GUINEA WORM:

SOCIO-CULTURAL STUDIES, MORPHOMETRY, HISTOMORPHOLOGY, VECTOR SPECIES AND DNA PROBE FOR *DRACUNCULUS SPECIES.*

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DECLARATION

I do hereby declare that except for references to other people's investigations which I duly acknowledged, this exercise is the result of my own original research, and that this thesis, either in whole, or in part, has not been presented for another degree elsewhere.

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DEDICATION

This thesis is dedicated to the Bimi family in memory of

our late father – ***Mr. Combian Bimi Dapaah (CBD),***

affectionately known as ***Nanaanbuang*** by his admirers.

You never limited us

with any preconceived notions

of what we could or could not achieve.

You made us to understand that the best kind of knowledge to have is that which is learned for its own sake, and that even the largest task can be accomplished if it is done one step at a time.

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PREFACE

This thesis is composed of eight main sections prefaced by an introduction and followed by an abstract of conclusions and recommendations. The first two of the main sections provide relevant background information about dracunculiasis (*Chapter 1*) and a literature review (*Chapter 2*). Chapter 3 presents a study of the socio-cultural practices of the local people and their belief systems that could aid in the transmission and sustenance of the disease. Chapters 4 and 5 present the morphometry and histomorphometry of the parasite respectively. Chapter 6 is an evaluation of the infection potentials of the various copepods found in the study area that could serve as vectors of the disease. A DNA probe for Dracunculus species is presented in Chapter 7. Finally, an overall discussion of all the aspects of the research is presented in Chapter 8. It is anticipated that material from all the chapters will be submitted for publication.

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Abstract

Dracunculiasis still remains as a disease of public health importance in Ghana, especially in the Northern, Volta and Brong-Ahafo Regions, despite over a decade of an Eradication Programme. In this study, attempts have been made to determine the problems associated with the eradication of Guinea worm in the Northern Region of Ghana, and suggestions provided to overcome them. Also investigated were the longitudinal anatomical variation of the parasite, the vector species and a DNA probe for *Dracunculus* species. It was found out that 43% of the populace still got afflicted with the malady during the last transmission season, with 33% suffering from the disease for the second consecutive year. The disease appears to be sustained by the very long transmission season, spanning over six months. The knowledge index of the local people with respect to the disease was quite high. Over 50% of respondents seem to be aware of disease causation, prevention, and management. Unfortunately, however, apathy, lack of motivation, and worker fatigue on the part of GWEP staff appear to be factors in the resurgence of the disease.

In the histomorphological part of the study, the longitudinal anatomical variation of the parasite was evaluated and its histomorpholgy illustrated. At maturity, the gut of the female guinea worm was found to be completely atrophied and the entire worm made-up of the larvae-filled uterus. Differences in the musculature from the anterior region, with much thicker muscles to the mid region were observed. The most important vectors of dracunculiasis in the study areas are: *M. keiferi* → *M. aspericornis* → *T. incisus* → *T. inopinus* → *T. oblongatus* .

To probe for molecular epidemiology of *Dracunculus* species, DNA sequence isolates from a number of Guinea worms originating from 9 African countries, Pakistan and Yemen were compared with a previously assembled isolate, 1819bp (not yet submitted to the Gene Bank) from Uganda. The 18S nuclear small subunit ribosomal RNA (SSU-rRNA) gene of Guinea worm from human and canine cases of dracunculiasis in Africa were isolated by PCR and sequenced. Phylogenetic analysis (of these sequences) and comparison with the sequences from Uganda revealed that *Dracunculus* isolates from human cases were indistinguishable from those of the dog case. These results certainly imply that incidental infections of Guinea worm could occur in certain domestic animals, and therefore, suggest that some domestic animals may serve as reservoirs for human Guinea worm infection.

Chapter 1

1.0 INTRODUCTION

At the dawn of the 21st Century, the terrible trinity of poverty, ignorance and disease, unfortunately still plagues most developing countries. Among these is Dracunculiasis or Guinea worm disease. This malady belongs to a group of diseases generally known as Tropical Diseases, based on their geographical occurrence in the tropics. Guinea worm is not a cause of mortality, but it can be a real burden in terms of morbidity and suffering for those affected. Dracunculiasis was estimated to affect several million people annually at the close of the 1970s. However, the international water decade (1981-1990), resulted in global decline in incidence by 1996 to less than 153, 000 cases worldwide, 78% of which were reported from Sudan (Kale, 1990). The disease is still found among the poorest rural communities in areas without safe water supplies in Sub-Saharan Africa and the Arabian Peninsula. Gradually worn down by penury, these underprivileged people find themselves trapped in a vicious circle of poverty and disease (Richards and Hopkins, 1989).

Dracunculiasis is caused by a long, string-like, female worm (*Dracunculus medinensis*). Its larval form infects an intermediate crustacean host (copepods, water fleas) that commonly infests shallow ponds or step wells used as sources of drinking water. Known to cause human suffering since ancient times, the infection was referred to by physicians as early as the Graeco-Roman era and by Arab physicians in medieval times. Common names include Guinea worm and Medina worm. Linnaeus classified the worm in the eighteenth century.

Fedchenko, a Russian naturalist, described the life cycle in 1869--the first time an invertebrate (arthropod) intermediate host was described for any parasitic disease of man.

This round worm is the largest of the tissue parasites affecting humans. The adult female, which carries from 1 to 3 million embryos, can measure up to 1 meter in length and 2 mm in diameter. The parasite migrates through the victim's body causing severe pain, especially in the areas around the joints. The worm eventually emerges (from the feet in 90% of cases), causing an intensely painful oedema, a blister and then an ulcer. When the worm perforates the skin, intolerable pain is accompanied by fever, nausea and vomiting (Muller, 1971).

Dracunculiasis (less commonly, dracontiasis, and rarely, dracunculosis) is contracted by drinking contaminated water. The offending parasite, historically the scourge of Islamic pilgrimage, is aptly called the Little Dragon of Medina or scientifically *Dracunculus medinensis* (Linnaeus, 1758; as cited in Macpherson, 1981).

From the middle ages through to the 18th century there were many varying opinions as to the nature of the "fiery serpents" - believed to be anything from exposed nerves to dead tissue. It was the celebrated Swedish naturalist, Carolus Linnaeus who first suggested that they were in fact worms. In 1870, Alexei P. Fedchenko became aware of the life-cycle of *Dracunculus medinensis* and identified copepods as its intermediate host. By the end of the 19th century, scientists had become well aware of how the disease was transmitted.

Dracunculiasis still represents a serious health risk for several millions of rural villagers in parts of Africa, the Middle East, and India. It affects only the rural poor who lack safe sources of drinking water for their households and places of work (agricultural plots).

Unlike most communicable diseases in developing countries, the greatest morbidity from Dracunculiasis occurs in adults. This may be the reason it has received less attention than those illnesses resulting in high morbidity and mortality in children. Because peak case rates often coincide with such agricultural activities as clearing land, planting, and harvesting, the disease is a major cause of agricultural work loss in many areas. Infected individuals are often crippled or disabled for many weeks each year from painful ulcers produced by the worms' emergence and complications resulting from secondary bacterial infections.

Dracunculiasis in Ghana declined substantially during 1992, the 3rd consecutive year of control. A total of 33,464 cases of Dracunculiasis were reported in 3,185 villages, compared with 66,697 in 3,718 villages in 1991 (a decline of 49.6%) and 179,556 in 6,873 villages (a decline of 81.4%) in 1989. In addition, when compared with 1991, the percentage reduction in cases reportedly by month increased from 20.1% in January to 34% in March, 59.9% in June, 81% in September, and 55.6% in December. During 1992, at least 84% of the villages affected reported surveillance findings to national authorities on time each month (within 20-30 days after the end of the reporting month), compared with 61% during 1991. Not withstanding the political will and massive international support, there is unfortunately an

apparent relapse and worker fatigue in the control efforts. This may account for resurgence of the disease in some communities (Annual Report, GWEP, Northern Region, 1997).

Increases in reported cases of Guinea worm in the country called for the intensification of efforts for the disease eradication. Thus, the Eradication Programme has been re-launched in a number of endemic communities. Notably, the Programme had to be re-launched in Gusheigu (in the Gushiegu-Karaga District of the Northern Region) in 1998 by the President of Ghana. Also, in Wusuta (Volta Region), and Asenyensua (Eastern Region), the GWEP had to re-launch the programme in 1999 due to re-emergence of the disease, and again at Chamba in the Nanumba district in 2001.

In July 1999, the Northern Region was still leading in the number of cases in the country. In the first quarter of the year, the region recorded 2,370 cases of Guinea worm. The Tamale Municipality recorded the highest number of 500 cases during the period. This was unfortunately, against the background of the government's commitment towards eradicating Guinea worm in the country by the year 2000.

1.1 JUSTIFICATION OF STUDY

Infection with *Dracunculus medinensis* is still widespread in the Northern Region of Ghana, where rural people drink from unprotected water sources such as ponds and small water catchments. The fact that the disease still exists in the country beyond the target date of global eradication warrants further investigations. The importance of investigating the socio-cultural factors that tend to sustain and maintain the disease transmission despite a decade of the Ghana Guinea Worm Eradication Programme (GGWEP) can therefore not be over-emphasized.

Understanding the local perceptions of disease causation and treatment of Guinea worm, morbidity and local knowledge of transmission and prevention is particularly crucial, especially those that might contribute to the persistence of the disease. Also, it would be helpful to know whether certain beliefs were more likely to contribute to Guinea worm infection. The level of acceptance of the interventions aimed at the disease such as the use of filters, chemical pond treatment, and community organization to dig and maintain wells/dams has to be evaluated. Answers to these issues would provide insights into how to reinvigorate eradication efforts in Ghana, which remains one of three most highly endemic countries for Guinea worm (the other 2 being Nigeria and Sudan).

Furthermore, recent advances in copepod systematics have refined the level of taxonomic resolution of these freshwater copepods and it is now known that *Mesocyclops leukarti* does

not occur in either Africa or India (Keifer, 1981; Van de Velde, 1984). There is, therefore, an obvious need to record these taxonomic changes, review earlier records, and update the nomenclature of the hosts where possible.

Also, the correct identification of the copepod intermediate hosts is important in mapping out the geographical distribution and spread of the disease. This is vital in the development of eradication programmes, which aim to combat the disease by vector control.

Lastly, since most of the key features used as morphological markers for systematic analysis of *Dracunculus spp.* are present only on the male worms, which are rarely ever available for study, there is the need to develop DNA probes for *Dracunculus spp.* for molecular characterization.

1.2 SPECIFIC STUDY OBJECTIVES

The specific objective of the project were to:

1. Evaluate the socio-cultural practices and beliefs of the local people that seem to maintain and sustain the guinea worm disease in northern Ghana.
2. Ascertain the morphometric correlates of *D. medinensis* in relation to the sex of patients.
3. Study the histomorphology of the female guinea worm
4. Document the true vectors of guinea worm in Northern Ghana.
5. Develop a simple and rapid molecular assay to determine if a dracunculid worm extracted from man in an area under control is *D. medinensis* or another species.
6. Ascertain possible species types of the parasite
7. Evaluate the zoonotic aspects of dracunculiasis for reservoir hosts
8. The complete gene coding of all possible species and sub-species (if any)

1.3 DEFINITIONS

Despite the relative simplicity of the life cycle and epidemiology of *Dracunculus medinensis*, some traditional terms need to be defined more precisely to promote communication and enhance the comparability of data over time and among different areas. The definitions that follow are based on current parasitologic and epidemiologic knowledge of the disease. As future research and experience adds to the understanding of dracunculiasis, these definitions may need revision. (Names and parts of the definitions in brackets indicate synonyms or optional inclusions).

1. **Active case**

   A person in whom an investigator, health worker, or other trained person sees the *Dracunculus medinensis* worm beneath or extending from the skin. Also, a person with an acute skin lesion from which the larvae of *D. medinensis* have been identified by a trained person using microscopy or other means.

2. **Presumptive case**

   [Retrospective or historical case] A person who reports having experienced the emergence of one or more *D. medinensis* worms within the past 2 years.

3. **Pre-patent case**

A person who has been infected with dracunculiasis or who has ingested infective larvae but who has not yet manifested clinical symptoms or signs of the disease such as blistering, laceration, and worm emergence. There is no specific diagnostic test to identify such cases at present.

4. **Affected community**

A local administrative or social unit (e.g., village, hamlet, town, city) in which indigenous active or presumptive cases of dracunculiasis, or both, have been reported during the previous 2 years.

5. **Incubation period**

The period of time between ingestion of infective *D. medinensis* larvae by a person and the onset of clinical symptoms.

6. **Peak patency period**

[French: period de mise en evidence communitaire] Period of the year during which more than 50 percent of all eases (in a community or defined geographic area) are reported. This definition applies to areas where incidence is seasonal.

7. Individual patency period

[French: period de mise en evidence individual] The interval in an individual case between the time of first parasitologic evidence of the worm beneath or extending from the skin (or onset of typical skin blister from which a worm will soon emerge) and the time of complete expulsion or extraction of the worm from the body.

8. Unprotected water source

Source of drinking water that contains copepods and that either allows partial or total immersion of an infected person in the water source or permits water runoff to enter. Such sources are also likely to contain unacceptable levels of microbiological contaminants.

9. Protected water source

Source of drinking water that prohibits the partial or total immersion of an active guinea worm case and contamination from ground runoff. Such sources are usually constructed so as to remain free from fecal pollution.

10. Infested water source

Source of drinking water containing copepod species capable of ingesting first-stage larvae (L_1) of *D. medinensis*.

11. Infective water source

Source of drinking water containing copepods infected with third-stage larvae (L_3) of *D. medinensis*.

12. Surveillance

The continuing collection, analysis, and feedback of epidemiologic data based on reporting or detection of cases of disease.

13. Passive case reporting

Recording of cases that voluntarily come to the attention of public health authorities. Passive reporting or surveillance is achieved through the establishment of routine reporting mechanisms and is useful for obtaining initial data on the distribution of dracunculiasis in an area.

14. Active case detection

Search for cases by representatives of the health system through a village-to-village or house-to-house survey or a sample of the population thought to be at risk. This method usually reveals more cases than does passive reporting.

15. Incidence

The rate of appearance of new cases in a defined population within a specified period of time, usually one (1) year.

16. Prevalence

The proportion of a given population showing patent dracunculiasis infection at a given point in time. Prevalence data may be of little use with regard to dracunculiasis because the period of patency is usually short and seasonal. Therefore, information collected during low transmission periods can be misleading. Prevalence should be measured at the period of peak patency.

17. Control

Reduction of disease incidence in a defined area over a period of 24 months through planned activities.

18. Elimination

Complete absence of new indigenous cases of patent dracunculiasis infection in a previously defined endemic area for a period of at least 24 months given the presence of an active surveillance system, including at least two annual village-to-village checks.

19. Eradication

Global elimination of human dracunculiasis infection.

Chapter 2

2.0 LITERATURE REVIEW

2.1 HISTORY AND IMPORTANT DATES OF DRACUNCULIASIS

2.1.1 History

Dracunculiasis is an ancient disease quoted by many classical authors and mentioned in the Old Testament (Numbers Chapter 21). This nematode was known as a parasite of humans about 1530 B. C. The aduceus, which is the symbol of a physician is the staff of Hermes and contains coiled serpents on a staff. The coiled serpents are believed to represent the Guinea Worm (Muller, 1971).

During the 1980s, the World Health Organization gave its backing to United Nations coordination mechanisms and, in collaboration with its Member States, drew up a list of attainable goals for the International Water Supply and Sanitation Decade. One of these goals was the eradication of dracunculiasis. This objective has been partially achieved. It was recently estimated that the prevalence of dracunculiasis has dropped from 10 - 15 million cases per year at the beginning of the 1980s to the present low figure of 1 million cases.

The Member States of the World Health Organization then requested that every effort be made to interrupt transmission of dracunculiasis before 1995. Once transmission ceases, there will be a three-year monitoring period. If during this time no further cases are detected,

WHO will issue an eradication certificate. For the last few years there has been a world eradication campaign (Abdou, 1982; Hopkins and Ruiz-Tiben, 1990).

2.1.2 Important Dates

Ever a subject of curiosity because of its apparently supernatural aspects, dracunculiasis has been documented since early history. In the 15th century BC, the first known mention of the disease is found in the ATurin Papyrus" which refers to the ancient Egyptian myth of the sun god Ra. A recent pathological examination of an Egyptian mummy clearly identified a calcified worm as *Dracunculus medinensis.* In the 14th century BC, the closing verse, of three stanzas of a poem in the Sanskrit book Rig-Veda, attributed to Vasistha, allude to the guinea worm. Also, in the 11th century AD, Abou Ali ibn Sina (known in the West as Avicena) gives detailed descriptions of the disease, its treatment, its evolution and the complications caused by the worm being ruptured during extraction. Dracunculiasis occurred frequently in Persia during this period (htt:p//aepo-xdv-www.epo.cdc.gov/wonder/PrevGuid/m0001960/m0001960.htm :1/18/00).

From the middle Ages through to the 18th century there were many varying opinions as to the nature of the "fiery serpents" - believed to be anything from exposed nerves to dead tissue. it was the celebrated Swedish naturalist, Carolus Linnaeus who first suggested that they were in fact worms. In 1870, Alexei P. Fedchenko became aware of the life cycle of *D. medinensis* and identified the copepod as its intermediate host. By the end of the 19th

century, scientists had become well aware of how the disease was transmitted and had started to advocate suitable protective measures. Between 1926 and 1931, dracunculiasis was totally eradicated from Uzbekistan following a series of effective health education, water purification and carrier control programmes in Boukhara and the surrounding areas. No recurrence of the disease has been recorded in this region since 1932. In the 1970s, dracunculiasis was eradicated in Iran. In 1984 dracunculiasis was eradicated in the Indian State of Tamil Nadu, by 1989 in Gujarat, and by 1991 in Maharashtra (htt:p//aepo-xdv-www.epo.cdc.gov/wonder/PrevGuid/m0001960/m0001960.htm :1/18/00).

2.2 EPIDEMIOLOGY AND TRANSMISION

This parasitic infection occurs most frequently in West Africa and in Western and Southern India, principally in the rural areas. Depending on the climate, dracunculiasis occurs in one of at least three seasonal patterns. In semi-arid areas, transmission occurs in the rainy season. Since the incubation period averages about 12 months, the transmission season remains synchronized with the annual period when the local environment is most receptive to the parasite. In areas where there are surface sources of water year-round, transmission usually occurs during the dry season, when the surface sources are scanty and most polluted. In some other areas, transmission may occur all year-round with very little seasonal variations. Because of its life cycle and the year-long incubation period, the transmission and clinical manifestations of dracunculiasis are highly seasonal.

Although the disease is easily diagnosed, and thus recognition of the problem is relatively simple, surveillance of dracunculiasis, paradoxically, is exceptionally poor. The lack of ongoing case information is due in part to the remote rural nature of affected populations, their limited attendance at government clinics, and in some countries, the low government priority accorded to dracunculiasis. This situation could continue since the absence of a specific curative or preventive drug or vaccine removes a treatment incentive for villagers to visit health centers where their infection might be diagnosed and officially reported. In most endemic zones, less than 5 percent of cases are reported. Millions of cases are thought to occur annually worldwide, but systematic epidemiologic surveillance is needed to produce a reliable estimate of the annual global incidence.

2.3 ETIOLOGY AND LIFE-CYCLE OF *DRACUNCULUS MEDINENSIS*

Although the discovery of the life cycle of *Dracunculus medinensis* has usually been credited to Fedchenko (1869), Manson-Bahr (1966) made the following statement: "Fedchenko (1869) is credited with the discovery of the transmission of the guinea worm, but probably, Manson was the original observer (1895). He believes that the stages figured by the former are those of *Cucullanus spp* (a parasite of fish), and not *D. medinensis*" (Hughes, 1967). Hughes however agrees that Fedchenko correctly postulated that human infection is caused by the ingestion of infected copepods in drinking water. This was obviously because he was already aware that the life-cycle of some parasitic helminths involved alternative hosts, and he was looking out for such a host for the Guinea worm.

Conversely, an investigator 75 years earlier did not know about alternative hosts, and so he (Manson) missed the complete story as unfolded by Fedchenko. Colin Chisholm (1795) also noted the occurrence of the "dracunculus or Guinea worm" and wrote: "The cause of this singular disease seems to be confined to the water of some wells". He described how filling up these wells prevented the spread of the disease, and continued; "In the water which contains the embryos of the dracunculi, the naked eye distinguishes innumerable animalculi, darting in every direction with astonishing force and rapidity; these on being subjected to examination with a small microscope, exhibit a very extraordinary figure, differing from any animalcule hitherto described". These were surely copepods. Thus, it was Fedchenko's better knowledge of parasitology and familiarity with the appearance of unparasitised copepods which enabled him to make the observations that had eluded Chisholm; that the Guinea worm embryos were inside copepods (Hughes, 1967).

Sir James Emerson Tennent gives another account of the mode of transmission of Guinea worm. As the Colonial Secretary of the British Government in Ceylon from 1845 to 1850, Sir James Tennent noted in the course of a description of "parasitic worms" found there, that "of these entozoa, the *Filaria medinensis* or Guinea worm which burrows in the cellular tissue under the skin, is well known in the north of the Island, but rarely found in the damper districts of the south and west. The natives of these areas attribute the occurrence to drinking the waters of particular wells". Sir James Tennent thus records the fact that the mode of infection/transmission seems to have been known on the African coast in at least the sixteenth century, and in Ceylon for some time before the establishment of British rule in the

eighteenth century, thereby giving additional support to Dr. Muller's contention that "the belief that transmission is associated with wells and water holes has been held since ancient times".

Sir James Tennent also goes further to suggest that "these pests in all probability received their popular name of Guinea worm from the narrative of Bruno or Braun, a citizen or surgeon of Balse, who about the year 1611 made several voyages to that part of the African Coast, and on his return, published, among other things, an account of the local diseases" (Gooneratne, 1969).

Bizarre as it appears, the life cycle of the Guinea worm, *Dracunculus medinensis* is actually very well adapted for the transmission of a parasite that utilises an aquatic intermediate host, which occurs principally in arid or semi-arid environments. Human infection occurs when copepods containing the infective stage larvae (L_3) in water are ingested. The copepods are killed by gastric juices in the stomach and the larvae are activated and liberated and quickly pass through into the duodenum of the human host within 4 hours after ingestion (Muller, 1982).

They proceed to the abdominal and thoracic cavities where they begin maturing in connective tissue. Male and female worms mate about 3 months after ingestion. The male worms are believed to die at 6 months of age and then become encysted, calcified, or are absorbed. However, Brandt *et al.*, (1990) demonstrated that male worms might survive for up to330

days in experimentally infected ferrets. The adult female worm, which measures about 70 cm long by 2 mm, lives in the connective tissues. At about 8 months female worms usually move down to the lower limbs, where the uterus containing first-stage larvae develops to fill

nearly the entire adult worm.

In experimentally infected animals, the larvae are seen to penetrate through the duodenal wall in 10 to 13 hours post ingestion, and migrate via the mesenteries to the abdominal and thoracic muscles by about the fifteenth day (Rab, 1989). The larvae do not grow during this period, but there is probably a moult between the fifteenth and the twenty-first days. As they develop further, they begin to migrate towards the connective tissues of the axillary and the inguinal regions where they mature into adult worms. The size of the worms at this stage remains small and mating occurs between 80 and 100 days after infection (Muller, 1982). The males, (size: 1 to 4cm long) move into the deeper tissues. Within eight months after infection, the gravid female is filled up with developing eggs and with first-stage larvae by the tenth month. At this time, the worm begins its migration usually towards the extremities and is ready to emerge from the body between 10 to 14 months after infection. Before, the 14th month, the gut becomes flattened and non-functional, and the whole worm is filled by the larvae-containing uterus (Muller, 1982).

When the worm (female) is ready to emerge, the anterior end of the worm provokes the formation of a painful burning blister in the human skin. The worm emerges when this blister ruptures (especially upon coming into contact with water). Infected people frequently try to relieve the burning sensation by immersing the affected part in water. Contact with water causes the worm's uterus to rupture and stimulates the worm to expel larvae into the water. The process is repeated intermittently over several weeks.

Numerous first-stage larvae (L_1) are expelled into the water in a milky white stream. Estimates of the number of larvae contained in the uterus of a single worm range from 1.4 to 3.0 million. Not all the larvae are released at once, and it has been shown that about half a million larvae are released on first immersion in water. Out of water, the anterior end of the worm then becomes flaccid and dries up. When the affected part comes into contact with water again more larvae are expelled through the broken end of the worm. The number of larvae expelled at each immersion decreases, and the worm is completely expelled within one to three weeks. This intermittent discharge and drying up of the blister aperture is an adaptation to increase the chances of some of the larvae finding copepods in the water. This process is one of the neatest adaptation in behaviour in all of the realm of biology, enabling a blind unmeditative burrowing worm to give her aquatic copepod-inhabiting offspring a fair chance in life, even in deserts (Rab, 1989; Muller, 1971).

The first stage larvae, (640 by 13μ) remain active in the pond for about one week. Following ingestion by a *Cyclops*, the larvae penetrate the gut wall of the copepod and reach its haemocoel within one to six hours. They moult twice inside the copepods and reach the infective third-stage (L_3) in 14 days. It is when man drinks this copepod-contaminated water that the cycle is repeated (Figure 1), (Rab, 1989; Muller, 1971; Brieger and Rosenweig, 1988).

The rate of development of the larvae in the copepod has been found to be temperature dependent. Temperatures above 24 °C and below 19 °C inhibit the growth of the larvae, which are then incapable of reaching the infective stage. It has also been observed that those larvae-containing copepods are sluggish in their movements and tend to sink to the bottom of the ponds, as compared to the non-infected ones. There is also some evidence to suggest that the life span of the infected copepod is shortened (Rab, 1989).

2.4 CLINICAL MANIFESTATIONS AND PATHOLOGY

In most patients the first physical sign is the local lesion, accompanied by an intense burning pain usually relieved by immersion of the affected limb in water. The blister fluid is bacteriologically sterile and contains numerous white cells and larvae. In uncomplicated cases, the worm emerges from the subsequent ulcer over a few weeks and then the lesion rapidly heals. If there is only one worm present, patency will last for only 4-6 weeks.

Unfortunately, secondary infection along the track of the worm in the tissues is very common, often with spreading cellulitis, and approximately 40% of patients will be totally incapacitated for an average of 6 weeks. More serious and permanent damage can follow the bursting of a worm in the tissues or as the result of bacterial infection. Dracunculiasis is unusual among infectious diseases in that the parasite does not appear to stimulate a protective response. Thus, that the same individual can be re-infected year after year.

2.5 DIAGNOSIS

2.5.1 Clinical and Parasitological Diagnosis

Infected people exhibit no signs or symptoms until the female worm matures and is ready to emerge. The first manifestation of dracunculiasis is localized swelling at the spot where the mature worm will emerge. In over 90 percent of cases, it emerges somewhere on the legs or feet, although worms may emerge from any part of the body. Intense burning or itching accompanies the swelling, which develops into a blister within 1 or 2 days. This blister ruptures several days later and becomes a superficial ulcer. Infected people often immerse the lesion in water in an effort to relieve discomfort. The worm's uterus expels larvae when the affected part is exposed to water, a process that may continue for several days to 3 weeks. Occasionally worms die before reaching the skin's surface and are absorbed, form aseptic abscesses, or become calcified, leaving cord-like masses. Generalized nonspecific symptoms may accompany the appearance of *Dracunculus* at the skin, but they are usually not severe. Such symptoms may include diarrhea, vomiting, skin rashes, or asthma.

The tissues near the blister become swollen, red, and very tender, probably as part of a largely allergic reaction. There is usually a secondary infection, which commonly spreads from the initial skin lesion to deeper tissues and may be accompanied by severe or fatal septicemia. Infected ankle and knee joints can become contracted, leading to permanent crippling. Even in cases uncomplicated by secondary infection of the ulcer, the affected person may find it very difficult to walk and thus must give up his usual labors. On average, about 4-6 weeks may elapse before an uncomplicated infection heals completely.

Less frequent are other severe conditions or death resulting from *Dracunculus* infection. These conditions include septic arthritis, tetanus, gangrene, pulmonary scarring, and ophthalmic disease. A person may be infected by several guinea worms at the same time. Although each infection lasts a year, no effective immunity develops, and people at risk may be repeatedly infected year after year. Because of its unusual manifestation, guinea worm disease is easily diagnosed once the worm is ready to emerge.

Patients in an endemic area usually have no doubt of the diagnosis, as soon as, or even before, the first signs appear. Local itching, urticaria, and a burning pain at the site of a small blister are usually the first signs of infection. The blister bursts in about 4 days and active larvae, obtained by placing cold water on the resulting small ulcer, can be recognized under a low-powered microscope.

2.5.2 Immunological diagnosis

Diagnostic tests to detect the presence of *Dracunculus* at earlier stages have not been developed for routine use. However, laboratory investigators have reported positive fluorescent antibody tests 6 months or more before emergence of the worm (Belcher, 1982). Another nonspecific aid to diagnosis may be eosinophilia of 10-15 percent. In general, however, very little biomedical research on dracunculiasis has been carried out, thus making interpretation of findings reported in the literature quite difficult. Immunological methods are not useful in practice. ELISA and SDS PAGE/Western blotting worked well in one trial for

patent infections (Bloch *et al.*, 1983) and the fluorescent antibody test using deep frozen first stage larvae diagnosed prepatent infections in monkeys (Muller, 1971).

2.6 TREATMENT

2.6.1 Surgery

Guinea worms have been wound out on sticks since antiquity (e.g. in the Rig Veda of about 1350 BC). Provided that bacterial infection or other complications have not occurred, regular winding out of the worm on a small stick, combined with sterile dressing and acriflavine cream, usually results in complete expulsion in about 4 weeks with little loss of mobility. Treatment should be commenced as soon after emergence as possible (Magnussen, *et al.*, 1997). Sometimes worms can be seen and surgically removed before emergence while there is no tissue reaction against them.

2.6.2 Chemotherapy

There is no evidence that any chemotherapeutic agent has a direct action against guinea worms. No drugs have proved effective in killing the adult worm prior to emergence, although some have shown experimental promise in reducing inflammation and facilitating extraction of the worm. Eberhard, *et al.*, (1989) came to the conclusion that it appears existing drugs commonly used to treat helminthic infections are poor candidates for use in the campaign to eradicate guinea worm disease. This was after they evaluated the potentials of 4 chemoprophylactic drugs for use in the treatment of dracunculiasis by the *Dracunculus insignis*-ferret model.

Niridazole, metronidazole, thiabendazole, levamisole, bitoscanate, and mebendazole have been tested in humans within the last 7 years. Of these compounds, only thiabendazole if given as a short (2-day) course of treatment, would make patient compliance more likely. One study reported that mebendazole (7-day course) resulted in significant symptomatic improvement. The action of niridazole appears to be largely one of reducing inflammation. For the most part, however, these drugs are expensive and may not be available locally. Large-scale controlled clinical trials have not been conducted, and the results of the smaller studies are difficult to interpret because of different patient selection methods, wide variation in criteria of efficacy, lack of control groups, and high drop-out rates.

The majority of infected people neither seeks nor receives medical care from qualified physicians or nurses. Patients often consult healers or resort to the traditional technique of extracting the worm by rolling it a few centimeters each day around a stick or gauze. Infection often results if the worm breaks. Some physicians prescribe chemotherapy and make multiple incisions under local anesthesia to extract the emerging worm. Antibiotics may be given to treat secondary bacterial infections, and tetanus prophylaxis is strongly advocated.

Although there are no drugs to treat or prevent dracunculiasis, transmission is easily interrupted by simple measures such as behavioural changes, protecting wells, tanks and water sources, filtering water before drinking it, or (when possible) treating water with

"Temephos" (a biodegradable organophosphorous compound) to kill the copepods. These have made it possible to eliminate the disease from many affected areas and to aim at total eradication.

However, many compounds, including thiabendazole, niridazole, metronidazole, mebendazole and albendazole, have been reported as hastening the expulsion of worms and may act as anti-inflammatory agents. Ivermectin had no action against pre-emergent worms (Eberhard, *et al.*, (1989).

2.7 SOCIAL AND ECONOMIC EFFECTS

Cases of Guinea worm disease have been declining in much of Africa since the inception of the Guinea worm Eradication Programs in 1983. Unfortunately, the infection still remains a serious public health problem in some countries. According to the US Centres for Disease Control and Prevention (CDC), Guinea worm is especially problematic in southern Sudan, where civil war has impeded efforts to eradicate it. There were 66,097 reported cases of the disease in Sudan in 1999. Outside of Sudan, there were 12,097 cases of Guinea worm during the first six months of 2000, 18 percent less than the 14,828 cases reported during the same period in 1999. However, there was a slight increase in the number of cases in Ghana during the first six months of 2000 (Ruiz-Tiben, August 2000 – Personal communication).

Guinea worm has been an officially reportable communicable disease in Ghana since 1960 (Diamensu and Nyaku, 1998). The Ghanaian government launched a Guinea worm Eradication Program in 1987. Despite the political will and massive international support, there is unfortunately an apparent lapse in the control areas. This allowed for resurgence of the disease in some communities. For 1997, the nation recorded 6,844 cases of dracunculiasis during the period January-August from 703 villages, an increase of 73% over the same period in 1996. The region most afflicted, the Northern Region, reported a total of 5,977 cases from 534 villages, representing an increase of 63.8% over 1996 when 3,902 cases were reported from 455 villages. These dramatic increases in reported cases of Guinea worm in the country called for the intensification of efforts for the disease eradication.

2.7.1 Cultural and Traditional Beliefs

In village life, an enigma truly remains as to why some individuals are Guinea worm-free, while the majority are annually afflicted, and even why some unfortunate few are so massively infested. Following the UNO Decade of Water, the question may be moot, but it does recognize fertile ground for the growth of cultural belief, or folk rationale, to "explain" dracunculiasis and place it in a belief system. In Ghana, women in endemic villages, although zealously indoctrinated by visiting health education officers, cannot easily accept that invisible objects (copepods) in drinking water cause the disease. Ostensibly, they acquiesce to the superposed dicta of the educator, but to them, a cultural matrix of "jealous rivals", "enemies", and "witches" is an equally plausible causation (Hunter, 1996).

A study in Imo State, Nigeria, showed that 35% of the disease victims implicated their enemies whilst 37% of them believed that the disease was an inherited family trait. Ironically, only 11% accepted the polluted water explanation (Nwoke, 1992). Local people often believed that the worm is a natural phenomenon, a "natural part of the body like a tendon" an innate part of the human anatomy (Adeniyi, *et al.*, 1992). This idea matches those of the early Persian, Arabic, and Greco-Roman physicians who reported that dracunculiasis was anatomically of nerve-like origin, resembling varices (veins) or a "piece of corrupted nerve".

Bierlich (1995), carried out an anthropological study of beliefs and practices concerning infection with Guinea worm (Nyarfu/Nierifu), in two rural Dagomba communities in the Northern Region of Ghana. He found that the local people do not attribute Guinea worm to water. The general understanding is that Guinea worm is an innate part of human anatomy. It is not seen as an alien presence in the body. Guinea worm is rather said to be 'in people's blood', and sooner or later to 'stand up'. Guinea worm is considered an 'inevitable' feature of living ('Guinea worm is in the human blood'). These observations are very different from those of biomedicine, which hold that 'Guinea worm is a disease'. Omar *et al.*, (1993) examined the knowledge, attitudes, practices, and beliefs of the people concerning Guinea worm disease and its transmission, prevention, and treatment. They found that most people could describe the cause and treatment. More than 58% thought dirty or bad water was the main cause, while up to 63.2% believed that contaminated drinking water was the cause.

Few people clearly understood how Guinea worm is transmitted. People received treatment from the nearest health unit (42%), traditional healers (34%), self-medication (16%), and drug shops (8%).

2.7.2 Socio-Economic Aspects of Dracunculiasis

The basic nature of Guinea worm disease in Ghana shows its long-overlooked serious clinical aspects, and the many environmental and social influences that explain its persistence in the face of control efforts. It is a neglected disease of remote rural areas. The socio-economic impact of the malady has, however, assumed a more quantifiable aspect in recent times. Although the disease is rarely fatal, and less than 1% of victims suffer permanent disability, it prevents large numbers of people from farming or attending school. It is a severely disabling parasitic disease, responsible for heavy economic losses and serious social repercussions in endemic areas (Ogunniyi and Amole, 1990; Chippaux *et al.*, 1992). The skin lesions and subsequent scars produced by the emerging mature female worm alone could be both a social stigma and embarrassment. Infected people are often incapacitated for several weeks by secondary infections associated with the emergence of the worm.

2.8 CONTROL MEASURES

Dracunculiasis transmission is considered to be "water-based". The cycle of transmission requires that; (1) infected individuals immerse the mature emerging female worm in water used for drinking, (2) suitable copepod species are present in that water source under optimal

conditions, and (3) someone drinks water containing copepods infected with mature *Dracunculus* larvae. Any break in this chain of events will interrupt transmission of dracunculiasis.

Effective personal protective or prophylactic measures that may be used include boiling the drinking water or straining 'it through a cloth to remove copepods. The utility of these measures for a large-scale attack on the problem is somewhat diminished by their dependence on intensive educational efforts and by their inconvenience or cost (e.g., farmers quenching thirst in fields, firewood needed for boiling water). Health education, including community organization, might also be employed to encourage residents of affected villages to prevent people suffering from the disease from entering, and thereby contaminating, sources of drinking water.

Effective control measures include the periodic chemical treatment of water used for drinking in affected villages to kill copepods. Temephos (Abate) is the insecticide most commonly used for this purpose. At concentrations of 1 part per million in stagnant surface sources of drinking water, temephos kills copepods; is harmless to vegetation and fish; and is tasteless, colorless, and odorless in drinking water; and has a wide margin of safety for ingestion by humans. Moreover, the compound has been used extensively in West Africa to control the blackfly vector of onchocerciasis.

Thus far, however, the most effective means of preventing dracunculiasis has been to provide safe water supplies. Such protected water sources prevent contamination of the drinking water by larvae from infected people, thereby breaking the chain of transmission. Provision of safe water via piped sources and protected bore and tube wells, along with the destruction or conversion of contaminated step wells, successfully eliminated dracunculiasis from large areas in the southern part of the Soviet Union in the 1920s and 1930s. In Nigeria, construction of piped water for a town of 30,000 people in the 1960s reduced the incidence of dracunculiasis from over 60 percent to zero within 2 years. In several other instances, dracunculiasis has been eliminated or drastically reduced as a side benefit of efforts to bring safe drinking water to rural populations who happen to suffer from the disease.

The current International Drinking Water Supply and Sanitation Decade offers a unique opportunity for an attack on dracunculiasis. In the context of the Decade, the provision of safe drinking water is intended for all by 1990. Hence, there is no need to justify providing safe drinking water solely as a means of eliminating dracunculiasis, only to encourage endemic countries to consider this disease when assigning relative priorities to areas where elimination of the disease would occur in addition to other benefits. Indeed, the number of villages in which dracunculiasis is endemic is estimated to be less than 10 percent of all villages targeted to receive safe drinking water during the Decade.

2.9 THE PARASITE

2.9.1 Morphology

Nematodes are ubiquitous, unsegmented, pseudocoelomate worms, and probably the most abundant multicellular animals living today. Among these are some of the major parasites of vertebrates. There is however a difficulty in describing or recognising nematodes species. This is generally due to their small size and in the extreme uniformity of both internal and external anatomy as well as morphology. Often, species determinants must be based on biochemical attributes or morphological details not readily visible, such as the size and placement of microscopic sensory structures or morphological details of the excretory system (Pechnenik, 1991).

Generally, the body organization of the Nematoda is similar throughout the Phylum. All actively moving nematodes have cylindrical bodies that usually taper at the anterior and posterior ends. The constancy of the structure of the nematodes is not limited to the general body shape, for no matter what the size of the worm, the form and arrangement of the internal organs is similar (Obeng, 1997).

The sexes are separate in the *Dracunculus spp*. The male is rarely seen. Records claim it to be about 40mm long and only 0.4mm in width. The female is the better known of the two. As an adult, it is one of the longest human nematode parasite, with an average length of about 60cm and a width of 1.5 - 1.7mm. The head end of the worm has glands and a thick protective cap (Obeng, 1997). According to Muller (1971) and Hunter (1996), the mature

female of the dracunculoid nematode, *D. medinensis,* measures 500-800 mm by 1.0-2.0 mm (Figure 2). The anterior terminal mouth has a triangular oval opening surrounded by a quadrangular cuticularized plate, and an internal circle of four (4) double papillae. The digestive system is simple with a bulbous oesophagus, long simple intestine and an anus. The worm feeds on fluids and juices in the body of the host. There is a simple nervous system, but no easily identifiable excretory system. The principal parts of the female reproductive system consist of two ovaries, each leading into an oviduct and a uterus that merge to form a vagina that is attached to an ovijector attached to the body wall at the vulva. It is suggested that females often reproduce without mating (Obeng, 1997), but this is well recognized to be incorrect (Mark L. Eberhard, 2001 – personal communication).

The vulva opens halfway down the body but is non-functional in the mature worm. The uterus has an anterior and a posterior branch; it is filled with 1-3 million embryos and occupies the entire body cavity (pseudocoel), the gut being completely flattened (Muller, 1971). The worm is pale white, thread-like and soft (Hunter, 1996). Males recovered from experimental infections in animals measure 15-40 x 0.4 mm. The tail has 4(36) pairs of pre-anal and 4-6 pairs of post-anal papillae; the sub-equal spicules are 490-750μm long with a gubernaculum measuring about 117μm.

2.9.2 Taxonomy

The phylogenetic systematics of *D. medinensis* has seen some form of metamorphosis, as has been the case for most organisms. In literature therefore, the Nematoda is sometimes listed as a class within another phylum, the Aschelminthes. The Aschelminthes are often referred to as the "Cavity worms" because they posses a pseudocoel. The majority of groups contained within the Aschelminthes (Nematoda, Rotifera, Gastrotricha, Kinorhyncha, Nematomorpha, Acanthocephala and Gnathostomulida) are now generally considered more distantly related, so that each class has been elevated to the phylum status (Pechenik, 1991). Thus, *D. medinensis* has been assigned the following taxonomic groups.

PHYLUM:	Nematoda
CLASS:	Secernentea.
SUBCLASS:	Spiruria.
ORDER:	Camallanida.
FAMILY:	Dracunculidae.
GENUS:	Dracunculus.
SPECIES:	medinensis.
SCIENTIFIC NAME:	*Dracunculus medinensis.*
COMMON NAME:	Guinea worm.

2.10 THE VECTORS OF *DRACUNCULUS MEDINENSIS*

The transmission of *D. medinensis* requires the involvement of a water flea as the intermediate host. This flea is a minute crustacean, the size of a pinhead, pear-shaped, a free-living fresh-water cyclopoid copepod, with different genera and species (Figure 2). *Dracunculus medinensis* however exhibits a high level of host specificity and only a few species act as vectors in nature. Cyclopoid copepods (Cyclopoida) are known as the intermediate hosts of more than ten genera of the Cestoda and Nematoda. Two among these are important parasites of man; *Diphyllobothrium latum* (the broad fish tapeworm) and *Dracunculus medinensis.*

Mesocyclops leukarti (Claus) is widely reported as the common intermediate host in India and Africa (Onabamiro, 1951; Muller, 1970). Other reported hosts include species of the genera *Mesocyclops* Sars, *Thermocyclops* Kiefer (Onabamiro, 1951) and *Metacyclops* Kiefer (Steib, and Mayer 1988). The correct identification of the copepod intermediate hosts is therefore not only important in mapping out the geographical distribution and spread of the disease, but also vital in the development of eradication programmes that aim to combat the disease by vector control (Boxshall and Braide, 1991).

Copepods (classified as Macrozooplankton, *Sensu stricto)* are subdivided into Calanoids, Cyclopoids and Harpacticoids. Of these groups, only cyclopoid copepods serves as intermediate hosts of *D. medinensis*. Recent progress in copepod systematics has refined the

level of taxonomic resolution of these freshwater copepods and it is now known that *Mesocyclops leukarti* does not occur in either Africa or India (Keifer, 1981; Van de Velde, 1984). There is, therefore, an obvious need to record these taxonomic changes, to review earlier records, and to update the nomenclature of the hosts where possible (Boxshall and Braide, 1991).

The various groups have been observed to have diverse feeding regimes, often modified in the course of development. Cyclopoids have an erratic jumping motion which makes them more conspicuous than the gliding motion of calanoids (McCullough, 1982). The broad taxonomic grouping of copepods is as follows.

Phylum:	Arthropoda
Subphylum:	Crustacea
Class:	Copepoda
Order:	Cyclopoida
Family:	Cyclopidae

Thus, cyclops belongs to the Cyclopoid copepods, one of the orders in the class Copepoda. The Copepoda is included in the subphylum Crustacea that also include such familiar invertebrates as crabs, shrimps and lobsters (Barnes, 1968). Freshwater Cyclopoid copepods are minute, pinhead-sized crustaceans which are biologically very successful, comprising

many genera with diverse feeding habits. They are found world wide and almost exclusively in standing or slow-flowing, marine, brackish and freshwater bodies. The free-living cyclopoid copepods (often referred to as cyclops), have pear-shaped bodies, comprising a cephalothorax, an abdomen and a telson with a tail which has two caudal rami. The sexes are separate and the eggs hatch into typical nauplius larvae, which are then succeeded by several metanauplius stages before moulting into the first of five successive copepodite stages (McCullough, 1982).

Muller (1971), listed 17 species of copepods that potentially act as intermediate hosts in different Dracunculiasis endemic areas. More recently Steib, (as cited in Olsen, 1993), working in Burkina Faso, added two more species (*Thermocyclops incisus* and *Metacyclops exsulis*) to the list of potential vectors. In Africa however, approximately 150 different species of freshwater cyclopoids have been described. Of these only 60 are prevalent in areas endemic for Guinea worm disease. Probably, only 34 of these species are frequent vectors. Generally, only members of the genera *Thermocyclops* and *Mesocyclops* are regarded as truly planktonic forms and can therefore act as the most important vectors of the disease (Kiefer, 1978; as cited in Oslen, 1993).

McCullough (1982), states that only large predatory species can readily ingest *Dracunculus medinensis* larvae and can therefore act as potential intermediate hosts. Of these carnivorous species, the older and larger copepodid stages are more predatory than the younger smaller

ones. Also, that in each endemic zone usually one of the local predator species is the dominant intermediate host by virtue of its preferred habitat and seasonal population dynamics or both.

Furthermore, Boxshall and Braide (1991), working on the freshwater Cyclopoid copepods of Nigeria, identified forty valid and four non-valid vector species of dracunculiasis. Species in the genera *Thermocyclops, Mesocyclops* and *Metacyclops* are often implicated as intermediate hosts of *D. medinensis.* Boxshall and his coworkers were however quick to point out that the often implicated (and misidentified) host, *Mesocyclops leukarti* does not occur in either Africa or India. This view is also held by Olsen (1993), who states in his key "Vectors of Guinea worm disease in tropical Africa - A key to the species of *Thermocyclops* and *Mesocyclops*", that of the 13 taxa of this genus described for Africa, most forms were recorded as *Mesocyclops leukarti,* which does not in fact occur in tropical Africa. Chippaux (1991: as cited in Boxshall and Braide, 1991), also identified 14 species of cyclopoids out of which 4 were suitable intermediate hosts for *D. medinensis.* Of these, *Thermocyclops oblongatus* appeared to be the most common, especially in ponds, followed by *T. neglectus* whilst *T. crassus consimilis* was considered a minor intermediate host. *T. emini* also played an important role as an intermediate host, especially in rivers at the beginning of the dry season when the rivers had stopped flowing (Boxshall and Braide, 1991).

Other species of copepods known to transmit *Dracunculus medinensis* are *Thermocyclops nigerianus and T. hyalinus* (Onabamiro, 1951). With respect to the food and feeding habits of copepods, the work of Klugh (1927), is believed to give a comprehensive review of the food of freshwater Entomostraca (Birge, 1897; as cited in Fryer, 1955).

Horse dung infusions (essentially protozoan cultures) appear to have been the chief source of food used in cultures. Coker (1933; as cited in Fryer, 1955), remarks that this medium is satisfactory for the rearing of *Acanthocyclops vernalis* and *Eucyclops (= serrulatus)*, but states, "we had reason to doubt its effectiveness with respect to the fertility of adults reared as to its general sustainability for *A. viridis*". Later Coker is said to have noted that he had satisfactory results by the addition of unicellular green algae and chopped fragments of the filamentous green alga *Mougeutia spp.* to the culture medium (Fryer, 1955). Fryer also revealed that individual species of copepods had preferred food material. The carnivorous species included *Macrocyclops albdus, M. fuscus, Acanthocyclops viridis, A. vernalis, Cyclops strenuus and Mesocyclops leukarti.* The herbivorous species were mostly from the genus *Eucyclops* and some *Microcyclops*.

The physico-chemical properties of copepod habitats depend on the general pattern of seasonal events. As Burgis (1971), points out, in the temperate lakes, the seasonal changes are due mainly to the incident solar radiation, and the consequent changes in water temperature leads to an alternate building-up and breakdown of thermal stratification. In the

tropical regions however, incident solar energy is high throughout the year, thus, diurnal stratification is of greater significance than seasonal changes. This phenomenon is pronounced in large bodies of water. In the small ponds that are often used as sources of drinking water, the almost constant mixing of the water produces an environment remarkably homogenous, with stable physico-chemical conditions. The only apparent seasonal changes are those associated with increased inflow during the rainy season and the consequent increase in volume, often coupled with increased turbidity (Fryer, 1955).

2.11 ZOONOSIS

Female worms of the genus *Dracunculus* have been reported as emerging from a wide range of mammals and reptiles from many parts of the world, both endemic and non-endemic for the human disease. Those found in reptiles clearly belong to other species but the situation in regard to those in mammals is not clear. For instance, Guinea worm is common in wild carnivores in North America and the species was named *D. insignia* by Leidy in 1858, and although distinct, there are few morphological features that separate this from the human species.

There have been two documented cases of clearly zoonotic infections, from Japan in 1986 and from Korea in 1926. In both cases the patients had eaten raw freshwater fish, which have been proved experimentally to be capable of acting as paratenic hosts. In most highly

endemic areas occasional infections in dogs and donkeys with what is presumably the human parasite have been reported but there is no evidence that they have any part in maintaining transmission. The parasite can still be found in dogs in the formerly endemic areas of Tamil Nadu in India and the central Asian republics of the former Soviet Union, but no new human cases have been reported, so it is not thought that this will be a problem once world eradication has been achieved.

2.12 GUINEA WORM ERADICATION PROGRAMMES (GWEPS)

2.12.1 Global Perspective.

Edingbola *et al.*, (1988), estimated the worldwide incidence of dracunculiasis at 5-15 million per year, all in the poor and remote rural area of Africa and the Indian subcontinent. The vagueness of this estimate is itself evidence of how little was correctly known about the prevalence and detailed distribution of the disease. However, the World Health Assembly Resolution of May 1986 (Res. No. 3G. 21) in support of the elimination of Guinea worm, and the World Health Organization Water and Sanitation Decade, (which ended in 1990), have helped focus attention on the disease and the problems involved in its study (Smith *et al.*, 1989).

In 1991, the Forty-fourth World Health Assembly declared the goal of eradicating dracunculiasis (guinea worm disease) by the end of 1995 (Resolution WHA 44.5). In 1988

the World Health Organization (WHO) Regional committee for Africa set itself the goal of eradicating the disease in all 17 remaining endemic countries (except Sudan which is in the WHO Eastern Mediterranean Region) by 1995 (Resolution AFR/RC38/ R13). Apart from these 17 African countries, Indian and Pakistan were the other countries still with the disease, which is only transmitted to humans when they drink water from stagnant ponds, step wells or cisterns (Hopkins and Ruiz-Tiben, 1991).

Beginning in 1986, 1987 and 1988 the Global 2000 Project of the Carter Presidential Centre assisted dracunculiasis eradication programmes in Pakistan, Ghana and Nigeria respectively, in collaboration with the ministries of health of these countries. The WHO Collaborating Centre for Research, Training and Eradication of Dracunculiasis at the Centres for Disease Control and Prevention also provided extensive technical assistance to these programmes. Eradication of dracunculiasis is particularly feasible for the following reasons:

1. There is no human carrier state beyond the one-year incubation period.
2. Transmission could be seasonal, depending on the climate and topography of the area in question
3. Active detection of individuals with worms protruding from skin lesions is a sensitive means of assessing the presence of the disease in the endemic villages.
4. The methods for controlling transmission are simple and can be targeted effectively.
5. The total estimated cost of global eradication is moderate - about US$ 75 million (Hopkins and Ruiz-Tiben, 1991).

Target dates for eliminating the disease were set at 1990 for Pakistan, 1993 for Ghana and 1995 for Nigeria. In Pakistan 408 villages with a total of 2 400 cases for 1987 alone was recorded, while 640 000 - 650 000 case from 6 000 villages were recorded from Nigeria. Ghana conducted a national search for cases in 1989/90, and found that almost 180 000 cases had occurred in the past year in about 6 500 villages. Each endemic country was mandated to decide on the most appropriate mix or combination of the available interventions. These included Health Education, Rural Water Supply, the use of Cloth Filters and Vector Control that it can afford or seek external help. No single intervention will work anywhere, and "problem" villages are to be dealt with as they become apparent.

With the eradication of the disease from India and Pakistan, the main focus of attention is now on Africa. In these countries, under the auspices of the WHO Regional Office for Africa, the respective ministries of health resolved in 1988 to eradicate dracunculiasis by the end of 1995. This has since been rescheduled year after year, with the focus now on December 2002.

2.12.2 The Guinea Worm Eradication Programme in Ghana

The campaign to eradicate the GWD in Ghana was launched by the government in 1989. This policy is based on the WHA Res. 39.21, which was adopted by the WHA in May 1986 and endorsed by Ghana as a member state of the WHO. The original target date of December 1993 was later shifted to December 1995. A myriad of problems including the 1994 ethnic

conflict in the Northern Region and operational fatigue as well as funding problems prevented the achievement of the target, even though it is reported to have achieved a 95% reduction in 1996. Against this background, the President was obliged to visit Gushiegu, the most endemic town in the country in 1997 to re-launch the programme. Unfortunately, the Programme had to be re-launched again in April 1999 in the Afram Plains District of the Eastern Region due to the unusually high number of reported cases. The new date for eradicating Dracunculiasis in the country was once again set at 31st December 1999; it has since been shifted to December 2002.

In his opening address at the 1998 annual National Review Meeting of the GGWEP on 27th September 1998, HE Vice President Professor John Atta Mills declared: "The scourge of Guinea worm has been a source of worry for Ghanaians, especially those who do not have clean water". He, however, acknowledged that the provision of good drinking water will not only eradicate the disease, but can also lead to a reduction in other water related diseases. The Vice President therefore called for a vigorous health education effort to eradicate the disease since the disease thrives in an ignorant and apathetic society.

The Plan of Action of the GWEP in Ghana states the methodology, strategy, targets and the estimated cost of achieving the objectives of total eradication of the disease by the year 1995. The programme in Ghana began identifying and training resident village volunteers (VVs) to report cases of Dracunculiasis in endemic communities in the early part of 1989 (Hopkins

and Ruiz-Tiben, 1991), control measures were directed towards freeing domestic water sources of copepods and preventing infected people from contaminating water supplies (Quashie, 1982).

The GWEP in Ghana is supported by the government as well as donor agencies such as the Carter Center in Atlanta Georgia, Bank for Credit and Commerce International (BCCI) and the Sasakawa Global 2,000 Project. Since the government recognized the need to eradicate dracunculiasis as a means of poverty removal/reduction, the National Guinea Worm Eradication Programme was incorporated into the Ministry of Health (MOH) with an autonomous administration headed by a National GWEP Coordinator. The mandate of the programme was to evolve a focal control strategy for eradicating the disease by 31st December 1995.

The intervention strategies adopted were based on seven points:

1. Surveillance.
2. Health Education (HE).
3. Provision of safe drinking water
4. Training.
5. Monitoring and evaluation.
6. Research.
7. Vector control.

The main emphasis as of July 1995 was all but research. An extraordinary level of public mobilization was achieved in June 1988 when the Head of State, Flt. Lt. Jerry John Rawlings spent eight day visiting 21 endemic villages in the highly infected Northern Region, promoting the goals of the national eradication campaign: an exceptional degree of involvement by any Head of State in combating any disease (Report on IDWSSD Impact on Dracunculiasis, October 20, 1989). Ghana also began distributing tens of thousands of copies of a manual to Secondary schools in 1989 for teaching about the guinea worm disease. Both Ghana and Nigeria are emphasising on Health Education, Community Mobilization and Rural Water Supply in their intervention so far. An extensive use of temephos (Abate) in selected villages began in 1991.

Chapter 3

3.0 SOCIO-CULTURAL STUDIES

3.1 MATERIALS AND METHODS

3.1.1 Study Locale

The study was carried out in 7 endemic villages in 4 districts, including Kukuo, Diare and Vognayili in West Dagomba, Gushiegu and Karaga in Gushiegu-Karaga, Savelugu in Savelugu-Nantong, and Nyankpala in the Tolon-Kumbumgu district in the Northern Region of Ghana (Figure 3). These villages had population sizes of 4,370; 9,514; 250; 7,511; 1,250; 27,478; and 6,776 respectively (National Population Census, 2000). Lying approximately 8-10° N latitude in the Savanna woodland, the region receives 40-45 inches (1015-1142mm) of rainfall annually. There is only one season of rain, mostly in April-October, associated with the air mass movements of the Inter-Tropical Convergence Zone (ITCZ). Data was collected over one month period (Mid-June to Mid-July1999), at the tail end of the most recent transmission season. These districts were specifically chosen for their high incidence of dracunculiasis. Complications caused by secondary infections were also common. These communities utilized water sources mainly typical of the traditional modes of water supply based on the collection and storage of surface water in ponds, large contaminated earthen dams, or reservoirs. These water sources are the essential points of dracunculiasis transmission in the dry season.

3.1.2 Retrospective Studies for Disease Prevalence

Data collection involved interview and focus group discussions. A standardized questionnaire was developed and used to ascertain the peoples' knowledge base with respect to perceptions on disease causation, prevention, treatment, and transmission (Appendix A). Here, face-to-face interviews were held with respondents. Disease prevalence was estimated by assessing the Guinea worm status of one person interviewed per household. Mostly, it was the head of household who were taken as respondents. However, in the absence of the head, a reliable informant was interviewed. In order to compare responses of individuals who have or are currently afflicted with Guinea worm disease with those who have never contracted it, the households, and therefore, the respondents were randomly chosen. All the interviewers spoke both Dagbani (the local language/dialect), and English. A total of 383 /households/people, made up of 226 males and 157 females living in the four districts were involved in the study.

A focus group discussion session was held in each village, with 8-15 adults (men and women) between 29 and 50 years of age, and 10 children, ages 11-15 years old. Information collected from respondents consisted mainly of: beliefs and practices, knowledge base concerning causes, mode of transmission, perceptions on curability and prevention, source of respondents' knowledge, source of water, and level of dependency on it. Risk factors (human practices and beliefs), hypothesized to have some relationship with the disease causation, were selected and grouped into demographic characteristics. Some socio-economic factors

examined included occupation and educational level of respondents. The relative prevalence of Guinea worm with respect to these factors and practices were then evaluated.

3.2 RESULTS

3.2.1 Prevalence of Guinea Worm Disease

Overall, 244 (64%), of the respondents reported to have had Guinea Worm Disease (GWD) at least once in the past two years, with 36% escaping infection. Out of this number, 43% of respondents had the disease in the current season (September 1998 - August 1999), with 24% and 32% having been afflicted in the previous year as well as both the previous and current year, respectively. With respect to gender, 226 (59%) of the victims were males, and 157 (41%) were females.

3.2.2 Seasonal Distribution of Guinea Worm Disease

Cases of dracunculiasis were recorded in 10 of the 12 months of the year. The highest number of cases was recorded in May (25%), while no cases were recorded in August and September. Notably, the disease appears to begin in October (0.8%), and gradually increases every month thereafter, peaking in May of the next year before sharply dropping in magnitude and disappearing completely by the end of July. Most cases were observed from December to May (88%), whilst the period July to November recorded only 5% of all cases. Thus, there appears to be a very long period of disease transmission, with a peak in April to May each year (Figure 4).

The negligible occurrence of dracunculiasis in the study area within the period July to October (0.2%), with a complete absence in August and September is a notable feature. It is

important to note that this is the peak of the rainy season in the region. Thus, most respondents might be harvesting and storing rainwater from their roofs for domestic use.

3.2.3 Occupational Distribution of Guinea Worm Disease

A majority of the Guinea Worm victims were farmers, with a considerable number being pupils, students, housewives, and traders. Thus, 94 (39%) were farmers, 41 (17%) pupils, 40 (17%) housewives, and 30 (12%) traders (Figure 5).

3.2.4 Perceptions of Disease Causation

To ascertain the knowledge base of the respondents regarding dracunculiasis, they were asked some pertinent questions such as; *"What do you think causes GWD?"*, *"How is GWD spread?"*, *" Can GWD be cured?"*, *and "Can GWD be prevented?"*. A majority of respondents (62%) alluded to the fact that dracunculiasis could be acquired by drinking water from a pond/dam. This state of awareness could be due to the active status of the Guinea Worm Eradication Programme (GWEP) in the region since being launched in 1989. Only 10% ascribed natural causes as the cause of the malady. Another 10% attributed causation to supernatural forces such as witchcraft and Juju. Almost every respondent assigned one reason or the other as the cause of the disease, with only 0.5% claiming they had no idea (Figure 6).

3.2.5 Perceptions of Disease Transmission

A large majority of the people (66%), felt that Guinea worm was transmitted by "entry into a

pond by an infected person". Not withstanding the active nature of the GWEP in the area, up to 19% of respondents still claimed they did know the mode of transmission of dracunculiasis. Other implicated routes of transmission (blood, dirt, sex, and hereditary), accounted for 12%. Only 0.5% thought communal eating habits could spread the disease. Insect vectors (flies and mosquitoes), were erroneously implicated by 2% of the respondents (Figure 7).

3.2.6 Perceptions on Disease Curability

Although there exists no known cure for Guinea Worm (at least for now), respondents attributed any remedy that could ameliorate the pain as cure. In this respect, 36% of the people ranked the use of the popular Tamale oil, (an oil formulated by GWEP purposely for use in facilitating and hastening worm expulsion) as the most effective cure for Guinea worm. As common as it is in most of rural Ghana, the use of herbs for the "treatment" of Guinea worm was anticipated, with 29% of the respondents employing herbs for remedies. Of those who relied on orthodox drugs, 20% resorted to the use of Paracetamol, with only 1% actually aware that the drug of choice, Ambilhar, could be used to ease the pain and hasten worm expulsion. Other methods (worm extraction, injections, going to the hospital, use of Maggie cubes, plastering blisters with ant-hill clay), accounted for 10%. Also, 2% believed that the disease could be cured/treated by a Juju-man, while 3% had no idea concerning cure (Figure 8).

3.2.7 Perceptions on Disease Prevention

Filtration of pond water before drinking was ranked as the most effective method of

preventing infection with Guinea worm. Up to 53% of the respondents knew this, with another 27% aware of boiling all drinking water as a preventive measure. The complete reliance on Borehole water for drinking to prevent infection was mentioned by 17%. Only 4% of the respondents were aware of regular use of Abate in ponds/dams as a method of breaking transmission (Figure 9).

3.2.8 Source of Knowledge

The respondents were probed to ascertain their sources of information/knowledge with respect to disease causation, cure, and prevention. A good number of them (50%) stated Guinea worm workers (coordinators and village volunteers) as their key source of information on preventive remedies. About 20% of respondents indicated that health workers other than the GW personnel were the source of their know-how on preventive measures (Figure 10).

Assuming the people had a zero knowledge base with respect to prevention before the onset of the GWEP, then it is quite possible the current 15% and 7% levels of awareness per relations and friends, respectively, could be due to a kind of snowball effect where information is passed on from person-to-person. This is quite an effective method of message transmission because of its multiplier effect with time. Unfortunately, however, teachers and

religious leaders were the least effective health educators on prevention against the spread of Guinea worm disease, accounting for only 8% and 0.3% respectively.

3.2.9 Respondents' Principal Sources of Water

The reliance on stagnant water bodies for water, by most of the respondents, was a common observation in this study. A vast majority of them (73%) depended on ponds/dams for their water supplies. Up to 58% of respondents were observed to depend on pond/dam water all-year-round. Also, 36% resorted to the use of ponds and dams for water only in the dry season (October - May), whilst 5% claimed they made use of pond water only during the rains when the nearby ponds are filled with water. Only 11% of the respondents had access to stand pipes as a principal source. Interestingly, this group was observed to be concentrated in Kukuo (a suburb of Tamale, the regional capital). The 16% of respondents who relied heavily on borehole water regrettably had to resort to the use of pond/dam water in the dry season either because the boreholes dried-up, or broke-down due to excessive pressure of usage or both (Figure 11).

3.2.10 Respondents' Alternative Sources of Water

In addition to their main sources of water supply, inhabitants made use of other sources in times of need. These ranged from those who had no alternative source (8%), to a few who could access potable water (7%). About 27% turned to boreholes for water as an alternative source. The salient observation here was that those who resorted to either standpipe water or

boreholes did so only when the ponds had dried up. This group preferred pond water to borehole and/or standpipe water primarily because of differences in the taste of the water.

Also, the distance of respondents' home/compound to the source of uncontaminated water could be a hindrance, and hence, tend to discourage patronage. The second plausible reason could be the inconvenience of having to form long lines for hours at pipe or borehole sites before getting water. Most of the people (39%), made use of rainwater by trapping and storing it for use (Figure12).

3.2.11 Common Activities At Pond/Dam Sites

The most common activity at the water-contact-site that could aid in the disease transmission was wading through the water during the process of fetching water. Over 50% of the people drawing water from the ponds/dams did actively wade into the water body before fetching water. Understandably, they believed others had contaminated the water at the periphery; thus, it is arguably wiser to fetch from the deeper waters.

This practice provides an ideal situation where blisters and/or emerging worms on the skin could get into contact with water. This gives the emerging worm a chance to expel larvae into the copepod-infested water. The copepod ultimately ingests the larvae and stands the chance of eventually infecting a person who consumes the water without boiling or filtering, unless, of course, the pond/dam has Abate added before the water was drawn.

This situation is further aggravated by the fact that majority of worms emerge at the lower extremities, (the most likely parts of the body that comes into contact with water while

wading). Also, those who engaged in washing clothing and/or cooking utensils at the water source (30%), were equally guilty of having to wade into the water during their water-related activity. Unwittingly, worms at anatomical sites other than the lower extremities also had a chance to infect copepods, and therefore sustaining the cycle as demonstrated by the 5% swimmers and 14% bathers who had intimate contact with the water source. These are, unfortunately activities that are highly implicated in Guinea worm transmission (Figure 13).

3.2.12 Socio-demographic correlates of dracunculiasis

To ascertain the dependence of Guinea Worm infection on some potential disease-related factors, a number of hypotheses were tested.

1. Prevalence of dracunculiasis does not depend on age or level of education.

Infections with Guinea worm disease were not observed to be age-related (P-value = 0.34). A stratified analysis of the data, however, implicated some age groups if the levels of education were considered as well. Among people with no formal education or only Primary School education, infection was age-dependent (P-value = 0.01). Conversely, among people with at least, a Senior Secondary School (High School) level of education, there was no statistical difference in the rate of infection within the different age groups. Thus, SSS (P-value = 0.90), and College level education (P-value = 0.63).

Likewise, while within the 10-29 years age group, infection was correlated with level of education (P-value = 0.003), this was not so in the other age groups. Thus, 0-9 years (P-value

= 0.53), 30-49 (P-value = 0.88), and 50+ years (0.90).

2. Prevalence of dracunculiasis does not depend on occupation.

There is a positive correlation observed between infection with Guinea worm and occupation (P-value = 0.04). Hence the disease has a predilection for people with certain occupations. In other words, some particular occupations predispose people to infection. Analysis of the data with respect to the different age groups, however, revealed no relationship between infection and occupation within some age groups. Within the 10-29 years age group, infection with dracunculiasis did not depend on ones' occupation (P-value = 0.12). This age group constitutes young people who are quite active and mobile. This high rate of mobility might lessen their level of dependency on the contaminated waters.

3. Infection with dracunculiasis does not depend on source of water.

From these results, it appears that infection with GWD did not only depend solely on the individual's source of water, but also, a function of both source of water and the degree of dependency on this water (Figure 11). Thus, among the investigated sources of water supply in the communities, only those who collected and depended solely on pond water throughout the year contracted infections more often (P-value = 0.002). Those who made use of pond water but did not rely on this source stood a lesser chance of infection (i.e., pond and stand pipe, P-value = 0.03; pond and bore hole, P-value = 0.02; stand pipe only P-value =0.74).

Notably, individuals who made use of both ponds and either standpipes or bore holes had a high probability of contracting infections. This could be due to the fact that, they depend

mainly on pond water during the dry season when water supply from the utility service is often quite erratic and unreliable.

3.3 DISCUSSION

The prevalence studies revealed that up to 43% of the populace still got afflicted with the malady during the last transmission season, with 33% suffering it for the second consecutive year. This supports the findings of Watts (1986), who found that there is no evidence of acquired immunity to infection, and residents in hyper-endemic communities remain susceptible to infection all their lives.

The seasonal transmission pattern appears to be an enhancing factor in sustaining the disease in the region. Apparently, there is a very long transmission season, spanning over six months with a peak in April to May. This would mean that, any eradication effort should aim at long and sustainable methods as against intensive short periods of vector control. Any shortfall or lapse in control measures would certainly lead to resurgence. This could be one of the factors that militate against the eradication of the disease in the region.

It is, however, important to note that the high levels of infection among farmers could be deceptive. This is more so because that is the main occupation of the local people. Likewise, the religious predilection or otherwise of infected persons could not be ascertained since over 90% of the local people in the study area are Muslims.

In this study, it was observed that the knowledge base of the local people with respect to the disease was quite high. Over 50% of respondents seem to be aware of disease causation,

prevention, and management. This could be due to the efforts of the Eradication Program in the region. Unfortunately, the status of infection is still relatively high (43%). A number of factors could be responsible for this. Obviously, ignorance and poor disease management habits on the part of some individuals play a very important role in sustaining and transmitting the disease. Also, apathy, lack of motivation, and worker fatigue could be implicated as the major players in promoting the resurgence of the disease.

Considering the source of knowledge of the people with respect to the disease causation, prevention and management, one is tempted to implicate the eradication program itself for not involving teachers and religious leaders in the eradication effort. These two groups could be very effective and reliable means of impacting and changing human behavior.

The observation that, infection with Guinea worm is not correlated with age and/or educational status is worth noting. This could imply that as one grew older, the level of education no longer plays a role in protection against infection provided the risk factor (exposure to infective source of water), is still present. This is more so since it is only within the 10-29 year age group that there is a negative correlation of infection with age. This is understandably the youth, who belong to a different era and are more likely to accept and adhere to disease prevention measures. Their belief systems are influenced by current events,

and they are more likely to depart from the old-fashioned beliefs of their parents which tend to maintain the disease transmission.

Not surprising, the children (0-9 years old), indicated no correlation with infection with age. This could be attributed to the fact that mothers are most likely not to give their infants pond water to drink. They are traditionally given boiled water or depend on breast milk for their water requirements.

Chapter 4

4.0 INVESTIGATION OF WORM MORPHOMETRY

4.1 MATERIALS AND METHODS

A total of 190 surgically removed gravid female guinea worms collected from various districts in the country were used in this study. These are mainly communities that have been declared as being endemic for the Guinea Worm Disease (GWD). Specimens were preserved in 10% formalin and brought to the laboratory for morphometric measurements. Only whole worms (noted to have both anal and buccal regions intact), were used in the studies. No fragmented or crushed specimens were used.

4.1.1 Determination of Worm Length

The lengths of the worms were measured using a thin inelastic thread. The reference end of the thread was knotted and the appropriate length determined by tracing the thread on the entire length of the specimens. The total length of thread so obtained was then stretched out on a metre-rule and the approximate length of the worm read off. Since the worms are purple-white or cream in colour, a black thread was used to produce an appropriate contrast for vision (Table 1 and Appendix B).

4.1.2 Determination of Worm Weight

Having measured the length of a worm, its weight was also taken by the use of a Mettler Teldo (PG503, 1996) weighing machine. This is an electronic device that reads up to four (4) decimal places. An empty petri dish was placed on the weighing stage and the machine re-zeroed. The specimens were then placed in the empty petri dish and the weight read (Table 1 and Appendix B).

4.1.3 Determination of Worm Diameter

Measurements of worm diameters were also carried out on a number of specimens and recorded appropriately. The measurements were done at the mid-sections of the worms and the average of four measurements of a given specimen was taken as the approximate diameter. These measurements were done, using an Olympus microscope (CH-2; Model-CHT, Olympus Optical Co. Ltd, Japan). This was done with the aid of a stage micrometer and a micrometer eyepiece. The actual measuring was done with the micrometer eyepiece, having calibrated it with the aid of the stage micrometer. Readings were taken at a magnification of 40 (Table 1 and Appendix B).

4.2 RESULTS

The mean length of the worms was 57.23cm, with the shortest worm measuring 30.5cm and the longest specimen measured up to 102.0cm. Considering worm weight, whilst the least was 0.118g, the highest was 2.260g.The mean worm weight was 0.901g (Table 1).

With respect to the morphometric relationships of *Dracunculus spp*., various correlation analyses were carried out with the aid of computer software - Microsoft Excel - 1997. Thus, the length-age correlation was computed to find out if there was any correlation between the length of a worm and the age of the patient. The computed coefficient of correlation was 0.068. Although this is a positive correlation, it shows a very weak association between the length of a worm and the age of the patient. Thus, the length of a worm does not in any way depend on the age of the patient.

Also computed was the weight-age coefficient of correlation. This depicted an even weaker relationship between the weight of a worm and the age of its victim. The calculated coefficient of correlation was 0.03 (about half that of the length-age correlation). The overall coefficient of correlation for weight-length relationship of Guinea worm was 0.47, showing a weak positive correlation between the weight and length of a worm. This revelation could mean that the diameter of a worm is an important factor with respect to its weight. Hence, a very long worm could have a small diameter and vice versa. Also, since these worms were

surgically removed from the patients, their intrinsic weights could depend on the state/stage of maturity and therefore the numbers as well as the total weight of the larvae present in the uterus.

Analysis of the data based on the sex of the patients was carried out. The coefficient of correlation for weight-length relationship of worms extracted from males was 0.43 (as opposed to 0.47 for both sexes combined). The worm mean length in this case was 57.71cm, with a median length of 57.4cm. Whilst the shortest specimens measured 30.5cm, the longest worm was 96.5cm. The mean weight was 0.875g (Table 1).

For specimens extracted from female patients, the weight-length coefficient of correlation was computed at 0.696 (as compared to 0.43 and 0.47 in the case of worms extracted from male patients only and for both sexes respectively). The mean, maximum and least worm lengths were 59.7cm, 102.0cm and 45.2cm respectively. Likewise, the mean, maximum and least worm weights were 0.839g, 1.961g and 0.602g respectively.

Generally, the mean diameter of the worms was computed as 1.32mm, with a modal thickness of 1.35mm. The smallest diameter of the specimens was 0.75mm, whilst the largest worm had a diameter of 1.96mm.

4.3 DISCUSSIONS AND CONCLUSIONS

From these analyses there appears to exist no relationship between the lengths and weights of *Dracunculus spp*. on one hand, and the sex of the patient. It is however, important to note that, there is an apparent correlation, though weak, for weight-length correlation in female patients (Correlation Coefficient = 0.696). Generally, however, there was no correlation with respect to the sex and age of the individual on the one hand, and worm length and/or weight on the other hand.

Analyses of the relationships between the three morphometric indices of the specimens (Length, Weight and Diameter), depicts positive correlations for the various paired variables. Thus:

1. The weight of the worm is associated with the length of the worm.
2. The weight of the worm is associated with the diameter of the worm.
3. The diameter and weight of the worm depends on its length.

All the computed coefficients of correlation were positive. The Length-Diameter Coefficient of Correlation was observed to be the least (0.653). On the other hand, the Coefficient of Correlation for Weight-Length and Weight- Diameter were 0.779 and 0.812 respectively.

From these analyses, it can be inferred that these three morphometric parameters are positively correlated. It is also important to note the positively strong correlation shown by the length-weight (0.779) and diameter-weight (0.812) variables. These relationships do not necessarily imply causation. Hence, it does not necessarily mean that the weight of a worm depends solely on its diameter and vice versa. Likewise, the weight of a worm cannot be said to depend on its length only.

Also, it is important to note that, the exact ages of these worms were unknown. Hence, it cannot be known whether worms recovered from women were more likely to be older and therefore more mature and/or larger/heavier.

Chapter 5

5.0 HISTOMORPHOLOGY OF *DRACUNCULUS MEDINENSIS*

5.1 MATERIALS AND METHODS

5.1.1 Tissue Preparation

Tissue preparations were carried out on alcohol-preserved specimens for histologic details. Microscopic examinations were carried out on sections (T/S) of the mature female worms (Figures 14 – 21). The specimens were first thoroughly rinsed with tap water. Pieces measuring 1.0 - 1.5cm long were then cut from the anterior, pharyngeal, mid and posterior regions of the specimens for serial sectioning. The cut pieces were washed in 10% Potassium Hydroxide (KOH) for 4-5 minutes. These were then rinsed with tap water and dehydrated at room temperature (29 °C) by passing it through a series of progressively more concentrated alcohol baths (i.e. $H_2O \rightarrow 30 \rightarrow 50 \rightarrow 70 \rightarrow 95\% \rightarrow$ absolute alcohol) for 45 minutes each. Placing in xylene briefly then cleared the dehydrated specimens.

The cleared pieces of worm were then impregnated with paraffin/candle wax for 24 hours at a temperature of 56°C. These wax-impregnated specimens were then imbedded in blocks of candle wax measuring 1cm by 1cm by 2cm each. Sections were then cut out using a large rotatory microtome (LR - 85, OSK - 9782; Yamato Kohki Industrial Co. Ltd, Japan). The 8-

micron (8μ) sections obtained were dewaxed/cleared in xylene and again passed through an alcohol series (i.e. absolute → 96 → 70 → 50 → 30% alcohol) to re-hydrate. The sections were over-stained in Borax Carmine and differentiated briefly in acid-alcohol before counter-staining in eosin. The sections were washed in 70% alcohol after each staining. After counter staining in Eosin, the preparations were again dehydrated briefly in 96% and absolute alcohol respectively and cleared in xylene. The preparations were mounted in dpx and examined under a microscope for morphologic details.

5.2 RESULTS

The histomorphology of *Dracunculus medinensis* (the anatomical composition of the worm), was observed to vary along the anterio-posterior axis. There is a progressive change in anatomy anterio-posterialy. As typical of all nematodes, the outer covering of the worm is a tough cuticle. The cuticle was found to be multi-layered, consisting of a cortical, a medial and a basal layer. A well-developed hypodermis, lying between the cuticle and the muscle layer was observed. The hypodermis is thickened in the dorsal and ventral positions to form the hypodermal cords. The muscle cells are the same from the anterior to the posterior regions, although the number and shape vary. They overlap, and depending on the region, they may look large or small (Figures 14 – 21).

5.2.1 The Cephalic Region

The anterior region was observed to be quite unique. The mouth is triangular in shape and leads into the esophagus, which has a trivoliate, cuticle-lined lumen. The contractile portions of the buccal musculature are well developed and distinct. The non-contractile segments of the longitudinal muscles are however not well differentiated in this region. There are no larvae, implying that the uterus does not extend to this segment of the worm (Figure 14).

5.2.2 The Pharyngeal Region

The esophagus is round, but assumes an oval shape towards the upper mid-section of the worm. The muscle cells of the pharyngeal region are well differentiated. Some cells of the non-contractile muscle cells are observed to be nucleated. The esophagus is perpendicularly oriented to the pseudocoel and lies along the axis of the lateral nerve cords. The syncytial hypodermis is also well differentiated here (Figure 15 and 16).

5.2.3 The Mid-Section

The shape at the pharyngeal region is completely modified or differentiated into a circular shape at the mid-section. The pseudocoel here is almost circular and its diameter is considerably increased. There are two bean-shaped identical layers of muscles cells that lie opposite each other. These are separated at either side by the lateral cords where the hypodermis projects into the body cavity. The thickness of the bean-shaped layer of muscle cells progressively decreases from the mid section to the posterior region. The gut is often difficult to see, and almost always displaced by the larvae-filled uterus, and pushed to one side of the body cavity. It is apparently free of any contents. The longitudinal muscle cells are well developed in this region. However, the non-contractile parts of the muscle cells are flattened against the contractile portions and not easily seen (Figure 17 - 20).

5.2.4 The Posterior Section

The characteristic circular shape at the mid-section is maintained at the posterior end of the worm. The thickness of the two bean-shaped muscles is, however, considerably reduced. The larvae filled uterus is still the prominent organelle in this region. The gut is however, markedly reduced to an orifice (Figure 21).

5.3 DISCUSSIONS AND CONCLUSIONS

At maturity, the gut of the female guinea worm is completely atrophied and the entire worm is completely made-up of the larvae-filled uterus. From this study, differences in the musculature from the anterior region, with much thicker muscles to the mid region was observed. The thicker muscles could be used for squeezing the larvae out into water by contractions. Thus, by intermittent active expulsions of larvae when exposed segment of worm or blister comes into contact with water. It appears the stimulus for contraction is provided by cold water. It has also been observed that the number of larvae expelled progressively decreases, with the highest coming from the first immersion/contact (Chandler, *et al.*, 1961). This contention is further buttressed by the fact that protruding/exposed segment of the worm dries up, and therefore is non-functional. This could account for the reducing numbers in the larvae expelled per immersion in water.

Chapter 6

6.0 VECTOR SPECIES OF *DRACUNCULUS MEDINENSIS*

6.1 MATERIALS AND METHODS

6.1.1 Sampling

Copepods (Figure 2), were collected form ponds/dams often used as sources of water. The collected water from these water bodies was observed to be used mostly for drinking (at home and on the farms), as well as for other domestic purposes. Samples were collected during peak seasons of the disease for two seasons. November 1998 to May 1999, and again from November 1999 to May 2000.

In sampling, a wide mouthed 4-liter plastic container was lowered into the water at the contact site and scooped. The water was filtered through a monofilament filter of 70μm mesh. The copepods so trapped were washed into a beaker. This was repeated four times and the collected samples fixed immediately in 10% formalin and brought to the laboratory for identification.

6.1.2 Identification

Copepods were identified using the key prepared by Olsen (1993). A phase-contrast microscope (Model: Optiphot-2; Nikon, Japan) was used. Only mature females (noted to be

carrying egg sacs), were examined and identified accordingly. The use of this key was supplemented by another key prepared by Boxshall and Braide (1991). The two keys use distinct morphological features on such parts of the body as the fifth pair of legs, the caudal rami, the antennae, the seminal receptacle, the maxillary palpi, the total body length and the relative lengths of the body segments.

The identification process involved handling the specimen in water-free glycerine on a clean slide under a microscope. The copepod was then teased with a dissecting pin until the structure under investigation was appropriately oriented for observation. The laboratory-bred copepods were identified before being evaluated for their infection potential.

6.1.3 Colony Maintenance

Specific cyclops species were reared in 500ml beakers in the laboratory at room temperature (26 - 29 °C). First, a single gravid female copepod was selected using a Pasteur pipette and washed several times with tap water in a petri-dish. The petri-dishes were placed over a dark background to facilitate easy access, handling and observation of the copepods. This gravid female was then transferred into a beaker containing 500ml tap water. The nauplii hatching from the eggs were fed on cow dung infusion and the green algae *Cladophora* spp. The water in the beakers was aerated for 5 minutes daily and changed every fortnight until the colony could provide enough adult copepods for infection with Guinea worm larvae.

6.1.4 Evaluation of Infection Potentials

Vectors of *D. medinensis* have hitherto been identified by elucidating the species of copepods (sampled from domestic water sources), which are noted to be naturally infected with Guinea worm larvae. As Yelifari (1997), postulates, the high mobility of the local people during the dracunculiasis transmission season presupposes that some of them could have been infected by water sources other than the ones investigated. It is therefore difficult to correlate occurrence of cyclopoid copepods in the local water sources with the prevalence of human infection in the catchment area. Hence, no valid conclusions as to the most important intermediate host(s) in these areas can often be drawn.

In this study, laboratory experiments were carried out to infect various species of copepods with first stage (L_1) guinea worm larvae (Figure 22). The larvae were obtained from matured female worms extracted from patients. Only pre-emergent whole worms were used in this study. The worms were extracted from patients by trained worm extractors after administering local anesthesia (Figure 24 - 28). To ensure viability of the larvae, the worms were immediately milked after extraction into filtered pond water. In addition to the tests on the laboratory-reared copepods, specimens obtained from the field were also tested.

Using a Pasteur pipette, 1-2 drops of the larvae-filled milky fluid were introduced into 500ml beakers containing 50 copepods of each species. Preliminary infectivity tests with composite samples showed that varying numbers of larvae could be ingested by different species and

different developmental stages of the vectors. These composite samples contained a mixture of copepods species sampled from the field, with various developmental stages of the vectors. Hence, the experimental set up was categorized into adults and copepodids. Each species had three replicates (adults only, copepodids only, and a mixture of adults and copepodids, each with a control. In this experiments, the following attributes were evaluated.

1. The ability of the various species of copepods to ingest the first stage guinea worm larvae (Figure 22).
2. The maximum number of larvae that adults and copepodids could ingest.
3. How long each developmental stage of copepods used in the experiment could stay alive with respect to the number of larvae ingested.
4. The ability of ingested larvae to develop from L_1 to the infective L_3 (Figure 23), in each species.

Samples of the "infected" copepods were observed under a microscope progressively (after 12, 24, 48, 72, hours; and 7, 10, 14, 21, 28 days etc), for the presence and viability of ingested larvae. A drop of ice water was put on the *Cyclops* on a microscope slide to immobilize it, leaving the active larvae that could then be seen in the haemocoel of the copepod. Observations for copepod mortalities were recorded accordingly.

6.2 RESULTS

6.2.1 Copepod Species

A total of 7 copepod species (4 *Thermocyclops* and 3 *Mesocyclops* spp.) were found in the study. The most common species in the study communities was *M. keiferi*. Whilst *M. keiferi* was present in all 5 communities, *M. aspericornis, T. incisus,* and *T. inopinus* were present in 3 communities each. Also, *T. oblongatus* was present in 2 communities, with *T. neglectus* and *M. major* occurring in only one community each. Table 2 shows a summary of copepod species found in the study area.

6.2.2 Infection Potentials

Four of the copepod species were observed to be capable of supporting development of guinea worm larvae (Table 3). All 4 species of copepods were exposed to first stage-larvae (L_1) of guinea worm. The number of larvae ingested as well as the ability of the copepod to stay alive and facilitate the development of the first-stage larvae into the infective third-stage larvae was evaluated. Among the adult copepods the larger *Mesocyclops* spp. were observed to be the most voracious. Up to 4 larvae could be ingested by a single copepod. Conversely, the smaller *Thermocyclops* spp. could ingest a maximum of only 2 - 3 larvae (Table 3).

Like the adults, the copepodid stages of the copepods did ingest the larvae. However, these could not ingest more than 2 larvae per copepodid (except the juveniles of *M. keiferi*).

Among both the adults and juveniles, infected copepods that were able to withstand the infestation for 72 hours (3 days), were observed to survive the infection. Also, in all 4 species of copepods, the larvae were able to moult and therefore to develop to the infective L_3 stage within 14-20 days (Table 3).

Although all copepod species studied did ingest Guinea worm larvae, very high mortalities were observed among the adults. *M. aspericornis* and *M. keiferi* ingested up to 5 larvae (81 and 75% respectively). However, by the third day (72 hours), only 12 and 6% of the infected copepods were still alive (i.e., 88 and 94% mortalities occurring). The smaller *Thermocyclops* spp. could ingest only between 2 and 3 larvae, with a corresponding percentage mortalities of 4 and 0% for *T. incisus* and *T. oblongatus* respectively (Table 3).

With respect to the copepodids, the maximum number of larvae ingested was 2. No remarkable mortalities were observed within the 72-hour critical period. The 2% mortality recorded was attributed to incidental death (Table 3).

6.3 DISCUSSION

Of all the 7 cyclops species found in the study area, the most common species was *M. keiferi*. In order of relative importance, therefore, *M. keiferi* → *M. aspericornis* → *T. incisus* → *T. inopinus* → *T. oblongatus* → *T. neglectus* →*M. major*. All but *M. aspericornis* have been found by a number of researchers to be naturally infected with Guinea worm larvae. Yelifari *et al.*, (1997), found *T. incisus, T. inopinus,* and *M. keiferi* sampled from water bodies in the Tamale municipality to be infected with Guinea worm larvae.

The adults of the relatively larger species (mainly *Mesocyclops* spp.), recorded very high mortality rates upon infection with the first stage larvae (L_1) of the parasite. The highest copepod mortality rate was recorded by *M. keiferi* (94%). However, the copepodid stages of these species were able to withstand infection for extremely longer periods. The smaller *genera* (mainly *Thermocyclops spp.*), did not record any remarkable mortalities on ingesting parasite larvae (L_1). The copepodid stages ingested mostly one larva each. This was comparable to the scenario in the *Mesocyclops* spp (Table 3).

The very high mortalities encountered in adults of the larger *Mesocyclops* spp. could be attributed to the fact that the ingested larvae draw nutrients from the copepods. Thus, hyper-infestation could lead to physiological upsets in the host as a result of the feeding regime of

the ingested larvae. This could further explain the observation by some researchers that, the ingested larvae feed on the ovaries of the copepod. What might actually be happening is that, by drawing nutrients form the copepod, its ovaries become atrophied and non-functional as a result of the physiological upset. Also, the development of the ingested first- stage larvae is observed to be faster in the adult copepods than in the copepodids. This could be due to the availability of nutrients, and the adult copepods have a greater reserve of nutrients upon which to draw. Also, the copepodid stages are themselves growing and thus require high nutrient amounts. In this fashion, development from the first stage larvae to the infective third stage larvae in adult and juvenile copepods infected at the same point in time definitely go out of phase, with larvae in adult vectors reaching the infective stage earlier than in the copepodids.

This apparent lag in development could result in differential manifestations of the disease (especially, the peak season), from year to year in the same locality. This becomes quite explicit, depending on the vector species that dominates in the community. This assertion is further buttressed by the fact that, usually in each endemic zone only one of the local predatory species of copepods is often found to be the dominant intermediate host by virtue of its preferred habitat, its seasonal population dynamics, or both (Onabamiro, 1954; McCullough, 1982).

The most important vectors of dracunculiasis in the study areas are: *M. keiferi* → *M. aspericornis* → *T. incisus* → *T. inopinus* → *T. oblongatus* . However, it is important to note that, the copepodids could be the main players in the transmission cycle since adults die massively (70-90%) upon ingesting the first stage larvae of the parasite. In the *Thermocyclops spp*, both adult and juvenile stages were observed to be equally efficient in habouring the larvae for the required periods needed for it to develop to the infective L_3 stage.

Chapter 7

7.0 MOLECULAR EPIDEMIOLOGY OF GUINEA WORM

Currently, the taxonomy and species classification of Dracunculid parasites known to infect both humans and other animals is based on type of host and morphological features of the parasite. The morphological markers are sufficient for systematic analysis of these parasites, however, most of the key features are present on the male worms, which are rarely, if ever, available for study. Therefore, these probably need to be supported by molecular characterization. Employing molecular probes, polymerase chain reaction amplification, and sequence-based identification is therefore, advantageous for substantiating epidemiologic relationships and diversity. Since this DNA speciation can be accomplished even on small portions of extracted female worms, it lends itself to taxonomic characterization.

As the number of genotypes of *Dracunculus* spp. responsible for human infections is still unknown, determining whether the epidemic under investigation is caused by multiple strains or a single species is often a dilemma. This makes it difficult to ascertain the source(s) of infections, and therefore does not allow for the formulation of needed prevention and control guidelines. Hence, there is the need for strain characterization of *Dracunculus spp.* for epidemiologic tracking (Lal and Tibayrenc, 1997).

The role of zoonotic infections of *Dracunculid* parasites in human infections is also yet to be ascertained. Thus, the species types and genotyping tools are needed for studies of the molecular epidemiology of dracunculiasis. Such tools would facilitate laboratory characterization of dracunculiasis cases and identification of infection sources. The variations or otherwise in genotypes may also shed light on the transmission dynamics of the *Dracunculid* parasites in different geographic areas and epidemiologic settings. An intragenotypic heterogeneity in the SSU-rRNA sequence by isolates could be a very useful tool for epidemiologic investigations.

The occurrence of multiple genotypes at the same geographic location or the distribution patterns of these genotypes would define the complexity of Guinea worm epidemiology. Divergence or aggregations of isolates from various geographic locations (countries) might confirm or dispel the idea of localized transmission cycles. Claims and allegations of imported cases could therefore be investigated and the true origins ascertained.

In this study, we report on the use of sequence data from the SSU-rRNA and the Internal Transcribed Spacers 1and 2 (ITS1 and ITS2) to distinguish among the species of Guinea worm. This represents a first step to determine genetic variation within isolates from specimens collected from humans and from some identified animals of various geographical locations. Relationship among species was inferred from DNA sequence data. Phylogenetic inference among *Dracunculus species* was done by examining a conserved region of the

complete gene sequence of the genomic DNA (the gene sequence of 18S-rRNA), and subsequently, the internal transcribed spacer 1 -ITS1 and ITS2 regions for sub-species (Figures 29 and 30).

DNA sequences of isolates collected from various geographical locations (mostly in Africa) were analyzed for sequence variability. In order to investigate the possibility of more than one species of *Dracunculus* in humans, Guinea worms originating from different African countries, Pakistan and Yemen were compared by sequence analysis of the nuclear small subunit 18S rRNA gene as well as the more variable ITS1 and ITS2 regions.

Since the highly conserved regions in the ribosomal repeat array can be used for study of relationships across phyla (Gerbi, 1985: as cited in Lal and Tibayrenc, 1997), the more variable ITS-1 and ITS-2 regions was targeted to investigate for sub-species (a lower taxonomic resolution/level). This is because the ITS regions does not encode for any product, permitting it to evolve at a faster rate than the ribosomal coding regions. The level of variation in this region therefore makes it suitable for detecting genetic diversity.

7.1 MATERIALS AND METHODS

7.1.1 Worm Collection

The specimens (Guinea worms), were collected mainly from villages in Northern Ghana, declared as endemic for dracunculiasis. Specimens from other African countries (Cameron, Niger, Nigeria, Togo, Sudan, Cote d'Ivoire, Ethiopia, and Burkina Faso) were also used in the investigation. Outside of the African continent, specimens from Pakistan and Yemen were used (Figure 31). These specimens were preserved in 10% alcohol (a few from Ghana were kept frozen). All specimens were pre-emergent worms extracted from patients (Figure 25). Two interesting cases of a "Red" worm (from Togo), and a worm of animal origin (from a dog in Ghana) were also used in the study.

7.1.2 DNA Extraction and Preparation

DNA was extracted from specimens following the procedure outlined in - " DNA Extraction from Animal Tissue" as per "Bio 101 FastDNA Kit" (Applied Biosystems: Appendix C). The extracted DNA was then purified using QIAquick kit (Appendix D), to remove PCR inhibitors.

7.1.3 Polymerase Chain Reaction (PCR) Amplification

To analyze the base sequence for speciation, the 18S rRNA region of the rRNA cluster was targeted (Figure 31). Extracted DNA was amplified using the forward primer NEMFG1 (5' TCT CCG ATT GAT TCT GTC GGC GAT TAT ATG), and the reverse primer CRYPTOR (5' GCT TGA TCC TTC TGC AGG TTC ACC TAC).

To type the isolates, however, the Internal Transcribed Spacer Region 1 (ITS1) was targeted. Here, DNA was amplified using the forward primer NAP9 (AAC AGG TCT GTG ATG CCC T), and the reverse primers 58S-R1 (TAG CTG CGT TCT TCA TCG ATC), 58S-R2 (TTG CTG CGT TCT TCA TCG ATC), and 58S-R3 (TAG CTG CGT TCT TCA TCG ACC).

PCR reaction mixtures consisted of 1X buffer, (10 mM Tris-HCl, 50mM KCl, 1.5mM $MgCl_2$, pH 9.0), 200µM of dNTP, 25pmol of each primer, 2.5 U of AmpliTaq Gold (Perkin Elmer), and 0.1-0.5µl of DNA, in each final volume of 50µl. Forty-five PCR cycles (94 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 90seconds) were carried out in an automated Thermal Cycler GeneAmp Perkin-Elmer 9700 with an initial hot start of 95 °C for 15 minutes, and a final extension at 72 °C for 10 minutes.

7.1.4 Verification of PCR Product

To visualize the products, the PCR products were run through 1% Agarose gel in TBE (gel electrophoresis), which was subsequently stained with Ethidium Bromide to ascertain DNA amplification or otherwise. A 100bp ladder was used as the standard (Figures 32 - 34).

7.1.5 DNA Sequencing

Amplification products were purified using spin columns (Stratagene kit), according to the instructions from the manufacturer (Appendix E), and eluted in 50µl of UV-H_2O. Sequencing reactions for speciation were performed with the purified products on both strands using the primers NEMFG1 and CRYPTOR, as well as the internal primers 5' CTG CCT TAT CAA CTT TCG ATG (NEM1F), 5' CAT CGA AAG TTG ATA AGG CAG (NEM1R), 5' GCG GTT AAA AAG CTC GTA GTT GG (NEM2F), 5' CCA ACT ACG AGC TTT TTA ACC GC (NEM2R), 5' GCG GCT TAA TTT GAC TCA ACA C (NEM3F), 5' GTG TTG AGT CAA ATT AAG CCG C (NEM3R), 5' CCG GGA CTG AGC CGT TTC GAG (NEM4F), and 5' CTC GAA ACG GCT CAG TCC CGG (NEM4R). However, sequencing reactions for typing isolates were performed with the purified products on both strands using the same primers employed in DNA amplification.

The products were purified with CENTRI-SEP Protocol (Applied Biosystems), to remove Dye Terminators prior to sequencing (Appendix F). The products were then dried with a DNA SpeedVac (Model 120, ThermoSavant), and re-suspended in acrylamide. Sequencing

was carried out with a 3100 Genetic Analyzer (Applied Biosystems). The base sequences were assembled and analyzed using Seqman.

7.2 RESULTS

The gene coding of isolates of human origin (*D. medinensis*) from the various locations were successfully extracted, amplified and sequenced. The Small Subunit Ribosomal RNA (SSU-rRNA) of these isolates was constructed/assembled (Appendix G).

All the isolates were observed to have identical sequences. PCR amplification of small subunit ribosomal (SSU-rRNA) gave a single product from each species about 1819 base-pairs long. Figure 32 – 34) illustrates an electrophoretic analysis (in 1% agarose gel) of PCR-amplified products of guinea worm SSU-rRNA.

Interestingly, the gene coding of an isolate of a worm of animal origin (a dog from Ghana), revealed an identical base sequence to sequences of isolates from worms extracted from humans. This implies that *D. medinensis* has low host specificity and can infect animals other than man. The "Red" worm of human origin (Togo) produced a similar base sequence as those of normal coloration (pale-white).

These results are quite interesting in relation to potential incidentalanimal infections with Guinea worm in endemic communities. The identical base sequences of the "Dog Guinea worm" to those extracted from humans implies that, the disease can be transmitted from man to dogs and vice versa. This is not a welcome revelation, especially at this point in time when global eradication efforts are intensified. This is more so because it is possible to distinguish *D. insignis* from *D. medinensis* at the SSU-rRNA locus.

Fortunately, a more suitable reverse primer from the base sequences obtained from the ITS studies was designed. This primer does not produce the undesirable problems such as the formation of dimmers that we had to contend with when we had to use CRYPTOR as the reverse primer. This primer is 18SR (GTT AAT GAT CCT TCC GCA GGT TCA CCT AC).

7.3 CONCLUSIONS

1. *Dracunculus medinensis* and *D. insignis* can be differentiated by the SSU-rRNA sequence. This may apply as well to other *Dracunculus* isolates from animals.

2. All *Dracunculus medinensis* isolates have the same sequence of the SSU-rRNA.

3. All the ITS1 sequences look identical, including the Dog isolate from Ghana (of course, it would have been more interesting if we found differences).

4. Thus, strain typing has to be done using other regions, if possible, to answer the question as to whether or not some animal isolates are really different from the human isolates.

5. Definitely, species that do not infect humans like *Dracunculus insignis* may be differentiated on the basis of the SSU rRNA.

6. It is possible to verify whether or not worms of animal origin are *D. medinensis* or otherwise based on the 18S (SSU rRNA) and the ITS1 regions.

7. **IMPLICATIONS:** Guinea worm can possibly be transmitted from man to animals (dogs), and vice versa.

Chapter 8

8.0 GENERAL DISCUSSION

In this study, it was found out that a considerably high percentage of the local people in the study communities could still fall prey to the guinea worm disease, even though there has been an active eradication programme in the area for over a decade. The evaluated incidence for the last transmission season was 43%, with 33% suffering from the disease for the second consecutive year. This collaborates the findings of Watts (1986) that there is no evidence of immunity to infection, and residents in hyper-endemic communities could remain susceptible to infection all their lives.

The seasonal transmission pattern appears to be a major factor in sustaining the disease in endemic communities. This could be due to the very long transmission seasons in some endemic communities. This would imply that, any eradication effort should aim at long and sustainable methods as against intensive short periods of vector control. Any shortfall or lapse in control measures would certainly lead to resurgence. This could be one of the factors that militate against the eradication of the disease in the region.

The observed high infection rate among farmers could be deceptive. This is because farming is the main occupation of the local people. Likewise, the religious predilection or otherwise of infected persons could not be evaluated because over 90% of the people in the study area are Muslims.

In this study, it was observed that the knowledge base of the local people with respect to the disease is quite high. Over 50% of respondents seem to be aware of disease causation, prevention, and management. This could be due to the efforts of the Eradication Program in the region. Unfortunately, the status of infection is still relatively high (43%). A number of factors could be responsible for this. Obviously, reluctance of the local people to comply with disease management and control practices could play a very important role in sustaining and transmitting the disease.

Considering the source of knowledge of the people with respect to the disease causation, prevention and management, one is tempted to implicate the eradication program itself for not involving teachers and religious leaders in the eradication effort. These two groups could act as very effective and reliable means of impacting and changing human behavior. It is therefore suggested that the GWEP in the country should co-opt these opinion leaders to intensity the eradication effort.

The observation that, infection with Guinea worm is not correlated with age and/or educational status is worth noting. This could imply that as one grew older, the level of education no longer plays a role in protection against infection provided the risk factor (exposure to infective source of water), is still present. This is more so since it is only within the 10-29 year age group that there is a negative correlation of infection with age. This is understandably the youth, who belong to a different era and are more likely to accept and

adhere to disease prevention measures. Their belief systems are influenced by current events, and they are more likely to depart from the old-fashioned beliefs of their parents, which tend to maintain the disease transmission.

Not surprisingly, the children (5-9 years old), indicated no correlation with infection with age. This could be attributed to the fact that this age group is likely to heed to instructions and advice from their parents, and therefore not drink from ponds.

The morphometric analysis of specimens for possible correlations revealed that there appears to exist no relationship between the lengths and weights of *Dracunculus spp.* on one hand, and the sex of the patient. The apparent correlation, though weak, for weight-length correlation in female patients (Correlation Coefficient = 0.696) could be due to hormonal factors on the part of the human host. Generally, however, there was no correlation with respect to the sex and age of the individual on the one hand, and worm length and/or weight on the other hand.

All the computed coefficients of correlation were positive. The Length-Diameter Coefficient of Correlation was observed to be the least (0.653). On the other hand, the Coefficient of Correlation for Weight-Length and Weight- Diameter were 0.779 and 0.812 respectively. It is suggested that an animal model be employed to investigate this further, since the parasite does not avail itself for scientific investigation in the human host due to the very long pre-patent period.

It can be inferred that these morphometric parameters are positively correlated. It is also important to note that the strong positive correlation shown by the length-weight (0.779) and diameter-weight (0.812) variables may not necessarily imply causation. Also, it is important to note that, the exact ages of these worms were unknown. Hence, it cannot be known whether worms recovered from women were more likely to be older and therefore more mature and/or larger/heavier.

This observation is further buttressed by the observation that, at maturity, the gut of the female guinea worm is completely atrophied and the entire worm is completely made-up of the larvae-filled uterus. The observed differences in the musculature from the anterior region, with much thicker muscles to the mid region could be an adaptation to enable the mature worm to expel. its larvae into water by muscular contractions. Thus, by intermittent active expulsions of larvae when exposed segment of worm or blister comes into contact with water. It appears the stimulus for contraction is provided by water. It has also been observed that the number of larvae expelled progressively decreases, with the highest coming from the first immersion/contact (Chandler, *et al.*, 1961). This contention is further buttressed by the fact that protruding/exposed segment of the worm dries up, and therefore is non-functional. This could account for the reducing numbers in the larvae expelled per immersion in water. It is the deposition of larvae into domestic water sources containing the appropriate vector species of the parasite, which ensures the transmission of the disease.

In this study, seven *Cyclops* species were found in the study communities. The most common species was *M. keiferi*. Ranking these gives the following in order of relative importance: *M. keiferi* → *M. aspericornis* → *T. incisus* → *T. inopinus* → *T. oblongatus* → *T. neglectus* →*M. major*. Among these, all but *M. aspericornis* have been found by a number of researchers to be naturally infected with Guinea worm larvae. Yelifari *et al.*, (1997), found *T. incisus, T. inopinus,* and *M. keiferi* sampled from water bodies in one of the study localities (Tamale) to be naturally infected with Guinea worm larvae.

It was observed that, the adults of the relatively larger species (mainly *Mesocyclops* spp.), recorded very high mortality rates upon infection with the first stage larvae (L_1) of the parasite. The highest copepod mortality rate was recorded by *M. keiferi* (94%). However, the copepodid stages of these species were able to withstand infection for extremely longer periods. The smaller *genera* (mainly *Thermocyclops spp.*), did not record any remarkable mortalities on ingesting parasite larvae (L_1). The copepodid stages ingested mostly one larva each. The very high mortalities encountered in adults of the larger *Mesocyclops* spp. could be attributed to the fact that the ingested larvae draw nutrients from the copepods. Thus, hyper-infestation could lead to physiological upsets in the host as a result of the feeding regime of the ingested larvae. What might actually be happening is that, by drawing nutrients form the copepod, its ovaries become atrophied and non-functional as a result of the physiological upset. Also, the development of the ingested first- stage larvae is observed to be faster in the

adult copepods than in the copepodids. This could be due to the availability of nutrients, since the adult copepods have a greater reserve of nutrients upon which to draw. Also, the copepodid stages are themselves growing and thus require high nutrient amounts. In this fashion, development from the first stage larvae to the infective third stage larvae in adult and juvenile copepods infected at the same point in time definitely go out of phase, with larvae in adult vectors reaching the infective stage earlier than in the copepodids.

This apparent lag in development could result in differential manifestations of the disease (especially, the peak season), from year to year in the same locality. This becomes quite explicit, depending on the vector species that dominates in the community. This assertion is further buttressed by the fact that, usually in each endemic zone only one of the local predatory species of copepods is often found to be the dominant intermediate host by virtue of its preferred habitat, its seasonal population dynamics, or both (Onabamiro, 1954; McCullough, 1982).

It is, however, important to note that, the copepodids could be the main players in the transmission cycle since adults die massively (70-90%) upon ingesting the first stage larvae of the parasite as a result of hyper-infestations. In the *Thermocyclops spp*, both adult and juvenile stages were observed to be equally efficient in habouring the larvae for complete development to the infective stage.

Studies to ascertain the molecular epidemiology of *Dracunculus species* revealed that:

1. *Dracunculus medinensis* and *D. insignis* can be differentiated by the SSU-rRNA sequence. This may apply as well to other *Dracunculus* isolates from animals.
2. All *Dracunculus medinensis* isolates have the same sequence of the SSU-rRNA.
3. ITS1 sequences of isolates from different geographical areas look identical, including the Dog isolate from Ghana. Of course, it would have been more interesting if we found differences.
4. Strain typing has to be done using other regions, if possible, to answer the question as to whether or not some animal isolates are really different from the human isolates.
5. Definitely, species that do not infect humans like *Dracunculus insignis* may be differentiated on the basis of the SSU rRNA.
6. It is possible to verify whether or not worms of animal origin are *D. medinensis* or otherwise based on the 18S (SSU rRNA) and the ITS1 regions.
7. This may imply that Guinea worm can possibly be transmitted from man to animals (dogs), and vice versa.

It is therefore recommended that further studies be carried out using suitable animal models to find out whether or not *D. medinensis* is capable of successful development in some animals.

TABLES

TABLE 1: WORM MORPHOMETRY

PARAMETER	MEAN	SMALLEST	LARGEST
	ALL WORMS		
Length (cm)	57.2	30.5	102.0
Weight (g)	0.901	0.118	2.260
	WORMS FROM MALES		
Length (cm)	57.71	30.5	96.5
Weight (g)	0.875	0.118	2.260
	WORMS FROM FEMALES		
Length (cm)	59.7	45.2	102.0
Weight (g)	0.839	0.602	1.961

TABLE 2: CYCLOPOID COPEPODS FOUND IN THE STUDY AREA

COMMUNITY	COPEPOD SPECIES FOUND	
	1998/99 SEASON	1999/2000 SEASON
NYANKPALA	*T. inopinus* *M. kieferi* *M. aspericornis*	*T. inopinus* *T. neglectus* *M. kieferi* *M. aspericornis*
DIARE	*T. incisus* *T. oblongatus* *M. kieferi*	*T. incisus* *T. oblongatus* *M. kieferi*
SAVELUGU	*T. incisus* *M. kieferi*	*T. incisus* *M. kieferi* *T. oblongatus*
KUKUO	*T. inopinus,* *T. incisus,* *M. kieferi* *M. aspericornis*	*T. inopinus,* *T. incisus,* *M. kieferi*
NANUMBA	*T. inopinus,* *M. major* *M. kieferi*	*T. inopinus,* *M. major* *M. kieferi* *M. aspericornis*

TABLE 3: INFECTIVITY STUDIES WITH ADULT COPEPODS

Copepod Species (Adult stages)	Ingested L_1		Maximum Number of L_1 Ingested				Larval Development ($L_1 \rightarrow L_3$)	
	YES	NO	1	2	3	4+	YES	NO
M. aspericornis	✓					✓	✓	
M. kieferi	✓					✓	✓	
T. incisus	✓				✓		✓	
T. oblongatus	✓			✓			✓	
(Copepodids)	**Ingested L_1**		**Maximum Number of L_1 Ingested**				**Larval Development ($L_1 \rightarrow L_3$)**	
	YES	NO	1	2	3	4+	YES	NO
M. aspericornis	✓			✓			✓	
M. kieferi	✓				✓		✓	
T. incisus	✓			✓			✓	
T. oblongatus	✓			✓			✓	

(Adult stages)	Percentage Ingesting "n" Number of Larvae				Percentage mortality within 12 hrs of ingesting "n" larvae			
	1	2	3	4+	1	2	3	4+
M. aspericornis	2	17	76	5	8	64	82	88
M. kieferi	5	20	68	7	12	70	82	94
T. incisus	44	53	3	0	0	2	2	4
T. oblongatus	40	60	0	0	0	0	0	0
(Copepodids)	**Percentage ingesting "n" Number of Larvae**				**Percentage mortality within 12 hrs of ingesting "n" larvae**			
	1	2	3	4+	1	2	3	4+
M. aspericornis	88	12	0	0	0	0	0	0
M. kieferi	91	9	0	0	0	0	0	0
T. incisus	95	5	0	0	0	0	0	0
T. oblongatus	100	0	0	0	0	0	0	0

FIGURES

FIGURE 1: LIFE CYCLE OF *DRACUNCULUS MEDINENSIS*

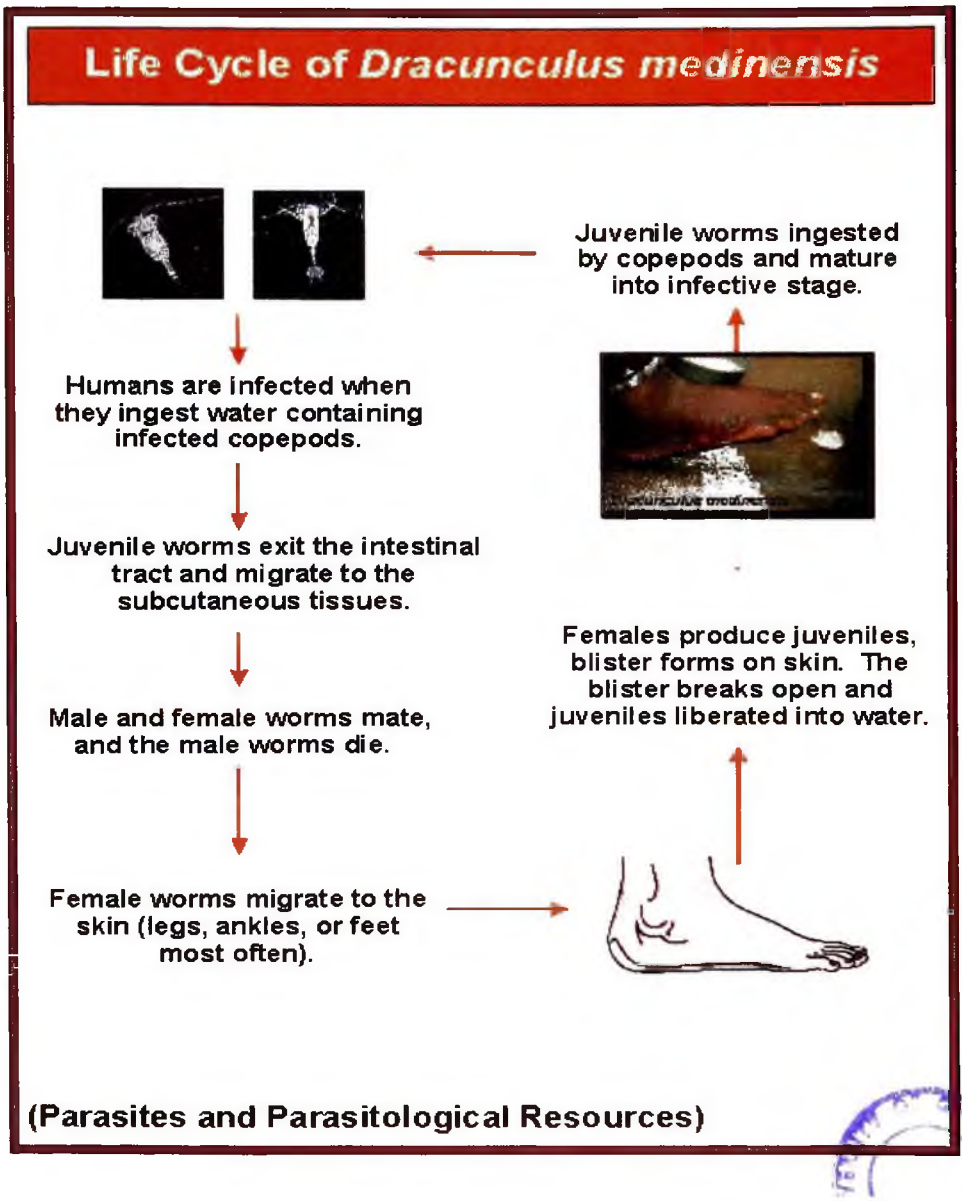


FIGURE 2: A COPEPOD

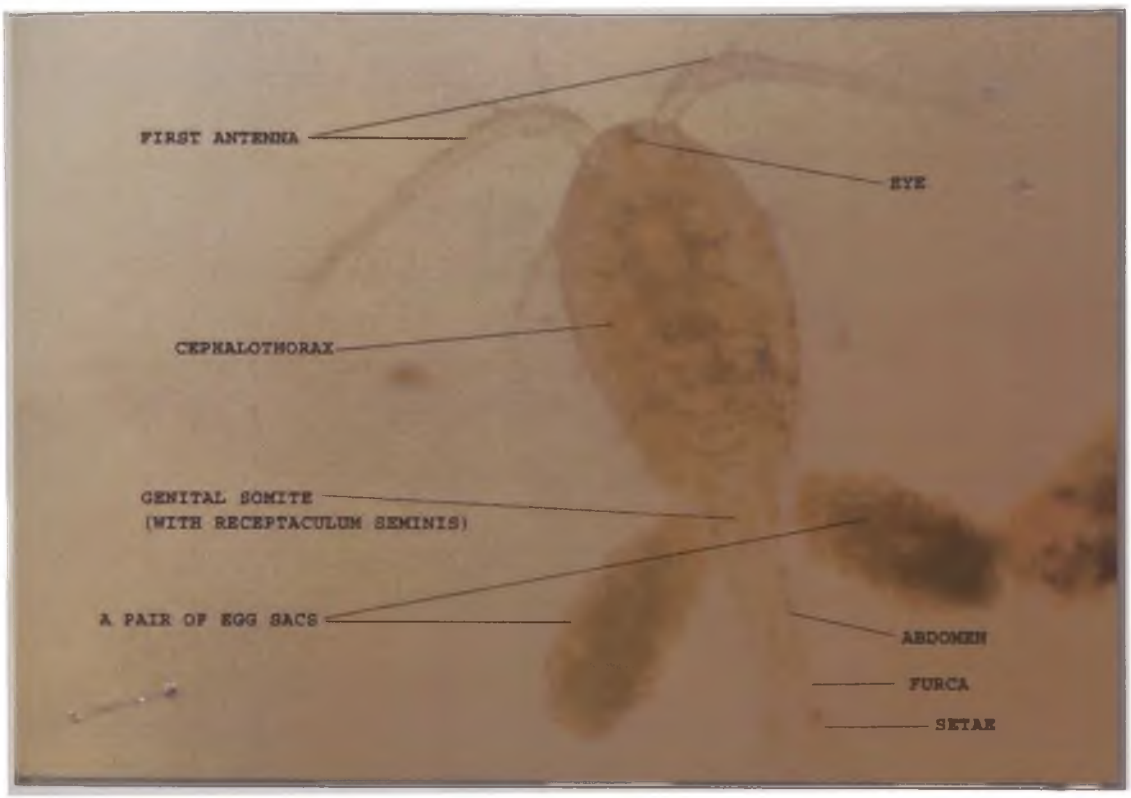


FIGURE 3: DISTRICT MAP OF GHANA

FIGURE 4: MONTHLY DISTRIBUTION OF GUINEA WORM DISEASE

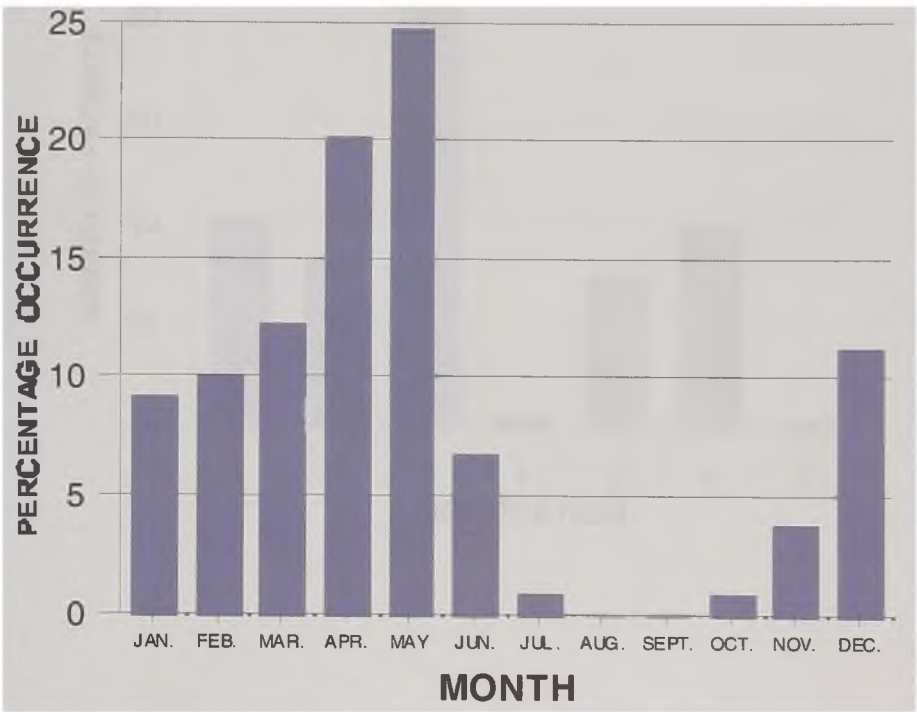
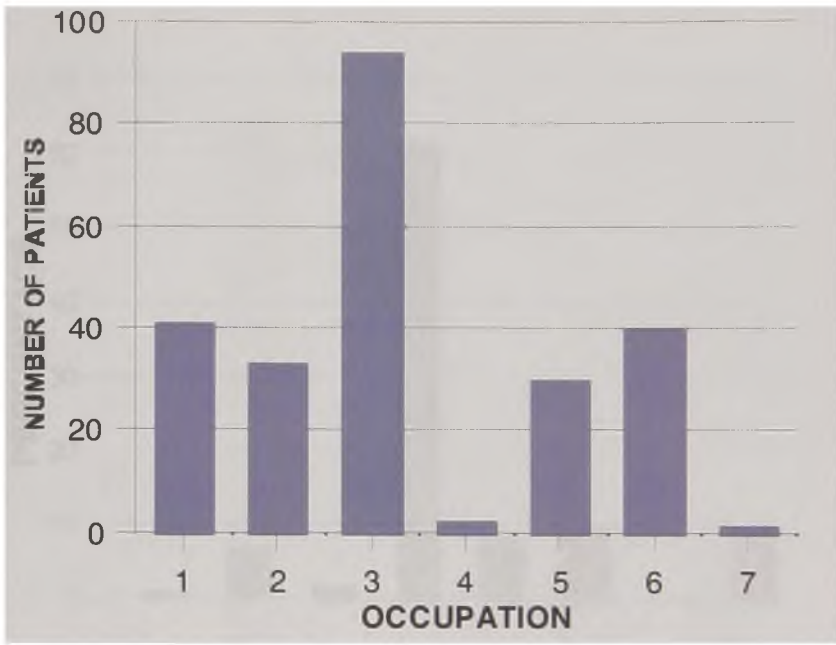


FIGURE 5: OCCUPATIONAL DISTRIBUTION OF PERSONS WITH DRACUNCULIASIS

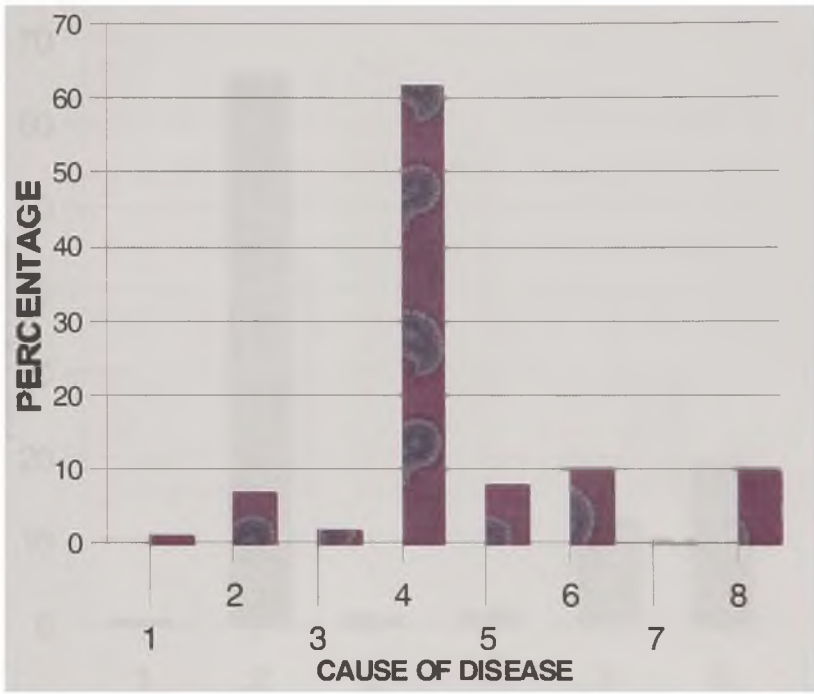


LEGEND:

1 = Pupil; 2 = Student; 3 = Farmer; 4 = Fisherman;

5 = Trader; 6 = Housewife; 7 = Others.

FIGURE 6: PERCEPTIONS OF THE CAUSES OF GUINEA WORM

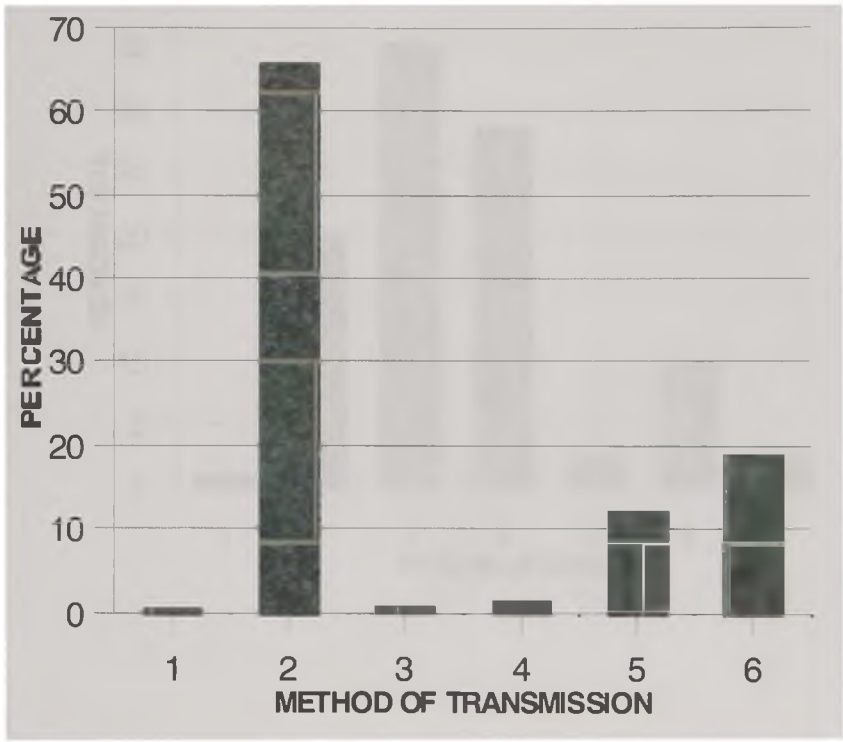


LEGEND:

1 = Walking bare footed; 2 = Wading in pond/dam; 3 = Witches;

4 = Pond water; 5 = Juju; 6 = God/Natural; 7 = Others; 8 = Don't know

FIGURE 7: PERCEPTIONS OF THE METHOD OF TRANSMISSION OF GUINEA WORM

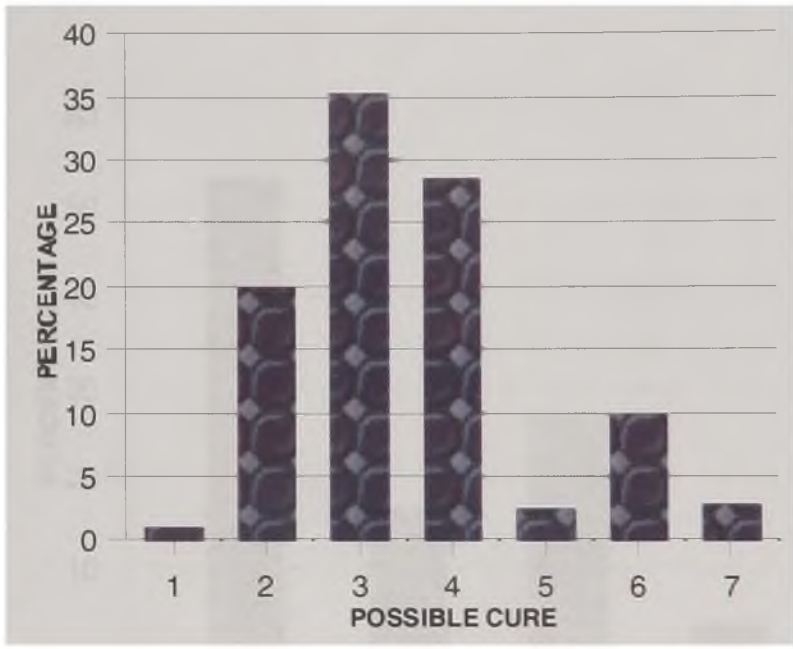


LEGEND:

1 = Eating with others; 2 = Entry of pond by infected person;

3 = Flies; 4 = Mosquitoes; 5 = Other; 6 = Don't know

FIGURE 8: PERCEPTIONS ON HOW TO CURE INFECTION

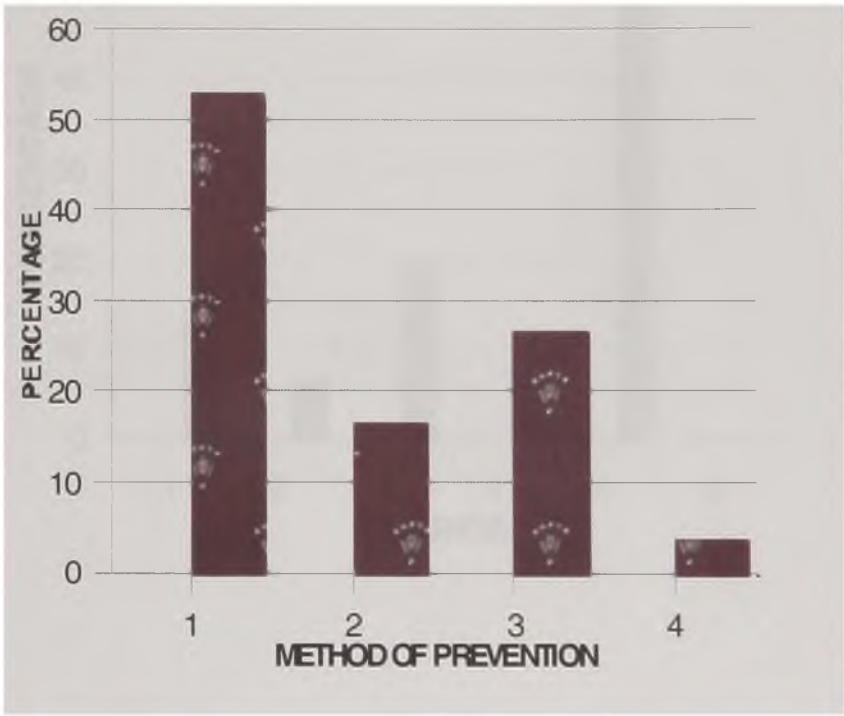


LEGEND:

1 = Ambilhar; 2 = Paracetamol; 3 = Tamale oil;

4 = Herbs; 5 = Juju-man; 6 = Other; 7 = Don't know

FIGURE 9: PERCEPTIONS ON DISEASE PREVENTION

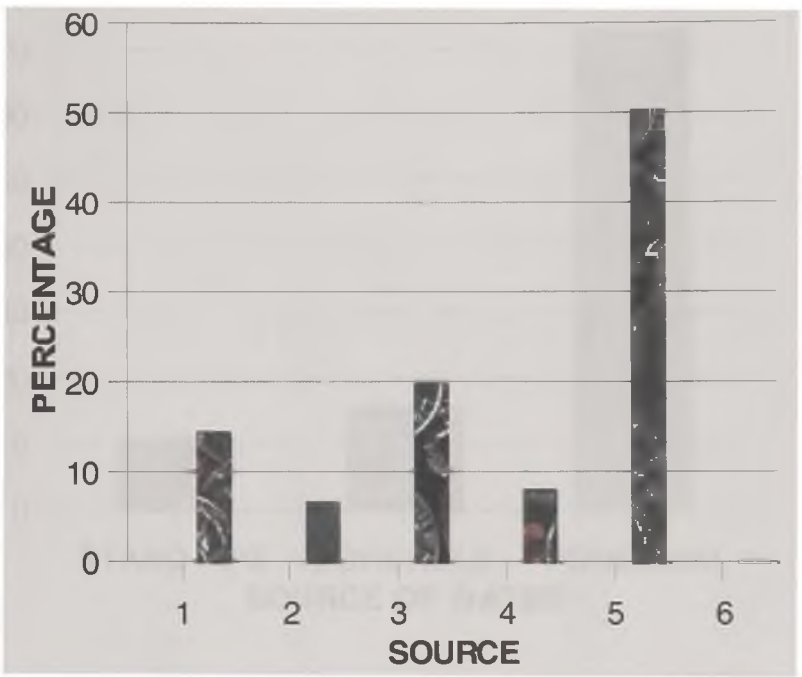


LEGEND:

1 =Filtering all pond water before drinking; 2 Drinking borehole water only;

3 = Boiling water before drinking; 4 = Regularly using Abate in ponds

FIGURE 10: SOURCES OF KNOWLEDGE ON GUINEA WORM PREVENTION



LEGEND:

1 = Relatives; 2 = Friends; 3 = Health workers; 4 = Teachers;

5 = Guinea worm Workers; 6 = Pastor/Imam

FIGURE 11: PRINCIPAL SOURCES OF WATER

FIGURE 12: ALTERNATIVE SOURCES OF WATER TO PONDS

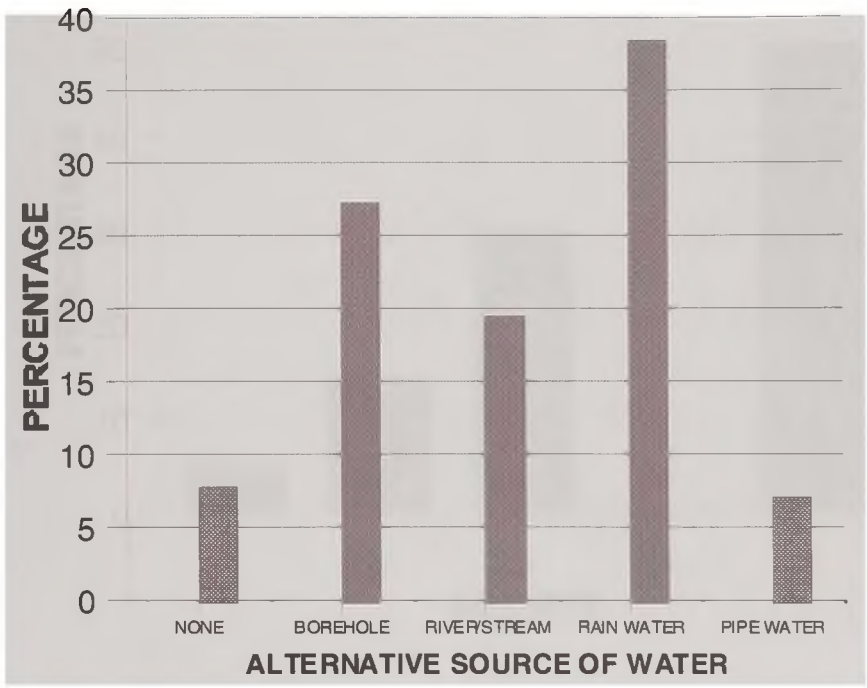
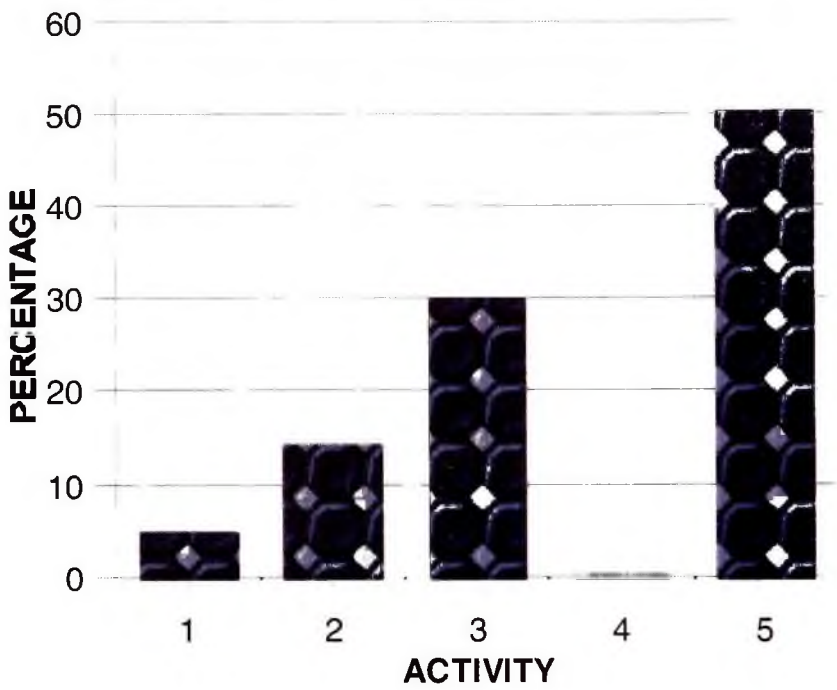


FIGURE 13: COMMON ACTIVITIES AT POND SITE



LEGEND:

1 = Swimming; 2 = Bathing; 3 = Washing clothing;

4 =Urination; 5 = Wading to fetch water.

FIGURES 14 – 21: T/S OF FEMALE *DRACUNCULUS MEDINENSIS*

FIGURE 14: CEPHALIC REGION OF GUINEA WORM

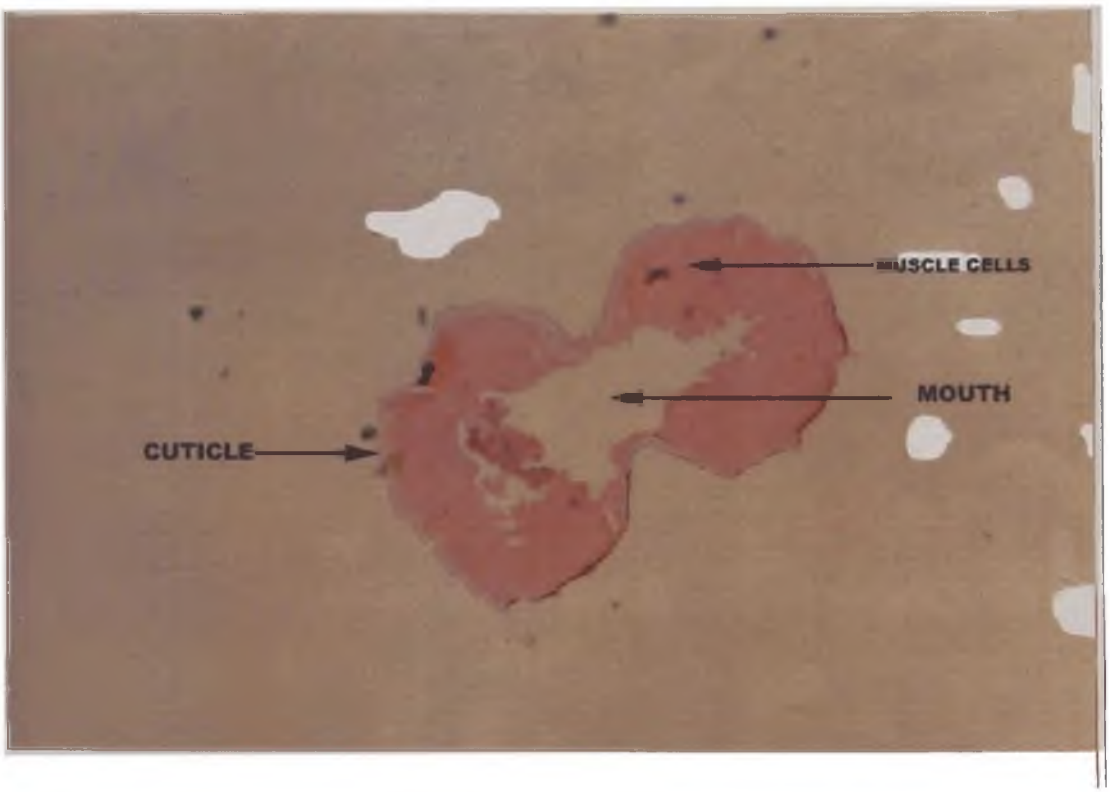


FIGURE 15: THE PHARYNGEAL REGION

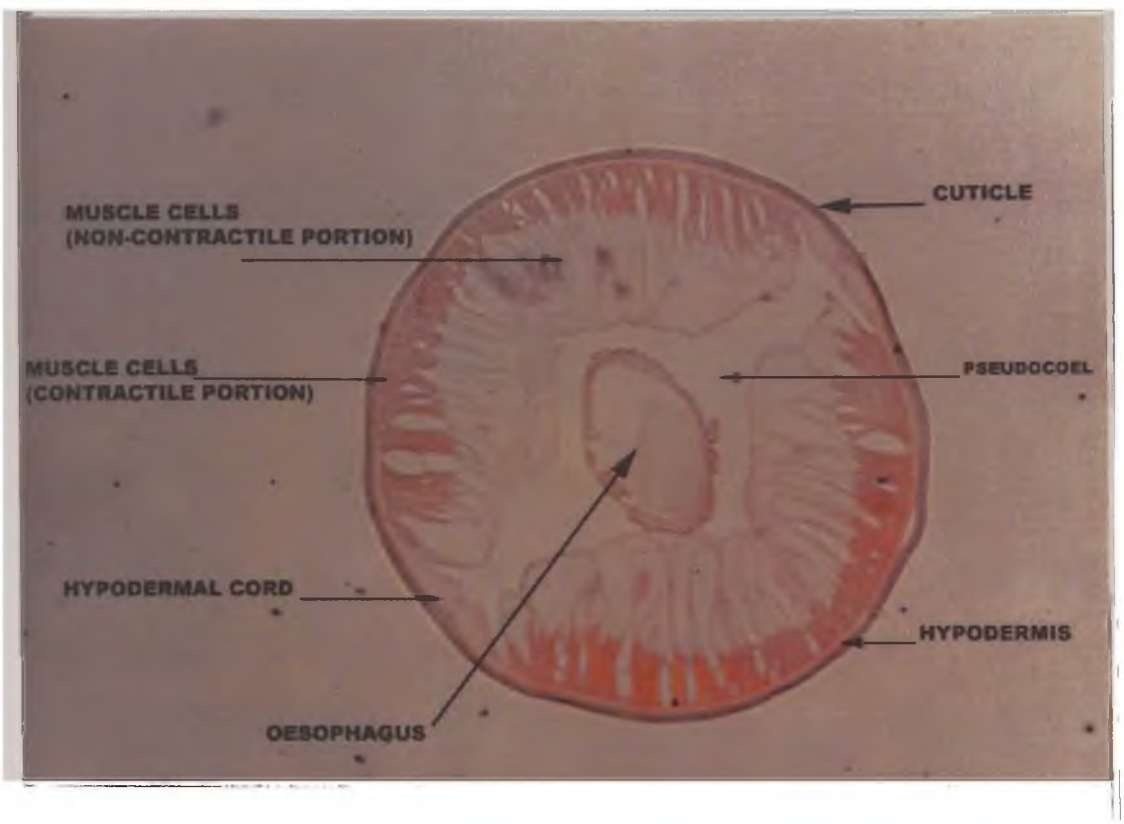


FIGURE 16: ENLARGENED VERSIONS OF:

1. The Muscle Cells
2. Hypodermal Cord
3. Cell Nucleus
4. Pseudocoel

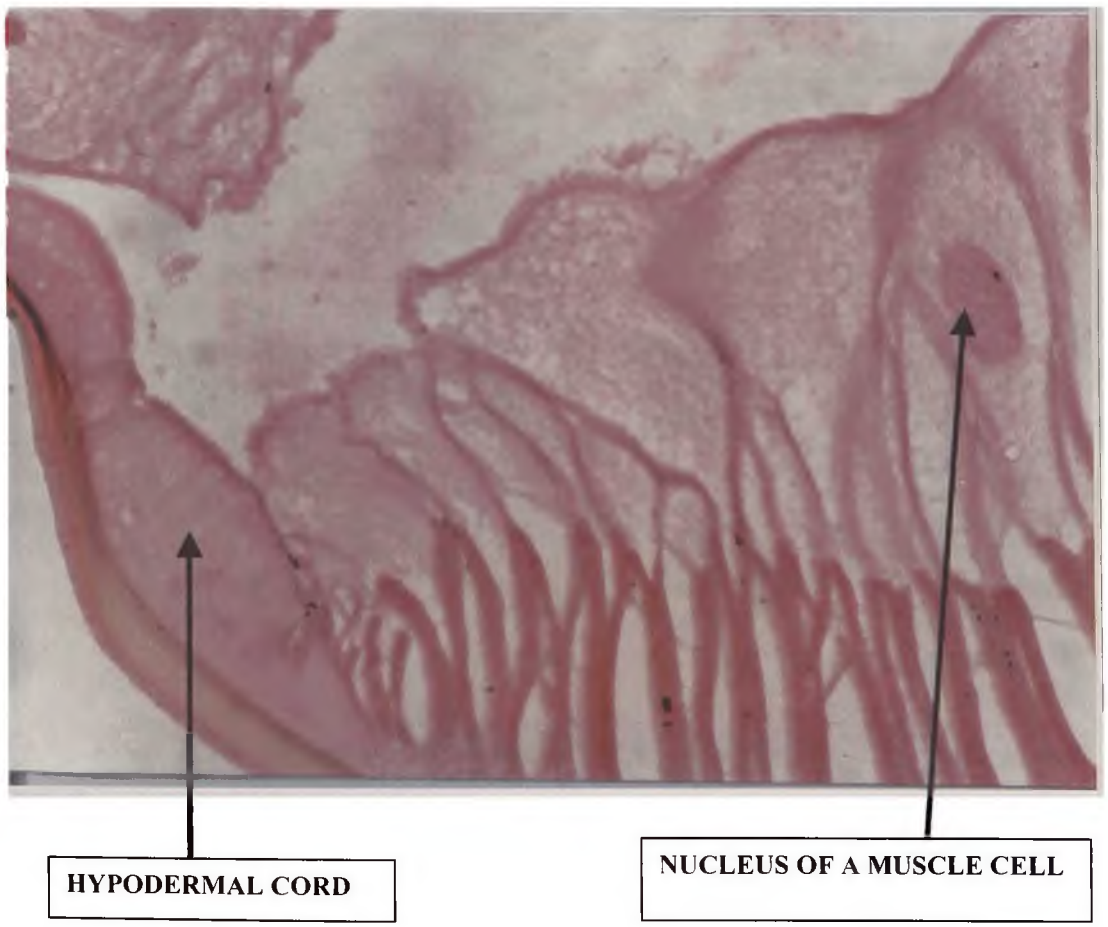
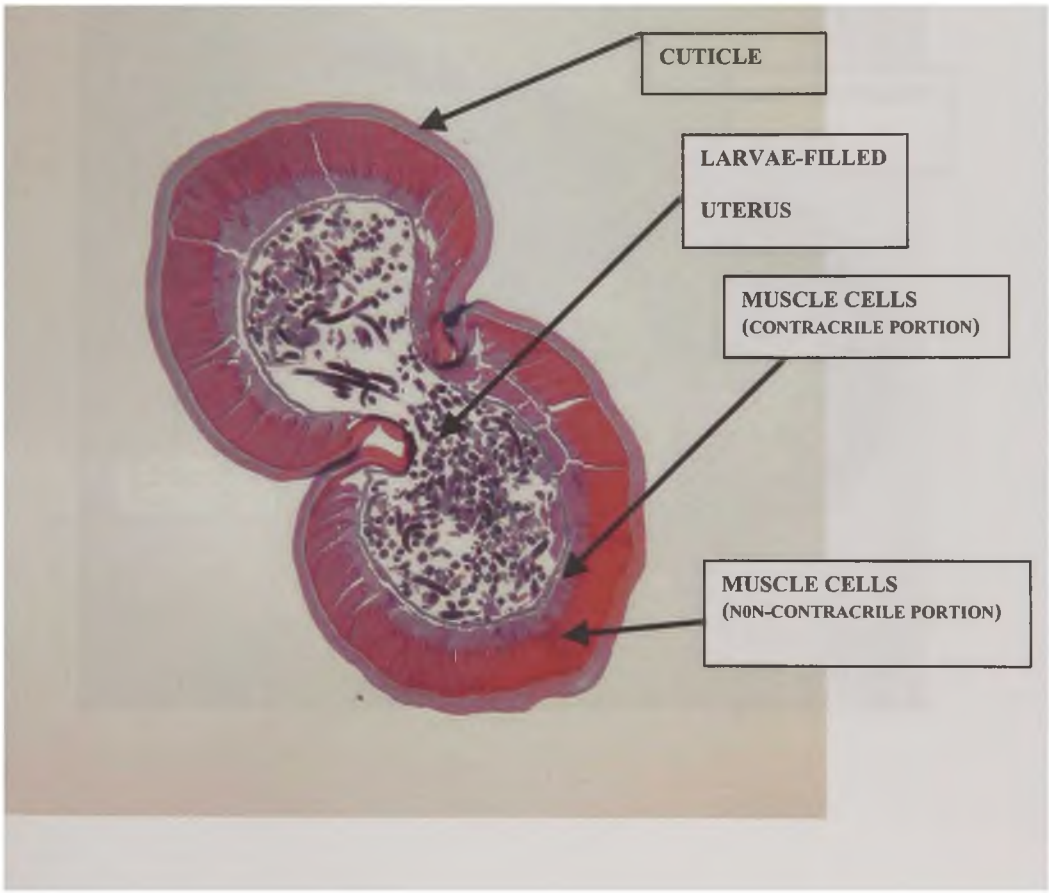


FIGURE 17: MID-ANTERIOR REGION



Note the relatively thicker musculature

FIGURE 18: MID-SECTION

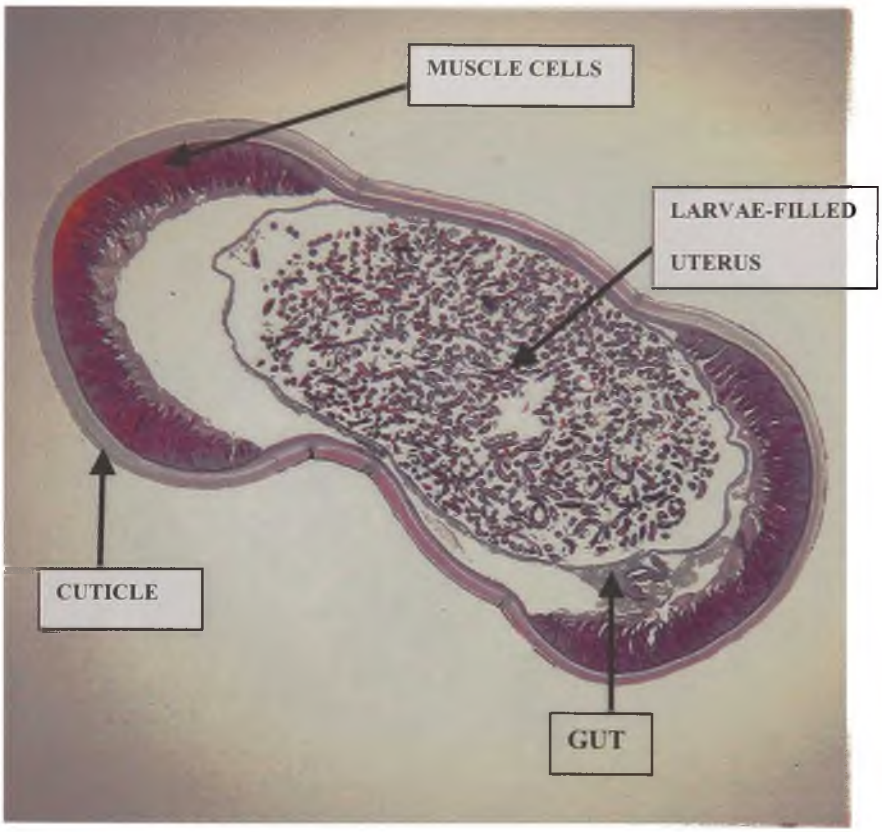


FIGURE 19: MID-SECTION PRESENTING THE MUSCLATURE

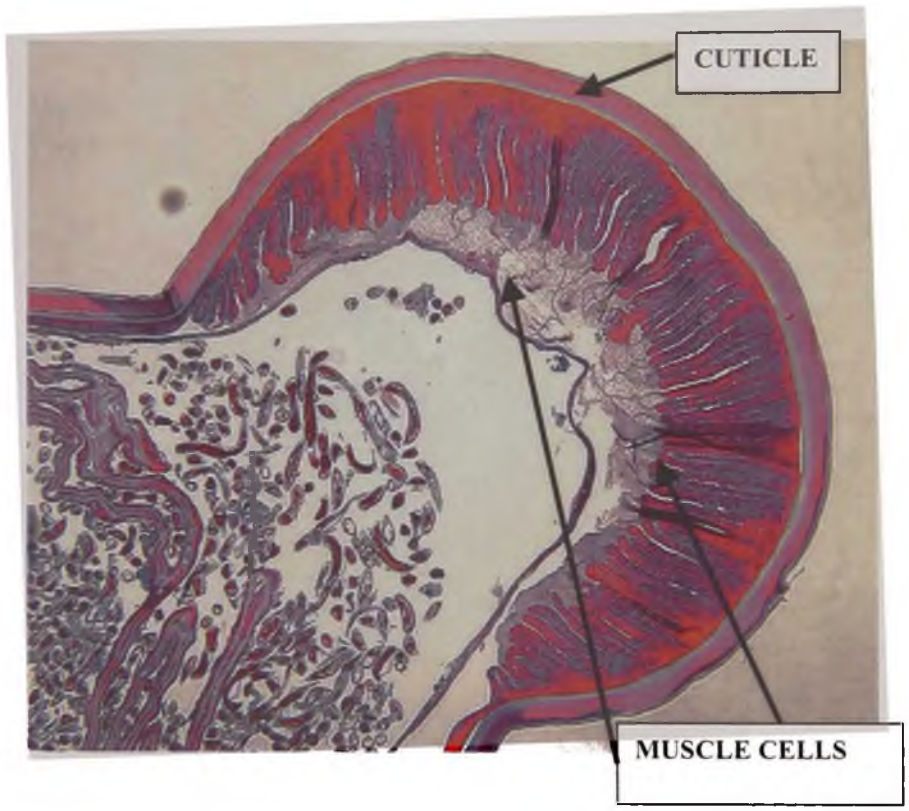


FIGURE 20: LOWER MID-SECTION

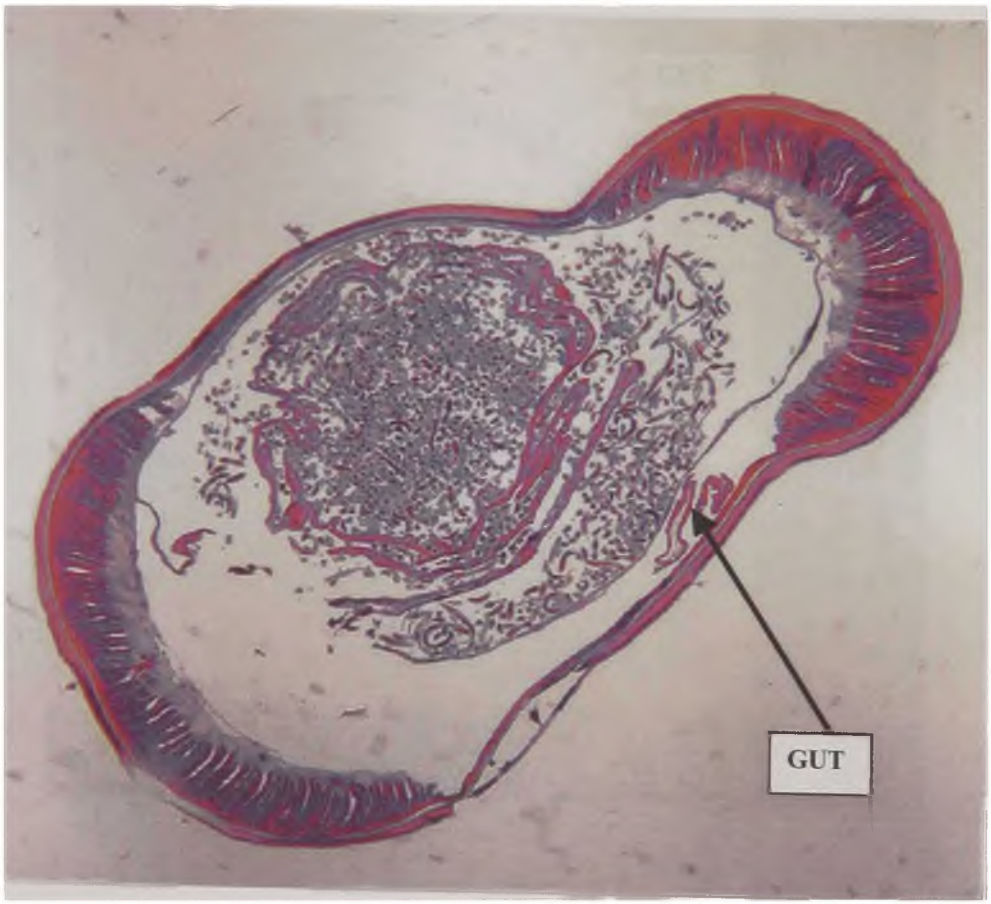


FIGURE 21: THE EXTREME POSTERIOR REGION OF GUINEA WORM

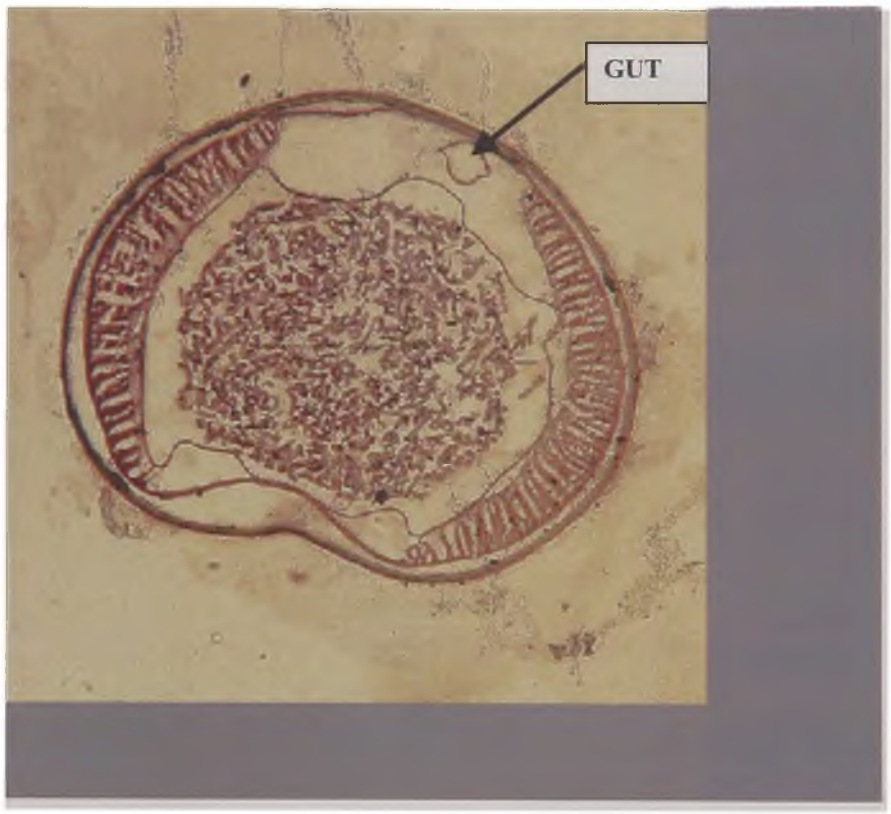
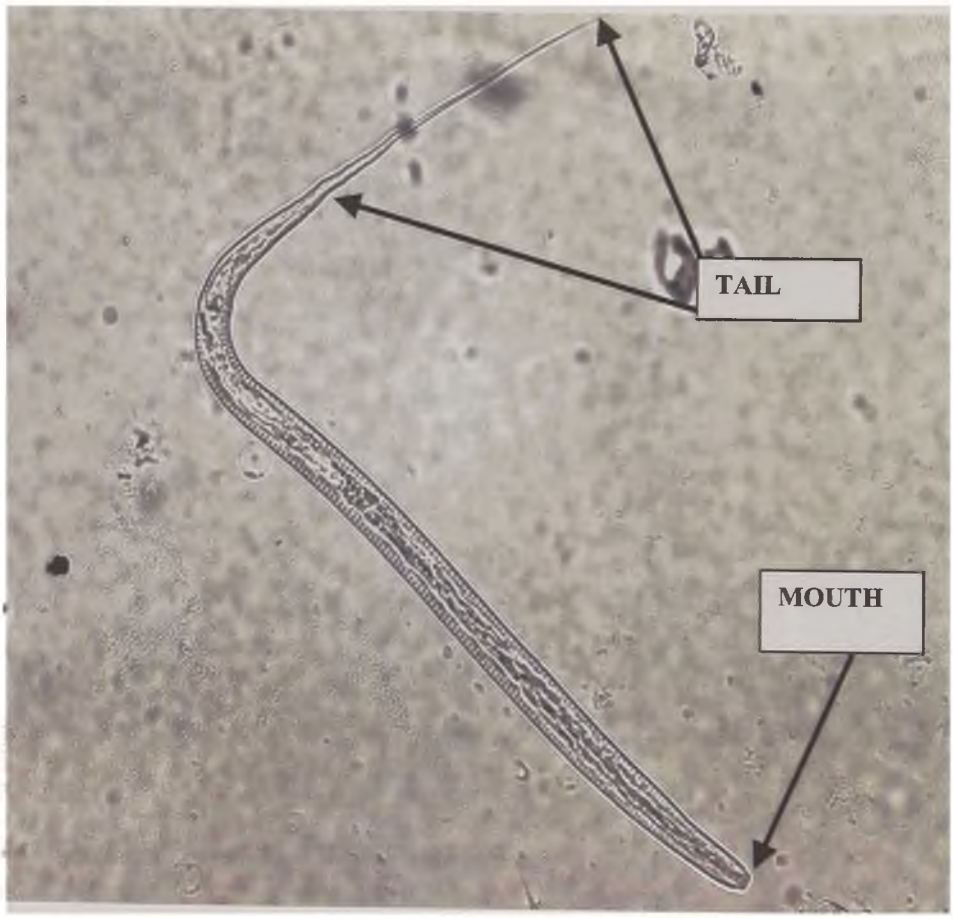


FIGURE 22:

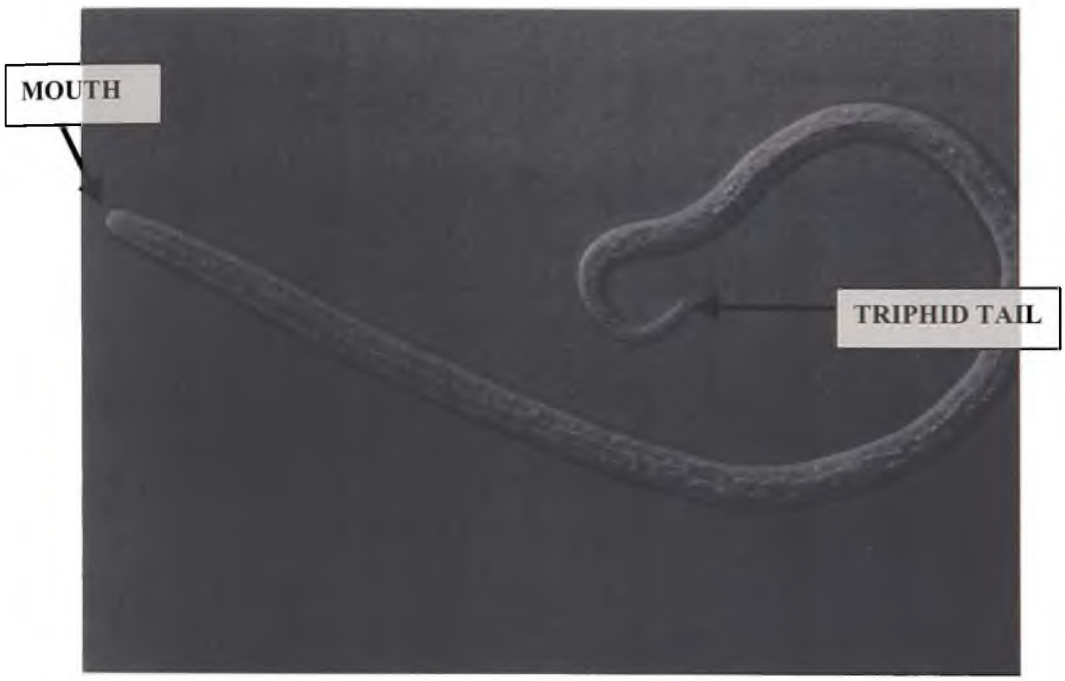
FIRST STAGE LARVA (L_1)



Note the rough cuticle.

FIGURE 23:

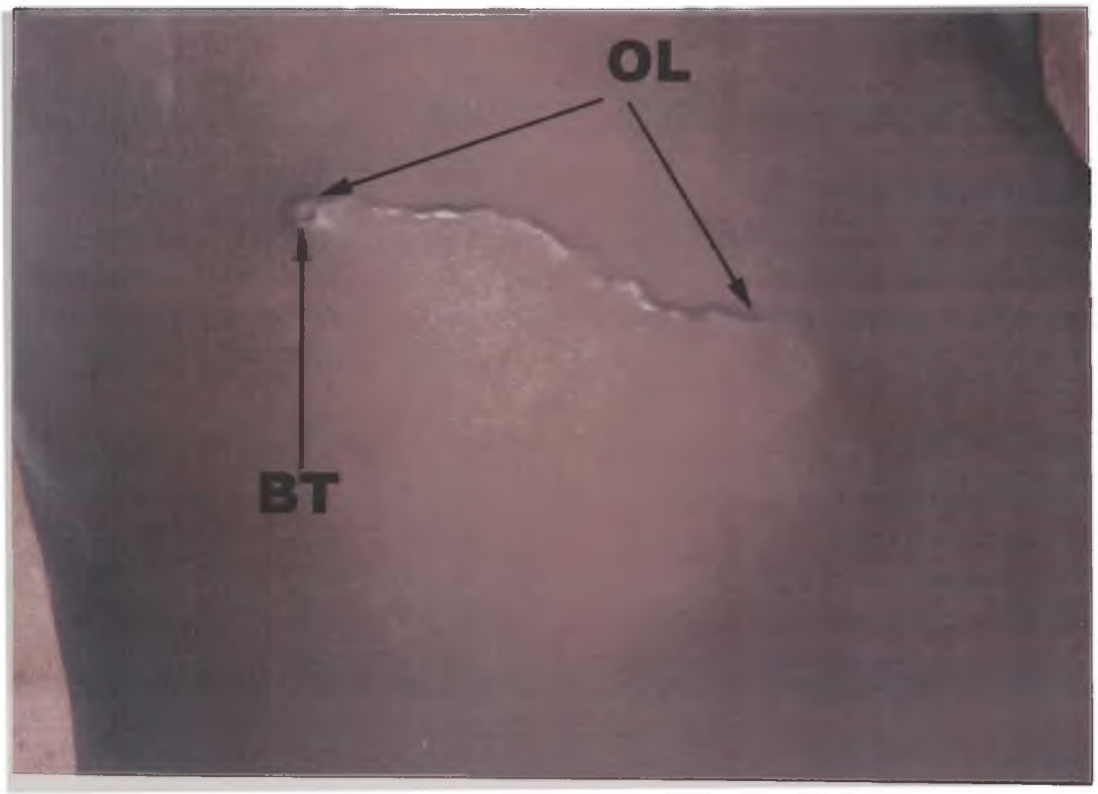
THIRD STAGE LARVA (L_3)



Note the rather smooth cuticle after molting.

FIGURES 24 - 28: CLINICAL PRESENTATIONS OF DRACUNCULIASIS

FIGURE 24: A GUINEA WORM ON THE CHEST OF THE PATIENT



LEGEND:

BT = Point of blister formation

OL = Outline of Guinea worm on a patient

FIGURE 25:
GUINEA WORM BEING EXTRACTED FROM THE LEFT FOOT OF A PATIENT

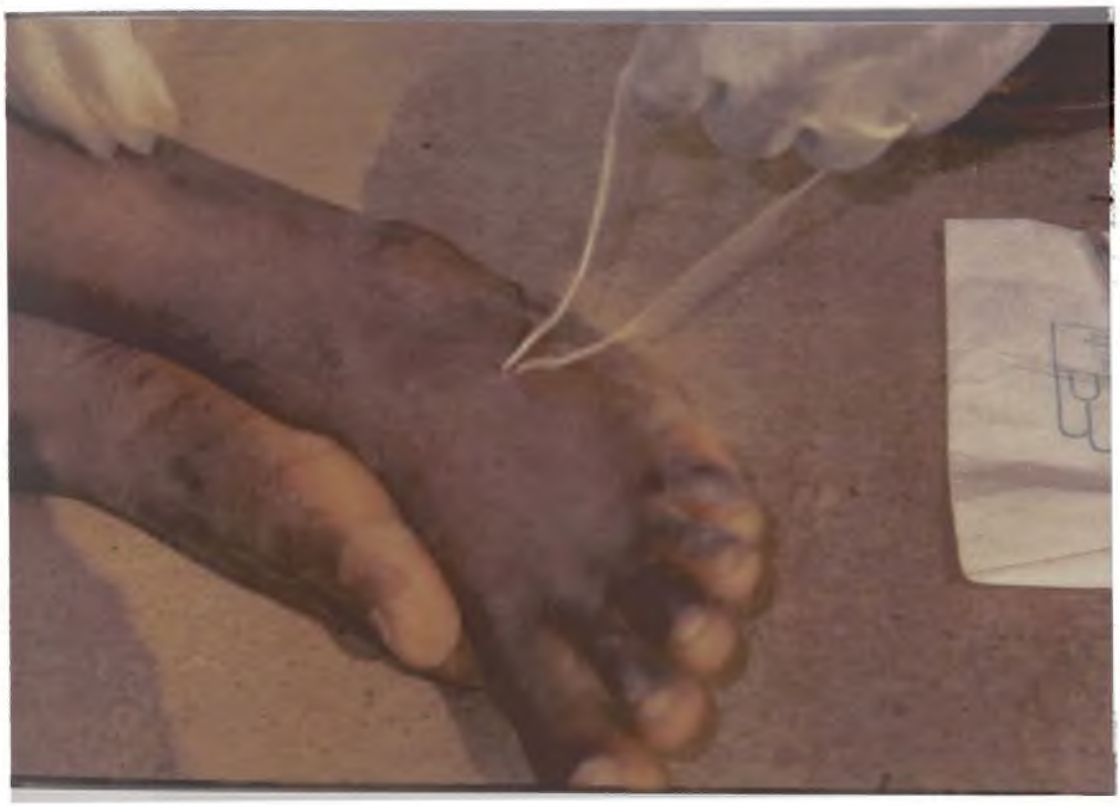


FIGURE 26:

GUINEA WORM BEING EXTRACTED FROM THE THORACIC REGION OF A PATIENT

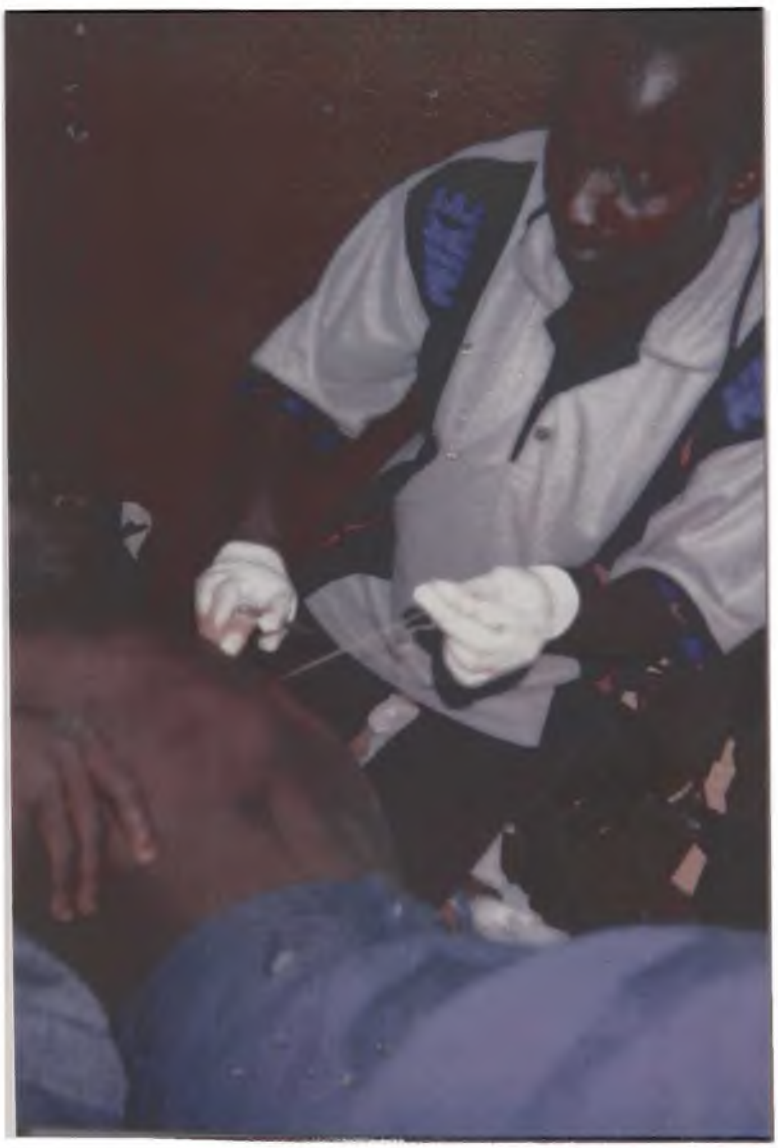


FIGURE 27:

GUINEA WORM BEING EXTRACTED FROM THE RIGHT HAND OF A PATIENT

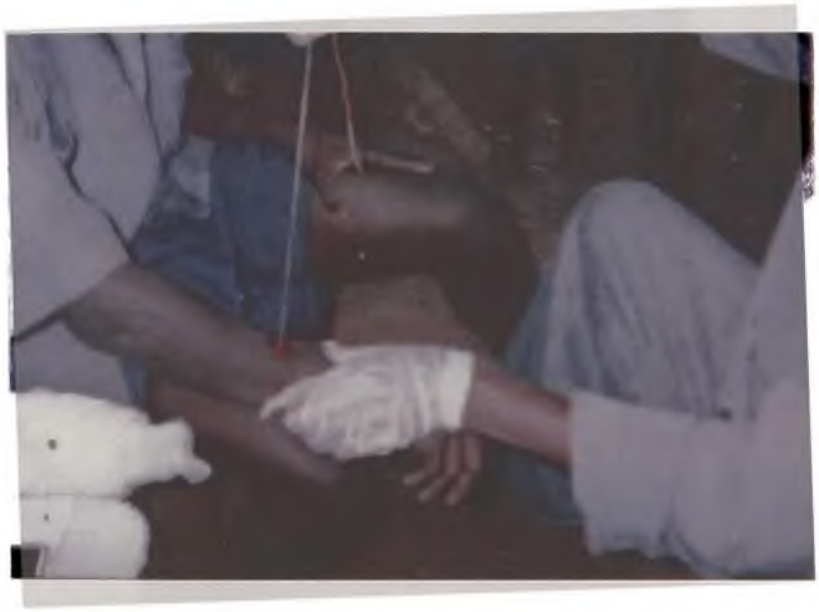
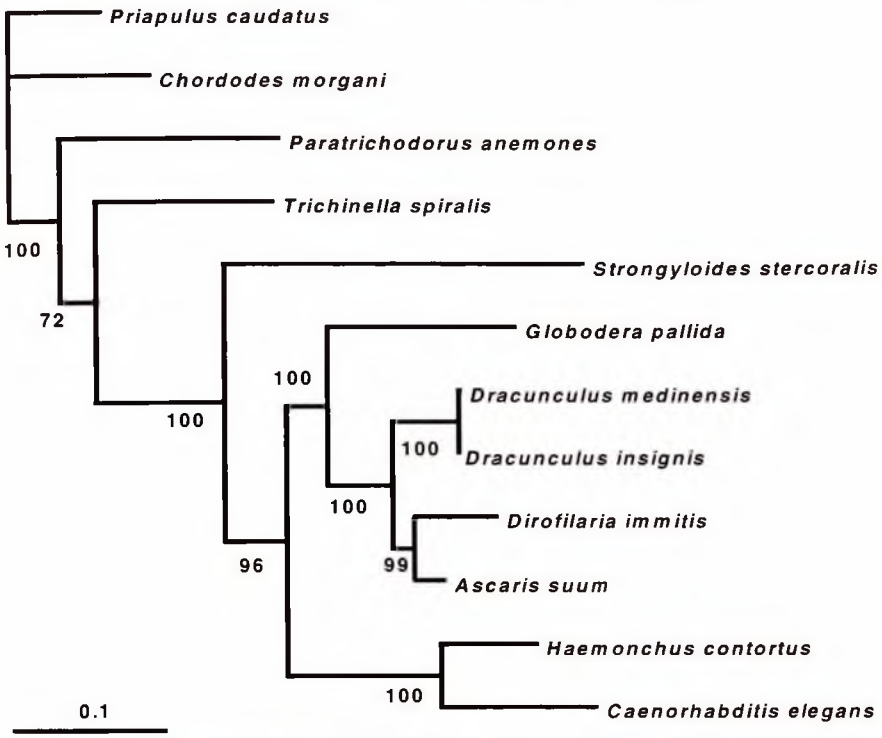


FIGURE 28:

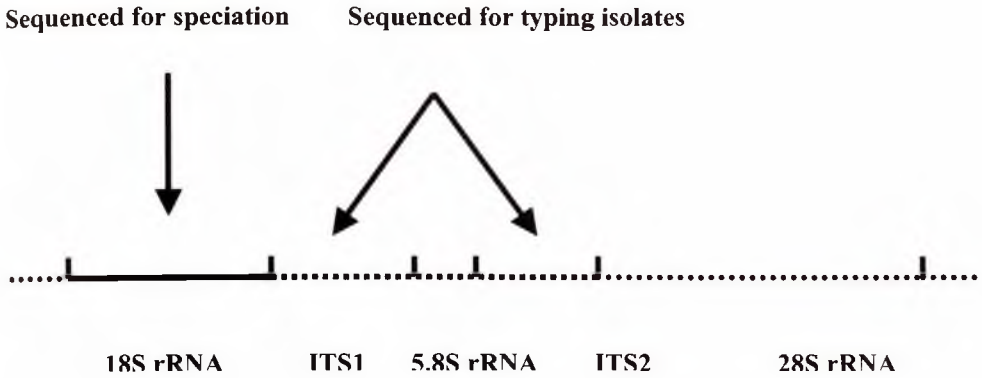
GUINEA WORM BEING EXTRACTED FROM THE PELVIC REGION OF A PATIENT

FIGURE 29:
Phylogenetic tree for small subunit ribosomal RNA sequences of *Dracunculus medinensis*, *D. insignis*, and selected *Nematoda*.



Quartets puzzling maximum likelihood results are shown using *Priapulus caudatus* and *Chordodes morgani* as outgroups. Numbers to the left of the nodes indicate the quartet puzzling support for each internal branch. Scale bar indicates an evolutionary distance of 0.1 nucleotides per position in the sequence. Vertical distances are for clarity only. GenBank accession numbers of the sequences used for analysis: *Ascaris suum* AF036587, *Caenorhabditis elegans* L04153, *Chordodes morgani* AF036639, *Dirofilaria immitis* AF036638, *Globodera pallida* AF036592, *Haemonchus contortus* L04153, *Paratrichodorus anemones* AF036600, *Priapulus caudatus* X87984, *Strongyloides stercoralis* M84229, and *Trichinella spiralis* U60231. The *Dracunculus* sequences were not yet submitted to GenBank. Both *Dracunculus* species form a sister group to a group formed by *Ascaris* and *Dirofilaria*.

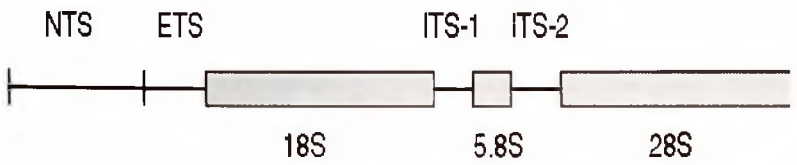
FIGURE 30A: **The map of the rRNA cluster**
[Showing the different parts of the genes that codes for ribosomal DNA (rDNA)]



SSU-rRNA (or 18S rRNA) – ITS1- 5.8S rRNA – ITS2 – LSU-rRNA (or 28S rRNA).

Figure 30b: Diagram of the ribosomal DNA gene.

The regions coding for the 5.8S, 18S, and 28S subunits of rRNA are shown by bars; NTS = non-transcribed spacer, ETS = external transcribed spacer, ITS = internal transcribed spacer regions.



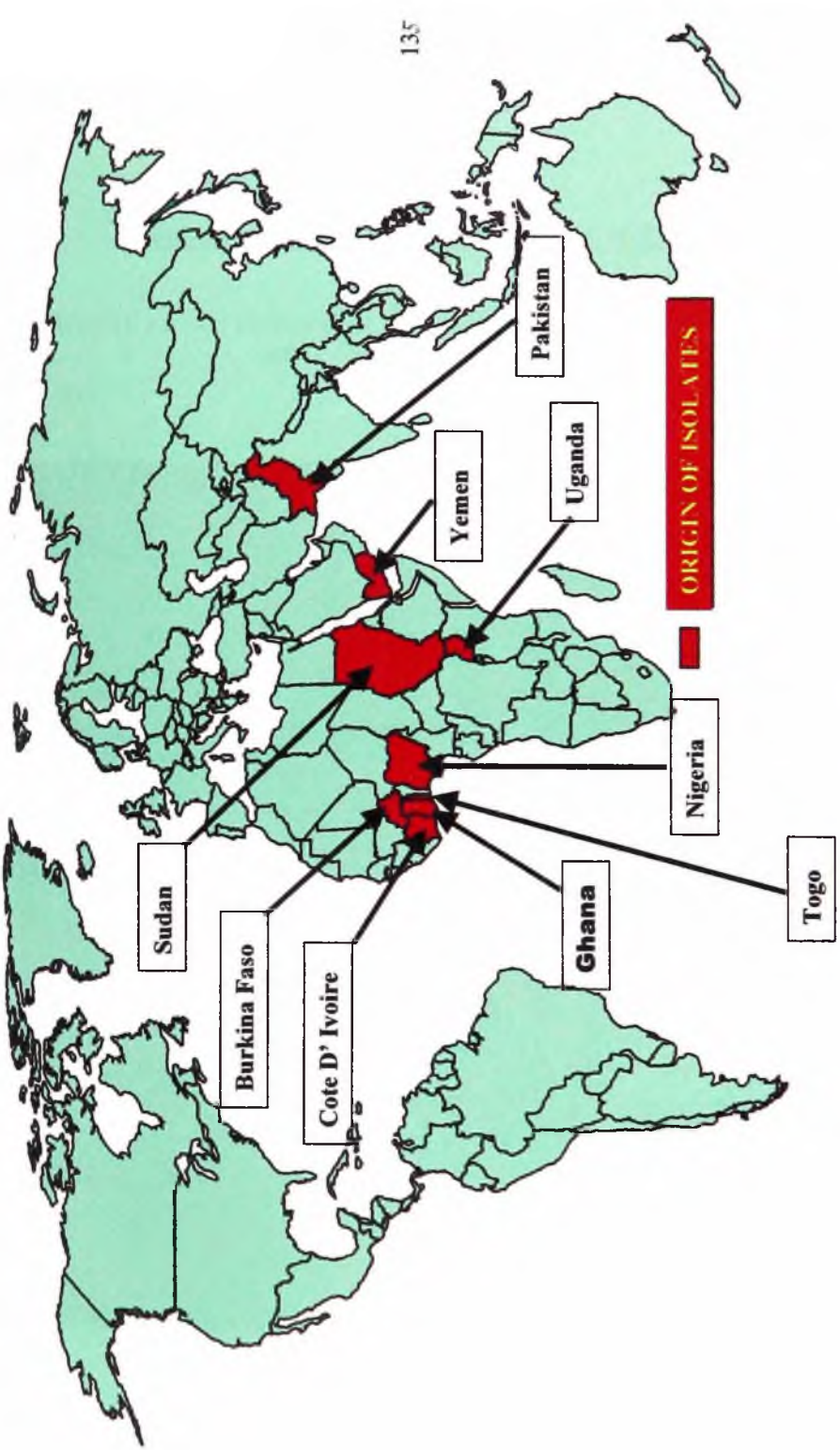


FIGURE 31: MAP OF THE WORLD

FIGURES 32 – 34: VERIFICATION OF PCR PRODUCTS

FIGURE 32: AN 1819bp PCR PRODUCT OF THE SSU rRNA (18S) OF *DRACUNCULUS MEDINENSIS*

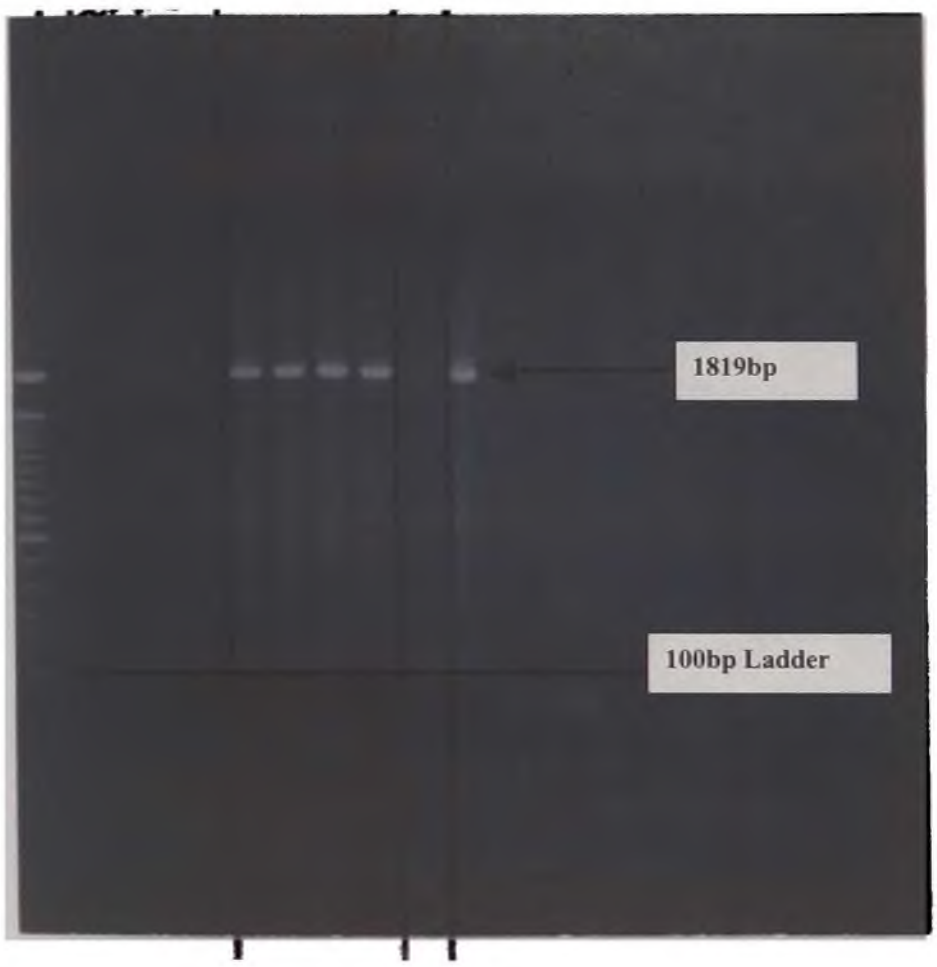


FIGURE 33: AN 1165bp PCR PRODUCT OF THE ITS1 rRNA OF *DRACUNCULUS MEDINENSIS*

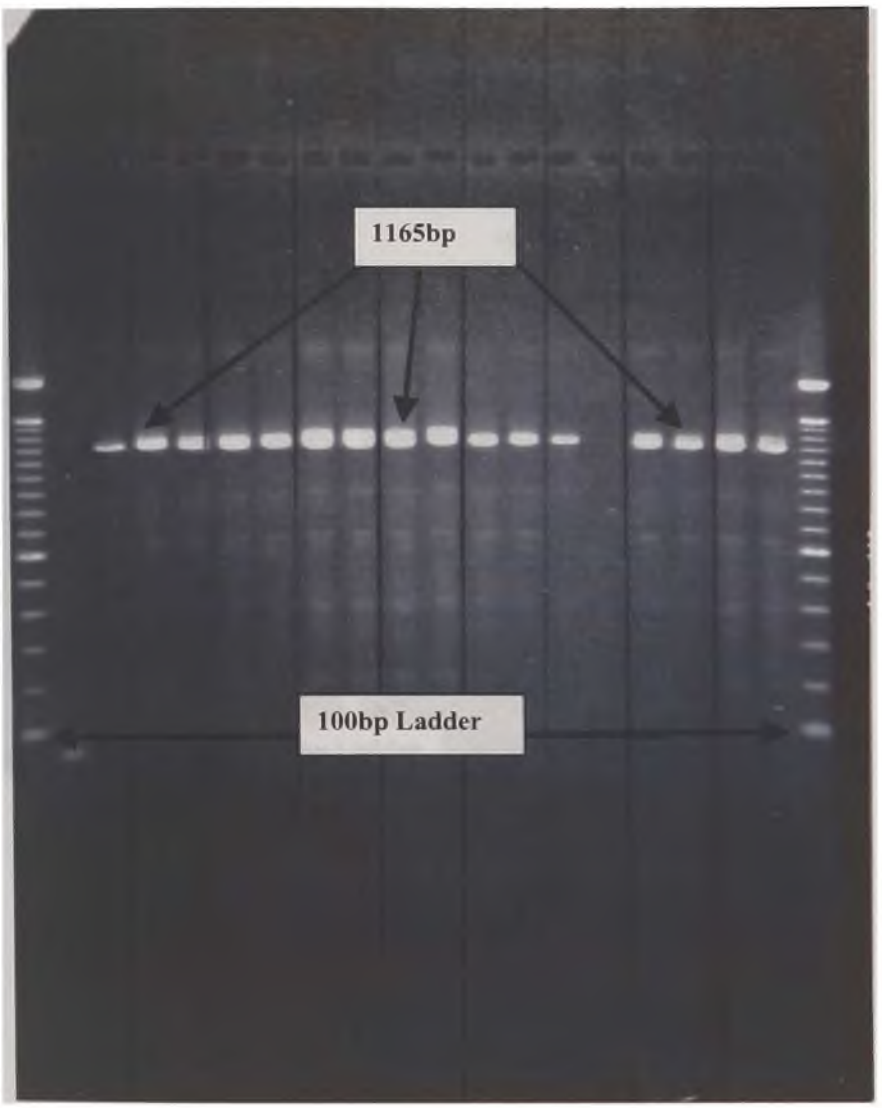
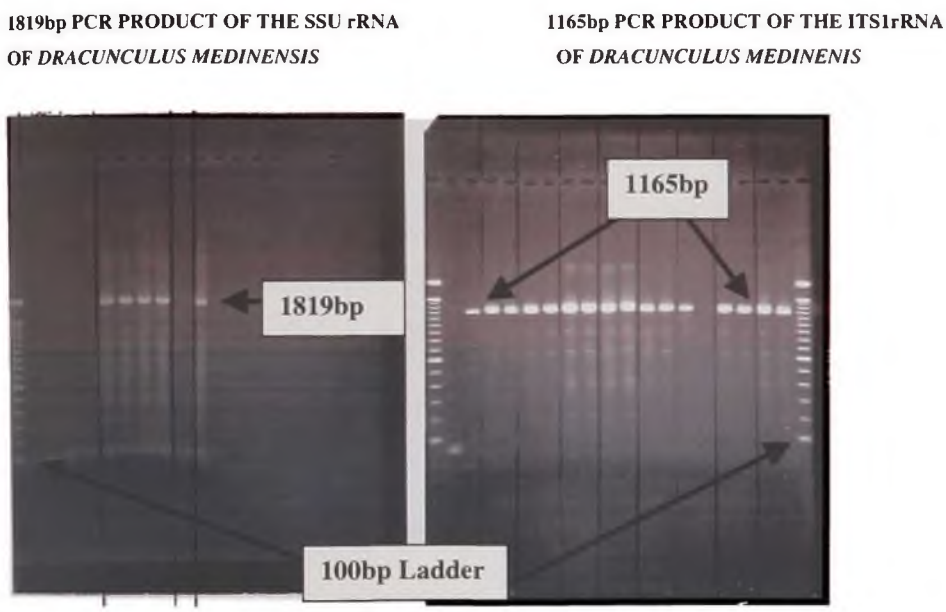


FIGURE 34:

RELATIVE SIZES OF AN 1819bp PCR PRODUCT OF THE SSU rRNA (18S) AND AN 1165bp PCR PRODUCT OF THE ITS1 rRNA OF *DRACUNCULUS MEDINENSIS*



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APPENDIX A

QUESTIONNAIRE FOR GUINEA WORM DISEASE (DRACUNCULIASIS) SURVEY

DISTRICT: .....

SECTION A: BACKGROUND INFORMATION

Q1. **Town/Village:** .....

Q2. **Sex:** 1. Male ☐ 2. Female ☐

Q3. **Age:** 1. 0 - 9 ☐ 2. 10 - 29 ☐ 3. 30 - 49 ☐ 4. 50 -69 ☐ 5. 70+ ☐

Q4. **Educational Background**:

1. None ☐ 2. Prim./J. S.S. ☐ 3. S..S.S. ☐ 4. College ☐ 5. Polytechnic ☐ 6. University ☐

Q5. **Occupation:**

1. Pupil ☐ 2. Student ☐ 3. Farmer ☐ 4. Fisherman ☐ 5. Trader ☐ 6. Housewife ☐
2. 7. Other (specify) .....

SECTION B: KNOWLEDGE BASE

Q6. Have you ever suffered from the Guinea worm disease before? 1. Yes ☐ 2. No ☐

IF YES TO Q6, Go to Q7,

IF NO TO Q6, Go to Q12

Q7. When was this? 1. This year only ☐ 2. Last year only ☐ 3. Last year and this year ☐

Q8. How many times? 1. Once ☐ 2. Twice ☐ 3. Three times ☐ 4. >Three times ☐

Q9. On which part(s) of your body did the worm(s) emerge?

1.Hand ☐ 2. Abdomen ☐ 3. Pelvic region/waist ☐ 4. Thoracic region/chest ☐

5. Thigh ☐ 6. Knee ☐ 7. Foot ☐ 8. Ankle ☐ 9. Other (specify) .....

Q10. During which month(s) did you get the disease?

1. Jan. ☐ 2. Feb. ☐ 3. March. ☐ 4. April ☐ 5. May. ☐ 6. Jun. ☐

7. Jul. ☐ 8. Aug. ☐ 9. Sept. ☐ 10. Oct. ☐ 11. Nov. ☐ 12. Dec. ☐

Q11. How long did it take for the worm(s) to completely come out?

1. One - two weeks ☐ 2. Three - four weeks ☐ 3. Two months ☐ 4. > Two months ☐

IF NO TO Q6,

Q12. Have you ever heard of the disease before? 1. Yes ☐ 2. No ☐

Q13. ***If yes to the above question***, indicate source (i.e, from whom did you hear this)?

1. Mother ☐ 2. Father ☐ 3. Friend(s) ☐ 4. Other family members ☐ 5. Teacher(s) ☐

6. Guinea Worm Workers ☐ 7. Pastor ☐ 8. Doctor/Nurse ☐ 9. Other (specify) .....

Q14. Has any member of your family had a Guinea worm disease before? 1. Yes ☐ 2. No ☐

Q15. ***If yes to the above question***, When was this?

1. This year ☐ 2. Last year ☐ 3. Last year and this year ☐

Q16. What do you think causes the guinea worm disease?

1. Walking bare footed ☐ 2. Wading in pond/dam/stream ☐ 3. Witches ☐

4. Drinking water from a pond/dam/stream ☐ 5. Juju ☐ 6. It is naturally in our bodies ☐ 7.

Other (specify) .....

Q17. A person who has Guinea worm may spread the disease by?

1. Eating with others ☐ 2. Washing the sore in a pond/stream/dam ☐ 3. Flies ☐

4. Mosquitoes ☐ 5. Other (specify) .....

Q18. Can guinea worm disease be cured? 1. Yes ☐ 2. No ☐

Q19. If yes to question 18, how can the disease be cured? By using

1. Ambilhar ☐ 2. Paracetamol ☐ 3. Tamale oil ☐ 4. Herbs 5. A Jujuman ☐

6. Other (specify) .....

Q20. **If yes to question 18,** How did you learn about this cure(s)?

1. Mother ☐ 2. Father ☐ 3. Friend(s) ☐ 4. Other family members ☐ 5. Teacher(s) ☐

6. Guinea Worm Workers ☐ 7. Pastor ☐ 8. Doctor/Nurse ☐ 9. Other (specify) .....

Q21. Can the disease be prevented? 1. Yes ☐ 2. No ☐

Q22. If yes to question 21, effective and affordable methods includes:-

1. Filtering all pond/stream/dam water before drinking ☐

2. Drinking Bore hole water ☐

3. By boiling water before drinking ☐

4. Abating ponds/dams ☐

Q23. How did you learn about this preventive measure(s)?

1. Mother ☐ 2. Father ☐ 3. Friend(s) ☐ 4. Other family members ☐ 5. Teacher(s) ☐

6. Guinea Worm Workers ☐ 7. Pastor ☐ 8. Doctor/Nurse ☐ 9. Other (specify) .....

Q24. If no to question 18, WHY? .....

SECTION C: HUMAN PRACTICES

Q25. Where do you get your drinking water from?

1. Stand pipe ☐ 2. Bore hole ☐ 3. A pond/dam/stream ☐ 4. Other (specify) .....

Q26. If pond/dam is not mentioned in Q25 above, do you use pond/dam water during any part of the year? 1. Yes ☐ 2. No ☐

Q27. If pond/dam mentioned in Q25 above, how much do you depend on the pond water? 1. 1. All year round ☐ 2. Only in the dry season ☐ 3. Only in the rainy season ☐

Q28. Which other source(s) of water do you use?

1. None ☐ 2. Bore hole ☐ 3. River/stream ☐ 4. Rain water ☐ 5. Pipe water ☐

Q29. Which of the following do you do in the water?

1. Swimming ☐ 2. Bathing ☐ 3. Washing clothes ☐ 4. Urinating ☐

5. Wading through to fetch water for domestic/farm use.

APPENDIX B

WORM MORPHOMETRY

WORM WEIGHT (g)	WORM LENGTH (cm)	WORM DIAMETER (mm)
1.217	62.7	1.35
0.677	37	1.2
0.397	45.2	0.75
1.1	65.1	1.23
1.049	53	1.35
0.549	37	0.88
0.437	37	1.15
1.8	77.8	1.961
1.55	69	1.425
1.675	55.2	1.44
0.831	63.4	1.25
1.159	70.8	1.475
1.246	73.3	1.375
1.021	50.6	1.65

APPENDIX C

DNA EXTRACTION FROM ANIMAL TISSUE

Bio 101 FastDNA Kit

a) **PULVERIZING OF TISSUE AND LYSING OF CELLS**

1. Wash tissue sample thoroughly in cold PBS to remove 70% EtOH..
2. Cut about 30–50mg (approximately 2-3cm) of worm and rehydrate in 1,000µl CLS-TC buffer for 18-24hrs at room temperature. Spin 1 minute at 10,000xg. Pipette off Buffer.
3. Add 500µl of CLS-TC buffer and homogenize with an electric homogenizer in a tube.
4. Add the resulting pulp of tissue to lysing matrix 4 (1/4" sphere + garnet + 1/4" cylinder
5. Add 850µl of CLS-TC buffer to the tube. Must leave a minimum air space of 250µl to allow for grinding action in FastPrep.
6. FastPrep at setting 5 for 20 seconds.
7. Spin 10 min at 10,000-x g (Jouan A-14 @ 10,700 rpm).
8. If intact tissue is seen, FastPrep again at setting 5 for 20 sec and spin again.

b) **BINDING OF DNA TO MATRIX, WASHING AND ELUTION**

9. Spin again in Micro-centrifuge for 5minutes at 14000-Xg to pellet protein and cell debris.
10. Transfer 600µl of supernatant to a clean tube containing 600ul of binding matrix.
11. Mix gently and incubate at room temperature for 5 min.
12. Spin 1 minute and discard supernatant.
13. Gently re-suspend pellet in 500µl of SEWS-M to wash DNA.
14. Transfer suspension to SPIN filter.

15. Spin 1 minute and discard contents of catch tube.
16. Spin again 1 minute to dry.
17. Transfer SPIN filter to a clean tube and add 100µl of DES to elute DNA.
18. Gently vortex to re-suspend and incubate at room temperature for 3 minutes.
19. Spin 1 minute.
20. DNA is in the flow through.
21. It may be necessary to perform QIAquick purification to remove PCR inhibitors. Use 5ul purified DNA/50ul PCR Reaction.

APPENDIX D

QIAquick-Spin: PCR Purification Kit

This protocol is designed for purification of single or double-stranded PCR products from primers, nucleotides and polymerases.

NOTE:

- **Add 220ml of ethanol (96-100%) to buffer PE before use.**
- **This protocol calls for the use of 2ml micro-centrifuge tubes, which are included in the kit.**
- **The entire procedure is conducted at room temperature.**
- **All spins are top speed for a conventional, tabletop micro-centrifuge.**

PROCEDURE:

1. Add Buffer PB (this should be 5 times the Volume of DNA), to the sample (extracted Genomic DNA). Thus, if Extracted DNA is 100µl, then add 500µl of Buffer PB. Mix thoroughly with micropipette.
2. Apply the above mixture to the supplied QIAquick-spin column (contained in a 2ml micro-centrifuge tube).
3. Centrifuge for 60 seconds at maximum speed.
4. Discard flow-through, from the catch-tube. Blot tube on tissue paper. Do not discard the 2ml micro-centrifuge tube. Insert the spin column back into the just used 2ml tube.
5. Wash QIAquick-spin column by adding 750µl of Buffer PE. Centrifuge for 60 seconds.
6. Discard buffer PE flow-through from the tube. Blot tube and insert spin column back into the tube.

7. Spin for an additional 60 seconds to get rid of excess buffer PE.
8. Now remove and place QIAquick-spin column in a clean (labeled), 1.5ml micro-centrifuge tube.
9. Elute DNA by adding 50µl of BE (10 mM Tris/HCl, pH = 8.5) or TE. Waite 2 minutes, and Centrifuge for 60 seconds.
10. DNA is in the flow-through.

APPENDIX E

StrataPrep PCR PURIFICATION

1. Add an equal volume of DNA binding solution to PCR product in a siliconized tube. Mix with the pipette. (Do not go above 350µl of each).
2. Transfer above solution/mixture to a micro-spin cup in a 2ml catch tube.
3. Spin at maximum speed for 30 seconds.
4. Discard binding solution, keep the micro-spin cup and catch tube.
5. Add 750µl wash buffer to the micro-spin cup and spin 30 seconds.
6. Discard wash buffer, keep the micro-spin cup and catch tube.
7. Spin the micro-spin cup in the catch tube again to remove residual alcohol.
8. Transfer the micro-spin cup to a siliconized tube. Discard tube.
9. Add 50µl de-ionized UV H_2O onto the fiber matrix at bottom of micro-spin cup.
10. Incubate 5minutes at RT.
11. Spin30 seconds.
12. Transfer the contents to a labeled siliconized tube.
13. For use in cycle sequencing, run a gel again to determine the amount of template (volume) to use in the sequencing reaction. This you determine by the intensity of the bands (i.e., 3, 5µl).

NB: The weaker the band, the more templates you need to use.

APPENDIX F

CENTRI-SEP PROTOCOL

(FOR PURIFYING PCR PRODUCTS FOR SEQUENCING REACTIONS)

Column Hydration

Gently tap the column to ensure that the dry gel has settled in the bottom of the spin column.

1. Remove the top column and reconstitute the column by adding 0.80ml of reagent grade water to buffer. Leave the column end stopper in place so column can stand up by itself. Replace the column cap and hydrate the gel by shaking and inverting the column or vortexing briefly. It is important to hydrate all of the dry gel.
2. Allow at least 30 minutes of room temperature hydration time before using the columns.

Removal of Interstitial Fluid

3. Remove air bubbles from the column gel by inverting the column and sharply tapping the column, allowing the gel to slurry to the opposite end of the column. Stand the column up and allow the gel to settle while in a microtube rack.
4. After the gel has settled and free of bubbles, first remove the top column cap, and then the bottom end stopper from the bottom.
5. Allow excess column fluid to drain (gravity) into a wash tube (2ml). If the fluid does not begin to flow immediately through the end of the column, use a 2ml latex pipette bulb to apply gentle air pressure to the top of the column filter. The column will stop draining on its own. Approximately 200-250µl will drain from the column. Discard this fluid.
6. Spin the column and wash tube in a variable speed centrifuge at 750Xg for 2 minutes to

remove interstitial fluid. For an Eppendorf microcentrifuge Model 5415C, spin at 3,000 rpm.

7. Approximately 300µl of fluid will be removed. If there is a drop at the end of the column, blot it dry. Discard the wash tube and the interstitial fluid. Do not allow the gel to dry excessively. **Process the sample within the next few minutes.**

Sample Processing

8. Hold the column upright and transfer 20µl of completed DyeDeoxy terminator reaction mixture to the top of the gel. Carefully dispense the sample directly onto the center of the gel bed at the top of the column, without disturbing the gel surface. Do not contact the sides of the column with the reaction mixture or the sample pipette tip, since this can reduce the efficiency of purification and possibly ruin the analysis due to excess dyes.
9. Place the column in the sample collection tube (1.5ml) and place both into the rotor. **Maintain proper column orientation**. The highest point of the gel media in the column should always point toward the outside of the rotor. Spin the column and collection tube at 3,000 rpm for 2minutes. The purified sample will collect in the bottom of the Sample Collection Tube. Discard the spin column and proceed with the Sequencing sample preparation procedure. **Protect sample from light with an aluminium foil.**
10. Dry the sample in a vacuum centrifuge. **Do not apply heat**.

APPENDIX G

BASE SEQUENCES OF ITS1 OF RIBOSOMAL DNA OF *DRACUNCULUS* ISOLATES

1. SPECIMEN OF HUMAN ORIGIN FROM BURKINA FASO

AACATGGTCGCATGCAATAACGCACCCTACTACACTGGGGACTCAACGTGCTATGTC
CATTGTCGAAAGGCATTGGTAACCCGTTGAAAATCCTCCGTGCTCGGGATAGGGAAT
TGCAATTATTTCCCTTGAACGAGGAATCCCTAGTAAGTGTGAGTCATCAGCTCACGC
TGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGGACTGAGCCGTT
TCGAGAAAAGCGGAGACTGCTGTATTGAAGCCGAATATTTGCGGTGGAAATACTCT
GGTGGAAATCGCCTTAATCGCAGTGGCTTGAACCGGGCAAAAGTCGTAACAAGGTT
TCCGTAGGTGAACCTGCGGAAGGATCATTAACGTATTTGGAAAGCATATTAAAATAC
GCGATATGATAACGATAGTTGTATCGAAATTGGTTTGAAGATTTTTCGATAAAGACG
AGAACGACGAATCTATATATCGCGACAATTATGTATAAACGTAATTTTATAAAAAAT
AATGCGTTTGATCAAAAGAATGATGCATGGGTCGTCTTCGCGTCGTCATCATMGTAT
GTCGTTGTAATGATGATGATGATGATGATGTAGTACTTATGCATGACGGTTAAT
GAATAACGCATATTTTGCATATACATATATTGCGTGATATTATTGTTGTTATATGATG
ATAACTCGTTAATTTCGTGAAAGAATTTTCAATATATATTTCGATACACTCGAAAAA
AATGTACAGAATAAAGAAGTAAAATGCGTGATTTTGTACAAATAACAGTGACACGG
TTGGCGTCTATACGTTGTTTAGTAGTTATTGCCCGACTGTCAGTAACGTTTGAACGAC
GGCGATATAGTTCTCGATGTGAGAGGAAATTTTCAAATTCGAGAATAGACTTAATAA
GTATTGCAGGGATACTGCCAACAAGAAAAAAATTCAATAAAGAAATTCATCTTAATT
AAGAATATGATAAAACGTTTCAAATAATGATATGTATATATTTTTGAAATGGATTGA
TGGGAGATAATGGCCCGAAAAATGAACTGTAGTATATCTTCATTTTCGTTATTATTAT
CACCCATCATCATCAAATATATATACAATTATTATTGTATAAGAAGCCAAAAGATGA
GACATTCTTAACAGA

2. SPECIMEN OF CANINE/DOG ORIGIN FROM GHNANA

AACATGGTCGCATGCAATAACGCACCCTACTACACTGGGGACTCAACGTGCTATGTC
CATTGTCGAAAGGCATTGGTAACCCGTTGAAAATCCTCCGTGCTCGGGATAGGGAAT
TGCAATTATTTCCCTTGAACGAGGAATCCCTAGTAAGTGTGAGTCATCAGCTCACGC
TGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGGACTGAGCCGTT
TCGAGAAAAGCGGAGACTGCTGTATTGAAGCCGAATATTTGCGGTGGAAATACTCT
GGTGGAAATCGCCTTAATCGCAGTGGCTTGAACCGGGCAAAAGTCGTAACAAGGTT
TCCGTAGGTGAACCTGCGGAAGGATCATTAACGTATTTGGAAAGCATATTAAAATAC
GCGATATGATAACGATAGTTGTATCGAAATTGGTTTGAAGATTTTTCGATAAAGACG
AGAACGACGAATCTATATATCGCGACAATTATGTATAAACGTAATTTTATAAAAAAT
AATGCGTTTGATCAAAAGAATGATGCATGGGTCGTCTTCGCGTCGTCATCATMGTAT
GTCGTTGTAATGATGATGATGATGATGATGTAGTACTTATGCATGACGGTTAAT
GAATAACGCATATTTTGCATATACATATATTGCGTGATATTATTGTTGTTATATGATG
ATAACTCGTTAATTTCGTGAAAGAATTTTCAATATATATTTCGATACACTCGAAAAA
AATGTACAGAATAAAGAAGTAAAATGCGTGATTTTGTACAAATAACAGTGACACGG
TTGGCGTCTATACGTTGTTTAGTAGTTATTGCCCGACTGTCAGTAACGTTTGAACGAC
GGCGATATAGTTCTCGATGTGAGAGGAAATTTTCAAATTCGAGAATAGACTTAATAA
GTATTGCAGGGATACTGCCAACAAGAAAAAAATTCAATAAAGAAATTCATCTTAATT
AAGAATATGATAAAACGTTTCAAATAATGATATGTATATATTTTTGAAATGGATTGA
TGGGAGATAATGGCCCGAAAAATGAACTGTAGTATATCTTCATTTTCGTTATTATTAT
CACCCATCATCATCAAATATATATACAATTATTATTGTATAAGAAGCCAAAAGATGA
GACATTCTTAACAGA

3. SPECIMEN OF HUMAN ORIGIN FROM PAKISTAN

AACATGGTCGCATGCAATAACGCACCCTACTACACTGGGGACTCAACGTGCTATGTC
CATTGTCGAAAGGCATTGGTAACCCGTTGAAAATCCTCCGTGCTCGGGATAGGGAAT
TGCAATTATTTCCCTTGAACGAGGAATCCCTAGTAAGTGTGAGTCATCAGCTCACGC
TGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGGACTGAGCCGTT
TCGAGAAAAGCGGAGACTGCTGTATTGAAGCCGAATATTTGCGGTGGAAATACTCT
GGTGGAAATCGCCTTAATCGCAGTGGCTTGAACCGGGCAAAAGTCGTAACAAGGTT
TCCGTAGGTGAACCTGCGGAAGGATCATTAACGTATTTGGAAAGCATATTAAAATAC
GCGATATGATAACGATAGTTGTATCGAAATTGGTTTGAAGATTTTTCGATAAAGACG
AGAACGACGAATCTATATATCGCGACAATTATGTATAAACGTAATTTTATAAAAAAT
AATGCGTTTGATCAAAAGAATGATGCATGGGTCGTCTTCGCGTCGTCATCATMGTAT
GTCGTTGTAATGATGATGATGATGATGATGATGTAGTACTTATGCATGACGGTTAAT
GAATAACGCATATTTTGCATATACATATATTGCGTGATATTATTGTTGTTATATGATG
ATAACTCGTTAATTTCGTGAAAGAATTTTCAATATATATTTCGATACACTCGAAAAA
AATGTACAGAATAAAGAAGTAAAATGCGTGATTTTGTACAAATAACAGTGACACGG
TTGGCGTCTATACGTTGTTTAGTAGTTATTGCCCGACTGTCAGTAACGTTTGAACGAC
GGCGATATAGTTCTCGATGTGAGAGGAAATTTTCAAATTCGAGAATAGACTTAATAA
GTATTGCAGGGATACTGCCAACAAGAAAAAAATTCAATAAAGAAATTCATCTTAATT
AAGAATATGATAAAACGTTTCAAATAATGATATGTATATATTTTTGAAATGGATTGA
TGGGAGATAATGGCCCGAAAAATGAACTGTAGTATATCTTCATTTTCGTTATTATTAT
CACCCATCATCATCAAATATATATACAATTATTATTGTATAAGAAGCCAAAAGATGA
GACATTCTTAACAGA