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Diagnosis of Schistosomiasis in Nubian Mummies

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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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By Hillary M. Olivier

Analysis of preserved soft tissue from naturally desiccated mummies has proven to be useful in understanding the emergence and development of diseases that continue to impact contemporary populations. In order to develop a better understanding of the relative sensitivity of tests conducted on these tissue types, we have tested a series of matched skin tissue and cranial contents samples from the NAX group (350-550 CE) cemetery population from Wadi Halfa in ancient Nubia. Each sample was homogenized, dialyzed, and lyophilized, and an enzyme linked immunosorbant assay (ELISA) was performed to diagnose *Schistosoma mansoni* infection and antigen concentration present in tissue samples. Antigen concentrations found in matched tissues were analyzed to determine the relationship between antigen levels detected in these types of dessicated tissue. Physiological and taphonomic causal mechanisms for variation between them is examined. A general model for the relative prevalence of infections tested in these tissue types will be developed. Further study with a larger data set may lead to conclusions about minimum antigen concentrations detectable in preserved samples as well as the aplicability of the relative prevalence figures presented here for other diseases.

Diagnosis of Schistosomiasis in Nubian Mummies

By

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George J. Armelagos

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## Diagnosis of Schistosomiasis in Nubian Mummies

### Introduction

The goal of this study is to determine whether or not it is possible to diagnose schistosomiasis in an archaeological population using enzyme-linked immunosorbent assay of preserved cranial contents. In addition to being detectable and measurable in preserved skin tissue, it seems likely that the same determinations of presence and concentration of schistosomes can be performed successfully using brain tissue. This investigation adds to previous research in an attempt to improve and expand the body of knowledge accessible through paleopathological research of parasitic infections.

In a bioarchaeological context, this study has as much to tell about the evolution of schistosomiasis as it has to tell about the social context of schistosomiasis infection in the past. This work adds to the history of the epidemiology of the disease as well as providing information about how the parasite may have evolved. The paleoepidemiological data drawn from this work also helps give a better understanding of the population in which the disease is being studied, here, the Nubians.

**Background**

Paleopathology:

Paleopathological research is a powerful tool for understanding the role of disease in archaeological populations and intersections of the past and present, in terms of health. More specifically, studying parasitological remains can suggest how a disease emerged and developed, and provide insight into the human-parasite connection (Gonçalves *et al*., 2003:104).

Paleopathology describes what public health issues a population may have faced (Miller *et al*., 1992: 556), and how disease distribution can reveal social disparities (Goodman 1998, 156). While such studies indicate the overall health of the population, they may also suggest the relative distribution of the disease based on sex and age-group. Paleopathological study of parasitic disease not only explains circulation and spread of the disease, to some extent, but also suggests how social standing is related to prevalence of infection using the bio-cultural approach.

Paleoparasitology has only become a recognized source of valid information in the past two decades. Originally, analysis of parasitic infection was based on coprolites and latrine sites, but mummies have proven to be important to the field as well. (Araújo *et al.*, 2000) There are several studies underway, including those of Andean and Egyptian mummies, which aim to compare ancient disease to contemporary trends and manifestations of disease (Aufderheide, 2000: 2573).

Schistosome infection has been of particular interest in paleoparasitological studies (see for example, Kloos and David 2002 or Miller *et al.* 1992) since it seems to have been prevalent in the past and is certainly still a public health risk at present. By

clarifying the historical context of schistosomiasis, scholars may gain a better understanding of its contemporary impact of the disease.

*Schistosoma mansoni*:

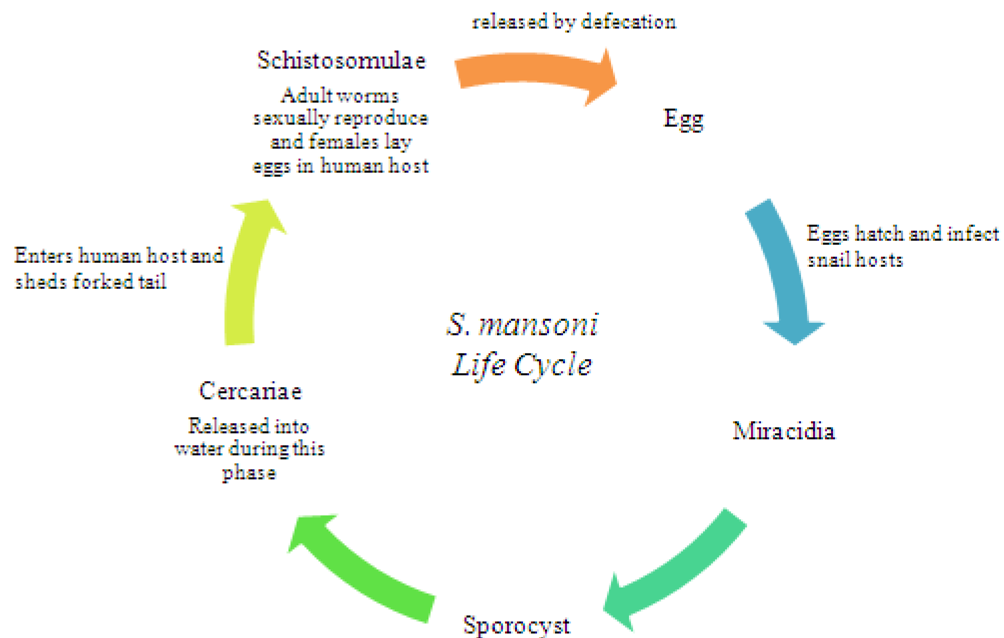
Schistosomiasis describes a disease resulting from parasitic infection by one of several species of *Schistosoma*. Among them, *S. mansoni*, *S. haematobium*, and *S. japonicum* are most well-known. As of 2007, 200 million people worldwide were thought to be carriers, and another 600 million were in danger of being infected. This study focuses on *S. mansoni*, which is most frequently found in South America, central and southern Africa, and Saudi Arabia. While humans are the primary hosts, *Biomphalaria* snails (Kloos and David 2002, 14) inhabiting the same bodies of water as the parasites act as a transitional host. (da Silva *et al.* 2007, 845)

Besides impacting the digestive system, specifically the intestines, the eggs or adult worms may migrate to other regions of the body, like the lungs, liver, and central nervous system. *S. mansoni* is less frequently associated with cerebral lesions that *S. japonicum*, but the spinal cord is known to be effected by *S. mansoni* and *S. haematobium*. (da Silva *et al.* 2007, 845)

The transmission of schistosomiasis involves a complex process including several transfer steps to intermediate host and from intermediate host to ultimate host (in this case, humans). The life cycle begins when eggs are released, in the case of *S. mansoni*, this usually occurs during defecation. When eggs hatch, they release miracidia, which proceed to occupy snails as their hosts. The miracidia passes into the sporocyst and then the cercariae phases, after which, it is released into the water by it snail host. The

cercariae enter human hosts through the skin, losing their forked tail during entry. At this point, the cercariae become schistosomulae. The schistosomulae migrate to the mesenteric venules, veins of the bowels and rectum. In the case of *S. mansoni*, the schistosomulae most frequently take up residence in the veins that drain the large intestine. It is possible, however, for *S. mansoni* to reside in the veins of the small intestine, though this area of infection is more frequently associated with *S. japonicum*. (United States Centers for Disease Control & Prevention) While present in the venules, the females lay eggs, approximately 300 per day (Boros 1989, 250), that are ultimately eliminated by the body, beginning the cycle of transmission anew.

Figure 1-
*S. mansoni* Life Cycle



Clinical Aspects of *S. mansoni*:

Besides the morbidity associated with schistosome infections, a number of other complications may arise as a result of exposure to the parasite. Initially, it is not uncommon to see dermatitis shortly after the cercariae enters the human host through the skin. This dermatitis can take the form of a small (1 to 2 mm) rash or, in more extreme cases, papules may form at the entry site and have the potential to become infected It is the chronic inflammation that often occurs as a result of collection of eggs that are not eliminated by defecation that causes the vast majority of the symptoms associated with the disease. The inflammation is brought on by the immune system's attempt to sequester the antigen by encapsulating it. (Boros 1989, 250)

It generally takes 4 to 10 weeks before any of the more severe symptoms are displayed in individuals experiencing acute schistosomiasis. Indications of acute schistosomiasis include fever, malaise, hepatosplenomegaly, and diarrhea, among many others. These symptoms will appear when the individual is most heavily exposed to cercariae and will pass when the chronic phase of infection is complete. (Boros 1989, 251) Chronic schistosome infection may also be marked by liver cirrhosis (Kloos and David 2002, 16).

Complications that more frequently lead to death are those that include the major body systems: neurological, pulmonary, cardiac, hepatic, and digestive (specifically intestinal). One potential cause of death in cases of major system involvement is hepatitis, but research has not yet shown any significant correlation between the number of eggs an individual is carrying with the expression of disease symptoms. (Boros 1989, 251)

It does appear that people living in an area where schistosomiasis is endemic have some resistance, most likely due to exposure from the fetal stage onward and infections beginning at a young age. Fetuses are potentially exposed to the antigen in the womb and some immunity would be passed down from the mother after birth, assuming she breastfeeds her infant. Additionally, after an individual from an area where schistosomiasis is endemic has been infected once, he or she is unlikely to experience any of the more severe symptoms of disease if reinfected. (Boros 1989, 251)

Bioarchaeology:

It is widely accepted that skeletal remains have the most information to offer regarding human variation and ways of living (Larsen 1997, 2), and the introduction of preserved soft tissues into bioarchaeological studies has great potential as well (e.g. Miller *et al.* 1992). Bioarchaeology is defined as many different types of study, but the most encompassing description from the department of archaeology at Cambridge University in the United Kingdom calls it "the study of all biological materials from archaeological contexts, especially flora (paleobotany) and fauna (paleozoology)" (Buikstra and Beck 2006, xvii).

In relation to paleopathology, bioarchaeology can speak volumes about a population: how it was impacted by disease, what the health status was overall, and what factors were influencing the spread of disease. Bioarchaeological materials can indicate disease prevalence, to describe what portion of the population was infected, but is unable to specify incidence (how many new cases occur) at a given time. (Larsen 1997, 64)

While bioarchaeology is a powerful and ever-improving area of study, even modern technology has not been able to solve some of the conundrums surrounding the study of human remains. One difficulty that is especially applicable to this study is the sometimes unknown composition of the sample death assemblage. Many groupings are made of individuals buried over an unknown period of time, not individuals who died in a relatively small time frame. Gender, social status, and age variation compound this issue. (Larsen 1997, 334) Because such concern cannot be fully addressed in the current study, it is important to bear in mind the potential impact of these limitations.

Sudanese Nubia in the Ballaña Period (350-550 CE):

The remains used in this study were excavated in the 1960s near Wadi Halfa in Sudanese Nubia. They are from the Ballaña Period (350 C.E. – 550 C.E.), after the Meroitic Era (350 B.C.E.-350 C.E.) and before the Christian Period (550 -1300 C.E.) (Armelagos 1969, 255). This population is known as the "X-group," or NAX, and all of the NAX mummies were naturally desiccated due to environmental conditions. (Miller *et al.*, 1992:555)

Bioarchaeology of Ancient Nubia and Ancient Egypt:

Use of the paleoepidemiological method has proven useful in decoding information held in archaeological materials and recreating the lives of Nubians from several time periods, including the X-group period (350-550 CE). This methodology takes into consideration the disease host, the disease itself, and the environment in which the host and the disease are interacting. It is by careful use of this technique that

paleopathologists have been able to gain some insight into the cultural conventions of Sudanese Nubians more than 2000 years ago. (Armelagos 1969, 255)

While some scholars suggest that the X-Group period was a time of deterioration and backsliding, closer examination has suggested that this is probably not the case. There is evidence that the period may have been one of advancement, at least in comparison to the previous Meroitic phase (350 BC- 350 CE). (Armelagos 1969, 259)

An issue that is of particular interest in the field is the possible correlation of social patterns to disease prevalence and distribution in the region. A line of evidence beginning before the X-group period and continuing up through present epidemiological research has been essential in tracing the paleoepidmiology of schistosomiasis in the region. From the disease's first known appearance of schistosome infection to modern population surveys and figures gathered by health organizations and medical researchers, there is a definite connection between paleoepidemology and current public health trends that must be clarified.

The oldest identified instance of schistosomiasis in humans was detected using the enzyme-linked immunosorbent assay (ELISA) in an Egyptian subadult who lived over 5,000 years ago. Scholars determined that this individual was suffering from an *S. haematobium* infection, but this information is still important to understanding the impact of *S. mansoni* in the region. The parasites are transmitted in much the same way and sociopolitical and cultural implications of both forms of schistosomiasis endemic in the region are most likely closely related to one another. (Kloos and David 2002, 16)

Scholars referencing the archaeological record and historical records of the region have determined that the way of life in the past is markedly similar to that of many

people currently living in the region. Several technologies are available (electricity, chemical farming aids, etc.) and have significantly impacted communities, but many of the same activities that put ancient Nubians and Egyptians at risk of becoming infected are still necessary for survival in modern times. Daily chores, farming duties, and leisure activities still require people to come in direct contact with water of infected or potentially infected bodies of water and waterways. (Kloos and David 2002, 17) The same intense contact necessary today was vital in ancient Nubia and Egypt. Not only is there evidence of infection among farmers and peasants, but cases of schistosome infections are also known to have occurred among the elites and royals (18).

After the Pharaonic Period, much less is known about the prevalence of schistosomiasis in the region. Very little evidence of schistosomiasis after about 35 C.E. has been uncovered, and what is known comes from Wadi Halfa, the same area where the NAX population lived. (Kloos and David 2002, 19)

Fortunately, the research in Wadi Halfa has been plentiful and informative. Miller *et al.* (1992) is one of the first samples from the regions to have significant epidemiological impact using techniques similar to those employed in this study. In a sample of 23 mummies from Wadi Halfa, 15 (7 male, 7 female, 1 unknown) were found to be infected. The epidemiological significance of this work is in the parallel that it may draw between ancient schistosomiasis infection and the disease burden of today. The most severe infections in this study were found in individuals identified as between the ages of 15 and 40. Both the sex and age trends in Miller *et al.* align with the modern distribution of schistosomiasis in Egypt.

In addition to recognizing what may be age and sex patterns in diseaserevalence, several scholars have also supported the idea that the socio-political status of the region was intimately linked with the health of the populations of Wadi Halfa and Kulubnarti. Relationships between the florescence of the northern kingdoms and the Wadi Halfa/Kulubnarti region seem to be negatively correlated. In other words, when the kingdom was in a peak period the southern regions had a decrease in overall health, while less success in the north meant that the health of the southern populations seems to have been generally better. (Goodman 1998, 156; Kloos and David 2002, 15) As paleoepidemiology reveals more information about disease and social patterns, a more cohesive picture of life in the region at the time can be built by incorporating historical information into paleopathological research.

Accounts of the infection in modern population simply reinforce how much more there is to learn about different forms of schistosome infection, particularly *S. mansoni*, which has been more prevalent than *S. haematobium* since the early 20$^{th}$ century. In the 1990s, the schistosome infection rate in Egypt was about 12%, but because of the environmental tolerance the *Biomphalaria* snail, it seems that this increase in prevalence of *S. mansoni* over *S. haematobium* is probably a modern phenomenon. *Biomphalaria* are better are surviving in polluted, under-oxygenated, slowly-moving water than the *Bulinus* snails that host *S. haematobium*. These special abilities may explain why *S. mansoni* has overtaken *S. haematobium* in modern times. Trends in prevalence and distribution for modern populations in the region also provide interesting points of comparison as scholars explore schistosomiasis in ancient times. As of the 1990s, more men than

women and more adolescents than other ages were infected. Not surprisingly, agricultural populations were more effected than other populations. (Kloos and David 2002, 19-20)

This study specifically builds on work done at both Arizona State University and Emory University to investigate the feasibility and accuracy of diagnosing schistosomiasis in ancient Nubian populations using the ELISA on different tissue sample types. The work of Amber Campbell, a PhD candidate at Emory University, uses skin tissue samples from some of the same individuals as those whose cranial material is used in this study. Campbell used the same technique as is utilized in this experiment and was able to get what seems to be a reasonable prevalence calculation from her data (the final prevalence calculations have not been completed or recorded as of yet). (personal communication) By comparing skin and cranial content assays from the same individuals, the relative sensitivity of the test may become more clear. A preliminary comparison to Campbell's study is given in the Discussion & Analysis.

Immunological Background:

The enzyme-linked immunosorbent assay operates on the basic principles of immunology. In order to understand how the procedure is able to function, several of the most foundational components of the immune system must be introduced. Both antigens and antibodies have key roles in the ELISA procedure. An antigen, also known as an immunogen, is a molecule, in this case the schistosome antigen, which binds to the corresponding antibody. An antibody, which is a type of protein, is comprised of two heavy and two light chains (each set is an identical pair). It recognizes its corresponding

antigen by its epitope and takes action to clear the antigen from the system. (Goldsby *et al.* 2003, G1)

The procedure used relies heavily on descriptions of circulating anodic antigens and their behavior. For example, Miller notes that these antigens are not found in *any* uninfected individuals, in the present or past (1992). Goncalves further remarks that these antigens are not destroyed by environment over time like other forms of parasite evidence, like eggs. (2003)

Miller, Armelagos, and several of their colleagues have provided evidence to support this form of antigen detection in both skin and cranial content samples. However, none of the individuals in the Miller *et al.* work had assay results for both skin and cranial samples (1992).

Enzyme-Linked Immunosorbent Assay (ELISA):

The ELISA technique was developed by Perlmann and Engvall at Stockholm University in the 1960s (Lequin 2005, 2415). The technique uses an antibody conjugated with an enzyme to produce color in a normally colorless substrate, called a chromogenic substrate. ELISAs can be used qualitatively or quantitatively, but this work utilizes the quantitative method involving a standard curve of varying concentrations of antibody so that the concentrations of the unknown samples can be determined. (Goldsby *et al.* 2003, 148)

The specific type of ELISA used in this study is known as the “sandwich ELISA,” and it detects that presence of antigen by fixing antibody to the sample well-plate and adding samples that contain antigen. The antigen in the unknown samples adheres to the

corresponding antibody, and another antibody is added. The second antibody is enzyme-linked and has the ability to bind to a different epitope on the antigen of interest. Unbound enzyme-linked antibody is removed from the samples. The enzyme-linked antibody reacts with the developing substrate to produce the color change that is ultimately measured to determine concentration in relation to the color change in the standard curve of known concentrations. (Goldsby *et al.* 2003, 149)

**Materials & Methods**

Sample Preparation:

For each sample, 150 mg of mummified cranial content were combined with 800 μL PBS solution in individually labeled tubes. Each of these was homogenized by hand, using a small spatula to achieve a roughly uniform particle size throughout the sample. After crushing each sample, the spatula was rinsed in 300 μl of PBS. This was done in order to maximize the amount of sample retained for concentration. The tubes used for rinsing were labeled to correspond to the sample from which the rinsed particulates came. *All* tubes were vortexed for approximately 2 minutes and then allowed to rest for 10 minutes. The samples were centrifuged at 2,800 rpm for 25 minutes. Supernatant from both of the tubes corresponding to each sample was combined in one small centrifuge tube per sample and 500 μl of TCA was added to each. The tubes were centrifuged again at 10,000 rpm for 30 minutes.

Each sample was dialyzed and lyophilized, both for 24 hours.

When preparing to perform the ELISA, the samples were re-dissolved in 250 μl of PBS.

Enzyme Linked Immunosorbent Assay (ELISA):

Approximately 24 hours before performing the ELISA, an Immulon II Elisa plate was coated with 5H11 antibody. 50 μl of a 5μg/mL solution of 5H11 antibody was prepared in sodium carbonate buffer (pH 9.6) added to each well. The plate was stored at 4°C overnight.

When ready to add samples, the coating solution was discarded and 100µL of PBS +0.3% Tween 20 was added to each well. This solution was allowed to block for ~1 hr at room temperature. After blocking, the plate was washed 5 times with PBS+0.05% Tween 20 and plate was patted dry.

A standard curve was prepared using soluble adult worm antigen preparation (SWAP), to provide the standard cutoff that determines whether an individual's values indicate infection or not. 10, 2.5, 0.625, 0.1562, 0.0390, and 0.010 µL/mL SWAP were prepared in 0.85% NaCl.

In the appropriate wells, 50 µl of processed standards and individual samples were added. Table 1 illustrates the standard and sample layout for this ELISA, with the row of standards highlighted and the sample number for each NAX individual given. The plate was incubated at room temperature for one hour and washed with PBS+0.05% Tween 20 five times, as in the previous step.

Table 1-
ELISA plate diagram

| | 1-2 | 3-4 | 5-6 | 7-8 | 9-10 | 11-12 |
|---|---|---|---|---|---|---|
| A | Std 10.0 | 359 | 564A-2 | 594 | 631 | 643A |
| B | Std 2.50 | 367 | 564Y | 608 | 632B | 643C |
| C | Std .6250 | 527 | 565A | 611 | 640B | 644 |
| D | Std .1560 | 537A | 568A-1 | 611-1 | 640B-1 | 646 |
| E | Std .0390 | 549 | 568A-2 | 612 | 640B-2 | |
| F | Std .010 | 552 | 581 | 618B | 640Y | |
| G | Std 0.0 | 561 | 585Y | 620Y | 643 | |
| H | BLANK | 564A-1 | 587A | 624 | 643-1 | |

To each well except the Standard 0.0 and the blank wells, 50 µl of 1:250 biotinylated 5H11 solution in PBS+0.3% Tween 20 was added. 50 µl of PBS+0.3% Tween 20 was added to the blank and standard 0.0, and the plate was left at room temperature for one hour.

After one hour, the plate was washed again with PBS+0.05% Tween 20 and patted dry.

A 1:500 avidin peroxidase solution in PBS+0.03% Tween 20 was prepared, and 50 µl was added to all wells except for the blank. 50 µl of PBS+0.3% Tween 20 was added to the blank well, and, again, the plate was allowed to incubate at room temperature for one hour.

After the incubation period, the plate was washed in the same manner as before to prepare for development.

To develop, 50 µl of TMB solution was added to each well and allowed to react for about 5-7 minutes. Upon adding TMB, the wells begin to turn blue if antigen was present in the sample placed in the wells. To stop the reaction when complete, 50 µl of acid (either 1:7 $H_2SO_4$ or 1:20 $H_3PO_4$) was added to each well. The acid changes the sample color from blue to yellow, and the plate can then be read using a 450 nm plate reader. The plate reader determines the values for the standard curve and the samples being tested, and graphs the standard curve to estimate the test's sensitivity.

**Results**

The results of the antigen test are reflected not only in the values produced by the plate reader analysis, but also in the color change that takes process during developing. In this particular experiment, observing the blue color change prompted by the addition of acid to the well plate was extremely important since the standard curve indicates that the test was not as sensitive as is desirable. The wells that turn to as deep a shade of blue as the standard values of 10.0 or 2.50 can be tentatively labeled as having tested positive.

In this particular ELISA test, the standard curve was not as consistent or as sensitive as possible. Figure 2 illustrates the standard curve values produced. Table 2 gives corresponding values for the standard curve.

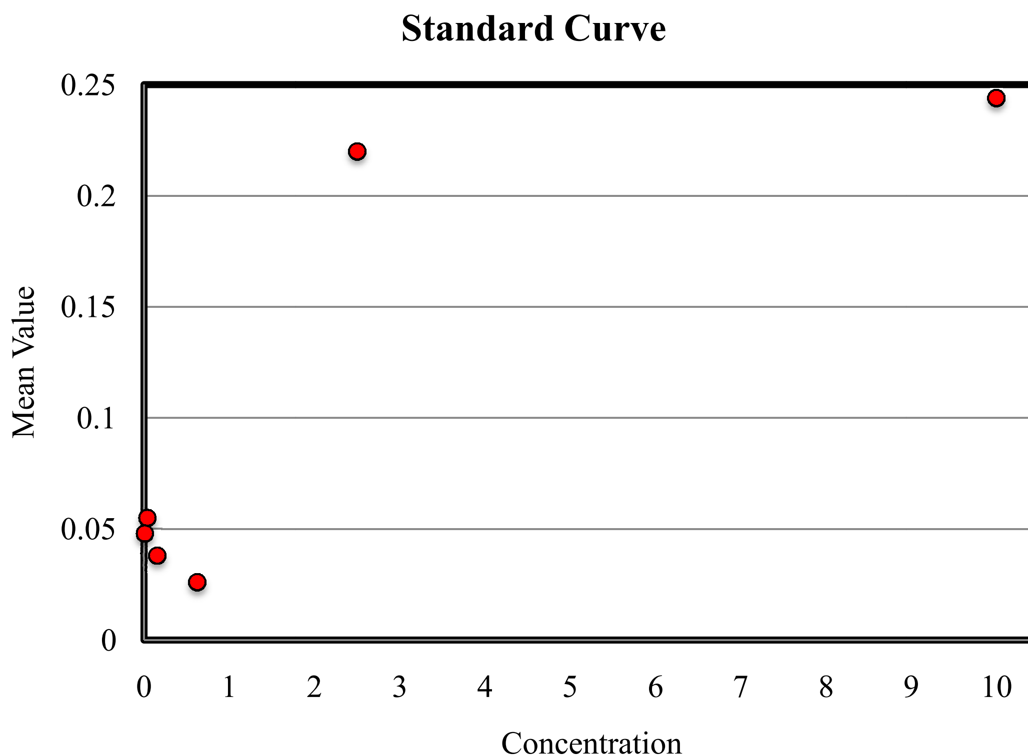



Figure 2- Mean Values of the Standard Curve Concentrations for ELISA

Table 2-
Standard Curve Values from ELISA

| Sample | Concetration | BackConcCalc | Values | Mean | Std.Dev. | CV% |
|---|---|---|---|---|---|---|
| St01 | 10 | ?<br>2.519 | 0.266<br>0.222 | 0.244 | 0.031 | 12.8 |
| St02 | 2.5 | 1.78<br>? | 0.057<br>0.383 | 0.22 | 0.231 | 104.8 |
| St03 | 0.625 | ?<br>? | 0.027<br>0.024 | 0.026 | 0.002 | 8.3 |
| St04 | 0.156 | 1.492<br>? | 0.043<br>0.033 | 0.038 | 0.007 | 18.6 |
| St05 | 0.039 | 1.829<br>1.617 | 0.063<br>0.046 | 0.055 | 0.012 | 22.1 |
| St06 | 0.01 | 1.911<br>? | 0.077<br>0.018 | 0.048 | 0.042 | 87.8 |
| Co01 | | | 0.111<br>0.078 | 0.095 | | |

Because of concern that the reagents were no longer viable, a second standard curve was generated using variable concentrations of the biotinylated 5H11. Duplicate samples of 4µl/ml, 8µl/ml, and 16µl/ml were used in order to determine if changing the concentration enhanced the test's sensitivity. The second curve showed that the reagents were still usable and that increasing the concentration of biotinylated antibody had no significant impact on the ELISA's sensitivity.

Because of the lack of sensitivity, results indicating positive or negative tests are based on the color change results given during development. The only sample that tested positively based on color change was NAX 620Y. Table 3 gives raw values, concentrations, mean concentration, standard deviation, confidence, and overall results for each of the samples as determined by the ELISA.

Table 3-
Raw values, concentrations, mean concentrations, standard deviations, confidence and overall results for individual samples from ELISA

| Sample | Values | Result | Mean | Std.Dev. | CV% | (+) or (-) |
|---|---|---|---|---|---|---|
| NAX 359 | 0.029<br>0.026 | ?<br>? | ? | ? | ? | ? |
| NAX 367 | 0.047<br>0.048 | 1.642<br>1.663 | 1.652 | 0.015 | 0.9 | (-) |
| NAX 527 | 0.072<br>0.085 | 1.885<br>1.948 | 1.917 | 0.044 | 2.3 | (-) |
| NAX 537A | 0.09<br>0.082 | 1.969<br>1.935 | 1.952 | 0.024 | 1.2 | (-) |
| NAX 549 | 0.094<br>0.084 | 1.984<br>1.944 | 1.964 | 0.029 | 1.5 | (-) |
| NAX 552 | 0.014<br>0.084 | ?<br>1.944 | 1.944 | 0 | 0 | ? |
| NAX 561 | 0.044<br>0.067 | 1.548<br>1.856 | 1.702 | 0.218 | 12.8 | (-) |
| NAX 564A-1 | 0.033<br>0.037 | ?<br>? | ? | ? | ? | ? |
| NAX 564A-2 | 0.001<br>0.009 | ?<br>? | ? | ? | ? | ? |
| NAX 564Y | 0.045<br>0.043 | 1.587<br>1.492 | 1.539 | 0.067 | 4.3 | (-) |
| NAX 565A | 0.066<br>0.08 | 1.85<br>1.926 | 1.888 | 0.054 | 2.8 | (-) |
| NAX 568A-1 | 0.043<br>0.05 | 1.492<br>1.697 | 1.595 | 0.145 | 9.1 | (-) |
| NAX 568A-2 | 0.057<br>0.049 | 1.78<br>1.681 | 1.731 | 0.07 | 4.1 | (-) |
| NAX 581 | 0.046<br>0.036 | 1.617<br>? | 1.617 | 0 | 0 | ? |
| NAX 585Y | 0.043<br>0.07 | 1.492<br>1.874 | 1.683 | 0.27 | 16 | (-) |
| NAX 587A | 0.047<br>0.045 | 1.642<br>1.587 | 1.614 | 0.039 | 2.4 | (-) |
| NAX 594 | 0.028<br>0.039 | ?<br>? | ? | ? | ? | ? |
| NAX 608 | 0.058<br>0.058 | 1.789<br>1.789 | 1.789 | 0 | 0 | (-) |

| Sample | Values | Result | Mean | Std.Dev. | CV% | (+) or (-) |
|---|---|---|---|---|---|---|
| NAX 611 | 0.018<br>0.001 | ?<br>? | ? | ? | ? | ? |
| NAX 611-1 | 0.027<br>0.029 | ?<br>? | ? | ? | ? | ? |
| NAX 612 | 0.059<br>0.083 | 1.798<br>1.939 | 1.869 | 0.1 | 5.3 | (-) |
| NAX 618B | 0.035<br>0.041 | ?<br>? | ? | ? | ? | ? |
| NAX 620Y | 0.154<br>0.199 | 2.185<br>2.362 | 2.273 | 0.125 | 5.5 | (+) |
| NAX 624 | 0.059<br>0.063 | 1.798<br>1.829 | 1.814 | 0.022 | 1.2 | (-) |
| NAX 631 | 0.023<br>0.011 | ?<br>? | ? | ? | ? | ? |
| NAX 632B | 0.02<br>0.05 | ?<br>1.697 | 1.697 | 0 | 0 | ? |
| NAX 640B | 0.002<br>0.002 | ?<br>? | ? | ? | ? | ? |
| NAX 640B-1 | 0.043<br>0.103 | 1.492<br>2.018 | 1.755 | 0.372 | 21.2 | (-) |
| NAX 640B-2 | 0.049<br>0.111 | 1.681<br>2.045 | 1.863 | 0.258 | 13.8 | (-) |
| NAX 640Y | 0.045<br>0.024 | 1.587<br>? | 1.587 | 0 | 0 | ? |
| NAX 643 | 0.067<br>0.103 | 1.856<br>2.018 | 1.937 | 0.114 | 5.9 | (-) |
| NAX 643-1 | 0.044<br>0.061 | 1.548<br>1.814 | 1.681 | 0.188 | 11.2 | (-) |
| NAX 643A | 0.015<br>0.029 | ?<br>? | ? | ? | ? | ? |
| NAX 643C | -0.005<br>0.002 | ?<br>? | ? | ? | ? | ? |
| NAX 644 | 0.001<br>0 | ?<br>? | ? | ? | ? | ? |
| NAX 646 | 0.04<br>0.041 | ?<br>? | ? | ? | ? | ? |
| | Outlier | ?= range? | | | | |

**Discussion & Analysis**

Interpreting the Results:

The results of the enzyme-linked immunosorbent assay are, at this point in testing, not conclusive, but there is important insight to be drawn from the data. Primarily, this work shows that there is a potential for making meaningful comparisons between skin and cranial tissue assays since both tests are possible. In the cranial content, some level of detection is possible using ELISA on a small scale. This is significant in bioarchaeology since it may lead to developing a technique that better conserves use and prevents destruction of irreplaceable tissues. Since the assay was able to produce some concentration data from some small samples, this study also emphasizes that in order to perfect the methodology, the concentration of the samples used must be optimized to a level that increases concentration without wasting the tissue samples. While there is much work left to be done, this study will inform and impact the design of future studies in the discipline.

Comparison of Crania and Skin Assay Results:

A primary goal of this research is to compare the accuracy of the technique using cranial content samples to the accuracy of the same technique with samples of skin from the same sample population. Due to sampling constraints and poor accuracy in the determination of individual sample concentrations, both of which will be discussed subsequently, it is not possible to determine which of the assays has greater potential.

One possible point of comparison at this stage is between those samples that were evaluated using both the cranial content and skin methods. (The skin tests were

performed in the same manner as described for the cranial tests in the Materials & Methods section. They were provided graciously by Amber Campbell from her doctoral research project.) Table 4 provides a comparison of the two methods and indicates whether the ELISA was positive or negative for each sample, for each method. Though the inaccuracy of the standard curve hinders in-depth analysis, it is evident that a few samples produced different results (positive in the skin ELISA, and negative in the crania ELISA) for each of the methods. This may be an artifact in the crania ELISA's relatively low sensitivity, or it may denote a legitimate antigen concentration in different body systems of affected individuals. Later discussion of the possible role and implications of the blood-brain barrier in the manifestations of schistosomiasis will delve further into this issue.

Table 4-
Comparison of Skin and Crania Assay Results

<table>
<tr><th>Sample</th><th>Skin</th><th>Crania</th></tr>
<tr><td>NAX 359</td><td>(-)</td><td>(-)</td></tr>
<tr><td>NAX 367</td><td>(-)</td><td>(-)</td></tr>
<tr><td>NAX 561</td><td>(-)</td><td>(-)</td></tr>
<tr><td>NAX 564A-1</td><td rowspan="2">(-)</td><td>(-)</td></tr>
<tr><td>NAX 564A-2</td><td>(-)</td></tr>
<tr><td>NAX 565A</td><td>(-)</td><td>(-)</td></tr>
<tr><td>NAX 568A-1</td><td rowspan="2">(-)</td><td>(-)</td></tr>
<tr><td>NAX 568A-2</td><td>(-)</td></tr>
<tr><td>NAX 581</td><td>(+)</td><td>(-)</td></tr>
<tr><td>NAX 594</td><td>(-)</td><td>(-)</td></tr>
<tr><td>NAX 611</td><td rowspan="2">(+)</td><td>(-)</td></tr>
<tr><td>NAX 611-1</td><td>(-)</td></tr>
<tr><td>NAX 631</td><td>(+)</td><td>(-)</td></tr>
<tr><td>NAX 643C</td><td>(-)</td><td>(-)</td></tr>
<tr><td>NAX 676A</td><td>(+)</td><td>(-)</td></tr>
</table>

Particularly of interest is evaluation of the possibility that concentrations in the skin and cranial content may indicate whether an individual was suffering from acute schistosomiasis (see Background for disease syndrome description) or neuroschistosomiasis.

Sampling Constraints:

The samples used in this work are composed of the preserved tissue and sedimentary contents of the cranial cavity in individual naturally desiccated mummies. Thus, there is some variability in the composition of each sample in terms of what proportion of the sample is from the individual and what proportion is from the earth.

In order to correct for this, the sample concentration may need to be significantly increased. A doctoral dissertation produced by Arizona State University graduate Annalisa Brigitte Alvrus under the supervision of Drs. Merbs, Williams, and Baker suggests that using a larger sample of cranial contents may be key in obtaining more sensitive results. Alvrus performed the ELISA using samples from the Nubian cemetery at Semna South, which dates back to the Meroitic era (350 B.C. to A.D. 350). (2006, iii) She separated the individuals into subadult, adult female and adult male categories and reported the mass and tissue type (brain, skin, and/or muscle) used. On average, for samples composed only of brain tissue, she used 279.2 mg in subadults, 274.8 mg in adult females, and 265.2 mg in adult males, with an overall average sample mass of 271.8 mg. (100-102) Table 5 indicates the number (n) of individuals tested using only brain tissue in each of the subgroups and reports the number of positive and negative ELISAs in each subgroup. In this study, the mean sample mass for cranial content used was 152.5

mg, approximately 119.3 mg less than the mean sample mass from Alvrus's study. These figures make increasing the mass of sample used per trial a likely means of improving the outcome of the ELISA, since increasing the amount used would significantly increase the concentration of the samples used in the assay.

Table 5-
Distribution and Results from Alvrus 2006, including mean sample sizes.

| Subgroup | n | + | - | Mean sample (mg) |
|---|---|---|---|---|
| Subadult | 13 | 6 | 7 | 279.2 |
| Female | 18 | 14 | 4 | 274.8 |
| Male | 23 | 18 | 5 | 265.2 |
| Total | 54 | 38 | 16 | 271.8 |

The Blood-Brain Barrier:

An initial concern in the development of this technique was the presence of the blood-brain barrier, which separates material dissolved in the blood from brain tissue and the spinal cord. While the barrier should not be seen as impermeable, it does have a marked impact on the speed at which compounds dissolved in the blood can diffuse into the brain. (Davson 1976, 1-2)

Additionally, *S. mansoni* is known to effect the brain only rarely in modern populations (da Silva *et al.* 2007, 845). There are essentially only two pathways by which the eggs may make their way to the brain of the infected individual. Transport by the arterial system or venous path, and direct deposition by adult worms migrating to the brain are both possible, but it is not well understood how the adult worms would reach

the brain to place the eggs. (847) When eggs are present in the spinal cord or the brain, either spinal cord neuroschistosomiasis or localized cerebral or cerebellar neuroschistosomiasis (CNS) can result, and they present with distinct signs in both cases. Spinal cord neuroschistosomiasis causes myelopathy, while CNS is marked by seizures and increased intracranial pressure, along with several other signs and symptoms (Nascimento-Carvalho & Moreno-Carvalho 2005, 179).

The precedent of finding eggs and worms in the spinal cord and brain make the blood-brain barrier an unlikely impediment, and there are other incidences of parasitic disease to suggest weakening of the blood-brain barrier during infection. Medana and Turner have shown that in the case of malaria a suite of systemic alterations may lead to an increase in blood-brain barrier penetrability. (2006, 555) Recent work of Tripathi *et al.* also suggests that red blood cells infected with *Plasmodium falciparum* decrease the blood-brain barrier's resistance to being breached (2007, 942).

Again, this is another issue where a comparison between the data for detection in skin tissue provides an interesting point of comparison. Since both Alvrus and Campbell have obtained data using brain, skin, and muscle in Alvrus' case and skin in Campbell's research, it seems that the blood-brain barrier should not present a problem in using the ELISA technique on multiple tissue types, including cranial material. What will be necessary in light of the effect of the blood-brain barrier is a careful modification of the sample size used to find a balance between ideal concentration and wasting limited tissue materials.

Future Directions:

Since there is evidence that all the reagents used are viable (see Results section), the next logical step in furthering this work is to attempt the same procedure as is described above with a larger sample (greater mass of cranial content from each individual). This would give the samples used in the ELISA a greater concentration, and perhaps yield more sensitive results.

An additional component of this study that also requires further investigation is construction of the standard curve for the ELISA. As described in the results section, increasing the concentration of biotinylated 5H11 in the standard curve had no real benefit. As the standard curve is a crucial part of being able to compare results from different ELISAs, it is critical that the inconsistencies be minimized so that a larger sample from the population may be considered in future work.

**Conclusions**

No firm conclusions may be drawn from this work alone, but this study did serve as a strong indicator that the principles and procedure for using enzyme-linked immunosorbant assay to evaluate disease prevalence and relative disease burden are sound. In light of Alvrus's recent work, it seems that increasing mean sample mass, and at the same time, sample concentration, will be sufficient to produce sensitive ELISA data.

Consideration of the ongoing impact of schistosomiasis on quality of life and morbidity worldwide makes the importance of studies that lead to a better understanding of the disease and its origins evident. The purpose of research such as this is essentially two-fold. While it is quite fascinating to better understand a people that have long since passed, the investigation does have significance for people in the here and now. Developing better measures of disease burden and clarifying the difference between the several manifestations of schistosomiasis will recreate the past and have a significant impact on the future.

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