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How to Design a Biomonitoring Study – A Practical Guide for Veterinary Professionals under a One Health Approach

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ABSTRACT

Currently, veterinarians can see their daily practice and medical tasks as constant opportunities for passive surveillance of One Health threats, such as infectious zoonotic diseases and chemical pollution effects on living beings. The present study aimed to provide a practical guide to designing a biomonitoring study during veterinary clinical practice without time-consuming procedures or significant costs. The constant access to several species' specimens provides the necessary samples to perform a biomonitoring study of environmental pollutants at the regional or national level. Generally, most health professionals know what to do (or where to find information) to report a disease outbreak. However, a summarized background to perform a biomonitoring study of a chemical hazard is missing. The authors of the current study provided a flow chart with the main steps to conduct a biomonitoring study in different fields of veterinary medicine. Thus, a biomonitoring study might give veterinarians (as other health professionals) a positive contribution to the clinical cases' resolution, while improving the general knowledge about the impact of environmental contamination on animals and human health.

Keywords: Contamination, Guideline, Monitoring, One Health, Pollution

INTRODUCTION

The One Health concept assumes that human health is deeply connected with animal and environmental health, as three main branches. Although it is not a new term, One Health principles have become more and more relevant and justified during the past few years due to several factors. These include the human population growth, the global movement of people, animals, and resources, the emergence and reemergence of new infectious diseases, natural habitat loss, and climate change, all affecting the three mentioned branches of One Health (Sleeman et al., 2019; Wilcox and Steele, 2021; CDC, 2022).

The One Health approach has continuously grown. Veterinarians can see their daily tasks as constant opportunities for passive surveillance of One Health threats, such as infectious diseases, antibiotic resistance, or environmental pollution. For instance, the practitioners have been detecting and reporting new disease outbreaks or pathogens in various hosts (companion animals, livestock, and wildlife). In the same way, they may verify the resistance of an isolated agent to antimicrobial treatment or chronic exposure to an environmental pollutant by pets and their owners (Kelly et al., 2017; Collignon and McEwen, 2019; Mackenzie and Jeggo, 2019; Sleeman et al., 2019).

The application of the One Health principles by veterinary professionals can provide information of considerable importance in pollution surveillance and ecotoxicology. Animals and humans often depend on the same environment and ecosystem, sharing potential chemical and physical hazards, such as organic and inorganic pollutants or radiation. Patterns of bioaccumulation and health effects of several xenobiotics (pesticides, drugs, inorganic compounds) are frequently shared by species from different taxa (including humans), just like other aspects of comparative medicine (Buttke, 2011). A substance's carcinogenic, hepatotoxic, or nephrotoxic potentials are often known and expected in several host species. The constant access to several species' specimens allows each veterinarian to perform a biomonitoring study of environmental pollutants in their operating region. Generally, veterinarians are trained to report a new outbreak or perform a study of disease prevalence, or at least they can find standardized procedures for these purposes (Buttke, 2011; Hryhorczuk et al., 2018). Notwithstanding, they may not have the autonomy to perform a biomonitoring study of a chemical hazard.

Therefore, the current study aimed to provide a practical guide to veterinary professionals from different fields, including wildlife) to design a biomonitoring study during their clinical practice without specific material or time-consuming procedures.

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DEVELOPING A BIOMONITORING STUDY

Step 1 – Defining a problem/xenobiotic

At first, as in many other research studies, veterinarians must define priorities for biomonitoring contaminants considering their location and the animal species they usually work with them. For instance, if veterinarians have been diagnosing and monitoring respiratory clinical cases in companion animals, this group of cases may represent an opportunity to assess the effects of indoor air pollution, a problem with repercussions for humans and companion animals' health (Lin et al., 2018). Moreover, food-producing animal veterinarians can perform biomonitoring studies to evaluate the composition of the pasture in terms of toxic plants (Green et al., 2023), heavy metals (Afzal and Mahreen, 2024) or mycotoxins (Gallo et al., 2015; Muñoz-Solano and González-Peñas, 2023), if a considerable number of cases in a short-time period, and with a compatible clinical presentation, has been registered. These are just some examples.

Step 2 – Choosing a bioindicator

Some veterinarians may work with more than one species or animal population daily. Before starting to implement their research project and data collection, researchers should do some literature review regarding the pathophysiology, exposure effects, and metabolism of the chosen xenobiotic because species-specific enzymes or metabolic pathways can influence the obtained results and prevent the authors from developing correct and practical conclusions. As an illustration, dogs (and other canines) can adequately metabolize and eliminate some persistent organic pollutants (POPs), such as organochlorine pesticides and polychlorinated biphenyls, which humans (and other mammals) accumulate throughout life (Shore et al. 2001; Ruiz-Suárez et al., 2015). In this case, despite sharing the same environment as the owners, dogs are not suitable sentinels of pollution. In contrast, in indoor environments, cats have been pointed out as better bioindicators of some POPs, such as polychlorinated biphenyls or polybrominated diphenyl ethers (Ma et al., 2022). Therefore, a companion animal veterinarian who intends to measure and study the chronic effects of POPs, must choose cats as sentinels instead of dogs.

Some aspects of the species or population may represent advantages for its use as a bioindicator (Talmage and Walton, 1991), as what happens in hedgehogs or shrews (Sánchez-Chardi, et al., 2009; Jota Baptista et al., 2023). For instance, living in a restricted region, representing local pollution, living long enough to show effects from the exposure, and for a comparison between periods or age groups, or presenting an adequate dose-effect relationship (Jota Baptista et al. 2022). On the other hand, some xenobiotics (as some POPs or metals) are frequently accumulated through food chains, which usually makes carnivores better bioindicators than the herbivores they prey, considering the same ecosystem. However, there is no total consensus or a fixed list of species that can or cannot be used as bioindicators. For instance, wild boars have been widely used, mainly because they are a game species (and, therefore, a possible source of contaminants to consumers). Nevertheless, their broad distribution and frequent migrations in most countries lead to a poor definition of the pollution source (Tataruch and Kierdoft, 2003).

Step 3 – Choosing samples

This step highly depends on what xenobiotic will be analyzed (Step 1), the species involved (Step 2), and also on the study's goals. All these aspects should be decided first, before choosing a sample type.

Furthermore, at this stage, vets may decide if the study will involve the use of invasive samples (such as blood, liver, or kidney biopsies, and other internal tissues) or non-invasive samples (such as hair, feathers, feces, or parasites). If the study is designed to include only alive individuals (instead of including dead animals), aspects of welfare and restraint may conditionate the use of invasive samples (Jota Baptista et al., 2022). Nevertheless, invasive samples are crucial to understanding some toxicological aspects, such as tissue bioaccumulation and distribution in the organism. One practical use of this information is to evaluate which internal tissues of game species and livestock usually accumulate toxic substances or drugs, and which are more frequently consumed, as some muscles compared to bones or offal (Jukna et al., 2006; Jota Baptista et al., 2024). Some POPs are often searched in the adipose tissue of marine mammals through skin biopsies (Baini et al. 2020); and lead is frequently measured in bones from recently-died animals or even specimens preserved in museums (Martiniaková et al., 2011; Lanocha et al., 2013; Baini et al., 2020).

Moreover, it is necessary to think about the objectives of the study. If the goal is to evaluate long-term exposure to a substance, some tissues may be better accumulators than others, such as bones for cadmium or lead (González-Reimers et al., 2023), or hair for mercury (Treu et al., 2018). On the other hand, blood is a good option if it is intended to clinically monitor patients through recovery time and evaluate their response (Jota Baptista et al., 2022). Finally, authors may intend to use biomarkers of effect (evaluate or measure the cellular and molecular consequences of exposure to a particular substance). In that case, the liver and kidney are organs that usually suffer changes due to chronic or acute exposure to toxic compounds and are usually used for measure catalase, superoxide dismutase, and lipid peroxidation, among other biochemical biomarkers of effect (García-Niño and Pedraza-Chaverri, 2014; Hampel et al., 2016). Nevertheless, it is crucial to highlight that this depends significantly on the xenobiotic and host, and it is always advised to review the literature before starting the work.

Step 4 – Sending the samples to the laboratory

Since the primary goal of this article is to provide brief guidelines to veterinary professionals to perform a biomonitoring study, a detailed explanation of the various methods available for the detection and quantification of xenobiotics goes far beyond the scope. It is, however, essential to select a good and experienced laboratory that is familiar with preparing the samples and dealing with different organic matrices. Deciding whether you want to detect or quantify a substance is also essential. For instance, detecting the substance may provide enough evidence to make practical conclusions if the goal is to study fecal antibiotic residues (Li et al., 2013). In contrast, measuring zinc or other elements in cattle to evaluate their actual bioavailability in a pasture may require a quantitative toxicological analysis (Rigueiro-Rodríguez et al., 2012).

Most toxicological analyses do not need special requirements and materials to collect and preserve the samples. Dinis-Oliveira et al. (2016) have published detailed guidelines for collecting each biological sample for toxicological analysis. In most cases, it is recommended to send frozen samples, collected without external contamination to proper sterile containers (such as dry tubes or plastic bags; without preservatives) and correctly identified. Notwithstanding, sampling guidelines should be checked for each xenobiotic and biological sample.

Whatever the method is used (gas chromatography, liquid chromatography, atomic absorption spectrophotometry, or induced-coupled plasma mass spectrophotometry), the laboratory must ensure suitable quality-control methods (the use of duplicates, blanks, and suitable reference matrices, Caballero-Casero et al. (2021), and enough sample must be provided by the clinician.

Step 5 – Results' interpretation

Interpreting results might be the most challenging part of a biomonitoring study, especially if the authors intend to compare exposure levels with effects. There is a lack of scientific literature on the effects of each relevant hazardous substance in each animal taxa and what to define as gold standard biomarkers for most relevant xenobiotics (Varea et al. 2020; Desforges et al., 2022). This may implicate an extensive literature search (references of similar studies in humans) and an exercise of presenting a hypothesis for further research (Jota Baptista et al., 2023). Authors should be aware that most lesions or biomarkers that may be associated with hepatotoxic, nephrotoxic, carcinogenic, and neurotoxic, among others, substances are not pathognomonic of a specific compound. Age, sex, chronic diseases, food habits, or environmental conditions may strongly influence the effect of xenobiotics in animals and provoke distinct individual or populational effects (Ortiz et al. 2022). Notwithstanding, each local study will represent scientific evidence and help build detailed and long-term surveys and metanalysis by the research institutions. A small and practical guideline for implementing biomonitoring studies for veterinarians is provided as a summary of this article (Figure 1).

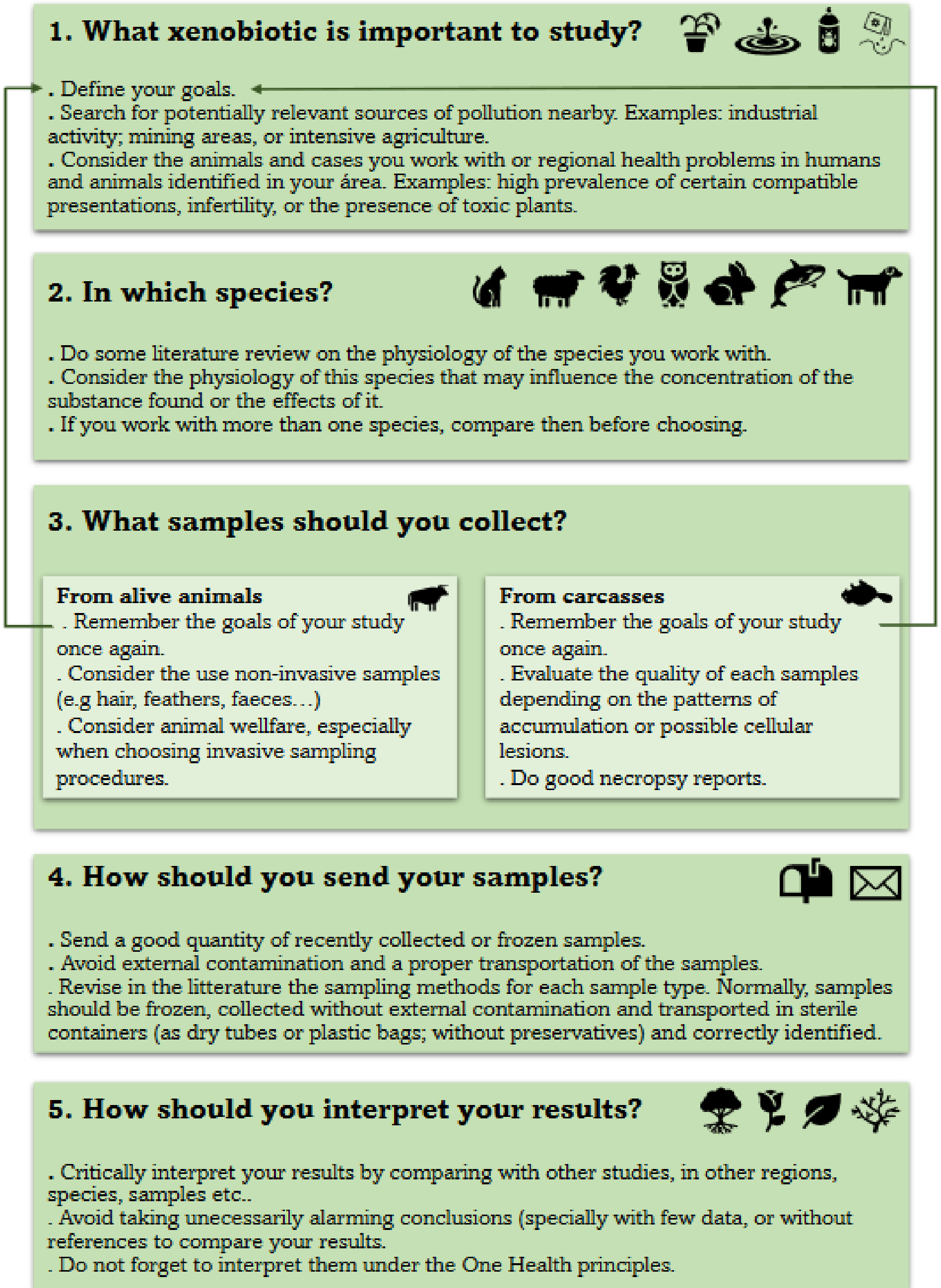

Figure 1. Guidelines for a quick implementation of a biomonitoring study by veterinarians

CONCLUSIONS

Veterinarians should never forget their crucial part in One Health. Therefore, each biomonitoring study should provide a concise interpretation of the results under a One Health approach. After all, as mentioned above, environmental contamination *sensu lato* is a global problem with remarkable One Health repercussions. Thus, veterinarians could analyse their daily practice, considering the species they work with and the xenobiotics of interest in their geographic region, and try to implement a biomonitoring study that might give a positive comprehension of their clinical cases, as well as improve the general knowledge about the impact of environmental contamination in animals and humans' health. The authors of the present study certainly believe these guidelines may also be applied to environmental researchers, biologists, or other health professionals (rather than veterinarians).

DECLARATIONS

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Availability of data and materials

The data of the current study are available by reasonable request.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues have been checked by the authors before submission.

Authors' contributions

The authors responsible for the conceptualization of the article were, Catarina Jota Baptista, Fernanda Seixas, José M. Gonzalo-Orden, and Paula A. Oliveira. Catarina Jota Baptista conducted the data collection and provided the original draft of the manuscript, while Fernanda Seixas, José M. Gonzalo-Orden, and Paula A. Oliveira revised it. All authors agreed with the final version of the manuscript.

Competing of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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