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ARCHAEOPARASITOLOGICAL INVESTIGATION OF A MUMMY FROM SICILY (18th-19th CENTURY AD)

ABSTRACT: The coprological analysis using parasitological methods of a mummy's intestinal contents from Sicily (Italy) revealed a severe trichurid infection (whipworm). The egg concentration was estimated at 34,529 eggs/gram of coprolite. We compared the eggs analyzed to those of other Trichuris species and concluded that the eggs were consistent with T. trichiura. The egg preservation was evaluated with the goal of establishing baseline data to compare with other archaeological contexts around the world. This taphonomic study shows that while most of the eggs were intact, many were folded and only a few still had their embryonic contents and polar plugs. Epidemiologically, whipworm is a common worldwide infection today and archaeological findings point to a similarly high prevalence in the past. Currently, and in the European archaeological record, T. trichiura infection is commonly associated with Ascaris lumbricoides (roundworm). The presence of T. trichiura and absence of A. lumbricoides has two possible explanations: either acquired immunity or the use of medicinal compounds.

KEY WORDS: Italian Mummy – Pathoecology – Archaeoparasitology – Coprolites

INTRODUCTION

We undertook the analysis of the intestinal contents of a mummy from Sicily to identify intestinal infections. Archaeoparasitology is based on the analysis of mummies, skeletal remains, coprolites, and archaeological sediments. Mummies of humans, companion animals, and other animals have all been analyzed for parasite remains (Reinhard, Araújo 2008). Other corporeal remains such as pelvic girdle sediments from burials can be a source of parasitological data (Le Bailly et al. 2006). The analysis of sacral sediments from excavated skeletons and from sacra curated in museums is also a growing source of archaeoparasitological data (Fugassa et al. 2007). The exploration of this new area of research is a relatively recent phenomenon. Coprolites are more frequently studied than the intestinal contents of mummies because the former are found in abundance in habitation sites in different parts of the world. The most abundant source of data comes from sediment samples: meaning soil excavated

from archaeological sites, ancient latrines, refuse deposits, streets, and other features found by archaeologists.

Mummies can tell us a lot about parasitism because the coprological findings indicate important elements of both the past behaviors and the living conditions of the subject (Reinhard 1992). The advantage of an analysis of the intestinal remains from a mummy compared to those from coprolites is that the biological origin of the infection is known. Also, the number of individuals from a cemetery allows for paleoepidemiological study. This is not always certain with coprolites and is rarely certain with latrine or trash sediments. In latrine deposits, coprolites from companion animals such as dogs can be confused with human coprolites. Additionally, it is rarely possible to determine the number of individuals responsible for a particular latrine deposit. Differential diagnosis of parasites from sediments is particularly challenging because sediments may contain the parasite eggs from a human source or from any other animal, domestic or wild, in the habitation. Importantly, mummies offer the opportunity to



FIGURE 1. A moment of the 2008 mummy expedition. Arthur C. Aufderheide and Dario Piombino-Mascali during the mummy sampling.

analyze parasites found in tissue samples as well as those found in fecal residues.

Italian mummies reflect a rich cultural heritage with an equally rich array of paleopathological conditions. Thousands of "natural" and "artificial" mummies dated as early as the 13th century have been found in Italy (Fulcheri 1991,

TABLE 1. Trichuris egg measurements in ocular units and microns measured at $400\times$.

Length (O.U.)	Width (O.U.)	Length (µ)	Width (µ)
21	12	52.5	30
23	11	57.5	27.5
21	11	52.5	27.5
22	12	55	30
21	10	52.5	25
20	10	50	25
21	11	52.5	27.5
21	12	52.5	30
21	11	52.5	27.5
21	11	52.5	27.5
21	11	52.5	27.5
22	11	55	27.5
21	11	52.5	27.5
21	12	52.5	30
22	10	55	25
21	11	52.5	27.5
21	11	52.5	27.5
21	12	52.5	30
20	11	50	27.5
20	12	50	30
	Avg	52.75	27.875
	Min	50	25
	Max	57.5	30

Fornaciari 2006). Favourable climatic and environmental conditions have often permitted spontaneous preservation while anthropogenic preservation is mostly associated with important public figures or members of the upper social echelons (Ascenzi *et al.* 1998, Aufderheide 2003). For example, in a study based in the inner Abruzzo region of Italy, 219 subjects, many of whom had become mummified, were recovered. Goiter, prostatic hyperplasia, arteriosclerosis, pneumonia, pulmonary silicoanthracosis, neoplasms and parodontal disease, fractures, tumors, and degenerative joint diseases have been recorded by Ventura *et al.* (2006).

Parasitism also plays an important role in adding to the overall paleopathological picture. According to Fornaciari *et al.* (2009), pediculosis has affected humans since ancient times. Head and pubic lice were ubiquitous during the Italian Renaissance (Fornaciari *et al.* 2009). The mummy of Ferdinand II of Aragon, King of Naples, was positive for both *Pediculus capitis* and *Pthirus pubis* as well as the toxic metal, mercury, most likely a treatment to counter the lice.

The general condition of the preserved parasite eggs themselves is also an interesting area for this analysis. The issue of taphonomy related to the decomposition of parasite eggs found at archaeological sites was first presented by Reinhard *et al.* (1986). However, the effects of decomposition on eggs within mummies have rarely been studied. Reinhard and Urban (2003) found that fish tapeworm eggs in Chilean mummies were more frequently deformed than eggs found in coprolites unassociated with mummies. We believe that it would be instructional to begin collecting baseline data on egg preservation from all archaeological contexts in order to develop an understanding of egg taphonomy in differing environments.

The coprolite in the present study was from a mummy labeled as Piraino 1, housed in the crypt of the so-called "mother church" of Piraino, province of Messina, north-east Sicily (Figure 1). The clothed body of Piraino 1 comprises an unidentified spontaneous-enhanced adult male mummy (Aufderheide 2003). Twenty-five other bodies of religious dignitaries - tentatively dating from the late 18th/mid-19th century AD - were also found in this crypt (Piraino Parish Archives, unpublished data). Visceral samples obtained via a minimal opening of the abdomen of Piraino 1 contained coprolites which were recovered and analyzed. In addition to finding coprolites, the body had upon inspection dental enamel hypoplasia, calculus and pleural adhesions. The diagnosis of plasmacytoma is likely as X-ray investigation revealed lytic lesions located on the skull, ribs and femur (Ortner 2003). Slight to moderate spinal arthritis was also noted. The inspection of this mummy took place in 2008 and was supported by the National Geographic Society.

MATERIAL AND METHODS

Our methods followed those published by Sianto *et al.* (2005) which are, in turn, based on the methods of Warnock and Reinhard (1992). A coprolite subsample, weighing 2.23

grams, was placed in a 300 ml beaker. The sample was rehydrated for one week in a 0.5% solution of trisodium phosphate. This process loosened the aggregated sample. One Lycopodium spore tablet (batch 212,761) containing about 12,500 spores was dissolved in 5 drops of 10% hydrochloric acid. This serves the purpose of quantifying the number of eggs per gram of coprolite. The Lycopodium solution was next poured into the trisodium phosphate and coprolite mixture. A magnetic stir bar mixed the contents of this solution. The solution was next poured into a new beaker through a wire mesh of 250 µm capturing seeds and other dietary components. The remaining liquid was centrifuged and decanted, leaving a plug of microscopic material. Slides were made using a drop of glycerin and microscopic material using the end of a wooden applicator stick to remove a sample from the centrifuge tube. Next, a cover slip was placed over mixture and nail polish sealed the slip and the slide. We scanned our slides at $250\times$, counting the parasite eggs and Lycopodium spores. Once 25 Lycopodium spores were counted the number of eggs in one gram of the coprolite was determined. One subsample of the microscopic remains was archived in a sealed 2 dr vial for future analysis in glycerin. The rest of the sample was processed in order to establish the pollen content.

To assess the condition of eggs, 100 eggs were assessed with regard to preservation. The conditions of eggs were classified into the following categories:

- pristine = polar plugs and larvae intact in an undamaged egg;
- quasi-pristine = larvae intact w/out polar plugs in an undeformed egg;
- 3) moderate = empty undeformed egg;
- 4) poor = empty deformed egg;
- 5) fractured = egg with a crack or tear;
- 6) fragmentary = egg broken in two or more pieces.

RESULTS

We located 25 *Lycopodium* spores and 154 *Trichuris* eggs (*Figure 2*). With these counts it was possible to calculate that 34,529 eggs/gram existed in the coprolite. The egg concentration formula was developed by Warnock and Reinhard (1992) and has been applied to coprolites by Sianto *et al.* (2005). The Piraino calculation was:

 $((154 \div 25) \times 12,500)) \div 2.23 = 34,529 \text{ eggs/gram}$

154 = eggs counted;

25 = *Lycopodium* spores counted;

12,500 = number of *Lycopodium* spores added to sample; 2.23 = weight of sample in grams.

Measurements of the eggs were taken without the polar plugs. We measured 20 eggs, with a calibrated ocular micrometer (*Table 1*). We compared these to Thienpont *et al.* (1979) to verify that the *Trichuris* eggs are specifically *T. trichiura* from humans, not *T. muris*, *T. vulpis*, or *T. Suis*, *T. ovis* from rodents, dogs and cats, pigs, or cattle

FIGURE 2. Only a few eggs contained larvae. In one case, the upper image, the egg was folded but the larva was intact. The two lower images show an egg with a well-preserved larva and one intact polar plug.

TABLE 2.	Trichuris egg	comparison	in a	variety	of hosts
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Parasp	Length (µ)	Width (µ)
T. trichiura	50-58	22–27
T. muris	67–70	31-34
T. vulpis	70–90	32–41
T. suis	50-68	21-31
T. ovis	70–80	30–42
	Parasp T. trichiura T. muris T. vulpis T. suis T. ovis	ParaspLength (μ)T. trichiura50–58T. muris67–70T. vulpis70–90T. suis50–68T. ovis70–80



FIGURE 3. Eight eggs showing that the majority of *T. trichiura* egg shells were well-preserved.



FIGURE 4. Most eggs were folded. This shows the appearance of deformed eggs.

and sheep respectively (*Table 2*). The eggs were all found to be consistent with *T. trichiura*.

A sample of 100 eggs showed them to be apparently well-preserved. No pristine eggs were found. Seven eggs were quasi-pristine (*Figure 2*). A large proportion of the eggs (42) were in moderate condition (*Figure 3*). However, deformed and empty eggs were more common than undeformed eggs (*Figure 4*). Forty-eight eggs were in

poor condition. Two fractured eggs (*Figure 5*) were found. Only one of the 100 eggs was fragmentary.

DISCUSSION

Humans worldwide host whipworm, *T. trichiura*. The adult worms look like a whip, thin at the anterior and thick at

TABLE 3: Find of *Ascaris* (A) and/or *Trichuris* (T), locality, country and date. Data presented here are from Leles *et al.* (2010). Bibliographic information is presented by Leles *et al.* (2010). Legend: *eggs most abundant of the two species. BP Before Present, BC Before Christ, AD Anno Domini.

Site	Area	Date	Material		Parasite	
				Α	Т	
Shillouro-kambos	Cyprus	8300–7000 BC	Skeleton	+	+	
Khirokitia, Larnaka	Cyprus	8300-7000 BC	Skeleton	+	+	
Ötzal	Austria	5300-5200 BP	Mummy	_	+	
Hulin, Central Moravia	Czech Republic	1600–1500 BC	Skeleton	+	+	
Drobintz girl Tollund and Grauballe	Prussia	600 BC	Mummy	+	+	
Man, Central Jutland	Denmark	3 nd -5 th century AD	Mummy	_	+	
Lindow Man	England	Iron Age	Mummy	+	*+	
Bobigny	France	2 nd century AD	Skeleton	+	+	
Karwinden Man	Prussia	500 AD	Mummy	+	+	
St. Isidoro's Collegiate-Basilica, Leon	Spanish	10 th –13 th century AD	Mummy	*+	+	
PUMII mummy Jiangling County,	Egypt	200 BC	Mummy	+	_	
Hubei Province	China	2300 years ago; Chu Dynasty, the Warring Stage (475–221 BC)	Mummy	_	+	
Hubei Province Ma-Wang-Dui, Changsha city,	China	167 BC	Mummy	_	+	
Hunan Province	China	2100 years ago (206 BC–220 AD during Han Dynasty)	Mummy	_	+	
Yangju	Korea	1411± 42 AD (Chosun Dynasty)	Mummy	+	*+	
Joseon Dynasty	Korea	Late 17 th Century	Mummy	+	+	
Orejas de Burro 1	Argentina	3720–3978 BP cal	Skeleton	_	+	
Gentio II Cave, Minas Gerais	Brazil	3490±120–430±70 BP	Mummy and coprolites	+	+	
Cerro El Plomo, Santiago Mummy pre-Columbian.	Chile	450 years	Mummy	_	+	
Murga culture	Peru	Colonial Period	Mummy	_	+	
Itacambira, Minas Gerais	Brazil	18 th century AD	Mummy	_	+	
Total of <i>T. trichiura</i> only					9	
Total of <i>A. lumbricoides</i> only					1	
Total of association of <i>T. trichiura</i> and <i>A. lumbricoides</i>					11	



FIGURE 5. Fracturing was exhibited by a few eggs. In the case of this egg, the shell fractured and filled with digesta.

the posterior. They are 30 to 50 mm long in size and they occupy the host's large intestine. Females lay anywhere from 3,000 to 20,000 eggs per day, which are passed with the host's feces. When a host ingests fecal-contaminated food or water containing embryonated, infective eggs, the young juveniles hatch in the host's stomach. Young adults penetrate the mucosa of the large intestine and initiate sexual reproduction and egg laying. Through fecal contamination, infection spreads easily within the human population (Leles *et al.* 2010).

Review of the archaeological record for geohelminth infections shows that *Trichiura* was a common parasite of the Italian Renaissance (Leles *et al.* 2010).

Piraino 1 exhibits a high infestation of *T. trichiura*. Indeed, the concentration of eggs is the highest recorded in the archaeological record. The 34,529 concentration value represents the daily output of 2 to 17 worms per gram of intestinal content.

The taphonomy of eggs in Piraino 1 shows some interesting trends. There were very few remains of decomposer organisms. Mites, whole and fragmentary, were observed microscopically (*Figure 6*). These were the only decomposer organisms evident in the analysis. No flies or beetles were found in the coprolites. Thus, only mites invaded the corpse after death and mite mouthparts are much smaller than the parasite eggs. Therefore, decomposition noted in the eggs is likely to be due to factors intrinsic to the physical and chemical changes in the digestive tract after death.

Even though many eggs were recovered from the mummy, very few were pristine. The loss of polar plugs and embryos was the norm. This suggests that protein components of whipworm eggs are subject to decomposition in mummies, possibly due to the continuing action of enzymes. Although many eggs were intact and undeformed, a remarkable number showed folding. This suggests that the eggs were compressed in the coprolite during the process of desiccation. The one fractured egg is noteworthy because it is filled with organic coprolite matrix (*Figure 5*). This indicates that it was fractured and filled with residue while it was inside of the mummy.

It is noteworthy that Piraino 1 was infected only with whipworm. Leles and colleagues (2010) note that in Europe, the archaeological record most commonly shows an association of ascarid and trichurid infection. The data is quite remarkable. In Europe, the association of *T. trichiura* and *A. lumbricoides* in archaeological sites is over



FIGURE 6. Mites, and fragments of mites. These infested the mummy after death. This image shows three mites, two of which are complete: that illustrates the density and preservation of these decomposer organisms.

78%, and nearly 90% during the Middle Ages. However, the association is less remarkable when only mummies or pelvic analysis of skeletons are considered (*Table 3*). For all cases worldwide, *T. trichiura* alone was reported from nine cases (43%), *A. lumbricoides* alone was found in just one case (5%) and the association of both species was reported from 11 cases (52%). When only European and Egyptian mummies are considered the association is high. For European cases only, *T. trichiura* alone was reported from 2 cases (18%), *A. lumbricoides* alone was found in just one case (9%) and the association of both species was reported from 8 cases (73%). This underscores the conclusion by Leles and coworkers that Europe saw a consistent association of the two species due to poor sanitation and crowding (Leles *et al.* 2010).

When the two species are not associated, it is more common to find whipworm without roundworm. Because the majority of skeletons and mummies are from adults and older children, it is tempting to suggest that acquired immunity may explain the reduction of parasitism levels from A. Iumbricoides. Although this may be true, it has been demonstrated that medicines are more effective against whipworm than roundworm (Leles et al. 2010). Natural and synthetic anthelminthics are more effective for roundworm than whipworm (for review see Leles et al. 2010). Fisher and colleagues have combined clinical observations with archaeological data to explain the greater prevalence of whipworm in some historic households in Albany, New York (Fisher et al. 2007). The latrines of higher-class individuals with access to vermifuges show a reduction of roundworm eggs relative to those associated with poor households. However, the numbers of whipworm eggs is comparable for all economic classes.

The reason vermifuges are more effective against roundworm than whipworm reflects the different habits of the parasites. Whipworms live in the large intestine and attach to the intestine by burrowing their anterior extremity into host mucosa. On the other hand, *A. lumbricoides* worms live freely in the gut lumen and maintain their place by muscular exertions against the peristalsis-induced flow of the intestinal contents through the intestinal tract. Anthelminthics work in different ways. Many traditional remedies paralyze the worms. Therefore, unattached worms are flushed out of the intestine. Consequently, since roundworms are not attached to the lumen they are more susceptible to the effects of anthelminthic compounds.

It is likely that Piraino 1 had access to medicinal compounds since pollen of *Polygala*, a plant traditionally used for treating lung disease and skeletal pain, was found to be present in the coprolite (Gastaldo 1987). It can be speculated from the presence of lytic lesions that this individual probably suffered from plasmacytoma, a condition which can lead to hyperviscosity syndrome, resulting in shortness of breath and chest pain. The presence of *Polygala* shows that he had sophisticated access to medicinal plants. It is very likely that anthelmintics were available to him as well, as natural remedies against worm

infestation are well-documented in Sicilian traditional medicine (Pitrè 2004).

In conclusion, Piraino 1 had a severe infestation of whipworm when he died. These parasites are relatively benign and cannot be put forward as a possible cause of death. It is likely that chronic fecal-borne parasitism was a common condition for Piraino 1 as it was for a majority of European communities in the Late Modern Era. The fact that only a single parasite species was found implies the use of anthelmintics able to control the other parasite species.

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