Human Intestinal Parasites From the Wushantou Site in Neolithic Period Taiwan (800–1 BC)

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Human intestinal parasites from the Wushantou site in Neolithic Period Taiwan (800-1 BC)

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Abstract

Here, we investigate the presence of parasitic infections in Neolithic peoples from Taiwan to provide insight into the health and cultural development of these populations. Analysis was conducted on 27 soil samples collected from the pelvic region of human skeletal remains, along with control samples taken from the skulls and feet excavated from the Wushantou site in southwest Taiwan. The samples were disaggregated, passed through micro-sieves, and visualized using light microscopy. Analysis revealed the presence of roundworm eggs (*Ascaris lumbricoides*) within the remains of one individual. The control samples were negative for parasites, suggesting a true infection in this individual and not later environmental contamination of the soil. This is the first discovery of ancient parasite eggs in prehistoric Taiwan. The low apparent prevalence of parasites in this population is discussed in the context of the environment during this time and the consequences of regional climate on preservation of parasite eggs.

Keywords: ancient disease; geohelminths; nematodes; palaeoparasitology; palaeopathology, Asia

1. Introduction

Austronesian-speaking groups are believed to have dispersed from the coastal region of southeast China to Taiwan during the Neolithic period sometime around 4000 BC (Bellwood

1988; Bellwood et al. 2006; Chang 1969, 1987, 1992; Hung 2013; Hung and Carson 2014; Tsang 1999, 2012). Here, they interacted with existing populations of the early and late Ta-Pen-Keng (TPK) culture dating from 4000-2200 BC who were already inhabiting the island (Tsang et al. 2006). By studying ancient parasites in these populations, we can improve our understanding of the health and lifestyle of these peoples, and provide the potential to find evidence for dispersal of new diseases. Past research using this approach has helped demonstrate the sequential nature of the prehistoric settlement of the Americas from Asia, the movement of people from medieval Europe to the Middle East with the crusades, and migration of Chinese laborers to the USA in the 1800s (Araújo et al. 2008; Mitchell et al. 2011; Montenegro et al. 2006; Reinhard et al. 2008). While a number of studies have identified the presence of parasites in East Asian populations during historic times, none have yet been found in individuals from prehistoric contexts. The identification of intestinal parasites that infected the earliest settlers of the region would help to provide a baseline for intestinal diseases in early populations, allowing further research as to how the prevalence of these diseases may have changed over time as populations increased and lifestyles changed.

In order to determine the kinds of parasites that infected early peoples in Taiwan, we investigated individuals buried at the Wushantou site, a late Neolithic settlement associated with the Dahu Culture, which occupied most of the southwestern part of the island. The Wushantou site is located on the Chianan plain, the largest on the island, which lies approximately 30 meters above sea level (Chen 2013; Lee 1999). The significance of this site is that it not only covers a wide area, but also is rich in artifacts that have remained relatively intact, and is characterized by continuous cultural strata. The importance of the Wushantou site has led to a number of investigations since the 1900s (Chen 2013; Kanaseki 1943;Mori 1902) and has helped significantly to better understand other sites in the region.

1.1 Neolithic Taiwan and the Austronesian-Speaking Peoples

It is generally agreed that TPK represents a new wave of migrants from the Chinese mainland (Chang 1992; Tsang 1999). Chang (1987) attempted to use TPK culture to rethink the interaction between early Neolithic period culture in southeast Fujian and Guangdong provinces with the western coast of Taiwan using linguistic data. He argued that Proto-Austronesians originated from the southeastern region of the mainland before migrating to Taiwan, dispersing prior to the late TPK cultural period. Austronesian languages, spoken by nearly 400 million people, form one of the largest language groups in the world. They are widely distributed throughout the islands of Southeast Asia, Madagascar, and the Pacific, with a few found in continental Asia. Similarly, Bellwood (1988) considered that the ancestors of Austronesian-speaking groups were Neolithic farmers from the southeastern coast of the mainland. Because of the need to expand their agricultural base, this led to population movements around 4000 BC and their arrival in Taiwan around 3500 BC (Bellwood 1988). After the TPK culture, the middle Neolithic period emerged around 2500 BC (Tsang 1999; Tsang et al. 2006) and distinct cultural traits began to develop in a number of localities (Liu 1992). Evidence of interaction during the late Neolithic period suggests the development of substantial exchange and trade relations. For example, green nephrite sourced to eastern Taiwan was used in the manufacture of characteristic ear pendants dating from 500 BC - AD 500 and that have been found distributed across sites in the Philippines, East Malaysia, southern Vietnam, and peninsular Thailand (Hung et al. 2007). Pottery collected in the Batanes Islands, northern Philippines, was also similar to those from eastern Taiwan, indicating a clear interaction between these two regions (Bellwood and Dizon 2005).

1.2 The Excavation of the Wushantou Site and the Culture

The Neolithic Wushantou site in southwestern Taiwan was dominated by the Dahu Culture from 1300 BC to AD 200, and was known for its black pottery (Tsang et al. 2006). Since black pottery is also typical in northern mainland China, the similarity in archaeological relics has led to the proposal that there was a strong influence from northern China (Kanaseki 1943). The Wushantou site (Figure 1), in Tainan City, has been excavated twice: in 1997-1998 and 2013-2014. Both of these were salvage excavations in which shell midden, bone tools, and pottery was discovered. According to Lee (1999) and Chen (2013), the site was radiocarbon dated to 800-500 BC. However, the pottery and other artifacts suggest the Wushantou site was in use for centuries longer, from 800 to around 1 BC. During the first season, there were 39 burials excavated, with 27 burials found in the second season. The pelvic sediment from the burials excavated in the second season was used for paleoparasitological analysis in this study.

2. Materials and Methods

Past paleoparasitological studies have shown that it is possible to recover parasite eggs from the remains of abdominal contents of individual burials (Bouchet et al. 2001; Fugassa et al. 2008; Mitchell et al. 2013; Rácz et al. 2015; Reinhard et al. 1992). After death, the intestines decompose and the contents become part of the soil that forms in the pelvis. While the intestinal worms themselves die and are broken down during this process, their eggs are often quite resistant to decay due to the chitin in their walls, and so may be preserved in this pelvic soil (Araújo et al. 2015). Therefore, in order to assess the presence of parasites within the buried individuals, samples of pelvic sediment were taken from the area just anterior to the sacrum of each individual. Samples of sediment were taken from the skull and the feet from the same individuals to be used as the control samples, as the eggs of intestinal parasites should not be present in these areas during life.

From each sediment sample, a subsample (1 g) was taken and converted to a solution (disaggregation) using distilled water. The test tube was shaken intermittently by hand to encourage mixing. The process was repeated with a parallel sample using 0.5% trisodium phosphate in the event that one method was more effective than the other (Anastasiou and Mitchell 2013). After one hour the process of disaggregation was complete, and the fluid was passed through microsieves of mesh size 300µm and 160µm. The sizes of most human intestinal parasite eggs in Asia have a diameter of between 10µm and 150µm (Bouchet et al. 2003). As a result, these eggs will pass through the 160µm sieve and be collected in the tray at the base of the stack of sieves. The sediment from the tray was centrifuged to concentrate it, the supernatant removed, and the sample suspended in glycerol before mounting on 20 slides in order to obtain a clear view of any eggs under the microscope. Based on standard parasitology sources (Garcia 2009; Gunn and Pitt 2012), the identification of the parasite eggs was made according to their morphology, dimensions, color, and special characteristics.

3. Results

Of the 27 burials analyzed, one was found to be positive for intestinal parasite eggs. The sediment from burial no.16 revealed four fertilized eggs of *Ascaris* sp. (roundworm) (Figures 2 and 3). Fertilized human roundworm eggs are oval and generally have the dimensions 45-75µm in length and 35-50µm in width, while unfertilized eggs are generally 85-95µm in length and 43-47µm in width (Garcia 2009). The control samples from the head and feet of the burial were negative for parasite eggs, which suggests that the eggs genuinely represent an intestinal infection in that person during life, and not later contamination of the soil by the feces of humans or animals. Since only a limited number of eggs were present in the sample, we are unable to determine if either of the disaggregation solutions was more effective in this archaeological context.

In our differential diagnosis we considered whether these structures were not parasite eggs, but instead fungal spores or pollen. However, there are no such structures in Taiwan with a similar appearance, as fungal spores from this island are generally much smaller than the dimensions of the structures we identified (Lin et al. 2004). There is such similarity in appearance between the eggs of roundworms that infect humans (*A. lumbricoides*) and pigs (*A. suum*), that it has been argued they may even be the same species (Leles et al. 2012). In a coprolite of unknown provenance, the identity of *Ascaris sp.* eggs can therefore only be made to the level of its genus. However, since these parasite eggs were present in the soil from a human pelvis, it is most likely that the parasites were of human origin and represent *A. lumbricoides*.

4. Discussion

The analysis of these 27 burials from the Wushantou site identified roundworm eggs in a single individual. This is the first research undertaken to identify whether ancient parasites were in fact present in prehistoric Taiwan. Below, we will consider the significance of this finding by first explaining what roundworm parasites are, and then comparing these findings with past research into ancient parasites in Asia. Finally, we consider the general effect of the environment in Taiwan on the preservation of parasite eggs in archaeological contexts.

4.1 Roundworm Infection in Modern and Ancient Humans

Adult roundworms are 20-30 cm long and live in the intestines of their hosts. During their life cycle they emit eggs that mix with the human intestinal contents. Once the feces have been passed from the body, the roundworm life cycle requires the eggs to mature in a warm, humid, and dark environment for 1-2 weeks before becoming infective (Garcia 2009:130-133; Gunn and Pitt 2012:127-129). Roundworms are typically spread by the contamination of food and water with human feces. A light infection with a few worms is unlikely to cause any symptoms in a well-fed individual, but a heavy infection of roundworms can lead to malnutrition, developmental delay, and stunted growth in children (Halpenny et al. 2012; Ngui et al. 2012). In modern times, roundworm is spread most easily in societies where sanitation is poor and human feces are used to fertilize crops (Jensen et al. 2008; Phuc et al. 2006; Ziegelbauer et al. 2012).

Parasite ecology is strongly affected by human hygiene, population density, food preferences, lifestyle, and social practices (Araújo et al. 2008; Mitchell 2015a; Mitchell 2015b; Reinhard et al. 1992; Yeh et al. 2014). Roundworm is thought to have infected humans throughout our evolution, and so co-evolved with us (Mitchell 2013). Indeed, the earliest known archaeological evidence for parasites in humans is a roundworm egg from the Paleolithic period in France, dating to 28,000-22,000 BP (Bouchet et al. 1996). Studies evaluating the impact of agriculture on parasite ecology have revealed significant increases in the prevalence of roundworm and other parasites within modern agricultural societies when compared to modern hunter-gather societies (Dounias and Froment 2006). Researchers from Japan have demonstrated the absence of parasitic infection prior to the establishment of agrarian societies (Matsui et al. 2003). The evaluation of sediment from the San-nai Maruyama site dated to 4000-1500 BC revealed the absence of roundworm eggs in the geological strata of non-agrarian Jomon groups, with *Ascaris* eggs becoming common after the introduction of agriculture during the Yayoi period (Matsui et al. 2003). It is known that cereals such as millet and rice have been found from this period of Taiwanese prehistory (Tsang et al. 2006), demonstrating that Neolithic Taiwan was populated by peoples who practiced farming.

However, the exhumed remains of a number of wild animal bones at the same site also shows that Dahu peoples seem to have practiced a mixed subsistence. It has been argued that the early Neolithic Austronesian-speaking peoples of Taiwan pursued a mixed coastal foraging and horticultural lifestyle (Chang 1981, 1987; Hung and Carson 2014), but by the late TPK culture, rice and millet farming were practiced in both small villages and larger settlements (Hung and Carson 2014; Tsang et al. 2006). As a result, it is believed that populations in Neolithic Taiwan had a broad-spectrum subsistence strategy. Lee (1999) has also used zooarchaeological data to argue that Dahu people relied heavily on hunting and gathering over and above a pure reliance on agricultural methods. In contrast, during the same period, peoples in mainland China had embraced a feudalistic system associated with stable sedentism. In general, such societies tend to be more densely populated than societies characterized by simple agrarian or hunting and gathering systems. Ancient parasite analysis from pelvic burial soil collected at the Zhengzhou Jinshui and Xinzheng Lihe sites, Henan Province, China, revealed five tombs where *Ascaris* eggs were identified (Wei et al. 2012). This indicates that in the spring and autumn period during the reign of the Zhou Dynasty (771-476 BC), the Zhengzhou region of central China was an area where infection with fecal–oral parasites was more widespread.

4.2 Evidence for Ancient Parasites Elsewhere in East Asia

Many intestinal parasites have been detected in archaeological contexts from different time periods across East Asia. If we consider the types of parasites that were present across the region, we can better interpret our findings in Taiwan. Some of these parasites are unique to humans and have infected them throughout their evolution; these are sometimes known as heirloom parasites. Others are zoonoses—parasites that are endemic to wild animals in the region, but which can infect humans if people consume or closely interact with those animals (Mitchell 2013; Sianto et al. 2009).

There are a great many zoonotic helminths in present-day East Asia, including *Clonorchis sinensis* (Chinese liver fluke), *Diphyllobothrium sp.* (fish tapeworm), *Paragonimus westermani* (oriental lung fluke), *Taenia asiatica* (Asian tapeworm), *Metagonimus yokogawai* (intestinal fluke), *Brugia malayi* (filariasis), and *Trichinella spiralis* (CSCP et al. 2006; Liu 2009; Youn 2009). Recent studies show that helminths such as *C. sinensis, Fasciolopsis buski*, and *P. westermani*, known to cause paragonimiasis, still cause epidemics in Taiwan (CSCP et al. 2006). Their transmission is normally associated with failure to thoroughly cook food, and with the consumption of pickled, salted, and raw fish or crustaceans infected with the metacercarial intermediate form of the parasite (CSCP et al. 2006).

Parasite infection surveys over the last century have provided insight into the impact of helminths on health in Taiwan and southern mainland China. Liu et al. (2008) examined the prevalence of parasitic infection in Taiwan and reported that the infection rate of *A. lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Ancylostoma duodenale* (hookworm), exceeded 90% in the first half of the twentieth century, while Chinese liver fluke showed significant epidemic infection rates of around 50% in some places during the 1980s. According to the national survey of parasite infection conducted in mainland China from 1988-1992, 62.6% of the population was infected with parasites, of which five species were nationally distributed: *A. lumbricoides, Enterobius vermicularis, T. trichiura, Giardia duodenalis* and *Entamoeba histolytica*, with prevalence rates of 47%, 26%, 19%, 3%, 1%, respectively. The Chinese liver fluke also ranked among the top 15 parasites found in humans, with a mean prevalence rate of 0.4% across mainland China, and a particularly high number of infections observed in Guangdong province of 1.8% (Liu 2009). It was estimated that 5-10 million of the population were infected with Chinese liver fluke at the time, 5 million of whom were from Guangdong province (Liu 2009).

A number of human mummies from mainland China have also been found to contain the remains of parasitic worms. Coprolite samples taken from mummies of the Ming and Song dynasties have found evidence of infection with *A. lumbricoides, C. sinensis, F. buski,* and *T. trichiura* (Li 1984). Another study undertaken on a female mummy from the Mawangtui tomb in Changsha, Han Dynasty (202BC-220 AD), found eggs of *Schistosoma japonicum*, *T. trichiura* and *E. vermicularis* in the coprolites (Wei 1973), while a similar study of a Han dynastic mummy from Phoenix Hill in Huibei Province observed showed the presence of *S. japonicum*, *C. sinensis*, *Taenia* sp. and *T. trichiura* (Wei et al. 1981). In Korea, the parasites *A. lumbricoides*, *T. trichiura, C. sinensis*, *M. yokogawai*, *Gymnophalloides seoi*, *P. westermani*, *Strongyloides stercoralis*, and *Trichostrongylus* sp. have been found in mummies, coprolites, and other archaeological finds (Seo et al. 2010; Seo and Shin 2015; Shin et al. 2009). In Japan, the eggs of ancient parasites identified include *A. lumbricoides*, *T. trichiura*, *M. yokogawai*, *C. sinensis*, *Taenia* sp. and *Diphyllobothrium* sp. (Matsui et al. 2003).

A general observation underpinning the paleoparasitological findings from mainland China is that they were confined to the low-lying wetlands of the Yangtze River, an environment that is favorable for flukes to complete their lifecycles. Research shows that the Wushantou site was surrounded by a tropical rainforest environment with significant marshes in the region (Lee 1999). While the palaeoenvironment data suggests that the ancient Chianan Plain in Taiwan would have been a favorable environment for flukes such as Chinese liver fluke, *F. buski*, and oriental lung fluke to complete their lifecycles, our study failed to uncover archaeological evidence of these species in Neolithic deposits from Taiwan.

4.3 Survival of Ancient Parasite Eggs at Archaeological Sites in Taiwan

We have demonstrated that a wide range of parasites were present in East Asia in the past, and that these were a mixture of heirloom parasites unique to humans and zoonoses that could infect humans after consuming the flesh of wild animals. We must then ask the question as to why only roundworm was found at the Wushantou site in Taiwan, and then only in one individual out of the 27 analyzed. One possibility is that there may have been very little parasitism in Taiwanese populations during the Neolithic period, while another explanation for our results is that the environment in Taiwan may have resulted in poor preservation of parasite eggs in this and other archaeological sites.

In Taiwan, the period from summer to early autumn is marked by tropical cyclones, with around 3-4 of these striking annually and causing severe flooding (CWB 2013). These cyclones can increase the annual rainfall to levels exceeding 2,500 mm (WRA of MEA 1996). As a result, the preservation of archaeological remains can suffer from the influence of different climatic effects. Skeletal remains from archaeological excavations may sometimes be poorly preserved due to their taphonomic environment, and it is understandable that the preservation of ancient parasites will be similarly affected. With such large volumes of water passing through the soil following storms, it is quite likely that many of the parasite eggs originally present may well have been washed away over time. We have studied a considerable number of burials at other archaeological sites in Taiwan for ancient parasites, but found them all to be negative (Table 1). As a result, we suspect that the heavy rain in Taiwan is a principle factor for the negative results of the paleoparasitological analysis in many of the individuals we have examined.

5. Conclusion

The discovery of roundworm eggs from Neolithic human burials at the Wushantou site in Taiwan provides the first evidence for ancient intestinal parasitism on the island. This demonstrates that this parasite was present in prehistoric communities that farmed, hunted, and gathered both marine and terrestrial resources here 2,500 years ago. Roundworm is spread by fecal contamination in food, and tends to become more common in populations with poor sanitation and who fertilize their crops with human feces. The range of species we identified was significantly less than has been found on mainland China, and after considering possible explanations, it seems likely that high levels of precipitation in Taiwan may have removed much of the ancient parasite evidence. As a result, roundworm may in fact have been just one of a number of species that infected early populations living on the island in prehistory. Future work may have better success if coprolites and latrine soil can be studied, as these sources of ancient feces often have better preservation of ancient parasite eggs than does pelvic soil from burials.

Table 1: Negative Results of Parasitological Study at Other Archaeological Sites in Taiwan

Sites	**Date**	**Time Period**	**Sampling area**	**Sample number**
Shiqiao (may also be spelled Shihchiao)	800 – 1 BC	Neolithic	burial soil	5
Shiqiao	AD 600 - 1000	Iron Age	burial soil	57
Qiwulan	AD 1000	Iron Age	burial soil	3

Figure 1. Location of Taiwan and the archaeological sites where palaeoparasitology was performed.

Map in black and white for use in the printed issue.

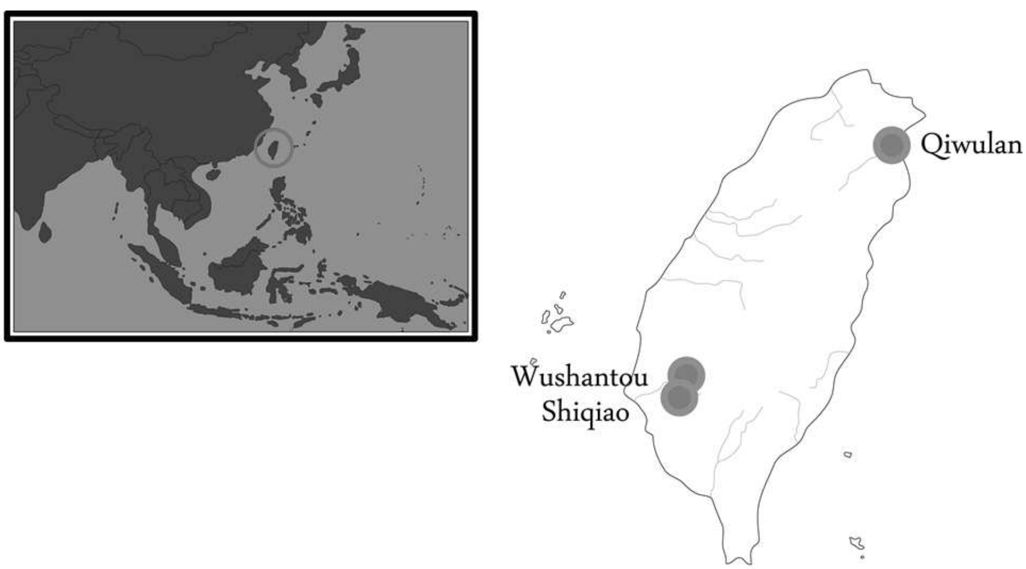


Figure 2. Damaged *Ascaris lumbricoides* (roundworm) egg with its mammillated surface coat. Dimensions 47x38µm.

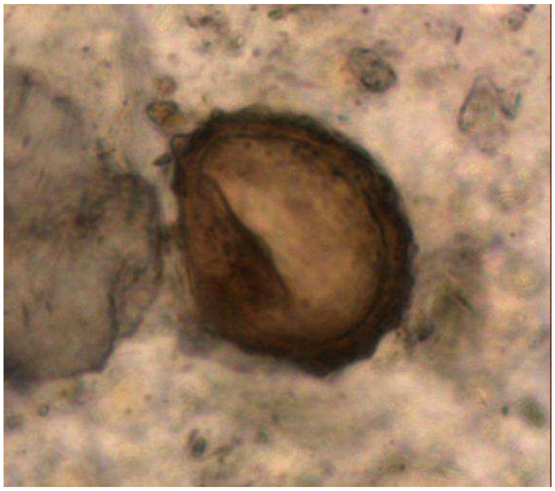
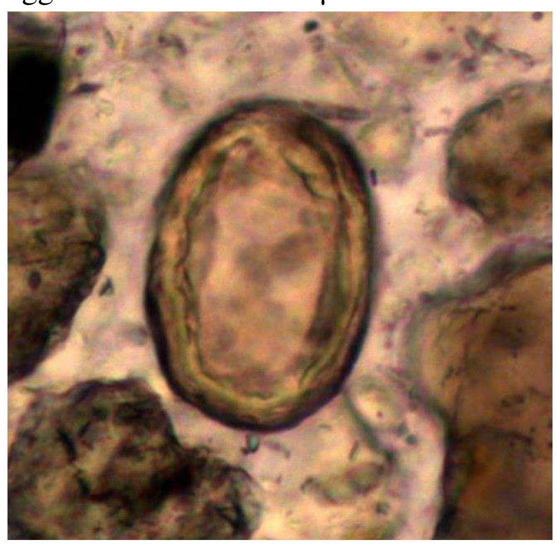


Figure 3. Damaged and decorticated roundworm egg. Dimensions 57×39 µm.



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