PREVALENCE AND PATHOLOGY OF HEART WORM (*Dirofilaria immitis*) INFECTION IN STREET DOGS AT DINAJPUR MUNICIPALITY AREA

A THESIS

BY

MD. ABU KAYES BIN AZIZ

REGISTRATION NO.: 1105023
SEMESTER: JANUARY – JUNE/ 2012
SESSION: 2011-2012

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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JUNE, 2012

Dedicated To My Beloved Parents

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The author
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ABSTRACT

This study was designed to investigate the prevalence and pathology of *Dirofilaria immitis* (canine heart worm) infestation in street dogs at Dinajpur municipality area, Dinajpur, Bangladesh. In a study of one year starting from July, 2011 to June, 2012 , a total of 100 dogs were observed and among of them 15, fifteen (9 male and 6 female of different age group and randomly selected) street dogs were collected from different locality of Dinajpur municipality area during Rabies Control Programme. A thorough necropsy examination was done and the characteristics clinical signs and gross lesions were recorded. During this investigation, it was observed that heart worm infection is common in street dogs. This study indicates that about 46.67% dogs were infected with *Dirofilaria immitis*. Using simultaneous clinical and histopathological examination and identification, a total of 15 street dogs were examined, among them 7 (46.67%) were positive with one or more species of microfilaria. Parasite *D. immitis* was found in 5 of 9 (55.56%) male and 2 of 6 female (33.33%) dogs so infestation of heartworm was higher in male than female dog. Prevalence also varied with the age of street dogs where adults were more susceptible than youngs. The prevalence of *D. immitis* in dogs > 9 years old was higher (66.67%) than in other age groups. Heart worm infection was recorded higher in poor body conditioned (65.5%) dogs than normal body conditioned (28.57%) dogs. Most of the heart worm infections apparently occurred in summer season. The highest seasonal prevalence was found 50% in summer and 42.85% in winter season. This study dictates that street dogs of Dinajpur district carried heart worm and it could be transmitted to human beings, thus it has zoonotic importance which may be the serious threat to public health.

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ABBREVIATION AND SYMBOLS

^0C	: Degree centigrade/celsius
^0F	: Degree fahrenheit
%	: Percentage
et al.	: And his associates
etc.	: Etectera
ELISA	: Enzyme linked immunosorbent assay
Fig.	: Figure
G	: Gram
HARD	: Heartworm associated respiratory disease
H & E	: Hematoxylin and Eosin
HSTU	: Hajee Mohammad Danesh Science and Technology University
HW	: Heartworm
J	: Journal
L	: Larvae
lbs	: Pounds
mff	: Microfilariae
min	: Minute
ml	: Milliliter
mm	: Millimeter
MS	: Master of Science
No.	: Number
OIE	: Office International des Epizootics
PBS	: Phosphate buffered saline
PCR	: Polymerase chain reaction
UV	: Ultraviolet
UK	: United Kingdom
USA	: United States of America
WHO	: World Health Organization
WSAVA	: World Small Animal Veterinary Association
WSPA	: World Society for Protection of Animals

CHAPTER I

INTRODUCTION

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INTRODUCTION

Dogs are the most successful canids, adapted to human habitation worldwide including Bangladesh. They have contributed to physical, social and emotional well-being of their owners, particularly children **(Dohoo *et al.*, 1998; Robertson *et al.*, 2000).** However, in spite of the beneficial effects, close bond between dogs and humans remain a major threat to public health, with dogs harboring a bewildering number of infective stages of disease causative agents transmissible to man and other domestic animals **(Robertson *et al.*, 2000; Molyneux, 2004).**

Street dogs (synonymously "stray dog" is also used) are ownerless native dogs (*Canis familiaris*) of mostly non descriptive nature which roam freely without human supervision. Street dogs, known in scientific literature as free-ranging urban dogs **(Daniels, 1983)** or urban free-ranging dogs **(Pal, 2001)** are unconfined dogs that live in cities. They live virtually wherever cities exist and the local human population allows. Street dogs may be pets which have strayed from or are simply allowed freedom by their owners, or may never have had an owner. Street dogs may be stray purebreds, true mixed-breed dogs, or unbred landraces such as the Indian pariah dog. Street dog overpopulation can cause problems for the societies in which they live, so campaigns to spay and neuter them are sometimes implemented. They tend to differ from rural free-ranging dogs in their skill sets, socialization, and ecological effects.

Street dog (Dogs found in public places irrespective of the level of care and level of supervision imposed upon them) is one of the important inhabitants in the environment of Bangladesh. In Dinajpur District, street dogs are scattered everywhere irrespective of urban and rural areas. Its lives are led at outdoor environment. Dogs live in unhygienic condition. Street dogs readily come into contact with the humans, especially the children and domesticated animals.

Street dogs impose a burden on the community in a number of ways. These pose serious human health, socio-economic and animal welfare problems in many countries throughout the world. A diverse range of zoonotic infections, including parasitic, bacterial, viral, protozoal and fungal diseases are transmitted from dogs to humans **(Acha and Szyfres, 2003; WHO, FAO and OIE, 2004).**

Street dogs are involved in the epidemiology of toxocariasis, visceral larva migrans, cutaneous larva migrans, strongyloidiasis, diphyllobothriasis, trichinosis, dirofilariasis, Rocky Mountain spotted fever, giardiasis, cryptosporidiosis, and a range of other diseases **(Robertson and Thompson, 2002 and Kahn, 2006).** Most important role of the dog is in the maintenance and transmission of echinococcosis and rabies **(Konno *et al.*, 2003; Hemachuda, 2005 and Kilic *et al.*, 2006).**

Sarcoptic mange is commonly reported in dogs and foxes and related to the human skin disease called scabies. Dog mange could readily be transferred from animal to humans **(Soglia *et al.*, 2007).**

Toxocara canis, hookworm, *Trichuris sp., Trichinella sp., Diphyllobothrium sp.* and *Dipylidium sp.* are common in dog **(Overgaauw, 2009).** *Toxocara canis* and *Ancylostoma caninum* have zoonotic importance as they cause visceral larva migrans **(Mitamura *et al.*, 2007)** and cutaneous larva migrans in man, respectively.

The impact of free-roaming dogs due to the spread of fatal diseases poses a significant threat to wild life conservation **(Patronek *et al.*, 1997; Manor and Saltz, 2004).** Free-roaming dogs may cause many other problems by fouling public places with excreta, creating undesirable noise, causing road traffic accidents, and placing stress on road users **(Dabritz *et al.*, 2006).**

To survive, street dogs need to avoid conflict with humans. However, Dog bites can occur when dogs are trying to mate or fighting among themselves, and pedestrians and other humans in the vicinity may be bitten by fighting dogs. In

addition, females with pups are often protective and may bite people who approach their litter.

Outbreaks of rabies are often traced to unvaccinated street dogs, one the most common carriers of the painful and often fatal disease.

Barking and howling and dog fights which invariably take place over mating can be very disturbing to people, and the smell of dog urine which is an unsavory product of territory marking can become quite pungent, especially among unspayed or neutered dogs, not to mention the presence of feces. To survive in modern cities, street dogs must be able to navigate traffic.

Heart worm (*Dirofilaria immitis*) infection is also common in dogs, cats, foxes and wild mammals and the predilection site of the parasite is in the right ventricle and pulmonary artery of the dogs **(Sabu *et al.*, 2005).** Dirofilariasis has a zoonotic importance and usually associated with pulmonary lesions in the human body **(Morchon *et al.*, 2010).**

Heartworm disease is of considerable economic importance affecting canine population around the globe. It is caused by the parasitic worm, *Dirofilaria immitis*, in arteries of the lungs of affected animals and occasionally in the right side of the heart **(American Heartworm Society, 2007).**

Mosquitoes are the intermediate host for *D. immitis*. Currently more than 70 species of mosquitoes have been recorded to transmit *D. immitis*. The type of mosquito present depends on locality and different species have different feeding habits **(Kittleson, 1998).**

The common species are *Armigeres sp.*, *Culex sp.* and *Aedes sp.* **(Vythilingam *et al.*, 2005).**

Several studies have proven that parasitism; particularly gastro-intestinal helminthiasis is the most commonly encountered disease and the major impediment to dog health all over the world **(Traub, 2003).** Parasites that live in

the internal organs of dogs can infect many different systems. Some parasites reside in only one area, such as the bowel or heart, whereas others can infect many different organ systems simultaneously throughout the body.

Most of the internal parasites affect the dogs sub-clinically with or without apparent clinical signs like lowered resistance to infectious diseases, retarded growth rate, reduced working efficiency and general ill health **(Taylor *et al.*, 2007).** Besides these, dogs may harbor a wide range of zoonotic parasites causing significant health risk to humans **(Craig and MacPherson, 2000).** They act as a usual connectors between people and nature as they thrive on food wastage around the tales (dustbins) in densely populated urban and peri-urban areas contributing high risk of parasitic zoonoses **(Khante *et al.*, 2009).**

Developing countries like Bangladesh, the number of street dogs that coexist with human being is high in most cities and villages which constitute a potential risk of infections for human beings. The distribution and intensity of parasitism in dogs are influenced by geographical, climatic, cultural and economic factors **(Robertson *et al.*, 2000).** Furthermore, the level of hygienic conditions, lack of veterinary supervision and less awareness concerning zoonotic diseases exacerbate the transmission of these diseases **(Traub *et al.*, 2002).**

Dirofilariasis is a disease of world wide distribution, but the most endemic areas are those with moderate, tropical and subtropical climates where mosquito populations are high and stable. Other regions with cold weather, with hot summers and with rivers, lakes and widely irrigated lands are also suitable for the development of the disease.

In view of the above facts, it is assumed that Dirofilariasis is one of the major problems for the street dogs in Bangladesh as Bangladesh has a subtropical climate with rivers, lakes and widely irrigated lands **(Maitby, 1986)** where mosquito populations are high and stable, but no attention has been paid to study the prevalence and its effects on the dog in Bangladesh.

Several studies have been carried out on gastrointestinal parasitism and other internal parasites of street dogs throughout the world but surprisingly in Bangladesh only few published data available in this regard **(Rahman, 1973 and Basu *et al.*, 2010).** Currently, there are no data available on the distribution, prevalence, parasitic burden and risk factors associated with heart worm (*Dirofilaria immitis*) of street dogs in Dinajpur.

Therefore, the current investigation has been undertaken to determine the prevalence, intensity and pathology of heart worm (*Dirofilaria immitis*) of street dogs at Dinajpur municipality area of Bangladesh with the following objectives:

i. To determine the prevalence of *Dirofilaria immitis* in street dogs at Dinajpur municipality area
ii. To study the gross and histopathological changes of the infected hearts which are burdened with worm load
iii. Categorization of the agents having zoonotic role

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Available and relevant literatures for the determination of Prevalence and pathology of canine heart worm (*Dirofilaria immitis*) in street dogs are reviewed in this part of the thesis after a brief overview on the history, identification, distribution, epidemiology, life cycle, mode of transmission, pathogenesis and pathology (gross and microscopic lesions), clinical manifestations, diagnosis and economic and zoonotic importance against the filarial nematodes.

2.1. Filarial nematodes of dogs

The filarial nematodes are characterized by their tissue tropism and their dependence upon blood-feeding arthropod vectors for transmission **(Macpherson *et al.*, 2000).** The most commonly reported species in dogs are; *Dirofilaria immitis, Dirofilaria repens, Acanthocheilonema reconditum, Acanthocheilonema dracunculoides, Brugia malayi, Brugia ceylonesis* and *Brugia pahangi* **(Irwin, 2002; Rishniw *et al.*, 2006 and SimÃ³n *et al.*, 2007).**

Dirofilaria immitis is responsible for heartworm disease in dogs, yet microfilaraemia associated with other filarial infections are commonly detected in blood films of dogs in tropical countries, which theoretically necessitates specific identification of the filarial parasite in order to exclude the non-pathogenic species. This requires experienced personnel and it may be difficult to detect multiple infections with more than one species of filarial worm **(Irwin and Jefferies, 2004).**

Despite the availability of published measurements of various microfilariae, the inaccuracy of morphological diagnosis was demonstrated by **Rishniw and colleagues (2006)** when microfilariae initially identified as *A. reconditum* were later characterised as *D. immitis* by molecular methods. Both *D. repens* and *Acanthocheilonema* spp. develop into adult worms in the subcutaneous tissue

resulting in skin nodules. Adults of *Brugia* spp. are usually recovered from the mandibular, retropharyngeal or axillary lymphatics. Most infections with *D. repens*, *Acanthocheilonema* spp. and *Brugia* spp. are of minimal veterinary clinical significance, however all canine filariae have the potential to infect humans and remain significant from a public health perspective.

Dirofilaria immitis infections in humans are very rare and are usually associated with pulmonary lesions or radiological coin lesions of the lung. The significance of *D. immitis* infection is the potential for a radiological misdiagnosis of primary or metastatic lung tumour, leading to thoracotomy for open lung biopsy or wedge resection of the lung to obtain the correct diagnosis **(Theis, 2005 and Foroulis *et al.*, 2005).**

Sporadic cases of immature heartworms in unusual locations in the human body such as the eye **(Moorhouse, 1978),** mesentery **(Tada *et al.*, 1979),** cerebral artery **(Dobson and Welch, 1974)**, spermatic cord **(Theis, 2001)** and liver **(Kim *et al.*, 2002)** have also been reported. *Dirofilaria repens* is a parasite of the subcutaneous tissue in dogs that can also accidentally infect humans, causing a condition referred to as subcutaneous dirofilariasis. It is considered to be a re-emerging zoonosis, transmitted by mosquitoes, endemic to Southern and Eastern Europe and Asia, particularly Sri Lanka **(Pampiglione and Rivasi, 2000),** Malaysia **(Shekhar *et al.*, 1996)** and India **(Sabu *et al.*, 2005).** The distribution of human cases of subcutaneous dirofilariasis appears to mirror the distribution of canine cases **(Pampiglione and Rivasi, 2000 and Sabu *et al.*, 2005).** Several genera of mosquitoes are competent vectors for *D. immitis* and *D. repens*, including *Culex*, *Aedes* and *Anopheles* **(SimÃ³n *et al.*, 2007 and Cancrini *et al.*, 2003).** *Acanthocheilonema reconditum* and *A. dracunculoides* rarely cause significant illness in dogs. Their importance lies in the fact that their microfilariae can be easily confused with those of *D. immitis* and *D. repens*. The adults from these species can be found in the body cavity and subcutaneous tissues of dogs. They are prevalent in the United States, Italy **(Cringoli *et al.*, 2001),** Egypt **(Hashem and Badawy, 2008)** and Africa **(Huynh *et al.*, 2001).** The intermediate host for *A.*

reconditum are *Ctenocephalides* (flea) and *Heterodoxus* (lice) **(Nelson, 1962),** and a biting fly, *Hippobosca longipennis* acts as intermediate host for *A. dracunculoides* **(Nelson, 1963).**

Dirofilaria spp., *Acanthocheilonema* spp. and *Brugia* spp. have all been reported in India **(Gogoi, 2002; Ananda *et al.*, 2006 and Dam and Das, 2006).** During one recent survey, post-mortem examination of 240 indigenous dogs at a local slaughterhouse (for dogs) in northeast India revealed 34% of dogs harboured heartworm infection **(Borthakur *et al.*, 2006).** The authors noted that among the heartworm-positive dogs, 35% had non-patent infections and none of the animals demonstrated overt clinical signs of disease on brief ante-mortem inspection. Both *D. immitis* and *D. repens* were isolated at post-mortem examination from 57% (4/7) and 14% (1/7) of dogs respectively in the central Indian state of Orissa **(Patnaik, 1989).** Two recent surveys of microfilaraemic dogs in **Kerala (Sabu *et al.*,2005)** and Karnataka States **(Ananda *et al.*, 2006)** in southern India, found only *D. repens* at a prevalence of 7% (n = 160) and 21% (n = 400) respectively. It is important to note however that these latter studies on *Dirofilaria* utilized morphological methods for diagnosis, which can be potentially misleading as microfilarial dimensions of both species of *Dirofilaria* often overlap. Moreover, it may be difficult to detect multiple infections with more than one species of filarial worm. Although minimally pathogenic in dogs, *D. repens* is zoonotic and a number of human cases of subcutaneous dirofilariasis in the medical literature of India have been reported in the same region **(Sabu *et al.*, 2005)** Despite the limited number of surveys performed, veterinarians in India strongly believed that heartworm is confined to the northeast and *D. repens* to southern India. This assumption is debatable since competent mosquito vectors for *D. immitis* are present throughout central and southern India. For example, *Aedes albopictus* **(Lai *et al.*, 2001; Cancrini *et al.*, 2003 and Tiawsirisup and Kaewthamasorn, 2007),** a competent vector for *D. immitis* is present in Maharastra, Karnataka and Pondicherry **(Kumar *et al.*, 2009),** and heartworm is yet to be reported in dogs from these areas. Moreover, the tropical climate of

these regions have average temperature ranges from 20°C in winter to 37°C in summer (**Current weather forecast and climatological information - India,** webcite) and this provides a suitable environment for *D. immitis* development within the vector **(Galliard and Dang, 1938; Kutz and Dobson, 1974 and Christensen and Hollander, 1978).** It is known that the development of *D. immitis* can also vary within mosquito species **(Frimeth and Hisao, 1982 and Lai *et al.*, 2000)** and the genetic diversity of different strains of the same species could therefore be accountable for the variation observed. A case of human pulmonary dirofilariasis due to *D. immitis* however, was reported in Mumbai in 1989, adding further doubt to its currently accepted geographical distribution **(Badhe and Sane, 1989).** Studies into the effect of temperature on larval development of Dirofilaria have largely focused on D. immitis with less information available on *D. repens* **(Medlock *et al.*, 2007).**

At present, 1.3 billion people worldwide are at risk of lymphatic filariasis and about 120 million people in 83 countries are affected. Amongst them, 45.5 million live on the Indian subcontinent **(World Health Organization report, 1994).** *Brugia malayi* is responsible for 10% of cases of zoonotic lymphatic filariasis in humans and is restricted to the tropics **(World Health Organization report, 1995).** Although the main reservoirs are populations of leaf-eating monkeys (*Presbytis* spp.) **(Orihel and Eberhard, 1998),** this filarial nematode has also been found to infect cats in Malaysia **(Abdullah *et al.*, 1993)** and Thailand **(Chansiri *et al.*, 2002).** *Mansonia, Aedes, Culex* and *Armigeres* are four genera of mosquitoes that are able to transmit brugian filariosis **(Regu *et al.*, 2005).** In people, the disease may range from causing few clinical symptoms, or sufferers may experience acute manifestations such as fever, rashes, orchitis, lymphadenitis and lymphangitis that, if progressing to chronic infection, will lead to lymphoedema or 'elephantiasis' **(Macpherson *et al.*, 2000).** Another species, *Brugia pahangi,* has not been recognized in natural infections in humans but is able to infect humans experimentally **(Edeson *et al.*, 1960).** *Brugia pahangi* was found mixed with other filariid species in 54.7% of dogs (n = 68) in Malaysia

(Mak *et al.*, 1980) and was isolated from 7.6% (n = 52) of cats in Thailand **(Nuchprayoon *et al.*, 2006).**

Brugia ceylonensis was first described from the lymphatics of dogs in Sri Lanka in 1962 **(Jayewardene, 1962)**. In a Sri Lankan survey of 65 dogs, 44.6% were positive for microfilaria; of these, 62% and 7% had single infections with *D. repens* and *B. ceylonensis* respectively, while 31% had mixed infections with both species **(Rajapakshe *et al.*, 2005).** An adult *B. ceylonensis* was recently isolated from the conjunctiva of a person in Sri Lanka **(Dissanaike *et al.*, 2000)** raising public health concerns about the zoonotic potential of this canine filaria.

Brugian filariasis accounts for approximately 5% of lymphatic filariasis cases in India where over 40 million people are estimated to be infected **(**Lymphatic filariasis, **webcite).** Recently, based on immunodiagnostic testing, 16/75 (21.3%) microfilaraemic dogs were shown to harbour *B. malayi* **(**Human filariasis parasite found in dogs, **webcite).** The role of dogs (and cats) as reservoirs of brugian filariasis has important implications for parasite control strategies. If canine and feline reservoir hosts exist in these areas, a more inter-sectorial approach to control may be required in addition to the traditional use of mass drug administration programs advocated by the World Health Organization. The species of *Brugia* recovered from dogs in Kerala therefore requires confirmation using molecular diagnostic tools, as it is possible that the immunodiagnostic tests utilized to diagnose infection in dogs cross-react with other *Brugia* spp. **(Supali *et al.*, 2004).**

2.2. Heartworm of dogs

Heartworm (*Dirofilaria immitis*) is a roundworm that is spread from host to host through the bites of mosquitoes. The heartworm is a type of filaria, a small thread-like worm that causes filariasis. The definite host is the dog, but it can also infect cats, wolves, coyotes, foxes and other animals, such as ferrets, sea lions and even, under very rare circumstances, humans **(American Heartworm Society, 2007).** The parasite is commonly called "heartworm"; however, that is a

misnomer because the adults actually reside in the pulmonary arterial system (lung arteries) for the most part, and the primary effect on the health of the animal is a manifestation of damage to the lung vessels and tissues **(Ettinger *et al.*, 2010).** Occasionally, adult heartworms migrate to the right heart and even the great veins in heavy infections. Heartworm infection may result in serious disease for the host.

2.3. Oetiology

2.3.1. Scientific classification of *Dirofilaria immitis*

Kingdom: Animalia

Subkingdom: Eumetazoa

(unranked): Bilateria

Superphylum: Platyzoa

Phylum: Nematoda

Class: Secernentea

Subclass: Spiruria

Order: Spirurida

Family: Onchocercidae

Superfamily: Filaroidea

Genus: Dirofilaria

Species: D. immitis

Binomial name

Dirofilaria immitis (Leidy, 1856)

2.3.2. Identification of *Dirofilaria immitis*

Dirofilaria immitis is a filarial parasite that causes heartworm infection in many mammals, notably dogs and cats **(Bowman and Atkins, 2009).** As a filarial worm, *D. immitis* belongs to the phylum Nematoda (roundworms), class Secernentea, order Spirurida, family Onchocercidae and superfamily Filaroidea **(Bandi *et al.*, 1999).** *Dirofilaria immitis* is found globally in tropical and temperate zones, with higher levels of infection in the United States, Japan, Australia, and Italy **(Venco and Vezzoni, 2001).**

Other filarial nematodes, including *Wuchereria bancrofti* and *Brugia malayi*, are known to cause human lymphatic filariasis (Elephantiasis), and *Onchocerca volvulus* is a causative agent for human river blindness disease.

Microfilariae (mff) are mobile vermiform embryos and are the microscopic immature stage of *D. immitis* circulating in the blood of the vertebrate host. They are not sheathed and have a straight tapered tail. Their size ranges from 286-340 µm in length and 6-7 µm in diameter. The physical characteristics of *D. immitis* microfilariae distinguish from other filarial species such as *Dipetalonema reconditum* **(Yabsley *et al.*, 2004).**

Dirofilaria immitis show sexual dimorphism; adult males range between 12 and 20 cm and are much shorter in length than the female worms, which can grow as long as 30 cm in length. Both sexes are thin, measuring less than 5 mm wide and white in color. They are cylindrical in shape and a psuedocoelon is present. Males have coiled (corkscrew) tails, whereas the females' tails are straight.

Dirofilaria immitis is a mosquito-borne nematode that causes a serious, fatal disease in dogs. Although this disease can be prevented with the use of anthelminthic drugs, but street dogs remain at risk because they are not given any preventative medicine **(Bowman, 2009). 2.4. Life cycle/pathogenesis.**

The life cycle of *D. immitis* involves both intermediate and definite hosts. Many species of mosquitoes can serve as intermediate hosts of *D. immitis*. Definitive hosts of *D. immitis* range from sea lions to ferrets; however, most research is focused on dogs and cats **(Geraci and St Aubin, 1987 and McCall, 1998).** The life cycle of *D. immitis* varies between dogs and cats **(Bowman and Atkins, 2009).** Regardless of definite host, the development of *D. immitis* within a female mosquito requires approximately 10 to 14 days after acquisition of a blood meal containing microfilaria. After larvae have developed to the third stage (L3) within the mosquito, the mosquito can infect a new definite host by transferring L3 during a subsequent blood meal. Within the definite host, L3 molt twice and mature to adult worms. Once microfilariae are produced in the host, mosquitoes can feed on the blood of the infected definite host and continue to spread the parasite.

Adult female heartworms range in size from 10 to 14 inches; male heartworms are smaller at 5 to 7 inches **(Haddock, 1987).** Adult heartworms tend to be found in the pulmonary arteries, sometimes moving into the right ventricle or right atrium of the heart **(Venco and Vezzoni, 2001).** In caval syndrome, heartworms are found in the caudal vena cava, leading to valvular insufficiency **(Strickland, 1998).** Ectopic locations for adult worms include the eye, brain, and peripheral vasculature in dogs and cats; however, ectopic locations are more common in cats than dogs **(Venco and Vezzoni, 2001).**

2.5. Epidemiology

2.5.1. Prevalence of *Dirofilaria immitis*

In areas endemic for *D. immitis*, prevalence in dogs that are not on heartworm preventive can exceed 50% and normally the prevalence in unprotected dogs is 25-50%. Feline heartworm prevalence is often 5-20% of the positive dog population **(Atkins *et al.*, 1998; Bowman *et al.*, 2007; Carleton and Tolbert, 2004; Hermesmeyer *et al.*, 2000; Miller, 1998; Nogami and Sato, 1997; Patton and McCracken, 1991 and Ryan and Newcomb, 1995).**

Endemic occurrence of *D. immitis* has been reported in the USA, Canada, South America, Africa, Australia, Asia and Europe **(Yildirim *et al.*, 2006).** The prevalence of heart worm infection in dogs appears to have increased in recent years. This is due to a lack of prevention procedures and weak knowledge of dogs' owners **(Lee *et al.*, 1996).** Heartworm infections in dogs in California were not diagnosed until the 1970s **(Weinmann and Garcia, 1974)** and a recent national survey showed dog heartworm in domestic dogs throughout the continental United States **(Bowman *et al.*, 2009).** At 3.9%, the southeastern states had the highest prevalence rate in the country while Oklahoma had a statewide prevalence of 2.1% **(Bowman *et al.*, 2009).**Recently a nationwide survey estimated the feline heartworm prevalence rate, determined by antigen testing, to be 0.6 times the canine prevalence **(Lorentzen and Caola, 2008).** Northern Florida is a high endemic area and many dogs are not on preventive. The prevalence in unprotected dogs is 25-50%. In Northern Florida, feline heartworms are found in 5% of the cat population in an animal shelter **(Levy *et al.*, 2003 and Snyder *et al.*, 2000).** Studies have demonstrated conflicting results when examining the cats' gender as a predisposing risk factor for *D. immitis.* Some researchers have found male cats to be at a higher risk for heartworm infection **(Kramer and Genchi, 2002 and Levy *et al.*, 2003)** whereas others reported gender not being a determining factor for infection **(Atkins *et al.*, 2000; Genchi *et al.*, 2008 and Liu *et al.*, 2005).**

2.5.2. Geographic distribution

D. immitis is spreading progressively from regions of subtropical climate to temperate areas. Within the last 20 years *D. immitis* has established itself in northeastern regions of the USA, parts of Canada, northern Italy, and northeastern France **(Slocombe, 1992 and Doby *et al.*, 1986).**

Although at one time confined to the southern United States, heartworm has now spread to nearly all locations where its vector, the mosquito, is found. Transmission of the parasite occurs in all of the United States (cases have even

been reported in Alaska), and the warmer regions of Canada. The highest infection rates are found within 150 miles of the coast from Texas to New Jersey, and along the Mississippi River and its major tributaries **(Ettinger *et al.*, 1995).** It has also been found in South America **(Vezzani and Carbajo, 2006),** southern Europe **(*The Merck Veterinary Manual*, 2006),** Southeast Asia **(Nithiuthai and Suwannee, 2003),** the Middle East **(Rafiee and Mashhady, 2005),** Australia, Korea, and Japan **(Ettinger *et al.*, 1995).**

Important questions about the spreading potential of *D. immitis*, namely whether genetic or climatic adaptations of parasite and intermediate host occur, remain unanswered at present. *D. immitis* is endemic in America, Africa, Asia, Australia, and southern Europe **(Fig.1, Schrey, 1996).** Unfortunately, literature references for Asia and Africa are incomplete, as canine filarial infections have not been differentiated to species level. Imported cases of *D. immitis* infections of dogs have been reported from the United Kingdom, the Netherlands, Sweden, Hungary, Switzerland, Austria, Poland, and Germany **(Schrey, 1996).** In a prevalence survey in Germany, no autochthonous *D. immitis* infections could be demonstrated, although the rate of infection in dogs with travel histories to endemic countries was high **(Schrey, 1996).** Thus 13% of dogs with right heart disease and travel histories to Africa, North America, Italy, Portugal, Spain, or Corsica were found to be infected with *D. immitis*. 10% of dogs imported into Germany from Italy, Spain, or Portugal were found to be infected with *D. immitis*. 12% of US Military dogs stationed in Germany were found to be infected with *D. immitis*.

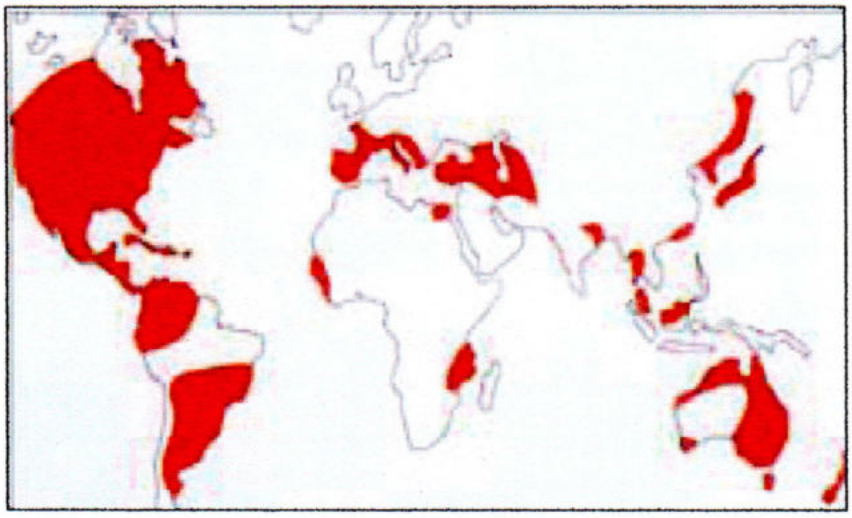


Fig.1. A diagram illustrating the global distribution of *D. immitis* infection in dogs

2.5.3. Hosts

Hosts of *Dirofilaria immitis* includes: **(American Heartworm Society, 2007).**

- Dog
- Cat
- Wolf
- Coyote
- Fox
- Ferret
- Sea Lion
- African Leopard *(Panthera pardus pardus)* **(Mazzariol *et al.*, 2010)**
- Human (rare)
- Beaver

2.5.4. Mode of transmission

There are three factors involving *D. immitis* transmission. First there needs to be an infected vertebrate with circulating microfilariae, most commonly a domestic dog, which is a definite host for *D. immitis.* Heartworm-infected canids have circulating microfilariae and are considered the natural reservoir for these

parasites. Although cats are naturally more resistant to *D. immitis*, feline infection is likely to occur anywhere the parasite is found in canids. Microfilaremia is uncommon in cats and therefore for a cat to become infected with *D. immitis* there needs to be an infected canid nearby **(Genchi *et al.*, 2008).** Environmental conditions must be appropriate for *D. immitis* larval development because environmental temperature affects growth within the mosquito and microfilariae. Finally, vector competent mosquitoes such as the *Culex* spp. must feed on both infected canids and susceptible hosts to effect transmission **(Nelson *et al.*, 2005a and Ralston *et al.*, 1998).** Almost 70 species belonging to the family Culicidae are considered potential vectors (*Aedes* spp., *Anopheles* spp., *Culex* spp., *and Mansonia* spp.).

To begin the cycle, a female mosquito feeds on a microfilaremic dog and the microfilariae are ingested during the blood meal. The microfilariae pass through the hemocoel and into the malpighian tubules of the mosquito. There they mature into first stage larvae (L1s) and molt to second stage larvae (L2s) within 10 days. Ten to fourteen days later, they become third stage larvae (L3s), which is the infective stage. The L3s migrate to the salivary glands and proboscis and when the mosquito feeds, the L3s are deposited into the skin and then enter the wound made by the mosquito. The larvae begin to develop and molt into the 4th stage larva in 3-4 days. Fourth-stage larvae migrate to the muscle fascia of the thorax and abdomen, molt, and then L5s enter the circulatory system over a period of 3 to 4 months. They are carried to the pulmonary arteries, heart, and lung via blood circulation 4 to 7 months of post infection. Once in the heart and lung they mature into adult worms approximately 6 months after infection if both male and female worms are present. Adult female worms begin to produce microfilariae 6 to 9 months (180 to 190 days) after the initial infection, which is the infectious period when *D. immitis* can be acquired by mosquitoes and then transmitted to other cats or dogs. In cats, arrested development of the worm is common because cats are not the natural host. Fewer worms reach maturity in cats compared to dogs. Only about 20% of *D. immitis* larvae become adult worms

in cats, whereas up to 75% of *D. immitis* larvae in dogs become adults. Microfilariae were shown to remain in blood circulation for up to two years in a dog that was experimentally inoculated with microfilaremic blood **(Underwood and Harwood, 1939).** Adult worms in dogs can live up to 5 to 7 years in dogs **(Newton, 1968)** and 2 to 3 years in cats. Aberrant migration of the parasite has been documented, resulting in disease associated with dysfunction of the target tissue **(Nogami and Sato, 1997).**

2.6. Course of infection

Heartworms go through several life stages before they become adults infecting the pulmonary artery of the host animal. The worms require the mosquito as an intermediate stage to complete their life cycles. The rate of development in the mosquito is temperature-dependent, requiring about two weeks of temperature at or above 27°C (80°F). Below a threshold temperature of 14°C (57°F), development cannot occur, and the cycle will be halted **(Knight and David, 1998).** As a result, transmission is limited to warm months, and duration of the transmission season varies geographically. The period between the initial infection when the dog is bitten by a mosquito and the maturation of the worms into adults living in the heart takes six to seven months in dogs and is known as the "prepatent period".

After infection, the third-stage larval heartworms (L_3) deposited by the mosquito grow for a week or two and molt to the fourth larval stage (L_4) under the skin at the site of the mosquito bite. Then, they migrate to the muscles of the chest and abdomen, and 45 to 60 days after infection, molt to the fifth stage (L_5, immature adult). Between 75 and 120 days after infection, these immature heartworms then enter the bloodstream and are carried through the heart to reside in the pulmonary artery. Over the next three to four months, they increase greatly in size. The female adult worm is about 30 cm in length, and the male is about 23 cm, with a coiled tail **(Johnstone and Colin, 1998).** By seven months after

infection, the adult worms have mated and the females begin giving birth to live young, called microfilariae.

The microfilariae circulate in the bloodstream for as long as two years, waiting for the next stage in their life cycles in the gut of a bloodsucking mosquito. When ingested by a mosquito, the microfilariae undergo a series of molts to the infective third larval stage, and then migrate to the salivary glands of the mosquito, where they wait to infect another host. The incubation period required to reach the stage where the microfilariae become transmittable to another host can be as little as two weeks or as long as six weeks, depending on the warmth of the climate, and the larval life cycle ceases entirely if the ambient temperature drops below 14°C (57°F).

2.6.1. Characterization of canine heartworm infection status

The life cycle of *D. immitis* is complicated, involving the use of an intermediate host and a definite host **(Bowman and Atkins, 2009).** Intermediate hosts include several species of mosquitoes, whereas definite hosts include dogs, cats, and other small mammals. Once a dog has been infected with L3 larvae through the bite of a female mosquito, approximately 5-7 months pass before the L3 larva develops into an adult. As an adult, the heartworm typically occupies the pulmonary arteries, and sometimes migrating into the right atrium and right ventricle **(Venco and Vezzoni, 2001).** Adult worms will reproduce sexually and produce microfilariae that pass into the blood stream. A mosquito picks up the microfilaria during a blood meal, whereby the microfilaria develops into the L3 larva stage.

2.7. Clinical signs of infection

Clinical signs associated with canine heartworm infection depend on worm burden. Although dogs can experience no signs, signs generally includes exercise intolerance, enlarged pulmonary arteries, and decreased blood flow to the lung **(Bowman and Atkins, 2009).** In regards to caval syndrome, canine

symptoms include heart murmur, sudden onset of lethargy, and hemoglobinuria **(Bowman and Atkins, 2009 and Strickland, 1998).** Feline heartworm burden tends to be limited, with cats experiencing signs when worms appear within the vasculature or at the time of worm death **(Bowman and Atkins, 2009 and Dillon, 1998).** Signs of feline heartworms include chronic cough, emesis, and rarely death. Many infected dogs and cats remain asymptomatic. Some infected dogs and cats may experience heartworm associated respiratory disease (HARD), a condition that is often misdiagnosed as asthma or bronchitis **(Bowman and Atkins, 2009).**

Dogs show no indication of heartworm infection during the six-month prepatent period prior to the worms' maturation, and current diagnostic tests for the presence of microfilariae or antigens cannot detect prepatent infections. Rarely, migrating heartworm larvae get "lost" and end up in unusual sites, such as the eye, brain, or an artery in the leg, which results in unusual symptoms such as blindness, seizures and lameness. But normally, until the larvae mature and congregate inside the heart, they produce no symptoms or signs of illness.

Many dogs will show little or no sign of infection even after the worms become adults. These animals usually have only a light infection and live a fairly sedentary life style. However, active dogs and those with heavier infections may show the classic signs of heartworm disease. Early signs include a cough, especially on exercise and early exhaustion upon exercise. In the most advanced cases where many adult worms have built up in the heart without treatment, signs progress to severe weight loss, fainting, coughing up blood and finally, congestive heart failure.

2.8. Pathology, diagnosis, treatment and prevention

Dog heartworm is a chronic, ultimately fatal infection of companion and freely roaming dogs and cats, caused by *Dirofilaria immitis* Leidy, and spread by the bite of over 60 species of mosquitoes **(Ludlam *et al.*, 1970).** *Dirofilaria immitis* is a nematode, filarid parasite in which the adults primarily infect the pulmonary

artery and right ventricle of wild and domestic canines. The female worms give birth to microfilariae that circulate in the bloodstream of the definite (dog) host. The intermediate host, a mosquito, ingests the microfilariae when she takes a blood meal.

The microfilariae develop to third stage larvae in the mosquito's malpighian tubules over a period of 10-15 days **(Foster and Walker, 2009).** The infective juvenile worms migrate to the head capsule of the mosquito and enter a definitive host when the mosquito feeds again. The larva stays near the bite wound for several days before molting. Over the next two to three months the larvae molts again and becomes an adult and migrates to the pulmonary artery. After another three to four months, the infection becomes patent and detectable, and following mating the adult females begin producing microfilariae.

Cats are also susceptible to infection with *D. immitis*, but are not competent hosts. A few *D. immitis* larvae can mature to adulthood in the aberrant cat host, but they are rarely able to reproduce and do not produce sufficient microfilaremia to infect mosquitoes **(Bowman, 2009).** Despite the low parasite load, infected cats can develop heartworm-associated respiratory disease, a serious condition caused by the migration of larvae through the lung **(Blagburn and Dillon, 2007).** Migration of the larvae throughout the cat can also result in sudden death **(Bowman, 2009).** Infection in cats, as in dogs, is ultimately fatal.

Depending on parasitic load, infected canines can experience four classes of disease. Precise numbers of worms that cause disease in dogs varies with the size and overall health of the animals. Smaller dogs and those that have other health problems are less tolerant of infection than larger dogs, and because of their smaller cardiovascular system, small dogs become symptomatic with lower numbers of *D. immitis*. Class one disease results in a subclinical infection. Class two is characterized by the onset of signs. The signs are generally mild and consist of a chronic cough, dyspnea, and reduced exercise tolerance. In class three disease, the dog shows more severe signs, including syncope, hemoptyses,

congestive heart failure, and ascites. Class four disease is the acute onset of heartworm disease and is also known as vena cava syndrome. If a dog is at this level of illness, surgery is the only option to remove the worms via the jugular vein. Without surgery the dog will die within 24 to 72 hours **(Bowman and Atkins, 2009).**

Occult infections are those in which the dog is infected with heartworm, but the infection is not detectable **(Bowman, 2009).** Several reasons for having an occult infection exist. The dog can have a single sex infection, in which only male or only female is present, resulting in no microfilariae production. Early infections reduce detectability before maturation of the worms and production of microfilarie. Cats have an especially strong immune response to the *D. immitis* worms, which typically results in maturation of a few adult worms, but no circulating microfilariae. Inconsistent use of avermectins by pet-owners can kill the circulating microfilariae, but not the adult worms. This irregular drug use likely accounts for the majority of occult infection. Occult infections are dangerous because the dog will not receive appropriate care and thus may contribute to the further transmission of disease.

2.8.1. Pathologic changes associated with *D. immitis* in dogs

Pathologic changes within the host system result from damage from adult worms, microfilariae, and juvenile migratory larvae. Adult worms and/or worm antigen lead to endothelial lesions, pulmonary thromboembolism, pneumonitis, pulmonary hypertension, cor pulmonale, and ultimately hepatic congestion, ascites, and immune-complex glomerulonephritis. In rare cases, individual worms are wound around the tricuspid valve or chordae tendinae. When there are high worm burdens (> 50 worms), worms migrate actively from pulmonary arteries into the right ventricle, right atrium and rarely, into the vena cava. This may lead to the acute 'vena cava syndrome', which is characterized by intravascular hemolysis, disseminated intravascular coagulation (DIC), and shock. Occasionally, ectopic infection is reported. Adult worms may be found in

the anterior chamber of the eye, the skin, or the CNS. During aberrant somatic migration, juvenile larvae become trapped in these locations yet grow to adult stages. Most often they are dead upon dissection **(Schrey, 1996).**

2.8.2. Heartworm disease

Heartworm infection can have different effects on cats and dogs. Dogs have higher parasite intensities, often reaching greater than 30 adult worms compared to cats that have only 1-3 adult worms **(Nogami and Sato, 1997 and Ryan and Newcomb, 1995).** Dogs are often microfilaremic versus cats are not usually microfilaremic and it is much easier to diagnose infection in dogs than in cats. Clinical signs in dogs includes coughing, exercise intolerance, dyspnea, hepatomegaly, syncope, and ascites. Clinical signs in cats include lethargy, coughing, anorexia, chylothorax, and vomiting. Commonly cats have dyspnea or respiratory distress similar to feline bronchial disease (asthma) and dogs experience right-heart failure **(Nelson *et al.*, 2005a, b).** Often times, there are no signs. When signs do occur, they are often the result of the arrival of L5s in the pulmonary arteries which leads to acute pneumonitis. Signs may also occur when the death of adult worms cause thromboembolism and anaphylaxis and may result in sudden death **(Dillon, 1998).**

2.8.3. Heartworm-associated pulmonary pathology

Although the presence of adult *D. immitis* in the pulmonary arteries and its associated arteritis and thromboembolic disease can explain some of the manifestations of canine and feline heartworm disease (HWD), the cause of other findings remains unclear. This is particularly true for cats, which frequently develop generalized severe bronchointerstitial disease and extrapulmonary signs in response to very low worm intensitites **(Atkins *et al.*, 2000).** In cats, even a single worm can produce fatal disease.

Browne *et al.* (2005) examined 630 cats from an animal control shelter and assigned them to three groups (HW-infected, HW-exposed, and HW-free) based

on serological tests and necropsy findings. Pulmonary lesions characterized by pulmonary arterial occlusive hypertrophy were common in cats with adult worms and in cats that were free of adult worms but with *D. immitis* antibodies, suggesting that even transient infection leave cats with long-lasting pulmonary pathology. Occlusive hypertrophy is a characteristic of feline heartworm disease and is defined as >95% occlusion of the arteriolar lumen by an increase in the thickness of the tunica media. Almost 80% of the HW-infected cats had hypertrophy, followed with the HW-exposed (50%) and HW-free (13%) and there was a significant association between the number of occluded vessels and HW status.

2.8.4. Pathophysiology

The presence of adult worms in the heart leads to thickening of vessel walls and subsequent pulmonary hypertension. Histopathological changes in the vessels of dogs and cats infected with heartworm include fibrosis, hypertrophy and thrombosis **(Browne *et al.*, 2005).** In both dogs and cats, sustained hypertension can eventually lead to right-sided heart failure. Pulmonary parenchymal damage also occurs as a result of thrombosis caused by the embolization of dead worms, larvae or microfilariae **(Castleman and Wong, 1982).** This can lead to severe lung damage in dogs and cats **(Maia *et al.*, 2011).** Not all worms successfully migrate to the heart; worms are occasionally reported in atypical locations such as the abdominal cavity, eye, spinal cord and brain **(Litster and Atwell, 2008).** Aberrant migration occurs more frequently in cats than dogs **(McCall *et al.*, 1992).**

Clinical disease in dogs is usually slowly progressive, with a gradual onset of signs consistent with right-sided congestive heart failure such as lethargy, cough and abdominal distension due to ascites **(Atkins *et al.*, 2005).** Dogs may also suffer from caval syndrome, whereby adult worms migrate from the pulmonary arteries to the right atrium leading to acute right-sided heart failure and death, if untreated **(Kitagawa, 2003).** Signs of caval syndrome include collapse, anemia,

hemaglobinuria, jugular pulsation and characteristic heart murmur. Signs of disease due to heartworm infection in cats are much more variable. Many cats do not show clinical signs, although infected cats may develop respiratory disease, neurological signs or vomiting, or may die suddenly **(Venco *et al.*, 2008 and Atkins *et al.*, 2000).** Dogs and cats may show signs even when infected by immature worms or a single adult worm **(Snyder *et al.*, 2000).** Right-sided congestive heart failure and caval syndrome have also been described in cats, but occur only rarely **(Iizuka *et al.*, 2009 and Small *et al.*, 2008).**

Dogs and cats may experience acute episodes of coughing, dyspnea or intermittent vomiting, known as feline heartworm-associated respiratory disease (HARD), at around three months post-infection **(Lee and Atkins, 2010).** Since most immature worms do not survive in cats after they reach the caudal pulmonary arteries, it is thought that this acute disease is related to the death and embolization of worms or worm fragments **(Lee and Atkins, 2010).** This induces a strong inflammatory response in the vessels and pulmonary parenchyma with subsequent infarction of the pulmonary parenchyma and circulatory collapse **(Lee and Atkins, 2010 and Venco *et al.*, 1984).** Other signs of HARD may include neurological signs (*e.g.* ataxia, head tilt, blindness, circling or seizures) and sudden death **(Lee and Atkins, 2010).** Dogs and cats infected with heartworm often self-cure; in a study of 34 naturally infected cats with subclinical disease, 28 (82 percent) self-cured, and 21 of these showed no clinical signs during the study **(Lee and Atkins, 2010).** However, it should be noted that four of the six cats that died during the study showed no overt clinical signs before death **(Lee and Atkins, 2010).**

Dogs and cats are not the only species susceptible to heartworm infection. Natural infections have been reported in wild felids, including the African leopard, tiger, bobcat, snow leopard and African lion; ferrets and wild mustelids; monkeys; and marine mammals and rodents. Dogs, however, are the most frequently infected and have the highest worm burdens **(McCall *et al.*, 2008; Mazzariol *et al.*, 2010 and Kemmerer, 1998).** Ferrets are also highly susceptible

to infection and even a few adult worms can cause severe disease or death **(Kemmerer, 1998).** Zoonotic infections have been reported worldwide and usually occur in heartworm-endemic areas.1, 6 Humans are aberrant hosts for *D. immitis* and migrating larvae typically cause subcutaneous, ocular or pulmonary disease syndromes **(Grieve *et al.*, 1983).** Pulmonary migration of worms usually causes respiratory signs and the subsequent pulmonary granuloma can be seen on chest radiographs as a "coin" lesion **(McCall *et al.*, 2008).** Worms may occasionally migrate to other locations, with uncomfortable consequences **(Theis *et al.*, 2001).** The immunopathology of heartworm infections are not well-understood. *In vitro* studies on peripheral blood lymphocytes in dogs and pulmonary intravascular macrophages in cats suggest that heartworms and microfilariae induce immunosuppression **(Grieve *et al.*, 1979 and Weil *et al.*, 1981).** However, it has been observed that worm burdens in dogs living in endemic areas seem to reach a natural limit and that worms survive longer in naturally infected cats than in cats experimentally infected with greater worm burdens than would occur naturally **(Grieve *et al.*, 1983 ; McCall *et al.*, 1992 and Venco *et al.*, 2008).** These observations, coupled with the fact that many cats self-cure, would suggest that the immune system is capable of mounting a response to heartworms or microfilariae to at least moderate the severity of infection.

Much of the recent focus on heartworm immunology and pathophysiology has been on the role of *Wolbachia*. *Wolbachia* is an intracellular gram-negative bacterium belonging to the order Rickettsiales and is an endosymbiont of some pathogenic filarioid nematodes **(Kramer *et al.*, 2008 and Taylor *et al.*, 2005).** Antibodies against *Wolbachia* surface protein (WSP) have been detected in naturally infected dogs and cats **(Kramer *et al.*, 2005 and Morchon *et al.*, 2004).** In experimentally infected cats, anti-WSP antibodies remain high after antibodies to *D. immitis* have waned **(Morchon *et al.*, 2004).** *Wolbachia* certainly plays a role in the pathogenesis of canine and feline heartworm infection, although the precise role is unclear. Treatment of experimentally infected cats with ivermectin increases anti-WSP titers even further **(Morchon *et al.*, 2004),**

suggesting that death of the worms releases *Wolbachia* organisms and stimulates a strong host immune response **(Morchon *et al.*, 2004).** Treatment with doxycycline and ivermectin prior to melarsomine administration reduces the severity of lung pathology in heartworm-infected dogs **(Kramer *et al.*, 2008 and Bazzocchi *et al.*, 2008).** In a study of naturally infected cats and dogs, there was no clear difference in lung pathology between animals with circulating anti-WSP antibodies or detectable WSP antigens in their lung and those that did not have detectable levels of WSP antigen or anti-WSP antibody **(Kramer *et al.*, 2008).** However, arterial lesions were found to be less severe and thrombi much less numerous in experimentally infected dogs treated with a combination of doxycycline and ivermectin prior to melarsomine administration than in dogs left untreated or those treated with melarsomine alone **(Kramer *et al.*, 2008).** These findings suggest that rather than contributing to pathology while worms are alive, *Wolbachia*'s contribution to the pathological effects of heartworm infection may be related to the host's immune response when worms die and release the bacteria.

2.8.5. Role of *Wolbachia*

Wolbachia pipientis is an intracellular bacterium that is an endosymbiont of *Dirofilaria immitis*. All heart worms are thought to be infected with *Wolbachia* to some degree. Research indicates the inflammation occurring at the die-off of adult heartworms or larvae is in part due to the release of *Wolbachia* bacteria or protein into the tissues. This may be particularly significant in cats, in which the disease seems to be more related to larval death than living adult heartworms. Treating heartworm-positive animals with an antibiotic such as doxycycline to remove *Wolbachia* may prove to be beneficial, but further studies are necessary **(Todd-Jenkins and Karen, 2007).**

2.9. Diagnosis

Three methods can be used for the diagnosis:

Microfilarial detection was accomplished most commonly in the past by the microscopic identification of microfilariae on a direct blood smear, above the buffy coat in a microhematocrit tube, using the modified Knott test, or after millipore filtration. The accuracy of these tests, typically used for routine screening or diagnosis of heartworm infection, is improved by multiple testing. The modified Knott test and millipore filtration are more sensitive because they concentrate microfilariae, improving the chance of diagnosis **(Ettinger *et al.*, 2010).** The direct smear technique allows examination of larval motion, helping in the distinction of *D. immitis* from *Acanthocheilonema reconditum*. This distinction is important because the presence of the latter parasite does not require expensive and potentially harmful therapy. However, the potential for amicrofilaremic infections is 5-67%. The number of circulating microfilariae does not correlate with the number of adult heartworms, so is not an indicator of disease severity **(Ettinger *et al.*, 2010).**

Antigen testing, in most practices, has supplanted or supplemented microfilarial detection. Combining the microfilaria and adult antigen test is most useful in dogs receiving diethylcarbamazine or no preventative (as macrolides as for example ivermectin or moxidectin typically render the dog amicrofilaremic). Up to 1% of infected dogs are microfilaria-positive and antigen-negative **(Ettinger *et al.*, 2010).** Immunodiagnostics (ELISA, lateral flow immunoassay, rapid immunomigration techniques) to detect heartworm antigen in the host's blood are now regularly used. The weakness of these tests is they only detect the antigens released from the adult female worm's reproductive tract, so will produce negative results during the first five to eight months of infection **(Ettinger *et al.*, 2010).** The specificity of these tests is close to 100%, and the sensitivity is more than 80% **(Atkins and Clarke, 2005).** A recent study demonstrated a sensitivity of only 64% for infections of only one female worm,

but improved with increasing female worm burden (85%, 88%, and 89% for two, three and four female worms, respectively). Specificity in this study was 97% **(Ettinger *et al.*, 2010).** False-negative test results can be due to low worm counts, immature infections and all male infections.

X-rays are used to evaluate the amount of lung damage caused by the presence of heartworms.

There are many blood tests for diagnosis of heartworm infection. A blood concentration test such as the Knotts method or membrane filtration can detect circulating microfilariae in the blood stream. *Dirofilaria immitis* antigen tests detect proteins shed from female adult worms. False-negatives occur if the dog or cat has an all male, a low worm burden, or juvenile worms. *Dirofilaria immitis* antibody tests indicate exposure to heartworms, but not necessarily current infection. Enzyme-linked immunosorbent assays (ELISA) or non-ELISA lateral flow tests can be used to detect *D. immitis* antigen or antibodies. Additional support for a diagnosis of *D. immitis* infection includes thoracic radiography and echocardiography, as well as combining or repeating antigen and antibody tests **(Berdoulay *et al.*, 2004; Nelson, 2008 and Snyder *et al.*, 2000).** A necropsy may provide a definitive answer; however *D. immitis* have been known to migrate to aberrant locations **(Nogami and Sato, 1997 and Oh *et al.*, 2008)** and necropsy may not detect immature infections.

There are three tests which can be used to determine if an animal is heartworm positive. In the simplest test, the animal is killed and the heart dissected. The animal is positive if adult worms are observed in the heart tissue. A second, non-lethal method is the modified Knott's test which looks for circulating microfilariae in the bloodstream **(Zajac and Conboy, 2006).** The veterinarian draws one ml of blood and lyses the red blood cells with a 1:10 dilution with formalin. The microfilariae are sediment stained with methylene blue. The numbers of microfilariae are counted. The final, most common test is a SNAP test which detects antigen given off by the female reproductive tract. This test is

commercially available. Three drops of whole blood or serum are added to four drops of the provided conjugate. This solution is mixed by inversion and poured into the test. When the liquid is absorbed across the test, the activator button must be fully compressed. The veterinarian must wait eight minutes for test results (IDEXX Laboratories, Inc.).

Problems exist for both lethal and non-lethal tests. Of the two non-lethal tests, the SNAP test is generally more reliable and accurate than the modified Knott's test. The modified Knott's test is now out-of-date for determining infection for several reasons. Occult infections in which there are no circulating microfilariae in the bloodstream could lead to false negatives. Additionally, the process of infection to production of microfilariae takes approximately 6-9 months. It only takes 4-5 months for nematodes to mature in the host, so using a SNAP test can detect the infection sooner than a Knott's test, shortening the window of false negative results **(Bowman, 2009).** Because the Knott's test only uses 1 ml of blood, it is has low sensitivity compared to the SNAP (**Venco** *et al.*, 2008) test which can detect the antigen from the reproductive tract of as few as one to three adult female worms. The antigen test detects a female reproductive tract antigen, so the SNAP test will only work once the females have matured. The antigen test does nothing to shorten the pre-patency period. If an animal is killed or has died, cardiac dissection can be performed at necropsy to visually inspect the pulmonary artery for adult worms. The problem with this method is that due to human error and the degree of decay of the carcass, it is possible to miss the worms.

2.10. Zoonotic importance

Microfilariasis has been reported in dogs in those areas of Italy **(Rossi *et al.*, 1996 and Pampiglione *et al.*, 1986)** in which the climate allows the development of a large population of mosquitoes (intermediate hosts). Dogs, cats, foxes and other wild carnivores (definitive hosts) constitute the sources of infection for humans **(Pampiglione *et al.*, 1995).** The adult worms reside in the subcutaneous

connective tissue **(Hubert, 1985 and Bredal *et al.*, 1998)**, whereas the microfilariae were present in the blood without showing a nocturnal periodicity **(Webber, 1955).**

Dirofilaria immitis infection in humans was usually associated with pulmonary lesions or radiological coin lesions of the lung **(Ciferri, 1982).** The significance of *Dirofilaria immitis* infection was the potential for a radiological misdiagnosis was of primary or metastatic lung tumour, leading to thoracotomy for open lung biopsy or wedge resection of the lung to obtain the correct diagnosis **(Theis, 2005 and Foroulis *et al.*, 2005).** Sporadic cases of immature heartworms in unusual locations in the human body such as the eye **(Moorhouse, 1978),** mesentery **(Tada *et al.*, 1979),** cerebral artery **(Dobson and Welch, 1974),** spermatic cord **(Thies, 2001),** liver **(Kim *et al.*, 2002)** and lung **(Pampiglione and Rivasi, 2001)** have also been reported. *Dirofilaria repens* was a parasite of the subcutaneous tissue in dogs that can also accidentally infect humans, causing a condition referred to as subcutaneous dirofilariasis.

2.10.1. Heartworm disease in humans

There are several isolated reports of *D. repens* infection of humans from France, Belgium, Italy, the United Kingdom, Slovenia, Austria, and Germany. However, clinical manifestations of *D. immitis* infections of humans are rare **(Schrey, 1996).** Worldwide, approximately 150 clinical cases in humans have been reported - mostly from the USA and Japan. In Europe, endemic infection and clinical disease were reported in Spain and Italy. Two cases of pulmonary *D. immitis* infections have been reported in Germany - both individuals had recently returned from Corsica **(Tornieporth *et al.*, 1990 and Wöckel *et al.*, 1993).** In the province of Salamanca (Spain), *D. immitis*-specific IgM and IgG antibodies were found in 9.3% of humans examined and specific IgE antibodies in 12.6% of humans examined **(Simon *et al.*, 1991 and Espinoza *et al.*, 1993).** The diagnosis of pulmonary *D. immitis* infections of humans is made by ELISA, electrophoresis, or ELISA-linked histopathologic staining of biopsy material.

Chemotherapy is, as a rule, not necessary, as worms are usually dead. The surgicalextraction of lung ma sses, where pulmonary dirofilariasis may be suspected, is a diagnostic and therapeutic necessity **(Tornieporth *et al.*, 1990 and Robinson *et al.*, 1977).**

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental animals, areas and duration

The experimental animals of this study were 15 street dogs. A total of 100 dogs were observed and among of them 15, fifteen (9 male and 6 female of different age group) dogs were collected. The street dogs were randomly captured and euthanized from different locality of Dinajpur municipality area and HSTU campus for necropsy during the rabies control programme. The animals were collected with protective clothing using sterile instrument and transferred in the laboratory of the Department of Pathology and Parasitology for necropsy and histopathological examination. The duration of this study was 1st July, 2011 to 30 June, 2012.

3.2. Selection of animal and Survey Design

Street dogs were selected as target animals. The sample size constituted 15% of the estimated 100 street dog population that were killed seasonally for Rabies control program in Dinajpur Municipality Area (DMA). A total of 15 (fifteen) street dogs were randomly captured and euthanized by intravenous injection with saturated Magnesium Sulphate ($MgSO_4$) solution. The capture was conducted under the permission of Dinajpur municipal and ethical committee of Dinajpur municipal and Hajee Mohammad Danesh Science and Technology University (HSTU). Demographic information like approximate age, sex, and body condition were carefully recorded in a data sheet. The dogs were categorized into young (<2years) and adult (5-9 years and >9 years) based on their approximate age which was estimated by examining the teeth described by **Cynthia *et al.* (2011).** The body conditions of the dogs were documented according to the guideline of **Laflamme (1997).** Immediately after euthanasia, carcasses were brought to the Pathology and Parasitology laboratory of HSTU

and the necropsy was conducted as per standard method described in **Coles (1986).**

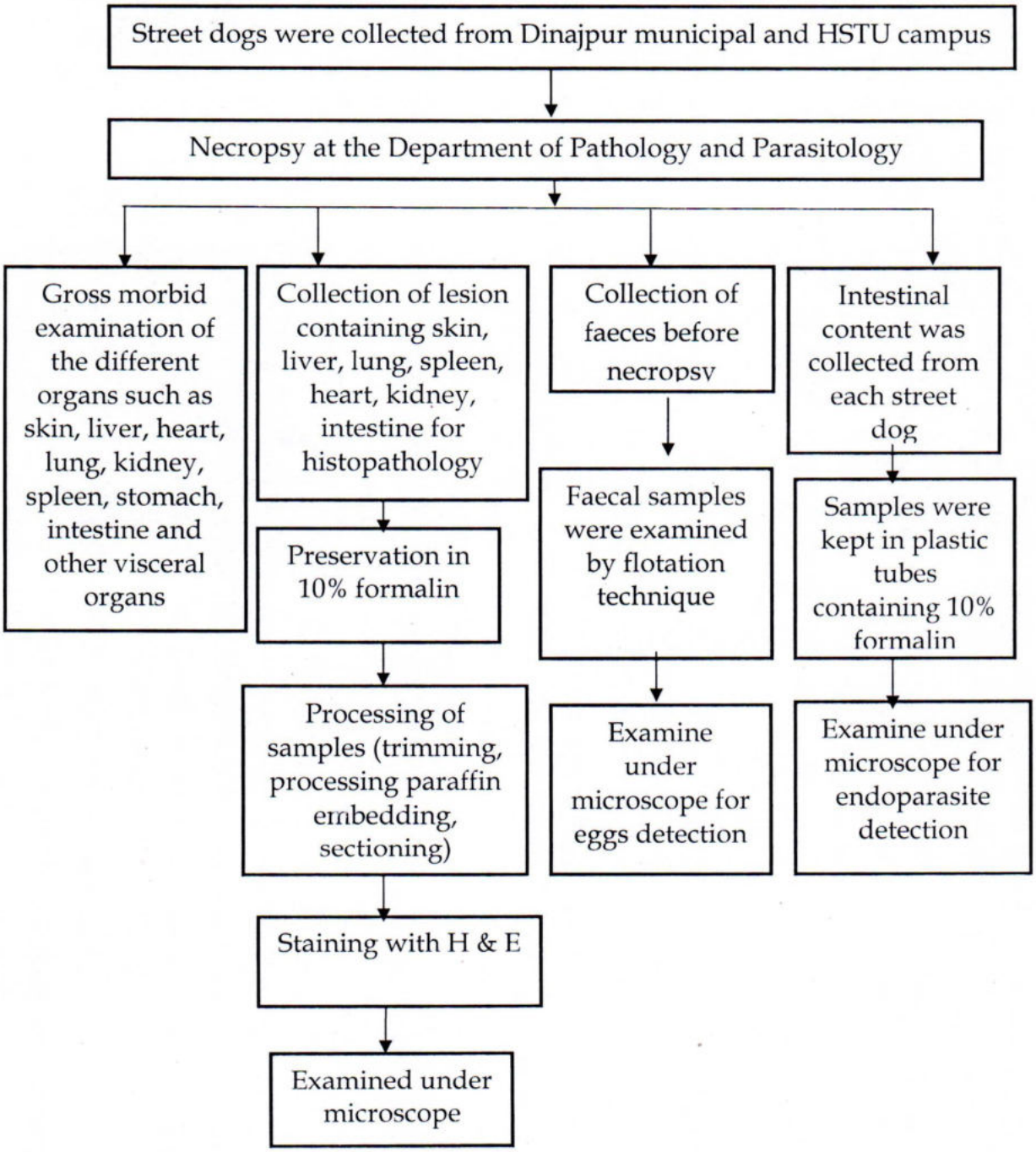
3.3. Analysis of the results

The examined data were compiled, tabulated and analyzed in accordance with the objectives of the study. The approximate percentage was calculated for each parameter. Finally data were analyzed and represented in tabular and graphical form.

3.4. Cleaning and sterilization of required glassware

Test tubes, glass tubes, glass slides, cover slips, beakers, pipettes, reagent bottles, glass bottle, spirit lamp, measuring cylinders etc. were used in this study. The conical flask, measuring cylinder, beakers, glass slides, cover slip, for slide preparation for histopathological study and staining of organisms after smear and pipettes, reagent bottle, glass tubes for different biochemical tests. New and previously used glassware were collected and dipped in 2% sodium hypochlorite solution and left there until cleaned. After overnight soaking in a household dishwashing detergent solution, the glassware were cleaned by brushing and washed thoroughly in running tap water and rinsed three times in distilled water. The cleaned glasswares were then dried on a bench at room temperature or in an oven at 50-70^{0}C.

EXPERIMENTAL DESIGN



Flow diagram of the experiment

3. 5. Clinical examination

The general health condition and sex of the street dogs were recorded. The sex of dogs was determined before and/or after killing. The dogs were observed to detect clinical signs. The clinical signs were observed from the visual examination of the street dogs.

3.6. Pathological examination

3.6.1. Necropsy of the street dogs

The necropsy examination was carried out in the Department of Pathology and Parasitology (HSTU).The postmortem examinations of all the cases were performed as soon as the Dogs were dead and brought to the department. At necropsy, gross tissue changes were observed and lesions containing tissues were collected for histopatholghy.

3.6.1.1. Materials required for necropsy examination

- Sample animals (Dogs)
- Scissors
- Forceps
- Gloves
- Musk
- Scalpel
- 10% formalin

Procedures

1. At first the animals were washed with the disinfectant
2. The animal was placed on post mortem table on dorsoventral position and observed for the external lesions, e.g. erosion, ulceration, wound in the skin, external orifice, discharge, raised part of the joint, prolaps of uterus, vagina, rectum etc were observed carefully.

3. An incision was made through mid-ventral line or through linea alba from chin to the anus.
4. Skinning of the animal was done in such way that the longisimus muscle was exposed.
5. The pectoral and serratus muscle were cut so such a way that the legs were fallen on the ground
6. Cutting down the medial thigh muscle in the groin region of both legs such way that coxo-femoral articulation was exposed and posterior leg was fallen on the ground
7. The sternum was hold in appropriate position.
8. Then the incision was made to the medio-lateral side of the rami
9. Mandibular muscle was cut and pulling out the tongue by holding the buccal cavity.
10. An assistant hold the tongue and cut down of the hyoid bone by a sharp knife.
11. Pulling out the tongue, pharynx, larynx from buccal cavity and cutting down the dorsal attachment of the tongue, trachea, esophagus and reach up to cariniform cartilage
12. An transverse incision was made on the xyphoid cartilage on the anterior abdomen
13. Through the costo-condral junction an incision was made to both side of sternum from posterior to anterior up to the cariniform cartilage
14. Severed the sterna attachment
15. Then breakdown the first 3-4 ribs were broken down in any sides to get enough space to enter into the thoracic cavity
16. Then examined thoracic cavity and the pleura for the presence of abscess, cyst, tumor, haemorrhage, fibrosis etc.
17. Diaphragm was examined for any lesions.
18. All the visceral organs like liver, spleen, lung, heart and kidney were examined for any gross lesions.

19. Brain tissues were examined by separating the head from alanto-occipital joint, skinning and by removing the brain from cranial cavity.

3.6.2. Gross lesion

Gross morbid lesions of different organs were observed after necropsy examination of the dogs.

3.6.3. Histopathological study

During necropsy, various organs having gross lesions were collected fixed in 10% formalin for histopathological studies. Formalin fixed tissue samples were processed and stained as per standard method **(Luna, 1968).**

3.6.3.1. Materials required for histopathology

- Samples (Skin, heart, lung, spleen, kidney, intestine etc)
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX).
- Microscope
- Microtome
- Water bath

3.6.3.2. Processing of tissue for histopathology

1. Collection of tissue and Processing

During tissue collection the following point were taken into considerations-

The tissues were collected in conditions as fresh as possible. Normal and diseased tissues were collected side by side. The thickness of the tissues were as less as possible (5mm approximately).

The tissues (skin, liver, heart, lung, spleen) were collected from the Dogs in the Histopathology Laboratory of the Department of Pathology and Parasitology, HSTU, Dinajpur.

2. Fixation: 10% formalin was added in the plastic container (10 folds of the tissue size and weight) and fixed for 3-5 days.

3. Washing: The tissues were trimmed into a thin section and washed over night in running tape water to remove formalin.

4. Dehydration: The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule.

- 50% alcohol – one hour
- 70% alcohol – one hour
- 80% alcohol – one hour
- 95% alcohol – one hour
- Absolute alcohol – three changes (one hour for each change.)

5. Cleaning: the tissues were cleaned in chloroform for 3 hours to remove ethanol (1 and half hr in each, two changes).

6. Impregnation: Impregnation was done in melted paraffin (56- 60°C) for 3 hours.

7. Embedding: Paraffin blocks containing tissue pieces were made using templates and molten paraffin

8. Sectioning: Then the tissues were sectioned with a microtome at 5-6µm thickness. The sections were allowed to spread on luke warm water bath (40-45 °C) and taken on a glass slide. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The slides containing sections were air dried and stored in cool place until staining.

3.6.3. 3. Routine Hematoxylin and Eosin staining procedure

3.6.3.3.1. Preparation of Ehrlich's Hematoxylin solution

Hematoxylin crystals	4.0 g
Alcohol, 95%	200.0 ml
Ammonium or potassium alum	6.0 g
Distilled water	200.0 ml
Glycerine	200.0 ml
Glacial acetic acid	20.0 ml

Hematoxylin was dissolved in the alcohol and the alum was dissolve in distilled water and mixed thoroughly. After these were in complete solution the glycerin and acetic acid were added.

3.6.3.3.2. Preparation of eosin solution

1% stock alcoholic eosin

Eosin Y, water soluble	1 g
Distilled water	20 ml
95% alcohol	80 ml

Eosin was dissolved in water and then 80 ml of 95% alcohol was added.

Working eosin solution

Eosin stock solution	1part
Alcohol, 80%	3 parts

0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

3.6.3.3.3. Staining protocol

The sectioned tissues were stained as described bellow:

- The sectioned tissues were deparaffinized in three changes of xyline (three minutes in each)
- Then the sectioned tissues were rehydrated through descending grades of alcohol as per following schedule.
  - Absolute alcohol – three changes (three minutes for each)
  - 95% alcohol - two minutes
  - 80% alcohol - two minutes
  - 70% alcohol - two minutes
  - Dipping with distilled water for 10 minutes.
- The tissues were stained with Harris hematoxylin for 2-10 minutes.
- Washed in running tap water for 10-15 minutes.
- Then the tissues were dipped in ammonia water (few dips).
- Stained with eosin for one minute.
- Differentiated and dehydrated in ascending grade of alcohol.
  - 95% alcohol – three changes (2-4 dips for each.)
  - Absolute alcohol – three changes (2-3 minutes for each)
- Cleaned in xyline: three changes (five minutes each).
- Tissues were mounted with cover slip by using DPX

- ❖ The slides were dried at room temperature and examined under a low (4X, 10X) and high (40X, 100X) power objectives.

3.7. Parasitological examination of faeces

3.7.1. Collection of faeces

Faecal samples were collected either directly from the rectum after killing of dog or from the street after defecation. Interstinal content was collected during the postmortem examination of the dog.

3.7.2. Microscopic examination of faeces

Equipment and appliances

- Beakers
- A tea strainer
- Stirring rod
- Test tubes
- Microscope
- Slides
- Coverslips
- Flotation fluid

Procedures

- ❖ The faecal samples were examined by floatation technique under standard protocol **(Fowler and Miller, 1999).**
- ❖ Approximately 3g of faeces was taken into a container.
- ❖ Then floatation fluid was added into the container which containing faeces.
- ❖ The faeces were mixed thoroughly with the floation fluid with stirring device.
- ❖ Then the faecal suspension was poured through a tea strainer into another container.
- ❖ The container was leaved to stand for 10 minutes.

- ❖ The test tube was filled with faecal suspension up to full.
- ❖ Then the test tube was stand in a test tube rack to stand for some minutes.
- ❖ A coverslip was placed on top of the test tube.
- ❖ Then the coverslip was placed on slides.
- ❖ The slides were examined under microscope for detection eggs in low and high magnifications.

3.8. Photography

All the characteristic pathological changes were subjected to gross photographs and the characteristic microphotographs were taken by personal Sony camera in the department of Pathology and Parasitology, HSTU.

CHAPTER IV

RESULTS

4.1. Prevalence of *Dirofilaria immitis* in street dogs

In order to detect the prevalence of *Dirofilaria immitis* in street dogs at Dinajpur municipality area during the period from July 2011 to June 2012, a total of 100 dogs were observed and among of them 15, fifteen (9 male and 6 female of different age group) dogs were randomly captured and euthanized from different locality of Dinajpur municipality area for necropsy during the rabies control programme (Fig.2).

The dogs were necropsized to observe the presence of *Dirofilaria immitis* in their heart and lungs producing characteristic lesions.

During this investigation, it was observed that heart worm infection is common in street dogs. The study indicates that about 46.67% dogs were infected with *Dirofilaria immitis*. Of a total of 15 street dogs tested, 7 were positive for *D. immitis* infection (46.67%). (Table1).

On the other hand, the prevalence of heart worm infection also varied with different variables like sampling season, age, sex and body condition of the street dogs.

Parasite *D. immitis* was found in 5 of 9 (55.56%) male and 2 of 6 female (33.33%) dogs, but without statistical difference between the sexes (Table 2, Graph 1). Sex specific prevalence showed that *D. immitis infection* was higher in male dogs.

Prevalence also varied with the age of street dogs where adults were more susceptible than youngs (Table 3). The prevalence of *D. immitis* in dogs > 9 years

old was higher (66.67%) than in other age groups, but without significance (Table 3, Graph 2).

However, heart worm infections attributed significant effect on the body condition of the study population (Table 4). It was revealed that nutritional condition of the dog had an effect with heart worm infection. Heart worm infection was recorded higher in poor body conditioned (65.5%) dogs than normal body conditioned (28.57%) dogs (Table 4).

Most of the heart worm infections apparently occurred more in summer season (Table 5, Graph 3). The highest seasonal prevalence was found 50% in summer and 42.85% in winter season.

4.2. Clinical examination

No abnormalities in animals with mild infection and some with moderately severe infection. Cachexia, syncope, coughing and exercise intolerance associated with labored breathing or crackles observed in severely heart worm affected dogs (Fig. 3, 4 and 5).

Other clinical signs typically appearing in this disorder are Gallop rhythm; heart murmur; Jugular pulse; muffled, decreased, heart sounds; peripheral venous distention, jugular distention; tachycardia, rapid pulse, high heart rate; weak pulse, small pulse; ascites, fluid abdomen; anorexia, loss or decreased appetite, not nursing, off feed; abnormal proprioceptive positioning, knuckling; ataxia, incoordination, staggering, falling; fever, pyrexia, hyperthermia; generalized lameness or stiffness, limping; generalized weakness, paresis, paralysis; inability to stand, downer, prostration; pale mucous membranes or skin, anemia; paraparesis, weakness, paralysis both hind limbs; underweight, poor condition, thin, emaciated, unthriftiness, ill thrift; weight loss; abnormal hindlimb

reflexes, increased or decreased; circling; dullness, depression, lethargy, depressed, lethargic, listless; head tilt; hindlimb hypoesthesia, anesthesia rear leg; seizures or syncope, convulsions, fits, collapse; abnormal lung or pleural sounds, rales, crackles, wheezes, friction rubs; coughing, coughs; dyspnea, difficult, open mouth breathing, grunt, gasping; hemoptysis coughing up blood; increased respiratory rate, polypnea, tachypnea, hyperpnea; dryness of skin or hair; pruritus, itching skin; rough hair coat, dull, standing on end; skin crusts, scabs; skin edema; skin erythema, inflammation, redness; skin necrosis, sloughing, gangrene; skin papules; skin ulcer, erosion, excoriation; observed in severely heart worm affected dogs (Fig. 3, 4 and 5). Animals are categorized as poor health which showed one or more signs of the above (Table.4).

4.3. Necropsy examination

At necropsy, examination of heart worms (*Dirofilaria* sp.) were found in street dogs. The parasites were seen in the right ventricle of heart (Fig. 6 and 8). Many adult male parasites were found from the right ventricle of heart and revealed male measuring 125 to 250 mm in length, posses tails that are blunted, armed with caudal alae, and are spirally coiled (Fig. 9). The females, which measure 250 to 300 mm in length, were larger. (Fig.9). The right ventricle and atrium were enlarged (Fig.7). Pulmonary emboli, containing dead parasites, were seen in a small number of pulmonary arterial branches (Fig.6), and some areas of the lung were congested. After a dog dies, the heartworms leave the heart chamber and can be found in the major arteries of the lung.

4.4. Histopathological study

The severity of cardiopulmonary pathology in dogs is determined by worm numbers, host immune response, duration of infection, and host activity level.

During the necropsy examination, adult *D. immitis* were found in the portal vein, right ventricle, and atrium of the heart and pulmonary trunk.

Heartworms cause the lining of the heart and pulmonary arteries to become rough and disrupt the flow of blood, the intima also thickened (Fig.11) compared with normal one (Fig.10).

Due to heart worms burden in heart chamber destruction in heart wall linings occurred and endothelial cells are separated and appear inflamed (Fig.12).

Microscopically, microfilarias were found throughout the vessels of heart (Fig.13).

After a dog dies, the heartworms leave the heart chamber and can be found in the major arteries of the lung.

The right ventricle and atrium were enlarged. Pulmonary emboli, containing dead parasites, were seen in a small number of pulmonary arterial branches, and some areas of the lung were congested; Congestions of cardiac muscle fibers was also observed (Fig.14).

Pulmonary hypertension initially induces a dilation of the right ventricle with a compensatory hypertrophy of the myocardium (Fig.15 and 16).

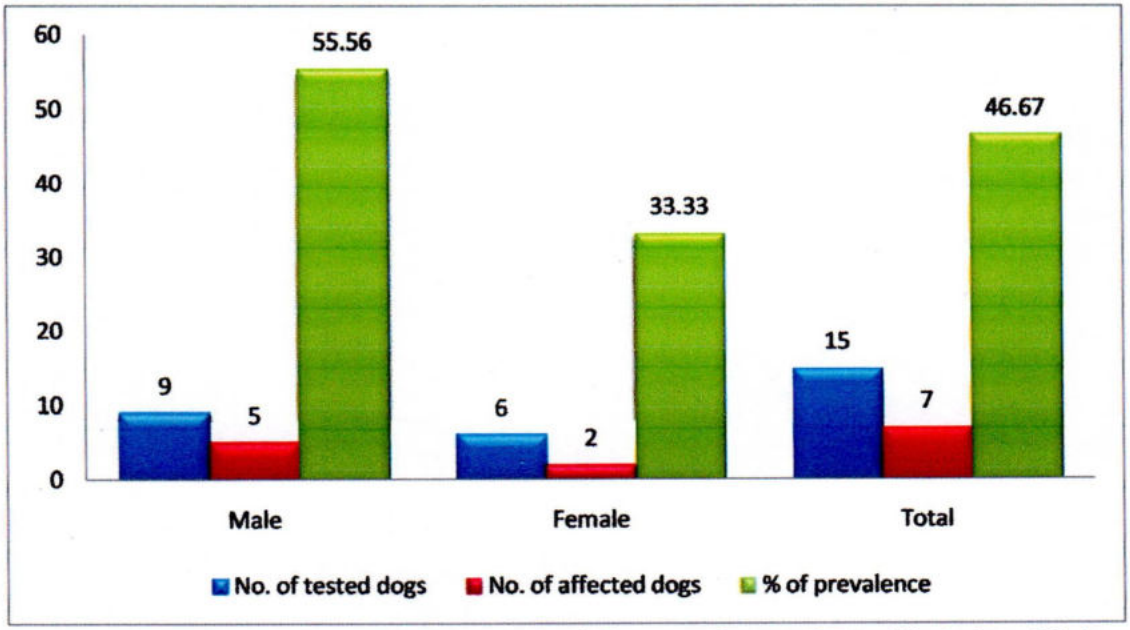
Endothelial damage leads to myointimal proliferation (Fig.17).

Table.1. Overall prevalence of *Dirofilaria immitis* in street dogs

No. of examined dogs	No. of affected dogs	% of prevalence
15	7	46.67

Table.2. The prevalence of *Dirofilaria immitis* in street dogs according to sex

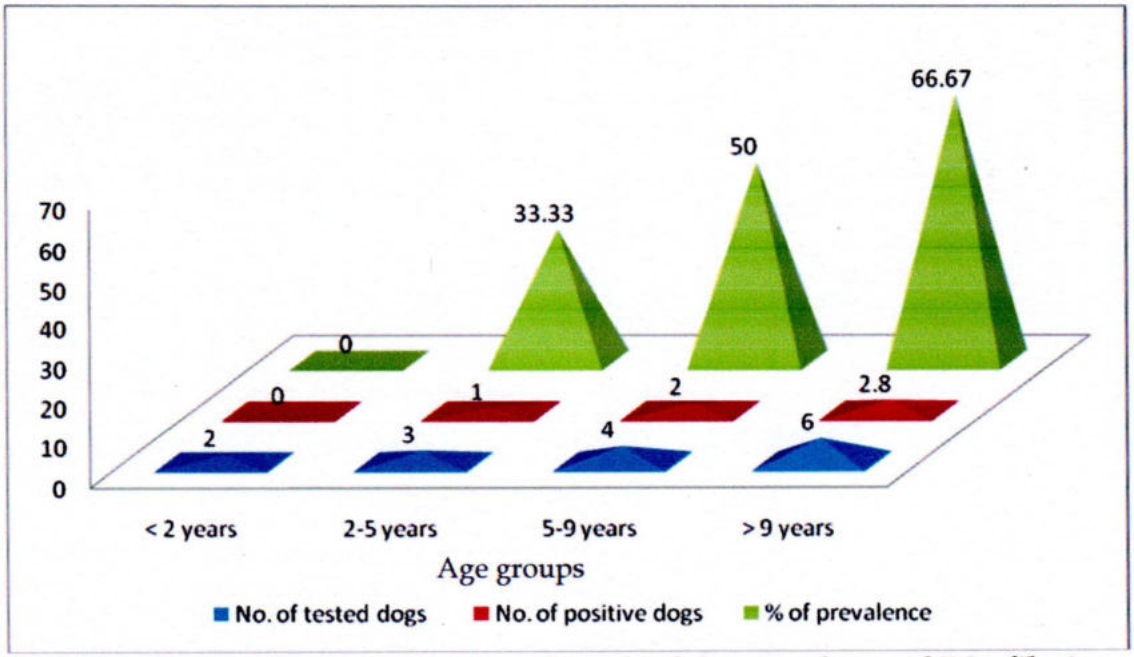
Sex	No. of tested dogs	No. of affected dogs	% of prevalence
Male	9	5	55.56
Female	6	2	33.33
Total	15	7	46.67



Graph.1. Graphical presentation of result of the prevalence of *Dirofilaria immitis* in street dogs according to sex.

Table.3. The prevalence of *Dirofilaria immitis* in street dogs according to age

Age groups	No. of tested dogs	No. of positive dogs	% of prevalence
<2 years	2	0	0
2-5	3	1	33.33
5-9	4	2	50
>9 years	6	4	66.67



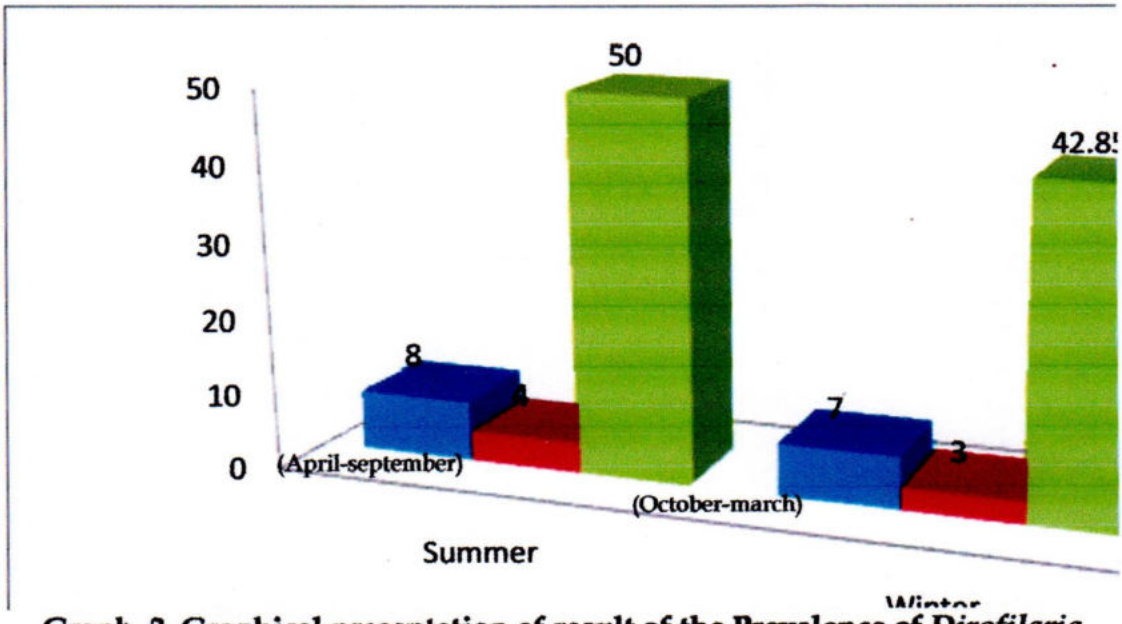
Graph. 2. Graphical presentation of result of the prevalence of *Dirofilaria immitis* in street dogs according to age.

Table.4. Health status related prevalence of *Dirofilaria immitis* in street dogs

Health status of the dog	No. of dogs examined	No. of dogs affected	Prevalence (%)
Poor health	8	5	62.5
Normal health	7	2	28.57

Table.5. Prevalence of *Dirofilaria immitis* in street dogs based on season

Season	No. of tested dogs	No. of affected dogs	Prevalence (%)
Summer	8	4	50
Winter	7	3	42.85



Graph. 3. Graphical presentation of result of the Prevalence of *Dirofilaria immitis* in street dogs based on season.

Fig.2. A street dog was captured at Dinajpur municipality area during the rabies control programme

Fig.3. A street dog at Dinajpur municipality area was coughing associated with labored breathing or crackles

Fig.4. Heartworm affected street dog at Dinajpur municipality area associated with dryness of skin, skin ulcer, erosion and cachexia condition

Fig.5. Heartworm affected street dog at Dinajpur municipality area associated with exercise intolerance, labored breathing, loss or decreased appetite, generalized weakness, dull and rough hair coat

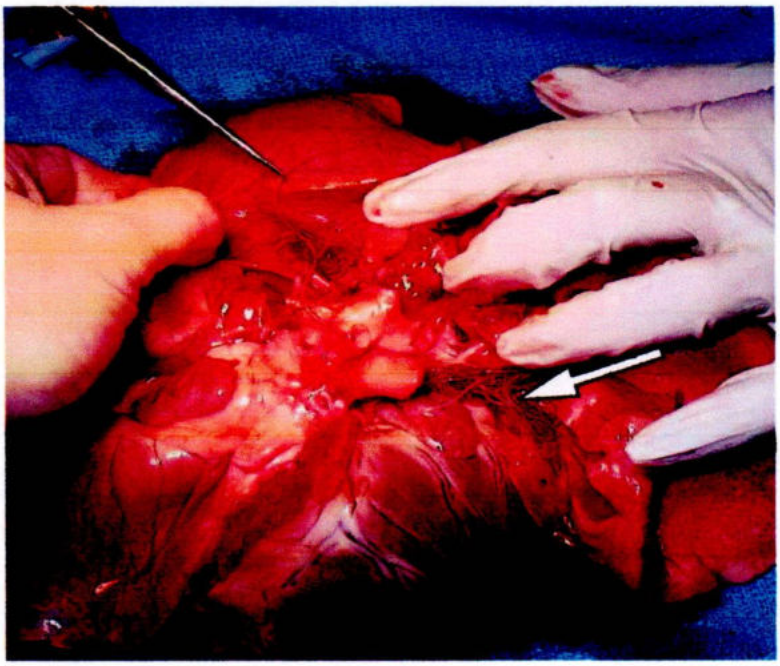


Fig.6. Dogs at Dinajpur affected with heart worm *(Dirofilaria immitis)*. The worms found in the right ventricle of heart and in pulmonary trunk during necropsy (Arrow)

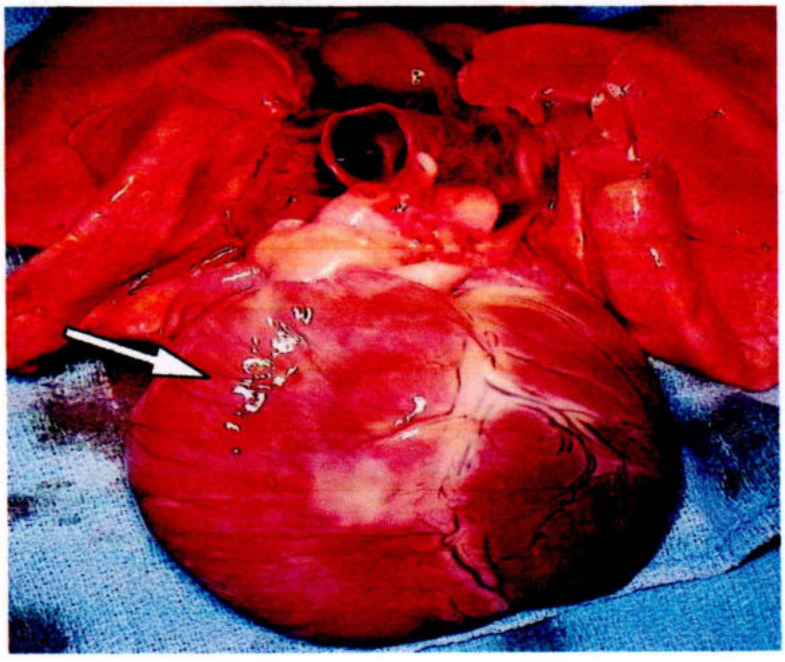


Fig.7. Heart with enlarged right ventricle and atrium affected with heart worm *(Dirofilaria immitis)*. Dilated right ventricle (Arrow)

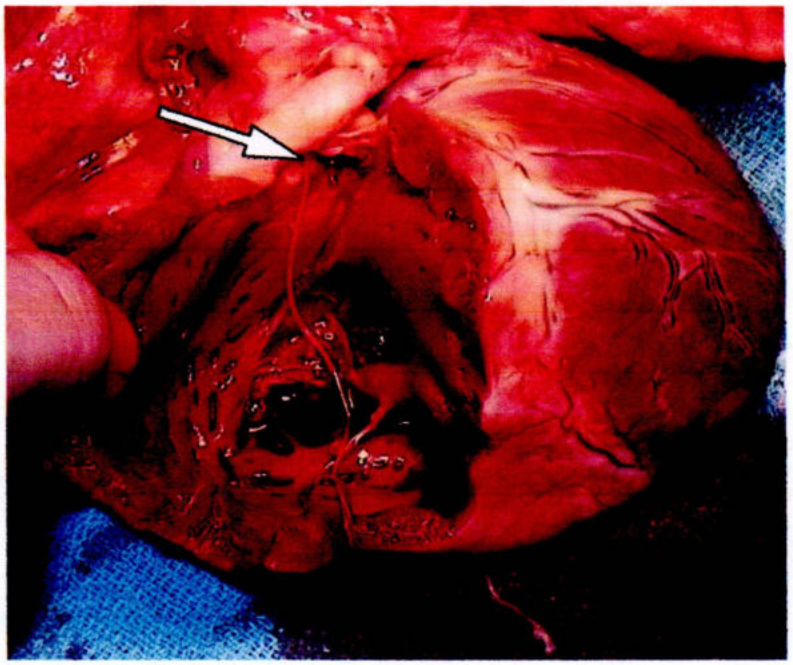


Fig.8. Dissection reveals greatly enlarged right ventricle and atrium. The right ventricle reveals heart worm (Arrow)

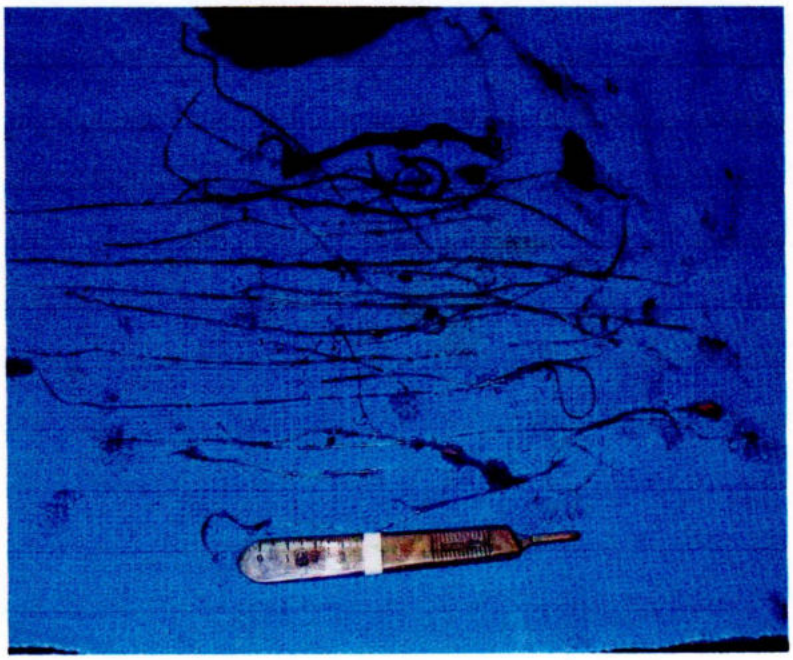


Fig.9. The canine heart worms *(Dirofilaria immitis)* as revealed from the right ventricle of heart

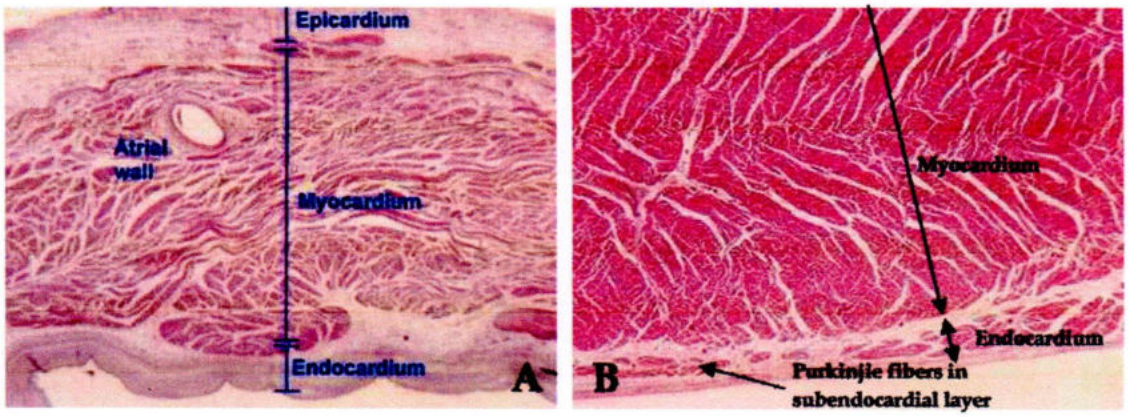


Fig.10. Cross section of normal heart wall stained with H & E (A, 4X and B, 10X) showing the components of the outer pericardium (heart sac), muscle layer (myocardium), and inner lining (endocardium)

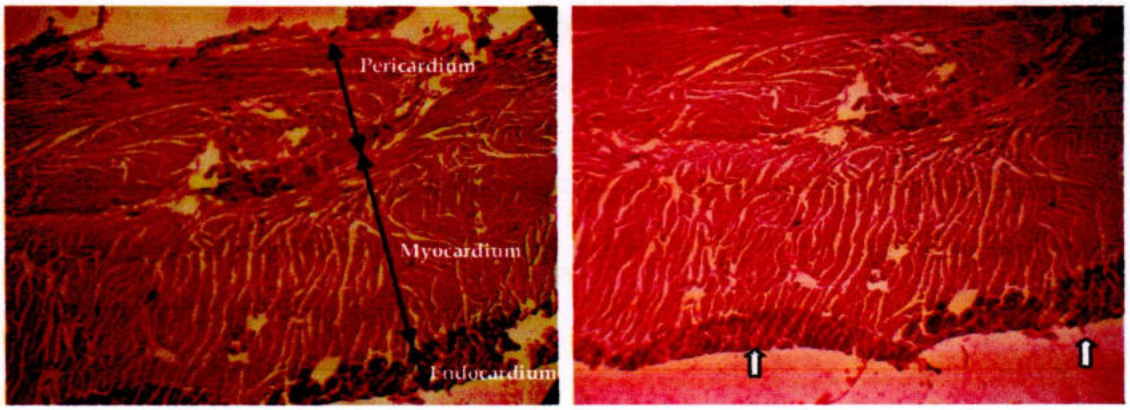


Fig.11. Cross section of infected heart wall stained with H & E (10X) showing the components of the outer pericardium (heart sac), muscle layer (myocardium), and inner lining (endocardium). Heartworms cause the lining of the heart to become rough and intima thickens (Arrows)

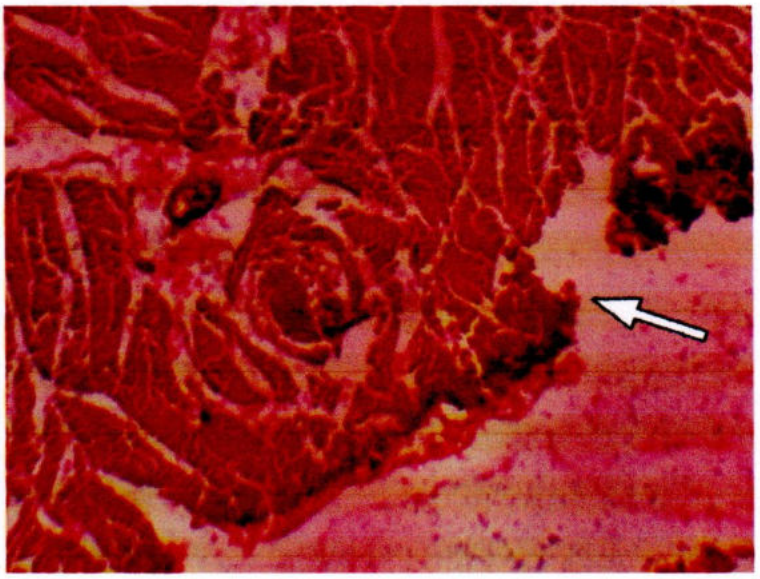


Fig.12. Destruction in heart wall linings due to worms load stained with H & E (40X). Endothelial cells are separated and appear inflamed (Arrow)

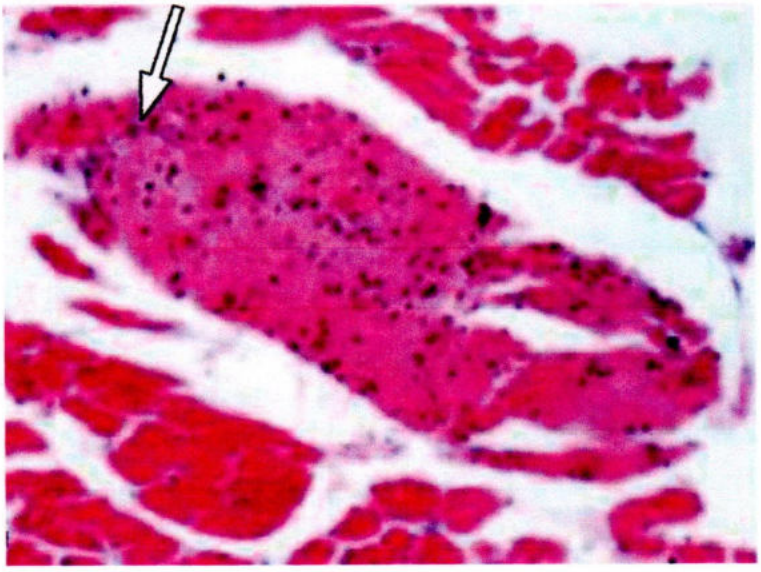


Fig.13. Microfilaria presentation in the vessels of heart stained with H & E (100X). Microfilarias were found throughout the vessels of heart (Arrow)

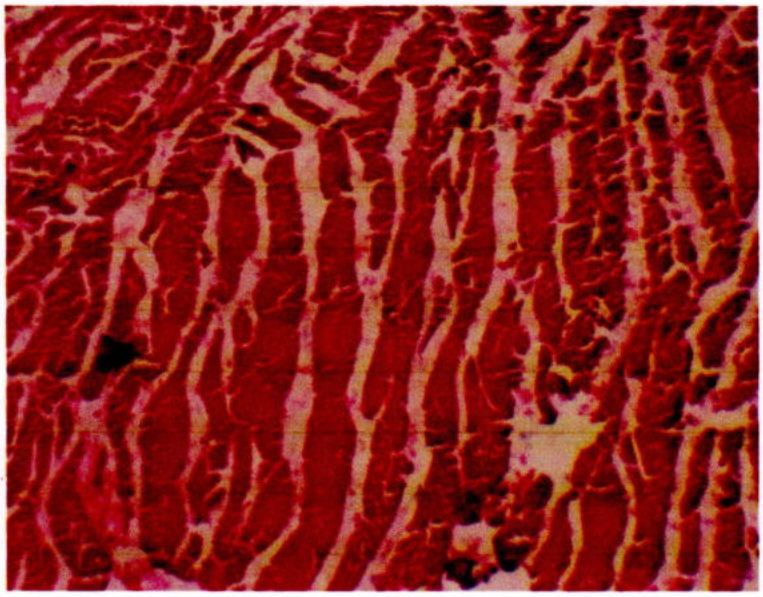


Fig.14. Congestions of cardiac muscle fibers stained with H & E (10X)

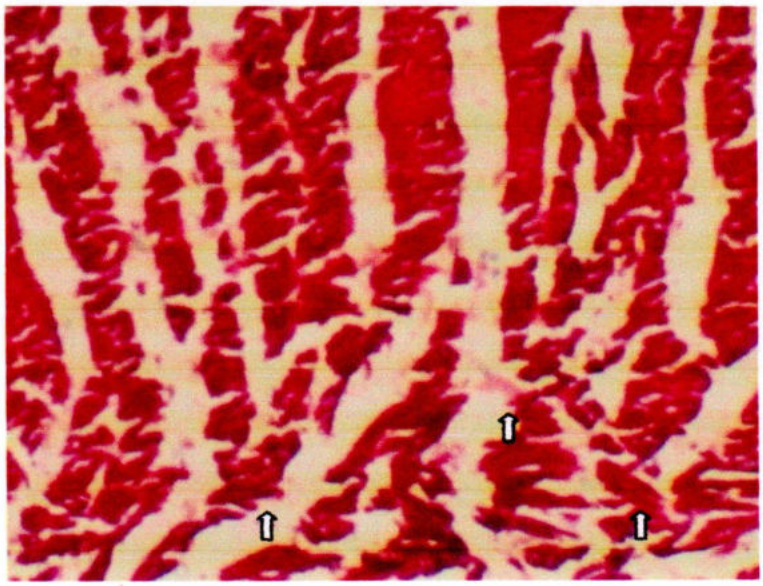


Fig.15. Hypertrophy of the myocardium (heart muscle layer) of right ventricle stained with H & E (40X). Heart muscles are hypertrophic and fragmented(Arrows)

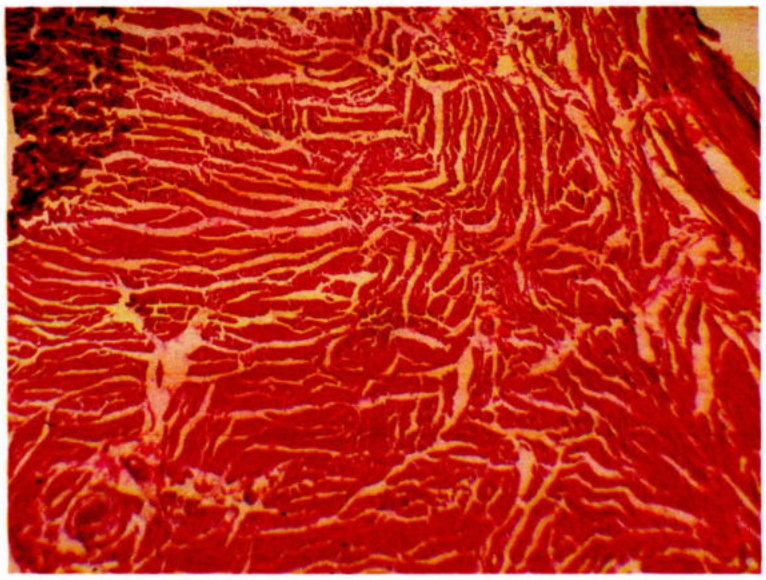


Fig.16. Hypertrophied right ventricular muscle layers stained with H & E (4X)

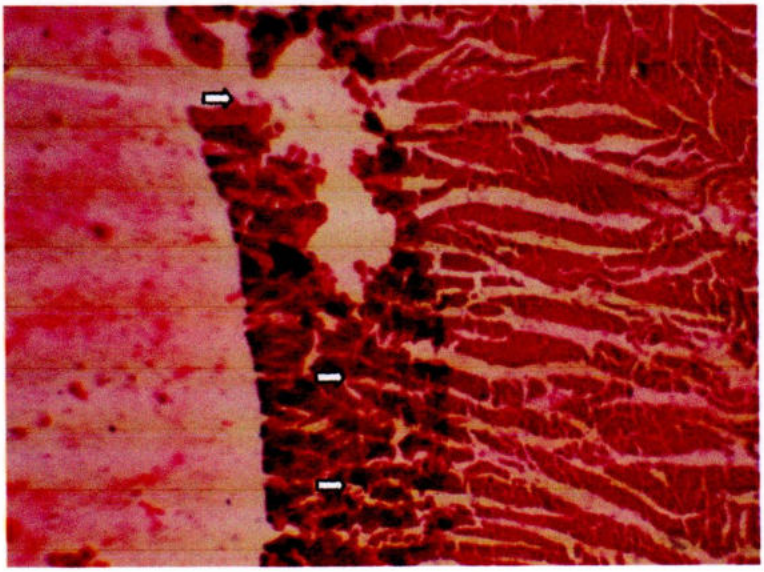


Fig.17.Destruction/damage of endothelial linings of endocardium stained with H & E (10X). Endothelial damage leads to myointimal proliferation (Arrows)

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

5.1. Prevalence of *Dirofilaria immitis* in street dogs

Dirofilariasis is a disease of world wide distribution, but the most endemic areas are those with moderate, tropical and subtropical climates where mosquito populations are high and stable. Other regions with cold weather, with hot summers and with rivers, lakes and widely irrigated lands are also suitable for the development of the disease. Female mosquitoes serve as an intermediate host by sucking blood from a dog with circulating *Dirofilaria immitis* microfilaria **(Montoya *et al.*, 1988).**

In view of the above facts, it is assumed that Dirofilariasis is one of the major problems for the street dogs in Bangladesh, as Bangladesh has a subtropical climate with rivers, lakes and widely irrigated lands **(Maitby, 1986)** where mosquito populations are high and stable, but no attention has been paid to study the prevalence and its effects on the dog in Bangladesh.

The results of this study showed that 46.67% of street dogs in Dinajpur were infected with adult heart worms and microfilariae of *Dirofilaria immitis*.

The overall prevalence (46.67%) of the heart worm infection of this study were in close consistency with the report of **Islam (2010)** who found 60% prevalence of such infection in necropsized street dogs from Dinajpur district of Bangladesh. **Basu *et al.* (2010)** reported 78.5% helminth infection through coproscopy in street dogs of Chittagong, Bangladesh. The earlier reports of **Komatangi (2005)** and **Minnaar *et al.* (2002)** were also documented similar type of prevalence in different corners of the world. Non descriptive street dogs are more prone to various filarial and helminth infections as they feed on rubbish bins, hardly dewormed **(Umar, 2009)** along with geographical or environmental factors might be accounted for higher frequency. Higher prevalence of parasites

indicated a continuous trend of such infection in the study area. The recorded species also have the zoonotic significance which constituted a great public health risk due to frequent contact between human and dogs (Ramirez-Barrios *et al.* 2004). However, the current investigation also revealed concurrent infection with more than one enteric helminth which is a usual scenario in street dogs all over the world **(Shimelis, 1994).**

In other country like in Iran, different prevalences were reported in previous studies: 60.8% in Shahsavar, 26.7% in Meshkin-Shahr and 8.4% in Tabriz **(Sadighian, 1969 and Javidi, 2003).** All the mentioned studies used the Knott test method. Dog heartworm has been increasing its range in the last 30 years in dogs **(Weinman and Garcia, 1974; Pennington *et al.*, 1970 and Kocan, 1976).**

In areas endemic for *D. immitis,* prevalence in dogs that are not on heartworm preventive can exceed 50% and normally the prevalence in unprotected dogs is 25-50%. Feline heartworm prevalence is often 5-20% of the positive dog population **(Atkins *et al.*, 1998; Bowman *et al.*, 2007; Carleton and Tolbert, 2004; Hermesmeyer *et al.*, 2000; Miller, 1998; Nogami and Sato, 1997; Patton and McCracken, 1991 and Ryan and Newcomb, 1995).** Endemic occurrence of *D. immitis* has been reported in the USA, Canada, South America, Africa, Australia, Asia and Europe **(Yildirim *et al.*, 2006).** The prevalence of heart worm infection in dogs appears to have increased in recent years. This is due to a lack of prevention procedures and weak knowledge of dogs' owners **(Lee *et al.*, 1996).** Heartworm infections in dogs in California were not diagnosed until the 1970s **(Weinmann and Garcia, 1974)** and a recent national survey showed dog heartworm in domestic dogs throughout the continental United States **(Bowman *et al.*, 2009).** At 3.9%, the southeastern states had the highest prevalence rate in the country while Oklahoma had a statewide prevalence of 2.1% **(Bowman *et al.*, 2009).**

The differences obtained in this and previous studies might be related to factors such as mosquito population density, mosquito fertility, mosquito species and

environmental temperature. Close to Iran, *D. immitis* was reported from Turkey with the prevalence of 9.3% in Ankara **(Oge *et al.*, 2003)** and 9.6% in Kaysari **(Yildrim *et al.*, 2006).** The highest infestation in Asia was reported in Japan with 62.8% prevalence **(Tada *et al.*, 1991).**

The prevalence in this study is higher than in Turkey and Korea (10.2%) but lower than in Japan (47%) and Taiwan (55%) **(Tada *et al.*, 1991; Lee *et al.*, 1996; Fan *et al.*, 2001 and Yildirim, 2006).**

The results of the present study revealed that there is no such significant difference in sex but apparently male dogs are more affected with heart worm. This finding is similar to other studies in Iran and USA, but a study reported from USA and another one from Spain showed significantly higher prevalence in male dogs **(Montoya *et al.*, 1988 and Wixsom *et al.*, 1991).** Since dirofilariasis has a higher prevalence rate in outdoor male dogs, this difference is probably due to different management methods of dog keeping.

The results of this study also showed that dogs above the age of 9 years were more infected than other age groups, but this difference was not significant. Similar findings were reported previously by several researchers in Iran and other countries **(Fan *et al.*, 2001 and Javidi, 2003).** Risk of infection in dogs probably lasts for whole life and the likelihood of acquiring *D. immitis* infection increases with the time of exposure to the mosquitoes. Thus, older dogs have more time and opportunities to become infected with heartworm **(Yildirim, 2006).** The results of **Ranjbar-Bahadori's study (2007)** showed that Knott method has a high sensitivity and specifity compared to commercial antigen detection kit. That study showed that although sensitivity of antigen detection test was higher compared to the Knott method, the differences were not significant.

In this study, only microfialariae of *D. immitis* were found in dogs from Dinajpur, but other authors reported presence of both microfilariae *Diptalonema reconditum* and *Dirofilaria immitis* in dogs from the other area **(Javidi, 2003).**

Heartworm infection in dogs has been diagnosed around the globe. Relocation of infected, microfilaremic dogs appears to be the most important factor contributing to further spreading of the parasite. The prevalence and spread of heartworm infection in Turkey and other countries has been comprehensively studied by both microfilarial detection and serological-molecular methods **(Simsek *et al.*, 2008 and Yildirim *et al.*, 2007).** An increasing number of cases are now being diagnosed in Turkey and Europe **(Genchi *et al.*, 2005 and Yaman *et al.*, 2009).** Thus, accurate clinical and histopathological diagnosis of *D. immitis* is important for development of an effective treatment programme.

5.2. Clinical examination

In the present investigation, symptoms of canine heartworm infection includes cachexia, syncope, coughing, exercise intolerance, sudden onset of lethargy associated with labored breathing or crackles, enlarged pulmonary arteries, and decreased blood flow to the lung which were similar to the findings by **Bowman and Atkins (2009).**

5.3. Necropsy examination

In the present investigation, heart worms (*Dirofilaria* sp.) were found in the right ventricle of heart. In Italy, **Tarello (2002)** reported a pruritic dermatitis characterized by the presence of erythema, papules, focal or multifocal alopecia, crusting and nodules infested with *Dirofilaria repens* microfilaria. Dirofilariasis has a zoonotic importance in human. **Muller (2002)** stated about 230 cases of human dirofilariasis have been reported worldwide. In almost all instances immature worms or unfertilized females have been isolated from lung by **Pampiglione and Rivasi (2001). Ciferri (1982)** showed that the most infections are asymptomatic, showing typical 'coin lesion' on chest radiography which were often mistakenly removed as neoplasm.

In the present investigation in canine dirofilariasis, the heart and lung were variably but consistently affected with changes consisting of dilatation,

hypertrophy, thrombosis, myointimal proliferation of the pulmonary artery, infarction, hemorrhage, hemosiderosis, chronic inflammation and fibrosis which were similar to the findings by **Grandi *et al.* (2007).** We additionally observed granulomatous reactions in perivascular connective tissue and bronchial epithelial changes, findings that were consistent with the presence of dead intrapulmonary *D. immitis* worms. On the other hand, changes were also observed in large pulmonary arteries, which were not infected with parasites.

In the present investigation, *Dirofilaria immitis* infection is characterized by several different clinical pictures, caused by both adults and microfilariae which were similar to the findings by **Grandi *et al.* (2007).**

5.4. Histopathological study

The severity of cardiopulmonary pathology in dogs is determined by worm numbers, host immune response, duration of infection and host activity level. In the present investigation during the necropsy examination, adult D. immitis were found in the portal vein, right ventricle and atrium of the heart and pulmonary trunk which were similar to the findings by **Tarello (2002); Sabu *et al.* (2005) and Venco and Vezzoni (2001).**

In the present investigation, it was observed that heartworms caused the lining of the heart and pulmonary arteries to become rough and disrupt the flow of blood, the intima also thickened which were similar to the findings by **Blagburn and Dillon (2007); Browne *et al.* (2005); Castleman and Wong (1982); Maia *et al.* (2011); Bowman (2009) and Nelson *et al.* (2005a, b).**Due to heart worm burdens in heart chamber endothelial cells are separated and appear inflamed.

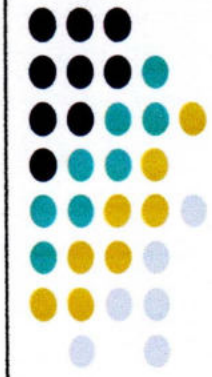
In the present investigation microscopically, microfilarias were found throughout the vessels of heart which were similar to the findings by **Browne *et al.* (2005); Castleman and Wong (1982); Lee and Atkins (2010) and Venco *et al.* (1984).** After a dog dies, the heartworms leave the heart chamber and can be found in the major arteries of the lung.

The right ventricle and atrium were enlarged. Pulmonary emboli, containing dead parasites, were seen in a small number of pulmonary arterial branches, and some areas of the lung were congested; Congestions of cardiac muscle fibers is also observed.Pulmonary hypertension initially induces a dilation of the right ventricle with a compensatory hypertrophy of the myocardium.

In the present investigation, we observed that heavy worm burdens cause endothelial damage which leads to myointimal proliferation; this finding was in agreement with the earlier reports by **Grieve *et al.* (1983); Schrey (1996); Theis (2005) and Foroulis *et al.* (2005).**

CHAPTER VI

SUMMARY AND CONCLUSION



CHAPTER VI

SUMMARY AND CONCLUSION

In Dinajpur, there are many street dogs freely roaming here and there. The population of the street dogs is still increases gradually due to lack of proper management system and insufficient and/or ineffective policy for the controlling of the street dogs. Thus it increases the risk of public health in Dinajpur as well as in Bangladesh. This study provides the evidence of several important parasitic and infectious diseases of dogs at Dinajpur municipality area. During this investigation, it was observed that heart worm infection is common in street dogs at Dinajpur municipality area. The study indicates that about 46.67% dogs were infected with *Dirofilaria immitis* and it could be transmitted to human beings. Due to the shortage of time and proper facilities, few other diseases of dogs was not be identified including rabies, canine distemper, toxoplasmosis, infectious canine hepatitis, echinococcosis, and sarcocystosis in street dogs.

A more detail and extensive study is essential to find clues of the above mentioned diseases. However, it could be concluded that:

- Public health is at great risk due to the increased number of street dogs.
- Street dogs are the great reservoir of zoonotic diseases.
- Research for the identification of all probable zoonotic diseases is a social demand.
- Policy for street dogs control as well as control of zoonotic diseases, sufficient financing and providing all infrastructures to stop zoonoses is prerequisite.

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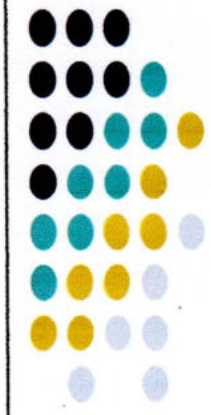

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