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Parasitology in an archaeological context: Analysis of medieval burials in Nivelles, Belgium

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Abstract
Coprolites were recovered from three burials near the Grand Place of Nivelles, Belgium. These remains yielded evidence of geohelminth parasitism. The evidence contributes to studies of differential parasite egg preservation related to the taphonomic conditions within the three burials. Using coprolite analysis techniques, parasite egg concentrations were quantified for each burial. Coprolites from the individual in Burial 122 were abnormally large and abundant, indicating an intestinal blockage. Additionally, this individual hosted an extremely high number of parasites evinced by the calculated parasite egg concentrations (*Trichuris trichiura* = 1,577,679 total eggs; *Ascaris lumbricoides* = 202,350 total eggs). Statistical analyses revealed a positive and significant correlation between *A. lumbricoides* egg and *T. trichiura* egg presence (eggs per gram [epg]: r^2 = 0.583; eggs per coprolite [epc]: r^2 = 0.71). Burial 122 coprolites show a statistically significant increase in egg concentration from the upper colon to the lower colon. Taking extreme parasitism into consideration, the possible causes of the intestinal blockage are discussed. We propose a synergy of high parasite burden and diet contributed to the intestinal blockage. Superior parasite egg preservation was observed in coprolites from Burial 122 compared to Burials 009 and 119. This is due to a variety of taphonomic factors, including a more limited percolation of fluid through the grave sediment.

Keywords: Archaeoparasitology, *Ascaris lumbricoides*, *Trichuris trichiura*, Nivelles, Pathoecology, Taphonomy

1. Introduction

The abbatial complex of Nivelles, erected in the 7th Century, was composed of three churches: Notre-Dame, St. Paul, and Saint-Pierre/Sainte-Gertrude (Figure 1). Notre-Dame was initially the abbey church and later became the parish church. The church of St. Paul housed a male community. Saint-Pierre/Sainte-Gertrude, named for first abbess Gertrude, was initially the funeral church. It later received St. Gertrude's body and became the main church.

Renovations at the Grand Place of Nivelles disturbed the subsoil in the historical heart of the city from early March 2009 until January 2011. Although some features excavated at Nivelles were known from ancient texts, many are new to the historical record of the region. Given the significant impact of the unearthing of such features, the Department of Archaeology of the Public Service of Wallonia intervened in the renovation efforts. The archaeological excavations uncovered seven distinct sets of features: 1) scattered features older than the abbey, 2) a tiler's work area, 3) a graveyard to the west, 4) St. Paul's church, 5) the church of Notre-Dame with its parish cemetery, 6) the abbey's district, and 7) parts of roads.

The cemetery west of the St. Pierre/St. Gertrude church (dating to approximately 1000 A.D.) drew attention due to its excellent state of preservation. Multiple burials and anaerobic conditions allowed for optimal preservation of organic materials.
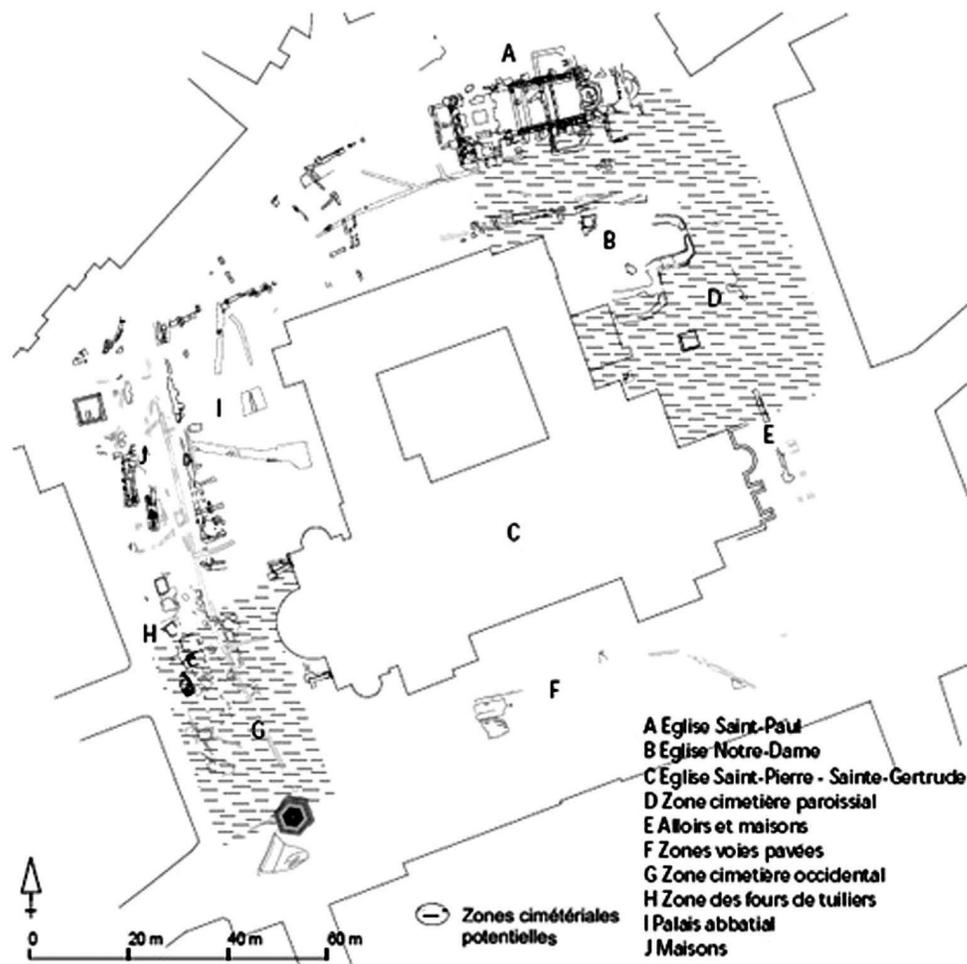
Figure 1. Archeological rendering of the seventh century abbatial complex and cemeteries at the Grand Place Nivelles.

Excavations of the cemetery led to further investigations of the health practices and lifestyles of the people who lived in medieval Nivelles.

The western burial ground is characterized by a short occupation period spanning from the end of the 10th to the middle of the 13th centuries (Figure 1). The physical construction of coffins found in the cemetery varied. Three burials were examined in the present study: Burial 009, Burial 119, and Burial 122 (Figure 2; Figure 3; Figure 4).

Multiple radiocarbon dates were obtained from bone fragments within the burials. The individual within Burial 009 died between cal A.D. 783 and cal A.D. 1018. The individual within Burial 119 died between cal A.D. 1052 and cal A.D. 1274. The individual from Burial 122 died between cal A.D. 1025 and cal A.D. 1159. In addition to the intestinal coprolites, the individuals from these three burials retained some preserved brain matter, skin fragments, hair, and bits of fabric.

The soils surrounding the burials were comprised primarily of clay. Burial 009 exhibited saturated sediment at the time of excavation (Figure 2). Burial 119 revealed an infiltration of sand (Table 1). Covered coffins were lidded with planks of solid wood (Figure 3; Figure 4). Burial 122 was tightly covered with a thick oak board, preventing the filtration of excess moisture, and was excavated into a matrix of low permeability (Figure 4).

Coprolites were recovered from each of the three burials for analyses. A single coprolite came from Burial 009. Three coprolites were recovered from Burial 119. Eight coprolites were discovered in Burial 122. Burial 122 samples were found in the lumbar region of the spine and within the pelvic girdle. The coprolites were arranged in a nearly linear fashion (Figure 4).

Eggs of two species of parasites were found: *Trichuris trichiura* and *Ascaris lumbricoides*. The eggs of *A. lumbricoides* and *T. trichiura* are quite distinct in morphology. Differences between eggs of both species are summarized in Table 2. Eggs of *A. lumbricoides* are ovoid in shape and measure 45 µm–75 µm × 35 µm–50 µm in size (Roberts and Janovy, 2009). The eggs of *T. trichiura* are lemon-shaped and measure 50 µm–54 µm × 22 µm–23 µm (Yoshikawa et al., 1989). Eggs of *T. trichiura* also possess polar plugs at either end. Eggs of *A. lumbricoides* lack any type of opercula. Both of these helminths are recurrent in the European archaeoparasitological literature (Jones, 1985; Aspöck et al., 1996; Kumm et al., 2010; Bartošová et al., 2011; Brinkkemper and van Haaster, 2012; Florenzano et al., 2012; Anastasiou and Mitchell, 2013b; Mitchell et al., 2013; Searcey et al., 2013; Reinhard and Pucu, 2013; Morrow et al., 2014).

The present study examined coprolites for quantifiable evidence of helminth parasitism. Taphonomic conditions were assessed to determine differential parasite preservation. Potential intestinal pathology, as related to cause of death, was assessed.

2. Materials and methods

All samples were processed for the recovery of pollen, parasite eggs, starches, and macroremains by the Palynology and

Figure 2. Burial 009 showing burial context.

Figure 3. Burial 119 showing burial context.

Archaeoparasitology Laboratory, University of Nebraska School of Natural Resources. The collections of these types of data are relevant to future reconstructions of diet, medicinal plant use, seasonality of death, and other aspects of pathoecology (Ferreira et al., 1983; Jones, 1985; Reinhard et al., 1986; Aspöck et al., 1996; Bouchet et al., 2003; Gonçalves et al., 2003; Santoro et al., 2003; Le Bailly et al., 2006; Fisher et al., 2007; Arriaza et al., 2010; Kumm et al., 2010; Araújo et al., 2011; Bartošová et al., 2011; Brinkkemper and van Haaster, 2012; Florenzano et al., 2012; Jiménez et al., 2012; Reinhard et al., 2012; Reinhard and Pucu, 2013; Searcey et al., 2013; Morrow et al., 2014). However, only the relevant archaeoparasitological methods and results are detailed within this publication.

2.1. Assessment of specimens

Laboratory processes began with the assignment of an identification number for each sample. Next, each coprolite was photographed, measured, and weighed (Table 3). Coprolites were examined for evidence of decomposition, such as the presence holes left by burrowing arthropods. Most of the coprolites were divided into two subsamples. One half of each coprolite was archived, while the other was processed. Coprolites that were too small to be divided for processing were utilized in their entirety. Each subsample used for processing was reweighed prior to rehydration (Table 3).

Samples 1–8 came from Burial 122 and were comprised primarily of organic material. Samples 9–11 came from Burial 119 and were comprised of organic material mixed with sand. The organic particles within sample 9 were separated from the sand and processed separately as samples 9a and 9b. The matrix sample, 9b, served as a control sample for the analysis. Sample 12 came from Burial 009 and was a mixture of clay and organic residue (Table 1; Table 3).

Figure 4. Burial 122 showing burial context.

Table 1. Contextual information regarding the burials and recovered parasite eggs.

Burial	T. trichiura		A. lumbricoides		Soil	Hydrology
	Presence	Preservation	Presence	Preservation	type	
009	+	Moderate	+	Poor	Clay	Standing water
119	+	Moderate	–	N/A	Sandy	Standing water
122	+	Good	+	Moderate	Clay	Dry

Table 2. Structural differences between two geohelminth parasite eggs.

Shell traits	A. lumbricoides	T. trichiura
Chitinous layer especially thick	+	–
Polysaccharide and protein layer	+	–
Polar plugs	–	+
Lipid layer with proteins	+	–

Table 3. Pre-processing measurements of coprolites.

Sample number	Diameter (cm)	Weight (g)	Subsample weight (g)
1	4.6	10.27	4.8
2[a]	2.8	7.31	2.9
3	3.0	1.24	1.1
4	5.7	4.81	3.2
5	3.8	2.26	1.5
6[a]	5.1	12.5	3.5
7[a]	2.5	6.73	3.1
8	2.5	5.22	1.4

a. Where samples were in pieces and the pieces were comparable in size, an average was taken.

Rózsa et al., 2000). To calculate the concentrations of microfossils in the samples, *Lycopodium* spores were added to each sample (Fugassa et al., 2006). A single *Lycopodium* tablet (batch 212761) contains approximately 12,500 spores. Tablets are available from B. E. Berglund and T. Persson, Laboratory of Quaternary Biology, Tornavägen 13, S-223 63 Lund, Sweden. Quantification is based upon the weight of the sample, the number of *Lycopodium* spores added, the number of *Lycopodium* spores recovered during analysis, and the number of microfossils observed.

After the addition of *Lycopodium*, the samples were disaggregated with a magnetic stirrer. The samples were then screened through a 250 μm mesh (Reinhard et al., 1986). The macroscopic remains on top of the screens were transferred to sterile filter paper, labeled appropriately, dried, and stored for analysis. The fluid passing through the screen was collected in a 600 ml glass beaker prior to being concentrated into 50 ml tubes via centrifugation. The concentrated microscopic remains were washed with distilled water.

2.3. Parasite analysis

It is standard practice to employ a sequential microfossil analysis in the study of coprolites. In order to retain *Lycopodium* spores for each type of microfossil analysis, researchers looked first for parasites, starch, plant tissues, animal hairs, phytoliths, and other identifiable remains. Afterward, the samples were processed for pollen (Reinhard et al., 2006, 2011).

Aliquots extracted from the concentrated microscopic remains were mixed with a drop of glycerin to create 2–5 microscope slide preparations per sample. These slides were examined for parasites via light microscopy with compound microscopes. Parasite concentration values were calculated using the formula: Concentration = $((p/m) \times a)/w$, where p = # of parasites counted, m = # of *Lycopodium* spores

2.2. Specimen processing

We attempted to rehydrate the subsamples in 0.5% trisodium phosphate for two days. After two days, the specimens had not rehydrated. They were monitored for five additional days. At the end of this seven day period, the coprolites were still in their original state. Drops of 38% hydrochloric acid were added until the rehydration solution was slightly acidic. The coprolites subsequently rehydrated within an hour. This suggests that calcium carbonate had entered the burial sediments there-by solidifying the samples.

The key to microfossil quantification, analysis, and interpretation is the addition of *Lycopodium* spores (Reinhard et al., 1986;

counted, a = # *Lycopodium* spores added, and w = weight of the subsample.

Photographs of relevant microfossils were taken at a total magnification of 400×. The exploratory data analysis of microfossil concentrations includes summary statistics, prevalence, Pearson's correlation, and linear regression.

3. Results

3.1. Taphonomy of source material

There were no indications of arthropod, nematode, or fungal decomposers. The control sample, 9b, contained no parasites eggs which suggests that parasite eggs were not part of the sediment into which the burials were dug. The calcification of the samples indicates an alkaline chemical environment within the burials.

Different burial conditions at the time of excavation contributed to the differential preservation of the geohelminth eggs recovered from the coprolites. Contextual information, along with preservation status for recovered parasites, is summarized in Table 1. In general, the eggs of *T. trichiura* were better preserved than those of *A. lumbricoides* within the burials in which they were concurrent. The overall egg preservation was best in Burial 122.

In Burial 122, *T. trichiura* eggs often retained intact polar plugs, though fully intact embryos were not encountered (Figure 10). By contrast, *A. lumbricoides* eggs in this burial were rarely intact (Figure 11). While in most cases the entire egg was present, though marred by large fissures and apertures, many fragmented eggs were observed.

Microscopic analyses of Burial 119 showed the presence of *T. trichiura* eggs in variable states of preservation. No *A. lumbricoides* eggs were observed.

Distortion and collapse of *T. trichiura* eggs were commonly observed in samples from Burial 009. Intact polar plugs were rarely observed from this burial. The *A. lumbricoides* eggs recovered from Burial 009 were noticeably damaged. During the analysis, *A. lumbricoides* egg fragments were frequently observed. The outer uterine layer was most often found to be intact while the chitinous and lipid layers of these eggs were corroded and fractured. Additionally, the majority of *A. lumbricoides* eggs were observed to have incurred damage to their internal structures. In general, the best preservation was evident in Burial 122, moderate preservation in Burial 119, and the poorest preservation in Burial 009.

3.2. Parasite egg concentrations

The total average concentration of parasite eggs in the samples from all three burials was N = 324,837 eggs per gram (epg) and N = 1,784,157 eggs per coprolite (epc) from all processed coprolites (Figure 9). More specifically, the total concentrations were broken down into groups by parasite; *T. trichiura* had N = 296,105 epg and N = 1,581,791 epc, while *A. lumbricoides* had N = 28,732 epg and N = 202,366 epc.

Summary statistics indicated a wide variation in the ranges of parasites found in subsamples. Extreme values were demonstrated by *T. trichiura* in sample 11 with a minimum value of 0 epc and sample 1 with a maximum value of 530,240 epc (Table 5). Correlations between total parasites, *T. trichiura* and *A. lumbricoides*, and their corresponding full and sample weights did not indicate any strong relationships. This non-significant relationship indicates successful random sampling. Correlations between *T. trichiura* egg and *A. lumbricoides* egg concentrations show a positive and significant relationship in both eggs per gram (r = 0.76; r^2 = 0.58) and eggs per coprolite (r = 0.84; r^2 = 0.71). This indicates a strong non-random association in the
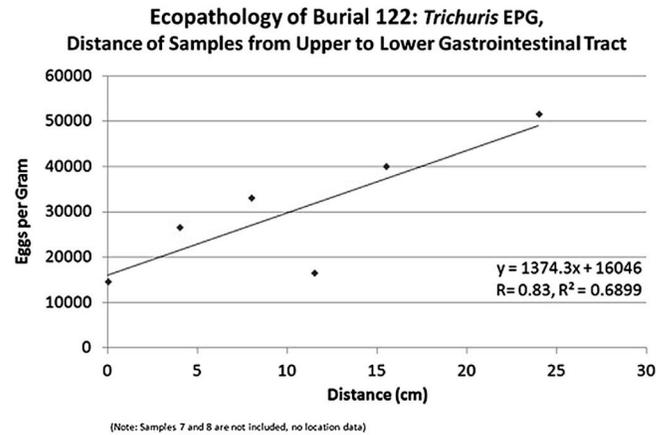

Figure 5. Spatial analysis of *T. trichiura* egg distribution per gram within Burial 122.
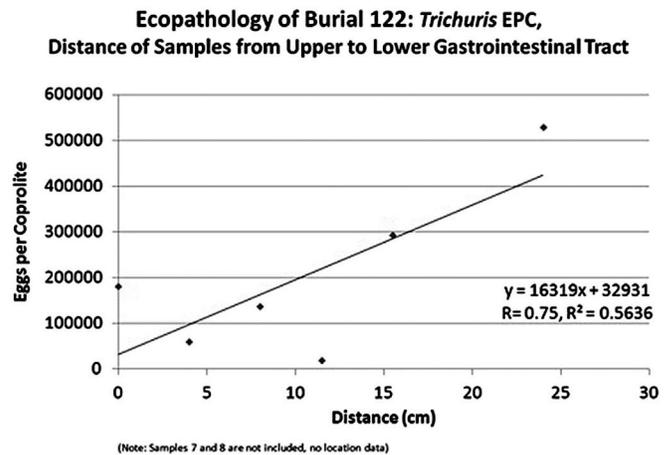

Figure 6. Spatial analysis of *T. trichiura* egg distribution per coprolite within Burial 122.

presence of both the *T. trichiura* and *A. lumbricoides* eggs. These data indicate that co-infection from exposure is not random.

Samples from Burial 119 yielded no evidence of *A. lumbricoides*. The highest mean value of eggs per coprolite was detected in Burial 122 with *T. trichiura* having a mean = 197,209 epc.

Based on the *T. trichiura* egg total (296,105 epg) found among all burials analyzed, the highest prevalence was found in Burial 122 (89%). Based on the *A. lumbricoides* egg total (28,732 epg) found among all burials analyzed, the highest prevalence was found in Burial 122 (99%). The calculated parasite egg concentrations for all Burial 122 coprolites (*T. trichiura* = 1,577,679 eggs per coprolite, *A. lumbricoides* = 202,350 eggs per coprolite) represent the highest values (Total parasite eggs = 293,402 epg; Total Parasite eggs = 1,780,029 epc) from any archaeological human analyzed to date (Fugassa et al., 2006, 2008; Jiménez et al., 2012; Kumm et al., 2010; Martinson et al., 2003; Morrow et al., 2014; Santoro et al., 2003; Searcey et al., 2013). The epg average for *T. trichiura* for Burial 122 is 33,090 epg of coprolite. This is very close to the highest previous record of 34,529 epg recorded by Kumm and colleagues for the Piraino 1 mummy from Sicily (2010). Piraino 1 was infected with *T. trichiura* only. A higher average value of 36,675 epg for Burial 122 is obtained when eggs of both species are considered.

The parasite egg counts obtained from the samples (Table 4; Table 5) indicate that the coprolites recovered from Burial 122 contained high amounts of *A. lumbricoides* eggs, with values
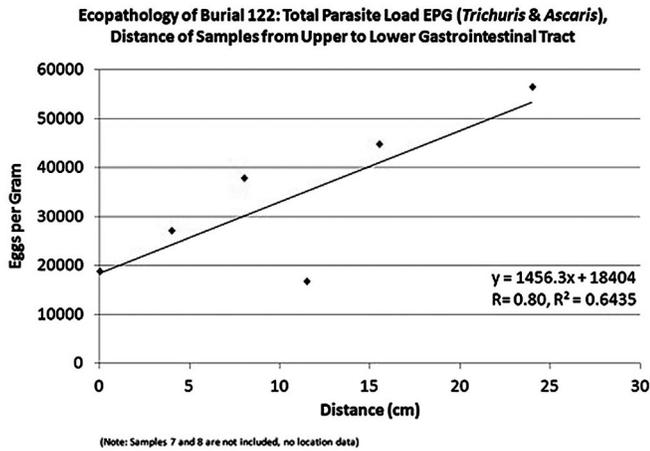
Ecopathology of Burial 122: Total Parasite Load EPG (*Trichuris* & *Ascaris*), Distance of Samples from Upper to Lower Gastrointestinal Tract

$$y = 1456.3x + 18404$$
$$R = 0.80, R^2 = 0.6435$$

(Note: Samples 7 and 8 are not included, no location data)

Figure 7. Spatial analysis of parasite egg distribution per gram within Burial 122.

Ecopathology of All Burial Sites: Coinfection of Parasite Microfossils EPG

$$y = 0.1035x - 159.22$$
$$R = 0.76, R^2 = 0.5835$$
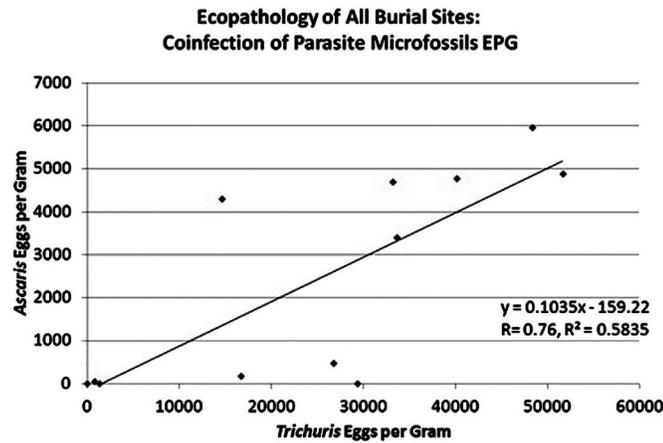
Figure 8. Co-infection of parasite eggs per gram as represented by Burials 009 and 122.

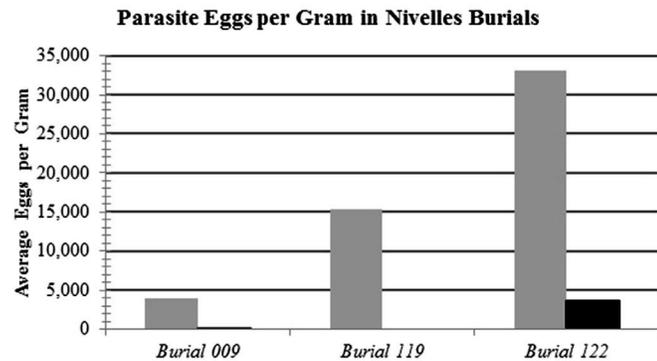**Parasite Eggs per Gram in Nivelles Burials**

Figure 9. Average number of eggs per gram as represented by all three burial sites. Gray bars represent *Trichuris trichiura* while black bars represent *Ascaris lumbricoides*.

for coprolites ranging from 211 epc to 53,712 epc and 170 epg to 5965 epg. An exceptionally high number of eggs of *T. trichiura* were noted in the coprolites, with values ranging from 20,642 epc to 530,240 epc and 14,610 epg to 51,630 epg.

Burial 122 demonstrated a positive and significant correlation between *T. trichiura* epg and the position of the coprolite along the length of the individual's gastrointestinal (G.I.) tract ($r = 0.83$ $r^2 = 0.69$) (Figure 5). A positive and significant correlation also exists between *T. trichiura* epc and position

Table 4. Parasite egg concentration values for Burial 122.

Sample number	A. lumbricoides		T. trichiura	
	eggs/gram	eggs/coprolite	eggs/gram	eggs/coprolite
1	4891	50,231	51,630	530,240
2	4779	34,938	40,119	293,268
3	170	211	16,647	20,642
4	4702	22,617	33,138	138,518
5	480	1084	26,712	60,369
6	4297	53,712	14,610	182,625
7	5965	21,834	48,291	176,748
8	3395	17,723	33,576	175,269

Table 5. Parasite egg concentration values for all burials.

Burial	Sample number	A. lumbricoides		T. trichiura	
		epg	epc	epg	epc
122	1	4891	50,231	51,630	530,240
	2	4779	34,938	40,119	293,268
	3	170	211	16,647	20,642
	4	4702	22,617	33,138	138,518
	5	480	1084	26,712	60,369
	6	4297	53,712	14,610	182,625
	7	5965	21,834	48,291	176,748
	8	3395	17,723	33,576	175,269
119	9	0	0	14,885	n/a
	9a	0	0	29,297	n/a
	9b	0	0	0	0
	10	0	0	1322	3887
	11	0	0	0	0
009	12	53	908	3951	68,196

of the coprolite along the length of the individual's G. I. tract ($r = 0.75$ $r^2 = 0.56$) (Figure 6). A positive and significant correlation also exists between total parasite load and position of the coprolite along the length of the individual's G. I. tract ($r = 0.80$ $r^2 = 0.64$) (Figure 7). The directionality of the distance measurements ran from the upper to lower regions of the G. I. tract (Figure 4). The relationship between parasite concentrations and distance indicates that eggs were concentrated in the lower portion of the individual's G. I. tract (Figure 4; Figure 5; Figure 6; Figure 7).

Co-infection of *T. trichiura* and *A. lumbricoides* was detected in Burial 122 and Burial 009 (Table 5; Figure 8). There is a significant positive correlation between *T. trichiura* and *A. lumbricoides* epg values in this study ($r = 0.76$ $r^2 = 0.58$) (Figure 8), i.e. infection intensity increases together.

3.3. Intestinal obstruction in the individual from Burial 122

The individual within Burial 122 was an elderly edentulous female. Tooth loss was not uncommon in medieval Europe. Esclassan et al. (2009) showed that one population of 58 individuals from medieval France all exhibited attrition on more than 90% of the teeth studied from these individuals. This demonstrates that diets from this time period contributed to tooth loss (Esclassan et al., 2009). The fact that osteophytosis was noted in the upper thoracic vertebrae coupled with the absence of teeth indicates that the individual was of an advanced age at time of death. The individual's skull had been crushed, probably post-burial. Coprolites from Burial 122 were found in the lumbar region of the spine and within the pelvic girdle (Figure 4).

Burial 122 coprolites were unusual in terms of their morphology and abundance. A total of 8 coprolites ranging in weight from 1.24 to 12.5 g and in size from 2.5 to 5.7 cm in diameter were removed from this burial for analysis. As reviewed by

previous researchers, typical coprolites in mummies range in weight from 1 to 6 g and in diameter from 2.5 to 3 cm (Reinhard et al., 2003). The coprolites from Burial 122 were abnormally numerous, heavy, and large (Table 3).

4. Discussion

4.1. What is known about parasite egg taphonomy and recovery?

Differential preservation of parasite eggs due to taphonomic conditions, both biotic and abiotic, has been recognized and reviewed by the field of archaeoparasitology. An array of processing methods has been developed to adjust for taphonomic conditions. In 1986, archaeoparasitologists from three labs in Germany, Brazil, and USA combined their experiences to address quantification and taphonomy (Reinhard et al., 1986). They also reviewed the extensive work of Andrew Jones's lab in England. An update of methods related to taphonomy was later published that included French perspectives on the field (Bouchet et al., 2003). Thus, the experiences from Europe and the Americas were presented. With these efforts, the authors intended to lay a groundwork for subsequent analysis. The original article, "Recovery of Parasite Remains from Coprolites and Latrines: Aspects of Paleoparasitological Technique" was very rarely cited, and has had little impact on the development of the field. It is useful to highlight aspects of this paper because some of the data presented therein are relevant to the recovery of parasite remains from skeletons and mummies. We present salient points of this study with reference to more recent papers and this analysis.

The foundation of much of this early work was the experimental desiccation of modern eggs to determine if the simple process of drying and rehydration resulted in alteration of egg form (Reinhard et al., 1986). The discovery of deformed hookworm and whipworm eggs from a dried mummy generated interest in this topic (Ferreira et al., 1983). Experimentally, parasite eggs responded based on the rigidity of their shells. Experiments with *T. trichiura* eggs showed that there was some shrinkage and minor wrinkling, but that no statistical differences were detected between the dimensions of the eggs before and after the experiments. Parallel experiments utilizing the two species of hookworms known to infect humans (*Necator americanus* and *Ancylostoma duodenale*) were conducted by Araújo (1988). This study showed that desiccation and rehydration often leads to egg deformation, but that the diagnosis of hookworm eggs is not significantly altered.

This issue was taken up recently. Using scanning electron microscopy, Shin and colleagues (2009) compared modern eggs with ancient eggs from Korean mummies for the species *T. trichiura*, *A. lumbricoides*, *Metagonimus yokogawai*, *Paragonimus westermani*, and *Gymnophalloides seoi*. The results of *T. trichiura* and *A. lumbricoides* are relevant to this study. Shin et al. (2009) found that the eggs of *T. trichiura* were generally well preserved but that the mucoid polar plugs were often lost in mummified eggs. They found that archaeological *A. lumbricoides* eggs were broken or flattened and that the details of the mammillated coats were not well preserved. These studies suggest that there are taphonomic processes that differentially interact with egg morphology such that *A. lumbricoides* are poorly preserved, even in mummies.

Using light microscopy, Kumm and colleagues (2010) quantified the preservation of *T. trichiura* eggs recovered from a coprolite in a Sicilian mummy. Nearly pristine eggs, exhibiting embryonic masses and one plug, accounted for 7% of the eggs observed. Of the *T. trichiura* eggs recovered in this study, 42% were empty and non-deformed and 48% were empty, deformed, and lacked polar mucoid plugs. One percent of the eggs were fragmented and 2% were fractured. It is noteworthy that one

egg, a mere fraction of a percent of the observed eggs, was fully embryonated. This shows that the desiccation process can occasionally allow for embryonic development to occur over several weeks (Kumm et al., 2010). Until this discovery, it was not recognized that eggs could mature within a mummifying corpse.

These studies show that taphonomic processes, perhaps combined with rehydration methods, result in the alteration of eggs even within the preserved intestinal contents of mummies. Recently, a case has been made that the analysis of mummified tissue may sometimes include sections of adult whipworms (Morrow et al., 2014). Parasite eggs existing within adult uteri are immature. Morrow et al. (2014) demonstrated that such eggs are deformed and do not exhibit ephemeral features such as mucoid polar plugs.

Reinhard et al. (1986) stated that preservation seems to be best in moist anaerobic environments or desiccating environments. The conclusion presented by Bartošová and coworkers (2011) is complementary. They stated "besides freezing and extremely dry conditions, moist anaerobic environment is ideal for preserving the structure of parasite eggs". However, both papers were addressing latrine sediments not associated with mummies and burials. It is worthwhile to explore studies specific to human remains.

Early researchers presented data that indicated pH had little effect on parasite egg preservation in sediments (Reinhard et al., 1986). This seems to bear out in more recent studies of mummies. Shin and coworkers (2009) report excellent preservation of parasite eggs and larvae in extremely alkaline environments within lime-soil mixture tombs in Korea where mummies preserved. The other pH extreme in preservation comes from bog bodies in Europe that were interred in acidic environments as reviewed by Searcey and colleagues (2013). The taphonomic distinction between acidic and alkaline environments relates to the preservation of parasite soft structures. Larvae and egg embryonic material preserved relatively well in the alkaline Korean soils but were generally absent in the acidic bog remains. Also, the mammillated coats of *A. lumbricoides* preserve less well in acidic bog conditions. In this study, the coprolites from all three burials were mineralized with calcium carbonate, which suggests that the preservation environment within the coffins was alkaline. Compared with the Korean study, it appears that alkaline environments with skeletal remains do not preserved more perishable parts of eggs such as embryos and mucoid plugs.

Rapid desiccation enhances preservation as demonstrated by excellent preservation of eggs and larvae in spontaneously mummified individuals (Arriaza et al., 2010; Araújo et al., 2011; El-Najjar et al., 1980; Kumm et al., 2010). The analysis of Ancestral Pueblo mummies by El-Najjar and colleagues (1980) is particularly important because they were able to recover delicate pinworm eggs. Certainly, freezing enhances the preservation of parasite eggs as found in the El Plomo mummy (Horne and Kawasaki, 1984) and the Tyrolean Iceman (Aspöck et al., 1996). These frozen mummies represent dry-freezing and ice-freezing environments. In general, recognizable helminth eggs can be recovered from seemingly any environment that allows for human mummification.

Recovering parasite eggs from skeletal remains is more challenging, and the range of preservation more variable, than with mummies. This is similar to the results from non-mummy contexts (Reinhard et al., 1986). This is highlighted by the review of Korean Joseon dynasty burial preservation (Seo et al., 2010). This study shows that eggs are common in tombs containing well-preserved mummies with textiles and other perishable artifacts. In tombs that contained skeletons without perishable offerings, parasite remains were not recovered. This sobering study highlights the challenge of recovering eggs from skeletonized burials. However, other analyses have been successful. Fugassa and colleagues

(2006) were able to recover parasite eggs from a sacrum of an Argentinian burial and even from sacra curated in museums. Most recently, Jaeger and colleagues (2013) were able to recover trace amounts of poorly preserved eggs from fragmentary burials in Brazil. These studies show that eggs can preserve in interments characterized by complete soft tissue decomposition.

European studies of skeletonized burials have been reviewed (Kumm et al., 2010; Leles et al., 2010). These reviews show that *T. trichiura* and/or *A. lumbricoides* eggs have been recovered from skeletons in Cyprus, the Czech Republic, and France dating as far back as Neolithic times. Negative studies do not necessarily reflect a lack of parasitism (Le Bailly et al., 2006). The absence of eggs may represent taphonomic conditions that did not preserve eggs within interments. Our study confirms that recovery of eggs associated with skeletons in burials is possible.

Some taphonomic factors were recognized by early researchers, especially from non-burial contexts. Fungal agents of decomposition were recognized in both Germany and the USA by early researchers. Archaeological trichurid eggs were observed covered with fungal mycelia and containing fungal spores (Reinhard et al., 1986). Recently, the fungal species *Pochonia chlamydosporia*, and two other nematophagous fungi were tested for their ability to attack trichurid and ascarid eggs (Araújo et al., 2008; Silva et al., 2010). In this experiment, eggs of the dog whipworm, *Trichuris vulpis*, were used. For both egg types, *P. chlamydosporia* caused morphological alterations of embryos and eggshells via hyphal penetration and internal egg colonization within three weeks of inoculation. It is noteworthy that trichurid eggs were more commonly attacked than ascarid eggs. Thus, the observation of fungal attack by archaeoparasitologists nearly three decades ago is supported by recent experimentation.

Another aspect of taphonomy that can inhibit egg recovery is the development of calcareous substrate in archaeological sites that can inhibit the recovery of eggs (Reinhard et al., 1986). In such conditions, the eggs rarely, if ever, exhibit embryonic masses or polar plugs for whipworm eggs and the ascarid mammillated coats are poorly preserved. The alkalinity of these substrates, as noted above, impacted the more perishable features of the eggs. Thus, both biotic and abiotic taphonomic factors were noted by earlier researchers. In the case of this analysis, the alkalinity of the grave sediments inhibited standard rehydration so the use of dilute hydrochloric acid, as described by Jones (1983), was used. Fungal attack was not evident in this analysis.

There was already a diverse array of methods available to archaeoparasitologists thirty years ago and by 1986 researchers emphasized quantification. "Quantification also provides a basis for an epidemiological approach and provides a basis for comparative study" (Reinhard et al., 1986:224). The methods developed by these researchers utilizing sediments are applicable to skeletons excavated from burials in which coprolites are not preserved. For sediments, several methods were tested including zinc chloride flotation, zinc sulfate flotation, sucrose solution flotation, and sodium chloride flotation. Differential gradient methods were tested including MIF-ether and discontinuous gradient sucrose solutions. None of these methods worked well. Geological sedimentation methods were tried and found to be ineffective. Screening methods were effecting but too time consuming (Reinhard et al., 1986).

Special methods were developed to recover eggs from calcareous sediments. Experimentation showed that palynological processing, excluding acetolysis, resulted in the recovery of *T. trichiura*, *A. lumbricoides*, *Schistosoma japonicum*, *Clonorchis sinensis*, and *Taenia pisiformis* (Reinhard et al., 1986). Recently, Florenzano and colleagues (2012) discovered that palynological processing, with shortened acetolysis, results in the excellent recovery of parasite eggs. An alternative method has recently been developed using rehydration and filtration to recover eggs (Anastasiou and Mitchell, 2013a). This method has been applied recently in burial context (Mitchell et al., 2013). The comparative condition of the eggs between the Anastasiou-Mitchell method and the Florenzano method are the same as founded by Reinhard and colleagues (1986). Palynological methods result in the recovery of ascarid eggs with mammillated coats while decorticated eggs were recovered from non-palynological methods. More comparative testing of these methods on the same samples needs to be done to determine if this difference is method-based or just a result of different sample sources. These methods rely on *Lycopodium* spore quantification as described by Warnock and Reinhard (1992).

Another effective method for sediments was innovated by Jones (1985). This method, Stolls dilution, requires just three grams of sediment and results in quantification without the need to add exotic spores. Recently, this method has been tested in a burial context and was found to be superior in recovering trace amounts of eggs (Fugassa et al., 2006). The German lab, directed by Herrmann, developed a method using a dilute detergent solution (Reinhard et al., 1986). After soaking for two days, the samples were screened, washed, and centrifuged. Then the samples were examined and eggs were counted. It was noted that these techniques are best applied to moist soils and that calcareous deposits need to be treated with dilute hydrochloric acid as a preliminary step.

A more limited range of coprolite analysis methods, relevant to mummy and burial contexts, were tested (Reinhard et al., 1986). Jones (1983) discovered mineralized coprolites and experimented with three methods to recover eggs. He found that dilute hydrochloric acid was effective in dissolving the soil matrix and liberating eggs. Regarding desiccated coprolites, zinc sulfate flotation and formalin-ether concentration methods were tested. These methods were unsatisfactory for the recovery of parasite eggs. Heavy density flotation media was found to distort eggs (Jones, 1983).

For desiccated coprolites, researchers determined that rehydration with 0.5% trisodium phosphate was an effective preliminary step (Reinhard et al., 1986). Subsequent to rehydration, two effective methods are available. One is the Lutz spontaneous sedimentation method, recently described by Camancho and colleagues (2013). The other is the disaggregation-screening method (Reinhard et al., 1987). Jiménez and coworkers (2012) compared these two methods with Sheather's heavy density technique. The Lutz and disaggregation-screening methods were equivalent and preferable to the Sheather's method.

For some mummies, intestinal contents can be solidified by fatty materials that permeate and congeal in the coprolites. Dilute potassium hydroxide is useful in dispersing this material (Reinhard et al., 2011). These methods rely on *Lycopodium* spore quantification as described for coprolites (Reinhard et al., 1986; Sianto et al., 2005).

4.2. Differential parasite egg preservation

Parasite eggs recovered from Burial 122 represented the best preservation among the three burials. This burial had a covering that consisted of a wooden plank and was dry at the time of excavation. The burial was excavated into stable, sterile substrate. The stability of this specific context resulted in the preservation of coprolites within the abdominal area. There was no evident breakdown of the coprolites. In our experience, the coprolites were more consistent with a desiccated corpse than a skeletonized burial. The preservation of a series of coprolites also signals that the process of body decomposition did not result in dispersal of the intestinal contents.

Figure 10. *Trichuris trichiura* from coprolite analysis of Nivelles burials.

Figure 11. *Ascaris lumbricoides* from coprolite analysis of Nivelles burials.

than those of *T. trichiura*, making them less likely to become distorted under mild stress. However, these same characteristics could make them more likely to break altogether under more severe stress. In conclusion, the preservation potential of parasite eggs is highly dependent upon both the environment from which the source material was obtained and upon the inherent structural components of the parasites themselves.

4.3. The medieval pathoecology and co-infection at Nivelles

The eggs of *A. lumbricoides* are notoriously resistant to environmental conditions including freezing, high heat, desiccation, and soil chemistry. The high egg production, 200,000 per female per day, along with the eggs' possession of adherent, mammallated coats, exacerbates the threat of contamination from fecal sources (Reinhard and Pucu, 2013). These characteristics give the parasite remarkable infective capabilities. Thus, a field fertilized with human feces easily becomes an infection hazard for people working in or consuming food from that field.

In contrast to ascarids reaching up to 49 cm in length, trichurids are smaller parasites with adults being approximately 3–5 cm in size (Roberts and Janovy, 2009). These worms have similar infection patterns to those of *A. lumbricoides*, but produce far fewer eggs, 5000–30,000 per female per day (Bogitsh et al., 2005, Bethony et al., 2006). Unlike ascarids, adult trichurids embed themselves in the lining of the intestinal mucosa, which protects them from the chemistry and mechanics of the intestinal lumen. Therefore, many anthelminthics are rendered ineffective for treating trichurid infection (Reinhard and Pucu, 2013).

The correlation between *T. trichiura* and *A. lumbricoides* egg concentration values in this study indicates co-infection. The fact that all of the sampled individuals were positive for geohelminths implies that Nivelles around the turn of the first millennium was an environment with a high potential for parasite infection.

The pathoecology of parasitism in Europe during the medieval period was defined by filth derived from feces. The archaeoparasitological record of Europe is characterized by the ubiquity of fecal-borne geohelminths such as the giant intestinal round worms or "maw worms" (*A. lumbricoides*) and whipworms (*T. trichiura*) (Bartošová et al., 2011; Brinkkemper and van Haaster, 2012; Florenzano et al., 2012; Reinhard and Pucu, 2013). The physical evidence of these parasites from archaeological contexts is complemented by their presence in medical records and other ancient texts (Cox, 2002).

Even in the stable mortuary environment of Burial 122, the preservation of *T. trichiura* eggs was much better than that of *A. lumbricoides*. *T. trichiura* eggs were well formed, with intact shells and some internal contents. None had embryos. Polar plugs had, by-and-large, disappeared (Figure 10). Within the same burial, *A. lumbricoides* eggs were rarely recovered intact. The outer mammillated coats were partially or completed absent. Many eggs were fractured (Figure 11).

Burials 009 and 119 did not yield as many helminth eggs as Burial 122, nor were the recovered eggs as well-preserved. Fully intact internal structures of both *T. trichiura* and *A. lumbricoides* eggs were not often observed. It was noted that some *A. lumbricoides* egg layers were better preserved than others within the same specimens. Eggs of *T. trichiura* were often collapsed or distorted and the polar plugs were seldom intact.

The excavations of 2010 were conducted during the winter, leaving Burial 009 and Burial 119 subject to flooding and standing water. Burial 122 was not flooded or exposed to standing water due to its location and substrate. However, during the 12th century, these burials were not continuously saturated. The exposure to standing water during excavation may have negatively affected parasite egg taphonomy. Burial 009 was surrounded by clay soil while Burial 119 was found in soil of a more organic nature. This may have also played a role in differential preservation. Clay soils seem to be preferable for helminth egg preservation, more so with *T. trichiura* than with *A. lumbricoides* in the present study.

Differences in preservation between *T. trichiura* and *A. lumbricoides* eggs as observed across all three burials could be due to the morphology of the eggs themselves. The eggs of *A. lumbricoides* have proportionately thicker chitinous layers and, overall, are larger in size. It is possible that these eggs are less flexible
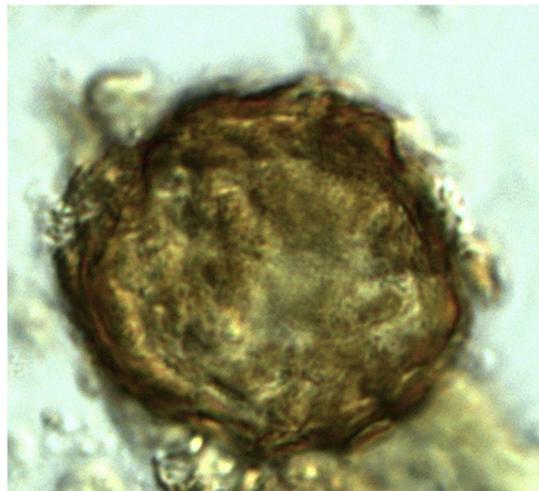
Parasitism has been recognized throughout written history, with the first descriptions coming from Egyptian medical documents dating from 3000 to 400 B.C. Other descriptions involving parasites were recorded by Greek physicians from 800 to 300 B.C. (Cox, 2002). In fact, both the Greeks and the Romans recognized *A. lumbricoides* and other parasitic helminths (Sandison, 1967). More definitive descriptions were recorded in later periods by Arabian physicians (Cox, 2002). Medieval physicians recognized parasitic helminth infections, but did not understand the organismal source (DeMaitre, 2013). All existing materia medicas and pharmacopoeias for Europe and the Mediterranean dating from the 5th century B.C. to the 19th century A.D. were summarized by De Vos (2010). This study concludes that certain plant species were used consistently to treat intestinal helminth infections.

European archaeoparasitological studies have revealed evidence of *A. lumbricoides* and *T. trichiura* infections dating as far back as the Paleolithic (Gonçalves et al., 2003). The eggs of these parasites were ubiquitous in the medieval environment as shown by analysis of archaeological sediments. In particular, *A. lumbricoides* was tremendously abundant, indicating the rampant filth of the period (Leles et al., 2010). Furthermore, A.K.G. Jones (1985) and Herrmann, 1985 and Herrmann, 1986 demonstrated that *T. trichiura* was a predictable part of medieval background fauna. Herrmann and Schulz (1986) defined the factors that caused this epidemic, which included common defecation areas, population structure, and relatively unhygienic conditions. These data are expanded by recent studies documenting the geographical breadth and temporal depth of the European geohelminth epidemic (Bartošová et al., 2011; Florenzano et al., 2012; Kumm et al., 2010; Mitchell et al., 2013; Morrow et al., 2014; Searcey et al., 2013). In medieval Europe, waste management was tightly tied to agriculture. The epidemic of geohelminths parasitism was undoubtedly tied to the recovery of feces from cess deposits used as fertilizer in near-by agricultural fields (Sterner, 2008).

Contemporary sanitization was limited during the medieval period. Feces were deposited in refuse pits, yards, in front of houses, or in the streets (Jones, 1985). Members of the lower social echelons often did not change clothes or bathe due to a lack of running water. Regular hand washing and rinsing of fruits and vegetables prior to consumption were uncommon practices. Water was also widely believed to be harmful to the human body (Vondruška, 2007). This lack of sanitation coupled with behavioral avoidance of hygienic practices created ideal microenvironments for the mechanical transmission of geohelminth eggs. Technological and horticultural advances after 1300 A.D. gave medieval agriculturalists the ability to produce more food, which led to a notable increase in Europe's population. Medieval agriculture relied on excrement, allowing for the continual rotation of geohelminth life cycles, making agricultural products reservoirs of infection. Despite most medieval urban residences having vegetable gardens, cities depended on rural agriculture.

Medieval fertilizers were composed almost exclusively of organic wastes such as biodegradable food scraps, human waste, and offal from urban regions. Waste was dumped in the unpaved city streets, and was either eaten by animals living in the city, or was soaked up into urban mud later used as fertilizer (Reinhard and Pucu, 2013; Sterner, 2008). As populations grew, sanitation crises emerged. Wastes could no longer be absorbed once cities became paved, leading to filth accumulation. Uncooked and unwashed vegetables, as well as unclean water, were major sources of parasite contamination. This environment greatly contributed to the emergence of diseases (Sterner, 2008) and inevitably created the constant risk of parasite contraction and reinfection.

4.4. Burial 122: extreme parasitism and pathology

The especially large coprolites observed in Burial 122 could be a synergistic result of extreme parasitism, diet, and pre-existing health conditions associated with aging. This individual was elderly as evinced by her bones and lack of teeth. Dietary analysis yielded evidence of a diet high in fiber, particularly in wheat glume or "chaff". There is also a large amount of what appears to be fine charcoal present in Burial 122 coprolites.

The abundance of chaff lends itself to the potential formation of a bezoar, a rare cause of intestinal blockage (Hall et al., 2011). A bezoar is an obstruction composed of incompletely digested material that forms within the intestine (DiMarino and Benjamin, 2002; Hall et al., 2011). Adhesions are the most common causes of bezoar formation (Hall et al., 2011), especially, in edentulous patients (DiMarino and Benjamin, 2002; Hall et al., 2011). Incomplete mastication increases the risk of intestinal obstruction because it is much more difficult for the gastrointestinal tract to chemically breakdown foods without an initial mechanical breakdown. Because the individual within Burial 122 was edentulous and was shown to have high fiber as part of her diet at the time of death, it is possible that a bezoar could have contributed to her impaction and subsequent death. Additionally, she may have suffered from a pre-existing intestinal condition, which would have compromised her natural ability to pass such a bolus.

Heavy parasite infections also contributed to this individual's intestinal blockage. Both *A. lumbricoides* and *T. trichiura* have been known to cause intestinal abnormalities in the event of heavy infections (Bethony et al., 2006). This individual's coprolites yielded egg concentration values of 1,577,679 total eggs for *T. trichiura* and 202,350 total eggs for *A. lumbricoides*. The numbers of *A. lumbricoides* represents the output of one female worm. Therefore, it is not likely that this parasite contributed to pathology. However, the numbers of *T. trichiura* are suggestive of heavy infection. Considering that whipworm females lay between 5000–30,000 eggs per day, it is probable that the individual in burial 122 was carrying between 53 and 315 female worms.

Despite laying far fewer eggs per day than *A. lumbricoides*, whipworms often cause more damage to the intestines by burrowing further into the intestinal walls. Colonic obstruction is a rare complication of extremely high infection. In addition, high burdens of whipworms may also result in pathology such as prolapsed rectum and intussusception (Fishman and Perrone, 1984; Bahon et al., 1997; Palmer and Reeder, 2001).

This individual's exceedingly high *T. trichiura* egg concentrations are unprecedented in the paleopathological and clinical literature. Heavy burdens of *T. trichiura* often leave hosts with decreased intestinal plasticity leading to problems with absorption and increasing the potential for intestinal blockages. The extreme parasitism found in this individual leaves little doubt that whipworms contributed to her death.

In conclusion, the death of the individual in Burial 122 was likely caused by a combination of dietary and disease factors. The advanced age of the edentulous individual, a diet largely comprised of wheat glumes, and an unprecedented whipworm infection were all factors that culminated in an intestinal obstruction. Glumes are not overrepresented due to differential digestion of softer seed parts. Extensive analysis of slides revealed no wheat starch. The high amount of non-masticated ingested fiber coupled with lowered intestinal plasticity due to extreme parasitism may have led to the formation of a bezoar, which caused the fatal intestinal obstruction within this individual buried in the west St. Pierre/St. Gertrude cemetery of the Grand Place Nivelles.

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