

**RESPONSES OF LARGE PRIVATE MANUFACTURING
COMPANIES TO THE HIV/AIDS PANDEMIC IN NAIROBI**

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**A research project submitted in partial fulfillment of the requirements
for the award of Master of Business Administration (MBA) degree,
Faculty of Commerce, University of Nairobi.**

March 2004

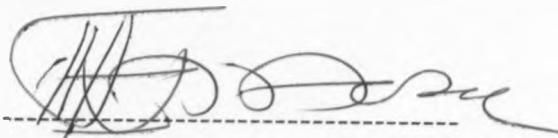
DECLARATION

The project is my own original work and has not been presented for a degree in any other university.

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This project has been submitted for examination with my approval as university supervisor.

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DEDICATION

To my dear parents, brothers and sisters, nephews and nieces for their immeasurable love, care and support.

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I am deeply indebted to Prof. E. Aosa, who worked patiently with me from the beginning of this study; constantly providing me with meaningful advice and criticism. His guidance facilitated the realization of this work.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
HIV	Human Immunodeficiency virus
STD	Sexually transmitted diseases
STI	Sexually Transmitted infections
NGO	Non-governmental organisation
TB	Tuberculosis
VCT	Voluntary counseling and testing
ILO	International Labour organisation
MOH	Ministry of Health
NASCOP	National AIDS and STDs Control Programme
NACC	National AIDS Control Council

ABSTRACT

Organisations exist in a complex commercial, economic, political, technological, cultural and social environment. This environment is turbulent and it is more complex to some organisations than others. The success of every organisation is determined by its responsiveness to the environment. To be able to retain competitive advantage, organisations need to examine their environment both internal and external and respond accordingly.

HIV/AIDS is one of the significant recent developments in the environment. Organisations are therefore expected to respond to the impact of HIV/AIDS. This study set out to investigate the response of companies to the pandemic. The objective of this study was to establish and analyse the responses that have been put in place to counter the devastating impacts of AIDS in organisations.

The study focused on large private manufacturing companies operating in Nairobi, Kenya. The research design used was a cross sectional survey. The population consisted of 250 companies and a sample size of 100 was used for this study. Data collection instrument was a structured questionnaire administered through drop and pick method and respondents were mainly personnel managers and CEOs. Data was analyzed using descriptive statistics. Frequencies and percentages were used to summarize and present collected data.

The study revealed that 86% of firms have responded to AIDS crisis. Some of the actions taken by businesses include promoting prevention and education, improving workplace policies to ensure rights for employees such as access to health care and counseling. The limitations were mainly on data collection, which was constrained by the attitude of some organisations owing to confidentiality of the subject as well as budget and time.

Though some businesses have responded to the AIDS crisis, many more are at early stages in establishing HIV/AIDS workplace prevention programmes or developing appropriate policies. Today the Kenyan government has not been actively involved in developing legal and regulatory guidelines for the business sector.

As a recommendation, a well crafted approach of public and private sector collaboration in workplace HIV/AIDS prevention and policy planning promises to address Kenya's AIDS crisis in a manner that will yield human, socioeconomic development, and private sector benefits in future.

CHAPTER ONE: INTRODUCTION

1.1 Background

Businesses exist in the context of a complex commercial, economic, technological, social, demographic, political and legal environment. This environment changes and is more complex for some organisations than others. How this affects the organisations could include an understanding of historical and environmental effects as well as expected or potential changes in the environmental variables. Many of these variables will give rise to opportunities and others exert threats on the organisation (Johnson and Scholes, 1999). Businesses need to formulate and adopt appropriate strategic responses for their survival. The management strategy used by businesses is a determining component of its responsiveness to environmental changes because it determines the way the management perceives environmental challenges, diagnose their impact on the business, decides what to do and implements the decisions.

The success of every organisation is determined by its responsiveness to the environment. To be able to retain competitive advantage, organisations need to examine their environment both external and internal and respond accordingly (Porter, 1985). The environment can be relatively stable or turbulent. In a stable environment, organisations are under no pressure to change. The major concern then is maintaining the firm's position against any competition. Turbulence in the business environment puts pressure on organisations to change so that they can effectively sustain their competitive advantage (Worley et al, 1996). In response to the increasing turbulence of the environment, systems have been forced to become progressively more responsive and more complex.

The origin of competitive advantage may be found in a firm's local environment (Porter 1985). Organisations are required to have a profound understanding of the industry they are in. This requires them to have a thorough knowledge of the critical success factors and the drivers of change in the industry. An organisation can further compete effectively when it identifies the strategic group it belongs to, within the industry, and therefore learn more about its closest rivals and the intra industry success factors and develop a focused strategy that will enable it to occupy attractive segments (Whipp and Pettigrew, 1993).

Fundamental changes have been experienced in the global business environment. HIV/AIDS is one of the significant recent developments in the external environment and the epidemic is already having devastating effects on economies and markets threatening the security and prosperity of our global society. It is now two decades since the AIDS epidemic first emerged and though we can point to some areas of the world where AIDS has been effectively addressed the global impact of the disease is deepening. The figures are grave. Over 42 million people around the world are infected with HIV and International Labour Organisation (ILO) estimates that at least 26 million workers in the prime labour force, aged 15-49 years, are infected with HIV/AIDS. The ILO has also calculated that the size of the labour force in high-prevalence countries will be between 10-34% smaller by 2020 than it would have been without HIV/AIDS (UNAIDS, 2000).

In the worst affected countries, AIDS is single handedly reversing the development gains of several decades. In southern Africa, life expectancy at birth climbed from 44 in the early 1950s to 59 in the early 1990s. With the

demographic impact of AIDS this is expected to drop to 45 sometime between 2005 and 2010 (Whiteside and Barnett, 2002). The impact caused by AIDS has reverberated through every sector of the society, from health to agriculture, education and the private sector, and is draining economies of the vital resources and contributions of a whole generation. Enterprises and national economies as well as workers and their families are feeling the effects. The epidemic cuts the supply of labour, with resulting loss of skills, experience and “institutional memory”. As a result, enterprises are losing productivity, public and private investment is being cut and employment opportunities are contracting, with resulting increase in informal activities and growing poverty.

According to survey of commercial farms in Kenya, illness and death have replaced old age as the leading reason for employees to leave service (Rugalema, et al. 1999). Reports from a single company in Kenya revealed that of 50 employees who died in 1998, 43 died of AIDS (Government of Kenya, 1996). The private sector is in a unique position to respond to the epidemic, because of its contacts with its employees and the wider business community, and the wealth of experience and skills it has accumulated. There is much that businesses can do, and the benefits go well beyond the workplace (UNAIDS, 2000).

Businesses are being subjected to the pressure of increasingly competitive national and global markets through globalisation and liberalisation of economies, combined with demands from investors and consumers for increased productivity, efficiency, innovation and quality of products and services. In addition, pressures are mounting for businesses to be more

responsible and accountable to their wider stakeholders; workforce, suppliers, communities, governments and the general public. Given this scenario and the known impact that HIV/AIDS has on business and its stakeholders, there is a clear requirement to respond. The challenge is clear; the response has been diverse, with a particular emphasis in the early stages of action on addressing and safeguarding core business activities through the protection and support of their own workforces. Increasingly, as businesses have become aware of the significance of other stakeholders in influencing the impact of HIV/AIDS on their ability to operate, they have begun to extend their responses to assist and collaborate in wider prevention and education initiatives (World Bank, 1996).

Hard-won gains in employment and social protection are being reversed because of the epidemic. At the enterprise level, the effects of AIDS include loss of earnings, loss of skills, reduced productivity and the loss of markets as the consumer base is whittled away. Concern about the disease and the impact on companies is now as widespread as the disease itself. HIV/AIDS affects employees at all levels, from top management to the shop floor and in the long run it also affects company profits. It makes strong business sense for companies to respond to the pandemic. The workforce is placed at increasing risks, with the epidemic disproportionately affecting people during their most productive years (UNAIDS, 2000).

1.2. Statement of the problem

Business environment can be relatively stable or turbulent and each level of turbulence has different characteristics and requires different strategy to match. The strategy in-turn has to be matched by appropriate organisational capability for survival, growth and development (Ansoff, 1990).

Fundamental forces of change have been experienced in the global business environment resulting in unprecedented competition. Organisations responding to these changes have realised that their existing strategies and configurations may no longer serve them well (Ansoff, 1990).

The Kenyan environment is not exempt from what the global scenes are experiencing. Organisations being environment dependent have to constantly adapt their activities and internal configurations to reflect the new external realities and failure to do this may put the future success of an organisation in jeopardy (Aosa, 1998). It is imperative that managers apply critical investigation into the realities of the changing environment of this millennium through enlightened diagnosis of the problem it poses. The political and economic environment for instance, can influence the lifestyles and the health of the people. The same environment should also be seen as a system, which calls for profound understanding in order to improve decision making and to recognise the links between the past, present and the future as well as local and global matters.

HIV/AIDS, being one of the significant recent developments in the environment is not purely a health issue; it is also an issue that goes to the very core of a business practices, a development challenge and the source of widespread insecurity. For many businesses the impact is already severely

constraining their ability to be competitive, while for others the potential risks are significant in both high and low prevalence regions. Building awareness of the severity of the impact of HIV/AIDS on business is one of the most important elements in assisting businesses to respond effectively. The effects are evident on two levels, the macroeconomic and the individual company levels, both of which require urgent responses if businesses are to remain competitive. It is expected therefore that businesses, especially have responded to the prevailing changes in the environment appropriately. The research question that this study addresses is; to what extent are the private manufacturing industries responding to the HIV/AIDS scourge?

1.3. Objective of the Study

This study has one objective. This is to establish the responses of manufacturing companies to the HIV/AIDS pandemic.

1.4. Importance of the study

The findings of this study are expected to be beneficial to businesses and other policymakers in clarifying the major policy issues related to HIV/AIDS in the workplace. The scholars and other researchers would find it useful by way of information and may be spurred to undertake more research in a related field.

1.5. Organization of the study

This study has five chapters as follows:

Chapter one

This introductory chapter gives background information of the interdependence of businesses with the external environment. The problem statement, aims and objectives of the study are outlined.

Chapter two

This chapter gives the literature review. It highlights the interrelationship between the environment, strategy and management. It discusses some of the intervention measures.

Chapter three

The chapter deals with the study design and sets out the various steps that were necessary in conducting this cross sectional study.

Chapter four

This chapter has the results, analysis and discussion.

Chapter five

The chapter contains the summary of the research findings, conclusion, limitations, and recommendations and outlines the opportunities for further research.

CHAPTER TWO: LITERATURE REVIEW

2.1. Introduction

A firm's external environment consists of interrelated factors that play a principal role in determining the opportunities, threats and constraints that the firm faces. Organisations external environment is turbulent, and for business survival appropriate strategies have to be instituted. Strategic responses call for organisations to craft their strategies to match the environment and also to transform or redesign their internal capability to match the strategy. This requires that its internal resources, which include both tangible and intangible, maintain a strategic fit in its value chain system. Failure to match an organisation's strategy to the environment will create a strategy gap, while an organisation that fails to match the internal capability to strategy will experience a capability gap. The challenge to organisation is to continuously match the environment, strategy and their internal capabilities in order survive, succeed and to remain relevant (Porter, 1985).

2.2. Organisations External Environment

The external environment provides many of the challenges that a particular firm faces in its attempts to attract or acquire needed resources and to profitably market its goods and service. This external environment includes economic forces, socio-cultural, demographic and technological, while its competitive environment includes competitors, customers and suppliers. This external component should have a strategic fit with the internal environment, which includes the organisation's system, policies, resource capability and its corporate culture (Pearce and Robinson, 1997). The external environment is turbulent and it is more complex for some

organisations than others and for survival, an organisation must maintain a strategic fit with the environment.

Businesses exist as open systems. The external environment provides resource inputs, which are important for business survival. The businesses in turn transform these resource inputs into products and/or services. These products and/or services exit into the external environment. The environment is a critical factor for any organisation's survival and success. It is a resource to be managed and to be shared, hence the need to manage the value chain system and establish collaborations, partnerships and to get involved in social responsibility to enrich this resource and enhance the corporate image of the organisation. In a report published by Pricewaterhousecoopers and Nation, it is noted that, many organisations are now more than ever being involved in social responsibility activities since a good corporate image can also be a source of competitive advantage. A sustainable competitive advantage is achieved when there is a strategic fit between the external and internal environment. It is the duty of strategic managers to understand the environment both external and internal and make decisions based on this analysis (Ansoff,1990).

2.3. Strategy and Strategic Management

Strategy is defined as “the direction and scope of an organisation in the long term which achieves advantage for the organisation through its configuration of resources within a changing environment to meet the needs of markets and to fulfil stakeholders expectations” (Johnson and Scholes, 1999, p14). Therefore strategy can be seen as matching the activities of an organisation to the environment in which it operates also termed as creating a strategic fit.

A good strategy is derived through a strategic analysis, which is concerned with understanding the strategic position of the organisation in terms of its external environment, internal resources and competencies as well as the stakeholders' expectations. Some of the organisations resources include product/service, human resource, capital and goodwill.

Strategy refers to the plan of action that a business adopts in using its resources and distinctive competencies to gain a competitive advantage over its rivals. The task of forming a strategy starts with hard analysis of the organisation's internal and external situation. A company's strategic action plan is dynamic, undergoing continuous review, refinement, enhancement and occasional major revision (Thompson and Strickland, 1993). Thus the design of business strategies is based on the conviction that a firm is able to anticipate future business conditions that will improve the performance and profitability.

Strategic management is defined as "a set of management decisions and actions that determine the long run performance of an organisation". It includes environmental scanning, strategy formulation, implementation and evaluation (Hunger and Wheelen, 1999, p3). Unlike other aspects of management, strategic management is different in the fact that it is concerned with complexity and ambiguous, non-routine situations with organisation-wide implications (Johnson and Scholes, 1999). This includes strategic analysis, formulation and implementation. Strategic analysis seeks to understand the organisations strategic position; formulation involves possible course of action, evaluating them and choosing the best option

between them and implementation is concerned with planning and putting into effect and managing the changes required (Johnson and Scholes, 1999).

2.4. Environment, Strategy and Capability

An organisation has two different but complementary strategies. The management role is to identify, plan and guide strategic responses and functional capability. The survival and success occurs when an organisation creates and maintains a match between its internal capability and strategy (Grant, 2000). The environment is not static but turbulent, discontinuous and uncertain.

Strategic management has helped organisations to be more proactive than reactive in coping with changes in the external environment. Certain business sectors have a direct commercial interest in HIV/AIDS through their core business operations, primarily the pharmaceutical companies and insurance industries. The most obvious is the pharmaceutical companies who are involved in the development of HIV/AIDS treatments and research. For instance, GlaxoSmithKline's "Positive Action" aimed at encouraging dialogue and Bristol Myers Squibb "Secure the Future" which has committed \$US100 million towards medical research and education (UNAIDS, 2002). The insurance industry has a direct commercial interest in HIV/AIDS given the impact on the well being of its clients and wider community and thus on the direct costs through insurance payments and future markets. This kind of approach, seeks to use core business practices to encourage other companies to respond, has considerable potential for replication elsewhere and banking sectors.

Given the increasing importance and complexity of the range of stakeholders in the global economy, businesses need to safeguard their direct business partners against the impact of HIV/AIDS in order to remain responsive and competitive and to maintain their reputation. Given the role that businesses might have played in HIV prevention, it is becoming apparent that the workplace is a major avenue for interventions (Rau, 2002). The business sector and its workplaces can play a key role in preventing the transmission of HIV and in caring for and supporting those affected. As people continue to die from the pandemic man has no option but to institute checks and balances, which must be seen to supplement all the efforts from the medical fraternity.

Every government the world over has been compelled to come up with strategies and mechanisms to combat this scourge. The notion that the pandemic could be confronted strictly as a health issue has been replaced by knowledge that it feeds on and reinforces existing laws and malfunctions in social and economic systems. Many of the more creative partnerships emerge from the realisation that HIV/AIDS marks a crisis that touches and implicates everybody. At company level such aggregate national losses are often hard to detect or else have yet to come and they may not always therefore convince businesses to act. But the business impact of AIDS is already visible in workplaces in many parts of the world something that worries managers from the shop floor to the top management.

2.5. HIV/AIDS Globally

HIV/AIDS has brought about a global epidemic far more extensive than it was predicted a decade ago. UNAIDS and WHO now estimate that the number of people living with AIDS now stands at 42 million (Table 1). The table also highlights the global figures, adults death in 2002 standing at 3.1 million which has big impact on businesses.

Table 1: Global Summary of the HIV/AIDS Epidemic, December 2002.

	Children < 15 years (Million)	Women (Million)	Adults (Million)	Total (Million)
People newly infected with HIV in 2002	0.8	2	4.2	5
AIDS death in 2002	0.6	1.2	2.5	3.1
Total number of AIDS deaths since the beginning of the epidemic	5.5	11.3	22.4	27.9
Number of people living with HIV/AIDS	3.2	19.2	38.6	42

Source: UNAIDS/WHO 2002

The world over has been affected by the epidemic, but of specific interest for this study is the Sub Saharan Africa, whose figures stand at 29,400,000 cases (Table 2). Given that most industries in this region are labour intensive compared to the other regions, this figure is devastating. Table 2 compares the figures with the rest of the world.

Table 2: Distribution of people estimated to be living with HIV/AIDS, December 2002.

Area	Population
North America	980,000
Caribbean	440,000
Latin America	1,500,000
Western Europe	570,000
North Africa & Middle East	550,000
Sub Saharan Africa	29,400,000
Eastern Europe & Central Asia	1,200,000
East Asia & Pacific	1,200,000
South & South East Asia	6,000,000
Australia & New Zealand	15,000

Source: UNAIDS/WHO 2002

2.6. HIV/AIDS Interventions in Kenya

Since 1984 when the first case of AIDS was identified in Kenya, the policy environment has gone through three broad phases. Each phase was unique in Kenya, but has reflected the experiences of other countries, which have had to confront the diseases. During the first phase, 1984-1987, there was a general sense that HIV/AIDS was not a serious problem for the country. The second phase, 1988-1991, saw a somewhat more realistic appraisal of HIV/AIDS as a potentially harmful health issue. Responsibility for managing the response remained with MOH, which became assertive in raising warnings about the consequences of the disease in Kenya.

Meanwhile, influential religious leaders spoke out against use of condoms for prevention. Policymakers hesitated to discuss AIDS because of the potentially harmful impact that such discussions might have on tourism (Lorch, 1993). The third phase, 1992-1995, marked another significant change in Kenya's policy environment. The government released its surveillance data and in April 1993 hosted the first National Conference on AIDS. The MOH declared AIDS a national crisis (Africa confidential, 1993). Socio-economic impact assessments were initiated by both the government (Nalo, 1994) and international donors (Forsythe, 1995).

The rising number of illnesses and deaths from AIDS across all population groups provoked pressure from businesses, the media, NGOs and professional societies for clear policy directions from the government. In the more recent years the government of Kenya has been playing a lead role in mobilizing financial human and technical resources to combat this epidemic. The government issued *Sessional Paper Number 4 of 1997* as the framework for its response to the epidemic (Government of Kenya, 1997).

The rapid increase in young adult deaths will have serious consequences for economic and social development. HIV/AIDS will increase the death rates at all ages. However, the impact will be more severe among young adults and young children under the age of five. Without AIDS and assuming decreased mortality due to other factors, the annual number of deaths among young adults, 15-49 years would increase slowly (due to the growing population) from about 52,000 today to 58,000 by 2005. However, AIDS has dramatically changed the scenario, quadrupling the number to 214,000 a

year by 2000 and increasing it to 270,000 by 2005. (Government of Kenya, 2001).

HIV/AIDS has affected life-underwriting practice especially in shortened average duration of policy and proposers being declined cover (Tari, 1998). A study done showed that few organisations in the insurance industry in Kenya have sound workplace policies on HIV/AIDS (Murambi, 2002). While they offer general medical insurance, this is not sufficient. The few organisations that have policies specific to HIV/AIDS tend to focus on education and awareness activities and on procurement of medication for their employees.

2.7. The Impact of AIDS on Business

The economic impact of HIV/AIDS on companies is manifested by reduced labour productivity through AIDS related deaths absenteeism and loss of skilled work force. Other effects include increased expenditures on staff recruitment and training, funeral expenses, medical costs and increased employee benefit. These costs could be enormous for firm depending on the HIV prevalence among its employees. According to the World Bank Strategy report (World Bank, 1996) a Kenyan company spent about US\$45 per employee per year for HIV/AIDS-related costs or 3% of company profits. A study of Auto Kenya, Western Wood, Kenya Transport (all fictitious names for anonymity) and Muhoroni Sugar Company showed that they spent Ksh. 1.1 million, Ksh. 2 million, Ksh 3.1 million and Ksh. 2.9 million respectively on HIV/AIDS related costs. The effects of absenteeism caused by HIV/AIDS morbidity and mortality are complex, comprising of both quantitative and qualitative effects. All these substantially affect labour

productivity and profitability of companies. A study on 16 businesses in Kenya reveals that HIV/AIDS absenteeism is the most significant cost on companies (Roberts, and Rau, 1994). Companies that will struggle most as a result of HIV/AIDS are those that are highly labour intensive, employ highly skilled workers, or offer comprehensive benefits to employees (Government of Kenya, 1996).

AIDS takes its toll in the workplace in a number of ways. The loss of experienced personnel- in Zambia for example a leading commercial bank has lost most of its senior management (Over, 1992). Absenteeism through AIDS related illnesses to care for others and to attend funerals- in Madras India industrial labour absenteeism is predicted to double in the next two years because of AIDS. (UNAIDS, 2000). Increased recruitment and training costs- in many developing regions finding qualified top management and skilled line workers to replace those who die or can no longer work can be extremely difficult. (UNAIDS, 2000). Increased labour turnover – productivity suffers during the time it takes to replace workers particularly among more skilled or senior workers. Lower productivity of new recruits- often it takes weeks for new employees to become as productive as those whom they replaced. For example in Mauritius it can take at least a year for a garment factory employee to be sufficiently skilled to work on a company's high-end clothing production line. (UNAIDS, 2000). Increased health care costs, including growing health staff, medical and insurance costs, death benefits, disability and pension payments.

2.8. Organisational HIV/AIDS –Related workplace policies and processes.

To strengthen workplace prevention efforts and to provide guidance on company operations, organisational policies related to HIV/AIDS are needed. Company policies demonstrate commitment to the principles and practices of the workplace and provide authoritative backbone to prevention efforts. Public health and business organisation alike have developed a number of workplace policy guidelines or recommendations to promote HIV prevention, the rights of workers, and a healthy workforce. Based on these, the following HIV/AIDS-related policies are recommended for formal adoption and practice (WHO/ILO, 1988).

- There will be no compulsory pre-employment or employee HIV/AIDS screening for employment, insurance, or other purposes.
- All medical information and records of employees will be kept confidential.
- Persons with HIV infection, including AIDS, will have the same rights, responsibilities, and benefits/opportunities as others with serious illnesses or disabilities.
- HIV infection is not a cause of termination of employment, and employees with HIV-related illnesses should be able to work as long as medically fit for available, appropriate work.

There appear to be relatively few private sector companies operating in sub-Saharan Africa that have formally adopted these WHO/ILO recommended policies.

2.9. Kenya's Manufacturing Sector

Kenya has the biggest formal manufacturing sector in East Africa (Abecor, 1988). This sector has grown over time both in terms of its contribution to its country's GDP and employment. Both public and private companies are present, but the public sector participation in manufacturing is much smaller than the private sector and is still decreasing due to government's change of policy. Within the private sector, companies are owned and operated by both local and foreign investors (Kenya Government Economic survey, 1991). Most of the foreign companies are subsidiaries of multinational corporations, while indigenous Kenyans, Indian Kenyans and Kenyans originating from other countries own the local companies. Large private companies are selected due to their leading role and value adding in terms of providing leadership, materials, advice and finance where appropriate.

CHAPTER THREE: RESEARCH METHODOLOGY

3.1. Introduction

The research methodology set out the various steps that were necessary to conduct the study. The main objective of this study was to establish the responses of large private manufacturing companies based in Nairobi to the HIV/AIDS pandemic.

3.2. Research Design

To establish responses of companies to the HIV/AIDS pandemic, information was obtained through questionnaires from across a number of manufacturing companies operating in Nairobi. The cross sectional survey was therefore the most appropriate design for this type of study.

3.3. Population

The population of study consisted of large private manufacturing companies in Kenya operating in Nairobi. Public sector and small manufacturing are excluded from this study. Kirkpatrick et al (1984) defined small companies as those employing less than 50 employees. For this study, large companies were defined on the basis of number of employees, which was 50 and above. The number of employees is a convenient proxy for size and a good indicator of the complexity of the management structure. The population consisted of 250 companies, obtained from both the members' list (Kenya Association of Manufacturers, 2002 and the Kenya Directory of Manufacturing Industries, 1997).

3.4. Sampling

The sampling frame was done with adequacy and resource considerations. By adequacy, we mean that a sample should be big enough to enable reasonable estimates of variables to be obtained, capture variability of response and facilitate comparative analysis. With this as well as budgetary allocation and time in mind, a sample size of 100 was considered adequate for this study.

3.5. Data collection

The study used primary data. Data collection instrument comprised of a questionnaire with open and close-ended questions. Respondents for this study were the CEOs of companies, personnel managers and any other top management. Data was collected through drop and pick method, which is a variation of mail survey method. The advantage of this method was that it was a low cost method and the contact respondents who were corporate executives were difficult to reach in any other way. There were also cases where we did not have specific persons to reach, and through this method, they were routed to the appropriate respondents.

3.6. Data Analysis

Before processing the responses, the completed questionnaires were edited for completeness and consistency across respondents. Responses were coded to facilitate basic statistical analysis. Descriptive analysis was used to summarize the data. Percentages, frequencies, pie charts, were used for presenting the data. Cross tabulations, frequencies and comparative analysis were used to measure overall ranking of the initiatives undertaken by the different organisations.

CHAPTER FOUR: RESEARCH FINDINGS

4.1. Introduction

This study has examined the responses of large private manufacturing companies in Nairobi. The objective of the study was to establish the organisations responses to the HIV/AIDS pandemic. This objective has been achieved through the respondents' organisations' profiles and their perceptions of the developments in the environment in which they are operating.

Questionnaires were distributed to a total of 100 private manufacturing companies. The response rate was 70%. General characteristics of these organisations were established by considering the type of ownership, length of period they have been in existence and the size of the workforce.

4.2. Organisations Profile

Type of ownership

Within the private sector, companies are owned and operated by both local and foreign investors (Government of Kenya, 1991). The type of ownership may affect the strategy formulation and implementation process. Most of the foreign companies are subsidiaries of multinational corporations and they borrow a lot from the parent company in terms of strategy formulations and resource base. The local companies are owned by indigenous Kenyans, Indian Kenyans and Kenyans originating from other countries. Respondents were asked to indicate whether their ownership was local, foreign or a mix of the two. The study revealed that of the 70 companies studied, 60% were

fully local, 27% were foreign owned and 13% had ownership drawn from both (Table 3).

Table 3: Type of Ownership

Ownership	Frequency	%
Local	42	60%
Foreign	19	27%
Local/Foreign	9	13%
Total	70	100%

Source: Research Data

Comparison between responses of local and foreign companies revealed that 100% of foreign owned companies consider HIV/AIDS to be a threat to their organisations and only 75% of local companies consider it as a threat. 75% of respondents from local firms indicated that that they have a policy on HIV/AIDS while all foreign companies' respondents have a written policy.

Staff establishment

The respondents were asked to indicate their staff establishment. The size of the workforce was varied, it was revealed that 26% of the companies had 50-99 employees; 28.5% had 100-199; 28.5% had 200-499 and 17% had 500 and above (Table 4).

Table 4: Staff Establishment

Staff Establishment	Frequency	%
50-99	18	26%
100-199	20	28.5%
200-499	20	28.5%
<500	12	17%
Total	70	100%

Source: Research Data

Period of existence

The respondents were also asked to indicate the period of existence, which is a strong indicator of the policies and guidelines of the organisations. All the respondents have been in existence for more than nine years, 14% are 10-19 years; 19% are 20-29 years; 27% are 30-39 years and 40% are 40 years and above. It was established that, firms that have existed for more than 20 years have well outlined HIV/AIDS policies as compared to the young ones.

Table 5: Period of existence

Duration in years	Frequency	%
0-9	0	0%
10- 19	10	14%
20-29	13	19%
30-39	19	27%
>=40	28	40%
Total	70	100%

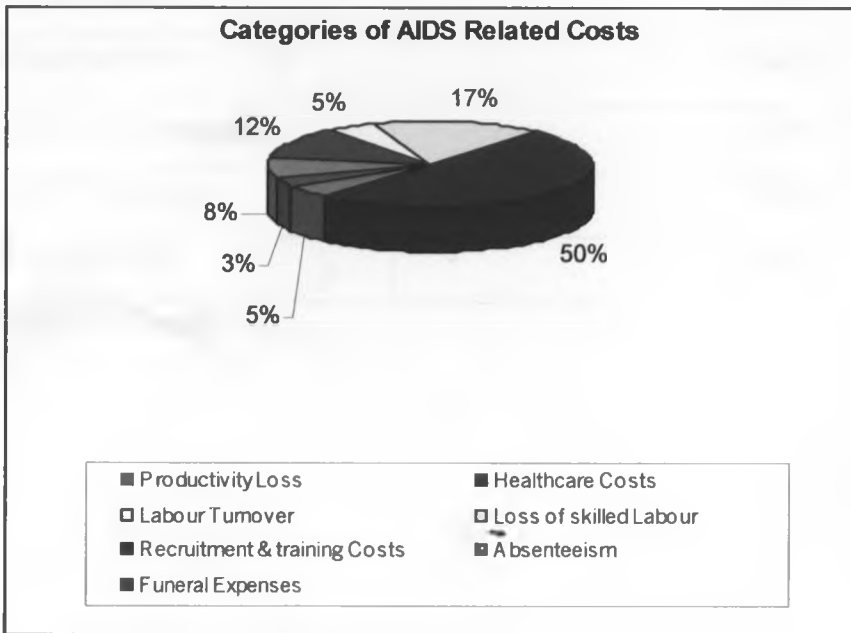
Source: Research Data

4.3. Impact of HIV/AIDS on organisations

As HIV prevalence rates continue to rise, labour productivity and savings affect different firms in various ways. From the respondents, 60% have been affected by the epidemic differently. It was noted that these are the firms with a workforce of more than 150 employees. 40% indicated that they have not been affected.

The economic impact of HIV/AIDS on companies is manifested by reduced labour productivity through AIDS related deaths absenteeism and loss of skilled work force. Other effects include increased expenditures on staff recruitment and training, funeral expenses, medical costs and increased employee benefit. These costs are enormous for firm depending on the HIV prevalence among its employees (Figure 1).

Figure 1



Source, Research Data

4.4. Interventions and Prevention Strategies

The study revealed that businesses have different strategies. Most businesses used single intervention method while others preferred to combine two or several methods. 19% offered informal education; 50% offered in-depth prevention education; 50% distributed company HIV/AIDS policy and updates; 38% offered counselling services; 94% distribute condoms; 63% offered STI diagnosis and treatment; 25% offered voluntary HIV testing and 58% offered HIV/AIDS treatment (Table 6).

Table 6: Types of HIV/AIDS Policies Offered

<i>Intervention/prevention</i>	<i>Frequency</i>	<i>%</i>
Informal Prevention education		19%
In-Depth Prevention education		50%
Distribution of company HIV/AIDS policy and updates		50%
Counselling services		38%
Condom distribution		94%
STI diagnosis and treatment		63%
Voluntary HIV Testing		25%
HIV/AIDS treatment		58%

Source: Research Data

4.5. Discussion

Comparison between responses of local and foreign companies revealed that 100% of foreign and local/foreign companies consider HIV/AIDS to be a threat to their organisations and only 75% of local companies consider it a threat. One factor that may explain the differences between the foreign and Kenyan companies is that the former are subsidiaries of bigger international companies. They also differ in the amount of management resources available to them. The foreign companies have access to managerial resources throughout the international corporate network.

The size of the workforce may affect the strategic management process of the organisation, due to the complexity of management. In this study companies with more than 50 employees were considered, reason being that the more the number of employees, the greater the impact of HIV/AIDS on the given organisation. The literature review revealed that the most affected organisations are those employing large numbers of workforce and are labour intensive (Rau, 2002).

Of the 70 firms sampled, 86% have already responded to the onset of HIV/AIDS pandemic. 19% of the firms offered only informal prevention activities in form of posters, pamphlets and/or factual presentations. This was however not a popular intervention method, since information could be obtained by employees from other sources such as the media. It was noted that the local owned firms were the ones using this tool. 50% offered in-depth prevention education (formal in-depth prevention activities and/or peer education). This is a popular method especially with the foreign owned

companies since deeper information is communicated using internal resources such as employees or in house formal training programmes. Condoms distribution is popular method across the board. This could be explained by the fact that they are cheaper and they also reach a wider community. About 60% of the companies offered STI diagnosis and treatment; 38% offered voluntary HIV testing and 58% treated HIV/AIDS mainly through outsourcing by providing drugs for opportunistic infections and anti-retroviral therapy. This was popular among the foreign owned firms and the reason for outsourcing was to maintain confidentiality.

50% of companies distributed HIV/AIDS policy and updates to their employees. This was to outline the guidelines to promote HIV prevention, the rights of workers as pertains to the HIV status. This was the practice by the foreign owned companies.

CHAPTER FIVE: SUMMARY, RECOMMENDATION AND CONCLUSION

5.1. Summary

The focus of this study was on the response of manufacturing firms to HIV/AIDS pandemic. The study endeavoured to achieve this through analysis of the policies that firms in the manufacturing industry have put in place in response to the pandemic. Consistent with the growing body of socio-economic research on AIDS in Africa, this study finds that AIDS is having a notable negative impact on business sector. 86% of the respondents have strategies and policies to curb the effects of the pandemic on the workforce.

The study revealed that companies have responded in various ways. The intervention strategies combine two more or programmes of the following popular methods: condoms distribution; STI diagnosis and treatment; HIV/AIDS treatment; in-depth prevention education and distribution of HIV/AIDS policy and updates.

5.2. Recommendations

At the company level, the benefits of HIV/AIDS prevention in the workplace will almost certainly outweigh the costs of unchecked spread of the disease. However, not all aspects of the recommended workplace policies are necessarily financially appealing in the short-term. All Kenyan companies should assess the potential impact of HIV/AIDS on business operations and profitability.

Non-medical costs are higher than medical costs (Robert and Rau, 1994). Organisations should therefore not only concentrate on cutting medical insurance. Although these costs may appear too great to bear, in the long term, they would be greatly minimised if appropriate measures are put in place.

5.3. Conclusion

The study findings revealed that organisations have been affected by the pandemic. Due to the interaction with the external environment, businesses have to respond to the changes for their success and survival. 86% of the companies have established measures to curb the effects and spread of the pandemic.

The findings showed that 14% of local organisations did not consider HIV/AIDS a threat to their organisations. This is because they have not encountered any cases of HIV yet. Although they may not be affected today, if no sound policies are put in place to counter its negative impact, the future of these organisations is bound to be grim, given the subsequent imbalance in the labour market.

5.4. Limitations of the study

The main limitation of this study was that data collection was constrained by the attitude of some organisations to the subject of the investigation- HIV/AIDS. Most people still consider HIV/AIDS a sensitive issue and could not divulge any information.

There was also the problem of non-response. 30% of those contacted did not respond and this would have made comparative analysis complete.

The budget and time taken to collect the data was limited. We would have wanted to contact large number of firms but this was constrained by lack of finance and time.

5.5. Suggestion for further studies

Further studies would be recommended given that HIV/AIDS is a recent threat in the environment of businesses. The cost-benefit of responses adopted by businesses may also be studied vis-à-vis those businesses that have not responded.

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APPENDICES: APPENDIX 1

Letter of Introduction

P.O BOX 66327

NAIROBI

Dear Sir/madam

**RE: RESEARCH ON THE RESPONSE OF PRIVATE
MANUFACTURING COMPANIES TO THE HIV/AIDS PANDEMIC
IN KENYA.**

I am a student at the University of Nairobi doing a masters degree in business administration (MBA). I am undertaking the above research project as part of the academic requirements.

You have been selected to form part of this study. This therefore, is to kindly request you to assist me collect the data by filling out the accompanying questionnaire.

This information shall be treated with the utmost confidentiality, and will be solely for this research. However, the findings of this research can be availed to you upon request. Your cooperation will be highly appreciated.

Thanking you in advance.

Yours faithfully,

Waita A.N

Student.

Prof. E. Aosa

Supervisor

APPENDIX 2

QUESTIONNAIRE

The questionnaire below has three parts. Section A is aimed at giving a general background of your company. Section B deals with the effects that HIV/AIDS has had on the company and section C deals with those interventions that the company has considered.

SECTION A: Company Data

1. What is the ownership pattern?

Foreign----- Local----- Foreign/Local-----

2. What is the age of your company?-----

3. What is the size of your workforce? -----

4. What is the age bracket of your workforce? -----

5. Is it mandatory for applicants and employees to be screened for HIV?

Yes /No

SECTION B:

1. Has the HIV/AIDS pandemic affected your company in any way? **Yes/No**

2. Explain how your company has been affected.

SECTION C

1. Has your company responded to the onset of the HIV/AIDS pandemic?

Yes/No

2. Explain how your company has responded to this pandemic.

3. Which components of HIV/AIDS does the company run?

(Add a check mark and/or explanatory note in the appropriate box)

	Company designs the program	Company uses some of its resources to manage the program.	Company seeks an outside agency for management.
1. Education materials and presentations.			
1a. Presentations on HIV/AIDS prevention.			
1b. Preparation and distribution of written materials.			
1c. Distribution of company HIV/AIDS policy and updates.			
2. Training of staff			
2a. Peer educators.			
2b. Supervisors and employee representative			

2c. Clinical Staff			
2d. Counsellors			
3. Condom distribution			
3a. Ordering supplies			
4. STI diagnosis and treatment			
4a. On-site facilities			
4b. Confidential record-keeping			
5. Counselling, HIV testing and support			
5a. Private facilities			
5b. Confidential record-keeping			
5c. Testing and diagnostic supplies			
6. HIV/AIDS treatment			
6a. Drugs for opportunistic infections			
6b. Antiretroviral drugs			
6c. Drugs to prevent mother-to-child transmission of HIV.			
6d. Private and confidential medical records.			

Others? Please specify.

.....

.....

.....

**A SURVEY OF THE PREVALENCE OF *Dirofilaria immitis*
(Heartworm) INFECTION IN DOGS ALONG THE KENYA COAST**

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NAIROBI

MSc 1997

By
Joseph A. Mutasa, BVM

A thesis submitted in partial fulfilment for the degree of Master of Science in
Clinical studies at the University of Nairobi

1994

DECLARATION

This is my original work and has not been submitted for a degree at any other university or institution



Joseph A. Mutasa, BVM

This work has been submitted to the university of Nairobi for examination with my approval



Prof. I.B.J. Buoro, PhD
Department of Clinical Studies
Faculty of Veterinary Medicine
University of Nairobi,
P.O. Box 29053,
Kabete, Kenya.

DEDICATION

This work is dedicated to my father, Augustine Mutasa; my mother, Aurelia Kasisi, my wife Lucy Kailembo and daughters, Jenniffer and Kemilembe.

ACKNOWLEDGMENTS

I am grateful to my supervisor Prof. I.B.J. Buoro of the University of Nairobi for his tireless guidance, devotion and tolerance throughout this work. I benefited a lot from his fruitful discussions, criticisms and encouragement all of which emerged as a testimony for his maturity in science.

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This work would have been difficult without the moral support of my wife Lucy.

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ABSTRACT

Dirofilaria immitis (*D. immitis*) also known as heartworm is the causative agent of dirofilariasis, a major condition in the dogs which is associated with debility and deaths in severe cases. It is known to occur in dogs and other animals particularly in coastal areas all over the world. The high prevalence of this parasite in several countries has necessitated the use of regular testing and chemoprophylaxis against it. In the majority of the remaining countries however, the status of this condition in dogs have not been published. Information on prevalence is important for setting up monitoring and control programmes of any disease, in any particular area. In the case of the Kenya coast, no work has been done to give an indication of the prevalence of *D. immitis* despite several anecdotal reports suggesting its existence there. This survey was therefore carried out with the primary objectives being (i) to determine the prevalence of *D. immitis* in dogs residing at the Kenyan coast (ii) to examine factors that may predispose dogs to heartworm infection (iii) to determine the occurrence of other filarids in the same dogs and (iv) to recommend appropriate control measures.

A total of 830 dogs of mixed breed and sex, aged above 6 months were selected from ten areas situated along the Kenya coast and examined for heartworm and other filarial infections by parasitological and serological methods and also by necropsy. In this survey, the prevalence rate of *D. immitis* was found to be 17.3%. Another filaria species, *Dipetulonema reconduum*, which is non-pathogenic to dogs was found to exist with *D. immitis* in examined dogs in almost equal proportions.

It was concluded from the results, that *D. immitis* is an important veterinary pathogen in the Kenyan coast and that its prevalence there warrants efforts to design appropriate control measures. In the view of this, routine annual testing of resident dogs for heartworm infection followed by chemotherapy in positive cases and also the use of regular chemoprophylaxis are recommended for the whole coastal area of Kenya.

CHAPTER ONE

1. INTRODUCTION

Dirofilaria immitis (*D. immitis*) Leidy 1856 (Nematoda, Filaridae: heartworm) is a filarid parasite that causes dirofilariasis or heartworm disease, a complex debilitating disease syndrome of dogs. The adult forms of this parasite typically lodge in the pulmonary arteries and right ventricle of the heart where they stimulate cardio-pulmonary related circulatory disturbances (Rawlings, 1986). They may also occur in aberrant locations such as the central nervous system, systemic arteries, skin/subcutaneous tissue and the anterior eye chamber (Rawlings, 1986). During heavy infections, the worms may be found in the thoracic posterior vena cava, their presence therein being associated with a more serious condition, the caval syndrome (Rawlings, 1986; Buoro, 1988); additionally, canine heartworm disease may be associated with glomerulonephritis (Nakagaki, Nogami, Hayashi, Hammerberg, Tanaka and Ohishi, 1993), encephalomyelitis and myositis (Cooley, Clemmons and Gross, 1987).

Although the domestic dog (*Canis familiaris*) acts as an important reservoir host of *D. immitis*, a wide variety of other mammalian hosts can be affected. The parasite has been recorded in the cat, ferrets, coyotes, foxes, California sea lion, jaguars, tigers, horses, monkeys and human beings (Soulshy, 1982). With the exception of domestic and wild canines, domestic felines, ferrets and California sea lion, all other species affected are considered aberrant hosts, incapable of serving as a reservoir of infection because in them, the life cycle of the parasite is terminated at the adult stage (Soulshy, 1982; Knight, 1987).

In many occasions, *D. immitis* has been observed to share an ecological niche with other less clinically significant filariids particularly, *Dipetalonema reconditum*

which is non-pathogenic (Martin and Collins, 1985; Boreham and Atwell, 1985; Owen and Slocombe, 1990; Ortega-Mora , Gomez-Bautista, Rojo-Vasquez, Rodenas and Guerrero, 1991; Patton and Faulkner, 1992). The importance of these other filarids is during clinical diagnosis, when their microfilariae must be distinguished from those of *D. immitis*.

D. immitis is ovoviviparous and release unsheathed larvae, the microfilariae, into the bloodstream (Soulshy, 1982). The vector involved in the transmission of the disease is the mosquito which acts also as an intermediate host. This biological association tends to limit the distribution of the disease seasonally and also geographically.

Historically, dirofilariasis is a disease of tropical and subtropical environments (Soulshy, 1982; Knight, 1987) but more areas with temperate climates are becoming involved as a result of increased movement of dogs from enzootic tropical areas to the colder areas. (Foreyt and Lagerquist, 1991). *D. immitis* is common and can be found throughout most of the temperate and tropical coastal zones of the world (Soulshy, 1982; Knight 1987). It is especially prevalent in Japan, Australia, the United states (Knight, 1983), and Canada (Owen and Slocombe, 1990). In these countries, prevalence rates ranging between 0.3% to 50% have been reported (Ishihara, Kitagawa, Ojuna, 1978; Carlisle and Atwell, 1984; Owen and Slocombe, 1990; Macv, Cheney and Taton-Allen., 1991; Patton and Faulkner, 1992).

Other reports of canine heartworm originate from France, Italy and Spain (Perez-Sanchez, Gomez-Bautista and Grandes, 1989; Ortega-Mora , Gomez-Bautista, Rojo-Vasquez, Rodenas and Guerrero, 1991), and Iraq, (Tarish, Al-Saqr, Al-Abbassy and Kadhim, 1986). In general, *D. immitis* is confined to those areas with high average ambient temperatures necessary for the completion of larval development in mosquitoes (Knight, 1983)

Information on canine heartworm disease in other parts of the world are either limited to few case reports or are completely absent. In Africa for instance, apart from reports by Heisch, Nelson and Furlong (1959) and Nelson, Heisch and Furlong (1962) who observed *D.immitis* in dogs in Pate island off the Kenya coast, and case reports of heartworm disease in dogs in the Republic of South Africa (Van Heerden, Verster and Gouws, 1980; Verster, Cilliers and Shroeder., 1991), Malawi (Fitzsimmons, 1964) and Beira in Mozambique (Verster *et.al.*, 1991), the prevalence of *D.immitis* in dogs has generally been considered insignificant. However, the current opinion held among workers is that *D.immitis* is worldwidely spread and is increasingly prevalent even in areas where it was absent. For instance, occasional cases dirofilariasis are being reported in imported dogs in the United Kingdom where the disease was formerly considered absent (Matic and Herrtage, 1987). Similarly, the first report of heartworms in the dog in Israel was made only recently (Zur and Bark, 1992).

Many factors can be considered to support the existence of *D.immitis* in the East African Coast. Average daily temperatures and humidity at the coast are high and consistent with the optimal requirements for both mosquito biological activities and also *D.immitis* larval development in the vector (Gillett, 1971; Subra, 1981; Russell, 1990). Mosquito abundance is further facilitated by the sanitary problems inherent in most African communities (Subra, 1981; Barnish, 1984). Inadequate shelter in these communities forces dogs to live outdoors where they become constantly exposed to potentially *D.immitis* infected mosquitoes (Mutero *et.al.*, 1984; Martin and Collins, 1985).

The presence of major mosquito-borne human diseases such bancroftian filariasis, yellow fever and malaria in the East African coast (Wijers and Kiilu 1977, Subra, 1981) and the fact that some of the mosquito species involved in the transmission of these diseases have also been found to transmit canine dirofilariasis elsewhere

(Subra, 1983; Russell, 1990), strongly suggest the possibility of existence of *D. immitis* in these areas in significant proportions

There have been several field reports of heartworms being encountered coincidentally during postmortem examination of dog carcasses, and these have usually originated from the Kenyan coast. Additionally, one of the two heartworm infections encountered in the Republic of South Africa was in a dog that had been recently imported there from Kenya (Van Heeden *et.al* , 1980). Yet, knowledge on the status of this condition in the dogs in Kenya has remained enigmatic as has been the strategies for controlling it.

Efficient chemoprophylaxis seen by many workers as the most reliable method of controlling canine heartworm diseases (Owen and Slocombe, 1990; Grieve *et.al.*, 1991; Paul, Todd, Acre, Plue, Wallace, French and Wallig, 1991; Shiramizu, 1991) requires routine testing programme, which in turn depends on knowledge of the biology of the worm, the mosquito and on the prevalence of heartworm disease in the area (Owen and Slocombe, 1990). On the other hand, routine chemoprophylaxis for canine heartworm disease is a costly practice (Owen and Slocombe, 1990), unacceptable from both technical and managerial point of views when the risk for the disease in the area is not known.

The primary objective of this study was to establish the existence and subsequently describe the prevalence and distribution of *D. immitis* in dogs along the Kenyan coast. Attempts were also made to evaluate the contribution of some of the important animal related factors such as breed, age sex and management factors such as provision of shelter and type of work assigned to dogs, on the disease status in the animals studied. Other filariids of dogs, particularly *D. reconditum* were also checked for, keeping in mind the potential implications they would pose in the diagnosis of heartworm diseases.

(Subra, 1983; Russell, 1990), strongly suggest the possibility of existence of *D. immitis* in these areas in significant proportions.

There have been several field reports of heartworms being encountered coincidentally during postmortem examination of dog carcasses, and these have usually originated from the Kenyan coast. Additionally, one of the two heartworm infections encountered in the Republic of South Africa was in a dog that had been recently imported there from Kenya (Van Heerden *et.al* , 1980). Yet, knowledge on the status of this condition in the dogs in Kenya has remained enigmatic as has been the strategies for controlling it.

Efficient chemoprophylaxis seen by many workers as the most reliable method of controlling canine heartworm diseases (Owen and Slocombe, 1990; Grieve *et.al.*, 1991, Paul, Todd, Acre, Plue, Wallace, French and Wallig, 1991; Shiramizu, 1991) requires routine testing programme, which in turn depends on knowledge of the biology of the worm, the mosquito and on the prevalence of heartworm disease in the area (Owen and Slocombe, 1990). On the other hand, routine chemoprophylaxis for canine heartworm disease is a costly practice (Owen and Slocombe, 1990), unacceptable from both technical and managerial point of views when the risk for the disease in the area is not known.

The primary objective of this study was to establish the existence and subsequently describe the prevalence and distribution of *D. immitis* in dogs along the Kenyan coast. Attempts were also made to evaluate the contribution of some of the important animal related factors such as breed, age sex and management factors such as provision of shelter and type of work assigned to dogs, on the disease status in the animals studied. Other filarids of dogs, particularly *D. reconditum* were also checked for, keeping in mind the potential implications they would pose in the diagnosis of heartworm diseases.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Morphology and Life cycle of *Dirofilaria immitis*

2.1.1 General Introduction

Dirofilaria immitis Leidy, 1856 is a filariid nematode belonging to the superfamily Filarioidea and family filariidae. Other genera of importance in this family are: *Dipetalonema*, *Brugia*, *Wuchereria*, *Parafilaria*, *Ornithofilaria*, *Onchocerca*, *Elaeophora*, *Suifilaria* and *Wehrdikhansia* (Soulshy, 1982). Among these, dogs are infected by *Dirofilaria immitis*, (*D. immitis*) *Dirofilaria repens* (*D. repens*), *Dirofilaria striata*, (*D. striata*) *Dipetalonema reconditum* (*D. reconditum*), *Dipetalonema grassii* (*D. grassii*), *Dipetalonema dracunculoides* (*D. dracunculoides*), *Brugia pahangi* (*B. pahangi*), *Brugia malayi* (*B. malayi*) and *Brugia pateti* (*B. pateti*) etc. Most of these have been reported to occur in some mosquito species such as are found in Kenya (Soulshy, 1982). With the exception of *B. pahangi* (Snowden, Hammerberge and Smallwood 1986), *D. immitis* is the only filarial parasite of dogs causing clinically significant disease. (Soulshy, 1982; Rawlings, 1986, Knight 1987).

The adult forms of *D. immitis* typically lodge in the pulmonary arteries and the right chamber of the heart hence the common name heartworms. In contrast to the heartworm, the adults of the other filariids do not reside in the cardio-pulmonary system. Instead, they have been reported to occur commonly in the subcutaneous tissue or in the peritoneum. However, their microfilariae, like those of *D. immitis* inhabit the blood stream (Soulshy, 1982). It is the microfilariae of *D. reconditum* and, in few cases, those of *D. repens* and *D. dracunculoides* that have been

encountered co-existing with those of *D.immitis* in several heartworm disease surveys (Martin and Collins, 1985; Owen and Slocombe, 1990; Ortega-Mora *et.al.*, 1991; Patton and Faulkner, 1992).

2.1.2 Morphology of adult *D. immitis*

Adult *D.immitis* (male and female) are slender and white in colour. The worms are relatively large, males measuring 15-19 cm. and females 25-31 cm. long (Fig.1) The oesophagus of the worm is 1.25-1.5 mm. long.



Figure 1 Male and Female adult heartworms. The male is smaller and has a spiral tail.

Source: Knight (1987)

The male (Fig.1) is identified further by the spirally coiled hind end with the tail bearing small lateral alae. In this part, (Fig.2), there are also four to six, but usually five, pairs of ovoid papillae, of which one pair is post-cloacal, two pairs of finger shaped papillae lateral and posterior to the cloacal opening, and three to four pairs of small conical papillae near the tip of the tail. The left spicule is 0.324-0.375mm. long and pointed, the right is 0.19-0.229mm. long and ends bluntly. The female (Fig.1), on the other hand, has a straight hind end while its anterior end bears a vulva situated just behind the end of the oesophagus (Fig.3).

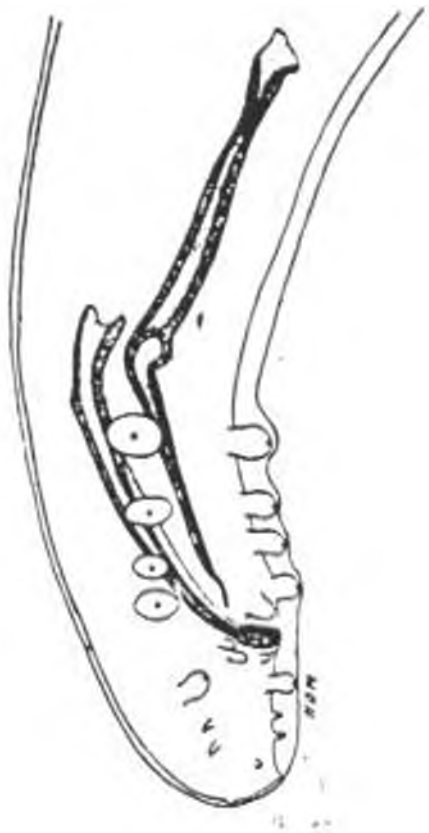


Figure 2. Hind end of male adult *D. immitis*
Source: Soulshy (1982)



Figure 3. Anterior end of female adult *D. immitis*
Source: Soulshy (1982)

2.1.3 Morphology of microfilaria of *D. immitis*

Adult female *D.immitis* are ovoviviparous and release vermiform, unsheathed embryos known as microfilariae into the blood circulation. These (Fig.4) have long, slender tail and are motile. *D. immitis* microfilaria measure between 286 and 340 μ in length with a mean of 314 μ (Knight, 1987). However, *D.immitis* microfilariae measuring as short as 276 μ and as long as 432.4 μ have been observed (Boreham and Atwell, 1985).

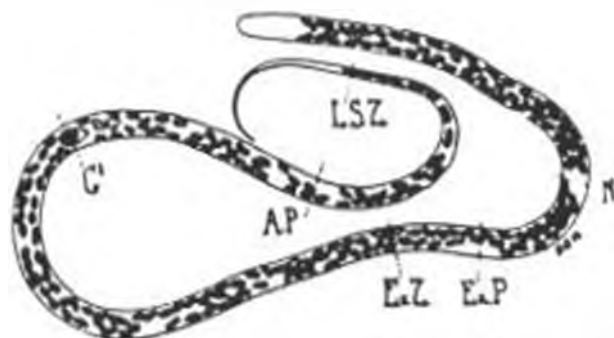


Figure 4. Microfilaria of *D. immitis* in a Knott preparation. The head (arrow) is tapered and the tail is straight.

Source: Knight, (1987)

It has become a custom to express the distance of certain fixed points from the anterior extremity as percentages of the total length when the size of a particular microfilaria species is being described. The fixed points used are: (Fig. 5) the position of the nerve ring (N), the excretory pore (EX.P), the excretory cell (EX.Z), the first genital cell (G.1), second genital cell (G.2), third genital cell (G.3), the

anal pore (A.P) and the last nucleus or last tail cell (L.S.Z.). Variations in mean total length and distance of these fixed points (Table 1) are used as criteria for identifying the species of a particular microfilaria



The fixed points for measurement of *Dirofilaria immitis* microfilariae.

- | | |
|----------------|--------------------|
| A.P. | anterior pore |
| Ex.P. | excretory pore |
| Ex.Z. | excretory cell |
| G ¹ | first genital cell |
| L.S.Z. | last tail cell |
| N. | nerve ring |

Figure 5. Microfilaria of *D. immitis* drawn to show position of fixed points.

(Source: Soulsby, (1982))

Table 1. Measurements (μ) of *Microfilaria* 'fixed points' for different filaria species of dog.

Species	N	Ex.P	Ex.Z	G.I.	A.P	L.S.Z	Range	Mean
<i>D. immitis</i>	23.8	32.7	38.6	67.9	82.0	92.9	107-122	313
<i>D. reconditum</i>	20.8	21.0	34.5	70.1	80.7	89.0	246-293	270.6
<i>D. repens</i>	23.0	30.0	33.0	62.5	70.0			290-10
<i>B. malawi</i>	24.5	35.0	40.0	64.0	83.0			220±20
<i>B. palei</i>	similar	to	<i>B. malawi</i>					
<i>B. pahangi</i>	22.0	31.0	33.0	68.0	80			280
<i>D. dracunculoides</i>	45*	66-70*			53-55+	20+	195-230	
<i>D. grassi</i>	-	-		-		-		570

*Measured from anterior end. + measured from posterior end Source: Soulshy, (1982)

2.1.4 Life cycle of *D. immitis*

The life cycle of *D. immitis* (Fig 6) has been described by Soulshy (1982), Kotani and Powers (1982), Rawlings (1986) and Knight (1987). Although the vector involved in the transmission of the disease, the mosquito, was discovered in 1901, the life cycle was not fully understood until fifty two years later (1953).

Molting and exsheathment of *D. immitis* occurs four times during the life cycle. The first and second molts occur in the mosquito while the third and fourth occur in the vertebrate host.

Transmission of infection depends on the ingestion of first stage larvae (L_1) microfilariae (in the blood meal) by a mosquito species capable of serving as an intermediate host. The ingested L_1 then burrow through the midgut to the malphigian tubules where, after two molts, infective third stage larvae (L_3) migrate

back to the mouthparts of the mosquito. The sequence of events, from infection to development of patency in the definitive canine host is shown in Table 2. The development of infective stage (L₃) can be completed within 10 to 14 days with ambient warm temperatures. In the cooler environments maturation to L₃ may be delayed up to 31 days. When mosquitoes take a blood meal they deposit the L₃ found in the saliva into the haemolymph produced by the puncture wound in the dog's skin. The larvae then find their way into the body of the dog through this bite wound.

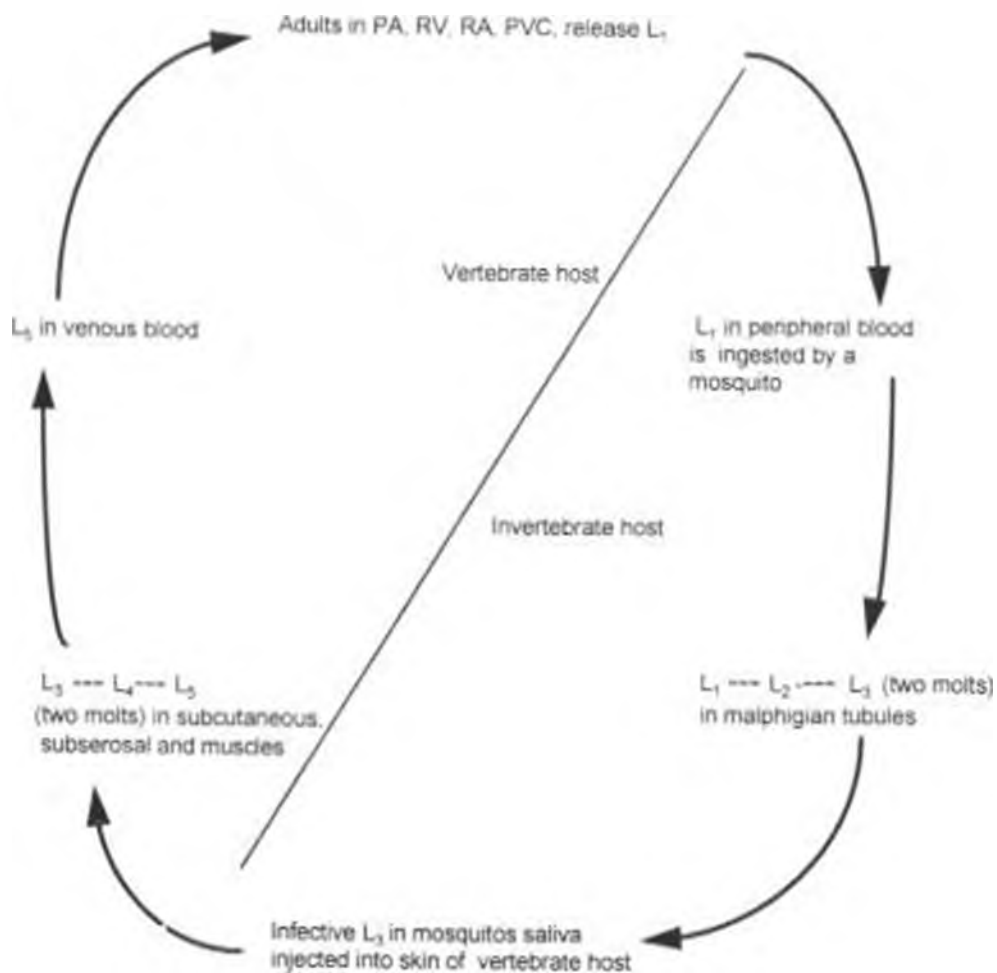


Figure 6. An illustration of the life cycle of *D. immitis*

PA: Pulmonary artery, RV: Right ventricle, RA: Right Atrium, PVC: Posterior vena cava. Modified from Rawlings, (1986)

Table 2. Time table for development of *D. immitis*

MOSQUITO INTERMEDIATE HOST L ₁ to L ₃ (Infective Larvae)	Days post-infection 10-14 (optimal conditions)
DEFINITIVE CANINE HOST	Days post-infection
precardiac	
L ₃ -L ₄ (third molt)	3-4
L ₄ L ₅ (fourth molt)	50-70
Cardiac	
L ₅ in the right ventricle and pulmonary artery	70-110
Patency (Microfilaraemia)	190 minimum

Adopted from Knight (1987)

The molt from the third to the fourth larva. stage occurs as early as 3 days postinfection. The fourth stage larvae then begin a subcutaneous migration toward the thorax. The fourth and final molt to the fifth (adult) stage occurs between 50 and 70 days postinfection. The juvenile fifth stage appears to have greater capacity for tissue penetration and, prior to their arrival in the pulmonary arteries between 70 and 110 days postinfection, they are found with increasing frequency in the skeletal muscles. Although the specific route of migration is ill-defined, access to the circulation appears to be via penetration of systemic veins. Once therein, the 2.5 cm long parasite is embolized to the pulmonary arteries. Young adults initially go to the caudal lung lobes, especially of the right side. Male and female worms reach maximal body length 5 months and 8 months respectively after infection. After additional three months, sexual maturity is attained. Females become gravid by 5 to 6 months. This results in the presence of circulating microfilariae (L₁) 6 to 7 months post-infection.

2.2 Epidemiology of *D.immitis* infection

Historically Canine heartworm disease was considered a condition of tropical and subtropical environments, however the disease is now recognised to be a worldwide problem (Knight, 1987). The heartworm parasite is known to commonly occur in coastal regions of the world, (Soulsby 1982; Carlisle and Atwell, 1984; Knight, 1987; Macy *et.al.*, 1991; Ortega-Mora *et.al.*, 1991;) It is most prevalent in Japan, Australia and the United States (Knight, 1983) and is also recognised as a major health problem of dogs in several European countries (Ortega-Mora *et.al.*, 1991), Canada (Owen and Slocombe, 1989) and Middle East (Tarish *et.al.*, 1986). Prevalance of *D.immitis* in dogs vary greatly between countries (Table 3) and even between different localities in the same country. For example, the prevalence of this parasite in dogs in Australia was reported (Carlisle and Atwell, 1989) to be less than 7% in Sydney and Victoria while some areas like Queensland and Darwin recorded up to 100% prevalence rates. Likewise, in the United States (Macy *et.al.*, 1991), a prevalence rate as high as 45% was reported in some areas of the Atlantic Coast and along the Mississippi and Ohio rivers, while in most other parts of the country the prevalence is considered to be less than 5%.

The General opinion currently held among workers (Matic and Herrtage, 1987; Knight, 1987; Russell, 1990; Foreyt and Lagerquist, 1991) is that canine heartworm disease is on the increase because of the reduction in Mosquito control as a consequence of widespread environmental concern about pesticides and also increased movement of infected dogs from endemic areas. Canine dirofilariasis is now being reported in the United Kingdom, (Matic and Herrtage, 1987), Israel (Zur and Bark, 1992), Republic of South Africa (Van Heerden *et. al.*, 1980; Verster *et.al.*, 1991) and Mozambique (reviewed by Verster *et.al.*, 1991), where the disease was formerly considered absent.

Increase in prevalence of canine heartworm disease has also been attributed to employment of ineffective chemoprophylaxis (Owen and Slocombe, 1990; Russell, 1990)

Table 3. Mean Prevalences of *D immitis* in dogs in some countries in the world

County	Prevalance (%)	Source
Japan	Up to 50	Ishihara <i>et.al</i> (1978)
Australia	> 30	Collins <i>et.al.</i> (1987)
United States	45	Macy <i>et.al.</i> , (1991)
Italy	24	reviewed by Ortega-Mora <i>et al.</i> , (1991)
Iraq	15	Tarish <i>et.al.</i> , (1986)
Spain	3.7	Ortega-Mora <i>et.al.</i> , (1991)
France	0.73	reviewed, Ortega-Mora <i>et.al.</i> , 1991
Canada	0.17	Owen and Slocombe (1990)

Perpetuation of the heartworm life cycle and prevalence of dirofilariasis in a given area depends mainly on the level of interaction between the reservoir of infection, the mosquito vector and a suitable definition host (Knight, 1987). Although the domestic dog (*Canis familiaris*) is a principle reservoir of *D immitis* (Soulshy, 1982; Knight, 1987), and other mammals probably acquire infection from mosquitoes that have fed on dogs, wild canidae may also become microfilaraemic and therefore capable of sustaining infection. As an example heartworm infection is reported to be well established in coyotes and red foxes residing in endemic areas (Knight, 1987).

Cats on the other hand have a very limited reservoir potential (Knight, 1987., Kendall et.al., 1991) and other hosts capable of supporting potent infections such as ferrets and the California Sea lion are too environmentally isolated to play a significant role. Horses, monkeys (Soulshy, 1982) and human beings (Alvarez et.al., 1990; Akao, Kondo and Fujita, 1991) are also affected but are considered to be aberrant hosts, incapable of serving as a reservoir of infection as they never become microfilaraemic (Soulshy, 1982; Knight, 1987). Vector competence is a multifactorial consideration affected by both intrinsic and extrinsic factors: among the former are included the mosquito host preferences, their ability to become infected and therefore transmit the filaria, (Sulaiman and Townson, 1980) and also the vectors longevity (Russell, 1990). The extrinsic factors include density of both vector and vertebrate populations, and environmental conditions.

There are reportedly approximately 3000 species of mosquitoes, out of these fewer than 70 have been identified as potential vectors of *D.immitis* (Knight, 1987, Russell, 1990). Even amongst these, only a few are considered effective vectors under natural conditions. For example, of the 28 susceptible species in the United States, *D.immitis* infective larvae have been found in the mouth parts of only 14 species collected in the field (Knight, 1987). Generally, mosquito species of genera *Aedes* (*Ae.*), *Culex* (*Cu.*), *Anopheles* (*An.*) and *Mansonia* can transmit *D. immitis*. Competent species have been found to be *Ae.aegypti*, *Ae.vigilax*, *Ae.notoscriptus*, *Ae.taeniorhynchus*, *Ae.sollicitans*, *Cu.pipiens quinquefasciatus*, *Cu.annulirostris*, *Cu.australicus*, *An.quadrifasciatus*, *An.annulipes* and *An.bradleyi* (Sauerman and Nayar, 1985, Bradley and Nayar, 1987; Russell, 1990; Parker, 1993). However, additionally, mosquito susceptibility to filarial infection is known to vary between as well as within the species (Sulaiman and Townson, 1980; Townson and Chaithong, 1991) and even between mosquitoes of same strain inhabiting different geographical locale (Knight, 1987).

In some mosquito species, the presence of a proventricular armature (e.g. *Cu. nigripalpus*) or the rapid coagulation of blood in the midgut (e.g. *Cu. pipiens*) prevents the majority of microfilariae from surviving ingestion or leaving the midgut. In other mosquitoes (e.g. some strains of *Ae. aegypti*) the microfilariae enter the malphigian tubules but fail to develop (Sauerman and Nayar, 1985). To be a successful vector of heartworm infection mosquitoes must readily feed on dogs. In most mosquito species a definitive host preference is present that is determined genetically (Takken, 1991). For instance, many culex species are ornithophilic whereas most Anopheles species feed on higher mammals. Indeed, some species feed on one kind of host only. Only female mosquitoes are blood sucking, a blood meal being a prerequisite for egg production (Knight, 1987, Takken, 1991). Those having a long breeding season and producing many clutches of eggs are known to feed repeatedly, thus increasing their importance as vectors. Some mosquito species for example *Ae. vexans* are known for their extensive flight ranges, an additional factor enabling them to greatly disseminate infection (Knight, 1987). This implies that even dogs living several miles from mosquito breeding site may be at considerable risk of *D. immitis* infection depending on the flight capability of the local vectors.

The relative importance of a mosquito species as vectors of *D. immitis* usually is based on two factors; relative abundance and the incidence of L₃ infection in mosquitoes. Chances of *D. immitis* transmission are increased by high densities of female mosquitoes susceptible to infection by the parasite. Since infected mosquitoes usually carry only one to three infective larvae (Knight, 1987), and constitute a relatively small percentage of the vector population, the rate of transmission is proportional to the density of mosquitoes and the number of bites they inflict. Risk of infection is therefore greatest in dogs that live outdoors because of being constantly exposed to potential mosquito vectors (Martin and Collins, 1985; Russell, 1990).

Generally, mosquito abundance is affected by temperature and availability of breeding habitats (Russell, 1990). Harsh conditions that weaken the mosquito and delay maturation of the infective larvae can depress transmission rates. Conversely, weather favourable to mosquito breeding may precede an increase in heartworm transmission (Knight, 1987). Insanitary conditions inherent in most developing countries especially due to improper disposal of waste water and the use of pit latrines, provide favourable breeding environments for mosquitoes (Barnish, 1984). Swamps, uncontrolled bushes and proliferation of irrigated agriculture (Russell, 1990) are usually associated with high mosquito densities. Natural habitats such as banana leaf axils or tree holes and coral rocks are common mosquito breeding places along coastal regions (Subra, 1981).

High average temperatures are reportedly necessary for the completion of larval development in the mosquito (Soulsby, 1982). Maturation to L₃ is temperature dependent and therefore a sustained drop in temperature below 60 F (15.6 °C) has been considered lethal to the developing larvae in mosquitoes (Matic and Herbage, 1987). However, recent studies (Townson and Chaitong, 1990) show that *D. immitis* microfilariae can survive greater extremes in temperature. Cryopreservation of L₃ is also possible (Minjas and Townson, 1980) but the infectivity of larvae in mosquitoes emerging from dormancy has not been determined.

Although the percentage of mosquitoes that become infected has been found to be directly proportional to the microfilaria densities in the carrier at the time of feeding (Samarawickrema *et al.*, 1985), moderate levels of microfilaraemia in the vertebrate host are more likely to lead to successful transmission of dirofilariasis than either very low or very high concentrations (Gillett, 1971; Knight, 1987). Dogs with microfilaraemias on the order of 20,000 microfilariae per millilitre or greater are

less efficient reservoirs of *D. immitis* infection than those with lower concentration (Knight, 1987). If the mosquito ingests few microfilariae these may be killed or digested along with a blood meal. If on the other hand there are very many microfilariae then the mosquito may die through gross damage to the wall of the gut (Gillett, 1971; Knight, 1987; Bradley and Nayar, 1987). In those engorged mosquitoes that survive a high intake of microfilariae, very few larvae actually complete development and become infective (Knight, 1987). On the other hand, mosquito may rid itself of all its filarial load and thus become non infective again (Gillett, 1971). This sometimes happens during a single feed. Newly acquired microfilariae may also be excreted as a bloody "diarrhoea" (Gillett, 1971).

D. immitis periodicity in peripheral venous blood of dogs is extremely variable and the patterns are so unpredictable that the classic terminology (nocturnal vs diurnal) cannot be applied consistently (Rawlings, 1986; Knight, 1987).

While susceptibility to heartworm disease is not significantly affected by the breed, sex or nature of the hair coat of the dog, there is a relationship between the age of the dog and development of the disease (Martin and Collins, 1985; Rawlings, 1986; Knight, 1987; Perez-Sanches *et.al.*; 1989; Tanaka and Atwell, 1991). It has been observed that *D. immitis* burdens generally increase with years of exposure. However, dogs aged between 3 and 10 years are the ones found frequently infected with heartworms in comparison to younger or older dogs (Perez-Sanches *et.al.*, 1989; Tanaka and Atwell, 1991).

Dogs living within endemic areas tend to have a relatively constant mean number of adult *D. immitis* as additional worms accumulate at a decreasing rate in such dogs that are exposed annually for several years (Knight, 1987). This suggests that there is development of immunity against *D. immitis* in such dogs. There is experimental evidence that protective immunity can function against larval

D. immitis (Grieve *et.al.*, 1988). As an example, dogs have been made immune to infection both by infecting with radiation-attenuated larvae and by chemically abbreviated infections (Scott, Ibrahim and Tamashiro, 1990; Frank and Grieve, 1991).

Transplacental transfer of microfilariae from *D.immitis* infected bitches to their puppies is possible (Rawlings, 1986; Knight, 1987) but microfilariae counts in this case are low, usually less than 30 per millilitre and seldom persist beyond 6 to 8 weeks (Knight, 1987). However, the immunological implications of *D.immitis* prenatal infections subsequent infections have never been determined.

2.3 Pathophysiology

The dog's response to *D.immitis* infection has been described and reviewed in considerable details (Rawlings, 1986; Knight, 1987; Buoro, 1988). Dirofilariasis is a multi organ disease, but with major pathological lesions being recorded in the cardiopulmonary system, kidneys and liver. Unusual migration of the heartworm may occur to such ectopic sites as the nervous tissues, muscles, skin and subcutis and systemic arteries where they are associated with various organ-related lesions. Except for cases of glomerulonephritis and allergic pneumonitis, the effects of heartworm infection are induced by adult worms only. Although the spectrum of heartworm disease presentation is broad, the common forms of its presentations are: corpulmonale, occult dirofilariasis, pulmonary pneumonitis, liver failure haemoglobinuria and nephropathy with proteinuria.

Heartworms have a predilection for the pulmonary arteries and are not ordinarily found in the chambers of the heart or systemic veins in living dogs. In heavy burdens, for example 50 heartworms in a 25 kilogramme dog, the worms extend

from pulmonary arteries into the right ventricle. Dogs with infection in excess of 50 heartworms commonly also have these worms in the right atrium, and as the number increases, they may extend into the caudal vena cava. Heartworms are found commonly in the right ventricle of dead dogs, due to postmortem incompetence of the pulmonary valve.

2.3.1 Cor Pulmonale

The primary lesions in canine heartworm disease are found in the pulmonary arteries and the lung parenchyma. The earliest lesions are limited to the small peripheral caudal pulmonary artery branches where the worms first come to rest. However, as the parasite grows, lesions also occur in the more proximal segments.

Contact between the parasite and the intima of the pulmonary arteries appear to be an important initial step towards the development of the endovascular lesions. Although the mechanisms leading to endothelial damage are not well understood, trauma is an obvious cause and the sequence of events follow the normal response to injury. Lesions are restricted to areas of heartworm attachment and are evident by scanning electron microscopy within 4 days of deposition and angiographically by 90 days post-infection.

Following trauma, the endothelial cells become swollen and develop wide intercellular junctions. Activated neutrophils and macrophages adhere to the endothelial surface and then linear strips of endothelium slough to expose the subendothelium. This stimulates platelets adhesion and activation. Although platelets adherence is vital in preventing extravascular blood loss, in some heartworm infected dogs major endothelial disruption can occur and therefore platelets participation is magnified (Boudreaux *et al.*, 1989). Platelets adherence and aggregation induce formation of thrombi. They additionally release a variety of

performed vasoactive substances. Leucocytes, particularly eosinophils constitute the major cellular part of the early lesion but later, as the lesion becomes more exuberant and coalesce, fibrosis becomes a prominent feature. Like platelets; the endothelial cells, vascular smooth muscles and macrophages secrete a fibroblast growth factor that may be responsible for the fibrosis of mature lesions.

Endothelial changes are followed by thickening of the intima, depolymerization of the internal elastic lamina and further invasion of the vascular wall by leucocytes. Beneath the affected areas, smooth muscles multiply within the tunica media and start to migrate toward the endothelial surface. This migration causes proliferation of the intima to form discontinuous rugose ridges with villous appearance. These villi-like structures are considered pathognomic for heartworm infection. Their number and complexity, are in direct relationship to the duration and severity of heartworm infection. However, these lesions remain vascularized and do not develop accumulations of cholesterol, fatty acids and calcium.

Further proliferation and encroachment of villi into the vascular lumen obstruct blood flow in the small arteries. On the contrary, the large distributing arteries actually dilate as pulmonary hypertension becomes increasingly severe. Dogs in end-stage heartworm disease with severe pulmonary hypertension also have hypertrophied arterioles. Since the worms never occupy vessels that are this small, arteriolar hypertrophy is considered to be a vascular response to regional increases in blood flow, resulting from widespread diversion of blood from obstructed arteries rather than a response from a direct contact with parasites.

Pulmonary blood flow is impeded primarily by the reduction in cross-sectional area of the arterial vascular bed, caused by obliterative endarteritis of small peripheral branches. Further obstruction may be a result of thrombosis and thromboembolism

(Hirano *et.al.*, 1992) In larger vessels, the lesions increase the thickness of arterial walls which become rigid and stiff. The decreased distensibility of the large arteries and obstruction in smaller arteries lead to pulmonary hypertension. The presence of heartworms in the pulmonary artery per se does not appear to cause any significant interference with blood flow.

Under normal circumstances, pulmonary arteries in the proximal portion of the lung remain partially perfused and are put in full use only when there is high demand for blood flow, for example during exercise. As heartworm infection impedes flow in increasing number of branches, the pulmonary vascular reserve diminishes. Eventually, the pulmonary arterial tree assumes the features of a system of rigid tubes and pulmonary vascular resistance becomes fixed. Consequently, more cardiac work is required in a severely affected dog before it can be active.

The right ventricle of the heart dilates, as a compensatory response to the increased load and in an effort to meet the cardiac output requirements. Ventricular dilation increases end-systolic volume, and this results in stretching of the free wall. When the ventricular radius increases, the tension within the wall also increases until when the ventricle cannot dilate further. At this stage, the ventricle must hypertrophy for it to cope with greater pulmonary vascular resistance and maintain the required cardiac output. The hypertrophied right ventricle apparently increases muscle mass more than it does to the coronary blood supply. In advanced cases of heartworm disease, therefore, ventricular aschemia develops which ultimately may lead into a low output congestive heart failure, as a result of the right ventricle's inability to generate and sustain the high perfusion pressures required to move blood through the lung. Dogs at this stage may experience syncope when over exercised. Thus, hunting dogs with severe heartworm disease are well known for exercise intolerance which renders them useless.

2.3.2 Occult dirofilariasis

Occult dirofilariasis defined as infection with adult heartworm but without detectable circulating microfilariae is reported to occur in about 10-67% (average of 25%) of all heartworm infections in dogs (Rawlings, 1986; Knight, 1987). Failure to develop microfilaraemia may be due to one of the following causes: prepatent infections, presence of unsex adult worms in light infections, suppression of microfilariae with microfilaricides, or immune-mediated clearance of microfilariae. About one third of amicrofilaraemic dirofilariasis cases are of the immune mediated type (Knight, 1987). The other forms of occult dirofilariasis seldom produce the disease and are therefore of less pathological significance. Gravid heartworms in immune-mediated occult infections release microfilariae but these are eliminated from circulation by the microfilarial-stage-specific hypersensitivity in the host. In such situations microfilaraemia does not develop or microfilariae disappear spontaneously after a variable period of patency (Knight, 1987).

There is some evidence that dogs which have been frequently exposed to heartworm infections have lower and shorter subsequent microfilaraemia compared with those on their first or with only few encounters (Knight, 1987). Grieve *et.al* (1986), observed the highest proportion of occult dirofilariasis in dogs older than 12 years as compared with younger ones. A single exposure to *D. immitis* infection may be adequate to sensitize the host (Knight, 1987). Live microfilariae are highly antigenic, and heartworm naive dogs can be sensitized by multiple intravenous inoculations of microfilariae (Grieve *et.al*, 1988; Carlisle *et.al.*, 1989, Frank and Grieve, 1991). In immune-mediated occult dirofilariasis, the microfilariae released become entrapped in the pulmonary capillary which is the first microvascular bed that the microfilariae traverse (Rawlings, 1986; Knight; 1987, Carlisle *et.al.*,

1989) In traversing the capillary bed, the passage of microfilariae is slowed and their motility impaired, thus facilitating contact with cells of reticuloendothelial system which ultimately sequester and destroy them.

Wong *et al* (1973), observed that the entrapped microfilariae were initially surrounded by polymorphonuclear cells, predominantly eosinophils, but later as microfilariae degenerated, the reaction became granulomatous with mainly mononuclear and giant cells

Eosinophils concentration may be the result of a primed immune response from adult heartworms or tissue invasion by their larvae or perhaps a more complex immune response. Neutrophils may be more important in both these responses as together with immune sera, they have been found to be cytotoxic to *D. immitis* microfilariae. The first stage larvae of *D. immitis* have a discontinuous outer cuticular layer forming crypts where neutrophils attach and release hydrogen peroxide and other products of degranulation which kill the microfilaria (Sutton, 1989). It has been hypothesized that IgM antibodies that are found localized in the crypts are directed against metabolic products which occur as globules in these crypts (Knight, 1987).

Crypts appear to be areas where the microfilaria is most vulnerable to neutrophil attack. Destruction is by antibody dependent cell cytotoxicity which is further enhanced by participation of complement. The importance of antibody in this case is demonstrated in the Pandit reaction whereby cells from uninfected, patent and occult dogs adhere equally well, but only occult serum is capable of promoting substantial *in vitro* adherence. The antibody responsible for the reaction is not only stage-specific but also filarial species specific. Both IgG and IgM are capable of participating in the reaction, but their relative importance is uncertain (Knight, 1987).

2.3.3 Allergic Pneumonitis

The sequestration and destruction of large numbers of microfilariae in the pulmonary capillary bed and alveolar septae of dogs with immune mediated occult dirofilariasis may result in severe allergic pneumonitis or an alveolar pattern presenting as severe respiratory distress.

This is similar to tropical (pulmonary) eosinophilia, or eosinophilic lung, a syndrome in human filariasis that resembles asthma. Recent studies have opposed the earlier held suspicion that the condition in human beings was due to infection by *D. immitis* (Knight, 1987). However, in heartworm infected dogs, like in human tropical eosinophilia subjects, the pathogenesis of allergic pneumonitis is attributed to the infiltration of eosinophils that surround the degenerating microfilariae in the pulmonary capillaries (Knight, 1987). In long standing occult heartworm disease, an interstitial pattern also may develop in the lung (Rawlings, 1986). Severe as it may be, allergic pneumonitis is a relatively uncommon presentation in dogs with heartworm disease (Knight, 1987).

2.3.4 Caval Syndrome

Liver failure was the original name for vena cava syndrome. It is less frequent in occurrence but represents a severe form of heartworm infection in dogs. Commonly, this syndrome affects dogs less than 3 years old, particularly those living in endemic areas, and has been induced experimentally by giving a heavy inoculum of 200 infective larvae. These observations imply mass migration of heartworms with rapid accumulation taking place commonly in dogs that have acquired little or no protective immunity at the time of infection. Naturally affected dogs are found with heavy heartworm infections usually in excess of 100 adult worms per dog (Rawlings, 1986; Knight, 1987; Buoro and Atwell, 1984; Buoro,

1988). In heavy infections with heartworms, adult worms occupy also the right atrium, venae cavae and hepatic veins and produce clinical signs recognized as the liver failure or caval syndrome. This is characterized by an acute onset and haemolysis causing gross haemoglobinuria and rapid death in cases not treated aggressively (Rawlings, 1986; Knight, 1987; Buoro, 1988).

It is not known why a majority of heartworms cluster in the right atrium and vena cavae but these may represent an overflow habitat for the large number of worms that are usually involved. However, the caval syndrome may also occur in association with low number of worms in the pulmonary arteries (Knight, 1987).

Although the pulmonary vascular disease precedes the caval syndrome, the most serious damage occurs in the liver. Changes in the liver are characterized by centrilobular congestion, with active, chronic phlebitis of the veins of the liver, which undergo fibrosis. Inflammatory cells, especially eosinophils infiltrate in and about these lesions. Adjacent hepatocytes may undergo necrosis in severe cases. Hepatic veins undergo cavernomatous changes with many dilated vessels replacing the centrilobular veins, these occur in conjunction with impaction of worms in posterior vena cava (Rawlings, 1986; Knight, 1987; Buoro, 1988).

Typically central venous pressure is elevated due, at least in part, to tricuspid valve regurgitation caused by accumulations of worms at the valve orifice. Thus, liver damage initiated by contact with worms may be worsened by passive congestion. Liver function can also be marginally depressed by chronic passive congestion secondary to the cardiopulmonary disease (Knight, 1987).

The caval syndrome has been described as an acute episode of intravascular haemolysis. Anaemia is a main feature in most, but not in all dogs with this syndrome. The anaemia is typically normochromic and normocytic, regenerative.

as both erythroblasts and reticulocytes are present in peripheral blood. Haemolysis is usually so great that pronounced renal haemosiderin deposits are seen in dogs with caval syndrome. Many affected dogs become icteric and have haemoglobinuria (Rawlings, 1986, Knight, 1987; Buoro, 1988). Haemoglobinuria is considered pathognomonic for the caval syndrome (Rawlings, 1986). Although a variable degree of haemolytic anaemia occurs also in chronic pulmonary heartworm disease (Kitagawa *et.al.*, 1992), haemoglobinuria is not a common feature in this case.

Disseminated intravascular coagulation (DIC) may occur in dogs with caval syndrome. But contrary to earlier propositions on the role of DIC in the mechanism of caval intravascular haemolysis, current evidence indicates that DIC occurs only coincidentally with caval syndrome (Rawlings, 1986; Knight, 1987). Dogs with caval syndrome have higher incidences of positive Coomb's reaction than do the heartworm infected dogs (Rawlings, 1986). However the Coomb's reaction does not correlate with anaemia which accompany caval syndrome, suggesting that immunological factors are not involved (Rawlings, 1986; Knight, 1987).

Increased osmotic and mechanical fragility of the red blood cells due to physical collision with heartworms, is the most likely explanation for the haemolysis. Following liver damage and dysfunction, less free cholesterol is esterified and the free cholesterol: ester ratio rises. Since cholesterol and phospholipids in the red cell membranes are exchangeable with plasma lipoproteins, the erythrocyte accumulates free cholesterol and becomes more fragile. Such cells become vulnerable to trauma which they encounter when passing through the tangle of worms impacting the heart and great vessels (Rawlings, 1986, Knight, 1987, Buoro, 1988).

2.3.5 Kidney disease

Kidney pathology is a common, though not inevitable sequela of a canine heartworm infection. Lesions reported include immune complex glomerulonephropathy, glomerulosclerosis, chronic interstitial nephritis, and amyloidosis (Rawlings, 1986; Knight, 1987). These lesions appear to be produced by live heartworm infections, in contrast to those produced by administration of adulticide and microfilaricide in infected dogs. Severity of kidney disease varies considerably, probably due to either the individual dog's immune competence or the level of infection. Renal dysfunction may also be produced by the heartworm-induced conditions of congestive heart failure and vena caval syndrome. Most of the evidence points to the glomerulus as the primary site for heartworm-related renal disease. However, it is the immune complex glomerulonephropathy that has received a fairly good coverage in studies compared with other renal lesions in canine heartworm disease. Yet, the incidence of renal disease attributable to heartworm infection remains unknown perhaps because the accompanying renal failure is insignificant (Buoro, and Atwell, 1983).

2.3.5.1 Immune-complex glomerulonephropathy

With the advent of new and more efficient diagnostic techniques, it has become clear that lesions in the glomerular basement membrane are mediated by the deposition of immune complexes (Rawlings, 1986; Knight, 1987; Nakagaki *et al.*, 1990; Nakagaki *et al.*, 1993). Nakagaki *et al.* (1990), reported varying degrees of glomerulonephritis in more than half of dogs infected with *D. immitis*. However, its pathogenesis has not yet been worked out in detail.

High levels of circulating immune complex (CIC) have been observed in dogs infected with *D.immitis* (Knight, 1987; Nakagaki *et.al.*, 1990) IgG, IgM and complement (C) have been demonstrated within the basement membrane by immunofluorescence as coarse granular patterns. The basement membrane is usually uniformly thickened undulated and with segmental splitting. As a result, glomerular capillary lumina become narrow. Additionally, narrow bands connecting the glomerular capillary endothelium with the cuticular indentations on the microfilariae can be viewed by electron microscopy. As in the case of microfilaricidal effects of activated neutrophils in immune-mediated occult dirofilariasis, the crypts provide an avenue for contact between the host and the metabolically active substances of the parasite. Although these have not been shown to be sources from which parasite antigens are deposited in the glomerular capillary basement membrane, the findings seem to link microfilariae even closer to the pathogenesis of the glomerular lesions (Knight, 1987).

Since the quantity of CIC appeared to be directly related to the intensity and duration of microfilaraemia, microfilarial antigens were thought to be involved (Knight, 1987; Nakagaki, *et.al.*, 1990) though to date no study has demonstrated the microfilarial antigens in the immune complexes. Moreover, some amicrofilaraemic dogs also have presented with glomerulonephritis and deposits of IgG in the kidney (Nakagaki *et.al.*, 1990). Additionally IgG deposition has not been demonstrated in about 25% of heartworm infected dogs with kidney lesions.

To what extent larval and adult heartworm antigens contribute to the formation of the immune complexes in the kidney therefore remains a research challenge. Adult heartworms are certainly a major source of circulating antigens, and their participation in the process is by no means insignificant. Recently it was found that CIC levels were significantly related to the adult worm burden (Nakagaki *et.al.*, 1990). Review by Nakagaki *et.al.* (1993) indicates that both large and small

immune complexes containing antigens are present in circulation of heartworm infected dogs. It was also found that large immune complexes are ingested by phagocytic cells in the circulation but that small ones were not phagocytized. Instead, they were deposited in the capillary walls.

2.3.5.2 Other Renal Lesions

Other renal lesions associated with heartworm infection include glomerulosclerosis, interstitial nephritis and amyloidosis and have been reviewed by Rawlings (1986)

Glomerulosclerosis in canine heartworm disease is characterized by intimal and medial changes associated with hyperplasia and infiltration of inflammatory cells. The lesion severity correlated well with microfilarial counts. The vascular lesions were speculated to be produced by the endothelial trauma from a large number of microfilariae and not by an immune response. However, some of these observations may have occurred due to old age.

In interstitial nephritis due to canine heartworm disease, there is usually infiltration of plasma cells, lymphocytes and macrophages in the cortical and medullary interstitium presenting as both focal and diffuse lesions. In one study, interstitial nephritis was present in 55% of dogs with heartworm infection and 59% of these dogs were over 4 years of age. Heartworm-induced interstitial disease can also be mediated by immune reactions.

Amyloidosis can be primary or secondary to chronic antigenic stimulation such as occurs in heartworm disease. However, no direct correlation of amyloidosis with sustained heartworm antigenic stimulation has been confirmed. When it occurs, amyloidosis produces a protein losing glomerulopathy which is associated with poor prognosis.

2.4 Clinical signs of heartworm disease

According to the descriptions and reviews by Rawlings (1986) and Knight (1987), the clinical signs of heartworm disease in dogs vary widely depending on the number of infecting adult heartworms, activity and immune status of the patient and the duration of infection. Ordinary house dogs may never show clinical signs except under heavy worm burdens. On the other hand, even a few heartworms may obviously compromise the performance of field or working dogs. Besides, signs of heartworm disease resemble those of other diseases and are therefore not pathognomonic.

In dogs with chronic pulmonary heartworm disease the onset of clinical signs is usually insidious. In all symptomatic dogs, coughing and dyspnoea are the most common signs. These become severe when the dog is exercised. Field or working dogs with heartworm disease have decreased exercise tolerance. Deep chest coughs may be a result of oedema around the pulmonary arteries caused by inflammation and also dead adult worms which subsequently cause thromboembolism and parenchymal disease around the airways. This reaction should be irritating enough to produce a cough. Laboured breathing may be due to extensive consolidation and fibrosis of the caudal lung lobes.

Less frequent clinical signs associated with dirofilariasis include haemoptysis and syncope. These are seen only in cases with advanced pulmonary vascular disease. The volume of blood loss in haemoptysis may be underestimated when it is swallowed by the patient. Haemoptysis may consist only of flecks of blood in sputum or can lead to exsanguination. Exsanguinating dogs may die in hypovolaemic shock. In small blood vessels disease such as shunts and aneurysms frequently develop in the caudal lung lobes. These, in combination with haemorrhage due to trauma from forceful coughing probably produce alveolar

haemorrhage. Haemorrhage may also be a result of DIC accompanying the pulmonary vascular disease.

Syncopal episodes appear to occur during an abrupt transition from rest to exercise and may be triggered by a sudden excitement. It is probably due to the inability to raise the cardiac output rapidly due to pulmonary hypertension and most likely this results in a transient systemic hypotension and cerebral ischaemia. Exercise intolerance in field or working dogs is due to the inability by the dog to elevate and sustain cardiac output through the diseased pulmonary system. During exercise, the poorly trained heartworm infected dog, unlike well trained infected counterparts, attempts unsuccessfully to elevate cardiac output by increasing the heart rate rather than stroke volume. Inability to exercise may also be related to hypoxaemia, as heartworm infected dogs have an exaggerated hypertensive response to alveolar hypoxia.

In the most severely affected dogs, right sided congestive heart failure (R-CHF) ultimately ensues. This has been commonly observed in large breed male dogs aged between 3 and 8 years and usually amicrofilaraemic. Right sided congestive heart failure may present as hepatomegally, ascites and sometimes with pleural effusion in conjunction with loss of lean body mass. Rarely does ventral oedema develop. Widening of the tricuspid valve follows right ventricular dilation and contribute to jugular pulsations. Dilation of the root of the main pulmonary artery may occur and very occasionally, produce pulmonary regurgitation and a faint diastolic murmur. Fixed splitting of the second heart sound may also be heard. In one study (Rawlings, 1986), most of the heartworm infected dogs that developed heart failure had had a prior exercise session. Congestive heart failure can also be associated with the death of many adult heartworms, either spontaneously or in response to arsenical therapy (Hirano et.al., 1992).

The caval syndrome is characterized by an acute onset of weakness, anorexia, depression and dyspnoea in dogs that had no earlier heartworm signs. Some of them may be reported to have been actively performing in the field just few days prior to acute illness. The mucous membranes are frequently pale and jaundiced and approximately one third of affected dogs have haemoglobinuria and bilirubinuria. Some dogs collapse and remain recumbent. Coughing, sustained exercise intolerance and ascites may occur in the caval syndrome but are not typical signs. Venous pressure is markedly increased and distends the jugular vein. The heartbeats may be forceful and a few dogs develop arrhythmias (Kitagawa et al., 1991). Although heart murmurs have been commonly described as atypical, they presented in 87% of the dogs with caval syndrome examined by phonocardiography (Rawlings, 1986). A few affected dogs may have haemoptysis.

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2.5 Clinicopathology

The haematological, serum chemistry, and urinalysis results of heartworm infected dogs reveal wide variations. Statistical comparison frequently do not show differences between pre-and post-infection values in individual heartworm infected dogs (Buoro, and Atwell, 1983; Rawlings, 1986; Davoust et.al., 1992). Even when statistical differences are present, variations between individuals is so great that clinicopathological changes cannot be used reliably to predict a disease in a specific patient.

2.5.1 Haematology

2.5.1.1 Red blood cells

Abnormal values in packed cell volume (PCV) and haemoglobin (Hb) levels occur in severe heartworm infections. However, even in these cases, the PCV may be only mildly decreased to 27 from the normal value of 38. Most of the severely affected dogs have normocytic, normochromic anaemia, poikilocytosis, increased erythrocyte sedimentation rate (ESR), and increased osmotic fragility, suggestive of regenerative haemolytic anaemia (Rawlings, 1986). The half-life of erythrocytes was also found to be reduced in such dogs (Kitagawa et.al., 1992). The reticulocyte count is low, and in the clinically sick dogs erythropoiesis may be depressed. Anaemia is pronounced in the caval syndrome where there is severe haemolysis (Rawlings, 1986; Buoro, 1988; Kitagawa et.al., 1992).

2.5.1.2 White blood cells

Generally, dogs with heartworm disease have normal leucocyte count (Rawlings, 1986). About 20% of patients however may have neutrophilia, some of these with mild left shift. But, as a rule, neutrophilia is observed in most cases of congestive

heart failure and pneumonia. Mild lymphopenia is common in canine heartworm disease and is probably related to the stress produced by the infection. Monocytosis which is frequently associated with stress-mediated neutrophilia is also not unusual in canine heartworm disease. This develops probably because of great demand for phagocytosis created by thromboembolism and death of adult heartworms. In patients with marked eosinophilia, blast transformed lymphocytes may be seen associated with immune stimulation.

In several different experimental studies on canine dirofilariasis (Rawlings, 1986), eosinophil levels have been seen to increase over baseline. In fact, eosinophilia and basophilia have frequently been considered to be part and parcel of heartworm infection. Basophilia is otherwise unusual in the dog's natural response to infections. One period of eosinophilia occurs during the first 40 days of infection, when the larvae are in a subcutaneous location, and between 70 and 100 days post-infection when young *D.immitis* adults emerge into the blood stream. Various studies reviewed by Rawlings (1986) have reported observing eosinophilia accompanying microfilaraemia. When stress from the infection develops, eosinophil levels tend to decrease.

But eosinophilia is also commonly observed in many other conditions of dogs including parasitic infections. Infection with *D.reconditum* in particular has been noted to produce even greater eosinophilia and basophilia levels than when infection is by *D.immitis* (Rawlings, 1986). Moreover, the definition of eosinophilia varies widely with minimum values being quoted as between 800 and 1500 cells per millilitre of blood (Rawlings, 1986).

2.5.1.3 Platelets

Platelets are likely to be central to the pathogenesis of many pathologic events associated with canine heartworm disease, including pulmonary thromboembolism,

pulmonary hypertension and pulmonary fibrosis (Boudreaux et.al., 1989). On the other hand, platelets survival is lower in heartworm infected dogs than in normal subjects, probably because of the high consumption of platelets on the damaged endothelial surfaces. For instance, mean platelet counts were observed to decrease from baseline values of 350,000 to 200,000 in dogs with heartworm infection (Rawlings, 1986). Platelet counts however, vary widely even in normal dogs and are frequently a reflection of the sampling technique.

2.5.2 Serum chemistry

Both beta and gamma globulin levels are frequently reported to increase over normal values in symptomatic and asymptomatic canine dirofilariasis (Rawlings, 1986; Davoust et.al., 1992). Total protein concentrations are frequently high, although severely ill dogs also have hypoalbuminemia, and the albumin: globulin ratio is decreased. Liver enzymes are elevated in less than 10% of the affected dogs. Some of these dogs have abnormally high serum aspartate aminotransferase or serum glutamicoxaloacetic transaminase (SGOT), serum alanine aminosaminase (SGPT), or serum alkaline phosphatase (SAP) levels (Rawlings, 1986).

Although serum enzyme changes do not appear to correlate with the disease state (Rawlings, 1986), they have been noted to decline after adult heartworms were removed. But elevation of the SAP level may also be associated with a fatty liver, Cushing's disease, or with steroid administration. Enzyme levels rise also in nearly half of the heartworm patients after arsenical treatment.

Liver function as evaluated by bromsulphalein (BSP) retention is slightly abnormal in about 20% of heartworm infected dogs (Rawlings, 1986). And when R-CHF accompany heartworm infection, 50% of affected dogs may have BSP retention

increased from 8 to 15% at 30 minutes. Azotemia is unusual and occurs in less than 5% of patients with heartworm disease. When azotemia is present, the disease is usually symptomatic.

In natural heartworm infections, serum free cholesterol levels increase while the cholesterol:ester ratio and lecithin-cholesterol acyltransferase activity decrease. In severe cases, these changes are more pronounced, with the free cholesterol levels being twice the normal values.

Arterial blood gas levels are normal in most heartworm infected dogs (Rawlings, 1986). Approximately 20% have mild compensated metabolic acidosis, and 30% have mild hypoxia. Some dogs with severe pulmonary disease, especially those with thromboembolism, develop hypoxaemia

2.5.3 Urinalysis

Mild proteinuria occurs in about 20% of *D. immitis* infected dogs and in 30% of those with related clinical signs (Rawlings, 1986). Proteinuria (albuminuria) is usually associated with hypoalbuminemia. However, these changes are generally not observed in experimentally infected dogs (Buoro and Atwell, 1983; Rawlings, 1986). Besides, most dogs with heart failure have proteinuria regardless of the aetiology. In old dogs, heartworm infection may coexist with renal disease and result into severe proteinuria and nephrotic syndrome associated with amyloidosis.

2. 6 Diagnosis of *D.immitis* infection

A diagnosis of heartworm infection is usually sought when there is a high probability of exposure, provocative clinical signs and clinicopathologic changes or a combination of these. Then, a definitive diagnosis may be arrived at by using one or more of the following methods: detection of microfilariae in the blood, observation of characteristic survey thoracic radiographic abnormalities, performing selective and non-selective angiography or by positive results of serologic tests. Heartworm infection can also be detected during postmortem examination of dog carcasses.

2.6.1 Detection of microfilariae in the blood

Typically, canine dirofilariasis is diagnosed by examination of venous blood samples for the presence of microfilariae (Rawlings, 1986; Knight, 1987). Finding microfilariae is the direct and conclusive method for determining that a filarial infection is present. Techniques for detecting circulating microfilariae include examination of wet blood smear, use of haematocrit capillary tube, or microfilariae concentration techniques employing either Knott or filter tests

2.6.1.1 Wet blood smear method

If many microfilariae are present in peripheral venous blood, the wet smear technique will reveal their presence and provide information that is useful in differentiating between *Dirofilaria* and *Dipetalonema* species. However, the absence of microfilariae in smears may be due to a low microfilarial concentrations and therefore may not necessarily mean absence of infection. Absence of microfilariae could also be because of occult infections. It seems that microfilarial counts should be greater than 1000 per millilitre to provide a positive diagnosis by a wet smear

technique (Rawlings, 1986). This technique which detects microfilarial motility has been found to be 10 to 50% less accurate than the concentration techniques (Rawlings, 1986). It is however, a quick and inexpensive method for screening for filarial infections.

2.6.1.2 Haematocrit capillary tube method

In this technique, microfilariae in a blood sample are normally spun down and can be examined at the buffy coat region where they remain motile for about 20 minutes. *D. immitis* microfilariae are less active than those of *D. reconditum* and they tend to collect at the bottom half of the capillary tube. Accuracy of this technique is similar to or lower than that of the wet blood smear technique and microfilarial counts of only a few hundreds per millilitre are frequently interpreted falsely as negative. Although this method has limited value for routine screening it can be used for in blood samples that are routinely submitted to the laboratory for PCV determinations. It is also a quick and inexpensive method (Rawlings, 1986).

2.6.1.3 Concentration technique methods

These include the modified Knott test and filtration tests. In the Knotts test, microfilariae in one millilitre of blood are concentrated and the red blood cells lysed to increase the likelihood of detecting even very low microfilaraemia. The lysate used is usually 2% formalin. This method is also used to differentiate microfilariae of different filarial species. Like the Knott test, the filtration tests are useful in diagnosing low microfilaraemias. The blood sample is injected through a filter chamber consisting of pores of approximately 5 microns in diameter and which are distributed sufficiently for efficient retention of *D. immitis* microfilariae. This technique like the Knott test also employs a lysate solution.

2.6.1.4 Comparison between techniques for detecting microfilariae

When the filter and Knott concentration tests were compared with the wet blood technique for their efficiency in detecting microfilariae in blood samples (Rawlings, 1986; Feldmeier et al., 1986; Knight, 1987), the concentration tests were found to be consistently more accurate especially with blood samples containing few microfilariae. Although results on which concentration test is superior have varied, it is generally agreed that any of the popular filter tests or the Knott test should be considered as equally reliable and accurate (Rawlings, 1986; Knight, 1987). Technical errors can occur especially with the filter tests (Martin and Collins, 1985; Rawlings, 1986). False positive results may occur with filter tests due to contamination of solutions and equipment. Up to 24% false positive results of this nature have been observed. Falsely negative results can originate from improperly placed filters. For instance in the Knott test, microfilariae may be lost in the supernatant during decantation, if centrifugation is not adequate, and this may result into false negative findings.

2.6.1.5 Microfilarial differentiation

Except in few cases when *D. dracunculoides* and *D. repens* were reported to occur in dog populations infected with *D. immitis* (Perez-Sanchez et al., 1989; Verster et al., 1991; Ortega-Mora et al., 1991), it is *D. reconditum* that is reported frequently to co-exist with *D. immitis*, sometimes in equal proportions (Martin and Collins, 1985; Boreham and Atwell, 1985; Perez-Sanchez et al., 1989; Owen and Slocombe, 1990; Ortega-Mora et al., 1991; Patton and Faulkner, 1992). Dogs infected with *D. reconditum* normally do not become ill, but may develop eosinophilia and basophilia similar to that found in heartworm disease.

In practice therefore it has become important that microfilariae of *D. immitis* be distinguished from those of *D. reconditum* (Soulshy, 1982; Boreham and Atwell, 1985; Rawlings 1986; Knight, 1987; Owen and Skxcombe, 1990). Apart from the differences in sizes which are used to differentiate microfilariae belonging to different filaria species (Table 1), microfilariae of *D. immitis* and those of *D. reconditum* can be distinguished further by criteria as summarized in Table 4.

Table 4. Major criteria for distinguishing microfilariae of *D. immitis* from those of *D. reconditum*

DIAGNOSTIC CRITERIA	SPECIES	
	<i>D. immitis</i>	<i>D. reconditum</i>
Number	Few to many	Few (< 2500 per ml)
Motility	Stationary	progressive
Head	Tapered	Blunt
Average length (range)	314 μ (286-340)	270 μ (250-292)
Average width (range)	6.8 μ (6.1-7.2)	5.2 μ (4.7-5.8)
Acid phosphatase stain	Excretory & anal pore	Diffuse
Brilliant cresyl blue stain	No hook	Cephalic hook

Source: Knight, (1987)

In practice, the tapered head of *D. immitis* and blunt head of *D. reconditum* (Fig. 7) are the most consistent features of the two species. This difference in head shapes, plus a size comparison are usually adequate to make a species differentiation (Knight, 1987)

Acid phosphatase staining is considered the most definitive differential technique (Chakifoux and Hunt, 1971, reviewed by Rawlings, 1986). Acid phosphatase activity is localized around the anal and excretory pores in *D. immitis* whereas

activity is seen throughout the microfilaria in *D. reconditum*. The characteristic cephalic hook of *D. reconditum* can also be highlighted by staining with brilliant cresyl blue and is a reliable differential feature.



Figure 7. Microfilariae of *Dirofilaria immitis* (A) and *Dipetalonema reconditum* (B) photographed at same magnification. In a Knott's preparation, *D. immitis* is larger and has a cephalic taper. Source , Knight, (1987).

Motility of microfilariae in fresh blood smears is a fairly accurate method of differentiating *Dirofilaria* from *Dipetalonema* species. *Dirofilaria* species undulate vigorously in a place whereas *Dipetalonema* species travel across the microscope field.

Dipetalonema reconditum microfilariae are usually fewer, with an average of only about 235 per millilitre in comparison with those of *D. immitis* which frequently are not less than tens to hundred thousand range per millilitre.

2.6.1.6 Microfilarial quantification

Quantification of microfilariaemia is a research tool and is not routinely indicated in clinical situations (Rawlings, 1986). Quantification can be useful in characterizing efficacy of microfilaricides, defining the cyclic nature of microfilarial concentrations, and studying the immune response to occult disease. When microfilariaemia is low, counting can be done directly on the Knott's stained sediment. A common technique for determining high microfilarial counts is to place 20 lambda (μm^3) aliquot samples of blood onto separate slides, count them, and the average obtained is multiplied by 50 to determine the number of microfilariae in a millilitre of venous blood.

2.6.2 Serologic tests for heartworm infection

Dogs infected with adult heartworms but without detectable circulating microfilariae (occult dirofilariasis), present a diagnostic challenge. During such situations, or when there is specific need for information pertaining to parasite antigenemia and host immune response, the use of immunodiagnostic techniques become appropriate. However, serologic tests are by no means a replacement for microfilarial detection and should be applied only to microfilaraemic dogs following screening by microfilaria-detecting methods (Atwell et al., 1988; Courtney et al., 1990). Commercially available serology kits detect either anti-*D immitis* antibody or antigenic component of the heartworm. Recently, the utility of filarial excretory-secretory (ES) products for diagnosis of heartworm disease has also been demonstrated (Frank and Grieve, 1991; Rojas et al., 1992). However, antibody detection assays such as the indirect fluorescent antibody (IFA) and the enzyme-linked immunosorbent assay (ELISA) have been found unsatisfactory for screening heartworm infection because they are frequently associated with false negative results (Knight, 1987). This inherent limitation is probably because antibody

detecting assays attempt to document the infection indirectly by examining the host response.

Much of the ambiguity associated with antibody detection has been overcome to a great extent by the more direct approach of demonstrating soluble parasite antigen in serum. But even the antigen detecting assays are not completely reliable for heartworm diagnosis and are sometimes falsely negative during light infections (Courtney et al., 1988).

2.6.2.1 IFA detection of antibody to microfilarial cuticular antigen

The IFA assay detects antibody specific to *D. immitis* microfilaria cuticular antigen. It was the first serologic test to be used widely for diagnosis of occult dirofilariasis (Rawlings, 1986; Knight, 1987; Wong and Thomford, 1991). In this test, fixed microfilariae are used to bind the anti-cuticular serum antibody which is labeled with a host specific antisera conjugated to a fluorescein. When results are based upon fluorescence of the cuticle rather than at the ends of broken microfilarial, IFA is both stage-specific for microfilariae and filarial species specific (Knight, 1987). Although it is known for a low overall sensitivity (Knight, 1987; Wong and Thomford 1991), IFA is a valuable diagnostic test for identification of immune-mediated occult dirofilariasis. Otherwise, the use of IFA has been replaced by other more sensitive tests.

2.6.2.2 Antigen-based ELISA detection of antibody to filarial somatic antigen

The principle of antibody ELISA (Ab-ELISA) is the same as that of IFA except that a semipurified antigen derived from adult parasites is used to bind anti-filarial antibody and an enzyme is substituted for a fluorescein. Enzymatic colour conversion of a substrate indicates a positive result. Like the IFA, Ab-ELISA must

incorporate the appropriate host species-specific antisera. The use of Ah ELISA has provided valuable information on *D. immitis* -host interactions. For instance, it is now known that a significant rise in antibody titre occurs about 3 months post-infection, and reaches a peak, with the onset of patency at 6 to 7 months (Knight, 1987). This assay is able to detect anti-*D.immitis* antibody even before the development of microfilaraemia.

2.6.2.3 Detection of parasite antigen in serum

The feasibility of detecting soluble *D.immitis* antigen in serum was first demonstrated by counterimmunoelectrophoresis (Knight, 1987). Following the development of monoclonal antibody to *D.immitis* derived antigen, the antigen ELISA (Ag-ELISA) test for detecting *D.immitis* antigen has become relatively easy to perform in a clinical setting. The specificity of the monoclonal antibody and sensitivity of the ELISA combine to make antigen detection by this method a very powerful diagnostic tool. The antibody employed binds to somatic antigens of both male and female adult heartworms but is most reactive with antigens of the female reproductive tract. Monoclonal antibodies do not bind to microfilarial cuticular antigen, and since, the amount of microfilarial antigens in circulation is below the threshold for detection, concurrent microfilaraemia does not affect the test results. Monoclonal antibody coated microbeads have also been utilized in a latex agglutination (LA) system (Collins et al., 1987).

Antigen detecting techniques have, to a great extent, overcome the drawbacks of antibody detection and are the preferred method for serodiagnosis of heartworm infection (Knight, 1987). Since it is *D.immitis* antigen that is being sought, the Ag-ELISA test can be used in any of the affected species without a necessity for modification.

False negative results have been encountered with the Ag-ELISA technique particularly with some infections consisting of few, for instance less than 5 adult worms or non-gravid female (Courtney *et.al.*, 1988). However, the parasite biomass in relation to host blood volume may neutralize the problems associated with light infections. False-positive results are rare and in most cases are probably due to technical error or host sensitization to murine antigen, since the monoclonal antibodies are of mouse origin (Knight, 1987). Cross reactivity with *D.reconditum* is not significant with this technique (Knight, 1987; *et.al.*, 1988).

Generally, antigen assays appear to be slightly less sensitive but much more specific than the antibody assays (Wong and Thomford, 1991). Detection of filarial antigen unlike antifilarial antibody, has a very high positive predictive value (Grauer *et.al.*, 1988; Courtney *et.al.*, 1988; Courtney *et.al.*, 1990). The qualitative relationship between antigenemia and number of adult heartworms is perhaps the most important feature of antigen detection. Correlation with the number of female heartworms is particularly good (Knight, 1987).

Since soluble *D.immitis* antigen does not reach detectable levels in the circulation until 6 months post-infection, just before patency, it becomes unlikely that prepatent infections will be detected by Ag-ELISA (Knight, 1987; Courtney *et.al.*, 1990). For instance, it was observed (Wong and Thomford, 1991) that commercial Ag-ELISA such as CITE and Dirochek® commonly missed relatively heavy infections (< 30 heartworms) in which worms were close to sexual maturity.

Latex agglutination and several Ag-ELISA kits are commercially available, and a considerable attention has been given to evaluating their diagnostic efficiency. Each has excellent specificity but sensitivity varies slightly (Courtney *et.al.*, 1988; Courtney *et.al.*, 1990; Wong and Thomford, 1991). Among them, Dirochek (Commonwealth Serum Laboratories) is currently the most widely used in many

different canine heartworm disease surveys. Although Atwell et al., (1988) observed only 73% as an overall sensitivity for Dirochek, indicating that it would not be a reliable screening test, more recent studies (Courtney et al., 1990; Macy et al., 1990) report Dirochek to have sensitivity in the range of 90 and 93% and specificity of up to 97%. However, none of the Ag-ELISA commercial kits is reported to be completely reliable especially in diagnosing occult dirofilariasis. Wong and Thomford, (1991) noted that even the most sensitive Ag-ELISA test could not detect 26% of the prepatent cardiac infections. With Dirochek for example, Atwell et al., (1988) and Courtney et al (1990) observed low sensitivity in the range of 53.3-65% when it was applied to microfilaraemic dogs.

Although it is generally accepted that the sensitivity and specificity of a test are constant values whereas the predictive values of positive and negative tests are variable that usually change with prevalence of infection, the sensitivity of heartworm immunodiagnostic test may differ between populations of dogs having different prevalences of the various types of heartworm infection (patent, immune-mediated occult, unisex and immature occult) (Courtney and Cornell, 1990).

It is then apparent and as has been observed by Foreyt and Lagerquist (1991) that prevalence of heartworm infection based on serologic surveys alone will tend to underestimate the real infection rate because low numbers of worms and immature worms often will not be detected.

2.6.3 Radiography

Chest radiographs can contribute important information of diagnostic and prognostic value on heartworm disease. Radiography can be used to confirm the presence of heartworm disease particularly when occult infection is suspected (Rawlings, 1986; Knight, 1987). Early signs of the disease may not be evident in survey

radiographs, but in later stages, diagnostic specificity is high. Lesions may be visible in survey radiographs as early as 4 to 5 months after infection especially in the caudal lobar arteries of the lung where earliest occupation and damage by heartworms occur. Best visualization is achieved by lateral projections of the chest, when impacted peripheral arteries in the caudal lobes are visible first as poorly defined linear densities having a slight peripheral flare. Similar changes may appear in the apical and cardiac lobar arteries if enough worms are present. As the parasites grow and additional parasites accumulate, radiographic lesions appear also in other parts of the lungs. In more advanced infection, the proximal segments of lobar arteries enlarge, become tortuous, and may become truncated. These features are considered pathognomonic for heartworm disease (Knight, 1987). Dilatation of the main pulmonary artery segment and right heart enlargement occur in association with severe heartworm disease and are always preceded by abnormal peripheral vascular patterns.

Although thromboembolic complications cannot be predicted from the appearance of chest radiographs, the relative risk of complications can be determined. Pulmonary parenchymal changes can also be visualized radiographically (Rawlings, 1986). A radiodense interstitial lung pattern radiating from the hilum may be so dense as to obscure the pulmonary arteries. This radiographic appearance is typical of dogs with allergic pneumonitis due to microfilarial hypersensitivity.

2.6.4 Echocardiography

The echocardiogram complements the chest radiograph by providing quantitative information on the dimensions of individual cardiac chambers (Rawlings, 1986; Knight, 1987). A functional evaluation can be made by assessing the dynamics of cardiac motion. The utility of echocardiography in the clinical evaluation of heartworm infected dogs has been demonstrated (Badertscher et.al., 1988). But apart from its usefulness in making certain management decisions such as locating worms within the right ventricle it has very limited application.

2.6.5 Electrocardiography

The electrocardiogram (ECG) is another aid to patient evaluation that does not provide information specifically diagnostic of heartworm disease (Rawlings, 1986; Knight, 1987). Cardiac rhythm disturbances are relatively uncommon with heartworm infection (Knight, 1987). Nevertheless in advanced cases with pulmonary hypertension the ECG may document right ventricular hypertrophy (RVH), although the absence of ECG criteria for RVH does not rule out either anatomic hypertrophy or pulmonary hypertension.

2.6.6 Necropsy

Necropsy is the most definitive test for heartworm infection (Foreyt and Lagerquist, 1991). Because adult *D. immitis* are relatively large, necropsy of the heart, pulmonary arteries, (Fig 8), thoracic vena cavae and lungs is customarily used to establish a dog's infection status.

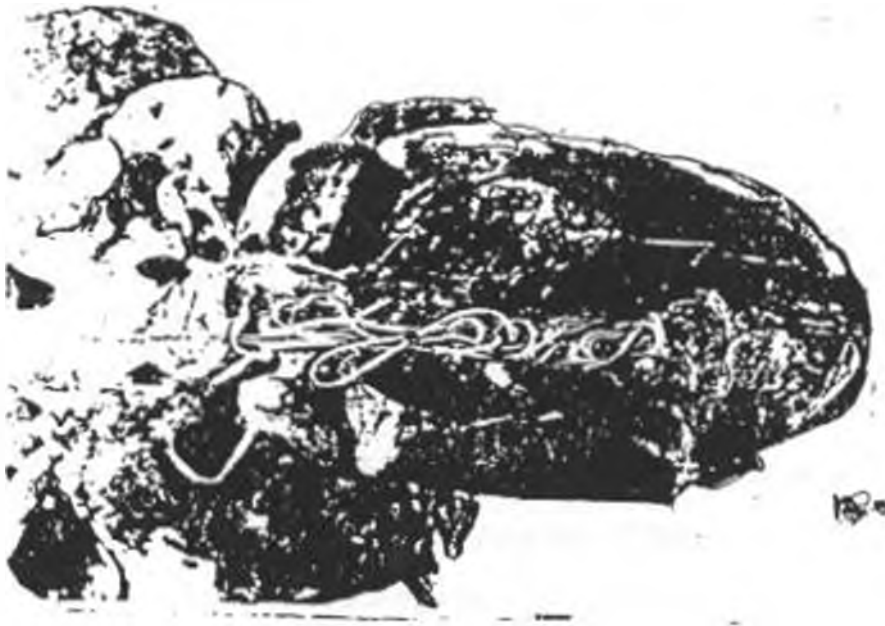


Figure 8 A mass of dead and live heartworms within the right caudal pulmonary artery which is characterized by oedema, inflammation, and haemorrhage. The dog was found dead.

Source: Rawlings, (1986).

However, conventional necropsy will not detect precardiac stages of *D. immitis*, especially those tissue-dwelling developing forms such as L3, L4, and early L5 (Courtney and Cornell, 1990). Some studies (Courtney and Cornell, 1990) have reported a small percentage (less than 1%) of microfilaraemic dogs that were apparently free of heartworms at necropsy. It is thought that such dogs had either eliminated adult worms while continuing to harbour microfilariae or that adult

worms existed at ectopic sites that were not examined by usual necropsy procedures, or perhaps both situations prevailed.

2.7 Management of canine heartworm infection

Management of canine dirofilariasis includes employment of chemoprophylaxis to prevent microfilarial development within the host during the mosquito season, chemotherapy to eliminate microfilariae or adult heartworms from the blood stream by administration of filaricides or surgery to remove the adult worms from cardio pulmonary system and ancillary medical therapy

2.7.1 Chemoprophylaxis and chemotherapy

Chemoprophylaxis is a method in which drugs are used to prevent development of microfilariae in the final host, while chemotherapy eliminates adult worms and microfilariae infection in dogs. Chemotherapy is complicated by the absence of a single filaricide that is effective against both precardiac and cardiac life cycle stages. Consequently, filaricide administration requires the use of stage-specific drugs. Usually, adulticide administration has to be followed in 3 to 4 weeks by a microfilaricide before commencing chemoprophylaxis (Knight, 1987; Hirano et al., 1992). Commonly used filaricides include thiacetarsamide, dithiazanine, levamisole, ivermectin, diethylcarbamazine (DEC) and the recently introduced, milbemycine oxime.

Since its introduction in 1947, thiacetarsamide has been the adulticide of choice (Knight, 1987). However, its activity on *D. immitis* is unpredictable (Vankan et al., 1987) and has a steep dose-response curve and narrow margin of safety (Knight, 1987). Pulmonary thromboembolism is the most frequent and potentially serious

sequela of thiacetarsamide therapy. Some degree of embolization is an inevitable consequence of destroying the adult worms. The most common manifestations of thiacetarsamide administration complications are fever, cough, general malaise, and in the most serious cases, haemoptysis. These signs usually occur 5 to 10 days after completing the treatment. Hepatotoxicity, if it occurs, leads to intermittent vomiting, anorexia and icterus. Renal dysfunction may also occur as thiacetarsamide is a nephrotoxin.

Dithiazanine is effective against the microfilariae of *D. immitis* but not the precardiac larvae or adult forms. It has been replaced by other drugs in the market.

Levamisole has less stage specificity than other filaricides and appears to exhibit some activity against microfilariae, precardiac larvae and adults of *D. immitis*, although it is commonly used as a microfilaricide only. Vomiting, central nervous signs and occasionally deaths may occur during levamisole therapy.

An ivermectin formulation has only recently become available for heartworm prevention in dogs. Ivermectin is capable of interrupting the life cycle of *D. immitis* at the microfilarial third and fourth stages, but has no apparent effect on adult worms (Knight, 1987). Its low toxicity to the host, safety of administration in the presence of microfilaraemia, and high potency against microfilariae, make it particularly useful for preventing heartworm infection. Prophylaxis with ivermectin can begin immediately, without regard for concurrent heartworm infection. It is normally given at a dosage rate of 6 microgrammes per kilogramme body weight but even higher doses are tolerated (Knight, 1987). Paul et al. (1991) observed that all dogs given chewable ivermectin tablets at a normal dose at 30 or 45 days post infection were free of heartworms at necropsy. An exception to the generally high tolerance of ivermectin is the unusual sensitivity of the Collie breed, this appears to be a breed idiosyncrasy (Knight, 1987). After ivermectin administration,

microfilariae are rapidly sequestered primarily in the microvasculature of lung, liver and kidney where they form microgranulomas.

Diethylcarbamazine is used as a microfilaricide for many filariids but not *D immitis*, for which it is used exclusively against precardiac larvae (Knight, 1987). The minimum effective dose is 5.5 milligrammes per kilogramme daily for 30 days when treatment is begun on the day of infection. As a practical matter under field conditions, treatment should start shortly before the anticipated beginning of the mosquito season and be continued for 60 days following the last exposure to potentially infected mosquitoes. The minimum effective dose possesses a wide margin of safety. Alternate-day administration on an extended basis is also protective (Knight, 1987). Four times the normal dosage started as late as 60 days post-infection may also be used in the event that prophylaxis has been interrupted or neglected.

Serious adverse reactions can occur when DEC is given to microfilaraemic dogs. This peaks at about an hour after administration. Usually there is tachycardia, pallor, dyspnoea and weakness. Although most dogs recover spontaneously within 24 hours, hypovolaemic shock associated with this reaction may lead to death within a few hours. Signs recur each time DEC is administered and are not dose-dependent. The severity of the reaction somehow correspond with the level of microfilaraemia but cannot be predicted from this. Associated abnormalities in haematologic and blood chemical values are in direct proportion to the severity of clinical signs. This reaction has come to be understood as systemic type I hypersensitivity or anaphylactic shock (Knight, 1987).

Milbemycin oxime analogs are antibiotics that are derived from *Streptomyces hygroscopicus aureolacrimosus*. The A₃ and A₄ milbemycine oxime analogs combination has been recently shown to have activity against the microfilariae and

the precardiac larvae of *D. immitis*, and at the target dose of 0.5 milligrammes per kilogramme body weight is effective in preventing the development of experimental heartworm infection in dogs when administered once at 30 or 45 days post-infection (Grieve *et.al.*, 1991; Shiramizu, 1991). Raynaud (1993), has also reported the use of melarsomine against both microfilariae and adult heartworms.

2.7.2 Surgery

The surgical extraction of heartworms from the right ventricle and pulmonary arteries is under investigations. Shiang *et.al.* (1987) observed that open ventriculotomy during cardiopulmonary bypass offers a more direct approach to adult worms and has advantages over the commonly used adulticide methods. Kitagawa *et.al.*, (1991) have also demonstrated a simpler type of surgery which has proved to be the treatment of choice for the caval syndrome. However, because of the demand of considerable technical expertise in thoracotomy, this method of treatment has not been adopted on a large scale (Knight, 1987).

2.7.3 Ancilliary medical therapy

Destroying the parasite is only one phase in the treatment of heartworm disease. Supportive therapy given before and after filaricide administration or surgical removal of worms may be very important for achieving a satisfactory result, particularly in advanced stages of infection. Along with exercise restriction, affected dogs may be given diuretics and digoxin in case of congestive heart failure. Anti-platelets drugs such as aspirin, ticlopidine and dipyridamole are recommended for use before and during treatment of heartworm disease (Knight, 1987; Boudreaux *et.al.*, 1991; Tarish and Atwell, 1993).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 An Overview of the areas selected for the survey

The Kenyan coastal strip is at an altitude of 16 meters above sea level, its climate being characterised by a mean annual rainfall of 1200–1600 millimeters with high daily mean temperatures (26–28 °C) and relative humidity (65–93%). Rarely do temperatures exceed 35°C. The main rain season is in the months of April, May and June. The land is semi arid with the natural vegetation varying widely from patches of rain forests to dry thorn bush. Deciduous woodlands alternate with dense bush. Dominant agricultural vegetation consists of coconut palm, cashew nuts, banana, sisal and sugar plantations.

A survey of the extent of *D. immitis* infection in dogs was conducted during the period November 1992 to June 1993 along the Kenyan coastal strip, in Mombasa, Kilifi and Kwale districts. The following ten areas within these districts were used as sampling points: Mombasa municipality (Area A), Bamburi Kennels (B), Mtwapa (C), Kikambala (D), Gongoni (E), Kibaoni (F), Kilifi (G), Majengo (H), Jaribuni (I), and South Coast (J) (Fig. 11). These were areas with a high human settlement density. With the exception of the Mombasa municipality and Bamburi Kennels, the areas surveyed comprised small villages with mainly peasantry agriculture and little social activity. Bamburi kennels offered boarding as well as training facilities for an average of 50 dogs every month. Dipping services were also provided for resident as well as neighbouring dogs. In each of the ten areas the following parameters were examined: dogs distribution per unit area, type of breed, level of management of dogs, presence of habitat favourable for mosquito breeding, the extent to which chemoprophylaxis against *D. immitis* was employed and microfilarial densities in dog samples.

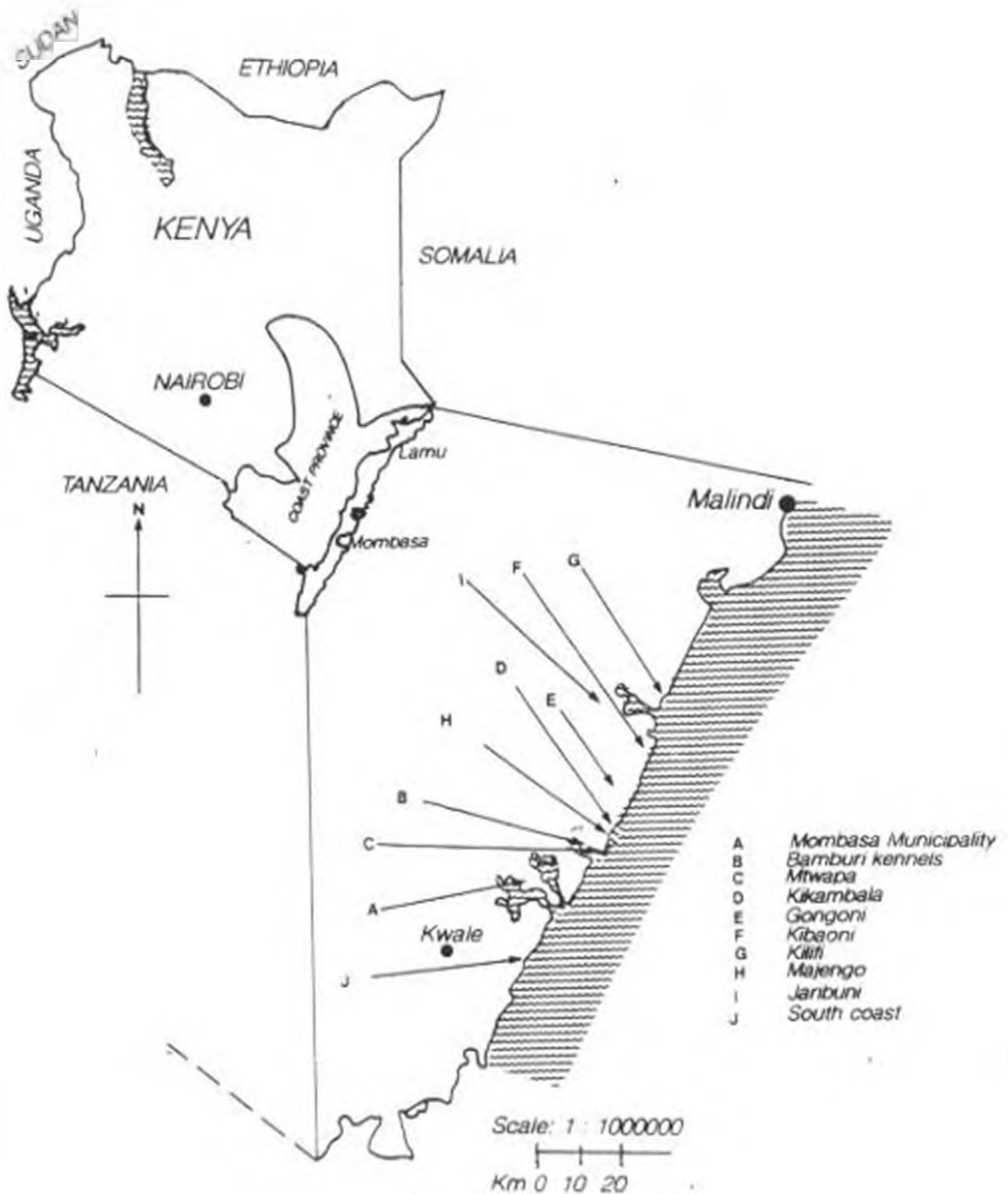


Figure 9 A sketch of the Kenyan coast showing the survey areas

3.2 Dog population and survey techniques

Dogs that were studied in area A are those which were attending to the Kongowea private veterinary clinic (5 Km. north east of the Mombasa municipality for various clinical procedures. Majority of them originated from the municipal environs and only few were referred cases from Kilifi, Malindi and South coast. In other survey areas dogs were seen by visiting individual homesteads or kennels.

Owners of the dogs were interviewed about the general health of their animals, type of accommodation provided, any recent loss of stamina, travel history, presence of a cough, any use of chemoprophylaxis (against *D. immitis*) and use (type of work, if any) to which a dog was put to. This was facilitated by checking clinic or health cards whenever they were made available. For the purpose of the survey, only dogs older than 6 months and which had not been on heartworm filaricides for a period of more than one year before this survey, were recruited. Chosen dogs were assigned serial numbers. These were included together with information on ownership as well as the name, breed, age and sex of the dog. Age was determined either through medical history or through dentition.

Study dogs were restrained and muzzled where necessary, before examination. The general health of the animal was assessed, this was then followed by a thorough examination of the cardiovascular and respiratory systems. Any abnormality encountered was noted. At the Bamburi kennels, dogs were additionally assessed for their training ability during the training sessions which took place daily from 8.30 a.m. to 10.00 a.m.

3.3 Parasitological and clinicopathological assessments

3.3.1 Blood sampling procedure

While the dog remained restrained, 10 ml. of blood was drawn from the cephalic vein using a sterile 10 ml. disposable syringe and a 21 G needle. The blood sample was then stored with EDTA in plastic bottles at 4 °C if it was not possible to process and examine it immediately. Each sample collected was labelled accordingly for identification. Blood sampling was done during the day time, between 8.00 am. to 6.00 pm. and the time at which the dog was sampled was recorded in every case. All samples were examined for microfilariae within 24 hours after collection, at the Kongwea veterinary clinic laboratory.

3.3.2 Parasitological examination of microfilariae

Three methods were used for assessing the presence or otherwise of circulating microfilariae. These were the thick wet smear, and the modified Knott's technique and the capillary haematocrit tube technique (Rawlings, 1986; Knight, 1987)

In the thick wet smear method, a drop of fresh blood was placed on a clean glass slide, and it was then covered with a clean glass cover slip. Microfilariae were then examined for their motility under low power magnification. Persistent undulating motion was considered an identification of the presence of microfilariae. Additionally, microfilariae with progressive motion were considered to be *Dipetalonema* species while those without progressive motion were judged to be *Dirofilaria*. In comparison, motility in wet blood smear due to *Trypanosoma congolense* would be limited to a relatively smaller microscopic field (Soulshy 1982).

In the modified Knott's test, 1 ml. of blood was haemolysed using 9 ml. of a 2% buffered formalin, the sample was then centrifuged at 1500 r.p.m. for 10 minutes after which the supernatant was discarded and the remaining sediment stained using a 1: 1000 dilution of new methylene blue dye. This was then mounted on a slide and assessed for microfilarial presence under X 10 objective lens magnification. When present, the morphological characteristics were assessed and the length determined using a micrometer inserted into the eye piece. When infection appeared to be light, microfilaria counts were performed on the whole sediment, however in apparently high densities, 20 different portions of the sediment were counted and the average value was then multiplied by 50 to give the number of microfilariae per ml. of blood (Rawlings, 1986). Relatively large microfilariae (286 – 340 μ in length) with tapered heads and straight tails were considered to be *D. immitis* while the relatively smaller ones (258 – 292 μ in length) with blunt heads and button -hook curved were considered to be *D. reconditum* (Rawlings, 1986; Knight, 1987). Microfilariae not conforming to the above morphological descriptions were identified by size assessment (Table 1) (Soulsby, 1982).

3.3.3 Haematocrit

Additional examination of samples for microfilariae was done during analysis for the packed cell volume (PCV) in some samples by capillary haematocrit tube method. In this method, a sample of blood was introduced into a clean capillary tube which was subsequently fitted into the microhaematocrit analyser (Compur microspin, Bayer, Germany) for determination of PCV. The resulting buffy coat was examined for microfilariae using X 10 objective lens magnification. The PCV of 135 samples representing 45 dogs from each of the three categories (i) those with *D. immitis* microfilariae, (ii) with *D. reconditum* microfilariae and (iii) without circulating microfilariae, were also examined.

3.3.4 Detection of occult infections using antigen- ELISA technique

Forty six dogs with histories and clinical signs related to heartworm disease for example weakness, coughing, dyspnoea, heart murmurs, etc. but whose blood samples were negative for *D. immitis* microfilariae upon parasitological assessment were suspected to have occult dirofilariasis. From these, sera were harvested and stored in 2 ml. plastic bottles at -21 °C for a period not exceeding 3 months. The sera were then tested for *D. immitis* adult antigen using an Antigen (Ag)- ELISA kit (Dirochek^R, CSL, Parkville, Australia). This commercial Ag-ELISA kit has a sensitivity in the range of 90 – 93% and a specificity of up to 97% (Courtney *et al.*, 1990; Macy *et al.*, 1991). Sera to be tested were allowed to thaw as was the Dirochek kit which had been stored at 4 °C, before undertaking the assay. Information on the procedure of carrying this assay which was contained in the instruction sheet accompanying the kit was adopted and applied as described. A serum sample was considered positive for heartworm antigen only if it turned blue within 10 minutes of commencement of the test procedure.

3.3.5 Necropsy

Twenty three dogs were necropsied and examined for adult heartworms. Of these, 16 were euthanised at the owner's request and 7 were carcasses that had died from an assorted number of causes. The carcasses were placed on the left lateral side, and the right hemithorax opened by incising the skin and resecting the ribs at the costochondral junctions. The lungs and heart were then exposed and were removed from the thoracic cavity. An incision was then made on the right auricle, and it was then extended to the right atrium, right ventricle, main pulmonary artery and its left and right branches. The incision was then continued to the level of arterioles. The right heart chambers and the main pulmonary artery and its branches were then

examined for the presence or otherwise of *D.immitis* and/or evidence of arterial pathology. When present, the *D. immitis* were harvested, counted, and identified as males if small and with spirally coiled tails. Otherwise they were judged to be females (Knight, 1987).

3.4 Statistical analysis

All data obtained from the survey were analysed, where appropriate, by using the Chi-square method, and one way analysis of variance (ANOVA). The difference in total prevalence between *D immitis* and *D. reconditum* microfilariae in dogs was analysed for significance using the Chi square method. This method was also applied to test the significance of breed and sex factors of dogs to the observed heartworm and *D. reconditum* infection status. Data on PCV values of dogs infected with *D. immitis*, *D. reconditum* and of those without filarial infection was analysed using ANOVA.

CHAPTER FOUR

4. RESULTS

4.1.1 Survey area

Except for the case of Bamburi Kennels (Area B) and some localities in the municipality (area A), all the other areas surveyed had poor sanitary conditions. The townships and suburban environments were dominated by poor, or absent drainage systems of waste water resulting in its accumulation in pools, along the streets as well as around residential abodes. This situation worsened when it rained. Used containers were found discarded and exposed in most of the residential areas. In both the urban and rural settlements pit latrines were evident. Often the poorly constructed human shelters were surrounded by unattended-to bushes.

4.1.2 Dog population and survey results

The distribution of dog breeds in the area surveyed is shown in Table 5. Apart from 13 dogs which were recently imported to area A, the rest of the dogs assessed were either native to their areas or had been brought there for some years back. In the Mombasa municipality and at Bamburi kennels the majority of dogs were of defined breeds such as Ridgebacks, Rotweilers, Dobermans, Labradors, Retrievers, Dachshounds, Alsatians, Pointers, Poodles, Terriers, Dalmatians, Great danes, Collies, Boxers, Pomeranians, Basset hounds, Whipets, Puli and their crosses. In comparison, the majority of dogs examined in the remaining areas were mongrels. As far as the ownership and the use to which a dog was put to (management) was concerned, there was a great discrepancy between the areas. Whereas the dogs in the Bamburi kennels and the Mombasa municipality were owned by well-of people

(some of them expatriates), the majority of rural dogs were owned by the peasantry. The former were able to care for their dogs, keeping them as pets and providing them with shelter, regular meals and health care while the peasants' dogs received little or no attention from their owners and were left to scavenge for food. Additionally, some of the urban dogs were used as guard dogs. These were owned by security firms or the police.

Table 5. Breed distribution of survey dogs

AREA	No of dogs examined	Breed of dogs	
		Pure and crosses	Mongrel
A	480	452	28
B	26	26	0
C	19	13	6
D	46	5	41
E	28	3	25
F	43	2	41
G	52	5	47
H	27	6	21
I	36	4	32
J	73	12	61
TOTAL	830	528	302

Some of the frequently observed clinical signs are shown in Table 6. They included arrhythmias and heart murmurs, coughing, weakness, loss of body condition, inappetance, dyspnoea, exercise intolerance and anaemia in that order. However, 36.8% of *D. immitis* infected dogs did not exhibit any clinical signs.

Table 6. Frequency of common manifestations observed in dogs infected with *D. immitis*

Manifestations	% infected dogs involved
Arrhythmias and heart murmurs	46.5
Coughing	43.0
Dullness	33.3
Poor body condition	25.0
Inappetance	22.2
Dyspnoea	19.4
Exercise intolerance	17.3
Anaemia	13.9
Skin nodules	2.8
Hind limb paralysis	1.4
Apparently healthy	36.8

4.2 Clinicopathology

4.2.1 Parasitological findings

Microfilariae were detected in 221 out of 830 dogs examined, constituting a total microfilarial prevalence of 26.6%. The prevalence of *D. immitis* and *D. reconditum* microfilariae in dogs in the surveyed areas is shown in Table 7. Microfilariae of both species occurred in all areas except in Bamburi kennels where *D. reconditum* was not detected. The prevalence of *D. immitis* microfilaraemia was 15.2% while that of *D. reconditum* was 10.7%. The difference was not found to be significant ($P > 0.005$). Overall 7 (0.9%) *D. immitis* microfilaraemic dogs had a concurrent *D. reconditum* infection while 3 (0.4%) had a mixed infection with *D. dracunculoides*. Excluding the mixed infections, 4 (0.5%) dogs had *D. dracunculoides* microfilariae while 2 (0.3%) had *D. repens* (Appendix 1).

Table 7. Prevalence of *D. immitis* and *D. reconditum* microfilaraemias in dogs for the different areas surveyed

AREA	Number of dogs examined	<i>D. immitis</i>		<i>D. reconditum</i>	
		No. dogs infected	% dogs infected	No. dogs infected	% dogs infected
A	480	103	21.5	59	12.3
B	26	1	3.8	0	0.0
C	19	1	5.3	2	10.5
D	46	4	8.7	2	4.3
E	28	3	10.7	3	10.7
F	43	2	4.7	3	7.0
G	52	3	5.8	6	11.5
H	27	1	3.7	1	3.7
I	36	2	5.6	4	11.1
J	73	6	8.2	9	12.3
TOTAL	830	126	15.2	89	10.7

The mean length of the microfilariae of *D. immitis* was found to be $310.3 \pm 16.4 \mu$ (range 279.0 – 436.2 μ). The median value was 312.9 μ . For *D. reconditum* the corresponding values were $269.1 \pm 17.4 \mu$, (241.6 – 327.1 μ) and 253.8 μ . A comparison of the three parasitological methods used in the detection of microfilariae, revealed the modified Knott's method to be the most efficient (Table 8).

Table 8. Detection levels of *D. immitis* microfilaraemic dogs by the three methods used in the survey

	<i>D. immitis</i> detection by Knott Technique	
	126 positive samples	704 negative samples
% detected by examining smears	68.3	87.5
% detected by haemocrit	74.5	79.2

As far as the apparent periodicity of *D. immitis* microfilaraemia is concerned, more positive cases appeared to be detected in the period between 8.30 to 10.55 and also between 16.00 to 18.55 hours as compared to the daytime intervening period (Table 9).

Table 9. Observed distribution of *D. immitis* microfilaraemic dogs in relation to time of sampling.

Time of sampling (hours)	No. of dogs examined		% of dogs with <i>D. immitis</i>
8.30 – 10.55	249	49	20
11.00 – 12.55	176	21	11.3
13.00 – 15.55	212	24	10.8
16.00 – 18.55	193	32	19.9
Total	830	126	15.2

The mean densities for the microfilaraemias for the two filarial species (*D. immitis* and *D. reconditum*) in the survey area are shown in appendix 2. The highest microfilaria density for both *D. immitis* and *D. reconditum* occurred in Kilifi. The overall mean microfilaria density in this survey was found to be 10,772/ml for *D. immitis* while that of *D. reconditum* was 302/ml. The difference was significant ($P \leq 0.005$).

4.2.2 Occult infections

Out of the 46 dogs tested for *D. immitis* antigen using *Dirochek*[®], 18 or 39% tested positive. This constitutes 12.5% of all *D. immitis* infections found in this study. Among those positive cases one was also examined postmortem and found with adult worms in the cardio-pulmonary system. More cases of occult dirofilariasis occurred in dogs older than 1 year (Table 10) as compared to younger dogs. The age bracket at which the highest number of occult infections occurred was (7-10) years. Dogs older than 10 years were not tested for occult dirofilariasis since the samples that were selected for this test did not include this age category.

Table 10. Prevalence of occult dirofilariasis observed in different age-groups of dogs

Age of dogs (years)	No. of samples tested*	No. of samples positive	% all occult cases
< 1	3	2	11.1
1	4	1	5.6
2-3	12	5	27.8
4-6	17	4	22.2
7-10	10	6	33.3
>10	0	0	0
Total	46	18 (39%)	

* *Dirochek*[®], CSL, Parkville, Australia

4.2.3 Haematocrit

The mean PCV for *D. immitis* infected dogs was 40.9 while that for *D. reconditum* infected dogs was 42.3. That for un-infected dogs was 40.1 (Appendix 3). There was no significant difference either between PCV values of *D. immitis* infected dogs and of those infected with *D. reconditum* ($P > 0.05$) or between microfilaraemic and amicrofilaraemic dogs ($P > 0.005$).

4.2.4 Necropsy

Adult *D. immitis* worms were found in 4 (3 males, 1 female) out of 23 dog carcasses examined at necropsy in this survey. Of the four, three had earlier on showed to be *D. immitis* microfilaraemic while one was amicrofilaraemic. The age of dogs affected ranged between 1 and 9 years and the breeds represented were: Alsatian cross (2) labrador (1) and mongrel (1). Whereas two of the affected dogs including the one which was amicrofilaraemic had presented various clinical signs, the rest were apparently healthy. The number of worms inhabiting the cardio-pulmonary system ranged between 2 and 20 per dog (average, 12 worms/dog). The lowest worm burden occurred in the amicrofilaraemic dog, the mongrel, in which the worms collected were both females. This one had also tested positive to Ag-ELISA. However, each of the remaining three carcasses had worms of both sexes. In all the cases, worms were found lodged in the pulmonary arterial tree and accompanied by pulmonary lesions. In only one dog (euthanised, Alsatian cross) did the worms occupy also the right heart chambers.

4.2.5 Overall prevalence of *D. immitis* in survey dogs

Out of 830 dogs examined for *D. immitis* infection, 144 (17.3%) were found to be infected by the parasite according to a combination of results obtained by parasitological methods, Ag-ELISA technique and necropsy examination of relevant samples.

Table 11. Overall prevalence of *D. immitis* in survey dogs

Technique of detection	Parasitological methods	Ag-Elisa	Necropsy
No. of microfilaraemic dogs	126	-	-
No. of amicrofilaraemic dogs, but positive sera	-	18	-
No. of dog carcasses with adult worms	-	-	*4
Total no. of dogs infected	144		
Total no. of dogs examined	830		
Total prevalence rate	17.3%		

* 3, Microfilaraemic; 1, Ag-ELISA positive serum

4.2.6 Host- parasite relationship

As far as host parasite interaction is concerned pure bred dogs and their crosses were more infected with *D. immitis* than were the mongrels ($P \leq 0.05$) (Table 12). 96 male dogs were found to be infected with *D. immitis* while only 48 females were infected by this parasite (Table 12). This difference was however not significant

($P > 0.05$). The effect of breed or sex of a dog to the status of infection with *D. reconditum* was similar to that observed with *D. immitis*. The relationship between age of dogs and infection status is shown in Table 13 and Figure 10. The peak level of infection was observed in dogs aged between 4 - 10 years. Dogs older than 10 years appeared to have slightly lower prevalence rate

Table 12: Prevalence of *D. immitis* and *D. reconditum* infection by sex and breed of dogs examined

Breeds	Male dogs				Female dogs	
	No. examined	% infected with		No examined	% infected with	
		<i>D. immitis</i>	<i>D. reconditum</i>		<i>D. immitis</i>	<i>D. reconditum</i>
Pure and crosses	358	21.8	9.2	172	20.3	7.2
Mongrels	199	9.0	11.0	101	12.9	4.0

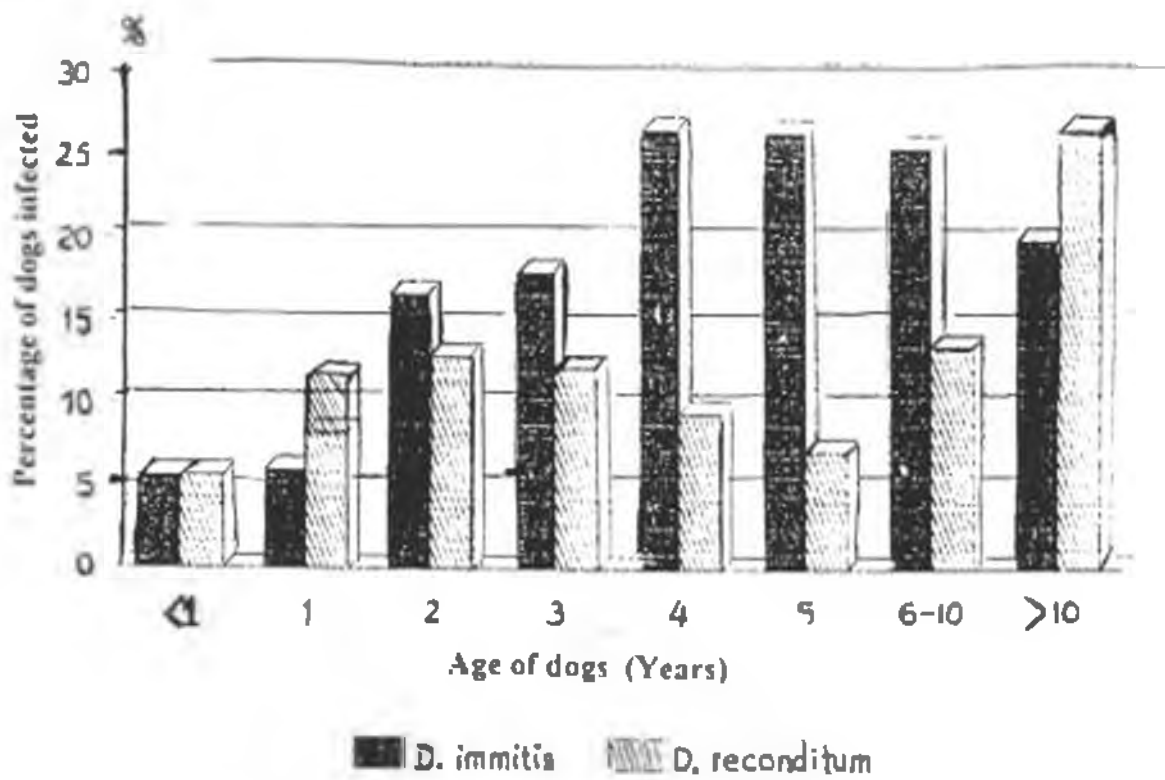


Figure 10: A Histogram of the distribution of *D. immitis* and *D. reconditum* infections in different age groups of survey dogs.

Table 13: Observed prevalence of *D. immitis* and *D. reconditum* infections in different age categories of dogs

Age in years	No of dogs examined	<i>D. immitis</i>		<i>D. reconditum</i>	
		No. of dogs infected	% of dogs infected	No of dogs infected	% of dogs infected
< 1	140	8	5.7	8	5.7
1	116	7	6.0	15	12.2
2	84	15	16.7	7	8.3
3	139	25	18.0	17	12.2
4	105	28	26.7	10	9.5
5	83	22	26.6	6	7.5
6-10	133	34	25.6	18	13.5
>10	30	6	20.0	8	26.7
Total	830	144	17.3	89	10.7

CHAPTER FIVE

5. DISCUSSION

Although canine dirofilariasis is a recognised important cause of morbidity and mortality in the dog, its real prevalence globally is a matter of conjecture. The reason for this is that while in some countries extensive surveys have been undertaken, in the majority of the remaining countries no such reports have been published. Information such as that is important both from the epidemiological point of view and also in allowing rational approaches as far as disease prevention is concerned. In the case of the Kenyan coast, anecdotal reports indicate presence of the disease in the area. However no study has been undertaken in this area to give an indication of its prevalence. This survey was therefore carried out to determine the prevalence of this condition in the Kenyan coast dog population and subsequently describe the relevant aspects pertaining to its diagnosis and control.

According to the results of this survey, *D. immitis* occurred in 17.3% of all dogs examined. This prevalence is similar to some of the reported rates in other canine heartworm surveys (Martin and Collins, 1985; Farish *et. al.*, 1986; Perez Sanchez *et.al.*, 1989; Owen and Slocombe, 1990; Macy *et.al.* 1991). However, it is quite below those prevalences reported in countries such as Japan (Ishihara *et.al.*, 1978), Australia (Collins *et. al.*, 1987), The United State (Macy *et al.*, 1991) and Italy (Ortega-Mora *et.al.* 1991,) where dirofilariasis is endemic. Variations such as these are not unusual since the prevalence of *D. immitis* in a particular area depends on several factors and especially the level of the existence of the reservoir of infection and the competence and relative abundance of mosquito vectors (Knight, 1987). In the areas surveyed, dogs and perhaps cats formed a major reservoir of the infection. However, in many areas, particularly the rural locations, dogs were greatly isolated in distance and this could have limited transmission of the disease among them. Unfortunately, the status of this condition in cats residing in the same area is not

known. Although cats are considered to be poor reservoir of the heartworm infection (Knight, 1987), the large number of stray cats observed during this survey, may have an enormous reservoir potential. There is a paucity of information on the relative effectiveness of the various mosquito species found in the Kenyan coast in relation to heartworm transmission. Although species such as *Ae. aegypti* and *Cu. pipiens quinquefasciatus* which are widely known to transmit *D. immitis* have been reported to occur in this area (Wijers and Kiilu, 1977; Subra, 1983), their relative abundance as well as competence in this particular area have never been established. These factors, according to Knight (1987), may vary depending on the geographical location.

As it had been noted earlier (Rawlings, 1986; Knight, 1987), no single or a combination of the commonly used diagnostic tests is able to establish current heartworm infection status with complete assurance. Thus each of the techniques used in this survey had some limitations. For instance, many blood smears that had not indicated presence of *D. immitis* microfilariae were later on found to have these parasites when haematocrit capillary tube and/or the modified Knott's test were used. On the other hand, some smears that had indicated motility and therefore considered heartworm positive were found to have *D. reconditum* only, upon re-evaluation using Knott's test. Yet, 18 positive cases or 12.5% of all heartworm infections which had previously been found to be amicrofilaraemic by Knott's test were detected when Ag-ELISA was applied. It has been observed that prepatent and light infections are sometimes missed even with the use of the best Ag-ELISA kit. (Courtney *et al.* 1988). Necropsy examination also may not reveal those worms occupying ectopic sites such as the brain (Courtney and Cornell, 1990).

In the areas where *D. immitis* shares ecological niche with filarids such as *D. reconditum* differentiation of microfilariae species is a pre-requisite for an accurate diagnosis of heartworm infection. In this survey, *D. reconditum* microfilariae

occurred in 10.7% of all survey dogs; with 7.3% of these infections existing together with *D. immitis*. Statistical analysis showed no significant difference ($P > 0.005$) between *D. immitis* and *D. reconditum* prevalence rates in the dogs examined. Similar findings were reported by Patton and Faulkner (1992). Although the prevalence of *D. reconditum* observed here is similar to that one observed in Canada (Owen and Slocombe, 1990), it is well above those reported in other countries where similar surveys have been conducted (Martin and Collins, 1985; Perez-Sanchez *et al.*, 1989; Ortega-Mora *et al.*, 1991; Patton and Faulkner, 1992). The fact that *D. reconditum* is a flea and tick-borne parasite (Soulshy, 1982) and because a great proportion of survey dogs had heavy flea and/or tick burdens, the high prevalence of *D. reconditum* observed here is not surprising. However other filariids, namely; *D. repens* and *D. dracunculoides* (Appendix 1) were present in survey dogs in insignificant proportions. Other workers (Perez-Sanchez *et al.*, 1989; Ortega-Mora *et al.*, 1991) have also reported very low prevalences for these parasites in Italy and Spain. Nelson *et al.* (1962) reported the occurrence in the Kenyan coast of other filariids of dogs such as *B. pahangi*, *B. pateri*, *B. malayi*, *D. striata* and *D. grassii* all of which were not encountered in this survey. It is possible that these are also present in insignificant proportions. Even in other canine heartworm surveys the occurrence of these filariids is rarely reported.

As far as the time of blood sampling is concerned, it appears that sampling during the morning period and late in the afternoon can result in more chances of detecting *D. immitis* microfilariae than when samples are collected during the intervening period. Although variations in *D. immitis* microfilaraemia in peripheral blood in relation to the time of the day have been frequently observed (Rawlings, 1986; Knight, 1987), the issue of microfilariae periodicity in heartworm infection is still not clear.

The host factors that have aroused particular attention in canine dirofilariasis are the age, breed and sex of dogs. Frequently, it has been observed that the risk for heartworm infection increases with advancing age (Knight, 1987; Tanaka and Atwell, 1991). In this survey, age was also found to play a significant role ($P < 0.005$). Like in previous reports survey dogs aged between 4 and 10 years were more infected with *D. immitis* than younger or older dogs. In addition, occult infections were observed more frequently in old than in young dogs similar to observations made by Grieve *et al.* (1986).

For the case of breeds, other workers (Martin and Collins, 1985; Knight, 1987) have reported no significant difference. However, in the present survey pure and crossed dogs were significantly more infected with *D. immitis* than the mongrels ($P < 0.05$). Although none of the previous studies on heartworm-breed interaction effect dealt with mongrels, it is unlikely that the observed difference in infection rates was due to a breed effect per se. Firstly, mongrels were fewer (36.8% of all survey dogs) than those dogs with defined breeds. Secondly, the majority of mongrels lived in isolated rural homesteads where the risk for contact between non-infected and heartworm reservoir dogs was undoubtedly minimal. Thirdly, mongrels were less cared for, in terms of health, food and shelter in which case their reservoir potential would have been reduced through frequent deaths. These reasons may explain why mongrels had lower *D. immitis* infection rate than other dogs. In contrast to observations made by Martin and Collins (1985) in which male dogs were more frequently infected by the heartworm than females, this was not the case in this survey and other such surveys (Knight, 1987; Perez-Sanchez *et al.*, 1989; Ortega-Mora *et al.*, 1991). In fact there was no significant difference ($P > 0.05$) in the infection status between males and females. Moreover, the roaming behaviour of entire males which was suggested by Martin and Collins (1985) as a reason for more exposure to potential heartworm vectors in this sex category as opposed to females could not be a major source of difference in this survey. In the

present case, male and female dogs alike were seen roaming at night in search for food and majority either slept outdoors or in houses without mosquito-proof facilities where both sexes probably encountered comparable number of mosquito bites.

It appears that occult dirofilariasis constitutes a significant proportion to the heartworm infections in this area. Although the prevalence of 12.5% observed for this category is somehow on the lower side of the global range of 10-67% for such infections (Knight, 1987) it is by no means negligible. Occult dirofilariasis in the Kenyan coast had also been studied before by necropsy (Dr. I.B.J. Buoro and Dr. M. Fazil (personal communication) and found to exist in significant proportion.

CONCLUSIONS AND RECOMMENDATIONS

The prevalence of heartworm infection should be used in determining whether routine annual testing or annual testing and prophylactic therapy should be recommended for a particular population. In view of the results in this survey, *D. immitis* is a major veterinary pathogen in the Kenyan coast and the prevalence observed warrants the designing of programmes to control it. Although there were marked variations in prevalences between different coastal areas that were surveyed the absence of restriction of dog movement between these areas necessitates application of similar control measures to all areas including those where low prevalence of the parasite was recorded. It is recommended that a programme be designed in the coastal areas of Kenya whereby dogs are annually tested for heartworm infection, positive cases treated and all covered by a chemoprophylactic regime. However, for such programmes to be successful, accurate diagnosis and the use of efficient chemoprophylaxis are the pre-requisites. In addition, dog owners have to be made aware of the importance of this condition in their areas

before these programmes are introduced to them. This is important because in almost all the survey areas only a very small proportion of dogs was reported to have been on chemoprophylaxis against the heartworm and even in these, the practice was erratic, mainly due to lack of concern by the dog owners about this condition.

Further studies are required to establish the prevalence of this condition in cats residing in the same area as well as competence and relative abundance of potential mosquito vectors. Such studies will provide more information on the epidemiology of this condition which in turn is necessary for designing efficient control programmes. Studies are also required to establish the status of this condition in the human population residing in the Kenyan coast. It has been shown in different geographical areas that human dirofilariasis is closely associated with heartworm infections in the canine population (Alvarez *et al.*, 1990). Interestingly, the prevalence of canine filariasis (all filarial infections) observed in this survey (26.6%) compares well with that of bancroftian filariasis in human subjects (22 per cent) reported by Wijers and Kinyanjui (1977) almost in the same area. This underlines the close relationship between canine and human filarial infections.

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APPENDIX

Appendix 1 Prevalence of Microfilariae of different filaria species in survey dogs

Filaria species	<i>D. immitis</i>	<i>D. reconditum</i>	<i>D. dracunculoides</i>	<i>D. repens</i>
No. of dogs infected	*126	89	+7	2
% all microfilaraemic dogs (221)	57	40	3	0.09
% all dogs examined (830)	*15.2	10.7	+0.8	0.02

* 7 (0.8%) *D. immitis* mixed with *D. reconditum*

+ : 3 (0.4%) *D. immitis* mixed with *D. dracunculoides*

Appendix 2: Mean densities for *D. immitis* and *D. reconditum* microfilarial counts in dogs observed in different survey areas.

AREA	<i>D. immitis</i>		<i>D. reconditum</i>	
	No samples counted	Mean microfilariae/ml	No. samples counted	Mean microfilariae/ml
A	20	11,200	20	370
B	0	0	0	0
C	3	4,500	2	216
D	5	7,000	2	178
E	3	10,200	3	415
F	4	3,800	3	119
G	5	20,600	5	445
H	5	13,500	1	304
I	2	9,350	4	280
J	5	16,800	5	394
TOTAL	52	10,773*	45	302*

* Mean of means for nine areas (B, excluded)

Appendix 3 Packed cell volume (PCV) for filaria infected and non-infected dogs

PCV%	Dogs infected with		Non- infected dogs
	<i>D immitis</i>	<i>D reconditum</i>	
25- 29	7	4	5
30- 34	11	5	9
35- 39	3	7	6
40- 44	6	10	13
45- 49	5	11	4
50- 54	8	3	6
55- 59	5	5	1
TOTAL	45	45	45