



Mini Review

Wildlife DNA Evidence: Recognition, Collection and Preservation

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Abstract

The power of discrimination, reliability, and authenticity of the DNA application provides a promising tool for forensic scientist to fight against wildlife crime. This mini review article is an update about the ability of standard methods use for biological evidence collection and also to highlight the problems, how DNA test results affected by handling methods (recognition, documentation, collection and preservation) in the wildlife crime cases for interest of justice.

Keywords: Forensic scientist, wildlife crime, biological evidence, justice.

Introduction

Like human forensic, wildlife forensic has the same task, to relate suspect, victim and crime scene in concert in a triangular manner with the physical evidence recovered from the crime¹. Technological advancement in human forensic provides a backbone for wildlife investigation, but their progress rate of advancements has been more gradual because lack of proper attention for many years makes it an isolated field².

Rather wildlife crime investigation is often lots more complicated as compared to others investigative science. There are a number of reasons like circumstances under which animal can be killed (legally or illegally), lack of proper species specific definition and lots more complication hindered for the fight against wildlife crime³.

A forensic crime investigation, set off with the basic steps, recognition, collection and preservation of biological evidences, is most important, but unfortunately it's getting less concern during handling and this is the keyhole of an investigation failure. Proper handling of evidences provides important investigation lead to forensic scientist to disprove an alibi or a connection of a suspect or victim to a crime scene^{4,5}.

During the past few decades, a significant boost up is recognized in the field of criminal identification system. Our judiciary system often much more relies on physical evidences as reliable sources, rather than eyewitness as unreliable and biased^{4,6}.

The prime aim of this article is to introduce the reader about the handling of biological evidences from a variety of wildlife crime investigation.

Strengthening of DNA analysis techniques with times brings great advantage for wildlife investigation⁷. Wildlife DNA forensic is well concerned and appropriates with the identification of biological evidences for determination of the species, gender, individual identity of samples, population, molecular taxonomy and relationship or relatedness of animals^{7,8}. It is very important to concern during sampling of biological evidences because DNA evidences are very easy to contaminate and degradable under environmental conditions⁹. So it is advisable to collect samples as soon as possible from a crime scene. A proper work is needed during sampling; items can be properly documented, sized, packed and stored for further examination at the wildlife laboratories¹⁰.

DNA source types

From 90's, DNA typing after getting good recognition in biological world contributes and constitutes a major advancement to forensic examinations. DNA analysis getting popularity, especially in the field of forensic science because of its remarkable sensitivity and power of discrimination¹¹. It is to be noted and considered in practice that the crime scene samples may contain less amount of DNA.

The quality and quantity of DNA that can be isolated from commonly encountered biological sources frequently varies and depending upon different environmental conditions¹². Tissue, blood and semen are the best source to obtain a DNA profile, whereas other biological fluid like gastric fluid, fecal matter, vomit, bone and hair, etc. have less percentage to isolate DNA and comparatively difficult to generate a genetic profile.

Many different sources of biological evidences related to wildlife crime are reported and submitted to wildlife forensic laboratories for examination. Most common biological sources are reported for DNA recoveries are as follows in table-1.

Table-1
Type of DNA evidence recovered during the investigation

Evidence sources	Possible location	DNA source	References
Weapon\ knife, tools, sticks	Handle\body	Blood\tissue	12, 13
Chopping board	Surface	Blood\tissue	14, 15
Traditional East Asian medicines	Medicines (tiger bone juice or rhino horn pills etc.)	Tissue	16-18
Traps (lag hold traps)	Handle\body	Blood\hair\tissue etc.	19-21
Clothing\Carpet	Surface	Hair\saliva\semen\ sweat\urine etc.	22
Artifacts or handicraft (Made by Hair or feathers)	Museum shops\costume	Feather\hair\shell etc.	23-26
Bite-marks	Skin\clothing	blood\saliva	27-29
Ivory idol\artifacts	Seals\hankos\chops	Bone fragments	30-32
Leather goods	Shops\museum\handbag\purses\shoes\ boots	Tissue	33-35
Caviar	Food supplements	Eggs	36,37
Horn\bones	Medicinal Products\Meusiums	Tissue	38,39
Cooked or processed meat	Tissue	Bone\tissue	40-44
Wool\fibers\fur\ Shahtoosh shawls)	Ready-made Shawls\Carpets	Hair	45,46
Egg	Illegal smuggling	Egg shells\eggs\ yolk	47,48
Claws left on tanned hides	Museum\ illegal smuggling	Pulp of Claws\ Tissue\Hair	49,50

Recognition of DNA evidences

Physical Evidence is a tangible object that connects or linked an offender\suspect or victim to the crime scene. Recognition is the ability of an experienced investigator who systematically evaluates the crime scene to identify substantiate or probative evidences amongst vast quantities of irrelevant, or unrelated items⁵¹. This is the first step of crime investigation, more crucial, challenging, and most complex part, help to determining which sample need to be further considered. All biological evidences which have DNA are a kind of physical evidence can be subjected to DNA testing⁵². Deposition of any biological evidence (tissue, blood, hair, bone, feces, urine, and saliva, etc.) to an individual’s body, clothing, any object, or at the crime scene, they adhere to the surface by absorption or adherence (liquid or solid respectively) and become stained⁵³. Most important prime aspects of crime scene investigation are to protect and preserve the crime scene with minimal disturbance.

Documentation of biological Evidences

If a DNA evidence or biological sample is not properly documented before their collection, its origin should be a question every time⁵⁴. So, all careful observations related to the location and condition of any biological evidence at the crime

scene thoroughly documented as soon as possible before its collection and preservation⁵⁵. Also note and gather identifying information from the first responding officer and veterinary personnel should also be documented. Proper documentation with maximum amount of information at the crime scene, postmortem room and forensic laboratory examination is essential which may help to make a permanent record of all the events⁵⁶ and it also helps the forensic expert to recall his memory during trial and can later be introduced in court.

Crime scene Evidence documentation

Videotaping the evidence at the crime scene, Photograph the evidence with its relative position before it is touched or collected. Document the location and condition of the evidence. Proper sketching with it’s the spatial relationship of the evidence relative to other objects in the scene. Complete description about collector, sample type, label, initial, and seal the evidence package.

Collection and preservation

Whenever any heinous crime is occurring, the presence of DNA evidence is obvious. Evidence integrity, both scientific as well as legally, is highly depends upon the skill of the first

investigating officer at the crime scene^{55,56}. The person who appointed as an investigative officer has properly trained with related guidelines for identification, collection, and preservation of DNA evidence because the integrity and ability of DNA results in court are highly idealized by handling of biological evidence⁵⁷. Once the whole crime scene has been properly documented, then the process of evidence collection should begin as per scientific guidelines. As soon as possible, collect all the evidences usually start with most fragile or most easily degradable.

Basically depend upon the kind and condition of physical evidence, most of the evidences will be collected in clean, sterile, paper, envelopes and bags. Whereas moist/wet biological samples from crime should be collected in clean sterile plastic containers. So, the main goal of the crime investigative officer to properly collect and preserve all biological evidences, it should be air dry the sample and freeze it as soon as possible⁵⁸. It is preferable to use paper for packaging because this is air permeable and prevent accumulation of water moisture inside the sealed envelopes⁵⁹. At collection time, if sample containing moisture, it should be guided that, as soon as possible, sample removed from plastic or paper package and allow to completely air dry in the absence of direct sunlight and humidity, at the secure location. Later the air dried, biological evidence should be stashed away in a facility with minimum extreme heat and humidity, which can cause DNA degradation⁶⁰.

Each evidence container should have all information regarding collector names, date, time, place, seal and complete description of evidence type. Once the sample collected, it should be delivered to the forensic agency with the proper chain of custody. Before the submission for forensic analysis, all the evidence should be stored in cool and dry environment. In forensic arena, every forensic scientist must focus and highlight the importance of effective crime scene processing. Improper collection means possibilities of contamination and that means biased or negative DNA results⁶¹⁻⁶². So, it is very essential that strict and proper guidelines should be followed to avoid contamination for better prevention and results.

In table-2 describe a collection and preservation methods of different biological sample.

Factor affecting DNA quality and quantity

Various factors are responsible for DNA degradation and affect the ability of DNA typing. A main leading factor which affects DNA includes sample quantity, time, temperature, humidity, sunlight, UV light, different substrate, chemical exposure, contamination (from bacteria or micro-organisms) etc.. Prolonged exposure of biological sample to the environment cause DNA degradation and become unsuitable for further scientific analysis. So, importantly, the prime goal of an investigator during handling (both collecting and preserving) of biological evidences to halt the degradation process have already in progress and limit any further future deterioration.

Contamination precaution

The main serious issue related to the handling of the biological DNA sample is the risk of contamination. There is always a high risk of DNA contamination during collection and transportation of evidence. So it is necessary to take preventive attention to reduce possible contamination during collecting and preserving DNA evidence. The main Contamination in DNA evidence is due to other biological source getting mixed with relevant^{63,66} or with surface contamination that come in contact with the sample or improper collection and preservation cause a high risk of biological activity (like microorganism or bacterial activity) will destroy the DNA authenticity⁶⁷. The following guidelines should be accompanied to keep off and reduce DNA contamination:

Secure and limit the scene to all important personnel. Always wear sterilized good quality gloves and change them often. Handle evidence carefully as possible. Avoid touching those areas, where the DNA evidence possibility exists. As the possible use disposable or single use items and clean/sterilized non disposable items before or after sampling. Avoid walking on or over evidence. Do not speak, sneeze, coughing at the crime scene as possible or over evidence. Always record and store individual scientific evidence with proper documentation in separate sealed envelopes or containers. Thoroughly air dries the evidence (in the absence of direct sunlight) before packaging. It should be recommended that avoid plastic packaging inner or outer covering and use paper bags, envelopes, or boxes and similar like materials for all forensic biological evidence. Packed each evidences separately to prevent cross contamination. Leave the scene if you become injured. A proper precaution taken and avoid direct body and oral contact with the tools used in an investigation that may be possibly have touched with contaminated surfaces. As possible, avoid eating, drink, chew gum, cigarette or tobacco at a crime scene.

Future prospective and recommendation

The prime goals of this review article are to identify the key needs in wildlife forensics. For improving the available tool techniques, the following suggestions are suggested.

It's preferred that the existing protocols for collection, preservation, transportation of biological evidence should be housed and organized at a single site. Proper techniques and scientific guidelines should be issued that contains all information regarding sampling or handling. It should be confirmed that a forensic investigator has knowledge about what kind of downstream forensic analytical methods have been used with collected samples (e.g., extraction methods, PCR-based method, elemental analysis), preservation or storage conditions (the long-term or short term storage, room temperature, or at 4°C, frozen at -20°C, -80°C), or the transportation conditions etc.

Table-2
Collection and preservation methods of DNA evidences

Sample type	Sample collection	Preservation or storage	References
Tissue	<p>Wear gloves always.</p> <p>A piece of Fresh tissue is removed with the help of scalpels or tweezers and place into a sample collection tube.</p> <p>Collect the tissue sample in sterilized airtight plastic collection tube\container without formaldehyde or formalin.</p> <p>Properly close the collection tube lid and place it in an evidence bag.</p> <p>Seal the bag and record sample details.</p> <p>Freeze the sample and ship in an ice-packed, sealed Styrofoam containers and handover to the forensic laboratory as soon as possible.</p>	<p>Freeze (for long preservation prefer to store below-80°)</p> <p>Or stored in 95-100% ethanol at ambient temperature</p> <p>(Keep away from direct sunlight and humidity.)</p>	52-62
Blood	<p>Wear gloves always.</p> <p>Use sterile, clean cotton cloth\swabs to collect samples.</p> <p>For fresh\wet blood, soak a small amount onto the swab.</p> <p>For dried blood, moisten the swab head with Milli-Q purified water then rub the swab across the dried blood.</p> <p>Dry swab sample and replace into the swab tube, seal and label.</p> <p>The control swab piece also collect to test for a contaminant DNA present on the swab or in the purified water.</p> <p>Air-dry and placed all the sealed swab samples into a clean paper bag\envelopes with sealed corners and record sample details.</p> <p>Do not use plastic containers.</p>	<p>Freeze or Dried blood sample stored at room temperature (if dried).</p> <p>Liquid blood refrigerate does not Freeze</p> <p>(Keep away from direct sunlight and humidity.)</p>	53-62
Saliva\ Buccal swab	<p>Use sterile, clean cotton swabs collect samples.</p> <p>From crime scene, collect tissue instead mouth swab (as possible).</p>	<p>Freeze or Stored at room temperature (if dried).</p> <p>(Keep away from direct sunlight and humidity.)</p>	51-62
Hair/Fur/Feathers/Scales	<p>Wear gloves always and use tweezers to pluck the hair with root.</p> <p>Collect at least 25 hair samples with hair follicles.</p> <p>Remember handle hairs by the tip, not from the root.</p> <p>Place hairs in a collection tube, airtight lid, and place tube in an evidence bag, seal and label.</p> <p>Handover to the Laboratory as soon possible.</p>	<p>Frizzed or store dry.</p> <p>(Keep away from direct sunlight and humidity.)</p>	53-64
Meat sample	<p>Wear gloves always.</p> <p>The entire meat sample should be collected and submitted to the forensic analysis agency.</p> <p>Freeze the sample and ship in an ice-packed, sealed Styrofoam containers.</p>	<p>Freeze (below-80°)</p> <p>(Keep away from direct sunlight and humidity.)</p>	53-65
Vomit	<p>Wear gloves.</p> <p>Sample of stomach contents placed into a collection tube.</p> <p>Close lid, place tube in an evidence bag, seal and label.</p>	<p>Freeze.</p> <p>(Keep away from direct sunlight and humidity.)</p>	53-65

Sample type	Sample collection	Preservation or storage	References
Feces	Wear gloves. Keep feces into a collection tube. Surface layer of feces contains the target DNA, therefore, carefully sampled. Close lid, place tube in an evidence bag, seal and label.	Frizzed or store in 95% ethanol. (Keep away from direct sunlight and humidity.)	53-66
Bone\ Teeth\ Ivory or tusk\ \Horn \shells	Wear gloves always. Place bone\teeth\horn\ivory directly into a collection tube, close and place tube in an evidence bag, seal and label. Pick up suspected bones and teeth with gloved hands and clean forceps. Submit whole bones. Cutting bones increase the possibility of contamination. Place teeth and bone samples in clear paper or an envelope with sealed corners. Bone and teeth evidence can remain at room temperature before shipping to the Laboratory for analysis.	Freeze or store in dry condition at room temperature. (Keep away from direct sunlight and humidity)	53-55,59-62,65,66
Caviar	Wear gloves and use a sterile spatula or tongue depressor to transfer of caviar (about a teaspoon) from the labeled tin into the labeled screw cap tube. Close the tube securely and seal it in the labeled plastic bag.	Refrigerate Do Not Freeze!	68

Standard guidelines for collection of evidence from crime scenes ought to be established. The best approach for searching the evidences from the crime scene is the use of technique and strategies grounded with established guidelines and experience. Law enforcement personnel appointed for the processes of crime scene investigation should be well trained and guided with proper guidelines used for collecting and handling wildlife forensic evidence.

Protocols need to be validated with a broad spectrum, there criteria should include specificity, sensitivity, maintenance of integrity, recovery efficiency, impact on analytical assays and techniques. Knowledge of the most effective technique for the collection and preservation is essential for improving the formulation of an operational plan and minimizing the effect of potential inhibitors.

Conclusion

Straightening of DNA application in criminal investigations provide promising tool to fight against wildlife crime. With the advent of DNA technology or because of the sensitivity, reliability and power of discrimination\exclusion, our judiciary system much more relies on DNA. However, DNA evidence that was not properly recognized, identified, documented, collected, preserved or packaged, transported raised questions about their authenticity and has zero value in criminal investigation. So it is very essential that strict and proper guidelines should be followed to avoid contamination for better prevention and results for interest of justice. In brief, Effective

handling of biological evidence means more chance of evidence to retain its original integrity until laboratory analysis, means more fruitful forensic results.

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