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### Original Article

## *Macracanthorhynchus hirudinaceus* Eggs in Canine Coprolite from the Sasanian Era in Iran (4<sup>th</sup>/5<sup>th</sup> Century CE)

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#### **Abstract**

Present paper is the second publication introducing the paleoparasitological findings from animal coprolites obtained from archeological site of Chehrabad salt mine in northwestern Iran. The current archeological site is located in northwest of Iran, dated to the Sasanian Era (4<sup>th</sup>/5<sup>th</sup> century CE). In the summer 2012 the carnivore coprolite was obtained within the layers in the mine and were thoroughly analyzed for parasites using TSP rehydration technique. Eggs of *Macracanthorhynchus hirudinaceus* were successfully retrieved from the examined coprolite and were confidently identified based on reliable references. Identifying of *M. hirudinaceus* eggs in paleofeces with clear appearance as demonstrated herein, is much due to appropriate preservation condition has been existed in the salt mine. The present finding could be regarded as the oldest acanthocephalan infection in Iran.

### Introduction

Paleoparasitological findings may illustrate the status of human and animal parasitic infections in past periods. Parasites, specifically helminth eggs, are detectable in ancient biological remains when morphological structures are not destroyed due to the taphonomic process over the time.

Extinct parasites and/or extinct hosts may bring difficulties to a precise diagnostic (1), but on the other hand it is possible to describe new species and parasitism evolution in disappeared hosts (2). Although a variety of remains in archeological sites can be studied for parasites, paleofecal samples are of the

most fruitful for parasites (3). A diversity of trematode, cestode and nematode eggs have been recovered from sediments, latrines and coprolites collected from archeological sites worldwide.

Acanthocephalan eggs have also been diagnosed in coprolites collected in some archeological sites (4). All acanthocephalan species are parasites and have a variety of carnivore hosts but some species can also be found in humans and other omnivorous animals. It is not easy to discriminate the origin of coprolites from humans or animals. In this regard, amongst all biological remains, mummified bodies are the most attractive items taking into account their preservation conditions allowing better parasite structures preservation and host diagnosis (5, 6). However, these unique and special kinds of samples are not common and may rarely be found in specific historic places in the world.

The ancient salt mine of Chehrabad in northwestern Iran is an exceptional site to perform paleoparasitological investigation in the country. *Taenia* sp. infection was identified in a salt mummy of Chehrabad (7) along with other human and animal helminth eggs from soil samples (8). Helminth parasites were also recorded in rodent coprolites (9).

Herein we report the finding of two well-preserved acanthocephalan eggs, identified as *Macracanthorhynchus hirudinaceus*, in coprolite, collected at Chehrabad archeological site.

## Materials and Methods

The sample No 2442-1(274) analyzed in this study has been collected during the excavation project in the summer 2011 at Chehrabad salt mine archeological site in northwestern Iran. The sample belongs to the Sasanian period of the salt mine and dates approximately in the 4<sup>th</sup>/5<sup>th</sup> century CE. The coprolite sent to paleoparasitological analysis was very small (weight 1.3g), so the whole piece was entirely rehydrated in 0.5% trisodium phosphate solu-

tion ( $\text{Na}_3\text{PO}_4$ ) according to standard recommendations (10, 11). Ten days after, 15 ml of rehydrated sample was examined under the light microscopy at magnification power of X100, X400 and X1000. In order to preserve the eggs, glycerin jelly-mounting method was applied (12). Eggs found were photographed using Olympus BX 41 microscope equipped with DP12 camera and measured by software Olysia zoom. Identification was based on morphologic and morphometric characters (13).

## Results

Based on morphological parameters described for mammalian feces (14, 15), the small coprolite was identified as an excrement of canine origin with distinct pointed ends, (Fig. 1). The substance of the feces was characterized by included hairs clearly visible especially at the two ends and the lack of larger botanical fragments.



**Fig.1:** Lateral view of the palaeofaeces (Photo: DBM/RUB/MFZ, N. Boenke)

Out of 170 slides prepared for microscopic study, two acanthocephalan eggs were found. Morphological characters of the eggs such as ovoid shape symmetrically, three-layered shell and an acanthor with the characteristic hooks inside the eggs that measured 82  $\mu\text{m}$  by 51  $\mu\text{m}$  and 84  $\mu\text{m}$  by 51  $\mu\text{m}$  (Fig.2) led to the diagnosis of the thorny-headed worm,

*Macracanthorhynchus hirudinaceus*. The cited range of the measurements for *M. hirudinaceus* eggs, 80  $\mu\text{m}$  - 100  $\mu\text{m}$  by 40  $\mu\text{m}$  - 50  $\mu\text{m}$  (13), fits to the eggs found in the coprolites. Polar parts of

the outer shell layer were seen partly broken in one (Fig. 2a), while the other one (Fig.2b) showed the layer more intact.

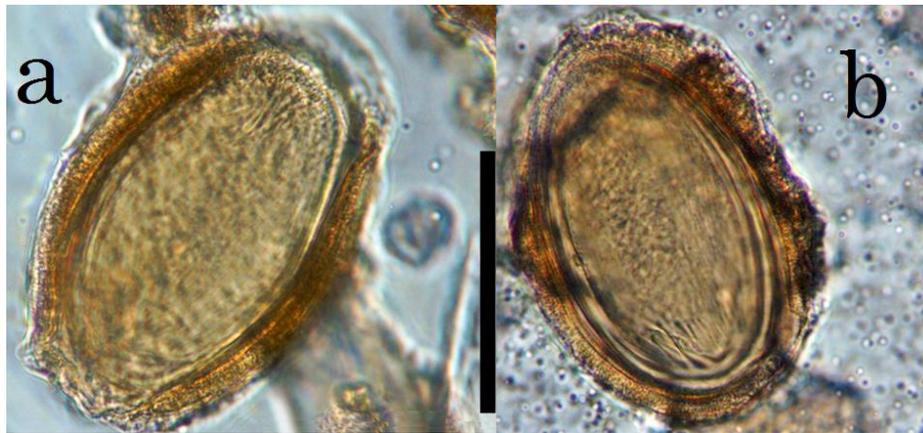


Fig. 2: Eggs of *M. hirudinaceus* retrieved from the coprolite analyzed in this study

## Discussion

Acanthocephalan or thorny-headed worms are obligate endoparasites of a wide range of final (definitive) hosts inhabiting aquatic and terrestrial ecosystems. Arthropods are the intermediate host (16). Humans may accidentally acquire two species of acanthocephalans, *Moniliformis moniliformis*, which is the common parasite of rats, and *M. hirudinaceus*, which can naturally infect pigs and wild boars (17). Recently it had been shown, that wild boars (*Sus scrofa*) frequently found in sugar cane fields of Khuzestan province in the southwestern Iran were infected by *M. hirudinaceus* with a high prevalence of 64% (18). Human infection due to *M. hirudinaceus* has been recorded from southeastern Asia, mainly from Thailand (19-21) and China (22). Intestinal perforation caused by this zoonotic helminth has been also detected in a 30-year old woman in Thailand (23). In Iran, acanthocephalan infection by *M. moniliformis* in humans was rarely recorded (24-26). The most recent case was observed in a 2-year old girl in the north east of the country (27). Although *M. hirudinaceus* is commonly known as a pig and wild boar para-

site it can be also found infecting carnivore mammals, including wild and domestic dogs (28) and stray dogs (29, 30). The current finding of *M. hirudinaceus* eggs at the archeological site may be due of an infected dog in the salt mine kept as a watchdog or other carnivores, such as a jackal or other wild canids, straying in the open galleries of the mine.

Due to the salty environment the Chehrabad archeological salt mine presents exceptional preservation conditions for biological remains, including helminth eggs, as it has been previously discussed for trematode eggs found in the same site (31). Moreover, thorny-headed worm eggs are known for their resistance to environmental changes due to the thick outer shell. Therefore, the combination of these two facts led to a very well preservation of the eggs. The present paper documents the presence of *M. hirudinaceus* infection in a canine animal 1500 years ago. This again exposes which parasites could have been a zoonotic disease to humans in Sasanian times.

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