Chapter

Noninvasive Sampling: Monitoring of Wild Carnivores and Their Parasites

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Abstract

This chapter aims to present the importance, advantages, and disadvantages as well as the different types of noninvasive samples that can be used to monitor the carnivorous fauna and the parasitic agents that can infect these animals. This issue is extremely relevant, since noninvasive sampling has been increasingly used in different scientific researches that study animals with elusive habits, such as carnivores, and that claim animal welfare, once these animals do not need to be observed or captured. It is still important to highlight the scarcity of studies on parasitic diseases in free-living carnivores, being needed that parasitological surveys be done frequently by the conservation unit managers also to monitor the infectious agents that may be being introduced into the ecosystem of carnivores due to anthropization.

Keywords: gastrointestinal parasites, wild carnivores, coproparasitologic, trichology, molecular biology

1. Introduction

1.1 Animal identification from noninvasive samples

The study of free-living wild animals is a challenge for researchers for several reasons, including obtaining biological samples from these animals. There are three main types of sampling: destructive sampling, which is a strategy whose biological samples, mainly tissue, are obtained from animals that have been killed; nondestructive sampling in which the animal is normally captured and biopsy or

blood collection techniques are performed in an invasive manner; and noninvasive sampling, that is, a strategy in which biological samples are obtained without the capture or manipulation of the animal [1].

Noninvasive samples are traces left by the animals in the places where they live, including hairs and loose feathers, feces, and other remnants of the diet [1, 2]. Noninvasive sampling is a strategy widely used by researchers in field studies, mainly biologists, since this method allows studies of free-ranging animals without the need to capture, manipulate, or even observe them [1, 3]. In this context, the analysis of noninvasive samples becomes an alternative with great cost-benefit for monitoring and, consequently, for the conservation of species, mainly free-living animals with nocturnal, elusive habits and that present low population densities, like carnivores, and those living in places of difficult access [4].

Among the different types of noninvasive samples, feces are ideal tools for indirectly analyzing free-ranging wild animals and the rocky areas, dry as well as frozen ecosystems provide the best conditions for stool preservation [4, 5]. By analyzing fecal material, it is possible to obtain information about the natural environment, including identification of the species that inhabits the region, composition of its diets, the function of that animal in the ecosystem, such as seed dispersal or population control of other animals, data on the taxa of prey ingested, especially in the case of carnivorous and omnivorous animals, and on the dynamics of gastrointestinal parasites in the environment and between animals; this is one of the major causes of mammalian fauna decline.

Thus, animal identification from the feces collected in the environment is very important and is possible by means of macroscopic analysis of fecal material [4], trichology of guard hairs [6], or using molecular biology techniques for the detection of animal DNA [1].

One of the ways of identifying animal taxa from noninvasive samples is by analyzing the morphology of feces or diet remains in these samples. The evidenced dietary components, such as claws, bones, teeth, and feathers, as well as the shape, size, and odor of feces are peculiar characteristics that can differentiate some animal groups. The use of morphological analysis in the study of feces of free-living wild animals is very advantageous, especially in field work, since it serves as an initial screening of the samples to be collected, allowing the classification of feces reliably, at least up to the category of order [7]. According to this author, this type of analysis is not such a safe resource for classification of samples up to family taxonomy, much less in gender or species. One of the disadvantages of using macroscopic analysis is that feces of free-living carnivores are exposed to suboptimal conditions for long periods of time and under the influence of different environmental circumstances, which may contribute to the loss of physical characteristics of the material and compromise a more reliable analysis of the sample [4].

In general, feces produced by carnivorous species have a cylindrical shape, are long (sausage type) with subdivisions, and presented one of the sharp ends. In the case of felids, in addition to the characteristics described above, it can be observed macroscopically that the feces tend to be more compact and have well-defined subdivisions and one of the ends is especially tapered and even slightly twisted. Stool diameter is also a very important feature to consider when estimating the size of the animal, that is, and to distinguish feces from small and large felids. In America, when fecal material is larger than 2.1 cm in diameter, they generally belong to large felids, such as *Puma concolor* and *Panthera onca* [4]. However, it is important to emphasize that the size and quantity of fecal cake produced by carnivores vary according to their age and type of feed intake. Therefore, large feces of carnivores that are still cubs can be easily confused with fecal material of carnivores of small size, for example.

Large carnivores are territorialistic animals, and when they are in reproductive or emotional times, they use different strategies to demarcate their territory [8, 9]. Usually, the territory marking is done through the urine (odoriferous marking), being this an important carrier of chemical information, or feces. A curious fact is that wild felines, such as *Panthera*, *Puma*, and *Leopardus*, do not bury their feces, contrasting with habits performed by domestic cats, which defecate in places where they can be buried [8]. This can be explained, since the burying of feces is related to the dominance or subordination of the animal. In this context, wild felines, which are dominant animals, do not hide their feces, leaving them visible for the demarcation of their territory [10].

Omnivorous animals' feces, such as canids, mustelids, and procyonids, fruit remains, seeds, insects, crustaceans, and plant remains are most commonly found. It is also worth mentioning that the fecal material of canids has a characteristic odor and, in the case of some species of canids such as *Chrysocyon brachyurus*, it has been verified that the diameter of the fecal material has been presented much larger than the feces of great felids, being these potential factors for the differentiation of these animal groups [4].

Using macroscopic analysis, the stool should be weighed with the analytical balance. Afterward, it is relevant that the researcher registers the color, presence of artifacts, and possible components of the diet, as well as the measurement of the length and diameter of all fecal propagules collected with the aid of a pachymeter. Subsequently, all the information obtained needs to be compared with the literature for the taxonomic classification of the fecal material author. Since there is little information about mammals' stool morphology and measurements in the literature, the comparison of all the feces collected is really difficult, especially in the cases of feces belonging to small neotropical wild felids.

Another mean of identifying animals from noninvasive sampling is analyzing lost hairs left in environment by trichology techniques. Hairs are keratinized epidermal attachments characteristic exclusively of mammals, being the second part of the body of the animal with greater durability [11]. Macroscopically, it is possible to distinguish two major regions in the hairs: the shield, characterized by being a longer and thicker distal region of the hair, and the stem, which is the sharpened portion and close to the bulb [12]. Morphologically, the hairs are composed of three layers: the cuticle, which is the outermost part; the cortex, which is the middle layer; and the medulla, which constitutes the innermost portion of the hairs [13, 14]. The cuticle consists of superimposed transparent keratin scales. From the base of the hairs, the distal portion of each scale lies on the proximal portion of the scale located above. Due to this conformation, the hairs have less resistance at their base when compared to their distal end [12]. The classification of the cuticular pattern can be established by the analysis of the imbrications, ornamentation, and continuity of the edges of the scales, shape, dimension, and oration of the scales [6]. In relation to the cortex, its thickness is what determines the width of the hair, being formed by keratinized, fusiform, small, and coalescent cells in a quasi-homogeneous hyaline mass with vacuoles and pigmented granules that can be organized as an amorphous mass [13]. Since the medulla is composed in a similar way to the cortex, however, its cells are clearly visible. Cells and air-filled spaces between intracellular connections are responsible for conferring the marrow characteristic [12]. The medulla can be classified according to its presence and continuity, number of rows, disposition and shape of the cells, and ornamentation of marrow margin [6].

The coat of most mammals is basically composed of two distinct types of hairs: the guard hairs, also called overhairs, which are the longest, smooth, and usually much pigmented, and the underhairs which are finer, shorter, and less pigmented

and can be curved or curled [15]. In general, underhairs are more numerous and cover most of the animal body; therefore, they have as main functions the mechanical protection against impacts and the thermal regulation of the mammalian body. The guard hairs mainly present sensorial functions, being constituents of antennas, mustaches, vibrissae, and other regions with tactile functions of the mammals [14].

Some characteristics of guard hairs are used to identify mammalian species, since the combination of the cuticle, medulla, and cortex presents morphological patterns that confer specific diagnostic characteristics to a particular mammal species [6]. The microstructure of guard hairs is a useful tool in the identification of mammalian species, being applied several areas of research such as forensic science, ecology, epidemiology, archeology, and paleontology [6]. In this context, guard hair analysis is increasingly being used by researchers in mammalian ecology studies, mainly in the identification of the predator and diet analysis from noninvasive samples collected in the environment [11]. In the case of carnivores, especially the felids, self-cleaning as a habit of corporal hygiene allows that in their fecal material, a great quantity of hairs, including those by the guard, are evidenced. In addition, the study of hairs deposited in museum collections in order to help the understanding and standardization of the nomenclature of the cuticular and spinal patterns, as well as the use of this material as reference, has been increasingly adopted in studies with noninvasive free-living animal samples [11]. It should be emphasized that the guard hairs of the ear, head, neck, paws, and tail have different microstructural characteristics from those of the hairs of the rest of the body, which are the majority, being detrimental to identifications.

The cuticle scales vary in size and shape depending on the region of the hairs being analyzed. Normally, at the extremities of the scales, they are small in size, whereas in the wider portion of the shield, the scales are larger and are arranged transversely to the larger axis of the hair. In the stem, in turn, a greater variety of cuticular patterns occur, being this a region of high diagnostic value and the best part of the hair for the differentiation of groups or species of animals. As for the medulla, the best region of the hair for its observation would be the broadest part of the shield [12].

In order to perform trichology, it is necessary to separate a portion of each sample [6]. First, the fecal samples have to be submitted to the washing, drying, and storage stages in the laboratory. The washing step is done in a sink with running water and the aid of fine-mesh tampons with 1 μm diameter for the separation of any type of hair and removal of the fecal material remaining. All hairs can be placed on a sheet of white paper labeled with the sample number for drying at 37°C in an oven. After drying, all the hairs, including the guard hairs, are stored in satin plastic bags. Afterward, the guards were separated on a white surface, which can be another sheet of paper, using two tongs, on a white-lined bench. The hairs can be separated into individual plastic bags according to their length and morphological similarities. In addition, it is also important to separate artifacts such as claws, feathers, seeds, scales, small bones, and other nondigestible materials. Then, some selected guard hairs recovered from the fecal samples are submitted to cuticle printing and medulla diafanization in order to find the predator's guard hair [6].

Among the advantages offered by trichology, also observed by our research group, is the identification of the animal species, both of the predator and of possible prey [6, 16]. In addition, it is possible to obtain this material for analysis from noninvasive samples, mainly feces, with no manipulation or encounter of the animal needed to obtain hairs. Another factor that contributes to the performance of trichology in the field of research is its cost-benefit, since the reagents and utensils used can be obtained easily and at a low price, such as nail polish, lathe, plastic bags, slides, and commercial hydrogen peroxide [16]. The disadvantages faced are

the difficulty of recovery of the predator's guards, since the volume of ingested hairs is significantly lower when compared to the ingestion of a prey, especially rodents. In this context, the macroscopic selection of the guardians of predators for analysis can often be ineffective. In the case of carnivores that eat other animals of the same order, trichology becomes a complementary tool, and other techniques are necessary for a more specific diagnosis. In addition, since the hairs are very delicate objects, it is necessary that the room where the researcher is working be isolated in order to prevent the hairs from dispersing in the environment [17]. Another obstacle would be the difficulty in producing high-quality slides for a reliable diagnosis of the species, especially with hairs obtained from fecal samples, that are often deteriorated or fragmented [6, 12, 15]. The most difficult cuticula pattern for our group to print was those with pavement wave type of scale imbrication, and the easiest ones were the hairs composed by imbricated foliaceous scales.

A third method that can be used in mammals' identification is the DNA analysis by molecular biology. The use of molecular markers for the study of free-living wild animals from noninvasive samples such as feces and dietary components has been increasingly applied in the research field, especially among carnivorous species with low population densities [1, 18]. From the molecular analysis, it is possible to obtain precise taxonomic information on the species, sexing, ecology, distribution, population estimates, and behavior of these animals, including their eating habits, reproductive preferences, and the pathogens that may be infecting these animals [1, 19, 20]. The main sources of DNA obtained from noninvasive samples are hairs, feces, urine, feathers, snake scales, skins, eggshells, and even skeletons. In the case of DNA analyses from fecal material, studies have shown that colon wall epithelial cells eliminated by the animal at the time of defecation are reliable sources of genetic material for identification and investigation of other information on the feces author [19, 21].

In relation to the molecular markers used in noninvasive samples for identification of the animal species, several primers have already been described, and many of them have been adapted, mainly from mitochondrial genes. Some characteristics of mitochondrial DNA such as the absence of recombination, high rate of evolution, and the large number of copies in the cell are the main advantages of its use as molecular marker, unlike nuclear genes [22]. The first molecular markers used to identify animal species were those named in the "universal primers" literature that amplify homologous fragments of several species, such as cytochrome b (CytB), which amplify fragments of 307 base pairs [21, 23, 24]. Another primitive also used was the cytochrome C and oxidase I subunit (COI), which amplifies about 650 base pairs and which was initially described to identify insects but which has also been widely used for the study of vertebrates [25]. In the case of animals inserted at high levels of the food chain, "universal primers" are poorly indicated for the identification of the predator because they also amplified nucleotide fragments of other animals, such as prey, and are therefore nonspecific [26–28]. All over the years, the mitochondrial genome has been extensively studied in the free-living mammals, such as the 16S region [28], the control region [29], ATP6 [28, 30], and 12S [27]. These genetic markers enhance the chances of success in polymerase chain reaction (PCR), since they amplify smaller DNA fragments and increases the probability of degraded DNA detection in noninvasive samples [28].

Despite all the advantages provided by the molecular methods for the study from noninvasive samples of wild animals in free life, these also present a series of limitations. Some of the obstacles faced in obtaining the DNA sequences of interest are the low quantity and low quality of genetic material in the samples, the extraction, and amplification method employed. This is because normally noninvasive samples of wild free-living animals are in the environment exposed to climate conditions,

which may cause degradation of the genetic material present therein [1]. Since the genetic material is very deteriorated, it is important not to dilute with pure water the reagents and the carnivore DNA in the tube when performing the PCR. The use of water to complete the required volume in several standard protocols can lead to a lower sample amplification rate. In accordance with our experience, it is therefore recommended that the water be completely withdrawn so as to increase the chances of DNA application in noninvasive samples and to obtain a minimum volume required for PCR, purification, and sequencing. In addition, the presence of genetic material from other organisms, such as prey, mainly on samples of large carnivores, plants in the case of omnivorous or herbivorous animals, as well as bacteria and fungi, may produce nonspecific bands or even void the amplification of samples in the PCR [1].

1.2 Parasitism in free-living wild carnivorous mammals

Over the years, mammalian fauna has been declining more and more throughout the world for several reasons. Some of these factors are run over, the growing rapprochement between wild and domestic predators and breeding animals, the expansion of the agricultural frontier, formation of cattle pastures, and deforestation, which reduce natural environments, as well as increase environmental pollution, fur trade, and lack of prey in the natural environment [31, 32]. Another factor that can culminate in the diminution of this fauna is the parasitism by different etiological agents, like microorganisms, helminthes, and even arthropods, highlighting the gastrointestinal parasitosis. Wild mammals are constantly subjected to environmental conditions that favor the spread of parasites, even when restricted to restricted areas and populations [33].

The relationship between the environment, parasites, and hosts is extremely dynamic and has many equilibrium points that were reached during long periods of evolution [34]. The environment is the place that presents biotic and abiotic resources that allow the encounter, the survival, and maintenance of the life cycle of parasites and hosts. The parasites have the capacity to infect a large number and variety of hosts and, therefore, have important functions in the structuring of the communities, exerting great impact on the biodiversity and ecosystem dynamics [35]. Wild animals (hosts), on the other hand, present different degrees of susceptibility for a particular parasite and, thus, interfere both directly and indirectly in the success of parasitism by different etiological agents in ecosystems [35, 36].

The susceptibility of hosts and the ability of parasites to invade and colonize them are related to several factors, including the taxonomy, morphology, body size, and eating habits of the host in question [36]. Normally, species of taxonomically related hosts are susceptible to infections by the same species of parasites [37]. Therefore, the greater the taxonomic distance, the less likely that host parasites have characteristics compatible with other potential hosts [36]. Other aspects that interfere in the parasite-host relationship are the body size and the morphology of the animals. The thickness of the tegument, for example, and volume of the organs influence the invasion and survival capacity of the parasites in the host organism, and body size has great importance in the selection of foods to be eaten as well as in the place where the animals go hunting [38]. The feeding of the hosts has direct and indirect relation with the susceptibility of the same to the parasitic infections. Carnivorous diets are harmful to infections by intestinal protozoa, whereas herbivorous diets increase their potential for infection. In addition, plant-rich diets may exhibit antiparasitic effects [39].

Gastrointestinal parasites are one of the groups of agents that are transmitted and transmitted from one host to another in protected areas through predation,

ingestion of water, or contact with contaminated soil. In general, helminths have been more reported than protozoa in free-living carnivores. This marked frequency of helminths in the different researches shows that the environment in which these animals circulate maintains favorable conditions for the maintenance of the cycle of nematodes, cestodes, trematoids, and acanthocephals, as well as the transmission of infective structures to these animals. The type of feeding ingested by the hosts can directly or indirectly affect the susceptibility of these animals to parasitic infections. Animals with meat-rich diets are more likely to have low prevalences of intestinal protozoa infections, whereas omnivorous or herbivorous diets increase the prevalence of infection by these agents. In addition, it is known that some plants that are ingested by animals may exhibit anthelmintic properties and the very friction of plant fiber may help omnivores and herbivores to purge helminth infections [36].

In Mexico, in tropical forests located in Veracruz and in the Yucatan Peninsula, 58.1% of positivity was reported in noninvasive fecal samples of wild felids identified using molecular techniques and with the aid of sniffing dogs. Two parasitological techniques were used, one of flotation and the other of spontaneous sedimentation [48, 49]. Among the parasites diagnosed were *Spirometra* sp. (33.5%), *Strongyloides* sp. (18%), *Physaloptera* sp. (11.4%), *Spirocerca* sp. (9%), Taeniidae (7.2%), phylum Acanthocephala (6.6%), *Ancylostoma* sp. (6.6%), *Toxocara* sp. (6%), ascarid-like eggs (3%), Coccidiasina oocysts (2.4%), *Capillaria* sp. (1.8%), *Gnathostoma* sp. (1.8%), *Uncinaria* sp. (1.8%), *Trichuris* sp. (1.2%), eggs of Anoplocephalidae (0.6%), and phylum Nematoda (0.6%). The authors observed that the parasitic communities of jaguar and puma were more similar between host species in the same forest type than among hosts inhabiting different forest types, which may have been influenced by the ecosystem differences and host evolutionary history, as well as disparate diet and habitat use of these two felines [40].

In Brazil, in Serra do Cipó National Park, 95% of positivity for gastrointestinal parasites was diagnosed in noninvasive fecal samples of *Chrysocyon brachyurus* and *Cerdocyon thous* identified macroscopically. The authors used three coproparasitological techniques, being two of spontaneous sedimentation [50, 51] and one of floatation [52]. The evolutionary forms detected in this study were mainly eggs of Trichuridae (68.4%). In addition, eggs of Ancylostomidae (52.6%), *Physaloptera* sp. (7.9%), Diphyllobothriidae (7.9%), Hymenolepidae (7.9%), *Toxocara* sp. (2.6%), Acanthocephala (2.6%), *Dipylidium caninum* (2.6%), *Isospora* sp. oocysts (2.6%), and *Strongyloides* sp. (2.6%) were also diagnosed. Despite the report of domestic dogs in Serra do Cipó National Park, signs of domestic dogs, such as feces, were found only in adjacent areas of the park where there are people communities. However, local residents reported seeing wild animals in the vicinity, indicating the possibility of a future proximity between these animals and perhaps their parasites [41].

After analyzing noninvasive fecal samples identified as felids by trichology in Serra dos Órgãos National Park, evolutionary forms of gastrointestinal parasites were detected in 88.6% of the feces analyzed using four different coproparasitological techniques, being two centrifuge floatations [53–55], one centrifuge sedimentation [51, 56], and one spontaneous sedimentation [57]. In this study, eggs of the Diphyllobothriidae family (65.8%) were the most detected parasites, followed by superfamilia Ascaridoidea (43.9%), nematode larvae (30.5%), Strongylidae order (21.9%), nonsporulated coccidian oocysts (9.8%), *Capillaria* sp. (7.3%), *Trichuris* sp. (6.1%), order Spirurida (4.9%), *Platynosomum* sp. (2.4%), and *Eimeria* sp. (1.2%). These results demonstrate that Serra dos Órgãos National Park presents all the elements necessary for maintenance of the biological cycles of different parasites, including those with complex biological cycles that include different types

of hosts. Moreover, the laboratory diagnoses on the fecal samples enabled indirect partial analysis on the park ecosystem, being these stages of the parasites usually detected in free-living wild animals' samples, such as felids [42].

Gastrointestinal parasite infections can determine weight loss, metabolic imbalance, reproductive problems, anemia, and dehydration. In severe cases, they may also cause fetal malformations, locomotor lesions, and even death of the animal [43, 44]. Due to the clinical manifestations of gastrointestinal parasites, many hosts present behavioral and functional changes within their community. A predator at the top of the food chain, for example, may exhibit a decrease in food intake and activity, including hunting [35]. Furthermore, anthropic actions may result in the introduction of etiological agents, which in certain circumstances determine emerging infectious diseases in wild animal communities [45]. However, according to [46], populations of wild animals are generally in balance with their parasitological fauna. However, environmental changes, especially anthropogenic ones, can determine the introduction of new infective agents and even stress factors that destroy this equilibrium by inducing pathological situations. Parasites can be considered excellent bioindicators of environmental impacts [47]. In this way it becomes relevant to perform routine coproparasitological surveys with noninvasive samples collected in trails of conservation units in order to indirectly check the health of the environment.

2. Conclusions

The study of wild carnivores through the analysis of noninvasive samples allows the identification of the animal species by different techniques, as well as their monitoring without the exposure of these animals to situations of risk, stress, or the use of chemical tranquilizers by the researchers to manipulate the animals. In addition, through the analysis of noninvasive samples, mainly feces, it is possible to detect structures of gastrointestinal parasites that may potentially be infecting these animals. It should be emphasized that this type of sampling causes minimal interference in the carnivores' habitat during the collection of the biological samples in the environment by the researchers. Besides that, noninvasive sampling is not detrimental to the ecological niche of these animals, cooperating to maintain the integrity of the fauna and where they live. This sampling strategy is mainly important when studying regions' considered biodiversity hotspots, such as Madagascar Island, which has a unique and a high richness of biodiversity, which includes more than 98 species of mammals [58]. It is also important to highlight that the approaches about the biodiversity conservation have changed over the years, which means that conservation strategies are needed since all the species have their own function and values in the ecosystem, but also because they play a role in providing benefits to people and to economy, known as ecosystem services, producing food and materials, for example.

Therefore, the association of the results obtained from the identification of wild or domestic carnivores that share habitats, sympatric species, and the investigation of gastrointestinal parasites in the noninvasive biological samples of these animals are fundamental for understanding the effects of possible diseases that can affect wildlife. Moreover, a constant research of the gastrointestinal parasites in conservation units and protected areas is extremely important to detect possible human interference through the presence of specific parasites or the introduction of parasite taxa not commonly reported in free-living wild animals by invasive host species or domestic animals. In addition, noninvasive sampling is fundamental for updating the records on the circulation of wild fauna in conservation units,

thus contributing to the creation or reformulation of management measures that aim, mainly, the preservation and perpetuation of these animal populations in the environment and also the non-entry and surveillance of animals that may be in the region and that do not belong to that ecosystem.

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Conflict of interest

There are no conflicts of interest.

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