Contents lists available at ScienceDirect

Acta Tropica

Short communication

First palaeoparasitological record of a dioctophymatid egg in an archaeological sample from Patagonia

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ARTICLE INFO

ABSTRACT

The collection of parasitological information from ancient material requires an exhaustive study of samples. In 2005, cestode and nematode eggs were found in a coprolite sample tentatively assigned to a canid. The sample was obtained from the layer of the archaeological site located in Cerro Casa de Piedra, Santa Cruz Province, Argentina, and dated from 6540 ± 110 years before present. The aim of the present work was to reexamine this fixed sample in order to confirm the presence of these parasites. The palaeoparasitological results support our previous findings. Interestingly, another parasite was also confirmed: a dioctophymatid nematode. *Dioctophyma renale* has been reported in several modern carnivores in the Southern Hemisphere but in ancient materials, it has only been reported in human coprolites from Switzerland. This report constitutes the first evidence of the presence of a dioctophymatid nematode parasite dioctophymatid nematode in American pre-Columbian times. The results obtained in this work show the importance of revising earlier palaeoparasitological results.

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1. Introduction

Currently, palaeoparasitological studies performed in Patagonia focus on obtaining data of the presence of parasites in diverse hosts and periods of time in history. The information obtained is integrated with other palaeoscatological data in the biological and anthropological context of the archaeological site studied. In this regard, many palaeoparasitological studies covering most of the Holocene and Pleistocene Transition of Patagonia and different hosts, including humans, have been reported (Beltrame et al., 2012; Ferreira et al., 2011; Fugassa et al., 2009, 2010; among others).

The nature of ancient environmental samples can often complicate the acquisition of palaeobiological information. This is true of ancient parasite remains. These are represented mostly by eggs, cysts and larvae of helminths that are recovered in small numbers and present few taxonomically relevant characters due to taphonomic modifications. Therefore, acquiring more empirical background depends on the processing techniques and on the observation by carefully selected methods and instruments.

Also, the sampling effort involves a balance between potentially obtainable information and the time and resources invested in the recovery of the data. Observer bias can also prevent correct taxonomic identification. Thus, the information available from a palaeoparasitological sample varies according to the context in which observation occurs. In this regard, after eight years of palaeoparasitological tests, we examined the possibility to detect differences in the palaeoparasitological results from a same sample. Accordingly, the aim of the present study was to reexamine the parasitic fauna present in coprolite samples. These data allow us to determine whether the extension of studies increases the information available and to critically review results previously obtained.

2. Material and methods

A coprolite from Cerro Casa de Piedra, cave 5, located in Perito Moreno National Park (47°57′ S and 72°05′ W), Santa Cruz Province, was reexamined. Cerro Casa de Piedra is a hill of volcanic origin in the river basin of Río Roble, and Burmeister lake, 900 m above sea level. On the north side, there is a set of caves and rock shelters (Aschero, 1982). The site is located in an ecotone between a forest of *Nothofagus* sp. and a bush steppe (Civalero and Aschero, 2003). Cerro Casa de Piedra 5 has a stratigraphic sequence that includes 19 levels, with human occupation between *ca.* 6780 and *ca.* 6540 before present (B.P.), between *ca.* 5170 and *ca.* 4330 B.P., and between *ca.* 2740 and *ca.* 2550 B.P. (Aschero, 1996). The
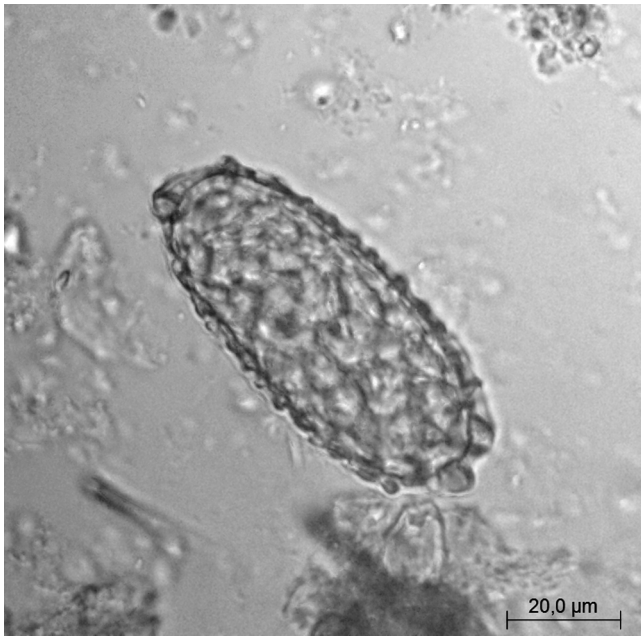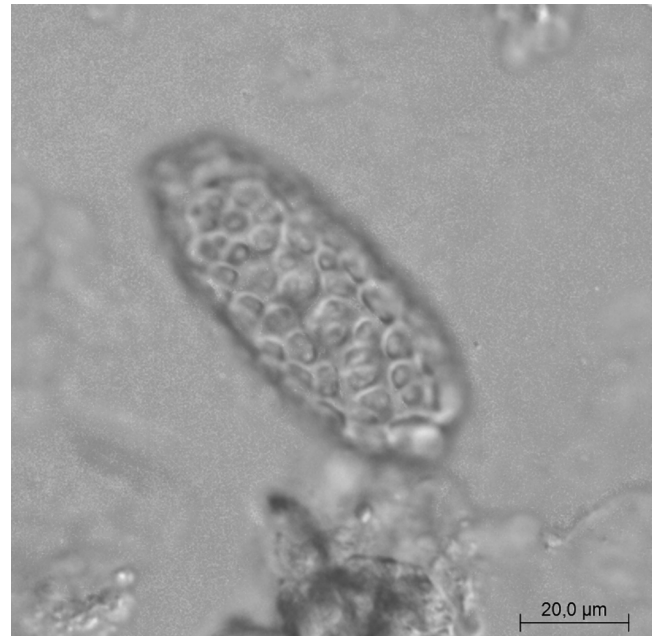
Fig. 1. Dioctophymatid egg found.

Fig. 2. Eggshell appearance.

coprolite, dated from 6540 ± 110 B.P., was collected from layer 4, and tentatively assigned to a canid by means of morphology (Chame, 2003), diet composition, and typical fox urine smell (Fugassa, 2006; Fugassa et al., 2006). To confirm the zoological origin of coprolite, DNA was extracted from hairs found in the sample, which may have been ingested by the animal during its grooming. Lysis was performed according to Allen et al. (1998), with some modifications. Briefly, a hair fragment was incubated in 50 µl of PCR-compatible buffer at 56 °C for 3 h, and proteinase k was inactivated at 95 °C for 15 min. An extraction negative control was added and the Authenticity Criteria to Determine Ancient DNA Sequences were followed (Hofreiter et al., 2001). PCR amplifications were performed using 0.5 units of Taq Platinum (Invitrogen), 5% of DMSO (Finnzymes), 2 mM of $MgCl_2$ (Invitrogen), 200 µM of each dNTP (Finnzymes) and 0.2 mM of each primer (Nyström et al., 2006; Roques et al., 2011). The gene fragments were sequenced and the sequences obtained were analyzed using BioEdit (copyright © 1997–2005, Tomm Hall). Sequence homology searches were performed using the BLASTN algorithm from the NCBI website.

The coprolite rehydrated in trisodium phosphate 0.5% containing formalin 5% and followed by spontaneous sedimentation (Lutz, 1919) in 2005 (Fugassa et al., 2006) was reexamined to extend and critically review the results obtained eight years before. Fifteen slides with one drop of glycerine were examined using a light microscope at 10×. Parasite eggs were measured and photographed at 40×.

3. Results and discussion

The morphological and dietary analysis of the coprolite seemed to indicate that it was deposited by a canid. However, DNA studies to detect a specific gene fragment from canids, felids and humans did not allow us to determine zoological identity with them because of the poor conservation of the sample, and thus fragmentation and low quantity of DNA.

In the current work, we found the same anoplocephalid cestode and nematode eggs reported by Fugassa et al. (2006). Also, we encountered a brown and elliptical egg with numerous hubs on the surface and apparently capped polar region (Figs. 1 and 2). Its measurements were 72.5 µm $\times 32.5$ µm, consistent with a dioctophymatid egg (Class Adenophorea, Enoplida, Dioctophymatidae) (Anderson, 2000). During the first study in 2005, we found three specimens of 70.0 µm $\times 30.0$ µm, 78.75 µm $\times 25.0$ µm and 67.5 µm $\times 37.5$ µm, respectively. The mean size of eggs was 72.19 ± 4.82 (67.5–78.75, $n = 4$) µm $\times 31.25 \pm 5.2$ (25–37.5, $n = 4$) µm. They had conspicuous opercular regions, compatible with *Ascaris* sp. because of their similarity to these infertile eggs (Fugassa, 2006). However, neither canids nor felids are hosts of *Ascaris* sp. eggs. In addition, they did not have all the typical morphological features.

The dioctophymatidae family includes the genera *Dioctophyma*, *Eustrongylides* and *Hystrichis* (Anderson, 2000). Generally, their life cycle includes the release of eggs from the urine and subsequent ingestion of eggs by oligochaetes and consumption by fish or amphibians as paratenic hosts or directly by the definitive host (Anderson, 2000). The best known species is *Dioctophyma renale*, which can parasitize several carnivorous mammals, and even humans (Gu et al., 2012; Ignjatovica et al., 2003; Ishizaki et al., 2010; Nakagawa et al., 2007; Woodhead, 1950). All members of the group have eggs with an irregular thick wall, and with one or two opercula (Anderson, 2000). Furthermore, it has been reported that *D. renale* lays eggs of different sizes according to the host (Miyazaki, 1991), and that such variations in size may depend on the developmental stage of the female (Pedrassani et al., 2009). The egg dimensions reported in this work are within the ranges of usual measures, except for the width, whose values are below those so far reported (Pedrassani et al., 2009). These differences could be due to taphonomic causes and the small number of eggs in faeces is probably due to contamination with urine.

D. renale has been reported in several South American carnivores such as *Chrysocyon brachyurus* (Beccaceci, 1990), *Cerdocyon thous* (Ribeiro et al., 2009), *Nasua nasua* (Milanelo et al., 2009) and *Galictis cuja* (Zabott et al., 2012). The only ancient record of a dioctophymatid corresponds to a human coprolite from the Neolithic period in Switzerland where zooarchaeological analysis showed a high consumption of fish and frogs (paratenic hosts) as well as the use of mustelids (definitive hosts) (Le Bailly et al., 2003, 2007). This report indicates the presence of a *Dioctophyma* parasite back in pre-Columbian times in America, suggesting that at least some of the findings could be from native wild carnivores. There is a zoonotic

risk to humans in consumption of paratenic hosts due to incomplete cooking. In this case, the contamination of the human occupation area with carnivore faeces containing *Dioctophyma* eggs, would not have involved a direct risk to the health of its residents.

This study highlights the importance of both expanding examinations and critically reviewing the findings from previous studies as a way to update the state of palaeoparasitological knowledge.

Acknowledgments

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