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IDENTIFYING BLOOD PROTEIN IN LOUISIANA CLAY SEDIMENT

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Arts

In

The Department of Geography
and Anthropology

by
Michelle D. Whipp
B.A., Louisiana State University, 2009
May 2012

ACKNOWLEDGEMENTS

First and foremost I thank my advisor, Dr. Rebecca Saunders, for whose ideas, support, plane tickets, and constant tinkering, allowed me to produce this. Thank you, Dr. Linda Scott Cummings and PaleoResearch Institute, for hosting, supporting, and teaching me. Thank you also to Dr. David C. Weindorf and the Soils Department at LSU for all the time, equipment and knowledge. Also, my committee deserves a huge thank you to Ms. Mary Manhein and Dr. Ginesse Listi.

Special thanks go to my peers, for I would be lost and crazy without the hours of thought-provoking discussion and constant support from the best graduate students in the world. In no particular order: Mark, Sophia, Stephanie, Lauren, Nicki, Mikeal, Amanda and Teresa, thank you for all the little things. There are of course others, but they know who they are.

Thanks to my family, those who are still with us and those who are not; Grandpa, Grandma, Mom, Dad, and too many to name but without each of whom, I would not be where I am today.

Thank you to the marvelous people at Ben Hur Farm, especially Mr. Mike.

I gratefully acknowledge receipt of funding from the Robert C. West/R.J. Russell Field Research Award as well as the LSU Department of Geography and Anthropology Research Materials Award.

Finally, friends not academically related, but just as important to me, Larissa and Family, and someone who sacrificed every moment I did, Bibi (Raed), deserves more thanks and credit than I can give.

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ABSTRACT

In modern forensics, soil from crime scenes is not tested for the presence of blood proteins. This is due to the belief that proteins degrade too quickly for detection. The purpose of this paper is to report on the research performed in an effort to counter that belief and in so doing, suggests changes in the current procedures.

Using pig blood as an analog for human blood, blood was deposited outside, at two separate locations roughly a half mile apart, at a southern Louisiana cattle farm. Over 18 months, samples were taken from cores at the deposition sites and these were tested for the presence of blood proteins at PaleoResearch Institute in Golden, Colorado. Crossover-Immunoelectrophoresis (CIEP) was used to test over 130 soil samples taken at three-month intervals.

Results indicate further research is warranted. The control samples tested negative, while intermittent weak positive results for blood proteins from the samples were obtained. The lack of interpretable patterning in the positive results, however, renders my results inconclusive. Further testing with more controlled variables is required.

CHAPTER 1. INTRODUCTION

The investigation of murder on a large scale, such as in war-torn countries, involves the collaboration of many forensic professionals including anthropologists, chemists, botanists, and psychologists. Forensic anthropology plays a particularly important role in such investigations, for instance, in helping to determine the biological profile of the victims of war crimes in mass graves. As a subfield of anthropology, archaeology also plays a crucial role in crime scene investigation. Forensic archaeology is vital in determining the contexts of a buried scene; careful excavation at a crime scene also allows for the establishment of a time line.

Forensic determinations are made more difficult when scenes have been purposefully cleansed. Tuller (2001) and Tuller and Saunders (2011), reacting to the cleansing of massacre sites in the former Yugoslavia, wondered whether blood proteins might remain in the soils to provide evidence where bodies and weaponry were removed. An execution site in Kosovo provided an excellent laboratory for such a study. Although there were no bodies at the scene, eye witness accounts, bullets and bullet casings, and bone and hair fragments were present. Tuller took soil samples in 1999, 1.5 years after bloodshed occurred, and analyzed the soil using cross-over immunoelectrophoresis (CIEP). The tests showed positive results for the presence of human blood protein. These findings were the springboard for my own research, in which I followed up on Tuller and Saunders' methods with a more controlled investigative procedure, detailed in the methods section below.

Using pig blood (from the Family Suidae) as an analog for human blood, I determined whether or not pig blood proteins are preserved in soil over a period of 18 months. My hypothesis is that pig blood proteins will remain in the soil, though there will be some movement, both vertically and horizontally, of blood proteins through the soil column due to

leaching or bioturbation. In contrast, the null hypothesis states that blood proteins will not be found, that biodegradation and other interacting forces will have destroyed blood proteins very quickly.

As discussed in more detail below, a number of researchers have been able to extract blood proteins from the surface of ancient stone tools, among other materials, indicating that blood proteins can last over thousands of years. Forensic scientists, however, generally discount the idea that blood proteins are preserved in soil for any length of time (as discussed in Tuller 2001). While discussions about the possibilities of protein survival have circulated in both forensic and archaeology communities, no body of literature exists to support these beliefs (as per my discussions with persons from both professions; see also Malainey 2011: 219). In particular, formal analyses of the decomposition and dissipation rates of blood proteins in soil have not been conducted. In this study, I will examine degradation and dissipation of blood proteins in Louisiana clay soil in two different environments; sunny pasture and shaded wooded, over a set period of time. Soil samples were collected from both environments every three months for 18 months and tested, using CIEP, for the presence of pig blood protein. The field situation is a proxy for an execution site where bodies are not present, but eye witness accounts or other evidence indicates that a crime has occurred.

Although my results were primarily negative, what I learned in terms of controls, environmental variables, handling, and processing of samples can contribute to a protocol for the collection, testing, and documentation of blood protein presence at execution sites. This study is important because there are no other controlled studies of the rate of blood protein degradation/dispersion (results on degradation and dispersion, of course, will be unique to the specific soil and environment in which this study was carried out).

CHAPTER 2. REVIEW OF LITERATURE

2.1 Background

Currently, forensic investigators do not test for blood residues in soils when the samples are believed to be more than a few months old. This is based on the assumption that degradation of blood proteins by microorganisms would preclude useful results (Tuller 2001). As noted, except for Tuller and Saunders (2011), a review of published literature reveals a complete lack of forensic research on the viability of blood residues in soils. Evidence exists, however, that blood protein residues survive for extended periods of time on artifacts such as stone tools and other archaeological materials in soils in varying environments (Allen et al. 1995; Borja et al. 1997; Culliford 1964; Downs and Lowenstein 1995; Gurfinkel and Franklin 1988; Hortolà 2002; Jahren et al. 1997; Kooyman et al. 1992, 2001; Loy 1983; Loy and Dixon 1998; Loy and Hardy 1992; Loy and Wood 1989; Loy et al. 1990; Newman and Julig 1989; Newman et al. 1996; Nolin et al. 1994; Petraglia et al. 1996; Tuller 2001; Vass et al. 1992; Yohe et al. 1991; Zimmerman 1973). Instructions from laboratories engaged in such analyses suggest collection of in-situ soils surrounding artifacts for further analysis to support findings on corresponding artifacts (PaleoResearch Institute Collection Manual 2006). This request indicates a belief that information can be ascertained from soils themselves and not just the artifacts. Such instructions notwithstanding, literature on protein analysis of soil in the absence of artifacts is scarce. With substantial archaeological evidence supporting the survival of blood proteins over hundreds to thousands of years, it stands to reason that the same techniques used in archaeological tests could be applied in a forensic context to identify the presence of modern blood residue.

In preparation for my research, I have reviewed the literature on protein analysis, and I have studied the various methods used to detect blood proteins. In so doing, I established that

CIEP is the best testing method for my particular research. The following discussion addresses the types of testing methods that have been utilized for identifying the presence of blood residues and my justification for selecting CIEP for this research.

2.2 Testing Methods

A number of testing methods exist for the determination of the presence of blood and its origin, whether human or non-human, on varying surfaces. These include various screening techniques, hemoglobin crystallization, and immunological tests.

2.2.1 Identifiable Blood Residue

The first type, screening techniques, tests for blood residue and includes simple procedures such as microscopy and colorimetric (analysis of color and shape of crystals that the residue produces) methods. Though these techniques have been used successfully on archaeological material (Loy 1983; Loy and Dixon 1998; Loy and Hardy 1992; Loy and Wood 1989; Loy et al. 1990; Hortolà 2002), they have limitations on species identification and have a likelihood of false positives with soil (Malainey 2011). Therefore, these techniques will not be used for this research.

2.2.2 Blood Protein

Testing methods for blood protein include crystallization of hemoglobin and immunological tests. Crystallization of hemoglobin identifies blood proteins to the species level, while immunological tests are origin-specific to the taxonomic level of family (Loy 1983; Loy and Wood 1989; Loy and Hardy 1992; Loy and Dixon 1998). The hemoglobin (Hb) crystallization method has met with a great deal of criticism for its lack of reliability (Hyland et al. 1990; Smith and Wilson 1992; Downs and Lowenstein 1995; Tuross et al. 1996). Critics include Smith and Wilson (1992:237), who suggested that proteins do not "survive the passage

of time in a homogenous and unchanged state that allows crystals to form." They also point out that crystals form imperfectly, making them quite difficult to identify to species. Hyland et al. (1990:106) also reported that the crystallization observed by Loy (1983) was possibly from "environmental contaminants such as salt crystals" and not hemoglobin. In addition, the technique is not necessary for my research because identification to the level of species is not required to rule out human versus faunal blood origins.

2.2.3 Immunological Tests

Immunological tests have been successful in the identification of blood residues in medical and archaeological contexts and have been used continuously throughout the twentieth century (Lee and Deforest 1976; Sensabaugh et al. 1971). Immunological tests use antiserum (human or animal serum containing antibodies developed to aid immunity) to test for the presence of antibodies. When a corresponding antiserum interacts with antibodies, a reaction occurs which is slightly different for each type of immunological test. Malainey (2011) summarized the many different immunological tests, reporting on the accuracy and effectiveness of the Ouchterlony method (OHC), Cross-over Immunoelectrophoresis (CIEP), and radioimmunoassay (RIA). These three types of immunological testing have been evaluated with varying results (Downs and Lowenstein 1995). For example, Downs and Lowenstein (1995:13) ran a blind study of 30 residue samples from projectile points from museums as well as from recently excavated sites. The samples were drawn from "darkened soils and cobbles from subsurface features, a dried mummy skin, and contents of a ceremonial shell" to test the sensitivity and discriminatory ability of each of the three immunological methods in blood protein recovery (Downs and Lowenstein 1995:14). The "sensitivity" of a test indicates the intensity of a signal needed to read positive, whereas the discriminatory ability is the ability of

the test to rule out possible false positives. Having a balance between these two factors is important for test reliability. Their results indicated that OHC had "no clearly positive reactions" due to its low sensitivity (1995:15). RIA had a "high sensitivity and high discriminatory ability reporting only a few positive results" in their study (1995:15). Finally, CIEP had a high sensitivity but low discriminatory ability, producing twice as many positive results as RIA, some of which may have been false positives. However, as detailed below, recent advances in CIEP methodologies, and the lower cost than RIA, have made this method more promising for forensic research and especially for this study.

The immunoassay tests described above are capable of detecting multiple antibodies in each test. Other immunoassay tests that are also able to detect multiple species (Loy 1983; Loy and Wood 1989; Loy and Hardy 1992; Loy and Dixon 1998) include: Enzyme-linked immunosorbent assay (ELISA) Gold Immuno Assay (GIA), GIA with silver enhancement, and the sandwich ELISA test. I am interested in determining the presence or absence of only one known, single species, thus CIEP has been chosen as the test to be used for this study.

2.2.4 Cross-over Immunoelectrophoresis

Cross-over Immunoelectrophoresis or CIEP, is a gel electrophoresis test. In gel electrophoresis, the antiserum and the sample serum are placed in wells created in a gel matrix. If the two sera are from the same taxon, a precipitate is formed between the two when an electrical current is run through the gel. Further explanation and information on the modern controls and protocols of CIEP are addressed in the methods section following. CIEP was first used in a forensic context in 1964 by Culliford and it has been applied extensively to archaeological materials and residues since 1989 (Allen et. al 1995; Culliford 1964; Newman

and Julig 1989; Downs and Lowenstein 1995; Kooyman et al. 1992, 2001; Newman et al. 1996; Petraglia et al. 1996; Yohe et al. 1991).

This method has incurred criticism in the past for either providing too many false positives or for having no positive results at all. The criticism has had a salutary effect, forcing adjustments and improvements to be made in sample preparation and other procedures (Malainey 2011). According to Shanks et al. (1999, 2001, 2004, 2005), inconsistent results were based on poor extraction procedures. The controls and protocols utilized in CIEP have now been greatly improved, increasing reliability (Malainey 2011; personal communication, Dr. Linda Scott Cummings, 2011). These advances include the testing of samples with varying dilutions of antiserum to account for false positives, as well as controlling the laboratory materials that contact samples causing false negative results. In addition to the improvements in reliability, CIEP was chosen as the test to be used for this study because it is less costly than other methods (Tuller 2001), it is very sensitive (Allen et al. 1995; Downs and Lowenstein 1995), and has shown positive, replicable results in archaeological contexts (Petraglia et al. 1996). In addition, CIEP was used in the only other study that assessed blood protein from soil (Tuller 2001; Tuller and Saunders 2012).

CHAPTER 3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Location Information

The test sites were located at the Louisiana State University Ben Hur Farm in southwest Baton Rouge, Louisiana (Figure 1 and Figure 2). I selected two different locations, a low wooded area and a sunny pasture, for the placement of blood samples in order to compare results between two different soil types and environments but otherwise similar geological and geographic factors. The wooded site was located on the western-most part of Ben Hur Farm, roughly 70 meters into a forested area which had a closed canopy composed primarily of deciduous secondary growth. The pasture site was located on the southern side of a hay barn in the center of the farm, 850 meters east of the wooded site. Both of these locations were selected after surveying the available farm land and taking soil cores to establish feasibility (some locations on the farm had soils so hard that I was unable to push a corer into the ground). In addition, the sites were selected based on the current usage of

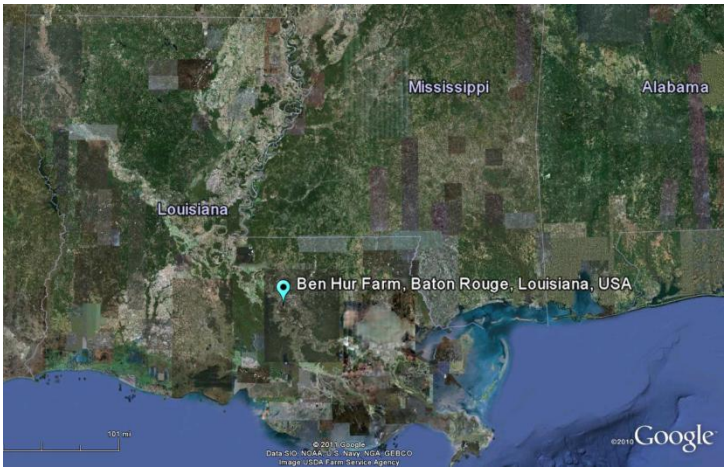

Figure 1. Ben Hur Farm, Baton Rouge, Louisiana, USA.

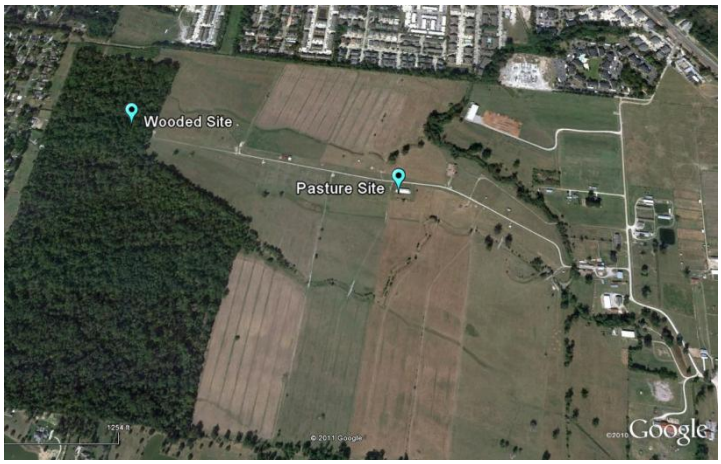

Figure 2. 1665 Ben Hur Road, Baton Rouge, Louisiana. 70820: Wooded and Pasture Sites.

Ben Hur as a working cattle ranch. All efforts were made to secure ground space that would not interfere with the activities of personnel as well as to protect the research equipment from curious cows. The altitude at both of the sites for this research was 23 feet above sea level, which is 15 feet below the city's average of 38 feet.

3.1.2 Soils

The single most major environmental variable controlled for in this study is the soil characteristics present in each area. This includes the mineralogical composition of the soils, their pH levels, permeability, and access to drainage. These factors provide the bases with which to address the test results in this research, as well as to provide data for further research in different soil types. The importance of soils information with regard to this research has to do with how the blood proteins interact with the soils. The soils located in this area and utilized in this study were specific to the region and have been detailed for future comparison studies.

The soils in the wooded area are defined as Schriever-Thibaut clays (websoilsurvey.nrcs.usda.gov). The Schriever-Thibaut soil series is comprised of very fine, primarily smectitic clay minerals, mixed with loam. As a group, they are "very deep, poorly draining, and slowly permeable soils that formed in clayey alluvium" (websoilsurvey.nrcs.usda.gov). Located east of the Mississippi River, these soils were formed in the lower parts of the Mississippi River alluvial plain during flooding and are found on flatlands with a slope of less than 1.0%. Today, these soils are now protected from major flooding of the Mississippi River by the Mississippi River levee. Due to the composition of the Schriever-Thibaut series and the weather of south Louisiana, these soils are commonly "saturated between 0 and 15 cm (0.5 feet) during the months of December through April, and moist in the subsoil layers below that" (https://soilseries.sc.egov.usda.gov/OSD_Docs/S/SCHRIEVER.html). These

attributes suggest that the Wooded Sites will likely be wetter than the Pasture Sites (although this was not the case when I took soil samples for soils analysis) and the soils may have more organic matter. Either or both of these factors could affect preservation. Otherwise, Schriever-Thibaut soils are similar in composition to Cancienne soils (found at the Pasture Site to be discussed in detail later); blood protein permeation and preservation may be similar.

In August, 2011, a soil survey was conducted by Dr. David Weindorf from the School of Plant, Environmental, and Soil Sciences at Louisiana State University Agriculture Center. Dr. Weindorf made the following observations for the soil at the wooded area: The parent material of the Schriever-Thibaut sediments is alluvium. The slope of the Wooded Site is less than 1.0%. In soil taxonomy terms, the soil in the Wooded Site is an Alfisol, which is a soil with a high aluminum and iron content. Alfisols are rich in nutrients for agriculture. Two soil horizons were accessed during the analysis, an A Horizon (the top layer) and the Bt Horizon (second layer, "t" indicates clay accumulation). The A Horizon is an Ochric Epipedon, meaning that it is a hard material of high color value and chroma, and the Bt Horizon is an argillic horizon with iron concretions. The A horizon occurs between 0-8 centimeters below surface (cmbs) with a clear boundary between it and the Bt horizon. The A Horizon is comprised of 26% clay (less than 10% sand and roughly 59% silt material); it has a silty loam texture, and a Munsell color of 10YR 4/2, Dark Grayish Brown. The overall structure is of moderate, well formed, fine (5-10 mm in diameter) peds, in a subangular blocky shape. Roots are common with diameters ranging from 1-5 mm. The pH level was moderately acidic (Table 1).

Table 1 displays the various elements of the soil from the analysis conducted by Dr. Weindorf and myself. Included is the weight of the soil sample wet, its volume, weight of soil after being oven dried, the amount of water from each sample, the calculated bulk density, the pH level of each sample, and the electrical conductivity (EC). The bulk density of a soil is an

indication of the particle sizes that make up the soil. A high soil bulk density indicates very fine particles (clay) whereas sand, which has a larger particle size, has a lower bulk density. This is important to know in order to assess how the hydraulic conductivity or water infiltration of the soil impacts the results of the tests (see below). EC (electrical conductivity) is presented as data in the table; however it was not used in the analysis.

Table 1. Soil Properties.

Soils Bulk Density Calculations								Soil Composition		
Soil Sample	Moist Soil (g)	Soil Volume* (cm3)	Oven Dry Soil (g)	Soil Water content (g/g)	Soil Bulk Density (g/cm3)	pH	EC (µS/cm)	Clay %	Silt %	Sand %
Wooded Horizon A	414.57	269.26	304.36	0.36	1.13	5.8	1797	26%	65%	<10%
Wooded Horizon Bt	506.78	269.26	399.68	0.27	1.48	3.8	688	44%	47%	<10%
Pasture Horizon A	453.15	269.26	335.85	0.35	1.25	6	1207	41%	50%	<10%
Pasture Horizon Bt	521.68	269.26	393.34	0.33	1.46	6.4	1044	50%	41%	<10%
* The soil ring is 7 cm height and 7 cm diameter										

The Bt horizon (a Bt horizon contains an accumulation of silicate clay "transported" down from upper horizons) exists from 8 cmbs to below 40 cmbs (soils were only sampled from 0-40 cmbs, so the C Horizon was not investigated). It has a 44% clay composition (less than 10% sand and roughly 46% silt material) and was also a silty loam, but this horizon is heavily mottled; Munsell colors of 10YR 5/3, Brown, and 7.5YR 4/4, Brown, predominated. Soil structure is of moderate, well formed, medium (10-20 mm in diameter) peds, in a subangular blocky shape. Again, roots are common with diameters ranging from 1-5 mm. The pH level is extremely acidic (Table 1).

Table 2. Wooded and Pasture Hydraulic Conductivity.

Wooded Hydraulic Conductivity			Pasture Hydraulic Conductivity		
Actual Infiltration Rate		Calculated Infiltration Rate	Actual Infiltration Rate		Calculated Infiltration Rate
Minutes	Inches	Inches per Hour	Minutes	Inches	Inches per Hour
1	0.9	54	1	0.6	36
2	1.3	39	2	0.78	23.4
3	1.5	30	3	0.85	17
5	1.6	19.2	5	1.15	13.8
10	1.8	18	10	1.45	14.5
15	2	8	15	1.72	6.88

Hydraulic tests were performed multiple times in August and September before and after rainfall, using an IN2-W Turf-Tec Infiltrometer provided by Dr. Weindorf. The hydraulic conductivity of the soil is an important measure for this study because it indicates general fluid penetrability. Table 2 shows that water has a high penetrability rate at shallow soil depths in the A horizons, and the rate decreases at lower depths in B horizons becoming stagnant. This would suggest likely movement of blood through the top layer of soil, then minimal to no movement after 8 cmbs. An alternative action is that the blood will become diluted in the Bt horizon due to the stagnant water in the soil.

The Pasture Site is on Cancienne silt loam soil. The Cancienne series consists of very deep, level to gently undulating, somewhat poorly drained mineral soils that are moderately to slowly permeable with slow runoff (websoilsurvey.nrcs.usda.gov). Like the Wooded Site, these soils formed in loamy and clayey alluvium. Here again, the slope of the research site is less than 1.0 percent.

Dr. Weindorf made the following observations during the soil survey: The Pasture Site is located on an old stream terrace with less than 1% slope. The parent material of the soil is alluvium. Cancienne soils are Vertisols, which are soils with a high content of expansive clay. The A Horizon at the Pasture Site is an Ochric Epipedon, and the Bt Horizon is an argillic horizon with slickensides (indicating a significant amount of swelling clay). The A Horizon is present between 0-30 (cmbs) with a clear boundary. Comprised of 41% clay (less than 10% sand

and roughly 49% silt material), it has a silty loam texture, and a Munsell color of 10YR (4/2), Dark Grayish Brown, with a mottling of 5YR (4/4), Reddish Brown. The overall structure is of weak, poorly formed, medium (10-20 mm in diameter) indistinct peds in a subangular blocky shape. Roots are common but less than 1 mm in diameters, pH level is moderately acidic at 6, and the Soil Bulk Density is 1.25 (g/cm^3) (Table 1).

The Pasture Horizon Bt exists from 30 cmbs to below 40 cmbs. It is a silty loam, comprised of 50% clay (less than 10% sand and roughly 40% silt material). The Munsell color is 10YR (5/3), Brown. There appears to be no mottling in this horizon. The soil structure is of moderate, well formed, coarse (20-50 mm in diameter) peds, in a subangular blocky shape. Once again, roots are few, with diameters less than 1 mm. The pH level is slightly acidic at 6.35, and the sediment has a Soil Bulk Density of 1.46 (g/cm^3) (Table 1). Results of the water infiltration tests performed at the Pasture Site indicate low hydraulic conductivity in both the surface (A Horizon), as well as in the subsoil (Bt Horizon) (Table 2).

The Cancienne soils, as in the Schriever-Thibaut series, are "drained; runoff is medium to slow and permeability is moderately slow." Unlike the Schriever-Thibaut series however, the saturation zone in the Cancienne soils is "perched above the clayey layers during December through April." (https://soilseries.sc.egov.usda.gov/OSD_Docs/C/CANCIENNE.html). The high saturation of these soils could affect the penetration and dispersion of blood deposited on them because of physics; greater saturation equals low permeation due to less penetrable space. Saturation might also cause blood to disperse out along the surface before penetrating due to the low permeation access, or, due to the greater density of blood to water, blood may leach further down, replacing the water.

In addition, an increase of moisture in the soil means an increase in microorganisms and, therefore, an increase in degradation of all biological material, including blood proteins (Tibbett

and Carter 2008:41). Presence and abundance of microorganisms is also dependent on temperature, which will be discussed in the climate section to follow.

3.1.3 Climate

The Ben Hur Farm is on the east bank of the Mississippi River, approximately 60 miles north of the Gulf of Mexico in southeastern Louisiana (Figure 1). This area has a sub-tropic climate, averaging a little more than 60 inches of annual precipitation, and ambient temperatures ranging between 60 to 40 degrees in January, and 91 to 72 degrees in August (Figure 3).

The Climate Center at LSU has had a weather station at Ben Hur Farm recording precipitation, air temperature, and soil temperatures since 1963. Information from this database has been recorded and applied to the analysis of the results. Soil temperatures were taken at a depth of 10 cm below surface.

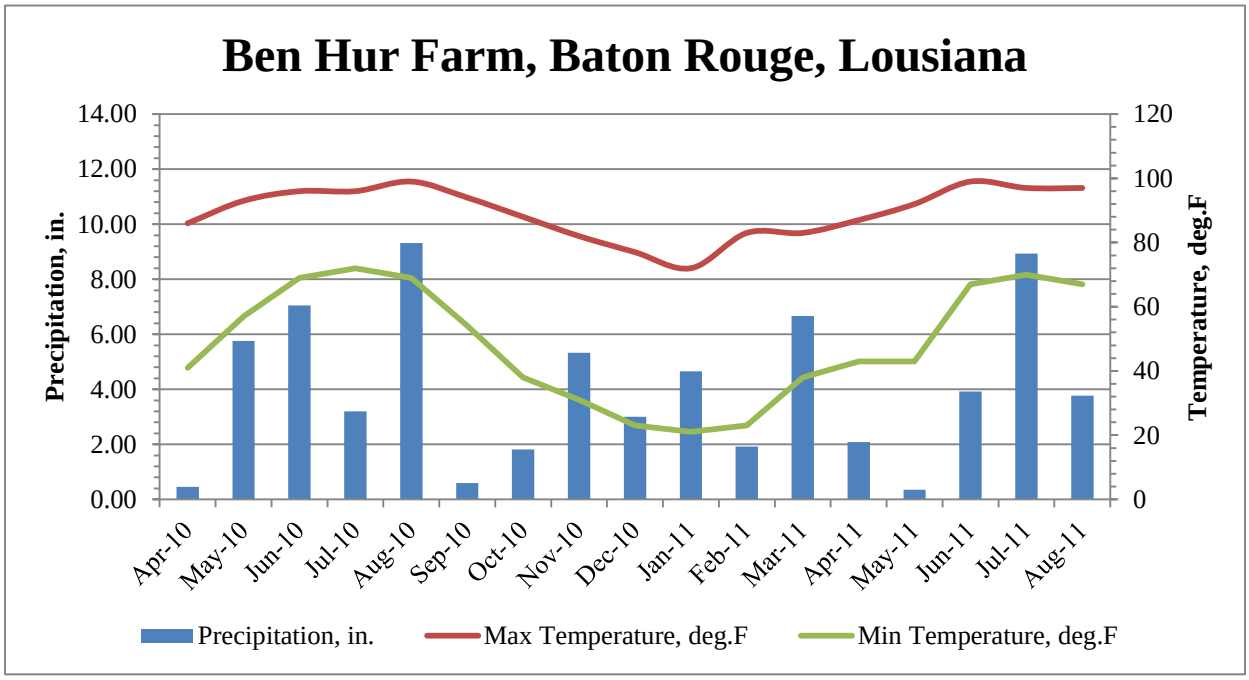

Figure 3. Ben Hur Climate, Graph Data from weather.lsuagcenter.com/reports.aspx.

3.2 Methods

3.2.1 Collection and Deposition

As noted, pig blood was used in this research as a proxy for human blood. Prior to obtaining the blood, I spoke with local funeral directors as well as to a phlebotomist about the possibility of acquiring human blood for research. They indicated that human blood procurement would require authorization from donors, and that the amount of blood I would require could not be obtained from a single source in a timely fashion without the use of an anticoagulant. Blood collected for medical purposes needs to be mixed with an anticoagulant in order to maintain its liquidity; without it, coagulation occurs, causing the blood to become a gelatinized solid. Refrigeration can slow coagulation, and I considered using it. However, recreation of a crime scene requires the smallest amount of interference with natural reactions, so anticoagulants and refrigeration were not used. These complications of blood acquisition from humans ultimately led me to the decision to use pig blood.

On April 7, 2010, four gallons of pig blood were obtained at Roucher's Meat Market & Slaughterhouse in Plaquemine, Louisiana. The collection process took approximately two hours and involved combining blood from four sows into two plastic containers. Blood began to solidify almost immediately. In order to minimize the solidification, the blood was immediately transported to Ben Hur Farm, Baton Rouge. The blood was then deposited onto the pre-selected sites as described below.

Deposition began at noon, starting in the wooded area. The temperature during deposition was approximately 80˚F with zero inches of precipitation preceding deposition on that day. To ensure that there would be enough treated area for sampling throughout the year, four discrete deposits were made in each area. On Wooded Sites A, B, and C, 0.5 L of pig blood was

Figure 4. Weed and Animal Barrier.

pushed through a geologic sieve (No. 45, 355 micrometer screen), roughly eight inches in diameter, (to remove clots and provide even distribution) onto the ground. Wooded Site D was deposited without the use of the sieve. At two sample sites (A and B), a physical, biodegradable, garden weed barrier (Figure 4) was placed on top of the blood to inhibit plant growth; on the other two (C and D), no weed barrier was used. On all sites, a round wire cage was pinned down using garden staples. This was to prevent animal disturbance. Though animal disturbance could be expected in actual execution sites, it was necessary for this study to reduce the number of variables affecting dispersion. Finally, each of the four locations at the Wooded Site was marked with flags indicating A, B, C, and D, respectively. While only sites A and B were used for coring, the presence of C and D sites was important as back up sites.

Figure 5. Clotting.

The blood deposition methods used in the Wooded Sites were duplicated for Pasture Sites A-D. In both areas, due to clotting, not all of the blood was immediately absorbed into the ground and instead remained on the surface (Figure 5). This led to concerns that blood would not filter down through the clay soil at all. This, however, also creates a likely analog for an exsanguinating body lying on the surface; in this case, too, it is probable that not all the draining blood would be readily absorbed into the ground before clotting occurred. Therefore, no attempt was made to encourage the blood to filter down (i.e., by piercing the soil, etc.).

3.2.2 Soil Sampling

Sampling dates were planned for one week and one month after deposition, and then at three-month intervals for a total of 18 months- eight sample days. In order for the methodology to be as replicable as possible, each month consisted of exactly 28 days, not a calendar month, and yet unforeseen circumstances precluded me from sticking to this exact schedule.

Experience with soils elsewhere on the farm and the relative lack of rainfall before the first sampling date led to the anticipation of very hard soil in the Pasture Sites. A preliminary test adjacent to the testing area confirmed that it would be impossible to penetrate with the corer. Thus, on April 13, one day prior to the first sampling date, approximately half of a gallon of water was poured on Pasture Sites A and B in order to soften the soil for sampling. This was not necessary at the Wooded Site as standing water was visible in the immediate area throughout the study. There was never standing water within the deposition sites themselves.

For each sample core taken, the following methods were used. A small cross cut was made into the weed barrier without removing it and with as little disruption as possible to the wire frame and surface vegetation. A ¾ inch soil corer was then inserted vertically through the cut in the weed barrier to the depth of the window on the corer (35 cm), and the corer was twisted and extracted (Figure 6). The initial goal of a 40 cm depth for every sample was not met due to the impenetrability of the soil on some sample days.

Figure 6. Pasture Core A 1.

To avoid slump and/or backfilling of the core hole after coring was complete, a ¾ inch PVC pipe, cut to slightly deeper than the core hole, was placed into the cored hole. A soda bottle cap was then placed onto the PVC pipe to prevent rain water from filling and excessively saturating the lower layers of the soil.

Table 3. Pasture Sample Cores.

Depth (Cent)	4/14/10	5/5/10	6/30/10	9/22/10	12/13/10	3/21/11	6/1/11	8/24/11
0-2		B 0-5		B 2	B 3	A 4	B 4	A 5
2-4	A 1		A 2	B 2	B 3	A 4	B 4	A 5
4-6	A 1		A 4-10	B 2	B 3	A 4	B 4	A 5
6-8				B 2	B 3	A 4	B 4	A 5
8-10		B 8-10		B 2	B 3	A 4	B 4	A 5
10-12	A 1	B 10-15	A 2	B 2	B 3	A 10-15	B 4	A 5
12-14			A 12-18	B 2	B 3		B 4	A 5
14-16		B 15-20		B 2	B 3	A 15-20	B 4	A 5
16-18				B 2	B 3		B 4	A 5
18-20	A 1		A 2	B 2	B 3		B 4	A 5
20-22		B 20-25	A 20-28	B 2	B 3		B 4	
22-24				B 2	B 3		B 4	
24-26				B 2	B 3		B 4	
26-28				B 2	B 3			
28-30	A 1		A 2	B 2	B 3			
30-32				B 2				
32-34				B 2				
34-36				B 2				

After stabilizing the core hole, the soil core was extracted onto clean plastic and photographed. On the first collection day, with the idea that only a very few samples could be run, samples were taken at pre-determined increments (Table 4; the block between 12 and 20 cm represents a portion of the core that was bagged in its entirety). Each time a core was taken, it was identified by site (Wooded vs. Pasture), location (A or B), and number of core from that location. By the second sample day, it was decided that a complete core should be collected--samples were cut into two centimeter increments and placed into labeled plastic bags. Between each coring, the corer was cleaned with distilled water, as were the knife and spoon used in cutting and moving the soil sample. Table 3 and Table 4 show the depths, location, and lengths of the samples taken.

Table 4. Wooded Sample Cores.

Depth (Cent)	4/14/10	5/5/10	6/30/10	9/22/10	12/13/10	3/21/11	6/1/11	8/24/11
0-2	A 1	B 1		B 2	B 3	A 4	B 4	B 5
2-4		B 0-5	A 2	B 2	B 3	A 4	B 4	B 5
4-6	A 1		A 4-10	B 2	B 3	A 4	B 4	B 5
6-8		B 10-15		B 2	B 3	A 4	B 4	B 5
8-10				B 2	B 3	A 4	B 4	B 5
10-12	A 1	B 10-15	A 2	B 2	B 3	A 10-15	B 4	B 5
12-14			A 12-18	B 2	B 3		B 4	B 5
14-16		B 15-20		B 2	B 3	A 15-20	B 4	B 5
16-18					B 3			B 5
18-20			A 2		B 3			
20-22	A 1	B 20-25	A 20-28		B 3			
22-24					B 3			
24-26					B 3			
26-28					B 3			
28-30	A 1		A 2		B 3			

Within 24 hours of collection, each sample was laid out in an environmentally semi-controlled room and dried at a temperature of 73˚F. The room had little direct air flow and no

sunlight. After each sample was air dried, it was returned to its bag: the bag was sealed and stored in the same temperature-controlled room. Some of the samples collected at later dates in the study were treated and stored differently, which will be addressed below. Through time, and especially once contact was made with PaleoResearch, the lab that eventually processed the samples, the methodology changed, as described in the Daily Notations in Appendix B.

3.2.3 Laboratory Testing

The CIEP test is a double-diffusion gel precipitation laboratory test which required specialized equipment and personnel. After communicating with laboratories throughout the United States, as well as professors at LSU, I was directed to the PaleoResearch Institute in Golden, Colorado. Dr. Linda Scott Cummings, Director of the Institute, agreed to assist in my research and to do the CIEP testing at a much reduced rate. Dr. Scott Cummings and the Institute lab technicians provided unequaled assistance, education, support, and generosity in processing all of my samples. Chad Yost, analyst, graciously guided me through each step of the CIEP testing and performed the reading of the results. Dr. Scott-Cummings provided the necessary materials for the testing and offered guidance throughout the entire research process.

Some sample processing was completed in Baton Rouge; all of the subsequent prep work performed on the samples was done under the direction of the laboratory specialists. In Baton Rouge, on July 15, 2011, 0.5 ml of soil from each sample was transferred into 1.5 ml polypropylene vials and mixed with 1 ml of Tris-Triton solution, which removes proteins from the soil samples. Tris-Triton solution is composed of Tris hydrochloride, which acts as a buffer to keep pH stable, sodium chloride, and Triton X-100, which is a detergent. This solution acts as a chemical disruptor to help break the hydrogen bonds binding the proteins to the soil (PaleoResearch Institute 2006). Once in solution, the samples were refrigerated for three days to

ensure complete saturation. After three days, I transported the samples to PaleoResearch Institute for analysis.

On July 18, 2011, at PaleoResearch Institute, the samples (still in the Tris-Triton chemical solution) were placed in an ultrasonic bath for five minutes because "previous studies have shown that use of physical disruptors (sound waves) result in recovery of more residual protein than just soaking in solution" (PaleoResearch Institute 2006). Thus, to ensure the best recovery of proteins, both chemical and physical disruptors were used (PaleoResearch Institute 2006). After sonicating the samples, each sample was centrifuged up to 3000 rpm but not less than 2500 rpm for 1 minute (to separate the soil components from the solution mixture within the vial).

CIEP was performed using an agarose gel plate as the medium. This medium is used as a conducting material which allows electrical current to move through it while keeping the antiserum and sample separate. Each gel plate was comprised of 15 ml of an agarose mixture made of sodium barbital, distilled water, and an agarose powder. The gel was formed on top of plastic Gel Bond Film (an agarose support medium), after which 32 cathodic and 32 anodic paired wells were created 5 mm apart to allow for a total of 32 samples per gel (Figure 7). During the electrophoresis, electric current flows through the gel and charges the wells positively and negatively causing the charged particles in the wells to react. These wells allow movement of the negatively charged protein in the cathodic wells toward the positively charged antiserum in the anodic wells. A positive reaction results in a visible line of precipitation between the cathodic and

Figure 7. Gel Plate with Cathodic and Anodic Wells.

anodic paired wells. A negative reaction has no line of precipitation. Control serums for this research were obtained directly from MP Biomedicals, LLC, the developing lab, and were used on each gel plate to provide comparison readings for the laboratory technician. The positive control was swine serum and the negative control antiserum was swine serum raised in goat to develop the antiserum. Control serums are used to validate the results of the samples being tested. For the control pairs, the positive control will have a strong line of precipitation present, whereas the negative control will have nothing. Well pairs 31 and 32 held the negative and positive control antiserum and serum, respectively, on each gel.

Using µl pipets, 0.5 µl of the sample solution from each sample vial was withdrawn and placed into the cathodic wells in the agarose gel plates and antiserum was placed in the corresponding anodic wells. Four separate batches of tests were performed with varying dilutions of antiserum: 1:3 (where one part antiserum, three parts Reverse Osmosis Deionized Water [RODI]), 1:6, 1:10, and 1:20. Testing the samples at different dilutions is performed in order to identify the strength of the positive signals as well as eliminate possible false positives. Each dilution ratio was developed using RODI and pig antiserum. Each dilution is interpreted differently. A lower dilution ratio (i.e., 1:3) creates a stronger antiserum, which is less precise and can result in false positives. A higher antiserum dilution increases specificity and reliability of positive results, but decreases the detection of trace amounts (Lab Report 11-104 from Paleo-Research Institute 2011). The industry standard for acceptance of a positive result requires a strong positive signal at the 1:20 dilution ratio.

The gel plates containing the samples and controls were then electrically charged in the Gel Electrophoresis Apparatus, in Barbital buffer (pH 8.6) for 45 minutes at a voltage of 130 to drive the antigens and antibodies towards each other (Figure 8). After removal from the electrophoresis bath equipment, each gel was rinsed with RODI water and then pressed for 10

minutes under the weight of a cinder block and large phone book to desiccate each gel. Next, each gel plate was soaked for a minimum of four hours in a salt water mixture of three liters H2O and 175.32 g NaCl. Again, the gels were rinsed RODI water and pressed for another 10 minutes. A final soak of each gel plate was performed in RODI water for a minimum of one hour, and then pressed for a final time. After the gel plates were completely dry, each one was stained blue to aid in analysis, which was done visually. Positive reactions appear as a line in the gel plates. Often UV illumination is used which causes the lines of precipitation to glow, making the positive reaction much more visible.

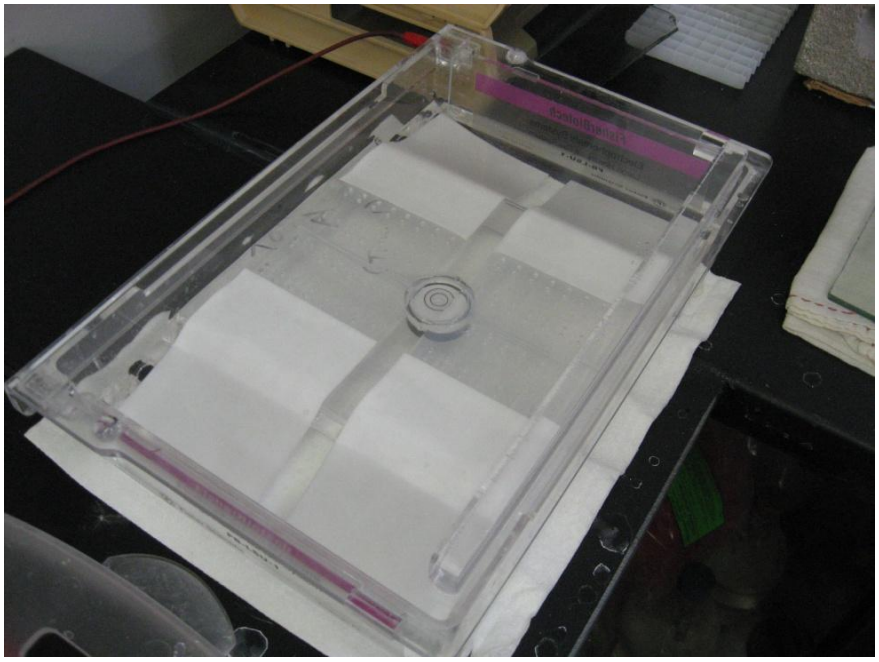

Figure 8. Electrophoresis Apparatus with Gel Plates and Electric Current.

CHAPTER 4. RESULTS

A total of 140 samples from Wooded and Pasture Sites, representing various depths, were tested for blood proteins. The results of this research are negative; that is, blood proteins were not recovered at industry accepted dilutions (1:20) (See Tables 5-12). However, blood proteins, possibly false positives, were detected at lower dilutions. These positive results were rated according to signal strength. Signal strength, defined as weak, moderate, strong and very strong, was measured visually by the laboratory technician on the basis of experience. I assigned these strengths arbitrary numeric values: inconclusive samples are annotated with "?"; samples determined to be negative were assigned the number "0." Positive results were assigned numbers"1" (weak positive), "2" (moderate positive), "3" (strong positive), and "4" (very strong positive). These numerical distinctions allow for the composition of the results in a quantitative manner. Blackened cells in the tables 4-13 indicate samples were either not taken, or were not tested. Samples from Sample Day 2 were not tested due to complications with the collection methods; therefore, no results are available (see Chapter 3). With reference to Table 7 and Table 8, the large number of blackened cells is due to a minimal number of samples being tested at a 1:6 dilution ratio. This dilution ratio was performed as an afterthought and was simply to provide further dilution ratio analysis. Therefore, not all samples were tested at the 1:6 dilution ratio.

Table 5 and Table 6 show the results of the Pasture and Wooded Sites, respectively, for the antiserum dilution of 1:3. At this dilution, all samples were negative for Sample Day 1 in both Pasture and Wooded Sites. Sample Day 3 at the Pasture Site had a weak positive on the surface (2-4 cm). Sample Day 4 from the Pasture showed a strong signal at the surface (0-2 cm), depths 8-10 cm, and 14-16 cm, but everything else was either weak or negative. Sample Day 5 showed results varying from negative (0) to very strong (4), with the samples from 12-20 cm

having the strongest signals. Sample Day 6 showed only a weak signal (1) at the surface (0-4 cm). Sample Day 7 was mostly inconclusive with one strong (3) positive at 12-14 cm deep. Sample Day 8 was also ambiguous with strong (3) positives at 4-6 cm, 10-12 cm, and 14-18 cm.

Table 5. Pasture Dilution Ratio 1:3, Characterized by Signal Strength.

Pasture	Dilution Ratio 1:3						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:3	3-1:3	4-1:3	5-1:3	6-1:3	7-1:3	8-1:3
0-2			3	3	1	?	0
2-4	0	1	0	3	1	?	0
4-6		0	0	1	0	?	3
6-8		0	0	2	0	?	1
8-10		0	3	0	0	1	?
10-12	0	0	1	3		0	3
12-14		0	1	4		3	?
14-16		0	3	4		1	3
16-18		0	1	4		1	3
18-20	0	0	0	4		1	?
20-22		0	0	1		1	
22-24		0	0	1		0	
24-26		0	0	1		0	
26-28		0		2			
28-30	0	0		3			
30-32							

For the Wooded Site Sample, again at an antiserum dilution of 1:3, Day 3 samples were negative until 16 cm below surface and then strong positive results were obtained at depths of 18-26 cm, a weak positive at 26-28 cm, and a very strong positive at 28-30 cm. Sample Day 4 showed a strong positive signal from 0-4 cm and 14-16 cm and varied with negative or weak signals in the rest of the samples. Like the Pasture samples, Sample Day 5 at the Wooded Site had positive results at every level (except 18-20 cm). Strong signals were obtained from 0-6 cm, 14-18 cm, and 22-30 cm, with weak to moderate signals at 6-14 cm and 18-22 cm. Sample Day 6 had a strong result at 6-8 cm and weak signals on either side at 4-6 cm and 8-10 cm. Sample Day 7 showed strong positive results from 6-8 cm, and weak to moderate signals from 8-16 cm. Sample Day 8 had only weak positive from 6-18 cm.

Table 6. Wooded Dilution Ratio 1:3, Characterized by Signal Strength.

Wooded	Dilution Ratio 1:3						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:3	3-1:3	4-1:3	5-1:3	6-1:3	7-1:3	8-1:3
0-2			3	3	0	0	0
2-4	0	0	3	3	0	0	0
4-6		0	0	3	1	0	0
6-8		0	1	1	3	3	1
8-10		0	0	1	1	2	1
10-12	0	0	0	1		1	1
12-14		0	1	1		1	1
14-16		0	3	3		2	1
16-18		3		3		0	1
18-20		3		0		0	0
20-22	0	3		2			
22-24		3		3			
24-26		3		3			
26-28		1		3			
28-30	0	4		3			
30-32							

Table 7. Pasture Dilution Ratio 1:6, Characterized by Signal Strength.

Pasture	Dilution Ratio 1:6						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:6	3-1:6	4-1:6	5-1:6	6-1:6	7-1:6	8-1:6
0-2							
2-4							
4-6					1		
6-8							
8-10		0	0	0	3	0	
10-12	0						
12-14		0					
14-16			0	0		0	
16-18							
18-20	0	0	3	0		0	
20-22							
22-24							
24-26			0			0	
26-28							
28-30		0		3			

Table 8. Wooded Dilution Ratio 1:6, Characterized by Signal Strength.

Wooded	Dilution Ratio 1:6						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:6	3-1:6	4-1:6	5-1:6	6-1:6	7-1:6	8-1:6
0-2							
2-4							
4-6					0		
6-8							
8-10		0	1	0	0	1	
10-12	0						
12-14							
14-16		0	0	0		0	
16-18						0	
18-20		0		1		0	
20-22	0						
22-24							
24-26							
26-28							
28-30	0	0		0			

Table 7 and Table 8 show the results of the Pasture and Wooded Sites, respectively, for the antiserum dilution of 1:6. At the Pasture Site, three strong positives were present at Sample Day 4; 18-20 cm, Sample Day 5; 28-30 cm, and Sample Day 6; 8-10 cm, with one weak signal at 4-6 cm on Sample Day 6.

The Wooded Site yielded only three weak positive signals: at Sample Day 4; 8-10 cm, Sample Day 5; 18-20 cm and Sample Day 7; 8-10 cm.

Table 9 and Table 10 show the results of the Pasture and Wooded Sites, respectively, for the antiserum dilution of 1:10. The Pasture Site samples returned only weak positives, but these

Table 9. Pasture Dilution Ratio 1:10, Characterized by Signal Strength.

Pasture	Dilution Ratio 1:10						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:10	3-1:10	4-1:10	5-1:10	6-1:10	7-1:10	8-1:10
0-2			0	0	0	0	0
2-4	0	0		0	0	0	0
4-6		0	0	0	0	0	0
6-8		1	0	0	1	0	1
8-10		0	0	0	0	0	1
10-12	1	0	0	0		0	1
12-14		0	0	0		0	1
14-16		0	0	0		0	1
16-18		0	0	0		0	1
18-20	0	1	1	0		1	0
20-22		0	0	1		0	
22-24		1		0		0	
24-26		0	0	0		0	
26-28		0		0			
28-30	0	0		0			

Table 10. Wooded Dilution Ratio 1:10, Characterized by Signal Strength.

Wooded	Dilution Ratio 1:10						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:10	3-1:10	4-1:10	5-1:10	6-1:10	7-1:10	8-1:10
0-2			0	0	0	0	0
2-4	0	0	0	0	0	0	0
4-6		0	0	0	0	0	0
6-8		0	0	0		0	0
8-10		0	0	0		0	0
10-12	0	0	0	0		1	0
12-14		0	0	0		0	0
14-16		0	0	0		0	0
16-18		0		0		0	0
18-20		0		0			
20-22	0	0		0			
22-24		1		0			
24-26		0		0			
26-28		1					
28-30	0	0		0			

were present for every sample day. These weak results were at: Sample Day 1; 10-12 cm; Sample Day 3; 6-8 cm, 18-20 cm, 22-24 cm; Sample Day 4; 18-20 cm; Sample Day 5; 20-22 cm; Sample Day 6; 6-8 cm; Sample Day 7; 18-20 cm; and Sample Day 8; 6-18 cm. The Wooded Sites also only returned weak positives. These results were at Sample Day 3; 22-24 cm, 26-28 cm, and Sample Day 7 at 10-12 cm.

Table 11 and Table 12 show the results of the Pasture and Wooded Sites, respectively, for the antiserum dilution of 1:20 over the length of the study. Pasture Site signals were weak positives at the following depths: Sample Day 3; 24-28 cm, Sample Day 4; 8-10 cm, 14-16 cm, and 24-26 cm, Sample Day 5; 12-16 cm, and 28-30 cm. Sample Day 6; 6-8 cm, and Sample Day 8; 6-8 cm, and 14-16 cm.

Finally, for the antiserum dilution at 1:20, the Wooded Site had only weak positive signal. These were visible from each sample day tested except Sample Day 8 and were located at: Sample Day 1; 28-30 cm, Sample Day 3; 10-16 cm, Sample Day 4; 2-8 cm, Sample Day 5; 12-14 cm, Sample Day 6; 6-8 cm, and Sample Day 7; 2-4 cm. (Note: Sample Day 2 was not processed.)

Table 11. Pasture Dilution Ratio 1:20, Characterized by Signal Strength.

Pasture	Dilution Ration 1:20						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:20	3-1:20	4-1:20	5-1:20	6-1:20	7-1:20	8-1:20
0-2			0	0	0	0	0
2-4	0	0	0	0	0	0	0
4-6		0	0	0	0	0	0
6-8		0	0	0	1	0	1
8-10		0	1	0	0	0	0
10-12	0	0	0	0		0	?
12-14		0	0	1		0	0
14-16		0	1	1		0	1
16-18		0	0	0		0	0
18-20	0	0	0	0		0	0
20-22		0	0	0		0	
22-24		0	0	0		0	
24-26		1	1	0		0	
26-28		1		0			
28-30	0	0		1			

Table 12. Wooded Dilution Ratio 1:20, Characterized by Signal Strength.

Wooded	Dilution Ration 1:20						
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Depth cm	1:20	3-1:20	4-1:20	5-1:20	6-1:20	7-1:20	8-1:20
0-2			0	0	0	0	0
2-4	0	0	1	0	0	1	0
4-6		0	1	0	0	0	0
6-8		0	1	0	1	0	0
8-10		0	0	0	0	0	0
10-12	0	1	0	0		0	0
12-14		1	0	1		0	0
14-16		1	0	0		0	0
16-18		0		0		0	0
18-20		0		0			
20-22	0	0		0			
22-24		0		0			
24-26		0		0			
26-28		0		0			
28-30	1	0		0			

At the end of the study, three control samples were collected at both the Pasture and Wooded Sites, a total of six controls. Each control was taken at a depth of 6-8 cmbs, and all of these controls came back negative for swine protein.

Although the ultimate results of this study are negative by industry standards, they can and should be discussed by addressing the major contributing factors. As stated in the Materials and Methods section, the soil characteristics from each area are the greatest contributors to this study. The mineralogical composition of this soil is moderately high in clay content while having minimal sand content. It is not clear how the pH levels of the soils affect the results, however it has been noted that both sites were moderately to highly acidic and could have played a role in protein degradation. Permeability and access to drainage are also greatly affecting factors as they would have allowed for the movement of the proteins through the soil layers or out of the sampling area. Each of these factors requires further attention and will be discussed more thoroughly in the following discussion chapter.

CHAPTER 5. DISCUSSION

Can pig blood protein be identified in Louisiana clay sediment over time?

The results of this study are negative. This is due to two major elements. The first is that there is no discernible pattern in the results (See Tables 5-12). This unpredictable sequence does not support any kind of usable inference about the detectability of blood proteins in these soils.

Second, the majority of the positive results obtained were at antiserum dilutions of 1:3 and 1:6--dilutions below industry-standard accepted levels. Few positive results were obtained using high antiserum dilutions of 1:10 and 1:20, which are necessary for acceptance. Samples are tested using dilutions of 1:3 and 1:6 because they are helpful in interpreting reliability and signal strength for results from higher dilutions. However, positive results generated using these low-ratio solutions are not generally reported by laboratories because of the likelihood of false positives. Positive results must be obtained from the higher ratio serums to be taken as reliable. Therefore, the results of this study overall are negative. However, as usual, results produced more questions; further research may help to identify sites more likely to preserve blood proteins as well as better handling procedures for samples.

Two points must be addressed with regard to the positive results. First, positive results obtained from the samples were a direct result of the blood deposition done for this study. That is, there is no possibility that pig blood was present at the test sites prior to my study. Secondly, since all of the results from the control samples were negative, it is unlikely that there is material in the soil matrix causing false positives in the study samples. No foreign contaminants that could cause a false positive were present in the samples. Thus, negative results from these tests notwithstanding, the major hypothesis, that blood proteins will be preserved in soils, warrants further research. Based on my results, I would recommend future research be conducted with

greater control of environmental variables, as well as more different handling procedures for samples.

Regarding the methods and materials, Cummings (personal communication, Dr. Linda Scott Cummings, PaleoResearch Institute) advises that handling methods do contribute to preservation; drying may have contributed to accelerated degradation of the protein residue, although this needs to be tested. Some preliminary observations can be made concerning the different handling procedures used in this study, however, Sample Day 1, 2, 3, 4, 5, and 6 were air-dried, and samples from Sample Days 7 and 8 were not. However, Sample 5 had more positive results than any other sample. Thus, there appears to be no direct correlation between air drying and reaction strength. On the other hand, refrigeration of the samples may have contributed to the positive results at the 1:3 dilution of the later samples, such as Days 5, 6, and 7; although, it is difficult to assess individual factors with so little control and considering the wide range of results. A way to minimize the effects of handling would be to begin the testing process (by adding the Tris-Triton solution) immediately after sample collection. This would inhibit residue degradation any further and could provide more reliable results. I did attempt one final round of sample testing that would address some handling issues. Along with Dr. Saunders, I bisected here-to-fore unsampled areas Pasture C and Wooded D, and took soil samples from the excavation walls. These were to be tested quickly. However, time and funding precluded the results from being obtained for this thesis. These samples have since been archived until further funding can be obtained. At present, one deposition area is still available for sampling in each area.

There are a number of environmental factors that may have affected the preservation and degradation of the blood proteins, as well as the dissipation and mobility of the proteins. These include climate and various soil characteristics. Climate surely affected the results of this study.

With respect to cadaver decomposition, colder temperatures inhibit biological decomposition (Tibbett and Carter 2008) and greater humidity results in increased decomposition compared to dryer and more arid environments (Tibbett and Carter 2008). It stands to reason that the same would apply to blood protein decomposition. With temperatures reaching into the high 90's in the summer months and up to nine inches of rainfall during those high temperature months (Figure 3), I believe humidity and water percolation hastened the degradation of the proteins or at least made them more difficult to detect. That said, a number of CIEP tests have been performed on objects that were subjected to extreme environmental (namely heat via oven) conditions and the tests yielded positive results (Malainey 2011).

Ultimately however, heat may have been one of the larger factors in degradation (in conjunction with water). In colder temperatures, blood can freeze and solidify, immobilizing the proteins. Curiously, the samples taken during the colder month (December 2010) had the greatest number and value in positive results (see Results Chapter and Tables 6-13). This may suggest that proteins degraded in the warmer months after the samples were removed from the ground, but did not degrade as much as when sampled in colder months. This leads to support for immediate testing of samples after removal from the ground or at least introduction of Tris-Triton solution to preserve the proteins at the time of sampling, especially during warmer months.

Because blood proteins are water soluble, during periods of higher precipitation, increased groundwater movement and higher water tables likely affect protein recovery. In saturated soils, blood proteins could be lost or degraded due to evaporation subsequent to sample drying. Proteins also likely dissolve into the ground water, making them much less detectable. Tuller (1991:28-29; see below) noted that blood proteins can bind with volatile fatty acids; thus, blood proteins in his samples from a massacre site in Kosovo may have been protected from

groundwater dissolution by the presence of volatile fatty acids. The greatest periods of precipitation during this study were April through August in 2010 and 2011 (Appendix A Table 13), where the fewest number of low-dilution positive results were obtained. The period with the least amount of precipitation was September 22, 2010 to December 13, 2010 which had only 7.55 inches of precipitation (Appendix A Table 13), and was the period of time with the greatest number of low-dilution positive results. Based on the positive results (of any strength and dilution) below 20 centimeters, the proteins had mobility and penetrated the soil in depth likely percolating with water. However, due to periods of increased saturation (water), access to drainage was minimal. That being said, protein contact with high levels of moisture from soil saturation likely increased degradation of the proteins themselves as they were not bound with non-water-soluble fatty acids as in Tuller 2001. Clearly, this information offers a point of further research for the future that must be addressed with more specialized tests to understand how climate, including heat and precipitation affects blood proteins in soil.

There are a number of other soil factors that may have impacted blood protein recovery. One factor is the possibility that root systems affect the behavior of the protein in the soils. However, based on the known nutrient needs of plants, there is no indication that plants would actually absorb blood proteins into their roots (personal communication, Dr. David Weindorf, LSU School of Plant, Environmental and Soil Sciences, 2011). However, it is possible that, in the act of consuming moisture in the soils surrounding the roots, a vacuum effect could be produced, causing the proteins to matriculate towards the roots, but not be taken up by the roots. If this were true, one might expect the bulk of proteins to be in the upper levels of the soil where the greatest content of root material exists. My results however, do not support this, so either this phenomena occurred on such a small scale as to not be detectable, or it did not occur in my test areas.

In a discussion of the preservation of blood proteins in soils, Tuller (2001: 27) noted that "soils high in sand and clay appear to help preserve protein from microbial attack better than other matrices (Cattaneo et al. 1993; Ensminger and Gieseking 1942; Loy 1983; and Pinick and Allison 1951)." Tuller also pointed out that "Loy (1983) suggested that positively charged blood proteins bind to negatively charged silica particles in clay and that this action helps to protect proteins from microorganisms that would feed upon them." Tuller's soils from Kosovo were comprised of between 39% and 46.5% clay, a clay content similar to my soils (Table 1) which range from 26% to 50%. Tuller also noted that research by Ensminger and Gieseking (1942), Pinick and Allison (1951), and Rice (1987) indicates protein preservation would be better in clays formed of illites and smectites, Tuller's study included smectite and chlorite clays with mica. The clays in this study are also smectites. Given my negative results, it is clear that clay mineralogy is only one of any number of factors affecting protein preservation.

Soil acidity may also affect blood protein preservation. While I am not aware of any studies directly addressing blood protein preservation and pH level, one might propose that high acidity would degrade blood proteins. Tuller 2001 reported essentially neutral pH levels for his soil samples-- 6.9 and 6.8—while the pH values for the soils in this study ranged from extremely acidic to slightly acidic (Table 1). Thus, pH would be a valuable control for future studies. The location of Tuller's study was on a hilltop whereas my study was a low lying alluvial plane. Comparatively, my samples are likely to have been saturated for periods of time, whereas Tuller's hilltop samples were well-drained. The area that Tuller's samples were collected from was also heavily wooded, somewhat comparable to my Wooded Site but I also had the luxury and access to a controlled Pasture Site for comparisons as well whereas Tuller did not.

The final environmental factor that can be compared to Tuller (2001) is climate. Kosovo is generally a temperate climate with the warmest months being July and August and averaging

around 86º F (http://www.kosovo-mining.org/kosovoweb/en/kosovo/climate.html). The coldest months are then in December and January, averaging around 14º F. These temperatures are cooler than in Louisiana where this research took place. Given that the best results obtained were in December (one of the coldest months of the year), it is possible that cooler temperatures result in better preserved proteins. Better results were obtained in the Kosovo samples (Tuller 2001) where a cooler climate exists.

Detailed precipitation records from Kosovo are difficult to obtain; however, the area where Tuller's research was performed was located in "a drier area of the country" which receives "a total annual precipitation of 600 mm [23.622 inches] a year" (http://www.kosovo-mining.org/kosovoweb/en/kosovo/climate.html). This is less than half of the average annual precipitation in Louisiana and is likely another contributing factor for the better preservation of proteins in the Tuller 2001 study than this one. It appears that the warmer and wetter climate of Louisiana did not provide the best preservation environment for blood proteins. Looking at the more positive results of Tuller 2001, it can be assumed that a cooler and dryer climate will provide more positive results as proteins are better preserved.

Storage time between sampling and testing for Tuller was six months for all of the samples where as my samples had a storage of times ranging between three weeks to 16 months. As presented in the methodology chapter, my samples were refrigerated during the storage time, while Tuller's were not. Also, Tuller laid out his samples to dry, three days after collection versus my same day drying for my earlier samples. My samples were dried over longer periods of time than Tuller's; multiple weeks versus a 12-hour period.

Though the results of this study are inconclusive, there is sufficient evidence to support the need for further studies using different controls. First and foremost, I would suggest testing for blood proteins in soil (of varying types) in controlled environments, where climate, soil

characteristics, and the presence of volatile fatty acids can be manipulated. Other studies should include testing different collection, sample handling, and storage methods, as well as time intervals between collection and testing. Testing for varying deposition methods in which the time between blood collection and blood deposition is minimal would be useful as well. Finally, the use of Tris-Triton solution toot sweet upon the collection of every sample has been learned to be imperative. This solution virtually stops the further degradation of proteins and offers a great degree of preservation of the samples for a long term study. Future studies should heed this particular aspect in order to ascertain the best and most reliable results.

CHAPTER 6. CONCLUSIONS

I hypothesized that CIEP would be an effective tool for identifying blood protein in a forensic context. In conjunction with Tuller (2001; Tuller and Saunders 2011), my results, while not unconditionally positive, do support further investigation and research would be beneficial. I continue to state that immunological assays should be applied to forensic and bioarcheological contexts and use. While further study is necessary, immunological assay tests, specifically CIEP, may provide a reliable test once methodologies in sampling have been perfected.

Based on earlier studies noted in this research, my results were not as had been expected. This can be speculated to be due to the handling and sampling methods of this particular project, especially the delay in refrigeration of samples taken early in the study. Considerations for the degradation/preservation of the proteins include: high saturation of the soils, limiting penetration and dispersion of blood and also causing an increase in microorganisms and thus the biological degradation of the proteins; high temperatures in Louisiana, also causing an increase in microorganisms and therefore biological degradation of the proteins; and the soils present in this study are not particularly conducive to the preservation of proteins.

From this research, some valuable points may be gleamed; the use of Tris-Triton immediately upon sample collection, refrigeration of any samples that may have a delay between sampling and testing, more controls must be implemented during testing to further understand the reach of obtaining positive results, and finally the confirmation that CIEP is a worthy immunological test which can be utilized for this purpose,

Identifying blood at outdoor crime scenes continues to be somewhat elusive. However, with additional research, it will hopefully not be long before what has been considered unachievable is attainable. Testing for the presence of blood and/or blood proteins in soils is a valid research goal.

REFERENCES CITED

Allen, J., M. E. Newman, M. Riford, and G. H. Archer
1995 Blood and Plant Residues on Hawaiian Stone Tools from Two Archaeological Sites in Upland Kāneʻohe, Koʻolau Poko District, Oʻahu Island. Asian Perspectives 34:283-302.

Borja, C., M. Garcias-Pacheco, E. G. Olivares, G. Scheuenstuhl, and J. M. Lowenstien
1997 Immunospecificity of Albumin Detected in 1.6 Million-Year-Old Fossils form Venta Micena in Orce, Granada, Spain. American Journal of Physical Anthropology 103:433-441.

Cattaneo, C., K. Gelsthorpe, P. Phillips, and R. J. Sokol
1990 Blood in Ancient Human Bone. Nature 347(6291):339 – 339.
1993 Blood Residues on Stone Tools: Indoor and Outdoor Experiments. World Archaeology 25:29-43.

Culliford, B. J.
1964 Precipitin Reactions in Forensic Problems. Nature 201:1092-1094.

Downs, E. F., and J. M. Lowenstein
1995 Identification of Archaeological Blood Proteins: A Cautionary Note. Journal of Archaeological Science 22:11-16.

Eisele, J. A., D. D. Fowler, G. Haynes, and R. A. Lewis
1995 Survival and Detection of Blood Residues on Stone Tools. Antiquity 69:35-46.

Gurfinkel, D. M. and U. M. Franklin
1988 A Study of the Feasibility of Detecting Blood Residue on Artifacts. Journal of Archaeological Science 15:83-97.

Hortolà, Policarp
2002 Red Blood Cell Haemotaphonomy of Experimental Human Bloodstains on Techno-Prehistoric Lithic Raw Materials. Journal of Archaeological Science 29(7):733 – 739.

Hyland, D. C., J. M. Tersak, J. M. Adovasio, and M. I. Siegel
1990 Identification of the Species of Origin of Residual Blood on Lithic Material. American Antiquity 55:104-112.

Jahren, A. H., N. Toth, K. Schick, J. D. Clark, and R. G. Amundson
1997 Determining Stone Tool Use: Chemical and Morphological Analyses of Residue on Experimentally Manufactured Stone Tools. Journal of Archaeological Science 24:245-250.

Kooyman, B., M. E. Newman, and H. Ceri
1992 Verifying the Reliability of Blood Residue Analysis on Archaeological Tools. Journal of Archaeological Science 19:265-269.

Kooyman, Brian, Margaret E. Newman, Christine Cluney, Murray Lobb, Shayne Tolman, Paul McNeil, and L.V. Hills
2001 Identification of Horse Exploitation by Clovis Hunters Based on Protein Analysis. American Antiquity 66(4):686 – 69.

Lee, H. C., and De Forest, P. R.
1976 A Precipitin-Inhibition Test on Denatured Bloodstains for the Determination of Human Origin. Journal of Forensic Sciences 21:804-809.

Loy, T. H.
1983 Prehistoric Blood Residues: Detection on Tool Surfaces and Identification of Species of Origin. Science 220:1269 – 1271.
1993 The Artifact as Site: An Example of the Biomolecular Analysis of Organic Residues on Prehistoric Tools. World Archaeology 25(1):44 – 63.

Loy, Thomas., and E.J. Dixon
1998 Blood Residues on Fluted Points from Eastern Beringia. American Antiquity 63(1):21 – 46.

Loy, T. H., and B. L. Hardy
1992 Blood Residue Analysis of 90,000-Year-Old Stone Tools from Tabun Cave, Israel. Antiquity 66:24-35.

Loy, T. H., Rhys Jones, D.E. Nelson, Betty Meehan, John Vogel, John Southon, and Richard Cosgrove
1990 Accelerator Radiocarbon Dating of Human Blood Proteins in Pigments from Late Pleistocene Art Sites in Australia. Antiquity 64(242):110 – 116.

Loy, T. H., and Andree R. Wood
1989 Blood Reside Analysis at Çayönu Tepesi, Turkey. Journal of Field Archaeology 16(4):451 – 460.

Malainey, Mary
2010 A Consumer's Guide to Archaeological Science. Springer, New York.

Marlar, Richard A., Banks L. Leonard, Brian R. Billman, Patricia M. Lambert, and Jennifer E. Marlar
2000 Biochemical Evidence of Cannibalism at a Prehistoric Puebloan Site in Southwestern Colorado. Nature 407(6800):74 – 78.

Maton, Anthea, Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, Jill D. Wright
(1993). Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall.

Newman, M. E., H. Ceri, and B. Kooyman
1996 The Use of Immunological Techniques in the Analysis of Archaeological Materials-A Response to Eisele; with Report of Studies at Head-Smashed-In Buffalo Jump. Antiquity 70:677-682.

Newman, M. E., and P. Julig
1989 The Identification of Protein Residue on Lithic Artifacts from a Stratified Boreal Forest Site. Canadian Journal of Archaeology 13:119-132.

Nolin, L., J. K. G. Kramer, and M. E. Newman
1994 Detection of Animal Residues in Humus Samples from a Prehistoric Site in the Lower Mackenzie River Valley, Northwest Territories. Journal of Archaeological Science 21:403-412.

Ouchterlony, O.
1968 Handbook of Immunodiffusion and Immunoelectrophoresis. Ann Arbor Science, Ann Arbor.

Petraglia, M., D. Kneeper, P. Glumac, M. Newman, and C. Sussman
1996 Immunological and Microwear Analysis of Chipped-Stone Artifacts from Piedmont Contexts. American Antiquity 61:127 – 135.

Romer, Alfred Sherwood, Parsons, Thomas S.
1977 The Vertebrate Body. Philadelphia, PA: Holt-Saunders International. pp. 404–406.

Sensabaugh, G. F., A. C. Wilson, and P. L. Kirk
1971 A & B Protein Stability in Preserved Biological Remains, Parts I and II. International Journal of Biochemistry 2:545-568.

Shanks, Orin C., Marcel Kornfeld, and Dee D. Hawk
1999 Protein Analysis of Bugas-Holding Tools: New Trends in Immunological Studies. Journal of Archaeological Science 26(9):1183-1191.

Smith, P.R., and M.T. Wilson
1992 Blood Residues on Ancient Tool Surfaces: A Cautionary Note. Journal of Archaeological Science 19:237 – 241.

Tibbett, Mark, and David O. Carter
2008 Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains. Boca Raton, CRC.

Tuller, Hugh
2001 Dirty Secrets: Blood Protein and VFA Analysis of Soil from Execution and Grave Sites in the Former Yugoslavia. Unpublished M.S. Thesis, Institute for Environmental Studies, Louisiana State University, Baton Rouge.

Tuller, Hugh., and Rebecca Saunders
2012 Dirty Secrets: Blood Protein and VFA Analysis of Soil from Execution and Grave Sites in the Former Yugoslavia. Journal of Forensic Sciences. Accepted for publication 2012.

Tuross, Noreen, Ian Barnes, and Richard Potts
1996 Protein Identification of Blood Residues on Experimental Stone Tools. Journal of Archaeological Science 23(2):289 – 296.

Vass, A. A., W. M. Bass, J. D. Wolt, J. E. Foss, and J. T. Ammons
1992 Time Since Death Determinations of Human Cadavers Using Soil Solution. Journal of Forensic Sciences 37:1236-1253.

Yohe II, R. M., M. E. Newman, and J. S. Schneider
1991 Immunological Identification of Small-Mammal Proteins on Aboriginal Milling Equipment. American Antiquity 56:659-666.

Zimmerman, M. R.
1973 Blood Cell Preservation in a Mummy 2000 Years Old. Science 180:303-304.

“Climatic Conditions.” Independent Commission for Mines and Minerals (ICMM), Kosovo. Web. 15 Mar. 2012. <http://www.kosovo-mining.org/kosovoweb/en/kosovo/climate.html>.

"Daily Summary Report for Baton Rouge." Louisiana Agriclimatic Information System. LSU Ag Center. Web. 20 Aug. 2011. <http://weather.lsuagcenter.com/reports.aspx>.

"Official Soil Series Descriptions (OSD)." NRCS Soils. Web. 06 Apr. 2011. <http://soils.usda.gov/technical/classification/osd/index.html>.

Web Soil Survey - Home. Web. 06 Apr. 2011. <http://websoilsurvey.nrcs.usda.gov>.

APPENDIX A: QUARTER CLIMATE DATA

Table 13. Quarter Climate Data Averages.

Dates Collected/ Time 13:00 - 12:00	Max Air Temp(°F)	Min Air Temp(°F)	Total Rain(in)	Max Soil Temp(°F)	Min Soil Temp(°F)	Max Relitive Humidity (%)	Min Relitive Humidity (%)
04/07/10 - 04/14/10	81	42	0.09	73	61	94	25
04/14/10 - 05/05/10	90	50	0.21	96	21	82	64
05/05/10 - 06/30/10	96	58	12.69	104	71	96	28
06/30/10 - 09/22/10	99	61	13.14	105	79	97	29
09/22/10 - 12/13/10	90	26	7.55	94	41	96	15
12/13/10 - 03/09/11	81	22	12.94	79	36	97	19
03/09/11 - 06/01/11	93	39	5.59	102	52	97	17
06/01/11 - 08/24/11	98	67	15.71	103	75	97	29

APPENDIX B: DAILY NOTES FROM SAMPLING

The following are notable comments from each sample day.

Sample Day 1, April 14, 2010, one week after deposition (7 days/1 week). Samples were taken from each site and labeled Site A, Core 1. A single section of centimeters 4-10 was also bagged for possible future use. The difference in the depth of samples taken from Pasture A (Table 3) and Wooded A (Table 4) was due to a worm that happened to be in the core at the desired location. Post sampling handling described above.

Sample Day 2, May 5, 2010, one month after deposition (28 days/4 weeks). Samples were taken from each site and labeled Site B Core 1. The change in sample amounts from two centimeter increments to five centimeter increments was the result of efforts to collect the maximum amount of usable soil for possible testing. However, because five centimeter increments could not be divided after collection due to the hardening of dry clay, none of these samples was testable in laboratory. On June 29, 2010, all samples from Days 1 and 2 were sealed and stored as previously indicated.

Sample Day 3, June 30, 2010, three months after deposition (84 days/12 weeks). Samples were taken from each site and labeled A Core 2, then were also laid out to be dried, in the same manner as samples from Day 1 and 2. On July 30 2010, the dried samples from Day 3 were sealed.

Sample Day 4, September 22, 2010, six months after deposition (168 days/24 weeks). Samples were taken from each site and labeled B Core 2. November 1, 2010, dried samples from Day 4 were sealed. Sample 4 was reopened November 15, 2010, laid out again for further drying because the samples still retained some moisture.

November 15, 2010 began refrigeration after being instructed by the staff at PaleoResearch Institute that refrigeration was the best way to minimize degradation of proteins.

Samples from Days 1, 2 and 3 were refrigerated, and samples from Day 4 were also refrigerated, after drying completely, on December 1, 2010. After this point, efforts were made to refrigerate the samples as soon as possible after drying.

Sample Day 5, December 13, 2010, nine months after deposition (250 days/36 weeks). Samples were taken from each site and labeled B Core 3, then laid out for drying. The initial scheduled date for this sample was December 14; however, due to unforeseen circumstances unrelated to the study, the sample was taken one day earlier. January 28 2011, sample 5 was sealed and refrigerated after drying.

Sample Day 6, March 9, 2011, 12 months after deposition (336 days/48 weeks). Samples were attempted from each site and took the label A Core 3. Unfortunately, because of heavy rainfall, the samples could not be taken. The silty clay was saturated and oozed out of the corer during extraction. After the inclement weather subsided, a new sample day was established, March 21, 2011, still 12 months after deposition, (348 days/49.71 weeks). Samples were taken from each site and labeled A Core 4, then laid out to dry briefly. March 29, 2011, samples from Day 6 were sealed and refrigerated.

Sample Day 7, June 1, 2011, 15 months after deposition (420 days/60 weeks). Samples were taken from each site and labeled B Core 4, then laid out to dry. June 8, 2011, samples from Day 7 was sealed and refrigerated.

Sample Day 8, August 24, 2011, 18 months after deposition (504 days/weeks 72). Samples were taken from each site and labeled Pasture A 5, Wooded B 5. Samples were not laid out to dry, but were sealed and refrigerated the same day as collection.

VITA

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