forensic sciences

Review

Wildlife Forensic Sciences: A Tool to Nature Conservation towards a One Health Approach

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Abstract: Wildlife forensics is a science field with a remarkable potential to provide accurate information regarding nature conservation and One Health. Wildlife crimes are now a persistent target of public opinion and the concern of conservation professionals, which is constantly putting pressure on governmental and non-governmental entities. Moreover, the cross-species transmission of pathogens is becoming more and more frequent, endangering the interconnected health of humans, animals and the environment (One Health). This review intends to briefly present and illustrate the potential of wildlife forensic sciences not only in crime solving, but also regarding health sciences and species conservation. By allowing target species identification, recreating the crime scene, and considering their contribution to diseases and ecosystems' surveillance, wildlife forensics are now constantly developing and improving. A cooperative and multidisciplinary approach (with the inclusion of forensic sciences) is necessary to avoid wildlife crimes, disease outbreaks, environmental disasters and to promote a sustainable and healthy future for all the living beings.

Keywords: fauna; forensics; One Health; illegal; crime; nature conservation

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1. Introduction

Wildlife forensic sciences can be defined as the application of different scientific fields to enforce the laws and solve illegal problems involving wildlife [1,2]. At first, wildlife forensic sciences may sound restricted to solve the illicit trade or criminal use of wild animals, helping to recreate the crime scene. However, the spectrum of uses of this complex scientific field is much broader, especially considering its impact on species conservation and health [1].

Wildlife biodiversity is now facing a massive decline and more and more species are becoming seriously threatened of becoming extinct, while others have already faced extinction. Some of the causes have a clear anthropogenic nature (including pollution, habitat loss, and different crimes against wildlife) [3]. Poaching (including coursing or bushmeat consumption), poisoning, illegal trade (including ornamental commercial items, pets, or traditional medicine ingredients), the introduction of invasive species, bioterrorism, illegal spills and animal cruelty are among the most common or prominent examples of wildlife crimes that threaten wildlife species worldwide (Figure 1).

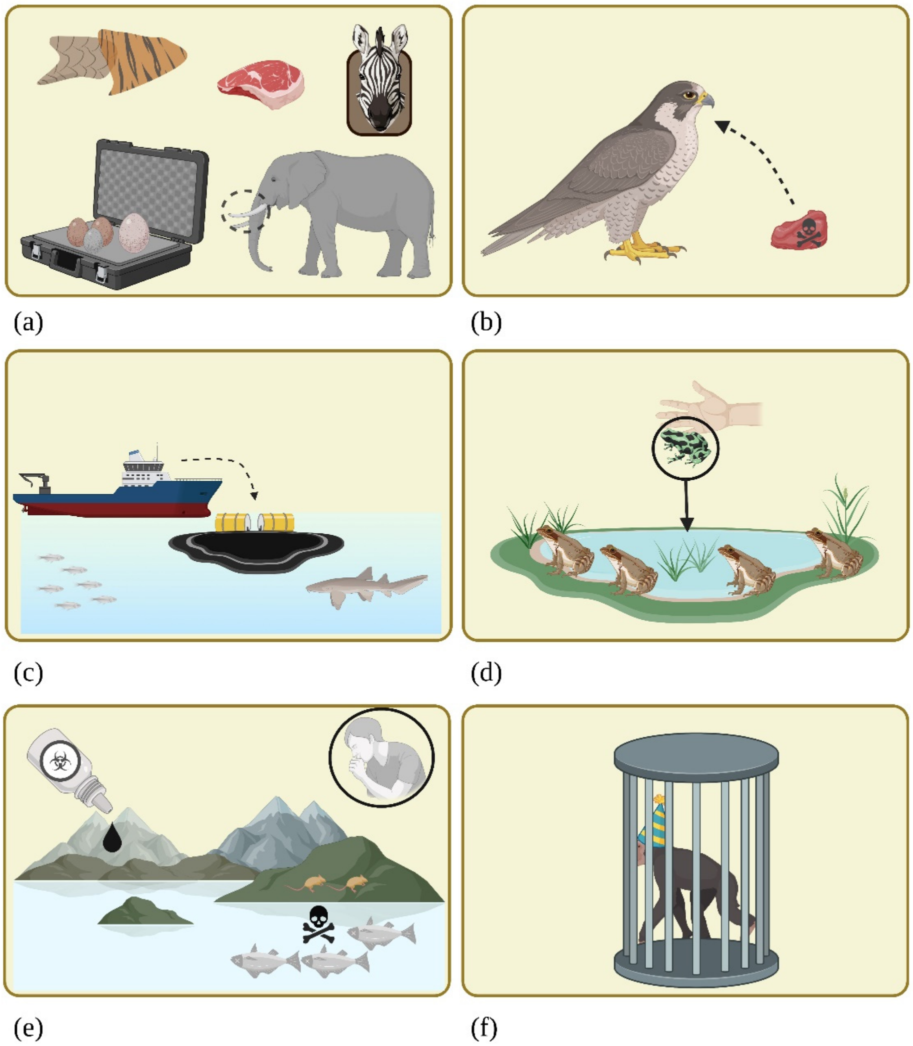
(a) (b) (c) (d) (e) (f)

Figure 1. Illustration of some wildlife crimes. (**a**) Poaching and illegal trade (of fur, eggs, ivory or other body parts); (**b**) poisoning; (**c**) illegal spill; (**d**) intentional introduction of invasive species; (**e**) bioterrorism; and (**f**) animal cruelty (created with BioRender.com, on 7 July 2022).

Wildlife forensic sciences usually comprise taxonomy, pathology, molecular biology and biochemistry, genetics and toxicology, among others (depending on the situation and the aims) [1,2,4–6]. In fact, wildlife forensic sciences may be implicated in such different tasks as species identification, prosecution of wildlife crimes, monitoring environmental changes and the study of disease epidemiology [1], which clearly illustrates its transdisciplinary nature.

One Health is a multidisciplinary and collaborative approach to global health, involving humans, animals and the environment, as three intimately connected branches. Although not recent, the One Health concept has continuously gained attention during the last few decades (due to global health threats we are already facing) [7–9] and progressively embraces new scientific fields, such as wildlife forensic sciences [10].

Therefore, this review aims to summarize the different contributions of wildlife forensic sciences in solving wildlife crime. Moreover, it aims to highlight their importance in a One Health approach, as well as to species and ecosystem conservation.

2. Methodology

Scopus®, Science Direct® and Google Scholar® were the main search tools used in this scoping review. Keywords included (but were not limited to) "wildlife forensic sciences"; "wildlife forensics"; "One Health"; "wildlife crimes"; "wildlife conservation". All the founded paper types (journal articles, book chapters, reports...) regarding wildlife forensics were included. Articles unrelated to the subject were excluded.

3. Species Identification

In wildlife forensic sciences, the identification process mostly refers to the taxonomic species involved in the crime, as well as its conservation and CITES status. Only after this process, does it become possible to determine the real consequences of the crime at a populational or even ecosystem level, to punish the responsible criminals for their actions and to practically intervene [1,11,12]. Despite the situation, methods to perform a suitable determination of the species can be divided into morphological and molecular.

3.1. Morphological Identification

Morphological identification is normally inexpensive and usually suitable to identify which species are involved in a crime scene. Some illegally traded body parts are very revealing, such as rhino horns or elephant tusks, but others may be challenging to identify. In those cases, expertise, comparative reference material and enough samples are essential to perform with success [11]. Physical identification methods include the analysis of footprints, external body parts and necropsies [1].

Footprints may provide the first non-invasive approach to wildlife crime. However, interspecific similarities and intraspecific differences in animal paws may strongly conditionate a definitive identification.

On the other hand, hair is a more significant finding to perform identification. Mammals have different hair segments, regarding length (cortex and medulla), the pattern of cuticle scale, diameter, and color. These aspects can be analyzed with compound optical or scanning electron microscopes [1,13]. Moreover, hair is considered as more resistant to post-mortem decomposition due to its content of keratin and dead keratocytes, even though some changes usually happen, such as root banding [14,15].

However, hair is not the only skin coating present in animals. For instance, reptiles and fish have epidermic and dermic scales, respectively, that cover their bodies. Reptiles have scales made of keratin. Fish may present different scale types, depending on the species. Bony fish have scales made of dentine and enamel (like teeth), while cartilaginous fish present placoid scales (spiny and small scales, usually called dermal denticles) [16,17]. Like hair, reptile scales present different colors, shapes and patterns that may work as a visual fingerprint [18]. Considering fish, scales have been used to distinguish genera, species and even local populations. Different fish taxa present distinct allometry, shape and patterns, depending on several aspects, such as the growth rhythm or swimming mode [19].

Furthermore, feathers are also valuable for wildlife forensic sciences. Feather nodes, barbs, barbules and pigmentation patterns are morphological details that can be used for identification. As an illustration, doves' feathers have nodes that have a distinctive "crocodile shape" associated with long barbs and barbules, which are pathognomonic of doves [20].

We have decided to mention internal organs and necropsies in Section 4, even though they may also play a complementary role in morphological identification [1]. However, most of the time, it is in determining the cause of death that necropsies are irreplaceable.

3.2. Molecular Identification

Mitochondrial genome and nuclear genome are the two major molecular tools used in wildlife forensic sciences in the identification of the species, population gender or (when possible) the individual, parentage analysis and phylogenetics [1,21].

Nevertheless, mitochondrial DNA is the most used choice in wildlife forensic sciences, due to the location in the cell and availability; and other features that make it more probable to be isolated from the evidence. Moreover, mitochondrial DNA presents regions with lower mutation rates, and regions with higher mutation rates; both are useful in identification. For instance, lower mutation rate regions (ribosomal RNA (12S)) will be helpful for higher-level taxonomic classifications (e.g., kingdoms, classes). Higher mutation rate zones (as cytochrome b (cytB)) will be used at closely related taxa (at a species level). The constant development and update of mitochondrial DNA databases is crucial to progressively pinpoint this comparative method and allow forensic scientists to identify individuals at a subspecies or populational level [11,22,23]. Moreover, mitochondrial DNA is also being used to study animals' age, longevity determinations and age-related diseases through a database (MitoAge®), with more than nine hundred species registered [24].

The use of nuclear DNA in wildlife forensic sciences is mostly dependent on Short Tandem Repeat (STR) analysis, based on an animal's genetic profile. Nuclear DNA markers include microsatellites, Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), and Short Sequence Repeats (SSRs). Once again, more relevant, and complete nuclear DNA databases are needed to properly generalize the use of these techniques in wildlife forensic sciences [21].

4. Characterization of Criminal Actions

4.1. Animal Death

In some wildlife crimes, the illegal act consists of intentionally or accidentally killing wildlife, due to the species, methods or purposes involved. In these cases, detailed history, necropsy and documentation are essential to perform a timeline of events and obtain proper conclusions regarding the cause of death. A pathologist with experience in wildlife and records of every observation are also very relevant [6].

Some crimes, such as poisoning, require complementary analysis (i.e., toxicology) to identify the substance responsible for the death or injury, in association with routine necropsy. This is important because poisoning may have no pathognomonic gross or histopathologic findings. Routine sample collections in those cases should include liver, kidney, urine, blood and stomach content. However, it is always advantageous to have a general idea about what substances should be screened based on the history, location or lesions found in the animal. In most substances, stomach content should be carefully analyzed because it may contain the bait and/or solid or liquid toxic substances [6]. An animal intoxicated by acetylcholinesterase inhibitors (such as organophosphates or carbamates) may show signs of convulsion, salivation, urination, defecation or emesis [6,25,26]. After death, analyses of the stomach content (to determine the substance) and brain (for acetylcholinesterase testing) confirm the cause of death. On the other hand, rodenticide poisoning (such as warfarin, brodifacoum or bromadiolone) usually cause external or internal signs of unproper coagulation (petechiae, bruises and other signs of internal bleeding). A determination of the anticoagulant substance on the liver confirms the diagnosis [6,27,28]. Considering wildlife, the intentional carbofuran poisoning of vultures (which are under threat in many regions of the world) in Kenya was detected. Carbofuran is known to be highly toxic to many species and it is prohibited as an agricultural pesticide in most countries [29].

When the cause of death is a gunshot, evident lesions may be present, such as lacerations, fractures, compressions and crushing wounds with or without cavitation. Embolization or secondary infection may also occur. A proper examination of these lesions may provide important forensic details. Entrance wounds are usually smaller, round and more discrete than exit wounds. Abrasion of the wound's marginal tissues may be visible. However, the maximum bullet diameter can only be accurately determined in the bone (if the bullet reaches this tissue), due to the laxity of the skin or other soft tissues [6,30]. Before the internal necropsy, radiographs should be taken to find all bullet fragments and determine the bullet direction. During the internal examination, angles and bevels in bones also help to describe the bullet's travel [31]. The collection of the fragments must be conducted carefully without damaging the surrounding tissues, and with plastic forceps. Then, those fragments should be measured or photographed with scale. Vials or small paper envelopes are suitable to store and send them to ballistic analysis (e.g., gun type, bullet caliber) [6,30].

Other causes of death in wildlife forensic sciences may include starvation, thirst or drowning. All of these require specific and challenging identification of primary and secondary alterations in the body to declare them as death causes. Even when detailed information is available at the crime scene, some of them may only be determined by exclusion. For instance, in a suspected drowning, it is difficult to establish if the animal was not already dead when the body entered the water. Fluid in the respiratory tract is indicative of a possible drowning. Moreover, the docimasia test (or hydrostatic test) may also help determine whether there was still air circulating in the respiratory lung when the animal died. A lung fragment is thrown into a water recipient, and, under normal circumstances, it should float due to the air present in the alveoli [32]. However, this is not pathognomonic of drowning. Histopathologic changes may include the enlargement of the alveoli, bleeding, the presence of foreign material in the deeper respiratory tract (alveoli) or concomitant swallowed water, to support this hypothesis. In wild birds, tiny connections between bones and air sacs may lead to the active entrance of water in the medullary space, in case of drowning. Nevertheless, it is implausible to be seen when water passively enters a carcass thrown into the water after death [6,33,34].

Whatever the cause of death, a complete and detailed forensic pathology report can provide an essential contribution in terms of legal and juridical compliance. Photo documentation of every lesion, foreign body or crime scene evidence (with proper light and measurements) is also crucial to support written descriptions [6]. Rulers and scales must be used to provide an adequate idea about the size and layout of a lesion or object. Some of these instruments are L-shaped and provide important geometrical references in photographs to avoid the misinterpretation or manipulation of them [35]. Everyone present at the crime scene and during the forensic procedure must be registered [6].

4.2. Other Crimes

Wildlife forensic sciences also play an essential role in explaining what happened to a live animal. Using animals for illegal purposes (e.g., pet trade, breeding, entertainment, ceremonial use, neglecting or even zoophilia) is an unfortunate reality that requires a detailed forensic investigation and, if possible, the identification of the people responsible. A medical examination of the confiscated animals may indicate their illicit use, any diseases, and help to decide where the animal should be placed (e.g., re-introduced to the wild or captivity) [36].

5. Timeline of Events

The determination of the post-mortem interval in a carcass is crucial to establishing a logical timeline of events in forensic investigations. Accuracy in this determination has been consistently high over the last decades, without major improvements. Using a combination of methods (rather than a single procedure) is highly recommended. Estimating the time of death is deeply dependent on several geographic, environmental or circumstantial factors. Therefore, the obtained post-mortem interval is always approximate. Even though most determination strategies have been developed for human bodies, an adaptation to animal species also allowed wildlife and animal pathologists to estimate post-mortem intervals in other species [37].

Different disciplines and methods contribute to this goal, such as gross pathology, histology, taphonomy, temperature-based methods, post-mortem chemistry, molecular methods, microbiology, radiology and entomology. A detailed explanation of the theoretical basis of every single forensic strategy to determine the post-mortem interval goes far beyond the purpose of this article, and are not specifically applied to wildlife forensics but to all other forensic areas instead. However, we have decided to present some examples [37,38].

Forensic entomology is a discipline that uses the time of insect colonization, insects' identification, and the development stage of these colonizers to estimate the post-mortem interval. Geographical and environmental factors (temperature, humidity) influence this colonization, depending on the insect species. Considering wildlife, the same species may present distinct insect colonization depending on the region of its death [37–40].

Forensic radiology is another discipline in forensic sciences that uses imaging techniques, including standard radiography, computed tomography, and magnetic resonance imaging. The application of these last two diagnostic imaging techniques to study dead carcasses is called virtopsy. Besides providing details about the cause of death or any lesions, some imaging findings are typical or very usual in a particular period after death. For instance, gas accumulation present in some body parts (chest, mediastinum, gastrointestinal tract) but absent in others may suggest a post-mortem interval [37].

Forensic taphonomy studies what happens to a body between death and the recovery of the body parts. Besides contributing to determining the post-mortem interval, forensic taphonomy may reveal other vital details in the crime scene, such as the movement of the carcass. Some Latin terms are commonly used in forensic taphonomy to describe alterations and help pathologists determine the post-mortem interval and correctly evaluate if changes in corps are ante or post-mortem. Rigor mortis happens when the body cannot break actin-myosin bridges (i.e., there is no longer adenosine triphosphate being produced) present in the muscle tissue, leading to generalized muscle contraction and inability to move joints. Pallor mortis happens when there is a collapse of capillary circulation, and the body becomes progressively pale, which may be difficult to evaluate in animals with dark pigmented skin. Livor mortis also occurs with the failure of blood circulation; by gravity, blood begins to settle to the lowest parts of the body; when livors are fixed, they can allow to determine if the corps was moved, or if the crime scene was manipulated. Algor mortis results in the continuous cooling of the body until reaching the environment temperature [41–46] and varies with environment temperature and contact with good heat conductors. Environmental conditions (temperature, humidity, contamination), the species involved, and concomitant lesions or diseases are among the factors that may influence this timeline of change [45–47]. As an illustration, when a dead body goes under dry environmental conditions (cool or warm) for several weeks, with very low humidity but proper ventilation, the body may suffer mumification, rather than a more usual decomposition, presenting distinct characteristics [37]. Furthermore, unfortunately, limited taphonomy information applied to wildlife species (such as birds of prey) allows such rigorous post-mortem interval determinations, as it is carried out in human forensics. Data on algor mortis are available from experimentally controlled studies for most game species, but vigorous activity of some animals right before death may conditionate determinations based on algor mortis and rigor mortis. Finally, the physical manipulation of carcasses may also alter the development of rigor mortis [40].

6. Wildlife Forensic Sciences Global Contributions

6.1. Nature Conservation

As illustrated above, wildlife forensic sciences are essential in describing a series of illegal events based on diverse evidence. Predicting and solving wildlife crimes contributes to the stability, health and well-being of wildlife populations. Five agencies unite themselves to create the International Consortium on Combating Wildlife Crime (ICCWC), responsible for fighting wildlife crimes globally. This effort includes the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the World Bank, the United Nations Office on Drugs and Crime (UNODC), Interpol, and the World Customs Organization (WCO) [12]. The UNODC 2020 report mentioned that approximately 6000 species have been seized, between 1999–2018, including mammals, birds, reptiles, corals and fish. There are no single species responsible for more than 5 per cent of the incidents [48]. Despite this number of agencies and organizations clearly focused in wildlife conservation, more than 70% of the cases of wildlife crimes become unsolved due to an inappropriate identification of species, items or crime evidence [1].

Wildlife crimes represent a significant part of the list of conservation threats that affect wild species. In almost every part of the world, it is possible to find traces of its existence.

However, some geographic regions clearly occupy the top positions, contributing more significantly to this serious biodiversity threat. Asia (and especially Southeast Asia) is a huge biodiversity hotspot, where several endemic animal and plant species are threatened by extinction, and is also a hotspot of wildlife illegal trade markets. Some colossal numbers of wildlife traffic are reported, such as 225,000 kg of ivory or 3800 bears that have entered the illegal trade market during the past 10 years [1,49]. The Golden Triangle (Thailand, Myanmar, Laos and China) is a global hub for trade in some of the world's most endangered species. Tigers, elephants, bears and pangolins are some of the most wanted species in Golden Triangle markets, according to the WWF [50].

Wildlife crime is a severe concern, threatening nature conservation, the economy, politics, health, security and culture, regardless of where we come from [12]. Stopping wildlife illegal acts requires intense monitoring and cooperation from different professionals and institutions to prevent more cases and legally punish their perpetrators. Particularly for the Golden Triangle, closing markets and increasing enforcement are among the proposed measures [50]. Wildlife forensic sciences are an integrated part of these procedures [51,52].

6.2. One Health

A not so recognized role of this scientific field is its contribution to achieving One Health. Reeve-Johnson and Bailey (2016) are two of the few authors that discuss the importance and possibilities of allying these areas of knowledge. These authors also raise awareness of translation research into One Health research, also considering forensics and crime [10]. Figure 2 illustrates the One Health concept and the application of different wildlife forensic sciences to distinct threats.

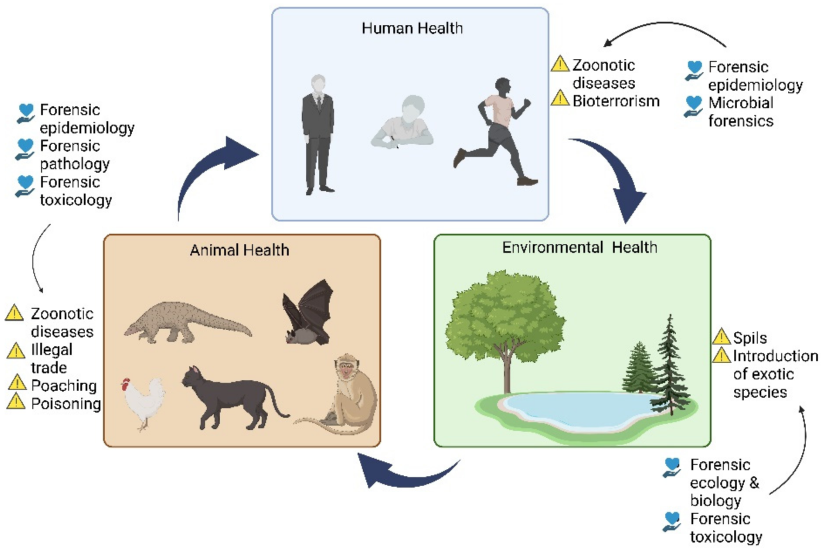

Figure 2. Application of different wildlife forensic sciences to the One Health concept. Besides the three branches of One Health (human health, animal health and environmental health) some illicit actions that mostly affect each part are represented, as well as the forensic science that may give an important contribution to its prediction or resolution (created with BioRender.com, on 22 September 2022).

Wildlife forensic sciences, such as forensic epidemiology, may give us a broad perspective about a disease outbreak (that might affect different species, including humans). Forensic sciences allow health professionals to classify an outbreak as natural, accidental (due to a possible non-compliance of health measures) or intentional (as bioterrorism). Moreover, new disciplines in forensic sciences are getting their relevancy, as microbial forensics. Recent discoveries and improvements in DNA sequencing have allowed a global analysis of entire bacterial communities and ecosystems. Bacterial genes from organisms, which are part of an ecological niche, have been analyzed by researchers for use in forensic investigations of different types [37]. Microbial forensics has been helping to identify the source of significant human and zoonotic disease outbreaks (such as Anthrax, HIV or West Nile Fever). Advanced technology and genetics are considered the main tools for these achievements.

Microbial forensics is undoubtedly an underdevelopment field with global potential, also due to its contribution to preventing and managing bioterrorism [53]. Biological weapons have been part of wars for a very long time, with a vast potential for harm to humans, animals and other living beings. Therefore, bioterrorism is clearly a One Health threat [54]. Bioweapons with zoonotic pathogens are 80% of the considered "priority bioterrorism agents" (Class A) [55]. Moreover, considering that more than 71.8% of zoonotic diseases originated in wildlife [49], the importance of wildlife forensic sciences in a One Health approach becomes even more evident.

Imbalances in the ecosystems may occur due to wildlife crimes, such as the illegal introduction of exotic species, mass poaching, illegal trade or an intentional spill. These will severely threaten the living beings (including humans) that are part of it, not only due to a possible introduction of new infectious agents (bacteria, viruses or fungi) in those populations [56], but also chemical changes in soils and water (such as oil mixtures) [57,58]. Wildlife forensic sciences may contribute to establishing a timeline of events and finding a primary cause for these health hazards (forensic ecology), leading to a better resolution or the prevention of these consequences [53].

7. Conclusions

The different fields and subjects that comprise wildlife forensic sciences have a remarkable impact on the different phases and procedures of wildlife crime. From species identification to establishing a timeline of events, many subdisciplines (as pathology, entomology, taphonomy or microbiology) contribute to answering the right questions in a crime scene.

Several species are very close to extinction and others may become so due to a variety of wildlife crimes. Thus, conservation actions and nature preservation may become ineffective if illegal trade, poaching or other illicit actions are not properly approached.

Humans are part of ecosystems, even though some societies have almost completely lost their direct connections with nature and other living beings. Therefore, the wider importance of wildlife forensic sciences regarding human and animal health (One Health) should not be underestimated.

A more cooperative approach of health sciences, forensic sciences, nature conservation and technology can positively contribute to a faster resolution of most wildlife and global health crimes. Big data analytics and/or artificial intelligence (already developed and applied in other areas) may also be applied in this field, contributing to a well-structured and organized chain of detection, detention, evidence collection, public health surveillance and communication. Multidisciplinary teams, with distinct professional backgrounds, are also important in extracting and interpreting information from various perspectives. Thus, with all this, it will be possible to stop wildlife crimes, and prevent disease outbreaks, environmental disasters or species extinction.

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