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GUIDELINES

Sampling guidelines for paleoparasitological and paleodietary studies

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ABSTRACT

Paleoparasitology is a discipline that searches for endo and ectoparasites helping in the identification of disease and migration. The success of these analyses is influenced by the quality of the samples. Thus, this manual aims to guide anthropologists, archeologists, paleontologists and other professionals involved in excavations and / or curators of museum collections to collect samples for studies of parasites and food remains in old materials. This text provides the guidelines to collect sediments from human burials, skeletons, coprolites, fauna, and structures used to dump waste, and how to avoid contamination and to store samples. The close association between sampling procedures and the laboratory study will enhance the quality of information to help in the reconstruction of past living conditions.

Keywords: Paleoparasitology, Paleopathology, Infectious diseases, Iberian Peninsula, Human migration, Archaeology.

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RESUMO

A Paleoparasitologia pesquisa evidências de endo e ectoparasitas que auxiliam na identificação de patologias e migrações. O sucesso destas análises é influenciado pela forma como são recolhidos os vestígios, pelo que este manual tem como objetivo orientar a recolha de amostras para estudos de parasitas e de vestígios alimentares em materiais antigos. Tem como público-alvo antropólogos, arqueólogos, paleontólogos e demais profissionais que realizam escavações e/ou gerem coleções museológicas. Fornece informações sobre a recolha de coprólitos e sedimentos em enterramentos humanos e de outros animais, em estruturas para despejo de dejetos e esqueletos depositados em coleções. Orienta o investigador para evitar contaminação e como armazenar corretamente as amostras. A estreita colaboração entre os procedimentos de amostragem e o estudo laboratorial permitirá ampliar o conhecimento sobre as condições de vida no passado.

Palavras-chave: Paleoparasitologia; Paleopatologia; doenças infecciosas; Península Ibérica; migrações humanas; Arqueologia.

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RESUMEN

La Paleoparasitología, es la disciplina que se encarga de estudiar las evidencias dejadas por endo y ectoparásitos, para ayudar en la identificación de enfermedades y migraciones. El éxito de estos análisis se ve influenciado por la forma en que estos vestigios son recogidos, pretendiendo con este manual dirigir la recogida de muestras para estudios parasíticos y de restos alimenticios en material antiguo. Tiene como meta orientar a antropólogos, arqueólogos, paleontólogos, y otros profesionales que realicen excavaciones o generen colecciones museológicas. Proporciona información sobre la recogida de coprolitos y sedimentos en enterramientos humanos y animales, en estructuras de descarga de desechos, y en esqueletos depositados en colecciones. Guía al investigador para evitar la contaminación, y cómo almacenar correctamente las muestras. La estrecha colaboración entre los procedimientos de muestreo y el estudio de laboratorio, ampliará el conocimiento sobre las condiciones de vida en el pasado.

Palabras clave: Paleoparasitología; Paleopatologia; enfermedades infecciosas; Península Ibérica; migraciones humanas; Arqueología.

Note: this is the translated manuscript of "Sianto L.; Santos, A.L. 2014. Manual resumido para a recolha de amostras para estudos paleoparasitológicos e de paleodieta. Cadernos do GEEvH, 3(2): 35-42". For this reason, it was not subjected to traditional peer-review.

Introduction

Paleoparasitology has its origins in paleopathology and can be defined as the field of science that searches and identifies parasites in archeological and paleontological material. Through the study of internal parasites, especially intestinal parasites and ectoparasites, it is possible to identify which infections a population was exposed during evolution (Araújo et al., 2003). By studying coprolites and sediments associated to skeletons and structures used to dump waste such as sewage systems, latrines or bedpans, it is possible to identify helminths and intestinal protozoans. Besides endoparasites, lice and ticks can be seen in preserved skin and hair of various animals, including from humans (Araújo et al., 2011).

The paleoparasitological research, developed especially in the last 35 years, extended the knowledge about host-parasite relations in different environments, and on the origins of zoonoses and other diseases (Sianto et al., 2009; Faulkner e Reinhard, 2014). It also helped to reveal food habits, cultural practices and contributed with new data to the interpretation of human migration, particularly by maritime routes (Sianto et al., 2012b; Reinhard et al., 2013).

Although there are many paleoparasitological studies made in Europe, data on the Iberian Peninsula are still scarce. To develop this research in Portugal, a laboratory was recently equipped at the Research Centre for Anthropology and Health (CIAS), located in the Department of Life Sciences at the University of Coimbra. This paper aims to provide guidelines for a more efficient sampling both during fieldwork and in stored specimens.

Sampling

During the excavation of faunal remains and human burials

The identification of parasites that infected one individual or a group of individuals is easily achieved by the analysis of the sediments associated with their skeletons. To evaluate intestinal parasites, the soil from the pelvic girdle should be collected, taking into account the body position in the grave. This is important because the deposition of parasites in the sediment follows the deposition of the viscera during the taphonomic processes. Thus, for instance, for an individual buried in lateral decubitus, samples should be collected in the most likely position for viscera deposition in the pelvic bones and soil below (Fig.1 point 1). If the skeleton is in supine position, it tends to accumulate parasite remains in the pelvis and sacral foramina (Fig.1 points 2 e 3).

Independently from the body position, it is also important to collect two samples for negative control (Fig.1 points 4 e 5) to attest that the parasitological findings are not the result of soil contamination. If the points indicated in the figure are not available, samples may be collected in other areas near to the skeleton.

Because several samples can be negative for parasites, it is important to collect samples from as many skeletons as possible.

The higher the number of samples, the higher is the chance of being able to carry out comprehensive studies at the population level. However, if this is not possible, it is preferably to collect a set of samples from individuals of different ages (infant [0-3 years], child [3-12 years]), adolescent and adult (male and female).

In faunal skeletons, the same logic adopted for sampling sediments from human inhumations should be applied. The study of parasites from domestic and wild animals helps to reveal unknown parasites (Araújo et al., 1989; Sianto et al., 2012a) and to identify which parasites were acquired from other animals or transmitted to humans during the evolutionary process (Leles et al., 2012). For more ancient periods, parasites of extinct species can be studied in fossilized coprolites (Dentzien-Dias et al., 2013; Hugot et al., 2014).

Skeletons previously excavated

Parasitological analysis is still possible on skeletons housed at institutions, by removing adhered sediment to the pelvic bones and sediment inside (filling) the sacral foramina. In these cases, it is important to collect sediment samples for negative control from other areas of the skeleton or, if not preserved after bone cleaning, soil from the archeological site should be provided for analysis.

Structures and containers (latrines and other sewer systems, pots, bowls, etc.)

Structures and containers may accumulate residues of food and parasites from humans and other animals (Harter et al., 2003; Bayman et al., 1996). In latrines, Roman sewers and other structures filled with faeces, sampling must be made according to stratigraphic layers. Thus, multiple sampling from the same structure may reveal differences in parasites and diet over time.

In specific situations, such as in mummification and / or embalming bodies, the viscera of the deceased were usually kept in pots (Aufderheide, 2003). Even if organic remains are not visible, sediment accumulated at the bottom of the pot should be collected nonetheless. The same procedures must be applied to food containers. Sometimes, to remove minor residues attached to the walls of the containers, acidic solutions must be applied. In this situation, it is advisable to contact the laboratory team before collecting the material.

The procedure of sampling sediments to be used as controls from these types of structures or containers varies according to the characteristics of the archaeological site and/or excavation. As a general rule, sediments should be collected from areas that are not associated to the samples but rather from a context that is similar to the one of the structures or containers, i.e. same layer or dating.

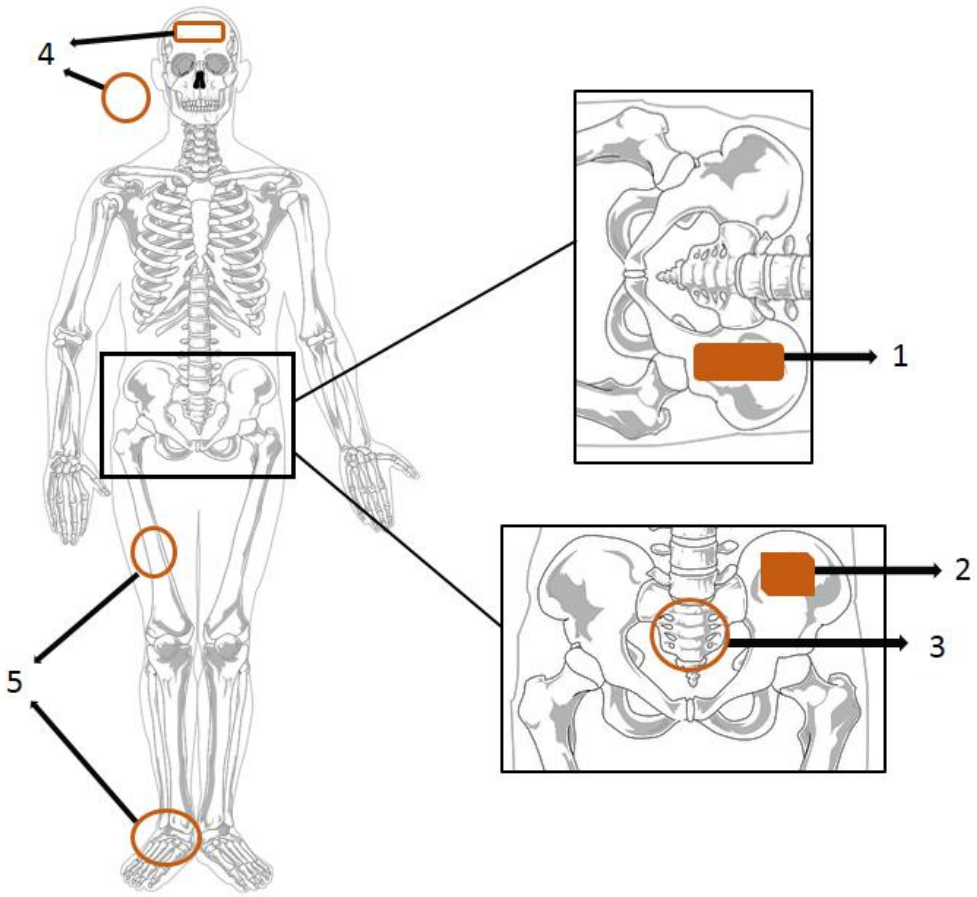

Figure 1 - Sampling points in burials and skeletons. 1) Pelvic region; 2) Surface of the illium; 3) Sacrum surface and from sacral foramina; Control soil samples: 4) near the skul and near the lower limb bones. Image from Pixabay.com adapted according to Sianto et al. 2013.

Coprolites

Coprolites may provide direct information about food habits and parasites. They can either be recovered directly from preserved bodies or be found in the soil during excavations. Coprolites must be photographed in situ, before being removed intact and with the utmost care in order to not compromise the original morphology and morphometry, which are helpful in the identification of its zoological origin (Chame and Sianto, 2011).

Coprolites must be collected and packaged separately unless several of them are actually found together. It is important to describe in detail the conditions under which the coprolites were found and if they were associated with other materials and/or structures. Negative controls are not required for each coprolite but it is advisable to collect soil samples from i) the surface of

the site; ii) the bottom of its sequence; and iii) the intermediate layer.

General Instructions

Sample labelling

Each sample must contain the following information: acronym of the archaeological site, sampling date, and the identification of the individual. For the site or for a set of samples occurring in the same location, GPS coordinates, the type of funerary structure, and its chronology (specifying if it was obtained by relative or absolute dating) must be indicated. It is also advisable to take pictures from the targeted anatomic regions before taking the samples.

Sample amounts

Ideally, it is important to collect around 50 ml (or 50 grams) of sediment but smaller samples can also be suited for analysis. During sampling, the researcher should use powder-free latex gloves (powder may interfere in dietary analysis) and disposable materials such as sticks and plastic spoons, for each sample.

Sample storage

Samples should be stored in clean plastic bags or plastic medical containers. In case of wet sediments, it is important to dry or refrigerate them to prevent the proliferation of fungi that may compromise paleoparasitological analysis. Another option is to add a preservative liquid to the sample such as ethyl alcohol 70% or a 10% formalin solution. In such cases, the label of the sample must indicate what product was added.

Before transporting the coprolites, each bag should be packed with bubble wrap or a similar material in order to preserve its morphology. To avoid contamination, do not use paper, cotton or any other kind of material in direct contact with the sample. Samples must be identified individually and accompanied by information that may be helpful to interpret the results. Such information includes, for example, skeleton or burial number, sampling location, associated materials, context disturbances, etc.

DNA analysis (ancient DNA)

Molecular analysis may be applied to ancient samples in order to detect fragments of parasitic DNA. These studies usually require greater care in the collection and storage of the samples as well as restrictions in the use of preservatives. Generally speaking, sampling requires us to be twice as careful about contamination and full-disposable materials such as gloves, coat, cap, mask, and others must be used, depending on the purpose of the study. Samples should be stored in sterile jars and kept refrigerated as soon as they are collected in the field. In addition, each laboratory may have specific protocols for receiving and processing samples, so prior contact for further information is advisable (Iñiguez, 2011).

Final Considerations

Parasitic studies are a successful tool to understand ways of lives, health and diseases of past populations but they are time-consuming. The average time for each soil sample analysis, from processing to results, is 120 hours, depending on the type of sample and on the results that are obtained. Not all samples reveal parasites or food remains useful to interpret ancient diseases. However, good results have been achieved in recent years. Improving collaboration between researchers, namely by collecting and sharing samples from various locations and timelines, will hopefully increase current knowledge about health, disease, food habits, and behavior in the past.

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