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Dysentery in the crusader kingdom of Jerusalem: an ELISA analysis of two medieval latrines in the City of Acre (Israel)

Piers D. Mitchell [a,*], Eliezer Stern [b], Yotam Tepper [b]

[a] Faculty of Medicine, Imperial College London, 7E Charing Cross Hospital, Fulham Palace Road, London, W6 8RP, UK
[b] Israel Antiquities Authority, P.O. Box 1094, Acre 24110, Israel

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Abstract

Dysentery was a significant cause of ill health during medieval times according to written sources of the period. However, it has been very difficult to detect the organisms that cause dysentery in archaeological samples from latrines and cesspools. This is because the cysts of the enteric parasites that cause dysentery are very small and usually fragment in the soil, in contrast to the robust ova of parasitic intestinal worms. This study uses a monoclonal enzyme-linked immunosorbent assay (ELISA) technique to identify the fragments of those enteric parasites that cause diarrhoea. Samples were taken from two excavated medieval latrines in the city of Acre. Coins and radiocarbon dating suggest that the latrines were in use during the thirteenth century CE. Positive results were noted for *Entamoeba histolytica* and *Giardia duodenalis*, while *Cryptosporidium parvum* was negative. This is the first time that either of these parasites have been identified in the Middle East using these methods.

Keywords: Crusades; Dysentery; ELISA; Parasites; *Giardia* sp; *Entamoeba* sp

1. Introduction

The armies of crusade expeditions to the Middle East often appear to have suffered from dysentery according to contemporary chronicles, and many soldiers died from it (Bachrach and Bachrach, 2005, 136; Mitchell, 2004, 1). In 1249 CE when King Louis IX of France led the seventh crusade armies, his diarrhoea was so frequent that he had part of his breeches cut away to simplify personal hygiene (Hague, 1955, 24). While written evidence such as this shows that infective diarrhoea was present in the Middle East in the crusader period, archaeological analysis has been unable to determine which microorganisms were responsible. While a number of palaeopathological studies of the health of crusader period populations have now been published (Mitchell, 2006a,b; Mitchell et al., 2006), intestinal parasitic disease is unlikely to result in lesions on skeletal remains. Microscopic analysis of human latrine soil from twelfth and thirteenth century sites in the Levant have also identified the ova of a range of parasitic intestinal helminths such as the whipworm, roundworm, beef/pork tapeworm and fish tapeworm (Mitchell and Stern, 2001; Mitchell and Tepper, 2007). However, the ova of these worms survive much better in the soil than do the organisms that cause dysentery. Such cysts are well known to degrade quickly once desiccated, making their identification challenging with conventional microscopic techniques. In consequence, the cysts of enteric parasites such as *Entamoeba histolytica*, *Giardia duodenalis* and *Cryptosporidium parvum* have not been noted.

In order to increase the sensitivity of tests for these organisms in an archaeological context a technique is required that can detect not just rare intact cysts, but also cyst wall fragments or even proteins secreted uniquely by these organisms. In this study we have employed a monoclonal enzyme-linked immunosorbant assay (ELISA) technique. This approach relies on antibodies specific for various stable proteins uniquely produced by each of those microorganisms that cause dysentery. Similar tests have been used with success in archaeological

* Corresponding author.
E-mail address: piers.mitchell@imperial.ac.uk (P.D. Mitchell).

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samples from North and South America and parts of Europe (Gonçalves et al., 2002; Gonçalves et al., 2004). Such an approach has never before been used on samples from the Middle East. It should indicate whether evidence for dysentery can be found, and which organisms were responsible.

2. Excavation of the latrines

Both sets of samples were taken from excavations in the city of Acre, now in Israel. This lay in the crusader kingdom of Jerusalem during the twelfth and thirteenth centuries.

The first set was from the latrine block of the Hospital of St. John. The Order of St. John was a religious order whose members cared for crusaders and pilgrims in their hospitals, but also fought with the king's army and defended a series of castles in the Frankish states of the Latin east. Historical records show that the latrine block was built of stone in the late twelfth century and fell into disuse in the late thirteenth century. It was constructed on four floors, included thirty-five stone seats, with an irrigation system using rainwater from the roof (Mitchell et al., in press). Its construction appears to have been solely for human use, rather than for the disposal of general waste or animal excrement. The samples for testing were obtained from the vault below the latrine seats and from the sewer drain from this area. Control samples were also taken from eighteenth century soil immediately above the latrines and from thirteenth century storage halls from the hospital complex.

The second set of samples was from a cesspool in the old city of Acre. This was a stone built, pear shaped construction with a narrow opening above, using masonry typical of the crusader period (Mitchell and Tepper, 2007). Its location within a building complex suggests that it was for use by the city inhabitants, and not just for the use of one religious institution as was the case for the first latrine. Since the cesspool was not in a specially constructed building, it is theoretically possible that both human and animal waste may have been emptied into it. Samples were taken from the base and middle of the cesspool, and from more recent soil above its entrance as a control sample.

Since latrines and cesspools can contain material that is not from the time period of their construction, we sought after further evidence to confirm our dating. Charred seeds and charcoal from the Hospital of St. John latrine sample underwent AMS radiocarbon dating at the Oxford Radiocarbon Unit, UK. All dates were calibrated and the ranges given to 95.4% probability. This has shown that the upper layers of soil in the hall date from the Ottoman period (date A: 1520–1610 CE) and were probably washed in with rain or blown in by wind after the latrines fell into ruin. However, the sample from the base of the hall was dated to the thirteenth century, when the latrines were in use (date B: 1260–1310 CE). A further sample of charcoal from the latrine hall drain was dated shortly before this (date C: 960–1020 CE), as one would expect for charcoal where dates represent when the analysed part of the original tree was growing. The layer of charcoal is presumed to have been deposited in the latrine as ash from fires resulting from the siege and loss of the city by the crusaders in 1291 CE. The cesspool was dated from artifacts found within it, so carbon dating was not necessary. It contained six coins minted during the twelfth and thirteenth centuries. They were deniers with origins in France, Sicily and Cyprus suggestive of use by crusaders. No other early or late medieval coins were present, which suggests that the cesspool had not been in use before or after the crusader period. This confirms the structural findings on excavation that suggest it was in use during the thirteenth century.

3. Method of analysis

All soil samples were disaggregated with a 0.5% aqueous solution of trisodium phosphate for 72 h. Samples of sediment were then taken for ELISA analysis, and further processed for microscopy.

For microscopy two processing techniques were used. The simpler method employed just zinc bromide heavy density flotation to concentrate any parasites present. The second method involved 5% sodium hydroxide to remove humic material, 30% hydrochloric acid to remove carbonates and 40% hydrofluoric acid to remove silicates, and centrifugation to concentrate any parasites. This was found helpful due to the large amount of fine silicates in Middle Eastern soils. The resulting material was viewed under ×100 and ×600 magnification. Details for these techniques can be found in standard publications (Warnock and Reinhard, 1992).

The monoclonal ELISA was performed on disaggregated soil samples using pre-made kits from TECHLAB® (Blacksburg, VA 24060, USA) for each organism under investigation. A positive test was demonstrated by the conversion of the well colour to a deep yellow, quantified with a spectrophotometer at 450 nm and compared with positive and negative controls in the kit. The kits used were *E. histolyitca* II, *Giardia*, and *Cryptosporidium* tests. Multiple cysts are required in each well to give a positive result. This varies from 10–15 cysts for *Giardia* to 78–156 cysts for *Cryptosporidium* (TECHLAB® kit booklets).

Sensitivity and specificity tests in modern clinical stool samples give some guide as to the likelihood of erroneous results in archaeological samples. After all, fresh stool contains a huge variety of different microorganisms. If the tests avoid giving false positive results with commensal gut microorganisms they are also likely to avoid false positives with commensal soil microorganisms. The results suggest that the ELISA technique is much more sensitive and specific than microscopy for detecting organisms responsible for dysentery. TECHLAB information booklets that come with the kits state 90% sensitivity and 100% specificity for the *Giardia* test kit, and 96.9% sensitivity and 100% specificity for *E. histolytica*. An independent assessment found the *E. histolytica* I test was 80% specific and 99% sensitive for *E. histolytica–dispar* complex, and the *E. histolytica* II test was 95% sensitive and 93% specific for differentiating the pathogenic *E. histolytica* from the non-pathogenic *E. dispar* (Haque et al., 1995). In simple terms, using modern faeces the ELISA test kits still miss