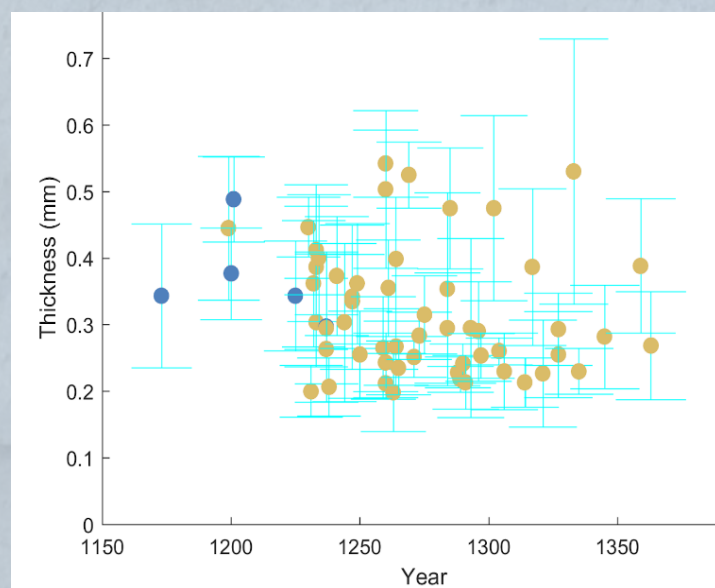


# Counting Sheep: Proteomic Analysis (eZooMS) of Legal Documents from Medieval East Anglia

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## INTRODUCTION

This project uses peptide fingerprinting (eZooMS) to analyze 123 parchment samples from 12<sup>th</sup>-14<sup>th</sup> century legal documents held in the Vanneck and Ely DC collections (Cam. UL). These manuscripts (mss) do not preserve text alone; they also record the lives of the animals whose skins supported the administrative scaffolding of medieval society. Understanding species choice can help us better map out the evolution of scribal practice in East Anglia. Due to its affordability and fragile surface, sheepskin parchment became standard for Western European legal documents by the late middle ages. However, this was not always the case:



**Fig. 1:** 13<sup>th</sup> century transition from mixed sheep & calf to exclusively sheep at Orval, Belgium

- blue = calf
- orange = sheep

From Ruffini-Ronzani et al 2021

**Q: does a similar transition occur at East Anglian sites?**



Adapted from "The World Factbook" (CIA)

## METHODOLOGY

### eZooMS

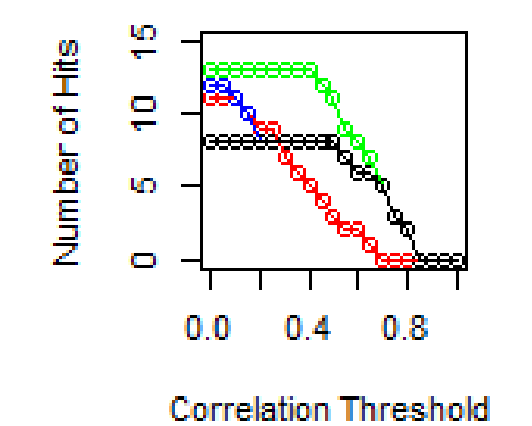
- Assembly of candidate mss; conservation assessments
- Photography and description
- Triboelectric collagen extraction
- Trypsin digestion
- Peptide extraction; spotting
- Analysis by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS)

### DATA ANALYSIS

Using **bacollite** to align theoretical reference peak positions with MALDI-TOF data:

- Generation of "theoretical" marker peptide masses
- Cross-correlation of MS and theoretical peaks
- Score assignment for each classification
- **Yields proposed species ID + confidence score**

Score for species 1 (Sheep) = 14.000000  
Score for species 2 (Cow) = 0.000000  
Score for species 3 (Goat) = 0.000000  
Score for species 4 (Deer) = 0.000000



## DISCUSSION

Data analysis is ongoing, but preliminary results suggest that the earliest (12<sup>th</sup> century) manuscripts from these East Anglian sites are all sheepskin, a result which differs from the Orval set. Continued data analysis will reveal whether this trend holds true throughout the sequence (up until the 14<sup>th</sup> century) and whether the seal tags and recycled parchment samples exhibit the same homogeneity.

Possible avenues of further work with these documents:

- Investigation of MALDI peak clustering as an indicator of collagen degradation
- How does the surface preparation of the parchment affect its fragility? Other methods (e.g., Raman, FTIR) could investigate possible presence of chalk + binder
- Paired studies involving attached beeswax seals

**Acknowledgements:** For their advice and expertise, Elizabeth Stazicker, Jiří Vnouček, Sarah Fiddymont, Anna Johnson, & Katrina Dean

**Bacollite** by Hickinbotham et al 2020, <https://t.co/13DKasOLQe>

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