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Evidence for the Endoparasite *Giardia Lamblia* in Human Paleofeces from Salts Cave. Mammoth Cave National Park, Kentucky

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EVIDENCE FOR THE ENDOPARASITE *GIARDIA LAMBLIA* IN HUMAN PALEOFECES FROM SALTS CAVE, MAMMOTH CAVE NATIONAL PARK, KENTUCKY

by

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Three desiccated human feces recovered from Salts Cave, Mammoth Cave National Park, Kentucky, were analyzed for evidence of parasitic infection, specifically *Giardia lamblia*. Positive detection of *Giardia* cysts was made using a direct immunofluorescent assay (IFA) technique. Scanning electron microscopy revealed what appear to be *Giardia lamblia* trophozoites. One additional fecal sample, analyzed for its dietary content, was composed primarily of *Helianthus annuus* (sunflower) and *Chenopodium berlandieri* (goosefoot). Over one hundred seeds of *Cucurbita pepo* (squash) were also contained within this specimen. This sample has been radiocarbon dated to 2420±90 B.P., falling into the cluster of previous dates from Salts Cave.

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CHAPTER I

INTRODUCTION

Giardia is one of the most common intestinal parasites in the world today. Affecting as many as 5 to 50% of persons in the developing world (Dupont and Sullivan 1986), the parasite may have been even more prevalent in prehistoric times, given the persistence of the cysts in water and food sources, and the lack of knowledge about vectors of transmission.

The examination of parasitic or dietary remains in feces can offer new insights into the relative health status of prehistoric individuals and may have implications for modern populations as well. The purpose of this study was to test for the presence of *Giardia lamblia* in human paleofeces. Confirmation of its presence in prehistoric populations may then be used as a starting point for subsequent studies aimed at understanding the relationship of seasonal patterns in dietary habits to the frequency of parasite infection.

In August 1992, the author, with the permission of the National Park Service, entered Salts Cave to recover human paleofeces. Four fecal specimens were collected from Dismal Valley in Upper Salts Cave (Figure 1). One was from a high ledge overlooking the "valley" area below and three others were from the lower area of

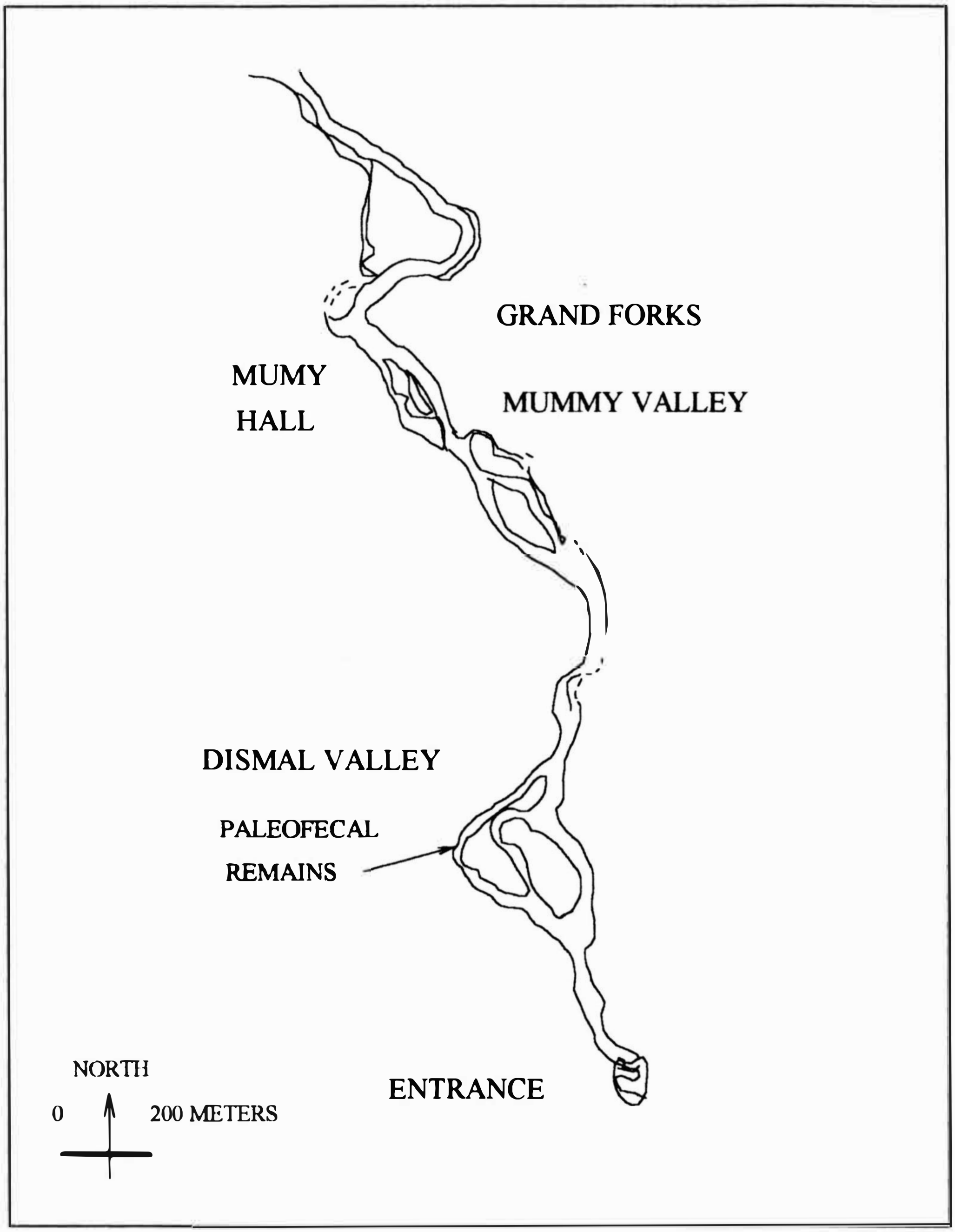

Figure 1. Map of Upper Salts Cave, Showing the Location of Recovered Paleofeces.

Dismal Valley. Three samples were tested for infection with *Giardia*. The fourth was retained for inspection of its dietary components and radiocarbon dating.

Salts Cave is located in Mammoth Cave National Park, Kentucky, midway between Louisville and Nashville, in the west-central part of the state. The region is known for its karstic terrain, marked by sinkholes, caves, and dissected uplands. Salts Cave lies in the bottom of a sink and is dry except for spring water trickling in at the entrance. It consists of three levels, referred to as Upper Salts, Middle Salts, and Lower Salts. Upper Salts, where the present study took place, is characterized by expansive rooms and passages in which the floors are covered in breakdown, or collapsed material from the cave ceiling, in the form of blocks, slabs, and chips. Middle and Lower Salts contain canyon-like passages which are occasionally disrupted by breakdown from Upper Salts.

Prehistoric utilization of Salts Cave has been documented since the late 1800's. Occupation of the cave seems to have been centered around mineral mining during the Late Archaic and Early Woodland time periods. Attempts at commercialization of Upper Salts during the 1920's and 30's were unsuccessful, leaving many items well preserved, and those in the lower passages largely undisturbed.

The obviously limited availability of fecal remains requires that research be interdisciplinary, providing information on a number of subjects, so that specimens are not needlessly depleted. The small number of specimens utilized in this study will allow others to remain *in situ*. While four samples do not provide a statistically representative sampling of diet and disease within a population, they are an important resource for future studies to which advanced technological methods can be applied.

Research on the prevalence of parasitic infection in human fecal remains from archaeological contexts is lacking. While a handful of parasitological studies do exist, most are on specimens from the southwestern United States where dry caves and rock shelters are common. Only two documented cases of the presence of *Giardia* in a prehistoric context can be found. Witenberg (1961) found cysts of *Giardia lamblia* in feces from a nearly two thousand year old mummy from a cave in Israel and Faulkner (1989) located cysts of *Giardia* in a 2500 year old specimen from Big Bone Cave, Tennessee. Past examinations of human paleofeces from Salts Cave have revealed only one specimen with a possible roundworm infection. S. Tankersley (1993) has found live tuberculosis in historic fecal material from Mammoth Cave, Kentucky. The absence of studies on *Giardia*, combined with recent developments in the identification of parasites from fecal samples, further emphasizes the need to apply these techniques to prehistoric contexts. Parasite infections have recently been located in mummy gut contents, dung and cesspit deposits, skeletonized burials, and soils associated with burials (Reinhard, Geib, Callahan, and Hevly 1992; Schelvis 1992; Warnock and Reinhard 1992; Holden and Nuñez 1993).

Subsistence and mobility changes during the Late Archaic/Early Woodland transition caused gradual population growth and subsistence changes that led to new settlement choices. Major river valleys eventually became filled to capacity, forcing fringe populations to move into smaller valleys or even forested upland environments, such as the area around Salts Cave. As populations grew, the energy required to obtain food resources also increased. Many investigators have suggested that at the time of the Late Archaic/Early Woodland transition, a decrease in mobility was occurring along with an increased dependence on plant husbandry, as indicated by the domestication of local cultigens and the infusion of plants from Mexico (Munson 1988; Watson 1988, 1989). If these models are accurate, one should expect to find the presence of cultigens and parasitic diseases in the fecal remains of sedentary or semi-sedentary groups.

The majority of food remains recovered from feces and excavated test pits in Salts Cave imply the use of storable food sources ideal for caving or for populations occasionally on the move. Storable foods would have been harvested in the fall, most likely between October and November, and may have been dried or parched before consumption or storage. The motivation for cultivation and domestication of these dietary items may have been due to their usefulness as easily stored and transported trail foods.

More than 300 years ago, the first description of the intestinal protozoan *Giardia* was made. In a letter to the Royal Society in 1681, Anton van Leeuwenhoek described his normally good health, but reported that he had been affected by a looseness of stools which sometimes lasted for three days. He was also unable to keep food in his body for longer than four hours. On several occasions he examined his own stools due to their watery nature (a sign of giardiasis). In his letter, he recorded the following description:

> ...wherein I have sometimes also seen animalcules a-moving very prettily; some of 'em a bit bigger, others a bit less, than a blood-globule, but all of one and the same make. Their bodies were somewhat longer than broad, and their belly, which was flatlike, furnisht with sundry little paws, wherewith they made such a stir in the clear medium and among the globules, that you might e'en fancy you saw a pissabed running up against a wall; and albeit they made a quick motion with their paws, yet for all that they made but slow progress (Erlandsen and Feely 1984:33-34).

Giardia lamblia (human strain) are protozoan flagellates which have two stages: (1) a mobile and actively feeding trophozoite (9-21 microns long and 5-15 microns wide) found on the surface of the small intestine, and (2) an infective oval-shaped cyst form which is smaller (8-12 microns long and 7-10 microns wide) and contains two to four nuclei (Dupont and Sullivan 1986). Clinical manifestations of *Giardia* infection range from asymptomatic cyst-passing with mild gastrointestinal complaints to severely acute cases involving explosive, watery and foul-smelling diarrhea with abdominal cramps as well as bloating, flatulence, anorexia, and nausea. Symptoms of acute or subacute infection may persist for several days to several months (Stevens 1985; Dupont and Sullivan 1986).

The period from initial exposure to infection varies from 3-42 days with an average of eight days. A mild infection may not produce intestinal symptoms. If untreated, giardiasis symptoms disappear and reappear; with treatment recovery is complete (Dupont and Sullivan 1986). So, even if a small outbreak occurred in a population, untreated giardiasis could remain with a group of people for an extended period.

One would expect to find this endoparasite in the archaeological record of Salts Cave since the probability of infection occurring is directly correlated with a decrease in mobility and an increasing dependence on cultigens. A more sedentary lifestyle or semi-sedentary livelihood is suggested by cultigens of this time. A population that is sedentary or semi-sedentary increases its risk of parasitic infection due to increased transmission vectors, such as a larger population constantly reinfecting itself through fecal-oral contact, uncooked foods containing cysts, or a contaminated water supply. The present study involves the analysis of four fecal samples from the interior of Salts Cave for the purpose of ascertaining whether *Giardia* is present.

CHAPTER II

STUDY AREA

Location

Salts Cave is located in Mammoth Cave National Park, Kentucky, approximately 1.5 km south of Green River and just inside the eastern boundary of Edmonson County. The Mammoth Cave Historic Entrance is about 2 km to the southwest. Encompassing 52,830 acres (Robert Ward, personal communication 1994), the Park lies in the most extensive limestone karst region of the United States. The solution of limestone by underground water has created the longest known series of caves in the world (Harris and Tuttle 1990). The Flint Ridge-Mammoth Cave System presently contains 345 miles of surveyed passageways, about twelve of which are open to the public. The interconnected passages of this system include the Flint Ridge System, made up of Salts, Austin, Unknown, Bedquilt, Crystal, and Colossal Caves; Proctor Cave; Morrison Cave; and the River System. It was not until 1972 that a connection was found between Mammoth Cave in Mammoth Cave Ridge and Salts Cave in Flint Ridge (Kennedy 1990).

Managed and protected by the National Park Service, Salts Cave is not open to the public. The cave is accessed through a sinkhole, which represents the upper portion of a valley which previously flowed toward Green River (Lobeck 1928), and one enters by unlocking an approximately one meter by one meter metal grate and lowering oneself onto breakdown, about 1.5 meters below ground surface (Figure 2). The entrance is wet, with a small waterfall during wet seasons. A steep descent is made into the vestibule area where the cave is dry.

Figure 2. Entrance to Salts Cave.

The temperature (approximately 59°F) and humidity of the cave are nearly constant (Watson 1969) and, because the cave interior is completely protected from elements such as wind, rain, temperature fluctuations, and sunlight, the preservation of prehistoric remains is often excellent.

Salts Cave was commercialized and shown to the public in the early 1900's (Watson 1969, 1974; Kennedy 1990), however, caves such as Mammoth, with better advertising and ease of access saw many more visitors. Consequently, the amount of disturbance in Salts Cave was lessened, allowing most prehistoric and historic artifacts and features to remain undisturbed. Even in areas where debris has been collected or exploited, a large amount of prehistoric material still remains (Watson 1985).

Environmental Setting

Kentucky is centrally located in the Deciduous Forest Formation of eastern North America. It contains parts of two forest regions, the Mixed Mesophytic in eastern Kentucky (east of the Pottsville Escarpment) and the Western Mesophytic in the rest of the state (Wharton and Barbour 1973). Mammoth Cave National Park lies within the Western Mesophytic region, although it lies on the western boundary of the Mixed Mesophytic, revealing a combination of both regions. Much of the Park area is presently covered in secondary growth represented by oak, hickory, sassafras, and dogwood (Watson 1969; Sutton & Sutton 1993). Archaeological investigations of the area suggest an oak-hickory woodland around Salts Sink during the first and second millennia B. C. (Watson 1974:235).

Fauna of the region include woodchucks (*Marmota monax*), Eastern Cottontail (*Sylvilagus floridanus*), White-tailed Deer (*Odocoileus virginianus*), Gray Squirrel (*Sciurus carolinensis*), Red Fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), and Virginia Opossum (*Didelphis virginiana*) (Sutton and Sutton 1993). Prehistorically, remains of deer, turkey, small mammals, insects, amphibians, reptiles, and river mussels have been recovered from excavations within the Vestibule of Salts Cave (Watson 1974).

Geology

Mammoth Cave National Park is in west-central Kentucky in a hilly plateau region of limestone bedrock, capped by insoluble clastic rocks, at the southeastern edge of the Illinois Basin. This plateau lies a few miles west of the Dripping Springs Escarpment. The sedimentary rock of the region gently slopes to the northwest. Insoluble rocks have been exposed in irregular ridges, leaving a rugged, hilly area called the Chester Upland. To the southeast lies the Pennroyal Plateau, caused by the erosion of insoluble rocks at the surface (Lobeck 1928; McGrain and Livesay 1962; Shimer 1972). The landscape comprised by the Chester Upland and the Pennroyal Plateau reveals the domination of solutional features, such as caves, closed depressions, sinkholes, and large springs (Harris and Tuttle 1990).

Underground water is necessary for cave formation, although an outlet at the surface must exist if there is to be active water movement. The Green River valley and its tributaries supply this outlet. As the various levels of a cave progressively form, the upper levels become drier due to water seeping into the lower areas (McGrain and Livesay 1962). The upper levels of Salts Cave were formed between 1 million and 10 million years ago and have been dry since water created lower levels, such as Middle and Lower Salts, in the limestone (Palmer 1981). This dry environment is highly conducive to the preservation of organic materials such as human paleofeces.

Cultural Context

Numerous Late Archaic/Early Woodland radiocarbon dates are available for material from Salts Cave (Watson 1969; Kennedy 1990). Ranging from approximately 3490 B.P. (1540 B.C) to 1920 B.P. (30 A.D.), the dates were obtained from paleofeces, charcoal, human tissue, and other material. Although the radiocarbon dates on paleofeces will be discussed in Chapter VII, they are noted here to demonstrate the relationship of Salts Cave occupation to the archaeology of other contemporaneous sites in Kentucky.

Mammoth Cave, Kentucky, located only a few miles from Salts Cave reveals a Terminal Archaic/Early Woodland culture very similar to that seen at Salts (Watson 1974). Indian Knoll, Kentucky, on Green River, had no pottery and was considered Late Archaic (Webb 1946). Newt Kash Hollow, Kentucky, dated to 2650±300 B.P. and 2600±300 B.P., also revealed no pottery (Seeman 1986). A large fragment of worked wood from Indian Avenue in Salts Cave, identified as a cradleboard, is similar to one from Newt Kash Hollow Shelter (Webb and Funkhouser 1936). However, Cloudsplitter, in Kentucky, dated to 2513±80 B.P., did have thick, grit tempered pottery (Seeman 1986:566). The dates obtained thus far from Salts Cave are comparable to dates associated with the above-mentioned sites.

The dates from Salts Cave span a possible 1570 year period, with a mean age of 2584 B.P. (634 B.C.) for all thirty eight dates, and 2480 B.P. (530 B.C.) on the twelve dated paleofeces. Therefore, prehistoric utilization of Salts Cave appears to coincide with the Late Archaic or Early Woodland period.

Activity in Salts Cave seems to have been centered on mining of gypsum and mirabilite (Watson 1969, 1974, 1986; Munson, Tankersley, Munson, and Watson 1989; Tankersley, Frushour, Nagy, Tankersley, and Tankersley 1994; Tankersley in press). This view seems warranted in light of similar activities taking place in Mammoth Cave and possibly in Wyandotte Cave in southern Indiana, due to large quantities of aragonite, chert, and epsomite (Watson 1969, Tankersley, Munson, Munson, Shaffer, and Leininger 1990; Tankersley and Shaffer 1990). Faulkner (1989) makes a similar interpretation for Big Bone Cave, dated from 3000 B.P. to 900 B.P., due to the abundance of sulfate minerals available.

Therefore, the presence of digging sticks, digging stick marks, knee prints where a miner presumably knelt while scraping sediment from cave walls, spoil piles, and gourd containers in Salts Cave, leads one to believe that the main purpose for entering the cave was to remove sulfate minerals. There is evidence that the collection of gypsum, mirabilite, and other cave minerals was an organized activity which allowed these minerals to be passed on to other regions through well-developed trade networks that occurred during the Terminal Archaic period (Munson *et al.* 1989).

The introduction of pottery is the most commonly used marker for the beginning of the Early Woodland period (Griffin 1967:180), however, its presence is seen at different times in different locations. Data suggest a date of around 1000 B.C. in the upper Ohio Valley and 500 B.C. in the lower Ohio Valley for the beginning of this period. Early Woodland pottery has been found in diverse topographic settings, including river floodplains, rockshelters, and upland forests (Seeman 1986:564). Evidence for pottery in Salts Cave is lacking, however. Young (1910:322) reports one pottery vessel from Salts Cave. The context is not clear, however, and one cannot rely on its presence to define cultural context.

Large specimens of *Cucurbita*, as have been recovered from Salts Cave, would have made durable light-weight containers. Such vessels appear to have been used during the same time period when the first ceramic vessels begin to appear in Terminal Archaic/Early Woodland contexts (Cowan 1993). It is probable that the inhabitants of Salts Cave made use of wood, gourds, and thick-shelled squashes for containers. If pottery was being used, it seems likely that evidence of a number of vessels would have been found somewhere in the cave in light of all the other materials found.

Presumably, if one were to go caving, a minimal number of items would be carried. One would not expect to find pottery, an item which is fragile and awkward to transport. On the other hand, squash or gourd could be used as containers. The insides could be eaten, leaving a sturdy, light-weight container for the collection of mirabilite (Watson 1969; Tankersley, Bassett, and Frushour 1985).

Perhaps the absence of pottery is related to the type of site. While a long-term occupation site should reveal pottery, one based on the procurement of minerals, possibly a satellite group associated with a larger settlement, may show signs of squash or gourd containers rather than pottery.

Salts Cave appears to have been utilized throughout the entire Late Archaic/Early Woodland transition. Thousands of datable prehistoric remains have been recovered from the cave, including a large number of human paleofeces which reveal the consumption of cultigens as well as gathered and hunted foods. Through radiocarbon dating of these items, one can arrive at an accurate time frame in which to place exploitation of the cave.

CHAPTER III

LITERATURE REVIEW

Early Studies

An understanding of the potential for recovering useful information from prehistoric feces existed as far back as the 1890's when Harshberger (1896:150) proposed that identifiable seeds consumed as food might be recovered from fecal remains. Young (1910:324), in his description of Salts Cave, mentions the examination of human feces, suggesting those sunflower seeds, hickory nutshell, and "watermelon" (actually squash), were important food sources for early cavers. Jones (1936) compared vegetal remains from feces, with plant materials from middens in Newt Kash Hollow Shelter, Kentucky. His observations of seed size led him to conclude that the seed remains present in the feces were domesticated and had been cultivated for large seed size. Wakefield and Dellinger (1936) studied the fecal contents of a mummy recovered from a rockshelter in the Ozark Mountains of Arkansas, identifying sumac and acorn. They unsuccessfully cultured microorganisms from the feces. Fecal contents from caves in eastern Kentucky were looked at by Webb and Baby (1957). They suggested that early cavers had a diet of goosefoot and sunflower seeds along with several kinds of insects. Early studies tended to be descriptive, focusing on what prehistoric individuals ate.

Scientific Trends

While Harshberger (1896) and Young (1910) broke open feces to view the contents and Jones (1936) crushed feces with a mortar and pestle, Neolitzky reconstituted dried intestinal contents with hydrogen peroxide as early as 1912 (Wilke and Hall 1975). However, it was not until forty years ago, that more advanced methods of analysis were introduced. Callen and Cameron (1960) showed the significance of rehydrating desiccated feces in a 0.5% trisodium phosphate solution. The solution allows fecal remains to gently break apart, eliminating the need for mechanical fragmentation. Original shape and size are maintained by using this procedure. Van Cleave and Ross (1947) had previously used this method to rehydrate desiccated zoological specimens. Other investigators, such as Yarnell (1969) and Fry (1977) have used dry separation analyses, in which, as in the early studies, samples were broken open and the contents examined.

A method for reconstructing prehistoric diet through fecal odor has recently been utilized by Moore, Krotoszynski, and O'Neill (1984). Through the use of headspace gas-chromatographic analysis, an association between dietary intake and fecal odor has been confirmed. Carnivore, herbivore, and omnivore feces each have distinct odors.

Martin and Sharrock (1964) were the first to use pollen analysis on human fecal material from the Glen Canyon area. Bryant (1974a) performed pollen analyses on coprolites from southwest Texas.

Radiocarbon dating has played a large role in the advancement of methods for studying paleofeces since its development in the 1940's. Faulkner (1989) documented radiocarbon dates from Big Bone Cave fecal specimens. Kennedy (1990) analyzed the radiocarbon dates from Salts and Mammoth Caves. Kennedy, Watson, Tankersley, and Ward (in review) report new accelerator radiocarbon determinations for these caves.

Advanced techniques, such as immunofluorescent assays, have been utilized in the last twenty years to better aid in diagnosing infection with *Giardia*. Ridley and Ridley developed an Indirect Immunofluorescence Test (Visvesvara and Healy 1984). Even more recently, a Direct Immunofluorescent Test, a simpler method, has been developed which allows antibodies to be directly conjugated with fluorescein (Sandy Greco, personal communication 1992).

Parasitological Studies

Organisms of the genus *Giardia* were first observed in 1675 by Anton van Leeuwenhoek who found cysts of *Giardia* in his own stool. In 1859, Lambl formally described the shape, size, and motile behavior of the parasite (Feely, Erlandsen, and Chase 1984). Light microscopic descriptions of the cyst and trophozoite have been published by Simon (1921) and Filice (1952).

Giardiasis affects millions of people in most parts of the world. Contaminated food, untreated surface-water polluted with cyst-containing animal feces, combined with inadequate filtration in water treatment facilities are the primary sources. In addition, public health authorities include giardiasis as a sexually transmitted disease (Stevens 1985).

According to Dupont and Sullivan (1986), the percentage of asymptomatic individuals excreting *Giardia lamblia* in industrialized countries is estimated to be between one and seven percent. The frequency for parts of the developing world is higher, ranging from 5-50% of individuals. The source for *Giardia* transmission in industrialized areas of the world is water contaminated by infected persons and probably lower mammals. Dupont and Sullivan (1986) considered the effects of *Giardia* in the developing world, regarding it as an important problem. Mothers and infants in Bangladesh were monitored for a year and *Giardia lamblia* was found to be excreted in 82% of the mothers and 42% of the infants. Many animals asymptomatically excrete an organism morphologically indistinguishable from *Giardia lamblia* strains associated with human infection. Large community-wide outbreaks of infection occur when central water systems become contaminated with *Giardia* cysts (Istre, Dunlop, Gaspard, and Hopkins 1984).

It has recently been realized that *Giardia lamblia* can be an important cause of mortality in the developing world, as well as in the Rocky Mountains of the United States and Canada, in day care centers in urban areas and in residential institutions for the mentally retarded (Dupont and Sullivan 1986). The presence of *Giardia* in remote and unpopulated areas of the Rocky Mountains suggests that surface water contamination by lower mammals is important to the epidemiology of infection in these areas. Beavers are suspect as vectors for such water contamination. Direct fecal-oral, person-to-person transmission of *Giardia* commonly occurs in both rural and urban settings where household or institutional crowding is combined with poor hygienic practices (Bemrick 1984).

In the United States, giardiasis occurs most frequently in travelers who have recently returned from endemic areas. Children are more likely to become infected with *Giardia lamblia* than adults, probably because of frequent hand-to-mouth activity. In addition, hypogammaglobulinemia (decreased gamma globulins in the blood, resulting in a state of immunodeficiency) also appears to predispose individuals to this infection. Gamma globulin is a protein formed in the blood that has the ability to resist infections. Importantly, giardiasis does not confer immunity and, therefore, reinfections may occur (Stevens 1985).

Many isolated incidents of hikers and campers becoming infected with *Giardia* from apparently clear surface water in remote rural areas have been reported. Swimming in contaminated water (including chlorinated pools) has been implicated as a source of infection. Certain foods may present a higher risk for contamination with *Giardia* cysts. Parasites are usually killed when food has been heated thoroughly, so infections occur mostly when underheated or raw foods are ingested (Bemrick 1984).

Giardia investigations proceeded at a constant rate until the mid 1960's, when research on this parasite decreased considerably. Work with *Giardia* was continued over the next decade by a small group, without much financial support. One reason for this lack of support was the belief that the organism was of no public health significance in the United States, or at least in developed countries. Many considered it an exotic parasite which sometimes produced diarrheic symptoms in travelers. *Giardia* research did progress and a large amount of significant information continues to develop (Bemrick 1984).

Paleofeces containing parasite products are known from several localities in the midwestern and eastern United States. Mites and eggs similar to those of the common intestinal roundworm (*Ascaris lumbricoides*) have been recovered in feces from Salts and Mammoth Caves (Fry 1974; Yarnell 1969). Evidence of infection with parasitic protozoa is known from several studies of prehistoric fecal material. In one such study, Witenberg (1961) examined two 1800 year old fecal samples from a cave in Israel and found cysts of *Giardia lamblia*. Faulkner, Patton, and Strawbridge Johnson (1989), in a study of material from Big Bone Cave, Tennessee, found eggs of *Enterobius vermicularis* in five of eight feces tested. Also seen were eggs resembling those of the intestinal roundworm, *Ascaris lumbricoides*, Rhabditiform larvae, and Ancylostomoid egg (human hookworm) with larvae. One specimen contained cysts of *Giardia intestinalis*.

Samuels (1965) found eggs of the human pinworm, *Enterobius vermicularis*, in one specimen and numerous nematodes (roundworms) burrowed into another specimen, from the Mesa Verde cliff dwellings. Reinhard, Ambler, and McGuffie (1985) looked at parasitism and its relation to diet at Dust Devil Cave in southern Utah. A correlation was shown between large quantities of *Chenopodium* seeds and an absence of parasitic roundworms. Williams (1985) found evidence for a possible calcified hydatid cyst from a Woodland burial in North Dakota.

While this represents only a small portion of related literature, it demonstrates the potential for archaeo-parasitological research. The recent development of techniques which aid in the detection of parasites further emphasizes the possibilities for applying these approaches in prehistoric contexts.

Dietary Studies

Before the introduction of maize (about 400 A.D.), gardens of the eastern woodlands of the United States contained only five species of plants associated with domestication: (1) bottle gourd (*Lagenaria sicaria*), (2) squash (*Cucurbita pepo*), (3) marshelder (*Iva annua*), (4) sunflower (*Helianthus annuus*), and (5) a starchy seed annual, goosefoot (*Chenopodium*) (Watson 1989).

The importance of food storage as early as 3,000 years ago is evident from a cache of five bags of domesticated chenopod, marshelder (sumpweed), sunflower, and *pepo* squash/gourd seeds found in a crevice in Marble Bluff rockshelter in Arkansas (Fritz 1993), a Terminal Archaic occupation from an upland environment. These seeds are larger than any others from the Terminal Archaic.

A sample of a basket of approximately 50,000 carbonized fruits of *Chenopodium berlandieri* recovered from Russell Cave, Alabama, represents the earliest known evidence for the deliberate storage of this plant species by prehistoric populations of the eastern woodlands (Smith 1984). Similar thin testa chenopod fruits have been recognized in Terminal Archaic (2700-2000 years ago) archaeobotanical collections from Salts Cave, Kentucky, suggesting the possible presence of a domestic chenopod by this early time (Yarnell 1983). Findings from Ash Cave, Ohio, also point to deliberate storage (Smith 1985).

Early Woodland seed size increased significantly from Late Archaic sizes, reflecting a corresponding increase in overall fruit size. Only five Early Woodland aged squash seeds were found at Cloudsplitter, but they are 30% larger in mean length and 37% larger in mean width than those from the Archaic (Smith and Cowan 1987).

At Rogers shelter in Missouri, 363 complete seeds produced measurements with mean dimensions approximately 25% larger than the Archaic seeds from Cloudsplitter (Fritz 1993). Similarly aged *Lagenaria* and *Cucurbita pepo* fruits recovered in Salts Cave provide evidence that large fruits were sometimes used as vessels (Watson 1969; Tankersley *et al.* 1985).

Numerous studies have been conducted in the Southwest. Over six thousand human coprolites, dating from 2500 B.C. to the present, were recovered from Lovelock Cave, Nevada, by Heizer (1967). Additional specimens were collected from Hidden cave, Humboldt Cave, and several other cave and shelter sites in this region. Reports detail the extremely diversified western Great Basin diet, consisting largely of aquatic items.

Previous Studies at Salts and Mammoth Caves

Prehistoric remains have been known to exist in Salts Cave since the late eighteenth century (Young 1910). One of the earliest accounts of Salts Cave, from Putnam (1875), discusses the "relics" observed there. Nelson (1917) excavated near the Historic Entrance to Mammoth Cave. He makes a brief mention of a visit to Salts Cave. Pond's work in 1937 relates the discovery of Lost John of Mummy Ledge, a prehistoric gypsum miner whose body was found by Grover Campbell and Lyman Cutliffe while exploring Mammoth Cave. Schwartz (1960) describes the history of Mammoth Cave National Park, particularly of Mammoth Cave.

Only a minimal amount of archaeological work had been done in the Mammoth Cave area prior to the 1960's. It was not until 1963 that any type of systematic study was undertaken in Salts Cave. Hall and Watson performed surface collection around Salts Sink, test excavations in the Vestibule of Salts Cave, and made detailed observations of remains found in the passages of Lower, Middle, and Upper Salts Cave (Watson 1969). The survey included the collection of desiccated human paleofeces which Callen investigated for evidence of dietary remains. Five fecal specimens were sent to the University of Michigan for radiocarbon dating (Watson 1974).

Further investigations in Salts Cave are reported in Watson's, *The Prehistory of Salts Cave* (1969). In this volume, Cockburn revealed that his examination of nine human paleofeces found no identifiable parasites. The Illinois Department of Public Health's examination of thirteen fecal specimens also found negative results, although one specimen had a moldy-looking growth. Kaiser identified mites of the genus *Protolaeps*.

Fry (1974) examined eight specimens from Upper Salts Cave for parasites and ova. One sample contained what appeared to be several eggs of *Ascaris lumbricoides*, or roundworm, which has been found in many parts of the world, thriving in warm, moist climates where soil is infected with feces.

Yarnell (1969, 1974b) looked at over 100 human fecal specimens, noting only minimal amounts of squash seeds. Remains ranged in size from .5mm long mites, to pieces of hickory nutshell up to one centimeter in diameter. Seventeen different plant foods were identified, including two nuts, six berries, and seven seeds.

The most abundant items were sunflower achenes (up to 10mm long), chenopod seeds, marshelder (sumpweed) achenes, and hickory nutshell. These four made up about 80% of the total bulk of the specimens. Two fecal specimens were composed almost entirely of hickory nutshell. The Salts Cave studies show that sunflower and marshelder achenes, maygrass and chenopod seed, and hickory nuts were important in the diet of the cavers (Yarnell 1974b).

Robbins (1974) and Yarnell (1974a) reported on the intestinal contents of a mummified body, Little Al, found in Salts Cave. Contents of Little Al's intestines were dominated by hickory nutshell (71% by weight) followed by sumpweed (25.4% by weight), carbonized material (2.3%, mostly hickory nutshell), chenopod (0.6%, about 100 seeds), and 0.6% unidentified material. There was no evidence of sunflower (Yarnell 1974a).

While removing tissue for dating, a small amount of fecal material was collected from the lower intestine of Lost John, a mummy found in Mammoth Cave. Food remains recovered include, from greater to lesser quantity: (a) sunflower, (b) sumpweed, (c) hickory nut, and (d) chenopod. By weight, sunflower pericarp (composed of 2000 sunflower achenes) made up 70% of the sample (Robbins 1974), contrasting with remains from the Salts Cave mummy.

Investigations have occurred on a smaller scale since the studies of the 1960's and 70's. Research has tended to focus on plant domestication in eastern North America and its chronology (Gardner 1987; Yarnell 1974b, 1983; Fritz 1990). Munson *et al.* (1989) and Tankersley (in press) have studied the systematic mining of mirabilite and gypsum from the walls of Salts and Mammoth Caves. S. Tankersley (1993) has shown evidence for live mycobacterium tuberculosis in the Euroamerican feces of TB patients in Historic Mammoth Cave. Tankersley *et al.* (1994) examined internal tissue from a mummy, Little Al, finding that the blood associated with these tissues suggests he died of an internal hemorrhage, possibly due to a fall from a high ledge. Kennedy *et al.* (in review) have reported four new radiocarbon determinations for Salts and Mammoth Caves.

CHAPTER IV

MATERIALS AND METHODS

Collection of Materials

The four prehistoric feces discussed in this study were collected by the author, in the company of several other researchers, in August 1992. Collection of the specimens occurred in an area known as Dismal Valley, approximately one thousand meters from the cave entrance. All specimens were photographed *in situ* and their respective locations recorded. Plastic bags were used to transport specimens from the cave interior to the research laboratory. Length and width measurements of each was made using a Vernier caliper. An Ohaus Port-o-Gram electronic scale was used to weigh the specimens. A brief description of each is provided below.

Specimen Descriptions

Specimen One

This specimen has the appearance of a coiled snake. It retains the shape of the lower bowel and is cylindrical. According to Faulkner *et al.* (1989; Fry 1985), this shape and form are indicative of a complete stool that is normal and healthy. The specimen was found lying on a large breakdown slab in Lower Dismal Valley, approximately one meter from a wall that supports a high ledge (or Upper Dismal Valley). Specimen one weighs 10.47 grams and has a length of 8.2cm and a maximum width (coiled) of 5.7cm.

Specimen Two

This specimen is slightly smaller than specimen one. It is cylindrical although it is twisted near the midsection. This specimen is narrower than the others, suggesting a possible association with a juvenile. It appears that part of the sample may have broken off on one end. This specimen was collected in Upper Dismal Valley, a long ledge two to three meters wide. The specimen was found lying on a piece of breakdown. The ledge is approximately 10-20 meters high and looks across lower Dismal Valley to a high-ledge mineral mine that appears impossible to access without an extended and sturdy ladder. Total weight of this specimen is 9.98 grams. A length of approximately 7cm and a width of 4.2cm at the widest portion (twisted area) were determined.

Specimen Three

This specimen was located approximately fifteen meters from the area used by researchers to descend into Lower Dismal Valley. The specimen was discovered between several pieces of breakdown. Specimen three is broad and cylindrical, with tapering at one end. The total weight for this specimen is 10.95 grams with a length of 9.02cm and a width of 4.2cm at the broadest section.

Specimen Four

This specimen, from Lower Dismal Valley, was collected several meters from a cedar climbing pole located below a high-ledge mineral mine. The largest of the samples, specimen four is somewhat ovoid with a taper at one end where it exited the colon. The broad and rounded shape of this stool indicates that it was probably soft when deposited. It is likely that this specimen represents a complete stool. The total weight of this sample is 13.8 grams. The approximate full length is 7.5cm, with the tapered end measuring 1.5cm. The maximum width, which is consistent for three-fourths of the specimen, is 5.25cm.

Rehydration

One of the most important steps in the analysis of paleofecal material is to determine that each specimen is of human origin. There are several criteria which allow for the differentiation of human from non-human feces with a degree of certainty. These include: (a) size and external morphology, (b) fecal content indicative of an omnivorous diet, (c) evidence of infection with human parasites, and (d) changes in the odor, color, and translucency of the feces in a trisodium phosphate solution. Changes in color and translucency of the solution used to reconstitute archaeological feces are the most widely acknowledged indicators of human origin. Human feces should turn the rehydrating solution an opaque dark brown-black and fecal odor should return (Fry 1977, 1985; Bryant 1974b).

Each specimen was photographed in the lab and the desiccated lengths, widths, and weights recorded prior to rehydration, or reconstitution.

Specimens one, two, and three were each bisected longitudinally and one half of each sample was placed in a glass beaker with a 0.5% solution of trisodium phosphate for approximately 72 hours, as suggested by Callen and Cameron (1960). Only one gram of specimen four was rehydrated so that a large portion of it could be retained for radiocarbon analysis.

Color, opacity, and odor were monitored at immersion and at 24 hour intervals for the following 72 hours. The paraffin covering the beakers was removed daily to determine if odor had become stronger. A Munsell soil color chart was referenced to record color changes, according to the method of Fry (1985). Beakers were gently agitated each day for approximately 10 seconds to aid in breaking up the fecal component.

A canine fecal sample was dried on a hot-plate and subsequently placed in a 0.5% trisodium phosphate solution for 72 hours to act as a control. The sample was likewise monitored from the beginning of immersion, at 24, 48, and 72 hour increments. A Munsell color chart was again used to describe color changes occurring in this specimen.

Specimens one, two, three, and four turned the rehydrating solution an opaque, dark brown-black color. Odor had become noticeable in the specimens after 48 hours, but intensified after 72 hours.

Several dry mounts were prepared from the remaining bisected samples of specimens one, two, and three for scanning electron microscopy (SEM). SEM work was performed at Wright State School of Medicine in Dayton, Ohio, by Dr. Frank Nagy (Department of Anatomy).

Parasitological Analysis

After reconstitution, each sample was washed with distilled water and fixed in 10% formalin based on the concentration method used by Ritchie (1948), for analysis of parasitic infection using immunofluorescence. The sample remained in formalin for 30 minutes so that the preservative was allowed proper fixation.

Meridian Diagnostics in Cincinnati provided a Direct Immunofluorescent Detection Kit which identifies the presence of *Giardia* cysts in stool specimens. This particular kit, Merifluor *Cryptosporidium/Giardia*, simultaneously detects *Cryptosporidium* oocysts and *Giardia* cysts in fecal samples.

A drop of formalin-fixed fecal material was placed in a treated slide well, along with a positive control provided by Meridian and a negative control from a canine. Two slides were prepared for each specimen using this procedure. Material was spread over the entire surface of the well and allowed to air dry at room temperature. One drop each of detection reagent and counterstain was applied to each slide well. The detection reagent contained a mixture of labeled monoclonal antibodies which are directed against the cell wall antigens of *Giardia* cysts. These monoclonal antibodies attach to *Giardia* antigens present in the specimen.

The reagents were mixed with an applicator stick and spread over the entire slide well. The slides were then incubated in a humidified chamber for 30 minutes, where they were also protected from light exposure. A wash buffer was used to rinse the slide well to remove excess reagent and counterstain, and excess buffer was removed by tapping the edge of the slide on a paper towel. The slides were not allowed to air dry. Finally, one drop of mounting medium and a coverslip were added to each well to prevent contamination between wells.

Each slide was examined at 100-200X for evidence of *Giardia* cysts under an epi-fluorescent microscope. Black and white negatives were made using Kodak 35mm T-Max 400 film to document the size and shape of fluorescing cysts and oocysts. Specimens were further examined using scanning electron microscopy to locate trophozoites.

Dietary Analysis

Specimens One, Two, and Three

Scanning electron microscopy was used to examine the dry mounts. Electron micrographs were taken of items resembling botanical remains.

Specimen Four

Specimen four was carefully dissected using a dental pick and tweezers, for visual inspection of intact food remains. Seeds were placed in vials according to various types. *Cucurbita pepo* and *Helianthus annuus* seeds were counted and length/width measurements were made on all complete seeds, using a Vernier caliper. Seed genus names were verified by Marjorie Schroeder (Botanical Laboratory, Illinois State Museum Research and Collections Center) using a light microscope. Black and white photos were made for documentation purposes using Kodak 35mm T-Max 400 film (Figure 3).

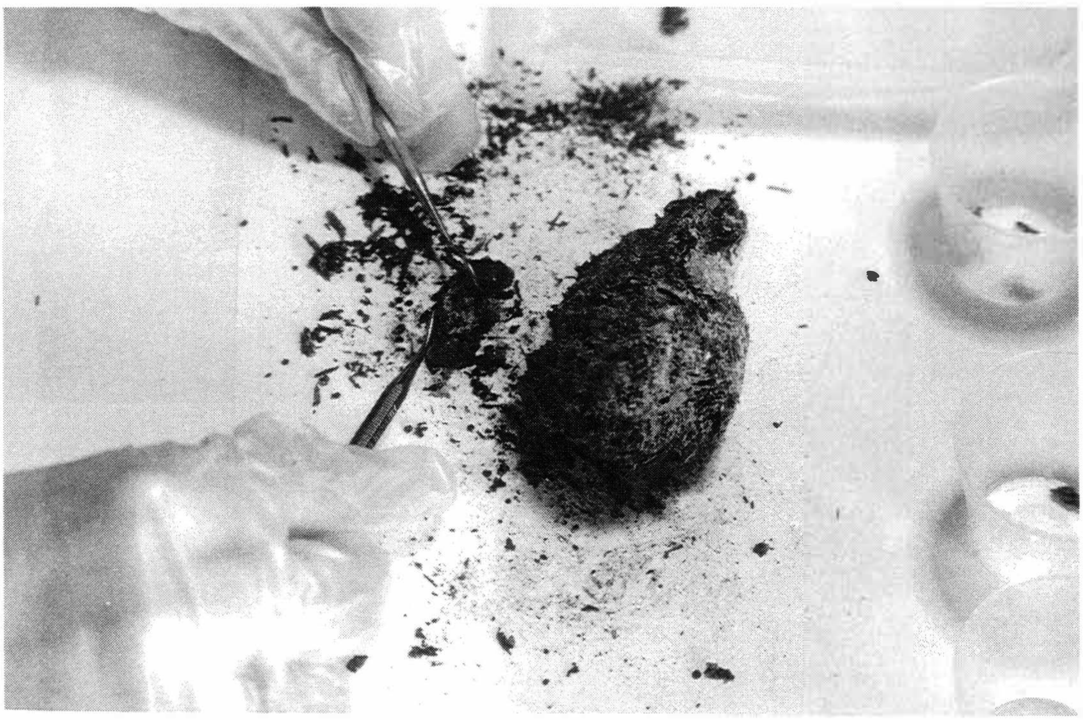

Figure 3. Photograph Showing the Dissection of Specimen Four.

ANOVA: Probability of Significant Difference tests were performed on complete *Cucurbita pepo* seeds from this specimen to determine seed variety. This was undertaken by Dr. Irwin Rovner (Department of Sociology and Anthropology, North Carolina State University).

Radiocarbon Assay

Approximately 11.3 grams of fecal material from specimen four, composed mostly of sunflower pericarp, was sent to Beta Analytic in Miami, Florida, to determine a corrected radiocarbon date.

CHAPTER V

PARASITOLOGICAL ANALYSIS

Results

Cysts similar to those of *Giardia lamblia* (also referred to as *Giardia duodenalis* and *Giardia intestinalis*) were observed in all specimens tested (specimens one, two, and three). The direct immunofluorescence (IFA) technique described in Chapter IV was used to confirm the presence of cysts. Slide wells prepared from each specimen contained cysts with a clearly defined fluorescing cell wall. Numerous cysts were detected in each specimen and, therefore, were considered positive for the presence of *Giardia lamblia*. The immunofluorescent assay procedure supports the identification of cysts of *Giardia lamblia*. *Giardia* cysts were oval in shape (resembling a football) and 8-12 microns long. Cysts were confirmed at 200X. Background material in the specimens was counterstained dull yellow to red.

The procedure utilized to detect *Giardia* cysts simultaneously detected for *Cryptosporidium* oocysts. Specimens two and three each exhibited more than one oocyst with an apple-green fluorescence. *Cryptosporidium* oocysts were round to slightly oval in shape and somewhat smaller (approximately half the size of *Giardia* cysts observed), with diameters of 2-6 microns. *Cryptosporidium* oocysts were confirmed at 400X. A brief discussion of *Cryptosporidium* and its association with *Giardia* will be addressed later in this chapter.

Scanning electron microscopy revealed *Giardia* cysts along with what appear to be trophozoites. Figure 4 shows cysts in specimen one, approximately 10 microns in length by 7 microns in width. Flagella-like structures are scattered about the surface. What appears to be a complete trophozoite, approximately 15 microns by 5 microns is located in the lower left corner.

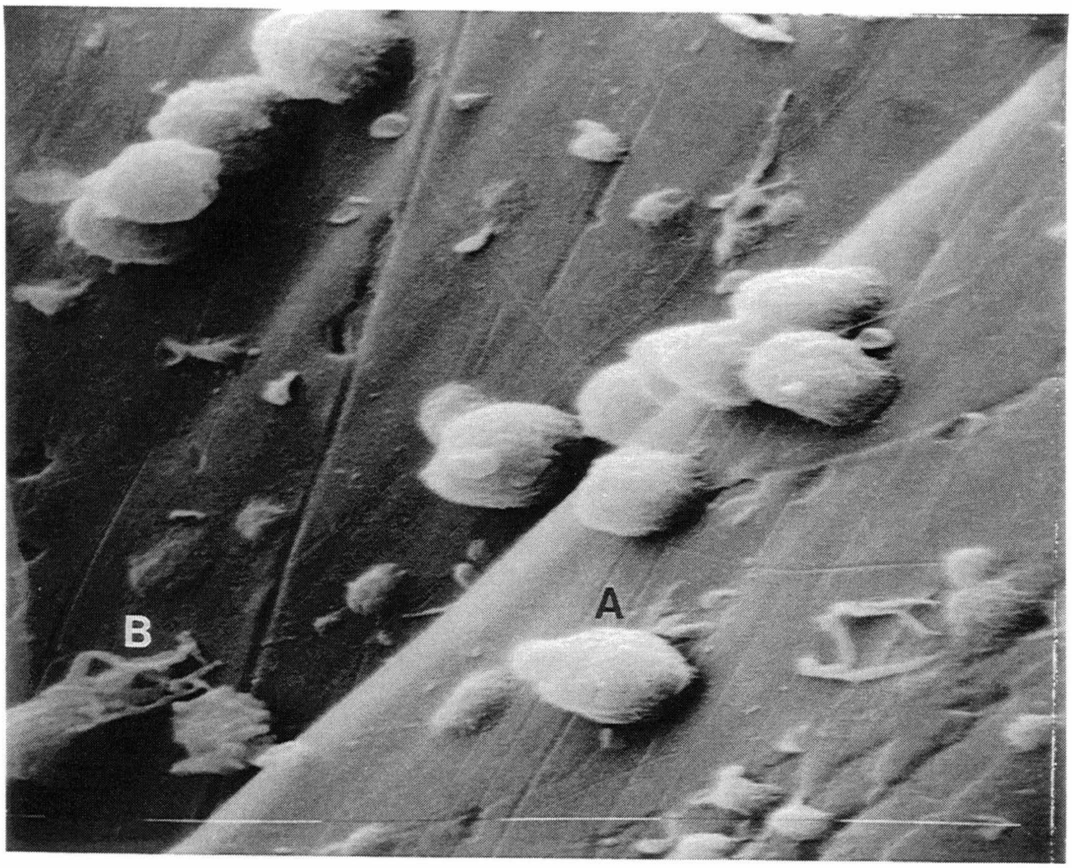

Figure 4. An SEM Photomicrograph of *Giardia lamblia* Cysts (A) and Possible Trophozoites (B) in Specimen One (White Bars = 10 Microns).

Some of the cyst-like objects appear to be transitional, possibly in a stage of encystation.

Usually trophozoites are not found in feces unless diarrheal disease is associated, although they can occur in non-diarrheic fecal samples (Wolfe 1978). Specimens one, two, and three were not diarrheic in consistency. Since these organisms may be 2400 years old, it is reasonable to expect that preserved cysts and trophozoites would not have the same appearance as recently cultured lab specimens. The somewhat distorted appearance of the organisms, along with the scattered flagella, suggest that extensive desiccation from the cave may have affected the characteristic morphology of *Giardia lamblia*.

Giardia Life Cycle and Epidemiology

The ingestion of *Giardia lamblia* cysts in fecally contaminated water or the fecal-oral transfer of cysts by an infected person results in giardiasis (Figure 5). When cysts enter the small bowel, they become trophozoites and attach to the bowel's epithelial surface with their sucking discs. The attachment of *Giardia lamblia* to the intestinal lumen causes destruction of the mucosal lining of the intestine, along with inflammation and irritation. All of these effects decrease food transit time through the small intestine and result in malabsorption. This malabsorption produces chronic gastrointestinal complaints such as abdominal cramps and pale, loose, malodorous, and frequent stools (from 2 to 10 daily), with concurrent nausea. Stools may contain mucus. Chronic giardiasis may produce fatigue and weight loss in addition to these

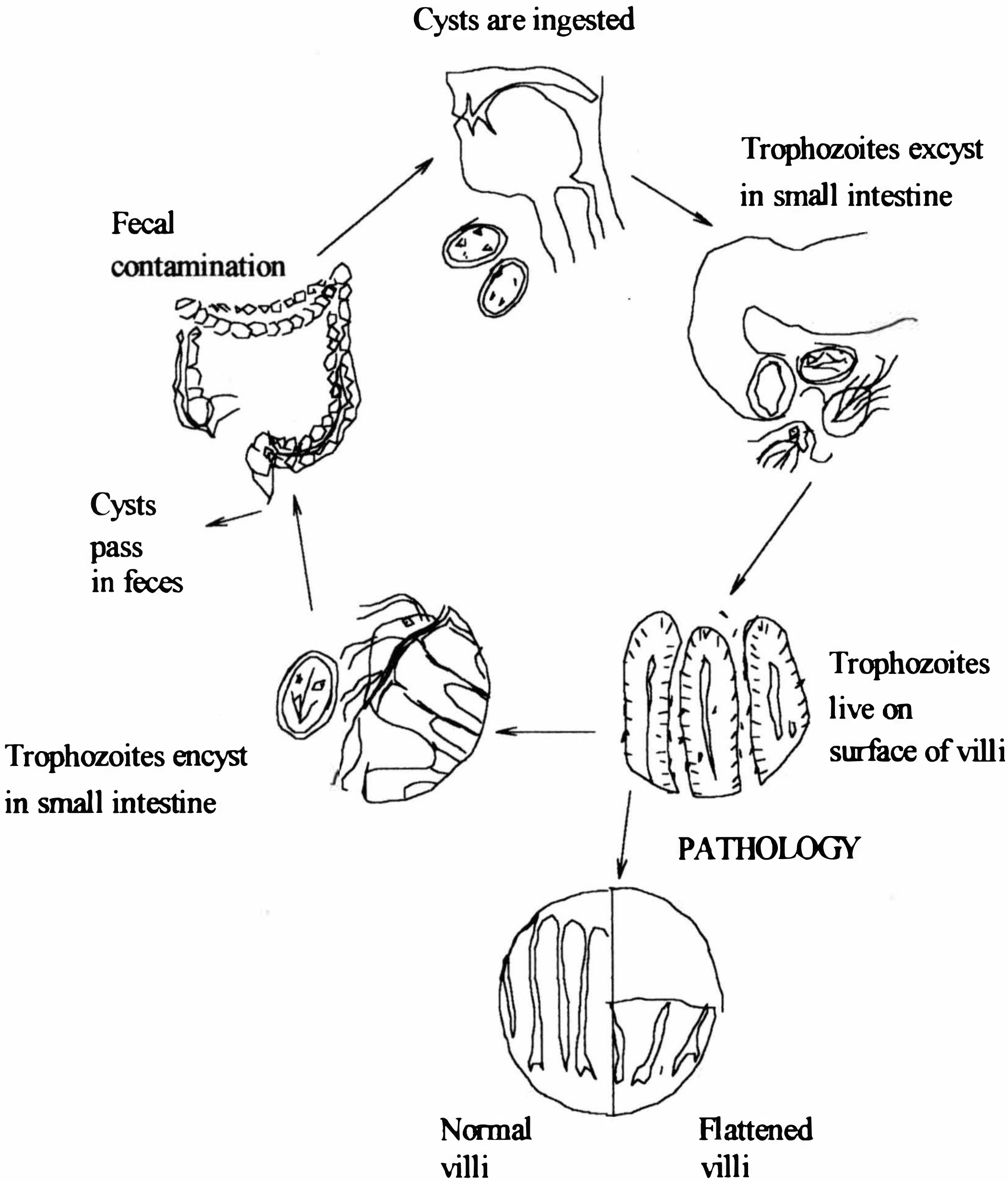

Figure 5. The Life Cycle of *Giardia lamblia* (Katz, Despommier, and Gwadz 1982).

Source: Katz, M., D. D. Despommier, and R. Gwadz (1982). Parasitic Diseases. P. 129. New York: Springer-Verlag.
Used with permission of Ian R. Gross, Rights and Permissions Administrator, Springer-Verlag New York Publishers, 5-9-94.

typical signs and symptoms. Following attachment, the trophozoites encyst, travel down the colon, and are excreted. Unformed feces that pass quickly through the intestine may contain trophozoites as well as cysts (Katz *et al.* 1982; Beaver, Jung, and Cupp 1984; Dupont and Sullivan 1986;), as seen in the photomicrograph of specimen one

When trophozoites detach from the mucosal surface and move into the large intestine, they lose their trophozoal characteristics of external flagella and ventral discs, and enter the cyst stage. As cysts, they assume a more oval shape and develop a thick external wall which protects them from destruction. The cyst is the infective stage, while the trophozoite is responsible for the outward signs of illness (Katz *et al.* 1982; Dupont and Sullivan 1986).

When intestinal transit time is accelerated by diarrheal disease, trophozoites are often passed into the large intestine and excreted in the stool before they can encyst. However, the trophozoite is incapable of survival for any significant period in the external environment and, even if ingested directly, is quickly destroyed by acid in the stomach. The cyst is capable of resisting destruction, even with considerable variations in temperature and pH. If ingested, the cysts can survive the low pH of the gastric juices, and are passed intact into the small intestine. This is where excystation and division take place, allowing cysts to resume the form of the pathogenically active trophozoite (Beck and Davies 1981).

The ability of *Giardia* to survive from one intestine to another depends on the resistance of the cyst cell wall to dehydration, penetration by disinfectants (such as chlorine), physical forces, and the action of gastric acid. The establishment of *Giardia* infection by ingestion of as few as 10 to 100 cysts demonstrates that the intake of even very small amounts of fecal material may be adequate for transmission of *Giardia*. *Giardia* does not always prompt its own expulsion and may remain a chronic infection in asymptomatic or intermittently symptomatic hosts who provide a continuing source of infection (Dupont and Sullivan 1986; Leech, Sande, and Root 1988).

Studies indicate that humans develop immune responses to *Giardia lamblia*. There is evidence that individuals infected with *Giardia* develop antibody responses to the trophozoites. Studies of several giardiasis outbreaks show that individuals repeatedly exposed to *Giardia lamblia* have a lower incidence of infection and symptoms than newly exposed individuals, suggesting that prior exposure imparts partial resistance to infection. One such incidence occurred at a mountain resort in Colorado where filtered water samples revealed *Giardia* cysts before and after treatment (Istre *et al.* 1984). Short-term residents (less than two years) had a significantly higher attack rate of illness than long-term residents. However, the low attack rate for long-term residents did not vary with the amount of water consumed, while it did with short-term residents. This is evidence for at least partial immunity.

Similarly, in a study of 33 lactating mothers and their infants in Bangladesh, 82% of the mothers and 42% of the infants excreted *Giardia lamblia* at least once during the study. Infection in infants produced symptomatic disease in 86% of the cases, while infection in the mothers was symptomatic in only one case. Forty-five percent of the mothers transiently developed low level anti-*Giardia* serum antibodies.

Seventy-three percent had demonstrable milk antibodies to *Giardia*, and 50% of non-infected infants drank milk containing anti-*Giardia* antibody. The results of this study suggest that in endemic areas partial immunity develops following exposure to the organism that protects against disease but not against infection by the parasite (Dupont and Sullivan 1986).

Cryptosporidium

Cryptosporidium spp. are protozoans that, as of 1986, occur on six continents, in developed and less developed countries, and in urban and rural areas. A self-limiting condition is produced in immunocompetent individuals with primary symptoms including explosive, watery diarrhea accompanied by vomiting, abdominal cramping and low-grade fever lasting two to fourteen days. In immunocompromised patients, symptoms are more severe and persistent and may be fatal in patients with Acquired Immune Deficiency Syndrome, or AIDS (Fayer & Ungar 1986).

The parasite is 2-6 microns in diameter and undergoes its life cycle within the microvillous border of the intestine. Infective oocysts are excreted in feces from the host. Fecal-oral contamination among humans and animals and ingestion of contaminated water appear to be the principal sources of transmission (Janoff & Reller 1987).

A correlation between *Cryptosporidium* and *Giardia* has not been demonstrated. However, numerous incidents of coinfection with both have been documented. Jokipii, Pohjola, and Jokipii (1985) found the presence of *Cryptosporidium* to be 6.9 times more frequent in patients with *Giardia* than in others. Intermittent infection with *Cryptosporidium* has occurred mainly in travelers and seems to be acquired from sources similar to those of *Giardia*.

Although the first published description of a parasite resembling *Cryptosporidium* was in 1895, the first isolation of *Cryptosporidium* in a human was not reported until 1976. Like *Giardia*, the protozoan was thought to be rare and insignificant. Few infections were diagnosed until the early 1980's, when cryptosporidiosis was reported to be a life-threatening infection in AIDS patients. By 1986, more than 110 patients with AIDS and cryptosporidiosis had been reported to the Centers for Disease Control. Interest in the epidemiology, diagnosis, and treatment of cryptosporidiosis has increased dramatically since this association was observed (Fayer and Ungar 1986).

Summary

Giardia cysts are extremely viable, and very resistant to disinfectants, but infection from contamination of water supplies can be solved by technology. A more critical problem is direct transmission of giardiasis by ingestion of fecally contaminated food or water. The source of such transmission lies within social institutions, cultural perceptions, and close social and sexual contact, which must be recognized.

Obviously we have no choice but to look at prehistoric *Giardia* from the sense of what we know about the modern parasite. Taking a closer look at how *Giardia* affects the lives of individuals infected today is important in considering what implications this parasite might have had for people living in the vicinity of Salts Cave. The specific pathogenic mechanisms of *Giardia lamblia* are not yet understood. The reasons why some individuals become acutely ill while others remain asymptomatic also remain to be explained. Importantly, immunity is not developed from past infection.

As for the recent association between AIDS and cryptosporidiosis, large-scale studies are in great need. Probably the largest concern is the control of the spread of infection by reducing the potential of cyst and oocyst ingestion from soil, water, foods cleaned with contaminated water, animals, and other humans.

CHAPTER VI

DIETARY ANALYSIS

Results

Representing diet accurately using fecal remains is a difficult issue. One specimen is obviously not a large enough sample to allow for interpretation of dietary patterns of the Salts Cave inhabitants. However, the specimen can, at least, point to some food preferences for cavers.

The ranking of food items in specimen four from greater to lesser quantity is as follows: (a) sunflower, (b) goosefoot, (c) squash, and (d) buttercup. Several hundred sunflower achenes were present, along with approximately 200 goosefoot seeds, over 100 squash seeds (95 of which were complete), and one seed of the buttercup family.

What is significant about the squash remains is that length and width measurements could be made on such a large quantity of seeds. Only nine of the sunflower achenes, on the other hand, were complete enough for measurements.

Seed Descriptions

Helianthus annuus (Sunflower)

Several hundred sunflower achenes were extracted from specimen four, nine of these were complete, or unbroken. Sunflower was the most abundant taxon by seed count. The majority of the sampling sent for radiocarbon dating (11.3 grams) was composed of sunflower achenes. Sunflower is a fall-maturing plant that is storable and easily transported. The seeds from this plant are oily and highly nutritious.

Mean sunflower achene size is 8.39 x 3.06mm with a range of 6.5-10.0mm x 2.5-4.0mm (Refer to Table 1). Sunflower achenes longer than 7.0mm are regarded to have been harvested from domesticated plants (Heiser 1985a). Sunflower, by weight, makes up nearly 85.5% of the sample.

Cucurbita pepo (Squash)

This species comprises the summer squashes, including pattypan and yellow crookneck; the vegetable marrows, such as zucchini; the winter squashes, such as acorn; and common orange pumpkins. Cucurbits are widely grown for their edible fruits and gourds (Heiser 1985b).

Specimen four contained 95 complete seeds along with approximately 20 additional fragmented seeds. These seeds were analyzed to attempt to recognize a particular variety. This is further discussed later in this chapter in the section entitled, "ANOVA".

Mean squash seed size is 12.60 x 7.95mm with a range of 9.0-15.0mm x 7.0-9.5mm, as shown in Table 1. Smith (1987:18) suggests that from 3,000 B.P. to the present, the average seed length values for *Cucurbita* exceed 11mm. A mean length of 11.3mm and a mean width of 7.3mm have been reported from previously analyzed

Salts Cave paleofeces (Yarnell 1974b). By weight, squash makes up about 13% of the sample.

The kernels of these seeds are edible and oily, but are covered with a placental tissue that is quite bitter. Boiling or roasting the seeds neutralizes the bitter compounds (Heiser 1985b). The seeds from this specimen did not appear to have been roasted.

Table 1

Measurements of Complete Squash and Sunflower Seeds
From Salts Cave Paleofecal Specimen Four

SEED TYPE	SEED COUNT	AVERAGE	RANGE
SQUASH	95		
length		12.60	9.0-15.0
width		7.95	7.0-9.5
SUNFLOWER	9		
length		8.39	6.5-10.0
width		3.06	2.5-4.0

Chenopodium berlandieri (Goosefoot)

Approximately 200 seeds of *Chenopodium berlandieri*, most likely of the species *jonesianum*, were located within specimen four. This species is also available in the fall and is storable through the winter (Watson and Yarnell 1966). It is palatable, nutritious, and easily transported. Although more than 200 seeds of *Chenopodium* were present, they only comprise about one percent of the sample by weight.

Ranunuculae (Buttercup)

Since only one seed of this genus was found, it is likely it was not intentionally ingested. *Ranunuculae* are generally summer-flowering plants. Common buttercup matures between May and September (Niering and Olmstead 1992).

Specimens One, Two, and Three

Scanning electron microscopy located seeds of blackberry (*Rubus*), squash seed coats, and what appeared to be hickory nutshell fragments.

ANOVA Statistical Analysis

ANOVA f tests were run on thirty complete squash seeds against four reference populations of *Cucurbita pepo* varieties: (a) sugarpie, (b) butternut, (c) pattypan, and (d) acorn. Sugarpie is a small seed pumpkin, while butternut, pattypan, and acorn are fairly standard garden-variety squashes. Obviously, a number of varieties were not tested for and this affects the significance of the results. ANOVA is a parametric test which should be applied to seed shape only, while ideally, non-parametric tests should be applied to seed size. The tests of seed size parameters clearly indicate that seed size is not normally distributed. Plot and correlation tests, as expected, revealed that large seeds tend to be longer and wider.

The tested seeds are consistently close to pattypan in both size and shape parameters, and are quite different from sugarpie and acorn. The size differences between butternut and pattypan seem to contradict previous finds. In an earlier study by Rovner (personal communication 1993), considerable overlap was seen between butternut and pattypan size parameters. In fact, at the level of individual seed identification, single pattypan seeds could not be distinguished from the butternut group. With regard to specimen four, pattypan is a far better match than butternut, as seen in Table 2.

Table 2

ANOVA: Probability of Significant Difference for Squash Seeds From Salts Cave Paleofecal Specimen Four

	SUGARPIE	BUTTERNUT	PATTYPAN	ACORN
AREA	100.00%	98.80%	33.85%	100.00%
PERIMETER	100.00%	38.31%	42.30%	100.00%
WIDTH	100.00%	99.99%	42.07%	100.00%
LENGTH	100.00%	37.82%	5.92%	100.00%
ROUNDNESS	99.46%	99.81%	84.55%	93.76%
ASPECT RATIO	99.98%	99.99%	41.05%	99.96%

ANOVA, or Analysis of Variance, shows the degree of probability, expressed as a confidence percentage, that a sample is different from the established data set against which it is tested. It is important to be aware that a statistical mean or median can be misleading. Reference populations represent a mean size for all samples, so any tested sample will invariably be above or below average (Irwin Rovner, personal communication 1993).

Archaeologically, large seed size tends to be interpreted as evidence of plant domestication or the use of irrigation. On the other hand, small seed size is often associated with drought conditions or plants which are not yet fully domesticated.

The seeds in this population indicate very little affinity to sugarpie, butternut, or acorn squash and are substantially more similar to pattypan. It should be noted that there still is a 30-40% probability of significant difference between the sample and pattypan reference populations.

Summary

The fecal contents from all four specimens indicate seasonal variation in diet. The small amount of evidence of seeds from specimens one, two, and three suggests a spring or summer utilization of the cave. Since blackberries mature in late spring and do not store well, it seems likely that the hickory nutshell associated with it matured in the fall and was stored through the previous winter and spring, when it was consumed.

The seed remains recovered from specimen four suggest a time of cave utilization in the fall or early winter. Since sunflower and goosefoot are fall-maturing crops, the seeds were probably eaten in the fall or early winter, unless stored. The squash remains of a variety similar to pattypan suggest a summer-maturing fruit. However, the *Cucurbita* seeds from specimen four were tested against only four reference populations, leaving the possibility that the seeds could actually be from a winter squash. It is equally possible that the cucurbit remains were harvested in the summer and stored for fall or winter use. Similarly, sunflower and goosefoot could have been stored until the following spring or summer, when the squash was mature.

Seed remains present in the feces represent a majority of storable foods rather than non-storable foods. All items recovered from specimen four (excluding the one seed of buttercup) were harvested from domesticated plants and represent storable food sources ideal for activities such as caving.

CHAPTER VII

RADIOCARBON ASSAY

Three types of datable artifacts have been recovered from Salts Cave: (1) cane torch fragments, (2) vegetal remains, and (3) human paleofeces (Watson and Yarnell 1966; Watson 1969; Kennedy 1990; Kennedy *et al.* in review). These materials often remain in excellent states of preservation due to the dryness and relatively constant temperature of the cave. Since these materials are organic, they can be radiocarbon dated directly (Kennedy 1990). Paleofeces offer much information regarding diet and disease. Dating these specimens can, therefore, determine when specific diseases prevailed and what types of foods were consumed.

Specimen four was chosen for radiocarbon analysis due to its larger weight of 13.8 grams (larger samples yield more reliable dates). This sample was collected several meters from a cedar climbing pole located below a high-ledge mineral mine. A corrected date of 2495±60 B.P. (B-47472) was obtained from the climbing pole (Tankersley *et al.* 1994; Kennedy *et al.* in review).

In January 1993, 11.3 grams of material from specimen four was sent to Beta Analytic in Miami, Florida, for radiocarbon analysis. A date of 2420±90 B.P. with a C^{13} corrected date of 2410±90 B. P. was returned. This sample falls well within the time frame of the six new dates from May of 1993 from researchers at Washington

University in St. Louis, and five older ones, dated at the University of Michigan in 1966 (Refer to Table 3).

Table 3

Complete List of Salts Cave Paleofecal Dates,
Arranged in Chronological Order

RADIOCARBON YEARS BEFORE PRESENT	PROVENIENCE	SAMPLE #	YEAR RUN
2240±200	Upper Salts, P54	M 1573	1966
2270±140	Upper Salts, P38	M 1777	1966
2350±140	Middle Salts, Blue Arrow, A60	M 1577	1966
2410±70	Upper Salts, P26	AA-10087	1993
2420±90*	Upper Salts, Dismal Valley, P72	Beta-60067	1993
2500±80	Upper Salts, Neville, K5	AA-10091	1993
2570±70	Middle Salts, Q5	AA-10086	1993
2570±140	Upper Salts, P63-64	M 1574	1966
2580±70	Upper Salts, P45	AA-10090	1993
2590±70	Upper Salts, P45	AA-10089	1993
2605±80	Upper Salts, P26	AA-10088	1993
2660±140	Middle Salts, Blue Arrow, A42	M 1770	1966

*Paleofecal date from specimen discussed in this text
Note: AA=University of Arizona; M=University of Michigan; Beta=Beta Analytic

The table represents a period of time during which the cave was occupied by humans. These dates are reported as radiocarbon years before 1950 (RCYBP). Wagner and

Watson are currently working on obtaining additional dates from Mammoth, Salts, and Lee Caves (Tankersley, personal communication 1994).

Only seven of the 55 dates submitted for Salts Cave are 3,000 B. P. or older and none of these is a paleofecal sample. The paleofecal dates represent a several hundred year period when humans were utilizing the cave. Considering all of the paleofecal dates from Salts Cave, there does not appear to be any difference between earlier or later dates and human activity in the three levels of the cave.

While it is thought that the cave entrance has always been accessible to prehistoric inhabitants of the region, it appears evident by the dates obtained so far, that Salts Cave was not visited before Late Archaic times and after the Early Woodland (Kennedy 1990).

CHAPTER VIII

INTERPRETATIONS AND CONCLUSIONS

Infectious disease results from the interaction of three main factors: (1) the host, (2) the parasite, and (3) the environment. A sedentary or semi-sedentary group can become infected over and over again with the same parasites since the ground on which individuals live can quickly become contaminated with feces, creating an almost direct route from one intestine to another. In many areas of the world, feces are dropped indiscriminately, which makes the continued transmission of the cysts inevitable. In a mobile group, these factors disappear and highly specific parasites have little chance of surviving unless they can travel with human populations. *Giardia lamblia* is capable of such travel. The parasite has been documented among hunting and gathering populations as well as sedentary village dwellers (Cockburn 1971).

While hunter-gatherers with high mobility should reveal a low incidence of parasitic infection, hunter-gatherer/gardeners with low mobility should have a higher incidence of infection. Infection could easily travel with a semi-sedentary group. The findings of this study are consistent with models of Late Archaic/Early Woodland culture which indicate decreasing mobility based on the consumption of cultigens and the presence of parasitic diseases.

The only other reports of cysts similar to *Giardia* in a prehistoric context are from one fecal sample from Big Bone Cave, Tennessee (Faulkner 1989, 1991), which revealed *Giardia* cysts (a radiocarbon determination from this sample yielded a date of 2550±80 B.P.), and two fecal samples from an 1800 year old mummy found in a cave in Israel (Witenberg 1961). Neither study mentions the presence of trophozoites. No other cases of *Cryptosporidium* from prehistoric times have been documented.

Jelliffe, Woodburn, Bennett, and Jelliffe (1962) studied the infections of the Hadza, a hunting people of northern Tanganyika (about 800 in number), who live isolated in the tsetse area of the savannah. The Hadza are very mobile and eat almost anything, including baboon, vulture, and hyena, and the food is usually roasted. Examination of 62 children found them well nourished, although Malaria parasites were present in 27%. Stool samples showed that three of the children had *Giardia*. There was no roundworm or hookworm, presumably because constant moving prevented transmission. In other words, the only infection found was that which could survive in a small population always on the move. While a large population could increase certain infectious hazards, it is not necessary to sustain *Giardia*.

The feces from Salts Cave probably represent special purpose diets composed of high-energy foods that are not directly comparable to feces recovered from open habitation sites. This has implications for attempts at estimating the significance of particular items in the diet of prehistoric cultures. The large amount of sunflower, squash, and *Chenopodium* in specimen four suggests that they are either an important part of the overall diet or an important source of nutrition for cavers. The representation of the dietary items in all four of the feces suggests that domesticated and/or cultivated foods are at least as important as food from gathering, such as blackberry and hickory nut.

The availability of seeds outside their growing seasons may have reduced mortality and allowed the movement of groups into areas not previously occupied. According to Smith (1989:1568), "...early gardens probably played a significant role in providing a dependable, managed, and storable food supply for late winter to early spring."

The fall harvested (storable) plant foods present in specimen four suggest that mining activities took place in the late fall or early winter. Recent studies reveal that *Giardia* cysts remain active longer in cold water, or, presumably, during the fall and winter (Erlandsen and Bemrick 1988). The regrowth of mirabilite generally occurs during the damp months of late winter and early spring (White 1969; Tankersley in press), the optimal time for miners to be utilizing the cave. Food-borne infections occur mostly with underheated or raw foods, such as those seen in the Salts Cave feces. Due to the coincidence of all the above factors, feces deposited during these times of the year seem more likely to contain *Giardia*.

This study represents the second documented evidence of prehistoric *Giardia* in the southeastern United States. Successful application of recently available immunological techniques, like immunofluorescence, to the identification of parasites recovered from an archaeological context, provide a basis for future studies at sites in the western United States, as well as other parts of the world. Future studies should examine the viability of the cyst wall of prehistoric *Giardia* to determine if the strain is as resistant to environmental and chemical factors as those represented in populations today. It is likely that advanced techniques for the recovery of parasite remains will continue to develop and that archaeological applications for parasitological study will expand.

Although fecal analysis is a valuable tool, there are limitations to its accuracy and usefulness. Generally, paleofeces are only found in dry caves and rock shelters which may represent seasonal occupations. Therefore, the types of cultures and environments that may be studied are limited. Likewise, the contents of feces do not necessarily represent accurate percentages of consumed items. While vegetal fiber is hardly affected by digestion, meat may leave little or no trace in feces. Even with these limitations, fecal analysis represents a useful method for determining past behavior.

The small sample utilized in this study allows only general conclusions regarding dietary patterns and parasitic infection during the Late Archaic/Early Woodland period in west-central Kentucky. However, given the presence of *Giardia* and *Cryptosporidium* in human paleofeces from Salts Cave, the data strongly suggest that populations of 2400 years ago were becoming increasingly sedentary, based on their food choices and the prevalence of parasitic infection.

APPENDICES

Appendix A

Report of Radiocarbon Dating Analysis

BETA ANALYTIC INC.

DR. J.J. STIPP and DR. M.A. TAMERS

UNIVERSITY BRANCH
4985 S.W. 74 COURT
MIAMI, FLORIDA, USA 33155
PH: 305/667-5167 FAX: 305/663-0964

REPORT OF RADIOCARBON DATING ANALYSES

FOR: Lisa K. Ruppert
Illinois State Museum

DATE RECEIVED: January 11, 1993

DATE REPORTED: February 4, 1993

SUBMITTER'S PURCHASE ORDER #

OUR LAB NUMBER	YOUR SAMPLE NUMBER	C-14 AGE YEARS B.P. ± 1σ	C13/C12	C13 adjusted age
Beta-60067	Salts Cave, KY Dismal Valley (peat)	2420 +/- 90 BP	-25.7 0/00	2410 +/- 90 BP

These dates are reported as RCYBP (radiocarbon years before 1950 A.D.). By international convention, the half-life of radiocarbon is taken as 5568 years and 95% of the activity of the National Bureau of Standards Oxalic Acid (original batch) used as the modern standard. The quoted errors are from the counting of the modern standard, background, and sample being analyzed. They represent one standard deviation statistics (68% probability), based on the random nature of the radioactive disintegration process. Also by international convention, no corrections are made for DeVries effect, reservoir effect, or isotope fractionation in nature, unless specifically noted above. Stable carbon ratios are measured on request and are calculated relative to the PDB-1 international standard; the adjusted ages are normalized to -25 per mil carbon 13.

Appendix B

Measurements of All Complete Sunflower Seeds From Salts Cave Paleofecal Specimen Number Four

Measurements of All Complete Sunflower Seeds From
Salts Cave Paleofecal Specimen Number Four

ENTRY NUMBER	LENGTH (mm)	WIDTH (mm)
1	8.75	3.00
2	10.00	4.00
3	10.00	3.00
4	9.00	3.00
5	8.00	3.00
6	7.50	3.00
7	6.50	3.00
8	8.75	2.50
9	7.00	3.00

Appendix C

Measurements of All Complete Squash Seeds From Salts Cave Paleofecal Specimen Number Four

Measurements of All Complete Squash Seeds From
Salts Cave Paleofecal Specimen Number Four

ENTRY NUMBER	LENGTH (mm)	WIDTH (mm)
1	13.00	8.50
2	14.25	8.50
3	14.25	9.00
4	13.00	7.75
5	11.25	8.25
6	13.00	9.00
7	13.00	7.75
8	12.50	8.25
9	14.25	8.75
10	12.50	8.50
11	14.00	8.25
12	13.00	8.25
13	13.75	8.25
14	13.50	8.00
15	15.00	9.00
16	12.50	7.50
17	13.00	8.50
18	12.50	8.50
19	13.50	8.50
20	12.50	8.50
21	12.00	8.50
22	12.50	7.50
23	12.50	8.00
24	13.00	8.50
25	13.00	8.50

Appendix C–Continued

ENTRY NUMBER	LENGTH (mm)	WIDTH (mm)
26	12.00	7.50
27	12.50	8.75
28	12.50	8.50
29	12.50	8.50
30	12.50	8.00
31	13.50	8.00
32	12.00	7.50
33	12.50	8.50
34	12.50	8.00
35	11.50	7.50
36	13.00	9.50
37	11.50	8.00
38	12.00	8.00
39	14.00	9.00
40	12.00	8.00
41	11.00	8.00
42	14.00	8.50
43	12.50	7.50
44	12.50	7.50
45	12.00	8.00
46	11.50	7.50
47	12.50	8.00
48	12.00	8.00
49	12.5	8.00
50	13.5	8.50

Appendix C–Continued

ENTRY NUMBER	LENGTH (mm)	WIDTH (mm)
51	12.00	8.00
52	12.00	7.50
53	13.00	9.00
54	13.00	8.00
55	13.50	8.00
56	13.50	8.50
57	13.50	7.50
58	13.00	7.50
59	13.00	8.00
60	11.50	7.50
61	13.50	8.50
62	12.50	8.00
63	12.00	8.00
64	14.50	8.00
65	11.0	8.00
66	11.50	8.00
67	12.50	8.50
68	12.00	7.50
69	14.00	8.50
70	13.50	8.50
71	12.50	8.50
72	13.00	8.50
73	12.00	7.50
74	12.00	7.50
75	13.50	8.00

Appendix C–Continued

ENTRY NUMBER	LENGTH (mm)	WIDTH (mm)
76	12.50	8.50
77	13.00	8.50
78	13.50	8.00
79	12.50	7.50
80	13.50	8.00
81	12.00	7.50
82	14.00	8.00
83	11.50	7.50
84	13.00	8.00
85	9.00	7.50
86	13.00	8.00
87	13.50	8.50
88	12.50	8.50
89	13.00	8.50
90	12.00	8.00
91	12.00	8.50
92	11.00	7.00
93	12.00	8.00
94	12.50	8.00
95	13.50	8.50

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