Intestinal parasites in a mid-14th century latrine from Riga, Latvia: Fish tapeworm and the consumption of uncooked fish in the medieval eastern Baltic region

Article *in* Journal of Archaeological Science · September 2014

DOI: 10.1016/j.jas.2014.05.001

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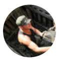

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Unformatted Copy of the following Article:

Yeh, H.-Y., Pluskowski, A., Kalējs, U., Mitchell, P.D. (2014) Intestinal parasites in a mid-14th century latrine from Riga, Latvia: fish tapeworm and the consumption of uncooked fish in the medieval eastern Baltic region. *Journal of Archaeological Science* 49: 83-89.

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Abstract

The aim of this study was to investigate faecal material from a medieval latrine in the coastal town of Riga (Latvia) in order to identify the intestinal parasites present within the population. We identified very large numbers of the eggs of three species of parasitic intestinal worms that affect humans - fish tapeworm (*Diphyllobothrium latum*), whipworm (*Trichuris trichiura*), and roundworm (*Ascaris lumbricoides*). The fish tapeworm evidence demonstrates that the population were eating large amounts of uncooked fish (perhaps raw, smoked, or pickled) since cooking prevents parasite transmission. ELISA analysis identified the presence of the parasite *Entamoeba histolytica*, which can cause dysentery in humans. We also noted two eggs of equid pinworm (*Oxyuris equi*) that affects horses and donkeys, demonstrating the presence of this parasite in farm animals of the region by the medieval period. We discuss the implications of these findings for our knowledge of intestinal parasites in coastal areas of the medieval Baltic region, of food consumption, of hygiene, and of the affects of the parasites upon the health of those living in medieval Riga, the most important city in the crusader state of Livonia.

Key Words:

Diphyllobothrium latum, Dysentery, Helminths, Palaeoparasitology, Protozoa

Highlights

1) Analysis of medieval latrine block from Riga with excellent organic preservation

2) Unusually high concentration of human intestinal parasite eggs present

3) The large numbers of fish tapeworm eggs noted

4) Indicates the consumption of significant amounts of raw, smoked, pickled, or dried fish by the population

1. Introduction

Riga was founded as a new port town in 1201AD during the Baltic Crusades. At this time armed forces from Denmark, Sweden, the Livonian Brothers of the Sword and the Teutonic Order invaded Estonia, Latvia, Lithuania and Prussia, with the aim of Christianising indigenous pagan societies, protecting existing converts and acquiring territory (Christiansen, 1997; Plakans, 2011). Riga became a significant Hanseatic port in the medieval eastern Baltic, as well as the most important power centre of the Livonian crusader state. The site therefore provides a good opportunity to study aspects of health in a medieval Baltic urban centre.

It is understandable that diet, food preparation methods, and environmental conditions varied significantly across Europe in the past, and these factors would have effected which diseases flourished in different regions. The study of the eggs and cysts of parasites from latrines at this site should demonstrate the types of helminths and protozoa present in the population of Riga. This has the potential to highlight how local dietary habits and food preparation techniques can enable particular diseases to become prevalent in a society.

1.1. Medieval Riga

The town of Riga was established as a frontier colony between two settlements occupied by indigenous Livs on the eastern bank of the Daugava. The *Chronicle* of Henry of Livonia documents the first houses in 1201 (Hellman 1989; Henry of Livonia, 2003). By the following year, the bishop had established his residence in Riga and the town became the base for subsequent crusading operations and the recruiting ground for the newly formed Sword Brothers, largely drawn from German merchant families in the colony. The construction of a series of walls enclosing the broader urban area resulted in the incorporation of the earlier Liv settlements within Riga's bounds by 1225 (Caune 2007, 156-167). Along with a substantial Russian community engaged in trade, the population of the town remained ethnically diverse, and the Livs in Riga also acted as essential middlemen between German merchants and the indigenous rural population (Šterns 2000). Politically the town was dominated by the German, Christian, militarised theocracy. During this time the colony became the most important Hanseatic port in the eastern Baltic, a vital hub connecting Latin Europe with Eurasia and particularly the Russian principality of Novgorod. It also developed a substantial hinterland, which connected the city with suburban sprawl and outlying rural settlements, pastures, fields and woods (Šterns 1998).

1.2. Archaeology of the 'Liv quarter' of medieval Riga

Archaeologists excavating in Riga in the 1960s in the area of Albert Square believed they identified one of the indigenous Liv settlements in the western part of the Old Town. The recovered material culture also suggested the contemporary presence of Rus (Smiltniece and Vijups 1998). Between November 2011 and May 2012, excavations between 13 Audeju Street and 33 Kalēju Street uncovered timber buildings and occupation deposits situated next to the town wall in the south-eastern corner of the city, part of the historic centre of Riga; a designated UNESCO World Heritage Site (No. 852) and national monument.

The excavations revealed 40m of the medieval defensive city wall along with the foundations of five stone arcades, alongside which lay the remains of the former Trauksmes street. These consisted of wooden planks which covered at least three sequential layers also made from timber. The remains of 17 wooden buildings were uncovered, along with several timber latrines and additional structures such as piles and the remnants of timber building components. More than 1400 artefacts were recovered (including jewellery, tools and household objects), several hundred articles and parts of leather items, pottery sherds and over 4000 animal bone fragments. The buildings had been constructing using indigenous building techniques consisting of logs with cross-joints, in contrast to the timber-framed structures imported by colonists. Virtually all the artefacts could be ethnically connected with the indigenous Liv population, in contrast to the imported material culture in other areas of the town and the settlement's character is comparable to the one uncovered between 1959-1964 in Albert Square (Caune 2007, 77-78). The excavation confirmed that this part of Riga would have been densely populated from the 13th to 14th century, but there is no convincing evidence that it was inhabited during the 12th century before the foundation of the town. The consistent integrity of the material culture in the Liv quarter suggests it remained ethnically distinct throughout the 13th and 14th centuries, within an otherwise 'Germanised' townscape (Svarāne 1998; Caune 2007).

Whilst a direct correlation between material culture and ethnicity is problematic (Curta, 2011), written sources also highlight distinctions between German-speaking colonists and the indigenous Livs. This has led some archaeologists to define medieval Livonia as a segregated society (e.g. Mugurēvičs, 1990), although this often carries connotations of a polarised struggle between Germans and non-Germans – of an ethnically motivated confrontation – which clearly was not present (Kļaviņš, 2009; Šterns, 2000). In this respect the excavations in Riga have reinforced the multi-cultural character of the most important trading centre at the heart of the Livonian crusader state.

2. Materials and context

During the excavations three latrines were uncovered and the earliest (sq. X14-16/Y11-13) was dated by dendrochronology to 1356 AD (Fig. 1). It is of course not necessarily the case that the contents of the latrine were dated to the same year as its construction, as it may have been emptied and reused for a number of years. The timber structure of this latrine, reinforced by vertical corner stanchions, measured 1.5 x 1.5 m with a height of 1.3 m, and samples were taken from the fill of its pit for bio-chemical analyses (Fig. 2). The material from the latrine dates from a time when Riga was a flourishing port: the 'golden age' of the eastern Baltic crusader states that saw extensive trade and economic growth.

3. Methods

In order to analyse the latrine soil for intestinal parasites, 1 gram of the sediment was disaggregated by adding it to 10ml of distilled water for 1 hour (Anastasiou and Mitchell, 2013). This suspension was passed through a column of sequential micro-sieves with mesh size of 300µm, 160µm and 20µm. Most parasite eggs have dimensions of 25-150µm so will be trapped on the 20µm sieve (Bouchet et al., 1999). After washing the sediment from the 20µm sieve the suspension was centrifuged to concentrate the sediment, excess water removed, and glycerol added prior to mounting on slides. In order to identify the parasite remains the slides were examined using digital light microscopy. They were identified based upon their shape, size, colour, and special characteristics when compared with standard parasitology sources (Garcia, 2009; Gunn and Pitt, 2012).

Disaggregated latrine soil that passed through the 20µm sieve was collected and analysed for evidence of the organisms that cause dysentery. Cysts of *Giardia duodenalis*, *Entamoeba histolytica* and *Cryptosporidium parvum* measure around 5-20µm in size (Garcia, 2009). Six samples of this fluid was tested with Enzyme Linked Immunosorbent Assay (ELISA) kits specifically designed by TECHLAB© to detect these species of dysentery organisms in faecal specimens (http://www.techlab.com). The test uses monoclonal antibody-peroxidase conjugate specific for proteins uniquely secreted by these organisms.

4. Results

4.1. *Helminths that Infect Humans*

Analysis of the sediment from the latrine showed an extremely high concentration of parasite eggs. The highest number was *Trichuris sp.* (whipworm) (Fig. 3) with around 3000 eggs in the ten slides examined. The next most frequent egg was *Diphyllobothrium sp.* (fish tapeworm) with around 2200 eggs in ten slides (Fig. 4). The third most frequent species identified was *Ascaris sp.* (roundworm) with around 550 eggs in ten slides (Fig. 5). Since the number of parasite eggs found was so large, the observation of ten slides was sufficient to develop a good understanding of the species present.

4.2 Protozoa that Infect Humans

ELISA analysis for single celled parasites tested for the presence of *Entamoeba histolytica, Giardia duodenalis* and *Cryptosporidium parvum* that cause dysentery. The test was positive for *Entamoeba histolytica* (1 sample out of 6) but negative for *Giardia duodenalis* and *Cryptosporidium parvum*. For a positive spectrophotometry result using this kit, the 450nm wavelength light absorbance reading must be at least 0.05 higher than the negative control, which was 0.035. The value for the positive test well was 0.092.

4.3 Helminths that Infect Animals

Two eggs of *Oxyuris equi* (equid pinworm, Fig. 6) were identified in the 10 slides examined, making it approximately 1000 times less frequent than the human species described above. This parasitic worm infects horses and donkeys. Two other parasite eggs were noted that are likely to represent parasites of animals, but could not be identified due to the overlapping characteristics of many such parasites that infect animals. Details of all parasite egg numbers and dimensions are given in table 1.

5. Discussion

This analysis of sediment from the latrine block from medieval Riga demonstrates the species of intestinal parasite that were present in the population living there. This reflects the kinds of food being eaten along with how it was prepared for consumption, and also indicates the levels of hygiene at the site. The unusually high concentration of eggs present indicates not merely good organic preservation at the site, but also heavy infestation of the population by these parasites.

The eggs of certain intestinal parasites that infect animals can appear similar to the eggs of different species that infect humans. This means that in contexts where the origin of the fecal material is unknown (e.g. a coprolite on an excavated midden) it may only be possible to suggest the genus rather than the species of the parasite. However, the archaeological context of this latrine makes it highly likely that the eggs represent the species of parasites that infect humans. The raised location of the latrine, with robust walls and toilet seat where people sat to defecate, makes it highly unlikely that animal dung would have been added to the latrine. Furthermore, the fact that from more than 5000 parasite eggs viewed during this analysis, only four eggs were of species that do not infect humans. In other words, it is likely that these eggs represent the human species of fish tapeworm (*Diphyllobothium latum,* which is still found in the region today), whipworm (*Trichuris trichiura*) and roundworm (*Ascaris lumbricoides*).

It is possible to determine the number of parasite eggs per gram of latrine soil using a variety of techniques (Jones, 1985; Reinhard et al. 1986). However, we feel that in this archaeological context the proportions of eggs of each species are a more helpful indicator. The number of eggs produced by a parasite may vary from one day to the next, and different parasites of the same species will produce different numbers of eggs to one another. This means that it is difficult to predict the number of worms present in the people who used this latrine based upon the number of eggs present per gram of soil. Furthermore, the concentration of eggs present in a latrine will be highly dependent upon the dilution effect of the rest of the material in that latrine. If non-faecal material is added to the latrine (such as for personal hygiene, or the disposal of general rubbish) then this will bulk out the non-faecal composition of the latrine and so dilute out the parasite eggs. This may be misinterpreted to indicate that the parasites were less common than they really were. The non-parasite components of faeces may decompose to a differing degree in contrasting environmental conditions. For example, in summer the activity of flies and fungi may encourage decomposition of the faeces faster than in the winter, so that layers of latrine soil may have the robust parasite eggs preserved at higher concentrations in summer layers than winter layers. The communal nature of latrine soil means that it is hard to differentiate many latrine users with a low parasite load from a few latrine users with high parasite load whose faeces are mixed in with others with no parasites at all. For all these reasons, we feel giving the number of eggs viewed in ten slides is a more helpful indicator of egg numbers in this context. These allow comparison of how common the eggs of each species were in this sample while controlling for the effects of dilution and decomposition, since these effects will be the same within the sample analysed.

The most notable finding of this study was the large number of eggs of fish tapeworm. This not only indicates that this population consumed plenty of fish, but also that the fish was not cooked thoroughly prior to consumption. This is because cooking kills the intermediate forms of the parasite (Gunn and Pitt, 2012, 104). It might have been eaten raw, smoked, or pickled. Zooarchaeological analysis at medieval Riga has shown the remains of many fish. The most common species were sturgeon, perch and haddock, while the remains of cod, salmon, pike and zander were also present (Hamilton-Dyer pers. comm.). Those species of fish that spend at least part of their development in freshwater lakes and rivers would have been at risk of acquiring the fish tapeworm larva. Moreover, documentary sources indicate that fishing in Riga was practiced by the indigenous population, rather than the German burghers (Caune and Ose, 2006, 468). When set alongside the evidence of fish bones and parasites, this reinforces the pivotal role of the Liv community in acquiring, handling, distributing and consuming fish. It is likely that fish came to play a pronounced role in the diet of the urban population given the introduction of a Christian fasting culture. Moreover, the Teutonic Order appears to have stimulated the development of a fishing industry in its eastern Baltic territories by the $14^{th}/15^{th}$ century (Orton et al., 2011).

In order to appreciate the role of fish tapeworm in the health of this society, it is helpful to understand the life cycle of the parasite. Fish tapeworms may reach up to 26 feet long, and spiral round inside the intestines. When an individual with fish tapeworms in their intestines goes to the toilet in a lake or river, the tapeworm eggs are contained within the faeces. In the presence of water, small coracidium larvae are released from the eggs. These larvae are eaten by freshwater crustaceans (copepods), and the development of the first stage larvae (procercoid larvae) occurs in the crustacean. During the next stage, the infected crustacean is eaten by a small freshwater fish (second intermediate host) following which the procercoid larvae released from the crustacean migrate into the fish's flesh. When the small freshwater fish is preyed upon by larger fish, the plerocercoids are infective for the definitive host such as humans if that fish is eaten without being cooked first (Garcia, 2009, 354). The requirements for each stage of this life cycle must have been present in medieval Riga for such strong evidence for fish tapeworm in the latrine. Many people today with a fish tapeworm do not suffer much in the way of health consequences. However, a heavy load of fish tapeworms worms can lead to anaemia from vitamin B12 deficiency (Garcia, 2009, 354). In view of the number of eggs noted in this latrine, we would suspect that a proportion of the population may have suffered with anaemia in this way.

Previously, fish tapeworm has been found at a number of sites in northern Europe from Austria, Belgium, Britain, France, Germany, Norway and Switzerland (Sianto et al., 2009; Le Bailly and Bouchet, 2013). However, the high density of fish tapeworm eggs in medieval Riga is quite notable. The parasite has also been found in crusader period latrines from the Frankish kingdom of Jerusalem in the Middle East dating to the 13th century, compatible with crusaders traveling from northern Europe to the Holy Land with fish tapeworms in their intestines (Mitchell et al., 2011). Fish tapeworm does not appear to have been present in southern Europe in the medieval period, indicating that conditions there were not suitable for its life cycle.

Despite the presence of the bones of cattle and pigs at the site, no beef tapeworm or pork tapeworm eggs were noted in the latrine. These two species of tapeworm are known to have been present across Europe by the medieval period (Bouchet et al., 2003; Anastasiou, in press). This suggests that the population of Riga may have cooked their beef and pork prior to eating it, as cooking kills the intermediate forms of this parasite. Hence a difference in culinary approach to fish, beef and pork would explain the contrasting prevalence of tapeworms present in the population.

Whipworm (*Trichuris trichiura*) and roundworm (*Ascaris lumbricoides*) were also found in significant numbers in the latrine, with the whipworm the more common of the two. These two parasites are spread from the fecal contamination of food, either by the use of human feces to fertilize crops or by cooks preparing food with unwashed hands. In contrast to fish tapeworm, they do not require an intermediate host to complete their life cycle. Adult whipworms are 3-5cm long but roundworms are much larger at 20-30cm long (Garcia, 2009). These parasites have been infecting in humans throughout our evolution, being present in both early Africa and people who then migrated to Europe (Bouchet et al., 2003; Mitchell, 2013). A heavy load of whipworm and roundworm can lead to malnutrition, reduced intelligence and stunted growth in children (Garcia, 2009, 130-33; Callender et al. 1998).

The cysts of *Entamoeba histolytica* were also found in this latrine. ELISA allows us to determine that the species of Entamoeba was the pathogenic form that causes dysentery in humans (*E. histolytica*), as other non-pathogenic species such as *E. dispar* appear identical on microscopy. The ELISA test detects proteins unique to the pathogenic species that causes dysentery in humans (Sharp et al. 2001). *E. histolytica* is a single celled parasite that inflames the lining of the intestines and causes bloody diarrhoea, abdominal pain, fever and dehydration for a number of days, and can sometimes be fatal. It is contracted by consuming water or food contaminated with infective cysts (Garcia, 2009, 256). *E. histolytica* has been found in other archaeological sites within Europe such as Belgium, Greece, France, Italy and Switzerland, and it has been proposed that the disease may have originated in Europe (Goncalves et al., 2004; Le Bailly and Bouchet, in press). It has also been found in a 13th century latrine in the Frankish Kingdom of Jerusalem in the Middle East, where crusaders from Europe stayed while on military expeditions (Mitchell et al., 2008).

In addition to the parasites that infect humans two eggs of *Oxyuris equi* were identified. This is a pinworm found in the intestines of horses and donkeys that causes anal itching (Bush et al., 2001; Hendrix and Robinson, 2012; Zajac and Conboy, 2012). This identification demonstrates that the equid pinworm was present in the region by the medieval period. In our opinion the extremely low numbers of eggs does not indicate that horse dung was deliberately added to the latrine, or else much higher numbers of eggs would have been seen. It may perhaps have found its way into the latrine on the shoes or hands of people working with horses immediately before using the latrine.

6. Conclusion

This latrine from the most important city in medieval Livonia is notable for the heavy concentration of the eggs of intestinal parasites. The vast majority were whipworm, fish tapeworm and roundworm indicating human infection by these parasites. The tapeworm eggs highlight how the consumption of raw, smoked, or pickled fish put the population at risk of fish tapeworm. The presence of whipworm and roundworm indicates a state of hygiene where the faecal contamination of food by these parasite eggs was frequent. The protozoan *Entamoeba histolytica* was also present in this population, which is one of the causes of dysentery. This would have caused outbreaks of bloody diarrhoea, and would have been much more pathogenic that the intestinal worms identified, since dysentery may be fatal. A tiny number of intestinal parasites of equids was also present.

The species of parasite found in different parts of medieval Europe varied considerably with geographic region and local dietary customs. While some parasites such as whipworm, roundworm and Entamoeba were found across Europe by this time, certain other species such as the fish tapeworm described here provide a vivid insight into the water-focussed culture of the coastal Baltic region during the medieval period.

7. Acknowledgements

The original excavation was funded commercially by F & F Buve, Riga, Latvia.

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9. List of Illustrations

Figure 1. A. Map of medieval Riga at the end of the 13th century with the excavation area highlighted by rectangle bottom right. B. Plan of excavation, black arrow shows location of latrine analysed.

Figure 2. Excavated latrine dated to 1356AD. Plan on right shows sampling locations.

Figure 3. *Trichuris trichiura* (whipworm) Dimensions 54x23 µm. Black bar indicates 20µm.

Figure 4. *Diphyllobothrium latum* (fish tapeworm) egg. Width 45µm (length unhelpful as operculum missing). Black bar indicates 20µm.

Figure 5. *Ascaris lumbricoides* (roundworm) egg with its mammillated surface coat. Dimensions 67x45µm. Black bar indicates 20µm.

Figure 6. *Oxyuris equi* (equid pinworm) egg. Dimensions 86 x42µm. Black bar indicates 20µm.

Table 1. Helminth Eggs Noted on Microscopy.

Table 1. Helminth Eggs Noted on Microscopy.

Parasite Species	Number of eggs in 10 slides	Length in µm	Width in µm	Host Species
Trichuris trichiura (whipworm)	3000*	Mean 54.2 SD 3.2	Mean 23.1 SD 1.2	Humans
Diphyllobothrium latum (fish tapeworm)	2200*	Not of use once operculum lost	Mean 47.0 SD 4.2	Humans, bears, dogs
Ascaris lumbricoides (roundworm)	550*	Mean 68.1 SD 3.4	Mean 48.4 SD 3.6	Humans
Oxyuris equi (horse pinworm)	2	Mean 86.9	Mean 42.8	Horses/donkeys

Note: 1) *indicates approximate numbers. 2) mean and standard deviation calculated from the first 20 eggs identified of the Trichuris, Diphyllobothrium and Ascaris.