SHORT REPORT

Archaeoparasitological Strategy Based on the Microscopic Examinations of Prehistoric Samples and the Recent Report on the Difference in the Prevalence of Soil Transmitted Helminthic Infections in the Indian Subcontinent

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Archaeoparasitology is a study to acquire data concerning the parasite infection of ancient people through the examination of the specimens obtained in the excavation sites. Although this research has achieved many successes worldwide, there has been few noteworthy reports from South Asia countries. In 2011 to 2016, we thus conducted parasite examinations on Indian archaeological specimens (n = 247) collected at excavation sites of Mature Harappan period (4600–3900 BP) and their contemporary rural Chalcolithic sites. To derive effective strategy of archaeoparasitological works in Indian Subcontinent, our data were analyzed together with previous clinical report on the soil transmitted helminth infection in the Indian Subcontinent. We propose that future paleoparasitological studies in India should be conducted more intensely on ancient specimens from the states of Assam, Bihar, Jammu and Kashmir, Tamil Nadu, Andhra Pradesh and West Bengal etc.

Introduction

About several thousand years ago, the urban societies flourished in the Gaggar-Hakra and Indus river basin and coastal regions of Northwest India as a symbol of the splendid Harappan civilization (Singh 2009: 132–181). Since the first Harappan site was discovered in the 1920s, a great deal of information has been revealed by many archaeologists' efforts. However, not all advanced techniques have been successfully applied to the research on the Harappan civilization. One such example is an archaeoparasitology.

Over the last half-century, archaeoparasitology has made significant contributions to archaeological science (Seo et al. 2014: 235–242; Seo et al. 2016: 555–563). By performing analysis on the parasitological samples from archeological sites, the ancient human population's parasitic infection pattern could be grasped vividly. By interpreting it from socio-cultural perspectives, the data became indispensable for archaeologists to understand the interaction between nature, mankind and society in history (Seo et al. 2014: 235–242; Seo et al. 2016: 555–563).

Despite these achievements, parasitological studies on ancient specimens have been rarely conducted in South Asia, especially in India. Given the fact that parasitic infections are closely related to the agricultural society (Reinhard 1988: 355–366) and that the farming was one of the most important parts of Indian history and culture, very few archaeoparasitological outcomes in South Asia is very surprising to us. For the past several years, we thus tried to do archaeoparasitological investigations on the samples of Mature Harappan (4600–3900 BP) and contemporary Chalcolithic period (Kenoyer 1998; Shinde 2002: 157–188) sites of India.

Materials and Methods

In 2011–2016, we took the samples at archaeological sites in India and microscopically examined ancient specimens for detecting ancient parasite eggs remained in them.

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We collected the soil sediments from excavation sites of Harappan or Chalcolithic periods, including Gujarat, Haryana, and Rajasthan in India. The detailed information of archaeology is presented in **Figure 1** and **Table 1**.

The specimens were sampled from the remains of fortification wall, mound, residential place, water channel, reservoir, bath, street sewage, drains, water line, and burials etc. considering that ancient parasite eggs were commonly reported to be found in the specimens of such facilities or structures (Seo et al. 2016: 555–563; Shin et al. 2009: 2534–2539; Shin et al. 2011: 3555–3559; Shin et al. 2013: 208–213; Shin et al. 2014: 569–573; Shin et al. 2015: 458–461; Kim et al. 2016: 80–86; Shin et al. 2018: e53). The surface soils were also obtained as negative control.

In the lab, the soil samples were rehydrated in 0.5% trisodium phosphate solution (Callen and Cameron 1960: 35–40; Criscione et al. 2007: 2669–2677). After the rehydrated solutions were filtered through gauze, they were precipitated for a day. The precipitates were then dissolved in 10% neutral-buffered formalin (20 ml) to be pipetted onto slides. We examined them using a light microscope (Olympus, Tokyo, Japan) (Seo et al., 2014).

Results and Discussion

Since parasitic eggs were not found in the surface soil, the contamination of the soil at the excavation site by modern parasite eggs could be ruled out. Nonetheless, in microscopic observations on 247 specimens of Harappan (n = 8) and Chalcolithic (n = 2) sites, we could not discover any ancient parasite eggs in them either.

In general, there were great urban areas in Harappan Civilization: Mohenjodaro, Harappa, Ganweriwala, Rakhigarhi, and Dholavira (Singh 2009: 147). The Harappan metropolitan areas had well-designed systems for drinking water, bathing, drainage, and sewage. The presence of toilets in the Harappan cities was also noteworthy. The Harappan toilets identified to date ranged from a simple hole above a cesspit to more elaborate system made of *big pot* on the floor and a *small jar* for washing-up. Archaeologists speculated that Harappan people must have cleaned the toilets and drains in each city district on a regular basis (Singh 2009: 148; Kenoyer 1998: 60). The waste water from the toilets was drained through the sewage chutes or pipes into open street drains, and finally emptied into the fields outside the city wall (Singh 2009: 148–149). Since well-organized city ruins (Rakhigarhi and Dholavira) were included in the survey at this time, we originally predicted positive results out of the ancient samples. However, we could not find parasite eggs in any sewage specimens of Harappan Civilization (Dholavira and Mitathal).

We also collected the specimens of the water facilities (water channels, water lines, bath drains, and reservoirs) in Dholavira, considering that they might have been contaminated by a discharged water from toilets. In parasitological examinations, however, no positive signs were detected in them either. Not only in such water facilities, the ancient parasite eggs were not found in the samples of the residential areas (Gilund, Balathal, Rakhigarhi, Mitathal, Karsola) or of graves (Rakhigarhi and Farmana) (**Table 1**). We wonder if such a low success rate

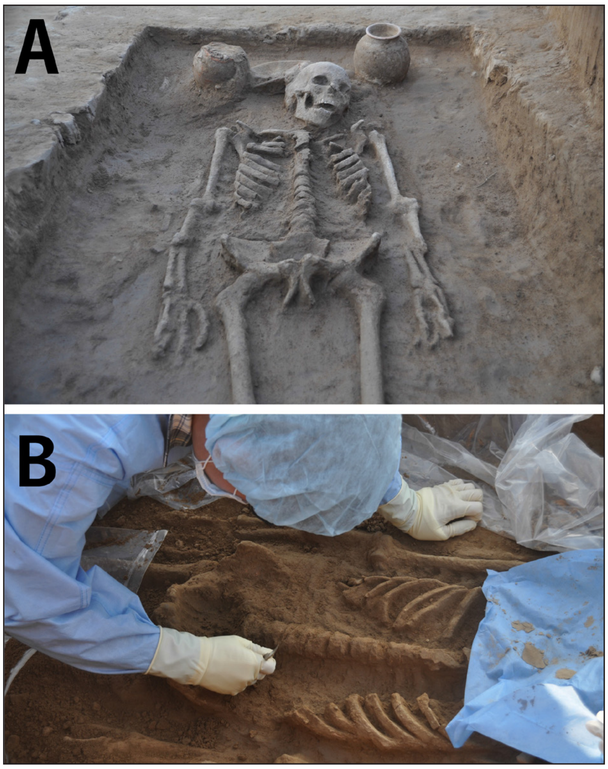

Figure 1: Parasitological sampling at prehistoric cemetery of Rakhigarhi site, the Harappan period megacity. **(A)** The skeleton at grave. **(B)** Parasitological sampling from hip bone.

Table 1: Specimens for Parasitological Examination in This Study.

Sites	Province	Locations	Used for	Period	Sample Number
Kotada Bhadli	Gujarat	Fortification Wall	Non-Residential	Harappan	7
		Surface Soil	NC	Modern	1
Gilund	Rajasthan	Index trench	Non-Residential	Chalcolithic	2
		Mound 1 south east	Residential	Chalcolithic	3
		Mound 1–1 to 1–7	Residential	Chalcolithic	7
		Surface Soil	NC	Modern	1
Balathal	Rajasthan	Section 1	Residential	Chalcolithic	5
		Surface Soil	NC	Modern	1
		Section 2	Residential	Chalcolithic	5
		Surface Soil	NC	Modern	1
Rakhigarhi	Haryana	Citadel Mound Locality 2	Residential	Harappan	6
		Mound 3	Residential	Harappan	3
		Surface Soil	NC	Modern	1
		Locality 2 Mound 6	Residential	Harappan	5
		Surface Soil	NC	Modern	1
		Y-B1–1	Residential	Harappan	1
		A	Residential	Harappan	3
		B	Residential	Harappan	15
		C	Residential	Harappan	10
		RGR7.2 A1 BR01	Grave	Harappan	1
		RGR7.2 A1 BR02	Grave	Harappan	2
		Surface Soil	NC	Modern	1
		RGR7.2 A2 BR14	Grave	Harappan	2
		RGR7.2 A2 BR08	Grave	Harappan	1
		RGR7.2 A2 BR09	Grave	Harappan	1
		RGR7.2 A2 BR11	Grave	Harappan	2
		RGR2.1 Ax7	Residential	Harappan	4
		South Res. Water Channel	Water Channel	Harappan	16
Dholavira	Gujarat	Surface Soil	NC	Modern	1
		Reservoir Bed	Reservoir	Harappan	6
		East Reservoir L2	Reservoir	Harappan	20
		Citadel Bath Drain	Bath Drain	Harappan	8
		Surface Soil	NC	Modern	1
		Lower town Street Sewage-1	Sewage	Harappan	1
		Surface Soil	NC	Modern	1
		Lower town Street Sewage-2	Sewage	Harappan	1
		Surface Soil	NC	Modern	1
		Middle town House/south drain soil	Drain	Harappan	1

(contd.)

Sites	Province	Locations	Used for	Period	Sample Number
		Surface Soil	NC	Modern	1
		Middle town street water line	Water line	Harappan	1
		Surface Soil	NC	Modern	1
		Surface Soil	NC	Modern	1
		Residence surface soul	NC	Modern	1
Mitathal	Haryana	Residence Drainage pipe	Drainage	Harappan	1
		Section A2	Residential	Harappan	8
		Surface Soil	NC	Modern	1
		Section 1	Residential	Harappan	1
		Section 2	Residential	Harappan	1
		Section 3	Residential	Harappan	1
		Section 4	Residential	Harappan	1
		Section 5	Residential	Harappan	1
		Section 6	Residential	Harappan	1
		Section 7	Residential	Harappan	1
		Y-B1	Residential	Harappan	8
Karsola	Haryana	Surface Soil	NC	Modern	1
		Y-B2-A	Residential	Harappan	6
		Surface Soil	NC	Modern	1
		Y-B3-B	Residential	Harappan	6
		Surface Soil	NC	Modern	1
		Y-C2	Residential	Harappan	1
		Surface Soil	NC	Modern	2
		X-B1	Residential	Harappan	10
		Surface Soil	NC	Modern	2
		X-B3	Residential	Harappan	16
		Surface Soil	NC	Modern	2
		Locality2	Residential	Harappan	14
		Surface Soil	NC	Modern	2
		Burial34	Grave	Harappan	4
Farmana	Haryana	Surface Soil	NC	Modern	1
		Surface Soil	NC	Modern	1
		Total			247

NC, Negative Control (Surface Soil).

of parasitological research in Indian archaeological specimens might have been due to the environmental condition of the sampling places that was not suitable for the preservation of ancient parasite eggs over 4,000 years. Or is it due to excellent hygiene of the Harappan cities, towns or villages?

To make a rational explanation of this phenomenon, we note a recent study on the published literatures to analyze the spatial distribution and prevalence of soil transmitted helminths (STH) in each province of India (Salam and Azam 2017: 201). In the study, the authors tried to identify all relevant publications (n = 480) pertaining to the STH infections in India, in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. They displayed infection rate of *T. trichiura* and *A. lumbricoides* in the mapping (Salam and Azam 2017: 201), providing invaluable information on the parasitic infections in India at a glance (**Figure 1**).

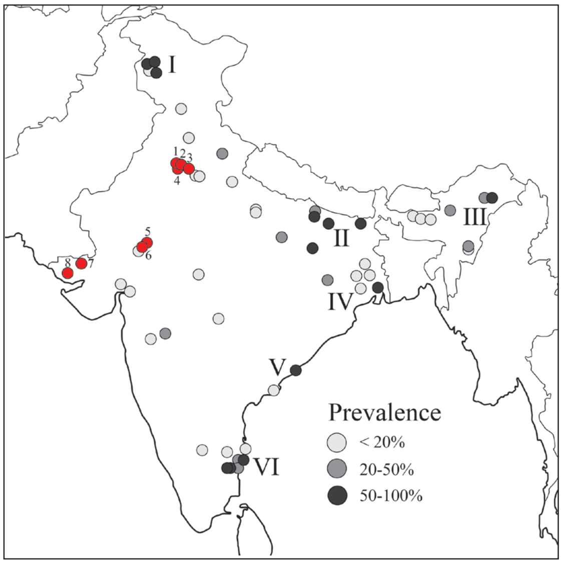

Figure 2: Gray-scale dots represent the recent report on the difference in the prevalence of Ascaris lumbricoides infection in the Indian Subcontinent (Salam and Azam 2017: 201). The closer the dot color is to black, the higher the infection rate. Areas where the infection rate is over 50% are as follows: I, Jammu and Kashmir; II, Bihar; III, Assam; IV, West Bengal; V, Andhra Pradesh; VI, Tamil Nadu. The red dots indicate where the archaeoparasitological samplings were conducted in this study, including: 1. Rakhigarhi, 2. Karsola, 3. Farmana, 4. Mitathal, 5. Gilund, 6. Balathal, 7. Dholavira, 8. Kotada Bhadli.

According to them, in all of India, *Ascaris* infection rates exceed 50 percent in several locations scattered across six states including Assam, Jammu and Kashmir, Tamil Nadu, Bihar, West Bengal and Andhra Pradesh, covering approximately 30% of India's total population (Salam and Azam 2017: 201). In case of *T. trichiura*, Assam and Andra Pradesh were the areas where the rate of infection exceeds 50 percent. Meanwhile, the areas such as Chandigarh, Delhi, Himachal Pradesh, Gujarat and Karnataka, Madhya Pradesh and Rajasthan showed a relatively low rate (20% or less) for *Ascaris* infection. This means that the provinces where we conducted the archaeoparasitological sampling (Rajasthan, Gujarat, and Haryana) belong to the area of extremely low STH infection rate in Salam and Azam's study (2017).

Why are the provinces of Rajasthan, Gujarat, and Haryana so low in STH infection rate? We note that the excavation sites we surveyed are arid or semi-arid places of low temperatures and humidity. As the general condition was not ideal for the survival of parasite eggs in the soil, the environment might lower the rate of STH in the regions. If we accept the assumption, future paleoparasitological studies in Indian subcontinent need to be approached in a totally different way than before.

In summary, the archaeological sites of Harappan civilization belong to the areas where paleoparasitological study has not been properly performed so far. In this study, we also failed to get positive results from the ancient samples of Harappan and Chalcolithic period sites in India. We thus hope to propose the strategy of future archaeoparasitology research in India. Since the modern STH infection rate in Bihar, Tamil Nadu, Jammu and Kashmir, Assam, West Bengal and Andhra Pradesh is higher than other states, future paleoparasitological studies should be conducted more intensely on ancient specimens from those provinces.

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Competing Interests

The authors have no competing interests to declare.

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