Basics of Archaeoparasitology

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Dedication

(To my mother)

Acknowledgement

To all my colleagues at faculty of medical laboratory sciences, Khartoum university.

Introduction

Archaeoparasitology, a multi-disciplinary field within paleopathology, is the study of parasites in archaeological contexts. It includes studies of the protozoan and metazoan parasites of humans in the past, as well as parasites which may have affected past human societies, such as those infesting domesticated animals.

Reinhard suggested that the term "archaeoparasitology" be applied to "... all parasitological remains excavated from archaeological contexts ... derived from human activity" and that "the term Paleoparasitology be applied to studies of nonhuman, paleontological material." Paleoparasitology includes all studies of ancient parasites outside of archaeological contexts, such as those found in amber and even dinosaur parasites.

The first archaeoparasitology report described calcified eggs of Bilharzia haematobia (now Schistosoma haematobium) from the kidneys of an ancient Egyptian mummy. Since then, many fundamental archaeological questions have been answered by integrating our knowledge of the hosts, life cycles and basic biology of parasites, with the archaeological, anthropological and historical contexts in which they are found. [(1)]

Parasitology basics:

Parasites are organisms which live in close association with another organism, called the host, in which the parasite benefits from the association, to the detriment of the host. Many other kinds of associations may exist between two closely allied organisms, such as commensalism or mutualism

Endoparasites (such as protozoans and helminthes), tend to be found inside the host, while ectoparasites (such as ticks, lice and fleas) live on the outside of the host body. Parasite life cycles often require that different developmental stages pass sequentially through multiple host species in order to successfully mature and reproduce. Some parasites are very host-specific, meaning that only one or a few species of hosts are capable of perpetuating their life cycle. Others are not host-specific, since many different hosts appear to harbor and pass on the infective stages of the parasite.

Most archaeoparasitology reports involve species which are considered to be true parasites of humans today. However, incidental parasitism (referred to by some authors as "pseudoparasitism", "false parasitism" or "accidental parasitism") occurs when a parasite which does not normally utilize a host for the perpetuation of its lifecycle is found in that host incidentally. One example is finding the eggs of Cryptocotyle lingua (a fish parasite) in the stomach contents of an Eskimo mummy. It is estimated that 70% of the "parasite" species reported from present-day humans are actually only incidental parasites. Some incidental parasites do cause harm to the infested pseudohosts.

Sources of material

In archaeological contexts, Endoparasites (or their eggs or cysts) are usually found in (i) fossilized human or animal dung (coprolites), (ii) the tissues and digestive contents of mummified corpses, or (iii) soil samples from latrines, cesspits, or middens (dumps for domestic waste). A cyst of Echinococcus granulosus was even retrieved from cemetery soil in Poland. Ectoparasites may be found on the skin or scalp, as well as wigs, clothing, or personal grooming accessories found in archaeological sites. Ectoparasite eggs may be found attached to individual hairs. The International Ancient Egyptian Mummy Tissue Bank in Manchester, England, provides tissue samples for a variety of uses, including parasitological studies.

Since 1910, parasite remains have been found in archaeological samples from Africa, the Americas, Asia, Europe, the Middle East, and New Zealand. The age of archaeological sites yielding human parasite remains ranges from approx. 25,000-30,000 years ago to late 19th-early 20th century. Parasite remains have also been found in domestic animal remains at archaeological sites.

Human skeletal remains may exhibit indirect evidence of parasitism. For example, hookworm (Ancylostoma duodenale) parasitism may lead to anemia, and anemia is one factor associated with the skeletal changes of cribra orbitalia and porotic hyperostosis. Thus, hookworm parasitism may be a causal factor in observed cribra orbitalia and porotic hyperostosis, though dietary factors may also lead to anemia.

Information on the presence of intermediate hosts, required for life cycle completion by many parasites, is also useful in determining the likelihood that a parasite may have infected a particular ancient society. One example is the identification of molluscan intermediate hosts of schistosomiasis in an Islamic archaeological context.

Artifacts depicting the appearance of individuals may also indicate cases of parasitism. Examples include the characteristic facial deformities of leishmaniasis found on pre-Columbian Mochica pottery, and morphological features of certain ancient Egyptian figurative art. Literary sources also provide valuable information regarding not only the parasites present in historic societies, but also the knowledge and attitudes that the people had towards their parasitic infestations. However, specific parasitological diagnoses reported in ancient and medieval texts must always be read with some degree of skepticism.

Techniques and methods

Parasite remains in archaeological samples are identified by a variety of techniques. Very durable remains, such as eggs and cysts, may remain intact for many thousands of years. In some cases, relatively intact soft-bodied adult helminthes and ectoparasitic arthropods have been found. All of these forms can be identified to the family, genus or species level by compound or electron microscopy. Petrographic techniques have been used for eggs of Capillaria hepatica found in cysts in the corpse of an adolescent from the late Roman period buried in Amiens (France). The authors stated that identification of tissue-dwelling parasites such as Capillaria hepatica in archaeological remains is particularly dependent on preservation conditions and taphonomic changes and should be interpreted with caution due to morphological similarities with Trichiuris sp. eggs

In cases where the intact bodies of parasites are not found, protein or DNA from the parasite may still be present. Antigenic and immunological assays (including enzyme-linked immunoassay - ELISA, and DNA sequencing are used to identify the source of these chemical remains, often to the species level.

Fundamental questions

Archaeoparasitological studies have provided information on many fundamental archaeological, historical, and biogeographical questions. These questions may be grouped into the following broad categories: past dietary and farming practices, animal domestication, migration patterns, climate change, sanitary practices, cultural contacts, ethnomedicine, and the overall health of various human societies. Archaeoparasitology data, combined with our knowledge of present host-parasite associations, also contributes to our understanding of the co-evolution of human host-parasite interactions. Our understanding of the geographic origins, evolution and biogeography of the parasites themselves and human diseases associated with them has also benefitted tremendously from archaeoparasitological studies. [(2)]

History of the parasitology

Humans are hosts to nearly 300 species of parasitic worms and over 70 species of protozoa, some derived from our primate ancestors and some acquired from the animals we have domesticated or come in contact with during our relatively short history on Earth. Our knowledge of parasitic infections extends into antiquity, and descriptions of parasites and parasitic infections are found in the earliest writings and have been confirmed by the finding of parasites in archaeological material. The systematic study of parasites began with the rejection of the theory of spontaneous generation and the promulgation of the germ theory. Thereafter, the history of human parasitology proceeded along two lines, the discovery of a parasite and its subsequent association with disease and the recognition of a disease and the subsequent discovery that it was caused by a parasite. This review is concerned with the major helminth and protozoan infections of humans: ascariasis, trichinosis, strongyloidiasis, dracunculiasis, lymphatic filariasis, loasis, onchocerciasis, schistosomiasis, cestodiasis, paragonimiasis, clonorchiasis, opisthorchiasis, amoebiasis, giardiasis, African trypanosomiasis, South American trypanosomiasis, leishmaniasis, malaria, toxoplasmosis, cryptosporidiosis, cyclosporiasis, and microsporidiosis.

During our relatively short history on Earth, humans have acquired an amazing number of parasites, about 300 species of helminth worms and over 70 species of protozoa. Many of these are rare and accidental parasites, but we still harbor about 90 relatively common species, of which a small proportion cause some of the most important diseases in the world, inevitably, these are the ones that have received the most attention. Since most of these parasitic diseases occur mainly in the tropics, the field of parasitology has tended to overlap with that of tropical medicine, and thus the histories of these two fields are intertwined. There is, however, much more to the history of human parasitology than this, and our understanding of parasites and parasitic infections cannot be separated from our knowledge of the history of the human race. In particular, the spread and present distribution of many parasites throughout the world has largely been the result of human activities, and the advent of AIDS has added a new chapter to the history of parasitology.

HUMAN EVOLUTION, MIGRATIONS, CIVILIZATION, AND PARASITIC INFECTIONS:

Human evolution and parasitic infections have run hand in hand, and thanks to the spinoffs from the Human Genome Project, we now know much more about the origins of the human race than ever before. Sometime, about 150,000 years ago, Homo sapiens emerged in eastern Africa and spread throughout the world, possibly in several waves, until 15,000 years ago at the end of the Ice Age humans had migrated to and inhabited virtually the whole of the face of the Earth, bringing some parasites with them and collecting others on the way. For the purpose of this review, the parasites that infect humans can be classified as heirlooms or souvenirs. Heirlooms are the parasites inherited from our primate ancestors in Africa, and souvenirs are those that we

have acquired from the animals with which we have come in contact during our evolution, migrations, and agricultural practices. The development of settlements and cities facilitated the transmission of infections between humans, and the opening up of trade routes resulted in the wider dissemination of parasitic infections. The slave trade, which flourished for three and a half centuries from about 1500, brought new parasites to the New World from the Old World; in more recent times, the spread of human immunodeficiency virus HIV and AIDS and the immunodepression associated with these conditions has resulted in the establishment of a number of new opportunistic parasitic infections throughout the world.

We are beginning to learn a lot about the past history of parasitic infections from studies of archaeological artifacts, such as the presence of helminth eggs or protozoan cysts in coprolites (fossilized or desiccated feces) and naturally or artificially preserved bodies; from such studies has emerged a new science, Paleoparasitology.

So vast is the field of human parasitology, and so many and far-reaching the discoveries made, that it is not possible to do justice to the whole subject. Therefore; only the most significant aspects and the most important parasites are considered under two major headings, the helminth worms and the protozoa.

EARLY RECORDS:

The first written records of what are almost certainly parasitic infections come from a period of Egyptian medicine from 3000 to 400 BC, particularly the Ebers papyrus of 1500 BC discovered at Thebes. Later, there were many detailed descriptions of various diseases that might or might not be caused by parasites, specifically fevers, in the writings of Greek physicians between 800 to 300 BC, such as the collected works of Hippocrates, known as the Corpus Hippocratorum, and from physicians from other civilizations including China from 3000 to 300 BC, India from 2500 to 200 BC, Rome from 700 BC to 400 AD, and the Arab Empire in the latter part of the first millennium. As time passed, the descriptions of infections became more accurate and Arabic physicians, particularly Rhazes (AD 850 to 923) and Avicenna (AD 980 to 1037), wrote important medical works that contain a great deal of information about diseases clearly caused by parasites.

In Europe, the Dark and Middle Ages, characterized by religious and superstitious beliefs, held back medical progress until the Renaissance, which released a flurry of activity that eventually led to the great discoveries that characterized the end of the 19th century and the beginning of the 20th. These discoveries included the demolition of the theory of spontaneous generation and the evolution of the germ theory by Louis Pasteur, the demonstration by Pasteur that diseases could be caused by bacteria, the discovery of viruses by Pierre-Paul Emile Roux, the introduction by Robert Koch of methods of preventing diseases caused by microorganisms, and the incrimination by Patrick Manson of vectors in the transmission of parasites. The great personalities of this period made discoveries in a number of fields, and their findings and ideas fed off one another. The names of Pasteur, Koch, Roux, and Manson occur time and time again in the history of parasitology and microbiology.

DISCOVERY OF THE HELMINTH WORMS:

Because of the large size of some helminthes, such as the roundworm Ascaris and the tapeworms, it is practically certain that our earliest ancestors must have been aware of these common worms. There is some evidence for this assumption based on contemporary studies of primitive tribes in Sarawak and North Borneo, where Hoeppli found that most people are aware of their intestinal roundworms and tapeworms. Some historians have identified references to helminth worms and their diseases in the Bible, but the relevant passages are open to several interpretations. Among the Egyptian medical papyri, the Ebers papyrus refers to intestinal worms, and these records can be confirmed by the discovery of calcified helminth eggs in mummies dating from 1200 BC. The Greeks, particularly Hippocrates (460 to 375 BC), knew about worms from fishes, domesticated animals, and humans. Roman physicians including Celsus (25 BC to AD 50) (244) and Galen (Galenus of Pergamon, AD 129 to 200) were familiar with the human roundworms Ascaris lumbricoides and Enterobius vermicularis and tapeworms belonging to the genus Taenia. Somewhat later, Paulus Aegineta (AD 625 to 690) clearly described Ascaris, Enterobius, and tapeworms and gave good clinical descriptions of the infections they caused. Following the decline of the Roman Empire, the study of medicine switched to Arabic physicians, including Avicenna, who recognized not only Ascaris, Enterobius, and tapeworms but also the guinea worm, Dracunculus medinensis, which had been recorded in parts of the Arab world, particularly around the Red Sea, for over 1,000 years.

The medical literature of the middle Ages is very limited, but there are many references to parasitic worms. In some cases, they were recognized as the possible causes of disease but in general, the writings of the period reflect the culture, beliefs, and ignorance of the time. The science of helminthology really took off in the 17th and 18th centuries following the reemergence of science and scholarship during the Renaissance period. Linnaeus described and named six helminth worms, Ascaris lumbricoides, Ascaris vermicularis (= Enterobius vermicularis), Gordius medinensis (= Dracunculus medinensis), Fasciola hepatica, Taenia solium, and Taenia lata (= Diphyllobothrium latum). Thereafter, more species were described until at the beginning of the 20th century, 28 species had been recorded in humans, a number that has now grown to about 300 species, including accidental and very rare records. Even if some of these are doubtful, at least 280 species are recognized by Ashford and Crewe in their annotated checklist.

Ascaris and Ascariasis

Ascaris lumbricoides, the large roundworm, is one of six worms listed and named by Linnaeus; its name has remained unchanged ever since. One billion people are now estimated to be infected with this worm. The adult worm lives in the intestine, and the female produces eggs that pass out with the feces, and the larvae within the eggs develop to the infective stage in soil. Humans become infected when food contaminated with infective eggs is eaten and the larvae emerge in the intestine. The worms do not mature immediately but migrate around the body, reaching the lungs, from which they are coughed up and swallowed and then develop into adults in the intestine. Ascariasis is an ancient infection, and A. lumbricoides eggs have been found in human coprolites from Peru dating from 2277 BC and Brazil from about 1660 to 1420 BC. In the Old

World, there are records of A. lumbricoides in a Middle Kingdom Egyptian mummy dating from 1938 to 1600 BC and from China in the Ming Dynasty between AD 1368 and 1644. The presence of this large worm, which reaches a length of 15 to 35 cm and is often voided in the feces or sometimes emerges from the anus, is very obvious. There are extensive written records including the Egyptian medical papyri, the works of Hippocrates in the fifth century BC, Chinese writings from the second and third centuries BC, and texts of Roman and Arabic physicians. Surprisingly, it was not until the late 17th century that the detailed anatomy of the worm was described, first by Edward Tyson, an English physician, and shortly afterward by the Italian Francesco Redi, who described the worms in his book Osservazioni Intorno Agli Animali Viventi che si Trovano Negli Animali Viventi, one of the first books on parasitology. These two publications, together with that of Tyson on the tapeworms of humans, can be considered to mark the beginnings of the subdiscipline of helminthology, which reached a peak in the 19th century. It was also during this period that the first real attempts were made to understand the infections caused by Ascaris and other worms and how they might be treated. In the meantime, the problem for those studying Ascaris and other parasitic nematodes was how the parasite's eggs infected a new host after leaving the original host. It was not until 1862 that transmission by ingesting eggs was demonstrated by the French medical scientist Casimir Joseph Davaine and later by the Italian scientist Giovanni Battista Grassi, who infected himself with the eggs of A. lumbricoides and subsequently found eggs in his feces. The life cycle in humans, including the migration of the larval stages around the body, was discovered only in 1922 by a Japanese pediatrician, Shimesu Koino, who infected both a volunteer and himself and realized what was happening when he found large numbers of larvae in his sputum. There are good accounts of the history of ascariasis by Groveand Goodwin.

Hookworms and Hookworm Disease

Human hookworm infections are caused by two species, Ancylostoma duodenale and Necator americanus, the former originating in Asia and the latter originating in Africa. The life cycles of the two worms are similar. Adult male and female worms live in the small intestine, where they can cause massive blood loss. Eggs pass out with the feces to contaminate the soil, where larvae emerge and molt to become infectious larvae that bore through the skin of a new host. In humans the larvae migrate to the lungs and trachea, from which they are swallowed before maturing into adults in the small intestine. Human hookworm infections have been associated with humans in the Old World for over 5,000 years. The presence of hookworm infections in pre-Columbian America is a fiercely disputed topic. Robert Desowitz has little doubt that hookworms were present before the arrival of Europeans, but Kathleen Fuller suggests that hookworms were introduced into the Americas after 1492. Palaeoparasitological evidence appears to back Desowitz's ideas since ova identified as Ancylostoma sp. have been found in a human coprolite dated from somewhere between 3350 BC and AD 480. Larval nematodes, possibly hookworms, have been found in fecal samples dated to about 200 BC from the Colorado Plateau. The introduction of hookworms into the Americas is discussed in more detail elsewhere.

The classical signs of hookworm disease are anemia, greenish yellow pallor, and lassitude. None of these symptoms is obvious or unambiguous, and the one distinctive feature exhibited by some

individuals, geophagy, is not necessarily associated with disease. Although worms must have been present in many civilizations, most infections have gone unnoticed such that early accounts of the disease interpreted in retrospect must be treated with caution. The greenish pallor called Egyptian chlorosis, first associated with hookworm infections by 19th century scientists, is not recorded in the early Egyptian papyri. It has been suggested that the enigmatic condition aaa that occurs in many papyri including the Ebers papyrus might refer to hookworms, but there is no real evidence for this. This subject is discussed when considering schistosomiasis below. There are references to yellowish pallor and geophagy in the works of Hippocrates and Lucretius, who noted the pallor seen in miners in about 50 BC. There are also references from the third century BC in China to laziness and a yellow disease. During the 18th and 19th centuries, there were increasing numbers of records from the West Indies and South and Central America. Worms were found in a human in 1838 by the Italian physician Angelo Dubini, and the connection between the worms and disease was finally established by Wilhelm Griesinger in 1854. Although the association between pallor and working in mines had been made by Lucretius, it was not until 1879 that the Italian veterinarian Edoardo Perroncito established the real connection while investigating the diseases of miners in the St. Gothard tunnel. Conditions in mines favor the development of larval hookworms that require warmth and damp. The fact that hookworm larvae enter the body by boring through the skin was not discovered until the end of the 19th century, when Arthur Looses accidentally infected himself. In the early part of the 20th century, hookworm disease was such a serious problem in the United States that the Rockefeller Foundation took on the task of controlling the disease, an activity that subsequently led to the establishment of a number of Schools of Public Health and the creation of the World Health Organization. There are good accounts of the history of hookworm disease by Ball, Foster, and Grove.

Trichinella and Trichinosis:

Trichinosis, also known as Trichinellosis and trichina infection, is caused by the intestinal nematode worm Trichinella spiralis, which requires two hosts in its life cycle. The female worms produce larvae that encyst in muscle, and a new host becomes infected when muscle is eaten. Because human infections are usually acquired by eating pork infected with the encysted larvae, this might have given rise to the Mosaic and Islamic traditions of avoiding pork, a practice that has also been attributed to tapeworm infection. The association between trichina infections and pigs has been long recognized, but the encysted larvae in the muscle were not seen until 1821 and even then were not associated with disease in humans. The discovery of the worm in humans in 1835 was made by James Paget, then a medical student at St. Bartholomew's Hospital in London and later knighted as a distinguished physician, but the definitive report was written by Richard Owen, who played down Paget's role and did not realize that the worm in human muscle was a larval stage of a nematode. The adult worms were discovered by Rudolf Virchow in 1859 and Friedrich Zenker in 1860, and it was Zenker who finally recognised the clinical significance of the infection and concluded that humans became infected by eating raw pork. The importance of these studies lies not only in the field of human parasitology but also in the more general field of parasitology concerned with the transmission of parasites between different animal species

and the importance of predator-prey relationships in such transmission. There are good accounts of the history of trichinosis by Bundy and Michael, Foster, and Grove.

Strongyloides and Strongyloidiasis:

Humans are hosts to two species of Strongyloides, S. stercoralis and S. fuelleborni, of which there are two subspecies, S. f. fuelleborni in Africa and S. f. kellyi in Papua New Guinea. As far as human disease is concerned, S. stercoralis is the more common and important species. Its life cycle is more complex than that of any of the other nematodes discussed so far and involves both parasitic and free-living generations. Adult parthenogenic female worms in the small intestine lay eggs that hatch within the host to produce first-stage larvae, which are passed out in the feces and adopt a free living existence in the soil. Here they molt to produce infective larvae that penetrate the skin and are carried around the body to the lungs and are swallowed and reach the gut in the same way as hookworms. Sometimes the larvae mature to the infective stage in feces on the skin and reinfect the host through the skin (autoinfection), or the larvae may mature to the infective stage without leaving the gut and penetrate the gut wall. Thereafter, in both cases, the infection proceeds as described above. In immunosuppressed individuals, larval stages can be found throughout the viscera. S. stercoralis also has an alternative free-living life cycle in the soil. Given the absence of eggs and the small size of the larvae, combined with confusion with other free living species of nematodes, it is not surprising that S. stercoralis was not recognized until 1876, when the larvae and the disease strongyloidiasis were both discovered by Louis Alexis Normand, a physician to the French naval hospital at Toulon. Normond later found adult worms and, not knowing what they were, sent them to Professor Arthur Réné Jean Baptiste Bavay at the French Conseil Supérieur de Santé, who realized that they were the adult worms of the larvae that were found in the feces. In 1883 the distinguished German parasitologist Karl Georg Friedrich Rudolf Leuckart discovered the alternation of generations involving parasitic and free-living phases. The discovery that infection occurred through the skin was made by a Belgian physician, Paul Van Durme, whose studies were based on the work of Looss, mentioned above, who had shown that A. duodenale infects its host in this way. It is now thought that Van Durme was probably working with A. fuelleborni, but the correct mode of infection had been established, and it was Looss who later succeeded in infecting himself by putting larvae of S. stercoralis on his skin and finding larvae in his feces 64 days later. Friedrich Fülleborn, working with dogs in Hamburg, described the phenomenon of autoinfection and discovered how S.stercoralis (and also Ancylostoma spp.) S. migrates around the body before ending up in the intestine. For over half a century, S. stercoralis received little attention until detailed studies on infections in prisoners of war who had acquired their infections in the Far East in the 1940s revealed disseminated infections in immunosuppressed patients. It was later found that Strongloides infections were more severe in patients infected with human T-lymphotropic virus type 1 and were at one time, but are no longer, regarded as major concomitants of AIDS. Strongyloides infections and strongyloidiasis are not well covered in the literature, but there is a good account by Grove.

Dracunculus and Dracunculiasis (Guinea Worm Disease)

The best-documented parasitic disease known from the earliest times is undoubtedly that caused by the nematode worm Dracunculus medinensis. Adult worms live in subcutaneous connective tissue, from which the female worm emerges to release thousands of larvae into water, where they are eaten by intermediate hosts, cyclopodid crustaceans, in which they mature into infective larvae that infect humans when the crustaceans are accidentally swallowed with drinking water. The large female worm, up to 80 cm in length, protrudes from the skin, usually of the leg, and causes intense inflammation and irritation, signs that are so unusual and unambiguous that ancient texts can be interpreted with some certainty. The earliest descriptions are from the Ebers papyrus from 1500 BC and include instructions for treating aat swelling in the limbs; they appear to refer to both the nature of the infection and techniques for removing the worm. This interpretation is widely accepted by most parasitologists, but there are difficulties in interpreting this particular text since the word aat may simply mean a swelling. Nevertheless, confirmation of the presence of this worm in ancient Egypt comes from the finding of a well-preserved female worm and a calcified worm in Egyptian mummies.

Dracunculiasis is one of the few diseases unambiguously described in the Bible, and most parasitologists accept that the “fiery serpents” that struck down the Israelites in the region of the Red Sea after the Exodus from Egypt somewhere about 1250 to 1200 BC were actually Guinea worms. The most authoritative interpretation of this biblical text, thought to have been written in the eighth century BC, is that by Gottlob Friedrich Heinrich Küchenmeister, a parasitologist, theologian, and Hebrew scholar, in his 1855 textbook translated into English as Animal and Vegetable Parasites. Assyrian texts in the library of King Ashurbanipal from the 7th century BC also refer to conditions that are obviously dracunculiasis, and later descriptions of dracunculiasis occur in all the major Greek and Roman texts and works by the Arab physicians the 10th and 11th centuries. Because there is reference to “Medina vein” in the Arab literature, some historians have suggested that the Arab physicians may have thought that the worm was actually a rotten vein, but most informed observers now agree that the Arab physicians were fully aware of the worm-like nature of dracunculiasis but not necessarily the actual cause of the disease.

Interest in dracunculiasis reemerged when the condition began to be recognized by European travelers visiting Africa (hence the common name, Guinea worm) and Asia. In 1674, Georgius Hieronymus Velschius initiated the scientific study of the worm and the disease it caused, and in 1819, Carl Asmund Rudolphi discovered adult female worms containing larvae, a discovery that was followed up in 1834 by a Dane known only as Jacobson. In 1836, D. Forbes, a British army officer serving in India, found and described the larvae of D. medinensis in water, and over the next few years several parasitologists, including George Busk, pursued the idea that humans became infected through the skin. It was not until 1870 that the whole life cycle, including the stages in the crustacean intermediate host, was elaborated by the Russian Alekej Pavlovitch Fedchenko. Fedchenko's observations gained wide acceptance after they were confirmed by Manson in 1894, and the whole life cycle was finally elaborated in 1913 by the Indian bacteriologist Dyneshvar Atmaran Turkhud, who succeeded in infecting human volunteers with

infected Cyclops. There are more detailed accounts of the history of Dracunculus by Foster, Grove, and Tayeh.

Filarial Worms and Lymphatic Filariasis (Elephantiasis):

Lymphatic filariasis is caused by infection with the nematode worms Wuchereria bancrofti, Brugia malayi, and B. timori, which are transmitted by mosquitoes. The discovery of the life cycle by Patrick Manson in 1877 is regarded as one of the most significant discoveries in tropical medicine, but in the context of the history of parasitology it is better perceived as a logical extension of much that had gone before. Like Dracunculus, the adult filarial worms live in subcutaneous tissues, but unlike Dracunculus, the larvae, called microfilariae, produced by the female worm pass into the blood and are taken up by a blood-sucking mosquito when it feeds. After development in the mosquito, the microfilariae are injected into a new host when the mosquito feeds again. One particular form of the disease that must have attracted the attention of our ancestors is elephantiasis, which is characterized by grotesque swellings of the limbs, breasts, and genitals. These deformities appear to have been described and depicted in drawings from the earliest times, but the interpretation of the early records must be viewed with caution. Lymphatic filariasis was, and is, common along the Nile and, although there are no written records, the swollen limbs of a statue of the Egyptian Pharaoh Mentuhotep II from about 2000 BC suggest that he was suffering from elephantiasis, and small statuettes and gold weights from the Nok culture in West Africa from about AD 500 depict the enlarged scrota characteristic of elephantiasis. Greek and Roman writers were aware of the differential diagnosis of the condition and used the term “elephantiasis graecorum” to describe leprosy and the term “elephantiasis arabum” to describe lymphatic filariasis; the Arabic physicians, including Avicenna, were also aware of the differences between leprosy and lymphatic filariasis. The first definitive reports of lymphatic filariasis only began to appear in the 16th century. Lymphatic filariasis is also known as “the curse of St. Thomas”, and on a visit to Goa between 1588 and 1592, the Dutch explorer Jan Huygen Linschoten recorded that the descendants of those that killed St. Thomas were “all born with one of their legs and one foot from the knee downwards as thick as an elephants leg”. Thereafter, there are numerous references to elephantiasis, especially in Africa but also in Asia, including China, where Manson was later to discover the life cycle of the parasite. Another pathological condition associated with lymphatic filariasis is chyluria, in which the urine appears milky. This condition was recorded by William Prout in his 1849 book On the Nature and Treatment of Stomach and Renal Diseases.

The larval microfilariae were first seen in hydrocele fluid by the French surgeon Jean-Nicolas Demarquay in 1863 and, independently, in urine by Otto Henry Wucherer in Brazil in 1866. It remained for Timothy Lewis, a Scottish physician working in Calcutta, to confirm the finding of microfilariae in urine and blood and to recognize their significance in elephantiasis. The adult worm was described by Joseph Bancroft in 1876 and named Filaria bancrofti in his honour by the British helminthologist Thomas Spencer Cobbold. The elucidation of the life cycle, one of the triumphs of parasitological research, was the work of Patrick Manson in 1877. This is widely regarded as the most significant discovery in tropical medicine, with implications that went far beyond helminthology into such diverse areas as malaria and the Arboviruses. The story of

Manson's discoveries has been told many times, but what is often omitted from the history of Manson's discoveries is the fact that he was aware of Fedchenko's earlier studies on the life cycle of D. medinensis and its transmission using an intermediate cyclopodid host. Fedchenko's observations stimulated Manson to seek an intermediate host but also led him astray when he tried to demonstrate that infection was caused by drinking contaminated water. Manson, then working in Amoy in China, found microfilariae in the blood of dogs and humans and hypothesized that these parasites in the blood might be transmitted by blood-sucking insects. Accordingly, he fed mosquitoes on the blood of his gardener, who was harboring the parasites, and found larval stages in the mosquitoes. However, Manson thought that the parasite escaped from the mosquito into water and that humans acquired infection from this contaminated water by drinking the contaminated water or via penetration of the skin. The actual mode of transmission was not established until suggestions made by the Australian parasitologist Thomas Bancroft were followed up by Manson's assistant George Carmichael Low, who demonstrated the presence of microfilariae in the mouthparts of mosquitoes in 1900. The history of lymphatic filariasis is well described in the works already cited in this section.

Loa and Loiasis (Eye Worm) and Onchocerca and Onchocerciasis (River Blindness):

Both loiasis, caused by infection with Loa loa, and onchocerciasis, caused by infection with Onchocerca volvulus, are filarial worms with life cycles similar to those described above. It is logical to consider these two conditions together because both affect the eyes and must have attracted the attention of early observers interested in sight and blindness. Surprisingly, there are no reliable early records. In loiasis the adult worm moves across the eye under the conjunctiva, an alarming experience that must have attracted attention of both sufferers and observers. An engraving by J. and T. de Bry made in 1598 was at one time thought to depict the extraction of a worm from the eye, but this has been hotly disputed, and it is now thought that this particular engraving represents a punishment for some offense rather than a treatment. The first definitive record is that of a French surgeon, Mongin, who, in 1770, described the worm passing across the eye of a woman in Santa Domingo, in the Caribbean, and recounts how he tried unsuccessfully to remove it. There are, however, less detailed earlier records of similar cases in 1768 and 1777 in an account of the history of French Guyane and Cayenne by Bertrand Bajon. In 1778, a French ship's surgeon, Francois Guyot, noticed that slaves in transit from West Africa to America suffered from recurrent ophthalmia and successfully removed a worm from one of them. The first English account of the removal of worms from the eye is that by William Loney in 1848; thereafter, there are increasing numbers of similar records. The microfilariae were discovered in 1890 by the ophthalmologist Stephen McKenzie and were sent for identification to Patrick Manson, who speculated that these might be the larvae of Loa loa. Loa infections are not confined to the eye, and there are also sometimes swellings on the arms and legs caused by the worm in its wanderings. These swellings, now known as Calabar swellings, were first recorded by a Scottish ophthalmic surgeon, Douglas Argyll-Robertson, in Old Calabar in Nigeria in 1895, but it was not until 1910 that Manson suggested that they might be associated with infections by Loa loa, an opinion shared by his colleague George Low. The transmission by biting flies, Chrysops spp., was unraveled by the British helminthologist Robert Thompson Leiper in 1912. There is an excellent account of Loa and loasis by Grove.

Onchocerciasis, caused by the filarial worm Onchocerca volvulus, is found mainly in Africa and in parts of South America and the Arabian peninsula, where it was introduced from Africa, and it was only when these regions were opened up by explorers that the disease was recognized. The most important signs are blindness, an unexceptional condition that might have been due to a number of causes, and scaly, itchy, nodular skin, which was unusual and was known locally in West Africa as kru kru or craw craw. The microfilariae live in the skin and were discovered by the Irish naval surgeon John O'Neill when examining skin snips from patients suffering from craw craw in Ghana in 1874. Some years later, in 1890, the adult worms were also discovered and identified by Patrick Manson. The role of the microfilariae in causing the skin lesions was established by Jean Montpellier and A. Lacroix in 1920, and the part played by microfilaria in blindness was finally elaborated by Jean Hissette in the Belgian Congo (now the Democratic Republic of the Congo) in 1932. O. volvulus is transmitted by sandflies, and their role in the transmission of onchocerciasis was discovered by the Scottish parasitologist Breadablane Blacklock in Sierra Leone in the mid-1920s. There are accounts of the history of onchocerciasis by Grove and Muller.

Schistosomes and Schistosomiasis:

Schistosomiasis, also known as bilharzia, is caused by infection with trematode worms belonging to the genus Schistosoma, of which the most important are S. haematobium, S. mansoni, and S. japonicum. The adult worms live in blood vessels associated with the intestine or bladder, and the females produce eggs that are passed out with the feces or urine. Larval stages, miracidia, emerge from the eggs when they reach water and bore into the intermediate host, a snail. After a period of multiplication in the snail, the next larval stages, the cercariae, emerge, and these are the stages that infect humans. The cercariae bore through the skin and transform into schistosomula that migrate through the body until they reach their final position in blood, vessels where they mature. The pathological effects of the disease are due mainly to immunological reactions to eggs that, instead of passing to the outside world, become deposited in different tissues; the effects depend on the tissues involved. In this context, it is interesting that schistosomiasis has been associated with carcinomas of the colon and bladder, one of the few examples of parasitic infections causing cancer (the others being the fluke infections opisthorchiasis and clonorchiasis. There is nothing special about the symptoms of schistosomiasis that might have attracted the attention of early observers except the bloody urine, hematuria, associated with S. haematobium infections, which is discussed below. There is no doubt that schistosomiasis is an ancient disease. In 1910, Marc Armand Ruffer found S. haematobium eggs in two Egyptian mummies dating from the 20th dynasty, 1250 to 1000 BC, a finding that is generally regarded as the beginning of the subdiscipline of Paleoparasitology. Thus, there is direct evidence that schistosomes were present in ancient Egypt, and there have been numerous attempts to find descriptions of this condition in the medical papyri. The most contentious word is aaa, which occurs in over 50 early papyri including the Ebers papyrus. In some medical papyri aaa occurs together with the initial hieroglyph suggesting a penis discharging what has been interpreted as blood. The juxtaposition is the papyri of aaa, antimony-based remedies, and possibly worms in the body suggests schistomiasis haematobia, and this interpretation is widely quoted in historical and parasitological textbooks. However, things are

probably not as simple as this because no passages from the papyri link aaa with the bladder or urine and the discharge from the penis might represent semen and not blood. This subject is discussed in more detail by Nunn and Tapp, who abandon aaa as a possible ancient Egyptian word for schistosomiasis. However, since schistosomiasis was almost certainly common and widespread in ancient Egypt, it is curious that the Egyptians did not have a word for it unless it was so common that it was ignored. In this context, it should be mentioned that there have been a number of other suggestions about what aaa might be, including hookworm disease, which is discussed above.

If we accept that there is no authoritative description of schistosomiasis in the earliest medical literature, the first definitive record must be that of an epidemic among soldiers in Napoleon's army in Egypt in 1798 by a French army surgeon, A. J. Renoult, who writes that “A most stubborn haematuria manifested itself amongst the soldiers of the French army… continual and very abundant sweats diminished quantity of urine…becoming thick and bloody”. Thereafter there are numerous reports of illnesses characterized by hematuria, particularly among armies including those involved in the Boer War (1899 to 1902). The worm S. haematobium was described by the German parasitologists Theodor Bilharz and Carl Theodor Ernst von Siebold in 1851. Bilharz, with Wilhelm Griesinger, made the connection with the urinary disease a year later. Although it was known that other flukes employed a snail vector, the search for the intermediate stages in the life cycle of S. haematobium took a long time and a number of experienced parasitologists including Arthur Looss, Prospero Sonsino, and Thomas Cobbold, working at the end of the 19th century, all failed to infect snails; it was not until 1915 that Robert Leiper demonstrated the complete life cycle in the snail host.

Our knowledge of the history of intestinal schistosomiasis caused by S. mansoni dates back to conclusions reached by Manson in 1902 that there were two species of Schistosoma in humans. Even though there had been similar suggestions by other workers, Manson's ideas were not universally accepted, and it was Leiper who firmly established the existence of S. mansoni as a separate species in 1915.

The third important species is the Asian form, S. japonicum. One aspect of schistosomiasis japonica is Katayama disease, an ancient disease that was properly recorded in Japan in the Kwanami district only in 1847 by Dairo Fujii in a report that did not become available until 1909. Fujii found people, cattle, and horses affected by wasting, abdominal swelling, and severe rashes on the legs, but he did not know the cause. By the time Fujii's paper had become available, another Japanese worker, Tokuho Majima, had found schistosome eggs in patients with Katayama disease, and he associated the pathological changes with the presence of the schistosome eggs. The worm itself, S. japonicum, was discovered and described by Fujiro Katsurada in 1904, and its development in the snail host was described by Keinosuke Miyairi and M. Suzuki in 1913, 2 years before Leiper independently described the life cycle of S. haematobium. Fuller accounts of the history of Katayama disease are given by Goodwin and Grove.

The 20th century has been marked by the discovery of further species of schistosomes, S. intercalatum and S. mekongi. The history of such an important disease as schistosomiasis

involves a great number of observations, events, and individuals; a detailed account of the history is given by Grove, and there are shorter accounts by Foster, Goodwin, and Hoeppli. A full bibliography is given by Warren, and an account of schistosomiasis in the context of British and American imperialism is given by Farley.

Liver and Lung Fluke Diseases:

Over 100 other species of flukes infect humans either as adults or as larvae, and only the most important ones are considered here. These are Paragonimus westermani, the lung fluke that causes paragonimiasis; Clonorchis sinensis, the liver fluke that causes clonorchiasis; and Opisthorchis spp., which cause opisthorchiasis. Virtually all the important discoveries about the parasites themselves were made during the period 1874 to 1918 as a result of observations on other parasitic flukes such as Fasciola hepatica in sheep and others of zoological rather than medical interest. The life cycles of these flukes are essentially similar to that described for Schistosoma spp. above, with the added complication that in some species, there is an additional intermediate host between the snail and the human in or on which the cercariae encyst. Humans become infected when they eat the infected second intermediate host. The various discoveries were made by a large number of people, often in obscure publications, and no attempt is made here to list the individual achievements; for this, the reader is referred to Groveand Muller. Our knowledge of the pathologic effects of clonorchiasis and opisthorchiasis has emerged gradually, with few historically interesting discoveries except the relatively recent finding of an association with the bile duct cancer cholangiocarcinoma.

The history of these infections as diseases begins with the discovery of the worms and continues with the elaboration of the life cycles. P. westermani was discovered in the lungs of a human by Ringer in 1879, and eggs in the sputum were recognized independently by Manson and Erwin von Baelz in 1880. Manson also suggested that a snail might act as an intermediate host, and a number of Japanese workers, including Koan Nakagawa, Sadamu Yokogawa, Harujiro Kobayashi, and Keinosuke Miyairi, reported on the whole life cycle in the snail Semisulcospira between 1916 and 1922.

The human liver fluke, C. sinensis, was first recognized by James McConnell in 1875, and the snail host was recognized by Masatomo Muto in 1918, but it was the discovery in 1915 by Kobayashi of a second intermediate host, an important food fish from which human infections are acquired, that had the greatest impact on our knowledge and control of this infection.

The first records of Opisthorchis infections in humans were made by Konstantin Wingradoff in 1892, and the snail and fish hosts and their roles in the life cycle were discovered by Hans Vogel in 1934.

Cestodiasis (Tapeworm Infections):

Humans can be infected by about 40 species of adult tapeworms and about 15 larval forms, mainly as accidental hosts. The most important cestodes belong to two groups, the taeniid and diphyllobothriid tapeworms. The characteristic taeniid adults, which can reach a length of several meters, live in the intestine attached by a scolex and shed mature proglottids (“segments”)

containing numerous eggs, which pass out into soil or water, where the eggs are released. When an intermediate host consumes the eggs, they hatch in the intestine, releasing larval stages, oncospheres that burrow through the gut wall to reach various tissues of the host, where they develop into encysted cysticerci or bladder worms. The life cycle is completed when undercooked or raw meat is eaten and the cysticerci are released and attach to the gut wall of the final host and develop into adult tapeworms. The two species in humans, Taenia saginata, the beef tapeworm and the larger of the two, and T. solium, the pork tapeworm, use cattle and pigs as their respective intermediate hosts. The scientific study of the taeniid tapeworms of humans can be traced to the late 17th century and the observations of Edward Tyson on the tapeworms of humans, dogs, and other animals. Tyson was the first person to recognize the "head" (scolex) of a tapeworm, and his subsequent descriptions of the anatomy and physiology of the adult worms laid the foundations for our knowledge of the biology of the taeniid tapeworms of humans. Although by this time it had become clear that there were differences between the broad tapeworm and the other tapeworms that we now know to be taeniids, the distinctions between T. solium and T. saginata were not obvious. These worms continued to be confused long after the work of Tyson, and although Goeze in 1782 had suspected that there were two species, it was not until the middle of the 19th century that Küchenmeister is credited with recognizing the differences between T. solium and T. saginata based on the morphology of the scolex. In 1784, the first indications that intermediate hosts were involved in the life cycles of taeniid tapeworms emerged from the detailed studies of the pork tapeworm by a German pastor, Johann August Ephraim Goeze, who observed that the scolices of the tapeworm in humans resembled cysts in the muscle of pigs. Some 70 years later, Küchenmeister, in much-criticized experiments, fed pig meat containing the cysticerci of T. solium to criminals condemned to death and recovered adult tapeworms from the intestine after they had been executed. Shortly afterward, in 1868 to 1869, J. H. Oliver observed that T. saginata tapeworm infections occurred in individuals who had eaten "measly" beef, and this was confirmed by the Italian veterinarian Edoardo Perroncito in 1877.

The adult stages of T. solium and T. saginata rarely cause any overt signs or symptoms, and there are no early descriptions of diseases that might be caused by these tapeworms. On the other hand, humans are host to two important kinds of larval tapeworm, cysticerci of the pork tapeworm T. solium and hydatid cysts of the dog tapeworm Echinococcus granulosus. The encysted larvae, cystercerci, of T. solium in the flesh of pigs, known as "measly pork," were well known to the ancient Greeks and are referred to by Aristotle (384 to 322 BC), who, in the section on diseases of pigs in his History of Animals, gives a detailed and accurate account of "bladders that are like hailstones". Although the cysts in the muscle cause no obvious illness in humans, cysts in the brain can cause symptoms resembling epilepsy, and these must have been apparent in early civilizations. However, there is nothing in the encyclopedic works of Hippocrates to suggest that the Greek physicians knew that humans harbored such cysts or suffered from any conditions associated with them. There is, however, indirect evidence from different cultures that people were aware of the possible dangers inherent in eating the flesh of pigs. Küchenmeister comments that infections with cysticerci are not found in those, such as Jews and Muslims, whose religious beliefs forbid the consumption of pork, but as we have already seen, similar arguments have been put forward with respect to Trichinella spiralis infections. There are accounts of what are possibly cysticerci in humans by Johannes Udalric Rumler in 1558,

Domenico Panaroli in 1652, and Thomas Wharton in 1656, but none of these observers realized that the structures they described were parasites. The first reliable accounts of cystercerci as parasites of some kind are by Philip Hartmann in 1688 and Marcello (Marcus) Malpighi in 1697, but the realization that these cysts were the larval stages of tapeworms had to await studies by Johann Goeze in 1784. The demonstration of the life cycle of T. solium shed new light on the nature of the human condition, cysticercosis, and it became apparent that humans could probably become infected with the larval stages of T. solium when they ingested the tapeworm eggs. Although the conclusive experiments could not be carried out for ethical reasons, many experiments with animals and observations of humans established without doubt by the middle of the 19th century that cysticercosis was caused by the ingestion of the eggs of T. solium. These observations had a massive impact on the control of tapeworm infections in humans by restricting the amount of meat of infected animals available for human consumption.

There are brief accounts of the history of cysticercosis by Nieto and more detailed accounts by Foster and Grove. There are also less easily accessible accounts by Vosgien, Henneberg, and Guccione.

The most serious human disease caused by a larval cestodes is echinococcosis, or hydatid disease, resulting from accidental infection with larval stages of the canid tapeworm, Echinococcus granulosus, which frequently occurs as an adult in dogs and as a larval cyst in wild and domesticated animals including sheep. The massive bladder-like hydatid cysts, particularly in the liver, were well known in ancient times, and there are references to such cysts in ritually slaughtered animals in the Babylonian Talmud and, in animals slaughtered for food, by Hippocrates in the fourth century BC, Arataeus in the first century AD, and Galen in the second century AD. There are also descriptions of hydatid cysts in humans in the Corpus Hippocratorum and in the works of Galen and in later European medical texts, in which they have variously been considered to be sacs of mucus, enlarged glands, distorted blood vessels, lymphatic varices, or accumulations of lymph. Francisco Redi in the 17th century was the first to appreciate the parasitic nature of these cysts, but credit for the hypothesis that these cysts were the larval stages of tapeworms goes to the German clinician and natural historian Pierre Simon Pallas, who showed this in 1766. It was not until 1853 that Carl von Siebold demonstrated that Echinococcus cysts from sheep gave rise to adult tapeworms when fed to dogs, and in 1863 Bernhard Naunyn found adult tapeworms in dogs fed with hydatid cysts from a human. There are good accounts of the history of hydatid disease by Foster and Grove.

Humans also harbor the adults of Diphyllobothrium latum, the broad or fish tapeworm that lives in the intestine. Eggs are passed out in the feces, and the first larval stage, the coracidium, develops within the egg and is eaten by a copepod, in which it develops to the second larval stage, the procercoid. When an infected copepod is eaten by a fish, the procercoid develops into the third larval stage, the plerocercoid, and when a human eats an infected fish, the plerocercoid develops into an adult tapeworm in the gut. The broad tapeworm was well known in antiquity and is mentioned, sometimes indirectly, in the major classical medical writings including the Ebers papyrus, the Corpus Hippocratorum, and the works of Celsus and Avicenna. However, there are no accurate early clinical records because there are few overt signs of the infection

apart from abnormal hunger, malaise, and abdominal pain. Early descriptions of the worm tend to be unreliable because, as has already been mentioned, there was considerable confusion with the two common species of Taenia. Nevertheless, by the beginning of the 17th century, it became apparent that there were two very different kinds of tapeworm (broad and taeniid) in humans. It is generally agreed that Diphyllobothrium was first recognized as being distinct from Taenia by the Swiss physician Felix Plater, who also provided the first descriptions of the disease at the beginning of the 17th the century. The first accurate description of the proglottids was by another Swiss biologist, Charles Bonnet, in 1750, but, unfortunately, the worm he illustrated had a Taenia scolex, a mistake he remedied in 1777. By the middle of the 18th century, it was apparent that infections with D. latum occurred in humans whose diet was mainly fish. However, it was not until the life cycles of other tapeworms of zoological interest had been elaborated that further progress became possible, since the existence of three hosts in the life cycle, human, fish, and copepod, confused the issue. An understanding of the life cycle of this parasite began in 1790, when the Dane Peter Christian Abildgaard observed that the intestine of sticklebacks contained worms that resembled the tapeworms found in fish-eating birds; however, it was some time before there was any significant advance in our understanding of the life cycle of D. latum. In the meantime, there were a number of misleading observations until 1881, when the German zoologist Maximillian Gustav Christian Carl Braun realized that the unsegmented tapeworms common in pike and other fish were the larval stages of D. latum and succeeded in infecting dogs with these plerocercoids; in 1882 he achieved similar results in humans. Braun suspected that this was not the whole story, but it was many years later that two Polish scientists, Constantine Janicki and Felix Rosen, working in Switzerland, incriminated copepods in the life cycle and showed that they fed on the eggs of the tapeworm and were then eaten by fish, which, in their turn, were eaten by humans. There are good accounts of Diphyllobothrium and dyphilobothriasis by Foster and Grove.

DISCOVERY OF THE PARASITIC PROTOZOA:

Because of their small size, it was not possible to recognize any protozoa until the invention of the microscope and its use by Antonie van Leeuwenhoek toward the end of the 17th century. The study of parasitic protozoa only really began two centuries later, following the discovery of bacteria and the promulgation of the germ theory by Pasteur and his colleagues at the end of the 19th century.

Amoebae and Amoebiasis:

Humans harbor nine species of intestinal amoebae, of which only one, Entamoeba histolytica, is a pathogen. The life cycle is simple. The amoebae live and multiply in the gut and form cysts that are passed out in the feces and infect new individuals when they are consumed in contaminated water or food. Most infections are asymptomatic, but some strains of E. histolytica can invade the gut wall, causing severe ulceration and amoebic dysentery characterized by bloody stools. If the parasites gain access to damaged blood vessels, they may be carried to extraintestinal sites anywhere in the body, the most important of which is the liver, where the amoebae cause hepatic amoebiasis. Supposed evidence that both the intestinal and liver forms of the disease were recognized from the earliest times is circumstantial because there are so many

causes of both the bloody dysentery characteristic of amoebiasis and the symptoms of hepatic amoebiasis that many of these records are open to other interpretations. With these reservations in mind, the earliest record is possibly that from the Sanskrit document Brigu-samhita, written about 1000 BC, which refers to bloody, mucus diarrhea. Assyrian and Babylonian texts from the Library of King Ashurbanipal refer to blood in the feces, suggesting the presence of amoebiasis in the Tigris-Euphrates basin before the sixth century BC, and it is possible that the hepatic and perianal abscesses described in both Epidemics and Aphorisms in the Corpus Hippocratorum refer to amoebiasis. Since epidemics of dysentery by itself are likely to result from bacterial infections and dysentery associated with disease of the liver is likely to be amoebic, later records are easier to interpret. In the second century AD, Galen and Celsus both described liver abscesses that were probably amoebic, and the works of Aretaeus, Archigenes, Aurelanus, and Avicenna toward the end of the first millennium give good accounts of both dysentery and hepatic involvement. As amoebiasis became widespread in the developed world, there were numerous records of "bloody flux" in Europe, Asia, Persia, and Greece in the Middle Ages. The disease appears to have been introduced into the New World by Europeans sometime in the 16th century, and with the later development of European colonies and increased world trade, there are numerous clear descriptions of both the intestinal and hepatic forms of amoebiasis. In the 19th century, several books mainly concerned with diseases in India, including Researches into the Causes, Nature and Treatment of the More Prevalent Diseases of India and of Warm Climates Generally by James Annersley, clearly refer to both intestinal and hepatic amoebiasis, and it is now generally agreed that this book contains the first accurate descriptions of both forms of the disease. The connection between amoebic dysentery and liver abscesses was described by William Budd, the English physician who discovered the method of transmission of typhoid. The amoeba itself, E. histolytica, was discovered by Friedrich Lösch (also known as Fedor Lesh) in 1873 in Russia, and Lösch also established the relationship between the parasite and the disease in dogs experimentally infected with amoebae from humans. Stephanos Kartulis, a Greek physician, also found amoebae in intestinal ulcers in patients suffering from dysentery in Egypt in 1885 and 1896 and noted that he never found amoebae from nondysenteric cases. Kartulis also showed that cats could be infected with amoebae per rectum and developed dysentery a finding discussed below. The authoritative report by William Thomas Councilman and Henri Lafleur, working at the Johns Hopkins Hospital in 1891, represents a definitive statement of what was known about the pathology of amoebiasis at the end of the 19th century, and much of it is still valid today.

It was pointed out above that humans harbor several species of amoebae. The most common are E. histolytica, which has just been considered, and a larger and superficially similar harmless species, E. coli; the presence of these two parasites confused early workers in this field. The first clues that there was more than one species in humans came from the work of Heinrich Iranaus Quincke and Ernst Roos working in Kiel in 1894, who observed that cats could only be infected per rectum or orally with cysts of amoebae that contained ingested red blood cells and not with those that did not, i.e., E. coli. Thereafter, the most contentious arguments relate to the various morphologically identical species and strains of E. histolytica, and their relationship to disease has only recently been resolved by using biochemical techniques that clearly show that the

presence of two common species, E. histolytica, which can cause disease, and E. dispar, which cannot.

The history of amoebiasis is well documented. The most comprehensive account of the early history is that by Dobell, and there are also good accounts of the early history by Bray, Foster, Kean, Scott, and Wenyon and reviews containing more recent information by Craig, Guirola, Imperato, Martínez-Báez, Stilwell, and Svanidtse.

Giardia and Giardiasis:

Giardia holds a special place in the science of parasitic protozoology because the parasite Giardia duodenalis, also known as G. lamblia or G. intestinalis, was the first parasitic protozoan of humans seen by Antonie van Leeuwenhoek in 1681. The life cycle of Giardia is very simple: the parasites multiply in the duodenum and form cysts that are passed out in the feces and infect new individuals when they are swallowed in food or water. Most infected individuals show few or no signs of infection, but in some, particularly children, there may be malabsorption, diarrhea, and abdominal pain. G. duodenalis was first seen by Leeuwenhoek and, interestingly, associated by him with his own loose stools. Leeuwenhoek's illustrations are not very informative, and the first good illustrations of Giardia are those of Vilém Lambl in 1859. The parasite received little attention until 1902, when the American parasitologist Charles Wardell Stiles began to suspect that there was a causal relationship with diarrhea. This was not followed up until the 1914 to 1918 World War, when soldiers with diarrhea were found to pass Giardia cysts that caused similar symptoms when administered to laboratory animals. In 1921, Clifford Dobell suggested that Giardia was a pathogen, and in 1926, Reginald Miller, a physician working in London, conclusively showed that some children infected with Giardia did suffer from malabsorption whereas others acted as unaffected carriers. It was not until 1954, however, that the detailed studies by the American physician Robert Rendtorff produced unambiguous evidence linking the parasite with the disease. In the 300 years since Giardia was first discovered, it has become recognized as a common parasite and potential pathogen worldwide; however, it is still not known how many species infect humans and what role, if any, reservoir hosts play in the epidemiology of the infection. Fuller accounts of the history of giardiasis are given by Wenyon and Farthing.

African Trypanosomes and Sleeping Sickness:

African trypanosomiasis is caused by infection with two subspecies of trypanosomes, Trypanosoma brucei gambiense, which causes Gambian or chronic sleeping sickness, and T. b. rhodesiense, which causes Rhodesian or acute sleeping sickness. The trypanosomes multiply in the blood and are taken up by tsetse flies when they feed. Within the tsetse fly, there is a phase of multiplication and development resulting in the formation of infective trypanosomes in the salivary glands of the fly, which are injected into a new host when the fly feeds. The infection itself causes a number of symptoms including anemia, wasting and lethargy, and, in some cases, if the parasites pass into the brain and cerebrospinal fluid, coma and death. There are similar parasites in wild and domesticated animals. The first definitive accounts of sleeping sickness are by an English naval surgeon, John Atkins, in 1721and Thomas Winterbottom, who coined the

term “negro lethargy” in 1803. An appreciation of the real cause of the disease was not possible until Pasteur had established the germ theory toward the end of the 19th century. Trypanosomes had been seen in the blood of fishes, frogs, and mammals from 1843 onward, but it was not until 1881 that Griffith Evans found trypanosomes in the blood of horses and camels with a wasting disease called surra and suggested that the parasites might be the cause of this disease. These observations led to the most important discoveries about human and animal trypanosomiasis shortly afterwards. In 1894, David Bruce, a British army surgeon investigating an outbreak of nagana, a disease similar to surra, in cattle in Zululand, was looking for a bacterial cause and found trypanosomes in the blood of diseased cattle; he demonstrated experimentally that these caused nagana in cattle and horses and also infected dogs. He also observed that infected cattle had spent some time in the fly-infested “tsetse belt” and that the disease was similar to that in humans with Negro lethargy and fly disease of hunters. Trypanosomes were seen in human blood by Gustave Nepveu in 1891. In 1902, Everett Dutton identified the trypanosome that causes Gambian or chronic sleeping sickness (T. b. gambiense) in humans, and in 1910 J. W. W. Stephens and Harold Fantham described T. b. rhodesiense, the cause of Rhodesian or acute sleeping sickness. Although Bruce had shown that trypanosome infections in cattle were acquired from tsetse flies, he thought that transmission was purely mechanical, and the role of the tsetse fly in the transmission of sleeping sickness remained controversial until Friedrich Kleine, a colleague of Robert Koch, demonstrated in 1909 the essential role of the tsetse fly in the life cycle of trypanosomes.

The persistence of trypanosomes in the blood and the existence of successive waves of parasitemia were described in detail by Ronald Ross and David Thompson in 1911, but the actual mechanism of what happens and how the parasite evades the immune response, now called antigenic variation, was not elaborated until the work of Keith Vickerman in 1969. The story of African sleeping sickness is told briefly by Hoare and in more detail by Foster, Nash, Lyons, Wenyon, and Williams.

South American Trypanosomiasis: Chagas ‘disease:

Chagas' disease is caused by infection with another trypanosome, Trypanosoma cruzi, transmitted by insects belonging to the order Hemiptera or true bugs, commonly known as kissing bugs because of their tendency to bite the lips and face. The transient trypanosome forms circulate in the blood and are taken up by a blood-sucking bug when it feeds. The parasites multiply in the gut of the bug, and infective forms are passed out in the feces while the bug is feeding on a new host and are rubbed into the bite. In the human host, parasites enter and multiply in a variety of different cells and eventually induce what are thought to be autoimmune responses that results in the destruction of both infected and uninfected tissues. The nature of the disease depends on the tissues and organs involved, and the most conspicuous forms are massive distension of the intestinal tract, especially the esophagus and colon, and destruction of cardiac muscle, which can result in death many years after the initial infection. T. cruzi infections are common in many mammals on the American continent, but the human disease now occurs only in South and Central America. The earliest indication that Chagas' disease is an ancient infection in South America comes from the examination of spontaneously mummified human remains

from Chile between 470 BC and AD 600 that show clear signs of the characteristic destructive nature of the disease. The use of immunological and molecular techniques has made it possible to detect the presence of T. cruzi without necessarily visualizing the parasites themselves. T. cruzi DNA has been detected in the heart and esophagus of mummified bodies from Peru and northern Chile dating from 2000 BC to AD 1400 and in samples from bodies in museums from northern Chile from about AD 1000 to 1400. The parasites themselves have also been identified by light and electron microscopy in a Peruvian mummy from the 15 to 16th century AD.

The history of T. cruzi and Chagas' disease really begins with a series of discoveries by the Brazilian scientist Carlos Chagas, between 1907 and 1912. Chagas not only discovered the trypanosome T. cruzi and demonstrated its transmission by bugs but also described the disease that affects some 18 million people and now commemorates his name. Chagas' first observation was that the blood-sucking bugs that infested the poorly constructed houses harbored flagellated protozoa and that when these flagellates were injected into monkeys and guinea pigs, trypanosomes appeared in the blood. Chagas later found the same trypanosomes in the blood of children with an acute febrile condition and suspected that blood-sucking bugs might also transmit the parasite to humans, but he thought that the trypanosomes were transmitted via the bite of the insect. It was the French parasitologist Emile Brumpt who demonstrated transmission via the fecal route. The links between infection with T. cruzi and the various signs of Chagas' disease, such as distended colon and esophagus and cardiac failure, were not determined until the work of Fritz Koberle in the 1960s. Exactly how the damage to heart and nerves is caused and what role the autoimmune component plays are still controversial. The history of Chagas' disease has been well documented by Scott, Lewinsohn, Leonard, Miles, and Wenyon.

Leishmania and Leishmaniasis:

Leishmaniasis, caused by several species of Leishmania, is transmitted by sandflies and occurs in various forms in the Old and New World. The parasites infect and multiply in macrophages and are taken up by sandflies when they feed. In the gut of the sandfly, the parasites multiply and reach the mouthparts, from where they are injected into a new host when the sandfly feeds again. The disease, leishmaniasis, takes a number of forms ranging from simple cutaneous ulcers to massive destruction of cutaneous and subcutaneous tissues in the mucocutaneous forms and the involvement of the liver and other organs in the visceral form.

From a historical viewpoint, it is easiest to consider the Old World forms first. Old World cutaneous leishmaniasis, known as oriental sore, is an ancient disease, and there are descriptions of the conspicuous lesions on tablets in the library of King Ashurbanipal from the 7th century BC, some of which are thought to have been derived from earlier texts from 1500 to 2500 BC. There are detailed descriptions of oriental sore by Arab physicians including Avicenna in the 10th century, who described what was (and is) called Balkh sore from northern Afghanistan, and there are later records from various places in the Middle East including Baghdad and Jericho; many of the conditions were given local names by which they are still known. Old World visceral leishmaniasis, or kala azar, characterized by discolored skin, fever, and enlarged spleen, is easily confused with other diseases, especially malaria. Kala azar was first noticed in Jessore in India in 1824, when patients suffering from fevers that were thought to be due to malaria

failed to respond to quinine; by 1862 the disease had spread to Burdwan, where it reached epidemic proportions. The cause remained unknown, and several eminent clinicians, including Ronald Ross, were convinced that kala azar was a virulent form of malaria. It was not until the parasite, L. donovani, was discovered in 1900 by Leishman and Donovan that the true nature of the disease became apparent.

The discovery of the parasites responsible for the Old World cutaneous disease is controversial, and a number of observers described structures that might or might not have been leishmanial parasites from oriental sores. Credit for their discovery is usually given to an American, James Homer Wright, although there is no doubt that they were actually seen in 1885 by David Cunningham, who did not realize what they were, and in 1898 by a Russian military surgeon, P. F. Borovsky. The discovery of the parasite that causes visceral leishmaniasis, L. donovani, is less controversial, and it is universally accepted that a Scottish army doctor, William Leishman, and the Professor of Physiology at Madras University, Charles Donovan, independently discovered the parasite in the spleens of patients with kala azar. It is fair to point out that Borovsky's discoveries were unknown to Wright and to Leishman and Donovan.

The search for a vector was a long one, and it was not until 1921 that the experimental proof of transmission to humans by sandflies belonging to the genus Phlebotomus was demonstrated by the Sergent brothers, Edouard and Etienne. The actual mode of infection, through the bite of the sandfly, was not finally demonstrated until 1941. The history of Old World leishmaniasis is described by Garnham, Manson-Bahr, and Wenyon.

In the New World, cutaneous and mucocutaneous leishmaniasis cause disfiguring conditions that have been recognized in sculptures since the 5th century and in the writings of the Spanish missionaries in the 16th century. It was originally thought that New World Leishmaniasis and Old World leishmaniasis were the same, but in 1911 Gaspar Vianna found that the parasites in South America differed from those in Africa and India and created a new species, Leishmania braziliensis. Since then, a number of other species unique to the New World have been described. Following the discovery of the sandfly transmission of Old World leishmaniasis, the vectors in the New World were also assumed to belong to the genus Phlebotomus, but in 1922 it was discovered that the genus involved was actually Lutzomyia. Over the last two decades, the complex pattern of species of parasite, vector, reservoir host, and disease has been painstakingly elaborated by Ralph Lainson and his colleagues.

Malaria:

Malaria is one of the most important infectious diseases in the world, and its history extends into antiquity. The disease is caused by four species of the genus Plasmodium, P. falciparum, P. vivax, P. ovale, and P. malariae. Similar parasites are common in monkeys and apes. It is now generally held that malaria arose in our primate ancestors in Africa and evolved with humans, spreading with human migrations first throughout the tropics, subtropics, and temperate regions of the Old World and then to the New World with explorers, missionaries, and slaves. The characteristic periodic fevers of malaria are recorded from every civilized society from China in 2700 BC through the writings of Greek, Roman, Assyrian, Indian, Arabic, and European

physicians up to the 19th century. The earliest detailed accounts are those of Hippocrates in the 5th century BC, and thereafter there are increasing numbers of references to the disease in Greece and Italy and throughout the Roman Empire as its occurrence became commonplace in Europe and elsewhere. Over this period, it became clear that malaria was associated with marshes, and there were many ingenious explanations to explain the disease in terms of the miasmas rising from the swamps.

Our scientific understanding of malaria did not begin until the end of the 19th century following the establishment of the germ theory and the birth of microbiology, when it became necessary to discover the cause of the disease that was then threatening many parts of the European empires. The discovery of the malaria parasite and its mode of transmission are among the most exciting events in the history of infectious diseases, and this topic has been reviewed many times, particularly by Bruce-Chwatt, Garnham, Harrison, McGregor, Poser and Bruyn, and Wenyon.

The life cycle is a very complex one that begins when an infected anopheline mosquito injects sporozoites, the infectious stages, into the blood of its host. Sporozoites enter and multiply in liver cells, and thousands of daughter forms, merozoites, are released into the blood. These merozoites invade red blood cells, in which another phase of multiplication occurs; this process is repeated indefinitely, causing the symptoms of the disease we call malaria. Some merozoites do not divide but develop into sexual stages, the male and female gametocytes that are taken up by another mosquito when it feeds; fertilization and zygote formation occur in the gut of the mosquito. The zygote develops into an oocyst on the outside of the mosquito gut, and within the oocyst there is another phase of multiplication that results in the production of sporozoites that reach the salivary glands to be injected into a new host. The parasites in the blood were first seen in 1880 by a French army surgeon, Alphonse Laveran, who was looking for a bacterial cause of malaria and who immediately realized that the parasites were responsible for the disease.

The discovery that the mosquito acted as a vector was due to the intuition of Patrick Manson. Manson had already demonstrated that filarial worms, also blood parasites, were transmitted by mosquitoes and postulated that the vector of the malaria parasite might also be a mosquito, partly because of his knowledge of the life cycle of filarial worms and partly because of the known association between the disease and marshy places in which mosquitoes breed. Manson was unable to undertake this investigation himself and persuaded Ronald Ross, an army surgeon, to carry out the work in India. The story of Ross' discoveries has been told many times and is not repeated in detail here, since there are excellent accounts by Ross himself and in the Ross-Manson collected letters and also by Bruce-Chwatt, Garnham, Harrison, Manson-Bahr, Nye and Gibson, Poser and Bruyn, and Russell. In 1897, Ross saw what we now know to be the oocysts of P. falciparum in an anopheline mosquito that had fed on a patient with crescentic malaria parasites (gametocytes) in his blood, but he was unable to follow this up at the time. Turning his attention to a bird malaria, P. relictum, he found all the stages of the parasite in Culicine mosquitoes that had fed on infected sparrows. In making this discovery, Ross acknowledged the work of a young Canadian, William George MacCullum, whose studies on the development of the sexual stages of a related avian parasite Halteridium (=Haemoproteus) columbae had led him to the conclusion that these parasites were similar to those in the blood of humans with malaria.

In the same year that Ross made his discovery, the Italian malariologists Giovanni Battista Grassi, Amico Bignami, and Giuseppe Bastianelli described the developmental stages of malaria parasites in anopheline mosquitoes; the life cycles of P. falciparum, P. vivax, and P. malariae were described a year later. For nearly 50 years, the life cycle in humans remained incompletely understood and nobody knew where the parasites, which could not be seen in the blood, developed during the first 10 days after infection. In 1947, Henry Shortt and Cyril Garnham, working in London, showed that a phase of division in the liver preceded the development of parasites in the blood. The final brick was put in place when an American clinician, Wojciech Krotoski, in collaboration with Garnham's team, showed that in some strains of P. vivax the stages in the liver could remain dormant for several months. Sadly, the discovery of the life cycle of the malaria parasite eventually led to acrimony between Ross and Manson and between the British and the Italians, something that still rumbles on a century later.

Toxoplasma, Toxoplasmosis, and Infections Caused by Related Organisms:

Toxoplasmosis is one of the most common and widespread parasitic infections but is relatively little known because in the majority of cases, infections are asymptomatic; however, it can be a serious cause of mortality and morbidity in fetuses and immunodeficient individuals. The parasite that causes the infection, Toxoplasma gondii, was discovered independently by the French parasitologists Charles Nicolle and Louis Herbert Manceaux while looking for a reservoir host of Leishmania in a North African rodent, the gundi Ctenodactylus gondii and by Alfonso Splendore in Sao Paulo in rabbits. At about the same time, Samuel Taylor Darling saw what probably similar organisms in a human were, and the first definitive observation of T. gondii from a child in connection with an infection was made by a Czech physician, Josef Janku, in 1923. Even then, T. gondii was largely regarded as an interesting curiosity until an association with human congenital disease was recognized in 1937 by Arne Wolf and David Cowen. This association was followed by the realization that T. gondii rarely causes disease even though it is a very common parasite of adults but that in pregnant women the parasite can cross the placenta and can damage the fetus. The early history of the discovery of T. gondii and toxoplasmosis is discussed by Wenyon and Dubey and Beattie.

While these developments were taking place, there were increasing numbers of records from virtually all species of mammals and many birds, but the nature of the parasite remained obscure until the life cycle had been worked out. The life cycle of T. gondii is a very complicated one and remained elusive until 1970, when scientists in Britain, Germany, The Netherlands, and the United States independently demonstrated that this parasite was a stage in the life cycle of a common intestinal coccidian of cats. In the most simple form of the life cycle, cats become infected when they swallow oocysts, the resistant infective stages containing sporozoites, which invade and multiply in intestinal cells, where sexual stages are produced, fertilization occurs, and oocysts are produced. However, there is an alternative life cycle. If the oocysts are swallowed by a mouse (or any other nonfeline host), multiplication occurs in the intestinal cells, but instead of sexual stages being produced there follows a disseminated infection during which resistant stages form in the brain and muscle. There is no further development in the mouse, but when the mouse is eaten by a cat, the life cycle reverts to its basic sexual pattern. Humans are infected in the same

way as mice if they consume oocysts, but they can also become infected by eating any kind of meat containing the resistant forms. It is therefore not surprising that the life cycle remained elusive until William McPhee Hutchison, working in Glasgow in 1965, showed that the infectious agent was passed in the feces of cats. At the time he thought that it was transmitted with a nematode worm, as happens with the flagellate Histomonas meleagridis and the nematode Heterakis gallinarum in fowl. Hutchison subsequently identified protozoan cysts in the feces as those of a coccidian related to Isospora, a common parasite of cats. In the meantime, other groups were following up Hutchison's 1965 observation of the presence of infectious agents in the feces of cats, and Hutchison's incrimination of the isosporan parasite of cats as T. gondii was independently confirmed by Jack Frenkel and Harley Sheffield in the United States, Gerhard Piekarski in Germany, and J. P. Overdulve in The Netherlands. The discovery of the T. gondii life cycle initiated a massive search for similar phases in the life cycles of other coccidian parasites, with the result that a number of protozoa that had not been properly identified were classified as stages in the life cycle of other poorly understood coccidians and that in many cases transmission depended on a predator-prey relationship. Humans are infected with two related parasites, Sarcocystis hominis and S. suihominis, acquired from beef and pork, respectively, and S. lindemanni, whose source is unknown. The early history of our knowledge of Sarcocystis is covered by Wenyon, and subsequent discoveries are described by Tadros and Laarman.

Humans are also hosts to three other species of coccidian, Isospora belli, Cryptosporidium parvum, and Cyclosporine cayetanensis that have in the past been regarded as rare and accidental curiosities but have recently been identified as pathogens in AIDS patients and other immunocompromised individuals. All have simple life cycles initiated by the ingestion of oocysts followed by multiplication and spread within the intestinal cells of the host and the eventual production of sexual stages, as for T. gondii infection in cats. C. parvum was discovered in 1912 by the American parasitologist Edward Ernest Tyzzer in the gastric glands of laboratory mice in which he had previously found another species, C. muris . C. parvum is not very host specific, and the first cases in humans were recorded in 1976 independently by Nime and Meisel. From 1981 onward, numerous new cases began to be recognized in AIDS patients. The oocysts Cryptosporidium are very resistant to chlorination, and the source of these infections is probably drinking water contaminated with cattle feces. Cryptosporidium infections are now known to be very common and have caused a number of epidemics in which the victims have experienced abdominal pain and diarrhea. In immunodepressed individuals, especially those infected with HIV, the infection can become disseminated to the liver, pancreas, and respiratory tract and can be fatal. There is an excellent history of human cryptosporidiosis by McDonald and a short but useful review by Dubey et al.

C. cayetanensis is another coccidian that is associated mainly with AIDS. In 1979, the English parasitologist Richard Ashford found an unidentified coccidian in patients in Papua New Guinea, but it received little attention until it was found again in the stools of patients with HIV by Soave et al. in 1986. In 1992, this parasite was named Cyclospora cayetanensis, and since then it has been identified as the cause of a number of outbreaks of diarrhea and fatigue in both immunocompetent and immunosuppressed individuals. Cyclospora infections are known to be transmitted in water and on fruit, but the original source is not known.

The last of this group of parasites, Isospora belli, discovered by Woodcock in 1915, is another coccidian frequently found in asymptomatic immunocompetent individuals but associated with diarrhea in AIDS patients. The whole subject of parasitic infections in immunocompromised hosts is discussed by Ambroise-Thomas.

Microsporidians:

Microsporidians are extremely common spore-forming parasites of vertebrates and invertebrates that were until relatively recently grouped with myxosporidians as cnidosporidians and classified with or close to the Sporozoa. We now know that the myxosporidians are more closely related to the Metazoa than the Protozoa and that the microsporidians are more closely related to the Fungi. Nevertheless, microsporidians are still regarded as the province of parasitologists and have become important as concomitant infections in AIDS patients. The life cycle of microsporidians is quite complex. The most conspicuous stage is the resistant gram-positive spore containing a coiled filament and an infective body, the sporoplasm. The host becomes infected when the spore is ingested or inhaled. The sporoplasm is extruded through the filament and penetrates a host cell, within which the organism multiplies and spreads to other cells; eventually, another generation of spores is produced. There are, however, many variations on this basic pattern. What are now thought to have been the spores of Nosma bombycis were described by Nägeli investigating an outbreak of a disease called pébrine in the silkworm Bombyx mori in 1857and studied in much more detail by Louis Pasteur in 1865 to 1870. During the 19th century, microsporidians attracted considerable attention mainly as parasites of invertebrates. Our knowledge of human microsporidiosis in the past is limited because of difficulties in interpreting various structures that might or might not have been spores, but from the second decade of the 20th century onward, there have been a number of sporadic reports of what might have been human microsporidial infections. The first case was probably that of Encephalitozoon chagasi in a newborn baby recorded in 1927, but the first authenticated record was not until 1959, when Hisakichi Matsubayashi and his colleagues in Japan found an Encephalitozoon sp. in boy with convulsions. Thereafter, there were reports of a number of sporadic cases of microsporidian infections in humans, but interest in this group really took off in 1988, when E. bieneusi was found in an AIDS patient. Since then, about 7 genera and 14 species associated with fulminating infections in immunodepressed patients and less serious infections in immunocompetent individuals have been described and the number of cases, particularly in AIDS patients, continues to rise. Despite their importance, very little is known about the transmission and epidemiology of the microsporidians. [3]

Archaeology

Archaeology, or archeology, is the study of human activity through the recovery and analysis of material culture. The archaeological record consists of artifacts, architecture, biofacts or ecofacts, and cultural landscapes. Archaeology can be considered both a social science and a branch of the humanities. In North America, archaeology is considered a sub-field of anthropology, while in Europe archaeology is often viewed as either a discipline in its own right or a sub-field of other disciplines.

Archaeologists study human prehistory and history, from the development of the first stone tools at Lomekwi in East Africa 3.3 million years ago up until recent decades. Archaeology as a field is distinct from the discipline of paleontology, the study of fossil remains. Archaeology is particularly important for learning about prehistoric societies, for whom there may be no written records to study. Prehistory includes over 99% of the human past, from the Paleolithic until the advent of literacy in societies across the world. Archaeology has various goals, which range from understanding culture history to reconstructing past lifeways to documenting and explaining changes in human societies through time.

The discipline involves surveying, excavation and eventually analysis of data collected to learn more about the past. In broad scope, archaeology relies on cross-disciplinary research. It draws upon anthropology, history, art history, classics, ethnology, geography, geology, literary history, linguistics, semiology, textual criticism, physics, information sciences, chemistry, statistics, paleoecology, paleography, paleontology, paleozoology, and paleobotany.

Archaeology developed out of antiquarianism in Europe during the 19th century, and has since become a discipline practiced across the world. Archaeology has been used by nation-states to create particular visions of the past. Since its early development, various specific sub-disciplines of archaeology have developed, including maritime archaeology, feminist archaeology and archaeoastronomy, and numerous different scientific techniques have been developed to aid archaeological investigation. Nonetheless, today, archaeologists face many problems, such as dealing with pseudoarchaeology, the looting of artifacts, a lack of public interest, and opposition to the excavation of human remains. [(4)]

History of archaeology:

The development of the field of archaeology has its roots with history and with those who were interested in the past such as kings who wanted to show past glories of their respective nations. The 5th-century-BC Greek historian Herodotus was the first scholar to systematically study the past and perhaps the first to examine artifacts. In the Song Empire (960-1279) of Imperial China, Chinese scholar-officials unearthed, studied, and cataloged ancient artifacts. The 15th and 16th centuries saw the rise of antiquarians in Renaissance Europe who were interested in the collection of artifacts. The antiquarian movement shifted into nationalism as personal collections turned into national museums. It evolved into a much more systematic discipline in the late 19th century and became a widely used tool for historical and anthropological research in the 20th century. During this time there were also significant advances in the technology used in the field.

Archaeology had its start in the European study of history and in people who were interested in the past. King Nabonidus (556-539 BCE), the last king of the Neo-Babylonian Empire, was interested in the past so he could align himself with past glories. He led a revitalization movement and rebuilt ancient temples. Early systemic investigation and Historiography can be traced back to the Greek historian Herodotus (c. 484-c. 425). He was the first western scholar to systematically collect artifacts and test their accuracy. He was also the first to make a compelling narrative of the past. He is known for his set of 9 books called The Histories, in which he wrote of everything he could find out about different regions. A few examples are he discussed the causes and consequences of the Greco-Persian Wars. He also explored the Nile and Delphi. However, scholars have found errors in his records and believe he probably did not go as far south down the Nile as he said he did.

Archaeology later concerned itself with the antiquarianism movement. Antiquarians studied history with particular attention to ancient artifacts and manuscripts, as well as historical sites. Their focus was to collect artifacts and display them in cabinets of curios and they usually were wealthy people. Antiquarianism also focused on the empirical evidence that existed for the understanding of the past, encapsulated in the motto of the 18th-century antiquary, Sir Richard Colt Hoare, "We speak from facts not theory". Tentative steps towards the systematization of archaeology as a science took place during the Enlightenment era in Europe in the 17th and 18th centuries.

During the Song Dynasty period (960–1279) in China, educated gentry became interested in the antiquarian pursuit of art collecting. Neo-Confucian scholar-officials were generally concerned with archaeological pursuits in order to revive the use of ancient Shang, Zhou, and Han relics in state rituals. This attitude was criticized by the polymath official Shen Kuo in his Dream Pool Essays of 1088. He endorsed the idea that materials, technologies, and objects of antiquity should be studied for their functionality and for the discovery of ancient manufacturing techniques, instead. Although a distinct minority, there were others who took the discipline as seriously as Shen did. For instance, the official, historian, poet, and essayist Ouyang Xiu (1007–1072) compiled an analytical catalogue of ancient rubbings on stone and bronze. Zhao Mingcheng (1081–1129) stressed the importance of using ancient inscriptions to correct discrepancies and errors in later historical texts discussing ancient events. Native Chinese antiquarian studies waned during the Yuan (1279-1368) and Ming (1368-1644) dynasties, were revived during the Qing dynasty (1644-1912), but never developed into a systematic discipline of archaeology outside of Chinese historiography.

In Europe, interest in the remains of Greco-Roman civilization and the rediscovery of classical culture began in the Late Middle Ages. Despite the importance of antiquarian writing in the literature of ancient Rome, such as Livy's discussion of ancient monuments, scholars generally view antiquarianism as emerging only in the middle Ages. Flavio Biondo, an Italian Renaissance humanist historian, created a systematic guide to the ruins and topography of ancient Rome in the early 15th century, for which he has been called an early founder of archaeology. The itinerant scholar Ciriaco de' Pizzicolli or Cyriacus of Ancona (1391—c.1455) also traveled throughout Greece to record his findings on ancient buildings and objects. Ciriaco traveled all

around the Eastern Mediterranean, noting down his archaeological discoveries in his day-book, Commentaria, that eventually filled six volumes.

Antiquarians, including John Leland and William Camden, conducted surveys of the English countryside, drawing, describing and interpreting the monuments that they encountered. These individuals were frequently clergymen – many vicars recorded local landmarks within their parishes, details of the landscape and ancient monuments such as standing stones – even if they did not always understand the significance of what they were seeing.

In the late 18th to 19th century archaeology became a national endeavor as personal cabinets of curios turned into national museums. People were now being hired to go out and collect artifacts to make a nation's collection more grand and to show how far a nation's reach extends. An example of this [clarification needed] is Giovanni Battista Belzoni who was hired by Henry Salt, the British consul to Egypt, to gather antiquities for Britain. In nineteenth-century Mexico, the expansion of the National Museum of Anthropology and the excavation of major archeological ruins by Leopoldo Batres were part of the liberal regime of Porfirio Díaz to create a glorious image of Mexico's prehispanic past.

Among the first sites to undergo archaeological excavation were Stonehenge and other megalithic monuments in England. The first known excavations made at Stonehenge were conducted by Dr William Harvey and Gilbert North in the early 17th century. Both Inigo Jones and the Duke of Buckingham also dug there shortly afterwards. John Aubrey was a pioneer archaeologist who recorded numerous megalithic and other field monuments in southern England. He also discovered and mapped the Avebury henge monument. He wrote Monumenta Britannica in the late 17th century, as a survey of early urban and military sites, including Roman towns, "camps" (hillforts), and castles and a review of archaeological remains, including sepulchral monuments, roads, coins and urns. He was also ahead of his time in the analysis of his findings. He attempted to chart the chronological stylistic evolution of handwriting, medieval William Stukeley was another antiquarian who contributed to the early development of archaeology in the early 18th century. He also investigated the prehistoric monuments of Stonehenge and Avebury; work for which he has been remembered as "probably... the most important of the early forerunners of the discipline of archaeology".He was one of the first to attempt to date the megaliths, and argued that they were a remnant of the pre-Roman druidic religion. Architecture, costume, and shield-shapes.

Excavations were carried out in the ancient towns of Pompeii and Herculaneum, both of which had been covered by ashes during the Eruption of Mount Vesuvius in AD 79. These excavations began in 1748 in Pompeii, while in Herculaneum they began in 1738 under the auspices of King Charles VII of Naples. In Herculaneum, the Theatre, the Basilica and the Villa of the Papyri were discovered in 1768. The discovery of entire towns, complete with utensils and even human shapes, as well the unearthing of ancient frescos, had a big impact throughout Europe.

A very influential figure in the development of the theoretical and systematic study of the past through its physical remains, was "the prophet and founding hero of modern archaeology," Johann Joachim Winckelmann. Winckelmann was a founder of scientific archaeology by first

applying empirical categories of style on a large, systematic basis to the classical (Greek and Roman) history of art and architecture. His original approach was based on detailed empirical examinations of artefacts from which reasoned conclusions could be drawn and theories developed about ancient societies.

In America, Thomas Jefferson, possibly inspired by his experiences in Europe, supervised the systematic excavation of a Native American burial mound on his land in Virginia in 1784. Although Jefferson's investigative methods were ahead of his time, they were primitive by today's standards.

Napoleon's army carried out excavations during its Egyptian campaign, in 1798-1801, which also was the first overseas archaeological expedition ever. The emperor took with him a force of 500 civilian scientists, specialists in fields such as biology, chemistry and languages, in order to carry out a full study of the ancient civilization. The work of Jean-François Champollion in deciphering the Rosetta stone to discover the hidden meaning of hieroglyphics proved the key to the study of Egyptology.

However, prior to the development of modern techniques excavations tended to be haphazard; the importance of concepts such as stratification and context were completely overlooked. For instance, in 1803, there was widespread criticism of Thomas Bruce, 7th Earl of Elgin for removing the "Elgin Marbles" from their rightful place on the Parthenon in Athens. The marble sculptures themselves were valued by his critics only for their aesthetic qualities, not for the information they contained about Ancient Greek civilization.

In the first half of the 19th century many other archaeological expeditions were organized; Giovanni Battista Belzoni and Henry Salt collected Ancient Egyptian artifacts for the British Museum, Paul Émile Botta excavated the palace of Assyrian ruler Sargon II, Austen Henry Layard unearthed the ruins of Babylon and Nimrud and discovered the Library of Ashurbanipal and Robert Koldeway and Karl Richard Lepsius excavated sites in the Middle East. However, the methodology was still poor, and the digging was aimed at the discovery of artefacts and monuments.

Development of archaeological methods:

The father of archaeological excavation was William Cunnington (1754–1810). He undertook excavations in Wiltshire from around 1798, in collaboration with his regular excavators Stephen and John Parker of Heytesbury. Cunnington's work was funded by a number of patrons, the wealthiest of whom was Richard Colt Hoare, who had inherited the Stourhead estate from his grandfather in 1785. Hoare turned his attention to antiquarian pursuits and began funding Cunnington's excavations in 1804. The latter's site reports and descriptions were published by Hoare in a book entitled Ancient Historie of Wiltshire in 1810, a copy of which is kept at Stourhead.

Cunnington made meticulous recordings of mainly neolithic and Bronze Age barrows, and the terms he used to categorize and describe them are still used by archaeologists today. The first

reference to the use of a trowel on an archaeological site was made in a letter from Cunnington to Hoare in 1808, which describes John Parker using one in the excavation of Bush Barrow.

One of the major achievements of 19th century archaeology was the development of stratigraphy. The idea of overlapping strata tracing back to successive periods was borrowed from the new geological and palaeontological work of scholars like William Smith, James Hutton and Charles Lyell. The application of stratigraphy to archaeology first took place with the excavations of prehistorical and Bronze Age sites. In the third and fourth decade of the 19th century, archaeologists like Jacques Boucher de Perthes and Christian Jürgensen Thomsen began to put the artifacts they had found in chronological order.

Another important development was the idea of deep time. Before this, people had the notion that the earth was quite young. James Ussher used the Old Testament and calculated that the origins of the world were on October 23 4004 BC.

Professionalization:

As late as the mid-century, archaeology was still regarded as an amateur pastime by scholars. Britain's large colonial empire provided a great opportunity for such 'amateurs' to unearth and study the antiquities of many other cultures. A major figure in the development of archaeology into a rigorous science was the army officer and ethnologist, Augustus Pitt Rivers.

In 1880, he began excavations on lands that came to him in inheritance and which contained a wealth of archaeological material from the Roman and Saxon periods. He excavated these over seventeen seasons, beginning in the mid-1880s and ending with his death. His approach was highly methodical by the standards of the time, and he is widely regarded as the first scientific archaeologist. Influenced by the evolutionary writings of Charles Darwin and Herbert Spencer, he arranged the artefacts typologically and (within types) chronologically. This style of arrangement, designed to highlight the evolutionary trends in human artefacts, was a revolutionary innovation in museum design, and was of enormous significance for the accurate dating of the objects. His most important methodological innovation was his insistence that all artefacts, not just beautiful or unique ones, be collected and catalogued. This focus on everyday objects as the key to understanding the past broke decisively with past archaeological practice, which had often verged on treasure hunting.

William Flinders Petrie is another man who may legitimately be called the Father of Archaeology. Petrie was the first to scientifically investigate the Great Pyramid in Egypt during the 1880s. Many theories as to how the pyramids had been constructed had been proposed (such as by Charles Piazzi Smyth), but Petrie's exemplary analysis of the architecture of Giza disproved these theories and still provides much of the basic data regarding the pyramid plateau to this day.

His painstaking recording and study of artefacts, both in Egypt and later in Palestine, laid down many of the ideas behind modern archaeological recording; he remarked that "I believe the true line of research lies in the noting and comparison of the smallest details." Petrie developed the system of dating layers based on pottery and ceramic findings, which revolutionized the

chronological basis of Egyptology. He was also responsible for mentoring and training a whole generation of Egyptologists, including Howard Carter who went on to achieve fame with the discovery of the tomb of 14th-century BC pharaoh Tutankhamun.

The first stratigraphic excavation to reach wide popularity with public was that of Hissarlik, on the site of ancient Troy, carried out by Heinrich Schliemann, Frank Calvert, Wilhelm Dörpfeld and Carl Blegen in the 1870s. These scholars individuated nine different cities that had overlapped with one another, from prehistory to the Hellenistic period. Their work has been criticized as rough and damaging — Kenneth W. Harl wrote that Schliemann's excavations were carried out with such rough methods that he did to Troy what the Greeks couldn't do in their times, destroying and levelling down the entire city walls to the ground.

Meanwhile, the work of Sir Arthur Evans at Knossos in Crete revealed the ancient existence of an advanced civilization. Many of the finds from this site were catalogued and brought to the Ashmolean Museum in Oxford, where they could be studied by classicists, while an attempt was made to reconstruct much of the original site. Although this was done in a manner that would be considered inappropriate today, it helped raise the profile of archaeology considerably.

Modern methodology:

The next major figure in the development of archaeology was Mortimer Wheeler, whose highly disciplined approach to excavation and systematic coverage in the 1920s and 1930s brought the science on swiftly. Wheeler developed the grid system of excavation, which was further improved on by his student Kathleen Kenyon. The two constant themes in their attempts to improve archaeological excavation were, first, to maintain strict stratigraphic control while excavating (for this purpose, the baulks between trenches served to retain a record of the strata that had been dug through), and, second, to publish the excavation promptly and in a form that would tell the story of the site to the intelligent reader.

The bomb damage and subsequent rebuilding caused by the Second World War gave archaeologists the opportunity to meaningfully examine inhabited cities for the first time. Bomb damaged sites provided windows onto the development of European cities whose pasts had been buried beneath working buildings. Urban archaeology necessitated a new approach as centuries of human occupation had created deep layers of stratigraphy that could often only be seen through the keyholes of individual building plots. In Britain, post-war archaeologists such as W. F. Grimes and Martin Biddle took the initiative in studying this previously unexamined area and developed the archaeological methods now employed in much CRM and rescue archaeology.

Archaeology increasingly became a professional activity during the first half of the 20th century. Although the bulk of an excavation's workforce would still consist of volunteers, it would normally be led by a professional. It was now possible to study archaeology as a subject in universities and even schools, and by the end of the 20th century nearly all professional archaeologists, at least in developed countries, were graduates.

New technology:

Undoubtedly the major technological development in 20th century archaeology was the introduction of radiocarbon dating, based on a theory first developed by American scientist Willard Libby in 1949. Despite its many limitations (compared to later methods it is inaccurate; it can only be used on organic matter; it is reliant on a dataset to corroborate it; and it only works with remains from the last 10,000 years), the technique brought about a revolution in archaeological understanding. For the first time, it was possible to put reasonably accurate dates on discoveries such as bones. This in some cases led to a complete reassessment of the significance of past finds. Classic cases included the Red Lady of Paviland. It was not until 1989 that the Catholic Church allowed the technique to be used on the Turin Shroud, indicating that the linen fibres were of medieval origin.

Other developments, often spin-offs from wartime technology, led to other scientific advances. For field archaeologists, the most significant of these was the use of the geophysical survey. This encompasses a number of remote sensing techniques such as aerial photography and satellite imagery. Light Detection and Ranging (LIDAR) is also used, a technology which measures the height of the ground surface and other features in large areas of landscape with resolution and accuracy that was not previously available. Archaeologists have also used subsurface remote sensing such as magnetometry using such things as ground-penetrating radar (GPR), enabling an advanced picture to be built up of what lies beneath the soil before excavation even commences. The entire Roman town of Viroconium, modern day Wroxeter, has been surveyed by these methods, though only a small portion has actually been excavated. The application of physical sciences to archaeology, known as archaeometry or archaeological science, is now a major part of archaeology.

Archaeology has also come to use Geographic Information System (GIS) technology, a system designed to capture, store, manipulate, analyze, manage, and visualize all types of geospatial data.

The discovery in 1991 in the Ötztaler Alpen of the prehistorical mummy of the so-called Man of Similaun introduced a new field of archaeological science. With the help of DNA Analysis the scholars could ascertain that Ötzi, as the mummy is called, doesn't belong to any known human population. Generally speaking, in the following years genetics have helped to understand the human migrations occurred during Prehistory. [(5)]

Paleoparasitology

Paleoparasitology is the study of parasites found in archaeological material. The development of this field of research began with histological identification of helminth eggs in mummy tissues, analysis of coprolites, and recently through molecular biology. An approach to the history of Paleoparasitology is reviewed in this paper, with special reference to the studies of ancient DNA identified in archaeological material.

In 1987 the first description of techniques used for the recovery of parasite eggs from archaeological materials was published54. Since that time, the exploration of archaeological remains has expanded and new techniques have been devised. The most important development during the past decade has been the application of molecular biology techniques for the recovery of ancient parasite DNA. Also, chemical digestion of archaeological sediments was introduced for the effective recovery of parasite eggs from all types of archaeological deposits. We review here the history of techniques within the context of the theoretical perspectives and the study goals of Paleoparasitology.

The Pioneering Period - Paleoparasitology (also archaeoparasitology) is the study of parasites in ancient material. The first report of ancient parasites is Ruffer's (1910) diagnosis of Schistosoma haematobium eggs in kidneys from Egyptian mummies. RUFFER57 used histological sectioning and staining for the identification of the eggs. Although a few other pioneering papers appeared in the first half of this century recording parasite eggs in archaeological material, the field really developed in the 1960s and was finally named in 1979.

Initially, parasitologists analyzing coprolites tried different flotation techniques. These are effective in unconsolidated sediments where parasite eggs are well preserved. However, standard clinical techniques were not effective when parasitologists turned their attentions to coprolites. Coprolites are desiccated and sometimes mineralized feces. To analyze coprolites, the trisodium phosphate rehydration technique was introduced. This technique was adapted from methods used to rehydrate desiccated zoological specimens in museums. Further experiments demonstrated that the trisodium phosphate technique was effective when applied to coprolites61. These studies showed that trisodium phosphate at 0.5% concentration in aqueous solution results in the reconstitution of the eggs and larvae of parasitic worms. It was shown that the egg shells and anatomical features of the larvae such as the esophagus and intestine are visible after application of trisodium phosphate. Thus, the application of this simple technique allows for the microscopic diagnosis of parasitic worms.

In the late 1960's and early 1970's, there was a burst of Paleoparasitology studies as the trisodium phosphate technique was widely applied to coprolites from Utah, Arizona, Colorado, and Nevada. These pioneering efforts can be characterized as a "discovery" phase. During this time, it was demonstrated that parasitism dates back to remote times and that prehistoric humans were hosts to a wide variety of parasites. In 1967, Aidan Cockburn pointed out that coprolite studies had a great potential for defining the evolution of infectious disease in relation to cultural evolution. He urged parasitologists to interpret their data from an epidemiological perspective. Cockburn's message reached paleoparasitologists in North and South America. Still, it took

several years before his recommendations were followed and broader paleoparasitological interpretations were scarce through the 1970's and 1980's. But, by the late 1980's, paleoparasitological data began to be interpreted to a greater extent.

Paleoepidemiology, 1980-1997:

After the pioneering and discovery periods in Paleoparasitology, researchers began struggling with new methodological questions. The consistent problems for paleoparasitologists are the diagnosis of the zoological origin of coprolites found in archaeological layers, and the diagnosis of the parasites themselves. A reference collection of desiccated feces of living mammals belonging to a national park in northeast Brazilian was prepared for comparison with the coprolites found at archaeological sites in the same region. The method shows good results when it can be applied. However, it is a long and tedious process to survey all animals to cover all the possibilities of fecal morphology.

Similar procedures are encountered regarding parasites. When the host is known, parasite check-lists are very useful. Morphometric parameters must be examined, as proposed by experimental Paleoparasitology. However, extinction and changes in the local fauna must be considered. Also, when eggs of a parasite not previously known to exist in humans are found, careful evaluation of the infection must be done to determine if a true case of parasitism is represented. This problem was encountered with acanthocephalan eggs in coprolites from Utah and Arizona. The eggs were identified as Moniliformis clarki. Analysis of the dietary constituents of the coprolites and the biology of M. clarki showed that this was a parasite of humans. Therefore, careful paleoparasitological analysis showed that M. clarki was a common parasite of prehistoric Indians before indigenous dietary patterns changed. In other cases, careful analysis reveals "false parasitim" when eggs of a parasite are consumed and passed through the intestinal tract without hatching.

During these two decades Paleoparasitology advances relied on morphological parameters of parasite remains. Light microscopy has been the main tool for scientists. However, other techniques including immunology and electron microscopy have been introduced. HORNE experimented with transmission electron microscopy (TEM). Although HORNE did not recommend that TEM replace light microscopy, TEM did allow for the identification of internal parasite egg structures. Scanning electron microscopy (SEM) is a useful diagnostic tool. In certain cases, fungal spores, and especially pollen can be confused with parasite eggs when only light microscopy is applied. SEM allows for the examination of surface features that can be used to distinguish them. Also, SEM is a very useful tool for the characterization of helminth larvae. Immunological tests have significant potential for Paleoparasitology. FOUANT was the first to apply immunological analysis to parasite remains. Her application of ELISA to possible Entamoeba histolytica cysts proved negative. Immunofluorescence stains were successfully applied to identify Giardia lamblia cysts in coprolites from Kentucky. In our opinion, immunology has a great potential for identifying parasite remains.

Quantitative studies also showed interesting epidemiological patterns. Ancient hunter-gatherers were shown to have a reduced parasite fauna relative to agricultural populations. Also, hunter-

gatherer parasitism is dominated by zoonotic species whereas agricultural populations had more human-specific parasites. Subsequent comparison of parasite prevalence in coprolites with bone lesion prevalence (porotic hyperostosis) in skeletons showed a correlation between parasitism and anemia51. Comparisons of the pathoecology of prehistoric agricultural villages showed that the level of parasitism was dependent on the local ecology, sanitation patterns, and house style. Detailed studies of house type through 10,000 years of prehistory in the southwest United States have shown that pinworm prevalence is related to the style of house construction which affects air flow. [(6)]

Paleopathology

The word paleopathology, derived from the Greek palaios, pathos, and logos, meaning literally "study of ancient diseases," was coined in 1892/3 by the German physicist R. W. Schufeldt and has appeared in the Standard Dictionary of Funk and Wagnall since 1895. However, it was Sir Armand Ruffer who made the word popular, using it in his extensive studies on Egyptian mummies. Paleopathology can be defined as the discipline that studies the diseases of past populations through the examination of ancient human remains, both skeletonized and mummified. [(7)]

Paleopathology is the study of past disease in human remains; it is a sub-discipline of bioarchaeology (study of human remains from archaeological sites). [(8)]

Paleopathology is the study of the evidence of trauma, disease, and congenital defects in human remains. Archaeologists, geneticists, and physical anthropologists, conduct paleopathology studies in order to evaluate the effects of disease upon ancient populations. Often, such research is conducted to garner more information about the biological and genetic characteristics of prehistoric or ancient populations, but sometimes paleopathology involves scientifically evaluating accounts of epidemics in historical records.

The earliest form of the science of paleopathology emerged in the 1600s. German naturalists, interested in newly discovered Egyptian mummies, carefully dissected and inspected the bones and tissues of specimen and attempted to compare the remains with contemporary cadavers. Early paleopathologists were able to identify striations and lesions on bone that indicated arthritis. In the late 1700s, scientists also cataloged distinctive marks on bone and tooth remains that were the result of deadly fevers.

Modern paleopathology is not limited to the study of mummified corpses. Various other types of remains, such as bone, teeth, blood, hair, fingerprints, and human waste, are actually utilized more frequently in research. As medical technology has become more advanced, scientists have been able to conduct paleopathological analysis on smaller amounts of biological material. With the aid of technology such as CAT scans and fiber optics, scientists are able to extract sample material without need of autopsy, thereby leaving remains relatively undisturbed and intact for future study.

The most common application of paleopathology is in studying patterns of disease in ancient individuals and populations. Osteologists (scientists who study bone remains) in the United States have devoted considerable effort to the study of the effects of European diseases on Native American population during the early colonial period (1492–1650). Analysis of remains not only shows the effect of disease upon individual specimen, but also adds to a larger understanding of the modes of transmission, virility, and mortality rates associated with epidemics.

Paleopathology can also be used in combination with some forms of population genetics. For example, comparisons of analyzed remains from diverse geographic regions or kin associations assists in distinguishing possible genetic traits that aid in a population's resistance to certain diseases.

Paleopathology is one of the few means scientists have at their disposal to gain clues about the diet, health, pathology, and general genetic trends of ancient populations. Scientists also use paleopathology as a means of collecting "census material" or information regarding a specimen's age, sex, stature, and cause of death. From this data, anthropologists can estimate the general demographic composition of populations without the benefit of sometimes confused, scattered, fragmentary, or inaccurate written records. Even information about social customs and medicinal practices can sometimes be determined thorough paleopathological research on human materials. For example, skulls found in South America suggest that ancient peoples may have attempted to perform a primitive type of neurosurgery to relieve fevers or brain swelling. High levels of arsenic and mercury in hair samples from medieval remains in France have raised questions about water contamination or possible medicinal use.

There are several limitations to paleopathological research. Since skeletal material is the most frequently recovered type of human remain, most paleopathology studies pertain to diseases that visibly alter or affect bone. Many diseases affect bone in similar ways, thus making it difficult and sometimes impossible to determine the exact disease represented. Many diseases do not affect bone at all, and are only evident in tissue, hair, or other rare remains. Paleopathology currently accounts for only a portion of the total diseases that ravaged past populations.

These very limitations, however, have yielded a paradoxical wealth of information. In the case of the disease tuberculosis, the limitations of paleopathology have provided a narrow scientific framework in which to study the disease without worry that data will grossly over-represent its threats and effects. Scientists know that tuberculosis remained a constant threat throughout the historic period until the advent of antibiotics and vaccines. Mentions of tuberculosis and tuberculin symptoms are present in medical writings from Ancient Greece and China, and the disease was still the focus of public health commissions at beginning of the twentieth century. However, tuberculosis is primarily a disease of the lungs, and only affects the skeletal system in 5–7% of all infected persons. Given the infrequency of bone degeneration associated with the disease, and the given limitations of paleopathological research itself, scientists did not expect to find numerous remains with evidence of tuberculosis. Yet, such remains have been discovered on every continent, in both prehistoric and historic burials, at a relatively frequent rate. Paleopathologists have concluded two possibilities. The first possibility is that the tuberculosis bacillus itself has altered and now produces a different pathological signature (e.g. that it used to affect bone more frequently) or that a certain strain was once more prevalent. The second possibility is that the disease was more widespread than scientists had previously estimated. Many paleopathologists theorize that both possibilities are factors in the history of tuberculosis.

The context of paleopathological research is not limited to the ancient world. However, modern research of this type often falls under the label of forensic anthropology—the branch of science most popularly known for its applications in crime investigation. (9)

Archaeogenetics

Archaeogenetics, a term coined by Colin Renfrew, refers to the application of the techniques of molecular population genetics to the study of the human past. This can involve:

•the analysis of DNA recovered from archaeological remains, i.e. ancient DNA;

•the analysis of DNA from modern populations (including humans and domestic plant and animal species) in order to study human past and the genetic legacy of human interaction with the biosphere; and

•the application of statistical methods developed by molecular geneticists to archaeological data.

The topic has its origins in the study of human blood groups and the realization that this classical genetic marker provides information about the relationships between linguistic and ethnic groupings. Early work in this field included that of Ludwik and Hanka Hirszfeld, William C. Boyd and Arthur Mourant. From the 1960s onwards, Luca Cavalli-Sforza used classical genetic markers to examine the prehistoric population of Europe, culminating in the publication of The History and Geography of Human Genes in 1994.

Since then, the genetic history of all of our major domestic plants (e.g., wheat, rice, maize) and animals (e.g., cattle, goats, pigs, horses) has been analysed. Models for the timing and biogeography of their domestication and subsequent husbandry have been put forward, mainly based on mitochondrial DNA variation, though other markers are currently being analysed to supplement the genetic narrative (e.g., the Y chromosome for describing the history of the male lineage).

The same expression was also used by Antonio Amorim (1999) and defined as: getting and interpreting [genetic] evidence of human history. A similar concept (even in a more ambitious form, as it included the recreation of inferred extinct states) was developed in the pre-DNA era by Linus Pauling and Emile Zuckerkandl (1963) . [(10)]

Methods:

Fossil DNA Preservation:

Fossil retrieval starts with selecting an excavation site. Potential excavation sites are usually identified with the mineralogy of the location and visual detection of bones in the area. However, there are more ways to discover excavation zones using technology such as field portable x-ray fluorescence and Dense Stereo Reconstruction. Tools used include knives, brushes, and pointed trowels which assist in the removal of fossils from the earth.

To avoid contaminating the ancient DNA, specimens are handled with gloves and stored in -20 °C immediately after being unearthed. Ensuring that the fossil sample is analyzed in a lab that has not been used for other DNA analysis could prevent contamination as well. Bones are milled to a powder and treated with a solution before the polymerase chain reaction (PCR) process. Samples for DNA amplification may not necessarily be fossil bones. Preserved skin, salt-preserved or air-dried, can also be used in certain situations.

DNA preservation is difficult because the bone fossilisation degrades and DNA is chemically modified, usually by bacteria and fungi in the soil. The best time to extract DNA from a fossil is when it is freshly out of the ground as it contains six times the DNA when compared to stored bones. The temperature of extraction site also affects the amount of obtainable DNA, evident by a decrease in success rate for DNA amplification if the fossil is found in warmer regions. A drastic change of a fossil's environment also affects DNA preservation. Since excavation causes an abrupt change in the fossil's environment, it may lead to physiochemical change in the DNA molecule. Moreover, DNA preservation is also affected by other factors such as the treatment of the unearthed fossil like (e.g. washing, brushing and sun dring), pH, irradiation, the chemical composition of bone and soil, and hydrology. There are three perseveration diagenetic phases. The first phase is bacterial putrefaction, which is estimated to cause a 15-fold degradation of DNA. Phase 2 is when bone chemically degrades, mostly by depurination. The third diagenetic phase occurs after the fossil is excavated and stored, in which bone DNA degradation occurs most rapidly.

Methods of DNA Extraction:

Once a specimen is collected from an archaeological site, DNA can be extracted through a series of processes. One of the more common methods utilizes silica and takes advantage of polymerase chain reactions in order to collect ancient DNA from bone samples.

There are several challenges that add to the difficulty when attempting to extract ancient DNA from fossils and prepare it for analysis. DNA is continuously being split up. While the organism is alive these splits are repaired; however, once an organism has died, the DNA will begin to deteriorate without repair. This results in samples having strands of DNA measuring around 100 base pairs in length. Contamination is another significant challenge at multiple steps throughout the process. Often other DNA, such as bacterial DNA, will be present in the original sample. To avoid contamination it is necessary to take many precautions such as separate ventilation systems and workspaces for ancient DNA extraction work. The best samples to use are fresh fossils as uncareful washing can lead to mold growth. DNA coming from fossils also occasionally contains a compound that inhibits DNA replication. Coming to a consensus on which methods are best at mitigating challenges is also difficult due to the lack of repeatability caused by the uniqueness of specimens.

Silica-based DNA extraction is a method used as a purification step to extract DNA from archaeological bone artifacts and yield DNA that can be amplified using polymerase chain reaction (PCR) techniques. This process works by using silica as a means to bind DNA and separate it from other components of the fossil process that inhibit PCR amplification. However, silica itself is also a strong PCR inhibitor, so careful measures must be taken to ensure that silica is removed from the DNA after extraction. The general process for extracting DNA using the silica-based method is outlined by the following:

1. Bone specimen is cleaned and the outer layer is scraped off
2. Sample is collected from preferably compact section
3. Sample is ground to fine powder and added to an extraction solution to release DNA

4. Silica solution is added and centrifuged to facilitate DNA binding
5. Binding solution is removed and a buffer is added to the solution to release the DNA from the silica.

One of the main advantages of silica-based DNA extraction is that it is relatively quick and efficient, requiring only a basic laboratory setup and chemicals. It is also independent of sample size, as the process can be scaled to accommodate larger or smaller quantities. Another benefit is that the process can be executed at room temperature. However, this method does contain some drawbacks. Mainly, silica-based DNA extraction can only be applied to bone and teeth samples; they cannot be used on soft tissue. While they work well with a variety of different fossils, they may be less effective in fossils that are not fresh (e.g. treated fossils for museums). Also, contamination poses a risk for all DNA replication in general, and this method may result in misleading results if applied to contaminated material.

Polymerase chain reaction is a process that can amplify segments of DNA and is often used on extracted ancient DNA. It has three main steps: denaturation, annealing, and extension. Denaturation splits the DNA into two single strands at high temperatures. Annealing involves attaching primer strands of DNA to the single strands that allow Taq polymerase to attach to the DNA. Extension occurs when Taq polymerase is added to the sample and matches base pairs to turn the two single strands into two complete double strands. This process is repeated many times, and is usually repeated a higher number of times when used with ancient DNA. Some issues with PCR is that it requires overlapping primer pairs for ancient DNA due to the short sequences. There can also be "jumping PCR" which causes recombination during the PCR process which can make analyzing the DNA more difficult in inhomogeneous samples.

<u>Methods of DNA Analysis:</u>

DNA extracted from fossil remains is primarily sequenced using Massive parallel sequencing, which allows simultaneous amplification and sequencing of all DNA segments in a sample, even when it is highly fragmented and of low concentration. It involves attaching a generic sequence to every single strand that generic primers can bond to, and thus all of the DNA present is amplified. This is generally more costly and time intensive than PCR but due to the difficulties involved in ancient DNA amplification it is cheaper and more efficient. One method of massive parallel sequencing, developed by Margulies et al., employs bead-based emulsion PCR and pyrosequencing, and was found to be powerful in analyses of a DNA because it avoids potential loss of sample, substrate competition for templates, and error propagation in replication.

The most common way to analyze a DNA sequence is to compare it with a known sequence from other sources, and this could be done in different ways for different purposes.

The identity of the fossil remain can be uncovered by comparing its DNA sequence with those of known species using software such as BLASTN. This archaeogenetic approach is especially helpful when the morphology of the fossil is ambiguous. Apart from that, species identification can also be done by finding specific genetic markers in an aDNA sequence. For example, the American indigenous population is characterized by specific mitochondrial RFLPs and deletions defined by Wallace et al.

A DNA comparison study can also reveal the evolutionary relationship between two species. The number of base differences between DNA of an ancient species and that of a closely related extant species can be used to estimate the divergence time of those two species from their last common ancestor. The phylogeny of some extinct species, such as Australian marsupial wolves and American ground sloths, has been constructed by this method. Mitochondrial DNA in animals and chloroplast DNA in plants are usually used for this purpose because they have hundreds of copies per cell and thus are more easily accessible in ancient fossils.

Another method to investigate relationship between two species is through DNA hybridization. Single-stranded DNA segments of both species are allowed to form complementary pair bonding with each other. More closely related species have a more similar genetic makeup, and thus a stronger hybridization signal. Scholz et al. conducted southern blot hybridization on Neanderthal aDNA (extracted from fossil remain W-NW and Krapina). The results showed weak ancient human-Neanderthal hybridization and strong ancient human-modern human hybridization. The human-chimpanzee and neanderthal-chimpanzee hybridization are of similarly weak strength. This suggests that humans and Neanderthals are not as closely related as two individuals of the same species are, but they are more related to each other than to chimpanzees.

There have also been some attempts to decipher aDNA to provide valuable phenotypic information of ancient species. This is always done by mapping aDNA sequence onto the karyotype of a well-studied closely related species, which share a lot of similar phenotypic traits. For example, Green et al. compared the aDNA sequence from Neanderthal Vi-80 fossil with modern human X and Y chromosome sequence, and they found a similarity in 2.18 and 1.62 bases per 10,000 respectively, suggesting Vi-80 sample was from a male individual. Other similar studies include finding of a mutation associated with dwarfism in Arabidopsis in ancient Nubian cotton, and investigation on the bitter taste perception locus in Neanderthals. [(11)]

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