DERMATOPATHOLOGICAL STUDY OF STEPHANOFILARIASIS (HUMPSORE) IN CATTLE AND ITS THERAPEUTIC APPROACHES

A THESIS

BY

LAILA AFIFA AZAD
REGISTRATION NO.: 1005030
SEMESTER: MARCH – AUGUST/ 2011
SESSION: 2010-2011

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

FEBRUARY, 2012

DEDICATED

TO
MY

BELOVED HUSBAND
AND PARENTS

ACKNOWLEDGEMENT

All praises are to the almighty God who enables the author to complete the research work successfully and to submit the thesis leading to Master of Science (MS) degree in Pathology.

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The author
February, 2012

ABSTRACT

A study was carried out on dermatopathological and therapeutic approaches of stephanofilariasis (humpsore) in cattle from March to December,2011 at Birgonj upozila of Dinajpur district.A total 1427 cattle were selected under farm and rural condition. Among them,381 cattle were affected with humpsore by using close visual inspection,palpation of skin surface and other clinicopathological techniques. The clinical and pathological features including topographic positions of the lesions as well as complications were recorded. The skins of 3 typically affected with stephanofilariasis were collected, preserved, processed, embedded with paraffin, sectioned and stained with haematoxylin and eosin for the histopathological study. The highest prevalence rates were recorded in dairy farm (31.63%), 10-15 age groups (83.00%) and male (31.57%). Crusts including the worm, were examined in the parasitology laboratory of HSTU for the identification of *Stephanofilaria* spp. Common predisposing factors were frequent rubbing by rope and metallic chain used for restraint, yoke used in draft animals, fences, barbed wire, manger protector and continuous ocular discharge. The more usual sites were the neck, withers / hump and ear. With an increase in the number of sites affected there was decrease in the number of animals. The most common clinical signs were the intense pruritis, ulcerated and non-ulcerated lesions with heavy accommulation of firm crusts. Histopathological changes were hyperkeratosis, presence of cross and longitudinal section of parasites. 5ml. of Ivermectin solution was injected under the lesion at different places of the sore. Three doses were repeated at weekly intervals in 20 cattle. An ointment containing Zinc oxide 45 gm. Potassium iodide 2 gm, Methylene blue 1 gm and Vaseline to make 100gm was applied simultaneously once on alternate day until complete cure of the lesions. The results indicate that humpsore is an important disease of cattle in the study area.

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INTRODUCTION

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INTRODUCTION

Hides and skins are valuable wealth of Bangladesh. Apart from meeting the internal demand, these also earn much foreign currency. Leather is the second highest foreign exchange earner of this country.(Ahmed,1982)

Several diseases have been found to affect the hides of cattle, most of which not only affect the general health, production, market value and working capacity of the affected animals but also lower the economic value of their hides. Moreover, some skin diseases of animals such as dermatophilosis (Weber, 1978) and ring worm (Anon, 1956) are transmissible to humans. In rural areas 80% of human ringworm may derive from animals in Britain (Anon, 1956).

Stephanofilariasis, a parasitic disease caused by worms of the genus *Stephanofilaria* which causes skin lesions characterized by alopecia and ulcerative nodular dermatitis in cattle, buffaloes and goats, among other mammals. In many situations, due to the obstruction of the lymph ducts, the wound may reappear. While recent studies characterize it as a zoonosis, its occurrence in humans is considered rare (Novaes *et al.,* 2006). Five species of the genus *Stephanofilaria* have been described as bovine parasites in a number of regions of the world; their intermediate hosts include *Haematobia irritans, Musca conducens, Musca planiceps* and *Musca autumnalis* (Riviera and Aycardi, 1985). *Stephanofilaria* can be characterized as the agent of a disease commonly observed in exuberant high-occupancy pastures with large quantities of wet feces, principally in the hot and rainy seasons (Sutherst *et al.,* 2006). These factors result in a greater proliferation of vectors. In this situation, the flies are attracted by exudates originating from open cutaneous lesions infested with adult worm parasites, with the consequent ingestion of microfilariae (Urquhart *et al.,* 1998). The final host is infected when the flies deposit larvae in the not injured skin, causing inflammation and

destruction of the hair follicles and epithelial cells (Urquhart *et al.,* 1998; Novaes; Miyashida, 2006).

Life cycle and vector of *Stephanofilaria assamensis* (Islam, l97l b,c,d), description of the parasite (Ra1 1957), artificial transmission (Ruq *et al.,* 1957), epidemiology (Huq 1953, Raliman 1957, Mia and Haque 1967) Hassan 1969 and 1970, Islam l97l b,c,d and 1977, Dewan l97l a,d,c), isolation of bacteria from the lesion (Dewan and. Rahman, 1970 and Dewan 1971b), clinical features (Huq 1953, Dewan l97l a and Islam 1971a), histological picture (Dewan 1975 and ilannan *et al.* 1977), treatment (Hassan and. Huq 1955, Fiassan 1969, Ahmed and. Ali 1973, Rahman and Khalecjue 1974, Baki and. Dewan 1975, Dewan and Baki, 1976 and. Baki and Hossain 1982 a) and. recurrence (Baki and L 1982 b) of humpsore have been investigated..

Treatment of Stephanofilariasis with antimosan has been attempted in Indonesia (Holz and Adiwinata, 1957), North west Germany (Dirkman and Radermacher, l96O a,b,c) and Comilla Bangladesh (Ahmed and All, 1973). These reports indicate that antimosan is effective against stephanofilaniasis but the time required in the treatment is protracted. Moreover, in neither of these trials the results of treatment were evaluated by histological study.

Thus this study on bovine cutaneous stephanofilariasis (Humpsore) of preliminary and basic survey in field conditions was under taken with the following objectives:

1. To determine the prevalence of the disease.
2. To determine the predisposing factors.
3. To study the clinical findings.
4. To determine the severity of the disease

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Oetiology

Stephanofilaria assamensis (Pande, 1936):

Stephanofilaria zaheeri (Singh, 1958):

Stephanofilaria andamani (Sinha andDas, 1958):

Stephanofilaria srivastavi (Bhattacharjee, 1967):

Stephanofilaria assamensis was the cause of humpsore in cattle was first reported in India (Pande, 1936).

2.1.1 Taxonomic position of the oetiologic agent

Kingdom: Animalia

Phylum: Nemathelminthes

Order: Filaroidea

Family: Filariidae

Genus: *Stephanofilaria*

Species: *Stephanofilaria assamensis*

2.2 Morphologic features of *Stephanofilaria assamensis*

The species was distinguished by the presence of lateral alae, smaller left spicule and an indistinct anus (Pande, 1936).

Head ornamented with spines. Head bare or with varied cuticular structures. Mouth surrounded by a chitinous ring or epaulette like structures. Mouth simple is not bounded by chitinous structure (Rahman *et al.*, 1996).

2.3 Life cycle

Eggs containing larva are laid to the subcutaneous tissues of the lesions which are ingested by *Musca conducens* in Bangladesh. After entry into the *Musca* the larvae migrate to the mid gut and then gradually move towards the proboscis, during which they moult twice to become infective larvae. Development to this stage takes 2-3 weeks time. Larvae hang out through the mouth, pass out of the fly and when these infected flies feed on tissue fluids on the injured hump region, the larvae escape and invade the surrounding tissues. By 6-12 months larvae become adults to lay eggs (Rahman *et al.*, 1996).

2.4 Epidemiological study of Stephanofilariasis:

2.4.1 Geographical distribution:

This species, along with its associated lesions in cattle, was reported from Bengal (Mohan, 1945, 1973), Bihar (Varma, 1956), Kutch (Pannu, 1958), Orissa (Das, 1955; Patnaik, 1964) and Gujrat (Hiregaudar and Chatupale, 1965). Beside humpsore, Pal and Sinha (1971) also found this parasite to cause lesions at the base of dewclaws of cattle. Similarly, the parasite has also been reported from humpsore like lesions in buffaloes (Patnaik and Roy, 1968; Sharma Deorani and Rao, 1980) and goats (Patnaik and Roy, 1968)

Ear-sore in buffaloes has been reported from Travancore (Menon, 1952), Madras, Bombay (Ahmed, 1961), Orissa (Patnaik, 1964; Das *et al.*, 1975b), Assam (Gopalkrishnan, 1951; Bhattacharjee and Das, 1967; Dutta, 1972), Madhya Pradesh (Bhopale, 1967; Agrawal, 1974), Andhra Pradesh (Rao *et al.*, 1979), Andaman Islands (Malviya, 1972; Sharma Deorani and Rao, 1980) and Uttar Pradesh (Patnaik and Khan, 1980).

Otitis externa in bullocks due to stephanofilariasis was reported from Assam by Roychoudhary and Chakravarty (1969) but the parasite was identified only to the generic level. Also, ear-sore in cows had been reported from Tripura, the parasite

being considered to be a variant of *S. zaheeri* (Barman Roy, 1970). More recently, Hiregaudar (1974) reported ear-sore in Nilgai (Boselaphus tragocamelus) due to a *Stephanofilaria* species.

The sporadic occurrence of the ear-sore caused by *Stephanofilaria assamensis* in20-50% of the buffaloes in the Nowgong district of Assam. Sometimes it can be' seen in an epidemic form but was rarely fatal. (Gopal krishan, 1949).

Stephanofilaria assamensis was very common in the plain cattle of Assam (Verma, 1953).

The micro-filaria of *Stephanofilaria assamensis* in cattle from material obtained from humpsore in Bengal was described for the first time (Sen *et al,* 1956). The occurrence of humpsore in the district of Purnea and particularly in the Forbesganj, Araria and Kishanganj areas of India was reported (Verma, 1956).

Incidence of humpsore in Bangladesh 25% and the percentage of infection is greater in the preserves, corrals and dairy farms than villages was reported (Rahman, 1957).

The prevalence of humpsore in cattle of Assam caused by *Stephanofilaria* assamensis (Hiregandar and Chatupale, 1965).

Humpsore affecting about 20% of cattle in Bangladesh,(Hassan,1969). It was found 3% of cattle were affected by stephanofilarial dermatitis in Northern Nigeria (Oduye,1971).

Musca conducens, (Walker, 185) in localities of Peninsular Malaysia where stephanofilariasis is endemic but also in 4 areas where the disease does not occur. (Fadzil, 1973)

Little work has been done on the epidemiology of this disease in young animals (Islam, 1979). *Stephanofilaria,* was found to be the cause of the skin lesions common in cattle in northern Australia (Johnson *et al.*, 1981).

2.4.2 Host ranges

It was reported that stephanofilariasis in goat had a greater tendency to spread over the body than in cattle (Kraneveld, 1935).

It was reported first in India that the *Stephanofilaria assamensis* was the cause of humpsore in cattle. (Pande, 1936)

The sporadic occurrence of the ear-sore caused by *Stephanofilaria assamensis* in20-50% of the buffaloes in the Nowgong district of Assam. Sometimes it can be' seen in an epidemic form but was rarely fatal (Gopalkrishan, 1949).

It was reported that stephanofilarial lesions on 214(97.72%) of 219 beef cattle. Only 28 (32.7%) of 66 Holsteins, 3(20%) of 15 Jersey, 9(12.5%) of Guernseys and 10 (33.3^{0}%) of 30 Herefords had the disease.(Hibler,1966).

The occurence of stephanofilariasis in domestic elephant in Assam was reported (Bhattacharjee, 1967).

The Stephanofilaria has also been reported from hump sore like lesions in goats (Patnaik and Roy, 1968).

It was reported that the occurrence of stephanofilarial dermatis among elephants in Assam (Bhattacharjee, 1970). The Stephanofilaria has also been reported from hump sore like lesions in buffaloes. (Patnaik and Roy, 1968;Sharma Deorani and Rao,1980.)

2.4.3 Predilection site of infection

Stephanofilaria assamensis had a special affinity for the hump of cattle, but the infection of the skin on the other parts of the body was not totally absent (Rahman, 1957).

A third species of *Stephanofilaria* from Navel sore in a buffalo calf from the Andaman Islands. (Sinha and Das, 1958)

The stephanofilarial lesions on the teat, udder, shoulder and medial canthus of the eye of Jersey cows in Denmark was observed (Hassan 1964).

A new species of *Stephanofilaria* resembling *Stephanofilaria assamensis* in leukoderma in muzzle of cattle (Kono, 1965).

It was observed that the hump sore lesions mostly on or around the hump (Mia and Haque, 1967).

The stephanofilarial lesion on the back and on the ventral surface of the body, anterior and posterior to the navel and on the sides of the abdomen of elephant. (Bhattacharjee, 1970).

The hump sore lesions as a chronic dermatitis affecting the hump,neck, ears and around the eyes (Dewan, 1971a).

Cases of chronic ulcerated growth around the dewclaws of cattle in India caused by *Stephanofilaria assamensis* (Pal and Sinha 1971).

In the USSR revealed that different *Stephanofilaria spp.* infected different areas of the skin, *Stephanofilaria dedoesi*, infected the skin around the neck, shoulders, and sternum, *Stephanofilaria stilesi* the abdominal region, concha auris and udder, *Stephanofilaria kaeli* chiefly the neck, *Stephanofilaria moni* the Muzzle, *Stephanofilaria assamensis* the neck and withers, *Stephanofilaria zaheeri* the ears, *Stephanofilaria andamani* the midventral abdominal region,*Stephanofilaria srivastavi* the back and *Stephanofilaria dinniki* the shoulders.(Ivashkin *et al.*, 1971).

The lesions may be present in different parts of the body, particularly the scrotum (Watrelot-Virieux; Pin, 2006), pelvic membrane (Novaes *et al.*, 2006), neck (Sutherst *et al.*, 2006), and udder (Silva; Braga; Fioravanti, 2001).

2.4.4 Breed, age ,sex and seasonal prevalence

Imported Shindi and Hariana cattle were more susceptible than the local breed. The disease was seldom observed in young cattle under the age of 7 years. Accidental infection was seen in only one bull calf. Male cattle were more prone'- to infection than female. Strong and healthy cattle were equally susceptible as weak animals. Severity of infection in different seasons varied slightly but cases were found to be aggravated with the onset of monsoon rains when flies are abundant (Rahman, 1957).

No animal below 2 years of age was found to be affected and higher incidences were recorded in Rainy season (Mia and Haque, 1967).

It was reported that ear-sores of buffalo in Assam became apparent in March or April and persisted until the end of October (Bhattacharjee and Dass, 1967).

It was stated that during the monsoon season (June to August) about 20% of cattle in East Pakistan were affected by a generally chronic dermatitis caused by *Stephanofilaria assamensis* (Hassan, 1970).

The disease occurred during all seasons of the year, with a highest incidence during spring and Summer (Dewan, 1971).

Cattle imported from West Pakistan were very susceptible but calves less than one year old were resistant (Dewan, 1971).

Topographical studies of the two infections suggest that *S. zaheeri* is a well adapted and thus a widespread parasite, while *S. assamensis* is confined mainly to the eastern parts of India. A humid climate with thick vegetation appears ideal for Stephanofilaria species in general and for *S. assamensis* in particular. Although altitude did not affect the prevalence of ear-sore (Patnaik, 1964), hump-sore has not been observed in hilly tracts (Patnaik, 1964; Dutta, 1972).

A high incidence of *S. zaheeri* and *S. assamensis*, respectively in the monsoon, with a fall during the winter. (Ahmed, 1961 and Datta1972).

The age of the host also influences these two infections. Calves below one year of age had not been seen with either ear-sore (Ahmed, 1961) or hump-sore lesions (Shamsul, 1971; Devan, 1971). However, more recently, have found a five months old female buffalo calf suffering with ear-sore lesions. They further observed a progressive increase in *S. zaheeri* infection with a maximum prevalence at four years of age. From then onwards there was a fall in the infection rate till the age of ten years, suggesting age resistance in buffaloes against *S. zaheeri* (Agrawal and Datt, 1978).

Subsequent observations in adult cattle at slaughter showed that lesion prevalence increased with age, was higher in bulls than in steers and cows, and was lower in animals with higher *Bos indicus* content (Johnson *et al.*, 1986).

National or even regional epidemiological information has not been found. Some authors, however, reported a greater frequency of this parasite in *Bos taurus* breeds and older animals in a study of herds in northern Australia. The same authors also report that the size of the lesion was proportional to the size and age of the animal and that females presented larger-diameter lesions than did the males (Sutherst *et al.*, 2006).

2.4.5 Transmission

Unsuccessful attempts were made by Rahman (1957) and Huq *et al.* (1957) to transmit hump-sore by collecting flies feeding on hump-sore lesions and transferring them immediately to abraded skin of healthy cattle or by placing scrapings from the lesions into abraded skin of the healthy cattle. However, in 1963, Srivastava and Dutt were able to demonstrate *Musca conducens* to be the biological vector for this parasite. Additional work, including experimental work, by Patnaik and Roy (1966), Chakravarty (1967), Shamsul (1971), Patnaik (1973) confirmed the findings of Srivastava and Dutt. Later, Dutt (1970) incriminated *Musca planiceps* as a biological vector for *S. zaheeri*. *Musca autumnalis* has also been found to harbour infective larvae referable to a Stephanofilaria species (Patnaik and Kumar, 1972). It is important to note that all the muscids so far confirmed as vectors for Stephanofilaria possess prestomal teeth on their labella which enable these flies to abrade the weakened or lacerated skin of the animal; blood has been found to be an essential prerequisite for oviposition by these flies, suggesting haematophagus feeding. However, a proboscis ill-adapted for blood sucking may account for the localization of the sore around the hump region where the skin of the draught animal is weakened or abraded by the constant friction of the yoke. This may be the reason why male animals are more affected (Halder, 1961). Likewise, *S. zaheeri* is confined to the ears of buffaloes probably because the skin on the inner side of the ear is thin and is easily abraded by the muscids. Both male and female buffaloes are equally predisposed to the infection (Gopalkrishnan, 1951).

2.4.6 Predisposing factors

Staphylococcus aureus create a favourable conditions for the development of *Stephanofilaria assamensis* in the skin (Dewan, 1971b).

A breach in the continuity of epidermis of the skin is the pre requisite condition for the subsequent development of hump sore (Dewan and Rahman, 1970 and

Dewan, 1975). In this study the most commonly observed predisposing factors for breach in the epidermis are as follows:

Inner canthus of the eye

Constant moistening of the skin by occular discharge and feeding of the discharge by flies.

Base of the horn

Frequent rubbing by rope used for restraint of the animal.

Base of the ear

Similar to base of the horn, ears also tied by rope causing injury to the skin and this is the common practice of controlling the animals.

Behind the poll

Continuous rubbing by the chain or rope used to control the animal at the house or during grazing.

Dorsal neck (Middle and posterior)

Frequent and continuous rubbing occured by yoke during ploughing, drawing cart and oil or molasses machine, chain or rope used for restraint and in the farm stanchion bar may damage the epidermis and when animals forcefully try to introduce their head with a view to eat better tall grass or cereal plants surrounded by fences made of bamboo or barbed wire.

Hump/Withers

Under farm cpnditions the manger at the open place of the barn for one to 2 years old animals is surrounded by metallic rod or bamboo in order to prevent soiling of the food in the manger by faeces and to prevent gathering during feeding time.

Nevertheless, the animals do enter forcefully and cause a severe injury over the hump, back, and even loin by node of the bamboo and rough surface at the joint between the rods.

Because of the presence of Onchocercial microfilariae in the dermo-epidermal space and absence of stephanofilarial microfilaria in the initial lesions of the humpsore. It is presumed that onchocercal parasites result in irritation and itching around the hump or neck during their movement and thus excited the animal to rub or scratch the area of skin to the posts,walls, trees, fences, stanchion bar etc. (Dewan 1971a and Haq *et al.,* 1977).

Abdominal wall or other regions: These sites were seen to injure by rough floor of farm, barbed wire, during fighting with each other or any other cause of injury.

Vector

Stephanofilaria assamensis was found in the intermediate host, *Musca conducens*. (Srivastava and Dutt, 1963)

Musca conducens was proposed as an intermediate host of *Stephanofilaria assamensis* (Patnaik and Roy, 1966).

Musca conducens was described as the intermediate host of *Stephanofilaria* resembling *Stephanofilaria assamensis,* causing leukoderma in muzzle of cattle. (Kono and Fukuyoshi (1967).

In Bangladesh, *Musca conducens*, (Walker, 1859, is the vector of *Stephanofilaria assamensis*, the causal agent of humpsore (Islam, 1971bcd).

It was proposed that the insect vector for *Stephanofilaria assamensis* was the biting fly, *Musca, conducens*.(Islam 1971 d)

The biting flies *Lyperosia titillans* and *Lyperosia irritants* were the most important vectors of various species of *Stephahofilaria* in the USSR.

2.5 Pathogenesis

Staphylococcus aureus, Staphylococcus albus, Staphylococcus epidermidis, Bacillus coagoulans, Bacillus circulans and Bacillus subtilis were isolated from naturally occurring humpsore lesions and concluded that *Musca conducens* may be unable to inject infective larvae of *Stephanofiaria assamensis* through healthy skin but may deposite them in the exudative lesions produced by another agent such as bacteria, or by injury. (Dewan and Rahman, 1970)

There are 4 stages of Stephanofilarial lesions. The lesions of subacute and acute stages look nearly similar and characterised by their surface, being somewhat devoid of hair, appearing granular and slightly raised, the cut surface clearly showing epidermal proliferations resulting in corrugations. The oozed serum forms crust on the surface of the lesions. In acute stage, the 4 stages of Stephanofilarial lesions .The lesions of subacute and acute stages look nearly intense surface granulations and epidermal proliferations are much more, and result in deep and close corrugations on the cut surface with thick layer of keratinized tissue and dry skin surface. The desquamative and reparative stage lesions are quite distinct and are completely devoid of hair. The lesions of the former stage have nongranular, moist and unevenly smooth surface, devoid of horny epidermis and absence of any epidermal proliferative changes in the cut surface. The later stage lesion can, however, be easily made out by the hardness and smoothness of the contracted cicatrial tissue (Sharma Deorani, 1967)

Bhattacharjee and Dass (1967) reported that the ear–sore of buffaloes in Assam began as small haemorrhagic spots resembling insect bites, serous discharge formed scabs and encrustations. The lesions became purulent extended upto the external auditory meatus and were covered with a thick creamy material having an offensive odour (Bhattacharjee and Dass 1967)

The humpsore lesions mostly on or around the hump. Single circular or irregular shaped lesion of varying size were found in most cases. The lesion was found to

commence as one small circle which gradually increased in size and covered with dark thick hard crust having cravices, and cracks. Slight to moderate bleeding surfaces were observed either the lesions were picked up by crows or rubbed on rough surfaces by the animals themselves for irritation.(Mia and Haque,1967).

The developmental process of humpsore may be divided into initial exudative and desquamative stages. Initial lesions revealed no microfilariae of Stephanofilaria but *Onchocerca spp.* were found. The initial lesion or a breach in the continuity of epidermis is thought to be a prerequisite condition for the subsequent development of humpsore (Dewan, 1975).

Stephanofilaria can be characterized as the agent of a disease commonly observed in exuberant high-occupancy pastures with large quantities of wet feces, principally in the hot and rainy seasons (Sutherst *et al.,* 2006). These factors result in a greater proliferation of vectors. In this situation, the flies are attracted by exudate originating from open cutaneous lesions infested with adult worm parasites, with the consequent ingestion of microfilariae (Urquhart *et al.*, 1998). The final host is infected when the flies deposit larvae in the not injured skin, causing inflammation and destruction of the hair follicles and epithelian cells (Urquhart *et al.*, 1998; Novaes; Miyashida, 2006). *Stephanofilaria* occupies the hair follicle and the dermic papilla through blood circulation, with the consequent formation of areas of alopecia, papules and intense itching, giving rise to lesions which may reach a diameter of 25cm (Smith; Jones, 1962).

2.6 Gross Pathology

The 4 stages of Stephanofilarial lesions on macroscopic examination. The lesions of subacute and acute stages look nearly similar and characterised by their surface, being somewhat devoid of hair, appearing granular and slightly raised, the cut surface clearly showing epidermal proliferations resulting in corrugations. The oozed serum on the surface of the lesions. In acute stage the intense surface granulations and epidermal proliferations forms crust are much more, and result in

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In general the lesions are characterised by pruritis, loss of hair exudation, granulation, ulceration and incrustation depending on the stage (Pande, 1935; Varma, 1956; Sharma Deorani, 1965, 1967; Dutta, 1972; Devan, 1975 and Dass *et al.,* 1975a)

The gross pathology of ear-sore is somewhat similar and includes congestion exudation, incrustation, loss of hair, ulceration, haemorrhage and depigmentation (Gopalkrishnan, 1951; Raghavachari and Reddy, 1957; Ahmed, 1961; Patnaik, 1964; Agrawal *et al.,* 1978 a)

2.7 Histopathological Features

The histological picture of humpsore lesions in cattle. epidermis had degenerated cells of the upper layer which were hyperkeratinized and the cell membranes of the stratum spinosum was disintegrated. The adnexa of the dermis was absent and the connective tissue had been broken down. The blood vessels were hypertrophied and hyperplastic and heavily infiltrated with lymphocytic macrophages (Mannan *et al.,* 1977)

Early studies on the histopathology of hump-sore or ear-sore were mainly directed to understanding the life cycle of Stephanofilaria species. Thus Pande (1935) observed adult *S. assamensis* worms in the papillary portion of the corium immediately underneath the rete malpighii, with the largest numbers of microfilariae in the dermis of cattle having hump-sore lesions. Halder (1961) reported adult worms to be in the dermis only, while the epidermis was filled with migratory macrophages and a certain number of lymphocytes(Sharma,1965; Deorani , 1967) described the histopathology of hump-sore by recognising lesions in four stages, i.e. subacute, acute, desquamative and reparative. The worms were found in both the dermis and the epidermis. Microfilariae were observed only in the desquamative and subacute stages. The reparative or healing stage was characterized by the presence of thick contracted scar tissue but the absence of worms.

The histopathology of ear-sore has been described by Bhattacharjee and Das (1967), Patnaik (1970a), Das *et al.* (1975b) and Agrawal *et al.* (1978 a,b). Worm sections were seen in the epidermis or the dermis, within hair follicles and sebaceous glands. Microfilariae infiltration, mainly with lymphocytes and

macrophages, beneath the epidermis, congestion, haemorrhage, hyperactivity of sebaceous glands or their degeneration, parakeratosis, acanthosis were the other main features observed. Agrawal *et al.* (1978a) found degenerated parasites in the dermis, with marked infiltration of mononuclear cells, eosinophils and plasma cells. They also observed 'microcavities' in the epidermis filled with macrophages, eosinophils and tissue debris, which was positive in the PAS test suggesting the debris to be from a degenerated worm. The finding led them to suggest that the ear-sore lesions may be allergic in nature and that the female *S. zaheeri* is destroyed or expelled more efficiently, thus altering the sex ratio of adult worms.

Dyskeratosis, a disorganised arrangement of epidermal cells with mitotic figures leading to a certain degree of anaplasia, has been reported by Dutta (1972) in hump-sore lesions. Similarly, Agrawal *et al.* (1978b) have found irregular proliferation of epidermis and hair follicles in two natural cases of earsore lesions in buffaloes, and suspected that the lesions might be heading towards neoplasia. As these reports have come from natural cases and from old animals, an etiology of the irregular proliferation or dyskeratosis is very uncertain.

The main histological abnormalities noted were liquifaction necrosis associated with inflammatory polymorphonuclear and eosinophilic infiltrate, with multifocal hemorrhage areas. When viewed, the parasite appeared flattened, with an irregular and undulating surface, numerous well-defined fixing points in its cuticle and reproductive and digestive tracts. These histological characteristics were similar to those reported by other researchers, who observed the worms surrounded by an inflammation zone containing eosinophiles, lymphocytes, neutrophils, histiocytes, and frequently, a layer of conjunctive tissue (Jones; Hunt; King, 2000). For these authors, the presence of eosinophiles in smears obtained from lesions containing *Stephanofilaria* sp is considered a histological characteristic pattern.

Another alteration that stood out in the histological samples was the presence of hyperceratosis and parakeratosis in the epidermis, in addition to severe dermatitis,

findings which were also taken by researchers as indicative of the disease (Jones; Hunt; King, 2000). Probably, these findings are related to the death of the parasite and the consequent sensitization of the host.

2.8 Clinical Findings

Humpsore lesion as an inflamed area, usually about 2 inches in diameter, with pin-point congested spots and slightly purulent yellow discharge. The surrounding area was considerably thickened and cracked (Huq, 1953).

The humpsore in cattle as a chronic granulomatous ulcerative condition of the skin (Varma, 1956)

Stephanofilaria assamensis had a special affinity for the hump of cattle, but the infection of the skin on the other parts of the body was not totally absent (Rahman 1957)

The stephanofilarial lesions on the teat, udder, shoulder and medial canthus of the eye of Jersey cows in Denmark (Hassan, 1964)

2.9 Diagnostic Approaches

4 microfilarae of *Stephanofilaria assamensis* in peripheral blood smears of 399 (9-83%) of 4,058 affected cattle with humpsore in Orissa, India (Dass,1955).

It was described for the first time the microfilaria of *Stephanofilaria assamensis* in cattle from material obtained from humpsore in Bengal (Sen *et al.*, 1956).

In most cases, the diagnosis is based on clinical signs, patient behavior and success of the adopted therapy. This is related to difficulty in isolating and identifying microfilariae, given the limited quantity of this parasite at the site of the lesion (Nooruddin, 1982; Huq, 1985; Urquhart *et al.*, 1998).

2.10 Differential Diagnosis

The rational diagnosis of late stage of humpsore lesions on clinical basis can be made readily because of their larger size, characteristic scabs/crusts formation, and typical locations. But smaller initial lesions where stephanofilarial parasites are not found (Dewan, 1975) can be confused with ringworm, and dermatophilosis. In this connection(Nooruddin,1962) proposed the following distinguishing features by which a rational diagnosis of initial lesions of humpsore can be made without difficulty.

Initial humpsore	Dermatophilosis	Ringworms
Occurs any season of the year.	Occurs most commonly in wet seasons of the year	Occurs most commonly in the dry seasons of the year
Animals above 1½ years of age are most commonly susceptible	Animals of all ages are susceptible (Khaleque, 1982).	Calves upto 1½ years of age are affected (Dey, 1982).
Alopecia is a very. characteristic feature	Alopecia is not a significant feature	Alopecia is a very characteristic feature
Pruritis is very common	Pruritis is absent	Pruritis is very common
No matted hair type of lesions	Isolated matted hair lesions are very common	No matting of hairs
Size of the initial lesions usually 3 cm in diameter	Size of the lesions vary from 1.3 to 6.0 cm (Khaleque, 1982).	The size of the circular lesions ranged from 0.4 to 4.5 cm. in diameter (Dey, 1982).
Initial lesions are usually one or two in number and occurs around the hump or neck	There is no usual location and number of lesions	There is no usual location and number of lesions.
Crusts are whitish	The Crusts are yellowish	The Crusts are whitish

grey to grey	brown to brown	grey to grey
Crusts are not membrane like and become powdery on scraping by scalpel	Crusts are membrane like and can be lifted by finger nails readily in later stage	Crusts are not membrane like and become powdery on scraping by scalpel
No spontaneous recovery occurs but crusts can be treated after treatment with no penetration of hairs.	In later stage the crusts separate from the skin but are held in -place by penetratings hairs	During recovery stage particularly spontaneous the crusts separate but no penetration of hairs occur.
No spontaneous epidermal growth and growth of new hairs can be seen beneath the skin	Spontaneous epidermal growth and growth of new hairs occur beneath the crusts	No spontaneous epider - mal growth and growth of new hairs occur beneath the crusts.

2.11 Therapeutical Findings

Stephanofilarial lesions have been removed surgically or by the use of cauterizing agents and afterwards treated as ordinary wounds (Mohan, 1945; Pannu, 1958; Mishra, 1969).

Antimony potassium tartrate (parentally or topically) and phenothiazine (4-8% ointment) have been claimed to be effective against hump sore lesions (Mohan, 1945; Pannu, 1958; Chakravarty, 1967; Pal and Sinha, 1971; Dutta and Hazarika, 1976). Hassan (1969) reported on the therapeutic value of 64 preparations in treating hump-sore including conch shell ash (sankamudra), lead monoxide, sulphur, tobacco, mustard oil and others containing calcium sulphate, quinine, alum, tar camphor and petroleum jelly. Diethylcarbamazine, Novalep , suramin,

benzene hexachloride, a tooth-paste prepared with tobacco leaves, molasses and other ingredients (Gudakhu), stibophen, diethylamine acetarsone, lithium antimony thiomalate, fouadin and an ethyl chloride spray have been tried in hump-sore with either partial or no effects (Chakravarty, 1967; Patnaik, 1970b). More recently a tobacco ointment (tobacco leaf decoction 8%, oil terebinth 2%, creosote I% in a petroleum jelly base) has been reported to cure 80% of cases of hump-sore and is considered to be the best remedy for the condition (Dutta andHazarika,1976).

Organophosphorous compounds like trichlorphon, malathion, fenitrothion have been tried successfully in treating stephanofilarial dermatitis by various workers (Srivastava and Malviya, 1968; Das et al., 1975b; Devan and Baki, 1976; Dutta and Hazarika, 1976; Das *et al.,* 1977; Agrawal and Dutt, 1977a; Patnaikand Khan, 1980).

Malathion has been shown to have a stephanofilaricidal effect in vitro (Agrawal and Dutt, 1977a). The additional insecticidal and fly repellent effects of the organophosphorous compounds increase their suitability for treating stephanofilarial dermatitis. These compounds have been used either in aqueous or in ointment form, the latter being more effective. Srivastava and Malviya (1968) used either 2% trichlorphon solution or 6% ointment on alternate days to a total of six applications. The solution did not cause complete recovery from hump-sore but the ointment was found completely satisfactory. Similar findings were reported by Patnaik (1970b) who classified the lesions according to duration and observed higher healing rates in mild cases (lesions up to 6 months old) and lowest in chronic cases (over 2 years) within a period of up to 40 days after treatment.

The treatment of humpsore with; Neguvon (Trichloroplion) either as a 6%solution in distilled water or as 6% Liniment in castor oil together with surgical removal of necrotic tissues from the wound borders. Out of 140 animals, 136 recovered completely. The castor oil preparation showed somewhat superior effect. The

drug proved to be safe, economic and highly effective (Rahman and Khaleque, 1974).

The application of Neguvon ointment (8% Neguvon, 4% sulphanilamide powder and 66% vaseline) twice daily to 30 hump-sore lesions. All animals recovered within 20-66 days of application. Histological studies after healing of the lesions revealed complete healing of the epidermis but no restoration of the hair follicle and adnexal structures. Hyperplasia of endothelial cells and proliferation of new capillaries associated with connective tissue are indicative of good healing response (Baki and Dewan 1975).

Application of Neguvon-sulphanilamide ointment (20% Neguvon, 5% sulphanilamide and 75% vaseline) may be effectively used against humpsore. The drug is to be applied topically once daily, the first application being made after thoroughly removing the scabs from the lesion. Maximum 20 applications may be needed for an exceptionally large and complicated lesion,(Dewan and Baki 1976).

The treatment of humpsore in cattle with various drugs with variable results. Sumithion as a 6% emulsion in liquid paraffin applied twice daily cured 6 of 10 animals in 16 days. Phenothiazine as a 4, 6 or 8% ointment applied twice daily cured 2 of 10 in 20 days, 9 of 14 in 14 days and 9 of 12 in 14 days, respectively. Powder containing 100 parts plaster of Paris, 20 parts of Alum, and 10 each of napthalene and quinine, applied daily cured one of 8 in 7 days. An ointment containin tobacco leaf decoction, 2% oil terebinth and 1% creosote, applied daily, cured 8 of 10 in 12 days. Diethyl carbomazine acid citrate at 5 ml. per 100 kg. body weight given as 3 injections at 3 day intervals cured none of 3 cattle (Dutta and Hazarika 1976).

The use of various formulations of Malathion, Neguvon and Sumithion against ear-sores of buffalo in India, They proposed that 6% ointment was superior to the 6% liniment (Patnaik and Khan 1980)

The recovery of humpsore lesions of 1000 cattle within 14 -34 days of treatment with ointment (15% Neguvon and 5^{0}% sulphanilamide in vaseline).Similar results also observed by the application of 20% Neguvon and 5% sulphanilamide ointment. Recovery occurs 5-20 days of treatment with no relapse (Baki and Hossain 1982a).

It was proposed that once the humpsore lesions were healed up by Neguvon ointment no recurrence would occur by the same parasites but reinfection could be the cause of recurrence (Baki and Hossain 1982b).

Treatment of stephanofilariasis lesions is laborious and may be time-consuming, making the continuation of the animal in the herd economically unviable (Novaes; Miyashida, 2006).

2.12 Economic importance of stephanofilariasis

Humpsore in general is chronic and mere healing up of the sore does not help much because the damage already caused to the hide is of paramount economic importance affecting the quality of leather and market value of the animals. Besides, for the tension due to itching and disturbance by flies, the working capacity of draft cattle and milk yield in milch cows are also considerably reduced (Ahmed and Ali, 1973).

Humpsore was one of the factors for stunted growth and general debility of the animals (Hassan and Huq, 1955).

Several diseases have been found to affect the hides of cattle, most of which not only affect the general health,production, market value and working capacity of the affected animals but also lower the economic value of their hides. Moreover, some skin diseases of animals such as dermatophilosis (Weber, 1978) and ring-worm (Anon, 1956) are transmissible to humans.

It may be added that because the discomfort and intense pain from the lesion result in stress and low food consumption, the negative impact on the production

of milk and meat is considerable (Nooruddin; Hoque, 1985; Novaes *et al.,* 1988; Silva; Braga; Fioravanti, 2001).

According to owners reports, cows with these stein udder lesions generally produce less milk, probably due to the discomfort caused by the constant presence of flies around the wounds, which is manifestedly translate into frequent abdominal kicking. They estimate that, after the animals´ recovery, milk production increased by approximately 10%. Although subjectively analyzed, this information reinforces the economic importance of the disease. Some authors relate a drop in leather quality to economic losses due to *Stephanofilariosis sp* (Nooruddin 1982; Huq, 1985, Urquhart *et al.,* 1998). An intense painful reaction in the areas of the lesions and stress to the animals due to the presence of flies result in significant losses when compared to other bovine skin damage. While these authors did not quantify these losses in monetary terms, in the present study it was clear that, when the problem occurs on a farm, these amounts may increase production costs to such an extent that the adoption of a therapeutic protocol such as the one described here is unviable.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental area

Humpsore was studied centralizing the Upozila Govt. Veterinary Hospital, Birgonj in Dinajpur, Bangladesh.

3.2. Experimental Animals

The clinically affected animals visited physically in different locations surrounding the Birgonj and the animals submitted to Upozilla Govt. Veterinary Hospital for the diagnosis and therapeutic purposes were considered as the experimental animals.

3.3. Experimental Duration/Study Period

The duration of the experiment was one year and conducted from March to December 2011. The total population of the clinical cases was 1,427 among which 381 cases of Stephanofilariasis were registered in this villages during the course of the experimental period.

3.4. Clinical examinations

The presented clinical manifestations of the Stephanofilariasis were recorded and the farmer's complaints in relation to the affection were also emphasized. The locations of the lesions, conditions, concurrent infection (s) and/ or complications of the cutaneous lesions were recorded. The seasonal occurrences of the disease were also considered.

3.5 Pathological Examination

The gross morbid lesions of the disease were systematically examined, noted and categorized. The suitable sizes of skins of 3 typically Stephanofilariasis affected cattle were collected from the live patients subjected for the diagnosis and treatment for further histopathological study.

The representative cutaneous tissues were collected and preserved at 10% formalin solution and subsequently processed, embedded with paraffin, sectioned and stained with haematoxylin and eosin for histopathological examination (Luna, 1968).

3.5.1 Collection of stephanofilariasis affected skin

- Surgical instruments were sterilized by boiling
- Restraining of animals was performed by casting
- Local anaesthetic was applied subcutaneously following site selection and waited for few minutes for anaesthetic action
- Folding of skin was done by artery forceps
- Excision of excess folded portion of skin and subsequently sutured with nylon threads
- Locally application of cotton admixing with Tincture of iodine as counter irritant/Tincture Benzoin Co.
- Antibiotic course was maintained and hygienic measures were suggested to avoid secondary complication
- Suture was removed after 7 days

3.5.2 Preservation of skins and tissue processing

- Collected skin samples were preserved at 10% formalin solution for at least 3 days
- Trimming of preserved samples was done at suitable sizes
- Overnight watering of tissues was done to remove formalin
- Dehydration was performed in a series of ascending grades of alcohol
    - 50% alcohol: 1hr
    - 70% alcohol: 1hr
    - 80% alcohol: 1hr
    - 95% alcohol: 1hr
    - 100% alcohol: 3 changes and 1 hr for each change
- Chloroform treatment: 2 changes and 1.5 hrs for each change

- ❖ Impregnation by paraffinization at melting point (56^0 C): 2 changes and 1.5 hrs for each change
- ❖ The cooked tissue samples were blocked
- ❖ Sectioning was done at 5-7 µm in thickness, placing on water bath, taking on a glass slide and air dry

3.5.3 Routine haematoxylin and eosin (H & E) staining procedures

Preparation of Ehrlich's Haematoxylin solution

Chemicals	Amount
Haematoxylin crystals	4.0 g
Alcohol, 95%	200.0 ml
Potassium or ammonium alum	6.0 g
Distilled water	200.0 ml
Glycerine	200.0 ml
Glacial acetic acid	20.0 ml

Preparation of Eosin stock solution

Chemicals	Amount
Eosin Y, water soluble	1.0 g
Distilled water	20.0 ml
Alcohol, 95%	80.0 ml

Preparation of Eosin working solution

Chemicals	Amount
Eosin stock solution	1 part
Alcohol, 80%	3 part

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

3.5.4 Protocol of haematoxylin and eosin (H & E) staining

- Xylene treatment: 3 changes and 3 minutes for each change
- Rehydration in descending grades of alcohol
    - 100% alcohol: 2 minutes
    - 95% alcohol: 2 minutes
    - 80% alcohol: 2 minutes
    - 70% alcohol: 2 minutes
    - Distilled water: 10 minutes
- Haematoxylin: 10-15 minutes
- Distilled water: 15 minutes
- Bluing in lithium carbonate: Few dips
- Eosin: 30 minutes
- Dehydration in ascending grades of alcohol
    - 80% alcohol: Few dips
    - 95% alcohol: Few dips
    - 100% alcohol: Few dips
- Xylene treatment: 3 changes and 3 minutes for each changes
- Mounting with Canada Balsam
- Examined under microscope using both low and high power objectives

3.6 Parasitological examination and examination of skin scraps

Parasitological examinations were performed to detect concurrent infections following a standard procedure (McLeod *et al.,* 1981).

3.7 Examination of skin scraps

Differential diagnosis to exclude mange was done by the examination of skin scraps of the suspected individuals and the skin scraps were processed with 10%, potassium hydroxide (KOH) solution, heated in spirit lamp and examined under

microscope with low and high power objectives following a standard procedure (McLeod *et al.,* 1981). The result was recorded.

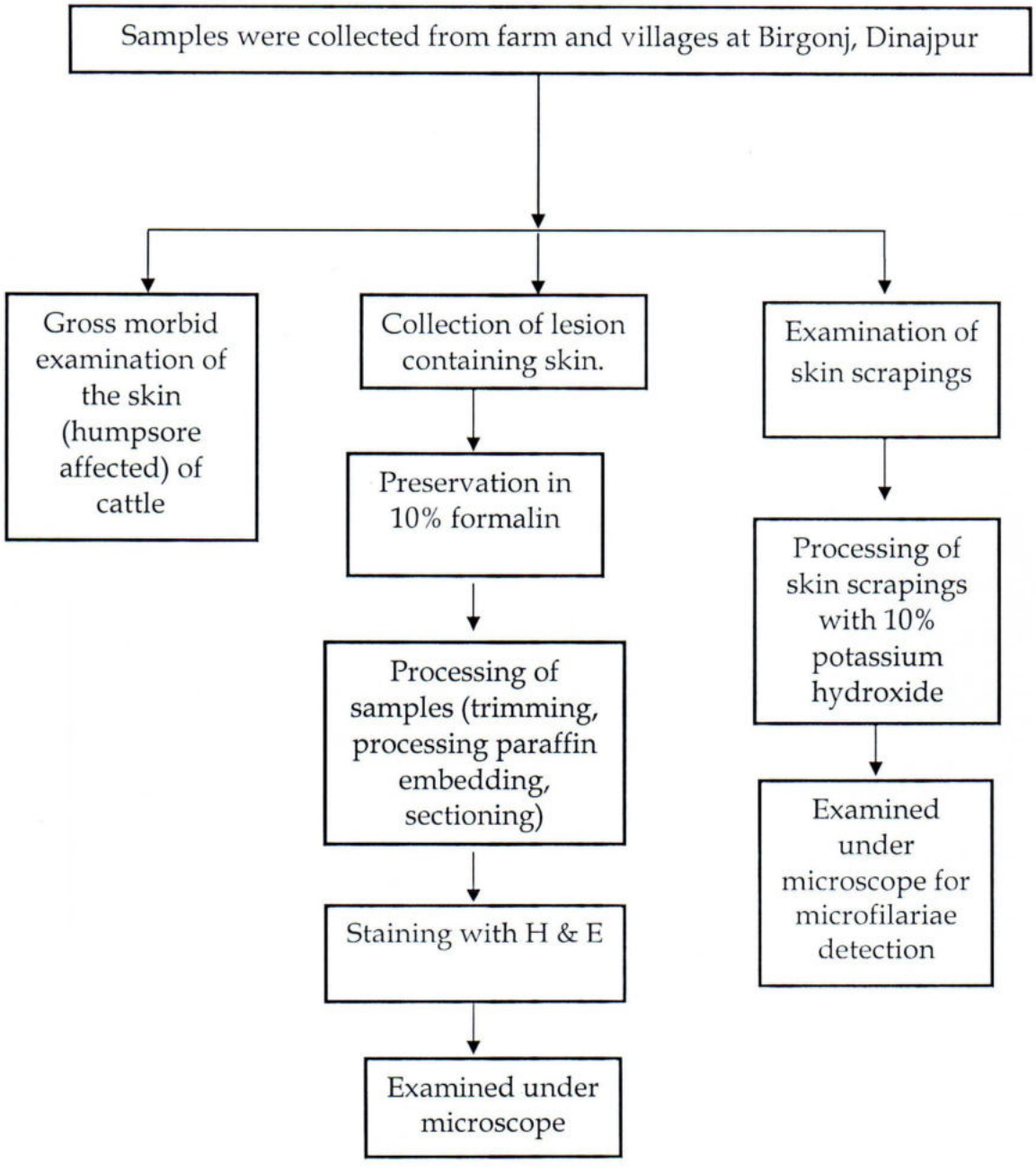
3.8 Grouping of animals for therapeutic purpose

Group	Drugs used	No. of animals
A	Ivermectin	3
B	Neguvon+Sulphanilamide	3
C	Cutaneous excision	3

3.9 Photography

The histopathological slides of normal and Stephanofilariasis affected cutaneous tissues were placed in microscope (Leica, Germany) and the respective microphotographs were taken directly by a digital camera (SAMSUNG ES55, 14.2 MEGA PIXEL, China) using both low and high objectives (X4, X10 and X40). The photographs were then placed in computer; image selection and magnification were further modified and placed in this thesis for better illustration of the results.

3.11 Experimental Design



Flow diagram of the experiment

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1 Predisposing Factors

In this study the most commonly observed predisposing factors for breach in the epidermis are as follows:

Inner canthus of the eye

Constant moistening of the skin by occular discharge and feeding of the discharge by flies.

Base of the horn

Frequent rubbing by rope used for restraint of the animal.

Base of the ear

Similar to base of the horn, ears also tied by rope causing injury to the skin and this is the common practice of controlling the animals, observed in this study.

Behind the poll

Continuous rubbing by the chain or raze mad to control the animal at the house or during grazing.

Dorsal neck (Middle and posterior)

Frequent and continuoues rabbi occured by yoke during ploughing, drawing cart and oil or molasses machine, chain or rope used for restraint and in the farm stanchion bar may damage the epidermis and when animals forcefully try to introduce their head with a view to eat better tall grasses or cereal plants surrounded by fences made of bamboo or barbed wire

Hump/Withers

Under farm conditions the manger at the open place of the barn for one to 2 years old animals is surrounded by metallic rod or bamboo in order to prevent soiling of the food in the manger by fasces and to prevent gathering during feeding time. Nevertheless, the animals do enter forcefully and cause a severe injury over the hump, back, and even loin by node of the bamboo and surface at -the joint between the rods.

4.2 Prevalence of humpsore

Table 1 summarises the results of the total survey. Stephanofilariasis was found in 381(26.69) of the 1427 bovine heads. Higher morbidity rate was recorded in Dairy Farm (31.63%) than rural areas (26.00%).

Table 1. Prevalence of Stephanofilariasis under Farm and rural conditions

Points	Animals Examined	Animals infected	Percentage
Dairy Farm	177	56	31.63
Villages (Rural house hold)	1250	325	26.00
Total	1427	381	26.79

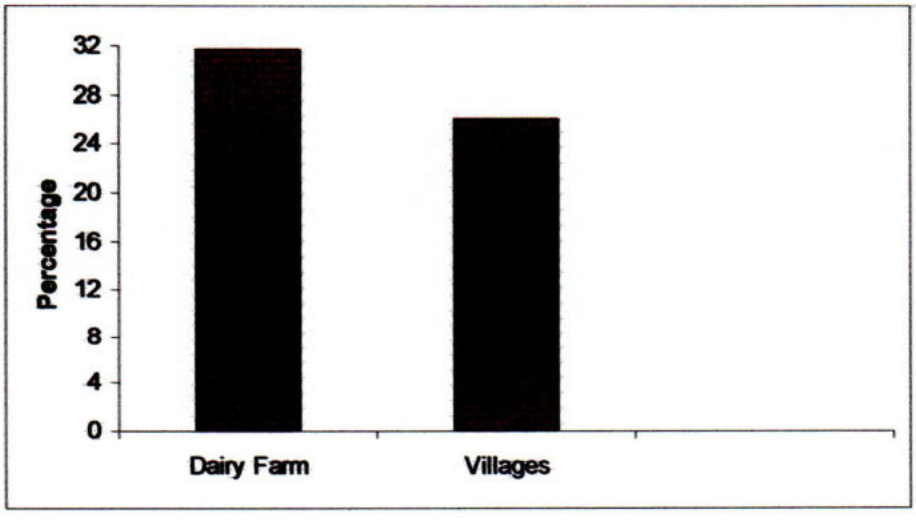


Fig. 1: Prevalence of Stephanofilariasis under Farm and rural conditions

Table 2. Prevalence of Stephanofilariasis in different types of cattle

Points	Animals examined	Animals infected	Percentage
Heifer calf	250	0	0
Bull calf	79	0	0
Heifer	216	0	0
Bull	300	250	83
Dry cow	211	125	59.24
Milch cow	215	6	2.79
Steer	156	0	0

Table 2 shows the prevalence of stephanofilariasis in different types of cattle (Fig.2). No stephanofilarial lesions was seen in either heifer or bull calves. Highest rate of incidence was recorded in bull (83.00%) followed by dry cow (59.24%), milch cow (2.79%).

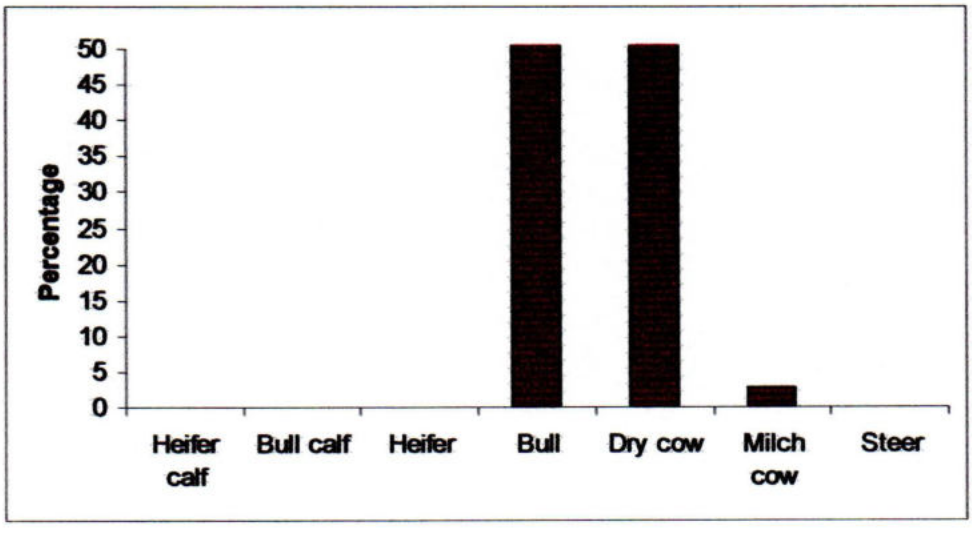


Fig. 2: Prevalence of Stephanofilariasis in different types of cattle

Table 3. Prevalence of Stephanofilariasis in both sexes

Points	Animals examined	Animals infected	Percentage
Male	950	300	31.57
Female	477	81	16.98

Male animals were mostly affected (31.57%) in compared to female (16.98%) (Table 3 and Fig. 3).

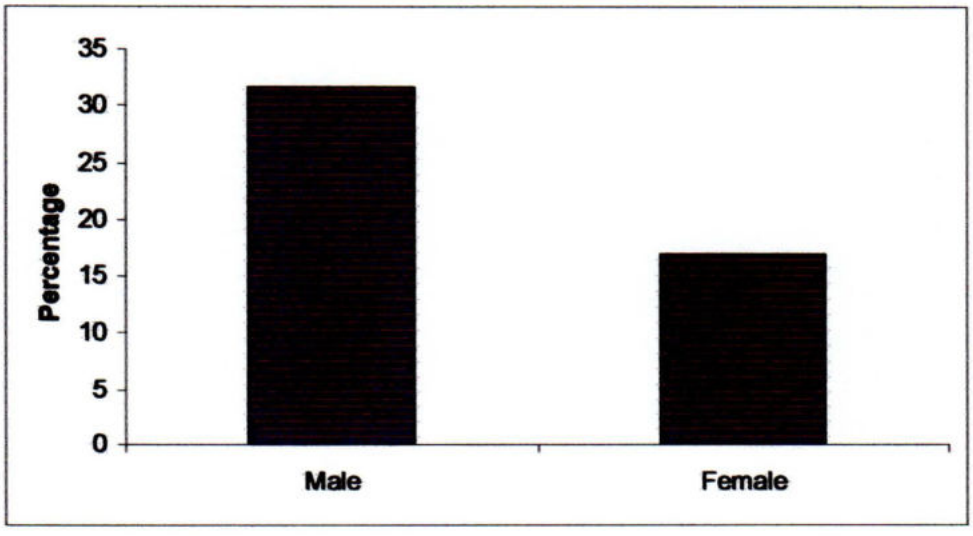


Fig. 3: Prevalence of Stephanofilariasis in both sexes

Table 4. Prevalence of Stephanofilariasis in various age groups of cattle

Points	Animals examined	Animals infected	Percentage
1-6 month	250	0	0
6-12 month	79	0	0
1-2 year	216	0	0
2-5 year	215	7	3.25
5-10 year	211	125	59.24
10-15 year	300	250	83.00
15-20 year	156	0	0

The minimum age of the affected animal was 11 years and the maximum was 18 years. The disease is most common in 10 to 15 years of age group (83.00%) followed by 5-10 years (59.24%), 2-5 years (3.25%) (table 4 and Fig.4).

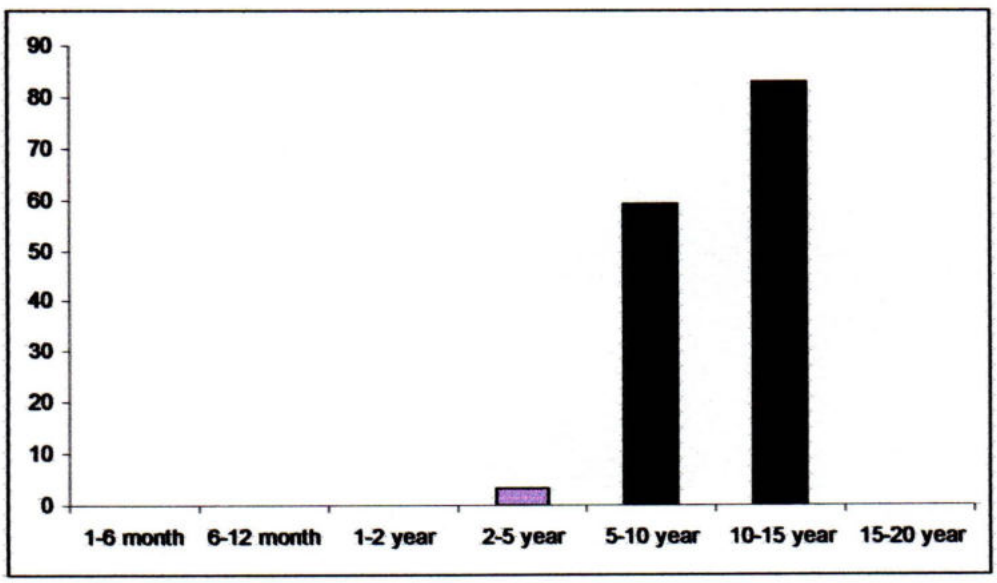


Fig. 4: Prevalence of Stephanofilariasis in various age groups of cattle

4.3 Clinical findings

A total of 961 stephanofilarial lesions available for study, were detected only by visual inspection of skin surface of the body. An intense pruritis in all the lesions was characterised by rubbing the affected part against the wall, pillar, posts, stanchion bar, trees, fences etc. A central ulcer or excoriation may be produced or enlarged the existing one by pruritis or rubbing disturbance. This disturbance can affect feeding and working time of the animal resulting in loss of weight and milk production. Excoriation caused by rubbing was seen complicated by screw-worm infestation, warts and yokegall.

The origin of the lesion was a breach in the skin which becomes enlarged by secondary bacterial infections (*Staphylococcus aureous, Staphylococcus albus etc.*). Gradually more tissue destruction occurs following deposition of stephanofilarial larvae together with rubbing the affected part. Oozing of fluid (serum, blood, mucopus) forms crusts and corrugations by epidermal proliferations.

In many instances the initial wound of the skin was covered by grayish white crust and frequently the crusts were excoriated and hence favoured the stephanofilarial infection.

The non ulcerated lesions frequently become excoriated favouring further deposition of stephanofilarial larvae and gradually obtains the characters of ulcerated lesions. Non-ulcerated lesions were comparatively much smaller than ulcerated ones. The lesions were partially or completely devoid of hairs.

The ulcerated lesions had central ulcers of various sizes and shapes surrounded by larger peripheral crusty areas in most of the lesions, however, entirely ulcerated lesions with slight crusting at the periphery also observed less frequently.

The central ulcers were seen to be depressed and roughly circular in majority of the lesions and irregular shaped ulcers such as transversely longitudinal,

triangular, rectangular, eliptical or variable shaped were observed in a good number of lesions. The size of the ulcers varied roughly from few mm to 7 cm in diameter. The boundary of the ulcers were always irregular. Sometimes two or more ulcers were seen to occur in one lesion. The discharge of the ulcers were purely blood, serum, or blood mixed serum. The surface of the ulcers were frequently hemorrhagic and moist but dry surface covered by thin blackish or brownish crusts produced by discharges were also the features of many ulcers. No offensive smell was noted. A number of flies were seen feeding on the discharges.

The colour of the crusty/scabby part was found to vary from grayish white to blackish and this part was raised from the ulcerated part or normal skin surface. It was thickened and the thickness varied from few mm to one cm. The scabs were very firm and cracked revealing blood inside the cracks and even a gentle pressure caused slight to moderate bleeding. The scabby lesions were very firm to touch and mostly freely mobile but immobile and painful lesions were also observed. The thickness of the crusty part of the lesions was up to 2.3 cm according to character and degree of crust formation, compared to the normal skin thick ness of about 2.5 mm.

The shape of the lesions was characteristically circular although roughly rectangular, triangular or irregular shaped lesions were not uncommon. The periphery of the lesions were regular. Occasionally the lesions at the poll and anterior or posterior neck were divided transversely into two identical halves. Although commonly encountered lesions were solitary, cence-coales of two lesions was not uncommon. Healed up lesions were covered by smooth black cicatrical tissue devoid of hairs.

4.4 Gross pathology

Presence of epidermal proliferations forms crust were much more, and result in deep and close corrugations on the cut surface with thick layer of Keratinized tissue and dry skin surface. The desquamative and reparative stage lesions were

quite distinct and were completely devoid of hair. The lesions of the former stage have nongranular, moist and unevenly smooth surface, devoid of horny epidermis and absence of any epidermal proliferative changes in the cut surface. The later stage lesion could, however, be easily made out by the hardness and smoothness of the contracted cicatrial tissue.Fresh wounds also produced due to pruritus.

The humpsore lesions mostly on or around the hump.Wounds also produced in different body parts including hump region. Single circular or irregular shaped lesions of varying sizes were found in most cases. The lesion was found to commence as one small circle which gradually increased in size and covered with dark thick hard crust having cravices, and cracks. Slight to moderate bleeding surfaces were observed either the lesions were eiher picked up by crows or rubbed on rough surfaces by the animals themselves for irritation.

4.5. Histopathology

Histological picture of skin biopsy collected from affected lesions of humpsore. Hyperkeratosis, proliferation of fibrous connective tissue and reactive cell infiltration were observed. Cross and longitudinal section of stephanofilarial parasites were seen in tissue sections which were encapsulated with fibrous connective tissue. Hyperkeratinization with distorsion of keratinized layer, proliferation of loose connective tissue predominantly collagenous fibers and reactive cells infiltration also observed.

4.6 Treatment

Group	Drugs used	No. of animals	Therapeutic response
A	Ivermectin	3	+++
B	Neguvon +Sulphanilamide	3	++
C	Cutaneous excision	3	+++ (?)

Fig. 5: Grouping of animals for therapeutic purpose

5ml of Ivermectin solution was injected under the lesion at different places of the body. Injections were repeated at weekly intervals in 3 cattle. At the end of 3rd week after first injection, the lesions in 3 animals were healed up by smooth black scar tissue. The irritation and itching had also considerably diminished. The larger lesions (10 to 14 cm diameter) in other 3 animals were also healed up similarly but had small moist ulcerated area at the centre. These animals were injected again on 4th time as before. On the 14 lesions which were injected thrice, exhibited re-growth of hairs over the peripheral part of the lesions leaving the centers hairless. In four of 3 animals which were dosed 4th time, the lesions appeared to have new growth of hairs around the periphery and the central ulcerated area appeared dry and blackish with no hairs. The two animals had lesions with no change in the centre but growth of hairs noticed at the periphery. These were not again injected due to want of Ivermectin solution. For the treatment of humpsore was given successful result on the experimental group .

Clinical signs of the affected animals

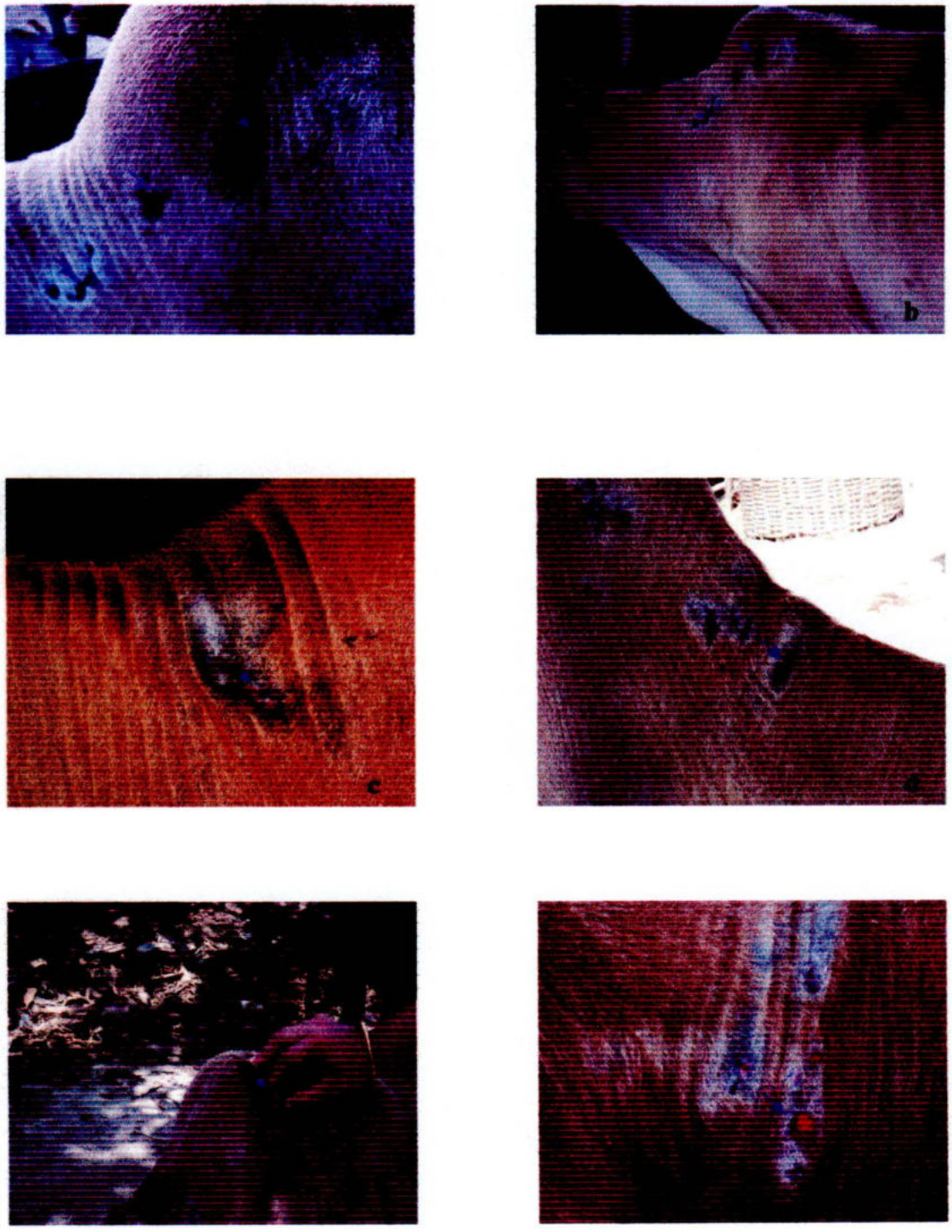


Fig. 5: (a & b) Wounds produced at different body parts including hump (arrows), (c) Humpsore with hyperkeratinization (arrow), (d) Wounds produced at different body parts including neck, (e) Humpsore with cutaneous erosion (arrow), (f) Widened area of humpsoreincluding keratinized and fresh wound areas (arrow)

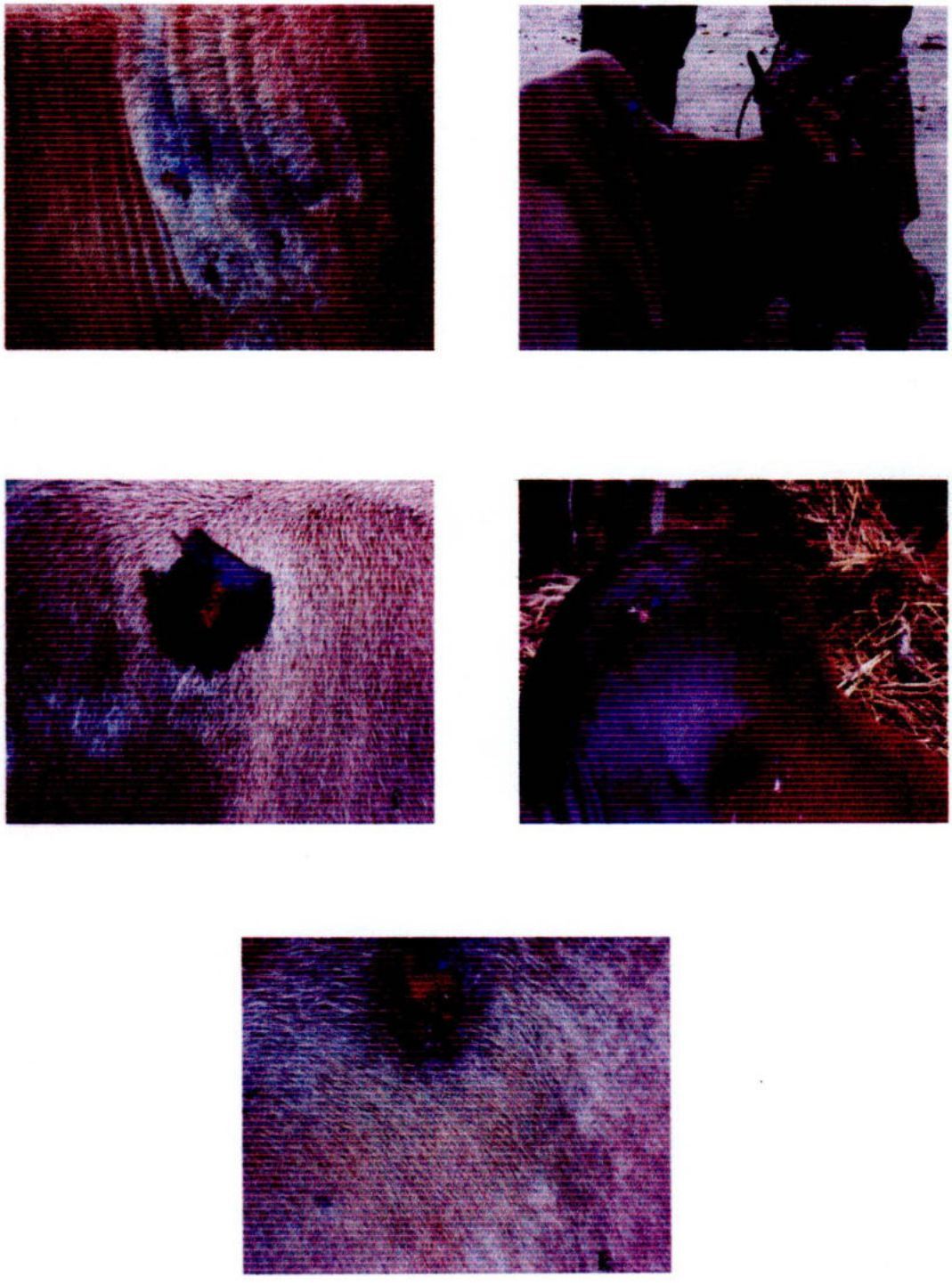


Fig. 6: (g) Widened area of hyperkeratinization (arrow), (h) Wounded area of humpsore produced in different body parts(arrow), (i) Fresh wound developed due to pruritus (arrow), (j) Humpsore with fly infestation (arrow), (k) Humpsore with cutaneous erosion produced due to intense rubbing with hard object and fly infestation

Oetiological agents

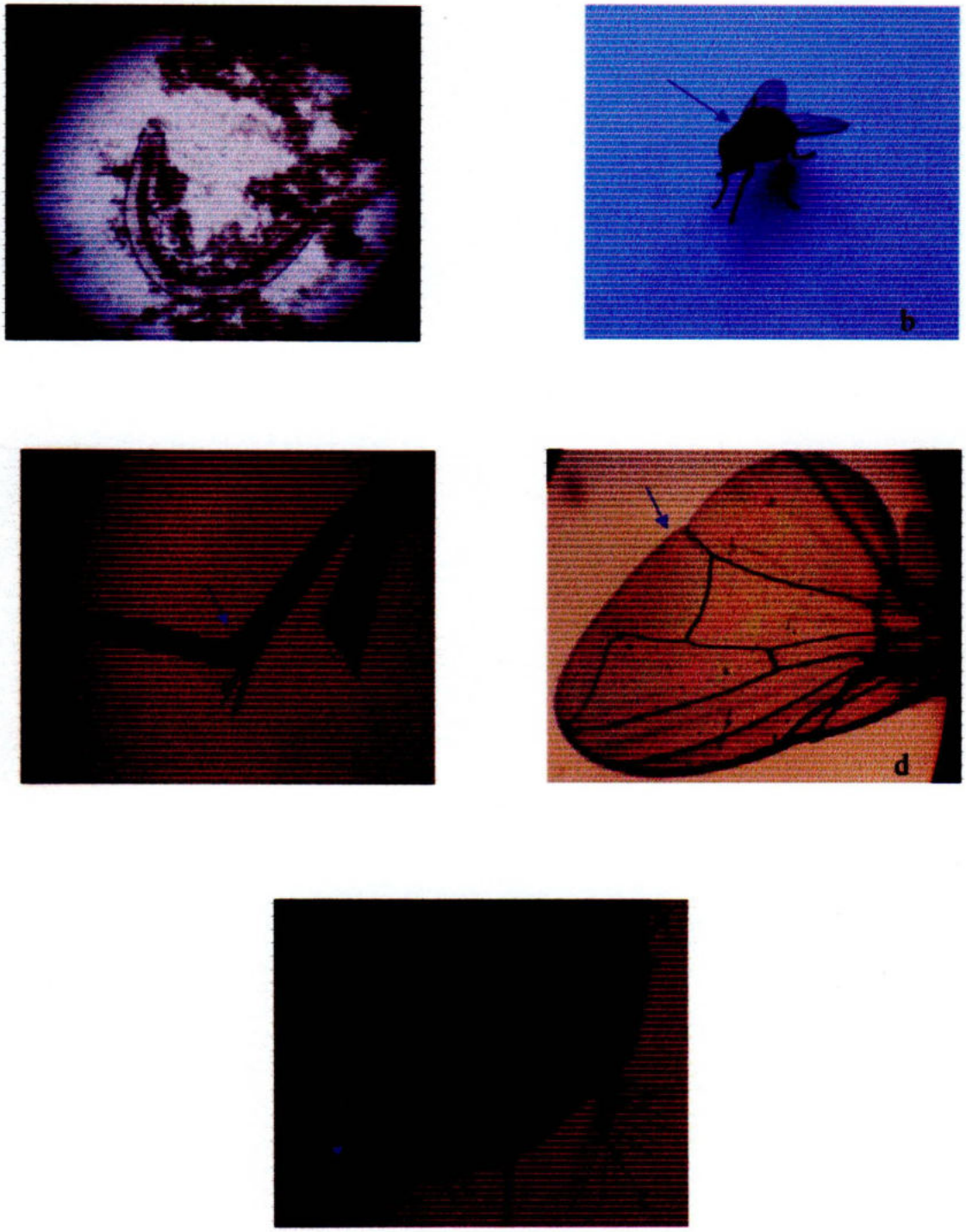


Fig. 7: (a) Microfilaria on stephanofilarial skin scraping, (b) *Musca* sp., (c) Leg-characteristics of the flies collected from the wounded area, (d) Wing- characteristics of the flies collected from the wounded area, (e) Mouthparts- characteristics of the flies collected from the wounded area

Histopathological Picture

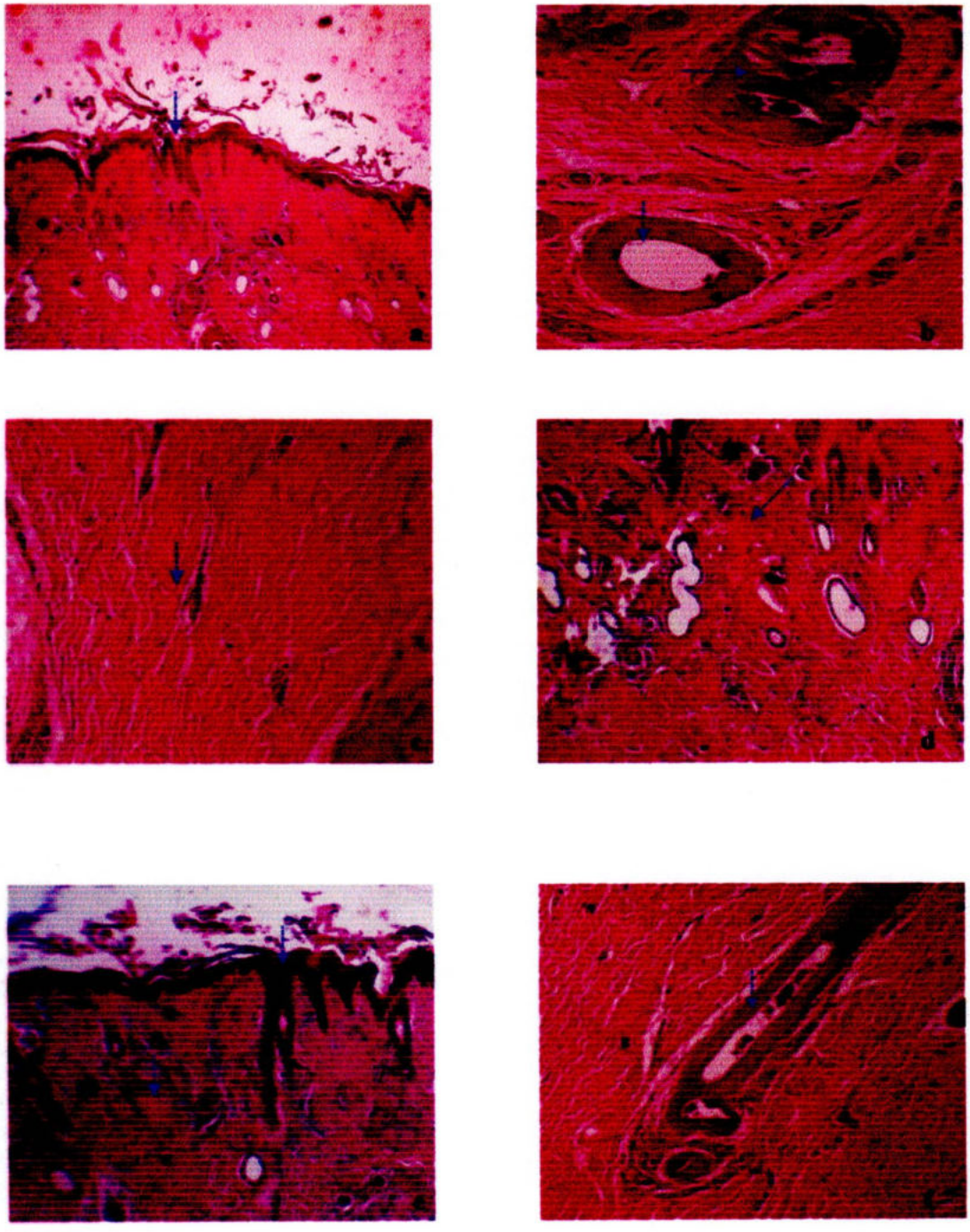


Fig. 8: (a) Hyperkeratinization with distorsion of keratinized layer (arrow), (b) Cross section of filarial worm (*Stephanofilaria* sp.) encapsulated with proliferating fibrous connective tissue and reactive cells (arrows), (c) Proliferation of loose connective tissue predominently collagenous fibers and fewer reactive cells (arrow), (d) Reactive dermis (arrow), (e) Hyperkeratinization, proliferation of glandular structures specially sebaceous gland and reactive cell infiltration (arrow), (f) Longitudinal section of parasite (*Stephanofilaria* spp.) surrounded by connective tissue fibers (arrow)

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

5.1. Clinical incidence

During the period of March-December, 2011, a total of 1427 cattle were examined and out of them 381 animals had typical lesions indicating 31.63% general incidence of the disease. This result simulate with the earlier reports of Md. Fazlul Haque (1982) who reported 31.10% incidence of the disease out of 4033 cattle in Mymensingh district. The rate of incidence of the disease in Birgonj were higher than that of other places under this experiment. This variation might be due to difference of geographical location, husbandry pattern and different examination technique.

The disease is more common in the farm than villages (Huq 1953, Rahman 1957 and Dewan 1971a). This was supported by the present observation of higher incidence in the farm (31.63%) than villages (26.00%). In farm condition particularly the Government ones close contact of the animals and negligent management of cutaneous abrasions could be the cause of higher incidence of humpsore under farm conditions of Bangladesh.

The overall incidence of humpsore recorded in this study was 21.44%. The incidence of the cutaneous stephanofilariasis recorded by other workers in Bangladesh are 20% (Huq, 1953), 25% (Rahman, 1957), 24% (Mia and Haque, 1967), 20% (Hassan, 1969) 20% and 30% (Islam), It appears that the presently recorded incidence rate might be comparable with the others although the present finding was only of the spring season of the year.

5.2. Gross lesion

In this study the characteristic gross lesions observed mostly on or around the hump. Single circular or irregular shaped lesion of varying size were found in most cases. The lesion was found to commence as one small circle which

gradually increased in size and covered with dark thick hard crust having cravices, and cracks. Slight to moderate bleeding surfaces were observed either the lesions were either picked up by crows or rubbed on rough surfaces by the animals themselves for irritation (Mia and Haque, 1967).

5.3. Distribution of lesion

It was observed the hump sore lesions mostly on or around the hump and also observed on the back and on the ventral surface of the body, anterior and posterior to the navel and on the abdomen which is agreement with the reports of Mia and Haque (1967), Bhattacharjee (1970), Dewan (1971a).

5.4. Histopathological examination

Samples were collected, preserved, processed and stained with hematoxyline and eosin stain for examination under light microscope. There was marked hyperkeratosis of stratum corneum of epidermis and discontinuity or loss of integrity of superficial layer of epidermis. There was severe dermatitis and also proliferation of fibrous connective tissue and reactive cell infiltration. These findings of this study correlate with the findings of Jones, Hunt, King (2000), their statement was hyperceratosis and parakeratosis in the epidermis, in addition to severe dermatitis, findings which were also taken by researchers as indicative of the disease. Probably, these findings are related to the death of the parasite and the consequent sensitization of the host. Presence of cross and longitudinal section of parasites.

There was an extensive proliferation of fibrous connective tissue (non neoplastic) at the reticular area of dermis. Some slides showed characteristic neoplastic cell islands. These statement correlate with the findings of Goldschimidt *et al.* (1988). Other important features were not possible to study due to limitaion of electron microscope and laboratory facilities.

5.5. Therapeutic Responses

For the study complete cure of stephanofilariasis caused by *Stephanofilaria assamensis* or clinical improvement in 18(90%) of the 20 cases by injecting ivermectin 3 to 4 injections at weekly intervals irrespective of the size and severity of the lesions. But according to Ahmed and Ali (1973) also reported clinical recovery or significant improvement of humpsore lesions by subcutaneous injection of antimosan but required longer time than our observation. Considoring the report of Dewan and Rahman (1970) that humpsore is complicated by secondary bacterial infections, the ointment we applied with a view to prevent such infections preven to improve the healing process while Ahmed and. Ali (1973) only applied 1% gentian violet solution.

Dirkman and Radermacher (1960) proposed that antimosan occasionally produced side effects such as diminution of milk yield, muscular tremors and inappetence. Similar to Ahmed and Ali (1973), no side effect in any of the cases during and after treatment with antimosan was observed in this study. Therefore, it may be considered that treatment of humpsore with antimosan together with the ointment is much better than laborious, and lengthy treatment with neguvon ointment or liniment (Patnaik 1970 Rahman and Khaleque 1974, Baki and Dewan 1975, Dewan and Baki 1976 and Fadzil, 1977).

Treatment with surgical excision has been recorded as an excellent achievement in humpsore. In this study, the percentage of recovery has been found highly significant (100%). Healing is rapid and the animals should be show-eligible in a few days but it create complication if lesions are present in different location of the body. In that case Neguvon ointment twice daily can be used and all the lesions recovered successfully with in a few days.

CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

Stephanofilariasis (Humpsore) in cattle with clinical observation was studied epidemiologically, clinically, pathologically and therapeutically. The annual incidences of bovine diseases were determined based on the types of clinical disorders, age and seasons, but stephanofilariasis in cattle was studied based on different epidemiological parameters such as season, sex, age and managemental systems. The topographic positions of the lesions, concurrent infections, histopathological examination as well as therapeutical efficiencies in relation to the stephanofilariasis were also included.

The highest prevalence of stephanofilariasis were observed in the dairy farm (31.63%) followed by 10-15 years age groups(83.00%) and male (31.57%), respectively.

The disease was clinically and pathologically characterized as dark thick hard crust having cravices and cracks with matted hairs, scab, pruritus, exudation and roughened coat with or without fly infestation. Histopathologically the diseases was characterized as hyperkeratosis,proliferation of loose connective tissue predominantly collagenous fibers and fewer in reactive cells and presence of cross and longitudinal section of parasite encapsulated with proliferating fibrous connective tissue. Thetherapeutical efficiencies of ivermectin and neguvon ointment used in the present study were found positive in all the treated animals.

The following conclusion was drawn based on the facts and findings studied throughout the course of the study.

- Stephanofilariasis was more prevalent in dairy farm cattle than the rural house hold cattle of Birgonj Upazilla.
- The comparatively more prevalence of stephanofilariasis were found in dairy farms, in adult and in male animals respectively.

- Hyperkeratinzation , distorsion of keratinized layer and proliferation of fibrous connective tissue were the general histopathological features
- Flyinfestations appeared as a common findings in the stephanofilariasis affected animals
- Ivermectin, and combined preparation of Neguvon-Sulphanilamide ointment (20%Neguvon,5% Sulphanilamide and 75% Vaseline) were the drugs of choice for the clinical improvement
- Antimosan could be used for the treatment of stephanofilariasis affected patients.

REFERENCES

REFERENCES

Agrawal, M.C. and Dutt, S.S. (1978) A note on the prevalence of *Stephanofilaria, zaheeri* infection in buffaloes of different age groups. Ind.J.Anim.Sci., 48(3),232-234. [Ahmed, K.A. (1982) Principal, Institute of Leather Technology, Hazaribagh, Dhaka-9, Personal communication.]

Agrawal, M.C., Vegad, J.L. and Dutt, S.C. (1978) Pathology of naturally occurring *Stephanofilaria zaheeri* infection in buffaloes. Ind. J. Anim. Sci., 48(4), 261-265.

Ahmed, S. and Ali, M.I. (1973) Treatment of humpsore with antimosan solution. Vet. Med. Rev., No.2, 136-142(Helm. Abst. (1975) 44(8), 3595.

Ali, A. (1973) Theory of Statistics. (First Edition) Dhaka Book Mart, 38, Banlabazar, Dhaka- 1, Pp. 350-351.

Anon, (1956) Animal Reservoir of Ring worm infection in Briain. Medical Research. Council. Vet. iR. ec. 68(23), 357.

Azimov, D.A., Dadaev, S., Sultanov, M.A. and Tukhmanyants, A.A. (1976) Some features of the ecology and biology of *Stephanofilaria stilesi* (Chitwood, 1934) in Southern Kazakhstan Uzbekiston SSR Fanla Akadeniyarining Dokladlari, No.8,53-54 (Helm. Abst. (1998) 47(7), 3098).

Baki, M. A. and Dewan, M.L. (1975) Evaluation of treatment of *Stephanofilariasis* (I-Humpsore) with Neguvon (Bayer) on (Bayer by clinicopathological studies. Bang.Vet. Jour., 9(1-4), 1-6.

Baki, M. A. and Hossain, M.I.(1982a) Field trials with Neguvon (Bayer) against humpsore in zebu cattle. Bang. J.Biol.Sci. (Submitted for publication).

Baki, M.A. and Hossain, M.I. (1982b) Preliminary studies on the cause of recurrence of humpsore (stephanofilariasis) in zebu cattle. Bang. J. Agril.-. Sci. (Submitted for publication).

Bhattacharjee, M. L. and Dass, D. D. (1967) "The study of ear-sore of buffaloes due to stephanofilariasis in Assam." Ind. Vet. J., 44(5), 400-402.

Bhattacharjee, M.L. (1967) Stephanofilarial dermatitis in Indian elephant.Curr. Sci., 36(21), 584-585 (Helm. Abst.(1968) -37(2), 915)

Bhattacharjee, M.L. (1970) A note of stephanofilarial dermatitis among elephants in Assam. Science and culture, 36(11): 600-601 (Helm. Abst. (1975) 44(2), 439).

Bhopale, K.K., Joshi, S.C. and Kamalapur, S.K. (1970) Occurrence of *Stephanofilaria zaheeri* in buffaloes of Madhya Pradesh. Orissa Vet. J., 5(2/3), 19-21 (Helra.Abst. (1973) 42(10), 3608).

Blood, D.C., Handerson, J.A. and Radostits, O.M. (1979) Veterinary Medicine, 5thEdn. Bailliere, Tindall and Cassell Ltd., London,Pp. 798-799.

Bonga, B. (1935) Extraction uit de maand-en jaarverslaggen der Gouvernements en provincials veeartsen.No.74 Eeneige. naardige worm van dermatitis verminosa bij buffalo(stephanofilariasis) Nederlandsch-Indische Bladen Voar Diertjeneerski undo on Dierenteett, 47(2), 107-108 (T, 4elm.Abst. (1935) 4(3), 272a).

Bubberman, C. and Kraneveld, F.C. (1933) Over een dermatitis squamosa crustosa et circumscripta bij het rund in Nederlandsch Indie, Genaand cascado. Indische Bladen Voor Diergeneenkinde, 45(4), 239-278 (Helm. Abst. (1933-34) 2(4), 272a).

Buckley, J.J.C. (1937) On a new species of stephanofilaria causing lesions in the legs of cattle in the Malay Peninsula, J. Helminth, 15(4), 233-242.

Chitwood, B.G. (1934) A nematode, *Stephanofilaria stilesi* new species from the skin of cattle in the united states. North Am. Vet., 15(6), 25-27 (Helm. Abst. (1934-35) 3(2), 86b).

Dadaev,S.(1978) The prevalence of the nematode *Stephanofilaria assamensis* Pande, 1936 in cattle in Uzbekistan. Uzbekistan SSR Fanlar, Akademiyasining, Dokladlari, No-7, 70-71. (Helm.Abst.1 (1979) 48(7), 3191).

Das, K.M. (1955) Occurrence of microfilariae of *Stephanofilaria assamensis* in the peripheral bloodof cattle. Ind.Vet. Jour., 32(2), 136-137.

Das, P.K. Tripathy, S.B. and Mishra, S.K. (1977) Study on the efficacy of some organophosphorus compounds in the treatment of stephanofilarial dermatitis in cattle.Orissa Vet. J., 11(3), 110-114(Helm.Abst.0978) 47(6), 2621).

Daulatram, J. (1950) Animal Husbandry Research Ann. Re-p. Ind. Coun. Agril.-Res., (1948-1949), 33-59.

Dewan, M.L. (19'71b) Experimental development of stephanofilariasis in zebu cattle. In materialy nauchnoi konferentsii povoprosam, veterinarii.Part I. Moscow, USSR, Veterinary Acad.USSR, 83-84 (Helm. Abst. (1973) 42(7), 2681).

Dewan, M.L. (1971c) Clinico-pathological characteristics and pathogenesis of stephanofilariasis in zebu cattle in East Pakistan. Ph.D. Thesis, Moscow Veterinary Academy, Moscow, USSR.

Dewan, M.L. (1975) Histopathology of stephanofilariasis (Humpsore) at different stages of its development. Bang.J. Agril. Sci., 2(1), 49-56.

Dewan, M.L. and Baki, M.A. (1976) Use of Neguvon (Bayer) for treatment and eradication of stephanofilariasis (Humpsore) in zebu cattle. Bang. Vet. Jour., 10(1-4), 47-50.

Dewan, M.L. and Rahman, M.M. (1970) Isolation of microorganisms from stephanofilariasis (humpsore) and their roles in the initiation of the disease. Bang. Vet. Jour., 4(1/4), 25-30.

Dewanpivi, L. (1971a) Clinical features of stephanofilariasis in zebu cattle in East Pakistan Veterinary, Moscow, No.2 PP-111-115.

Dey, A.S. (1982) Epidemiological and clinical study of ringworm in cattle M.Sc. (Vet.Sc.) Thesis, BAU, Mymensingh.

Dikmans, G. (1934a) Observation on stephanofilariasis in cattle. Proc. of the Helm.Soc. Washington, 1(2), 42-43 (Helm.Abst. (1934-35) 3(5), 379f).

Dikmans, G.(1934b) Nematodes as the cause of a recently discovered skin disease of cattle in -the United States. North Am. Vet., 15(6), 22-25 (Helm. Abst. 1934-35" 3(2), 86a).

Dirkman, G. and Radermacher, F. (1960a) Erste Ergebnisse der Allgemein behandlung der stephanofilariose ("Summer Wounden") des Rindes mit Antimosan and Neguvon. Deutsche Tierarztliche Wochenschrift, 67(3), 70-72) (Helm. Abst. (1961) 30(4), 2189).

Dirkman, G. and Radermacher, F. (1960b) First results of general treatment of stephanofilariasis (summer lesions) in cattle with Antimosan and Neguvon.Vet. Med. Nachr., No.2.

Dirkmans, G. and Radermacher, F.(1960c) Further experience in general treatment of stephanofilariasis (summer sore) in cattle with Antimosan and Neguvon.Vet.Med.1qachr.No-4.

Djaenoedin, R. and Adiwinata, T.R. (1953) Stephanofilariasis IX. *Stephanofilaria* sp. pada kulit kerbau.Hemera 30 a.Buitenzory, 60(7/8), 203-215 (Helm.Abst. (1953), 22(4), 34.8a).

Dutt, S.C. (1971) Preliminary studies on the life history of *Stephanofilaria zaheeri*. Ind. J. Helmth., 22(2),139-143.

Dutta, P.A. and Hazarika, R.N.(1976). Chemotherapy of stephanofilarial dermatitis, in bovines. Ind. Vet.J., 53(3),221-224.

Fadzil, M. (1973) *Musca conducens*, Walker, 1859- Its prevalence and potential as a. vector of *Stephanofilaria kaeli* in peninsular Malaysia.Kajian Veterinarinaire, Malaysia-Singapore 5(2), 27-37.

Fadzil, M. (1974) Studies on the epidemiology of stephanofilariasis in West Malaysia. M.Sc. Thesis, University of Science, Malaysia.

Fadzil, M. (1975) The development of *Stephanofilaria kaeli*,Buckley, 1937 in *Musca condi-Icens* Walker, 1859. Kajian veterinar, 7(1), 1-7.

Fadzil, M.(1977) *Stephanofilaria kaeli* infection in cattle in Peninsular Malaysia-Prevalence and treatment.Vet.Med.Rev.No.1, PP. 44-52(Helm.Abst. (1978) 47(6), 2334).

Fadzil, M., Cheah, T.S. and Subramaniam P.(1973) *Stephanofilaria kaeli* Buckley, 1937 as the cause of chronic dermatitis in the foot of a goat and on the ears and teats of cattle in West Malaysia.Vet.Rec., 92(12), 316-318.

Fouad, K., Saleh, D.I., Khaniis, Y., Sl-iournan, T. and Fahmy, L. (1974) Further investigations on the so-called oedematous skin disease of buffaloes and cattle. J.Egyptian Vet. Med. Assoc., 34(3/4) Special issue, 154-171 (Helm.Abst.'1976) 45(3), 1041).

Gnedina, M.P. (1950) The occurrence of *Stephanofilaria stilesi* in cattle.

Skryabina, 4, 82-84 (Helm.Abst. (1959) 27(3/6), 1462r).

Gopalakrishnan, V.R. (1949) Stephanofilariasis among buffaloes in 227 Assam. Ind. Vet. Sci. and A.H. 18(4), 227-231.

Haq, S., Rahman, M.H. and Dewan, M.1. (1977) A study on the incidence of Onchocercal parasites in cattle in Bangladesh. Bang. Vet. Jour., 11(1-4), 63-65.

Hasan, H.K.D. (1964) "On behanding of nengorm of stephanofilariose has kvaeg mud anychophylex beo" Medlemslad for den Danske Dyrlaege foreigning, 47(9), 103-406.

Hassan, Z. (1969) Studies on treatment and transmission of humpsore. Pakistan J. Sci., 21, 160-166.

Hassan, Z. (1970) A brief note on parasitic diseases of domestic livestock in East Pakistan. Pak. J. Sci., 22, 111.

Hassan, Z. and Huq., M.M.(1955) Preliminary observation on the treatment of humpsore. Proc. 8th Pakistan Science Conference, Part III, Pp. 21 (1954-1955).

Hibler, C.P. (1966) Development of *Stephanofilaria stilesi* in the hornfly. J. Parasit., 52(5), 890-898.

Hire, gandar, U.S. and Chatupale, W.V.(1965) Incidence of *Stephanofilaria assamensis* Pande, 1936, in Gujrat state. Ind. Vet.J., 42(10), 808-809.

Holz, J. and Adiwinata, R.T.(1957) Investigation on the treatment of stephanofilariasis in cattle with antimosan. Vet.Med.Rev. Nachr., No.l.

Huq, M.M., Hussain,Z. and Ansari,M.Y. (1957) Humpsore in cattle. Proc. of the 9th Pakistan Science Conference. Part III Pp.131-133 (Helm.Abst.(1960). 29(2), 552).

Huq, M.M.(1953) Humpsore of cattle in East Bengal.Proc.of the 5th Pakistan Science Conference, Pp. 180(1953).

Ihle, J.E.W., Ihle-Landenberg and M',E1-(1933) Over een dermatitis squamosa crustosa circumscripta bij het rand in Nederlandsch-Indie,genaarnd Cascado.II. *Stephanofilaria dedoesi* (N.gen. N.sp.), een nematode uit de huid van voor.Diergeneeskunde, 45(4), 279-283 (Helm.Abst.(1933-34). 2(4), 272b).

Islam, A.W.M.S. (1971a) Stephanofilariasis in zebu cattle in Bang-ladesh.Veterinariya. Akademiya, 124-125(Helm. Abst. (1973) 42(5), 1808).

Islam, A.W.M.S. (1971d) Biology of *Stephanofilaria assamensis* and epizootiology of stephanofilariasis in cattle of Bangladesh. Veterinariya, 3, 112.

Islam, A.W.M.S. (1977) Stephanofilariasis (Humpsore) in cattle of Bangladesh. Incidence of humpsore in different districts and geographical areas of Bangladesh. B,.1ng.J.Agril.Sci., 4(2), 127-128.

Islam, A.W.M.S.(1971c) Biology of *Stephanofilaria assamensis* and the epizootiology of infection in zebu cattle.Veterinariya, Moscow, 115,112-113.

Islam, M.S. (1971b) Study of the life-cycle of *Stephanofilaria assamensis* in conditions of Bangladesh. Veterinariya Akademiya, 122-124 (Helm. Abst. (1973) 42(7), 2932).

Ivashkin, V.M., Khromova, L.A. and Shmitova, G.Y. (1963) Stephanofilariasis in cattle, Veterinarian, 40(8), 36-39 (Jelm. Abst. (1964) 33(3), 2217).

Ivashkin, V.M., Maklakova,L.P. and Golovanov,V.I.(1969).Stephanofilariasis of the skin of the inner surface of the ear in cattle. Trudy nauch, Konf.Parazit.

UKSSR (6th), Part II, Pp. 23-24. (Helm.Abst.(1970) 39(4), 3723).

Ivashkin, V.M., Shmitova, G.Y.A. and Koishibaev,G.K.(1971).Stephanofilariasis of herbivorous animals. Veterinarian, Mosk. 48 (3)9 66-68(i-ielm. Abst. (1971) 40(4), 44/1-9).

Ivashkin, V.M., Timofeeva, T.N. and Khromova, L.A. (1961) Stephanofilariasis in cattle. Trudi GelmintoloSiches Koi laboratorie. Akademiya Nauk, SSII, 11, 109-114 (Helni.Abst. (1964) 33(2), 713).

Khaleque, M.A.(1982) Epidemiological, clinical and therapeutic studies of bovine dermatophilosis. M.Sc.(Vet.Sc.) Thesis, BAD, Mymensingh.

Kono, I and Fukuyoshi,S.(1967) Leucoderma of the muzzle of cattle induced by a new species of *steohanofilaria*. Jap. J. Vet. Sci., 29(6), 301-313 (Helm. Abst. (1971) 40(2), 2946).

Kono, I. (1965) Leucoderma of the muzzle in cattle induced by a new species of stephanofilaria. Jap.J.-Vet.Sci. 27(l),33-39.

Kraneveld, F.C. (1955b)f Stephanofilariasis VII.Een door di *Stephanoilaria dedoesi* veroor zaakte ooraandoening bijbuffels. (Voorloopige mededeelin, Nederlandsch-indisch; b Bladen Voor Diergeneeskunde en Dierenteelt, 47(6), 310-316.(Helm. Abst. (1935) 4(5), 595a).

Kraneveld, F.C. and Djaenoedin,R. (1937) Stephanofilariasis VIII. orienteerent onderzock betreffende de therapic van het bijden. Nederlandsch-Indische Bladen Voor Dierdeneeskude in Dierenteelt, 49(5/6), 332-348(Helm. Abst. (1937) 6(5), 576a.

Kraneveld, F.C.(1935a) Stephanofilariasis VI. Enkele bijzonderheden betreffende het bijden. Nederlandsch-Indische Bladen Voor.Diergeneeskunde en Dierent eelt, 47(3/4), 183-186 (1-lelm. Abst. (1934) 4-(3), 272c).

Lahman, H.H. (1057) Observations of the mode of infection of the East hump of cattle by *Stephanofilaria assamensis* in Jast Pakistan.J.Parasit., 43(41), 434-435.

Levine, N.D. and Morrill,C.C.(1955) Bovine stephanofilarial dermatitis in Ittinois. J.Am. Vet. Iled. Assoc., 127(945)?528-530.

Loke, Y.W. and Ramachandran, C.P. (1967) The pathology of lesions in cattle caused by *Stephanofilaria kaeli* ; Buckley,1937- 2 J.Helminth, 41(2/3) 161-166.

Maddy, K.T.(1955) Stephanofilarial dermatis of cattle. North Am.Vet., 36(4), 275-278. (Helm. Abst. (1955) 24(2), 236d).

Malviya, H.C.0972) Stephanofilarial infection in cattle and buffalo in Andaman Islands. Indian journal of Helminth., (Publ.1974)24(2)t 68-71(Helm. Abst.(1975) 44(6), 2237).

Mannan, M.A., Mia, M. Khan,M.-tl.B. and Talukdar, A.111.0977) Histological investigation of the normal and pathological(humpsore) skin of the hump of cattle. Bang.Vet.Jour.11(1i/4), 49-53.

Rahman H. M., Ahmed, S and Mondal, M. N. H (1996) Introduction to Helminth Parasites of Anilams and Birds in Bangladesh, 1st edi. November. Published by Tahsina Mostafa.

Menon, T.C.(1952) Contagious otorrhoea, in cattle.Ind.Vet.J.,29(1)'s 75-78 "Helm.Abst.(1952) 21(2) 92c.).

Mia,A.S.and Haque,A.(1967) Skin diseases in cattle.Pak.J.Vet.Sc. 1(3), 76-83.

Mishra, S.P. (1969) Treatment of earsore in buffaloses. Ind.vet. J., 46(7), 632.

Mohan,R.D.,"1945) Humpsore and its treatment. Ind Farming, 6(2), 55-56 (Helm.Abst. (1945) 14(2 & 3), 1o6a).

Mohler, J.R.(1935) Zoological Division. Report of the Chief of the Bureau of Animal Industry, United States Department of Agriculture, (1935) Pp. 48-55(Helm.Abst.(1935) 4(4), 435a).

Muchlis, A. and Soetijono,P. (1973) A short report on the use of Asuntol ointment in the treatment of Cascado and hoof myiasis. Vet.Med.Rev., lqo.2, 134-135.

Nooruddin, M.(1982).Department of Veterinary Medicine & Surgery, B.A.U. Mymnensingh, Personal communication.

Odaye,0.0. (1971) Stephanofilarial dermatitis of cattle in Nigeria.J.Comp.Path.,81 (4), 581-583.

Pal, A.K. and Sinha, P.K. (1971) S*tephanfila assamu* as the cause of common chronic ulcerated growth at the base of the dewclaws in cattle in West Bengal.Ind.Vet.J.,48(2), 190-193

Pande, P.G.(1936). On the identity of the nematode worm recovered from humpsore of cattle in India. Ind.J.Vet.Sci. & A.H., 6(4), 34-6-351.

Pannu,H. (1958) Humpsore in Kutch.Ind.Vet.J., 35, 172-173.

Patnaik, B. and Khan, M.H. (1980) Chemotherapeutic trials against the *stephanofilarial otitis* externa in buffaloes. Ind. Vet. J., 57(5), 368-372.

Patnaik, B. and Kumar, V.(1972) A research note on the occurrence of juveniles of Stephanofilaria,, the causative parasites of ear-sore in buffaloes, in *Musca autumnalis* de Green, 1967. Ind.J. Anim. Sci., 42(5), 351-352.

Patnaik, B. and Roy, S.P. (1966) On the life cycle of the filaroid, *Stephanofilaria assamensis*, Pande (1936), in the arthropod Vector Musca conducens, Walker, 1959, Ind.Jour.Anim.Hlth. 5(2), 91-101.

Patnaik, B. and Roy, S.P. (1968) Studies on stephanofilariasis in Orissa. II. Dermatitis due to *Stephanofilaria assamensis* Pande, 1936, in the Murrah Buffalo. (Los bubalis) with remarks on the morphology of the parasite. Ind.J.Vet.Sci., 38(3), 455-462.

Patnaik,B.(1970) Studies on stephanofilariasis in Orissa.V.Treatment and control of humpsore in cattle due to *Stephanofilaria assamensis*. Ind.J.Anim.Sci.,40(2),167-175.

Patton, W.S. (1933) Studies on the higher diptera of Medical and Veterinary - importance. The genus *Musca linnaeus*. Ann. Trop. Med.Parasit., 27, 135.

Rahman, A. and Khaleque, A. (1974) Treatment of humpsore with Neguvon in local cattle of Bangladesh. Vet. Med. Rev., No-4y379-362.

Ramachandran, C.P., Loke, Y.W. and liagendram,C.(1966) Studies on *Stephanofilaria ka*eli in cattle. Med.J. Malaya, 20W1, 344-347.

Rao, B.V. Reddy, P.R., Reddy,K.S. and Patnaik,D.(1979) On the occurrence of *Stephanofilaria assamensis* var. *bubalensis* in Cuddapah district, Andhra Pradesh.Ind.Vet.J., 56(3),250-251.

Round, (1964) A new species of Stephanofilaria in skin lesion from the black rhinoceros (*Diceros bicornis L.*) in Kenya. J. of Helminth, 39(1/2), 887-96.

Varma, A.K. (1956) Some photomicrographs depicting the pathology of filarial humpsore in cattle. Ceylon Vet. J., 4(1), 11-13.

Varna, A.K. (1953) (1) occurrence of *Elaeophora poeli* in Indian buffaloes, (2) Onchocercosis of the aorta of cattle in India, (3) Stephanofilariasis in cattle in India. Trans. Royal Soc. Trop. Med. Hyg. 47(1), 11 Eelm. Abst. (1953) 22(1) 54f).

Weber, A. (1978) Dermatophilosis in animals and man. Berliner and Munchener Tierarzliche, Wochenschrrift, 91(17), 341-345 (Vet. Bull. (1980) 49(2), 749).