Paleoparasitology in Iran: A Review

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SUMMARY

Paleoparasitology was created to trace and recover the natural development of parasites as well as the origin of infectious diseases. Paleoparasitology is defined as the study of parasites in ancient material and their interactions with hosts and vectors. Advances in the field have helped to open up new prospects for anthropologists, archaeologists, biologists and medical scientists. In recent years, Iranian parasitologists and biologists have developed immense interest in this field. One of the first human settlements on earth was established in Iran and there is extensive evidence of early human life in this ancient land. Therefore, the aim of the review was to assess paleoparasitological research conducted in Iran in order to facilitate the discovery of the origin of infectious diseases in the region. English and Persian electronic databases including Web of Science, Sciencedirect, PubMed, Scopus, Google Scholar, Iran Doc, SID, Iran Medex and Magiran were employed as search engines (up to 2017) using the keywords: Iran, Islamic Republic of Iran, Parasitology, Parasites and Archaeology. According to the current review, the results of the parasitological study revealed the incidence of human and animal parasitic infection in Iran dating back to 8100 BC.

Keywords: Iran, paleoparasitology, parasites, parasitology, archeology.

INTRODUCTION

In the present century, a branch of science and several techniques have been proposed to study the relationships between hosts and vectors of parasites through examination of the remains of ancient materials in archaeological sites, habitats and ecosystems. This branch of science is called paleoparasitology. This field of science is one of the sub-branches of paleontology [1]. In fact, paleoparasitology was developed to trace and recover the evolution of parasites and the origin of infectious diseases. Paleoparasitology, which is the study of parasites in ancient material, has produced new data on the evolution, ecology, paleoepidemiology and phylogenetics of infectious diseases [2]. Paleoparasitology investigations started in 1910, when Ruffer succeeded in separating the egg of *Schistosoma haematobium* from two mummies dating back to more than three thousand years ago. He developed a method for refreshing, staining and preparing a tissue sample isolated from mummies dating back to several thousand years ago [3]. In 1979, Ferreira finally christened this field as paleoparasitology [4].

Paleoparasitology makes it possible for issues such as host-parasite relationships, amount of disease and prevalence of parasites, food habits, amount of pollution, health of various human populations, effect of different cultures, evolutionary process of parasites, pathological lesions induced by parasites and effects of climate change on ecosystems to be studied [5-9]. The development of the field has helped to broaden the understanding of anthropologists, archaeologists, biologists and medical scientists on the etiology of diseases induced by parasites. Furthermore, this field has also facilitated the discovery of the origin of infectious diseases. For example, a cysticercosis case was found in a mummy 200-100 B.C. years in Egypt [10]. Researchers speculated neurocysticercosis as the possible cause of Julius Caesar's (100 to 44 B.C.) epilepsy [11]. Iran is one

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of the first human settlements on earth and there is several evidences of early human life in the ancient land of Iran. Therefore, the aim of the review was to evaluate paleoparasitological researches conducted in Iran in order to facilitate discovery of the origin of infectious diseases in the region.

METHODS

To evaluate paleoparasitological researches conducted in Iran, the present review was designed based on published articles, which were limited to Persian and English papers. The English databases evaluated included Web of Sciences, Scopus, ScienceDirect, Pubmed, Google scholar, Embase. In addition, the Persian databases employed included Scientific Information Database (SID), Magiran, Iran Medex and Iran Doc.
These databases were evaluated without time restriction. They were searched employing medical subject heading (MeSH) terms such as: "Iran" [MeSH Terms], "Islamic Republic of Iran" [MeSH Terms], "Parasitology" [MeSH Terms], "Parasites" [MeSH Terms], and "Archeology" [MeSH Terms]. Inclusion criteria in the current review included published papers based on the discovered parasites in Iran. Accordingly, the study of Mowlavi et al. (conducted in Iran based on discovered parasites in France) was excluded (12).

Sites and materials in paleoparasitology
Initially, many biologists believed that ancient parasites do not become fossil and their traces cannot be found in ancient remains; however, this idea has changed following the discovery of animal and plant parasite fossils belonging to millions of years ago [13-16].
Sites and materials with maintainability of ancient remains are used for sampling and searching of ancient parasites. Sites such as caves and human settlements (places where human wastes are deposited), and dry places such as desert and ancient tombs are suitable places for the search of ancient parasites. In addition, a series of materials such as mummified corpses, hair, skin, feathers, fossils and fossilized feces (surrounded by salts)

Table 1 - A quick review on the parasites detected in the archaeological sites of Iran using the paleoparasitological methods.

Studies	*Sites/Locations*	*Materials/Samples*	*Discovered Parasitic Eggs*	*Old*	*Year*
Nezamabadi et al. (30)	Chehrabad salt mine in Zanjan	Mummy remains	*Taenia spp.*	Third century BC	2012
Nezamabadi et al. (31)	Chehrabad salt mine in Zanjan	Coprolites of human, animal, or undefined origin	*Taenia/Echinococcus spp., Ascaris spp, Trichuris spp., Enterobius vermicularis, Oxyuris equi, Dicrocoelium spp.*	2500 and 1500 years BP	2011
Mowlavi et al. (34)	Yasouj	Three burials and one burial jar	*Dicrocoelium dendriticum*	2600 to 2200 BC	2012
Bizhani et al. (38)	Kiasar archaeological site	Pelvic bones from an adolescent male	*Dicrocoelium dendriticum*	247 BC–224 AD	2015
Mowlavi et al. (32)	Chehrabad salt mine in Zanjan	Feces of rodents	*Syphacia species, Trichosomoides crassicauda, Trichuris species*	565 CE	2014
Paknazhad et al. (29)	Tehran	A female sacrum and pelvic bones	*Enterobius vermicularis*	7000 years	2014
Mowlavi et al. (33)	Chehrabad salt mine in Zanjan	Paleofeces of the carnivore coprolite	*Macracanthorhynchus hirudinaceus*	4th and 5th century CE	2012
Makki et al. (35)	Shahr-e Sukhteh*	Sheep and carnivore coprolites	*Taenia spp., Capillaria spp., Dicrocoelium dendriticum*	3200-1800 BC	2017
Makki et al. (36)	Shahr-e Sukhteh*	Sacrum and pelvic bones excavated from a grave	*Physaloptera spp. (Nematoda: Physalopteridae)*	2800-2500 BC	2017
Paknazhad et al. (37)	East Chia Sabz archaeological site	Pores and surface of a dog pelvic bone	*Taeniid and Ascarid*	8100 BC	2017

*Meaning burnt city in Persian.

are used to search for the remains of ancient parasites [1, 5, 7, 17-20].

Techniques and methods in paleoparasitology

From the onset of studies on paleoparasitology until the last 30 years (before the advent of cellular and molecular techniques), most studies were conducted employing the flotation technique such as zinc sulfate flotation, sucrose flotation, and/or zinc chloride flotation, and then, assessing the samples employing light microscope [21, 22]. The most common technique for the hydration of samples is Trisodium phosphate (Na3PO4) method. In this method, 0.5% concentrated Na3PO4 is used to separate the egg of parasites and larvae of worms from materials and mineral salts. The samples are then examined using a light microscope. In some studies, acids such as hydrochloric acid are employed to increase demineralization of the samples [5, 20, 23].

Furthermore, transmission electron microscopy (TEM) is employed to observe the structure of the eggs and the larvae. This technique differentiates between the eggs of parasites and other microscopic organisms. TEM is a powerful tool for paleoparasitological investigations and study of the morphology of eggs [24]. Furthermore, immunological tests were employed to recognize ancient parasites in some studies [25]. Today, with the discovery of ancient DNA (aDNA) and RNA (aRNA) using PCR, a new window has been opened for scientists in paleoparasitological studies [26-28].

Paleoparasitological researches in Iran

Several studies have been conducted on ancient parasites in some countries. In recent years, Iranian parasitologists and biologists have shown immense interest this field. A series of studies have been conducted in recent years. Table 1 shows a quick review of the parasites discovered in the archaeological sites of Iran employing the paleoparasitological methods. Also, Figure 1 shows the geographical map related to the archaeological sites in Iran.

Tehran city

In 2014, a parasitological study was conducted by Paknezhad et al., in Tehran city (capital of Iran). In the study, which was conducted close to a sewage construction and civil water project, an ancient pottery was discovered in the soil. During further evaluation, a female skeleton was found in the ancient pottery dating back to 7000 years ago. Several soil samples were obtained around the sacrum and pelvic bones. An *Enterobius vermicularis* egg attached to the sacral area of the skeleton was discovered in the rehydrated soil samples (using trisodium phosphate method) by microscopic evaluation. In fact, Paknazhad et al.,

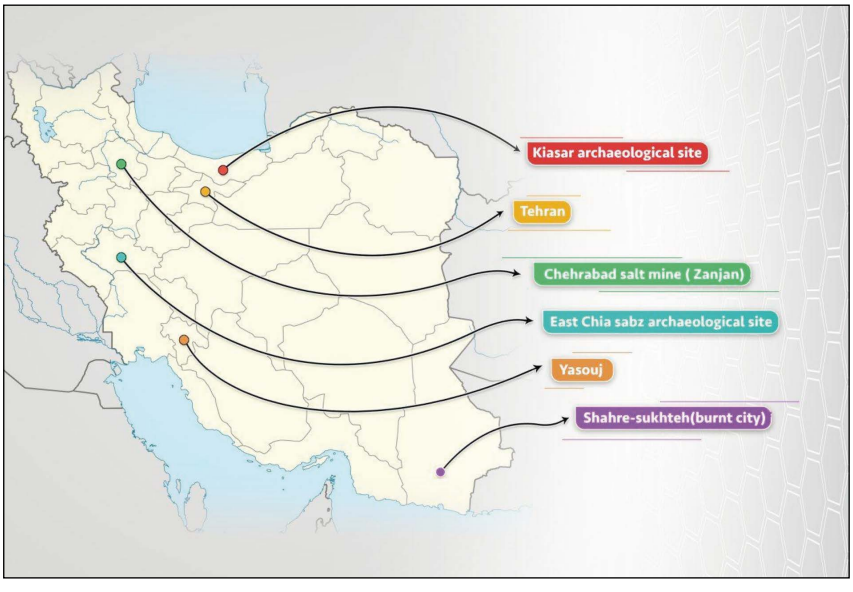

Figure 1 - The geographical map related to the archaeological sites in Iran.

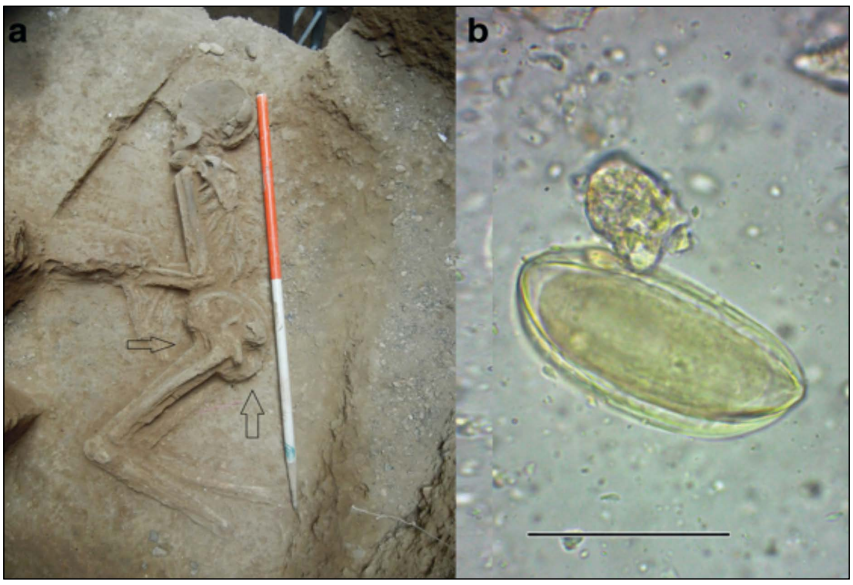

Figure 2 - a) The skeleton of the female discovered in the Tehran archaeological site (b) E. vermicularis egg detected by Paknezhad et al. (29).

evaluated 488 slides and one egg similar to those of oxyurid worms was identified. Archeological and parasitological result of the study has not only shown the oldest possible incidence of a human pinworm infection in the city and Iran as a whole, but also has confirmed human settlement in the city in the 5th millennia B.C (29). Figure 2 shows the skeleton of the female discovered in the Tehran archaeological site as well as the *E. vermicularis* egg found in the area.

Chehrabad salt mine

In 2012, Nezamabadi et al., succeeded in identifying the eggs of a parasitic worm employing paleoparasitological tests in one of the mummies discovered in Chehrabad salt mine in Zanjan (northwest of Iran, the site place of the Iranian salt man). This study provided the first information related to parasitic diseases in ancient Iran. In one of the pioneer studies carried out on ancient parasites in the Middle East, 12 eggs belonging to *Taenia* spp. were found in the salt man dating back to the third century B.C. This study presented the first recovery of parasites in ancient Iran. This finding provided the earliest evidence of ancient intestinal parasites in Iran [30]. In the other investigation conducted in 2011 (published in 2013), Nezamabadi et al., examined the coprolites of human, animal, and/or the undefined origin obtained from the salt mine. They discovered *Taenia/Echinococcus spp., Ascaris spp., Trichuris spp., Enterobius vermicularis, Oxyuris equi* and *Dicrocoelium spp*. [31].

Furthermore, in 2014, Mowlavi et al., reported the egg of ancient parasites in the feces of rodents in the archaeological site of Chehrabad salt mine in Zanjan. They reported that these feces probably belonged to a particular species of rats. These researchers succeeded in recovering six eggs belonging to *Syphacia* species, fifteen eggs belonging to *Trichosomoides crassicauda* and one egg belonging to *Trichuris* species employing trisodium phosphate method. Rodent paleofeces excavated from Chehrabad salt mine archeological site successfully revealed the spread of three common helminthic infections amongst rats in ancient Iran [32].

In the other research, Mowlavi et al. published a second report on paleoparasitological results from animal coprolites in 2015. The samples were obtained from the archeological site of Chehrabad salt mine in Zanjan city. In fact, the carnivore coprolite was obtained from the layers in the salt mine in 2012 and was discovered to be related to Sasanid era (4th and 5th century CE). Then, the animal was evaluated for parasites using trisodium phosphate rehydration method. After examination of the samples, *Macracanthorhynchus hirudinaceus* eggs were obtained from the animal. The current result could be considered as the old-

Figure 3 - Dicrocoelium egg detected in the Kiasar archaeological site by Bizhani et al. (38).

est acanthocephalan infection in Zanjan and Iran as a whole. Because of the appropriate preservation condition in the salt mine, these eggs had a clear appearance in paleofeces (33).

Yasouj city

In 2012, excavations were done in the cemetery belonging to the Bronze Age (2600 to 2200 B.C.) in Yasouj city (southwest of Iran), Kohkilooye and Boyer-Ahmad province. Mowlavi et al. examined 1100 rehydrated soil samples employing trisodium phosphate method. In one of these graves, two eggs were found. The morphological features of these eggs such as brown color, thick wall, lack of window in the eggs as well as the size of the eggs convinced the researchers that the eggs were that of *Dicrocoelium dendriticum*. The presence of nomadic tribes in the area for a long time as well as the location of the exploration site close to the Achaemenes capital (Persepolis) and petroglyphs in Persepolis confirmed the prevalence of animal husbandry at that time. Results of this nature can provide interesting information on the distribution of parasites in the former Iranian eras [34].

Shahr-e Sukhteh (Burnt city)

Shahr-e Sukhteh, which means burnt city in Persian, is regarded as the junction of trade routes in the Bronze Age. It extends to the Iranian plateau and is located in Sistan and Baluchistan province (southeast of Iran). In 2017, Makki et al. investigated sheep and carnivore coprolites obtained from the city (an archeological site dating back to about 3200-1800 B.C.) using trisodium phosphate solution. After evaluation of the samples employing light microscope, *Taenia spp., Capillaria spp.* and *Dicrocoelium dendriticum* eggs were found. Also, while some other objects similar to *Toxocara* species and Anoplocephalidae were discovered. The results showed the first paleoparasitological results related to the Bronze Age in eastern Iran that revealed the true identity of the people, their economic activities and communication as well as appropriate condition for zoonotic helminthiasis life cycle in the city [35].

In the other study, in 2017, Makki et al. evaluated the soil samples obtained from the sacrum and pelvic bones of a skeleton discovered in a grave related to Shahr-e Sukhteh. They examined 320 samples and only one parasite-infected individual was identified and reported. *Physaloptera* spp. eggs were found in the sample (dating back to the Bronze Age). Today, this parasite rarely induces human helminthiasis, but it might not have been so rare in ancient Iran. This study was the first report related to ancient human physalopterosis in Iran, particularly in the Middle East [36].

East Chia Sabz archaeological site

The East Chia Sabz archaeological site (Western Iran) is located in Seymareh valley and dates back to the Pre-Pottery Neolithic era, about 8100 B.C. In a research, in 2017, Paknezhad et al. analyzed the soil obtained from the pores and surface of a dog pelvic bone in this archaeological site using trisodium phosphate method and reported taeniid and ascarid eggs obtained from the samples. The present results significantly show the natural prevalence of cestode and nematode parasites among dogs dating back to the Pre-Pottery Neolithic era, about 8100 B.C. In addition, these eggs can be used to contribute more facts on the paleoparasitological documentation of the Fertile Crescent and track the oldest parasitic infections in the Iranian plateau [37].

Kiasar archaeological site

This site is located on the Caspian Sea littoral of Iran in Mazandaran province (northern part of

Iran) dating back to 250 B.C. (the Parthian dynasty). In 2015, Bizhani et al. analyzed samples obtained from soil layers attached to pelvic bones using trisodium phosphate method. The microscopic evaluation of the ten examined samples showed that one case (an adolescent male) was parasitized by *Dicrocoelium dendriticum* eggs [38].

CONCLUSION

The results of the current paleoparasitological review showed the incidence of human and animal parasitic infection in Iran dating back to 8100 B.C. The parasitic infection identified include *Taenia/Echinococcus spp.*, *Ascaris* species, *Trichuris* species, *Oxyuris equi*, *Dicrocoelium* species, *Syphacia* species, *Trichosomoides crassicauda*, *Enterobius vermicularis*, *Macracanthorhynchus hirudinaceus*, *Capillaria* species and *Physaloptera* species. In consonance with the review results, some of the parasites were discovered in others countries such as Brazil, Argentina, Chile, Peru and China as well as North America [2, 39, 40]. Because of the diversity of the species and ecosystems, habitats, caves, catacombs (Dakhma), temples, tombs and ancient castles around Iran as well as the presence of valuable and unique sites and biological treasures such as Burnt City and the salt men mummies, the country can be considered as a fascinating country for paleoparasitological researches [41, 42].

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Conflict of interest
The authors declare no conflict of interests.

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