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Early evidence for travel with infectious diseases along the Silk Road: intestinal parasites from 2,000 year old personal hygiene sticks in a latrine at Xuanquanzhi relay station in China.

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Abstract

The Silk Road has often been blamed for the spread of infectious diseases in the past between East Asia, the Middle East and Europe. While such a hypothesis seems plausible, there is actually very little concrete evidence to prove that diseases were transmitted by early travellers moving along its various branches. The aim of this study is to look for ancient parasite eggs on personal hygiene sticks in a latrine at a large relay station on the Silk Road at Xuanquanzhi (111 BC-AD 109), at the eastern margin of the Taklamakan Desert in north-western China. We isolated eggs of four species of parasitic intestinal worms: Chinese liver fluke (*Clonorchis sinensis*), *Taenia sp*. tapeworm (likely *Taenia asiatica, T. solium or T. saginata*), roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*). The Chinese liver fluke requires wet marshy areas to sustain its life cycle and could not have been endemic to this arid region. The presence of this species suggests that people from well-watered areas of eastern or southern China travelled with their parasites to this relay station along the Silk Road, either for trade or on government business. This appears to be the earliest archaeological evidence for travel with infectious diseases along the Silk Road.

Background

1.1 Parasites as Markers of Migration with Diseases

It is well known that people in the past travelled long distances for trade, exploration, political delegations, military campaigns, or just to find a new place to live. Those who happened to be infected with diseases at the time would have carried those organisms with them. The Silk Road has often been blamed for the spread of infectious diseases such as bubonic plague, leprosy and anthrax by travellers between East Asia, the Middle East and Europe (Monot et al. 2009; Schmid et al., 2015; Simonson et al. 2009). However, such theories are generally based upon either the modern distribution of disease strains, or archaeological samples from Europe that match modern East Asian strains (Morelli et al., 2010; Wagner et al., 2014). There is very little firm archeological evidence to prove that early travellers moving along the various branches of the Silk Road did spread diseases. These diseases could potentially have reached Europe from East Asia via other routes, such as through India to the south or Mongolia and Russia to the north.

It is often very challenging to identify clear examples of the spread of ancient disease with long distance travel. Intestinal parasites can be a useful approach to investigate this, as some species are only endemic in a restricted geographic region due to the nature of their life cycle and the intermediate hosts that may be required for the parasite to mature into a form that can infect humans. Examples where such parasites have successfully demonstrated migrations include the various routes taken during prehistoric settlement of the Americas (Araújo et al. 2008), crusaders travelling from medieval Europe to the Middle East (Mitchell et al., 2011), African parasites in France around 1500 (Bouchet et al, 2002), and Chinese laborers moving to California in the 1880s (Reinhard et al., 2008).

If we could find evidence for parasites at locations on the Silk Road well outside their endemic area, we could prove for the first time that travellers along the Silk Road really were responsible for the spread of infectious diseases along this route in the past.

1.2 The Gansu Corridor and the Silk Road

Gansu Province in north-west China contains the Hexi Corridor, a 1000 km band of territory stretching from the banks of the Yellow River at its south east to the Tarim Basin at its north west, where it is dominated by Taklamakan Desert. The corridor is bordered by the Gobi desert, the Qilian Mountains and the Beishan Mountains (Ma et al., 2004). This corridor formed a section of the Silk Road, an ancient network of thoroughfares extending 4,000 miles, interconnecting the ancient communities of East Asia, Central Asia, Western Asia and the Near East. The Silk Road was used by merchants, pilgrims, monks, soldiers and nomads to travel between China and the Mediterranean Sea, so contributing to cultural exchange between ancient civilisations (Liu, 2010; Boulnois and Mayhew, 2005; Hansen, 2012). The Silk Road came to prominence during the Han Dynasty (202 BC – AD 220). According to Sima Qian's biography of Dayuan in *The Records of the Grand Historian* (*Shiji*), Zhang Qian was a Chinese envoy appointed by Emperor Wu of Han to the Western Regions (*Xiyu*), around Xinjiang and Central Asia. Zhang Qian explored the sections of the Silk Road through military missions (Boulnois, 2005).

1.3 The excavation of the Xuanquanzhi site and the Han dynasty

Xuanquanzhi relay station is a site that has been designated a Major Historical and Cultural Site Protected at the National Level in China. It is located in the town of Dunhuang, a key stopping point on the Silk Road within the Hexi Corridor (Fu, 2005; Bonavia et al., 2004) (Fig. 1). According to documents and the archaeological stratigraphy, the relay station was built in 111 BC and used as such until AD 109, before being later transformed into a beacon tower during the Six Dynasties (AD 220 or 222–589) (He, 2000). The final syllable *zhi* (置) in the name Xuanquanzhi designates a site in charge of a horse-delivering post (Lien, 2015). The postal relay system relied on horses as a means of communication between local governments and the seats of the imperial court, such as Chang'an and Luoyang in the Central Plain of China, both of which were major capitals during the Han Dynasty. The relay station not only conveyed messages but also hosted visitors (He, 2000; Lien, 2015).

Between October 1990 and December 1992, the Gansu Institute of Cultural Relics and Archaeology conducted a comprehensive excavation at Xuanquanzhi (Fig. 2). Many artifacts were excavated including bamboo and silk used for writing, bones of livestock, linen fabrics, paper and stationery (He, 2000). These findings were among the most important archaeological discoveries in China at the time.

2. Methods

The hygiene sticks were excavated from the latrine at the Xuanquanzhi site in 1992 (Fig. 3) and have been stored at the Gansu Institute of Cultural Relics and Archaeology. These sticks were made of wood or bamboo wrapped with cotton cloth, to be used as personal hygiene sticks for wiping the skin around the anus after passing faeces into the toilet (Fig. 4). Hygiene sticks for wiping, sometimes also referred to as bamboo slips, have been described in ancient Chinese texts and also found at archaeological sites before (Gansu Provincial Institute of Cultural Relics and Archaeology, 1991; Wang, 2010). Seven sticks from Xuanquanzhi were found to have preserved faeces adherent to the cloth. Faeces from 6 sticks was combined to make one sample, while one stick with more faecal matter preserved comprised the second sample. The dried faeces was disaggregated using distilled water, and the process was repeated with a parallel sample using 0.5% trisodium phosphate in case one method was more effective than the other (Anastasiou and Mitchell, 2013). After one hour of disaggregation, the samples were fully suspended and were passed through micro-sieves of mesh size 300µm and 160µm in order to separate parasite eggs from the rest of the soil particles. Since the dimensions of the eggs of most intestinal worms that parasitize humans in Asia range between 10µm and 150µm, sieving the samples through micro-sieves will separate the eggs from soil sediment particles that are larger than the size of the eggs (Yeh et al., in press). The fine particles that passed through the sieves were concentrated by centrifugation and the supernatant removed.

Egg counts per gram of soil can be determined using a number of methods (Reinhard et al., 1986). In our study we counted the number of eggs in 0.2-gram sediment samples and multiplied the number of eggs observed by five. Doing this avoided the need to use *Lycopodium sp.* spores and prevented potential bias resulting from processing an aliquot of disaggregated fluid from a larger original sample, since the eggs may not be uniformly distributed throughout the disaggregation fluid (Yeh et al., 2015). Finally, the samples were mixed with glycerol, mounted on slides and analysed with light microscopy. The identification of the parasite eggs was based on their morphology, dimensions, colour and special characteristics, in accordance with standard parasitological sources (Garcia, 2009; Gunn and Pitt, 2012).

3. Results

Samples from the hygiene sticks were positive for the eggs of whipworm (*T. trichiura*), roundworm (*A. lumbricoides*), *Taenia sp.* tapeworm (compatible with *T. asiatica, T. solium or T. saginata*), and Chinese liver fluke (*C. sinensis*) (Table 1). Whipworm was identified by its lemon shape, brown colour, polar plugs and dimensions. Roundworm was identified by its oval shape, brown colour, dimensions and mammillated coat. *Taenia sp.* tapeworm was identified by its round shape, thick wall with striations, brown colour, and dimensions. The sample collected from the single latrine stick also contained one egg of Chinese liver fluke (*C. sinensis*) (Fig. 8a). This was identified by the oval shape, a thick light-brown wall, its dimensions (normal range 27µm to 35µm long and 12µm to 19µm wide), the operculum that covers the anterior end with a conspicuous rim protruding from the side of the egg, and a small knob or a little curved spine on the abopercular end (Garcia, 2009; Gunn and Pitt, 2012). A modern example of a Chinese liver fluke egg is given as a comparison (Figure 8b). While all four species of parasite were found in the samples disaggregating using distilled water, only whipworm, roundworm and *Taenia sp.*

tapeworm were identified in the samples disaggregated in trisodium phosphate.

4. Discussion

4.1 Chinese Liver Fluke and Long Distance Travel

Perhaps the most significant finding from these surprisingly well-preserved personal hygiene sticks is the presence of Chinese liver fluke (*C. sinensis*). This species of flatworm has been endemic to the marshy and humid areas of south and central China for thousands of years (Seo and Shin, 2015). A study undertaken on a Han Dynastic mummy from Phoenix Hill in Hubei Province in central China revealed the presence of *C. sinenisis*, *Schistosoma japonicum, Taenia sp.* and *T. trichiura* (Wei et al., 1981). Similarly, coprolite samples from mummies of the Ming and Song Dynasties found in East China contained the eggs of *C. sinenisis, A. lumbricoides, Fasciolopsis buski,* and *T. trichiura* (Li, 1984). What is so important is the location where the liver fluke was found.

Chinese liver fluke could not have been endemic at Dunhuang in the arid north-west region of China, as the parasite requires a wet marshy environment for its life cycle. The flukes mature in bile ducts of the liver and can produce up to 4,000 eggs per day for at least six months (Schmidt and Roberts, 2006). If an infected person goes to the toilet in fresh water, these eggs gain entry to a suitable snail, which acts as the intermediate host. The larval forms develop in the snail before a further period of development in fresh water fish, which can then be eaten by humans. Infection can lead to abdominal pain, diarrhoea, jaundice, and liver cancer (Qian et al., 2015). According to a national survey of parasitic infection conducted in mainland China in 1988-1992, the majority of people infected by Chinese liver fluke lived in Guangdong Province, south China, with a prevalence of 1.82% (Yu et al., 1994). It has been estimated that between 5 and 10 million people were infected with Chinese liver fluke at the time of this survey, 5 million of whom were from Guangdong Province (Liu, 2009).

The relay station is at least 1,500km away from any region where the parasite is endemic today (Fig.1), and 2,000km from Guangdong Province where most cases are found today. The presence of this parasite indicates the migration of people from a well-watered area of China to this relay station, perhaps participating in trade along the Silk Road, or on government business.

4.2 Sanitation, Hygiene and Health

Roundworm and whipworm are thought to have infected humans throughout our evolution (Mitchell, 2013; Reinhard et al., 2013). They are both spread by the contamination of food with human faeces, often through the use of faeces as a crop fertiliser or during the preparation of meals with unwashed hands (Phuc et al., 2006; Ziegelbauer et al., 2012). Adult roundworms are 20-30 cm long and live in the intestines, while adult whipworms are 3-5 cm long. A light infection may not cause any symptoms, but a heavy infection with these parasites can lead to malnutrition, reduced intelligence and stunted growth in children (Halpenny et al., 2012; Ngui et al., 2012).

According to the *Book of Han* (also known as the *History of the Former Han*), human latrines in the Han Dynasty were sometimes used as a source of crop fertiliser (Wu and Liu, 2013; Yi, 2005). The whipworm and roundworms eggs found on the personal hygiene sticks suggests that visitors to the station had been eating food contaminated by human faeces, providing insight into the efficacy of hygiene practices in ancient China.

Interestingly the eggs of pinworm (*Enterobius vermicularis*) were not found on the hygiene sticks. Since the eggs are laid at night on the skin outside the anus by the adult worms, there was potential for us to find eggs on the hygiene sticks used to wipe this area. Pinworm eggs have been found in a mummy dating from 202 BC- AD 220 (Han Dynasty) at Changsha in China, so we know the parasite was present in the China at that time (Wei, 1973). Their absence in our samples may indicate that the parasite was not present in those who used the sticks, or that the fragile eggs did not survive well in this latrine environment.

4.3 Pig Viscera Consumption and Cooking Methods

We found several specimens of *Taenia sp.* tapeworm, and due to its geographic location it may well represent the Asian tapeworm (*Taenia asiatica*), pork tapeworm (*T. solium*) or beef tapeworm (*T. saginata*). Asian and pork tapeworm are spread by eating raw or undercooked pork viscera, while beef tapeworm is spread by undercooked beef. Humans are the only definitive host for *T. asiatica* (Ale et al., 2014; Eom et al., 2009).

During the Han Dynasty, pigs were the predominant farm animal consumed, with horses and cattle being largely used for transport and agriculture (Li, 1997; Yu, 2010). Written records indicate that the culture of raising pigs was introduced to the region around Xuanquanzhi by the Chinese, in particular through the presence of the military, since the northwestern region was not initially populated by Chinese (Wei, 2010). According to historical documents written on Han Dynasty bamboo slips, pigs were raised in captivity in pigsties and not allowed to graze freely, since laws existed to protect crops from being destroyed by grazing pigs (Hou, 2012). The excavation of Mawangdui in central China included tombs of upper-class people of the Han Dynasty, and there were many highly valuable artifacts that were foods made from viscera such as liver, stomach, lungs and spleen, used as burial goods (Hunan Provincial Museum, 1973; Hunan Provincial Museum, 1974). These findings suggest that among people at the time, the consumption of viscera was common. In historical documents such as the *Book of Han*, references to eating sliced liver, bacon, raw and roasted meat, and viscera from dogs and pigs indicate their popularity amongst the Chinese population at the time (Wu and Liu, 2013; Jang, 1995). Eating raw or undercooked viscera of pigs would explain why we found eggs of *Taenia sp.* tapeworm on the personal hygiene sticks at Xuanquanzhi relay station. The fondness of ancient Chinese people for pork might suggest that Asian or pork tapeworm is the more likely diagnosis than beef tapeworm.

5. Conclusion

The parasites recovered from the personal hygiene sticks found in the latrine of the Xuanquanzhi relay station during the Han Dynasty provide important information concerning the hygiene conditions, food consumption and migration patterns of people living in ancient China. Of particular note, the presence of Chinese liver fluke demonstrates the long distance migration of people from a well-watered area of southern or eastern China to this relay station on the Silk Road, perhaps associated with trade or government business. Our findings lay support to previous hypotheses suggesting that travel along the Silk Road was responsible for the spread of infectious diseases between East Asia, the Middle East and Europe 2,000 years ago.

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List of Figures

Figure 7. *Taenia sp. egg* (likely *T. asiatica, T. solium* or *T. saginata*). Dimensions: 31x30 µm. Scale: black bar indicates 20 µm

Figure 8a. *Clonorchis sinensis* egg (Chinese liver fluke). Operculum located at right hand end of egg, the adjacent rim, and small nob at the abopercular end of the egg (to the left). Dimensions: 29x16 µm. Scale: black bar indicates 20 µm. Figure 8b. Example of modern Chinese liver fluke egg as comparison.

List of Tables

Table 1: Parasite egg concentrations on the latrine sticks.

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