Basics in Wildlife Forensic Sciences

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Introduction
Who I am

• Working as a wildlife veterinarian for 20 years

• Trained state veterinary officer

• Currently pathologist at Research Institute of Wildlife Ecology

• Our lab does 1000+ necropsies on wildlife and zoo-animals (50:50) per year, with numerous cases of (suspected) Wildlife crimes each year
Aim of Talk

• Give general overview of Wildlife Forensic Science
• What is achievable/possible from scientific side
• Shortcomings/Pitfalls
• Suggestions

Overall aim of work package:

Standard Operating Procedures (SOP) Handbook
“With a value of between $7 billion and $23 billion, illegal wildlife trafficking is the fourth most lucrative global crime after drugs, humans and arms”

World Economic Forum
Rural Crimes

“Criminological research has historically been based on urban metropolises in developed countries. Minimal research focuses on crimes in rural settings, particularly in crimes involving species, such as poaching, coursing and the rustling/theft of farm animals. The absence of research establishing patterns of crimes in rural areas brings into question the relevance and effectiveness of existing policy and methods for preventing rural crime.

Rural crime is severely underreported in the UK with gross underestimation of the emotional and financial costs to rural communities. (…) By establishing the facts relating to species crime in the UK it is possible to better identify solutions for rural communities. (…)”

© 2017 by D. Delpech, University College London, Dep. Security and Crime Science at Meeting of SWFS (Society for Wildlife Forensic Science)
Possible Pitfalls

Prevention and persecution of Wildlife Crimes on a broader transnational scale hampered by

- Varying legislations
- Varying standardisations
- Varying knowledge
- Varying responsibilities
- Varying focuses (e.g. species)
- Prosecutors with high workload
- Prosecutors with minimum knowledge in wildlife crimes
  
  => specialised prosecutors in some countries
- …
General Considerations in Wildlife Crimes
What is Wildlife Crime?

- Killing of wildlife
  - under unlawful conditions
  - by inappropriate means or methods
- Damaging health of wildlife
- Illegal keeping/trading of wildlife
What is Wildlife Forensics?

**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:** Application of science to criminal investigations. Collecting, Preserving, Analysing, Reporting on EVIDENCE

Interdisciplinary!
What we are not talking about in the AlpBioNet2030 project
What we are not talking about in the AlpBioNet2030 project
What we will mostly deal with in AlpBioNet2030 project
What is Wildlife Forensics in our context?

Wildlife as *victims*

Wildlife as "offenders"

Wildlife

livestock
Some remarks on life animals:
“Clinical cases”

• Rare (e.g. non-lethal gunshot wounds, snaring...)
• Usually relevant only in “flagship species” (lynx, bears, raptors,...) not in game species
• Specialized veterinarians in zoos, wildlife rehabilitation centres etc.
• Same forensic techniques as in dead animals applicable
• Report Clinical observations: Gait, respiration, excretion, unusual behaviour,...
Causes of Death in Wildlife I

Not necessarily comparable to human cases!

Natural causes

- Disease (more widespread than most expect)
- Starvation/decrepitude
- Predation
- Intra-/interspecific fights
- Ingestion of toxic plants
- Natural Disasters (Avalanches, lightning strike, thunderstorms, flooding…)
- ...

Rule out
Human induced „legal“ causes

- Vehicle/train/aircraft/windmill/power line collisions
- Legal hunting
- Fireworks etc.
- ...

Rule out

Provide possible recommendations for mitigation measures! (e.g. prevention of collisions)
Human induced illegal causes

- Illegal Hunting/Trapping: Protected species
  Poaching
  Wrong time of year
  Not expertly manner, e.g. Bow hunting

- Poisoning (incl. secondary Poisoning)

Confirm

How can this be achieved?
Obvious or not?

Obvious causes  
  e.g. aircraft collision

Nevertheless detailed forensic investigation pivotal
  • Which species?
  • Species a local resident? (close to airport)
  • Evaluation of damage to aircraft

Less suspicious  
  e.g. carcass of deer in avalanche, carcass of raptor under windmill

Highly suspicious:  
  e.g. stained carcass
Obvious or not?

How to discern?
Tasks in Wildlife Forensic Investigations

- Visiting crime scene
- Collating on site information and data
- Sampling and Transportation

- Necropsy/Pathological Investigations
- Additional investigations
- Storage

- Filing reports
- Appearance in court

Specialists and collaborations needed!
Techniques & Methods in Wildlife Forensic Investigations

- Pathological investigations (including histology)
- Radiography/Imaging
- Microbiological investigations (bacteriology, virology, parasitology)
- Toxicological investigations
- Chemical investigations (incl. haematological investigations)
- Immunological investigations
- Genetic investigations
- Age determination
- Botanical investigations
- Entomological investigations
- ...

- Not all can be done by one institution
- Costs!
- Clear questions by authorities

=> Establishing collaborations is essential!
People involved in Wildlife Forensic Cases

- Prosecutors
- Law enforcement officers/investigators/state veterinary officers
- Scientists (veterinarians, biologists, toxicologists etc.)
- Stakeholders (hunting agencies, farmers, game wardens, National park authorities, NGOs…)
- Newsman
- Public

May have differing primary interests!
Passing of Information

Wildlife Crimes often get high media attention and are highly emotional in public perception.

Passing of information critical:

- Strict chain of reporting (who passes what information when to whom)
- Labs must stay “neutral” (no information except to authorities)
- Press releases in any kind of media by stakeholders etc.? 
- Role of social media?
- Trained authorities representatives, e.g. press officers

Leaking may hamper persecution and conviction!
Respect Hierarchy

Prosecutor

↓

Law enforcement

↓

Labs

↓

Stakeholder

↓

Public

May want to influence or interfere

Who pays?
At the Crime Scene in Wildlife Forensic Cases
Finding dead Animals

Detection for most cases (regardless of cause of death) unlikely (at least in smaller sized animals)!

=> Decay, removal of carcass by scavengers, hunters

Passive <=> Active - targeted surveillance

Public Awareness

Experiment by Wobeser and Wobeser 1992
– 250 dead chicks placed in a 1 ha area over 5 day period
– 10 % of area searched daily for 10 days
– Only 1 intact chick recovered
– Only the removal of 1 chick by a scavenger was observed

=> A massive mortality of 250 chicks/ha was missed despite intense surveillance
At the Crime Scene I

Managing crime scene I

Ideal conception:
Crime Scene Investigator and trained person (e.g. state veterinary officer) at crime scene => transport of whole carcass to forensic lab

2nd best practise:
Trained person (e.g. vet with knowledge in crime scene management) at crime scene => transport of carcass to forensic lab

3rd best practise:
Trained person (Vet) does on site necropsy at crime scene => transport of samples to forensic lab

.....
At the Crime Scene II

Managing crime scene II

- Evidence Collection
- Documenting: Standard protocols, photos, videos
- Taking Samples => whole carcass best!
- Correct handling, packaging, labelling, transport & storage
- Chain of custody!

Specialists/Trained personnel available?
At the Crime Scene III

Systematic investigations

• Detailed inspection of the surroundings
• Other dead animals in vicinity (regardless of species)?
• Signs of fights/struggling/predator encounter (e.g. entangled animals)
• Accumulation of faeces (may indicate a period of immobility)
• Check environmental conditions
• Blood stains/Discoloration of soil etc.
• ....
At the Crime Scene IV

Photos/Videos:

- Ensure exact date and time stamp set on camera
- Take pictures (wide-angle) of surroundings first as reference (landmarks)!
- Keep original files, never delete, rename, photoshop!

Protocol

Unique protocol available for Wildlife Crimes?

Should include e.g.:
- Description of location (open field, wood etc.)
- Relevant nearby structures (windmills, powerlines, roads…)
- Recent weather data
- Events like fireworks etc.
- …
At the Crime Scene V

Why photos may assist (or bias) pathologists

Why is this probably electrocution?

... and this is not?
Not all birds killed on utility structures are electrocutions. This is entanglement.
Considerations for on-site necropsies

- Size of animal
- State of carcass
- Terrain (transportation)
- Available personnel
- Weather/time of year
- (day of week)
- …
Investigation Techniques in Wildlife Forensic Cases

Part I: Pathological Investigations
Part II: Additional Investigations
General Considerations on Wildlife Forensic Techniques

- Credibility of methods applied
- Approved methods?
- Scientifically sound?
- Standardisations?
- Reproducibility?
- Must stand in court!
- Cost effectiveness!
- ...
Pathological Investigations I

Pivotal part in Wildlife Forensic Investigations!

... more than just dead bodies!
Dead animals are packages of information and future opportunities!

Macroscopic examination => Necropsy

Microscopic examination => (Patho)Histology
Pathological Investigations II

Aim of Necropsy

Determination of

• Cause of death
  Manner (e.g. blunt trauma)
  Reason (e.g. car)

• Cause of ill health

• Underlying pathology

• ….

As soon as possible!
Minimum requirements for “first responding” forensic lab

- Fully established Veterinary Pathology Lab with ability for both necropsy and histology
- Experts able to assess and evaluate necessity of further investigations (and argue necessity to prosecutor – Costs!) familiar with legal realms in country
- Radiography
- (Parasitology)
- Existing network/collaborations with other labs
Standard operating procedures

• Standard protocol: Protocols for companion animals/livestock usually need adaptations

• Pathologists involved must have awareness/knowledge of presentations of shooting wounds, poisonings etc.

• Hygiene: Staff protection
  Dissemination of pathogens
  Disinfection
  Disposal of carcass
Pathological Investigations V

Routine macroscopic examination

- Body condition/Weight/Age/Sex

- External Examination: Body condition, skin, pelage/plumage, body orifices, signs of injuries/predation…

- Dissection and removal of inner organs: „clean“ organs first, digestive tract as last step

- Inspection of internal body cavities

- Dissection and evaluation of inner organs: Size, weight, shape, colour, texture…

- Sampling for additional investigations
Pathological Investigations VI

Histopathological Investigations I

Fixation in Formalin → Embedding in wax → Cutting → Mounting on slide

Various staining methods available for questions pending: Can be done at any time!

Magnification up to ca. 1000x
Histopathological Investigations II

- Rule out/confirm disease
- Ageing of tissue damage (e.g. wounds) and inflammations (acute, subacute, chronic,…)
- Exact analysis of swellings (tumour, old trauma,…)
- Specific histopathological findings (e.g. lead intoxication)
- …. 
Pathological Investigations VIII

Special questions during necropsy in Wildlife Forensic Cases I

Species determination

- Morphological features (corpse, bones, hairs, feathers,…)
  Reference collections in museums!
- Genetics

Age Determination

- Size of individual
- Ossification
- Teeth: Dentition/Eruption
  Assessment of wear
  Incremental lines in Cementum (microscope)
- Coloration of pelage/plumage
- Eye lens weight
Pathological Investigations IX

Special questions during necropsy in Wildlife Forensic Cases II

Determination of Post-mortem Interval

- Livor Mortis: Hypostasis of blood
- Rigor Mortis: Rigidity of muscles
- Algor Mortis: Cooling of body
- Decomposition
- (Vitreous Humor [liquid in eye bulb]: Potassium Concentration)

Attention: Times are highly variable depending on many intrinsic and extrinsic factors! Scientifically not well established in distinct animal species!
Pathological Investigations X

Special findings in Wildlife Forensic Cases I: Bullet wounds

Normally: Massive trauma, bleeding, fractures, pieces of bullet in carcass

But: Can also be only subtle

Use plastic forceps for recovery of bullets!

Bullet entrance “wound”
Mammals: Shots in hypoderm (under hide)
Birds: Shots penetrate deeper in body

• Only subtle trauma/bleeding (mammals)
• Often no vital organs affected (Exception: Shooting at close distance)
• Number of shots found in carcass no hint on shooting distance
  — May vary depending on shot-type and size, choke in shotgun barrel etc.)
  — Multiple firing?
Special findings in Wildlife Forensic Cases:
Predation - post mortem scavenging - vehicle collision?
Pathological Investigations XIII

Interpreting results

- Rims of wound
- Bleeding

Exert caution identifying and attributing predation by macroscopic findings alone!

Intra vitam (bleeding rims)

Post mortem (no bleeding)
Genetic Investigations

Dr. Steven Smith
Head of Genetics
Department of Integrative Biology and Evolution
University of Veterinary Medicine Vienna
Radiography (X-Rays)

- easy detection of
  - bullet (fragments)/shots
  - fractures/dislocations
  - bone density
  - ...

Before or after necropsy ?!??

Keep original digital radiography files, never delete, rename, photoshop!
Additional Investigations II

Other imaging techniques

• Magnetic resonance imaging (MRI)
• Computed tomography scan (CT)
• Ultrasonography (US)
• ...

+ 3 dimensional images (MRI, CT)
+ High contrast
- Experts needed for interpretation
- Expensive
Almost 100% of wildlife harbour endo-/ectoparasites

Infestation may have adverse effects on health

Analysis of
- Faeces
- Skin
- Blood (smears/whole blood)
- Histologic samples
- ...
Additional Investigations IV

Bacteriological & Virological Investigations

Direct: Detection of potential pathogens in samples (blood, tissue, swabs, soil, water etc.)
- Incubation on agar plates (Bacteria)
- PCR (Bacteria, Virus)
- Electron microscope (Virus)
- …

Indirect: Detection of antibodies against potential pathogens in blood/tissue
- NOT necessarily prove of acute disease!
- Many techniques available (ELISA, Gel diffusion precipitation….)
Additional Investigations V

Toxicological Investigations

Detection of various potentially toxic substances: Pesticides, heavy metals, drugs...

Methods available:
- High Performance Liquid Chromatography (HPLC)
- Atomic Absorption Spectroscopy (AAS)
- Gas Chromatography Mass Spectrometry (GC-MS)
- …

Specialised labs
Check with lab for
- kind of sample
- sample preparation
- Costs!
Botanical Investigations

Analysis of plant(-parts) on/in animal

- Stomach content (herbivores) often partially digested!
  => Poisonous plants?
  => Rumen acidosis?
  => Individuals diet

- May give clues to origin of animal

Specialists in plant systematics and plant(-parts) taxonomic investigations rare!
Additional Investigations VII

Immunological, Cytological & Clinical Chemistry Investigations

Analysis of blood cells, enzymes, electrolytes etc. => Information on immune-system and physiological status of animal

Often not feasible/valid in animals dead for longer period => Mainly in clinical cases
Additional Investigations VIII

Entomological Investigations

Clues on

• Origin of animal
• May help calculating minimum post mortem interval

Age of Blowfly larvae

Succession of insects on carcass

Influenced by season, environmental conditions/weather etc.

Process samples in hot water and store in ethanol as soon as possible!
Stable Isotope Analysis

Use of stable isotope-ratios of C, H, O, N and Sr in tissues (hairs/feathers/bones) to determine

- feeding ecology
- physiological status
- water use
- movement patterns
- Origins
- ...

Specialised labs needed

Holds huge potential for future studies and investigations!
Taking Samples in Wildlife Forensic Cases
Sampling I

Best sample: Whole Carcass

If not feasible:
Samples of - tissues
  - organs
  - fluids (blood, urine,..)
  - hairs/feathers
  - bones/teeth
  - …

Trained personnel needed!
Sampling II

Appropriate containers: Different samples require different containers!

Carcass: Sealed plastic bag, absorbing material and hoofblock
Thermal packs?

Tissue samples/organs/fluids:
- Tight sealing containers
- Outer box
- Absorbing material

Hairs/feathers: Paper envelops!

Swabs: Various types for analysis in question (microbiology, genetics etc..)
Sampling III

Inappropriate Containers....
Sampling IV

Storage and Treatment of Carcasses/Samples I

Appropriate preservatives/fixatives: formalin, alcohol, transport medium for microbiology…

Identification: Correct tagging of samples

Transport and storage

If in doubt check with lab!
## Storage and Treatment of Carcasses/Samples II

<table>
<thead>
<tr>
<th>Method</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chilled at +4°C</td>
<td>• Good preservation for few days</td>
<td>• Autolysis continues slowly</td>
</tr>
<tr>
<td>Frozen</td>
<td>• Indefinite storage • Pathogens usually not killed</td>
<td>• Artefacts • histological examination usually not feasible • Pathogens usually not killed</td>
</tr>
<tr>
<td>Formalin</td>
<td>• Indefinite storage • Pathogens usually killed</td>
<td>• Affects appearance of organs • Genetic studies difficult • Pathogens usually killed</td>
</tr>
<tr>
<td>Ethanol/Methanol</td>
<td>• Indefinite storage • Pathogens usually killed</td>
<td>• Affects appearance of organs • Genetic studies difficult • Pathogens usually killed</td>
</tr>
</tbody>
</table>

If in doubt check with lab!
Epilogue
Further Reading

Do not consult books on human forensics unbiasedly!
Download the latest SWFS Newsletter
August 2017

The Society for Wildlife Forensic Science (SWFS)

Welcome to the Society for Wildlife Forensic Science (SWFS). We are an international organisation that supports practitioners and promotes best practice in wildlife forensic science.

This site contains information on wildlife forensics for the scientific community and others interested in using forensics to tackle wildlife crime. For the latest updates from the Society, please read our current newsletter.
Take home messages

• Wildlife Forensic Investigations need experts and time
• Many techniques available
• Many things to be considered, if in doubt check
• Wildlife Forensic Investigations must bring together specialists from several scientific disciplines

AS WELL AS

local authorities/stakeholders/NGOs etc.

BRIDGING THE GAP

=> That’s why we are here!
What to discuss in workshop

• Aims/needs of SOP handbook
• Contents of SOP handbook (requirements)
• Existing collaborations
• Common strategies
• Shortcomings (authorities/availability of experts/labs/personnel)
• ....
Questions ?