feathers are plucked from the body with gloved hands. Do not use scissors etc. to cut the hairs since the best results are obtained with the hair roots (and possible some skin cells) still attached to the sample. Both hairs and feathers must be stored in a paper envelope or bag and stored and shipped at room temperature. Placing these samples in a tight sealing plastic container will increase the likelihood of degradation by mould, rendering the samples useless for genetic analysis!

Blood: In very fresh carcasses (and of course living animals), blood may be collected from the carcass with sterile veterinary equipment and stored in a tube with anticoagulant (EDTA, not heparin). Blood tubes should be stored chilled at +4°C or less. Avoid repeated thawing and refreezing since DNA degradation will occur. Samples should be shipped via express mail in a cooler with ice packs.

In most wildlife crime cases, however, it will not be possible to take blood samples at the spot. In such cases, blood may be collected during necropsy. Wet blood can be transferred onto swabs or special filter paper (e.g. Whatman Paper®) and stored in the swab-tube or a paper envelope (filter paper) at room temperature. Dried blood on the carcass or on any object of interest can be collected with a slightly moistened swab and placed in a swab tube or paper envelope after drying.

Blood stains on ice or snow can be collected with the original material in a tight sealing container. Samples should remain frozen. Again, avoid repeated thawing and refreezing since DNA degradation will occur. Samples should be shipped via express mail in a cooler with ice packs.

Saliva: Saliva on any object or on a wound of an animal supposedly killed by a predator can be sampled using swabs. After air drying at room temperature the swab should be placed in the swab tube and may be stored and shipped at room temperature.

Urine: Fresh urine can be sampled in a tight sealing container (minimum 50ml) and should be frozen at -20°C as soon as possible. Samples should be shipped via express mail in a cooler with ice packs. Dried urine on any object can be collected with a slightly moistened swab and placed in a swab tube or paper envelope after drying at room temperature. Urine on snow or ice should be collected in a manner similar to blood stains on these materials.

Faeces: Wet faeces should be sampled in a tight sealing, shatter-proof container and frozen at -20°C as soon as possible. Dry faeces should be stored and shipped in a paper bag or a breathable container at room temperature.

6.7 Botanical Investigations

6.7.1 General

Plants are ubiquitous in nature and the analyses of plant and plant parts have become recognised as important tools in human forensic cases in recent years. For example, linking a suspect and/or a victim to a certain crime scene may be achieved by comparing the plants at the crime site with plant parts found on the body and on clothing, shoes and other possessions of the individuals involved in the case.

In suspected wildlife crime cases, botanical investigations may be of similar assistance as described above, but they may also assist in addressing other questions. At least in herbivorous animals, analysis of the gastric contents and/or the faeces may reveal not only the individual's diet and thus possible malnutrition by ingestion of inadequate food (e.g. containing only very low energy), it may also be possible to link the death of an individual to the ingestion of toxic plants. Surprisingly, the latter does occur in wild animals, e.g. elks being supposedly killed by ingesting a toxic species of lichen or roe deer becoming moribund and dying because of ingestion of a certain variety of rape (*Brassica napus*). When screening gastric contents for possible toxic plants, it should be remembered that not each part of a toxic plant may contain the toxins and that not all plants toxic in one species are detrimental in others. For example, English yew (*Taxus baccata*) is highly toxic in horses whereas other herbivorous species are less at risk.

In carnivorous animals, ingestion of plants or plant parts (especially fruits) may occur at regular intervals in some species (e.g. wolf) and almost never in other species (e.g. lynx). However, analysis of the gastric contents of wild carnivores for botanic material may give clues to conditions like abnormal (feeding-) behaviour, starvation, inability to hunt and others.

Further, analyses of the gastric contents may help linking an animal to a certain region if (recent) translocation of the animal or the carcass is suspected. Finding plants in the gastric contents that do not grow in the wider surroundings of the place where an animal was found should raise suspicion.

6.7.2 Methods

Identification of plants and plant parts can be done by analysing their morphological characteristics using the naked-eye and a microscope or by genetic analysis. However, the latter can be cost- and time-consuming due to the limited DNA databases for plants and the fact that gastric contents will normally comprise many different plant species, making genetic analyses very complex.

6.7.3 Sampling

- Reference plants from the crime site, e.g., should be collected as complete as possible and then dried by compressing them flat between two sheets of paper weighed down by large books, for example. After drying, the specimen can be stored at room temperature.
  Alternatively, the reference material can be stored in ethanol or in FAA solution containing 10% formalin (37%), 10% concentrated acetic acid and 80% ethanol (50%).
- Samples of plant parts (e.g. seeds, fragments) found on the animal or on clothing, shoes etc. should be collected with forceps and stored in paper envelops or in vials containing ethanol or the FAA solution mentioned above.
- Samples of gastric or intestinal contents are usually taken during necropsy. The material should be stored refrigerated (+ 4°C) in tight sealing, shatter-proof containers or plastic bags.
- For DNA analysis, preferably the leaves of a plant are collected and stored in a silica gel desiccant at room temperature or refrigerated. Do not use alcohol as a preservative.

As with all samples of evidence, thorough labelling and strictly adhering to the chain of custody when transferring the botanical evidence to other institutions is required.

Analysing plants by their morphological features requires a well-trained botanist. When examining gastric or intestinal contents of an animal, the analyst will have to deal with plant parts that are at least partly digested. Specialists in this kind of investigations are rare and it may take some efforts to find an expert. However, this can be worthwhile in wildlife crime cases and universities and natural history museums may assist in the search.

6.8 Entomological Investigations

6.8.1 General

Numerous species of insects feed on dead organic material. Analyses of the insects that colonize a carcass may help to determine the time of death of an animal. This procedure is well established in human forensic science and gaining increasing attention in veterinary forensics, since the principles in human and animal cases apply equally.

6.8.2 Methods

6.8.2.1 Age of Blowfly (*Calliphoridae*) larvae

Blowflies are among the first insects that arrive on a carcass (often within minutes) depositing their eggs especially on the natural body openings (ears, nose, genital region, anus, etc.) and on wounds. Development of the eggs into various succeeding larval stages (maggots), pupae and the adult fly follows a more or less predictable pattern and time scheme. However, it must be taken into account that the development is highly dependent on environmental conditions (temperature, humidity, precipitation, etc.) and hence information on these parameters is paramount when analysing the insects. For example, development will be delayed by cold weather, but the development will not exceed a certain speed even under ideal conditions for development. Consequently, it is possible to determine the minimum time elapsed since death. It should be noted, however, that under certain circumstances, blowflies will deposit their eggs on living animals, especially on (necrotic) wounds at body areas that the animal is not able to groom with its mouth or legs. In debilitated individuals, egg deposition may also occur on body openings while the animals are still alive.

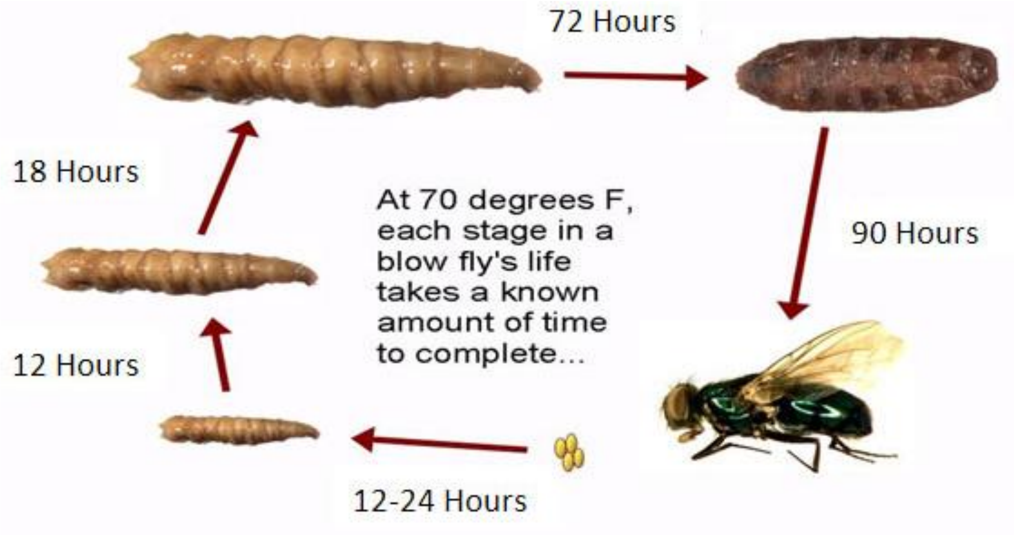

Figure 24: Development of different blowfly larvae stages on carcass under ideal circumstances (Source: Internet)

6.8.2.2 <u>Succession of insects on carcass</u>

With continuing decomposition of a carcass, various species of insects will colonize and feed on the remains at certain stages. While blowflies will be among the first, beetles will feed on a carcass at late stages of decomposition (e.g. skeletonisation). As with blowfly larvae, the exact successional colonization by insects is predictable but highly dependent on environmental factors, habitat type, season and other factors, and records of these parameters must be considered when determining the post mortem interval.

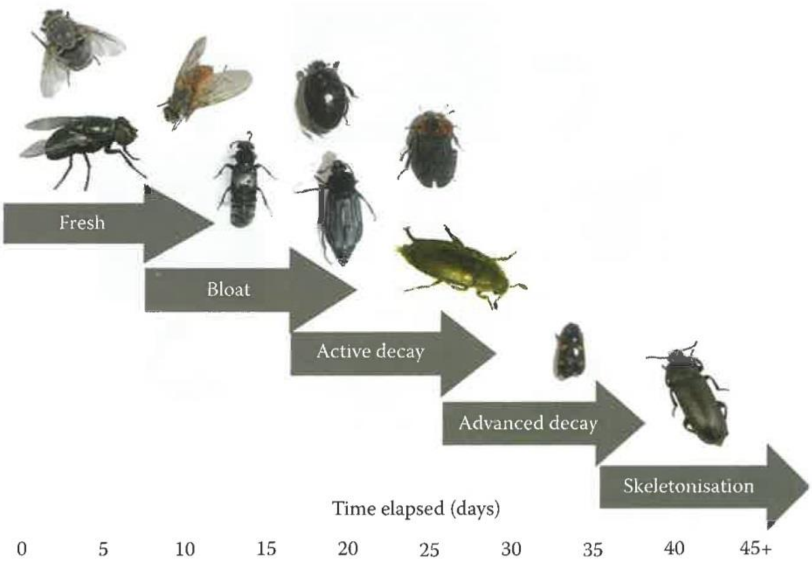

Figure 25: Approximate successional colonisation of a carcass by different insect families (from Cooper & Cooper, 2013).

In both methods, correct identification of the insects and the interpretation of the results that may give a minimum post mortem interval must be done by an experienced forensic entomologist. Universities, CSI units of the police and natural history museums may assist in searching for a forensic entomologist.

6.8.3 Sampling

Any insect activity on a carcass should be recorded during the crime scene investigation and documented with overview and close-up photos. Further, any dead insects on the carcass should be documented since this can be a valuable hint on a poisoning occurrence. These insects may be used for toxicological analyses, especially when the carcass is too degraded for toxicological analyses.

Correct sampling of insects or insect stages is somewhat tricky and should be done by trained personnel, either during crime scene investigation or during necropsy. In the latter, however, it must be considered that the development of blowflies will continue during transport of the carcass depending on transport conditions.

Both live and dead specimens of insects and insect-stages (especially the oldest stages) should be collected for entomological analyses. Use forceps or a small moistened brush for collecting. Samples from different body regions should be collected in separate vials. For each sample, records must be kept detailing date and time of collection and location from which the sample was taken (head, wound etc.).

While dead insects can be placed directly into vials containing 70% - 90% ethanol, samples of living beetles and other crawling insects can be stored in normal containers without preservative and may be killed by storing them in a freezer. Make sure to store living beetles separately to prevent cannibalism.

Adult blowflies are of little to no value since it cannot be discerned if they have hatched from the carcass or recently arrived at the site. Life stages of blowflies (eggs, larvae, pupae) should be split in two lots: One half should be kept alive for continuing development to facilitate species identification when the adult fly hatches. Eggs should be placed in aerated containers (e.g. vials covered with two layers of paper towels secured with elastic band) containing wet paper towels or other material that prevents drying-out during transport plus organic material (e.g. a piece of meat) for feeding.

The other half should be killed by placing them either directly into vials containing 70% - 90% ethanol (pupae) or by immersing them in near-boiling water for several seconds before storing them in vials containing ethanol (larvae). If no hot water is available store them in ethanol directly.

Samples of soil near the carcass may also contain valuable information, since third stage larvae migrate from the carcass to dry places to pupate.

Entomological evidence should be transported to the laboratory as soon as possible and living samples should be cooled (+4°C) throughout the transport. As with all samples of evidence, thorough labelling (time, place, and location of sampling site on carcass) and strictly adhering to the chain of custody is required.

6.9 Immunological, Cytological & Clinical Chemistry Investigations

These types of investigations are widely used in clinical veterinary medicine to assess the physiological- and health-status of an individual. They comprise mainly the analyses of

- Blood cells and other blood components (e.g. proteins)
- Enzymes
- Electrolytes, minerals, trace elements
- Hormones

The application of these investigation techniques in wildlife forensic cases, however, is only of limited value in most cases. As soon as a carcass begins to decompose (which is essentially right after death), the values obtained for many of the parameters will become inadequate and inconclusive. Decomposition will affect many of the parameters (especially proteins like enzymes) and thus will greatly alter the values. Further still, almost all parameters greatly vary between and within species. Age, sex, nutritional status, reproductive status and other individual constituents affect the parameters and thus many reference values have to be available to infer the health status of an individual through these investigation techniques. Reference values do exist for domesticated animals but data for wildlife is often lacking.

These limitations will constrain haematological and clinical chemistry investigations to wildlife crime cases where live animals are involved or to animals that have been found moribund and samples can be taken from a very fresh carcass. Samples should only be taken by a trained veterinarian since knowledge of the varying requirements like type of blood vials, preservation, handling and storage is vital.

6.10 Isotope Analyses

Isotope Analysis or Stable Isotope Analysis, as it is sometimes called, refers to a relatively new method that determines the varying ratios of stable elements in tissues (especially bones, teeth, hairs, feathers). It is used in various fields of science including archaeology, ecology, geology and others. The main elements used in isotope analysis are carbon (C), hydrogen (H), oxygen (O) and nitrogen (N).

In short, the chemical elements in a biological tissue (e.g. bone) are found in a certain ratio of their isotopes (e.g. 99% ^{12}C and 1% ^{13}C). This ratio reflects the location where an animal lived when the tissue was formed and its diet because distinct geographic regions and distinct diets exhibit varying ratios of the different isotopes of chemical elements. Thus, isotope analysis can help determine the origin of an animal and its possible movement patterns. Further it can be used in studies on feeding ecology and other physiological traits.

In forensic science, the stable isotope ratios of hairs, bones, or tooth enamel have been used to determine the geographic origin of individual humans, animals and animal products as well as the origin of plants, especially drugs.

Isotope Analysis is done by mass spectrometry and specialised laboratories are needed for measurement and the interpretation of the results.

7 REPORTING

7.1 General considerations

An accurate final report (“Necropsy Report”) of all investigations in a suspected wildlife crime case is of utmost importance. Ideally, it collates and interprets all findings gathered during the whole process of the investigations and draws clear, concise, methodical, understandable and verifiable conclusions.

The report will play a key part in any trial associated with the respective legal case and decisions will be made upon it. It is a vital document that will be available to the law enforcement agencies, prosecutors, defence attorneys, the judge and, if applicable, the jury in court and thus will be investigated (and challenged!) throughout the legal process. It shall therefore provide a clear, professional and well-balanced opinion on all evidence available in the case.

7.2 Format of report

The report ideally follows a concise and comprehensible format since not all legal authorities and the people in court will have a deep understanding of the medical and medico-legal aspects in wildlife crime cases. Thus, while using medical terminology when describing the findings, switching to lay terminology in the sections of the report dealing with the interpretation of the results is advisable.

A template for a report format may look as follows:

<u>Heading</u>

This includes date, case number, investigating agency and –officer, as well as information on the leading veterinarian including name and contact details.

<u>Subject of exam</u>

Provides information on and full description of the animal(s) species, sex, age and any characteristic colourings or other marks. The basis for age determination (dentition, tooth wear, plumage coloration and others) should be stated.

Reason for examination

A short statement on the exact reason for the examination and the report (e.g. “suspected illegal shooting”) should be made here.

Abbreviations

If (medical) abbreviations are used in the report, a key of them must be provided.

Definitions

Any terms that may not be common to non-experts should be defined in this section, especially when the terminology is unique in animals.

Method of arrival and packaging

A brief description of the means of transportation and transfer of the carcass and any other material upon hand-over at the laboratory (packaging, mode of transport, condition of material, e.g. cooled or refrigerated).

Crime Scene findings

Relevant findings at the crime site, either provided by the primary investigating officer or by an on-scene investigation by the veterinarian should be included here. Photographs, videos and other material and information like weather data and others should be described and listed in this section.

Examination results and findings

The findings of all investigations and testing should be precisely described here, using descriptive qualifiers if applicable (e.g. “mild” to severe”). Use subsections for each investigation (e.g. necropsy, histopathology, radiology, microbiological-, entomological-, genetic-investigations etc.). For a better understanding, include all relevant photographs and diagrams that were made during the investigation, especially those of the necropsy.

Summary of findings

A summary of all findings should be reported in this section. It is recommended to use lay terms, as this chapter will probably gain the highest attention of the readers.

Conclusions

Clear, professional and concise conclusions based on all findings collated must finalize the report. Each detail in the section “Summary of findings” should be addressed. As a rule, absolute statements should be made with caution and it is advisable to rather use terms as “consistent with”, “probable” or even “unknown”.

This section should include:

- Cause of death (e.g. gunshot wound, blunt force trauma etc.)
- Manner of death (e.g. natural, accidental, non-accidental etc.)

Signature of leading (veterinary) investigator

8 FURTHER READING

For those interested in further and more in-depth information on the topics presented in this handbook, the readers may refer to the textbooks listed below that were consulted when preparing this handbook. The textbooks on veterinary forensics mostly address cases of domestic animal abuse and neglect but many of the forensic techniques described can be applied to wildlife crime cases. Most of the textbooks are solely available as English editions, but you may check if other editions in your preferred language are available. Make sure to consult the latest editions of the books since the discipline of forensics is rapidly evolving!

Aside from the textbooks, hundreds of specific articles dealing with wildlife forensics have been published in scientific journals, not all of them as open access, however. Due to the sheer amount of them and the fact that many of the (older) articles have been cited in the relatively new textbooks listed below, we refrained from listing them here.

A recent issue of the scientific journal "Veterinary Pathology" also focussed on veterinary forensic pathology with several articles on various aspects of the forensic work. It can be accessed publicly via this link: https://journals.sagepub.com/toc/vetb/53/5

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Wolfram Meier-Augenstein: Stable Isotope Forensics: Methods and Forensic Applications of Stable Isotope Analysis
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9 WEBLIOGRAPHY

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www.wildlifeforensicscience.org

International Veterinary Forensic Sciences Association:
www.ivfsa.org

American Association of Forensic Science:
www.aafs.org

Forensic science resources on the internet – website updated and maintained by The Gelman Library at George Washington University:
www.istl.org/03-spring/internet.html

PAW UK Forensic Working Group: Providing forensic advice and support to wildlife crime investigations:
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www.fbi.gov/about-us/lab/forensic-science-communications

Firearms ID – Information on ammunition identification, gunshot residue, shotgun pattern testing, etc.:
www.firearmsid.com

Crime Scene Investigations:
www.feinc.net/cs-inv-p.htm

Forensic Magazine – with full issues, articles, products, and services and recent court rulings:
www.forensicmag.com

Forensic Pieces – training and consultations offered in a broad range of forensic sciences:
www.forensicpieces.com

Veterinary Forensics Consulting:
www.veterinaryforensics.com/

Crime & Clues –Information for crime scene investigation and forensic science:
www.crimeandclues.com

Crime Scene Investigator EDU – Become a crime scene investigator:
www.crimesceneinvestigatoredu.org

Forensic Science for Beginners:
http://www.exploreforensics.co.uk

Blood stain tutorial
www.bloodspatter.com

Forensic entomology:
http://www.forensic-entomology.com/

Entomology supplies and collection information:
www.texasento.net/equip.htm
www.bioquip.com

HAIRbase™ – a website containing a database of various animal hairs to support forensic analysis:
web.me.com/kwpmiller/HAIRbase/Welcome.html

Arrowhead Forensics- crime investigation and forensic supply company:
www.crime-scene.com

Evident Crime Scene – crime scene and evidence collection products:
www.evidentcrimescene.com

Fitzco, Inc. – crime scene and evidence collection products:
www.fitzcoinc.com

Lynn Peavey – crime scene and evidence collection products:
www.lynnpeavey.com

Sirchie – crime scene and evidence collection products:
www.sirchie.com

Tri-tech USA – Crime scene and evidence collection products with veterinary forensic kit:
www.tritechusa.com

CSI Gizmos – Templates for creating your own evidence markers, scales and arrows.
www.csigizmos.com

Rite in the rain - All weather writing equipment
www.riteintherain.com

10 ANNEX

10.1 To do lists

- Capacity Building
- Necropsy

10.2 Flow Chart

- Investigation scheme

10.3 Protocols/Records

- Comprehensive Reference Lists of National and Regional Legal Authorities, of National and Regional Veterinary Authorities and of Potential Stakeholders (to be filled out by handbook users according to their needs)
- Comprehensive Reference List of Forensic Laboratories (to be filled out by handbook users according to their needs)
- CSI Form
- Evidence Log
- Chain of custody Form
- Photo Log
- Sampling during necropsy Log

10.4 Packaging Lists

- CSI
- Field Necropsy

10.5 Pros and Cons of Usual Sample Storing Methods

TO DO LIST
CAPACITY BUILDING

prior to the occurrence of suspected wildlife crime cases

- **Study this handbook in its entirety to obtain an overview of the traits relating to suspected wildlife crimes ☺**
- Identify the respective national and regional legal- and veterinary authorities
- Identify potential stakeholders (hunting agencies, conservation organisations, NGOs)
- Identify an appropriate first responding veterinary facility (and the facilities for ancillary investigations)
- Familiarize yourself with your national/regional legal aspects regarding wildlife and wildlife crimes
- Establish a working relationship with all parties and institutions potentially involved in the persecution of wildlife crimes
- Discuss their respective requirements and needs in a forensic investigation
- Discuss a preliminary working- and communication-plan and the respective protocols in case of the occurrence of a suspected wildlife crime case
- Discuss the potential costs of a forensic investigation and the meeting of costs

TO DO LIST
NECROPSY

All veterinary pathological facilities will have their distinct protocols according to their needs and practical experience and, although undoubtedly adhering to best-practise standards, these protocols may vary slightly between institutions. We therefore refrain from presenting a necropsy protocol-template but rather point out some issues that should be obeyed during the forensic necropsy process.

- Adopt your necropsy protocol according to the needs and requirements of a forensic investigation

- Take photographs of all steps during necropsy, beginning with reception of carcass (packaging, earmarks etc.). Use tags and rulers for best documentation and start with overview photos before going into detail. Take photographs also of non-significant findings (e.g. an organ without any alteration),

- Consult CSI form and photos of crime scene for full background history of case

- Recover and retain all potential pieces of evidence like snares, traps and others still attached to the carcass. Handle them with care and use gloves (Fingerprints).

- Recover and retain all markings of the carcass like rings in birds, microchips, telemetric collars

- Record storage conditions of carcass prior to necropsy

- Record body-measurements and –weight as well as those of relevant organs

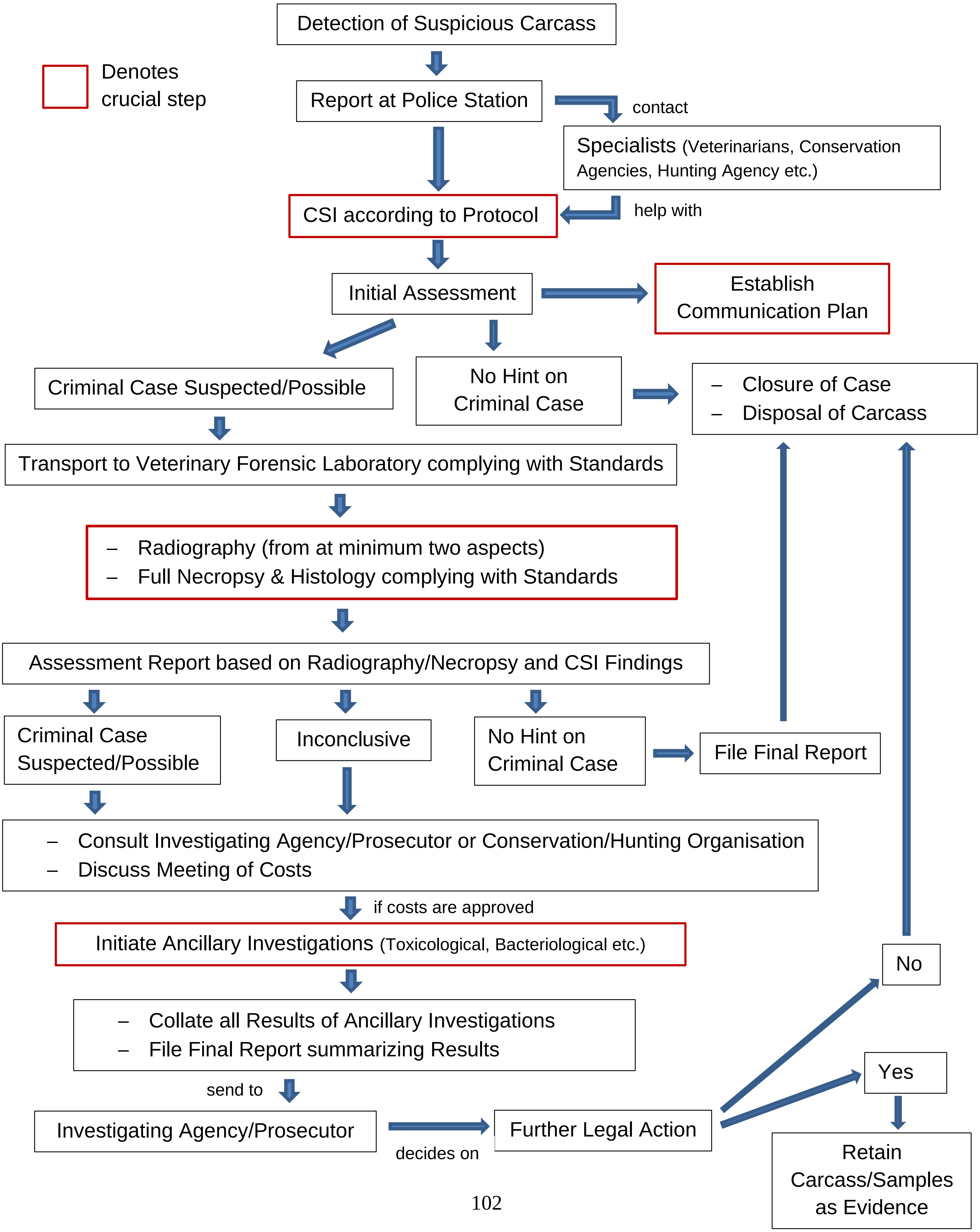
- Perform a FULL, THOROUGH and EXHAUSTIVE NECROPSY & HISTOPATHOLOGY, even if the cause of death seems obvious

- Bear in mind that autolysis may be prominent in dead wildlife. Do not confound the alterations caused by this process with those of other origin.

- Pay attention to signs of (see text):
    - Shooting
    - Snaring
    - Poisoning

    - Blunt trauma indicative of vehicle collision, wind turbine/power line collision etc.
    - Electrocution (in birds)
- In shooting incidents, recover and retain at least the larger bullet fragments. Use plastic forceps, padded forceps or gloved fingers for recovery
- Select best samples for ancillary investigations and fill out sampling log
- Initiate ancillary investigations deemed necessary
- Try to establish a post mortem interval
- Determine cause, mechanism and manner of death

FLOWCHART INVESTIGATION SCHEME



Comprehensive Reference List of National and Regional Legal Authorities, National and Regional Veterinary Authorities and Potential Stakeholders

(to be filled out by handbook users according to their needs)

NATIONAL LEGAL AUTHORITY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

REGIONAL LEGAL AUTHORITY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

NATIONAL VETERINARY AUTHORITY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

REGIONAL VETERINARY AUTHORITY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

POTENTIAL STAKEHOLDER (Hunting Agency/Conservation Organisation)	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

POTENTIAL STAKEHOLDER (Hunting Agency/Conservation Organisation)	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

POTENTIAL STAKEHOLDER (Hunting Agency/Conservation Organisation)	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

Comprehensive Reference List
of Forensic Laboratories

(to be filled out by handbook users according to their needs)

VETERINARY PATHOLOGICAL LABORATORY	
Name	
Address	
Contact Person	Name: Tel.: e-mail
Remarks	

VETERINARY IMAGING FACILITY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

FORENSIC TOXICOLOGICAL LABORATORY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

VETERINARY BACTERIOLOGY LABORATORY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

VETERINARY VIROLOGY LABORATORY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

VETERINARY PARASITOLOGY LABORATORY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

GENETIC LABORATORY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

BOTANIC LABORATORY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

FORENSIC ENTOMOLOGY LABORATORY	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

(additional laboratory)	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

(additional laboratory)	
Name	
Address	Street: Postal code, city:
Contact Person	Name: Tel.: e-mail:
Remarks	

CSI Form

CSI LEADING AGENCY:
CASE NUMBER:
START OF CSI **Date:** **Time:**

HEAD INVESTIGATING OFFICER	
Name:	
Contact Details	Tel.: e-mail:

SUPPORTING INVESTIGATOR	
Name:	
Affiliation:	
Professional Background: ☐ Veterinarian ☐ Biologist ☐ other:……………………	
Contact Details	Tel.: e-mail:
Remarks:	

SUPPORTING INVESTIGATOR	
Name:	
Affiliation:	
Professional Background: ☐ Veterinarian ☐ Biologist ☐ other:……………………	
Contact Details	Tel.: e-mail:
Remarks:	

INITIAL REPORTER	
Name:	
Contact Details	Address: Tel.: e-mail:
Affiliation: (e.g. local hunter, landowner, NGO Staff, passer-by etc.)	
Date of report: **Time of Report:** **Police Station:**	
Date and Time of Detection of Carcass(es):	
Details of Report:	

ENVIRONMENTAL FACTORS AT SCENE	
Current Ambient Temperature: **°C**	**Current approx. Humidity:** **%**
Current Weather: □ Mostly Clear Sky □ Partly cloudy □ Overcast □ Rain □ Snow □ Strong Wind	
Recent Significant Weather Events: (e.g. thunderstorms, heavy precipitation, gales …) Date: Event: Date: Event: Date: Event:	
Other Relevant History: (human activities in area, recent unusual events, deaths in livestock or other wildlife…) Date: Event: Date: Event: Date: Event:	

<table>
<tr><th colspan="3">DETAILS OF SCENE</th></tr>
<tr><td colspan="3">Location:</td></tr>
<tr><td colspan="3">GPS Coordinates:</td></tr>
<tr><td colspan="3">Type of landscape: (forest, open field, clearing, rocky terrain, ravine etc.)</td></tr>
<tr><td>Landowner</td><td colspan="2">Name:
Address:

Contact Details Tel.:
E-mail:</td></tr>
<tr><td>Hunting Permit Holder</td><td colspan="2">Name:
Address:

Contact Details Tel.:
E-mail:</td></tr>
<tr><td colspan="2">Proximity to nearest relevant man-made Structures</td><td>☐ Road with regular traffic: ………..m
☐ Wind Turbine: ………..m (if birds are involved)
☐ Power line: ………..m (if birds are involved)
☐ Others: …………………….. ………m</td></tr>
<tr><td colspan="3">Search Radius Around Carcass: ……… Meters ☐ with Search Dogs ☐ without</td></tr>
<tr><td colspan="2">Relevant Conspicuous Features at/near Crime Scene

(take photos!)</td><td>☐ Foot Imprints ☐ Tire Imprints
☐ Cartridges ☐ Ground/Soil Disturbances
☐ Blood Stains ☐ Hair/Feather Accumulation
☐ Other Biologic Samples/Items: ………………………………….
……………………………………………………………………....
☐ Others: ………..………………………………………………….
…………..……………………………………………………………...</td></tr>
<tr><td colspan="2">Photos/Videos of Crime Scene
Photo Log Nr. ……………..</td><td>☐ Overview ☐ Mid-Range ☐ Close-Up
☐ Others: ……………………………………………………….</td></tr>
</table>

BRIEF OVERALL DESCRIPTION OF SCENE

DETAILS ON AFFECTED ANIMAL(S)
Use multiple copies of this page if numerous carcasses are found (one for each carcass) **Species: ……………………………………………………** **Carcass Condition*:……..** *****(1 = fresh to 5 = completely decomposed)
Positioning of Body:
External signs on Carcass: ☐ Obvious Wounds Location: ☐ Wound suspicious of shooting Location: ☐ Discoloration suspicious of Poisoning Location: ☐ Other Signs:

Insect Activity	☐ Non	☐ Mild	☐ Moderate	☐ Severe
Rigor Mortis	☐ Non	☐ Mild	☐ Moderate	☐ Severe

Photos/Videos of Carcass **Photo Log Nr. …………..**	☐ Overview ☐ Mid-Range ☐ Close-Up ☐ Others:………………………………………………………………..

INITIAL ASSESSMENT OF CAUSE OF DEATH

☐ **“Legal” Human Induced Killing**:

☐ Road Kill ☐ Electrocution ☐ Wind Turbine ☐ Legal Shooting

☐ Others: ……………………………………………………………………………………

☐ **“Illegal” Human Induced Killing**:

☐ Shooting ☐ Poaching ☐ Snaring/Trapping ☐ Poisoning

☐ Others: ……………………………………………………………………………………

☐ **Natural Cause**:

☐ Starvation ☐ Disease ☐ Predation ☐ Fighting

☐ Natural Disaster:

☐ Avalanche ☐ Landslide ☐ Flooding ☐ Forest fire ☐ Lightning strike

☐ Others: ……………………………………………………………………………………...

☐ **Non-Specifiable**

Remarks:

INITIAL ESTIMATION OF APPROXIMATE TIME OF DEATH

Based on Findings at Site and Witness Statements

☐ Hours: ……....... ☐ Days: ……….. ☐ Weeks: …………. ☐ Months: ……………

☐ Indeterminable

Remarks:

EVIDENCE COLLECTED

☐ Yes => Fill in Evidence Log ☐ No

INTERVIEWS WITH WITNESSES/POTENTIAL INFORMANTS
Statements on separate Protocols ☐ Local Hunter(s) ☐ Landowner ☐ Other Locals ☐ Others: ………………………………………………………………………………………

FURTHER ACTIONS TAKEN

REMARKS

FORMS FILLED: ☐ Evidence Log ☐ Photo Log ☐ Sampling Log ☐ Chain of Custody Forms ☐ Witnesses/Informants Reports ☐ Others: …………………………………………	
END OF CSI: **Date: ……………………** **Time: ………………………**	
Name Supporting Investigator: **…………………………………………….**	**Signature**
Name Supporting Investigator: **…………………………………………….**	**Signature**
Name Leading Investigator: **…………………………………………….**	**Signature**

Evidence Log

Page….of…..

Investigating Agency: ………………………………………………………………………

Case Number: …………………………………………………………………………......

Head of Investigations: ……………………………………………………………………..

Tel.-Nor.: ………………………………………………………

E-mail: ………………………………………………………

Remarks:

Item ID	Date & Time	Location	Description	Purpose	Storage Conditions	Signature

Chain of Custody Form

Each item of evidence that is moved for investigation purposes must have its own Chain of Custody Form that is passed with the item.
Photocopies of this form must be made each time the item is transferred, one to be kept by the transferring institution and one to be sent to the leading investigating authority in order to keep them updated on the deposition of the item!

Case Number: ……………………………………………………………

Original Item ID: ………………………………………………………

Description: ………………………………………………………………………………..…
……………………………………………………………………………………………..…
……………………………………………………………………………………………..…

Date & Time	**Released by** Institution & Deliverer	**Received by** Institution & Recipient	**Purpose**	**Storage Conditions**	**Release Sign**	**Receipt Sign**

Photo Log

Page….of…..

Investigating Agency: ……………………………………………..………………….

Case Number: …………………………………………………………………………......

Photos taken during: ☐ CSI ☐ Necropsy ☐ other: ……………….

Photographer: ……………………………………………………………………………

Remarks:

Photo ID	Date	Description

Sampling at Necropsy Log

Page….of…..

Investigating Agency: …...…………………………………………………………………

Case Number: ……………………………………………………………………….....

Samples Taken by: ………………………………………………………………………

Date	Purpose	Tissue	approx. Amount	Storage Conditions	Remarks

PACKAGING LIST CSI

Forms

- CSI form
- Photo Log
- Chain of custody form

General Equipment

- Writing utensils (Waterproof Pens, Pencils, permanent markers)
- Clipboard (preferable with rain cover)
- Protective plastic sheets to keep documents dry
- Mobile
- Notebook
- Camera and spare memory cards
- Spare batteries and chargers for all electronics
- GPS unit
- Torch/Head Lamp
- Scene lightning equipment
- Multi-tool/knife
- Waterless hand wash
- Desinfectant

Personal Protective Equipment (PPE):

- Disposable coverall
- Gloves
- Respirator mask
- Goggles, protective glasses and/or shield
- (Rubber boots and/or coverboots)

CSI Equipment

- Barrier tape
- Evidence flags/markers
- Evidence tape and sealing tape
- Forensic scales and rulers
- Swabs for genetic sampling
- Fingerprint sampling equipment
- Magnifier
- Tape measure
- Forceps

Evidence sampling

- Tight sealing (body) bag
- Envelops (for hairs/feathers and non-biological material)
- Other Packing and sampling equipment as needed: Ziploc bags, plastic bags, tight sealing containers,…
- packing tape
- Rubber bands

PACKAGING LIST FIELD NECROPSY

Forms

- Necropsy form
- Sampling at Necropsy Log
- Photo Log
- Chain of custody form

General Equipment:

- Sturdy cases/duffle bags/rucksacks for carrying equipment
- Folding camp table
- Large plastic sheets
- Packing equipment: cooler box, cardboard box, Ziploc bags, packing tape
- Writing utensils (Waterproof Pens, Pencils, permanent markers)
- Clipboard (preferable with rain cover)
- Protective plastic sheets to keep documents dry
- Mobile
- Notebook
- Camera and spare memory cards
- Spare batteries and chargers for all electronics
- GPS unit
- Torch/Head Lamp/Scene lighting equipment
- Multi-tool/knife

Personal Protective Equipment (PPE):

- Disposable coverall
- Gloves
- Respirator mask
- Goggles, protective glasses and/or shield
- Apron
- Rubber boots and/or coverboots

Other Personal Equipment:

- First Aid Kit
- Drinking water
- Food/Snacks
- Spare clothes
- Tarps and rope to create a tent to ward off rain or sun

Necropsy Kit:

- Metal ruler and measuring tape
- (Spring) scale
- Knives and knife sharpener
- Scalpel handle and disposable blades or disposable scalpels
- Forceps - various
- Scissors - various
- Strong scissors to clip bones
- Saw for cutting bones
- Sharps disposal unit
- Cutting board
- String

Sample Collection Equipment:

- Labels
- Manila labels
- Syringes & Needles - various
- Serum collection tubes
- Blood collection tube
- Sterile, tight sealing plastic bottles of various sizes
- Sterile cryovials
- falcon tubes
- Sterile plastic bags (zip-lock bags)
- Sterile swabs
- Capillary tubes
- Viral transport medium
- RNA later solution (or equivalent lysis buffer)
- FTA cards
- Glass microscope slides, coverslip and slide storage box
- Staining kit
- Plastic container with 10% neutral buffered formalin
- Plastic container with of 70- 90% ethanol
- Disposable transfer pipette
- 12 volt portable centrifuge
- Cooler and ice packs or dry ice
- Liquid-nitrogen dry shipper or Dewar
- Tube holder
- Sample storage box

Clean-up Equipment:

- Water
- Plastic buckets
- Condoms (no joke, they make perfect devices for carrying water)
- Soap, detergent, hand sanitizer
- Disinfectants
- Spray bottle
- Scrub brush
- Heavy duty rubbish bags
- Paper towels

PROS AND CONS OF USUAL SAMPLE STORING METHODS

Method	+	-
Chilled at +4°C	• Good preservation for few days	• Autolysis continues slowly
Frozen at -20°C	• Almost indefinite storage • Pathogens usually not killed (thus can still be analysed)	• Artefacts • histological examination usually not possible anymore • Pathogens usually not killed (thus sample may be hazardous)
Formalin	• Indefinite storage • Pathogens usually killed (thus sample is safe to handle)	• Affects appearance of organs • Genetic studies difficult • Pathogens usually killed (thus cannot be analysed)
Ethanol/Methanol	• Almost indefinite storage • Pathogens usually killed (thus sample is safe to handle)	• Affects appearance of organs • Genetic studies difficult • Pathogens usually killed (thus cannot be analysed)

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Interreg AlpineSpace Alpbionet 2030 project

ISBN: 978-3-200-06798-1