# Tiina Äikäs, Ulrich Bergmann & Anna-Kaisa Salmi
# AN ATTEMPT TO USE BLOOD RESIDUE ANALYSIS TO IDENTIFY SACRIFICIAL PRACTICES AT SIEIDI SITES

Abstract
In autumn 2012, samples were taken from three sieidi stones in order to test blood residue analysis as a method for identifying marks of Sámi sacrificial practices. Prior to the fieldwork, test samples were taken from stones that the authors smeared with blood and left outside first for six and then for five more months. Altogether 19 samples from sieidi stones were analysed. Prior research has not concentrated on blood traces on stones that are exposed to sunlight. The analyses using mass spectrometry revealed that blood proteins could be identified in the test stones. There were nevertheless indications of a rapid disintegration of the blood proteins when exposed to open-air conditions. All the samples from sieidi stones gave a negative result. Currently it is unclear whether the degradation of blood proteins has exceeded the detection limit of the method, or whether sacrificial practices at those sites did not include blood offerings.

Keywords: blood residue analysis, blood protein, mass spectrometry, sieidi, Sámi sacrificial practices

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## SACRIFICIAL PRACTICES AT SIEIDI SITES

The sacrificial practices of Sámi ethnic religion[1] took many forms, including, for example, sacrifices at dwelling places (Rydving 1993: 97–8; Halinen 2010: 41–2). However, in this article we concentrate on the sacrifices that took place at *sieidi* sites (the orthography of North Sámi language is used in this text). A *sieidi* is a Sámi sacrificial place that is most often a natural object unmodified by humans. In different Sámi areas, the traditions of what constituted a *sieidi* varied somewhat, but in the part of Lapland confined by the present borders of Finland (henceforth referred to as Finnish Lapland), our area of study, a *sieidi* was usually a boulder or a large rock formation (Äikäs 2011: 18). Curved wooden poles are also known from written sources, but only rare examples have survived (Andersson 1914: 44; Paulaharju 1979 [1939]: 200; cf. Svestad 2011: 47).

Archaeological excavations conducted in recent years at *sieidi* sites in Finnish Lapland have unearthed bone material dating from the 11th century to the 17th century AD (Äikäs 2011: appendix III). However, this does not mean that the making of sacrifices ceased after that time. Written sources and a still living oral tradition inform us that sacrifices were offered to *sieidis* even in the 20th century (Paulaharju 1932: passim; Kjellström 1987). In addition, evidence of modern visits, such as coins, candles, snuff, and fishing equipment, have been found in the course of archaeological fieldwork (Äikäs 2011: 118–23; 2012). There is thus a long tradition of sacrifices at *sieidi* sites.

Judging by the archaeological material, reindeer has been the animal species most commonly sacrificed in Finland. Birds, especially capercaillie, were also sacrificed. In addition, some fish, bear, and sheep or goat bones have been found at the excavated *sieidi* sites in northern Finland

(Äikäs 2011: 42; Salmi et al. 2011: 199–222). The distribution of species differs between sites, perhaps reflecting the special function of the *sieidi* site (Äikäs et al. 2009). Sacrifices were often connected to means of livelihood; some sites were said to be better for fishermen, whereas others were more suitable for hunters or reindeer herders (e.g. Paulaharju 1932: passim).

In addition to the *sieidi* sites with animal bones as indicators of past offering practices, we also conducted excavations at sites that yielded no ancient bone material whatsoever. Nevertheless, these sites are also well known *sieidis*, mentioned in written sources, and there were marks of recent visits at these sites indicating their continuing importance. One possible explanation for the lack of bone material is that sacrifices at these sites, which included Porviniemi (number 1000000537 in the Finnish National Register of Monuments and Sites) and Kirkkopahta (1000009401) in the municipality of Muonio, as well as Dierpmesvárri (1000016716) in Enontekiö (Eanodat), did not consist of whole animals or animal parts. There might be various explanations for these 'empty' sites. Sacrifices at *sieidi* sites took a myriad of forms. Sometimes live animals were left at the sites, sometimes only parts of the animals, such as a head or antlers, and sometimes a sacrificial meal was eaten and its remains were left at the *sieidi* (Äimä 1903: 115; Paulaharju 1932: passim; Itkonen 1948: 311–2). There were also occasions when the stone was smeared with animal fat or blood (Itkonen 1948: 311–2; Schefferus 1963 [1673]: 177–8). Evidence for the last-mentioned activities cannot be obtained through archaeological excavation, which provoked our interest in exploring methods that could provide evidence for these kinds of practices. One possibility lies in identifying traces of blood residues on the surface of the stone.

## BLOOD RESIDUE ANALYSIS AS A METHOD FOR IDENTIFYING ANCIENT BLOOD REMAINS

Different methods, such as biochemical tests, hemoglobin recrystallization, electrophoresis, and immunological methods have been employed in the analysis of ancient blood protein residuals (Downs & Lowenstein 1995). Many attempts to identify ancient blood protein from archaeological finds and sites were made during the 1990s (e.g. Cattaneo et al. 1993; Loy 1993; Downs & Lowenstein 1995; Fiedel 1996; Tuross et al. 1996), while only a handful have been made after the year 2000 (e.g. Williamson 2000; Solazzo et al. 2008; Heaton et al. 2009).

The reliability of these methods has been the subject of continuous discussion. Critical voices have pointed out the problems of false positive results, lack of suitable controls, and evidence for rapid degradation of proteins in buried objects (Heaton et al. 2009 with references). Although many studies report that blood protein residuals are easily detected from fresh samples by different techniques, the analysis of archaeological objects has provided contradictory results (Downs & Lowenstein 1995). This has probably something to do with the degradation of blood proteins with time. Different proteins can survive for thousands of years in different environmental conditions (Loy 1993). However, the amino acid chains of the proteins break into shorter chains with time, which prevents biochemical and immunological reactions (Gurfinkel & Franklin 1988; Downs & Lowenstein 1995; Fiedel 1996). The rate of protein degradation depends on taphonomic factors, such as weather, soil conditions, and microbial activity (Cattaneo et al. 1993; Downs & Lowenstein 1995). It has also been suggested that natural UV radiation destroys immunoreactivity (Tuross et al. 1996). Cattaneo et al. (1993) experimented on blood residue survival in outdoor conditions by burying blood-stained objects for different periods of time and testing them with immunological methods. Their results suggest that water leaching through the ground may play a role in the loss of blood from blood-stained objects. They furthermore suspect that conditions sheltered from direct rainfall are better for the survival of blood proteins. Most of the work has been done on objects buried in the ground, but some sheltered open-air sites, such as rock paintings located in semi-caves, have also been investigated with success (e.g. Loy et al. 1990; Williamson 2000).

In this study, we used mass spectrometry (MS) in an attempt to identify blood protein from stones exposed to open-air conditions. First, we conducted an experiment by smearing rocks with reindeer blood and leaving them under open-air conditions for different periods of time. Subsequently we used MS to determine whether the blood proteins had been preserved well enough

to be identified with this method. In the second part of our study, we applied MS to samples taken from three *sieidi* stones to see whether any blood protein related to ancient blood offerings could be identified. We chose MS because the technique – unlike DNA sequencing and immunological methods – does not require specific antibodies or primers for each target protein and, furthermore, does not suffer from crossreactivity. Moreover, even peptides of degraded proteins can be identified with MS. This was especially important, as we were dealing with sites that had been exposed to open-air conditions.

Mass spectrometry was developed in the beginning of the 20th century, but it became feasible for the analysis of proteins only 20 years ago, when suitable ionization techniques were developed (Karas et al. 1987; Fenn et al. 1989). Depending on the instrument and the experimental set-up, one can achieve high sensitivity (low attomol range) and obtain protein sequence information that may allow identifying the biological species. However, discriminating blood proteins from different species might become difficult if the most abundant proteins, which are likely to be detected with the highest sensitivity, are conserved between species. On the other hand, MS is fast and easy to carry out and does not require species-specific reagents, such as the primers required for DNA detection by the polymerase chain reaction or specific antibodies for immunological assays. For the analysis described here, for several reasons, the detection of full-length proteins was not attempted. Intact proteins might be more difficult to extract from the mineral matrix, large molecules cannot be detected and sequenced with the same sensitivity as smaller ones, and if the protein is partially degraded, molecular weight information is less informative.

Instead, it is more suitable to digest the proteins into peptides with a specific protease, usually trypsin. The peptide masses can be measured with high sensitivity, and it is easy to obtain sequence information. Even degraded proteins are still likely to yield sufficient peptides to allow identification. And most importantly, the accurate masses of tryptic peptides are specific for a given protein and can be used for identification without the need to obtain sequence information (Peptide mass fingerprint PMF). Solazzo et al. (2008) were able to identify myoglobin peptide sequences, dating to c 1200–1400 AD and deriving from potsherds, to species with the aid of mass spectrometry. Furthermore, Heaton et al. (2009) succeeded in detecting a range of proteins in their experiment on desorption electrospray ionization mass spectrometry (DESI-MS).

## MATERIAL AND METHODS

### *Background of the research*

Our interest in studying those ritual practices that left no bone material at *sieidi* sites, in this case blood offerings, was aroused by our excavations at *sieidi* sites from which no bone material was found. These excavations were a part of the 'Human-animal relations among Finland's Sámi 1000–1800 AD: DNA and stable isotope analyses of bones from ritual sites' project funded by the Academy of Finland. This research is a continuation to those excavations, but was conducted when the authors were no longer participating in the project. Hence this research should be seen as a preliminary test for methods between the first author's doctoral and post-doctoral research. The fieldwork was part of a field trip that was organized in order to return the offered bones that were studied during the Academy project, but otherwise no additional funding was available for this research.

### *Test samples from modern blood remains*

Preliminary tests for the survival of blood residue were made during 2011 and 2012. For the tests, three stones of different stone types were obtained from the Department of Geology, University of Oulu. The sample stones were gneiss, granulite, and quartzite. The stones were brushed with reindeer blood so that half of the surface remained clean for control samples. Since no fresh reindeer blood was available, frozen blood (in which 0.05 % of salt and stabilizer E450 had been added) was used instead. It was allowed to defrost gradually so that it did not precipitate, and then approximately 50 centilitres of the blood was applied to the surface of the stones. The use of reindeer blood instead of some other animal blood was considered essential in order to estimate whether identification of the animal species is possible.

The test was initiated on February 16, 2011. The stones were left outside in a sheltered loca-

tion where they were covered from rain, but not from wind-blown snow. The stones were also exposed to sunlight. The stones were analysed in three stages after six weeks, three months, and six months. Hence the samples were subjected to different weather and light conditions. The test samples were taken from all stones, after which the stones were returned outdoors.

After the last samples in August 2011, the stones were moved from their sheltered location to a place in which they were exposed to all weather conditions. They were left here for an additional five months. The final samples were taken on January 27, 2012.

### *Samples from* sieidis *in Näkkälä, Sieiddakeädgi, and Koskikaltiojoen suu/ Nitsijärvi*

In the next stage of the research, samples were taken from three *sieidi* sites. Prior to the fieldwork, permission was obtained from the National Board of Antiquities. In addition, the Sámi Parliament (Sámediggi) was informed about the project.

Based on previous excavations, three sites were selected for sampling. At this point, we wanted to concentrate on sites at which the location of ritual activities could be identified based upon finds of animal bones. This limited the number of sites selected. The sites were Näkkälä (47010001) in Enontekiö, Sieiddakeädgi (890010016) in Utsjoki (Ohcejohka), and Koskikaltiojoen suu (148010327) in Inari (Anár) (Fig. 1). These locations were selected because previous excavations had confirmed that the places were used as sacrificial sites. Animal bones were found at each of the sites, and both written records and the oral tradition pertaining to these sites speak of sacrifices of reindeer and fish (e.g. Paulaharju 1932: 31, 35, 41). There are, however, no specific descriptions of blood sacrifices mentioned in connection to these sites. Regarding the site of Koskikaltionjoen suu, Paulaharju (1932: 35) writes that it was smeared so that it was yellow. However, this colour would seem to indicate the use of fat rather than blood. Unfortunately, our previous fieldwork did not include any sites associated with blood sacrifices.


*Fig. 1. Map of the sites selected for sampling. Map: T. Äikäs.*

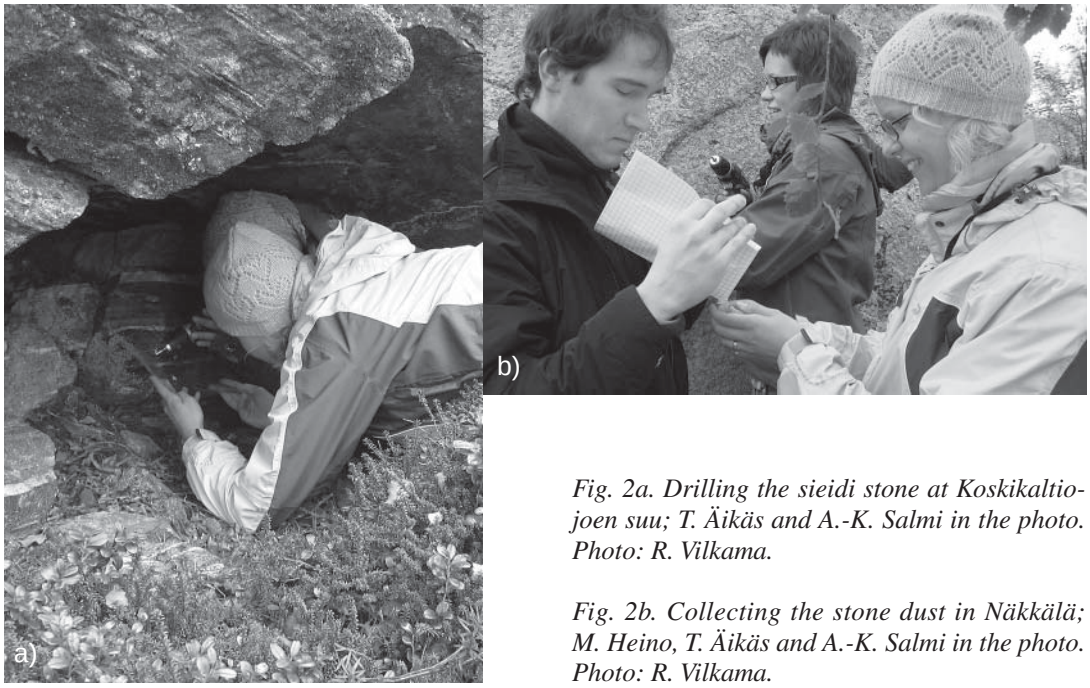


*Fig. 2a. Drilling the sieidi stone at Koskikaltiojoen suu; T. Äikäs and A.-K. Salmi in the photo. Photo: R. Vilkama.*

*Fig. 2b. Collecting the stone dust in Näkkälä; M. Heino, T. Äikäs and A.-K. Salmi in the photo. Photo: R. Vilkama.*

The fieldwork was conducted between August 31 and September 2, 2012. During the fieldwork, a number of samples (ranging from five to seven) were taken from each stone. For ethical reasons, we preferred to take a small number of samples in order to interfere with the sacred site as slightly as possible. Samples were taken from the side of the boulder where the archaeological evidence for sacrifice was strongest. In addition, one or two control samples were taken from those sides of the stone where no archaeological evidence of sacrifice had been found. When possible, the samples were taken from sheltered spots in the stone. The area sampled covered no more than one square centimetre of the stone's surface. Samples were taken using a drill equipped with a diamond bit. Lichen was removed from the surface of the stone prior to the drilling. The drilling extended at most to a depth of one millimetre. The drilled stone dust was collected on a piece of paper and then moved to a container (Figs. 2a & 2b). Each sample weighed c 5 mg.

At Näkkälä, the bone finds consisted mostly of reindeer (*Rangifer tarandus*), but skull bones of brown bear (*Ursus arctos*) were also found. The dating of the bones ranged from cal. AD 1165/1260–1220/1290[2], indicating the minimum period of use. In addition, an undated bone button and some coins of the modern period were found. (Äikäs 2011: appendix II & III; Salmi et al. 2011: 199–222.) In the account offered by Samuli Paulaharju, antlers and lower limb bones of reindeer were brought to the site, and the stone was smeared with fish fat (Paulaharju 1932: 40). However, the only signs of fish encountered in the course of our work were some fragments of relatively fresh fish skin and six fish scales. Based on the bone finds, the sacrifices were concentrated close to the stone – no more than three metres away from it – on its eastern and southern sides. These are the sides from which the nearby Lake Näkkäläjärvi is visible.

At Näkkälä, five samples were taken from the northern, eastern, and southern sides of the stone. In addition, two control samples (N1 & N2) were taken from the western side of the stone. All surfaces of the stone were smooth and relatively even, making it impossible to acquire samples from sheltered locations. Sample N3 was taken from the northern side of the stone from a place in which there are traces of dark red colour in the surface of the stone. It has been hypothesized that the colour might be remains of a rock painting, but this seems unlikely, as the red area is similar to other geological features in the stone (Lahelma 2012: 19). Beneath the sampled spot lies an area (#3) earlier subjected to excavation, from which a single fragment of reindeer bone was found

*Fig. 3. Sample spots on the eastern side of the Näkkälä sieidi. Photo: T. Äikäs.*

(Puputti 2008: appendix I). Samples N4, N5, and N6 were taken from the eastern side of the boulder, and N7 from the side facing south. These faces of the boulder have received attention even in contemporary times, as indicated by coins left in the cracks of the stone (Fig. 3). Moreover, most of the animal bones were found adjacent to these parts of the boulder.

At Sieiddakeädgi, the identified bone material consisted only of reindeer. The bones dated between 1165/1260 and 1510/1660 cal. AD. In addition, three coins from the end of the 19th century, some green 19th century bottle glass, and modern (post-1950s) coins were found. (Äikäs 2011: appendix II & III; Salmi et al. 2011: 199–222.) Relatively few bones were found close to the *sieidi* stone, with most of the bone material being found between five and ten metres away from the stone downhill on the western side of the *sieidi*. The location and mixed stratigraphy of the bones indicates their removal from the stone at some time after the original sacrifice (Äikäs 2011: 136–8; Salmi et al. 2011: 227). Based on both the location of the bones found close to the *sieidi* and the direction in which the bones had been moved, it looks like the southwestern part of the stone was mainly used for sacrifices.

At Sieiddakeädgi, altogether five samples were taken. Control sample S1 was from the southern side of the stone, which had yielded no finds during the excavations. The dominant feature of the *sieidi* stone in Sieiddakeädgi is a cave-like hole in the southwestern part of the stone. The hole has been described as almost big enough for a man to crawl in (Paulaharju 1932: 31). Sample S2 was taken inside this hole, close to its opening. Sample S3 was also taken close to the opening, but from outside the hole. Sample S5 was again from inside the hole, from its ceiling, where erosion has formed small, circular depressions. The importance of this hole is evident from oral tradition and from the coins left inside. Sample S4 was taken further away from the hole, from the northwestern side of the stone. This was the furthest place in the north side from which bone material was found.

The *sieidi* of Koskikaltiojoen suu (henceforth referred to as Nitsijärvi, as the locals call it) is a so-called *rauk*, a stone pillar, the surface of which is strongly weathered. Rather than a single stone, it is in fact a formation consisting of several stones. The finds of bones identified at Nitsi-

järvi included reindeer and capercaillie (*Tetrao urogallus*). In addition, oral tradition mentions fish sacrifices. The datings of the bones ranged between cal. AD 1270/1400 and 1510/1800, thus being among the most recent dates acquired so far (Äikäs 2011: appendix II & III; Salmi et al. 2011: 199–222.) The finds were concentrated on the southeastern side of the stone. The fact that the topography of the surroundings somewhat hindered opening large excavation areas on other sides of the stone might have affected the excavation results. The eastern side of the stone, facing the River Koskikaltiojoki, has been used for fat sacrifices even in recent times, when a Nenets, Anastasia Lapsui, smeared the *sieidi* with seal fat in 2008 (Mattus 2008: 30–2).

The samples from Nitsijärvi consisted of six actual samples and one control sample. The control sample, K1, was taken from the western side of the stone, from which no finds came to light during the excavations. The rest of the samples were from the eastern and southern sides of the stone. On the southern side, a part of the stone juts forward and forms a small shelter. Sample K2 was taken from the jutting, roof-like formation, whereas sample K3 was taken from under the roof, from the 'back wall' of the shelter. Bone material from the southern side of the stone derived partly from this shelter and partly from the southeastern corner, from under some smaller stones. Sample K4 was taken from the last-mentioned part, from the surface of the *sieidi*, while samples K5, K6, and K7 were taken from the eastern side of the stone. This side was actually formed by a group of smaller stones. On this side, there is also a smaller, shed-like structure, where the above-mentioned smearing with blubber took place. Sample K5 was taken from a place where the surface of the stone seemed oily, and where lichen of a strong yellow-green colour grows around the sampled spot. Samples K6 and K7 were taken from both sides of this area under the roof-like stones. Sample K7 is from a place where there is a hole between smaller stones, allowing a view 'through' the *sieidi*.

### *Methods*

The analyses of both test samples and *sieidi* samples were conducted in the manner described here. Ground stone material, typically 5–10 mg, was suspended in 100 µl of 6 M guanidinium hydrochloride (GuHCl) and reduced with 20 mM dithiothreitol (DTT) for 30 minutes at room temperature. The reaction was stopped by incubating with 40 mM iodoacetamide at room temperature for 30 minutes. The suspension was diluted with 200 µl of 50 mM ammonium bicarbonate in 10/90% (v/v) acetonirile/water. The pH was raised with 20 µl of 0.5 M ammonium bicarbonate and trypsin (Roche, proteomcis grade) was added to 5 ng/µl. The suspension was incubated overnight at 37°C and purified with C18 solid phase extraction pipette tips (Supelco) in the following way: the suspension was centrifuged (5 min, 13000 rpm), acidified with 5% (v/v, final concentration) TFA, and 20 µl, in the case of 'weak' samples the whole solution, was passed over the tip. The tip was washed three times with 0.1% TFA, prior to elution into 10 µl of matrix solution (see below), or alternatively, into 10 µl of 90%/10% (v/v) acetonitrile/water, containing 0.1% TFA.

0.5 µl of the sample was applied to an 800 µm anchor chip plate (Bruker) and allowed to dry. If the sample was in matrix-free solution, the spot was overlaid with 0.5 µl of matrix solution (0.8 mg/ml in 90%/10% (v/v) acetonitrile/water, containing 0.1% TFA and 1 mM $(NH_4)_2HPO_4$).

Mass spectra were measured on a Bruker UltrafleXtreme MALDI-TofTof instrument in reflector mode between m/z 700 and 3500 by collecting sufficient shots to maximize the signal/noise ratio (typically between 2000 and 10000 shots). From this spectrum, peaks were selected for MS/MS measurements (PSD Lift mode) and MS/MS data were combined with the parent's spectrum for database search. Spectrum processing and database search were done with Biotools (Bruker) and MASCOT (Matrix science). The search conditions were: NCBI database with no species restrictions, compulsory carbamidomethyl modification on cysteins, optional methionine oxidation, mass tolerance 100 ppm, and MSMS tolerance 0.5 Da.

## RESULTS

### *Control samples from test stones*

The samples stored in dry conditions presented no problems in obtaining mass spectra with the simple protocol used for this study. However, although blood contains a large number of different proteins, only hemoglobin alpha and beta chains were found

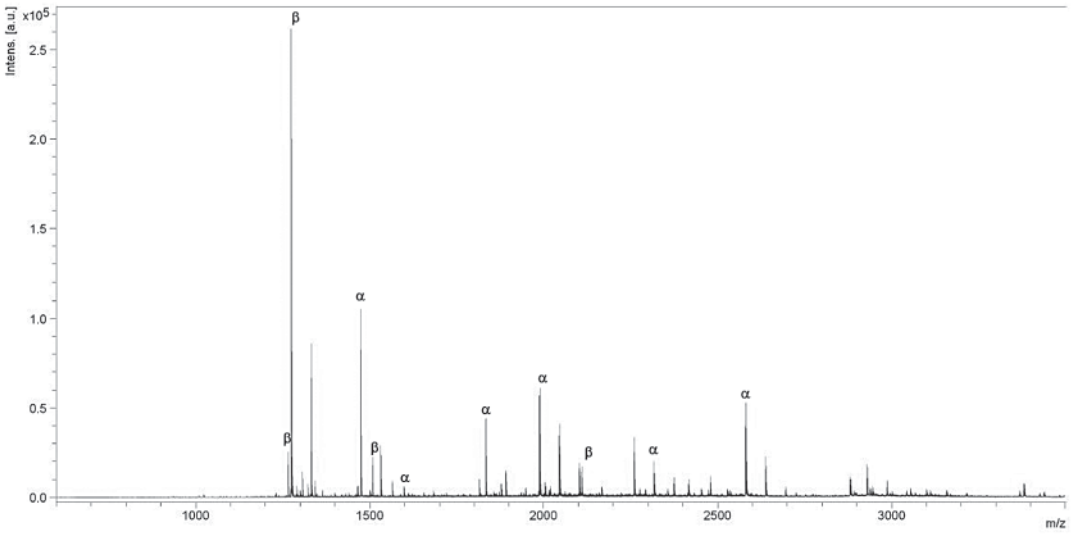


*Fig. 4. MALDI-ToF spectrum of control samples stored outside for six months in dry conditions. Main peaks are labeled to show how peptides from alpha or beta hemoglobin are represented.*

(Fig. 4). At this stage, reindeer (*Rangifer tarandus*) is unambiguously identified as the source of the hemoglobin from the peptide mass fingerprint (PMF) without the need to obtain sequence information. For the alpha chain, the European elk (*Alces alces alces*) would fit the data equally well, but the beta chain data are specific for reindeer (Fig. 5).

After unsheltered exposure, the situation is changed considerably. Samples 1 and 3 appear to be at the detection limit, while sample 2 still provides spectra with sufficient signal to noise ratio fairly easily. Surprisingly, all signals for the alpha hemoglobin were lost, while three signals for the beta chain are still preserved (Fig. 6). A

a) Mascot results

| | Accession | Mass | Score | Description |
|---|---|---|---|---|
| 1. | gi\|122476 | 15092 | 100 | RecName: Full=Hemoglobin subunit alpha; AltName: Full=Alpha-globin; AltName: Full=Hemoglobin alpha chain |
| 2. | gi\|110831902 | 15237 | 100 | RecName: Full=Hemoglobin subunit alpha; AltName: Full=Alpha-globin; AltName: Full=Hemoglobin alpha chain |
| 3. | gi\|66865970 | 15234 | 74 | alpha hemoglobin chain [Cervus elaphus canadensis] |
| 4. | gi\|122678 | 16213 | 61 | RecName: Full=Hemoglobin subunit beta; AltName: Full=Beta-globin; AltName: Full=Hemoglobin beta chain |
| 5. | gi\|302425109 | 15239 | 52 | RecName: Full=Hemoglobin subunit alpha-2; AltName: Full=Alpha-2-globin; AltName: Full=Hemoglobin alpha-2 chain |

b) Mascot results with unmatched peaks from search a

| | Accession | Mass | Score | Description |
|---|---|---|---|---|
| 1. | gi\|122678 | 16213 | 70 | RecName: Full=Hemoglobin subunit beta; AltName: Full=Beta-globin; AltName: Full=Hemoglobin beta chain |
| 2. | gi\|357635919 | 34825 | 64 | polysaccharide deacetylase [Streptococcus macacae NCTC 11558] |
| 3. | gi\|99082017 | 22732 | 47 | unnamed protein product [Ruegeria sp. TM1040] |
| 4. | gi\|335277169 | 25159 | 46 | GntR family transcriptional regulator [Propionibacterium acnes 6609] |
| 5. | gi\|291570063 | 5991 | 46 | hypothetical protein [Arthrospira platensis NIES-39] |

c) Detailed search results for the top hits

Hemoglobin alpha chain gi|122476

| Meas. M/z | Calc. MH+ | Sequence |
|---|---|---|
| 1071.556 | 1071.554 | MFLSFPTTK |
| 1238.681 | 1238.685 | AHGEKVANALTK |
| 1474.737 | 1474.728 | VGGNAPAYGAEALER |
| 1598.882 | 1598.890 | FLANVSTVLTSKYR |
| 1833.897 | 1833.892 | TYFPHFDLSHGSAQVK |
| 1987.995 | 1987.998 | AAWGKVGGNAPAYGAEALER |
| 2311.170 | 2311.168 | AVGHLDDLPGTLSDLSDLHAHK |
| 2356.146 | 2356.147 | TYFPHFDLSHGSAQVKAHGEK |
| 2527.261 | 2527.265 | VGGNAPAYGAEALERMFLSFPTTK |
| 2580.356 | 2580.353 | AVGHLDDLPGTLSDLSDLHAHKLR |

Hemoglobin beta chain gi|122678

| Meas. M/z | Calc. MH+ | P | Sequence |
|---|---|---|---|
| 936.477 | 936.494 | 0 | AAVTGFWGK |
| 1115.572 | 1115.569 | 0 | VDEVGAEALGR |
| 1207.690 | 1207.691 | 0 | VVTGVANALAHR |
| 1265.825 | 1265.830 | 0 | LLGNVLVVVLAR |
| 1274.740 | 1274.726 | 0 | LLVVYPWTQR |
| 1342.740 | 1342.732 | 1 | VKVDEVGAEALGR |
| 1507.830 | 1507.813 | 1 | VVTGVANALAHRYH |
| 1785.925 | 1785.938 | 1 | VLDAFSDGLKHLDDLK |
| 2109.017 | 2109.018 | 1 | LSELHCDKLHVDPENFR 6: Carbamidomethyl |

*Fig. 5. Database search with the spectrum shown in Fig. 4. a: Top five hits from the search with full spectrum. 1 (reindeer [Rangifer tarandus]), and 2 (European elk [Alces alces alces]) are significant but cannot be distinguished from each other. Reindeer beta hemoglobin is in position 4.; b: Search with data not matched to alpha hemoglobin. Reindeer beta hemoglobin shifts into first position, but the score is too low for reliable identification.; c: Comparison of experimental data with the theoretical mass data for the reindeer hemoglobin tryptic peptides.*

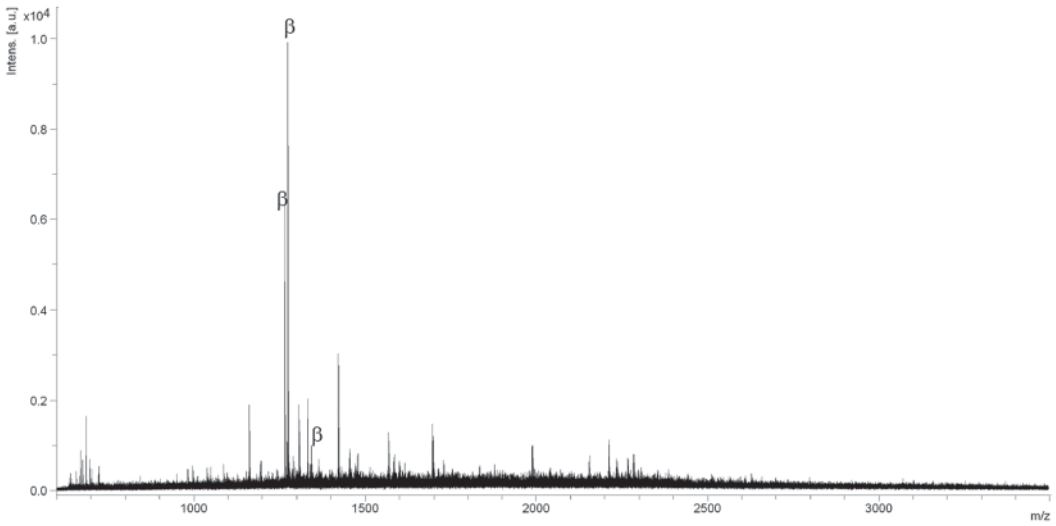


*Fig. 6. MALDI-ToF spectrum of control samples stored outside for six months in dry conditions, followed by five months fully exposed to rain and snow. The three detected beta hemoglobin signals are indicated.*

database search with PMF data no longer yields a confident hit. The top score is beta hemoglobin from a whale species (*Physeter catodon*, sperm whale). The reason for the unsecure hit is that sequence coverage has dropped from over 60% to about 40%, and the weaker signal strength has reduced mass accuracy and increased the risk for background peaks giving false positive results. It is still possible to obtain MS/MS spectra and identify the peptide sequence with high confidence (Fig. 7), but the two peaks with sufficient intensity are from highly conserved parts of the beta hemoglobin sequence and could originate from about 50 different species.

### *Samples taken from* sieidi *sites*

All samples taken from *sieidi* stones were negative for blood proteins. No traces of proteins could be identified by conducting similar analyses as on the test stones. There were no differences between the samples taken from the control side of the *sieidi* and from the side of the sacrifices.

## INTERPRETATION AND CONCLUSIONS

### *Detection of blood in stone samples*

The aim of this study was to develop methods for blood detection from *sieidi* stones and other archaeologically interesting rock formations that are exposed to sunlight. Mass spectrometry is probably the most suitable technique for sensitive protein detection. Immunological methods can reach similar or better sensitivity, but they require specific antibodies for each target protein, and cross reactivity and false positive results will remain a problem. Also for the other major alternative approach, DNA analysis by either polymerase chain reaction or advanced sequencing, the target molecules are likely to be degraded in an archeologically relevant time frame, and it remains doubtful whether blood samples can be analysed in that way after several hundred years of open-air exposure.

The second question discussed here is whether the animal species can be determined. This has previously been reported from burial sites, where myoglobin could be sequenced by FT-ion trap mass spectrometry to reveal the animal species (Solazzo et al. 2008). The advantage of mass spectrometry over PCR in this matter is that no species-specific primers are required and a single experiment can yield an unbiased result. On the other hand, PCR or advanced DNA sequencing is significantly more sensitive, but it will only be possible to prove that material from a certain animal species has been in contact with the site, not to identify which biological material has been the source of the nucleic acid.

Our pilot experiments suggest that blood can be detected for a long time if sites are not exposed to rain or otherwise immersed in water. Whether this is sufficient to answer the archaeological

questions posed remains to be addressed with further experiments. Naturally, at the current stage it cannot be determined whether the negative results obtained from the field samples are due to protein loss or insufficient sensitivity, or whether the samples happened to be collected from sites with no blood exposure.

However, it appears that stone sites exposed to water are probably not suitable specimens for archaeological blood detection with the method described here. Partially sheltered sites are more promising. This may change if sensitivity can be improved. We used MALDI ionization with time of flight detection as the most sensitive method available in our laboratory and because the detection limits are not dependent on the mass range. Our results will be useful for further development. We established that hemoglobin is the most promising target, with beta hemoglobin the best preserved protein, yielding at least two peptides for sensitive detection. However, to identify the species, more peptides need to be recovered. Other mass spectrometers, potentially more sensitive ones such as triple quads or ion traps, could be tuned for suitable ions to improve detectability and species identification. Another question is the influence of different stone materials. Our preliminary studies showed a significant difference between materials, but currently it is not clear whether this is an experimental artefact or whether certain types of stone can provide some protection and preserve the proteins better, for example, by preventing them from washing out (stronger ionic interactions) or by absorbing them more efficiently (more porous structure).

Another field for improvement is sample preparation. The protocol described here is straightforward and can be expected to yield reasonable protein recovery and efficient proteolytic digestion. However, it is adapted from protocols opti-

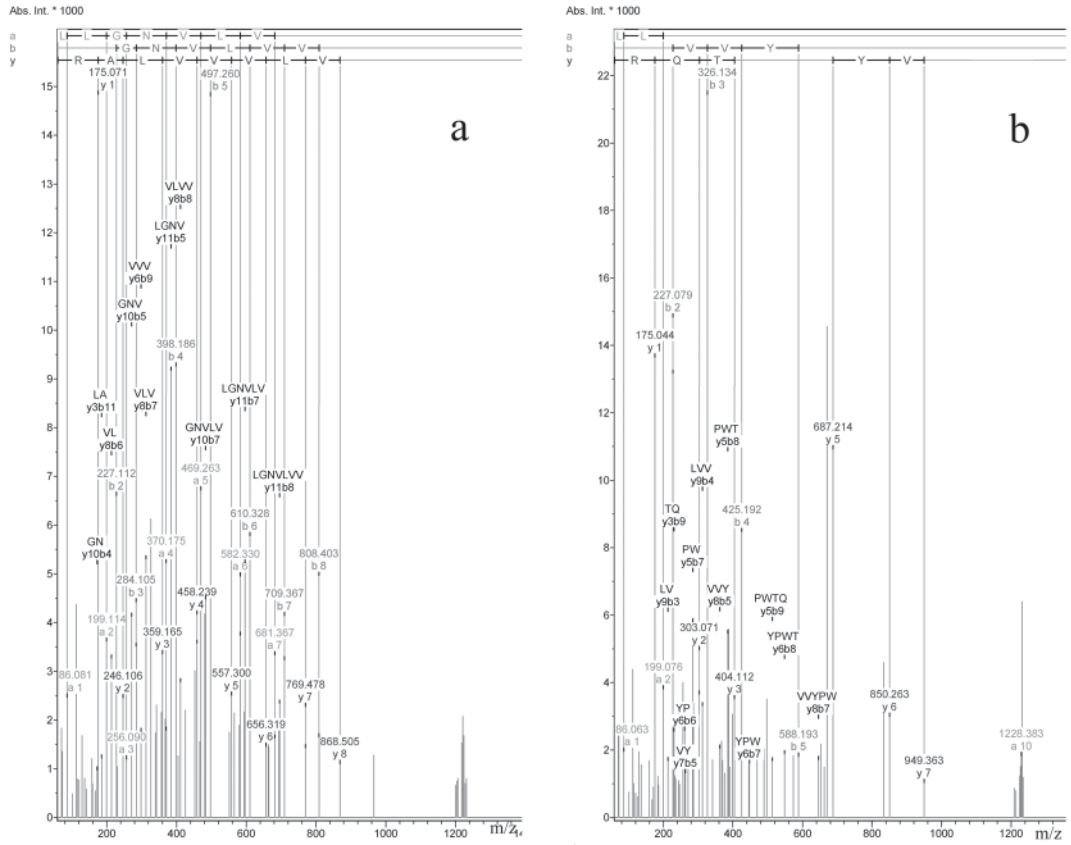


*Fig. 7. MSMS spectrum for the two major beta hemoglobin peaks from the spectrum shown in Fig. 6: a: parent ion 1265.8; b: parent ion 1274.7. The data provides sufficient sequence information to identify beta hemoglobin unambiguously, but the sequence is identical in about 50 different species (not shown).*

mized to detect proteins from electrophoresis gels and buffered solutions. Whether this is the optimal way to recover blood from a stone remains to be investigated. Another option is to use LC-MS as an additional step to maximize sample use and reduce signal suppression by the sample matrix. Such experiments will be carried out in the future with more aged control samples.

### *The meaning of the results for archaeological interpretation*

From an archaeological perspective, the absence of blood proteins might indicate either the fact that blood was sacrificed a long enough time ago for the blood proteins to degrade in sunlight and rain, or the fact that blood was never smeared on the surface of these *sieidi* stones. Because of the sacred nature of *sieidi* stones, we investigated only a selected area of the stone's surface with small sample areas. Hence it is possible that blood could have been found from other parts of the stone's surface. The results from the test stones nevertheless indicate that the protein degrading in exposed weather conditions takes place quickly in terms of an archaeological timeframe. Hence the methodology would need to be improved in order to use it for *sieidi* stones or other similar stone surfaces.

As we have seen in the case of these three *sieidi* stones from which there are marks of sacrificial practices, the absence of blood proteins does not always indicate the absence of ritual practices. As stated earlier, sacrifices took many forms and some of them might have left no marks in the archaeological material. In addition to the material sacrifices, there was a myriad of immaterial aspects in ritual practices at *sieidi*s. These included embodied experiences, emotions, feelings of time and continuity, and social relations (Salmi et al. 2011). In the course of the long history of the *sieidi* sites, the associated ritual practices may have taken many different forms, and some evidence pertaining to earlier practices may also have been cleaned away (Äikäs et al. 2009).

Our experiments in using mass spectrometry in blood residue analysis of *sieidi* sites showed that the method is potentially useful, but needs to be adjusted due to the challenging conditions caused by exposure to direct sunlight and altering weather conditions, particularly rain and snow. One must also bear in mind that the absence of blood proteins on a *sieidi* does not indicate a lack of sanctity, since the ritual practices also included many immaterial aspects.

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## NOTES

[1] We use the term ethnic religion here, since it does not carry such negative connotations as the term primitive religion. Ethnic religion is better suited than pre-Christian religion to the situation in which Christian elements and ethnic traditions lived side by side for a long time (see, e.g. Äikäs & Salmi in press). The term indigenous religion could also be used, but it can have connotations of a static religion without contacts to the outside world (Mebius 2003: 12–3). We are aware of the fact that also the term 'religion' is an etic, theoretical concept and doesn't necessary describe the Sámi worldview.

[2] Calibrated with the accuracy of 2δ using the OxCal v3.10 program, based on the calibration curve offered by Reimer et al. (2004). All datings were conducted by the Laboratory of Chronology, Finnish Museum of Natural History, University of Helsinki.

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