Universiteit Leiden
The Netherlands

Malaria in bone. Hunting for Hemozoin

Schats, R.; Dalebout, H.; Heijs, B.

Citation
Schats, R., Dalebout, H., & Heijs, B. (2019). *Malaria in bone. Hunting for Hemozoin*. Retrieved from https://hdl.handle.net/1887/83212

Version: Not Applicable (or Unknown)
License: Leiden University Non-exclusive license
Downloaded from: https://hdl.handle.net/1887/83212

Note: To cite this publication please use the final published version (if applicable).

MALARIA IN BONE

Hunting for hemozoin

Rachel Schats, Hans Dalebout, Bram Heijs

INTRODUCTION

Malaria had and still has a detrimental impact on human health. While almost certainly also being a disease of great importance in the past, as of yet, there is no fully satisfactory biomolecular method to detect malaria in human skeletal remains. Therefore, we will discuss a new method, pioneered by Yale University, for identifying malaria in the archaeological bones which targets the insoluble crystal named hemozoin.

HEMOZOIN

Hemozoin is a waste product of the malaria parasite formed by the digestion of haemoglobin in the red blood cell (fig. 1). As heme is toxic, the parasite converts it into a insoluble crystalline form. Hemozoin can be detected with MALDI-TOF-MS (matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry).

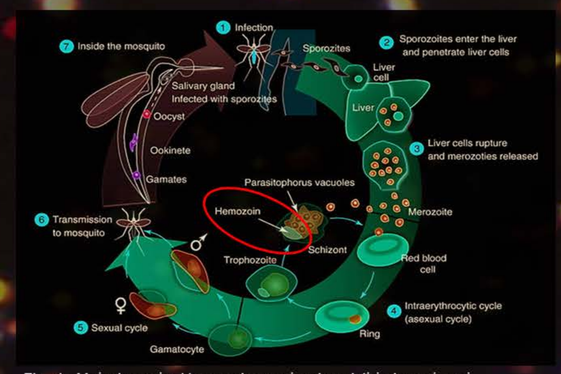

Fig. 1: Malaria cycle. Hemozoin production visible in red oval

MATERIALS AND METHODS

To study the feasibility of using hemozoin for the identification of archaeological malaria, initial test were performed using synthetic hemozoin (InvivoGen) to 1) study the measurability of hemozoin, 2) investigate the use of matrix (4-HCCA) to improve ionisation, 3) assess the detection limit, and 4) research different solvents (MQ and $CHCl_3$). Solutions were spotted (0.5 µl) on a ground steel plate and spectra were recorded using an rapifleX mass spectrometer controlled by flexControl 3.4 Build 135 (Bruker Daltonics). Analysis was done with flexAnalysis.

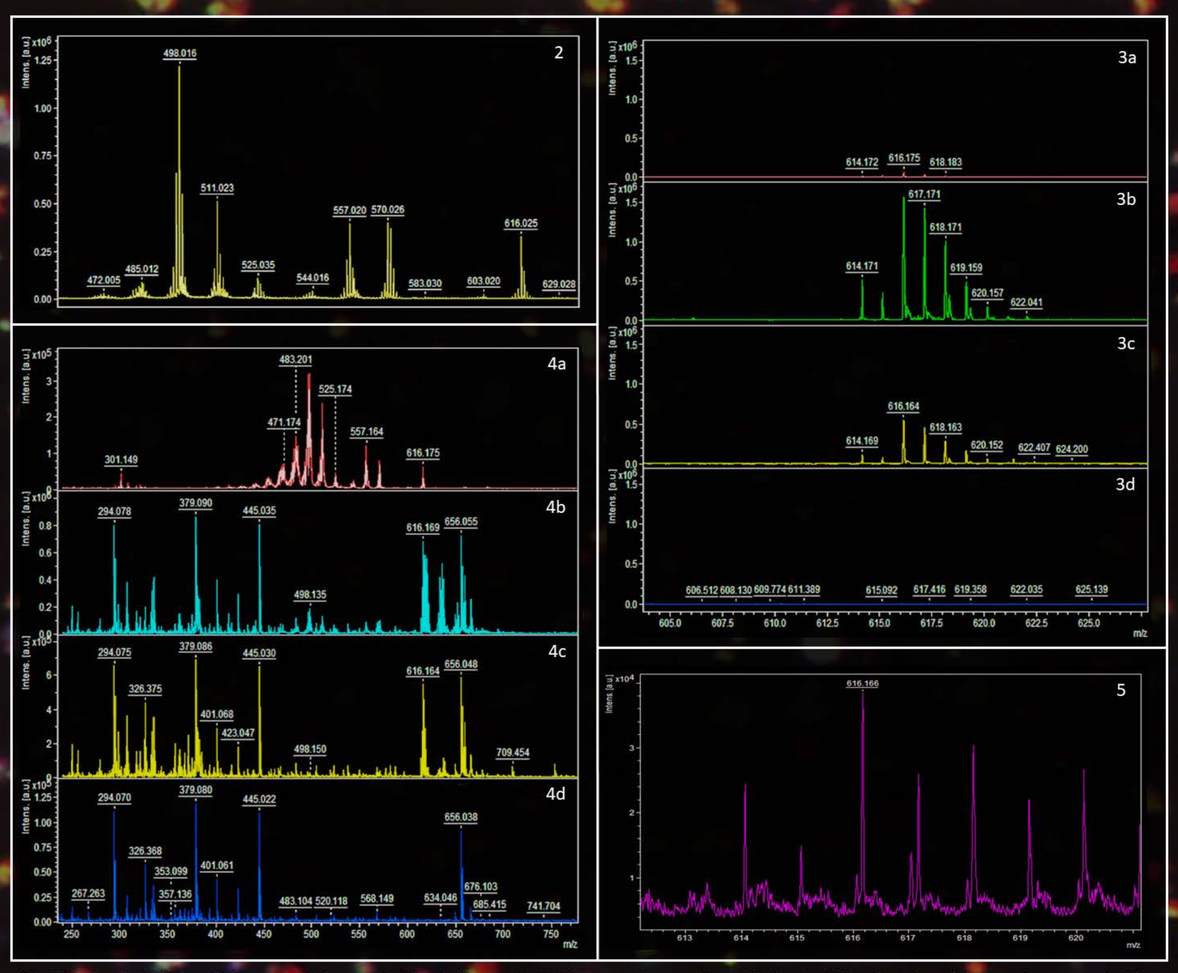

Fig. 2-5: Results initial analysis synthetic hemozoin. Fig. 2: Hemozoin MS signature (no matrix used). Fig. 3: 616 *m/z* region, a) hemozoin, no matrix, b) hemozoin in CHCl3 with matrix, c) hemozoin in MQ with matrix, d) matrix (4-HCCA). Fig. 4: Suppression of fragment ions with matrix, a) hemozoin, no matrix, b) hemozoin in $CHCl_3$ with matrix, c) hemozoin in MQ with matrix, d) matrix (4-HCCA). Fig. 5: 616 *m/z* at 50 pg (signal to noise = 14).

RESULTS

Measurability: The RapiFlex mass spectrometer hemozoin spectra indicate that hemozoin can be detected well and the spectra are comparable to those published in literature. The characteristic 616 *m/z* peak as well as the peaks from the fragment ions (484, 498, 512, 557 *m/z*) are visible (fig 2).

Matrix: The use of matrix (4-HCCA) improves ionization (fig. 3a-c). However, the fragment ions seem to be suppressed by the use of matrix (fig. 4a-c).

Detection limit: Using matrix, it is possible to detect as little as 50 pg of hemozoin in the sample.

Solvents: Hemozoin was suspended in MQ water and solubilised in chloroform. Hemozoin solved in $CHCl_3$ results in slightly higher signal intensities (fig. 3b-c).

DISCUSSION AND CONCLUSION

Hemozoin is well detectable using MALDI-TOF-MS. The use of matrix improves the signal intensity of the 616 *m/z* peak, but has a suppressive effect on the fragment ions. The low detection limit suggests that this method is suitable for archaeological skeletons as amounts of hemozoin are likely to be low. Future research will apply this method to past human remains.

This research is funded by the Dutch Research Council (NWO) and the Elise Mathilde Fund/ Leiden University Fund.

LUMC
Universiteit Leiden
Archaeology