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Original Article

Paleoparasitological Findings from Rodent Coprolites Dated At 500 CE Sassanid Era in Archeological Site of Chehrabad (Douzlakh), Salt Mine Northwestern Iran

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Abstract

Background: In this paper, paleoparasitological findings from rodent excrements obtained from Chehrabad Salt Mine archeological site located in northwest of Iran are demonstrated and discussed.

Methods: Chehrabad Salt Mine archeological site located in northwest of Iran, dated to the Achaemenid (mid 1st mill. BCE) and to Sassanid (3rd cent. - 7th cent. CE) period, is a unique study area to investigate parasites in the past millenniums in Iran. Rodent coprolites obtained from this archeological site were thoroughly analyzed for parasite eggs using TSP re-hydration technique.

Results: Specimen analyzed were attributed to juvenile and adult rats based on their apparent morphology comparing with the modern dried pellets of Muridea family. Helminth eggs retrieved from two positive pellets were identified as *Trichosomoides crassicauda*, *Syphacia* sp. and *Trichuris* sp.

Conclusion: The present paper discusses the first paleoparasitological findings of rodent gastrointestinal helminthes in Iran along with possible favorite items to rats in ancient Chehrabad Salt Mine.

Introduction

Although all endoparasites and ectoparasites are detectable in archeological contexts, helminth eggs with sturdy structures can be retrieved more easily from paleofecal samples (1). From another perspective, access to organic remains, like excrements in archeological sites, necessarily depends on the host population density in a certain space and time. Regarding to this issue, the order Rodentia corresponds to approximately 42% of mammalian biodiversity in the world, including 2277 extant species (2).

Therefore, rodent excrements are expected to be found in almost every archeological excavation projects performing in prehistorical and historical sites worldwide. Few available publications on rodent's paleoparasitological data emphasize the importance of specimen collection in archeological excavation concerning rodents' biological remains.

Recovering of some helminth ova of rodents, including *Trichuris sp*, Taeniid, Anoplocephalidae, *Capillaria hepatica*, *Trichosomoides crassicauda* as well as Oribatid mites in pellets and sediments obtained from Alero Mazquiarán archeological site in Argentina is highlighted in the literature (3). More records of helminths eggs in rodent coprolites dated to the Holocenic period were reported simultaneously from the same country (3, 4).

In Iran, paleoparasitology has recently appeared by identification of *Taenia* sp. eggs in Zanjan salt mummy number 5 (5) and consequent recovery of six different ova in soil samples, obtained from Chehrabad salt mine archeological site (6).

Herein, paleoparasitological findings from rodent excrements obtained in this site are demonstrated and discussed.

Materials and Methods

Chehrabad Mine is one of the rare examples of antique rock salt exploitation worldwide. The rock salt layers being exposed to the surface by a salt dome, a diapire (Fig. 1), have been exploited in large underground working chambers during two main eras, the Achaemenid period, 6th and the 5th century BCE and later during the Sassanid period beginning in the 3rd century. The Sassanian exploitation ended in the 7th cent and 6th century CE (7-9). All the samples being investigated have been recorded in stratigraphical context, which can be dated by the succession of strata, archaeological artifacts and AMS 14C (8).

Rodent coprolites, number, 263,264,265 and 266, dated at 565 CE (Sassanid era), obtained from "Douzlakh"archeological site located in Zanjan Province, Iran (Fig.1, down) were transferred to the Laboratory of Helminthology, School of Public Health, Tehran University of Medical Sciences, Iran in May 2012. For retrieving helminth eggs, excrements were separately rehydrated in 0.5% trisodium phosphate aqueous solution in a 2 ml micro centrifuge tubes, for 5 days according to previous researchers (10, 11). To avoid fungi growing, 2 drops of 10% formaldehyde solution were added to each container. From each rehydrated specimen, twenty slides were carefully examined under the light microscope. Identified eggs were finally photographed and measured.

Results

Coprolites were primarily grouped to three small, and a little larger one compared with others. Samples appearance with regards to complementary information accumulated during the study, led us to assign the coprolites most probably to rats (*Rattus* sp.). All four analyzed pellets were obtained from a 5 square centimeters, which could be most probably belonging to merely one rat colony. Moreover, the smaller coprolites could have been laid by juvenile rats.

Of the pellets number, 263,264,265 and 266, two cases, 264 and 266 were found parasitized. Parasite eggs were identified as *Syphacia* sp. (Genus *Syphacia* Seurat, 1916), *Trichosomoides crassicauda* (Bellingham 1840, Railliet 1895) and *Trichuris* sp. (Family Trichuridae, Roederer 1761). Six *Syphacia* sp. eggs and fifteen eggs of *T.crassicauda* were retrieved from the pellet No: 264, while the analysis of the pellet number 266 demonstrated merely a single *Trichuris* sp. egg. Table 1, shows the number and the appearances of the coprolites and the measurements of the identified parasite eggs (Fig. 2).

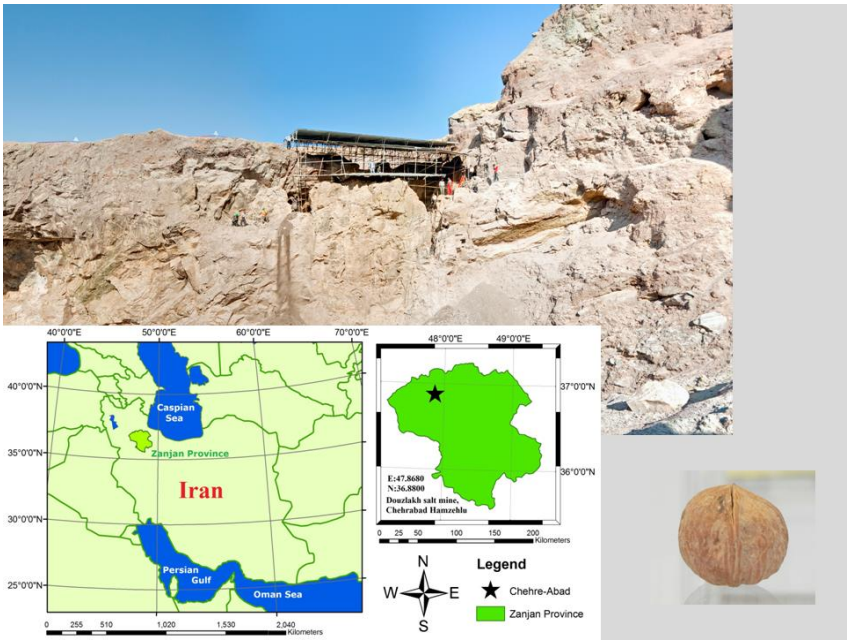

Fig. 1: Location of Chehrabad archeological site in Iran

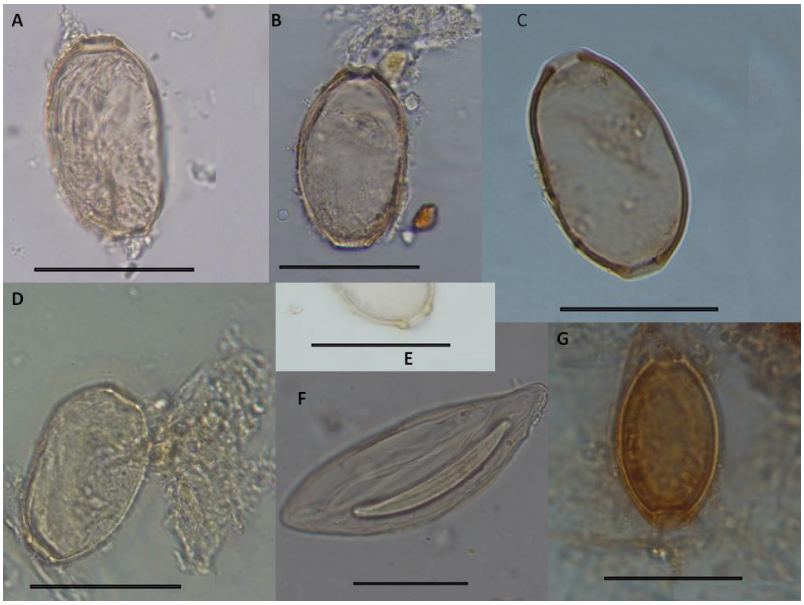

Fig. 2: Helminth ova identified in examined pellets

Table 1: Examined coprolites and the measurements of retrieved helminth ova

Coprolite (appearance)	Identified eggs	Egg measurement (µm) Length range mean(n)	width range mean(n)
263 (small)	-	-	-
264 (small)	*Trichosomoides crassicauda*	73.46 ± 7.38 (15)	44.93 ± 6.22 (15)
	Syphacia sp.	129.66± 4.54 (6)	48± 6.78 (6)
265 (small)	-	-	-
266 (large)	*Trichuris* sp.	63.79	37.93

Discussion

Proven life in a certain paleoenvironment will theoretically support the expectation of obtaining biological remains in the designated place. Preservation condition including, extreme moisture, great aridity, and natural refrigeration that may lead to rapid interruption of decay in organic remains (11) are fundamental issues in paleoparasitological investigations. According to available evidences, several helminths eggs of prehistoric and historic times have been recovered from archeological remains in African continent, Middle East, Southeast Asia ,American continent and Europe so far (12, 13). Moreover, natural situations like salt mine, glacial ice and amber are known to be able for preserving the biological remains including paleofeces, fungi and arthropods over a long period of time (14-16).

Chehrabad salt mine archaeological site is a unique historical preservation complex of ancient Iran in which paleoparasitological investigations can be taken placed successfully. In the present study, rodent coprolites obtained from this historical site were studied for parasite eggs. The origin of the coprolites was attributed to *Rattus* spp, the most well-known vertebrate pests worldwide (17) with horrible background of Black Death (18). Presence of rats in ancient salt mine of Chehrabad would be undoubtedly expected with regards to salt miners activities, employing domestic animals and ordinary keeping of food items in the site in that time. Attractive smell and debris of barbequing beef meat "Kebab" attributed to acquired taeniasis by the salt mummy No 5 in Chehrabad archeological site (5) could have been a cause of attracting rats into the salt mine. The walnut of the salt mummy No.1 found in the same location (Fig 1, down left), could be also in favor of rat infestation in surrounding environment.

Of two parasitized coprolites examined in this study, eggs of the nematodes, *Trichosomoides crassicauda*, *Syphacia* sp. and *Trichuris* sp. have been identified. In the literature, *Trichuris* sp. eggs have been reported more frequently, among which, in a particular study, out of 297 different identified eggs, finding of 267 eggs of *Trichuris* sp. is exemplary (4). Another helminth eggs that recovered in this study was *T. crassicauda*. This rat bladder worm is a hair like nematode residing in urinary tract of rats transmitting by female rats to their offspring (19) which may lead to a very high infection rate in crowded colonies. Identification of these eggs in a coprolite could be justified by urine contaminated feces explained earlier by previous researchers in Argentina (3).

Fifteen eggs of Trichosomoididae family Hall, 1916, more likely *T. crassicauda* were detected in the coprolite No.264. Morphological characters, including bipolar plugs, remaining

of embryo inside the egg (Fig. 2. A) , emerging position of the embryo from the shell (Fig 2 B,D),and prominent swelling of egg shell adjacent to poles (Fig. 2, E) have been clearly illustrated. Mean length and width were measured as 73.46 ± 7.38 and 44.93 ± 6.22 (n=15), with SD ≥ 6, for both dimensions, indicating the eggs size variation like the similar measurements 55-80 × 30-48 and 60-70 × 30-35 µm with a wide deviation analyzed for *T. crassicauda* eggs previously (20, 21). It should be noted that, modern occurrence of *T. crassicauda* have been studied since early 20th century in the world (22, 23) and recently in Iran (24).

Detection of *Syphacia* sp. eggs, most likely *S. muris* (Fig 2, F) was another finding in the present study that has been recovered from the same pellet (No. 264) harboring *T. crassicauda* eggs. Identification of these two different types of eggs in a single pellet could not be interpreted as co-infection in rats since the examined coprolite might have been contaminated by the urine of the other parasitized rats, as stated above. The rodent coprolites obtained from South American archaeological sites have been shown infected with the eggs of Anoplocephalidae cestodes, *Pterygo dermatitis* sp. of the family Rictulariidae, *T. crassicauda*(3), *Trichuris* sp., *Paraspidodera* sp. of *Aspidoderidae* and *Eucoleus* sp. of the family Capillariidae (4) so far. The earlier study, in which the eggs of *Taenia* sp., *Trichuris* sp., *Ascaris* sp., *Oxyurid* eggs and *Dicrocoelium* sp. were recovered from different strata of Chehrabad archeological site (6) no signs of rodent parasites have been detected.

Conclusion

Paleoparasitological findings in rodents should be merely concluded from an assured rodent sample, like pellets. Rodent paleofeces excavated from Chehrabad Salt Mine archeological site were successfully remind the circulation of three common helminthic infections amongst rats in ancient Iran. The eggs of *T. crassicauda*, *Syphacia* sp. and *Trichuris* sp. revealed from excavated biological remains elsewhere in the world so far, were identified in two parasitized pellets of rats analyzed in this study.

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