STUDIES ON STEPHANOFILARIASIS IN QUEENSLAND

Thesis submitted by
Stephen James Johnson BVSc BSc

for the degree of Doctor of Philosophy in the Department of Tropical Veterinary Science, Faculty of Science at James Cook University.

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PREFACE

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ABSTRACT

Stephanofilariasis is an infection of the skin of cattle and less commonly other ruminants, with small filarial nematodes of the genus *Stephanofilaria*. This disease was discovered in Queensland and Australia for the first time in 1980 and this thesis outlines the results of the subsequent investigation of determinants of the disease, its pathogenesis and control, and the life cycle and morphology of the parasite which induces disease in Queensland.

The disease was found in cattle and was manifest as circumscribed hairless lesions in the skin. The surface of the lesions was usually slightly raised, dry and hyperkeratotic with small areas of dried blood. Less commonly, the lesions were cracked and scab encrusted.

Lesions were restricted to four sites: the medial canthus of the eye, where all infected animals had lesions; the neck, where older animals had lesions (41% of steers, 35% of cows and 4% of bulls); the sternum, (18% of bulls, 0.6% of steers and 1% of cows); and head, where 0.6 to 1% of animals were infected independently of sex, breed or age. Whether the location of lesions reflected preferred vector biting sites or a greater physiological receptivity of infected skin to infection than skin in other locations was not determined. In either case, the location of the lesion on the host animal is unlikely to be a useful character for determining the species of *Stephanofilaria* found in the present study.

Adult parasites were found either in small groups in cysts formed from the remnants of hair follicles or singly in the epidermis and superficial dermis. Microfilariae were found in the superficial areas of the dermal papillae adjacent to

the epidermis. The presence of adult parasites elicited a severe local inflammatory reaction comprising lymphocytes, histiocytes and eosinophils and resulted in the destruction of hair follicles and associated sebaceous glands.

Alopecia is a constant feature of stephanofilarial lesions yet its pathogenesis has not been determined. In histological sections, most adults were entwined in cysts formed in the base of hair follicles but adults of both sexes were found in the epidermis and superficial dermis.

Microfilariae recovered from the skin of cattle in this study were confined within semi rigid vitelline membranes. Small spherical bodies, probably yolk remnants, were also confined within the membranes. Live microfilariae were actively motile within the membrane but they were unable to effect any progressive movement of the entire structure when suspended in saline. Furthermore, no microfilariae were recovered in the saline recovery technique. These findings strongly suggest that the microfilariae of this *Stephanofilaria* sp. are incapable of independent migration. Therefore, adult females would have to migrate through the superficial dermis and epidermis to discharge microfilariae in areas accessible to potential vectors. The males found in this situation may have been migrating in search of females. If these adults and maturing larvae re-entered other hair follicles then the repeated invasion of hair follicles and destruction of the germinal matrix cells would cause the local alopecia which is characteristic of stephanofilarial lesions.

The histopathology of the lesions examined in this study showed differences from those of lesions produced by fly feeding alone and also to those described for lesions induced by

hypersensitivity to other biting flies. This, and the successful transmission of the parasite to produce a characteristic lesion strengthens the conclusion that lesions previously called "buffalo fly lesions" are stephanofilarial dermatitis. The following determinants of stephanofilariasis were observed in abattoir and field studies.

(a) Sex: Prevalence of lesions was highest in bulls, intermediate in steers and lowest in cows for all breeds and in all areas.

(b) Age: Prevalence of lesions increased with increasing age, independently of sex or breed. Animals as young as 37 days of age were found infected.

(c) Breed: Prevalence of lesions was lower in *Bos indicus* animals than in *B. taurus* animals. Within *B. indicus* genotypes, animals with 75% Brahman content or >7/8 Sahiwal content were significantly more resistant to development of lesions than animals with lower Brahman or Sahiwal content.

(d) Season: In some areas, the prevalence of lesions was higher in winter (June-August) than at other times.

(e) Coat colour: In some years prevalence of lesions was lower in animals with lighter coat colour.

(f) Distance from the coast: Prevalence of the disease tended to decrease with increasing distance from the coast. Prevalence ranged from 95% on Cape York Peninsula to less than 5% in southern Queensland where the occurrence was sporadic.

(g) Range of *Haematobia irritans exigua*: The occurrence of stephanofilariasis corresponded with the geographical range of *H. i. exigua*.

It was concluded that *H. i. exigua* is the vector of stephanofilariasis in Queensland for the following reasons;

(a) It was shown to be capable of ingesting microfilariae, supporting development of the larvae and transmitting infective third stage larvae to cattle.

(b) Females taken from infected cattle were found to contain developing larvae of the parasite

(c) It is the only haematophagus fly with a distribution matching that of the parasite.

Stephanofilarial larval development occurred in the abdominal haemocoel of the fly but a few larvae were found enclosed within membranes attached to the fat body of the vector. Most infected flies (91%) contained only a single developing larva of *Stephanofilaria* sp. and the maximum number of larvae found in a single fly was four.

Adults and microfilariae of the parasite were found in a previously uninfected animal 35 days after wild-caught flies were first allowed to feed on that animal and 19 days after the last fly died. This indicates a prepatent period of less than 35 days for this parasite. Developing larvae were found within lesions suggesting that the larvae of this *Stephanofilaria* sp. may not undergo an extensive or prolonged somatic migration.

The adults of the Australian species of *Stephanofilaria* are small. The males are 2.3-3.2(2.8)mm long and 50-80(68) microns wide and the females are 3.8-6.4(5.2)mm long and 62-93(81) microns wide.

The mouth is surrounded by 15-16 peribuccal spines, the cuticular striations lack a posterior frill, and the males have a spicule ratio of 6.8:1.

The Australian species thus differs from all other species in the genus in lacking cephalic spines and therefore probably represents a hitherto undescribed species.

Prolonged treatment with avermectin caused a significant regression in lesion size and lower prevalence of lesions in treated animals than in control animals. Similar treatment with closantel was ineffective.

A single dose of either avermectin, levamisole or morantel was ineffective against adult *Stephanofilaria* sp. whereas a single dose of oxfendazole appeared to have limited efficacy.

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1000 GENERAL INTRODUCTION

The common alopecic lesions around the eyes and on the necks of cattle in northern Australia have traditionally been ascribed to the feeding activities of the buffalo fly, *Haematobia irritans exigua* (see Roberts, 1941). Furthermore, the presence of numerous or extensive lesions on cattle has been viewed as evidence of susceptibility of the affected cattle to buffalo flies (Eastaway, 1974). However, the prevalence of these lesions in cattle appeared to vary with the breed and sex of the animal and was not always correlated with buffalo fly numbers (Eastaway, 1974; Holroyd et al, 1984).

Initial histopathological examinations to define the aetiology and pathogenesis of the lesions revealed that many lesions contained small filarial nematodes of the genus *Stephanofilaria.* These parasites were previously unknown in Australia but were recognised as a cause of skin lesions on cattle and other ruminants in other countries (Bubberman and Kraneveld, 1933a; Dikmans, 1934a; Pande, 1935; Buckley, 1937; Gnedina, 1950; Singh, 1958; Dirksen, 1959; Round, 1964; Kono, 1965; Oduye, 1971). This parasitic disease therefore became a focus of attention in the search for an understanding of buffalo fly lesions on cattle in northern Australia. This interest was given added impetus when the subsequent revelation of the presence of the disease in Australia caused a temporary embargo on live cattle imports from Australia to several Asian countries.

Interestingly, the horn fly, *H.i.irritans*, long considered the same species as the buffalo fly, was known as a vector of *Stephanofilaria stilesi* in north America (Hibler, 1966) and Russia (Ivashkin et al, 1971) but was not known to cause discrete feeding lesions as the buffalo fly had been presumed to do in Australia.

These discoveries therefore added a new dimension to the original enigma of lesions on cattle and their relationship to the buffalo fly. Hence an investigation of the distribution, prevalence, pathology, epidemiology, life cycle, taxonomy, treatment and control of stephanofilariasis in Queensland was undertaken. This thesis reports the results of those investigations.

2000 REVIEW OF THE LITERATURE

2100 TAXONOMY

Ihle and Ihle-Landenberg (1933) erected the genus *Stephanofilaria* to contain a single species *dedoesi* which had been recovered from lesions in the skin of cattle in Indonesia. The authors listed the generic characters as; a mouth with cuticular rim carrying very delicate teeth; a cuticular convex wall with a crown of cuticular spines close to the mouth rim; female with anterior vulva, a short vagina with two posteriorly directed uteri, ovoviviparous and without an anus; and male with unequal spicules.

Several species have subsequently been described in the genus based on various morphological and biological characters. Chitwood (1934) described *S. stilesi* from north America on the basis of asymmetry of cephalic armature, size of left spicule of the male and distance of vulva from anterior end of the female.

Pande (1936) described *S. assamensis* from India on the basis of size, length of left spicule of the male and distance of vulva behind the anterior end of the female. Buckley (1937) described *S. kaeli* from Malaysia although admitting that the new species may have been synonymous with *S. dedoesi*. He separated *S. kaeli* on the basis of the number of preanal papillae in the male and location of the lesion on the host animal. Singh (1958) described *S. zaheeri* from specimens recovered from lesions on the ears of buffaloes in India. Although closely resembling

S. assamensis it was differentiated on the basis of size of female, presence of an anus and length of left spicule and number of caudal papillae in the male.

Ueno and Chibana (1977) described *S. okinawaensis* from lesions on the muzzle and teats of cows in Japan on the basis of cephalic armature, length of spicules, size of microfilaria, and site of lesion in host animal. Ivashkin et al (1971) erroneously referred to this species as *S. roni*. *S. dinniki* Round, 1964 was the first species described from a non domestic animal. The species was recovered from skin lesions in the black rhinoceros in Africa and was readily differentiated from the other members of the genus on the basis of curved tail in both sexes, distance of vulva from anterior end of female and length of male spicules.

Two additional species names, *S. andamani* (see Sinha and Das, 1958) and *S. srivastavai* (see Bhattacharjee, 1967) were proposed for specimens recovered from buffaloes in the Andaman Islands and from elephants in India respectively. No taxonomic descriptions have been published in support of either name, and no type specimens designated. They are therefore *nomina nuda*.

Many of the original descriptions were based on small series of parasites and minor differences in one or two morphological measurements were considered adequate grounds for erection of a new species. In several of the species, many more specimens have subsequently been recovered from different hosts, different lesion sites or from different geographical areas. Examination and measurement of these additional specimens has provided more taxonomic data for comparison of species within the genus.

Little work has been done on *S. dedoesi* and no further descriptions have been published. The specimens recovered from buffaloes in the eastern Indonesian islands (Djaenoedin and Adiwinata, 1953) were not considered to be *S. dedoesi* but a species resembling *S. stilesi*.

The subsequent identification of *S. stilesi* in Russia and the examination of numerous specimens (Gnedina, 1950; Ivashkin et al, 1961) has confirmed the status of *S. stilesi* as a separate species.

Several authors (Rahman, 1957; Patnaik, 1964; Hiregaudar and Chatupale, 1965; Patnaik and Roy, 1968; Mohan, 1973; Chatterjee, 1984) have examined numerous *S. assamensis* recovered from lesions in various sites in cattle and from lesions in other host species. The measurements obtained were within the range of measurements for several other species in the genus. The recovery and examination of further specimens from lesions in buffaloes in close contact with infected cattle in India (Patnaik, 1964; Patnaik and Roy, 1968; Malviya, 1972; Agrawal and Dutt, 1980) has cast doubt on the status of *S. zaheeri*. Patnaik and Roy (1968) have suggested synonymy of *S. zaheeri* with *S. assamensis*. The identification by Rao et al (1979) of a single adult recovered from an ear lesion of a buffalo as *S. assamensis var bubalensis* was not supported on biological or morphological grounds and only added further confusion to the taxonomy of this group. The only difference between the two species appears to be in the number of peribuccal and cephalic spines; both being more numerous in *S. zaheeri* than *S. assamensis*, but this feature has not been adequately examined in either species.

In his description of *S. kaeli*, Buckley (1937) stated that there was a possibility that *S. kaeli* may be the same species as *S. dedoesi*. The examination of further specimens of *S. kaeli* by Ramachandran et al (1966) has extended the range of the measurements of many characters of *S. kaeli* into the range of *S. dedoesi*, *S. assamensis* and *S. zaheeri*. The close geographical proximity of all 4 species would suggest probable synonymy of at least some of them.

The position of *S. okinawaensis* has similarly been placed in some doubt following the examination of the extra material of *S. assamensis*, *S. zaheeri* and *S. kaeli*. Work on the life cycles of *S. assamensis*, *S. kaeli* and *S. okinawaensis* has shown all 3 species have the same vector, *Musca conducens* (see Srivastava and Dutt, 1963; Fadzil, 1973; Kono and Fukuyoshi, 1967) and that all require the presence of an existing wound for successful transmission. The subsequent recovery of adult parasites from lesions on the muzzle and feet of a cow in India (Chatterjee et al, 1982) suggested that the location of the lesion on the host animal would not be a reliable taxonomic character. Dunn (1978) has questioned the legitimacy of many of the described species and suggested probable synonymy of all the Old World species.

2110 ***Conclusions***

S. assamensis, *S. kaeli* and *S. okinawaensis* cannot be separated morphologically from *S. dedoesi*. All 4 species occur in the same hosts, have the same vector, and occur in close geographical proximity to each other. They should probably be synonymised.

Until details of the cephalic ornamentation of *S. zaheeri* have been confirmed, the status of this species must remain in doubt. *S. stilesi* and *S. dinniki* appear to stand as distinct species.

The range of measurements for each species, compiled from the data of all authors, is shown in Table 2.1.

2200 HISTORY, DISTRIBUTION, PREVALENCE AND HOSTS

2210 S. dedoesi

In Indonesia, skin lesions on stock are known locally as "cascado". A parasitic aetiology was first proposed by de Does (1911) when he recorded adult parasites in histological sections taken from a lesion on the prepuce of a steer from south Sulawesi. A fungal aetiology was proposed by Doeve (1931) when he found fungal elements in lesions in cattle from Sulawesi. He called the fungus *Penicillium bovis* but was unable to reproduce the disease by scarification of normal skin with cultures of the fungus.

In a preliminary communication, Bubberman and Kraneveld (1933a) recorded finding adult filaria in cascado lesions in cattle. In a more detailed paper in the same year (1933b) they reported the disease to be present in cattle in Sulawesi and Sumatra and the lesions as occurring on the neck, dewlap, withers and adjacent to the medial canthus of the eye. Doeve (1933) continued to propose alternate aetiologies for cascado lesions although admitting that a fungal aetiology was probably not important.

With the publication of the description of *S. dedoesi* Ihle and Ihle-Landenberg, 1933 the filarial aetiology of the disease was established. In a series of short papers, Bubberman

and Kraneveld (1934a; 1934b; 1934c) recorded the disease in goats,

Table 2.1. Measurements of taxonomic characters of *Stephanofilaria* spp.

	S. dedesi	*S. kaeli*	*S. assamensis*	*S. zaheeri*	*S. okinawaensis*	*S. dimniki*	*S. stilesi*
MALE							
length	2.3 - 3.2*+	2.4 - 3.2*	2.5 - 6.0*	2.5 - 4.8*	2.7 - 3.9*	2.6 - 3.1*	2.6 - 3.7*
width	.07 - .09	.071-.10	.08 -.126	.067-.150	.062-.127	.072-.086	.04 -.126
left spicule	.226-.230	.150-.230	.133-.230	.160-.220	.147-.173	.530-.750	.273-.376
right spicule	.045	.041-.055	.042-.063	.040-.060	.039-.050	.062-.115	.030-.055
anus - tail	.022-.032	.03 -.035	.025-.03	.02 -.032	.028-.039	.018-.029	
post anal papillae	2 pairs	3 pairs	2-3 pairs	3 pairs	2-3 pairs	2-5 pairs	2-3 pairs
pre anal papillae		6-7 pairs	6-8 pairs	6-8 pairs	6-7 pairs	5-6 pairs	5-6 pairs
ad anal papillae				2 pairs			1 pair
peribuccal spines		15-18	14-18	18-24	15	11-12	18-19
cephalic spines		16-17	18-24	28-32	18-20	8	4-6
median papilla					present		
FEMALE							
length	6.1 - 8.5	6.9 -9.94	7.0 -12.7	7.5 -13.6	7.0 - 9.1	4.6 - 5.7	3.7 -6.86
width	.156-.172	.116-.150	.105-.208	.150-.207	.139-.220	.087-.119	.062-.120
ant-vulva	.049-.057	.062-.098	.075-.113	.075-.120	.063-.082	.009-.014	.077-.090
ant-nerve ring	.065		.063-.099	.060-.100	.063-.082	115-.144	
oesophagus			.252-.450	.110-.240		.144-.173	
vagina					.20 -.30	.180-.224	.20
peribuccal spines		15-18	14-18	23-24	14-20	11-12	19
cephalic spines		19-23	16-24	28-32	20-21	8	4-5
cuticular frills	present	present	present	present	present	absent	absent
lateral alae	absent	absent	absent/ present	absent	absent	absent	present
lateral line		present		present	absent		present

* Ranges presented incorporate values from all papers containing taxonomic data

\+ All measurements are in mm.

and in cattle in Java and north Sulawesi. Kraneveld (1935a) reported the disease in different breeds of cattle in Java, Sumatra and Billiton.

The first record of the disease in buffaloes in Indonesia was in lesions on the ears of animals from Sumbawa (Kraneveld, 1935b). Djaenoedin and Adiwinata (1953) recorded lesions on the neck of buffaloes from the same area although they considered the parasite to be closer to *S. stilesi* than *S. dedoesi.* In a study of buffaloes in south Sulawesi and adjacent islands, Seijffers (1965) found between 70% and 90% of animals with ear lesions and 40% with lesions on the neck, head, dewlap and sternum. Adult parasites recovered from these lesions were identified as *S. dedoesi.*

2220 S. stilesi

The first record of the disease in north America was from lesions on the scrotum of two bulls (Dikmans. 1934a). Following the identity and description of the parasite involved (Chitwood, 1934) the disease was recorded from several western areas of the United States of America (Dikmans, 1934b; 1948). Prevalence in some herds in north America was given as 80%-90% (Maddy, 1955). The disease has been found in 89.8% of cattle in the Hawaiian Islands (Alicata, 1947), in Canada (Maddy, 1955; Dies and Pritchard, 1985) and in Guyana in South America (Craig, 1976).

After the initial report of stephanofilariasis in Russia (Gnedina, 1950) the parasite was identified as *S. stilesi* (Ivashkin et al, 1961) and the distribution extended in southern Russia (Ivashkin et al, 1971). In addition to lesions on the ventral midline, between 1.5% and 39% of cattle in southern Kazakhstan had

ear lesions (Azimov et al, 1976). Anisimova (1984), in an abattoir survey in Dagestan, Russia, found 12% (2.8-40%) of cattle infected with both *S. stilesi* and *S. assamensis*.

2230 S. assamensis

Skin lesions on working bullocks in India had long been known (Nunn, 1890) and were called Calcutta sores or Dum Dum sores. The lesions occurred predominantly on the hump and a mycotic aetiology was suspected on the basis of inoculation experiments (Dey, 1927). A filarial aetiology was proposed by Pande (1935) and later confirmed by the same author (1936) with the description of the parasite involved.

Within India the prevalence of infection varies between regions and seasons and with sex and age of the host animal. In Gujarat State, lesions were found on only adult cattle (Hiregaudar and Chatupale, 1965) whereas in Orissa the prevalence increased with age (Das et al, 1975b). Animals as young as 6.5 months (Patnaik, 1968) and 7 months of age (Dutta and Hazarika, 1973) have been found infected. Overall prevalence in cattle in India has been reported as low as 11.42% (Das et al, 1975b), 12.5% (Patnaik, 1968) or 30.5% (Dutta and Hazarika, 1973) whereas Pande (1935) reported the prevalence to be as high as 90%.

Prevalence was higher in males than females (Patnaik, 1968; Dutta and Hazarika, 1973; Das et al, 1975b) and highest in July to September and lowest in March and April (Patnaik, 1968; Dutta and Hazarika, 1973). Lesions caused by *S. assamensis* have been recorded in Murrah buffaloes and goats (Patnaik, 1966; Patnaik and Roy, 1968; Rao et al, 1979) and Indian elephants (Chatterjee et al, 1982; Chatterjee, 1984).

In Pakistan, the overall prevalence in cattle varied from 2.5% (Rahman, 1957) to 20% (Hassan, 1969; 1970) and 24% (range 5%-54%) (Mia and Haque, 1967). In Bangladesh, prevalence of infection was 30% (2%-60%) (Islam, 1979) and in southern Russia between 4.8% and 37% of cattle were found infected (Dadaev, 1978). In the Andaman Islands, 60% of cattle had humpsore lesions (Malviya, 1972) and 15%-23% of buffaloes had lesions on the navel flap (Sinha and Das, 1958; Malviya, 1972).

2240 S. kaeli

Typical skin lesions known as krian sore or filarial sore and caused by *S. kaeli* occur on the feet, ears and teats of cattle in peninsular Malaysia (Buckley, 1937; Fadzil, 1975; 1977). Prevalence of infection was 16% with animals as young as 18 months of age infected (Fadzil, 1977). A lesion has been reported from the foot of a goat in the same area (Fadzil et al, 1973).

2250 S. zaheeri

Examination of lesions on the pinna of the ear of buffaloes in India revealed the presence of stephanofilarial nematodes similar to *S. assamensis* (see Gopalakrishnan, 1948) which were subsequently separated as *S. zaheeri* Singh, 1958. Prevalence of lesions has been reported as 5-6% (Das et al, 1975c; Sharma et al, 1985), 20%-50% (Gopalakrishnan, 1948), 52% (Shastri, 1973), 25%-96% (Agrawal and Dutt, 1977b; 1978), 70% (Malviya, 1972) and 70%-80% (Ahmed, 1961). In most areas, lesions are restricted to buffaloes (Shastri, 1973; Agrawal and Dutt, 1977b), but occasional lesions have been found on cattle (Roychoudhury and Chakrabarty, 1969) and Nilgai (*Boselaphus tragocamelus*) (see Hiregoudar, 1974).

Prevalence of infection increases with age (Agrawal and Dutt, 1978; Sharma et al, 1985) and lesions develop and are active in the wet season and become quiescent in the dry season (Gopalakrishnan, 1951; Bhattacharjee and Dass, 1967; Patnaik and Khan, 1980).

2260 S. dinniki

Infection with *S. dinniki* appears restricted to the black rhinoceros (*Diceros bicornis*) in South Africa and Kenya (Schulz and Kluge, 1960; Tremlett, 1964). No data on prevalence of lesions have been published.

2270 S. okinawaenis

Kono (1965) first reported the disease in cattle in the Amami and Ryuku Islands of Japan. Ueno and Chibana (1977) described the causative parasite as a new species and Ueno et al (1977) and Kono (1980) reported the disease in cattle in the southern Nansei Islands with 66% of animals over 5 months of age being infected.

2280 Undetermined species

Stephanofilarial infection of cattle has been reported from several areas of the world but either parasites have not been recovered or specific identification has not been determined.

The earliest reports were from Germany (Dirksen, 1959; Schulz and Schafer, 1965; Dahme and Weiss, 1983) where a stephanofilarial aetiology was proposed for the locally known Summer sores on the teats of 5% of dairy cows. Similar lesions have been

reported from Denmark (Hyldgaard-Jensen and Moller, 1961), Norway (Bakken, 1980a,b) and Finland (Sarrkila, 1982;1983). Adult parasites have not yet been recovered.

Lesions in 3% of cattle encountered during an abattoir survey in Nigeria were diagnosed as stephanofilariasis by histopathological examination (Oduye, 1971).

2300 PATHOLOGY

2310 Macroscopic appearance

In all areas and hosts, stephanofilarial lesions occur in two macroscopic forms. In the non traumatised or quiescent state the lesions are circumscribed, raised, dry, hairless, hyperkeratotic or scab encrusted. In the traumatised or active state the surface is raw or cracked with haemorrhage and exudation of serum (Nunn, 1890; Dey, 1927; Bubberman and Kraneveld, 1934c; Kraneveld, 1935b; Dikmans, 1934b; Pande, 1935; Ragavachari and Reddy, 1957; Dirksen, 1959; Schulz and Kluge, 1960; Patnaik, 1964; Seijffers, 1965; Loke and Ramachandran, 1967; Oduye, 1971; Ueno et al, 1977; Chatterjee et al, 1982; Nooruddin and Hoque, 1985).

2320 Histopathology

Histological changes involve hyperkeratosis, parakeratosis, acanthosis and fibrosis. Cellular reaction is predominantly mononuclear with prominent perivascular cuffs of lymphocytes, monocytes, plasma cells, histiocytes and occasional multinucleate giant cells. Eosinophils are common in superficial areas and neutrophils can be numerous in traumatised lesions. Adults are found either singly in epidermis or in cysts in the base of hair

follicles or rete pegs. Microfilariae either enclosed within vitelline membranes or free are found in superficial areas within the lesions (de Does, 1911; Bubberman and Kraneveld, 1933b; Buckley, 1937; Levine and Morrill, 1955; Holz and Adiwinata, 1957; Sinha and Das, 1958; Hyldgaard-Jensen and Moller, 1961; Tremlett, 1964; Kono, 1965; Deorani, 1965; 1967; Loke and Ramachandran, 1966;1967; Bhattacharjee, 1967; Bhattacharjee and Dass, 1967; Jubb et al, 1985; Oduye, 1971; Pal and Sinha, 1971; Das et al, 1975a; Dewan, 1975; Mannan et al, 1977; Smith et al, 1972; Bakken, 1980b; Patnaik, 1982; Sarrkila, 1983; Agrawal and Shah, 1984).

*2330 **Site of lesion***

The site of the lesion on the host animal has been ascribed taxonomic significance within this genus.

*2331 **S. dedoesi***

"Cascado" lesions occur most commonly on the withers of cattle (Bubberman and Kraneveld, 1933b;1934c) but lesions have been recorded on head, medial canthus of eye, dewlap and ears (Kraneveld, 1935b; Seijffers, 1965).

*2332 **S. stilesi***

In north America lesions are restricted to the ventral midline (Dikmans, 1934b; Levine and Morrill, 1955) whereas in Russia lesions are also found on the ears of cattle (Azimov et al, 1976; Anisimova, 1984).

*2333 **S. assamensis***

"Humpsore" is so named because lesions most commonly occur on the hump of cattle but lesions have been reported from medial canthus of eye, neck, poll, base of horns and ears, muzzle, back, base of tail, legs, abdominal wall and feet (Patnaik,

1968; Pal and Sinha, 1971; Mohan, 1973; Das et al, 1975b; Chatterjee et al, 1982; Agrawal and Shah, 1984; Anisimova, 1984; Karim, 1984; Mitra et al, 1984; Nooruddin and Hoque, 1985; Chatterjee et al, 1986). In buffaloes, lesions have been found on the navel flap (Sinha and Das, 1958), and in Indian elephants lesions have been found on the feet (Chatterjee, 1984) and flank (Bhattacharjee, 1967).

2334 ***S. kaeli***

Typical "krian sore" lesions are found on the feet of cattle (Buckley, 1937; Loke and Ramachandran, 1967) but lesions have been found on the teats and ears (Fadzil et al, 1973).

2335 ***S. zaheeri***

"Ear sore" lesions have only been described from the pinna of the ear (Ragavachari and Reddy, 1957; Patnaik, 1964, 1982; Bhattacharjee and Dass, 1967; Agrawal et al, 1978).

2336 ***S. dinniki***

Lesions have only been reported from the posterior aspect of the shoulder (Schulz and Kluge, 1960; Tremlett, 1964).

2337 ***S. okinawaensis***

Lesions in Japan were found on the muzzle and teats (Kono, 1965; Ueno et al, 1977).

2338 ***Undetermined species***

"Summer sores" in northern Europe were found on the teats (Dirksen, 1959; Hyldgaard-Jensen and Moller, 1961; Bakken, 1980a; Sarrkila, 1982). Lesions in Nigerian cattle were on the shoulder, thorax, abdomen and ventral midline (Oduye, 1971).

2339 Conclusions

In different countries each species has a predeliction for certain sites on the body of the host animal although most species appear capable of establishing in skin on almost any part of the body of the host. Little has been published on the factors that determine the location of stephanofilarial lesions on the host animal. Somatic migration by larvae of *Stephanofilaria* spp. is not known and is thought not to occur because larval stages are commonly found within the lesions (Hibler, 1966; Patnaik and Roy, 1966).

Therefore the site of the lesion may be determined by the site of inoculation of infective larvae. This could therefore involve feeding preferences of the respective vectors and hence this aspect of stephanofilarial infection will be discussed further under the section dealing with vectors (2500).

2400 MICROFILARIA

There has been some confusion in the literature concerning the size, form and site of microfilariae of *Stephanofilaria* spp.

2410 Size and form

Buckley (1937) in his descriptions of *S. kaeli* referred to two forms of microfilariae, a small form up to 140 microns in length and a larger form up to 220 microns in length. The small microfilariae were enclosed within vitelline membranes within the uteri of adult females and in smears taken from the surface of lesions. The larger forms were free and occurred in lesion smears as

well as in the blood stream of the host. Buckley suggested that the small forms were laid by the adult females in an immature form and later matured to become the larger forms. A similar explanation was proposed by Deorani and Tewari (1968) and Chatterjee et al (1982) to explain the presence of two forms of microfilariae in *S. assamensis* lesions.

Morphology of microfilariae has usually been described in conjunction with adult taxonomy and has largely been restricted to measurement of length. The published values for microfilarial length are shown in Table 2.2. Microfilariae of all species are less than 150 microns in length.

Within the uteri of gravid females, microfilariae are enclosed within spherical vitelline membranes (Ihle and Ihle-Landenberg, 1933; Pande, 1936; Buckley, 1937; Levine and Morrill, 1955; Singh, 1958; Ueno and Chibana, 1977). A similar membrane also encloses microfilariae within the tissues of the host indicating true ovoviviparity in females of *Stephanofilaria* spp. (see Bubberman and Kraneveld, 1933b; Ramachandran et al, 1966; Hibler, 1966; Kono and Fukuyoshi, 1967; Deorani and Tewari, 1968).

Microfilariae of *Stephanofilaria* spp. have not been adequately described. With the possible exception of *S. stilesi* it is not currently possible to separate the species on the basis of their microfilariae. The small size and presence of vitelline membranes appear to be the most reliable means of separating microfilariae of *Stephanofilaria* spp. from those of other filarioid genera.

Table 2.2. Length in microns of microfilariae of *Stephanofilaria* spp.

	LENGTH
S. dedoesi	140*[a]
S. stilesi	45-60[b]
S. assamensis	93-148[c]
S. kaeli	109-140[d]
S. zaheeri	85-140[e]
S. okinawaensis	100-110[f]
S. dinniki	120-150[g]

* Ranges presented incorporate values of all the authors listed under the appropriate subscripts.

a Ihle and Ihle-Landenberg 1933

b Hibler, 1966

c Pande, 1936; Srivastava et al, 1967; Patnaik and Roy, 1968; Chatterjee, 1983

d Buckley, 1937; Fadzil, 1975

e Singh, 1958; Patnaik and Roy, 1968; Das et al, 1975c

f Ueno and Chibana, 1977

g Round, 1964

2420 Site

Das (1955) and Ahmed (1961) reported the presence of microfilariae which they considered to be those of *Stephanofilaria* sp., from the blood stream of cattle and buffaloes infected with *Stephanofilaria* sp. in India. Gopalakrishnan (1948) reported that microfilariae were common in the blood stream of infected animals in India but he did not identify the microfilariae involved. Ragavachari and Reddy (1957), Hiregaudar and Chatupale (1965), Hassan (1969), Mohan (1973) and Chatterjee (1983) were unable to find microfilariae in the blood stream of animals with stephanofilarial lesions in India.

Holz and Adiwinata (1957) and Dies and Pritchard (1985) in histopathological studies of lesions considered that the microfilariae were in the lymphatic system of the host.

In *S. assamensis*, *S. zaheeri* and *S. okinawaensis* infection, free microfilariae have been reported in exuded fluids on the surface of lesions (Kono and Fukuyoshi, 1967; Das et al, 1975c; Chatterjee, 1983). In *S. kaeli* a mixture of free and unhatched microfilariae have been reported from the surface of lesions (Ramachandran et al, 1966; Fadzil et al, 1973) whereas microfilariae of *S. stilesi* do not appear on the surface of lesions and remain in the tissues enclosed within their vitelline membranes (Ivashkin et al, 1963; Hibler, 1966).

The measurements presented in Table 2 show that *Stephanofilaria* spp. have microfilariae smaller than 150 microns in length. All species are ovoviviparous and microfilariae are deposited in the superficial tissues of the lesions caused by the adult parasites. In some species the microfilariae appear in exuded

fluids on the surface of the lesions. Larger free microfilariae encountered within the skin or blood stream of bovine hosts have undoubtedly been microfilariae of other filarioids such as *Onchocerca* spp., *Setaria* spp. or *Elaeophora* spp.

2500 VECTORS

Rahman (1957) found nematode larvae in unidentified flies taken from "humpsore" lesions. Srivastava and Dutt (1963) found that 0.7%-1.3% of wild caught *Musca conducens* contained developing larvae of *S. assamensis*. Subsequent surveys by Malviya (1972) and Patnaik (1973) found 4.2% and 7.0% respectively, of wild caught *M. conducens* with developing larvae of *S. assamensis*. Patnaik and Roy (1966) and Patnaik (1973) fed *M. conducens* on *S. assamensis* lesions and found that larval development was in the gut of the fly for 6-9 days, then in the thorax for 7-10 days and finally in the head for 5-8 days. Total development time was 23-25 days at 25 degrees C. and infection was successfully transmitted by *M. conducens* with adult parasites being recovered after 6 weeks.

Kabilov (1980) working in southern Russia found developing larvae of *S. assamensis* in *Lyperosia titillans*, however, this record must be regarded as doubtful as the flies were taken from cattle that apparently had lesions of both *S. assamensis* and *S. stilesi*. Ivashkin et al (1963) had found *L. titillans* to be the vector of *S. stilesi* in southern Russia with larval development occurring in the haemocoel of the fly. Ivashkin et al (1971) and Azimov et al (1976) identified *Haematobia irritans* and Azimov et al (1976) also identified *Stomoxys calcitrans*, as additional vectors of *S. stilesi* in southern Russia.

Hibler (1966) identified *H. irritans irritans* as the vector of *S. stilesi* in north America.

Kono and Fukuyoshi (1967) recorded *M. conducens* as the vector of *S. okinawaensis*. Working with *S. zaheeri*, Dutt (1970) recorded *M. planiceps* and Patnaik and Kumar (1972) recorded *M. autumnalis* as vectors. Fadzil (1973;1975) established that *M. conducens* was also the vector of *S. kaeli* in Malaysia. Partoutomo et al (1981) found developing larvae which they considered to be *Stephanofilaria* sp., in *M. conducens*, *Siphona exigua* and *Sarcophaga* sp. However, the figures accompanying these records are unconvincing. The larva reputed to be from *Siphona exigua* is unrecognisable and the one recovered from the proboscis of *Sarcophaga* sp. is a third stage trichostrongylid larva.

Rahman (1957) failed to achieve transmission of *S. assamensis* infection by confining infected and uninfected cattle together in the presence of wild caught flies. Hassan (1969;1970) and Patnaik (1973) achieved transmission by providing artificial wounds for the infected flies to feed on. Dewan and Rahman (1970) isolated several species of bacteria from active humpsore lesions and inoculation of normal skin with cultures of *Staphylococcus aureus* and *S. albus* predisposed to *S. assamensis* infection in the field.

M. conducens, *M. planiceps* and *M. autumnalis* are not true biting flies and are unable to pierce intact skin (Soulsby, 1982). All feed on body secretions and are readily attracted to moist wounds. Microfilariae would have to be present on the surface of lesions to be ingested by these flies. Similarly, infective third stage larvae could not be inoculated

through intact skin, which would explain the failure of Rahman (1957) to transmit *S. assamensis* infection in the absence of skin wounds.

Such an epidemiology would indicate that one of the primary determinants for infection by *S. assamensis*, *S. kaeli*, *S. zaheeri*, *S. dedoesi* and *S. okinawaensis*, is the presence of a pre-existing wound. The position of a suitable wound would therefore determine the position of the resulting stephanofilarial lesion. Thus the location of the lesion on the host animal would be a doubtful taxonomic character for this group.

S. stilesi is the only species known with certainty to be transmitted by biting flies. Microfilariae of this species do not appear on the surface of lesions but remain within their vitelline membranes beneath the intact epidermis. Therefore only a true biting fly would be capable of ingesting microfilariae and inoculating infective third stage larvae through intact skin. The site of inoculation of the third stage larvae could be determined by vector feeding preferences and the site of the resulting lesion may not be of taxonomic significance.

2600 TREATMENT

2610 Organophosphates

The most widely used organophosphate anthelmintic has been trichlorphon (Neguvon). Parenteral administration has shown equivocal results (Dirksen and Radermacher, 1960; Srivastava and Malviya, 1968). Topical applications of trichlorphon solutions and ointments have given more consistent results. Preparations containing 4% trichlorphon gave a rate of cure of 57-66% (Patnaik,

1970), 6% solutions cured 70-100% (Srivastava and Malviya, 1968; Patnaik, 1970; Pal and Sinha, 1971; Rahman and Khaleque, 1974; Fadzil, 1977; Patnaik and Khan, 1980) and solutions greater than 10% trichlorphon gave 98-100% rate of cure (Ivashkin et al, 1971; Fadzil, 1977).

Baki and Dewan (1975) and Dewan and Baki (1976) found that the addition of sulphanilamide powder to trichlorphon preparations resulted in a 100% rate of cure of stephanofilarial infection.

Topical application of coumaphos (Asuntol) gave 73% (Patnaik, 1970) and 100% (Muchlis and Soetijono, 1973) rates of cure. Agrawal and Dutt (1977a), Das et al (1977) and Patnaik and Khan (1980) found topical application of maldison to be ineffective but Dutta and Hazarika (1976) claimed a 60% rate of cure with topical application of a 6% solution.

Topical application of fenvalerate was ineffective at low concentrations (Agrawal and Dutt, 1977a) but solutions containing 4-6% fenvalerate gave rates of cure between 58% (Dutta and Hazarika, 1976) and 100% (Das et al, 1977).

Patnaik (1970) achieved 44% rate of cure with topical application of 2% fenthion and Das et al (1977) and Tripathy et al (1985) achieved 100% and 70% rates of cure respectively with prolonged application of 4% and 6% solutions of chlorfenvinphos.

2620 ***Non organophosphates***

Subcutaneous injection of Antimosan solutions resulted in partial resolution of some lesions and complete cure of others (Holz and Adiwinata, 1957; Patnaik, 1970; Ahmed and Ali, 1973;

Nooruddin et al, 1984). Phenothiazine and diethylcarbamazine have shown equivocal results (Patnaik, 1970; Pal and Sinha, 1971; Dutta and Hazarika, 1976; Roychoudhury and Chakrabarty, 1969).

Numerous other compounds have been used for treatment of stephanofilariasis and the results are shown in Table 2.3.

2630 Conclusions

Many of the treatments reported were evaluated on small numbers of animals and in the absence of control groups. Efficacy was usually assessed in terms of clinical improvement of lesions and few authors assessed parasite numbers before or after treatment. Variability in results using the same drug resulted from different treatment intervals and different drug mixtures, many of which contained more than one efficacious compound.

2700 GENERAL CONCLUSIONS

Stephanofilarial infection of domestic animals has been recognised for over fifty years and a body of literature has been amassed on the subject. However, there are several areas that warrant further work.

(a) The taxonomy of the group is imprecise and the status of several species is doubtful. There is an urgent need for a generic revision using more modern taxonomic methods.

(b) The disease has been largely ignored in those western countries in which it occurs and confirmation of its occurrence in Europe is needed.

(c) No data have been reported on the economic impact of this disease on livestock production or use, despite frequent statements in the literature that the disease is viewed

seriously in many Asian countries. Such data would provide the basis for cost benefit analyses of proposed treatment and control measures.

(d) While numerous small scale treatments have been evaluated, no work has been reported on the control of this disease. Vectors are known in most areas but control of the disease by vector control has not been investigated. The species infecting cattle and buffaloes in Asia require a pre-existing wound for infection and in most areas the majority of lesions are restricted to a single common site on infected animals. This would indicate that in these areas, certain sites in cattle and buffalo are prone to injury. Simple wound preventative measures could be investigated as a means of controlling stephanofilarial infection in these areas.

Table 2.3. Efficacy of various compounds against stephanofilarial infection.

Drug	Route of Administration	Efficacy	Author
Acriflavin	T*	40%	Agrawal and Dutt, 1977a
Anthiomaline	T	0%	Agrawal and Dutt, 1977a
Anthisan	T	88%	Mia and Haque, 1967
Antimony tartrate	T	100%	Pannu, 1958
Fenitrothion formula	T	70%	Das et al, 1975c
Florocid	T	100%	Sen and Das, 1976
Formalin	T	Improvement	Mishra, 1969
Levamisole	P,O	100%	Ueno and Chibana, 1980; Mitra et al, 1984
	T	0% 100%	Roy et al, 1987 Tripathy et al, 1985
Methyridine	T	0%	Agrawal and Dutt, 1977a
Promintic	T	0%	Agrawal and Dutt, 1977a
Sodium Arsenite	T	Improvement	Kraneveld and Djaenoedin, 1937
Suramin	T	0%	Srivastava and Malviya, 1968
Tartar emetic	T	100%	Gopalakrishnan, 1948
Tetramisole	T	100% 0%	Karim, 1984 Roy et al, 1987
Tobacco extract	T	80%	Dutta and Hazarika, 1976
Vaseline	T	47%	Patnaik, 1970

* T = topical P = parenteral O = oral

3000 ABATTOIR SURVEY

3100 INTRODUCTION

The discovery of stephanofilarial parasites in skin lesions in cattle on one property in north Queensland prompted a survey to determine the distribution and prevalence of infection in other areas, and to establish the relationship between stephanofilarial infection and the commonly encountered buffalo fly lesions in cattle. Large coastal abattoirs were selected because they processed large numbers of cattle predominantly from areas to the north and west of the abattoir and extending the width of the state. Also the slaughtering procedure permitted ease of inspection of cattle and sampling of lesions.

3200 MATERIALS AND METHODS

3210 Animals

The cattle originated from properties throughout Queensland and were examined at abattoirs in Mareeba, Cairns, Townsville, Rockhampton and Brisbane. Cattle were selected at random depending on availability during visits for sampling. A maximum of 20 animals were examined from most properties surveyed. More cattle from some properties in the more remote areas were sampled in order to achieve sufficient numbers of cattle from these areas. In order to be 95% confident of being within 1% of the true proportion of

infected animals, a sample size of 10,000 animals (or 0.1%) was selected (Cochran, 1963). This sample size was achieved by surveying 0.1% of the animals from each region of the state.

3220 Abattoir procedures

Animals were examined immediately after slaughter and prior to skinning. The month of slaughter, the shire and property of origin, and the breed, sex, age, and presence of lesions were recorded for each animal examined. The breed was classified on appearance; recorded categories being Brahman (*Bos indicus*), Crossbred (*Bos indicus* - *Bos taurus* crossbred), Hereford, Shorthorn and other *Bos taurus* breeds. For the statistical analysis, these categories were reduced to 3, namely, Brahman, Crossbred and *Bos taurus* (Hereford, Shorthorn and other).

Sex was recorded as bull, cow or steer.

Age was determined by examination of incisor teeth as described by Ladds et al (1979). For analysis 3 major age groups - less than 2.5 years, 2.5 to 5 years, and greater than 5 years - were used. In some northern abattoirs insufficient time was available for satisfactory examination of incisor teeth for age. As a result, age data were not collected for 2,446 cattle and these animals were omitted from statistical comparisons involving age.

At least one lesion from each affected animal was sampled by removal of a section of skin approximately 1.5 cm by 2-3 cm. Smaller lesions were excised completely. Samples were placed in either normal saline or a fixative solution (either 10% formalin, Carnoys solution or 70% alcohol).

Cattle were grouped according to season when slaughtered into 3 groups namely, autumn (March to May), winter (June to August), and spring (September to November). Cattle were not examined during the months December, January and February, because export abattoirs in northern Queensland are closed during these months.

3230 Laboratory procedures

Samples in normal saline were cut into 3 mm wide strips and left for 24 hours at room temperature, after which an equal quantity of 20% formalin was added to preserve the skin and any emerged parasites. Samples collected into preservative and those preserved after the saline recovery technique, were processed by routine histological techniques, stained with haematoxylin and eosin and examined microscopically.

3240 Statistical analysis

Animals were assigned to one of 5 regions on the basis of location of the property of origin. The regions were delineated as 4 latitudinal zones along shire boundaries. The south west corner (region 4) was separated as a region in which the disease was largely absent. The regions are shown in Figure 3.1.

For analysis, the presence of stephanofilarial lesions was coded 1, their absence coded 0. Standard least squares theory for non-orthogonal data (Harvey 1960) was applied and identified the main effects of shire, sex, breed and age. The analysis was performed separately for each of the 5 regions because of confounding between the factors across regions and because a different operator collected some of the data in one region.

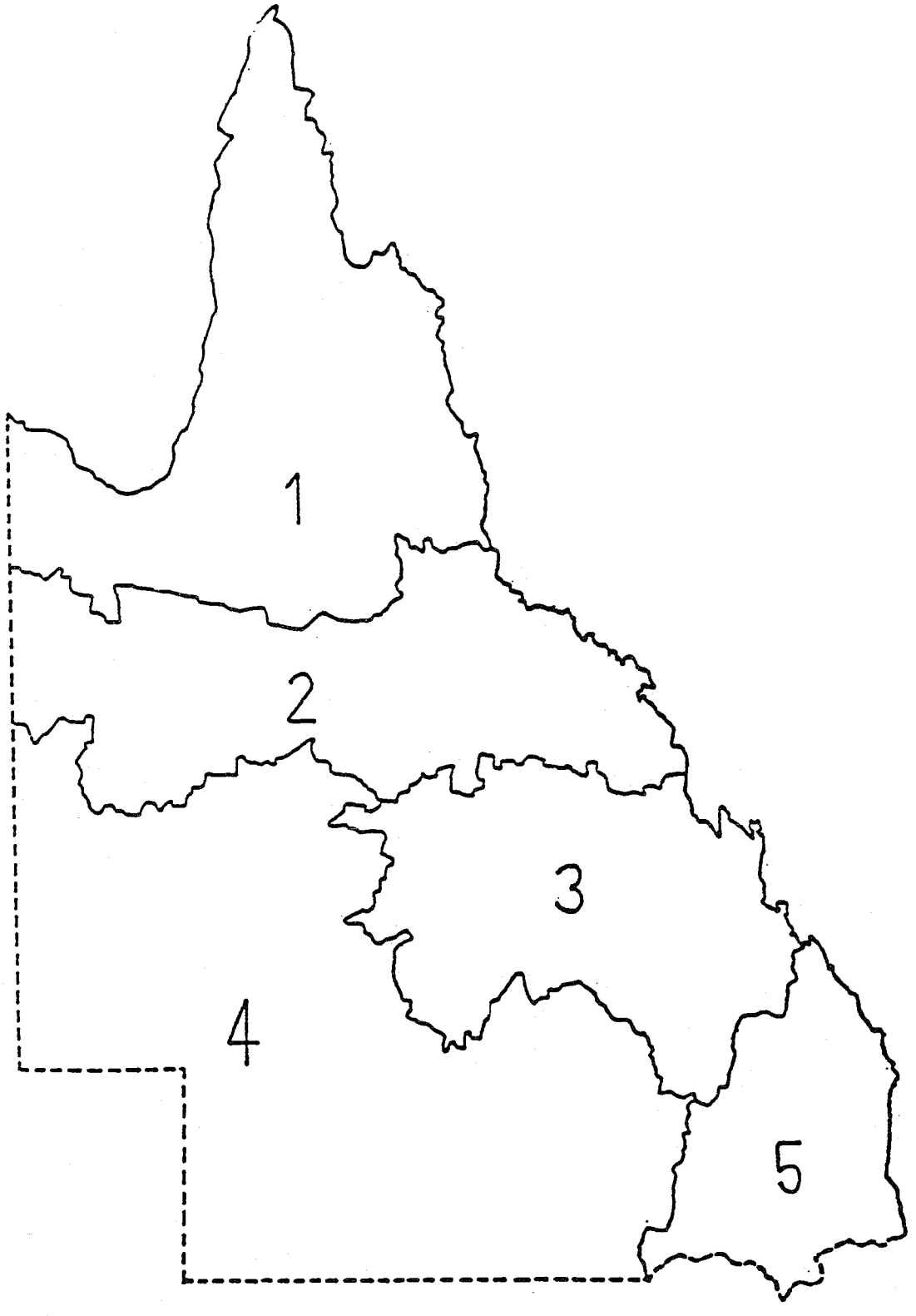


Figure 3.1. Map of Queensland showing regions used in statistical analysis of abattoir survey data.

The sex by breed, sex by age, and breed by age interactions were individually tested for significance in regions 1, 2 and 3 and were found to be unimportant. In regions 1, 2 and 4 the effect of age was estimated only from animals with age recorded. The effects of other factors in these regions were estimated ignoring age.

The effect of season of slaughter was estimated after adjusting for other factors in each region.

3300 RESULTS

3310 Laboratory diagnosis

The saline recovery technique applied to 60 samples yielded parasites from 12 (20%). Subsequent histological sectioning of these tissues revealed parasites in 19 (32%). However, 6 lesions (10%).that yielded parasites in saline showed no evidence of them on histopathology. The combined result of saline recovery plus histological examination was detection of parasites in 25 (41%) of the lesions.

Histological examination of all 4374 lesions sampled revealed parasites in 1310 (29%). No other aetiological agents were detected in any of the lesions examined and, throughout the State, parasites were found wherever lesions were found. A random sample of 528 lesions, 167 (31%) of which had shown parasites on the initial histological examination, were re-examined. On the second sectioning parasites were detected in 183 (34%) of the lesions, but only 135 (25%) revealed parasites in both examinations. Thus parasites were detected in 215 (40%) of the lesions when 2 sections were examined.

To check the reliability of duplicate histological sectioning, a second block was cut from 1160 of the known positive lesions and sectioned. Seventeen of the larger lesions were recut twice making a total of 2337 sections. On re-examination, 546 (23%) showed no evidence of parasites.

3320 Distribution

There were significant differences between percentage of animals with lesions in different regions and between shires within regions ($P<0.01$). The distribution of the disease is shown in Figure 3.2.

3321 Region 1

The disease was present throughout region 1. The maximum prevalence of 95% occurred in cattle from central Cape York Peninsula and in excess of 80% of cattle were infected in the shires extending south and west of the Atherton Tableland. In the drier southern and south western areas adjoining the Gulf of Carpentaria, 50% of cattle had lesions. On the Atherton Tableland and adjacent wet tropical coast, prevalence of lesions was 35%-37%.

3322 Region 2

The disease was present throughout the region with a tendency for prevalence of lesions to decrease with increasing distance from the coast. Throughout most of the region between 40% and 60% of cattle had lesions.

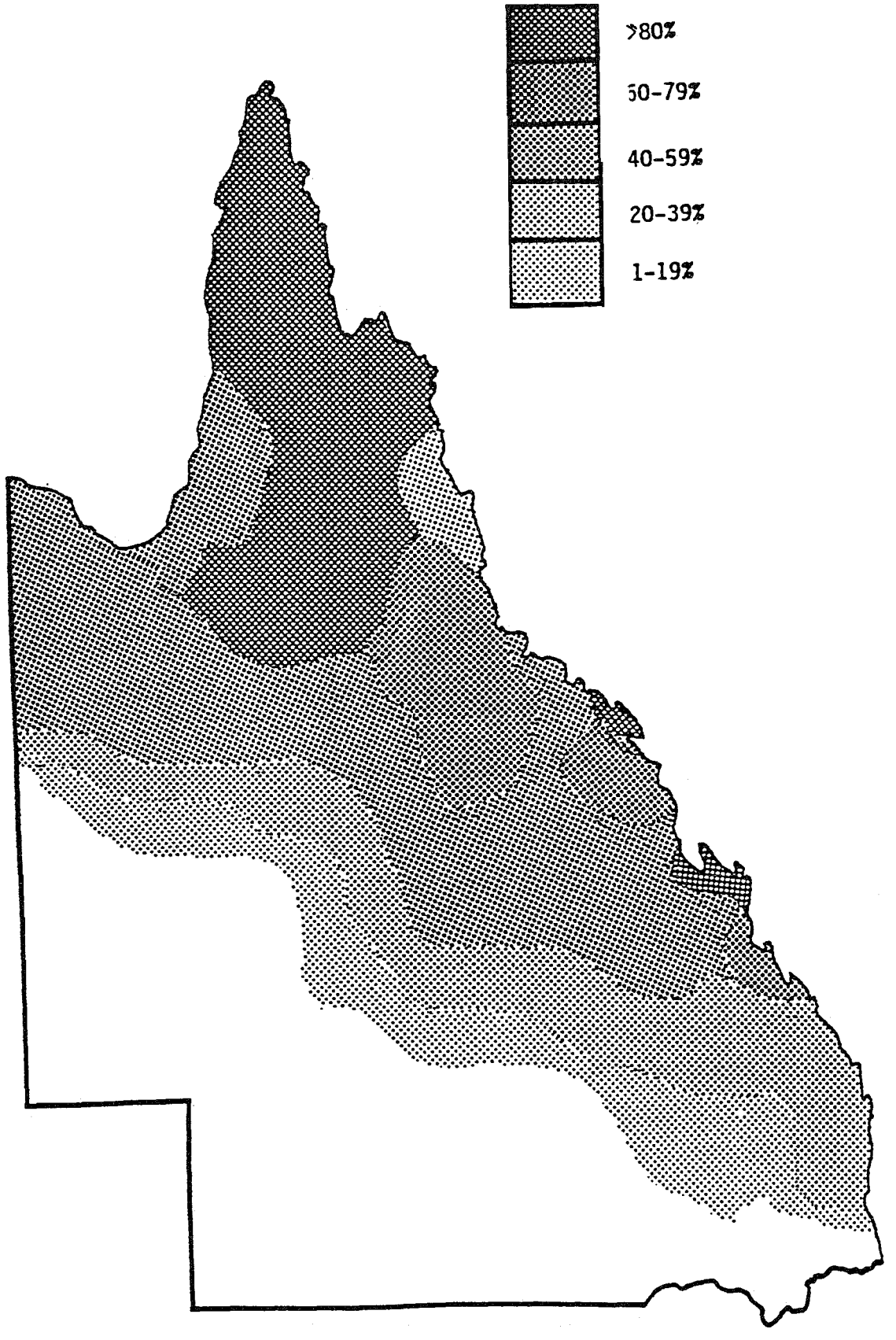


Figure 3.2. Map of Queensland showing the distribution and prevalence of stephanofilariasis.

3323 Region 3

The disease was present throughout the region with the maximum prevalence (81%) on the coastal plain north of Rockhampton. Throughout most of the region the prevalence of lesions was between 40% and 60% with a marked decrease in far western and southern areas of the region.

3324 Region 4

Within this region the disease was present only along the eastern limits of the region adjoining the endemic areas of regions 2, 3 and 5.

3325 Region 5

The southern limit of the disease occurred in region 5. Prevalence of lesions decreased with increasing latitude and there was a sporadic occurrence of the disease in southern areas of the region.

3330 Prevalence

Throughout the state, 10,543 cattle were examined, of which 4103 (38%) had at least one lesion presumed to be stephanofilarial. The prevalence of the disease in different regions is shown in Figure 3.2.

3331 Prevalence in relation to sex

Throughout the state bulls had a higher prevalence of lesions than steers and cows ($P<0.01$) with the exception of the south east (region 5) where insufficient bulls were examined. There was a tendency for steers to have a higher prevalence of lesions than cows, but this was only significant in regions 2 and 3 ($P<0.05$).

Prevalence of lesions in relation to sex is shown in Table 3.1.

Table 3.1. Prevalence of stephanofilarial lesions in slaughtered cattle in relation to sex.

	Region 1		Region 2		Region 3		Region 4		Region 5	
	n	% p*	n	% p	n	% p	n	% p	n	% p
SEX										
Bull	111	82a+	124	62a	118	68a	11	25a	12	1a
Steer	658	68b	1947	56b	1428	48b	415	3b	815	3a
Cow	701	64b	988	49c	1533	39c	540	6b	1142	2a
TOTAL	1470		3059		3079		966		1969	
STANDARD# ERROR	1.12		0.87		0.79		0.70		0.36	

* values presented are mean values adjusted for other variables

\+ Means not followed by the same letter differ significantly ($P<0.05$)

n number of animals examined

\# standard error of general mean

3332 Prevalence in relation to breed

Prevalence of lesions was significantly less in *Bos indicus* animals (P<0.01). The higher prevalence in brahman type animals in region 2 and in crossbred animals in regions 3 and 5 resulted from the uneven distribution of breeds within these regions. In these regions *B. indicus* animals occur predominantly in areas where stephanofilariasis occurs and *B. taurus* animals are largely restricted to areas free of this disease.

Differences in prevalence in relation to breed are shown in Table 3.2.

3333 Prevalence in relation to age

Prevalence of lesions increased significantly (P<0.01) with increasing age in all regions. The high prevalence in young animals in region 1 was because 68% of the young animals surveyed in this region came from the most heavily infected area.

Differences in prevalence in relation to age are shown in Table 3.3.

3334 Prevalence in relation to season

In the more heavily infected areas of the far north there were no differences in prevalence of lesions between seasons. In areas where the prevalence of lesions was less than 50% there was a significantly higher prevalence in June to August (P<0.01).

Differences in prevalence of lesions in relation to season are shown in Table 3.4.

Table 3.2. Prevalence of stephanofilarial lesions in slaughtered cattle in relation to breed.

	Region 1		Region 2		Region 3		Region 4		Region 5	
	n	% p*	n	% p	n	% p	n	% p	n	% p
BREED										
Bos indicus	85	63a+	284	68a	700	32a	12	11a	63	2a
Crossbred	834	66a	1948	54b	982	58b	88	9b	176	11b
Bos taurus	551	76b	827	71a	1397	48c	866	11a	1730	2a
TOTAL	1470		3059		3079		966		1969	
STANDARD# ERROR	1.12		0.87		0.79		0.70		0.36	

* values presented are mean values adjusted for other variables

\+ means not followed by the same letter differ significantly ($P<0.05$)

n number of animals examined

\# standard error of general mean

Table 3.3. Prevalence of stephanofilarial lesions in slaughtered cattle in relation to age.

	Region 1			Region 2			Region 3			Region 4			Region 5		
	n	%	p*	n	%	p	n	%	p	n	%	p	n	%	p
AGE															
≤2.5 years	60	61ab+		88	43a		859	38a		187	9a		1070	2a	
2.5-5 years	77	59a		454	56b		609	43b		134	11a		461	5b	
≥5 years	592	69b		922	65c		1611	50c		535	8a		438	4ab	
TOTAL	729			1464			3079			966			1969		
STANDARD# ERROR	1.42			1.19			0.79			0.50			0.36		

* values presented are mean values adjusted for other variables

\+ means not followed by the same letter differ significantly ($P<0.05$)

n number of animals examined

\# standard error of general mean

Table 3.4. Prevalence of stephanofilarial lesions in slaughtered cattle in relation to season of slaughter.

	Region 1		Region 2		Region 3		Region 4		Region 5	
	n	% p*	n	% p	n	% p	n	% p	n	% p
SEASON										
Autumn	218	73a+	699	61a	914	39a	283	10a	1097	0a
Winter	795	73a	1469	67b	962	53b	209	21b	142	2a
Spring	359	75a	856	68b	1185	41a	474	9a	728	3a
TOTAL	1372		3024		3061		966		1967	
STANDARD# ERROR	1.12		0.87		0.79		0.70		0.36	

* values presented are mean values adjusted for other variables

\+ means not followed by the same letter differ significantly ($P<0.05$)

n number of animals examined

\# standard error of general mean

3400 DISCUSSION

Recovery of parasites by the saline recovery technique was not reliable. Only adults and occasional larvae were recovered; microfilariae were apparently non motile which is not surprising in view of their enclosure within vitelline membranes. Most adult parasites detected in histological sections were entwined in cysts in the superficial dermis. Detection of parasites by histological examination alone was also not entirely satisfactory with up to 25% of single sections of known infected lesions being negative for parasites. Accurate determination of sexual maturity was not always possible on a single section and therefore, it was often not possible to distinguish between adults and developing larvae. The presence of either was taken to indicate stephanofilarial infection. The density of adult parasites within stephanofilarial lesions has been shown to be as low as 1 per cm^2 (Hibler, 1966). Such a low density combined with the small size of adults and microfilariae would explain the failure to detect parasites in all histological sections.

In the absence of alternate aetiological agents, all lesions consistent with the macroscopic appearance of stephanofilarial lesions were presumed to be stephanofilarial in origin. These included lesions known commonly as buffalo fly lesions.

Examination of Queensland Department of Primary Industries stock inspector returns for the years 1975 to 1984 showed the distribution of *H.i.exigua* to be as shown in Figure 3.3.

The area of endemic buffalo fly distribution closely matched the distribution of stephanofilarial infection. *H.i.irritans* has been shown to be the vector of *S. stilesi* in America (Hibler, 1966) and Russia (Ivashkin et al, 1971). The close association between the distribution of *H. i. exigua* and the distribution of stephanofilariasis in Queensland suggests that this fly is most probably the principal vector of the disease.

The lower prevalence on the wet tropical coast and adjacent Atherton tableland resulted from several factors. On the wet tropical coast, only a few animals were sampled, of which all were of high *B. indicus* content and thereby more resistant to infection. On the Atherton tableland, the cattle surveyed were predominantly *B. taurus* dairy animals which originated from properties on which buffalo fly infestations are effectively reduced by treatment (C. R. Parke, personal communication).

The sporadic occurrence of the parasite in the south east of the State probably resulted from the sporadic occurrence of *H. i. exigua* in this area during the years 1974 - 81 (Williams et al, 1985). Coastal areas of New South Wales appear favourable for the spread of *H. i. exigua* (Sutherst and Maywald, 1985) therefore, the parasite is likely to occupy the same area.

The higher rate of infection in males probably resulted from the preference of buffalo flies for male animals (Roberts, 1941; Holroyd et al, 1984). Dobson et al (1970), working with *H. i. irritans* found that testosterone-treated steers had a significantly higher fly burden than control animals. It is thus possible that differences in hormone levels

between bulls and steers result in bulls carrying a higher fly burden and therefore having a higher rate of infection than steers.

The observed lower rate of infection in *B. indicus* animals could reflect a longer evolutionary contact of *B. indicus* than *B. taurus* animals with the parasite or its vector. *Bos indicus* herds are infested with fewer flies of both *H. i. irritans* (see Tugwell et al, 1969) and *H. i. exigua* (see Doube, 1984) than predominantly *B. taurus* herds. In the present study, however, it was not possible to define the contribution made by parasite or vector resistance to the lower prevalence of infection in *B. indicus* than *B. taurus* animals and, consequently, no speculation as to the historical origin of the Queensland species can be made on these grounds.

Increased infection rate with increasing age was observed in this study and also in India by Das et al (1975b) for *S. assamensis* and by Agrawal and Dutt (1978) for *S. zaheeri.* This suggests that animals remain susceptible to reinfection throughout their adult life. Too few animals less than 2 years of age were encountered during the survey to permit meaningful interpretation of the dynamics of infection during this period.

The higher prevalence of lesions in cattle slaughtered in winter than in other seasons in regions 3 and 4 probably resulted from large seasonal fluctuations in the vector population. In these areas maximum buffalo fly populations on cattle occur from late summer through to autumn (Doube, 1984; Holroyd et al, 1984). Increased transmission of the parasite during this period would result in increased numbers of lesions in cattle in winter.

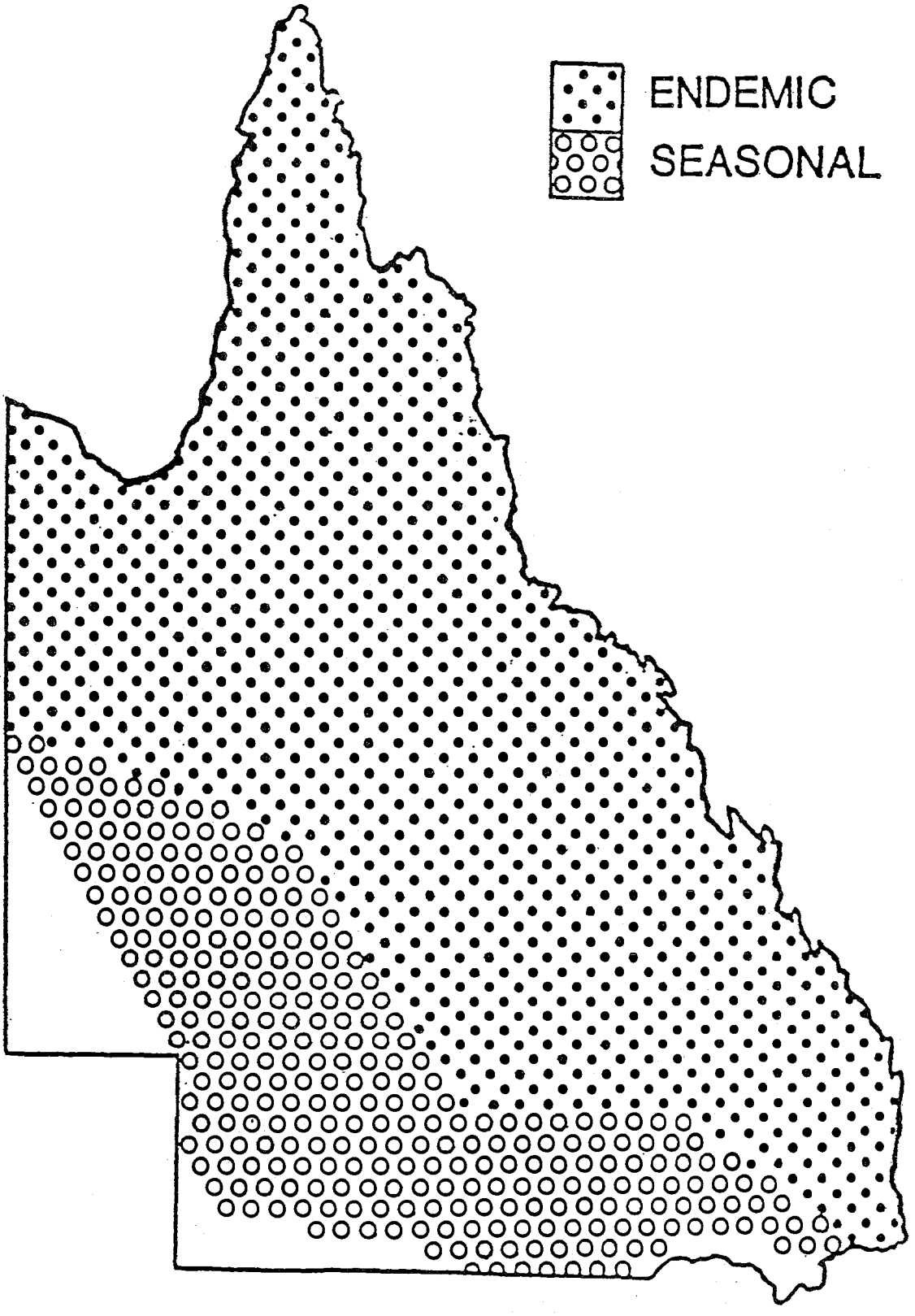


Figure 3.3. Map of Queensland showing endemic buffalo fly distribution and area of seasonal extension as compiled from Queensland Department of Primary Industries, stock inspector returns, 1975-1984.

4000 PATHOLOGY

4100 INTRODUCTION

Dermal "buffalo fly" lesions in cattle in Queensland have traditionally been ascribed to the feeding activities of the buffalo fly. These lesions have most commonly been recorded from the area adjacent to the medial canthus of the eye and along the neck although occasionally lesions have been seen in other areas (Roberts, 1941). Similar skin lesions, possibly caused by the feeding behaviour of *Haematobia meridiana*, have been reported in cattle in South Africa (Newsholme et al, 1983).

During the abattoir survey for this disease (3000) many of these lesions were found to contain *Stephanofilaria* sp. Infected and apparently uninfected lesions were visually indistinguishable. This macroscopic and histopathological study was undertaken to define the pathological features of stephanofilariasis and in an attempt to elucidate an aetiology for those lesions in which *Stephanofilaria* sp. parasites could not be detected. In addition, laboratory reared *H. i. exigua* were fed on an animal uninfected with *Stephanofilaria* sp. in an attempt to produce skin lesions resulting from fly feeding. Additional observations on the development of lesions in young animals were made during the field trials discussed later (5000; 8000).

4200 MATERIALS AND METHODS

4210 Histopathological examinations

Skin samples were taken either as biopsies or at slaughter, within 15 minutes of death. All samples were immediately fixed in 10% buffered neutral formalin and sections were processed by routine methods and stained with haematoxylin and eosin.

4220 Aetiology and pathogenesis of lesions

Buffalo flies were reared at the laboratory using the method of Thomas and Davis (1984) modified to permit female flies to oviposit directly onto the faeces produced by the stalled animal. The faeces containing the eggs was held for 48 hours, formed into pats, placed on sand in trays and held for a further 7 days at 25^{o} C. Pupae were recovered by flotation in water.

A steer which had been reared in an area free of buffalo flies and was free of skin lesions was confined in a fly proof room similar to that used for the laboratory colonisation of *H. i. exigua*. Two thousand recently emerged *H. i. exigua* were released onto the animal on day 1. Each day for 30 days thereafter, an additional 400 freshly emerged adults were released onto the animal in order to maintain a constant population of 1500 - 2000 flies on the animal. The animal was examined daily for the development of lesions and after 35 days was removed from the room and all lesions were biopsied and examined histologically.

4300 RESULTS

4310 Macroscopic appearance

In young animals, the parasites initially become established adjacent to the medial canthus of the eye on the edge of the hairless area normally present in cattle (Figure 4.1). The lesions began as small focal areas of swelling, with scab formation and loss of hairs (Figure 4.2). Continued reinfection of the site resulted in spread of the lesion anteriorly and medially (Figures 4.3,4.4) becoming extensive in older animals (Figure 4.5). Occasionally these lesions extended dorsally around the eye (Figure 4.6). Lesions on the neck began as small circular hairless areas and with increasing age of the animal, these lesions increased in number (Figure 4.7) and or size, reaching up to 30cm in diameter (Figure 4.8).

The surface of most lesions was dry and hyperkeratotic with occasional areas of dried blood (Figure 4.4). A few lesions were ulcerated with either a raw (Figure 4.9) or extensively cracked and scab encrusted surface.

In some older animals the lesions appeared to regress with a loss of hyperkeratosis and scab formation to give a smooth and often polished surface (Figure 4.10). Little or no regrowth of hair occurred in these lesions.

4320 Site of lesions

During the abattoir survey reported in 3000 the presence of lesions was recorded for 4 anatomical regions, namely, head, eye (adjacent to medial canthus), neck and sternum. Prevalence of infection at each site with respect to age, sex and breed is

recorded in Tables 4.1, 4.2 and 4.3 respectively. All animals with the disease had lesions adjacent to the medial canthus of the eye. Lesions on the neck occurred in 35% of infected animals and with a prevalence that increased with age (Table 4.1), was higher in steers and cows than in bulls (Table 4.2), and was lower in *Bos indicus* animals (Table 4.3).

Sternal lesions were found predominantly in males with 18% of infected bulls having lesions in this site (Table 4.2) The lesions occurred on the ventral midline at the level of the xiphisternum and occasionally extended posteriorly towards the prepuce (Figure 4.11). They were present only in adult animals (Table 4.1) and occurred with similar frequency in all breeds (Table 4.3).

Head lesions were found in only 0.6% of infected animals and were situated on the midline of the face between the eyes (Figure 4.12). There were no differences in the prevalence of head lesions with age (Table 4.1), sex (Table 4.2) or breed (Table 4.3).

4330 Histopathology

Consistent changes in the epidermis of infected skin were hyperkeratosis and acanthosis (Figure 4.13). Changes seen in the dermis were fibrosis, hyperaemia, and inflammatory cell infiltration. Cellular infiltrate was predominantly mononuclear comprising lymphocytes, monocytes and histiocytes (Figure 4.14). Eosinophil granulocytes were scattered throughout the dermis with increased numbers in close proximity to adult parasites (Figure 4.15).

Table 4.1. Location of stephanofilarial lesions in relation to age

	SITE OF LESION							
	Eye		Neck		Head		Sternum	
	n	%	n	%	n	%	n	%
AGE								
<2.0 years	300	100	36	12	5	2	0	0
2.0-4.0 years	529	100	160	30	3	1	0	0
4.0-7.0 years	1037	100	536	52	10	1	42	4
>7.0 years	113	100	75	66	0	0	7	6
TOTAL	1979	100	807	41	18	1.0	49	2.0

n number of infected animals with recorded age with lesions in the site

% percentage of animals with recorded age with lesions in the site

Table 4.2. Location of stephanofilarial lesions in relation to sex

	SITE OF LESION							
	Eye		Neck		Head		Sternum	
	n	%*	n	%	n	%	n	%
SEX								
Bull	274	100	12	4.3	3	1.0	50	18.2
Steer	2279	100	807	35.4	15	0.6	15	0.6
Cow	1550	100	650	41.9	9	0.5	6	0.3
TOTAL	4103	100	1469	35	27	0.6	71	1.7

n number of infected animals with lesions in the site

* percentage of animals of the same sex with lesions in the site

Table 4.3. Location of stephanofilarial lesions in relation to breed

		SITE OF LESION							
		Eye		Neck		Head		Sternum	
	n	%*		n	%	n	%	n	%
BREED									
Bos indicus	525	100		110	21	2	0.3	11	2.0
Crossbred	2135	100		722	33	18	0.8	26	1.2
Bos taurus	1443	100		637	44	7	0.4	34	2.3
TOTAL	4103	100		1469	35	27	1.7	71	0.6

number of infected animals with lesions in the site

* percentage of animals of the same breed with lesions in the site

Infiltration by neutrophils was a feature of only those lesions in which the epidermis had been lost (Figure 4.16) In all lesions there was a marked depletion or complete absence of hair follicles and associated sebaceous glands. Apocrine sweat glands which occurred at a deeper level in the dermis were usually unaffected.

An examination of parasites within sections revealed that 47% of cysts contained only females. 43% contained males and females, 8% contained only males and in 2% the sex of the parasites could not be determined. Of the single parasites found in the epidermis and dermal papillae, 67% were gravid females, 27% were males and the sex of the remaining 6% could not be determined.

Rarely adult parasites were found in intact hair follicles (Figure 4.17) but more usually they occurred in cysts formed from the remnants of hair follicles (Figures 4.13, 4.15). Adults not in cysts were found in the epidermis and superficial dermal papillae (Figure 4.18) and necrotic tracts indicating recent migration were commonly encountered (Figure 4.19).

Microfilariae were deposited in the superficial areas of the dermal papillae close to the stratum germinativum of the epidermis (Figure 4.20) and in close proximity to superficial capillaries (Figure 4.22). Occasionally microfilariae were found in the epidermis of the rete pegs (Figure 4.21) or deeper in the dermis adjacent to adult cysts. Each microfilaria, together with a series of small spherical bodies, was enclosed in a spherical membrane 50-80 microns in diameter (Figure 4.22).

Similar membranes were present enclosing microfilariae in the uteri of gravid females (Figure 4.23). Microfilariae of *Onchocerca* sp. were also present in the dermis of some lesions (Figure 4.24).

Presence of adult parasites in the base of hair follicles elicited a severe local inflammatory response involving lymphocytes, histiocytes and eosinophils (Figure 4.15) and resulted in the destruction of the hair bulb and sebaceous glands. In most lesions the presence of microfilariae evoked a minimal response by inflammatory cells (Figure 4.25). Dead and dying microfilariae were phagocytosed by histiocytes and multinucleate giant cells.

Parasites were rarely encountered in lesions in which the epidermis had been extensively breached and in which secondary bacterial infection led to heavy neutrophil infiltration. When parasites were detected in such lesions, they were in areas of the lesion with intact epidermis or where neutrophils were few in number.

Parasites were not detected in all lesions. Constant histopathological changes in those lesions in which parasites were not detected were acanthosis, variable inflammatory cell infiltration, and loss of hair follicles and sebaceous glands (Figures 4.26, 4.27). In lesions which had regressed macroscopically, the epidermis remained slightly acanthotic and mononuclear cell infiltration was often within normal limits but hair follicles and sebaceous glands failed to regenerate (Figure 4.28).

Following release, the flies rested along the dorsal midline and down onto the upper flanks and the shoulders. Occasionally a single female fly was observed adjacent to the medial canthus of an eye. Five days after release of the first flies there was an increased flaking of loose keratinised material from the skin overlying the transverse processes of the cervical and anterior lumbar vertebrae. By day 9 small amounts of exuded serum were present in the same areas and by day 14 some discrete scabs had formed. By day 18 a small excoriation had developed on the left side overlying the base of the thirteenth rib. This excoriation increased in size until by day 30 it was 90 cm long and 10 - 20 cm wide.

The animal was removed from the room on day 35 and brushed to remove adherent material. The only lesion present was the ulcerated area on the left side. Histological examination of this lesion revealed ulceration of the epidermis and heavy exudation of neutrophils and eosinophils (Figures 4.33; 4.34). Scattered remnants of epidermis remained but there was no evidence of acanthosis. The adnexa were intact and hair shafts were present in many follicles even in ulcerated areas. Small aggregations of lymphoid cells were present in perivascular areas in the basal dermis but the predominant cell in the superficial dermis was the eosinophil.

The lesions examined in this study were macroscopically indistinguishable from the lesions that were thought to have resulted from hypersensitivity to, and irritation from, the bites of buffalo flies. The failure to detect parasites in some lesions meant, that for these lesions, an alternate aetiology was possible.

However, pathological changes in lesions in which parasites were not detected were similar to those of lesions in which parasites were detected. A constant finding in all lesions was acanthosis accompanied by the destruction of hair follicles and sebaceous glands. This similar pathology suggested a common aetiology for infected and apparently uninfected lesions.

If infected and apparently uninfected lesions had arisen from a common aetiology, then two explanations were possible. The first was that all lesions were stephanofilarial in origin and that the technique used in the study had failed to detect parasites in some of them or that the parasites were no longer present in some lesions. The second explanation was that all lesions were the result of hypersensitivity to fly bites and that some lesions were incidentally infected with *Stephanofilaria* sp. parasites. The first explanation could only be resolved by the use of a more sensitive diagnostic test. The second explanation was tested by comparing the pathology of the lesions examined in this study with the pathology of known hypersensitivity lesions and by feeding uninfected flies on an animal and examining any resultant lesions.

The pathological changes present in the lesion produced by the uninfected *H. i. exigua* were different from those seen in the stephanofilarial lesions surveyed in this study. The most obvious difference was the lack of epidermis with retention of all adnexa in the fly feeding lesion (Figure 4.33). In addition, the predominant inflammatory cell in the fly feeding lesion was the eosinophil (Figure 4.34) whereas in stephanofilarial lesions, monocytes, histiocytes and lymphocytes predominated. During the survey occasional lesions were found in which the epidermis was eroded (see Figure 4.16), however, there was always a complete loss of hair follicles and sebaceous glands and acanthosis of residual epidermis.

Further evidence for these differences was obtained from a comparison of the fly feeding lesion with the stephanofilarial lesion produced under under similar experimental conditions using infected flies (see 6330). This stephanofilarial lesion was examined 36 days after release of infected flies. In that lesion the epidermis was intact and markedly acanthotic (Figures 4.29; 4.30) and the cellular infiltrate was predominantly mononuclear. Although all adnexa were present, there was evidence of early necrosis of hair follicles and sebaceous glands resulting from invasion by parasites (Figures 4.30; 4.31; 4.32). If such a lesion was continually reinfected resulting in increased density of parasites within the lesion, then there may be increased destruction of hair follicles.

Irregular skin lesions resulting from hypersensitivity to bites of *Stomoxys calcitrans* have been reported in Australian cattle (Moorhouse, 1972). In these lesions, intraepidermal blisters were formed which led to sloughing of the

epidermis. The predominant inflammatory cell he found associated with the lesions was the eosinophil granulocyte. Similar pathological changes were found in the lesions in South African cattle caused by the feeding activity of *H. meridiana* (see Newsholme et al, 1983). Also, extensive, irregular, raw lesions have been reported in some cattle on first exposure to buffalo fly feeding but most of the lesions occurred on the shoulder (Roberts, 1941). In the present survey no lesions were found on the shoulder.

Occasional microfilariae of *Onchocerca* spp. were found within the dermis of lesions examined in this study. Cattle in northern Australia are commonly infected with *Onchocerca* spp. (see Ladds et al, 1979) and microfilariae can be abundant in skin on the dorsal and ventral midlines (Beveridge et al, 1981). No lesions were observed on the dorsal midline during this survey and lesions on the ventral midline were confined to a small area in some bulls

In cattle, the inflammatory reaction to the presence of microfilariae of *Onchocerca* spp. is minimal (Jubb et al, 1985) and macroscopic lesions are not seen (Hussein et al, 1975). Therefore, no aetiological significance can be attributed to the presence of microfilariae of *Onchocerca* spp. in these lesions. They were regarded as an incidental finding in this study.

Therefore, these 4 factors, namely; the demonstrated ability of stephanofilarial infection to produce characteristic lesions, the inability of uninfected flies to produce such lesions, the lack of other aetiological agents and the subsequent demonstration of parasites in almost all lesions

(5000; 8000), all strengthen the conclusion that buffalo fly lesions are synonymous with stephanofilarial dermatitis.

Holz and Adiwinata (1957) and Dies and Pritchard (1985) considered that microfilariae were in superficial lymph vessels. By contrast, in this study, no association was found between the parasites and the lymphatic system of the host. In histological sections microfilariae appeared to be free in dilated vessels. However, on close examination it was observed that microfilariae were always accompanied by a series of small spherical eosinophilic bodies (Figures 4.20, 4.22) and that the dilated vessels were, in fact, the enclosing vitelline membranes. The not uncommon finding of microfilariae within the epidermis of the rete pegs (Figure 4.21) would also preclude a lymphatic site.

The finding by Dies and Pritchard (1985) that adults are also occasionally in lymph vessels, is not supported by the findings of this study. The adults enter the base of hair follicles (Figure 4.17) and, after the loss of the hair shaft and germinal epithelium, the follicle remains as a cavity containing the parasites. During processing, parasites are occasionally lost from these cavities and the remaining artifacts may have been mistaken by Dies and Pritchard for dilated lymph vessels.

Alopecia is a constant feature of stephanofilarial lesions yet its pathogenesis has not been determined. During histological sectioning, most adults were found entwined in cysts formed in the base of hair follicles. Fewer adults of both sexes were found in the epidermis and superficial dermis. The predominance of gravid females in the epidermis suggests that they may migrate in this manner to discharge eggs in superficial areas accessible to potential vectors. The males found in this situation may have been

migrating in search of females. If these adults as well as maturing larvae re-entered other hair follicles then the repeated invasion of hair follicles and destruction of the germinal matrix cells would cause the local alopecia which is characteristic of stephanofilarial lesions.

The presence of a vitelline membrane enclosing the microfilaria indicates ovoviviparity in females of this *Stephanofilaria* sp. All other species in this genus are known to be ovoviviparous (2410). If, as suggested later, such membranes preclude independent migration of microfilariae within the skin of the host, then the membranes would act as egg shells. For the purposes of this study the entire structure was termed an egg. Potential vectors would have to be capable of breaching the epidermis to ingest these eggs. Each egg contained numerous small spherical bodies which stained eosinophilic in sections stained with haematoxylin and eosin. They were easily mistaken for erythrocytes, thereby creating a false impression of the microfilariae occurring in small capillaries. They were readily distinguished from erythrocytes by special staining (Figure 4.22). The function of the small eosinophilic bodies is unknown; they are most probably yolk remnants.

The suggestion by Buckley (1937), Deorani and Tewari (1968) and Chatterjee et al (1982) that large free microfilariae within the dermis of cattle were mature forms of *Stephanofilaria* sp. microfilariae is unlikely for the following reasons.

(a) All *Stephanofilaria* spp. have microfilariae smaller than 150 microns in length (Table 2.2).

(b) All *Stephanofilaria* spp. are ovoviviparous and microfilariae in the skin are contained within vitelline membranes.

(c) Such microfilariae were encountered in this study and were microfilariae of *Onchocerca sp.*

The site of the lesion on the host animal has been ascribed taxonomic significance within this genus. In this study, lesions were restricted to medial canthus of eye, head, neck and sternum. All infected animals had lesions adjacent to the medial canthus of the eye with some animals also having lesions in one or more of the other sites. No other species in the genus occurs in lesions restricted to these sites on the host animal. In a study of buffaloes in eastern Indonesia, Seijffers (1953) found lesions near the medial canthus of the eye, and on the neck, head and chest but most animals (70-90%) had lesions on the ear as well. Parasites recovered from these lesions were identified as *S. dedoesi* which, in cattle in other areas of Indonesia, occurs most commonly in lesions on the withers (Bubberman and Kraneveld, 1933b).

The only other species that is usually restricted to lesions occurring in one of the sites found in this survey, is *S. stilesi*. In north America *S. stilesi* occurs in lesions on the ventral midline, extending posteriorly as far as the scrotum (Dikmans, 1934b; Levine and Morrill, 1955). It has not been reported from lesions on the head, neck or eye, although there is a report from southern Russia of this species occurring in lesions on the ears of cattle (Azimov et al, 1976). The factors that determine the location of stephanofilarial lesions on the host animal are not known, therefore little taxonomic significance can be attributed to the site of the lesion for this *Stephanofilaria* sp.

The results of the abattoir survey (3000) indicated a close association between the distribution of stephanofilariasis and the distribution of *H. i. exigua*. Such an association would indicate that this fly is the most likely vector in Australia.

Somatic migration by larvae of *Stephanofilaria* spp. is not known and is thought not to occur because all larval stages are commonly found within the lesions (Hibler, 1966; Patnaik and Roy, 1966; see 6340). In those species with non biting fly vectors, it has been shown that the presence of a pre-existing wound is necessary for transmission (Hassan, 1969;1970; Patnaik, 1973) and that the resulting stephanofilarial lesion developed at the site of the wound. For those species transmitted by true biting flies a pre-existing wound would not be necessary (Hibler, 1966). Therefore, the position of a resultant lesion could be determined by a vector preference for feeding at the site.

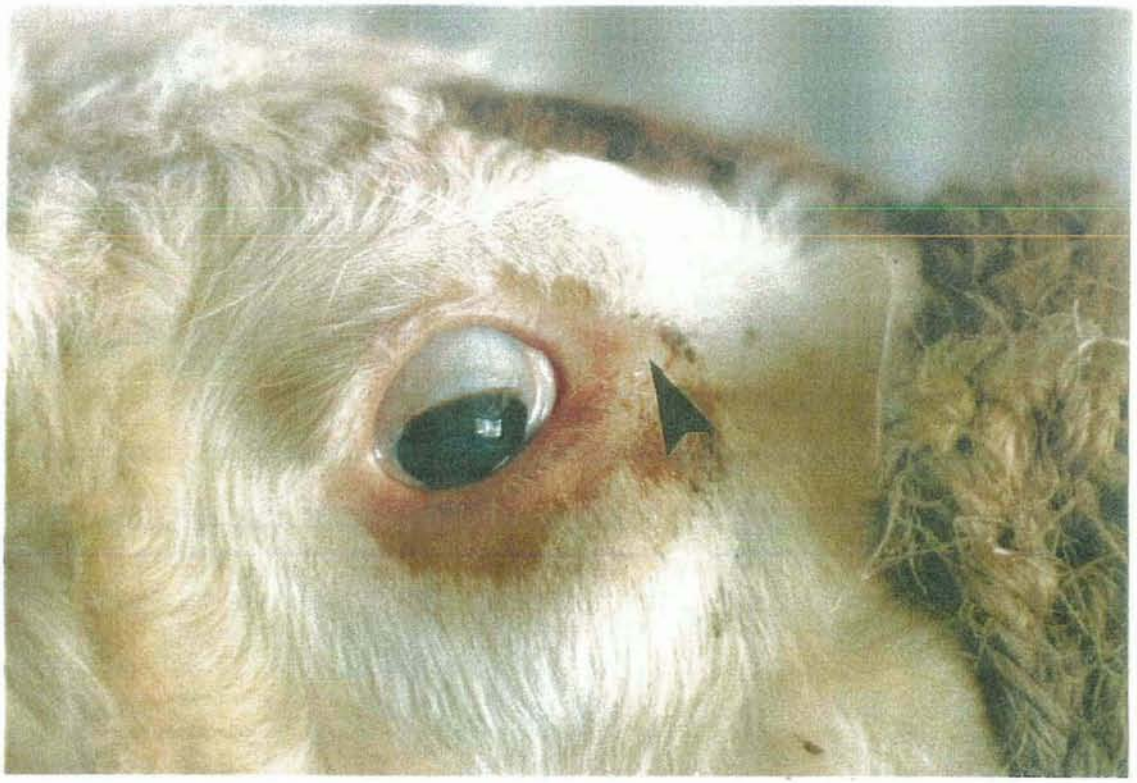
In this study all infected animals had eye lesions, indicating that *H. i. exigua* may have a strong preference for feeding at this site in cattle, independently of sex, breed or age of the host. Conversely, the low prevalence of head lesions suggests that the fly could have a low preference for feeding on the front of the head of cattle. The prevalence of neck lesions was interesting. The higher prevalence of these lesions in older animals suggests that the vector shows an increasing tendency to feed along the neck of older cattle. The lower prevalence in *Bos indicus* animals is most probably a reflection of the overall resistance of *B.indicus* type animals to stephanofilarial infection. The lower prevalence of neck lesions in bulls may be a result of a vector preference to feed at sites other than the neck in these animals.

Sternal lesions occurred exclusively in adult animals and predominantly in bulls, independently of breed. These lesions were always located over the xiphisternum on the ventral midline, which in bulls is the area that makes frequent and vigorous contact with the base of the tail of cows during mating. During the present survey it was noticed that older uninfected bulls frequently had the skin overlying the xiphisternum denuded of hair presumably from frequent sexual activity. *H. i. exigua* may prefer to feed on areas of skin with little or no hair

The preferred feeding sites of *H. i. exigua* on cattle are not known. Although *H. i. exigua* were released onto animals during this study, little useful information on fly feeding preference was gained because fly behaviour appeared to change in response to the artificial lighting used. Unfortunately, *H. i. exigua* abandoned animals that were housed in fly proof accomodation illuminated by natural light. It was interesting to note that laboratory reared flies rarely fed at the medial canthus of the eye whereas many wild caught females released onto animals under the same artificial conditions did so. Therefore to what extent site of lesion is determined by vector feeding preference or by other physiological factors that operate locally in the skin of cattle, cannot be determined.

Figure 4.1 Head of *Bos taurus* calf showing hairless area (arrowed) normally present on the medial canthus of the eye.

Figure 4.2. Young *Bos indicus* calf showing early stephanofilarial lesion (arrowed) developing on the edge of the hairless area on the medial canthus of the eye.



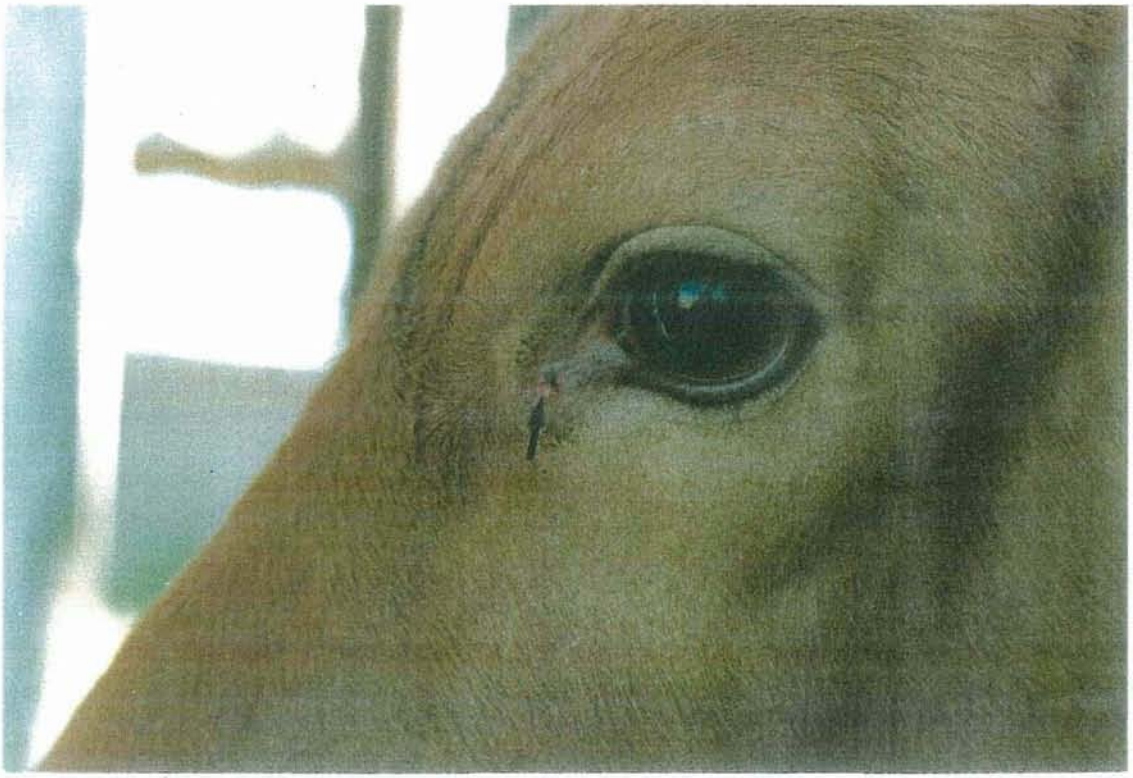
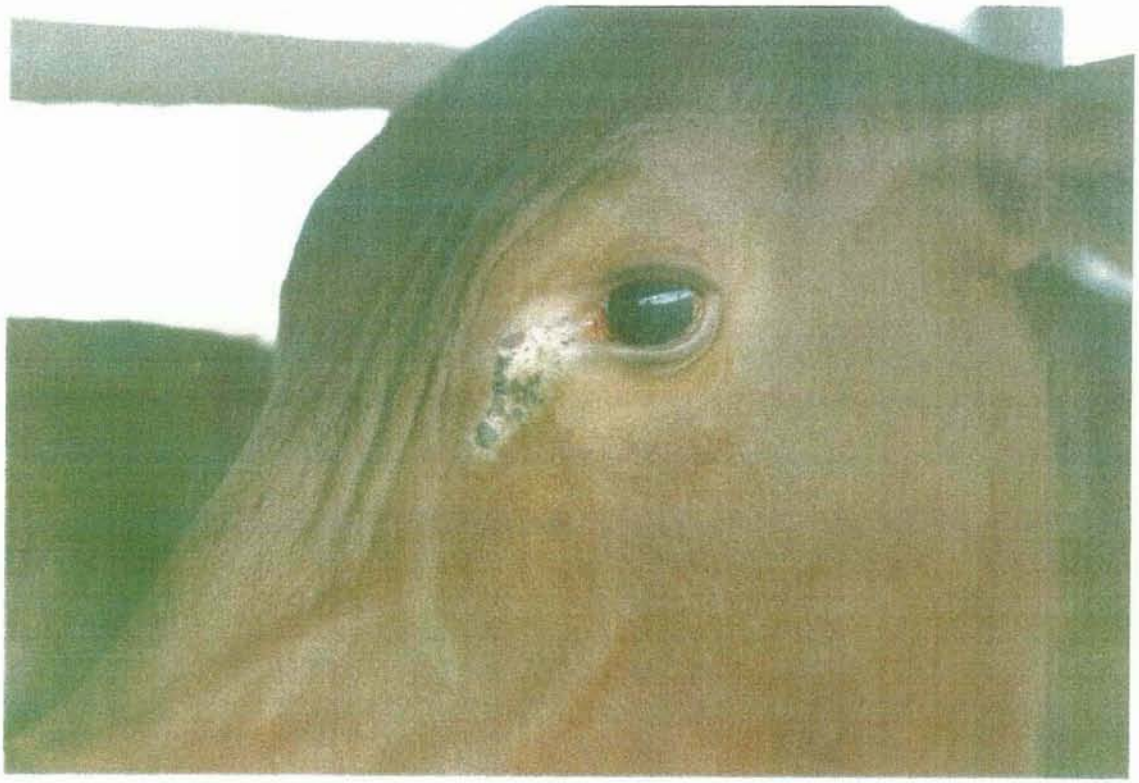


Figure 4.3. Head of *Bos indicus* calf showing stephanofilarial lesion progressing anteriorly from the medial canthus of eye.

Figure 4.4. Head of a young *B. indicus* animal with a stephanofilarial lesion expanding from the medial canthus of the eye.



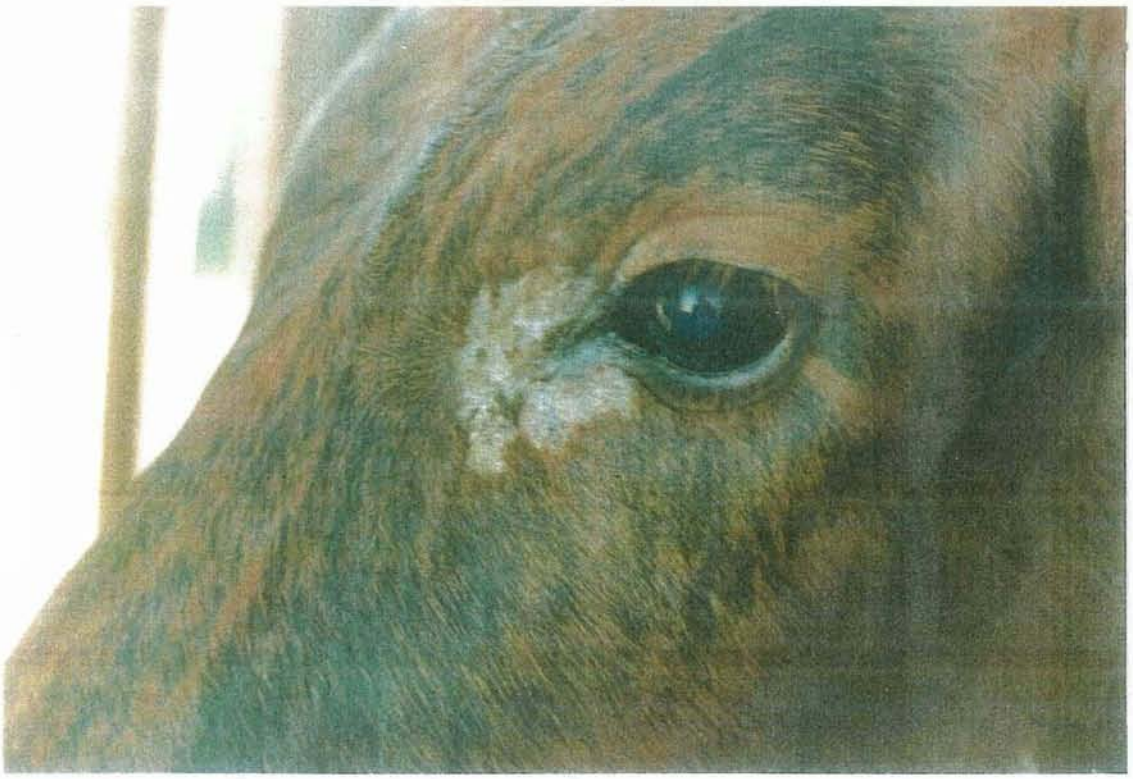
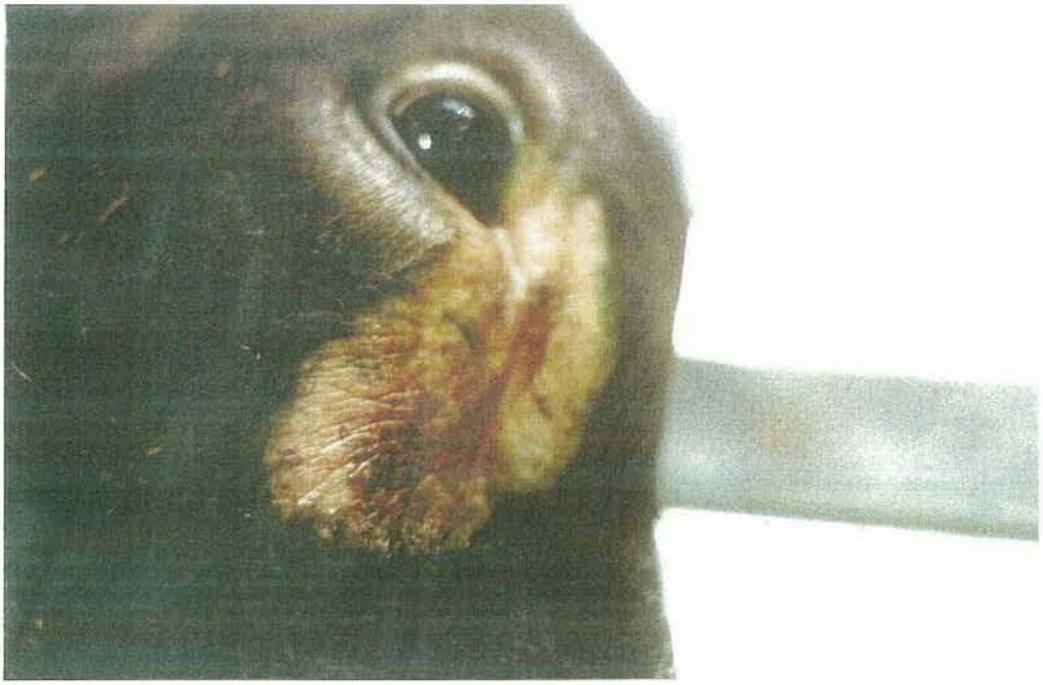


Figure 4.5 Head of *Bos taurus* bull showing an extensive stephanofilarial lesion near the medial canthus of eye.

Figure 4.6. Head of a *B. indicus* steer with a stephanofilarial lesion extending dorsally around the eye.



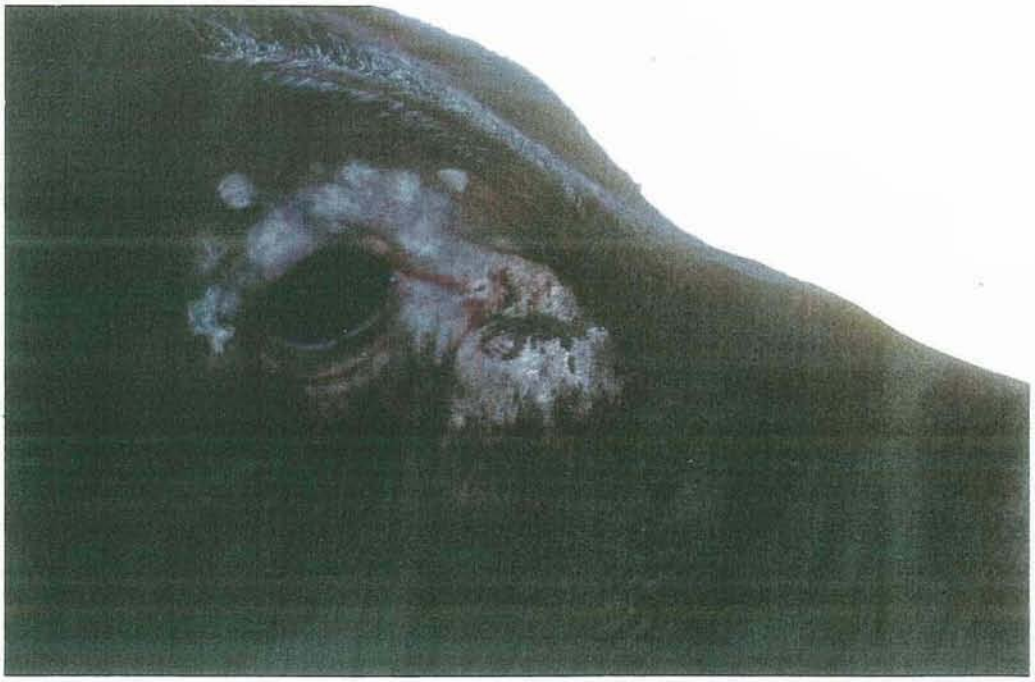


Figure 4.7. *B. indicus* bull showing multiple stephanofilarial lesions (arrowed) on the neck.

Figure 4.8. Neck of 6 year old *Bos indicus* bullock showing extensive stephanofilarial lesions.

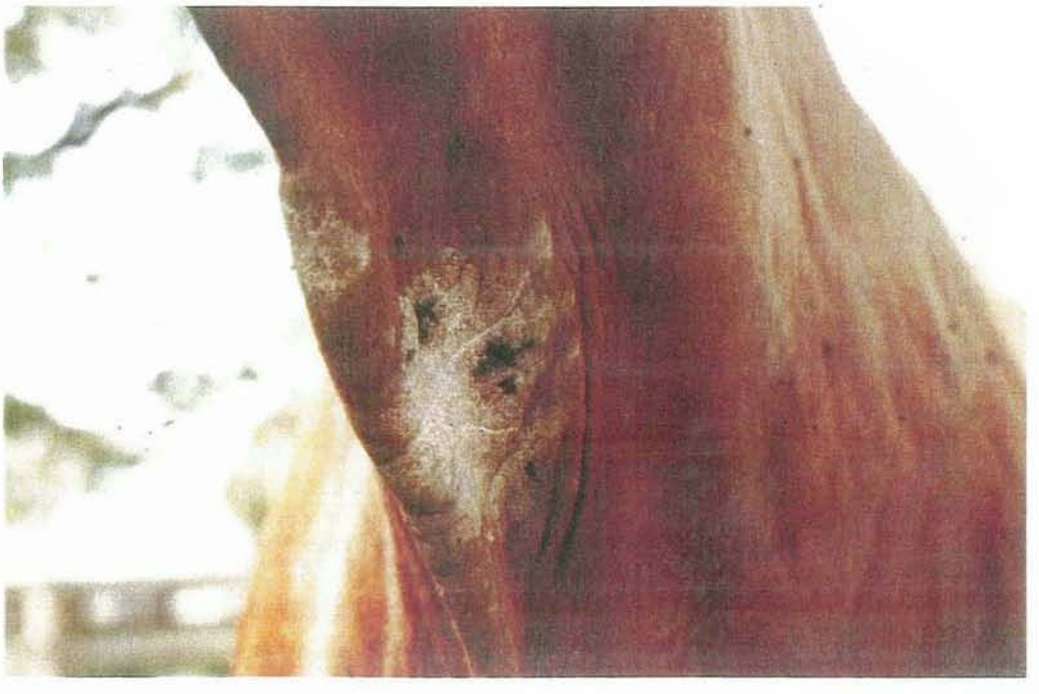
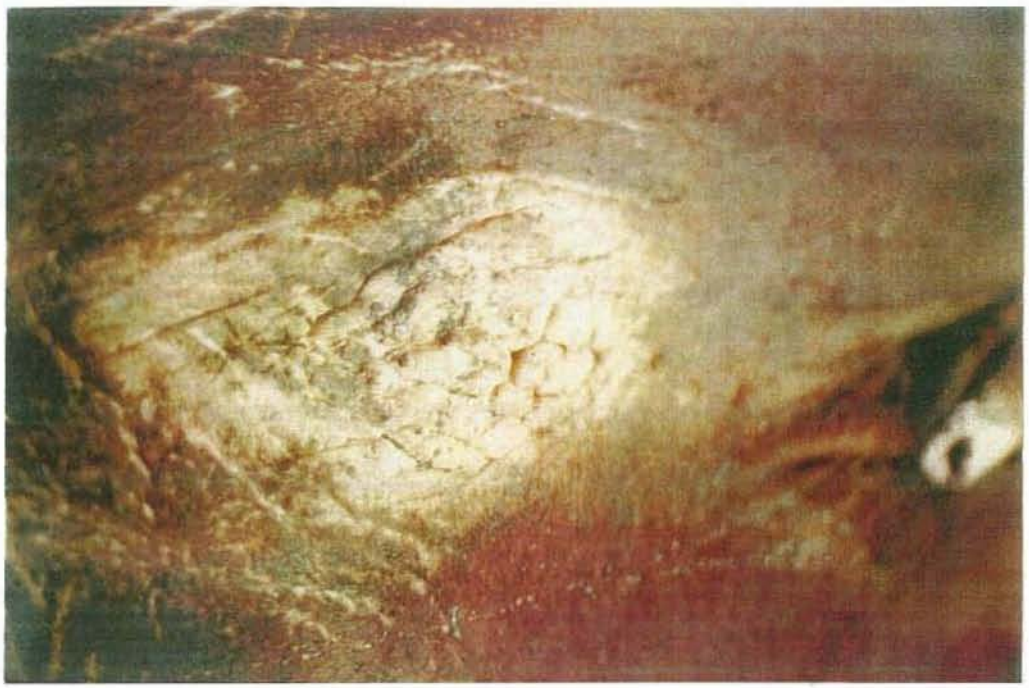


Figure 4.9. *Bos indicus* cow with raw ulcerated stephanofilarial lesions on the neck.

Figure 4.10. Neck of a *Bos indicus* steer showing a regressed stephanofilarial lesion. the surface of the lesion has become smooth and there is little or no regrowth of hair.

Figure 4.11. Stephanofilarial lesion anterior to the prepuce of a *Bos indicus* bull.

Figure 4.12. Head of a *Bos indicus* bull showing a stephanofilarial lesion on the dorsal midline of the face.



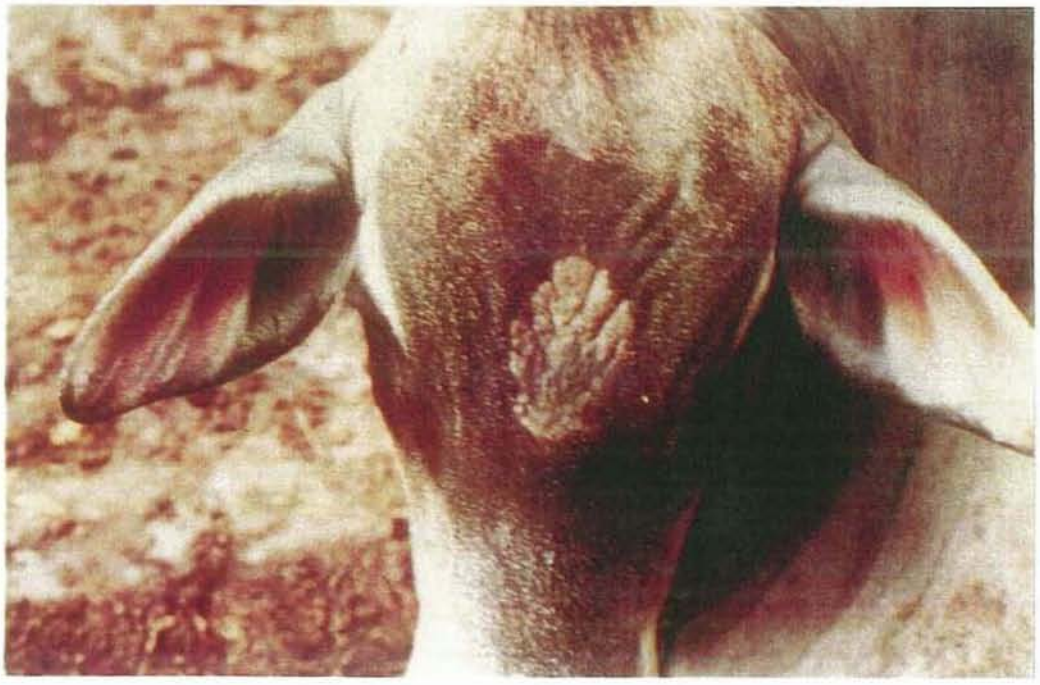
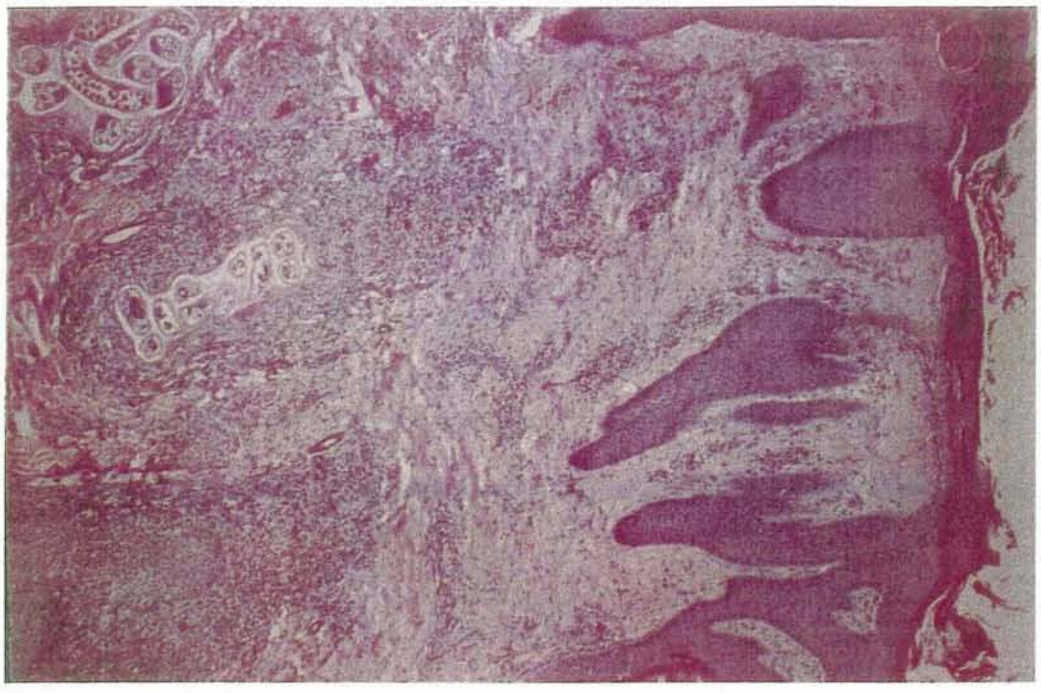


Figure 4.13. Section of skin showing hyperkeratosis, acanthosis, loss of hair follicles and sebaceous glands and containing adult *Stephanofilaria* sp. in cysts surrounded by inflammatory cells. Haematoxylin and eosin x 25.

Figure 4.14. Skin section from a stephanofilarial lesion showing mononuclear cell aggregation. Haematoxylin and eosin x 50.



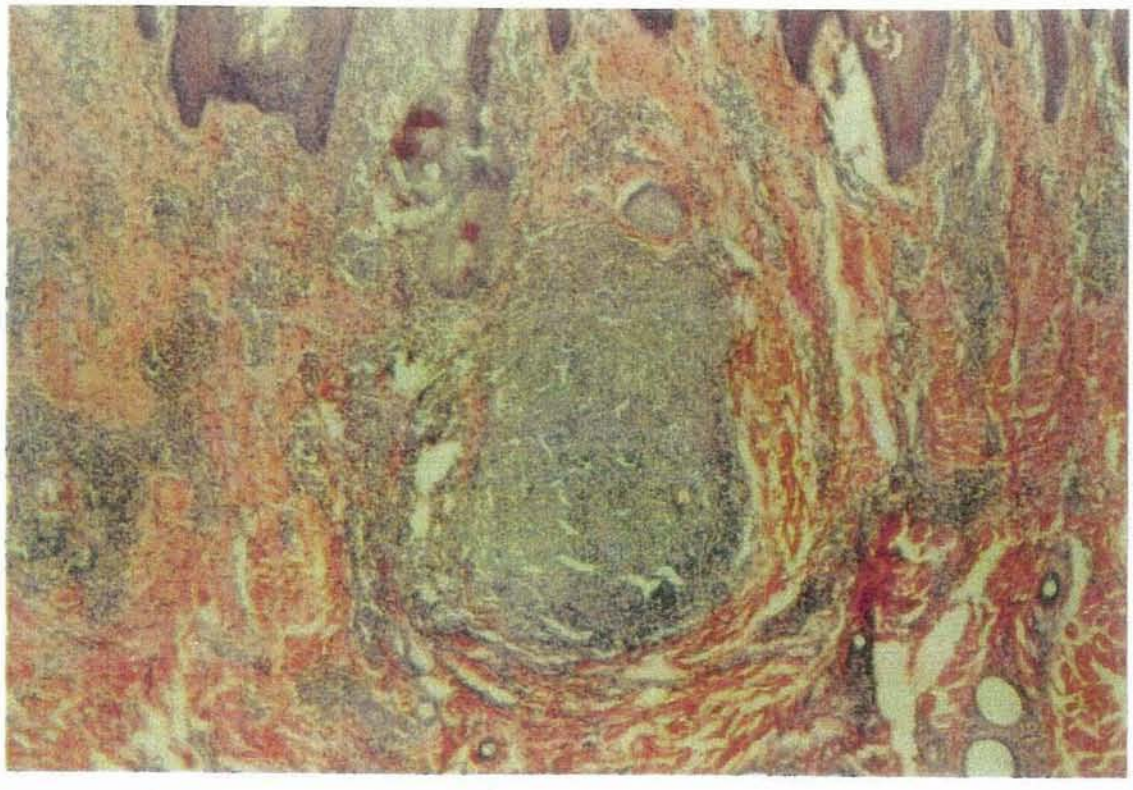
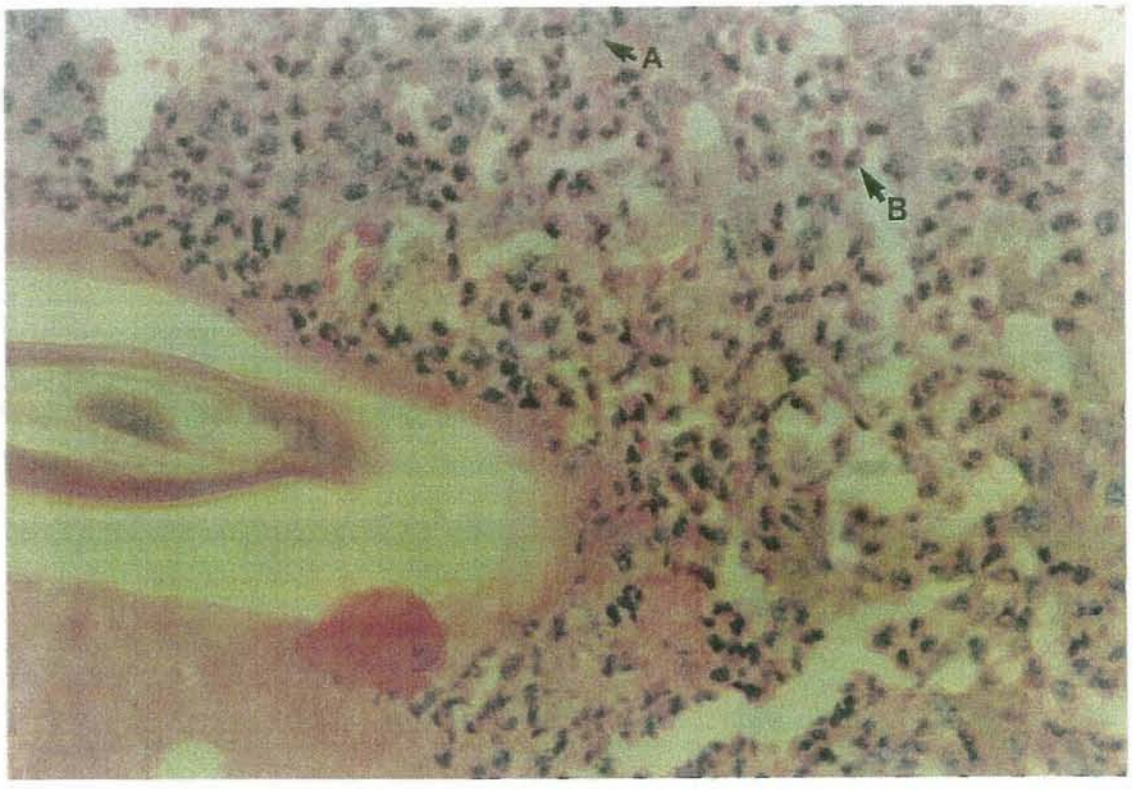


Figure 4.15. Section of same lesion shown in Figure 4.14 showing adult *Stephanofilaria* sp. within a cyst and surrounded by (A) histiocytes and (B) eosinophils. Haematoxylin and eosin x 560.

Figure 4.16. Section of a stephanofilarial lesion with ulcerated surface showing focal loss of epidermis and heavy neutrophil infiltration (arrowed). x 25.


A
B

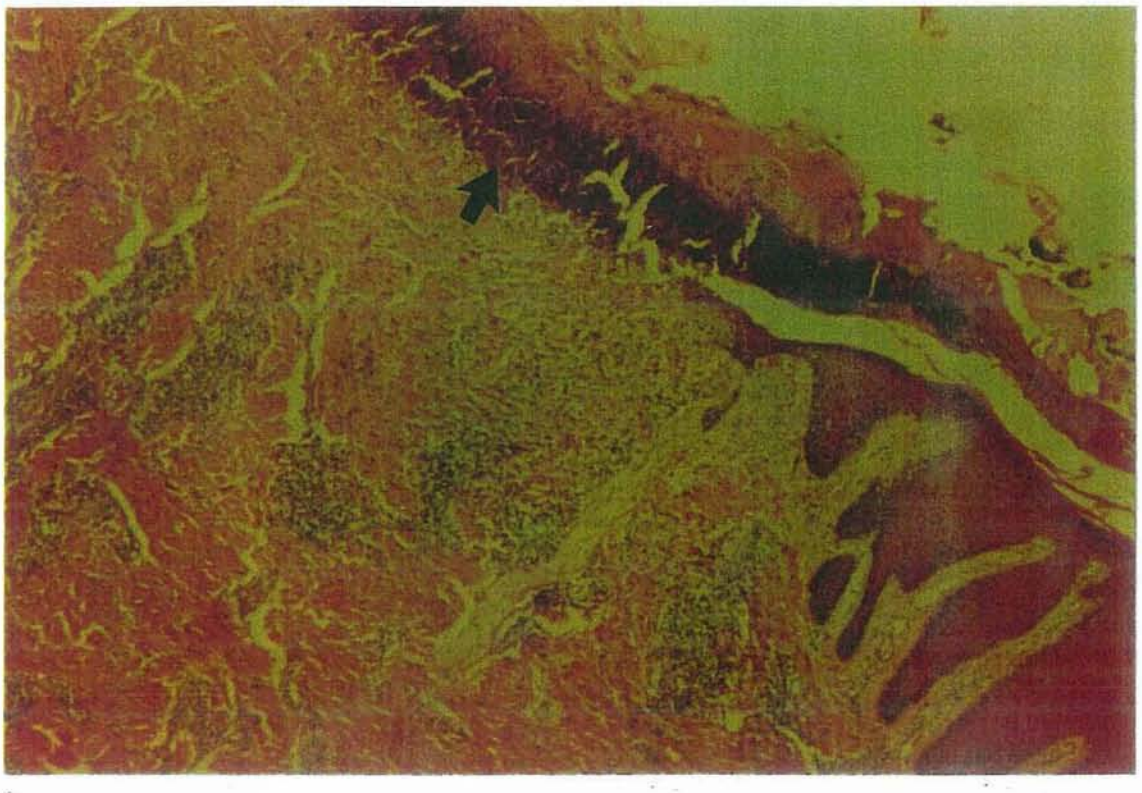
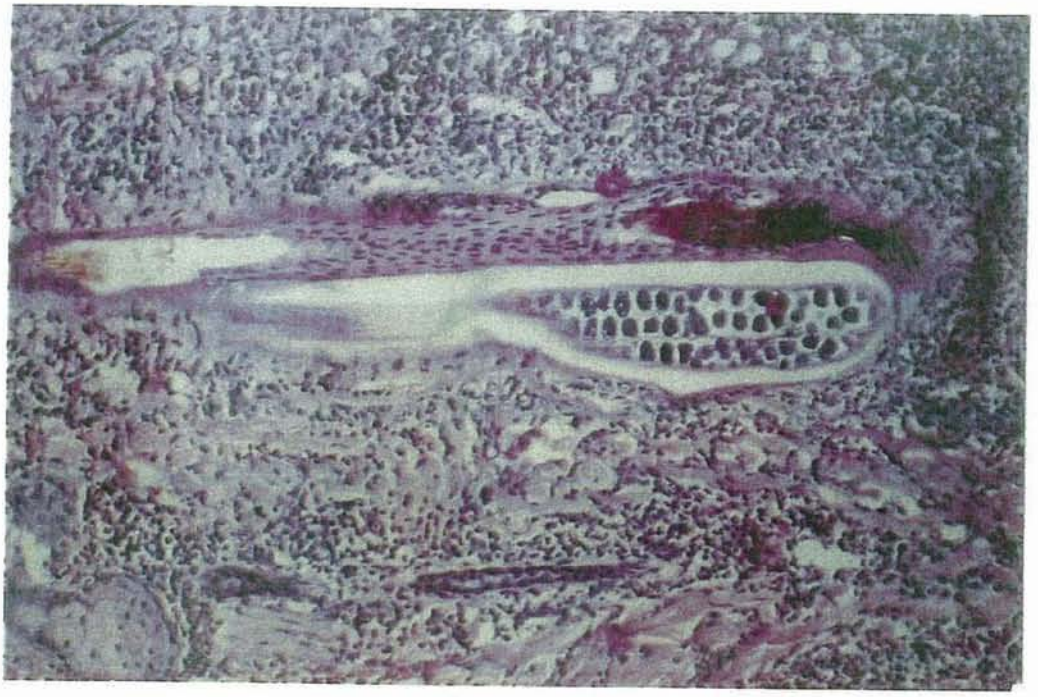


Figure 4.17. Section of skin with adult *Stephanofilaria* sp. adjacent to hair shaft. Haematoxylin and eosin x 240.

Figure 4.18. Section of skin with adult *Stephanofilaria* sp. within the epidermis. Haematoxylin and eosin x 300.



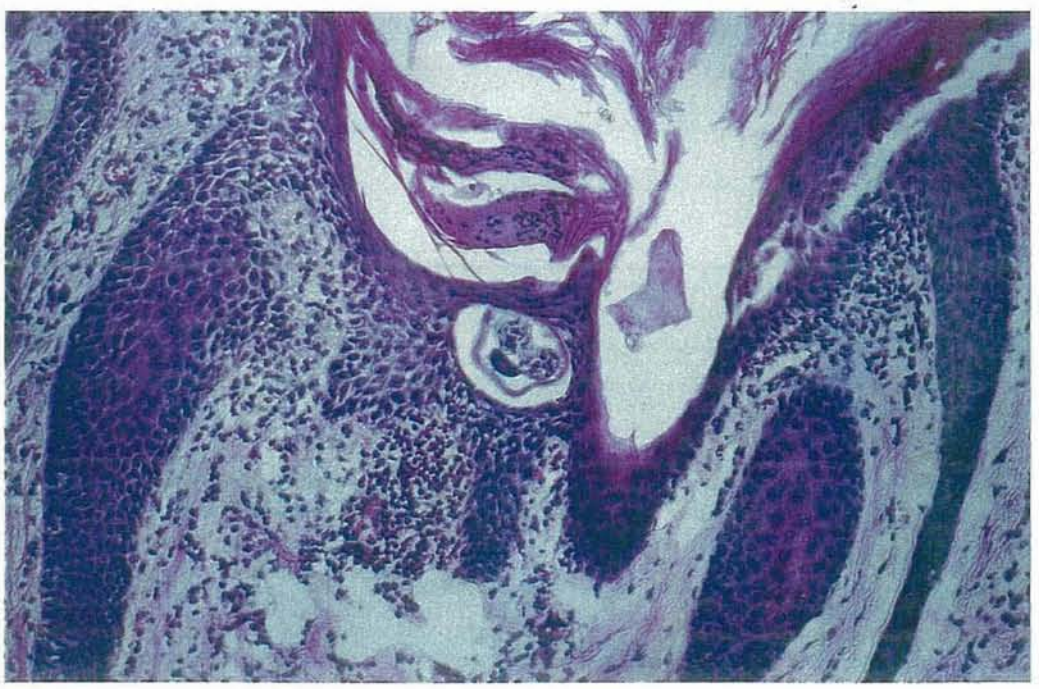
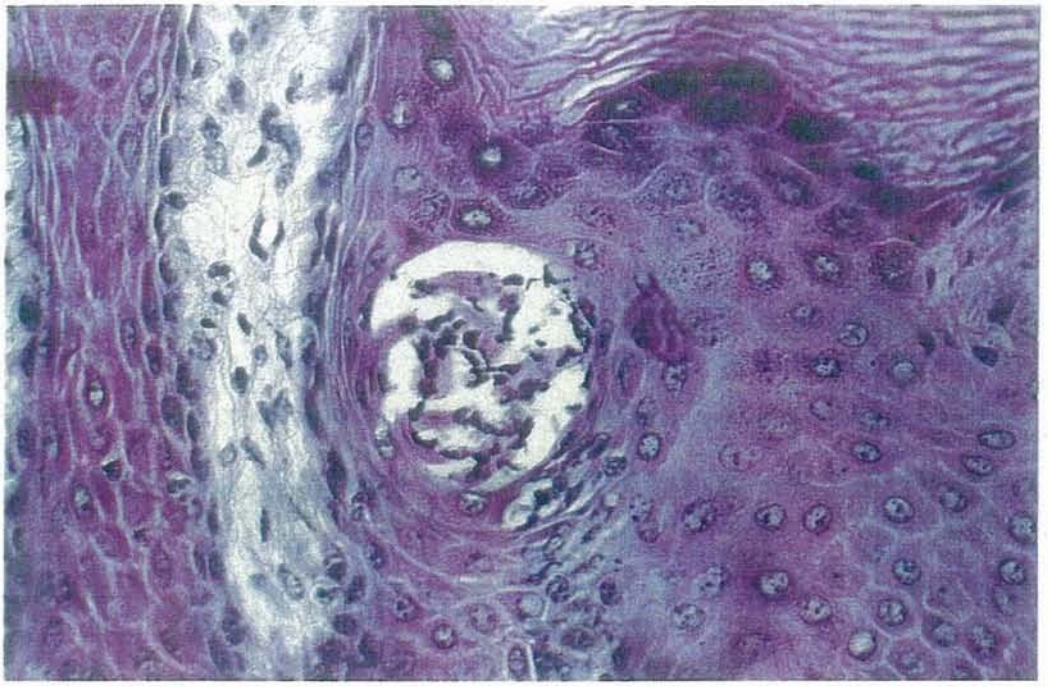


Figure 4.19. Section of skin with necrotic tract in superficial epidermis. Haematoxylin and eosin x 500.

Figure 4.20. Section of skin with microfilariae (arrowed) of *Stephanofilaria* sp. in superficial areas of dermal papillae and epidermis. Haematoxylin and eosin x 160.



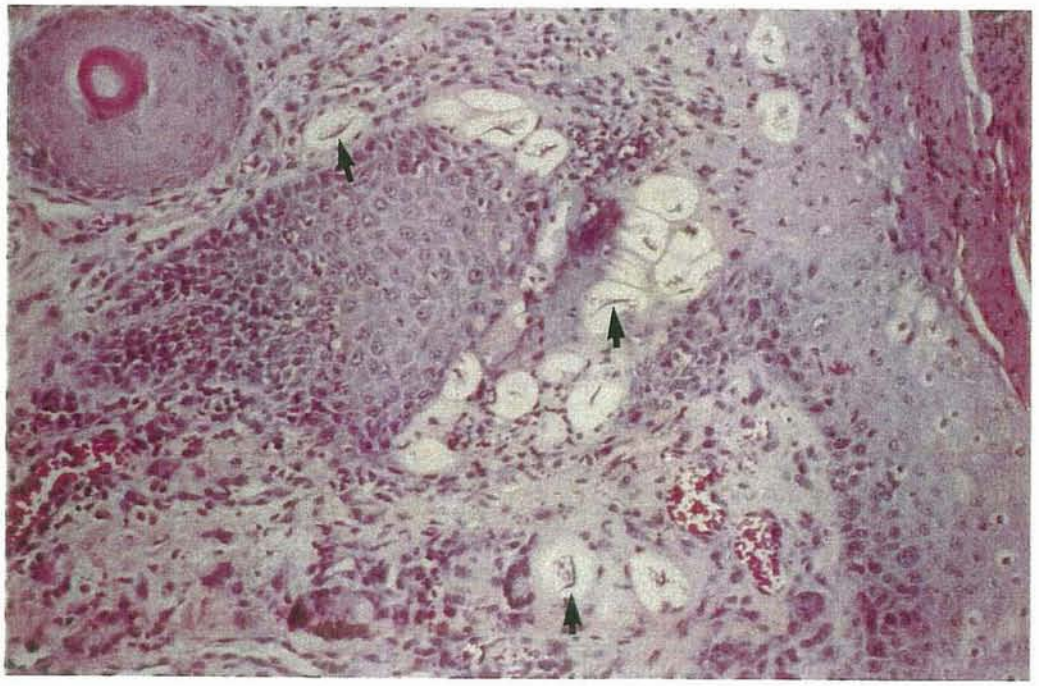
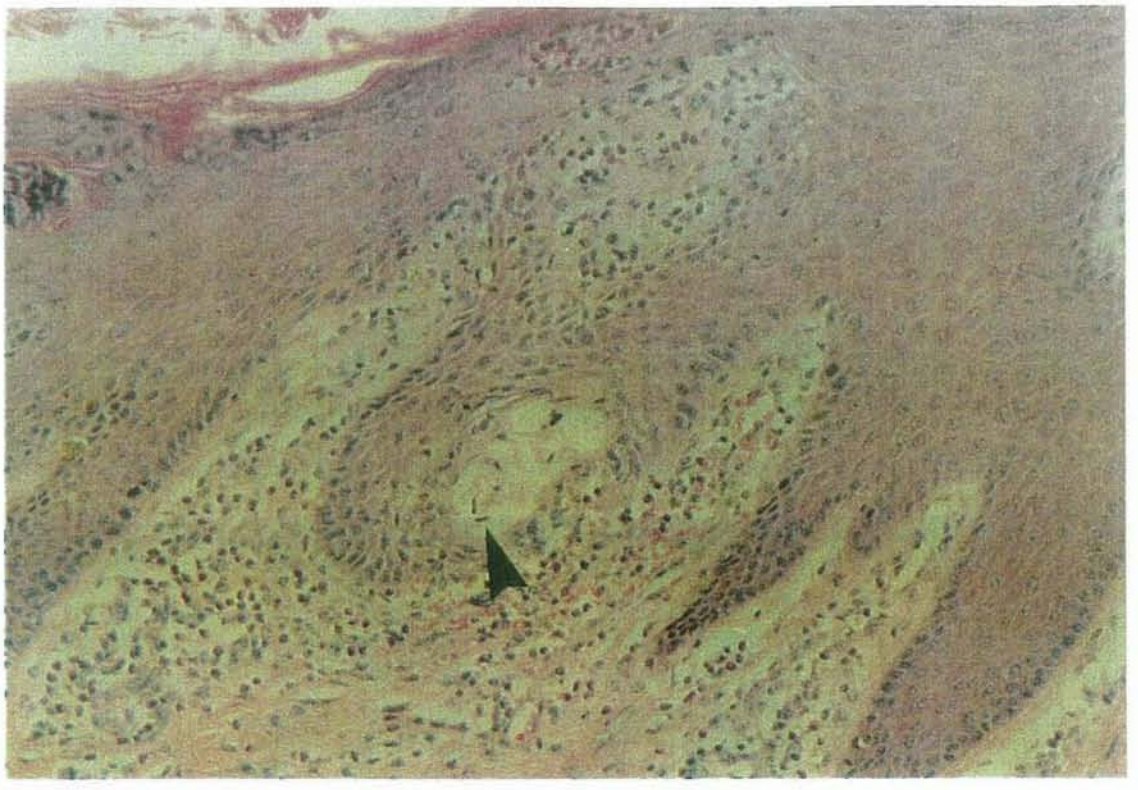


Figure 4.21. Section of skin with microfilariae (arrowed) of *Stephanofilaria* sp. within the epidermis of a rete peg. Haematoxylin and eosin x 160. The presence of numerous eosinophils is probably in response to the recent migration of adult/larval parasites as evidenced by the remnants of necrotic tracts (a) in the epidermis.

Figure 4.22. Microfilariae of *Stephanofilaria* sp. (A) with numerous spherical PAS positive bodies and adjacent to superficial capillaries (B). P.A.S. x 600.



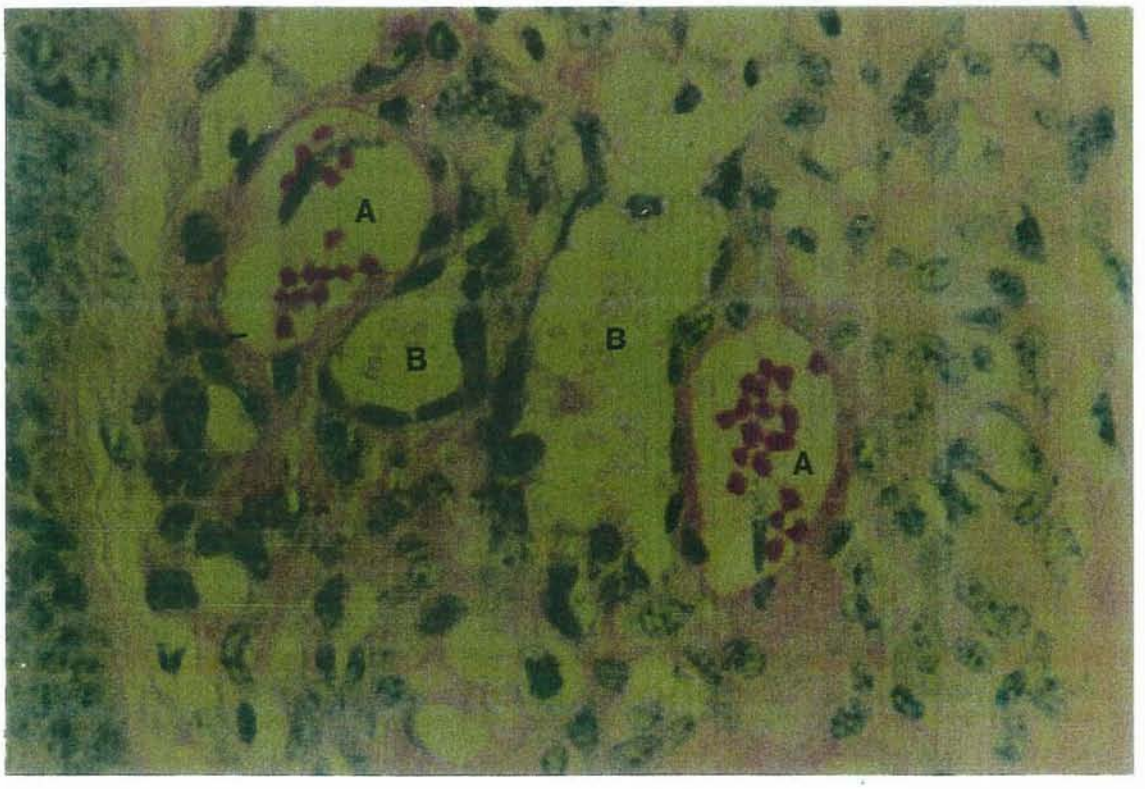
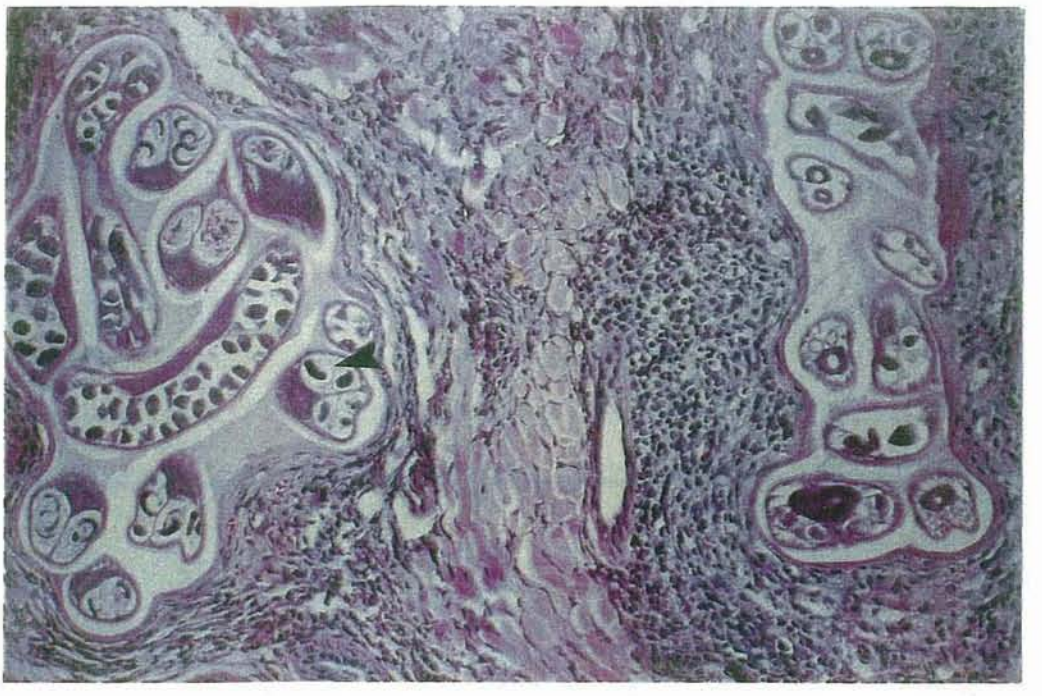

A
B
B
A

Figure 4.23. Cross sections of gravid female *Stephanofilaria* sp. showing microfilariae enclosed within vitelline membranes (arrowed) inside the uteri. Haematoxylin and eosin x 220.

Figure 4.24. Section of skin with microfilariae (arrowed) of *Onchocerca* sp. lying free within the superficial dermis. Haematoxylin and eosin x 300.



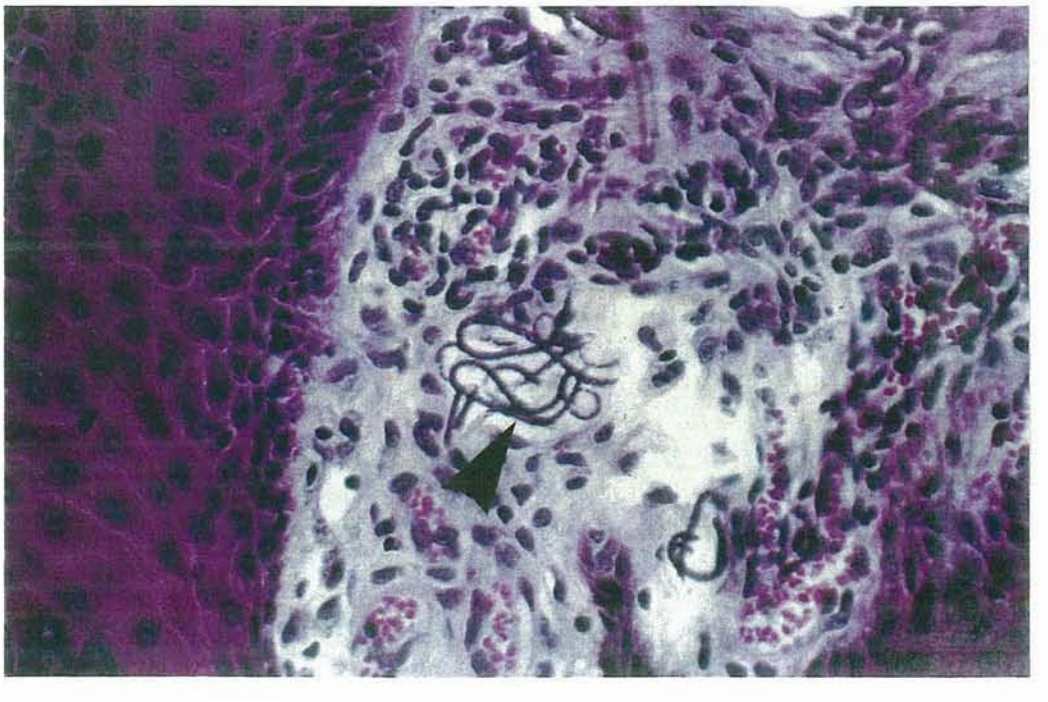
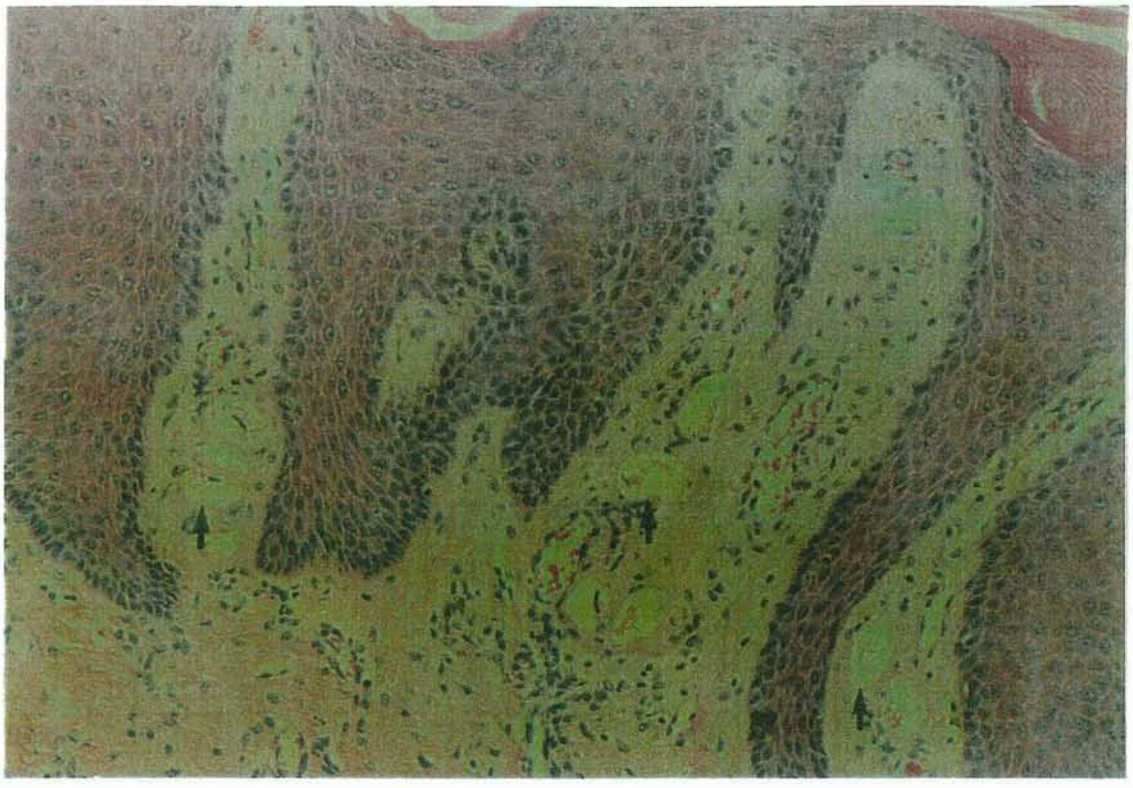


Figure 4.25. Minimal inflammatory cell reaction to the presence of microfilariae of *Stephanofilaria* sp. (arrowed) within superficial dermis. Haematoxylin and eosin x 180.

Figure 4.26. Section of a stephanofilarial lesion in which parasites were not detected. Histological changes evident are hyperkeratosis, acanthosis, extensive inflammatory cell infiltration and loss of hair follicles and sebaceous glands. x 30.



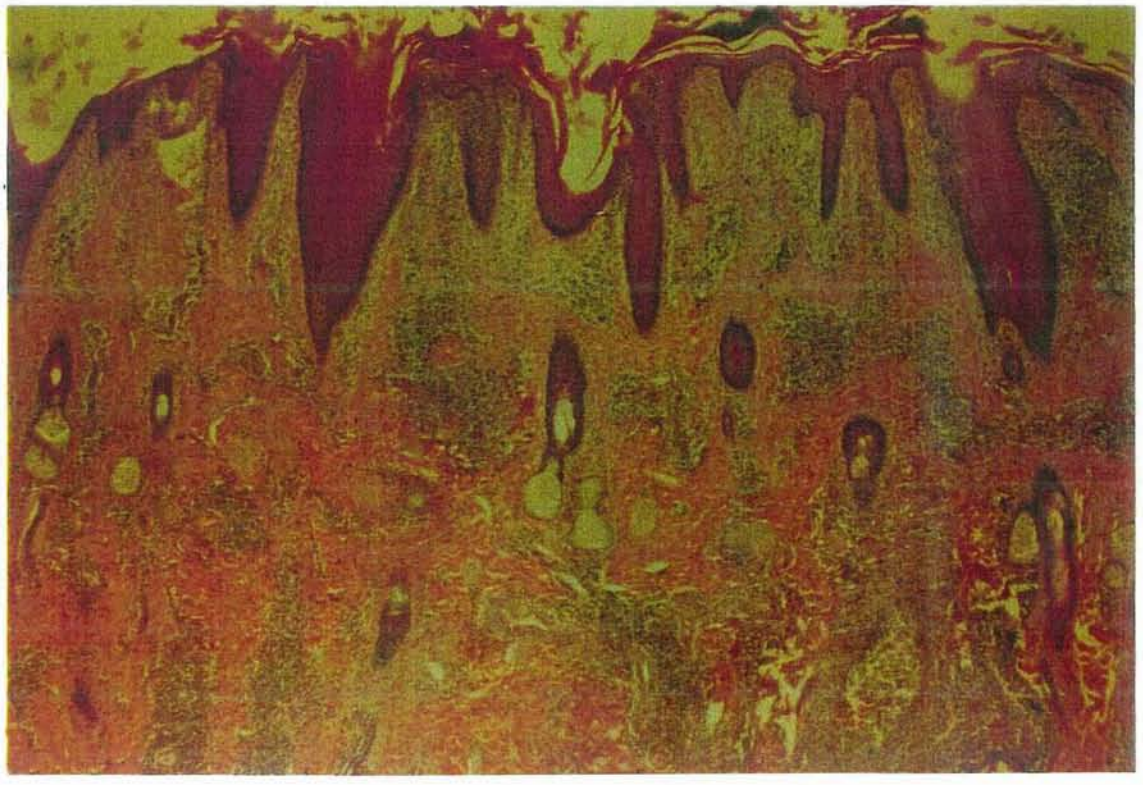
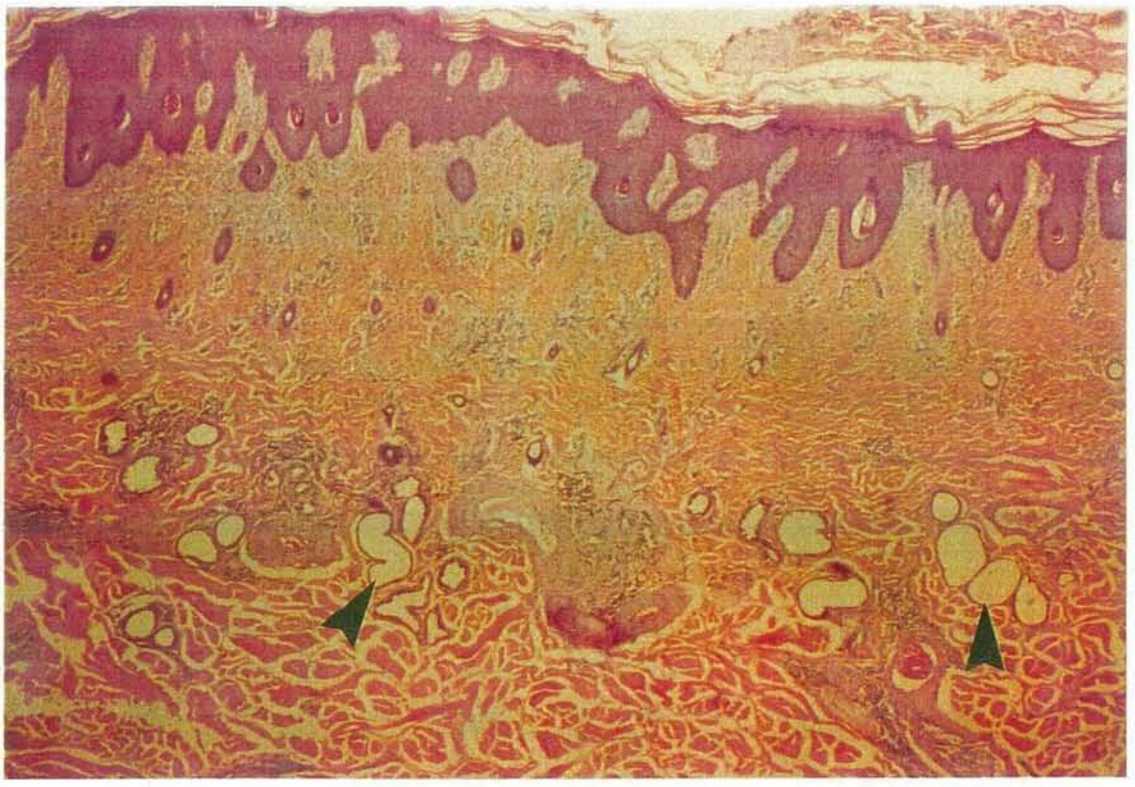


Figure 4.27. Section of a lesion showing acanthosis, absence of hair follicles and sebaceous glands, minimal inflammatory cell infiltration but retention of aprocrine sweat glands (arrowed). The changes suggest that the lesion was probably stephanofilarial in origin. x 25.

Figure 4.28. Section of a lesion showing thickened epidermis, fibrosis of superficial dermis and complete absence of hair follicles and sebaceous glands. The changes suggest that the lesion was probably stephanofilarial in origin. x 25.



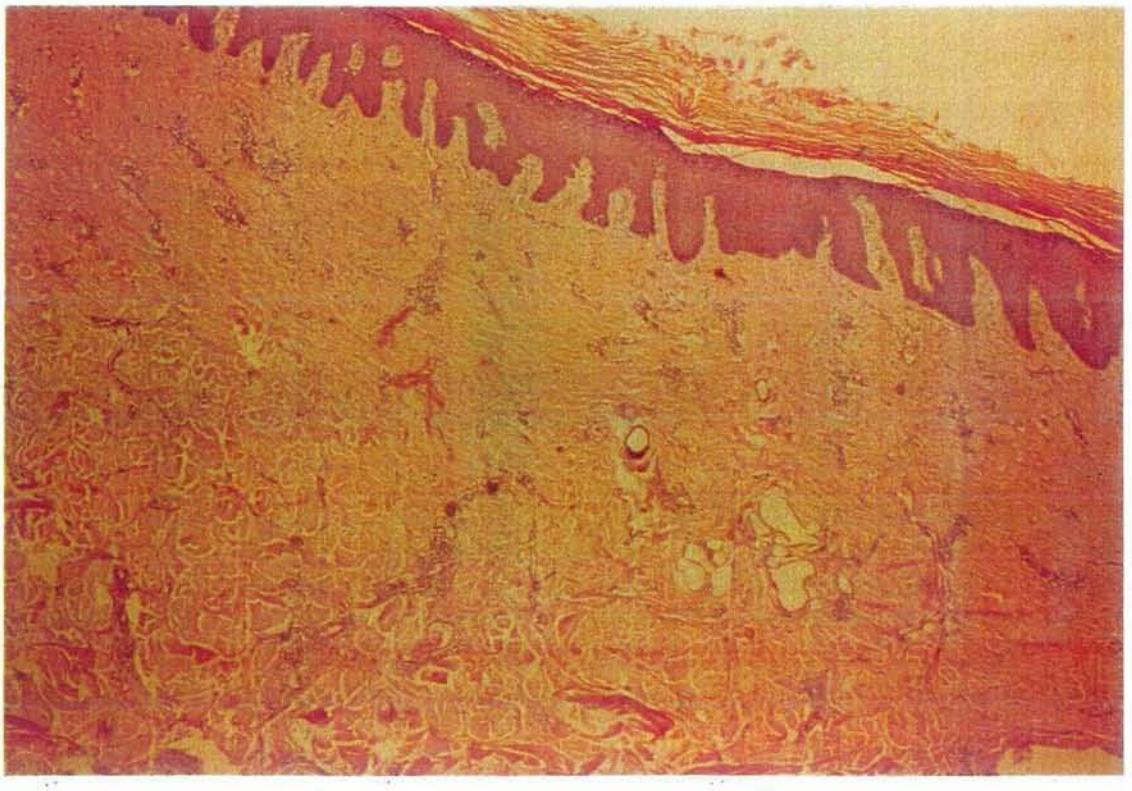
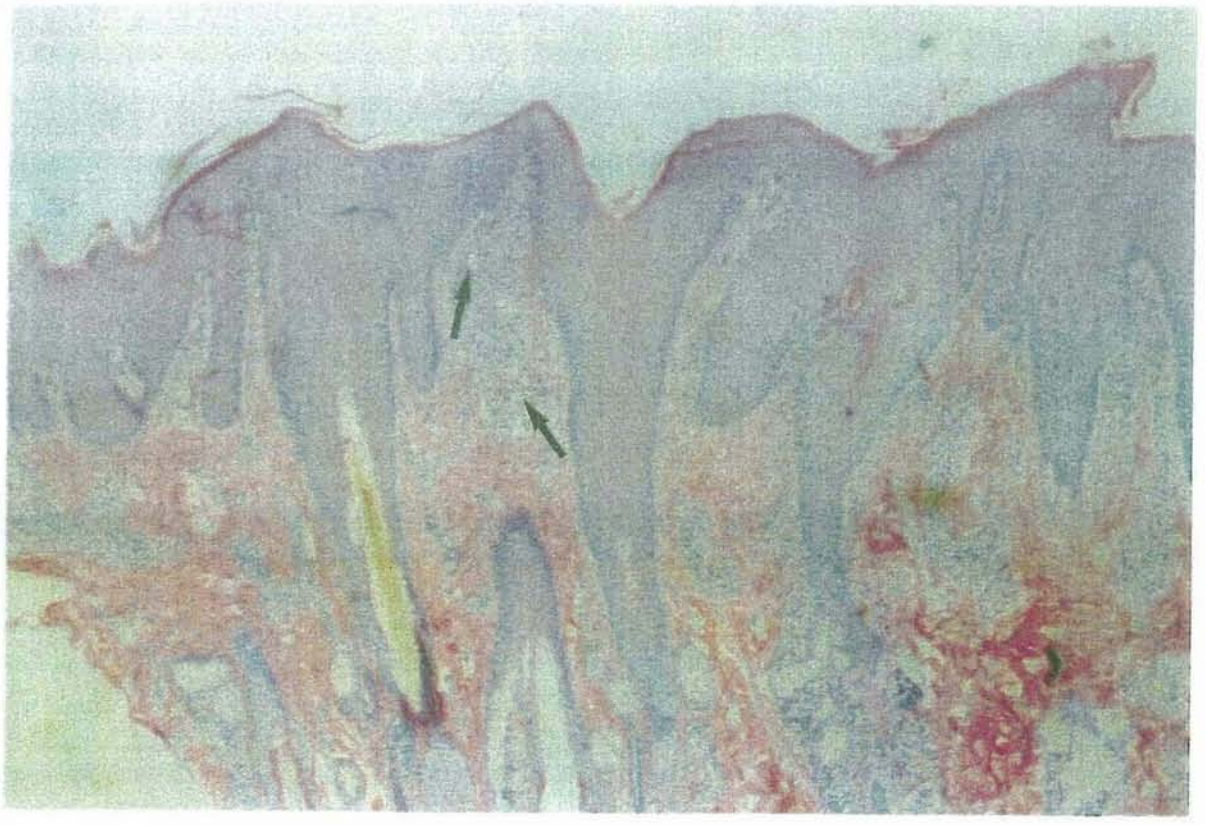


Figure 4.29. Section of stephanofilarial lesion 36 days after feeding infected *H. i. exigua* (see 6330). Note the intact epidermis, marked acanthosis, intact hair follicles, infiltration by inflammatory cells and presence of microfilariae (arrowed). Examination at higher power revealed that the inflammatory cells were mononuclear. x 25.

Figure 4.30. Section of the same stephanofilarial lesion 36 days after feeding infected *H. i. exigua* showing marked acanthosis and early necrosis of a hair follicle (arrowed). (a) adult parasite (b) necrotic tract (c) hair shafts. x 25.



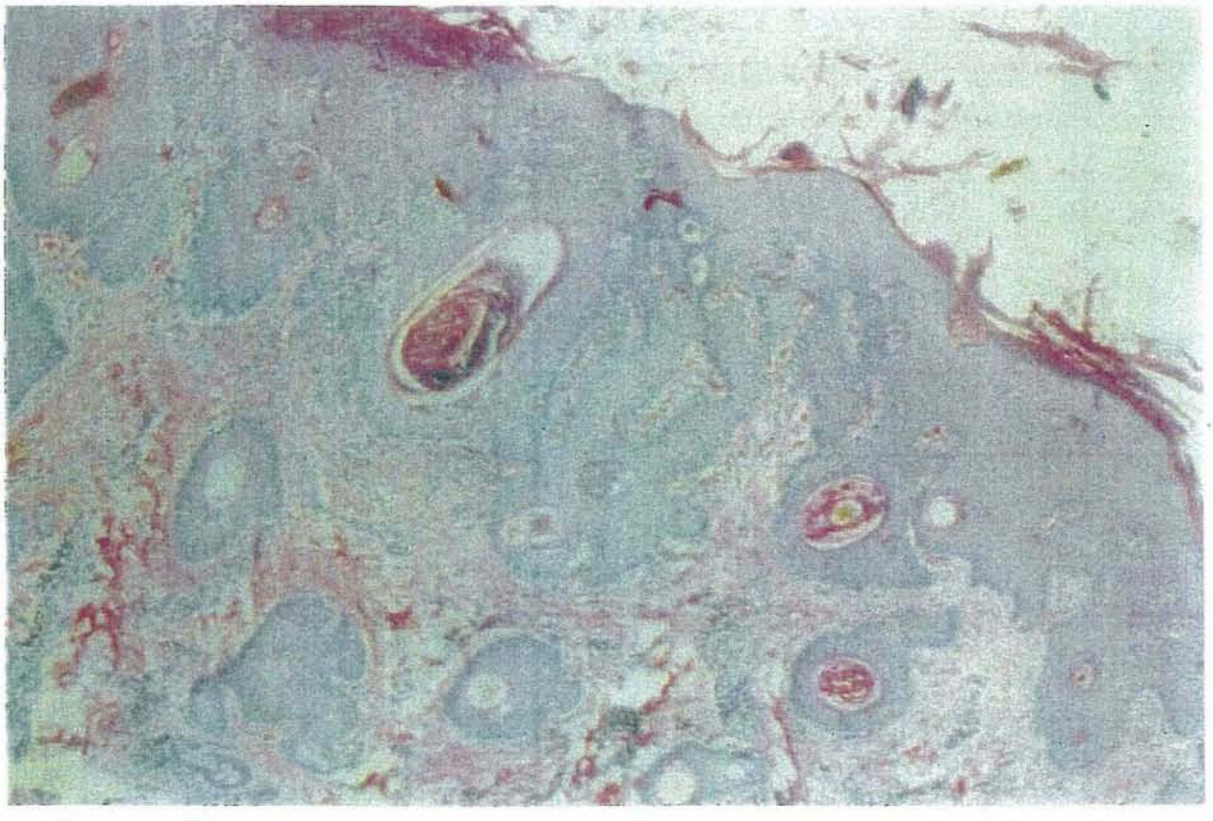
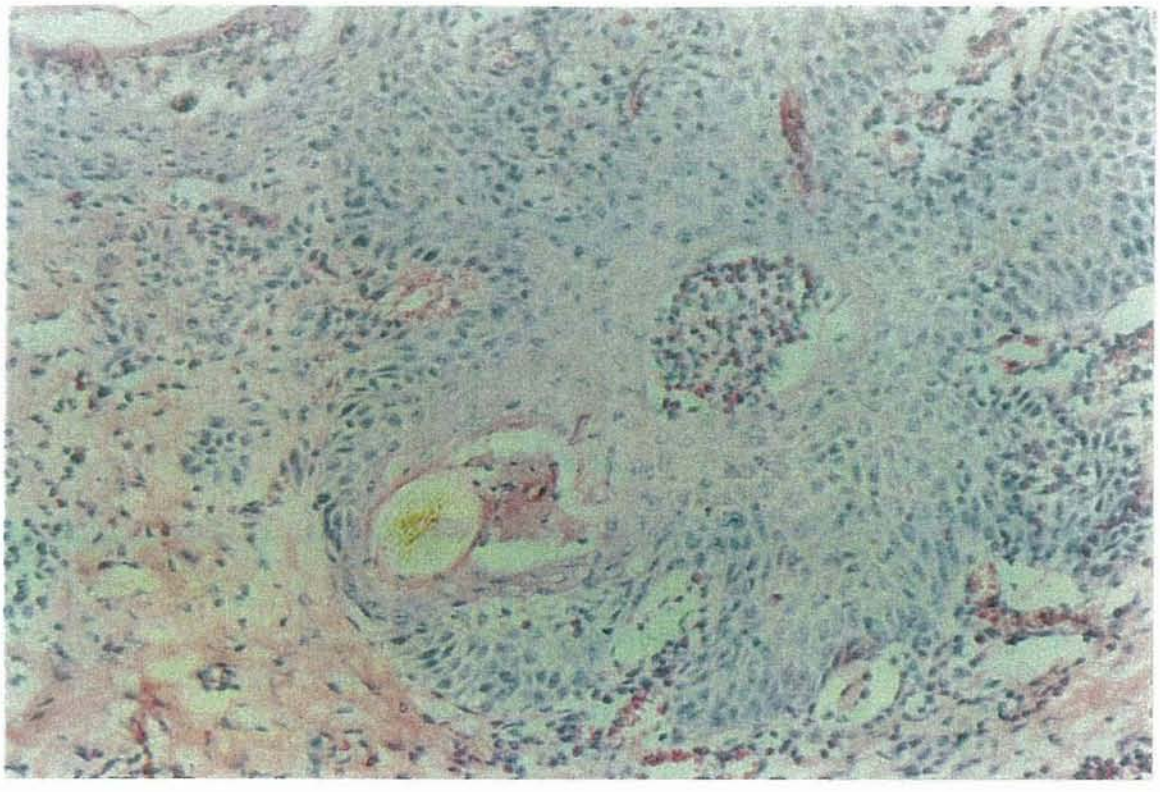


Figure 4.31. Higher power view of the same lesion (see 6330) showing early hair follicle destruction. Note the necrotic tract containing eosinophils and the cellula debris and early necrosis of germinal matrix cells surrounding the hair shaft. x 220.

Figure 4.32. Oblique section of the same stephanofilarial lesion (see 6330) 36 days after feeding infected *H. i. exigua* showing more advanced destruction of a hair follicle and sebaceous glands. (a) intact hair follicle and sebaceous glands (b) necrotic hair follicle surrounded by histiocytes, monocytes and lymphocytes. x 25.



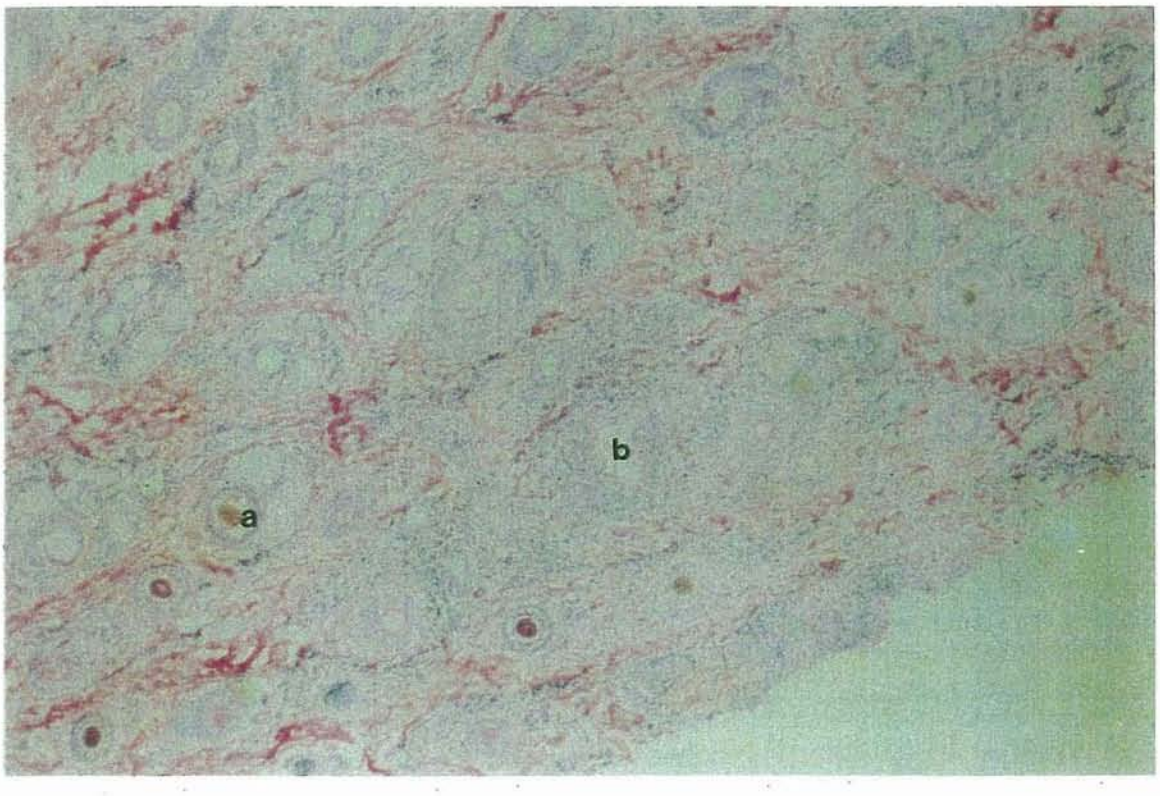
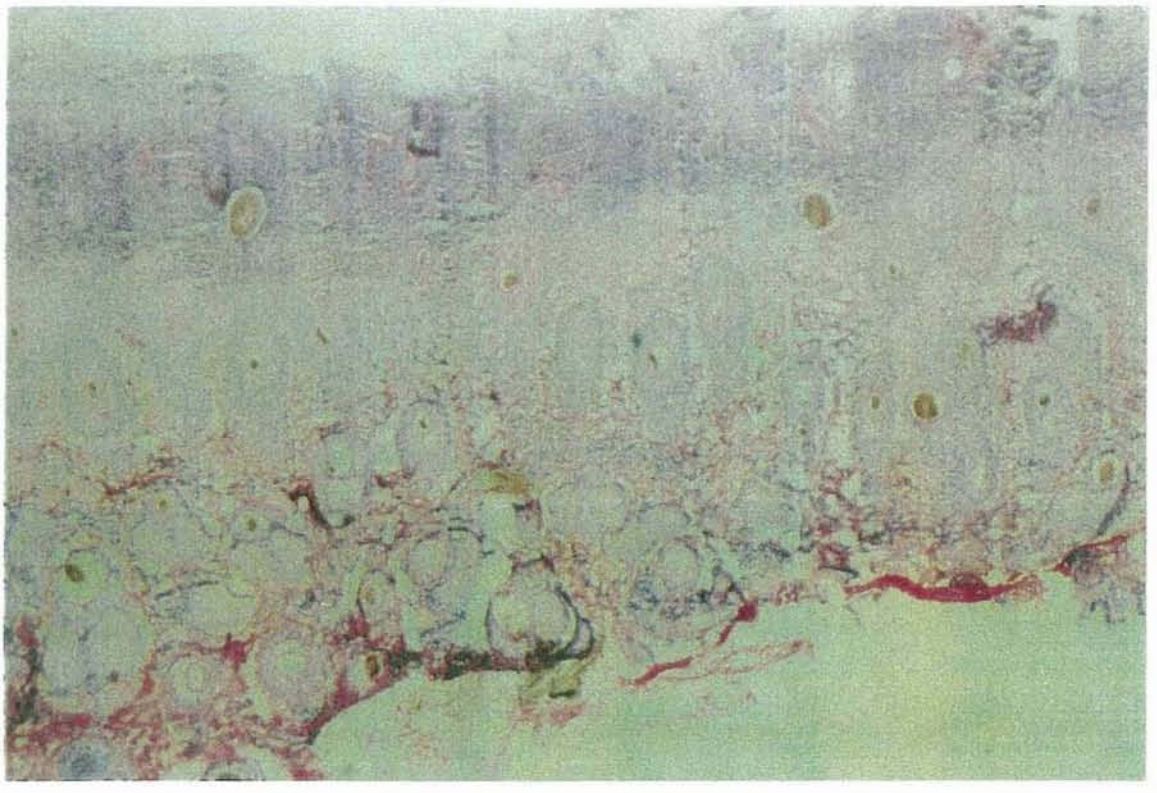
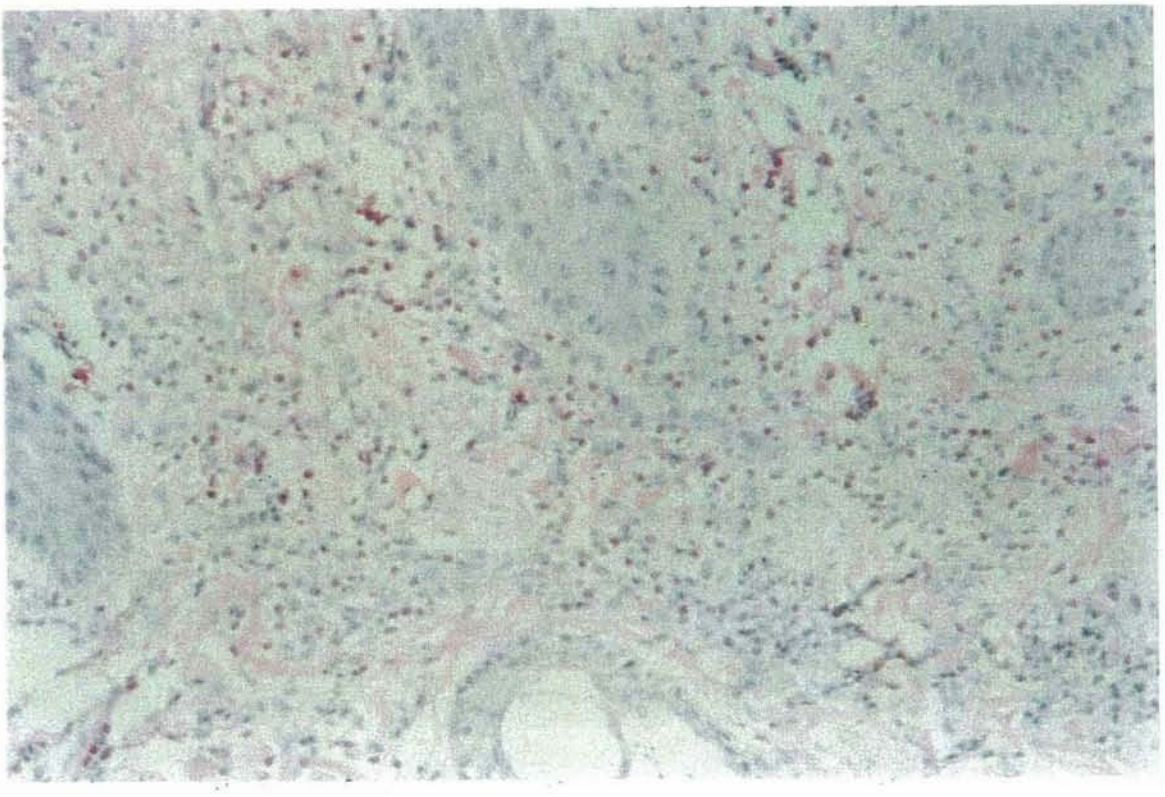

b
a

Figure 4.33. Section of a lesion produced by feeding 1000 2000 uninfected *H. i. exigua* on a steer for 35 days (see 4220). Note the loss of epidermis, superficial encrustation and intact adnexa. x 25.

Figure 4.34. Section of the same lesion shown in figure 4.33 showing predominance of eosinophils in superficial dermis. x 250.





5000 EPIDEMIOLOGICAL STUDIES

5100 INTRODUCTION

During the abattoir survey (3000), few animals less than 18 months of age were examined, because most of the abattoirs used in the survey killed exclusively adult cattle. Breed was assessed visually to indicate Brahman content but separation of animals into Brahman and crossbred types was often imprecise. Because of these deficiencies, observations were made on properties to complement the abattoir survey by monitoring development of dermal stephanofilarial lesions in young animals and in animals of known genotype. Consequently, the coat colour of individual animals was recorded to assess whether some of the differences in prevalence of lesions had resulted from differences in vector feeding preference influenced by coat colour.

5200 MATERIALS AND METHODS

5210 Animals

Bos indicus calves of known genotype were examined before and after weaning at Swans Lagoon beef cattle research station and Expedition, which are adjoining properties situated 100 km south of Townsville.

5211 Swans Lagoon

Before weaning the calves were run with their dams in herds of mixed genotypes and after weaning all calves were

run together. Five genotypes were present, namely 1/2 Brahman (50% Brahman - 50% Shorthorn), 3/4 Brahman (75% Brahman - 25% Shorthorn), 1/2 Sahiwal (50% Sahiwal - 50% Shorthorn), 3/4 Sahiwal (75% Sahiwal 25% Shorthorn) and High Grade Sahiwal (93% Sahiwal - 7% Shorthorn).

Animals were classified into 3 groups based on coat colour, namely light (white, grey and yellow), medium (red and light brown) and dark (brown, dark red and black). Cattle with more than one colour were classified on the basis of the predominant colour.

Cattle were bred as single sire groups and mating was restricted to produce births in the months November to January. All calves were weaned in early June at between 4 and 7 months of age except in 1983 when, because of adverse pasture conditions, the calves were weaned earlier than usual in mid April.

5212 ***Expedition***

On this property the calves were all 3/4 Brahman and were bred in multiple sire groups. The calves examined in this study were born between 12 June and 12 July 1982 and were unweaned during the period of observations.

5220 ***Recovery of parasites***

Lesions developing adjacent to the medial canthus of the eye were sampled by surgically removing a section of skin 3-4 mm thick and 5-10 mm square from the centre of the lesion. These biopsy samples were digested in 3.0% collagenase for 24 hours at 37^{o}C and the residue was scanned under low power magnification for the presence of only adult parasites. For recovery of microfilariae, the digest residue was sieved through a 0.15mm diameter mesh to remove larger debris, then concentrated

by centrifugation at 400 "g" for 3 minutes. The resulting pellet was layered onto the surface of a column of two discontinuous Percoll gradients. The Percoll was mixed with 2.5 M sucrose to give solutions of 5% Percoll (density 1.037) and 10% Percoll (density 1.055). The column containing the digest residue was centrifuged for 15 minutes at 400 "g". The band at the interface of the 5% and 10% Percoll layers was collected with a fine pipette. resuspended in distilled water, then centrifuged at 400 "g" for 3 minutes. The resulting pellet was placed on a slide . under a coverslip and examined for the ovoid eggs containing microfilariae that are characteristic for *Stephanofilaria* spp. The sensitivity of this technique was not quantitated and, therefore, the limitations of the technique were never established. The technique was used only as a diagnostic aid in that it concentrated the eggs thereby enabling a more rapid diagnosis of lesions as infected or uninfected with *Stephanofilaria* sp.

*5230 **Statistical analysis***

The data were analysed using prevalence of lesions as the dependent variable. In the first year at Swans Lagoon and all observations at Expedition, prevalence of lesions between the genotypes and coat colours was compared using a chi squared test. In the remaining 4 years of the Swans Lagoon data the method used was analysis of variance (Harvey, 1982) of prevalence of lesions (1 = lesions present, 0 = lesions absent) using the following independent (predictor) variables and their interactions;

sex	(1 = male, 0 otherwise)
breed 1	(1 = 1/2 Brahman, 0 otherwise)
breed 2	(1 = 3/4 Brahman, 0 otherwise)
breed 3	(1 = 1/2 Sahiwal, 0 otherwise)
breed 4	(1 = 3/4 Sahiwal, 0 otherwise)
colour 1	(1 = light, 0 otherwise)
colour 2	(1 = medium, 0 otherwise)
birth date	(days from January 1)
birth weight	
weaning date	(days from January 1)
weaning weight	
average daily gain	(birth to weaning)

The selection of the "best" set of independent variables was facilitated by the use of a stepwise regression procedure (McCullagh and Nelder, 1983). Analysis, using the general purpose statistical package, GLIM (Baker and Nelder, 1978), was carried out for each set of lesion prevalence data (each year) and associated independent variables.

5300 RESULTS

5310 Development of lesions

Examination of the calves revealed that initial stephanofilarial lesions developed in the skin adjacent to the medial canthus of the eye and were usually bilateral. Over the 5 years, a total of 1612 calves with ages ranging from 2 to 12 months, was examined and only eye lesions were found.

5320 Diagnosis of lesions

A small number of animals from Swans Lagoon was selected at random in each of the years 1983, 1984 and 1985, and a stephanofilarial lesion from each animal was biopsied and examined for the presence of only adult parasites. In 1983. 20 animals were sampled and adult parasites were recovered from 15 (75%). In 1984 and 1985 those lesions in which adults were not found were screened for presence of microfilariae of *Stephanofilaria* sp. with the result that in 1984 93% (14/15) contained parasites and in 1985, 87.5% (7/8) contained parasites.

5330 Prevalence of lesions - Swans Lagoon

Prevalence of lesions was not affected by birth weight, weaning date, weaning weight or average daily gain in any of the years

5331 1981

An opportunity was taken to examine the 1981 calf crop on 10 November 1981 when the animals were between 9 and 12 months of age. The overall prevalence of ocular stephanofilarial lesions was high with 161 (71%) of the 227 animals examined having lesions. Interestingly, the prevalence of lesions between the genotypes differed significantly. Within the Brahman (B) genotype, 81% of the 1/2 B group had lesions but only 48% of the 3/4 B group had lesions and in the Sahiwal (S) genotype, 91% and 92% of the 1/2 S and 3/4 S groups respectively had lesions but only 59% of the high grade Sahiwal (>7/8 S) group had lesions (Figure 5.1).

Prevalence of infection did not differ between the 1/2 B, 1/2 S and 3/4 S groups (X^2_2 = 1.01, P > 0.05) or between the 3/4 B and 7/8 S groups (X^2_1 = 1.27, P > 0.05). There was, however, a large difference between the 2 sets of groups (X^2_1 = 38.87, P < 0.01).

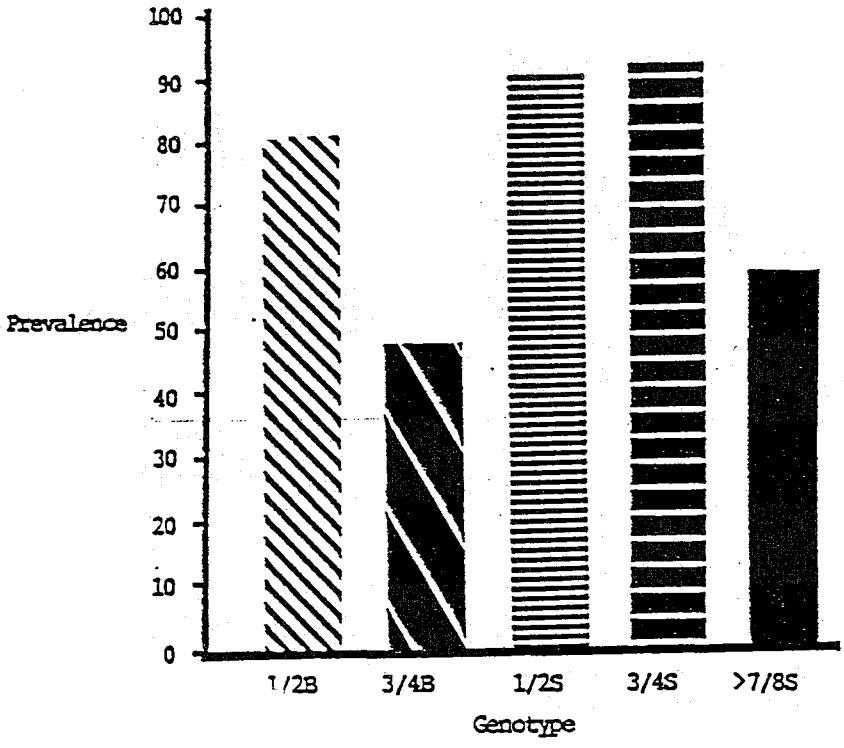


Figure 5.1. Prevalence of stephanofilarial lesions in relation to genotype of *Bos indicus* calves 9 to 12 months of age at Swans Lagoon in 1981. B = Brahman, S = Sahiwal.

5332 1982

Sixty-four unweaned calves from the 1982 calf crop were examined on 23 April, 1982 and animals as young as 80 days of age had ocular lesions of stephanofilariasis. The calves were weaned on 1 June and all 244 were examined for lesions 9 days later when their average age was 181±33(SD) days. The model of prevalence of lesions was as follows,

$$\text{Prevalence of lesions} = \underset{(\text{S.E. } 0.289)}{1.53} + \underset{(\text{S.E. } 0.0938)}{0.296\ \text{breed 1}} + \underset{(\text{S.E. } 0.0904)}{0.0024\ \text{breed 2}} + \underset{(\text{S.E. } 0.0913)}{0.324\ \text{breed 3}} + \underset{(\text{S.E. } 0.1032)}{0.338\ \text{breed 4}} - \underset{(\text{S.E. } 0.000758)}{0.00266\ \text{birth date}}$$

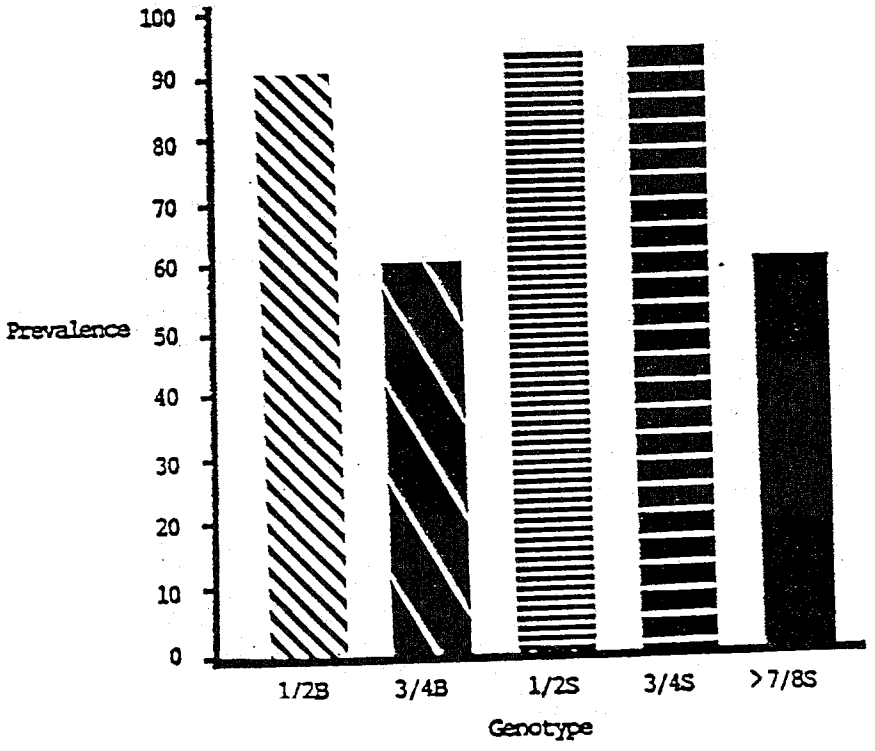


Figure 5.2. Prevalence of stephanofilarial lesions in relation to genotype of *Bos indicus* calves aged 181±33(SD) days at Swans Lagoon in 1982. B = Brahman, S = Sahiwal.

The goodness of fit of the model was 21.7%. Values of the predicted prevalence are shown in Figure 5.2. As in 1981, the prevalence of lesions of the 3/4 B and >7/8 S groups were similar and differed significantly from that of the 1/2 B, 1/2 S and 3/4 S groups. Prevalence did not differ with sex or coat colour.

5333 1983

Two hundred and seven unweaned calves from the 1983 calf crop were examined on 1 April 1983 and animals as young as 69 days were found with ocular lesions of stephanofilariasis. The calves were weaned on 18 April and all 480 were examined for stephanofilarial lesions on 11 August when their average age was 257±21(SD) days. The model of the prevalence of lesions was as follows,

$$\begin{aligned}\text{Prevalence of lesions} = {} & 0.909 \ (\text{S.E. } 0.273) \\ & + 0.334 \ \text{breed 1} \ (\text{S.E. } 0.066) \\ & + 0.316 \ \text{breed 2} \ (\text{S.E. } 0.068) \\ & + 0.317 \ \text{breed 3} \ (\text{S.E. } 0.064) \\ & + 0.250 \ \text{breed 4} \ (\text{S.E. } 0.065) \\ & + 0.116 \ \text{sex} \ (\text{S.E. } 0.033) \\ & - 0.294 \ \text{colour 1} \ (\text{S.E. } 0.058) \\ & - 0.0052 \ \text{colour 2} \ (\text{S.E. } 0.0452) \\ & - 0.00111 \ \text{birth date} \ (\text{S.E. } 0.00078)\end{aligned}$$

The goodness of fit of the model was 16.7%. Males had a significantly higher prevalence than females in all genotypes and coat colours ($P < 0.01$). The >7/8 S group had significantly lower prevalence of lesions than the other genotypes, independently of sex or coat colour ($P< 0.01$). Within each genotype light coloured animals had a lower prevalence of lesions than medium or dark coloured animals ($P< 0.01$). Prevalence of lesions in relation to genotype, sex and coat colour are shown in Table 5.1.

Table 5.1. Prevalence of stephanofilarial lesions in relation to genotype, sex and coat colour of *Bos indicus* calves aged 257±21(SD) days at Swans Lagoon in 1983.

	MALE			FEMALE		
	Coat Colour			Coat Colour		
	Light %p*	Medium %p	Dark %p	Light %p	Medium %p	Dark %p
GENOTYPE						
1/2 Brahman	69.7	98.6	99.1	58.1	87.0	87.5
3/4 Brahman	67.9	96.8	97.3	56.3	85.2	85.7
1/2 Sahiwal	68.0	96.9	97.4	56.4	85.3	85.8
3/4 Sahiwal	61.3	90.2	90.7	49.7	78.6	79.1
>7/8 Sahiwal	-	65.2	65.7	-	53.6	54.1

* values presented are mean values adjusted for other variables

5334 1984

Two hundred and five unweaned calves of the 1984 calf crop were initially examined on 16 April and the youngest animal found with ocular lesions of stephanofilariasis was 72 days old. All 222, 1984 calves were weaned on 5 June and examined for lesions when their average age was 170±23(SD) days. The model of prevalence of lesions was as follows,

Prevalence of lesions = 1.452 (S.E. 0.444) + 0.457 breed 1 (S.E. 0.097) + 0.202 breed 2 (S.E. 0.0945)

+ 0.371 breed 3 (S.E. 0.0949) + 0.230 breed 4 (S.E. 0.0981) - 0.002593 birth date (S.E. 0.00122)

The goodness of fit of the model was 14.3%. Prevalence of lesions was not affected by sex or coat colour and, as in previous years, the 3/4 B and >7/8 S groups had lower prevalence of lesions than the other groups. Prevalence of lesions values in relation to genotype are shown in figure 5.3.

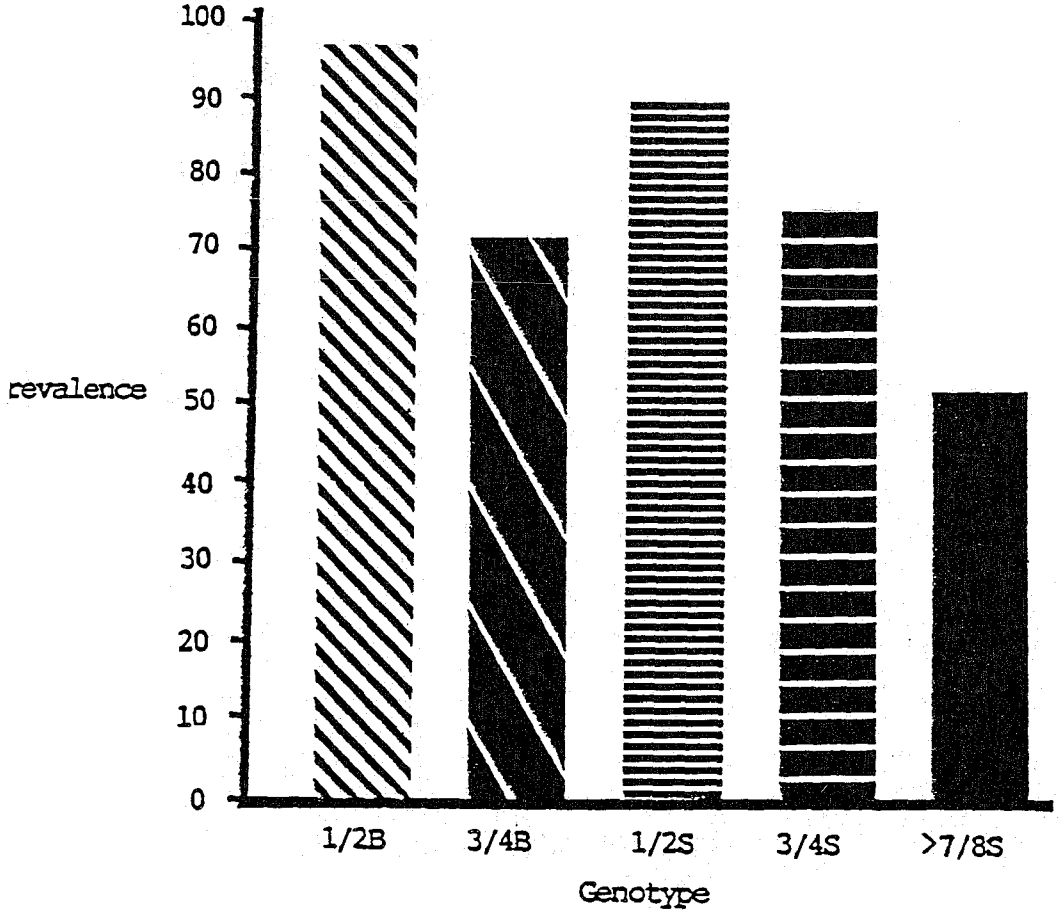
5335 1985

The 390, 1985 calves were weaned on 3 June and examined for ocular stephanofilarial lesions on 30 July when their average age was 240±22(SD) days. The model of prevalence of lesions was as follows,

Prevalence of lesions = 1.530 (S.E. 0.248) + 0.108 breed 1 (S.E. 0.06) - 0.00229 breed 2 (S.E. 0.063)

+ 0.107 breed 3 (S.E. 0.057) + 0.0392 breed 4 (S.E. 0.064) - 0.251 colour 1 (S.E. 0.048)

- 0.0622 colour 2 (S.E. 0.0396) - 0.00191 birth date (S.E. 0.0645)



igure 5.3. Prevalence of stephanofilarial lesions in relation to genotype of *Bos indicus* calves 170±23(SD) days old at Swans Lagoon in 1984. B = Brahman, S = Sahiwal.

'able 5.2. Prevalence of stephanofilarial lesions in relation to genotype and coat colour of *Bos indicus* calves 240± 22(SD) days old at Swans Lagoon in 1985.

	COAT COLOUR		
	Light %p*	Medium %p	Dark %p
GENOTYPE			
1/2 Brahman	74.6	93.5	99.7
3/4 Brahman	63.6	82.5	88.7
1/2 Sahiwal	74.5	93.4	99.6
3/4 Sahiwal	67.7	86.6	92.8
>7/8 Sahiwal	-	82.7	88.9

* figures presented are mean prevalence values (%p) adjusted for other variables

The goodness of fit of the model was 14.7%. Prevalence of lesions was not affected by sex of the animals. Light coloured animals had significantly fewer lesions than medium or dark coloured animals ($P < 0.01$) and, as in previous years, the 3/4 B and >7/8 S group had lower prevalence of lesions than the other groups (Table 5.2).

5340 ***Prevalence of lesions - Expedition***

Forty-nine unweaned calves were available in this group and were examined for stephanofilarial lesions on 4 occasions, namely; 5 November. 30 November and 30 December 1982 and 19 January 1983. On the first observation all calves were free of lesions and on the second examination only one animal had an ocular stephanofilarial lesion.

By 30 December, 32% of the calves had developed stephanofilarial lesions and the prevalence of the lesions was significantly affected by coat colour ($X^2_2 = 11.81$, $P < 0.01$). On 19 January, the overall prevalence of stephanofilarial lesions had increased to 69% and was once again significantly affected by coat colour of the animal ($X^2_2 = 11.50$, $P < 0.01$).

On both these latter dates there was no difference in the prevalence of lesions between medium and dark coloured animals but there was a significant difference between these groups and the light coloured group (30 Dec. $X^2_2 = 11.77$; 19 Jan. $X^2_2 = 10.63$, ($P < 0.01$). Values for prevalence of lesions in relation to coat colour of Expedition calves are shown in Table 5.3.

Table 5.3. Prevalence of Stephanofilarial lesions in relation to coat colour of *Bos indicus* calves of the same genotype and aged between 117 and 221 days at Expedition in 1982-1983.

	COAT COLOUR						
	Light		Medium		Dark		
	n	%	n	%	n	%	
STEPHANOFILARIAL LESIONS							DATE
present	0	0	0	0	0	0	5 November
absent	27	100	11	100	12	100	
present	0	0	0	0	1	8	30 November
absent	27	100	11	100	11	92	
present	3	11	6	55	7	58	30 December
absent	24	89	5	45	5	42	
present	13	48	9	82	12	100	19 January
absent	14	52	2	18	0	0	

5400 DISCUSSION

The observation in calves that the initial lesions of stephanofilariasis developed adjacent to the medial canthus of the eye was consistent with the findings of the abattoir survey (3000) which showed that all infected animals had such ocular lesions. Possible factors that determined the location of the parasites in this site have been discussed in 4400.

The failure to detect parasites in some lesions probably resulted from examination of insufficient biopsy material. Also the sensitivity of the microfilarial recovery technique was not known. The finding of parasites in 84% of lesions strengthens confidence in the conclusion reached after the histopathological examination of lesions (4000) that all were stephanofilarial in origin.

The significantly lower prevalence of lesions in calves with higher Brahman content was consistent with the findings in adult cattle in the abattoir survey (3000). In all years this resistance of *B. indicus* type animals to stephanofilarial infection was expressed independently of other variables such as sex, age, coat colour or weight. It is possible that this resistance may have been partly due to a resistance of *B. indicus* type animals to the likely vector, *Haematobia irritans exigua*. Tugwell et al (1969) working with *H. i. irritans* and Doube (1984) working with *H. i. exigua* found that *Bos indicus* animals were infested with fewer flies independently of coat colour. However, Holroyd *et al*, (1984) in a trial with adult cattle at Swans Lagoon found that prevalence of lesions was not associated with numbers of flies or with coat colour.

This suggests that prevalence of lesions is determined by host resistance to the parasite rather than host resistance to the vector.

The role of coat colour in the attraction of *H.i.irritans* has yet to be fully elucidated. Franks et al, (1964) compared attractiveness of coat colour to *H.i.irritans* and found that more flies occurred on darker coloured animals than on lighter coloured animals. Similarly, the significant effects of coat colour on prevalence of lesions in this survey at Expedition in 1982 and at Swans Lagoon in the years 1983 and 1985, could best be explained by a vector preference for host factors associated with dark coat colour. Whether such vector attraction to dark coat colour resulting in presumably higher transmission rate of stephanofilariasis to these dark animals is more pronounced in years with high fly numbers or low fly numbers is not known. It was interesting, however, that the most resistant group were the >7/8 S and that all of this genotype were darker coloured animals.

Within this genotype *B. indicus* "content" appeared to be a stronger determinant of resistance to stephanofilarial infection than coat colour was , thereby supporting the earlier suggestion that prevalence of lesions was determined by host resistance to the parasite more than by host resistance to the vector.

The significantly higher prevalence of lesions in males than females in 1983 was consistent with findings in adult cattle in the abattoir survey (3000). By contrast, no sex effect on prevalence was observed in the other years when animals were younger at time of observation and therefore less sexually mature than in 1983. These results thus could be supported by the postulation of Dobson et al (1970) that sex differences were the result of vector attraction to testosterone levels of host animals.

6000 VECTOR STUDIES

6100 INTRODUCTION

The known vectors of *Stephanofilaria* spp. are all muscid flies. *S. assamensis, S. kaeli, S. okinawaensis* and *S. dedoesi* are transmitted by *Musca conducens* (see Patnaik and Roy, 1966; Fadzil, 1973; Kono and Fukuyoshi, 1967; Partoutomo et al, 1981), *S. zaheeri* by *M. planiceps* (see Dutt, 1970) and *M. autumnalis* (see Patnaik and Kumar, 1972) and *S. stilesi* by *Haematobia irritans irritans* (see Hibler, 1966), *Lyperosia titillans* and *Stomoxys calcitrans* (see Azimov et al, 1976)

The abattoir survey (3000) had shown the distribution of stephanofilarial infection in Queensland. A comparison of that distribution with that of *H. i. exigua* established from stock inspector returns indicated a close association between the two. Therefore, wild caught flies, particularly *H. i. exigua*, were taken from infected cattle. Some were examined for the presence of developing larvae of *Stephanofilaria* sp., and others were fed on known uninfected animals in order to transmit the parasite. In addition, laboratory reared flies were fed on stephanofilarial lesions in an attempt to provide details of the life cycle of the parasite in the vector.

6200 MATERIALS AND METHODS

6210 Vector collections

Flies were collected from infected cattle by means of a sweep net or an aspirator. Particular attention was given to flies

resting along the ventral flanks of cattle because it was observed that engorged and gravid female *H.i.exigua* preferred to rest in this area on cattle. Infected cattle were yarded into an open crush and allowed to settle and then flies that alighted on stephanofilarial lesions were caught with an aspirator.

Regular monthly collections of flies were made from cattle at the C.S.I.R.O. Lansdown research station, 40 km south of Townsville. The flies were placed in small gauze-covered cages and chilled for transport to the laboratory.

At the laboratory flies to be dissected were killed with chloroform, identified into species and sorted into sexes. For dissection, each fly was cut into 3 sections, head, thorax and abdomen. Each section was carefully dissected and the contents separated in a drop of physiological saline on a microscope slide, placed under a cover glass and examined for the presence of developing larvae of *Stephanofilaria* sp.

Morphological examinations were made on live specimens where possible. For more detailed examinations larvae were killed and fixed in AFA solution and mounted in chlorolactophenol on a slide under a coverslip. Larval measurements were made on live specimens using an ocular micrometer and are given in microns. Larvae recovered from flies in this study were sufficiently sluggish to permit accurate measurement by this method. Drawings were made using a camera lucida and with the aid of photographs.

6220 Life cycle studies

Buffalo flies were reared at the laboratory using the method outlined in 4220. Newly emerged flies were

anaesthetised with carbon dioxide and sorted into sexes before feeding on a stephanofilarial lesion.

For feeding, 30 flies were enclosed in a 25 mm diameter cylindrical plastic cage with the ends covered in gauze. One end of the cage was held onto the surface of a lesion for 30 - 45 minutes in the morning and afternoon of the same day and the flies allowed to feed until fully engorged. Following the afternoon feed the flies were transferred to a gauze-covered plastic cup, 90 mm in diameter and 100 mm deep. They were held at a constant temperature of either 25 or 30° C and 80% relative humidity and given continuous access to a pad soaked in citrated bovine blood containing 1000 units of penicillin, 1.0 mg streptomycin and 250 units of nystatin per ml. Each day post feeding (PF), all flies that had died during the previous 24 hours as well as 2 of the surviving flies were dissected and examined for *Stephanofilaria* sp. larvae. Developing larvae were recovered from biopsies of lesions using the methods outlined in 3230 and 7200.

*6230 **Transmission trials***

Wild caught *H. i. exigua* taken from cattle infected with *Stephanofilaria* sp. were fed on 2 known uninfected cattle. In the initial trial, the flies were confined within a gauze covered cage which was strapped onto a clipped area on the neck of one animal. In a subsequent trial, the flies were released onto a second animal held in a fly proof room similar to that used for the laboratory colonisation of *H. i. exigua* (see Thomas and Davis, 1984). In both trials the animals were then held in the fly proof accomodation for a further 35 - 40 days and monitored for

development of lesions

At the end of this period biopsies were taken and examined for the presence of parasites.

6300 RESULTS

6310 Vector examinations

Flies swept from infected cattle and aspirated from lesions were almost exclusively *H.i.exigua.* The only other species of fly taken was *M. vetustissima* and 32 adults of this fly were dissected but none was found to contain larvae of *Stephanofilaria* sp.

A total of 3292 female and 1237 male *H.i.exigua* was dissected of which 96 (2.91%) females and no male flies were found containing developing larvae of *Stephanofilaria* sp. The maximum number of larvae found in a single fly was 4 but most infected flies (91%) contained only a single *Stephanofilaria* sp. larva (Table 6.1).

TABLE 6.1. Number of larvae of *Stephanofilaria* sp. found in female *Haematobia irritans exigua* taken off infected cattle in the dry tropics of north Queensland.

Number of *Stephanofilaria* larvae per fly	1	2	3	4
Number of infected flies	88	6	1	1
percentage	91%	7%	1%	1%

The rate of infection of female *H.i.exigua* feeding on infected cattle at Lansdown, ranged from 0% to 9.3% (Figure 6.1). but only in August was there a significant difference in the rate ($P < 0.01$, $X^2_{.01} = 24.73$).

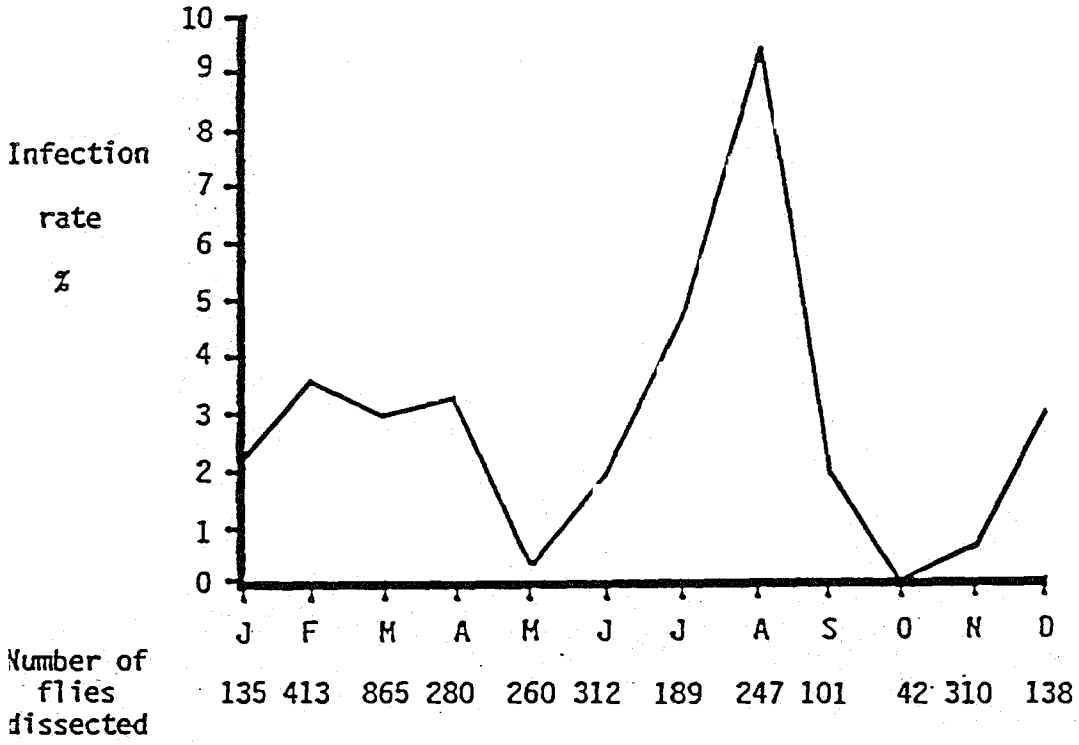


Figure 6.1. Rate of infection of female *Haematobia irritans exigua* with larvae of *Stephanofilaria* sp. in different months of the year at Lansdown in North Queensland.

Sixty-nine percent of larvae detected were in the abdomen of the flies. Almost all the larvae appeared to be free

within the haemocoel of the fly, but in 4 flies, a single larva was found enclosed within a connective tissue capsule attached to the fat body of the fly (Figure 6.2).

The only first stage larva found in wild caught flies was a late first stage larva, 120 long and 35 wide. First, second and early third stage larvae were only found within the abdomen of infected flies whereas late third stage larvae were found in the head and thorax as well.

Only 11% of larvae were found within the thorax of infected flies and all were advanced third stage larvae. Twenty percent of larvae detected were in the head of the vector fly, either free within the haemocoel or less commonly in the proboscis (Figure 6.3) and all were advanced third stage larvae.

6320 Life cycle studies

During the course of this trial 1000 female *H. i. exigua* were fed on *Stephanofilaria* sp. infected lesions. Although most flies fed on the lesions, 90% of them died within 36 hours and none survived beyond day 11PF. Only 5 flies were found infected with developing larvae of *Stephanofilaria* sp. A single third stage larva 300 long and 30 wide was found on day 9PF in a fly held at 25^{o}C and a larva 500 long and 38 wide was found on day 10PF in a fly held at 26^{o}C.

Subsequently, 3 of a group of 30 flies held at 30^{o} C were found infected. The first, dissected on day 3PF contained a first stage larva 60 long and 25 wide. The second, dissected on day 5PF, contained a late first stage larva 210 long and 42 wide and the third fly, dissected on day 7 PF contained a third stage

larva 625 long and 45 wide. All larvae recovered were found free within the abdominal haemocoel of the flies.

*6330 **Transmission trials***

Wild caught flies confined in cages held onto the neck of an animal were reluctant to feed and all died within 12 hours of capture. Over several months, 3000 *H. i. exigua* were fed on an animal using this method but no lesions developed and no parasites were detected either at the feeding site or at the medial canthus of the eye.

Wild caught, potentially infected flies released onto an animal confined in the enclosed room fed successfully and survived for up to 16 days. Most of the flies remained on the dorsal midline and down the shoulders of the animal, but a small number of gravid females were continually present feeding at the skin adjacent to the medial canthus of each eye. By the third day after release numerous pin point haemorrhages were present on the hairless skin at the medial canthus of the eyes. These feeding marks persisted while the flies remained on the animal but had largely disappeared by day 20, 4 days after the last fly died. By day 30 after release a few haemorrhagic spots were still evident and small hairless areas had developed along the margins of the hairline near both eyes.

Collagenase digest of a single biopsy from around each eye taken on day 35 yielded 5 adults and several microfilariae of *Stephanofilaria* sp. Histological sections of the remaining skin taken 24 hours later revealed 2 further adults and numerous microfilariae. Histopathological changes in these lesions have been discussed under Pathology (4400).

*6340 **Stephanofilaria sp. larval morphology***

Microfilariae recovered from the skin of cattle were confined within semi rigid spherical vitelline membranes. Live microfilariae were actively motile within the membranes but they were unable to effect any progressive movement of the entire structure when suspended in saline.

The earliest stage found in a fly was pear shaped, 60 long and 25 wide. In this larva, the developing intestine was not fully differentiated and occupied most of the body cavity (Figure 6.4A).

In larvae between 150 and 200 long, the anterior end of the intestine had begun to form into an oesophagus and the anal cell area had constricted off into a bulbous mass of cells (Figure 6.4B). Larval development beyond this stage was largely confined to an increase in body length accompanied by a gradual reduction in the width of the intestine (Figure 6.4C).

Infective third stage larvae recovered from the proboscides of infected flies, measured 760-890 in length and 30-35 wide and contained a well differentiated oesophagus and nerve ring (Figure 6.4D) and a patent intestine and anus (Figure 6.4E). Cephalic ornamentation became evident in third stage larvae and initially comprised a fine peribuccal elevation and in late third stage larvae a single prominent spine developed adjacent to the peribuccal elevation.

Third stage larvae recovered from lesions on cattle ranged in size from 874 to 982 long and 37.5 to 39 wide. They were morphologically similar to infective third stage larvae recovered from *H. i. exigua*. Fourth stage larvae recovered ranged from 1030 to 1800 long and 42 to 75 wide. In third stage females

the genital anlage began to develop in the region of the vulva (Figure 6.5A). The ovaro-uteri developed posteriorly in fourth stage larvae (Figure 6.5B) and in larvae 1440 - 1500 long had extended half the length of the body. In third stage males the genital primordium developed as a hook shaped mass of cells 135 - 150 behind the anterior end (Figure 6.5C) and the spicular primordia developed as clusters of cells on either side of the rectum (Figure 6.5D).

Fifth stage females recovered had a complete genital tract but lacked developing microfilariae. They were morphologically similar to gravid females but were smaller in size, ranging from 2740 to 4060 long and 50 to 62 wide. The smallest male recovered was 2170 long and 52.5 wide with left spicule 300 long and right spicule 45 long.

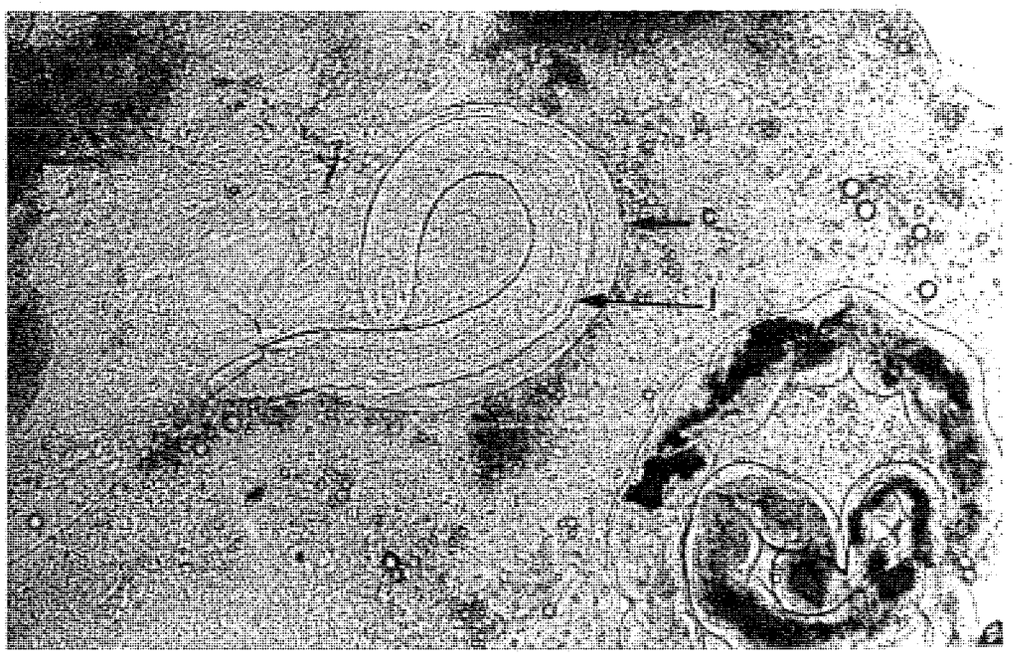


Figure 6.2. Third stage larva of *Stephanofilaria* sp. enclosed within a connective tissue capsule from the abdomen of a female *Haematobia irritans exigua*. c = capsule; l = larva.

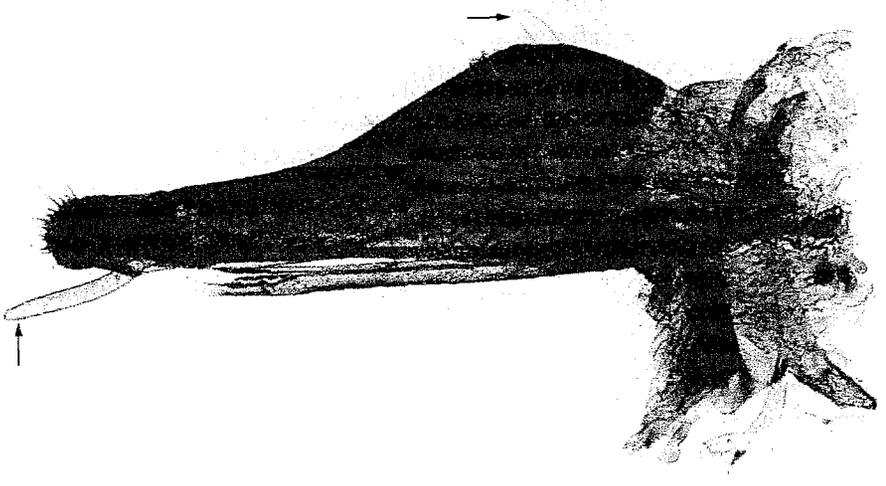


Figure 6.3. Proboscis of female *Haematobia irritans exigua* containing an infective third stage larva of *Stephanofilaria* sp. (arrowed).

Figure 6.4. Developing larvae of *Stephanofilaria* sp. recovered from *Haematobia irritans exigua*. (A) early first stage. (B) late first stage. (C) second stage larva from abdominal haemocoel. (D) anterior end of infective third stage larva. (E) posterior end of same.

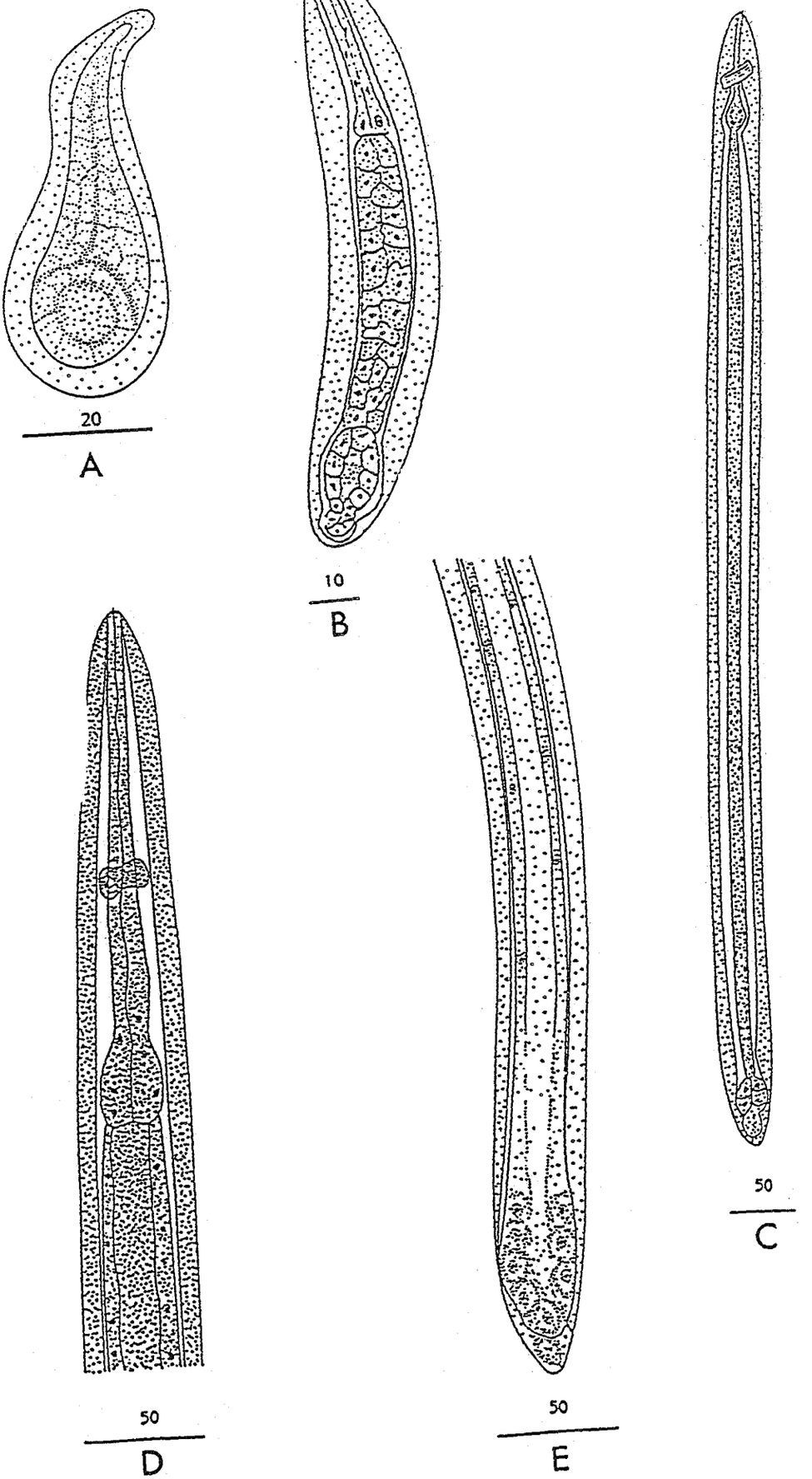
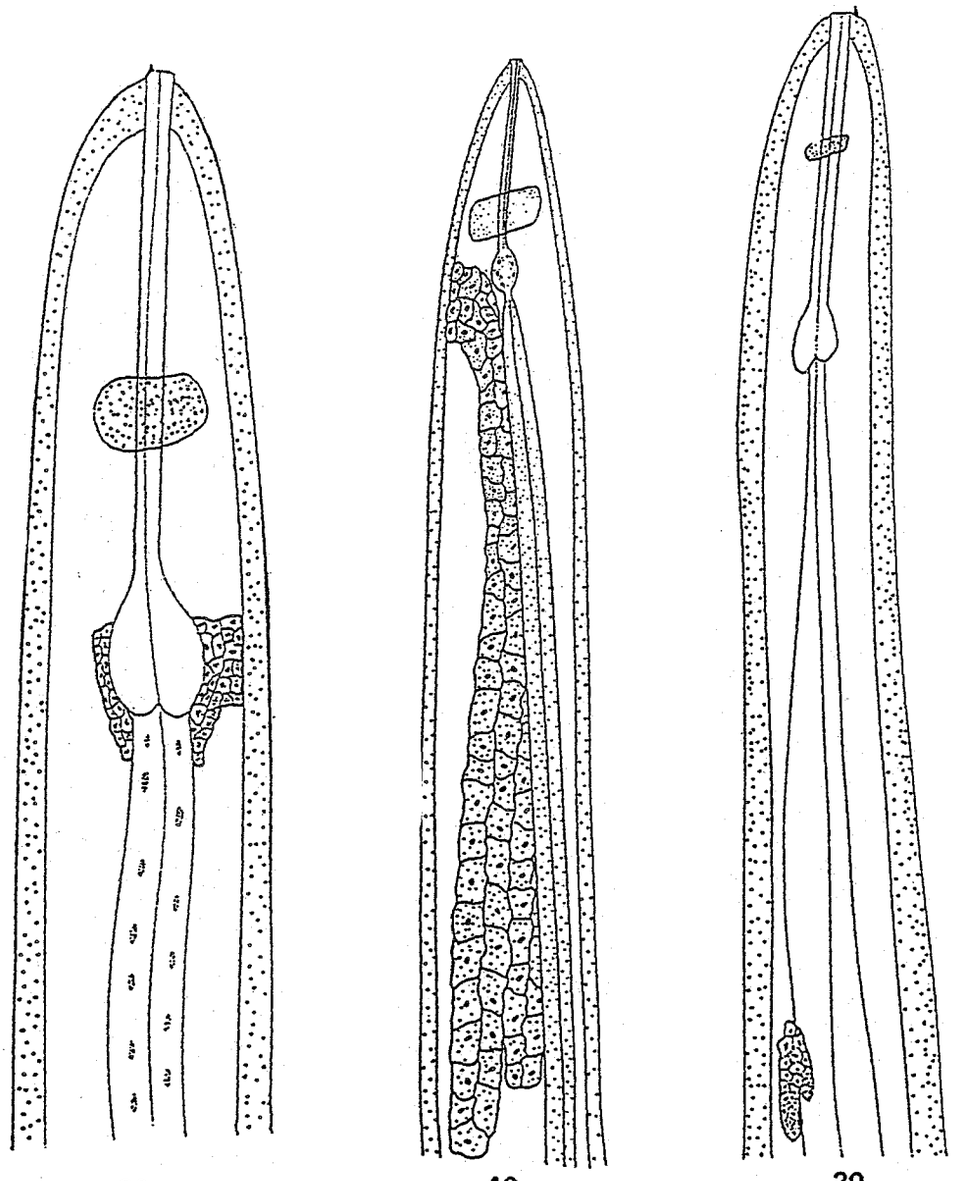

20
A
10
B
50
C
50
D
50
E

Figure 6.5. Developing larvae of *Stephanofilaria* sp. recovered from the skin of cattle. (A) Anterior end of third stage female. (B) anterior end of fourth stage female. (C) anterior end of third stage male. (D) posterior end of same.


30
A
40
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20

The close association between the distribution of stephanofilarial infection and the distribution of *H.i.exigua* suggested that this fly was a likely vector of *Stephanofilaria* sp. in north Queensland. The closely related *H.i.irritans* has been shown to be the vector of *S. stilesi* in north America (Hibler, 1966) and Russia (Ivashkin et al, 1971).
A developing larva of an unidentified *Stephanofilaria* sp. has been found in *H. i. exigua* in Indonesia (Partoutomo et al, 1981). Also, in common with *S. stilesi*, microfilariae of the Australian *Stephanofilaria* sp. are confined within vitelline membranes beneath intact epidermis of the skin of the host (4000). Any potential vector must therefore be capable of piercing intact skin to liberate and ingest the microfilariae. *H. irritans* is a true biting fly and capable of piercing intact skin (Harwood and James, 1979).

The monthly variations in the rate of infection of the vector are interesting. The low value for October undoubtedly arose from examination of insufficient flies. The rise in the rate in Winter (July - August) was interesting in that it occurred at a time when the number of flies on cattle in the area would have been at its lowest (Holroyd et al, 1984). The result remains an enigma, however, a couple of explanations are possible.

Firstly, an increased rate of infection of the vector could have resulted from an increased density of microfilariae in the skin of the host. The density of microfilariae was not measured in this study, however, there

appeared to be no qualitative evidence of increased numbers of microfilariae in the the large number of skin sections examined in the abattoir survey. Secondly, such an increase could have arisen from an increased feeding activity by the vector during this period. If, as reported by Holroyd et al (1984), the vector populations are lowest at this time, then there may have been reduced competition for feeding sites on the lesions thereby resulting in more flies successfully feeding on infected skin.

The finding of developing larvae in wild caught nd laboratory reared flies fed on lesions confirms that *H. i. exigua* is capable of ingesting microfilariae and supporting development of the larvae. The successful transmission of the parasite using wild caught *H. i. exigua* confirms the ability of this fly to also transmit infective third stage larvae to cattle. Therefore, *H. i. exigua* is a vector of *Stephanofilaria* sp. in Australia.

Other vector species are possible, but no other haematophagous fly has a distribution that closely matches that of the parasite. The most likely additional vector is *Stomoxys calcitrans* . It is a haematophagous muscid fly that feeds on cattle and is a vector of *S. stilesi* overseas (Azimov et al, 1976). However, it prefers to feed on the legs of cattle (Seddon, 1967; Moorhouse, 1972) and has a cosmopolitan distribution. Therefore, whilst it may be capable of acting as a vector of *Stephanofilaria* sp. in Australia, it is unlikely to be of epidemiological significance for transmission of this parasite in north-eastern Australia.

M. vetustissima, which was also taken on cattle in this survey, has only small prestomal teeth and is unable to

pierce intact skin (Hughes et al, 1972). *M. vetustissima* is therefore unlikely to be a vector of this parasite, even in areas where this fly is more common on cattle.

Biting midges (Diptera: Ceratopogonidae) are haematophagous and feed on cattle in Australia (Muller and Murray, 1977; Kay et al, 1978). However there are no species that are known to feed on cattle that have a distribution matching that of this parasite. *Culicoides peregrinus* , *C. schultzei* and *C. fulvus* are all restricted to the north of the continent and *C. brevitarsis, C. marksi, C. victoriae* and *Lasiohelea townsvillensis* have distributions that extend well into New South Wales and Victoria (Debenham, 1979).

Mosquitoes such as *Culex annulirostris* and *Anopheles bancrofti* are known to feed on cattle in Australia (Doherty et al, 1972; Muller and Standfast, 1986), but neither species has a distribution (Muller and Standfast, 1986) matching that of stephanofilariasis.

The rate of infection of wild caught female *H. i. exigua* with larvae of *Stephanofilaria* sp. agrees closely with that reported for *H. i. irritans* taken off range cattle infected with *S. stilesi* (Hibler, 1966). However, in this study the data presented for rate of infection of flies were based largely on the presence of second and third stage larvae. The small first stage larvae were probably overlooked during the dissections. Therefore,the rates of infection in the field were probably higher than that shown by this study.

The nil rate of infection found in male *H. i. exigua* in this survey is similar to the rate of infection of male *H. i. irritans* with *S. stilesi* in north America (Hibler, 1966). The feeding habits of male *H. i. exigua* are not known but in *H. i. irritans*, female flies take 1.5 times more blood meals per day than males do and feed 1.7 times longer than males do (Harris et al, 1974). However, if such differences in feeding behaviour were applied to *H. i. exigua*, it could only partially explain the lifference in rate of infection between the sexes for this fly. It is likely that other factors operate to limit the intake or development of *Stephanofilaria* sp. larvae in males of *H. i. exigua*.

Larvae recovered in this study appeared morphologically similar to larvae of *S. stilesi* (see Hibler, 1966), however, the cellular detail illustrated by Hibler was not seen in this study. The time taken for development of the larvae in the vector was much shorter in this study than that recorded by Hibler (1966) for *S. stilesi*, but the difference was probably a result of the higher temperature used for incubation in this study.

The finding of some larvae enclosed within membranous structures in the vector is interesting. Similar membranes have been found enclosing developing larvae of *S. stilesi* in *L. titillans* (see Ivashkin et al, 1963) and *S. assamensis* larvae in *M. conducens* (see Patnaik and Roy, 1966). Fadzil (1973) illustrated developing larvae of *S. kaeli* within such membranes in *M. conducens*, but he failed to mention the membranes in the text. The origin of these membranes is obscure. Patnaik and Roy (1966) thought them to be the cast cuticle of

previous larval stage, however, this is unlikely to be the origin of the membranes seen in this study as they were part of the fat body of the fly. Similar membranes are present around developing larvae of *Parafilaria bovicola* in *M. xanthomelas* and *M. lusoria* in south Africa and Nevill (1981) considered them to be remnants of fat body cells. It is possible that microfilariae of the *Stephanofilaria* sp. examined in this study occasionally penetrate fat body cells in the abdomen of the vector but the results of this study indicate that most larvae develop free within the haemocoel of the vector.

The recovery of gravid adults and numerous microfilariae 35 days after first release of infected vectors onto the host indicates a prepatent period of 35 days or less for this parasite. Hibler (1966) recorded the development of a lesion on a host animal 14 days after feeding infected *H. i. irritans* infected with *S. stilesi* on the animal, but he did not examine the lesion for parasites at that stage. Alopecia was not evident in this trial until day 30 following release of the infected flies. This was probably a result of a small number of parasites being confined to the normally hairless area of skin adjacent to the medial canthus of the eye. Alopecia would have been evident at an earlier stage in Hiblers trial because he fed his flies on fully haired skin on the ventral midline. It is therefore possible that the prepatent period of some *Stephanofilaria* sp. could be as short as 14 days.

The finding of third and fourth stage larvae in digests of lesions together with the short prepatent period, indicate that larvae of the Australian *Stephanofilaria* sp.

may not have an extensive or prolonged somatic migration. Adults of the *Stephanofilaria* sp. appear to reach sexual maturity before attaining maximum size, a feature reported for other filarioids (Orihel, 1961; Schacher, 1962; Spratt and Varughese, 1975).

7000 **TAXONOMY**

7100 INTRODUCTION

The taxonomic descriptions of some of the earliest described *Stephanofilaria* spp. were brief and based on small series of parasites. Moreover the adults are small and details of their external morphology are difficult to see, even at the highest magnification possible using light microscopy. Identification of species based on simple linear measurement of the parasites and their structures has failed to provide a reliable means of separating all the currently recognised species (2110).

Geographical location and site of the lesion on the host were also used to distinguish species but proved unsatisfactory, adding further to the present confusion in identification of *Stephanofilaria* spp.

A solution to these problems was introduced by Ueno and Chibana (1977). They used scanning electronmicroscopy (SEM) to obtain precise morphological information for their description of *S. okinawaensis*. However, they made no comparative SEM examinations of material from other areas in support of their new species.

For these reasons SEM was used in the present study to facilitate description of the Australian *Stephanofilaria* sp. SEM was also used to redescribe *S. dedoesi* obtained from lesions on the shoulders of cattle in Sulawesi as *S. dedoesi* is the type species of the genus.

Adult parasites were recovered by cutting freshly collected lesions into strips 10mm wide and 3-4mm thick which were then either soaked in normal saline at room temperature for 24 hours or were digested. For digestion, the strips of skin were incubated at 37°C for 18-24 hours in a solution of cell culture medium 199 containing 20mM Hepes buffer to which was added 100 IU penicillin per ml, 1 mg streptomycin per ml, 2 microgram amphotericin per ml, 0.3mg $NaHCO_3$ per ml and 3mg collagenase per ml.

Skin that had previously been fixed in either 70% alcohol or Carnoy's solution was washed in running water for 24 hours then placed in distilled water to which was added 10mg pepsin per ml and sufficient hydrochloric acid to give a final pH of between 2 and 4. These pepsin digests were then incubated at 37°C for 24 hours or until digestion of the skin was complete.

Residues of digests were examined using a dissecting microscope. Adult worms recovered from unfixed skin were rinsed twice in phosphate buffered saline (PBS) and then fixed in either 70% alcohol, 10% buffered neutral formalin or acetic acid-formalin-alcohol (AFA) solutions for 24 hours. After fixation the worms were stored in 70% alcohol containing 5% glycerine or in 10% buffered neutral formalin.

Worms examined under light microscopy were cleared in lactophenol. Drawings were made using a camera lucida and measurements were made with an ocular micrometer or from camera lucida drawings. All measurements are given in micrometres unless otherwise stated and are listed as a range followed by the mean in parentheses.

Specimens for SEM were post fixed in 1% buffered osmium tetroxide for 1.5 hours and then washed in PBS. Some specimens were given additional washing by gentle agitation in a sonicator. All specimens were then dehydrated by immersion in 30% alcohol for 12h, 50% alcohol for 4h, 70% alcohol for 4h and finally into 95% alcohol. They were then critical point dried, coated with gold and examined under a scanning electron microscope.

7300 DESCRIPTION

7310 Stephanofilaria sp. from Australian cattle

7311 General description

Adults small, slender, creamy white. Head with prominent elevated peribuccal collar supporting 15-16 spines (Figure, 7.1). Cephalic ridge lacking spines (Figures 7.1, 7.2). Pair of large semicircular, amphids laterally interrupting cephalic ridge, Large pair of cephalic papillae, remaining papillae not observed (Figures, 7.1,7.2). Cuticle bearing numerous fine striations throughout (Figure, 7.3) except for anterior end (Figure, 7.4) and distal tail (Figure, 7.5). Cuticular striations without spines or a posteriorly directed frill. Prominent raised lateral ala commencing 80-150 short of anterior end (Figure, 7.6) and extending the full length of the body (Figures, 7.3,7.7).

7312 Male

Body 2.3-3.2(2.8) mm long with maximum diameter 50-80(68). Nerve ring 60-62(61) from anterior end. Oesophagus undifferentiated, 85-90(87) long; tail slightly curved

12.5-15(13) long with 7 pairs of ventrolateral preanal papillae a single median papilla, one pair of adanal and one pair of postanal papillae (Figures, 7.7,7.8).

Left spicule protrusible, 262-350(299) in length, slender with expanded base and tapering to a point at the distal end. Right spicule, 37.5-50(44) in length, strongly curved and grooved and with a bifid distal end to accommodate left spicule (Figure, 7.9). Spicule ratio 5.2 - 9.3 (6.8):1. A short gubernaculum in close appostion to right spicule (Figure, 7.9).

7313 ***Female***

Body 3.8-6.4(5.2) mm long with maximum diameter 62.5-93.5(81). Nerve ring 62-75(67) from anterior end. Oesophagus undifferentiated, 120-132(124) in length. Vulva crescentic (Figure, 7.10), opening 81-118(95) from anterior end (Figure, 7.11). Vagina vera and vagina uterina short, bifurcating into two uteri. Uteri extend proximally to convoluted ovaries in tail region (Figure, 7.12). Ovoviviparous. Intestine leading into short rectum and an anus (Figure, 7.12).

7314 ***Microfilaria***

Body 47-73(60) long with maximum width 3.3-6.2(4.3). Anterior end slightly expanded and posterior end tapering to a fine point (Figure. 7.13). Microfilaria contained within a round to oval shaped vitelline membrane 62-90(72) long by 47-75(60) wide (Figure, 7.13). Vitelline membrane also enclosing numerous small spherical bodies.

7320 Stephanofilaria dedoesi

A small series of adult *S. dedoesi* were supplied by Dr. P. Stevenson, Research Institute for Animal Diseases, Bogor, Indonesia. The adults were recovered in saline from scrapings of a lesion on the shoulder of a cow in north Sulawesi. A prolonged delay before fixation of the worms resulted in autolysis of the internal soft tissues and only cuticular structures were suitable for examination. Preliminary washing of these specimens before fixation was omitted and therefore some of the external structures were obscured by adherent material.

7321 External morphology

Head (Figure 7.14), with circumoral elevation bearing 15-16 peribuccal spines. Cephalic ridge bearing 20 large cephalic spines interrupted laterally by a pair of amphids. Four submedian cephalic papillae.

Cuticle with numerous striations and an indistinct recessed lateral line (Figure 7.15). Striations in the anterior one third of the body bear a posterior serrated frill (Figures 7.15; 7.16). On the remainder of the body striations are smooth (Figure 7.17). Distal tail with a small circular area containing 2 phasmids (Figure 7.18).

7322 Male (2 specimens)

Specimen 1. Body 2.7mm long with maximum width 87.5. Left spicule 175 long and right spicule 51 long.

Specimen 2. Body 2.7mm long with maximum width 150. Left spicule 187 long; right spicule 50 long.

*7323 **Female (7 specimens)***

Body 5.2-7.6(6.3)mm long with maximum width 114-165(141). Vulva situated 50-87(68) behind anterior end. Posteriorly, an anus present (Figure 7.18).

*7400 **DISCUSSION***

The parasites examined in this study were classified as Filarioidea on the basis of, location in the connective tissue of the host, adults lacking a buccal cavity, male with unequal spicules and female with anterior vulva and producing microfilariform larvae (Anderson et al, 1974). They were further classified to the genus *Stephanofilaria* on the basis of being small worms from the subcutaneous tissues of Bovidae, having an oral opening surrounded by numerous cuticularized spines, large amphids, simple oesophagus, male with a gubernaculum and female with vulva adjacent to nerve ring (Ihle and Ihle-Landenberg, 1933; Anderson and Bain, 1976)

The currently recognised species fall into 3 morphological groups. The 5 Asian species, *dedoesi*, *kaeli*, *assamensis*, *zaheeri* and *okinawaensis* form a distinct group characterised by a complete circle of 16-32 cephalic spines, cuticular striations possessing a posterior serrated frill, male with left spicule between 130-230um in length and female with vulval opening close to nerve ring.

S. dinniki is distinct from the *dedoesi* group and is characterised by cephalic spines reduced to 4 pairs spaced equally around the head, smooth cuticular striations, male with left spicule between 530-750um in length and female with vulval opening in close proximity to anterior end.

S. stilesi is also distinct with cephalic spines abbreviated to a single asymmetrical patch of 4-6 spines, smooth cuticular striations, male with left spicule between 275-376um in length and female with vulval opening near nerve ring. The measurements of the taxonomic characters used to separate the species groups are shown in Table 7.1.

The Australian parasite differs from all other species in the genus in the absence of cephalic spines. In all other characters, the Australian sp. is closest to *S. stilesi* and it would be best placed with *stilesi*.

The number of cephalic spines on the species within the *dedoesi* group appears variable. The small series (9 worms) of *S. dedoesi* examined in this study all possessed 20 cephalic spines. A similar number has been reported for *S. assamensis* (Patnaik, 1964) and *S. okinawaensis* (Ueno and Chibana, 1977), but only 16-17 for *S. kaeli* (Buckley, 1937) and 28-32 for *S. zaheeri* (Singh, 1958; Patnaik 1964; Patnaik and Roy, 1968). The variations in the number of cephalic spines reported for *S. kaeli* and *S. zaheeri* need to be confirmed using SEM. The very close geographical proximity of *S. kaeli* and *S. dedoesi* together with the coincidence in other characters as well as a common vector, *M. conducens*, would indicate that *S. kaeli* cannot be retained as a species separate from *S. dedoesi*.

In this study, the counting of peribuccal spines under light microscopy was imprecise as the spines could not readily be distinguished from the supporting collar, and the enfoldings of the collar between the spines were easily mistaken for additional spines. A graphic illustration of the imprecision of light microscopy in this context is the report of Johnson et al (1981)

(appendix) that the *Stephanofilaria* sp. from Australia has an asymmetrical patch of 3 - 5 cephalic spines, whereas in this study SEM clearly showed the absence of cephalic spines. Thorough cleaning of specimens was essential even for SEM examination of this structure (compare figure 7.1 with 7.14).

Table 7.1. Measurements of taxonomic characters used in separation of morphological groups of *Stephanofilaria* spp.

	TAXONOMIC CHARACTER				
	cephalic spines	cuticular frill	left spicule	anterior vulva distance	anterior nerve ring distance
SPECIES GROUP					
dedoesi group					
dedoesi	20	present	.175-.230	.049-.087	.065
kaeli	19-23	present	.150-.230	.062-.098	-
assamensis	16-24	present	.133-.230	.075-.113	.063-.099
okinawaensis	18-21	present	.147-.172	.063-.082	.063-.082
zaheeri	28-32	present	.160-.220	.075-.120	.060-.100
dinniki					
dinniki	8	absent	.530-.750	.009-.014	.115-.144
stilesi group					
stilesi	4-6	absent	.273-.376	.077-.118	-
Aust. sp.	0	absent	.262-.350	.081-.118	.062-.075

All 3 species that have now been examined by SEM have 15 or rarely 16, peribuccal spines. It is therefore possible that, with the exception of *S. dinniki*, this may be a standard configuration within the genus and that reported variations in the number of these spines may have arisen from difficulties in accurately seeing this structure under light microscopy.

Cuticular striations are a feature of all species in the genus but only the *dedoesi* group have terminal frills associated with the striations. The restriction of the cuticular frill to the anterior one third of the body in the *S. dedoesi* examined in this study was similar to that figured for *S. okinawaensis* by Ueno and Chibana (1977). The Australian species was similar to *S. dinniki* and *S. stilesi* in the absence of cuticular frills. The prominent lateral ala seen in the Australian species was also present in *S. kaeli*, *S. zaheeri* and *S. stilesi*; was apparently absent in *S. okinawaensis* and was not described in *S. assamensis* and *S. dinniki*. A depression above the lateral cords was detected in one of the *S. dedoesi* examined in this study (Figure 7.15).

Adults of the Australian species could not be differentiated on the basis of size from other *Stephanofilaria.* spp. Males of all species are similar in size and, whilst females within the genus are more variable in size, the Australian species was similar in size to *S. dinniki* and *S. stilesi.*

Pande (1936) ascribed taxonomic significance to the distance of the vulva from the anterior end of the female, whereas Chitwood (1934), Buckley (1937) and Ueno and Chibana (1977) considered this character to be unimportant.

With the exception of *S. dinniki* in which the vulval orifice is much closer to the anterior end (Table 7.1), none of the remaining species can be differentiated on the basis of this character.

There is uncertainty in the literature concerning the number and position of caudal papillae in the males of *Stephanofilaria* spp. All species are reported to possess 2-3 pairs of postanal papillae but there are variable accounts of the number and arrangement of preanal papillae. Ihle and Ihle-Landenberg (1933), Chitwood (1934) and Pande (1936) did not record preanal papillae in their descriptions of *S. dedoesi*, *S. stilesi* and *S. assamensis*, respectively. However, whether they are absent or were overlooked will only be known after further observations are made, preferably using SEM. Both specimens of male *S. dedoesi* examined in the present study became distorted during dehydration and drying and a view of the ventral tail area was not possible during SEM examinations.

In *S. kaeli*, *S. zaheeri*, and *S. dinniki* the preanal papillae were arranged in 2 ventrolateral rows. In the first 2 of these species, the papillae were arranged symmetrically in pairs but in *S. dinniki* the papillae were arranged asymmetrically. Arrangement of the papillae in *S. stilesi* from Russia is unclear. Gnedina (1950) illustrated the caudal end of the male with symmetrical preanal papillae whereas Ivashkin et al, (1961) considered the papillae to be asymmetrically arranged. Perhaps, these conflicting accounts result from the variability of this character between specimens, a feature also observed in the Australian species examined in this study.

However, as with many of the surface features of adults within this genus, papillae are difficult to see using light microscopy because of their small size. It is likely, therefore, that uncertainty over the number and position of preanal papillae will only be resolved when all species have been examined by SEM.

Ihle and Ihle-Landenberg (1933) failed to find an anus in adult *S. dedoesi* and listed the lack of an anus as a generic character. These authors were not able to distinguish the structure using light microscopy but specimens of *S. dedoesi* examined by SEM in the present study, possessed an anus. The lack of an anus would exclude *S. dedoesi* from the Filarioidea.

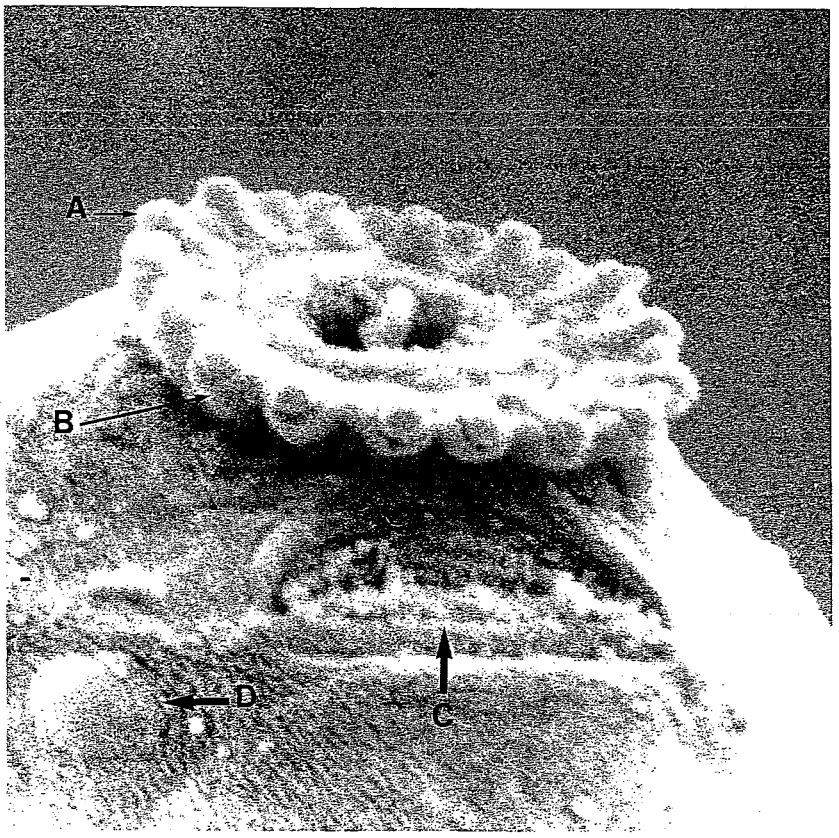
The microfilaria of the Australian species was indistinguishable from published descriptions of the microfilaria of *S. stilesi* (Chitwood, 1934; Hibler, 1966). The recording by Chitwood (1934) of the length of the microfilaria as 680um was obviously an error and probably should have been 68um. The microfilariae of these 2 species are less than half of the size of those of any other species in the genus (Table 2.3).

The external morphology of the *S. dedoesi* examined in this study is indistinguishable from that figured for *S. okinawaensis* by Ueno and Chibana (1977) which was also based on examination by SEM. Given the similarities in other taxonomic characters between the 2 species, as well as both species having the same vector, *Musca conducens* (see Ueno and Fukuyoshi, 1967; Partoutomo et al, 1981), it is therefore suggested that *S. okinawaensis* be synonymised with *S. dedoesi*.

In most characters the Australian *Stephanofilaria* sp. closely resembles *S. stilesi*, but the difference in the cephalic armature indicates that this Australian nematode is a hitherto undescribed species. It would not be advisable to elevate it to specific status without a detailed comparison with *S. stilesi*. Despite several requests I have not been able to obtain specimens of *S. stilesi* for such a study.

Figure 7.1 Head of adult *Stephanofilaria* sp. from Australia. (A) peribuccal spines (B) supporting collar (C) amphid (D) cephalic papilla. SEM.

Figure 7.2 Oblique enface view of adult *Stephanofilaria* sp. from Australia. (A) mouth (B) peribuccal spines (C) amphid (D) cephalic papilla (E) cephalic ridge. SEM.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B
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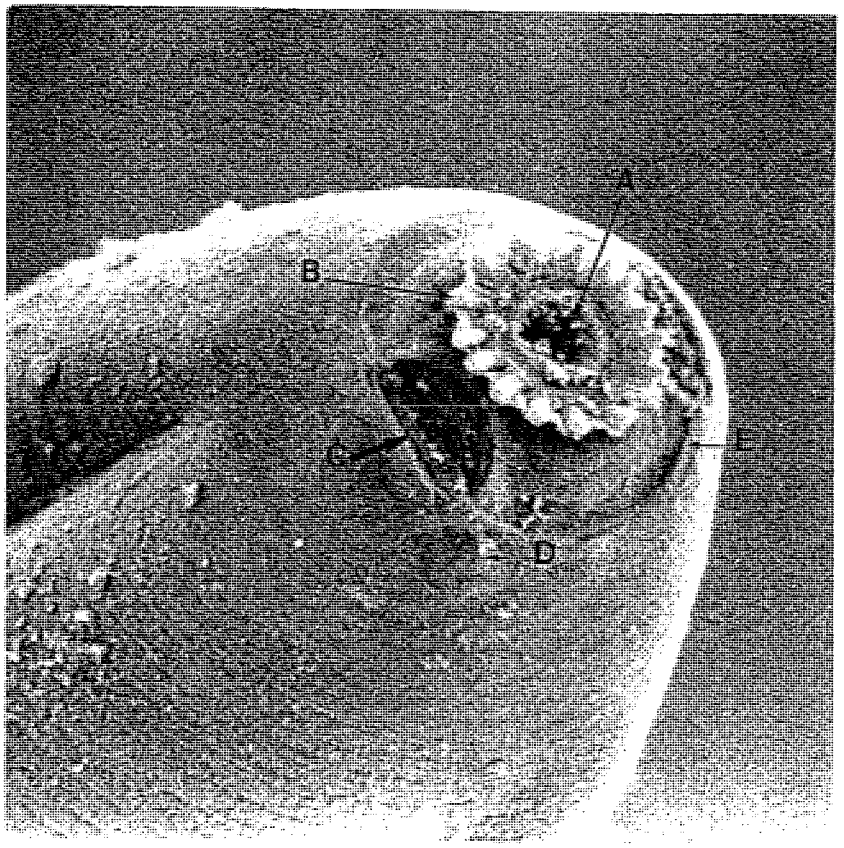
C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igure 7.3 Cuticle of adult *Stephanofilaria* sp. from Australia showing striations and prominent lateral ala. SEM.

Figure 7.4 Anterior end of adult female *Stephanofilaria* sp. from Australia showing absence of cuticular striations immediately posterior to head. SEM.

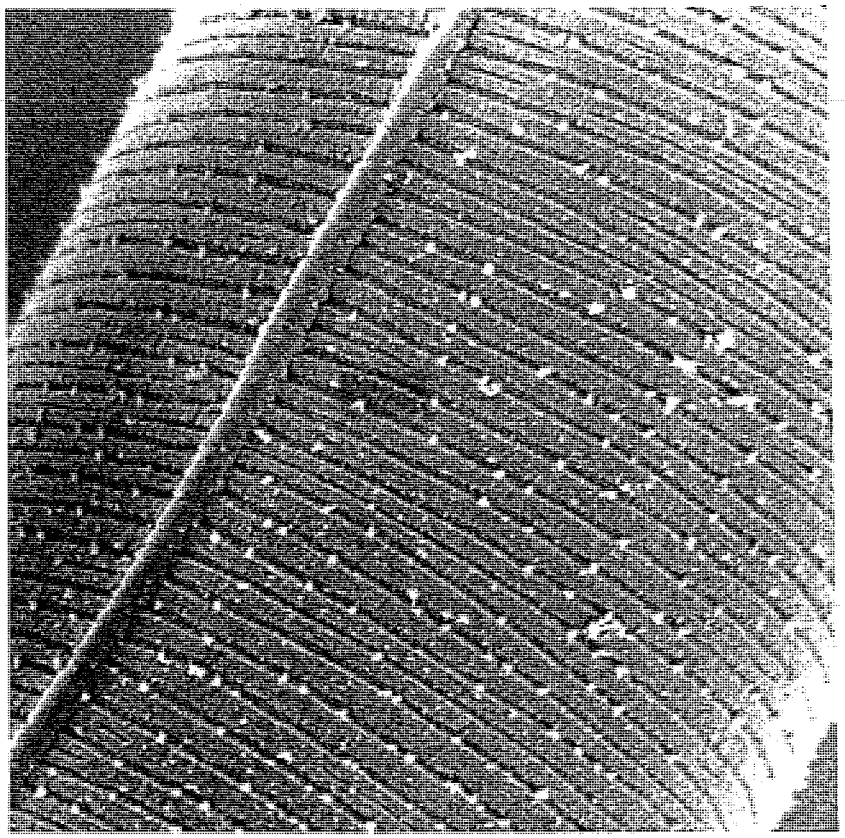
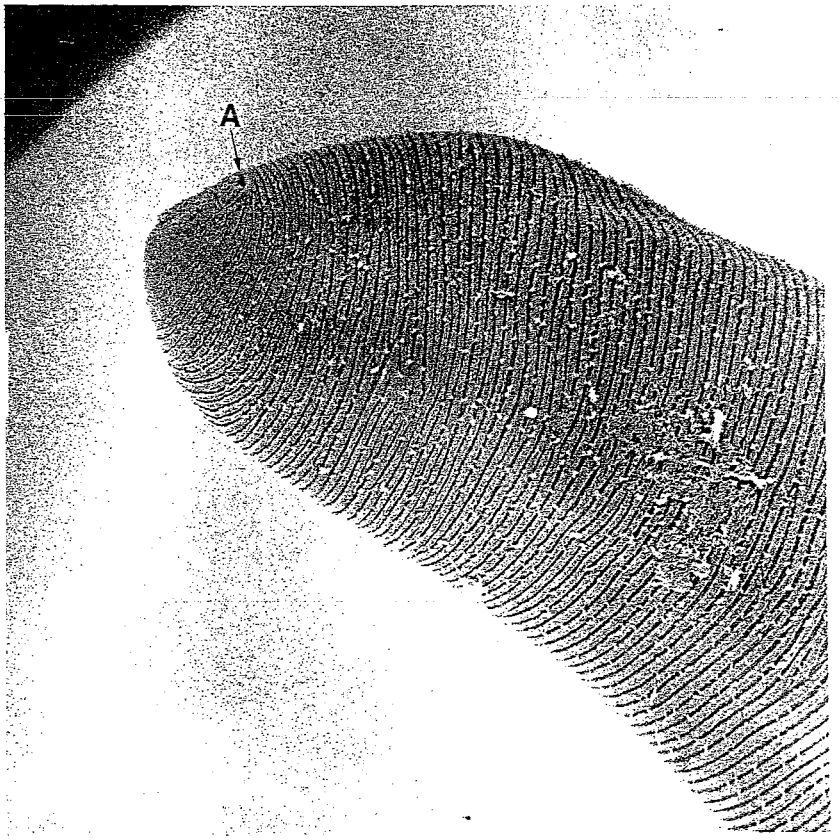


Figure 7.5 Tail of adult female *Stephanofilaria* sp. from Australia showing lack of cuticular striations on distail tail region. (A) anus. SEM.

Figure 7.6 Anterior end of adult female *Stephanofilaria* sp. from Australia. (L) lateral ala (V) vulva. SEM.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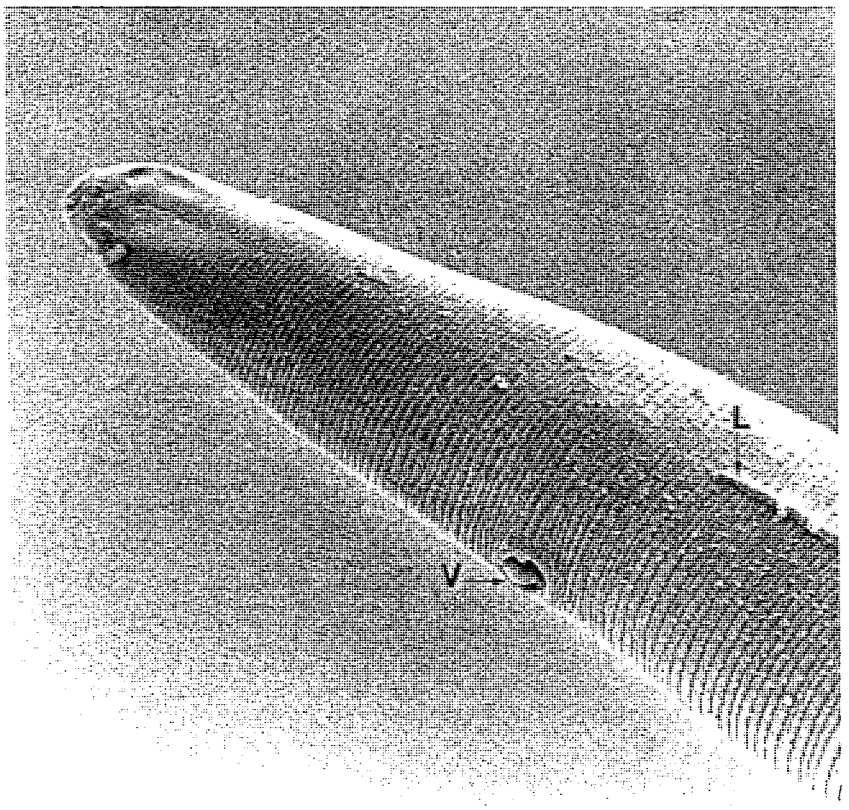
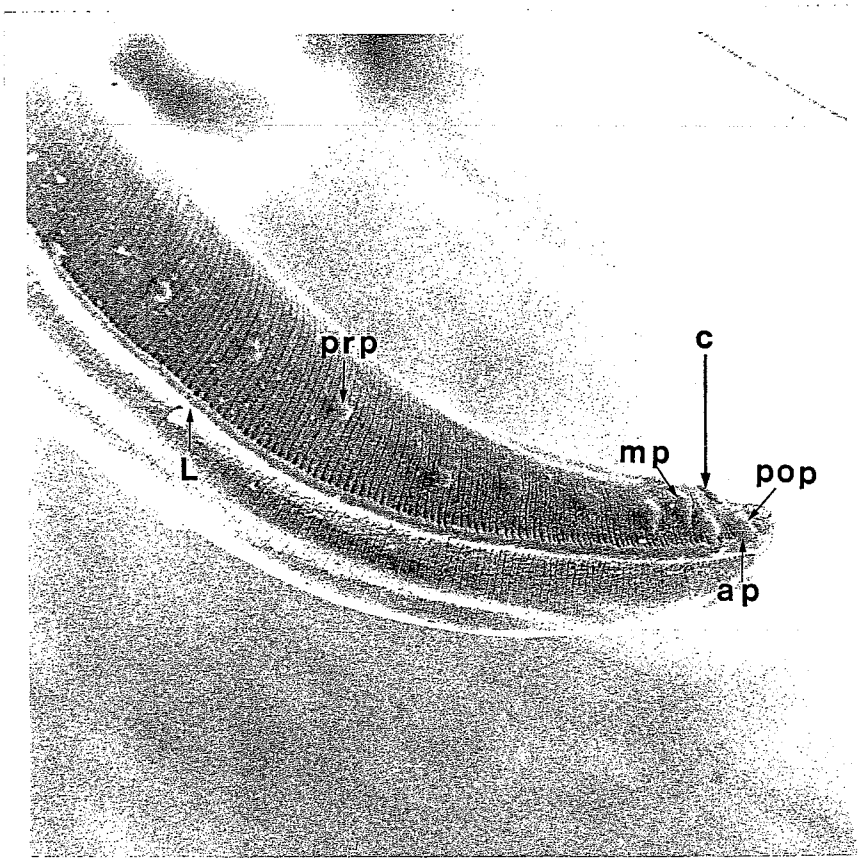

L
V

Figure 7.7 Lateral view of tail of adult male *Stephanofilaria* sp. from Australia. ap. adanal papilla; c cloaca; L lateral ala; mp median papilla; pop postanal papilla; prp preanal papilla. SEM.

Figure 7.8 Ventrolateral view of tail of adult male *Stephanofilaria* sp. from Australia. ap adanal papilla; c cloaca; L lateral ala; mp median papilla; pop postanal papilla; prp preanal papilla. SEM.


prp
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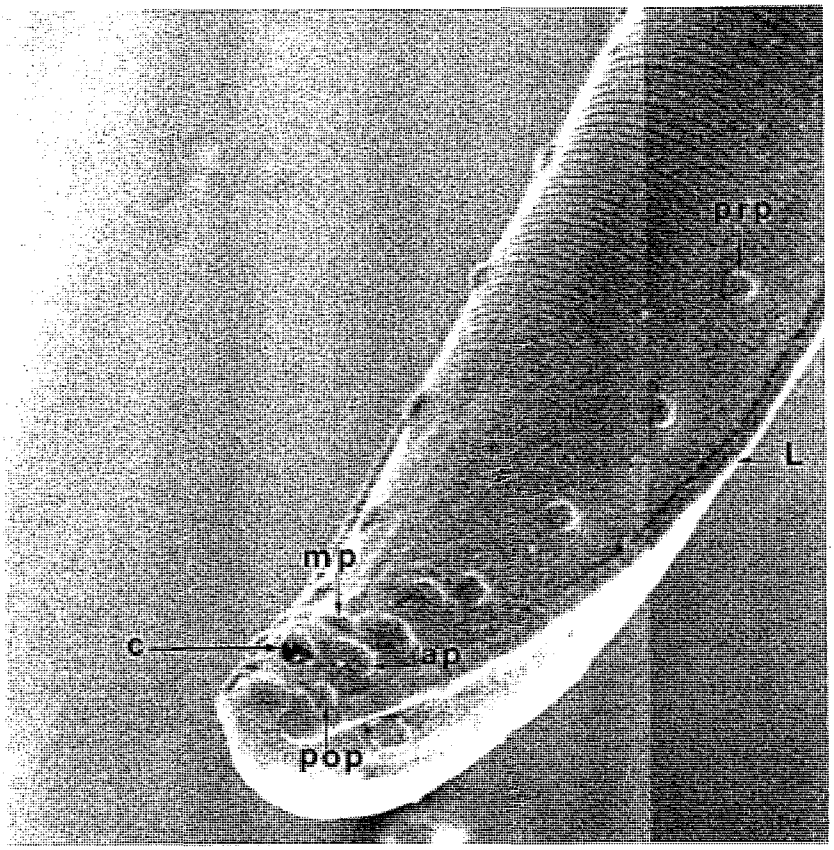
prp
L
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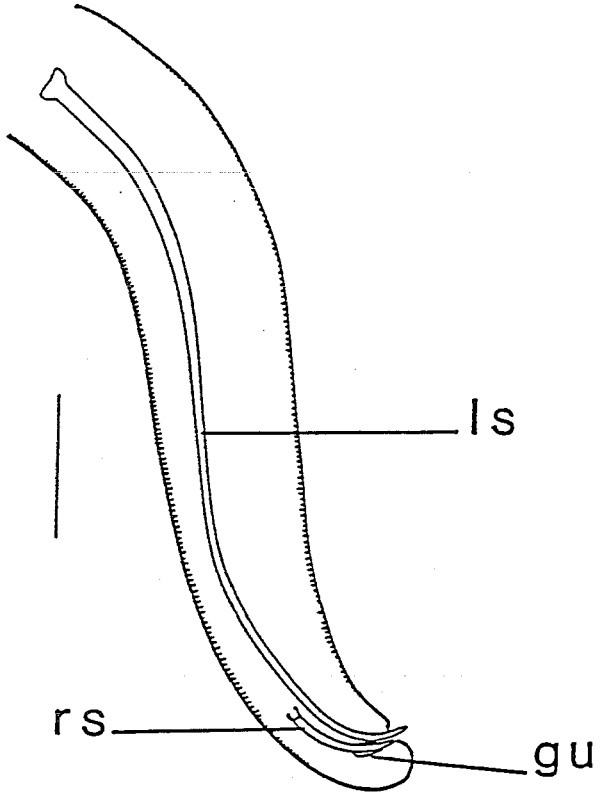
pop

Figure 7.9 Posterior end of adult male *Stephanofilaria* sp. from Australia. gu = gubernaculum; ls = left spicule; rs = right spicule. Scale line = 50 um.

Figure 7.10 Vulva of adult female *Stephanofilaria* sp. from Australia. SEM.


ls
rs
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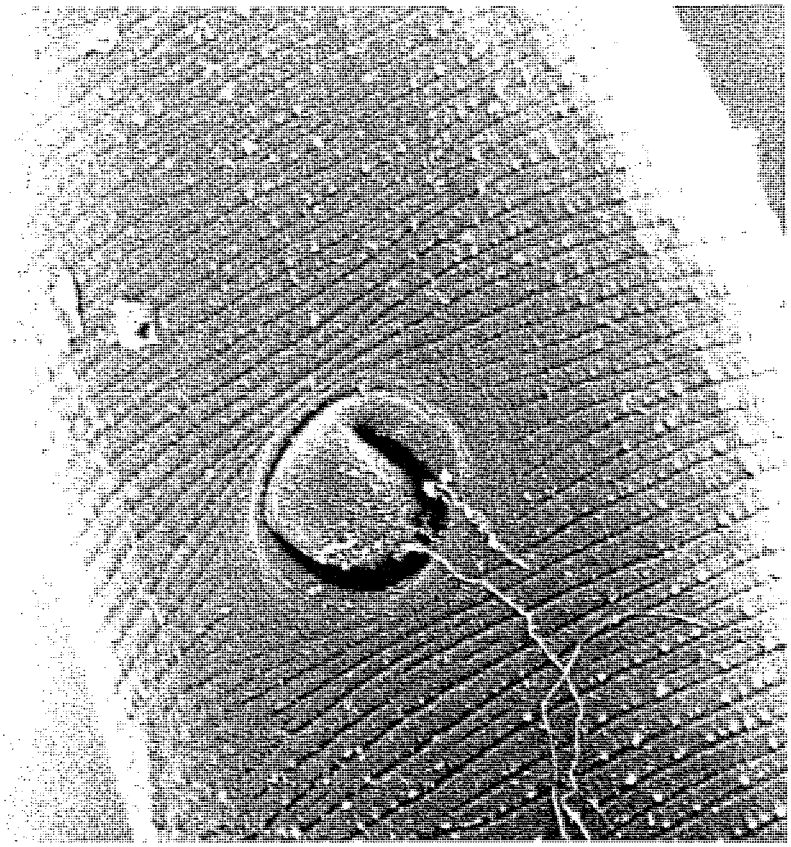


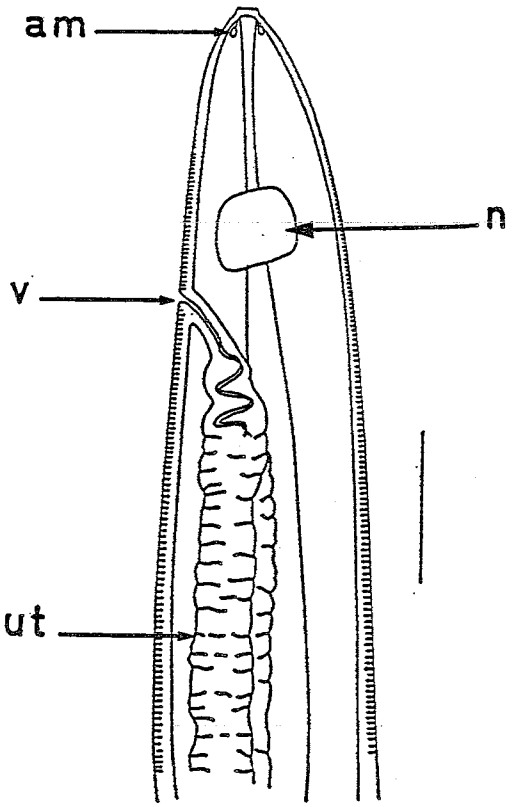


Figure 7.11 Anterior end of adult female *Stephanofilaria* sp. from Australia. n = nerve ring; v = vulva; ut = uterus; am = amphid. Scale line = 50 um.

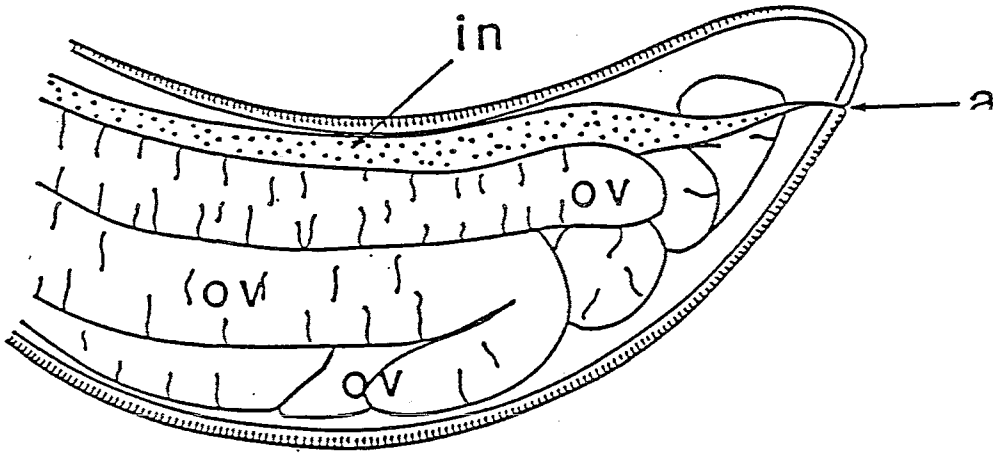
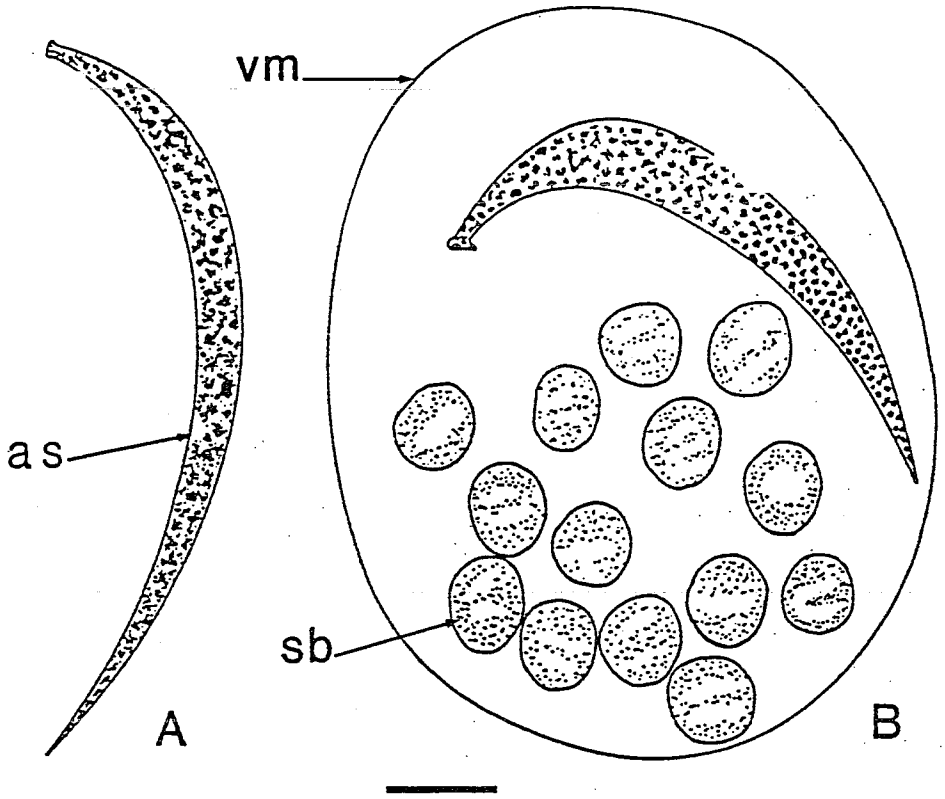
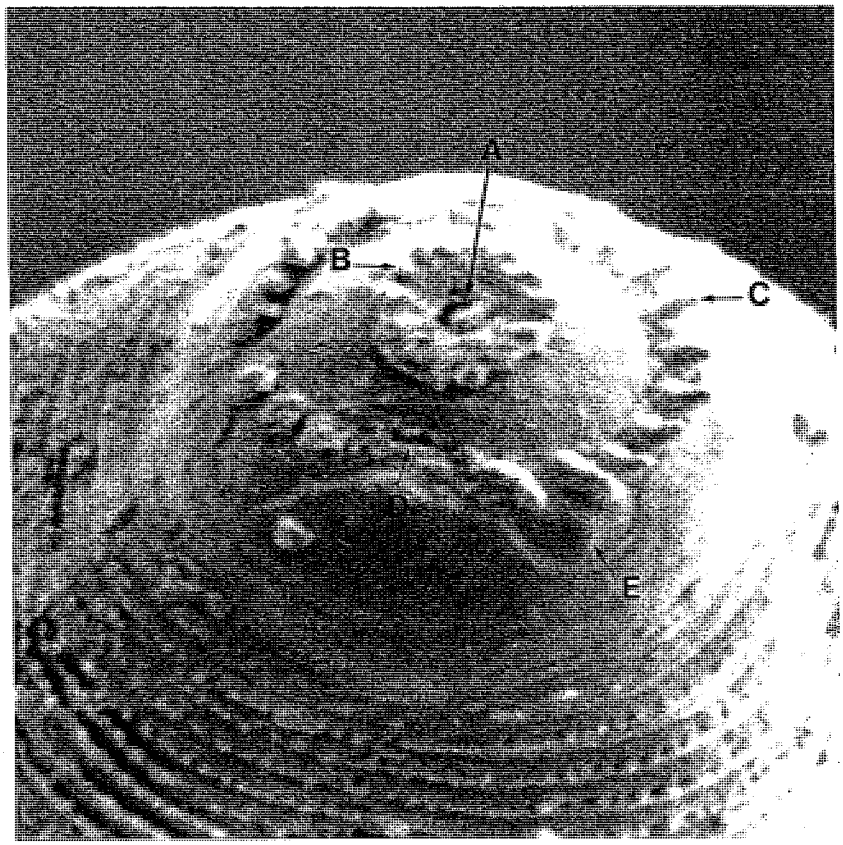


Figure 7.12 Posterior end of adult female *Stephanofilaria* sp. from Australia. a = anus; in = intestine; ov = ovary. Scale line = 50 um.

Figure 7.13 Microfilaria of *Stephanofilaria* sp. from Australia (A) free (B) enclosed within vitelline membrane. as = anal space; sb = spherical body; vm = vitelline membrane. Scale line = 10 um.

Figure 7.14 Head of adult female *Stephanofilaria dedoesi* from the skin of a cow in Sulawesi, Indonesia. (A) mouth (B) peribuccal spines (C) cephalic spines (D) amphid (E) cephalic papilla. SEM.


vm
a s
sb
A
B


A
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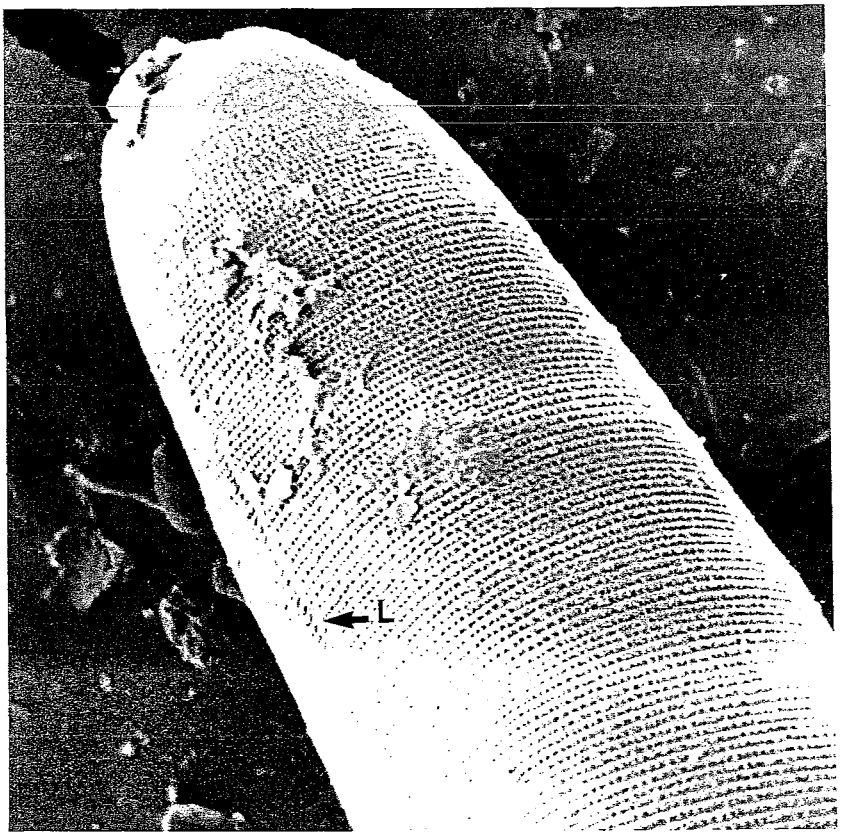
E

Figure 7.15 Anterior end of adult female *Stephanofilaria dedoesi* from the skin of a cow in Sulawesi, Indonesia. L depression over lateral cords. SEM.

Figure 7.16 Cuticle of adult female *Stephanofilaria dedoesi* from the skin of a cow in Sulawesi, Indonesia, showing posterior serrated frill on cuticular striations in anterior 1/3 of body. SEM.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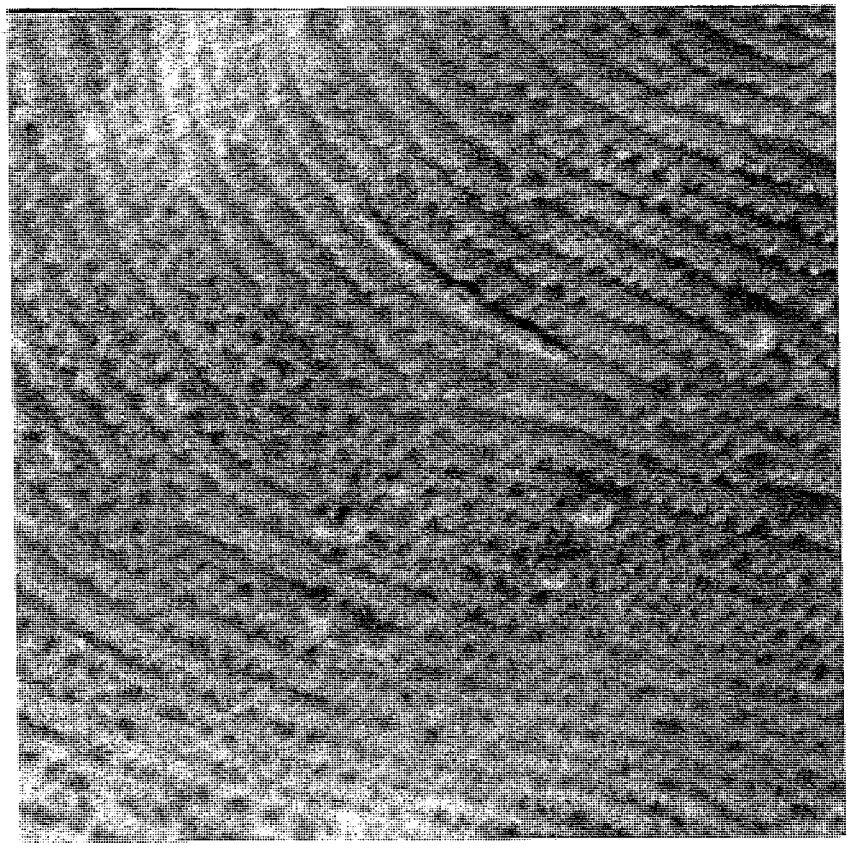
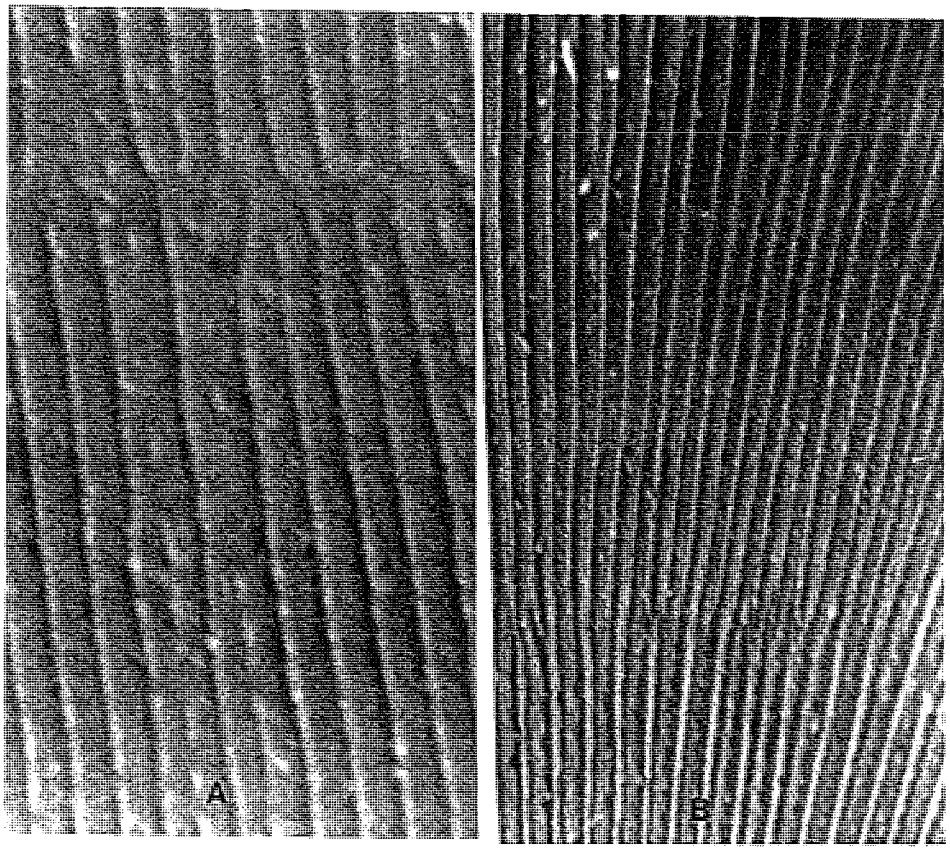
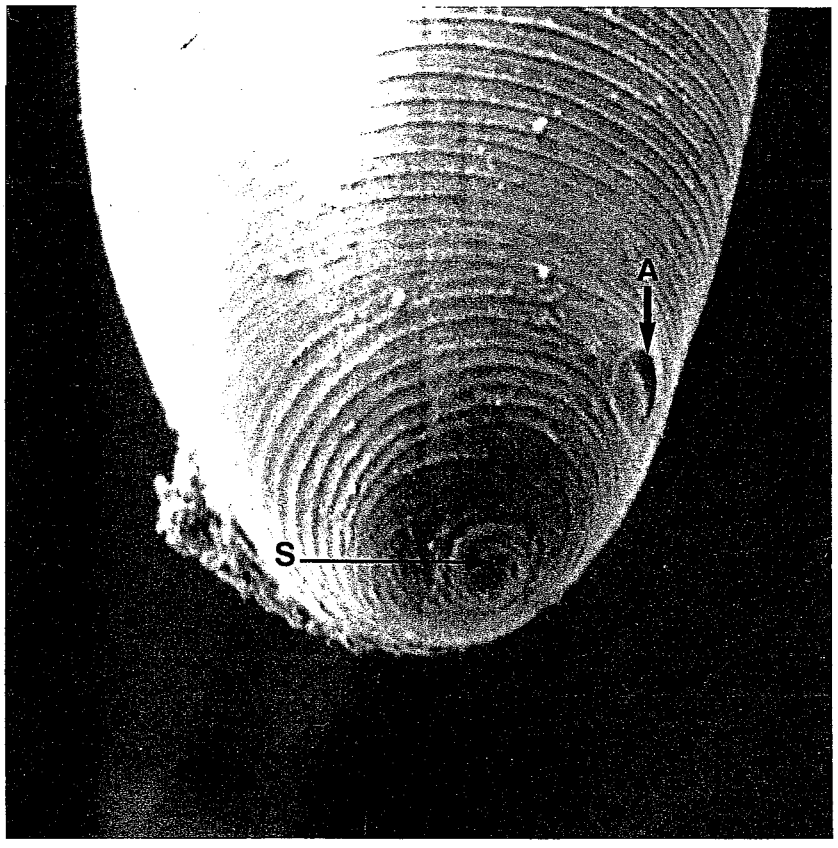


Figure 7.17 Cuticle of adult female *Stephanofilaria dedoesi* from the skin of a cow in Sulawesi, Indonesia, showing smooth cuticular striations. (A) mid body (B) posterior body. SEM.

Figure 7.18 Tail of adult female *Stephanofilaria dedoesi* from the skin of a cow in Sulawesi, Indonesia. (A) anus (S) phasmids SEM.


A
B


A
S

8000 TREATMENT AND CONTROL

8100 INTRODUCTION

Stephanofilarial infection was shown to be widespread in the Queensland cattle population (3000) and in endemic areas calves become infected early in life (5000). The effects of stephanofilarial infection on growth rate are not known. Although numerous treatments against stephanofilariasis have been evaluated (2000), little work has been done on the broad spectrum anthelmintics currently in use in the Australian cattle industry.

If treatment of stephanofilarial infection was to be undertaken in large numbers of cattle in the field in Australia, then a suitable diagnostic test would be necessary to monitor for effectiveness of treatment. Surgical biopsy and recovery of parasites would only be possible on small numbers of cattle. A disadvantage of this diagnostic technique to the stud industry would be permanent scarring of the biopsy site.

It was shown earlier in this study that the lesions were caused by infection with *Stephanofilaria* sp.(4400; 6330). Successful treatment of the lesions should therefore either result in resolution of the lesions or at least prevent an increase in the size of lesions. Resolution of lesions would only occur in those animals in which infection was recent and pathological changes were not extensive and irreversible. The most suitable animals in which to assess efficacy of treatment by this method would be young animals with recently established infection and with lesions that were increasing in size.

In this study several drugs were screened for efficacy against stephanofilarial infection. In the initial trials the efficacy was assessed by monitoring changes in lesion size but in the subsequent trials an attempt was made to measure parasite mortality.

8200 EXPERIMENT I

In this trial levamisole was used in young weaned calves. A treatment interval of 27 days was chosen because this was the interval used in the industry for the control of gastrointestinal helminths. An exception occurred at the fourth treatment when animals were treated by mistake after only 12 days.

8210 Materials and methods

8211 Animals

Bos indicus calves of 2 Brahman (B) genotypes, 1/2 B and 3/4 B were used. The calves were from the 1982 calf crop at Swans Lagoon for which materials and methods have been presented in 5211.

8212 Experimental design

Seventy-three calves were stratified by sex, weight and genotype and allocated at random into one of 4 groups, namely, 1/2 B treated, 1/2 B control, 3/4 B treated and 3/4 B control. All animals were weighed and the treated animals were administered 9 mg per kg of levamisole phosphate by subcutaneous injection on each of the following dates, 7 May, 3 June, 1 July,

13 July, 12 August, 9 September and 8 October.

The animals were visually inspected for the presence of stephanofilarial lesions at the final treatment (8 October) and subsequently on 10 November 1981 and 10 February and 17 March 1982.

8213 Statistical analysis

The data were analysed using the method outlined in 5230.

8220 Results

8221 Prevalence of lesions

Prevalence of lesions was not affected by the sex, birth weight, weaning date, weaning weight or average daily gain of the animals on any of the dates.

8222 8 October 1981

Only 70 animals were examined on this date and the model of the prevalence of lesions was as follows:

Prevalence of lesions	=	0.289 (SE 0.093)	+	0.277 genotype (SE 0.110)	+	0.294 treatment (SE 0.110)

The goodness of fit of the model was 17.6%. In both the control and treated groups, the 3/4 B animals had a significantly lower prevalence of lesions than the 1/2 B animals. Within each genotype, the treated group had a significantly lower prevalence of lesions than the control group ($P < 0.01$). Prevalence values in relation to genotype and treatment are shown in figure 8.1.

8223 11 November 1981

This observation was conducted 34 days after the final treatment and all 73 animals were examined. The model of the prevalence of lesions was as follows:

Prevalence of lesions	=	0.305 (SE 0.088)	+	0.391 genotype (SE 0.102)	+	0.237 treatment (SE 0.103)

The goodness of fit of the model was 22.4%. Although the prevalence of lesions in the treated groups had increased slightly since the cessation of treatment, it was still significantly lower than the control groups. As at the previous date, the prevalence of lesions in the 3/4 B animals was significantly lower than that of the 1/2 B animals (Figure 8.1).

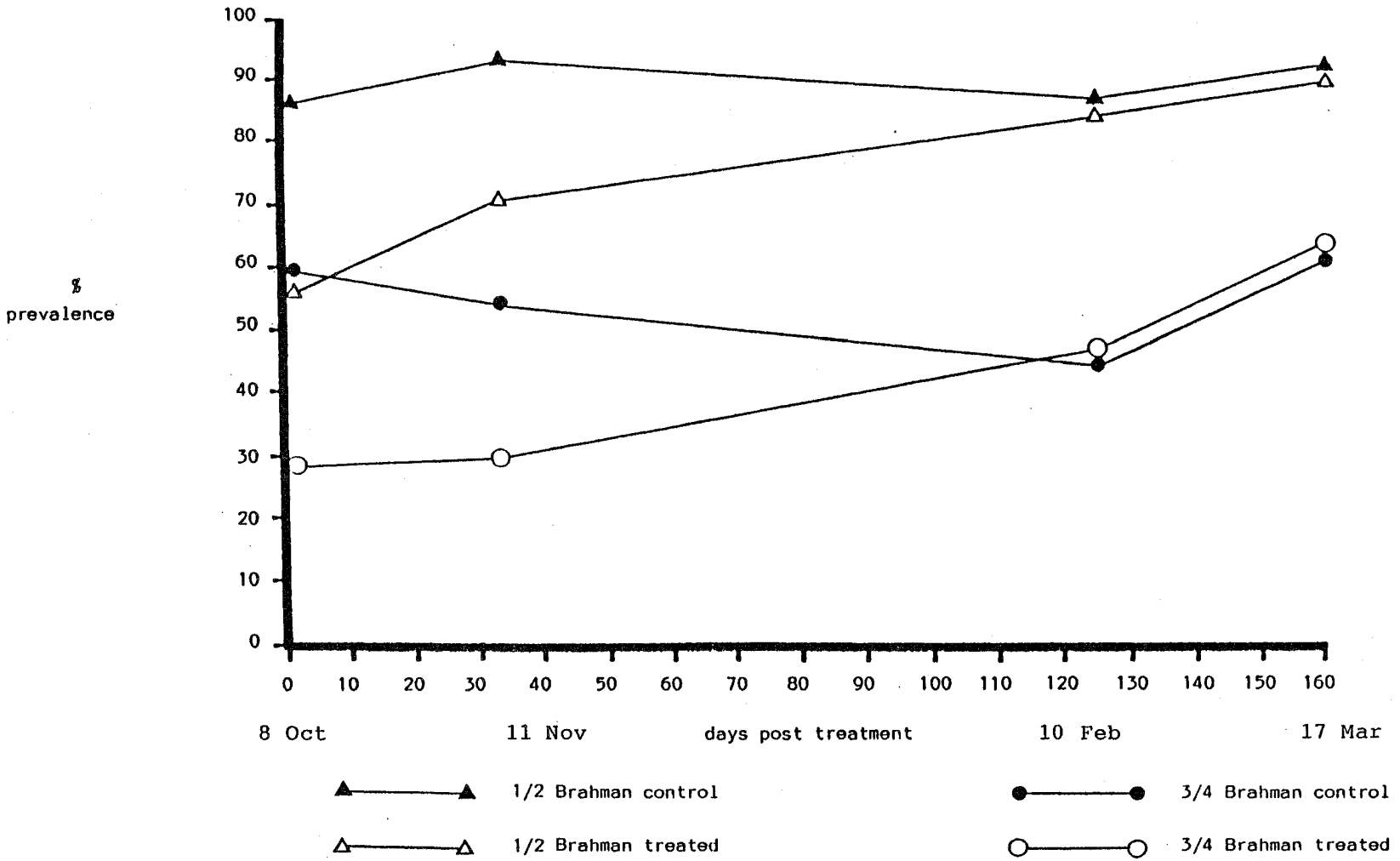
8224 10 February and 17 March 1982

Seventy-two and 73 animals were examined on 10 February and 17 March 1982 respectively and the models of the prevalence of lesions were as follows:

Prevalence of lesions	=	2.760 (SE 0.972)	+	0.425 genotype (SE 0.0976)	-	0.0668 birth date (SE 0.00276)
Prevalence of lesions	=	3.100 (SE 0.883)	+	0.310 genotype (SE 0.088)	-	0.00717 birth date (SE 0.00251)

The goodness of fit of the models was 30.5% and 26.9% respectively. There was no longer any difference in prevalence of lesions due to treatment but differences in prevalence due to genotype were still evident (Figure 8.1). Birth date had entered the models indicating an increase in the prevalence of lesions in the older animals.

Figure 8.1 Change in the prevalence of stephanofilarial lesions in young *Bos indicus* animals of known genotype following 7 treatments with levamisole over 6 consecutive months.


%
prevalence
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
8 Oct
11 Nov
days post treatment
10 Feb
17 Mar
1/2 Brahman control
3/4 Brahman control
1/2 Brahman treated
3/4 Brahman treated

8300 EXPERIMENT 2

In the second trial avermectin was assessed in young animals. The results of the levamisole trial suggested that change in lesion size may be a useful monitor of efficacy of treatment. In this trial parasite numbers were measured in some animals for comparative purposes. An additional group of animals was treated with closantel for reasons not associated with this trial. This drug was not selected for assessment against *Stephanofilaria* sp. for reasons discussed later (see 8500), however, the results are included here for comparative purposes.

8310 Materials and methods

8311 Animals

One hundred and eighty-seven calves of known *B. indicus* genotype from the James Cook University farm "Fletcherview", located 20^{o} 03' South 146^{o} 20' East, were used in the trial. The calves were bred as single sire mating groups and were present as 4 genotypes, namely, 3/4 Africander, 3/4 Brahman, 3/4 Sahiwal and Reciprocal (3/4 Africander x 3/4 Brahman). Before weaning the calves were run with their dams in herds of mixed genotypes and after weaning all calves were run together.

8312 Experimental design

The calves were stratified on genotype, sex and age and allocated at random into one of 3 groups, namely avermectin group (A), closantel group (B) and controls (C). Calves in group A were treated with 200 ug per kg of avermectin B_1 by subcutaneous injection, calves in group B were dosed orally with 7.5 mg per kg of closantel and calves in group C were left untreated.

Treatments in both groups commenced on 25 and 26 February 1985 and were continued on the following dates, 19 March, 24 April, 4 June, 9 July, 19 August, 16 September, 14 October and 13 November. On each occasion that the animals were treated, each animal was weighed and visually inspected for the presence of ocular lesions of stephanofilariasis. On 19 March only 77 (41%) of the animals were able to be examined by the author. The remaining 110 animals were treated by a cooperator but no data on size of lesions were kept in order to maintain uniformity of examination procedure. Nineteen animals were removed from the trial after the July treatment for reasons not associated with the trial. In June, July, September and November between 1 and 3 animals evaded muster and were not treated or examined. The size of each stephanofilarial lesion was recorded as the maximum diameter of the lesion in centimetres.

8313 Parasite recovery

Surgical biopsies were taken from some lesions on 19 July and 9 December and examined for the presence of adults and microfilariae of *Stephanofilaria* sp. by the method outlined in 5220.

8314 Statistical analysis

The data were analysed for prevalence of lesions, size of lesions, change in size of lesions, weight of the animal and average daily weight gain of the animals. Standard least squares theory for non-orthogonal data (Harvey, 1960) was applied and identified the main effects of treatment, genotype, sex and age. All interactions were tested and found to be not significant.

8321 Prevalence of stephanofilarial lesions in relation to treatment

At the commencement of the trial the overall mean prevalence of lesions was 24%, although only 77 animals were examined at this time. The youngest animal found infected at this date was 37 days of age. By the third treatment date, all 187 animals were examined and the overall mean prevalence of lesions was 54%. There were no differences between the mean prevalence of lesions in any of the groups.

By the fourth treatment the prevalence of lesions had increased in all groups and the overall mean prevalence of lesions was 91%. Once again there were no differences between prevalence of lesions in any of the groups.

At treatment 5 the prevalence of lesions in the avermectin treated group had begun to decrease and was significantly less than that of the closantel treated group and the control group. The overall mean prevalence of lesions remained at 91% for the duration of the trial and the prevalence of lesions of the closantel treated group and the control group did not differ significantly from each other throughout the trial.

In the avermectin treated group, the prevalence of lesions showed a gradual decline throughout the remaining treatments and was always significantly lower ($P < 0.01$) than that of the other 2 groups. At the completion of the trial after 9 treatments the avermectin treated group had a mean prevalence of lesions of 76%.

Values for the prevalence of lesions in relation to treatment are shown in Table 8.1.

8322 Size of stephanofilarial lesions in relation to treatment

The size of stephanofilarial lesions increased in all groups over the first 3 treatment periods and there were no differences between the mean size of lesions of any of the groups.

By the fourth treatment, the mean size of lesions in the avermectin treated group had remained constant and was significantly smaller than that of the closantel treated group or the control group ($P < 0.01$).

After the fourth treatment, the mean diameter of lesions of the avermectin treated group decreased to 0.4 cm and remained at this level for the remainder of the trial. In the closantel treated group the mean diameter of lesions increased throughout the trial and by the completion of the trial had reached 1.3 cm. The mean diameter of lesions in the control group was highest in the fourth month of the trial, decreased slightly after this to remain constant at 1 cm until the sixth month, and then increased in the final 2 months.

Table 8.1 Prevalence of stephanofilarial lesions in young *Bos indicus* animals treated with avermectin B1 and closantel over a period of 10 months at Fletcherview in 1986.

	Mar		Apr		June		July		Aug		Sept		Oct		Nov	
	n	%p*	n	%p	n	%p	n	%p	n	%p	n	%p	n	%p	n	%p
Treatment group																
avermectin	22	44a+	57	51a	58	85a	59	75a	55	66a	53	73a	55	70a	55	76a
closantel	23	24a	60	56a	59	89a	60	94b	55	91b	54	97b	55	97b	55	100b
control	32	24a	70	46a	69	94a	64	92b	58	87b	58	94b	58	91b	56	97b

* values presented are mean values adjusted for other variables

\+ means not followed by the same letter differ significantly ($P < 0.01$)

n number of animals examined

%p % prevalence of stephanofilarial lesions

Values for the size of stephanofilarial lesions in relation to treatment are shown in Table 8.2.

This difference in the mean size of lesions between the avermectin treated group and the other groups was reflected in a significant difference in the change in size of lesions between the avermectin group and the other groups.

Over the complete period of the trial, the closantel treated and control groups showed an increase in size of lesions, whereas the avermectin treated group showed a decrease in size of lesions. A similar trend occurred in the period following the fourth treatment to the end of the trial, although the changes in size of lesions in each group were less in this period. The values for change in size of lesions in relation to treatment are shown in Table 8.3.

8323 Growth rate of animals in relation to treatment of stephanofilariasis

The mean weights of the animals in the 3 groups were the same at the beginning of the trial but by the completion of the trial, the mean weights of the animals in the avermectin and closantel treated groups were significantly higher ($P < 0.05$) than that of the animals in the control group (Table 8.4).

However, an analysis of the average daily weight gain of the animals in the 3 groups revealed that those in both the avermectin and closantel treated groups grew significantly faster than those in the control group ($P < 0.01$) and that there was no difference between the average daily weight gain of animals in the avermectin treated group and that of animals in the closantel treated group (Table 8.5).

Table 8.2 Size of stephanofilarial lesions in young *Bos Indicus* animals treated with avermectin B1 and closantel over a period of 10 months at Fletcherview in 1986.

	Mar		Apr		June		July		Aug		Sept		Oct		Nov	
	n	s*	n	s	n	s	n	s	n	s	n	s	n	s	n	s
Treatment group																
avermectin	22	0.2a+	57	0.7a	58	0.8a	59	0.4a	55	0.3a	53	0.4a	55	0.3a	55	0.4a
closantel	23	0.08a	60	0.9a	59	1.2b	60	0.9b	55	1.1b	54	1.0b	55	1.1b	55	1.3b
control	32	0.1a	70	0.7a	69	1.5c	64	1.0b	58	1.0b	58	1.0b	58	1.1b	56	1.2b

* values presented are mean values adjusted for other variables

\+ means not followed by the same letter differ significantly ($P < 0.01$)

n number of animals examined

s diameter of lesion (cm)

Table 8.3 Change in the size of stephanofilarial lesions in young *Bos indicus* animals treated with avermectin B1 and closantel over a 10 month period at Fletcherview in north Queensland.

	Treatment period			
	A		B	
	n	a	n	a
Treatment Group				
avermectin	22	-0.5a[+]	53	-0.3a
closantel	23	0.7b	55	0.4b
control	24	0.7b	56	0.5b

a change in lesion diameter (cm)

[+] means not followed by the same letter differ significantly ($P < 0.01$)

A full 10 month treatment period (February - November)

B final 6 month treatment period (June - November)

n number of animals examined

Table 8.4 Weight of young *Bos indicus* animals treated with avermectin B1 and closantel over a 10 month period at Fletcherview in north Queensland.

	March		July		November	
	n	wt*	n	wt*	n	wt*
Treatment Group						
avermectin	59	120a[+]	59	168a	55	172a
closantel	60	119a	60	165a	55	170ab
control	70	118a	69	165a	56	163b

* values presented are mean values adjusted for other variables

[+] means not followed by the same letter differ significantly ($P < 0.05$)

n number of animals examined

wt weight in kg

Table 8.5 Average daily weight gain of young *Bos indicus* animals treated with avermectin B1 and closantel over a 10 month period at Fletcherview in north Queensland.

	Treatment period			
	A		B	
	n	adg*	n	adg
Treatment Group				
avermectin	55	.21a+	55	.026a
closantel	55	.21a	55	.024a
control	56	.19b	56	.006b

* values presented are mean values adjusted for other variables

\+ means not followed by the same letter differ significantly ($P < 0.01$)

n number of animals examined

A full 10 month treatment period (February - November)

B final 6 month treatment period (June - November)

adg adjusted average daily weight gain (kg)

*8330 **Recovery of parasites***

In July, after 4 treatments had been given, a biopsy was taken from a single lesion from each of 39 animals and examined for *Stephanofilaria* sp. parasites. Thirty of these animals were selected at random and the remaining 9 were a group of Africander and Sahiwal females removed from the trial at this time for reasons not associated with the trial.

Parasites were recovered from 32 (82%) of the animals but within treatment groups, 45% of avermectin treated animals, 94% of closantel treated animals and 100% of control animals yielded parasites (Table 8.10). Adult parasites were recovered from 15 animals and the remaining 17 animals yielded only microfilariae.

In December, one month after the final treatment, 60 animals were selected at random and a biopsy taken from one lesion from each animal. Parasites were detected in 40 (66%) of the animals and, within treatment groups, 24% of avermectin treated animals, 93% of closantel treated animals and 100% of control animals yielded parasites (Table 8.10). Adult parasites were recovered from 31 animals and 9 animals yielded only microfilariae.

Biopsies were taken from sixteen animals on both occasions and of these, 6 were from the avermectin group, 6 from the closantel treated group and 4 were from the control group. On each occasion only one animal of the 6 from the avermectin treated group yielded parasites although it was a different animal on each occasion. In the closantel treated animals all 6 produced parasites in July but only 5 produced parasites in December. All 4 control animals produced parasites on both occasions.

Table 8.6 Recovery of *Stephanofilaria* parasites from lesion biopsies of young *Bos indicus* animals treated with avermectin B1 and closantel at Fletcherview in 1986.

	July		December	
	n	% infected	n	% infected
Treatment Group				
avermectin	11	45	25	24
closantel	17	94	15	93
control	11	100	20	100

n number of animals examined

8340 Size and prevalence of stephanofilarial lesions in relation to genotype

During the course of the treatment trial, examination of the animals for lesions suggested that there was a variation in the prevalence and size of lesions between the different genotypes. Analysis of the data has confirmed these differences.

8341 Prevalence of stephanofilarial lesions

Insufficient numbers of some genotypes were examined in March and April but numbers were increased in the June examination. At this time the Brahman animals had a significantly lower prevalence of lesions than the Sahiwal and Reciprocal animals which had a lower prevalence of lesions than the Africander animals ($P < 0.01$).

By the time the animals were examined in July the prevalence of stephanofilarial lesions had increased in all genotypes and only the Brahman animals retained a prevalence significantly lower than the general mean prevalence of lesions ($P < 0.01$).

This pattern of a lower prevalence of lesions in the Brahman animals was maintained throughout the remainder of the trial period. At the conclusion of the trial in November, 100% of Africander animals, 90% of Sahiwal animals, 92% of Reciprocal animals and 79% of Brahman animals had stephanofilarial lesions.

Values for prevalence of lesions in relation to genotype are shown in Table 8.6.

Table 8.7 Prevalence of stephanofilarial lesions in relation to genotype of young *Bos indicus* animals at Fletcherview over a 10 month period in 1986.

	Month																			
	Mar		Apr		June		July		Aug		Sept		Oct		Nov	
	n	%p*	n	%p	n	%p	n	%p	n	%p	n	%p	n	%p	n	%p
Genotype																				
Africander	-	-	24	91a[+]	24	100a	24	92a	16	99a	16	97a	16	98a	16	100a
Sahiwal	-	-	62	52b	61	90a	63	90a	56	87a	55	90a	56	89a	56	90ab
Brahman	22	10	22	9c	23	72b	22	76a	22	55b	22	70b	22	70b	22	79b
Reciprocal	54	51	79	60b	78	95a	74	88a	74	84a	72	88a	74	88a	72	92a

* values presented are mean values adjusted for other variables

[+] means not followed by the same letter differ significantly ($P < 0.05$)

n number of animals examined

%p % prevalence of stephanofilarial lesions

8342 Size of lesions in relation to genotype

Throughout the trial, the Brahman animals had significantly smaller lesions than all other genotypes ($P < 0.01$). Africander animals tended to have the largest lesions and there was no difference in size of lesions between Sahiwals and Reciprocals.

Size of lesions increased rapidly in all genotypes in Autumn (April - May), decreased slightly in winter (June - July), then slowly increased in spring and early summer (August - November).

Values for size of lesions in relation to genotype are shown in Table 8.7.

8350 Size and prevalence of stephanofilarial lesions in relation to sex

Throughout the trial the adjusted mean prevalence of lesions did not differ between male and female animals and although males tended to have slightly larger lesions, the difference in size of lesions was not significant. Values for prevalence and size of lesions in relation to sex are shown in Table 8.8.

8360 Size and prevalence of stephanofilarial lesions in relation to age

Throughout the trial period there was a consistent and significant trend for prevalence and size of lesions to increase with increasing age of the animal (Table 8.9).

Table 8.8 Size of stephanofilarial lesions in relation to genotype of young *Bos indicus* animals at Fletcherview in north Queensland.

	Month													
	Mar		Apr		June		July		Aug		Sept		Oct		Nov	
	n	s*	n	s	n	s	n	s	n	s	n	s	n	s	n	s
Genotype																
Africander	1	0.05	24	1.5a	24	1.9a	24	1.0a	16	1.3a	16	1.2a	16	1.2a	16	1.5a
Sahiwal	-	-	62	0.8b	61	1.3b	63	1.0a	56	0.8b	55	0.9ab	56	1.0a	6	1.1b
Brahman	22	0.01	22	0.2c	23	0.4c	22	0.2b	22	0.2c	22	0.2c	2	0.3b	22	0.4c
Reciprocal	54	0.3	79	0.9b	74	1.2b	74	0.7c	74	0.8b	72	0.8b	74	0.9ca	72	1.0b

* values presented are mean values adjusted for other variables

\+ means not followed by the same letter differ significantly ($P < 0.05$)

n number of animals examined

s lesion diameter (cm)

Table 8.9 Size and prevalence of stephanofilarial lesions in relation to sex of young *Bos indicus* animals at Fletcherview in 1986.

	Male			Female		
	n	%p*	s*	n	%p	s
Month						
March	38	32	0.2	39	29	0.1
April	91	48	0.8	96	54	0.8
June	91	91	1.2	95	88	1.1
July	86	87	0:8	97	87	0.7
August	85	79	0.9	83	83	0.7
September	84	87	0.8	81	85	0.7
October	85	85	0.9	83	87	0.8
November	83	91	1.1	83	92	1.0

* values presented are mean values adjusted for other variables

n number of animals examined

s diameter of lesion (cm)

%p % prevalence of stephanofilarial lesions

Table 8.10 The partial regression coefficient (b) and the standard error of b (S.E.b) for the variable, number of days from 1 January, 1985 to the birth date of each animal in relation to the prevalence and size of stephanofilarial lesions at Fletcherview 1986.

	Lesion prevalence		Lesion area	
	b	S.E.b	b	S.E.b
Month				
March	-.0056	.0025	ns	-
April	ns	-	ns	-
June	-.0022	.0010	-.0123	.0034
July	ns	-	-.0064	.0022
August	-.0055	.0014	-.0120	.0024
September	-.0037	.0013	-.0079	.0023
October	-.0036	.0013	-.0089	.0021
November	-.0035	.0011	-.00814	.0022

ns not significant

8400 EXPERIMENT 3

The third trial was conducted to screen some anthelmintics for efficacy against adults and microfilariae of *Stephanofilaria* sp. Avermectin, levamisole, morantel and oxfendazole were used because all are used in the cattle industry for control of gastrointestinal helminths.

8410 Materials and methods

8411 Animals

Fifty - five calves of known *B. indicus* genotype from the James Cook University farm "Fletcherview" were used. Materials and methods are similar to those given in 8311.

8412 Experimental design

The animals were stratified on genotype, sex, age and size of lesion and 40 animals were allocated at random into 4 equal groups. The remaining 15 were left as untreated controls. Calves in group A were treated with 200 ug per kg of avermectin B1 by subcutaneous injection, calves in group B were dosed orally with 4.53 mg per kg of oxfendazole, calves in group C were dosed orally with 10 mg per kg of morantel and calves in group D were given 8.9 mg per kg of levamisole by subcutaneous injection.

For detection of parasites, each animal had both eye lesions surgically removed. One lesion was digested in collagenase and the other fixed in neutral buffered formalin and processed for histological examination. Half the animals in each treated group were biopsied on day 7 post treatment and the remainder on day 14 post treatment. Four control animals were biopsied on day 1, 5 on day 7 and the remaining 6 on day 14.

8420 ***Results***

On days 7 and 14 post treatment adults and microfilariae of *Stephanofilaria* sp. were recovered from 100% of animals treated with avermectin, morantel and levamisole, and from 60% of animals treated with oxfendazole. Parasites were recovered from 100% of control animals on each sampling day. Differences due to treatment were not significant on either occasion (Day 7, $X_4^2 = 8.29$, $P > 0.05$; Day 14, $X_4^2 = 9.10$, $P > 0.05$).

Many adults and most microfilariae were alive when recovered by digestion and no evidence of dead and dying parasites could be found in histological sections.

8500 ***DISCUSSION***

The results of the first 2 experiments suggested that both levamisole and avermectin B1 had some efficacy against stephanofilarial infection, whereas orally administered closantel had none.

No data were recorded for prevalence of lesions during the levamisole trial. However, the animals were randomly divided into groups at the commencement of the trial and therefore, the significantly lower prevalence of lesions in the treated group at the end of the treatment period suggests some efficacy against this parasite. The results of the third trial indicate that a single dose of levamisole has no effect on adults or microfilariae of *Stephanofilaria* sp.

Ueno and Chibana (1980) and Mitra et al (1984) found levamisole to be effective against stephanofilarial

infection although lesions re-occurred within 8 weeks of cessation of treatment. Lesions also occurred on the animals in the first trial in this study within 8-10 weeks of cessation of treatment but this was undoubtedly due to infection acquired post treatment.

Levamisole affects acetylcholine mediated neurotransmission in nematodes causing a reversible paralysis (Anderson and Waller, 1983). Paralysed nematodes in the gastrointestinal tract are removed by peristalsis. No mechanism exists for rapid removal of paralysed nematodes from within the skin and they would remain *in situ* and recover from the paralysis. Cell mediated immune responses of the host may be more effective against paralysed nematodes but this is a prolonged process and no evidence of it was found in the sections examined histologically.

The lower prevalence of lesions in the treated animals may have resulted from repeated administration of the drug. A possible explanation is that levamisole is active against the developing stages of the parasite and repeated treatments prevent reinfection of the lesions. Natural attrition of the adults, particularly if longevity is short (Tripathy and Das, 1987), would result in loss of infection and resolution of the lesions. If the prepatent period of the parasite was less than 35 days (see 6330) then a shorter treatment interval may have been necessary in this study to prevent maturation of all larvae.

Therefore, it is possible that the lesions remaining in this trial had reduced parasite numbers resulting in

partial resolution of the lesions. Complete resolution of the lesions may not have been possible if the pathological changes present were irreversible.

Avermectins have been shown to act against several stages of some filarial nematodes. Efficacy has been demonstrated against adult *Parafilaria bovicola* (Swan et al, 1983), against larval *Dirofilaria immitis* (Blair and Campbell, 1980) and *O. lienalis* (Lok et al, 1987). Avermectin B1 causes paralysis of nematodes by effecting a release of gamma aminobutyric acid (Pong et al, 1980) which results in blockage of transmission between neurons in the ventral nerve cord (Kass et al, 1980). Unlike levamisole, the paralysis resulting from avermectin B1 appears to be irreversible (Fritz et al, 1979).

In the second trial in this study prolonged avermectin B1 therapy resulted in fewer lesions and smaller lesions but it did not give complete resolution of lesions. The results of the single dose trial indicate no efficacy of avermectin B1 against adults of *Stephanofilaria* sp. This is supported by the finding of adults in 24% of lesions at the completion of the prolonged therapy trial.

Avermectin B1 has been shown to prevent development of *H. i. irritans* larvae in the manure of treated animals (Miller et al, 1981; Schmidt, 1983), but its effects on adults are not known. It is therefore possible that numbers of *H. i. exigua* were reduced on avermectin treated animals during this trial resulting in reduced transmission of *Stephanofilaria* sp. to these animals. Avermectin B1 may also affect the feeding behaviour of *H. i. exigua* towards treated animals thereby reducing transmission of *Stephanofilaria* sp. to them.

Morantel has similar kinetics and mode of action to levamisole and is therefore unlikely to be effective against skin dwelling parasites for the same reasons. Closantel binds strongly to plasma proteins and is most effective against blood feeding parasites (Hall et al, 1981). It was always unlikely to be effective against tissue dwelling parasites and is not used in cattle and therefore, was not selected.

Oxfendazole acts against nematodes by inhibiting the fumarate reductase system and by prevention of microtubule formation (Anderson and Waller, 1983). It was ineffective in the single dose trial but may be more effective if a more intense or prolonged activity against the metabolism of the parasite could be achieved.

The effects of treatment on growth rate of the animals in the second trial are interesting but are probably not associated with treatment of stephanofilarial infection. The factors affecting the growth rate of young animals in the field under the conditions of this trial are multiple. Whilst it could be assumed that most of these factors would have operated equally on all groups in the trial, the 2 drugs used were broad spectrum anthelmintics and both gave increased average daily weight gain over that of untreated animals. This result was almost certainly due to control of parasites other than *Stephanofilaria* sp. because closantel therapy was ineffective against this parasite.

The epidemiological data from Fletcherview on prevalence and size of stephanofilarial lesions in relation to genotype, sex and age of the host animal were in agreement with the findings at Swans Lagoon and Expedition (5000). A constant feature of this disease on all properties was the increased resistance to

infection in animals with increased Brahman content.

The finding of an infected animal as young as 37 days of age is consistent with the short prepatent period for this species found earlier in this study (6330).

No anthelmintic was completely successful against this disease in these trials. Therefore, in order to achieve satisfactory control of this disease with any of these drugs, it may be necessary either, to commence treatment before calves become infected, to shorten the interval between treatments or to increase the dose rate used. Concurrent vector control to prevent reinfection would most likely be necessary.

9000 GENERAL DISCUSSION

One of the initial objects of this investigation was to establish the aetiology of the lesions around the eyes and along the neck of cattle in northern areas of Australia. These lesions had been traditionally ascribed to the feeding activity of the buffalo fly, *H. i. exigua* even though the occurrence of the lesions on cattle was not always directly correlated with buffalo fly activity (Eastaway, 1974; Holroyd et al, 1984). *H. i. exigua* also occurs on cattle throughout Asia but there are no reports in the literature of it causing discrete feeding lesions on cattle in these areas as it was presumed to do in cattle in Australia. The closely related *H. i. irritans* from southern Russia and North America was also not known to cause such feeding lesions on cattle. Therefore an alternate aetiology seemed possible.

Initial histological examination of lesions revealed the presence of *Stephanofilaria* sp. parasites in some lesions. The gross and microscopic appearance of infected lesions was similar to that of apparently uninfected lesions and suggested a common aetiology. However, even with repeated histological sectioning of lesions, parasites could be found in only 40%-50% of lesions. Recovery of parasites by saline recovery technique was equally inconclusive.

It was not until the digestion technique was developed and younger animals examined, that parasites were detected in almost all lesions. In the endemic area of north Queensland, 75-100% of lesions on animals aged between 8 and 12 months were found

to contain parasites. It was therefore tentatively concluded that the aetiology of the lesions was stephanofilarial infection and not buffalo fly feeding.

Subsequently it was shown that uninfected *H. i. exigua* were unable to produce the characteristic lesions seen on cattle whereas similar flies infected with larvae of *Stephanofilaria* sp. did so. Further evidence for this conclusion was obtained during the treatment trials where administration of anthelmintics such as levamisole and avermectin appeared to inhibit development of lesions in treated animals exposed to normal field populations of buffalo fly whereas lesions developed normally in untreated animals.

The prevalence of stephanofilarial infection was found to vary with the sex, age and breed of the host animal. Prevalence of infection in adult animals was highest in bulls, intermediate in steers and lowest in cows. No differences in the prevalence of stephanofilarial infection occurred between the sexes in sexually immature animals.

The higher prevalence of infection in bulls than in cows and steers suggests that rate of infection was influenced by testosterone levels. This may have arisen from an increased attraction of the vector, *H. i. exigua*, to animals with higher levels of testosterone (Dobson et al, 1970) or from a higher innate resistance to infection in cows and steers than in bulls. A higher prevalence of infection in bulls than in cows has also been reported for *S. assamensis* infection in India (Patnaik, 1968; Dutta and Hazarika, 1973; Das et al, 1975b). However, transmission of *S. assamensis* infection is by a non biting fly

through pre-existing wounds (Hassan, 1969;1970; Patnaik, 1973) and therefore the increased infection rate in bulls may be associated with increased wounds on bulls used for harness work.

In this study it was found that not only was the prevalence of infection significantly lower in *B. indicus* type animals than in *B. taurus* type animals but that the prevalence of infection varied between genotypes within *B. indicus* type animals. Animals with greater than 75% Brahman content were significantly more resistant to stephanofilarial infection than animals with a similar Sahiwal or Africander content. A similar level of resistance to that shown by 75% Brahman animals was achieved by Sahiwal type animals only when the Sahiwal content was greater than 90%. Whether this resistance was an innate resistance to the parasite or to the vector or to both is not known. Whilst it is known that *B. indicus* type animals are infested with fewer buffalo flies than *B. taurus* type animals in herds containing both genotypes (Doube, 1984), no data have been published on variations in buffalo fly numbers between genotypes within *B. indicus* type animals. However in all groups of cattle examined in the field in this study, the infection rate ranged from 60% to 100%. It is therefore unlikely that lack of exposure to infected vectors was a limiting factor in the transmission of this parasite. Variations in prevalence of lesions were therefore most probably due to innate resistance of the host animals and this innate resistance was influenced by sex and breed of the host animal.

The surveys of young animals in the field revealed that in 1982 at Expedition and in 1983 and 1985 at Swans Lagoon, prevalence of infection was lower in light coloured animals than in darker coloured animals. This could be explained by the findings of

Franks et al (1964) who found that darker coloured animals were infested with more *H. irritans* than lighter coloured animals.

However, in all years of the survey at Swans Lagoon the most resistant group were those animals with 95% Sahiwal content and all of these animals were dark coloured. Therefore the *B. indicus* genotype was a stronger determinant of resistance to stephanofilariasis than coat colour.

In this study, lesions were found on only 4 sites on the host animal, namely, adjacent to medial canthus of the eye, on the front of the head, on the neck, and on the ventral midline overlying the xiphisternum. There is no evidence in the literature that larvae of *Stephanofilaria* spp. undergo a somatic migration. In *S. assamensis* infections (Patnaik, 1973) and *S. stilesi* infections (Hibler, 1966) lesions developed at the site at which infected flies were fed. In this study, animals as young as 37 days old were found infected, which indicated a much shorter time for development in the host for larvae of *Stephanofilaria* spp. than for larvae of other filarioids such as *Parafilaria* spp. (see Bech-Nielsen et al, 1982) and *Dirofilaria* spp. (see Kume and Itagaki, 1955). In this study the parasite was transmitted to an uninfected host and fully grown adults and numerous microfilariae were found 16 - 35 days later. This indicated a prepatent period shorter than 35 days for this *Stephanofilaria* sp. Also developing larvae were found within lesions in this study supporting the finding of Hibler (1966) working with *S. stilesi*.

Therefore it appears possible that development of *Stephanofilaria* spp. larvae in the host and resultant lesion formation occur locally at the site of inoculation by the vector.

All cattle examined in this study had lesions adjacent to the medial canthus of the eye and in young animals, the infection initially became established at this site.

Therefore, either *H. i. exigua* has a strong preference for feeding on the skin adjacent to the medial canthus of the eye of cattle or the skin in this site is physiologically more favourable to *Stephanofilaria* sp. than is the skin in other areas of the body.

With increasing age of the host animal there was an increase in the prevalence of neck lesions which suggests that, in adult cattle, the neck is an additional preferred feeding site for the buffalo fly. The almost exclusive occurrence of sternal lesions in adult bulls suggests that the hairless area overlying the xiphisternum resulting from frequent sexual activity, is also a favoured site for feeding by the buffalo fly.

Results obtained in this study support the conclusion that *H. i. exigua* is the principal vector of *Stephanofilaria* sp. in Australia. The close association between the distribution of the disease and the distribution of this fly indicated that it was the most likely vector. In addition, histological examination of lesions showed that adults and microfilariae occurred beneath intact epidermis and that therefore, only a true biting fly would be capable of ingesting microfilariae. Not only is *H. i. exigua* a true biting fly (Soulsby, 1982), but it is also one of the predominant flies occurring on cattle in northern areas of Australia (Seddon, 1967; Eastaway, 1974; Doube, 1984; Holroyd et al, 1984). *Musca vetustissima* can occasionally be common on cattle in Australia but it is a non biting fly and occurs throughout most of mainland

Australia (Hughes et al, 1982). No *Stephanofilaria* sp. larvae were found in this fly in this study. *Stomoxys calcitrans* is a biting fly that has the potential to transmit *Stephanofilaria* sp. in Australia. However, it prefers to feed on the legs of cattle and occurs widely throughout mainland Australia (Seddon, 1967). It is therefore unlikely to be epidemiologically significant for the transmission of this parasite in Australia.

H. i. exigua is the only haematophagous fly associated with cattle with a distribution matching that of stephanofilarial infection. It was the only fly taken from stephanofilarial lesions in the field in this study and only females of this species were found containing developing larvae of *Stephanofilaria* sp. Furthermore, it was shown to be capable of ingesting microfilariae, supporting development of the larvae and transmitting infective third stage larvae to the host. All of this was added evidence that this fly is the vector of stephanofilariasis.

The rate of infection of female flies ranged from 1%-9% and averaged 2.91%. No male flies were found infected which is similar to the findings of Hibler (1966) with *S. stilesi* transmission by *H. i. irritans*. Either male *H. i. exigua* feed at different sites on the host than female flies do, or other factors operate to limit the uptake or development of larvae of *Stephanofilaria* sp. in males of *H. i. exigua*.

The developing larvae of the *Stephanofilaria* sp. found in this study appear similar to those of *S. stilesi* (see Hibler, 1966). Few descriptions have been published of the larvae of other species in the genus. The characteristic circumoral elevation is present on second and third stage larvae.

The prominent single spine on the head of the infective third stage larva is apparently lost in later stages because it is not present in the adult parasite. A similar spine is present on infective third stage *S. stilesi* and is lost during the moult to the fourth stage (Hibler, 1966). This spine may be used to pierce the proboscis of the fly to enable escape into the tissues of the host during feeding by the fly.

Considerable confusion has existed in the taxonomy of *Stephanofilaria* spp. and no morphological or biological characters have been found to reliably differentiate all species within the genus. This has led to the description of several species of doubtful status and thereby further compounded the problem of species differentiation. It is testimony to this confusion, that, despite 7 species having been described and a further 2 proposed, no author has attempted to produce a key for separation of species within the genus.

The examination of the species found in this study as well as *S. dedoesi*, by scanning electron microscopy has shown that cephalic ornamentation appears to be the most reliable morphological character present in both species. Assuming the synonymy of *S. assamensis*, *S. kaeli* and *S. okinawaensis* with *S. dedoesi*, then the following key can be constructed to separate the species in the genus.

1. Cephalic armature consisting of peribuccal and cephalic spines (cephalic spines may be reduced) 2

   cephalic armature represented by peribuccal spines only, cephalic spines absent. left spicule 262-350 um long. Host cattle ..... Australian species.

2. Cephalic spines forming a complete circle interrupted laterally by amphids and numbering in excess of 16. Cuticular striations on anterior body bearing posterior serrations .. 3

cephalic spines reduced to 8 or fewer arranged symmetrically or asymmetrically around the head. Cuticular striations without posterior serrations 4

3. Cephalic spines numbering 16-24. Left spicule of male 130-230um long. Host cattle, buffalo, goat. .... *dedoesi*

cephalic spines numbering 28-32. Left spicule 160-220um long. Host buffalo. .... *zaheeri*

4. Cephalic spines 8 in number, arranged symmetrically as 4 pairs around the head. Left spicule 530-750um long, tail curved. Host, Black Rhinoceros. .... *dinniki*

cephalic spines numbering 4-6 and arranged as a single asymmetrical patch on the ventrolateral side of the head. Left spicule 270-376um long. Host cattle. .... *stilesi*

Examination of the remaining species by SEM would be necessary in order to confirm the reliability of such a key and also may reveal morphological characters of other species that could be used to strengthen the key.

The origin of the *Stephanofilaria* sp. found in this study is not known. The parasite would have almost certainly entered Australia in infected cattle or buffaloes, either at the same time, or subsequent to, the arrival of the vector *H. i. exigua*. Several shipments of cattle and buffaloes from Indonesia and Malaysia were imported into the Northern Territory early in the nineteenth century (Seddon, 1967). However, no *Stephanofilaria* sp. resembling the species found in this study has been reported from Asia and if it does occur there, then it has been overlooked.

The finding of *Stephanofilaria* spp. larvae in both *M. conducens* and *H. i. exigua* in Indonesia (Partoutomo et

al, 1981) is interesting and suggests the possibility of 2 *Stephanofilaria* spp. in that area. *S. dedoesi* causes the typical 'cascado' lesions on the withers of cattle in Indonesia (Bubberman and Kraneveld, 1933b; 1934c). These lesions appear identical to those of 'humpsore' in cattle in India caused by the almost certainly synonymous *S. assamensis*. The vector of *S. assamensis* is *M. conducens* (see Patnaik, 1973) and therefore the vector of *S. dedoesi* is also likely to be *M. conducens* as found by Partoutomo et al (1981). But the *Stephanofilaria* sp. larva recovered by the same authors from *H.i.exigua* was possibly that of a species other than *S. dedoesi*.

Cattle in Indonesia also have stephanofilarial lesions adjacent to the medial canthus of the eye (Kraneveld,. 1935b). The cattle from which Partoutomo et al took the infected flies, had eye lesions in addition to shoulder lesions (Partoutomo, personal communication). The eye lesions on those cattle were likely to be caused by the same *Stephanofilaria* sp. as found in this study and the larva recovered from *H. i. exigua* by Partoutomo et al was probably a larva of this *Stephanofilaria* sp.

Such a postulate will only be proved by the recovery and examination of *Stephanofilaria* spp. parasites from eye lesions in cattle in Asia.

Examination of the literature had shown that numerous compounds have been used for the treatment of stephanofilariasis, but that many of the drugs evaluated were either toxic or no longer readily available. Few of the newer anthelmintics have been assessed for efficacy against this disease and therefore levamisole, avermectin, morantel and oxfendazole were screened for efficacy against stephanofilariasis.

Levamisole had been shown to be effective against *S. okinawaensis* in Japan (Ueno and Chibana, 1980) and *S. assamensis* in India (Mitra et al, 1984). The trial conducted in this study suggested that prolonged administration of levamisole may have some efficacy against stephanofilarial infection in young animals. Prolonged therapy with Avermectin resulted in a lower prevalence of lesions in treated animals than in untreated animals, fewer parasites in treated animals than in untreated animals and a reduction in the size of lesions in treated animals compared to an increase in the size of lesions in untreated animals. Oxfendazole had limited efficacy whereas the other anthelmintics screened were ineffective.

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STEPHANOFILARIASIS IN CATTLE

S.J. JOHNSON, R. J. PARKER, J.H. NORTON, P.A JAQUES
AND A. A. GRIMSHAW

SUMMARY: a species of Stephanofilaria closely resembling S. Stilesi is recorded as the cause of lesions on the head, neck and sternum of cattle in northern Queensland. Macroscopic and histopathological descriptions of the lesions are given, together with a brief description of the parasite.

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The distribution and prevalence of stephanofilariasis in cattle in Queensland

S J JOHNSON, R J ARTHUR, and R K SHEPHERD

SUMMARY: A total of 10,543 cattle from 1,386 farms throughout Queensland was examined at abattoirs and the presence of stephanofilarial lesions was related to property of origin, sex, breed, and age of the slaughtered cattle and season of slaughter.

The mean prevalence was 38% and within the infected area this varied from less than 5% in south east Queensland to 95% on Cape York Peninsula. The prevalence of lesions was higher in bulls than in cows and was least in steers. *Bos indicus* animals had a lower prevalence than *B. taurus.* Prevalence increased with age. The distribution of the disease closely matched that of the buffalo fly, *Haematobia irritans exigua*.

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Prevalence of Stephanofilariasis in Young *Bos indicus* Cattle in Northern Australia

S.J. JOHNSON and M.A. TOLEMAN

ABSTRACT

Four consecutive annual calf crops of known genotype from a single property in northern Australia were examined for the presence of stephanofilarial lesions. The animals ranged in age from 60 to 348 days at the time of examination. Initial lesions of stephanofilariasis developed adjacent to the medial canthi of the eyes; animals as young as 69 days of age were found infected. In all years, prevalence of lesions increased with age for all genotypes, and was significantly lower in genotypes with higher *Bos indicus* content ($P < 0.01$) . Males had a higher prevalence of lesions than females in only one year, and in two of the years, dark-coloured animals had a higher prevalence of lesions than light-coloured ones.

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