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Hemorrhagic fever virus, human blood, and tissues in Iron Age mortuary vessels

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ABSTRACT

This study identifies and interprets the proteins present on sherds from six ceramic mortuary vessels from a burial mound near the Heuneburg, an early Iron Age (750–400 BCE) hillfort in southwest Germany, using a novel adaptation of proteomic analysis that identified 166 proteins with high confidence. Surprisingly, among the identified proteins were peptides from Crimean-Congo hemorrhagic fever virus (CCHFV), a pathogen previously unknown in this geographic region and time period, as well as peptides from human blood and tissues. These results highlight the first example of a viral cause of death of at least one high-status individual from the Iron Age west-central Europe and provide the first archaeological evidence for the interment of human organs in mortuary vessels in the region. We also demonstrate the suitability and value of a proteomics approach for discovery-based residue analysis of archaeological ceramic vessels and reveal how identification of adsorbed proteins can provide insight into prehistoric mortuary practices.

1. Introduction

The Heuneburg in the German state of Baden-Württemberg is one of the most extensively excavated early Iron Age hillfort settlements in Europe (Arnold, 2010; Arnold et al., 2003, 2001, 2000; Fernández-Götz and Krausse, 2012) (Fig. 1). The discovery of white-washed walls of sun-dried mudbrick—a feature typical of Mediterranean architecture—and imported ceramics demonstrate the site's importance in the early Iron Age. In addition to data from the settlement, the associated burial monuments provide information about the inhabitants of this promontory above the upper Danube River and hinterland.

The mounds closest to the hillfort were explored in the late 19th century, the 1930s, and the 1950s, yielding numerous burials containing gold ornaments, metal drinking vessels, and other grave goods (Arnold, 2010). The Iron Age agro-pastoral society in this region had a hierarchical social structure based on complex kin relationships in which some individuals acquired elite status as the result of both inheritance and personal achievement (Arnold and Murray, 2017; Fernández-Götz and Krausse, 2012; Krausse et al., 2016; Kurz and Schiek, 2002). The production and consumption of alcoholic beverages, symbolized by feasting equipment found in burials as well as settlement contexts, was one of the ways elites established and maintained socio-political alliances (Arnold, 1999).

In order to understand the life history of the Heuneburg as reflected in its mortuary monuments, excavations in 1999 and 2002 conducted by archaeologists from the University of Wisconsin-Milwaukee, the University of Mississippi, and the State Monuments Office of Baden-Württemburg/Tübingen focused on Tumulus 17 (T17) and Tumulus 18 (T18) in the Speckhau group, the first mounds under 50 meters in diameter to be systematically

Abbreviations: 1D, 1 dimensional gel electrophoresis; ABC, ammonium bicarbonate; AcN, acetonitrile; CCHFV, Crimean Congo Hemorrhagic Fever Virus; CHAPS, 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate; DI, de-ionized water; DTT, dithiothreitol; FDR, false discovery rate; IAc, iodoacetamide; ITMS, ion-trap mass spectrometry; LC-MS/MS, liquid chromatography tandem mass spectrometry; m/z, mass-to-charge; MWCO, molecular weight cut-off; RP-HPLC, reversed-phase high performance liquid chromatography; T17, Tumulus 17; T18, Tumulus 18; TCEP, tris2-carboxyethyl phosphine; TFA, trifluoroacetic acid.

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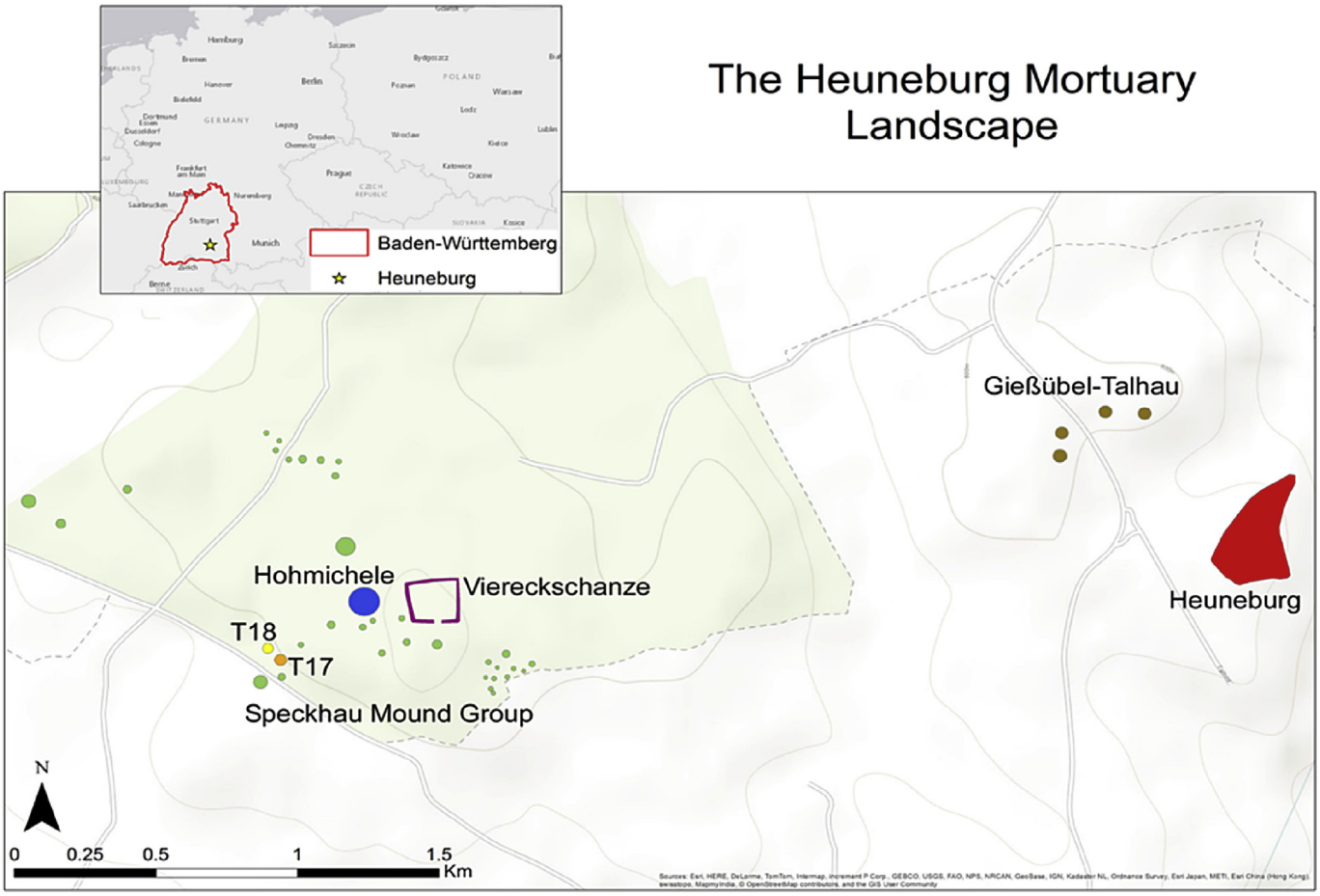

Fig. 1. Map showing location of Landscape of Ancestors project in southwest Germany and within the Heuneburg area. (Image produced by K. Garstki and provided by B. Arnold).

excavated. Only 15–20 meters apart, they were each about 20 meters in diameter and originally about three to four meters high. The central chamber of T17 was 5 × 5 meters in size, one of the largest for this time period. The radiocarbon dates and grave goods indicate a use life of 150 years, from about 600–450 BCE (Arnold, 2010) (during Hallstatt D, Table 1). A subset of the ceramic vessels recovered by the "Landscape of Ancestors" project in 1999 and 2000 associated with the central interment of T17 were analyzed, as part of exploratory data gathering, by proteomic techniques at the University of Texas Medical Branch in Galveston. The results of this analysis provide new and exciting information about the possible contents of vessels included in a high-status burial in the Heuneburg mortuary landscape as well as one of the individuals inhumed there.

1.1. Early Iron Age mortuary ritual in southwest Germany

Dominant mortuary ritual changed over the period in which T17 and T18 were in use. Cremation was common in the early Iron Age Hallstatt C period (800–650 BCE), followed by a period in which cremation and inhumation were practiced. Inhumation became the dominant rite in the subsequent Hallstatt D1 through La Tène A period (600–400 BCE) (Table 1). Entomological evidence suggests that the bodies of some of the elites were prepared for display, possibly while being transported to the burial site and/or exhibited during a regional funeral procession (Stegmaier, 2009, 2008; Stegmaier and Amendt, 2012, 2010).

The contents of ceramic vessels associated with burials have not been systematically studied despite being more numerous than metal vessels. Pollen residue analysis of metal vessels has revealed evidence of alcoholic beverages, primarily honey mead, so the assumption was that ceramic vessels also contained alcoholic beverages (Moe and Oeggl, 2014; Rösch, 2005, 1999). The most common alcoholic beverages were assumed to have been honey- or grain-based, as demonstrated by the recovery of a bronze cauldron containing mead in a secondary burial in T17 (Rösch, 2017). This is the first analysis of ceramic remains from the T17 central cremation which tests this assumption.

Table 1
West-central European Iron Age chronology.

Hallstatt A/B	Urnfield Period (1200–800 BCE)
Hallstatt C	Early Hallstatt Period (800–650 BCE)
Hallstatt D	Late Hallstatt Period (650–450 BCE)
La Tène A/B	Early La Tène (450–275 BCE)
La Tène C	Middle La Tène (275–150 BCE)
La Tène D	Late La Tène (150–25/15 BCE)

1.2. T17 proteomic analysis of potsherds

The use of proteomic technologies in archaeological and paleontological applications has only developed in the last few years. Recent studies include identification of harbor seal muscle proteins on a potsherd from Alaska dating to 1200 CE (Solazzo et al., 2008), proteins from fossilized Siberian bison bone dating to 58,000 BP