DETECTION OF TETRACYCLINE IN NEW KINGDOM NUBIAN REMAINS FROM TOMBOS: Beer or Microbes?

Abagail Breidenstein[1], Michele Buzon[2], and Maureen Devlin[1]

[1]Department of Anthropology, University of Michigan, Ann Arbor, Michigan

[2]Department of Anthropology, Purdue University, West Lafayette, Indiana

INTRODUCTION

- Tetracycline is a broad-spectrum antibiotic produced by bacteria of the genus *Streptomyces* (Madigan, 2006); chelates calcium > labels bone growth
- Previously detected in archaeological bone sections, possibly from contaminated grains used for beer & bread
  - Found Nubians and Egyptians from Roman Period to Christian Era (Basset, 1980, Farbits, 2008, Hummert & Van Gerven, 1982, Margolis et al, 2013)
- Source: airborne yeast or leftover malted grains > cooking or fermentation process stresses bacteria > produces tetracycline
- Positive detection contested as contamination from ubiquitous soil bacteria (Pipenbrink 1986, Keith & Armelagos et al, 1988)
- Contamination and diagenesis are true concerns; should be carefully investigated

Project Purpose:

- **Detect presence of tetracycline in Nubian skeletal remains from New Kingdom era Tombos using fragmentary femora and previously proven methods**

Consumption of a broad-spectrum antibiotic has implications for health and disease status in ancient populations

MATERIALS

- Sample excavated from the Nubian archaeological site of Tombos, in modern day Sudan, (Fig. 1)
- New Kingdom colonial town (1,550 – 1,050 BCE), occupied through 3rd Int. and Napatan Periods; Egyptian imperialism
- Sample of commingled adult femura; most burials looted in antiquity; Nubians and Egyptians likely
- Individuals most likely belonged to the middle class, ie. administrators or high class artisans > skeletal indicators of relatively low activity levels found in intact burials (Schrader, 2012)

Fig 1: Map of Ancient Nile Valley

- All femura demonstrate various degrees of taphonomic damage and fractures; fragmentary
- 16 individuals were selected on the ability to section the femur diaphysis at an estimated mid-shaft

METHODS

To determine mid-shaft, remains were measured using standard femur landmarks (Steele 1969, Buikstra, Ubelaker 1994). Regression formulae used to determine maximum femur length range to estimate male, female, or indeterminate sex (Bidmos, 2008, 2009). Maximum femur length is the best estimator for sex determination in Tombos Nubian remains (Table 1):

Table 1: Determination of sex based on Maximum Femur Length

Females	**< 410 mm**
Indeterminate	**410 - 440 mm**
Males	**> 440 mm**

10cm piece sampled from each individual, 5cm flanking mid-shaft. Samples were embedded in epoxy for thin sectioning. Samples were sectioned at 1mm slices across entire length for comparison of remodeling throughout 10cm sample. Sections were mounted with epoxy on slides, then ground and polished to ~100µm with an electric grinder. Tetracycline labeled osteons were viewed under ultraviolet light at 490nm with a Nikon Eclipse Ni-U Microscope.

RESULTS

To positively detect the presence of tetracycline, we hypothesize the chemical will be incorporated at mid-shaft where the most bone turnover occurs in adult long bones due to max mechanical loading (Fig. 2).

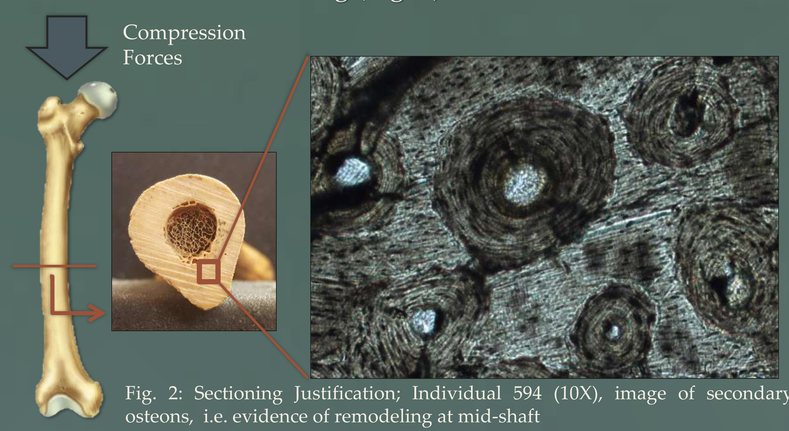

Fig. 2: Sectioning Justification; Individual 594 (10X), image of secondary osteons, i.e. evidence of remodeling at mid-shaft

- Eight individuals were assigned sex; other eight were too fragmentary (Table 2):

  3 Male, 3 Female, 1 Indeterminate (8 unknown)

- **14 individuals showed likely evidence of tetracycline-labeled osteons** (Table 2)

Table 2: Sample List with Results

Individual No.	Estimated Max. Femur Length◊	Estimated Sex	Tetracycline Presence❖
641-122	*	-	Yes
641-123	448.61 mm	Male	Yes
641-132	439.67 mm	Indeterminate	Yes - Extensive
641-183	*	-	Yes
641-216	406.77 mm	Female	Yes
641-219	445.00 mm	Male	No
641-228	420.06 mm	Indeterminate	Yes
641-349	*	-	Yes - Moderate
641-350	399.02 mm	Female	Yes - Moderate
641-354	*	-	No
641-399	408.27 mm	Female	Yes
641-400	*	-	Yes
641-412	447.59 mm	Male	Yes - Moderate
641-594	*	-	Yes - Moderate
641-743	*	-	Yes
641-800	*	-	Yes

◊ Maximum Femur Length determined by Bidmos (2008, 2009)

* Measurements incomplete, mid-shaft estimation with help from Dr. Kathleen Alsup

❖ Presence of tetracycline scored as positive if any **intact osteons** were fluorescent

IMAGES

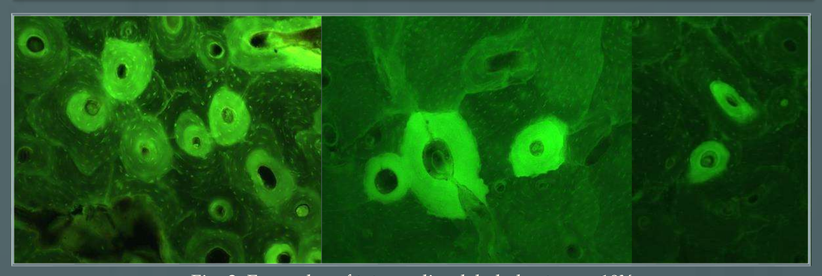

Fig. 3: Examples of tetracycline-labeled osteons, 10X

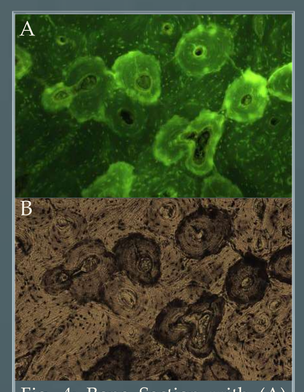

Fig. 4: Bone Section with (A) and without (B) UV light viewing, 10X

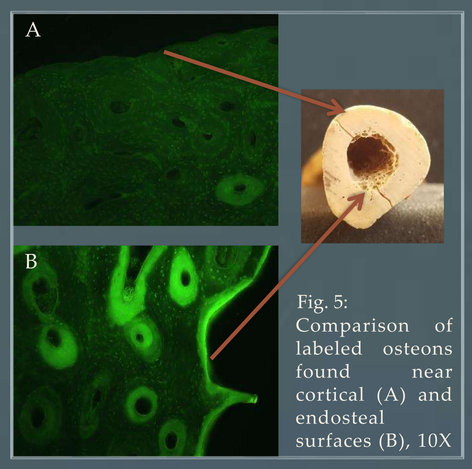

Fig. 5: Comparison of labeled osteons found near cortical (A) and endosteal surfaces (B), 10X

DISCUSSION & CONCLUSIONS

- Analysis shows **likely evidence for the presence of tetracycline** in the diets of people living in Tombos over 3000 years ago
- Consumption of a broad spectrum antibiotic has implications in discerning health profiles gathered from the bioarchaeological record
- Expect to see **decrease in disease prevalence and/or severity** > tetracycline is an effective therapeutic agent against various bacterial infections
- Commingled sample obscures any correlations between disease rates, severity, and progression; lacking context
- Despite small sample size, equal distribution of sex (3 males, 3 females), suggests **no difference** in the presence of the antibiotic
- All samples were adult, unable to make conclusions about age differences and presence of tetracycline
- Despite signals, still **very possible it is diagenesis** (microbial and chemical degradation of bone tissue)
  - Canaliculi, Canals of Volkmann, Haversian canals = means of egress into tissue when buried (see images below)
  - Microorganisms could be responsible for labeling osteons, burial context would have been aerobic

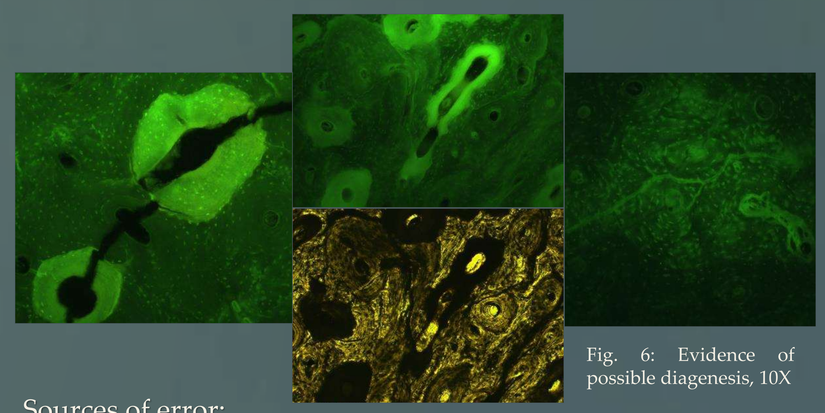

Fig. 6: Evidence of possible diagenesis, 10X

Sources of error:

- Sectioning methods – bad adherence = fluorescence noise, thick sections obscured true fluorescence
- Small sample size (N=16), no negative control
- Commingled remains lack useful context

Further Directions:

- Extraction of tetracycline from bone samples, test efficacy
- Test associated soil samples (or available proxy for similar site) for tetracycline

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