UNIVERSITÀ DEGLI STUDI DELLA TUSCIA
DIPARTIMENTO PER LA INNOVAZIONE NEI SISTEMI BIOLOGICI, AGROALIMENTARI E FORESTALI

Understanding Medieval livestock diversity in Viterbo's Tuscia using aDNA data

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Osteological samples were selected from three sealed contexts in Tuscia (Fig.1), dated to the 14th century by stratigraphic position and archaeological materials: two are waste pits in urban (Cencelle) and rural (Celleno) areas, while the third (S. Valentino) is a bovine carcass burial from a former monastic settlement, later reused for agricultural purposes.
Different bones were selected from these archaeological sites to understand the Medieval livestock diversity in Viterbo's Tuscia. The analyses were carried out in the ancient DNA (aDNA) Laboratory of DIBAF (Tuscia University).

Fig.1 Sampling sites

Materials and methods

N.	Species *	Archeological sites	Reads (M)	PMD>1
7	*Bos taurus*	Celleno - Butto 'leggendario'	40.840	40322
2	*Bos taurus*	Cencelle, Sett. II - Cisterna	25.915	5004
4	*Bos taurus*	Cencelle, Sett. II - Cisterna	0.025	77
6	*Bos taurus*	Cencelle, Sett. XI	8.573	162
8	*Bos taurus*	Soriano - S. Valentino	15.636	14902
28	*Ovis vel Capra*	Celleno - Butto 'leggendario'	21.699	692763
25	*Ovis vel Capra*	Celleno - Butto 'leggendario'	24.877	5501
26	*Ovis vel Capra*	Celleno - Butto 'leggendario'	41.766	37768
22	*Ovis vel Capra*	Cencelle, Sett. II - Cisterna	0.461	17660
24	*Ovis vel Capra*	Cencelle, Sett. XI	14.469	3050
9	*Sus domesticus*	Cencelle, Sett. II - Cisterna	0.002	1
12	*Sus domesticus*	Cencelle, Sett. II - Cisterna	0.000	0
13	*Sus domesticus*	Cencelle, Sett. X	0.001	0
14	*Sus domesticus*	Cencelle, Sett. XI	0.601	41

Tab. 1 Samples, archaeological sites, coverage and *post mortem* damage (PMD)
* Colours refer to archaeozoological identification

DNA was extracted and quantified, genomic libraries were prepared and sequenced at a coverage of 1.5 Gb per sample (10Mreads PE 150bp). A metagenomic analysis was carried out (Fig.2) for an early identification of the species: reads were aligned with many livestock species reference sequences. After that, each sample was aligned to the specific reference sequences. Further analyses were carried out to evaluate the damage pattern typical for aDNA (PMD), and thus exclude contamination of exogenous DNA, for example during the excavations or laboratory practices (Tab.1). Subsequent analyses were aimed at determining the sex of the animals to better understand the exploitation mode of the period. Mitochondrial DNA (mtDNA) is usually less difficult to amplify than nuclear DNA, so variants on mtDNA and on Cytochrome B (CytB) were investigated to establish the haplogroup and thus the origin of the samples.

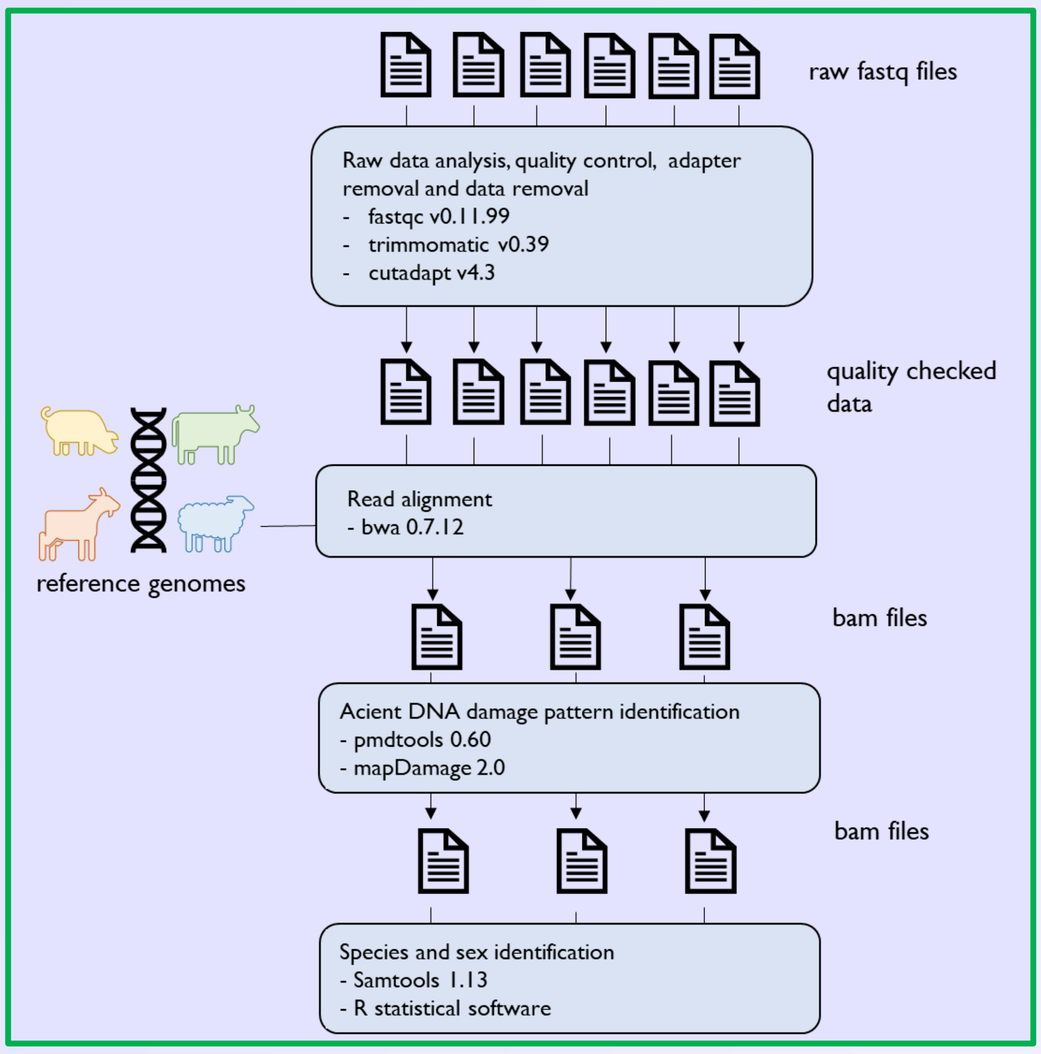


Fig. 2 Analysis workflow

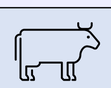
Results and discussion

Sus scrofa findings were inconclusive because they were degraded by the practice of double boiling, providing indirect confirmation of the species.

Species identification
Reads were aligned to the reference genome for the identification of the species of interest, to validate the morphometric analysis of the bones (Tab.2). In the specific case of the sheep and goat finds, the reads were aligned to both the *Ovis aries* and *Capra hircus* genomes. Four sheep and one goat were identified, while for cattle the identification of the morphometric analysis was confirmed.

Sex determination
Three out of five *Bos taurus* finds had adequate coverage and were found to be male; the *Ovis aries* finds were both male and female; the only *Capra hircus* find was found to be female.

Mitochondrial DNA (mtDNA)
It was possible to establish the haplogroup of only one sample of *Ovis aries*, which was found to belong to the HB haplogroup, the most common in Europe.

Analysis				
SPECIES IDENTIFICATION	4/5	1/5	3/5	0/4
SEX DETERMINATION	3♀ 2♂	♀	3♂	-
mtDNA	HB Haplogroup	-	-	-

Tab. 2 Results for each species

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