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RESEARCH NOTES

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Chinese Liver Flukes in Latrine Sediments From Wong Nim's Property, San Bernardino, California: Archaeoparasitology of the Caltrans District Headquarters

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ABSTRACT: Parasitological analysis of 5 sediment samples from latrine deposits spanning the time period from about 1880 to the 1930s are presented. Two sediment samples are from a latrine used by European-
?1 Americans. Three sediment samples are from latrines used by Chinese-Americans on the property of Wong Nim, an important member of the
?2 Chinese community. Two of the Chinese latrines were positive for human parasites. The human parasites encountered include the human whipworm (*Trichuris trichiura*), the giant intestinal roundworm (*Ascaris lubricoides*, c.f.), and the Chinese liver fluke (*Clonorchis sinensis*). Evidence of the liver fluke is especially important. This parasite cannot complete its life cycle outside of its endemic range in Asia because suitable intermediate hosts are not present in the American continents. Its presence signals that at least some of the Chinese-Americans who used the latrines were immigrants who were infected in Asia and then sustained infections while in the Americas.

Parasitological analysis of archaeological sediments can provide insights into human transhumance (Ferreira et al., 1984; Araújo et al., 1988; Reinhard et al., 1987; Reinhard, 1992; Matsui et al., 2003). The latter authors presented evidence of diplomatic legations in Japan that
?3 were parasitized with nonendemic species. Hevly et al. (1979) and Reinhard et al. (1987) reported finding *Trichuris trichiura* in a nonendemic region near modern Flagstaff, Arizona. Ferreira et al. (1984) discovered *Diphyllobothrium pacificum* eggs in coprolites recovered from a Chilean inland site, and this indicates that the prehistoric people who deposited the coprolites used both the coast and inland areas. Araújo et al. (1988) and Ferreira and Araújo (1996) used hookworm evidence to trace prehistoric long-distance migrations. Here, we present evidence of transcontinental introduction of the Chinese liver fluke to California with historic migrations. This study confirms an earlier, unpublished report of Chinese liver flukes from a historic Chinese community in Sacramento, California (Hall, 1982).

Chinese populations moved into San Bernardino in 1867 (Costello and Hallaran, 2004; Costello et al., 2006). By 1880, the countywide Chinese population was about 150. Initially, they lived in various places throughout the town of San Bernardino. They farmed, operated laundries, worked in restaurants and hotels, and were employed as domestic servants or farm laborers. In 1878, the city prohibited laundries within the town limits, and, subsequently, a Chinese quarter was established. By the turn of the twentieth century, as many as 600 Chinese lived in Chinatown. Initially, Chinatown was virtually all male. It was composed of shops, boarding houses, gambling parlors, a temple, labor contractors, and other establishments. By 1893, Chinatown had electricity, and it had piped water by 1900. Human waste disposal was managed by construction of backyard latrines.

Three artifact-filled latrines were discovered during excavations. They were located on property purchased by California-born Wong Nim in 1900. Wong Nim was born in Alameda County, California, and moved to San Bernardino about 1875. He was successful. He first worked as a laundryman but eventually opened a mercantile shop and acted as a labor contractor. He also opened a temple. Wong remained on the corner of Third and B Streets until his death at age 89 in 1941. At that time, he had earned the honorary title of ''Mayor of Chinatown.'' When the State of California purchased Wong Nim's property in 1943, all of the remaining buildings on his property were demolished. At least 1 latrine (number 1035) was filled at this time with debris from the abandoned buildings.

The privies were used by people who built residences and businesses on Wong's original property. However, Wong's house, store, and temple were located a half block away from the latrines and associated houses. It is possible that the latrines were communally used by several Chinese households and businesses.

Processing of the latrine sediment samples was done in 2001, following the methods of Reinhard et al. (1986), Warnock and Reinhard (1992), and Sianto et al. (2005). Sediment was removed from each sample bag. The sediment was freed of large fragments of detritus. From the loose sediments, 30 ml were removed. Next, 3 *Lycopodium* sp. spore tablets were added to each 30-ml sample (about 1,250 *Lycopodium* sp. spores were added to each ml of sediment). For this analysis, *Lycopodium* sp. spore batch 212761 was used. Previous analysis has shown that ~12,500 spores are present in each tablet (values presented from different analyses of tablets are 12,432, 12,489, and 12,542). The tablets were dissolved in a few drops of hydrochloric acid in 300-ml beakers. Next, the 30-ml aliquots of sediment were added to the beakers with 50 ml of distilled water. Subsequently, 20 ml of 10% hydrochloric acid in distilled water was added to dissolve calcium carbonates in the sediment. More water was added until the reaction between the acid and the carbonates in the sediment stopped.

Once the calcium carbonates were dissolved, the samples were treated with the swirl technique. The contents of the beaker were swirled until all particles were in suspension. The beaker was placed on a flat surface for 30 sec. After 30 sec, the fluid was poured through a 300-μm mesh. This was repeated twice. The macrofossils on the mesh were examined for night-soil indicators, especially the presence of *Rubus* sp. seeds. Next, the screened fluid was concentrated by centrifugation in 50-ml centrifuge tubes. The sediments were washed 3 times in distilled water.

Preliminary microscopic examinations were made of the samples to determine if further chemical processing was necessary. It was found that the high content of fine silicates required further processing, so 20 ml of 40% hydrofluoric acid were added to each tube, and the sediments were thoroughly mixed in the acid. The samples were left in the hydrofluoric acid for 24 hr and were stirred occasionally during this period. Next, the sediments were concentrated by centrifugation. The acid was replaced by water, and the sediments were reexamined. The vast majority of silicates were dissolved, and microscopic examination was deemed to be possible. The sediments in the tubes were then washed 3 times in distilled water.

Drops of the sediments were transferred to glass microscope slides with Pasteur pipettes. The sediment drops were mixed with glycerin and cover-slipped. For each sample, a total of 25 *Lycopodium* sp. spores was counted along with all parasite eggs found in the process of counting the spores. A count of 25 spores represents 0.02 ml of the sediment sample. After counting, at least 3 more microscope preparations were counted to assess the presence of trace amounts of parasite eggs.

We quantified the parasite eggs for 0.02 ml of processed sediment for each sample in order to standardize the results of each analysis in terms of parasite eggs per ml of sediment. For latrine contexts, we found that 0.02 ml is sufficient to identify parasite egg quantities as low as 50 eggs per ml. We then scanned an additional 0.06 to 0.08 ml of processed sediment to identify trace amounts of parasite eggs.

The concentrations of eggs of each species were calculated using the following formula: concentration = $[(p/m) \times a]/v$, where p is parasite eggs counted, m is marker *Lycopodium* sp. spores counted, a is marker *Lycopodium* sp. spores added, and v is volume of sediment.

Identification of the species of the parasite eggs was done by morphological analysis. In the case of trichurid eggs, the dimensions of the eggs were taken and compared to those of trichurid species from a variety of hosts, including humans, domestic animals, and rodents that commonly infest habitations, outbuildings, and yards. Operculated eggs

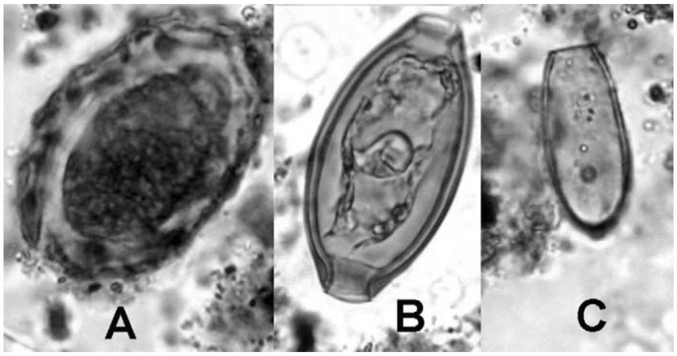

FIGURE 1. Helminth eggs found in the San Bernardino Chinatown latrines. (**A**) *Ascaris lumbricoides* (c.f.); (**B**) *Trichuris trichiura* (c.f.); (**C**) *Clonorchis sinensis*. Note: "c.f." = compares favorably, meaning that the morphology is consistent with human parasites.

were compared to the morphology of a variety of cestode and trematode genera. These included species of *Clonorchis*, *Paragonimus*, *Fasciola*, *Diphyllobothrium*, and *Dicrocoelium*.

Based on many years of experimentation (Reinhard et al., 1986; Warnock and Reinhard, 1992; Sianto et al., 2005), we have found this method to be superior to all clinical methods for recovery of parasite eggs from latrine sediments (soil derived from feces). This is because parasite eggs in latrine soils do not respond to flotation in the same way as modern eggs. Parasite eggs are trapped in calcium carbonate deposits and must be freed by chemical means. The calcium carbonate deposits are a special problem in latrines because people added lime to the latrines when they were in use.

We reanalyzed the processed sediments in 2003 to verify the diagnoses based on observations of more eggs. A third analysis was done in 2005 to photograph the eggs.

Samples from the Euro-American latrines were negative for parasite eggs. Two of three samples from Chinese-American latrines were positive for parasite eggs (Fig. 1). Latrine 1056 was the earliest latrine and was used from the 1880s to about 1900. Latrine 1058 was built in 1900 and filled in 1910; number 1035 was the final latrine and was used from 1910 to 1944.

Latrine 1035 yielded 1,065 ascarid eggs and 710 whipworm eggs per ml of sediment, while latrine 1056 contained 3,374 ascarid eggs and 3,552 whipworm eggs per ml of sediment. These fecal-borne roundworm eggs are nearly ubiquitous in historical town sites. These numbers are not high for latrine sediments and are relatively normal for historical sediments. At low or moderate infections, these parasites rarely cause severe disease. No eggs were found in latrine 1058. The whipworm and ascarid eggs were morphologically identical to eggs of *Trichuris trichiura* and *Ascaris lumbricoides*, respectively. We acknowledge that *Ascaris suum* of pigs is morphologically identical to *A. lumbricoides*. We believe that the eggs are from *A. lumbricoides* because pigs were
?4 not present at the site and the latrines were used for human waste. There has been a debate concerning the value of egg measurements for diagnosis of *T. trichiura* and *Trichuris suis*. Horne and Tuck (1996) argued that this diagnosis is not possible with archaeological remains. In contrast, Fernandes et al. (2005) presented the majority view that whipworm egg dimensions can be obtained from archaeological sediments for diagnosis.

The most interesting discovery in both of these privies was the delicate eggs of *Clonorchis sinensis*, the Chinese liver fluke. The discovery of these eggs shows that the Chinese immigrants in California brought with them at least 1 species of parasite from Asia. Latrine 1035 contained 710 *C. sinensis* eggs per ml, and latrine 1056 contained 533 eggs per ml.

The 3 latrines were used at different times by the same Chinese community. It is noteworthy that the earliest latrine deposits (1880–1900) and the latest latrine deposit (1910–1941) were positive for parasite eggs, but the 1900–1910 latrine sediment contained no eggs. The absence of evidence of parasitism in the middle period is unexplained.

The Chinese liver fluke, like most trematodes, has a multihost life cycle, which includes fishes and snails. These intermediate hosts have important roles in the life cycle of the parasite. The parasite goes through asexual reproduction in the snails. Thus, the number of parasites produced by a single egg is amplified by the snail stage of the life cycle. The fish is important in conveying the parasites to their definitive host, a fish-eating mammal. The definitive host is the animal that harbors the sexually active stages of the parasite. If the parasite survives the culinary preparation of the fish, it will eventually migrate to the liver of the definitive host and live there for many years, mating and laying eggs. The eggs pass through the bile duct into the digestive tract and are passed with feces.

However, the introduction of this parasite to California was a dead end. The intermediate snail hosts to which it is adapted in Asia are absent in the Americas. The archaeological identification of parasite eggs in latrines that date from the late nineteenth century to the early twentieth century shows that the parasite was possibly introduced by immigrants, who may have lived long lives with their infections during this period. If infections were light, the human hosts probably had no symptoms or mild indigestion with light abdominal pain. If they had heavy infections, then they could have suffered from pronounced abdominal pain, diarrhea, jaundice, hepatomegaly, and/or anorexia. In chronic cases, liver cancer could have resulted from infection. No traditional Chinese remedies for this ailment are known to us. However, many bottles from various medicines were found in the Chinese latrines (Costello et al., 2006). These may be related to the symptoms of parasitic disease.

The absence of *Taenia solium* is important for this community. Two backyard roasting ovens were found near the latrines. The historical evidence and animal bone analysis show that these ovens were used for roasting pigs for weekend parties, ceremonies, and feasts. One of the ovens was in use by 1880. It was probably used by one or more stores to cook meat (Costello et al., 2006). By the 1920s, its use had likely declined but not ceased. When this portion of Wong Nim's property was leveled in the mid-1920s, a new roasting oven was built. This new roasting oven (1036), apparently still important for festival events, was built south of Wong Nim's building, adjacent to the Kuan Yin Temple. This was where people gathered for celebrations and where a pig would be cooked, offered at the altar, and then consumed. The absence of *T. solium* eggs in latrines spatially associated with pig-roasting ovens shows that the preparation of the pigs killed any tapeworm cysts.

Asia is home to many parasites that did not exist in the New World, even after European colonization. These parasites included the Chinese liver fluke (*C. sinensis*), the intestinal fluke (*Fasciolopsis buski*), the oriental lung fluke (*Paragonimus westermani*), and the Asian blood fluke (*Schistosoma japonicum*). These parasites were present in Asia in ancient times, as indicated by archaeoparasitology in Korea, China, and Japan. They infected many people, and, undoubtedly, some immigrants entered the Americas with these parasites. For this reason, the analysis of sediments from the San Bernardino Chinatown latrine is particularly important in demonstrating the cross-continental introduction of parasites into North America. For Chinese liver flukes, this was an ecological dead end.

It may be of further interest to mention that clonorchiasis is, to this day, very much a clinical problem in Asian/Chinese immigrants (Stauffer et al., 2004). We may think this a recent problem, but as this paper points out, it is not.

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