INVESTIGATION OF CUTANEOUS AND GASTROINTESTINAL DISORDERS OF STREET DOGS AT DINAJPUR DISTRICT

A THESIS

BY

MD. MOMINUL ISLAM

SEMESTER: MARCH – AUGUST/ 2010
REGISTRATION NO.: 0905079
SESSION: 2009-2010

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR.

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AUGUST, 2010

DEDICATED

TO
MY

BELOVED PARENTS

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The author
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ABSTRACT

Cutaneous and gastrointerstinal disorders of the street dogs were investigated at Dinajpur districts. In a study of one year starting from July/2009 to June/2010, a total of fifteen street dogs were collected from the Municipal, Dinajpur and HSTU campus during Rabies Control Programme. A thorough necropsy examination was done and the characteristics clinical signs and gross lesions were recorded. Different organs including skins were collected, preserved and processed for histopathological examination. Skin scraps also examined for mite infestation. The most common cutaneous lesions observed were alopecia (93.33%) followed by scale/crust (73.33%), hyperkeratosis (66.67%), puritus (40%), erythema (40%), hyperpigmentation (40%) and seropurulent discharge (20%). The incidence of heart worm (*Dirofilaria* sp.) (60%), hook worm (*Ancylostoma* sp.) (100%) and ascariasis (*Toxocara* sp.) (40%) were detected. Demodex mite, hyperplasia, hyperkeratosis, parakeratosis and mononuclear cell infiltration in dermis were recorded in the histopathological study. Demodectic mite was detected from the skin scrapings. The study dictates that street dogs of Dinajpur district carried heart worm, hook worm, ascariasis, mite infestation and various dermapathological disorders. Among them heart worm, hookworm, ascariasis and mite infestation have zoonotic importance.

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ABBREVIATION AND SYMBOLS

^0C	: Degree centigrade
%	: Percentage
ACL	: Acral lick dermatits
et al.	: And his associates
etc.	: Etectera
ELISA	: Enzyme linked immunosorbent assay
Fig.	: Figure
g	: Gram
H & E	: Hematoxylin and Eosin
HSTU	: Hajee Mohammad Danesh Science and Technology University
lbs	: Pounds
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
PD	: Pyotraumatic dermatitis
UV	: Ultraviolet
UK	: United Kingdom
USA	: United States of America
WHO	: World Health Organization
WSPA	: World Society for Protection of Animals

CHAPTER I

INTRODUCTION

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Dogs were domesticated from wolves as recently as 15000 years ago (Morey, 2006), or perhaps as early as 100000 years ago based on recent genetic fossil and DNA evidence (Savolainen *et al.*, 2002; Lindbald-Toh, 2005). Evidence suggests that dogs were first domesticated in East Asia, possibly China, and the first people to enter North America took dogs with them (Savolainen *et al.*, 2002). The World Health Organization (WHO) and World Society for Protection of Animals (WSPA) classify dogs based on the level of dependence on human care (that is food, shelter and human companionship) and also on the level of restriction or supervision imposed by humans (WHO and WSPA, 1990).

Dogs perform a range of cultural, social, and economic functions in society. These are kept as pets and companions, for hunting, as guards, draught animals, for food, or for commercial purposes. In some areas of Eurasia and North America dogs are used to carry goods and to pull sledges and carts. Large breeds in developing countries were raised to guard livestock, premises and agriculture crops. It also perform some specialized tasks such as leading the blind, detection of illegal goods, tracing criminals, and as an aid to detect certain illnesses in humans (Willis *et al.*, 2006). Dogs play an important role in society, enhancing the psychological and physiological well being of many people (DiSalvo *et al.*, 2005).

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 and cats 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; Kahn, 2006). Most important role of the dog is in the maintenance and transmission of echinococcosis and rabies (Konno *et al.*, 2003; Hemachuda, 2005; Kilic *et al.*, 2006).

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

Heart worm (*Dirofilaria immitis)* infection is 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).

Toxocara canis, hookworm, *Trichuris* sp. *Trichinella* sp. *Diphyllobothrium* sp. and *Dipylidium sp.* are also 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 amd 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).

Thus, the present study was carried out to identify the causal agents or diagnosis of diseases of street dogs with the following objectives.

1. Investigation of causal agents of diseases of street dogs
2. Categorization of the agents having zoonotic role
3. Parasitological examination of faeces and skin scrapings

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

In this study, available and relevant literatures are reviewed with special emphasized on the gross and histopathological changes of collected skins. A number of studies have been conducted on different skin diseases of dogs highlighting the parasitic infestation after a brief overviewed on the zoonotic importance, etiology, epidemiology and prevalence.

2.1. Prevalence of skin diseases in dog

Delmage (1972) stated that alopecia was hairlessness and may be caused by various skin diseases, deficiency diseases and abnormal function of adrenal cortex. Some of the alopecia had also neurological basis and some dogs tended to shed hairs in a warm climate

Sischo *et al.*, (1989) made a diagnosis of skin diseases in dogs. The 10 most common diagnoses were fleabite allergic dermatitis, skin cancer, pyoderma, seborrhea, allergy, demodectic acariasis (demodicosis), sarcoptic acariasis, immune-mediated skin disease, endocrine related skin disease, and acral lick dermatitis

Pratibha *et al.*, (2000) was examined 40 dogs of different breeds suffering from various clinical skin disorders. Skin samples were collected for bacteriological, fungal and mite examinations. The outcomes were 17.5% and 5% of the cases due to fungal dermatitis and bacterial infection, respectively. Male dogs suffered more (75.0%) than females (25.0%).

Kumar and Thakur (2001) were examined 193 different dog breeds to determine the incidence of various skin diseases of dogs in the vicinity of Ranchi, India. Results revealed that 49 cases were positive for ringworm (45%) infection, mange (3.59%), eczema (2.55%) and other skin diseases (2.34%) were also present. Highest incidence of dermatomycosis was reported during summer and lowest in winter. The species of dermatophytes of higher prevalence that affect dogs were *Microsporum gypseum* (8.62%), *Trichophyton mentagrophytes* (18.01%), *Trichophyton rubrum* (14.64%) and *Microsporum canis* (14.64%).

Mazzei ***et al.,*** **(2002)** was carried out a clinical epidemiological and retrospective study for the diagnosis of dermatitis. The alopecic, scaly, lichenified, erythematous and hyperkeratotic lesions were restricted to the skin fold areas, with severe pruritus in most cases. Other underlying diseases, especially allergic dermatitis, were identified in 88% of the cases

Chakrabarti ***et al.,*** **(2002)** was conducted an epidemiological study on 757 dogs with complaints of hair fall and skin lesions during April to September 1996 at Kolkata metropolis and its surroundings in India. Of the alopecic dogs, 38 were males and 18 were females (67.85 and 32.15%, respectively). Parasitic alopecia (42.86%) predominated over fungal (14.29%) and hypothyroidism (10.71%) alopecia. Alopecia due to tick infestation (0.92%) predominated over demodectoic and scabietic alopecia (0.79%)

Hill ***et al.,*** **(2006)** were made a survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice in the UK. In dogs and exotic species, pruritus was the most common presenting sign, accounting for 30 to 40 per cent of the dermatological consultations. In dogs, the most common final diagnoses were otitis, pyoderma, anal sac impaction, flea infestation and atopic dermatitis.

Menelaos (2006) was examined a total of 209 dogs (121 male and 88 female with 81 young and 128 adult dogs) and 52 cats (23 male and 29 female with 36 young and 16 adult cats) with dermatological lesions were examined for the presence of dermatophytes. The prevalence of dermatophytosis was 18.18% in dogs and 71.05% in cats.

2.2. Ectoparasites

2.2.1. Demodectic mange

Demodex canis was the main causative agent of canine demodicosis, an inflammatory parasitic skin disease characterized by the presence of large numbers of demodecid mites **(Scott *et al.*, 2001).** A short-bodied unnamed *Demodex* mite has also been associated with canine demodicosis **(Mason, 1993; Chen, 1995; Chesney, 1999; Saridomichelakis *et al.*, 1999).** Recently, a new species of demodex mite has been described and implicated as a causative agent of the generalized form of canine demodicosis **(Mueller and Bettenay, 1999; Hillier and Desch, 2002; Robson *et al.*, 2003).** This demodecid mite, named as *Demodex injai*, was a long-bodied species. Adult male *Demodex injai* mites were more than twice the length of *Demodex canis* male mites, and the adult female mite was approximately 50% longer than the *D. canis* female mite. This mite seems to show a preference for the dorsal trunk and, histologically, it appears to inhabit hair follicles and sebaceous glands **(Desch *et al.*, 2003).**

Baker (1975) reported the occasional development of hyperpigmentation of the skin of dogs with demodectic mange. The hyperpigmentation appeared to be the result of increased melanocyte activity in the epidermis. The dermis about infested hair follicles contained many histiocytes packed with melanin granules

Nayak *et al.*, (1997) reported the Prevalence of canine demodicosis in Orissa (India). Demodicosis was detected in 1697 (3%) cases. There was no difference in the occurrence of the disease in male (51%) and female (49%) dogs. The disease was recorded in 60%, 23% and 17% of dogs up to 1 year, 1-2 years and above 2 years of age respectively, suggesting that young dogs up to 1 year of age were more susceptible.

Tamura *et al.*, (2000) conducted a research work on the dual infection with a short-bodied demodex mite and *Demodex canis*. The unidentified short-bodied mites were 97.5-167.5 (139) micro m in body length. The posodoma was 55-100 micro m, and the blunt-ended opithosoma was 40-67.5 micro m. Skin lesions took the form of scaly dermatitis with patches of pustules around the ears, eyes, lips, chin and legs.

Mahato *et al.*, (2005) was studied the occurrence of demodicosis in dogs from November 2002 to June 2003. A total of 240 dogs with skin lesions accompanied with pruritus from dog wards, private clinics in Kolkota and households in West Bengal, India, were selected for this study. Skin scrapings from the dogs revealed *Demodex canis*. Only 36 dogs were positive for generalized demodicosis (10.56%). Out of the 36 cases, 20 and 16 cases were male dogs and bitches, respectively (55.5% and 44.5%, respectively). Demodicosis occurred predominantly in dogs below 2 years old (66.66%).

Kalyan *et al.*, (2005) conducted a surveillance of mange mite infestation in 120 dogs in Aizawl, revealed 46.6% infestation, of which 64.3% were found positive for sarcoptic mange and 35.7% for demodectic mange. The overall occurrence of mite infestation was higher in winter season. Haematological study showed significant ($P<0.05$) neutrophilia and leucocytosis in dogs infested with sarcoptic mange, whereas low haemoglobin level and total erythrocytic count along with eosinophilia were evident in dogs infested with demodectic mange.

Canine demodicosis can present as a generalised or localised condition. It may be either juvenile or adult in onset. Juvenile onset demodicosis, as in this case, has a better prognosis than adult onset demodicosis, which was often associated with other primary systemic diseases such as hyperadrenocorticism **(Henfrey, 1990; Lemarie, 1996).**

Localised demodicosis was a mild clinical disease, most cases resolving without treatment. However, the generalised form was a severe disease with a guarded prognosis **(Scott, 1979b; Folz, 1983).** The history in this case would suggest that in the twelve months prior to presentation the localized form was present around the mouth. Examination of skin scrapings of the lips at initial presentation yielded *Demodex canis* mites.

Demodicosis has been induced in dogs and horses following the use of glucocorticoids **(Scott and White, 1983; Sundberg *et al.*, 1994).** In a retrospective study of adult onset demodicosis **(Lemarie *et al.*, 1996)** 24% of the dogs had been treated with glucocorticoids.

Evaluation of skin scrapings was used to determine the end point of treatment. Use of visual clinical improvement was an unreliable indicator of cure as some animals can be clinically normal for some time before elimination of mites, as was seen here **(Paradis and Laperriere, 1992; Miller *et al.*, 1993).** Treatment should continue for four weeks after negative skin scrapings were obtained, as in this case **(Ristic *et al.*, 1995; Medleau *et al.*, 1996).**

2.2.2. Sarcoptic mange

Sarcoptic mange was probably under diagnosed in dogs **(Carlotti and Bensignor, 1997)** due to mild signs in some cases. Furthermore, clinical lesions may be atypical and localized, particularly in dogs that receive flea-control products regularly. Very little mention of localized sarcoptic mange was found in the veterinary literature **(Curtis and Paradis, 2003),** and this skin disease was usually described as having a tendency for lesions to spread over the body **(Carlotti and Bensignor, 1997; Bourdoiseau, 2000; Scott *et al.*, 2001).** The current study describes atypical cases of canine sarcoptic mange, characterized by their localization to one body area. Such localized presentations were also described in human beings and include crusty lesions, restricted to the scalp, the face, the arch of the foot, a digit or a nail **(Chosidow, 2000).**

Pin et al., (2006) reported 10 cases of localised sarcoptic mange in dogs. In each case, lesions were localised to one precise area of the skin. Affected areas were the feet (one case), the face and/or the pinnae (six cases), the abdominal skin (one case), the flank (one case) and the lumbar area (one case). The types of lesions were erythema, papules, lichenification, scales, crusts and alopecia.

Canine scabies was a parasitic skin disease caused by the mite *Sarcoptes scabiei* var. *canis*. The classic presentation was an intensely pruritic dog with alopecia and crusting involving the ear pinnal margins and pressure points, but some dogs have the more vague presentation of a generally pruritic dog with few or no skin lesions **(Griffin, 1993).** Scabies can, therefore, closely mimic other causes of pruritus such as atopy, food allergy and flea allergy. The diagnosis of scabies can be challenging, as mites were found in only 20–50% of skin scrapings from infested dogs **(Bornstein *et al.*, 1996).**

Scabies has been reported in 10-50% of humans after contact with infected dogs **(Charlesworth and Johnson, 1974; Ruiz- Maldonado *et al.*, 1977).** The infection was usually self-limiting **(Estes *et al.*, 1983).** Spread to in-contact dogs has been reported in 75% of cases **(Baker and Stannard, 1974).** In this case the in-contact dog and humans were not clinically affected by *Sarcoptes scabiei.* It was possible that the infrequent use of selamectin in both dogs may have limited the spread of the mite.

Lesions usually become generalized although the occasional case may have lesions that remain localised (Anderson. 1979), as in seen in this case.

Secondary superficial pyoderma, as seen here, was common **(Griffin, 1993).**

Diagnosis of sarcoptic mange can be difficult with 50-65% of skin scrapings being negative **(Bornstein *et al.*, 1996).** Scraping was diagnostic in this case but other methods can be used to aid diagnosis including faecal flotation for egg detection **(Folz, 1984).** ELWASA testing for scabies antibody was useful in the diagnosis of this condition with a sensitivity of 84-92% and specificity of 89-96% **(Curtis, 2001, Lower *et al.*, 2001).** Antibodies were not detectable until three to five weeks post infection **(Bornstein and Zakrisson, 1993).**

Histopathology can be diagnostic but only if mites can be found **(Morris and Dunstan, 1996).** The pinnal-pedal reflex, as seen in this case, was a useful tool in the diagnosis of scabies as it can be elicited in between 75-90% of dogs **(Mueller *et al.*, 2001).** However up to 7% of normal dogs will exhibit the reflex.

2.3. Endoparasites

Tarello (2002) was described the first case of canine subcutaneous dirofilariasis in this region of the Middle East. The lesions consisted of multifocal alopecia; erythema on the head, neck and hind limbs; hyperkeratosis and acanthosis on the lumbosacral region; papulae on the abdomen; chronic otitis and generalized pyoderma intertrigo. Other clinical signs included anorexia, fever, lethargy, lameness, pruritus and conjunctivitis. A diagnosis of subcutaneous dirofilariasis was made, based on the skin lesions; the presence of *Dirofilaria repens* microfilariae in the blood and a negative test for circulating *Dirofilaria immitis* antigens

Barutzki and Schaper (2003) were investigated 8438 dogs and 3167 cats samples from the years 1999 until 2002. 2717 dogs (32.2%) and 771 cats (24.3%) have been infected with endoparasites. In the infected dogs the following parasites have been identified: Class Nematodea: *Toxocara canis* (22.4%), *Toxascaris leonina* (1.8%), Ancylostomatidae (8.6%), *Trichuris vulpis* (4.0%), *Capillaria* spp. (2.3%), *Crenosoma vulpis* (0.9%), *Angiostrongylus vasorum* (0.3%); Class Cestodea: Taeniidae (1.2%), *Dipylidium caninum* (0.4%), *Diplopylidium/Joyeuxiella* (0.1%), Mesocestoides (0.2%), *Diphyllobothrium latum* (0.1%); Class Sporozoea: *Sarcocystis* spp. (9.0%), *Cystoisospora* spp. (22.3%), *C. canis* (8.0%), *C. ohioensis* (17.0%), *Hammondia/Neospora* (1.7%); Class Zoomastigophorea: *Giardia* spp. (51.6%)

Irwin (2004) stated that *Dirofilaria immitis* was responsible for heartworm disease in dogs, yet microfilaraemia associated with other filarial infections was 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.

Cisek *et al.*, (2004) was examined to determine the prevalence of *Toxocara canis* in dogs and red foxes in area of north-west Poland. The dog coproscopy was provided according to Willis-Schlaff method. In examined area dogs were infected with *Toxocara canis* from 2.67 to 55%. The highest prevalence was observed in Gorzów Wielkopolski in its neighborhood (villages) and in urban places. The lowest extensity (2.67%) was determined in urban area of Słupsk city. In examined forest regions the prevalence of *Toxocara canis* in red foxes was 43%.

Kim and Huh (2005) were examined the intestines and hearts of dogs for *Toxocara canis, Toxascaris leonina,* and *Dirofilaria immitis,* after necropsy between June 26 and September 29, 2004 in Chuncheon, Korea. Of the 662 dogs examined, 6 were infected with *Toxocara. canis* (0.9%), 86 with *Toxascaris leonina* (13.0%). Fifty dogs were infected with *Dirofilaria. immitis* among 500 dogs examined (10.0%). Five were co-infected with *Toxocara canis* and *Toxascaris leonina,* and three were co-infected with *Toxascaris leonina* and *Dirofilaria immitis.*

Ho *et al.,* (2006) were examined intestinal helminth and protozoan infection in the quarantined dogs in Taiwan using fecal examination between January to December, 2004. Of the 376 dogs imported from 11 countries, 63 (16.8%) were found to be infected with at least one species of intestinal parasite. The parasites detected were oocysts of *Isospora canis* and eggs of *Toxocara canis, Trichuris vulpis* and hookworms. Of the 63 infected dogs, 11 were found to have a mixed infection of two different species of parasites.

The filarial nematodes were 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 were; *Dirofilaria immitis, Dirofilaria repens, Acanthocheilonema reconditum,* *Acanthocheilonema dracunculoides, Brugia malayi, Brugia ceylonesis* and *Brugia pahangi* **(Irwin 2002; Rishniw *et al.*, 2006 and Simon *et al.*, 2007).**

Miro *et al.*, (2007) were analyzed 1161 faecal samples from animal shelters, showing a 28% prevalence for different intestinal parasites: *Giardia duodenalis* (7%), *Cystousopora* spp. (3.8%), *Toxocara canis* (7.8%), *Toxoscaris leonina* (6.3%), Ancylostomidae (4%), *Trichuris vulpis* (3.3%), Taenidae (2.9%) and *Dipylidium caninum* (0.9%).

Musa and Sadek (2007) stated that cystic echinococcosis due to *Echinococcus granulosus* was a serious public health and livestock economy problem in Libya. Kato thick smear examination of 50 street dogs stools showed that they had *Echinococcus granulosus* (58%), *Taenia* spp. (14%), *Diplydium caninum* (16%), *Toxocara canis* (121%) and 20% were parasite-free.

Batchelor *et al.*, (2008) investigated a total of 4526 dogs for parasitic infection. The most common parasite was *Giardia* spp., which was found in 380/4526 dogs (8.4%, 95% CI 7.6-9.2%). Surprisingly, *Cryptosporidium* spp. infection was detected in only 29/4526 (0.6%, 95% CI 0.4-0.9%). *Toxocara canis* was found in 63/4526 dogs (1.4%; 95% CI 1.1-1.8%).

Daryani *et al.*, (2009) were examined a total of 27 adult and 23 young dogs with 11 adults (40.7%) and 19 youngs (82.6%) being infected with *Toxocara canis* with an overall prevalence of 60%. There were significant differences in the prevalence of infection between adult and young dogs ($p = 0.003$). There were no significant differences in the prevalence of infection between male and female dogs ($p > 0.05$).

Roddie *et al.*, (2008) stated that *Toxocara canis,* the common intestinal nematode of dogs and foxes, was the parasite responsible for human toxocarosis. It has recently been shown that dogs may harbour eggs of the parasite in their fur. To further investigate this claim a population of 100 stray dogs was examined to establish the prevalence and intensity of adult toxocaral worm infection in the intestines and eggs harboured in the hair. Sixty-seven percent of dogs were found to have *T. canis* eggs on their hair with a mean egg retrieval of nearly 584 eggs per gram from positive dogs. Thirty-nine percent of dogs were found to have adult *T. canis* worms in their intestine, although a significantly higher percentage of puppies (80%) were infected with worms than adults (22.5%). Puppies also had more worms per infection than adults and have a strong positive correlation between egg and worms numbers whereas adults did not.

2.4. Bacteria

Pyotraumatic dermatitis (PD), also called acute moist dermatitis or 'hot spot', was defined as an acute and rapidly developing surface bacterial skin infection secondary to self-inflicted trauma **(Scott *et al.*, 2001; Kunkle 1979; Medleau and Hnilica, 2001).** It was a common clinical syndrome in the dog but there were only two papers describing the histopathology **(Reinke *et al.*, 1987; Schroeder *et al.*, 1996)** and minimal other refereed literature on possible causes and results of bacterial culture. An abundance of underlying causes capable of starting an 'itch-scratch cycle' have been suggested including ectoparasites (flea bite hypersensitivity, lice and ticks), allergic skin diseases, anal sac problems, otitis externa, foreign bodies in the coat, irritant substances on the skin, dirty unkempt coat, poor grooming and musculoskeletal disorders **(Mason and Lloyd, 1989; Foster and Foil, 1987).**

Diagnosis of PD was often based on history and clinical signs. Scrapings and cytology were used to rule out other causative factors (Rosencrantz, 2000) as in this instance. Histopathology was not performed, as this was the first occurrence of PD in this patient. However, **Rienke *et al.*, (1987)** have recognised a deep form of PD with suppurative, necrotising folliculitis. Histopathology should therefore be performed in a recurrent case.

PD may occur at flea feeding sites **(Curtis, 2001)** and flea-bite hypersensitivity was a common cause of this condition **(Scheidt, 1988; Sousa and Medleau, 1992).** No further diagnostic tests to confirm flea bite hypersensitivity were conducted as most cases of PD respond to clipping, cleaning and topical treatment irrespective of the underlying cause **(Kunkle, 1979; Mason 1991),** as was seen here.

Pyoderma was a pyogenic bacterial infection of the skin **(Ihrke, 1996)** and was one of the most common causes of skin disease in dogs **(Scott *et al.*, 2001; Ihrke, 1983).** *Staphylococcus intermedius* was the primary bacterial pathogen of the skin of dogs and the most common isolate in canine pyoderma **(Ihrke, 1987; White *et al.*, 2005).**

Pseudomonas aeruginosa was a frequent pathogen in dogs with chronic otitis externa and otitis media **(Harris. 1978; Cole *et al.*, 1998),** but was infrequently isolated from skin infections **(Scott *et al.,* 2001).** This bacterial organism may be isolated from the skin of dogs with chronic deep pyoderma where it was typically associated with infection with other pathogens such as *S. intermedius* and *Escherichia coli* **(Krogh and Kristensen, 1981; Seol *et al.,* 2002).** In a more recent report, *P. aeruginosa* was isolated from 42 (7.5%) of 561 skin samples submitted to a diagnostic laboratory during a 6-year period, and it was the only organism isolated from 14 (33.3%) of these 42 samples **(Petersen *et al.,* 2002).**

Paul and Wortman (1993) stated that bacterial infections of the skin comprise a significant proportion of the disorders seen in the practice of dermatology. Common primary bacterial infections of the skin due to gram-positive and gram-negative bacteria include impetigo, ecthyma, cellulitis, erysipelas, folliculitis, furunculosis, carbuncles, erythema, pitted keratolysis, and *Pseudomonas* toe web infection. Less common infections include blistering distal dactylitis, necrotizing fasciitis, botryomycosis, blastomycosis-like pyoderma, trichomycosis axillaris, erysipeloid, ecthyma gangrenosum, rhinoscleroma, and infections due to *Pasteurella multocida* and *Vibrio vulnifcus.*

Pododermatitis was a common and potentially debilitating disease of dogs **(White, 1989).** Although pedal involvement was a component of many canine skin diseases, some animals present with lesions confined solely to the foot **(Ihrke *et al.,* 1985).**Common causes of pododermatitis in the dog include, but were not limited to, trauma **(Anderson, 1980),** microbial disease **(Mason, 1991),** ectoparasitism **(White *et al.,* 1993),** foreign-body reactions **(Shaw, 1987)** and immunological skin diseases **(White, 1989).**

2.5. Virus

Kaldrymidou *et al.*, (2001), studied seven cases of canine papilloma histopathologically using light and electron microscopy. Papillomavirus was detected immunohistochemically and by the polymerase chain reaction (PCR) method. The outcome of the studied was found as classical squamous papilloma in four dogs, inverted papilloma in one dog and fibropapilloma in two. Virus particles were detected electromicroscopically in two cases.

2.6. Fungus

Dermatophytoses were infections of the skin and its appendages in humans and animals caused by a group of closely related species of fungi of the genera *Microsporum, Trichophyton and Epidermophyton* **(Bourdeau, 1993; Chester, 1979; Badillet, 1977).** Dermatophytes form enzymes which can digest the complex protein keratin. Their activity was confined to the keratinized levels of the epidermis, hairs and claws **(Foil, 1993).** The incidence of canine dermatophytosis varies geographically, seasonally and with natural reservoirs **(Lewis *et al.*, 1991).** It was considered generally to be an uncommon fungal skin disease of dogs and was either misdiagnosed or overdiagnosed in canine practice **(Bourdeau, 1993).**

It was important to consider that dermatophytosis in the dog can be caused by dermatophytes other than *Microsporum canis* **(Foil, 1993).** Over 20 species have been reported to infect domestic dogs. For example, *Trichophyton mentagrophytes, Trichophyton erinacei, Microsporum persicolor,* *Microsporum gypseum, Trichophyton verrucosum, Trichophyton terrestre, Microsporum cookei, Microsporum distortum, Trichophyton rubrum, E. floccosum* and *Microsporum nanum* have been incriminated as responsible for the development of skin diseases in the dog **(Faggi *et al.*, 1987; Kushida *et al.*, 1975).** *Trichophyton mentagrophytes* and *Microporum gypseum* were found in the soil and *Trichophyton verrucosum* has been incriminated in cases which have had direct or indirect contact with cattle **(Wright, 1989).**

2.7. Zoonotic importance

Ancylostoma caninum is one of the most common parasitic infections of domestic dogs **(Blaghburn *et al.*, 1996)** and is a good model for studies on human hookworm disease. Percutaneous exposure to infective sheathed third stage larvae has been implicated as the most common route for infection in human, although oral infection of *Ancylostoma caninum* **(Landmann and Prociv, 2003)** and *Ancylostoma ceylanicum* **(Yoshida *et al.*, 1971).** Cutaneous larva migrans or creeping eruptions is a hypersensitivity reaction caused by migrating hookworm larvae of *A. caninum* **(Chaudhry and Longworth 1989; Malgor *et al.*, 1996).** Cutaneous larva migrans is commonly reported in travelers returning from the tropics **(Miller *et al.*, 1991; Jelinek *et al.*, 1994; Tremblay *et al.*, 2000).** Infections are acquired from exposing unprotected skin to contaminated moist or wet sandy or wet loan soil **(Beaver *et al.*, 1984c).** A pruritic, erythematous popular rash usually occurs within several hours of larval penetration **(Bouchaud *et al.*, 2001).**

Procive and Croese (1990) stated an epidemic of eosinophilic enteritis (93 cases) has occurred in Townsville, northern Queensland, Australia. A hookworm was found attached to a resected, inflamed ileal segment from 1 patient but the species could not be identified. An adult hookworm of species *Ancylostoma caninum* was recovered at colonoscopy from the terminal ileum of a later patient. All of 38 patients interviewed in an epidemiological survey described behaviour which could have exposed them to infective larvae of this widespread dog parasite.

Microfilariasis has been reported in dogs in those areas of Italy **(Rossi *et al.,* 1996; 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; 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; Foroulwas *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 lungs **(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.

Acral lick dermatitis (ALD), also called acral lick granuloma or acral pruritic nodule **(MacDonald *et al.,* 2000),** was a common and often frustrating disease characterized by compulsive licking of the lower portion of a limb and development of a firm, proliferative, ulcerative and alopecic plaque **(Gross *et al.,* 2005).** Although there were reports of ALD-like lesions in various species, including cattle, zoo and exotic species, and humans, the disease was primarily seen in middle aged or older dogs **(Yeruhum *et al.,* 1992; Kellner *at el.,* 1992).**

Saari *et al.,* (2001) stated canine scabies or sarcoptic mange was caused by an epidermal mite, *Sarcoptes scabiei*. Dogs were usually infested by the canine variant of the mite, *S. scabiei* var. *canis*. Wild raccoon dogs (*Nyctereutes procyonoides*) and foxes (*Vulpes vulpes*) were commonly infested by *S. scabiei* var. *vulpes* in Finland, and these species serve as a source of scabies for dogs. Canine scabies was characterized by intense pruritus and the skin lesions, which were usually present on the ear margins and on the ventral trunk

Nodular dermatophytosis in dogs was reported to be most commonly caused by *Microsporum gypseum*, followed by *Trichophyton mentagrophytes* and *Microsporum canis* **(Carlotti *et al.,* 1999).** A similar presentation was also observed in human beings, where it was more often caused by zoophilic or geophilic fungal species **(Nelson *et al.,* 2003).**

Wolfe and Wright (2003) stated that toxocariasis in man is traditionally thought to be contracted through the ingestion of eggs from contaminated soil. The disease may manifest itself in different syndromes such as ocular larval migrans, visceral larval migrans and covert toxocariasis. Hair was collected from 60 dogs from various places in Ireland and the UK and examined for the presence of *Toxocara canis* eggs. *T canis* eggs were found in the hair of 25 per cent of the dogs; in total, 71 eggs were recovered, of which 4·2 per cent were embryonated and 23·9 per cent were embryonating. The maximum densities of the embryonating and embryonated eggs were 180 and 20 eggs per gram of hair, respectively, much higher than the densities reported for soil samples. It is suggested that dogs infected with *T canis* may infect people by direct contact.

Inpankaew *et al.*, (2007) were examined fecal samples from 204 humans and 229 dogs from 20 different temples. Human and dog stool samples were examined for intestinal parasites. Hookworms were the most common parasite in dogs (58.1%) followed by *Trichuris* (20.5%), *Isospora* (10%), *Giardia* (7.9%), *Toxocara* (7.4%), *Dipylidium caninum* (4.4%) and *Spirometra* (3.1%). *Blastocystis hominis* (5.9%) was the most common parasite in humans followed by hookworms (3.4%), *Giardia* (2.5%), *Strongyloides* (2%) and *Cryptosporidium* (1.5%).

Cornegliani, *et al.*, (2009) stated that dermatophytosis was a common zoonotic disease, and one of its clinical presentations in the dog was nodular dermatophytosis (kerion). Nodular dermatophytosis in 23 dogs of different breed, age and sex with single or multiple nodules was described. Twelve dogs had a single nodule, and 11 dogs showed multiple lesions

2.8. Histopathological lesions

Baker (1969) described the histopathological lesions of canine demodecosis (*Demodex canis*), was postulated that the disease was primarily a chronic inflammatory response to the mechanical presence of mites in the hair follicles. A pustular reaction follows follicular rupture due to their over-distention. A small minority of cases were possibly allergic reactions to the parasite

Rojko *et al.,* (1978) were correlated clinical and histological data on skin biopsies from 238 dogs with various cutaneous disorders. Biopsies from normal dogs were used to establish normal histological anatomy. Moderate to marked acanthosis usually was present. Parakeratosis was more common than hyperkeratosis. Epidermal degeneration associated with neutrophil infiltration and intraepidermal pustule formation were seen in 22 dogs (73%). Ulceration or parakeratosis, or both, were in nine of the latter 22 dogs.

Reinke *et al.,* (1987) was examined the skin of 17 dogs with pyotraumatic dermatitis for histopathological feature. Two patterns were seen. The first pattern was a superficial, ulcerative, inflammatory process of undetermined cause and pathogenesis. Current recommended treatment, which includes corticosteroids, was believed to be appropriate for such lesions. The second pattern, suppurative folliculitis, was considered to be localized pyoderma.

Casewell *et al.,* (1997) conducted a research work on dogs skin for the temporal changes in the histologic lesion of canine demodicosis. Mural folliculitis was a common consistent histologic lesion of canine demodicosis. Five dogs with demodicosis were examined biopsied biweekly for upto 14 weeks.

Kagawa *et al.*, (1998) showed a granulomatous lesions in the skull skin and gingiva of the left mandible from an 8-year-old, female mongrel dog. The lesions were macroscopically seen as grayish white papular granulomas, and microscopically consisted to numerous swollen macrophages and a few neutrophils without fibrocaseous necrosis. Macrophages contained many small oval or round-shaped yeast-like cells and a few rod-shaped organisms indicating a narrow based budding in their cytoplasm.

Tarantino *et al.*, (2001) investigated a dog skin for the *Leishmania infantum* and *Neospora caninum* in Italy. . Histopathology of cutaneous lesions revealed a suppurative, diffuse dermatitis with numerous intracellular protozoa.

Senter *et al.*, (2002) was conducted a retrospective histopathological study on skin biopsy specimens from 111 dogs with diseases associated with parakeratotic hyperkeratosis to determine whether intracorneal vacuoles were present. Additional criteria evaluated were the size and location of the vacuoles and the degree of parakeratosis. Cases examined included dogs with primary idiopathic seborrhoea, necrolytic migratory erythema (NME), *Malassezia* dermatitis, zinc-responsive dermatosis, hereditary nasal hyperkeratosis of Labrador Retriever dogs, thallotoxicosis. Thirty-seven cases (37/111, 33%) had intracorneal vacuoles, including nine cases of primary idiopathic seborrhoea (9/29, 31%), 10 cases of NME (10/18, 56%), five cases of *Malassezia* dermatitis (5/19, 26%), five cases of zinc-responsive dermatosis (5/36, 14%), five cases of hereditary nasal hyperkeratosis (5/5, 100%).

Holm *et al.*, (2004) was made a clinical diagnosis of acute pyotraumatic dermatitis in 44 privately owned dogs. Males exceeded females ($P = 0.0348$) and lesions were more common in dogs aged 4 years or less ($P < 0.0001$). Histopathologically, the dogs could be separated into four patterns by the presence or absence of eosinophils and/or folliculitis. Eosinophils have not previously been recorded in pyotraumatic dermatitis but were seen in 29 cases. Acute folliculitis was seen in 20 cases.

Heijden *et al.*, (2005) features on tick-bite lesions were evaluated in capybaras naturally infested with *Amblyomma cajennense* and *Amblyomma dubitatum* ticks. Gross appearance of tick bite site was characterized by a mild swelling and erythema. Microscopic examination revealed the cement cone, a tube-like homogenous eosinophilic mass penetrating deep into the dermis. This structure was surrounded in the dermis by a cellular infiltrate and free eosinophilic granules and was associated to edema of variable intensity. Necrosis was a common feature deep in the dermis particularly at the far end of the eosinophilic tube. Hyperplasia, cellular edema and occasionally necrosis of keratinocytes could be seen at both sides of the ruptured epidermis. Cellular infiltrate was constituted overwhelmingly by polymorphonuclear leukocytes with eosinophilic granules.

Radbea (2005) described the histopatholgical lesions of dog skin due to the combined action of *Demodex canis* and *Staphylococcus intermedius*. Results showed the presence of *Demodex canis* mite in the pilous follicle, but not found in the subcutaneous gland, monocytes and eosinophil. Incidence of hyperkeratosis was also observed

Solanki and Hasnani (2006) described the microscopic examination of skin scrapings from 330 dogs with dermatitis presented to the College Clinic from January to September 2004 revealed an overall demodicosis prevalence of 25.45% (84 cases). Localized demodicosis was characterized by alopecic erythematous patches with or without fine silvery scales on periocular area, head, neck and legs. Lesions like pruritus, encrustation, complete alopecia, hyperpigmentation, folliculitis and wrinkling of the skin of the leg, head and trunk were the characteristic signs of generalized demodicosis.

Nishifuji *et al.,* (2007) was described a 3.5-year-old, male West Highland white terrier as having hyperplastic dermatosis by clinical and histopathological findings. Controlling of Malassezia overgrowth by antifungal drugs provided a temporal improvement of the skin lesions, but the disease was deteriorated within the next 2 months despite the negative demonstration of the yeasts. Induction of recombinant canine interferon-gamma (rCaIFN-gamma) therapy resulted in almost complete cure of the skin lesions within 2 months after the initiation of the therapy.

Mauldin *et al.,* (2008) stated that all dogs had strikingly similar histopathologic changes that consisted of mild to moderate laminar orthokeratotic hyperkeratosis with an absence of epidermal hyperplasia and dermal inflammation. All affected dogs had retained and convoluted membranes with crystalline structures in the stratum corneum. Scattered keratinocytes in the granular cell layer had prominent, clear, membranebound, cytoplasmic vacuoles. This unique hyperkeratotic/scaling disorder in Golden Retrievers has distinctive clinical, histologic, and ultrastructural features, which are consistent with a primary cornification defect.

Miranda *et al.,* (2009) reported the histopathological findings of 86 skin lesions of dogs with sporotrichosis from Rio de Janeiro. Suppurative granulomatous inflammation was the predominant finding and was observed in 76 (88.37%) cases. Plasma cells surrounding the suppurative granulomas were detected in 68 (89.5%) cases and an inflammatory infiltrate at the periphery of these granulomatous lesions was observed in 63 (82.9%). Fungus-specific staining revealed yeast cells compatible with *Sporothrix schenckii* in 36 cases. These fungal elements were only detected in lesions characterized by suppurative granulomatous inflammation.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

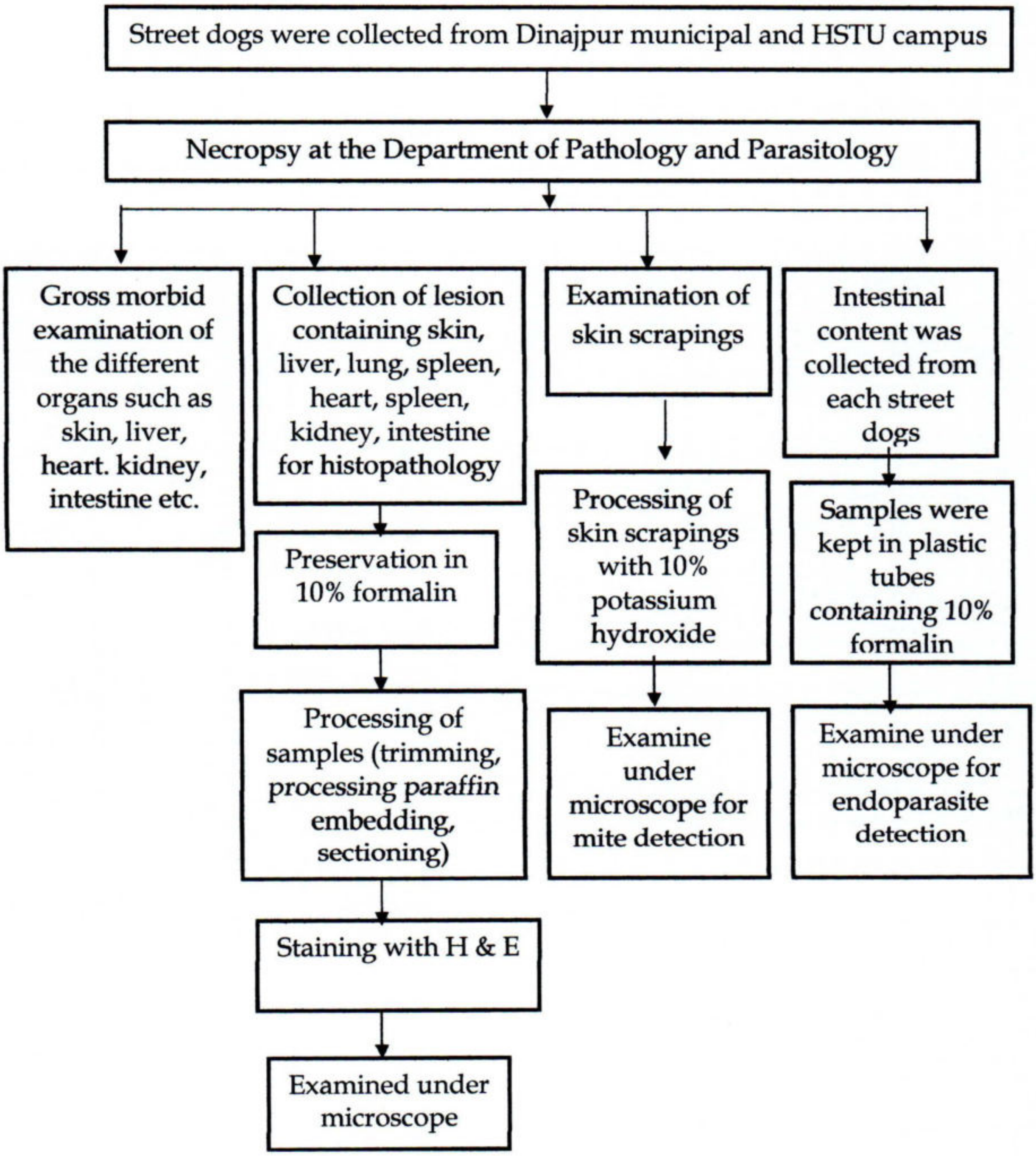
3.1. Experimental animals, areas and duration

The experimental animals of this study were 15 street dogs. The street dogs were collected from the different region of Dinajpur Municipal and HSTU campus during rabies control program. 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, 2009 to 30 June, 2010.

3.2. 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 glassware 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. 3. 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.

3.4. Pathological examination

3.4.1. 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.

3.4.1.1. 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, 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.

3.4.2. Gross lesion

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

3.4.3. 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).

3.4.3.1. Materials required for histopathology

- Samples (Skin, heart, lungs, 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

3.4.3.2. Processing of tissue for histopathology

1. Collection of tissue and Processing

During tissue collection the following point were taken into consideration-

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 changes.)

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.

3.4.3. 3. Routine Hematoxylin and Eosin staining procedure

3.4.3.3.1. 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.

3.4.3.3.2. 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.

3.4.3.3.3. 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 (10X) and high (40X, 100X) power objectives.

3.5. Parasitological examination of faeces

3.5.1. 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.

3.5.2. 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.

3.6. Examination of skin scrapings

Skin scrapings were taken from the selected area of the affected street dogs.

Equipment and appliances

- Handle and blade
- 10% Potassium hydroxide
- Microscope
- Slide
- Spirit lamp

Procedures

- The area was selected for scrapings.
- The skin was wiped gently with sterile water.
- Then the scraping was done with the scalpel blade. The surrounding tissue was tensed with fingers of other hand.
- The scraping was taken until oozing occurs.
- Then the scraping tissue was placed on the slide.
- Then one or two drop of 10% potassium hydroxide was added to the tissue.
- Heat was given gently for dissolving the tissue.
- Then the slide was examined under microscope in 40x objectives.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1. Clinical examination

The incidences of various dermatological disorders in relation to sex were shown in Table 1. The cutaneous lesions were recorded as alopecia, scales and crusts, hyperkeratosis, pruritus, erythema, hyperpigmentation, and seropurulent discharge (Fig. 1, 2, 3, 4, and 5). Alopecia was considered to be a most important cutaneous lesion (93.33%) in the examined street dogs, followed by scale/crust (73.33%), hyperkeratosis (66.67%), puritus (40%), erythema (40%) and seropurulent discharge (20%), respectively. Eight species were female and seven male. Dermatological disorders in respective to sex were not observed in this study.

The site of the cutaneous lesions was shown in Table 2. Cutaneous lesions localization was variable in street dogs but most of the lesions were located in dorsum, abdominal skin, neck, shoulder, limbs, and flank region.

4.2. Necropsy examination

At necropsy examination heart worms (*Dirofilaria* sp.) were found in street dogs. The parasites were seen in the right ventricle of heart (Fig. 6). The adult parasite was stained with lactophenol and revealed males measuring 125 to 250 mm in length, posses tails that are blunted, armed with caudal alae, and are spirally coiled (Fig. 6). The females, which measure 250 to 300 mm in length, were larger. (Fig. 7).

4.3. Histpathological study

Demodectic mange was detected in three dogs. Gross pathological examination revealed alopecia, erythema, thickened skin and scale formtion. The lesion was mostly confined in the skin of dorsum, neck and back. Histopathologically, cross sections of mite were found in the hair follicles of skin (Fig 8 & 9). Folliculitis with infiltration of neutrophils was also seen.

There was diffuse mononuclear cell infiltration in the skin (Fig. 10). The infection was very deep and resulted infiltration in dermis. In one cases furunculosis was present with the neutrophilic infiltration.

The stratum corneum was increased in thickness. Hyperkeratosis was present with minimal epidermal hyperplasia (Fig. 11 & 12). Orthokeratotic hyperkeratosis was present in 11 dogs. Parakeratosis with nucleated thickness of stratum corneum was present in 8 dogs (Fig. 11& 12).

The thickness of noncornified epidermis was increased due to increased number of epidermal cells. Epidermal hyperplasia was accompanied by ballooning degeneration of the epidermis (Fig. 13). There was formation of rete ridge that appear to project downward into the dermis.

4.4. Parasitic examination

In this study, eggs of hook worm (*Ancylostoma caninum*) were detected in the faeces (Fig. 14). All the dogs examined in this study were infected with the hook worm. Egg of ascarids (*Toxocara canis*) was also found in the faeces of dog.

4.5. Examination of skin scrapings

In skin scrapings, the elongate demodectic mites were present in this study (Fig. 15).

Table-1: Clinical features of cutaneous lesions as observed in dogs

Clinical features	Case															Disorders in percentages (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
	F	F	M	F	M	M	M	F	M	F	F	M	F	M	F	
Alopecia	+++	++	+++	+++	0	++	++	+++	+	++	+++	+++	+	+	++	93.33
Pruritus	0	+	++	+	0	+	0	+	0	0	+	++	0	0	0	40
Hyperkeratosis	++	+	++	+++	0	0	+	++	0	+	+	++	0	0	+	66.67
Erythema	0	0	+	0	+	0	0	+	0	++	0	0	+	+	0	40
Scale/Crust	++	+	+++	+	0	0	+	++	++	0	++	+	0	+	+	73.33
Seropurulent discharge	0	+	+	0	0	0	0	0	0	0	0	+	0	0	0	20
0 = absent; + = mild; ++ = moderate; +++ = marked/severe; F=Female; M= Male.																

Table 2. Distribution of cutaneous lesions in dog

Case	Site
1	Abdominal skin
2	Dorsum
3	Dorsum, hind limb
4	Left flank
5	Shoulder, Head
6	Neck, abdomen
7	Front limb, shoulder
8	Abdominal skin
9	Dorsum
10	Dorsum, Hind limb, thigh
11	Neck, Shoulder
12	Dorsum, Abdominal skin
13	Flank
14	Scrotum, hind limb
15	Dorsum, Abdominal skin

Fig. 1. Alopecia with scale/crust formation in the street dog at Dinajpur

Fig. 2. Alopecia with erythema in the skin of dog at HSTU campus

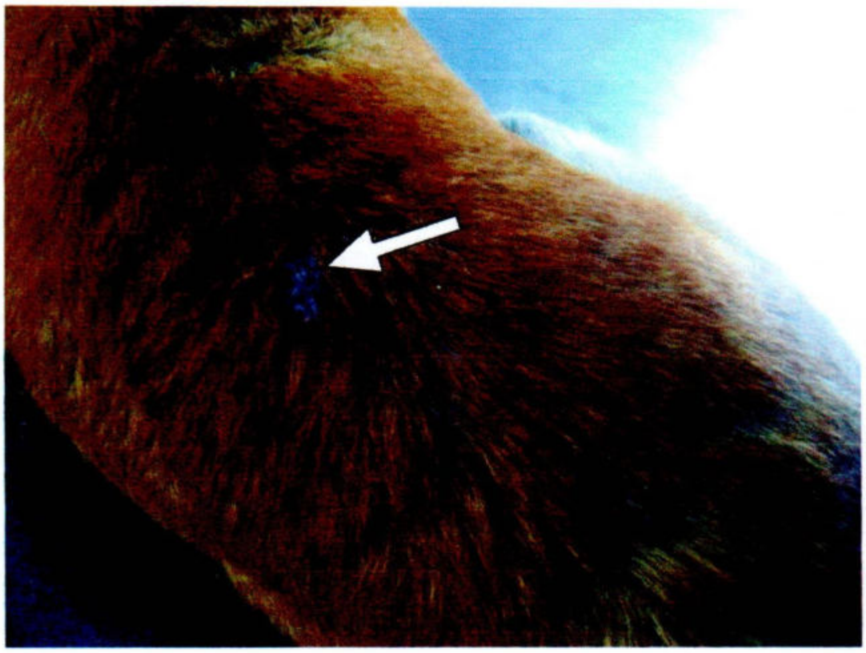


Fig. 3. Pruritus in the skin of dog at Dinajpur

Fig. 4. Serous exudates in alopectic dog at Dinajpur

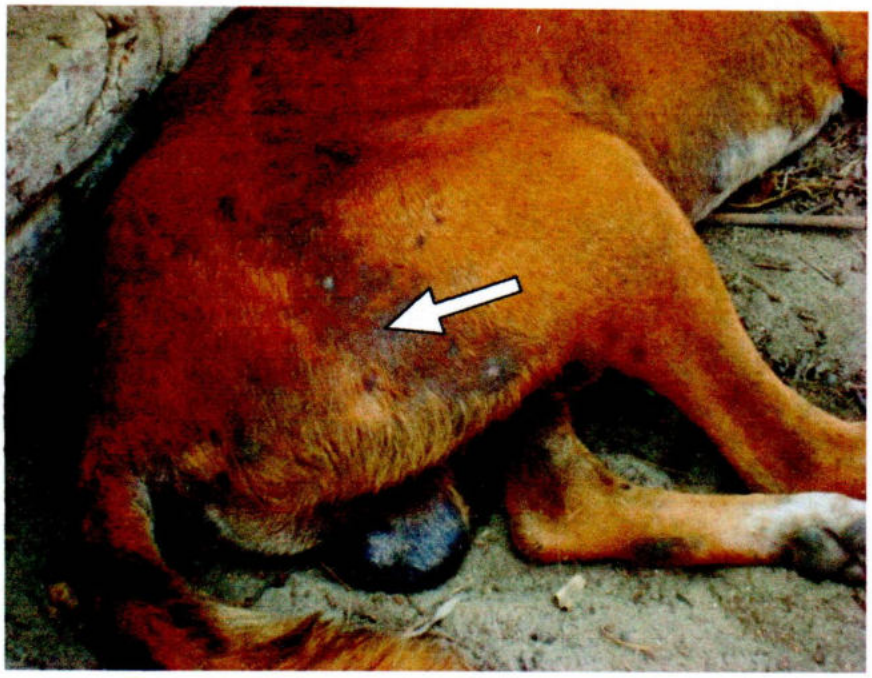


Fig. 5. Alopecia with crust formation in the skin of scrotum and thigh

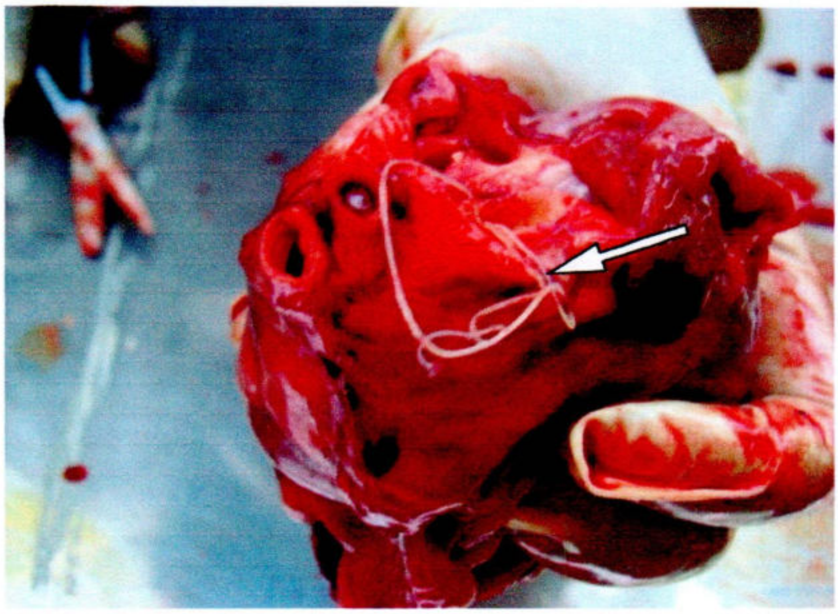


Fig. 6. The dogs at Dinajpur affected with heart worm (Dirofilaria immitis). The worm found at the right ventricle of heart during necropsy (Arrows)

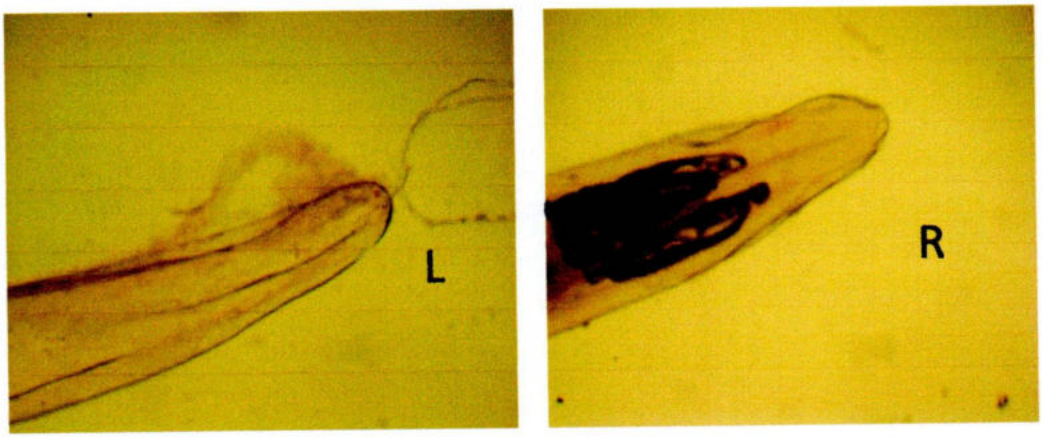


Fig. 7. The anterior (L) and posterior part (R) of canine heart worm as revealed from the right ventricle of heart and stained with lactophenol

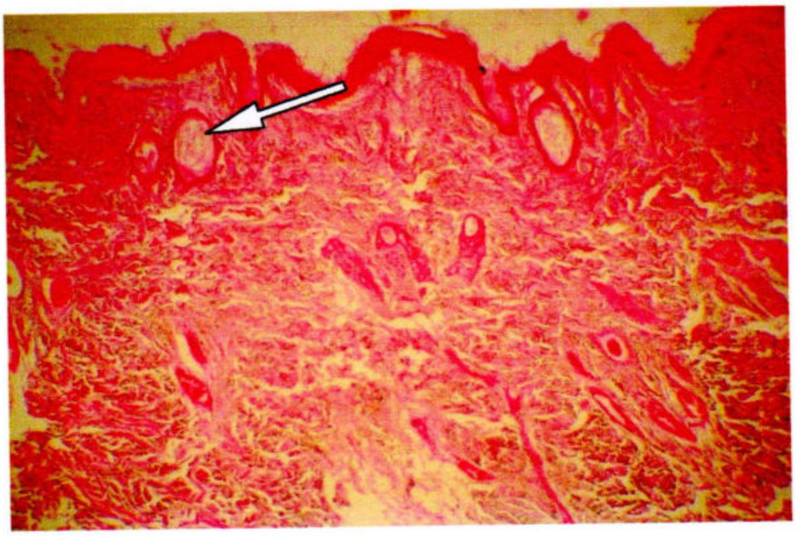


Fig. 8. Demodectic mite as detected in the section of skin stained with H & E (10X). The mite present within the hair follicle (Arrows)

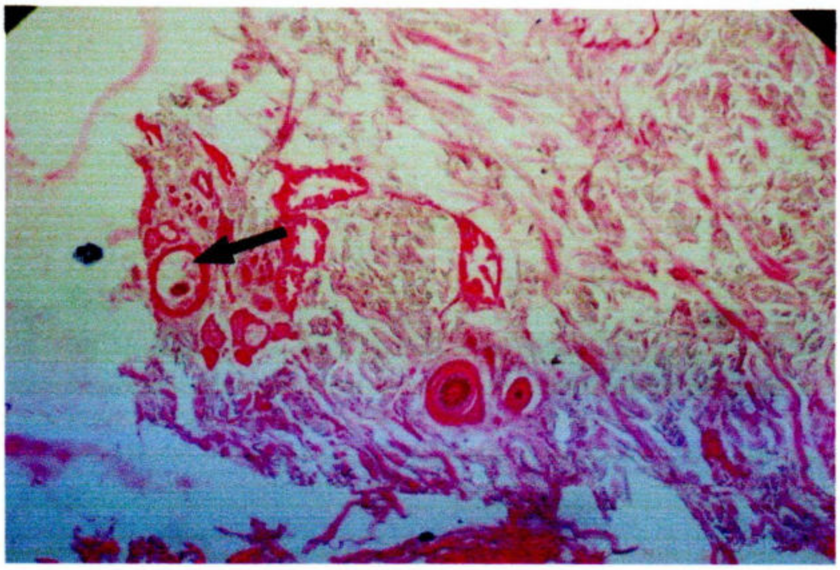


Fig. 9. Section of skin from the dorsum and stained with H & E (4X) revealed demodectic mite within the hair follicle (Arrows)

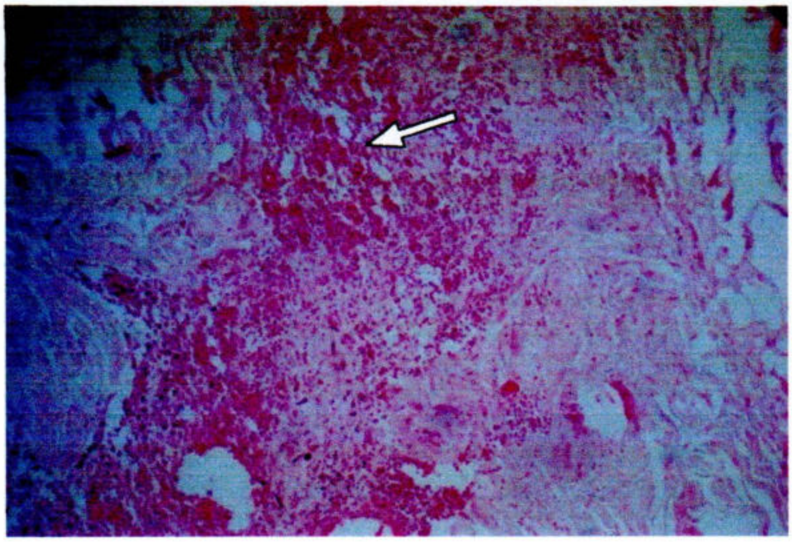


Fig. 10. Section of skin stained with H & E (10X) infiltration of mononuclear cell in the dermis (Arrows)

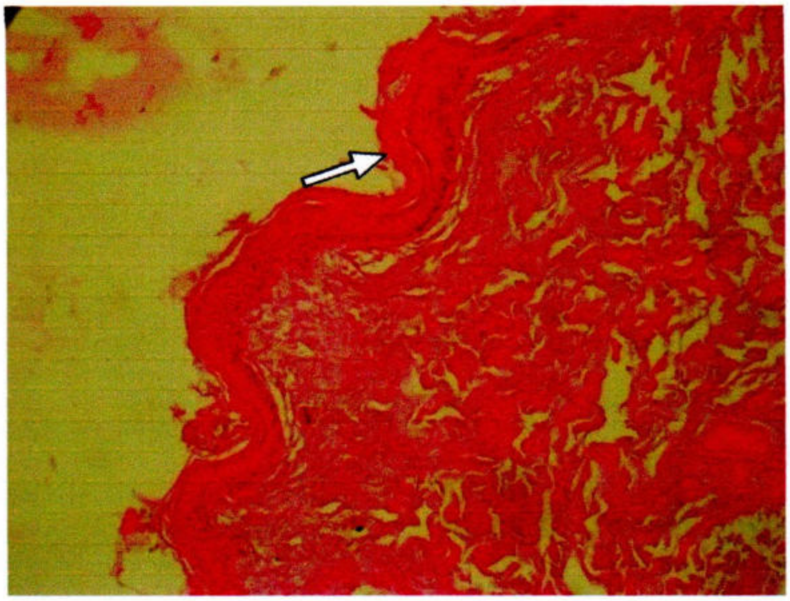


Fig. 11. Section of abdominal skin obtained from a dog and stained with H & E (10X) revealed hyperkeratosis and parakeratosis in the stratum corneum (Arrows)

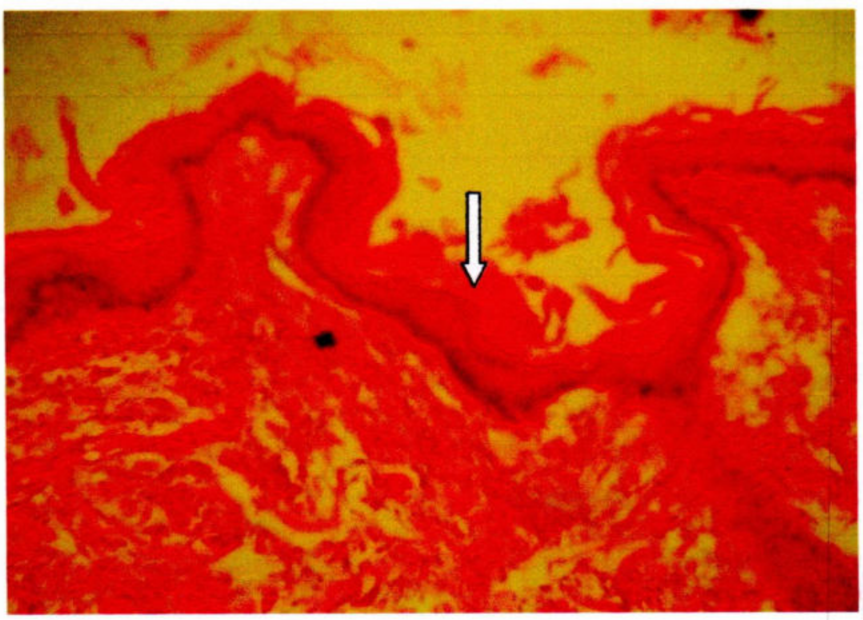


Fig. 12. Section of dorsum skin obtained from a dog and stained with H & E (10X) revealed hyperkeratosis in the stratum corneum (Arrows)

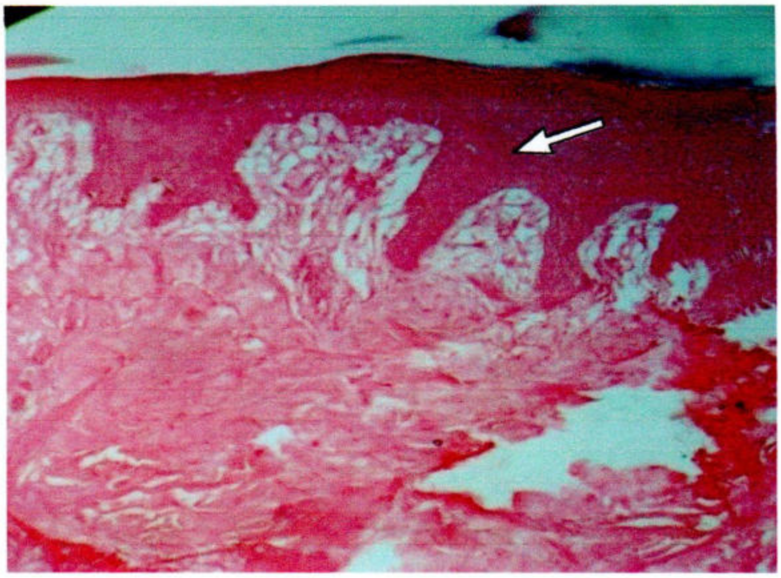


Fig. 13. Section of skin stained with H & E (10X). There is increased number of epidermal cells in the epidermis revealed as epidermal hyperplasia (Arrows)

Fig. 14. Egg of *Ancylostoma caninum* detected by faecal examination (40X)

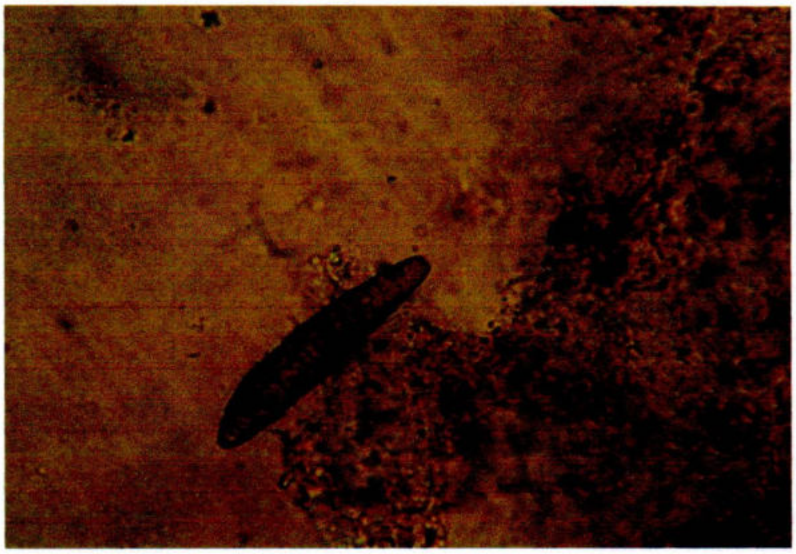


Fig. 15. Demodectic mite in skin scraping (40X)

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

5.1. Clinical examination

In the present study, the cutaneous lesions in street dogs were recorded as alopecia, scales and crusts, hyperkeratosis, pruritus, erythema, seropurulent discharge. The most important cutaneous lesions were alopecia (93.33%), followed by scale/crust (73.33%), hyperkeratosis (66.67%), puritus (40%), erythema (40%) and seropurulent discharge (20%). The results indicate that the dogs may live where there are poor nutritional management, worm infestation, etc. Sischo *et al.*, (1989) was recorded dermatitis, skin cancer, pyoderma, seborrhea, allergy, demodectic acariasis (demodicosis), sarcoptic acariasis, and acral lick dermatitis as common skin diseases in dog.

In this study, the Cutaneous lesions localization was variable in street dogs. This was similar to the findings by Pin *et al.*, (2006) who stated that the localization of cutaneous lesion was variable in dogs caused by mite infestation.

5.2. 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 lungs 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.

5.3. Histopathological study

In this experiment, cross sections of mites were found in the hair follicles of the thickened skin. The species of the mite was not identified in this study. This finding was in agreement with the earlier report by Scott *et al.*, (2001) and Jekl *et al.*, (2006). Caswell *et al.*, (1997) was identified canine demodicosis in a dog with the histopathological lesion of interface mural folliculitis, suppurative folliculitis and furunculosis.

There was diffuse mononuclear cell infiltration in the dermis could be due to chronic dermatitis. The infection was very deep and resulted infiltration in dermis. Bardagi *et al.*, (2007) was reported neutrophilic dermatosis in a dog.

In the present study, hyperkeratosis was seen with minimal epidermal hyperplasia in the skin of street dogs. The hyperkeratosis could be due to inflammatory conditions in the skin. This finding was in agreement with the earlier report by Mauldin *et al.*, (2008) who reported hyperkeratosis in 45% dogs. Whereas, Rojko *et al.*, (1978) recorded hyperkeratosis in 70 dogs and parakeratosis in 47 dogs suffered from various clinical dermatological disorders.

In this study, parakeratosis with thickning of stratum corneum was seen in the epidermis of skin in the street dogs. In some cases parakeratois were present with the hyperkeratosis. Senter *et al.*, (2002) reported marked parakeratosis in dogs skin. However, Scott *et al.*, (2000) stated that parakeratosis implies altered epidermopoiesis secondary to inflammatory, hormonal, developmental or neoplastic causes. Whereas, Mauldin *et al.*, (1997) stated that Surface and infundibular parakeratosis is a characteristic of Malassezia dermatitis in the skin of dogs.

In the present investigation, epidermal hyperplasia was seen accompanied by ballooning degeneration of the epidermis. Epidermal hyperplasia results might be due to as a consequence of neoplastic diseases. Muller *et al.*, (1989) describes the similar findings of epidermal hyperplasia. Nishifuji *et al.*, (2007) stated epidermal hyperplasia in a 3.5 years male dog as a consequence of *Malassezia* infection.

5.4. Parasitic examination

In this study, egg of ascarids (*Toxocara canis*) was found in the faeces of street dogs. Cisek *et al.*, (2004) also examined the eggs of *Toxocara canis* in dogs and red foxes in northwest Poland and stated as most dangerous parasite for carnivores. Ascariasis (*Toxocara canis*) has zoonitic importance for human. Overgaauw *et al.*, (2009) stated that patent *Toxocara* infections in dogs and cats were considered as a public health risk because of their zoonotic potential. The hair contamination with *Toxocara* eggs found in the studies of Wolfe and Wright (2003); Roddie *et al.* (2008) was used to suggest that this probably the main route of transmission to the human. Lim (2008) reported visceral larva migrans in humans, forming eosinophilic inflammation such as eosinophilic abscess or granuloma in the liver and lungs by the *toxocara canis*.

In this experiment, Eggs of hook worm (*Ancylostoma caninum*) were detected in the faeces of street dogs. Blaghburn *et al.,* (1996) reported *Ancylostoma caninum* as a major parasite of dogs in the U.S.A. and other parts of the world. Georgi and Georgi, (1991) stated that hook worm infestation can caused moderate to severe iron deficiency anemia, hypoproteinemia and bloody diarrhea that can be fatal to puppies and immunosuppressed dogs. Miller *et al.,* (1991) stated that *Ancylostoma caninum* was also a zoonosis to humans due to skin penetration by the soil-dwelling infective larvae resulted in cutaneous larva migrans. Eosinophilic enteritis due to the potential larval development to the adult stage in the human gut as stated by Prociv and Croese, (1990) and Croese *et al.,* (1994).

5.5. Examination of skin scrapings

In the present study, the elongate demodectic mites were present in skin scrapings of street dogs. The species of the mite was not identified in this study. Mahato *et al.,* (2005) and kalyan *et al.,* (2005) reported 10.56% and 35.7% demodectic mange in skin scrapings of dogs, respectively. On the other hand, Malmasi *et al.,* (2009) identified *Demodex canis* by microscopic examination in skin scrapings. Whereas, Chen (1995) detected both *Demodex canis* and short tailed demodectic mite from the skin scrapings.

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 District and HSTU campus. Among them Demodectic mange, Heart worm, and Ascariasis 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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