



Paleoparasitological analysis of the extinct *Myotragus balearicus* Bate 1909 (Artiodactyla, Caprinae) from Mallorca (Balearic Islands, Western Mediterranean)

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ABSTRACT

Myotragus balearicus (Artiodactyla, Caprinae) is an extinct caprine endemic of the Eastern Balearic Islands or Gymnesics (i.e., Mallorca, Menorca and surrounding islets, Western Mediterranean Sea). In spite of its small size, c. 50 cm height at the shoulder, it was the largest mammal inhabiting these islands until the human arrival, and it had peculiar short legs and frontal vision. It disappeared between 2830 and 2210 cal BCE. The coprolites here studied were recovered from Cova Estreta, in Pollença, Mallorca. The samples were subjected to microscopic examination and enzyme-linked immunosorbent assays (ELISA) for *E. histolytica*/*E. dispar*, *Giardia intestinalis* and *Cryptosporidium parvum*. This study provides new paleoparasitological data from an extinct animal species of the Holocene period. The microscopy revealed one sample containing uninucleated-cyst of *Entamoeba* sp., whereas ELISA detected nine positive samples for *Cryptosporidium* sp. The finding of these protozoans can help in the discussion of its extinction cause and demonstrates the antiquity and the evolutionary history of host-parasite relationships between protozoa and caprines since the Messinian.

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1. Introduction

Myotragus balearicus Bate 1909 (Artiodactyla, Caprinae) is an endemic caprine that inhabited the Eastern Balearic or Gymnesic Islands (i.e., Mallorca, Menorca and surrounding islets) during the Pleistocene and Holocene, until the human arrival. It is a small species that despite its size, which did not exceed 50 cm, was the largest mammal inhabiting these islands. It became extinct sometime between 2830 and 2210 cal BCE, most probably between 2639 and 2280 cal BCE [1]. Classically, two main hypotheses to explain this extinction have been developed: a human-related or a climate-caused extinction [2,3].

Coprolites, desiccated or mineralized feces, are sources of valuable information about extinct animals. They have been studied to proxy the diet of *M. balearicus* through the pollen analyses [4], the plant epidermis [5,6] and the fossil DNA [3]. The pollen grains contribute to elucidate the diet, as well as to reconstruct the paleoenvironment. According with Welker et al. [3], a climate change in the region affected the distribution of *Buxus balearica*, a plant that was an important part of the diet of *Myotragus* on surroundings of Cova Estreta [2,4], although Bover et al. [1] discuss both the incidence of this assumed climatic event on the distribution of the Balearic box and the dependence of *Myotragus* on this plant over all the island.

Paleoparasitology studies the parasites in the archaeological and paleontological record from various sources, including coprolites. The study of parasites in ancient remains has been done for many years in human populations from different locations, and with DNA and immunological techniques it starts to increase evidence of blood and intestinal protozoan infections [7]. The search for parasites in animal remains is an important contribution for this science, even if it is to know about their origin [8] or in relation to zoonoses [9]. Importantly

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when it comes to extinct animals the evidence of parasites in coprolites are rather scarce [10].

This article aimed to investigate parasites in coprolites of extinct goat, *M. balearicus*, using conventional parasitological technique and immunological analysis.

2. Material and methods

A total of twenty-five coprolites of *M. balearicus* were analyzed. They were obtained from the Vertebrate Collection of the Institut Mediterrani d'Estudis Avançats and registered on the paleoparasitological collection *Coleção de Coprólitos e Material de Interesse Paleoparasitológico Luiz Fernando Ferreira*, Laboratório de Paleoparasitologia, Escola Nacional de Saúde Pública Sérgio Arouca (ENSP)/Fiocruz, Rio de Janeiro, Brazil, by the numbers A1100–A1124. Some of the coprolites were fragmented or even pulverized due to their fragility, but the majority of them preserved their morphology. All of them were analyzed. Two techniques were used in the search for parasites, light microscopy and enzyme-linked immunosorbent assays (ELISA), described below.

2.1. Paleontological context

The samples come from Cova Estreta, Pollença, Serra da Tramuntana, Mallorca (Fig. 1). It is a small cave with an entrance 3 m wide, 3.5 m deep and 4 m maximum height, and has other compartments inside. All the studied coprolites came from the surface of the excavation square M4 (Fig. 2). A coprolite obtained in the source package of the coprolites here analyzed has been 14C dated in 4950 ± 38 BP (Wk-33010, 3800–3650 2σ cal BCE; [6]).

2.2. Microscopy

The samples were firstly processed to facilitate visualize on the light microscope. The fragments of desiccated coprolites were immersed in 0.5% trisodium phosphate aqueous solution for 72 h, then homogenized according to rehydration technique described by Callen and Cameron [11], which recovers the parasites remains. The spontaneous sedimentation technique proposed by Lutz [12] was performed with a slight modification. As the samples had very little and thin sediment, we deleted the step that is traditionally used for filtration with gauze, and so, make a better use of the material without unnecessary losses.

After this procedure, 20 parasitological slides of each sample were mounted using a sediment drop with a glycerin and analyzed on light microscope (Nikon Eclipse E200) at 100× and 400× magnification. All



Fig. 1. Coprolites of *Myotragus balearicus* on the surface of the excavation site.

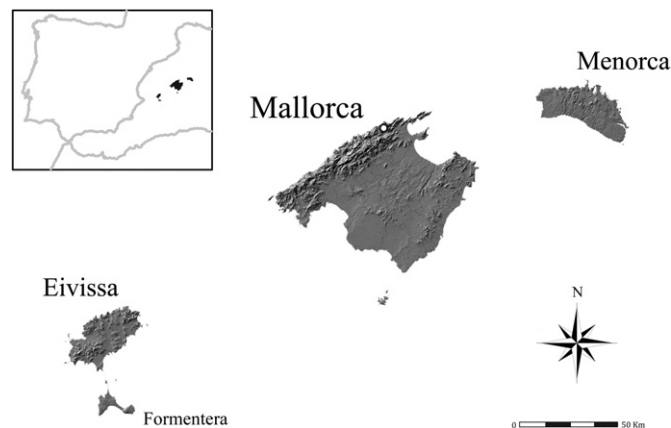


Fig. 2. Location of Cova Estreta, Serra da Tramuntana, Mallorca.

the remains found were measured. Digital images was taken using the Image-Pro Express 6.3 software that captures the image from Infinity1 camera connected to an light microscope with direct transmission of the image to the computer.

2.3. Enzyme-linked immunosorbent assays (ELISA)

After the procedure made for microscopic analysis, aliquots were separated into graduated conical-bottom plastic tubes with screw-on caps and stored in a refrigerator (2–5 °C) to prevent the growth of fungi and bacteria.

During the process to prepare the samples for the tests no formalin solution was added as it is commonly used for the preservation of fungi in archaeological and paleontological material. So there was no interference within the antigen-antibody reaction [13,14].

All 25 samples were submitted to 3 commercially available kit enzyme-linked immunosorbent assays (ELISA) (brand IVD Research®). The tests were for rapid detection of *E. histolytica*/*E. dispar* antigen in fecal samples, and for qualitative determination of *Cryptosporidium parvum* and *Giardia intestinalis* antigens in stool samples, one for each protozoa. The tests were applied to the samples according to the manufacturer's instructions. The main technique's principle consist in trap the antigens by specific antibodies in the microwells, and then made a successor chain of antibody and anti-antibody associated with peroxidase. In the presence of this complex, the chromogen added changes its color indicating the positive samples.

3. Results

The results are depicted in Table 1. The analyses by light microscopy, revealed a positive sample with the presence of a cyst in goat coprolite, *M. balearicus*, measuring $22 \times 22 \mu\text{m}$ with a visible nucleus, identified morphologically as *Entamoeba* sp. (Fig. 3).

Enzyme-linked immunosorbent assay revealed nine samples positive for *Cryptosporidium parvum* and none positive for *G. intestinalis* and *E. histolytica*/*E. dispar*. The results were validated by the positive and negative controls.

Table 1
Parasitological and immunological diagnosis (enzyme-linked immunosorbent assays) for enteric protozoa in coprolite samples from the extinct goat, *Myotragus balearicus*.

Method	Results
Microscopy	Uninucleated-producing <i>Entamoeba</i> (n = 1)
ELISA	
<i>Entamoeba</i> / <i>E. dispar</i>	Negative
<i>Cryptosporidium parvum</i>	Positive (n = 9)
<i>Giardia intestinalis</i>	Negative

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