

**IDENTIFICATION, BIONOMICS AND CONTROL OF  
INFECTIVE LARVAE OF COMMON NEMATODES  
OF DOMESTIC RUMINANTS**

By

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**THESIS**

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requirement for the degree

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**1995**



**To My Mother**

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I hereby declare that the thesis entitled "IDENTIFICATION, BIONOMICS AND CONTROL OF INFECTIVE LARVAE OF COMMON NEMATODES OF DOMESTIC RUMINANTS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

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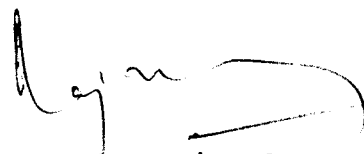
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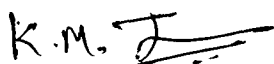
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**ABBREVIATIONS USED**

an	-	anus
bc	-	buccal capsule
ex.p	-	excretory pore
g.pr	-	genital primordium
int	-	intestine
nr	-	nerve ring
oes	-	oesophagus
rec	-	rectum
T.S	-	tail sheath
T.T	-	tail tubercle
u/um	-	micrometre

# *Introduction*

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## INTRODUCTION

The domestic ruminants play a major role in the rural economy of Indian Agricultural Sector. Next to land, the domestic ruminants are the precious possession of peasants. It is a well known fact that the gross income from domestic ruminants is derived from milk and milk products, meat and meat products, wool and pashmina, hides, skins, bones, hooves etc. and also the indispensable draft power and manure for agricultural operations and transportation in rural India.

But profitable animal husbandry is often impeded by different pathogens like bacteria, viruses and parasites. Among the parasitic diseases, nematodiasis is the common disease entity in domestic ruminants. From the tip of the hoof to the brain and nose to anus of an animal occur different nematode parasites, which adversely affect the production of milk, meat, wool and hide and even cause acute clinical diseases leading to death and thus heavy loss to farming industry.

Domestic ruminants acquire nematode infections by different ways. Infection by ingestion of infective larvae is the most common one. The eggs and larvae of some of the common nematodes are passed out mainly through faeces. They develop into infective larvae in ambient environmental conditions in faeces. This faeces contaminate the surroundings and pasture



land and the infective larvae migrate from the faecal pad to grass. The larvae remain viable in the soil or pasture for varying periods depending upon the climatic conditions and season. Their bionomics also vary in different times of a day. These larvae are ingested by the domestic ruminants from animal house and mainly from pasture during grazing. In a nutshell, the infection is called manure cum pasture borne nematode infection. This is a serious problem in temperate climate countries, because of the prevalence of ambient climate and season for the development of infective larvae in that countries. It leads to severe economic loss by way of loss of production and also due to mortality in domestic ruminants. There are so many reports from various parts of the world about the heavy economic loss due to pasture borne nematode infections. Research works on the bionomics and control of infective larvae and also on the pasture borne nematode infections are being carried out at various centres.

In a subtropical country like India, which has variable climatic conditions, the possibility of pasture borne nematode infection is high especially in free grazing animals. The factors like lowlying imperfectly drained, unhygienic grazing areas and the use of faeces, dung and slurry as manure which may contaminate the pasture, help the propagation and survival of infective larvae in pasture, which may subsequently infect the animals grazing it. But unfortunately there are no data

available on economic loss incurred by pasture borne nematode infections. Reports of systematic studies on parasitic epidemiology, bionomics and control of infective larvae of nematodes affecting domestic ruminants are also scanty.

Among the states of India, Kerala has a unique topography and climate. The rainfall is occurring from June to November comprising the South-West and North-East monsoon followed by the hot dry season from December to May. So during rainy season and upto February the entire state is covered by lush growth of grasses and other plants which are grazed by domestic ruminants. This climate is most favourable for the development and existence of infective larvae of nematodes in the pasture. So here in Kerala the infection recurs repeatedly through out the year. Hence the control of nematode infection in domestic ruminants is a difficult task for which a thorough knowledge on the specific identity of the infective larvae of nematodes occurring in domestic ruminants in different season and their bionomics is a must. With this aim in mind the present study to identify the infective larvae of nematodes commonly occurring in domestic ruminants and their bionomics such as phototropism and viability was undertaken. To formulate an economically feasible control measure against the pasture borne nematode infections of ruminants, the assessment of ovicidal and larvicidal property of the common fertilizers such as urea, ammonium sulphate, quick lime and ash was also undertaken during the study.

# *Review of Literature*

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## REVIEW OF LITERATURE

### Prevalence of common nematodes of domestic ruminants

There are many reports on prevalence of common nematodes in domestic ruminants like cattle, buffalo and goat in India. The reports were based on the results of faecal examination, faecal culture and also on the collection of worms on post-mortem.

#### Cattle

Vaidyanathan (1942) reported Strongyloides papillosus in 60 per cent of 150 young calves but none were found in 400 animals over two years of age. He described the various free living stages of Strongyloides papillosus. Sarwar (1945) also recorded this nematode from dairy calves at Izatnagar and Muckteswar.

The incidence of strongyle infection was found to be 29.8 per cent in cattle as per data furnished by Rajamohanam and Paily (1971) on parasitic cases treated in Veterinary Hospital, Trichur for 4 years from 1966-1969.

Sukumara Pillai (1980) reported that the non-descript and crossbred calves in Kerala were infected with eight species of gastro-intestinal nematodes viz. Strongyloides papillosus,

Neoscaris vitulorum, Cooperia sp., Haemonchus contortus, Bunostomum phlebotomum, Oesophagostomum radiatum, Trichostrongylus colubriformis and Trichuris globulosa and of which Strongyloides papillosus was the commonest. He also described and illustrated the infective larvae of above species except Neoscaris vitulorum and Trichuris globulosa.

According to Borkakoty et al. (1984) the incidence of Haemonchus, Strongyloides, Mecistocirrus, Cooperia and Neoscaris ranged from 13 to 34 per cent in calves in Kamarup district of Assam.

Muraleedharan et al. (1990) studied the spatial and seasonal incidence of gastro-intestinal parasites of cattle in Mysore and Mandya districts of Karnataka and found that Haemonchus, Bunostomum and Oesophagostomum infections were predominant there in hot season.

Raote et al. (1991) reported helminthic infections in cattle in Bombay and found that various nematodes occur in them and they were Strongyles (33.2%), Trichuris (20.07%) and Toxocara (1.82%) species. They also found Toxocara vitulorum more in January and Strongyles throughout the year.

Borthakur and Das (1994) reported from Assam that calves were more susceptible to nematodes than the higher age groups and the nematodes encountered were spp. of Haemonchus,

Oesophagostomum, Cooperia, Trichostrongylus, Mecistocirrus and Bunostomum.

Dixit and Sahasrabudhe (1994) investigated the prevalence of gastro-intestinal helminths in cattle in Jabalpur. The results showed that Haemonchus was most common followed by Trichostrongylus and Oesophagostomum in cattle. In calves (0-3 months of age) Toxocara vitulorum was most common followed by Strongyloides.

Mathur et al. (1994) reported that June to September were the months of high risk for nematode infections in dairy animals in West Bengal and the eggs per gram showed the predominance of Haemonchus and Cooperia. In native and crossbred cattle there Mecistocirrus and Trichostrongylus spp. were predominant whereas Haemonchus, Cooperia and Trichostrongylus were predominant in buffaloes. Strongyloides sp. were prevalent in calves of all types of dairy animals with its peak in June to October.

### **Buffalo**

Patnaik and Pande (1963) in a survey of the helminth parasites in 27 one month old buffalo calves in India, the following species were recorded in decreasing order of frequency and relative pathogenicity. Neascaris vitulorum, Strongyloides

papillosus, Paracooperia nodulosa, Cooperia laterouniformis, Bunostomum phlebotomum and Setaria labiatopapillosa.

Sharma and Pande (1963) found on autopsy of 103 buffalo calves Paracooperia nodulosa, Oesophagostomum radiatum, Strongyloides papillosus, Trichostrongylus colubriformis, and Gaigeria pachyscelis, in addition to many other parasites.

Bhopale et al. (1971) in a survey of helminth parasites in buffalo calves in Mhow region of Madhya Pradesh recorded the following species of nematodes as of common occurrence, viz., Oesophagostomum radiatum (58.6%), Paracooperia nodulosa (26.08%), Bunostomum phlebotomum (23.9%) and Thelazia rhodesi (23.9%).

Baruah et al. (1981) reported that 52.5 per cent of 1151 buffalo calves examined were found to be infected with Neascaris vitulorum in Hissar. He also reported other nematodes like Strongyloides papillosus, Trichostrongylus axei and Dictyocaulus viviparus.

Gupta and Chhabra (1990) revealed the occurrence of Toxocara vitulorum (41.2%), Strongyloides sp. (25.9%) and Strongyles (3.8%) in young buffalo calves in Haryana.

Muraleedharan et al. (1990) studied the spatial and seasonal incidence of gastro-intestinal parasites of buffaloes in Mysore and Mandya districts of Karnataka and found that

Haemonchus, Bunostomum and Oesophagostomum infections were predominant there in hot season.

According to Agnihotri (1993) 66.66 per cent of buffalo calves in Tarai region of Uttar Pradesh were infected with one or more intestinal parasites. They were Toxocara vitulorum (48.87%) Strongyloides papillosus (35%) and Strongylids (6.25%).

Dixit and Sahasrabudhe (1994) while investigating the prevalence of gastro-intestinal helminths in buffaloes in Jabalpur found that Haemonchus was most common followed by Trichostrongylus and Oesophagostomum. In calves (0-3 months of age) Toxocara vitulorum was most common followed by Strongyloides.

Rasool Saheb and Md. Hafeez (1994) reported seven species of nematodes occurring in buffaloes in Andhra Pradesh. They were Toxocara vitulorum (5.57%), Haemonchus contortus (4.33%), Bunostomum phlebotomum (4.95%), Oesophagostomum radiatum (3.78%) and Setaria cervi (11.63%).

#### Goat

Based on the larval counts, Tripathi (1970) recorded the following spp. of nematodes of goats viz., Haemonchus spp., Strongyloides spp., Trichostrongylus spp. and Oesophagostomum spp.



The incidence of strongyle infection was found to be 68.7 per cent of parasitic cases treated in goats at Veterinary Hospital, Trichur during 4 years period from 1966-1969 as per data furnished by Rajamohanan and Paily (1971).

Sathianesan and Peter (1971) studied the incidence of gastro-intestinal nematode infections in goats in Kerala by examining entrails of indigenous goats slaughtered in various parts of the state and by faecal examination and faecal culture in the case of farm bred goats. Eight hundred and sixty five entrails and 236 faecal samples were examined and 11 species of nematodes including two new species were encountered. This study revealed that 97.8 per cent of the indigenous goats and 89 per cent of the farm bred goats examined were positive for one or more species of gastro-intestinal nematodes. In indigenous goats, the highest incidence was for Haemonchus contortus (75%) and the lowest for Gaigeria pachyscelis (9.1%), whereas in the farm bred goats Strongyloides papillosus had the highest incidence (72.8%) and Trichostrongylus axei the lowest (18.2%) and they did not notice any significant seasonal variation in the incidence.

Misra (1972) reported that the epidemiological study of parasitic gastro-enteritis of goats in Bhubaneswar revealed 28 species of helminths in 85.0 per cent of the goats examined and the incidences of gastro-intestinal nematodes encountered were Gongylonema verrucosum (16%), G. pulchrum (12%), Haemonchus

contortus (80%), Mecistocirrus digitatus (12%), Trichostrongylus colubriformis (23%), Oesophagostomum columbianum (71%), Trichuris ovis (66%) and Trichuris globulosa (30%).

According to Shastry and Ahluwalia (1972) the incidence of various gastro-intestinal nematodes encountered in goats in Mathura (Uttar Pradesh) were Haemonchus contortus (86%), Trichostrongylus spp. (41%), Mecistocirrus digitatus (1%), Bunostomum trigonocephalum (56%), Oesophagostomum spp. (56%) and Trichuris ovis (72%).

Sinha and Sahai (1973) reported the incidence of Oesophagostomum columbianum (14.4%), O. venulosum (27.1%), Haemonchus bispinosus (67.2%) and H. contortus (74.2%) of 160 goats out of 162 examined in Patna.

According to Bali and Singh (1977) the incidence of Haemonchus contortus in goats in Hissar was 63.42 per cent.

The gastro-intestinal nematode parasites recorded in goats at Coimbatore (Tamil Nadu) were Haemonchus contortus (90%), Trihostrongylus axei (50%), T. colubriformis (80%), Gaigeria pachycelis (20%), Bunostomum trigonocephalum (20%), Oesophagostomum sp. (90%), Trichuris sp. (80%), and Skrjabinema ovis (10%) (Chellappa and Gopalakrishnan, 1977).

According to Masud Ahamed and Ansari (1987), among the nematodes they recovered from goats in Aligarh, Haemonchus contortus was having the highest incidence followed by Oesophagostomum columbianum and Trichuris ovis with lowest incidence. Similarly in goats in Tarai region of Utter Pradesh according to Upadhyay and Bhatia (1987), the incidence of Haemonchus contortus (74.09%) was highest followed by O. columbianum (43.37%). The youngest age group of goats showed only Strongyloides papillosus infection alone.

According to Sahai et al. (1990) the incidence of various gastro-intestinal nematodes in black Bengal goats in West Bengal was: Oesophagostomum columbianum (51.68%), O. asperum (27.08%), O. venulosum (7.92%), Trichuris globulosa (37.91%), Gaigeria pachycelis (14.16%) and Haemonchus contortus (32.08%). Study on the seasonal incidence of gastro-intestinal nematodes of goats showed highest incidence during winter months, 79.41 per cent followed by 76.40 per cent in monsoon and 72.28 per cent in summer months.

### **Identification of infective larvae**

Dikmans and Andrews (1933) while giving a detailed description of the infective larvae of the common gastro-intestinal nematodes of sheep pointed out that owing to overlapping, neither the length of the tail nor that of the tail sheath of infective larvae was reliable as a distinguishing

feature for an accurate diagnosis particularly in mixed cultures, where several species of smaller Trichostrongylids are involved. So they further stated that other characters must also be taken into consideration.

According to Gordon (1933) infective larvae of species of Ostertagia may be differentiated from those of Trichostrongylus by their total length. Alicata (1935) pointed out that tail of the infective larvae of Strongyloides papillosus of sheep and Strongyloides ransomi of swine are tripartite not bipartite. Andrews (1935) had given a key for the identification of the infective larvae of several gastro-intestinal nematodes of sheep with illustrations of their anterior ends.

Ortlepp (1939) described that the morphology of the various larvae of Bunostomum trigonocephalum were practically identical with that of Gaigeria pachyscelis. Anantaraman (1942) described the various free living stages of Oesophagostomum radiatum of cattle. Krug and Mayhew (1946) reported that the first larval sheath of Bunostomum phlebotomum (cattle) was not shed, so that the third stage larva had two sheaths. They further stated that the second and third stages were not sharply differentiated. Sprent (1946) also described all the three free living stages of Bunostomum phlebotomum.

Basir (1950) mentioned that no differences could be detected between the newly hatched larvae which developed into free living adults and those which directly formed infective larvae in Strongyloides papillosus. As the first moult approached however, the genital primordium in those destined to become infective larvae remained unchanged, whereas in those destined to become free living adults, divided into a number of cells and increased in length.

Keith (1953) while giving various details like measurements, photomicrographs and a key of infective larvae stated that for identification of the common gastro-intestinal nematodes of cattle, the appearance and length of the tail sheath were more reliable than the length and breadth of the larva. According to Kharichkova (1953) the infective larvae of Oesophagostomum radiatum and O. venulosum could be distinguished from the difference in the number and shape of their intestinal cells.

Hansen and Shivnani (1956) stated that the specific identity of the third stage larvae of nematodes parasitising beef cattle could be established on morphological characteristics. Thomas (1957) speciated the infective larvae of various spp. of Nematodirus on the basis of the measurements of the body, tail and tail sheath. According to Supperer (1958), the infective larvae of Bunostomum phlebotomum possess two sheaths and their oesophagus was divisible into two sections,

a glandular and a muscular section which enabled them to be easily distinguished from the larvae of other nematodes in cattle.

Zeletzki (1959) gave an illustrated account of the development of the parasitic first to third stage larvae of Strongyloides papillosus and according to him the first stage possessed a rhabditiform and the second stage an elongated filariform oesophagus. Donald (1963) described the infective larvae of Haemonchus similis and compared the same with infective larvae of H. contortus and H. placei. As per Isenstein (1963) the infective larvae of Cooperia oncophora were larger than that of C. curticei and C. pectinata. Gevrey *et al.* (1964) reported that gastro-intestinal strongyles of ruminants could be identified more accurately from their infective larvae than from their eggs. Sahai (1966) described and compared the morphology of eggs and larvae of Haemonchus contortus and H. bispinosus. All stages of H. bispinosus differ in size from the corresponding stages of H. contortus and the infective larva had a more whip like tail. Niec's (1968) identification of the infective larvae of strongyles of sheep and cattle was based mainly on their sheath length. He also made use of the measurements of other parts of the body and also the morphology of the larvae for this purpose.

Sathianesan *et al.* (1968) had made a detailed study of free living larval stages of various common nematodes of

goats and they had compared their findings with that of certain previous workers. Similarly Tripathi (1968) made a detailed study of infective larvae of various common gastro-intestinal nematodes of goats at IVRI. Like the previous worker Hulinska (1969) had also differentiated the infective larvae of common gastro-intestinal nematodes of sheep based on the morphology and body measurements. Padmavathi et al. (1971) also studied the morphology of infective larvae of Haemonchus contortus and H. bispinosus from sheep.

Hentriksen (1972) described and illustrated with photomicrographs, the morphology of infective larvae of commonly occurring gastro-intestinal strongyles of cattle from faecal culture.

Pacenovsky and Krupicer (1972) cultured the third stage larvae from faeces of cattle and described them with figures. Measurements were compared with those given by other authors.

Chauhan et al. (1973) described and illustrated the infective larvae of 7 species of gastro intestinal nematodes from Bubalus bubalis in India the nematodes involved being Oesophagostomum radiatum, Skrjabinagia boevi, Bunostomum phlebotomum, Trichostrongylus colubriformis, Paracooperia nodulosa and Strongyloides papillosus. Pandey (1973) also described the characteristics with measurements of the infective

larvae of the common strongyles of cattle. Borgsteede and Hendriks (1974) also did the same.

Sathianesan and Peter (1976) described and illustrated all the free living stages of Oesophagostomum asperum of goats and differentiated them from that of O. venulosum. Later Hariantha Rao and Venkataratnam (1977) also described the free living larval stages of Oesophagostomum asperum of goats.

Sathianesan and Peter (1977) described in detail all the three free living larval stages of Haemonchus contortus with their measurements, behaviour and duration of each stage.

Okpala et al. (1978) also described the infective larvae of the common strongyles of cattle with their measurements and gave a key for identification. Sathianesan and Peter (1979) described all the free living larval stages of Trichostrongylus colubriformis of goats. Nascimento et al. (1984) gave the morphometrics of L2 and L3 of Gaigeria pachyscelis of goats. Berrie et al. (1988) also gave differential features of infective larvae of Oesophagostomum radiatum, Haemonchus placei and Cooperia pectinata of cattle. Gamble et al. (1989) and Lichtenfels et al. (1990) made a detailed scanning electronmicroscopic study of the third stage larvae of Haemonchus contortus and the latter also gave a detailed description of its morphology.



## Bionomics of infective larvae

### Phototropism

Stewart and Douglas (1938) studied the bionomics of Trichostrongylus axei eggs and larvae and found infective larvae to be negatively geotropic, but neutral to light. Rogers (1940) noticed some favourable influence of light on the activity of larvae on pasture. Sprent (1946) confirmed that third stage larvae of Bunostomum phlebotomum were positively phototropic. Rees (1950) found light to be the prime factor determining the time of day for the maximum amount of migration and they reported that early morning and evening were the most favourable times for migration, fewer larvae being recovered during the day and at night.

According to Soliman (1953) only first stage larvae were positively phototropic. Silangwa and Todd (1964) did not notice any influence of light on the migration. Soulsby (1965) noticed the phototropic behaviour of infective larvae of gastro-intestinal nematodes. Deodhar (1966) found that mild light had a favourable influence on the migration of larvae. Tripathi (1969a) did not notice any evidence of phototropism in the infective larvae of the common nematodes.

Misra and Ruprah (1972) noticed a favourable influence for the mild day light on infective larvae.

According to Voznyi (1978) light had no effect on migration of Chabertia larvae.

Mittal (1987) also did not notice any influence of sunlight or shade on the activity of nematode larvae on pasture.

### Viability

Anantaraman (1942) reported that infective larvae of Oesophagostomum radiatum survived three months in water at room temperature. According to Goldberg (1951) the infective larvae of Oesophagostomum venulosum of sheep and goats could be kept alive in water as long as five months.

Premwati and Lal (1961) reported that infective larvae of Oesophagostomum columbianum of sheep could survive for 50 days at summer, 108 days at winter temperatures, 105 days at 30°C and a few minutes at 50°C.

Chhabra and Singh (1965) noticed that infective larvae of Oesophagostomum venulosum and O. columbianum survived for 36 days at 32°C. According to Daskalov (1965), the larvae of Haemonchus contortus, Bunostomum trigonocephalum and Oesophagostomum spp. survived at about 28°C in faecal cultures for 40, 15 and 220 days respectively. Narain (1965) found that infective larvae of Bunostomum trigonocephalum survived in tap water at 30°C for about 38 days. Zhidkov (1965) reported that the infective larvae of Bunostomum and Oesophagostomum survived

for 4 months and 12 months respectively under room temperature in West Siberia. Agrawal (1966) stated that longevity of Oesophagostomum columbianum infective larvae was 110 days at 30°C.

Altaev (1967) stated that infective larvae of Trichostrongylus skrjabini at room temperature in water lived for seven months in Russia. Sathianesan (1968) reported that infective larvae of Haemonchus contortus, Oesophagostomum columbianum, O. asperum, Bunostomum trigonocephalum and Trichostrongylus colubriformis of goats survived for 12-13 weeks, 16-17 weeks, 12-15 weeks, 4-5 weeks and 10-11 weeks respectively in aquarium water at room temperature.

Tripathi (1969b) found that the infective larvae of Haemonchus contortus, Oesophagostomum spp. and Trichostrongylus spp. survived for 40, 55 and 70 days respectively at 32 ±2°C in water under room temperature at Izatnagar.

Ravishankar (1970) reported that infective larvae of Oesophagostomum columbianum survived best at higher humidities and lower temperatures in water. Ahluwalia (1974) reported that infective larvae of Cooperia curticei remained alive upto 52 days at 32°C in water. Jehan and Gupta (1974) found that the longest period of survival of infective larvae of Haemonchus contortus was 120 days at 30°C in water. Sood and

Charanjitkaur (1975) reported that the survival of infective Haemonchus contortus larvae in water at 30°C was 110 days. According to Todd et al. (1976) the third stage larvae of Haemonchus contortus survived 64 days at 30°C (average temperature) in sheep faecal pellets.

Nath (1977) reported that Strongyloides papillosus infective larvae survived 35°C for 12 days in water. Hariantha Rao and Venkataratnam (1977) found that the infective larvae of Oesophagostomum asperum survived in water for 45 days under room temperature. Tripathi (1977) reported that Haemonchus contortus infective larvae survived for 98 days at 30°C in water.

Misra (1978) found that infective larvae of Haemonchus contortus to survive for 9 weeks at 30°C in water. Delgado (1982) observed that L<sub>3</sub> of Cooperia spp., Oesophagostomum spp. and Bunostomum sp. suspended in water with mean temperature ranging from 21.9 and 34.6°C survived for 26 weeks, 22 weeks and 10 weeks respectively in Cuba.

Rose and Small (1984) reported the infective larvae of Trichostrongylus vitrinus survived for upto 16 months on the herbage of grass plots. According to Boag and Thomas (1985) the infective larvae of Haemonchus contortus, Trichostrongylus spp. and Cooperia spp. survived in distilled water at 30°C for 91, 78-95 and 62-131 days respectively.

Verma et al. (1986) found that survival of free living infective larvae of Strongyloides papillosus in 3 mm of water at room temperature (25-29°C) was not longer than 17 days. Harbinder Singh et al. (1994) reported that in in vitro studies on the longevity of different larval stages in tap water at 28 ± 1°C, the infective larvae of Haemonchus contortus survived for 105 days.

### **Control of infective larvae**

Assessment of the ovicidal and larvicidal property of the fertilizers such as urea, ammonium sulphate, quicklime and ash

#### **Ovicidal property**

Sarimsakov and Nikolski (1961) reported that the development of the eggs and larvae of Bunostomum trigonocephalum was impeded by sheep urine and by excess moisture. Abdul Qadir (1971) found that urea at a concentration of 0.1 per cent completely inhibited the development of ruminant nematode eggs to infective larvae.

Persson (1973) observed that both staked and unslaked lime had an ovicidal effect on the eggs of Ostertagia ostertagi and Cooperia oncophora at both 1 per cent and 5 per cent concentrations at 20°C but the time required was little longer in 1 per cent concentration. Abdul Qadir (1976b) in another

experiment found that urea at the rate of 1 per cent concentration in soil in infected pasture land have 82.4 per cent and 58.9 per cent of ovicidal effect against the gastro-intestinal nematodes of goats and calves respectively.

Edirisinghe and Ihalamulla (1976) observed in 10 and 5 per cent concentrations of urea and ammonium sulphate, decortication of the infective eggs, temporary reduction of larval motility and hatchability and no ovicidal effect against the eggs of Ascaris lumbricoides.

Chongrak Polprasert and Gamboa Valencia (1981) observed the ovicidal property of the lime at concentration of 1.9 per cent faeces to be 26.5 per cent against Ascaris lumbricoides ova.

Hamdy et al. (1984) reported that ammonium sulphate was less effective as an ovicide against Ascaris suum eggs. Helle et al. (1989) observed that urea 2-4 per cent solution sprinkled over faecal cultures had 100 per cent ovicidal property against the common gastro-intestinal nematodes of sheep.

According to Agarwal et al. (1991) the ovicidal effect of urea was higher than that of ammonium sulphate against the eggs of Haemonchus contortus.

### Larvicidal property

Boloanta et al. (1968a and 1968b) found that out of the four fertilizers viz., potassium chloride, superphosphate, urea and ammonium nitrate, only the latter could destroy the strongyle and lung worm larvae.

Mango (1971) found ammonium sulphate to be larvicidal against infective larvae of hook worms of dog.

Persson (1973) observed that slaked and unslaked lime (both 1% and 5% concentration) had larvicidal effect on the larvae of Ostertagia spp. and Cooperia spp. at 20°C but the time required was little longer in 1 per cent concentration. Abdul Qadir (1976a) found urea at 1 per cent concentration in soil to be 72.7 per cent larvicidal against the infective larvae of common Trichostrongylids of sheep.

Ramajomartin (1981) found urea to be 68 per cent larvicidal against Ostertagia ostertagi and did not find any effect for lime against the larvae. Hamdy et al. (1984) observed that ammonium sulphate and urea reduced the longevity of Ancylostoma duodenale larvae.

## *Materials and Methods*

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## **MATERIALS AND METHODS**

### **Prevalence of common nematodes of domestic ruminants**

Investigation of prevalence of common nematodes of ruminants was carried out by recognising faecal eggs and infective larvae after coprological examination and copro culture respectively.

#### **Coprological examination**

The faecal samples for coprological examination were collected from animals brought to veterinary hospitals and slaughter houses at Trichur and Mannuthy and they were collected directly from the rectum to avoid extraneous infections.

The examination of faeces for eggs was done by following the routine concentration technique of centrifugation cum sedimentation technique.

#### **Copro-culture**

The modified Veglia's method (Sathianesan and Peter, 1970) was followed for copro-culture. A clean dry glass bottle measuring 14 cm in height and 5.5 cm diameter was used for this purpose. The faecal sample which were found to be positive for strongyle and strongyloides eggs were mixed well or powdered well in case

of pellet faeces by trituration and transferred into the bottle without soiling the sides of the bottle. Faeces should contain optimum moisture when cultured for which required water was added in case of dry faeces or excess water was removed by adding charcoal or by using blotting paper in case of watery faeces. Then the bottle was closed and kept under room temperature. The bottle cap was just loosened at intervals to let out the injurious gas as a result of fermentation and the culture was examined at regular intervals to note the presence of infective larvae developed. The larvae developed moved upwards from the faecal pad and got collected in the water droplets formed on the sides of the bottle by the condensation of vapour emanated from the faeces as a result of fermentation heat. The presence of larvae could be detected microscopically and sometimes even macroscopically when the larval colonies were large, from their wriggling movement.

#### **Recovery of infective larvae from faecal culture**

The culture bottle was kept horizontally over a table and small quantity of water was then dropped on to the inside of the bottle containing the larval colonies. The bottle was then rolled over the table gently, so that the water added washed all the sides of the bottle and all the larvae got collected in that water. Care being taken to avoid the water touching the faecal pad. The water containing the larvae was then pipetted out into a cavity dish for identification.

## **Identification of infective larvae**

A drop of water containing sufficient number of infective larvae was pipetted out from the cavity dish on to a slide. It was then gently heated to immobilize and gradually kill the larvae to facilitate the examination. Then it was covered with a cover slip. The sides of the cover slip was then sealed with molten paraffin to avoid evaporation. The larvae were then examined under a light microscope and identified from their morphological peculiarities and measurements of various parts of the body.

### **Measurements**

Measurements of various body parts were taken from micrometry and camera lucida drawings.

### **Preservation of infective larvae**

The infective larvae were preserved in 5 per cent warm formalin.

### **Diagrams**

All the diagrams of infective larvae were drawn with the use of a camera lucida.

## Photomicrographs

Photomicrographs were taken from the immobilized fresh infective larvae, which were collected from fresh faecal culture.

## Scanning electromicroscopic study of infective larvae

For studying the superficial details of the body of the larvae they were subjected to scanning electron microscopic study. For this the larvae were processed by the following procedures.

### a. Preparation of larvae

Freshly collected live larvae were cleared of all the adhering dirt and debris by repeated washings with distilled water.

### b. Killing of larvae

The larvae were killed by immersing in boiling water for 10 seconds. Heated larvae were then cooled quickly by the addition of ice. The larvae were then pelleted by centrifugation at 3000 rpm for 5-10 mts. The supernatant fluid was discarded slowly and the larval pellet was separated out. Centrifugation was a must in every step of processing to pellet the larvae and thus avoid the loss of larvae.

### c. Fixation of larvae

The killed larvae were fixed in 20 volumes of 4 per cent glutaraldehyde. This was kept at room temperature atleast for 24 hours.

### Preparation of fixative

The fixative, 4 per cent glutaraldehyde was prepared by mixing 16 ml of 25 per cent glutaraldehyde (available solution) and 84 ml of 0.1 M phosphate buffer solution (pH 7.4). The phosphate buffer solution was added drop by drop with constant stirring into the glutaraldehyde to prevent precipitation.

0.1 M phosphate buffer solution (pH 7.4) was prepared by mixing 19 ml of stock solution A (0.2 M solution of sodium dihydrogen phosphate dihydrate i.e. 31.2 g in 1000 ml distilled water) and 81 ml of stock solution B (0.2 M solution of Di-sodium hydrogen phosphate anhydrate i.e., 28.5 g in 1000 ml distilled water). Then it was diluted to a total of 200 ml with distilled water.

### d. Rinsing

The fixed larvae were rinsed thrice each time 10-15 mts with 0.1 M phosphate buffer solution to remove the fixative.

#### e. Washing

The larvae were washed thoroughly with distilled water for 30 mts to remove the buffer salts and other electrolytes present in the fixative.

#### f. Dehydration

The larvae were dehydrated in 30, 50, 70, 85 and 95 per cent ethanol with several washes in 100 per cent ethanol. Each washing was carried out for 10-15 minutes.

#### g. Critical point drying

After dehydration in ethanol, the pelleted larvae were placed on small coverslip and air dried. Few drops of isoamyl acetate solution was added to the larvae and the cover slip with larvae was placed in a tissue cage (perforated metal containers). The cage was then placed in the bomb of critical point drier. The bomb ~~was~~ sealed off, and its temperature was raised above the critical point of carbondioxide (31°C). The liquid carbondioxide changes to gas without change of volume. The gaseous CO<sub>2</sub> ~~was~~ then allowed to escape from the bomb. Thus the larvae were completely dried with no water particles left in any of the larvae.

#### **h. Mounting**

The dried coverslips containing larvae were mounted in metal stubs or specimen holders with suitable glues (colloidal silver glue).

#### **i. Gold coating**

The stubs were placed into the sputter coater and the larval surfaces now get coated with gold (evaporated conducting coat). The purpose of gold coating is to overcome the problem of charging when the specimen is examined in the SEM because the biological materials are non conductors when dry.

#### **j. Examination**

The larvae were examined in a Hitachi model 530 scanning electron microscope at 15 KV. The stub containing larvae was placed inside the specimen chamber of SEM and then vacuum was created. The images of the larvae were displayed in the screen of the SEM and the detailed external morphology was studied. Scanning electron micrographs were also taken by using Ilford 35 mm black and white film.

## **Bionomics of infective larvae**

### **Phototropism**

Faecal samples found positive for gastro-intestinal nematode infections on coprological examination were collected from cattle, buffalo and goat in sufficient quantities and were divided into two equal sub samples. Each of the sub sample was then kept for culturing following the technique described above. One of the culture was kept exposed to light and the other was either covered with a black paper or kept in a dark room to prevent the exposure to light. The former was the control for the experiment. Both exposed and unexposed cultures were examined after seven or eight days both macroscopically and microscopically to note the movement of larvae on the sides of the bottle. The distance of vertical migration of larvae from the faecal pad to the sides of the bottle was measured and compared. The infective larvae were then collected from the bottle, examined and identified.

### **Viability**

The larvae were washed out with aquarium water from the culture bottles and this washing was transferred into a suitable petridish or cavity dish and kept closed to avoid drying, under room temperature. The larvae were examined daily and noted their activity. To replace the evaporated water sufficient



quantity of filtered aquarium water was added whenever required. The observation repeated till all the larvae were found dead and thus the longevity (viability) of the larvae was determined.

### **Control of infective larvae**

Assessment of ovicidal and larvicidal property of fertilizers viz., urea, ammonium sulphate, quicklime and ash

#### **Ovicidal property**

Faecal samples found positive for gastro-intestinal nematode infections on coprological examination were collected from cattle, buffalo and goat in sufficient quantities and were divided into five equal sub samples. Each of the sub sample was then thoroughly pulverised and then mixed with each of the chemicals at a particular strength, starting with 1 per cent and then kept for culturing following the technique already mentioned, keeping one sub sample as untreated control culture. At the end of a week, the larvae developed in each of the culture bottle were recovered separately and their count was also taken. By taking the difference between the number of larvae collected from treated samples and that from untreated sample, the ovicidal property of each chemical at the particular strength was assessed. The experiment was repeated in the same way with the same chemical at higher strengths, viz. 2.5

per cent and 5 per cent and the efficacies at that strengths for the chemicals were also assessed.

Ovicidal property of the above fertilizers was also assessed by mixing the eggs harvested from fertilized female worms, directly with fertilizer solutions without faeces in a petridish starting with 1 per cent of the fertilizer. The egg culture with fertilizers at above strength and control culture were kept at room temperature and examined daily under binocular microscope to note the hatching of eggs. The experiments were repeated in the same way with the same chemicals at higher strengths (2.5 and 5%) and the efficacy was assessed and compared with the control.

#### Larvicidal property

Equal number of live larvae (50) were suspended in 10 ml of water contained in a small petridish, 5 such preparations were made. To these preparations, sufficient quantities of each of the fertilizer (urea, ammonium sulphate, quicklime and ash) was added to give a required strength (0.1%, 0.5% and 1%) starting with 0.1 per cent. The fifth preparation was kept as untreated control. Petridishes were covered and kept for further observation. The activity of the larvae in each of the solutions was observed daily for 5 days. The number of live larvae was counted in all the petridishes. By taking the difference between the number of live larvae in each of the

treated sample and that of the control sample, the efficacy of each fertilizer was arrived at that particular strength. The experiments were repeated with fertilizers at the rest of the strengths.

## *Results*

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## RESULTS

### Prevalance of common nematodes of domestic ruminants

#### Coprological examination

A total of 732 dung samples collected from cattle, buffaloes and goats were examined for nematode eggs and 352 dung samples (48.08%) were found positive for one or more nematode infections. The results animal-wise were (Table 1) cattle 176 (38.26%) out of 460, buffaloes 23 (31.94%) out of 72 and goats 155 (77.50%) out of 200. Highest incidence was noticed in goats out of all animals affected. Incidence was highest for strongyles (62.0%) out of all types of nematodes encountered and that was also in goats.

#### Copro-culture examination

The prevalence of different species of strongyles and strongyloides involved in the infections of animals found infected on coprological examinations was studied by culturing their faecal samples for larvae. The results are presented in Table 2 and their specific identification is dealt with in a separate chapter.

The number of faecal cultures examined were 176 from cattle, 23 from buffaloes and 155 from goats. The species of nematodes involved were Haemonchus contortus (57.95%),

Table 1. Results of coprological examination for eggs

Sl. Species No. of animals examined	Total number		Type of positive infections										
	Examined	Found positive		Strongyle		Strongyloides		Trichuris		Ascarid		Mixed	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Cattle	460	176	38.26	142	30.86	66	14.34	30	6.52	17	3.69	52	11.3
2. Buffalo	72	23	31.94	17	23.60	8	10.95	-	-	6	8.53	8	11.1
3. Goat	200	155	77.50	124	62.00	70	35.00	31	15.50	-	-	52	26.0

Table 2. Results of copro-culture for larvae

Sl. No.	Species of animals examined	No. of faecal cultures	Species of infective larvae obtained																			
			<u>Haemonchus contortus</u>		<u>Oesophagostomum radiatum</u>		<u>Oesophagostomum columbianum</u>		<u>Oesophagostomum asperum</u>		<u>Bunostomum phlebotomum</u>		<u>B. trigonocephalum</u>		<u>Cooperia punctata</u>		<u>Trichostrongylus colubriformis</u>		<u>T. axei</u>		<u>Strongyloides papillosus</u>	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1.	Cattle	176	102 57.95	84 47.19	- -	- -	- -	32 18.18	- -	- -	52 29.5	28 15.9	56 31.8	76 43.19								
2.	Buffalo	23	15 65.20	12 52.17	- -	- -	- -	- -	- -	- -	6 26.08	- -	10 43.47									
3.	Goat	155	112 72.25	- -	84 54.19	42 27.09	- -	16 10.32	- -	56 36.12	35 22.58	80 51.61										

Oesophagostomum radiatum, (47.19%), Bunostomum phlebotomum (18.18%), Cooperia punctata (28.5%), Trichostrongylus colubriformis (15.9%), Trichostrongylus axei (31.8%) and Strongyloides papillosus (43.19%) in cattle, Haemonchus contortus (65.20%), Oesophagostomum radiatum (52.17%), Trichostrongylus colubriformis (26.08%) and Strongyloides papillosus (43.47%) were the species of nematodes encountered in buffaloes. The species of nematodes encountered in goats were Haemonchus contortus (72.25%), Oesophagostomum columbianum (54.19%), Oesophagostomum asperum (27.09%), Bunostomum trigonocephalum (10.32%), Trichostrongylus colubriformis (36.12%), Trichostrongylus axei (22.58%) and Strongyloides papillosus (51.61%). Among all the species of nematodes involved, the incidence of Haemonchus contortus was highest in all animals. It was lowest for Bunostomes in cattle and goats and for T. colubriformis in buffaloes.

### Identification of infective larvae

#### Cattle

Haemonchus contortus, Oesophagostomum radiatum, Trichostrongylus colubriformis, T. axei, Strongyloides papillosus, Cooperia punctata and Bunostomum phlebotomum were the species of infective larvae identified from the copro culture of dung samples of cattle. Their biometrical details are given in the Table 3.



Table 3. Comparative biometry of infective larvae of common nematodes of domestic ruminants - Cattle

Sl. Infective No. larvae	Body		Oesophagus	Intestinal cell			Distance from anterior end				Genital primordium		Rectum	Tail	Tail sheath
	Length	Width	Length	No.	Long	Wide	Nerve ring	Excretory pore	Genital primordium	Anus	Length	Width	Length	Length	Length
	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
1. <u>Haemonchus</u> <u>contortus</u>	675- 780	19.5- 24	127- 157	16	47.5- 52.5	6- 8.5	85- 95	102- 106	325- 395	565- 615	10.5- 12	4.5- 6	21- 32.5	55- 66.5	63.5- 77
a. <u>H. contortus</u>	650- 751	-	122- 150	16	-	-	-	-	310- 385	-	-	-	-	54- 68	65- 78
2. <u>Oesophago-</u> <u>stomum</u> <u>radiatum</u>	765- 876	24- 32	135- 165	16- 20	42.5- 57	10.5- 15	85- 104	102- 118	326- 405	530- 650	9.5- 14	4.5- 6	25- 35	59- 72	142- 176
b. <u>O. radiatum</u>	750- 866	-	133- 160	16	-	-	-	-	300- 406	-	-	-	-	55- 75	136- 185
3. <u>Cooperia</u> <u>punctata</u>	780- 842	28- 32	137.5- 165	16- 24	52- 78	8- 10	98- 108	110- 118	382- 435	600- 705	10- 15	6- 8	28- 36	56.5- 69.5	52.5- 63
b. <u>C. punctata</u>	760- 865	-	147- 170	16- 24	-	-	-	-	372- 443	-	-	-	-	52- 75	42- 65
4. <u>Trichostron-</u> <u>gylus axei</u>	630- 720	22.5- 27.5	135- 167	16	52- 65	9.5- 11	77- 87.5	87- 95	320- 375	560- 622	11.5- 12.5	3.5- 4.5	26- 30	58- 65	27- 35
b. <u>T. axei</u>	604- 745	-	150- 181	16	-	-	-	-	322- 424	-	-	-	40- 72	25- 40	

Contd.

Table 3 (Contd.)

5.	<u>T. colubri-</u> <u>formis</u>	645- 750	21- 24.5	154.5- 172	16	52.5- 70	7.5- 10.5	87.5- 110	98- 117	330- 385	570- 630	6.5- 10	3.5- 5	24.5- 27.5	55- 63	26.5- 40
c.	<u>T. colubri-</u> <u>formis</u>	674- 749	-	150- 180	16	60	10	-	-	340- 404	-	-	-	-	58- 70	25-
6.	<u>Bunostomum</u> <u>phlebotomum</u>	460- 630	20- 24.5	135- 162	16	30- 33	6- 9.5	76- 86	85- 102	228- 290	350- 540	9- 12	2- 4	25- 30	48- 65	65- 95
b.	<u>B. phlebotomum</u>	462- 624		130- 152	16	-	-	-	-	210- 300	-	-	-	-	42- 63	61- 96
7.	<u>Strongy-</u> <u>loides</u> <u>papillosus</u>	540- 625	14- 20	220- 260	16- 24	25- 35	5.5- 10.5	70- 80	84- 92	285- 320	535- 590	5.5- 8.5	2- 3.5	14- 17.5	75- 95	Nil
b.	<u>S. papillosus</u>	523- 674	260- 255	-	-	-	-	-	-	-	-	-	-	-	-	-

Measurements from: a - Soulsby (1965); b - Hansen and Shivnani (1956); c - Dikmans and Andrews (1933)

Infective larva of Haemonchus contortus (Plate I, Figs.1, 2, 3 and 4)

It was easily identified by the presence of a sharp kink in tail sheath just posterior to the end of tail proper. The larva was of a medium length with a gradually tapering head end and was measuring 675 to 780  $\mu\text{m}$  in length and 19.5 to 24  $\mu\text{m}$  in width. The buccal cavity was globular in shape. The esophagus was 127 to 157  $\mu\text{m}$  long and was constricted in the region of the nerve ring. The nerve ring and excretory pore were situated at 85 to 95  $\mu\text{m}$  and 102 to 106  $\mu\text{m}$  respectively from the anterior end. The intestinal cells were 16 in number and rectangular in shape and the cell walls were distinctly seen. The length and width of the intestinal cells were 47.5 to 52.5 and 6 to 8.75  $\mu\text{m}$  respectively. The genital primordium was located at 325 to 395  $\mu\text{m}$  from the anterior end and it measured 10.6 to 12  $\mu\text{m}$  and 4.5 to 6 in length and width respectively. The length of rectum was 21 to 32.5  $\mu\text{m}$ . The anus was located 565 to 615  $\mu\text{m}$  from the anterior end. The tail of the larva proper was 55 to 66.5  $\mu\text{m}$  long and pointed, the tail sheath was 63.5 to 77  $\mu\text{m}$  in length.

Infective larva of Oesophagostomum radiatum (Plate II, Figs.1, 2, 3 and 4)

The tail sheath of the larva was characteristically long. The body was 765 to 876  $\mu\text{m}$  long and 24 to 32  $\mu\text{m}$  broad. The buccal cavity was tubular and its opening into the

oesophagus was through a complicated chitinised region. The sheath was wringled, which being clearly noticeable on the inner edge of the larva lying in a curved fashion. The oesophagus was 135 to 165  $\mu\text{m}$  in length. The nerve ring and excretory pore were situated at 85 to 104 and 102 to 118  $\mu$  respectively from the anterior end. The intestinal cells were clearly demarcated, 16-20 in number, elongated in shape with well defined nuclei and the length and breadth were 42.5 to 57  $\mu\text{m}$  and 10.5 to 15  $\mu\text{m}$  respectively. The genital primordium was located 326 to 405  $\mu\text{m}$  from the anterior end and its length and width were 9.5 to 14  $\mu\text{m}$  and 4.5 to 6  $\mu\text{m}$  respectively. The rectum was 25 to 35  $\mu\text{m}$  long. The anus was situated 530 to 650  $\mu\text{m}$  from the anterior end. The tail of the larva was simple and blunt at the tip and 59 to 72  $\mu\text{m}$  long and the tail sheath was seen projecting beyond the tail of larva for about 142 to 176  $\mu\text{m}$  from the anus.

**Infective larva of Cooperia punctata (Plate III, Figs.1, 2, 3 and 4)**

The larva measured 780 to 842  $\mu\text{m}$  in length and 28 to 32  $\mu\text{m}$  in breadth. The buccal cavity was pear shaped. The length of oesophagus was 137.5 to 165  $\mu\text{m}$ . The nerve ring and excretory pore were situated 98 to 108  $\mu\text{m}$  and 110 to 118  $\mu\text{m}$  respectively from the anterior end. The intestine was composed of 16-24 spindle shaped cells which measured 52 to 78  $\mu\text{m}$  in length and 8 to 10  $\mu\text{m}$  in width. The genital primordium was located at 382 to 435  $\mu\text{m}$  from the anterior end and the length and breadth were

10 to 15 um and 6 to 8 um respectively. The rectum was 28 to 36 um in length. The tail of the larva proper was 56.6 to 69.5 um long. The tail sheath which was gradually tapering into a very short filamentous portion and was 52.5 to 63 um long. The anus was situated 600 to 705 um from the anterior end.

Infective larva of Trichostrongylus colubriformis (Plate IV, Figs.1, 2, 3 and 4)

The characteristic feature of this larva was that it had two tubercles at the end of the tail. It was 645-750 um long, and 21 to 24.5 um broad. The oesophagus was 154.5 to 172 um long. The nerve ring and excretory pore were situated at 87.5 to 110 um and 98 to 117.5 um respectively from the anterior end. The intestinal cells were 16 in number. Each cell was 52.5 to 70 um x 7.5 to 10.5 um in size. The genital primordium was located 330 to 385 um posterior to the anterior end and it measured 6.5 to 10 um x 3.5 to 5 um. The rectum was 24.5 to 27.5 um long. The anus was situated at 570 to 630 um from the anterior end. The tail was 55 to 63 um long and was bearing 2 tubercles at the tip. The tail sheath was short and stumpy measuring 26.5 to 40 um long.

Infective larva of Trichostrongylus axei (Plate V, Figs.1, 2, 3 and 4)

It could be differentiated from T. colubriformis by the absence of tubercles at the tail tip. The total length of larva was 630 to 720 um and width 22.5 to 27.5 um. The oesophagus was 135 to 167 um long. The nerve ring, excretory pore, genital primordium and anus were located 77 to 87.5 um, 87 to 95 um, 320 to 375 um and 560 to 622 um respectively posterior to the anterior end. The length and width of genital primordium were 11.5 to 12.5 um and 3.5 to 4.5 um respectively. The rectum measured 26 to 30 um in length. The intestinal cells were 16 in number and were elongated rectangular. They measured 52 to 65 um in length and 9.5 to 11 um in width. The tail was 58 to 65 um and the tail sheath 27 to 35 um long and both were bluntly rounded terminally.

Infective larva of Bunostomum phlebotomum (Plate VI, Figs.1, 2 and 3)

It could be easily identified by its small size and two portions of oesophagus a glandular portion and a muscular portion.

The larva measured 460 to 630 um in length and 20 to 24.5 um in width. The buccal cavity was very small and somewhat funnel shaped. The oesophagus was 135 to 162 um long with a

crescent shaped cuticularised structures at the anterior end and a conspicuous bulb in the posterior portion. The nerve ring, excretory pore, genital primordium and anus were located at 76 to 86  $\mu\text{m}$ , 85 to 102  $\mu\text{m}$ , 228 to 290  $\mu\text{m}$  and 350 to 540  $\mu\text{m}$  respectively from the anterior end. The intestinal cells were 16 in number and 30 to 33  $\mu\text{m}$  x 6 to 9.5  $\mu\text{m}$  in size. The genital primordium was 9 to 12  $\mu\text{m}$  long and 2 to 4  $\mu\text{m}$  wide. The rectum was 25 to 30  $\mu\text{m}$  in length. The tail of larva was 48 to 65  $\mu\text{m}$  in length and bluntly rounded at the tip. The tail sheath was 65 to 95  $\mu\text{m}$  long and filamentous terminally.

**Infective larva of Strongyloides papillosus (Plate VII, Figs.1, 2, 3 and 4)**

It could be easily identified by the absence of sheath, forked tail tip, very long oesophagus extending for 36 to 44 per cent of the body length and very slender body. It was 540 to 625  $\mu\text{m}$  long and 14 to 20  $\mu\text{m}$  wide. The oesophagus was about one third of its entire length ranging from 220 to 260  $\mu\text{m}$  in length. The nerve ring, excretory pore, genital primordium and anus were situated 70 to 80  $\mu\text{m}$ , 84 to 92  $\mu\text{m}$ , 285 to 320  $\mu\text{m}$  and 535 to 590  $\mu\text{m}$  respectively posterior to the anterior end. The genital primordium was 5.5 to 8.5  $\mu\text{m}$  long and 2 to 3.5  $\mu\text{m}$  wide. The rectum was 14 to 17.5  $\mu\text{m}$  long. The tail was 75 to 95  $\mu\text{m}$  long and with a bifid tip. The intestine was with small, thin, elongated, 16 to 24 cells measuring 25 to 35  $\mu\text{m}$  and 5.5 to

10.5 um in length and breadth respectively. The walls were not clearly seen.

### Buffalo

Four species of nematodes were found to occur in buffaloes in the present study by identifying the infective larvae. They were Haemonchus contortus, Oesophagostomum radiatum, Trichostrongylus colubriformis and Strongyloides papillosus. The biometrical details of each larva are given in Table 4. The larvae were identical in morphology with that of cattle origin except in very minor differences in measurements for which the Table 4 may be referred.

### Goat

Seven species of nematodes were found to occur in goats by identifying the infective larvae obtained on culturing the faeces of goats found infected on coprological examination for eggs. They were Haemonchus contortus, Oesophagostomum columbianum, O. asperum, Trichostrongylus colubriformis, T. axei, Bunostomum trigonocephalum and Strongyloides papillosus. The biometrical details of the larvae are furnished in Table 5. The morphological details of H. contortus, T. colubriformis, T. axei and S. papillosus were identical to those of the same species of cattle origin. The body measurements were also almost similar for these species of



Table 4. Comparative biometry of infective larvae of common nematodes of domestic ruminants - Buffalo (Bubalus bubalis)

Sl. No.	Infective larvae	Body		Oesophagus	Intestinal cell			Distance from anterior end				Genital primordium		Rectum	Tail	Tail sheath
		Length	Width	Length	No.	Long	Wide	Nerve ring	Excretory pore	Genital primordium	Anus	Length	Width	Length	Length	Length
		u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
1.	<u>Haemonchus contortus</u>	650- 750	21- 24.5	136- 155	16	34- 52	9.5- 12	86- 97	92- 100	275- 295	524- 620	8.5- 11.5	3.5- 5.5	20- 28	56.5- 67	60- 75
a.	<u>H. bubalis</u>	550- 627	-	136- 150	16	-	-	88- 95	90- 99	272- 302	-	-	-	-	50- 64	45- 48
2.	<u>Oesophago-stomum radiatum</u>	785- 896	24.5- 30	150- 170	16- 20	28- 48	8.5- 12.5	98- 105	106.5- 113	345- 370	545- 670	10- 14	4- 6.5	27- 34	62- 76	133- 167
a.	<u>O. radiatum</u>	780- 918	-	150- 190	20	-	-	102- 106	112- 115	360- 380	-	-	-	-	68- 81	153- 170
3.	<u>Trichostrongylus colubriformis</u>	650- 720	21- 25	140- 157	16	36.5- 65	8- 11.5	82- 87	85- 98	270- 330	520- 610	6.5- 10	3.5- 5	21- 27	55- 24	25- 35
a.	<u>T. colubriformis</u>	474- 612	-	135- 152	16	-	-	80- 88	85- 95	230- 306	-	-	-	-	44- 60	20- 30
4.	<u>Strongyloides papillosus</u>	490- 620	14- 17.5	210- 250	16- 24	27- 36	5- 10	77- 85	84- 97	290- 318	410- 520	4.5- 8	2- 3.5	14- 17.5	87- 92	Nil
a.	<u>S. papillosus</u>	470- 610	-	208- 246	16	-	-	76- 85	85- 96	285- 316	-	-	-	-	85- 90	Nil

a - Chauhan et al. (1973)

cattle and goat origin. For minute deviations refer the Table 5. The details of rest of the species are furnished here under.

Infective larva of Oesophagostomum columbianum (Plate VIII, Figs.1, 2, 3 and 4)

This was almost similar to O. radiatum of cattle but its tail was slightly shorter than that of O. radiatum. The larvae measured 780 to 830 um long and 29.5 to 34 um wide. The buccal canal opened into the oesophagus through a complicated chitinised armature which formed a triangular area with the base directed anteriorly. The oesophagus measured 147 to 165 um long. The nerve ring excretory pore, genital primordium and anus were situated at 80 to 92 um, 92 to 112 um, 372 to 410 um and 565 to 620 um respectively from the anterior end. The intestine consisted of 16 to 20 triangular well demarcated cells. They measured 32-42 um long and 10.5 to 12 um wide. The genital primordium was 12 to 14 um long and 6 to 8 um wide. The rectum was 25 to 35 um long. The tail was simple and blunt measuring 73 to 85 um long and the tail sheath was 135 to 157 um long.

Table 5. Comparative biometry of infective larvae of common nematodes of domestic ruminants - Goat

Sl. Infective No. larvae	Body		Oesophagus	Intestinal cell			Distance from anterior end				Genital primordium		Rectum	Tail	Tail sheatg
	Length	Width	Length	No.	Long	Wide	Nerve ring	Excretory pore	Genital primordium	Anus	Length	Width	Length	Length	Length
	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
1. <u>Haemonchus contortus</u>	675- 810	21- 25	135- 157.5	16	45- 45	3.5- 7	86- 92	105- 110	320- 395	540- 650	11.5- 14	4- 7	27- 38	51- 65	68- 78
a. <u>H. contortus</u>	650- 820	20- 30	119- 166	16	45- 53	5- 10	86- 92	105- 113	305- 405	531- 679	10- 14	5- 8	24- 40	52- 68	67- 78
b. <u>H. contortus</u>	645- 765	-	120- 151	16	48	5	-	-	310- 388	-	-	-	-	52- 74	70- 80
2. <u>Oesophago-stomum columbianum</u>	780- 830	29.5 34	147- 165	16- 20	32- 42	10.5- 12	80- 92	92- 112	372- 410	565- 620	12- 14	6- 8	25- 35	73- 85	135- 157
a. <u>O. columbianum</u>	775- 840	30- 32	140- 170	24	30- 35	10- 12	75- 95	92- 110	370- 422	555- 615	12- 14	6- 8	20- 35	70- 90	137- 153
b. <u>O. columbianum</u>	718- 926	-	150- 175	16	65	15	-	-	270- 415	-	-	-	-	60- 80	130- 160
3. <u>Oesophago-stomum asperum</u>	840- 980	34- 45	157- 185	24	33.5 37	8- 12	90- 108	110- 130	380- 460	530- 720	10- 15	5- 9	27- 38	85- 92	142- 167
a. <u>O. asperum</u>	799- 1025	35- 45	157- 190	24	32- 37	5- 10	83- 112	108- 137	385- 495	556- 762	12- 16	4- 7	30- 45	68- 100	136- 162
c. <u>O. asperum</u>	895- 920	23- 26	152- 182	20	-	-	69- 75	87- 94	315- 381	591- 627	-	-	-	114- 132	117- 125

Contd.

Table 5 (Contd.)

4.	<u>Trichostr- onyglus colubriformis</u>	640- 765	21- 27	135- 162	16	48- 57	6.5- 11.5	87- 108	95- 115	315- 392	557.5 660	5- 10	3- 4	19- 25	58- 78	27- 37
a.	<u>T. colubri- formis</u>	560- 780	20- 30	130- 170	16	45- 60	8- 13	80- 114	90- 122	325- 400	559- 680	8- 12	4- 5	20- 26	50- 62	25- 40
b.	<u>T. colubri- formis</u>	600- 784	-	150- 182	16	-	-	-	-	348- 404	-	-	-	64- 74	10- 18	
5.	<u>T. axei</u>	660- 720	24.5- 27	143.5 150	16	52- 56	8.5- 13	76- 82	85- 90	336- 350	560- 620	10- 12	2.5- 4	27- 33	59- 65	27- 30
a.	<u>T. axei</u>	665- 700	27- 29	140- 148	16	50- 51	10- 13	75- 80	87- 89	340- 360	570- 605	12- 13	3- 4	30- 33	64- 66	28- 30
6.	<u>Bunostomum trigono- cephalum</u>	484- 652	24.5- 30	135- 160	16	28- 35	5- 9	76- 85	84- 105	256- 296	352- 530	12- 15	3.5- 5	20- 27	52.5- 65	86- 98
a.	<u>B. trigono- cephalum</u>	476- 664	20- 30	130- 170	16	30- 32	7- 10	74- 87	82- 105	250- 300	341- 550	10- 17	2- 5	22- 30	50- 67	80- 102
b.	<u>B. trigono- cephalum</u>	510- 698	-	146- 176	16	-	-	-	-	231- 370	-	-	-	58- 76	20- 32	
7.	<u>Strongy- loides papillosus</u>	570- 630	17.5- 21	225- 256	16- 24	25- 35	5- 8	76- 80	87- 94.5	290- 320	520- 580	7- 9	2- 3.5	14- 17	80- 98.5	Nil
a.	<u>S. papillosus</u>	550- 640	17- 22	220- 260	24	-	-	75- 80	85- 90	290- 327	556- 610	7- 9	2- 4	15- 18	77- 97	Nil
b.	<u>S. papillosus</u>	600- 680	-	225- 262	-	-	-	-	-	395- 415	-	-	-	77- 109	Nil	

a - Sathianesan and Peter (1968); b - Tripathi (1968); c - Harianth Rao and Venkataratnam (1977)

Infective larva of Oesophagostomum asperum (Plate IX, Figs.1, 2, 3 and 4)

This larva was similar to O. columbianum but larger and the tail sheath was longer. The larvae measured 840 to 980  $\mu\text{m}$  long and 34 to 45  $\mu\text{m}$  wide. The buccal cavity was tubular and extended to oesophagus which was 157 to 185  $\mu\text{m}$  long. The nerve ring, excretory pore, genital primordium and anus were located at 90 to 108  $\mu\text{m}$ , 110 to 130  $\mu\text{m}$ , 380 to 460  $\mu\text{m}$  and 630 to 720  $\mu\text{m}$  respectively from the anterior end. The intestine was composed of 24 cells which were rectangular, well demarcated and measuring 33.5 to 37  $\mu\text{m}$  long and 8 to 12  $\mu\text{m}$  wide. The genital primordium measured 10 to 15  $\mu\text{m}$  long and 5 to 9  $\mu\text{m}$  wide. The rectum was 27 to 38  $\mu\text{m}$  long. The tail measured 85 to 92  $\mu\text{m}$  and was bluntly rounded at the tip. The tail sheath was 142 to 167  $\mu\text{m}$  long.

Infective larva of Bunostomum trigonocephalum (Plate X, Figs.1, 2, 3 and 4)

It was almost similar in appearance to Bunostomum phlebotomum of cattle but some of the body measurements were slightly greater than that of B. phlebotomum. Body was 484 to 652  $\mu\text{m}$  long and 24.5 to 30  $\mu\text{m}$  wide. The buccal cavity was small and funnel shaped. The oesophagus was shorter and with a posterior bulb. It measured 135 to 160  $\mu\text{m}$  long. The nerve ring, excretory pore and genital primordium were situated at

76 to 85 um 84 to 105 um and 256 to 296 um respectively from the anterior end. The intestinal cells were 16 in number, each measuring 28 to 35 um long and 5 to 9 um wide. The genital primordium was 12 to 15 um long and 3.5 to 5 um wide. The rectum was 20 to 27 um long. The tail was 52.5 to 65 um and tail sheath 86 to 98 um in length and it was filamentous terminally. The anus was situated at 352 to 530 um from the anterior end.

### **Key for identification of infective larvae**

**Haemonchus contortus**: (cattle, buffalo & goat)

Globular buccal capsule; kink in tail sheath just posterior to tail, tail sheath ending in a fine whip-like filament.

**Oesophagostomum radiatum**: (cattle & buffalo)

The buccal capsule tubular; oesophageal armature present; tail sheath very long fine filamentous 16-20 intestinal cells.

**Oesophagostomum columbianum**: (goat)

Similar to O. radiatum but tail sheath slightly shorter.

O. asperum: (goat)

Similar to O. radiatum and O. columbianum but larger in size, with longer tail sheath, intestinal cells 24.

Trichostrongylus colubiformis: (cattle, buffalo & goat)

Buccal cavity absent; 2 tubercles at the tail tip, tail sheath stumpy.

Trichostrongylus axei: (cattle & goat)

Similar to Trichostrongylus colubiformis except for the absence of tubercles at the tail tip.

Bunostomum phlebotomum: (cattle)

Larva with smallest body; esophagus club shaped.

Bunostomum trigonocephalum: (goat)

Similar to B. phlebotomum; but for the slightly greater body measurements.

Cooperia punctata: (cattle)

Buccal cavity pear shaped; tail sheath short and whip like.

Strongyloides papillosus (cattle, buffalo and goat)

Slender; naked; oesophagus is very long and filariform, tail tip bifid.

**Scanning electronmicroscopic study of infective larvae**

Ultrastructural characteristics of infective larvae of some of the gastro-intestinal nematodes of domestic ruminants were studied under SEM. The processing of larvae for SEM was done as described in materials and methods. The following are the observations made on different infective larvae under SEM.

Strongyloides papillosus (Plate XI, Figs.1, 2 and 3)

At the head end there was a peribuccal ring-like structure having a number of prominences. The oesophagus was very clearly seen and it was very thick occupying about 3/4th of the thickness of the body. There were two gutter-like areas of varying length on the right and left side of the oesophagus. Numerous fissures were seen at about the middle of the body. The tail end was tridigitate and each portion was bulbous terminally. The larva was free of sheath.

Cooperia punctata (Plate XII, Figs.1 and 2)

The sheath of the larva was seen very loose, particularly at the anterior end. A distinct depression was also



seen on the sheath at the anterior end. Numerous transverse striations were seen on the sheath. The head end of the body was cone shaped bearing a long arrow-like structure directed anteriorly. Transverse striations were seen on the larva also. Oesophagus was very clearly demarcated. The tail was simple. The tail of the sheath ended in a spoon-like structure at the tip.

**Trichostrongylus axei** (Plate XIII, Figs.1 and 2)

The sheath of the larvae was very loose enclosing the larva, in the form of an inverted test tube. There was a distinct depression in the sheath a short distance from the anterior end of the sheath. The head end of the larva was narrow with two elongate arrow-like projections directed anteriorly. At the base of the projections on the head end, a flat prominence was also seen. The oesophageal lumen was clearly seen. The cuticular striations were absent. The tail end was with a small pointed spur-like structure. A central dark, dense, longitudinal line was seen on the larva at the posterior region which may be a cuticular ala. Two round cuticular thickenings were seen at about the middle of the tail sheath which was also pointed terminally.

**Oesophagostomum radiatum** (Plate XIV, Figs.1, 2 and 3)

The sheath of the larva was very loose at the anterior end. The larva was seen close to one side of the sheath.

The head end of the larva was with two horn-like projections. Distinct longitudinal ridges were seen on the larva in the anterior region. Transverse striations were absent. Oesophagus was indistinct. The tail sheath was very long and whip-like. The tail tip was not well demarcated.

**Haemonchus contortus** (Plate XV, Figs.1, 2 and 3)

The larval sheath was not loose as in the preceding cases but it was seen projecting beyond the head end of the larva. Longitudinal ridges were seen on the cuticle of the larva. A central dark longitudinal line was seen throughout the body. The oesophagus was not well demarcated. The tail end of the larva was pointed. The tail sheath end was also pointed.

**Bionomics of infective larvae**

**Phototropism**

A total of five experiments were conducted by culturing faecal samples collected from three different species of animals viz., cattle, buffalo and goats. The cultures were kept in bottles as described in materials and methods. Always two cultures were kept, one unexposed to light and another exposed (control). After seven days the cultures were examined. In both the cultures larvae could be seen migrated into the water droplets formed near the neck region of the bottles,

Table 6. Results of experiments for phototropism

Sl. No.	Faecal sample of	Date of faecal culture	Date of larval examination	Upward migration of larvae in		Species of larvae in both light unexposed culture and exposed culture	Result
				----- unexposed culture cm	----- exposed culture (control) cm		
1.	Goat	27.3.95	3.4.95	9	9	<u>S. papillosus</u> , <u>H. contortus</u> <u>T. axei</u>	no phototropism
2.	Goat	8.4.95	15.4.95	8.5	8.5	<u>T. axei</u> , <u>T. colubriformis</u> <u>O. columbianum</u>	no phototropism
3.	Calf	14.4.95	21.4.95	8.7	8.7	<u>H. contortus</u> , <u>T. axei</u> <u>C. punctata</u>	no phototropism
4.	Cow	2.5.95	9.5.95	9	9	<u>O. radiatum</u> , <u>H. contortus</u>	no phototropism
5.	Buffalo calf	6.5.95	13.5.95	8.5	8.5	<u>O. radiatum</u> , <u>T. colubriformis</u>	no phototropism

macroscopically and microscopically. Then the larvae were collected into separate containers and speciated. The results are presented in Table 6. From the results it could be seen that the activity or migration of the larvae was not influenced by the presence or absence of light indicating that the larvae did not show phototropism, irrespective of the species of nematodes or species of animals involved.

### **Viability**

The viability of infective larvae of all the species of nematodes encountered in all the three domestic ruminants viz., cattle, buffaloes and goats in all the seasons was assessed. For this the larvae were kept in water in suitable containers well closed to avoid evaporation, under room temperature as described in materials and methods. The larvae were microscopically examined at regular intervals and noted the presence or absence of activities of the larvae. Whenever stirring of the water was required to make the larvae active, that was also done. Observation was continued till all the larvae were found dead. It was repeated for all the species of larvae from all the animals for all seasons. The results are presented in Tables 7 and 8 indicating the period of viability and percentage of viability respectively. The longest viability was noticed in winter. The longest viable larvae were that of *Oesophagostomum* species closely followed by *Haemonchus*.

Table 7. Showing period of viability of infective larvae

Sl. No.	Species of		Viability in days in			
	Animals	Infective larvae	Winter	Average	Summer	Average
Cattle						
1.		<u>Haemonchus contortus</u>	80-90	85	75-82	79
2.		<u>Oesophagostomum radiatum</u>	85-94	90	65-75	70
3.		<u>Trichostrongylus colubriformis</u>	75-82	79	67-74	71
4.		<u>Trichostrongylus axei</u>	70-80	75	63-72	68
5.		<u>Cooperia punctata</u>	65-73	69	54-62	58
6.		<u>Bunostomum phlebotomum</u>	34-43	39	28-32	30
7.		<u>Strongyloides papillosus</u>	16-18	17	14-16	15
Buffalo						
1.		<u>Haemonchus contortus</u>	72-86	79	68-77	73
2.		<u>Oesophagostomum radiatum</u>	86-97	72	75-84	80
3.		<u>Trichostrongylus colubriformis</u>	67-82	75	56-70	63
4.		<u>Strongyloides papillosus</u>	17-19	18	13-17	15
Goat						
1.		<u>Haemonchus contortus</u>	95-102	99	80-92	86
2.		<u>Oesophagostomum columbianum</u>	105-114	110	91-98	95
3.		<u>Oesophagostomum asperum</u>	84-97	91	62-78	70
4.		<u>Trichostrongylus colubriformis</u>	72-86	79	66-74	70
5.		<u>Trichostrongylus axei</u>	72-80	76	60-70	65
6.		<u>Bunostomum phlebotomum</u>	37-46	42	30-35	33
7.		<u>Strongyloides papillosus</u>	17-20	19	10-14	12

The shortest viability was noticed for the nude Strongyloides papillosus larvae irrespective of the seasons.

For the percentage of viability also the same trend as for the period of viability was noticed. The first and second place went to oesophagostomes and Haemonchus respectively and the strongyloides was in the last place. In addition to the above observation, the following observations were also made during the viability study. One observation was about the movement of larvae. The larvae were showing vigorous swarming movements when transferred into a petridish or cavity dish from the faecal cultures. This activity continued one to three weeks depending on the life span of larvae. During this period the larvae remained in the top of water column. Afterwards the movement became sluggish. Then the larvae were moving downwards and remaining in the middle of water column for one or two weeks. Finally they settled to the bottom of the container and started showing wriggling movement for one week. Thereafter the movements also ceased and the larvae remained tightly coiled or semi coiled at the bottom of the container. But when agitated or stirred the larvae were seen moving again. Gradually the larvae straightened and floated over the contained water indicating death of the larvae. But the Strongyloides papillosus larvae even if they were straight and motionless, sometimes, started moving when stirred. So it was felt

Table 8. Showing percentage of viability of infective larvae

animals	Species of infective larvae	Season	Viability in percentages in different weeks																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Cattle																			
1.	<u>H. contortus</u>	Summer	100	80	73	69	62	52	46	28	16	5	2	Nil					
		Winter	100	95	86	75	70	64	60	50	42	28	18	10	Nil				
2.	<u>O. radiatum</u>	Summer	100	90	82	73	65	56	45	28	15	8	4	Nil					
		Winter	100	95	90	85	76	69	62	45	38	32	25	10	2	Nil			
3.	<u>C. punctata</u>	Summer	100	90	80	75	50	45	20	8	Nil								
		Winter	100	90	85	70	60	48	36	25	10	2	Nil						
4.	<u>T. colubriformis</u>	Summer	100	92	82	75	65	40	35	20	5	1	Nil						
		Winter	100	95	86	78	60	50	42	35	25	16	4	Nil					
5.	<u>T. axei</u>	Summer	100	95	85	70	60	45	36	25	15	5	Nil						
		Winter	100	95	90	80	70	60	45	30	24	18	10	Nil					
6.	<u>B. phlebotomum</u>	Summer	100	80	55	30	Nil												
		Winter	100	85	65	45	25	15	Nil										
7.	<u>S. papillosus</u>	Summer	80	15	Nil														
		Winter	75	25	Nil														

Contd.

Table 8 (Contd.)

## Buffalo

1.	<u>H. contortus</u>	Summer	100	90	80	72	56	42	35	25	20	10	Nil			
		Winter	100	95	85	75	60	55	45	30	25	15	10	3	Nil	
2.	<u>O. radiatum</u>	Summer	100	95	80	70	65	60	55	40	30	20	10	Nil		
		Winter	100	98	85	80	75	65	57	45	35	25	15	6	1	
3.	<u>T. colubriformis</u>	Summer	100	85	70	65	55	40	28	15	7	Nil				
		Winter	100	90	80	76	60	55	36	25	16	9	3	Nil		
4.	<u>S. papillosus</u>	Summer	70	10	Nil											
		Winter	95	30	Nil											

## Goat

1.	<u>H. contortus</u>	Summer	100	90	82	70	65	50	42	34	26	15	10	5	1	Nil	
		Winter	100	98	86	80	72	62	56	45	36	22	27	15	10	4	Nil
2.	<u>O. columbianum</u>	Summer	100	95	86	72	66	60	55	45	30	20	16	10	5	Nil	
		Winter		98	90	85	78	70	66	60	52	40	36	27	20	12	8
3.	<u>O. asperum</u>	Summer	100	85	76	70	65	52	40	25	10	6	1	Nil			
		Winter	100	90	82	76	68	56	50	40	36	22	16	10	4	Nil	
4.	<u>T. colubriformis</u>	Summer	100	87	72	66	50	45	36	22	10	5	Nil				
		Winter	100	92	85	76	65	54	40	32	25	12	6	2	Nil		
5.	<u>T. axei</u>	Summer	100	80	68	54	42	36	20	12	5	Nil					
		Winter	100	85	76	65	56	47	32	26	18	10	4	Nil			
6.	<u>B. trigonocephalum</u>	Summer	100	82	50	26	Nil										
		Winter	100	87	68	46	26	18	Nil								
7.	<u>S. papillosus</u>	Summer	70	25	Nil												
		Winter	90	37.5	Nil												



necessary to agitate the larval suspension to make sure whether the larvae were alive or dead.

Another observation was about the food reserve. The food reserve in the intestinal cells got depleted with the aging of the larvae. As a result the intestinal cells gradually became indistinct and got filled with a smaller vacuole at first which became larger afterwards by coalescing with adjacent smaller vacuoles. At the end, the larvae became pale and shrunken and the sheath became loose, ultimately the larvae died and floated in the water.

### **Control of infective larvae**

**Assessment of ovicidal and larvicidal property of common fertilizers viz., urea, ammonium sulphate, quick lime and ash**

#### **Ovicidal property**

The comparative ovicidal property of each fertilizers at 1 per cent, 2.5 per cent and 5 per cent was assessed by mixing it with faecal cultures as well as with egg cultures as described in materials and methods and the results are given in Tables 9 to 12.

Table 9. Showing the comparative ovicidal property of urea, ammonium sulphate, quick lime and ash against the common strongyles and strongyloides of ruminants at 1 per cent level based on the infective larve obtained when treated with faecal culture

Sl. No.	Dung sample of	Urea		Ammonium sulphate		Quick lime		Ash		Control No. of larvae	Infective larvae species
		No. of larvae	% of efficacy	No. of larvae	% of efficacy	No. of larvae	% of efficacy	No. of larvae	% of efficacy		
1.	Goat	0	100	0	100	43	28.33	54	10.00	60	<u>H. contortus</u> , <u>S. papillosus</u>
2.	Bullock	0	100	0	100	235	40.35	362	8.12	394	<u>T. axei</u> , <u>H. contortus</u>
3.	Cow	0	100	0	100	14	30.00	17	15.00	20	<u>H. contortus</u> , <u>O. radiatum</u>
4.	Goat	0	100	0	100	73	32.40	92	14.81	108	<u>S. papillosus</u> , <u>O. columbianum</u>
5.	Calf	0	100	0	100	34	37.03	46	14.81	54	<u>S. papillosus</u>
6.	Buffalo (she)	0	100	0	100	36	37.93	52	10.34	58	<u>O. radiatum</u> , <u>H. contortus</u>
7.	Cow	0	100	0	100	65	45.83	98	8.33	120	<u>O. radiatum</u> , <u>T. axei</u> , <u>H. contortus</u> ,
8.	Goat	0	100	0	100	197	43.22	342	1.44	347	<u>T. colubriformis</u> <u>S. papillosus</u>
9.	Calf	0	100	0	100	342	34.23	480	7.69	520	<u>Cooperia</u> , <u>T. axei</u>
10.	Calf	0	100	0	100	56	42.85	85	13.26	98	<u>H. contortus</u> , <u>S. papillosus</u>
Average		0	100	0	100	109.5	36.52	162.8	5.62	172.5	
% Efficacy		100		100		36.52		5.62			

Table 10. Showing the comparative ovicidal property of urea, ammonium sulphate, quick lime and ash against the common strongyles and strongyloides of ruminants at 2.5 per cent level based on the infective larve obtained when treated with faecal culture

Sl. No.	Dung sample	Urea		Ammonium sulphate		Quick lime		Ash		Control No. of larvae	Infective larvae species
		No. of larvae	% of efficacy	No. of larvae	% of efficacy	No. of larvae	% of efficacy	No. of larvae	% of efficacy		
1.	Bullock	0	100	0	100	56	48.14	86	20.37	108	<u>Cooperia</u> , <u>O. radiatum</u>
2.	Cow	0	100	0	100	95	51.03	12	21.64	194	<u>T. axei</u> , <u>T. colubriformis</u>
3.	Goat	0	100	0	100	338	65.33	770	21.02	975	<u>S. papillosus</u> , <u>T. colubriformis</u>
4.	Goat	0	100	0	100	150	57.98	186	19.88	357	<u>O. columbianum</u> <u>O. asperum</u>
5.	Calf	0	100	0	100	38	54.21	64	22.89	83	<u>T. axei</u> , <u>H. contortus</u> <u>Cooperia</u>
6.	Cow	0	100	0	100	16	61.90	33	21.42	42	<u>O. radiatum</u> , <u>H. contortus</u>
7.	Calf	0	100	0	100	52	56.66	96	20.00	120	<u>S. papillosus</u> , <u>H. contortus</u>
8.	Goat	0	100	0	100	121	49.58	185	22.91	240	<u>S. papillosus</u> , <u>H. contortus</u>
9.	Goat	0	100	0	100	58	53.60	98	21.60	125	<u>O. columbianum</u> , <u>T. colubriformis</u>
10.	Bullock	0	100	0	100	65	55.77	118	18.62	145	<u>T. axei</u> , <u>Cooperia</u> <u>O. radiatum</u>
Average		0	100	0	100	98.9	58.6	187.8	21.38	238.9	
% Efficacy		100		100		58.6		21.38			

Table 11. Showing the comparative ovicidal property of urea, ammonium sulphate, quick lime and ash against the common strongyles and strongyloides of ruminants at 5 per cent level based on the infective larvae obtained when treated with faecal culture

Sl. No.	Dung sample	Urea		Ammonium sulphate		Quick lime		Ash		Control No. of larvae	Infective larvae species
		No. of larvae	% of efficacy	No. of larvae	% of efficacy	No. of larvae	% of efficacy	No. of larvae	% of efficacy		
1.	Goat	0	100	0	100	8	75.00	16	50.00	32	<u>T. colubriformis</u> <u>O. columbianum</u>
2.	Goat	0	100	0	100	0	100.00	8	52.94	17	<u>S. papillosus</u> , <u>H. contortus</u>
3.	Goat	0	100	0	100	56	80.07	135	51.95	281	<u>H. contortus</u> , <u>O. columbianum</u>
4.	Calf	0	100	0	100	28	67.05	42	50.58	85	<u>H. contortus</u> , <u>T. axei</u> ,
5.	Cow	0	100	0	100	22	77.08	47	51.04	96	<u>O. radiatum</u>
6.	Goat	0	100	0	100	34	77.33	68	54.66	150	<u>S. papillosus</u> , <u>T. colubriformis</u>
7.	Bullock	0	100	0	100	32	74.60	57	54.76	126	<u>Cooperia</u> , <u>T. axei</u>
8.	Calf	0	100	0	100	0	100.00	21	54.34	46	<u>S. papillosus</u>
9.	Goat	0	100	0	100	38	78.40	87	50.56	176	<u>S. papillosus</u>
10.	Goat	0	100	0	100	16	71.42	26	33.57	56	<u>H. contortus</u>
Average		0	100	0	100	23.4	78.02	50.7	52.40	106.5	
% Efficacy		100		100		78.02		52.40			

Table 12. Showing the ovicidal properties of fertilizers such as urea, ammonium sulphate, quick lime and ash at the strengths of 1, 2.5 and 5 per cent solutions tested in egg culture (without faeces)

Sl. No.	Egg culture	1% solution				2.5% solution				5% solution				Control
		Urea sulphate	Ammonium sulphate	Quick lime	Ash	Urea sulphate	Ammonium sulphate	Quick lime	Ash	Urea sulphate	Ammonium sulphate	Quick lime	Ash	
1.	<u>H. contortus</u> (goat)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
2.	<u>T. colubriformis</u> (goat)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
3.	<u>O. radiatum</u> (cattle)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
4.	<u>O. columbianum</u> (goat)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
5.	<u>H. contortus</u> (cattle)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
6.	<u>B. trigonocephalum</u> (goat)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
7.	<u>H. contortus</u> (goat)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
8.	<u>O. asperum</u> (goat)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
9.	<u>H. contortus</u> (cattle)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D
10.	<u>O. columbianum</u> (goat)	D	D	ND	NC	D	D	ND	NC	ND	ND	ND	NC	D

D - Development of egg to first stage larva in the solutions

ND - No development

NC - Not clear

### Urea

In faecal cultures, urea was found to have 100 per cent ovicidal property at all the three strengths viz., 1, 2.5 and 5 per cent. No development of egg was noticed in the urea treated faecal cultures even at the lowest strength of 1 per cent. But when the eggs were treated directly with urea solutions at the same strengths, the ovicidal property was noticed at 5 per cent level only. In 1 and 2.5 per cent levels the eggs hatched, but the first stage larvae remained viable only for a short period of 2-3 days.

### Ammonium sulphate

It behaved exactly the same way as urea in both the situations. It manifested 100 per cent ovicidal property even at 1 per cent level when mixed with faecal cultures. But when it was directly treated with eggs it also showed ovicidal property only at 5 per cent strength. In 1 and 2.5 per cent strengths though the eggs hatched the larvae did not live for not more than 2-3 days as in urea.

### Quick lime

The ovicidal property of quicklime was comparatively poor in faecal cultures; its efficacy being 36.52, 58.6 and 78.02 per cent at 1, 2.5 and 5 per cent strengths respectively. Its behaviour was exactly the opposite of that of urea and

ammonium sulphate when it was directly treated with eggs and it showed 100 per cent ovicidal property even at 1 per cent strength in this situation.

#### **Ash**

It showed less ovicidal property than quicklime in faecal cultures. It possessed 5.62, 21.38 and 52.40 per cent ovicidal property at the strengths of 1, 2.5 and 5 per cent respectively. But the fate of eggs directly treated with ash could not be studied as the eggs were not clearly visible because of turbid nature of solution.

#### **Larvicidal property**

The comparative larvicidal property of the above fertilizers at various strengths (0.1, 0.5 and 1%) were assessed by adding the required quantity of the fertilizers to the larval suspension containing known number of larvae and counting the number of larvae remained active for the next five days as described in materials and methods. The results are presented in Table 13.

Urea and ammonium sulphate and ash did not show any larvicidal effect even at the highest strength of 1 per cent. But quicklime was 100 per cent larvicidal at 1 per cent strength, below which it was ineffective.

Table 13. Showing the larvicidal properties of fertilizers such as urea, ammonium sulphate, quick lime and ash at the strength of 0.1, 0.5 and 1 per cent solutions tested in larval suspension

Sl. No.	Infective larvae	0.1%				0.5%				1%				Control
		Urea sulphate	Ammonium sulphate	Quick lime	Ash	Urea sulphate	Ammonium sulphate	Quick lime	Ash	Urea sulphate	Ammonium sulphate	Quick lime	Ash	
1.	<u>H. contortus</u> (cattle)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
2.	<u>S. papillosus</u> (goat)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
3.	<u>S. papillosus</u> (goat)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
4.	<u>H. contortus</u> (goat)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
5.	<u>O. radiatum</u> (buffalo)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
6.	<u>T. axei</u> (cattle)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
7.	<u>S. papillosus</u> (calf)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
8.	<u>T. colubriformis</u> (goat)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
9.	<u>Cooperia</u> , <u>T. axei</u> (cattle)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM
10.	<u>S. papillosus</u> (goat)	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM

N.M - No mortality upto 5 days

M - Mortality



# *Plates & Figures*

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PLATE I

- Fig.1. Infective larva of Haemonchus  
contortus (x 160)
- Fig.2. Head end (x 250)
- Fig.3. Tail end (x 250)
- Fig.4. Camera lucida drawings

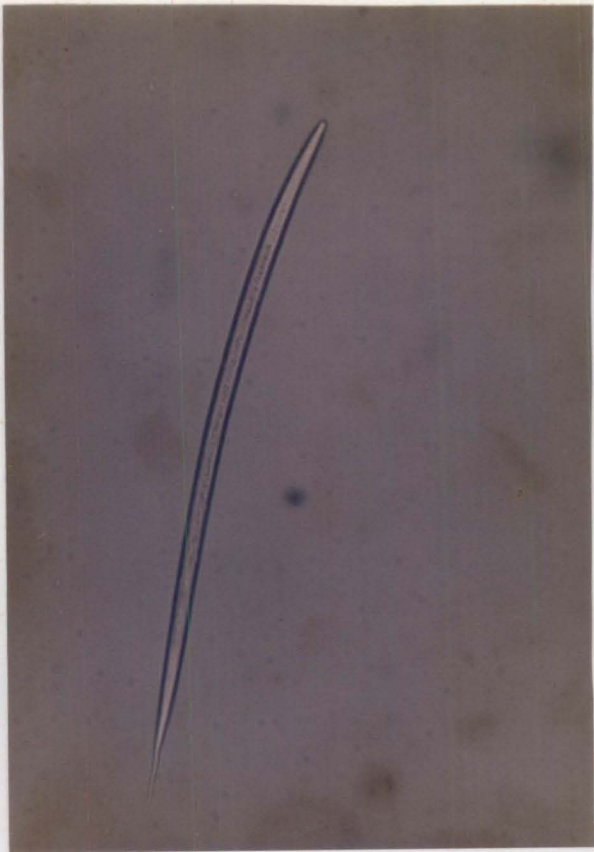


Fig. 1

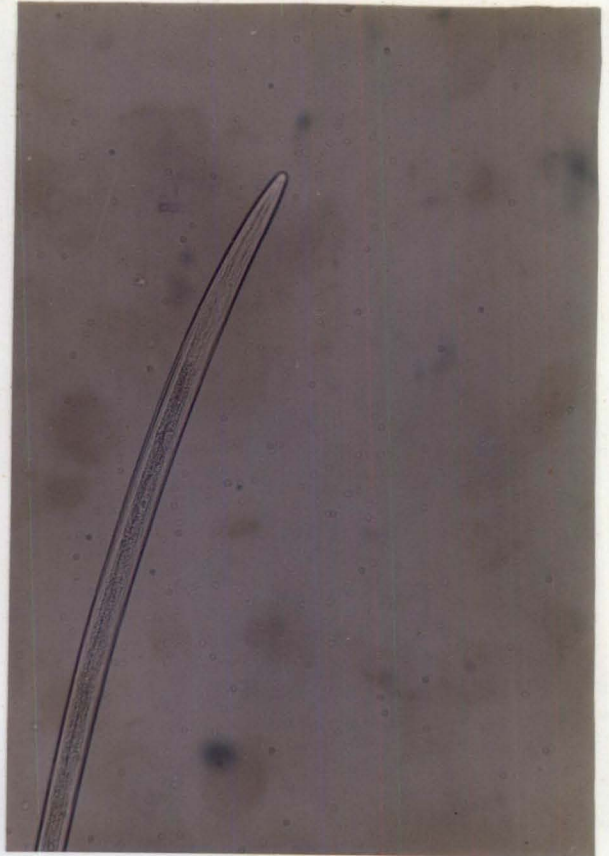


Fig. 2



Fig. 3

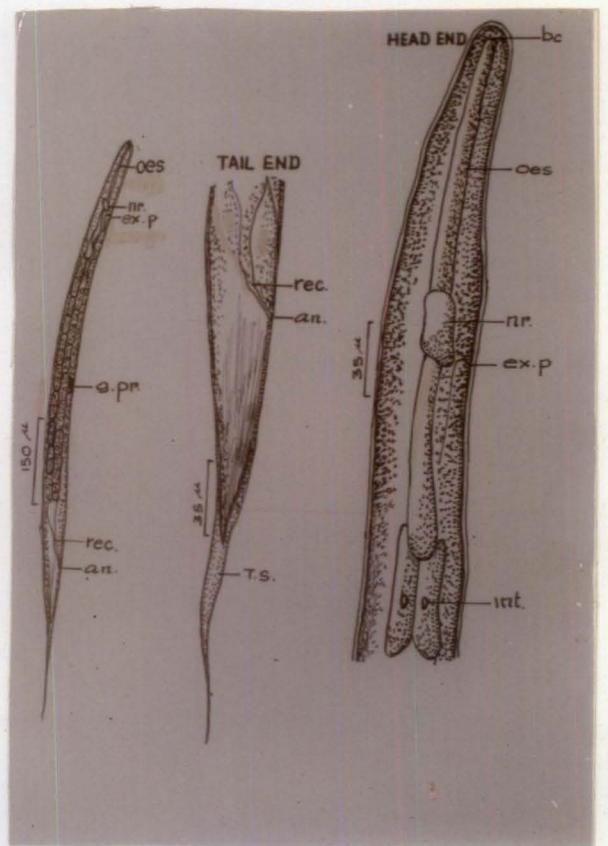


Fig. 4

PLATE II

- Fig.1. Infective larva of Oesophagostomum radiatum (x 160)
- Fig.2. Head end (x 400)
- Fig.3. Tail end (x 400)
- Fig.4. Camera lucida drawings

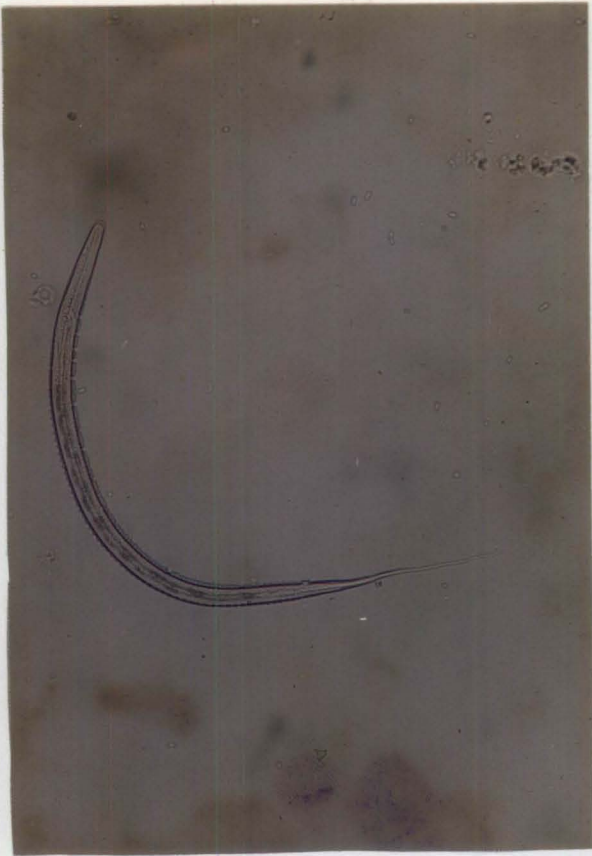


Fig. 1

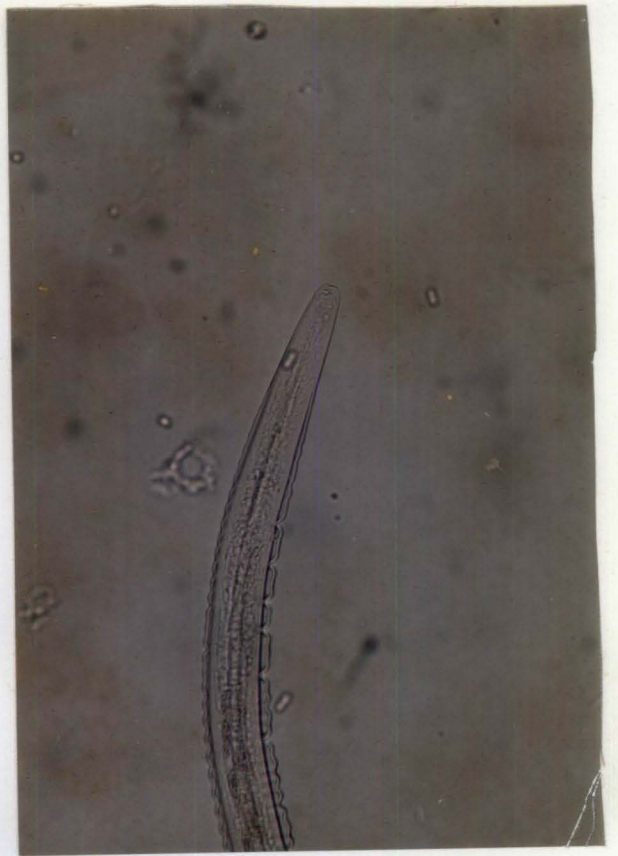


Fig. 2



Fig. 3

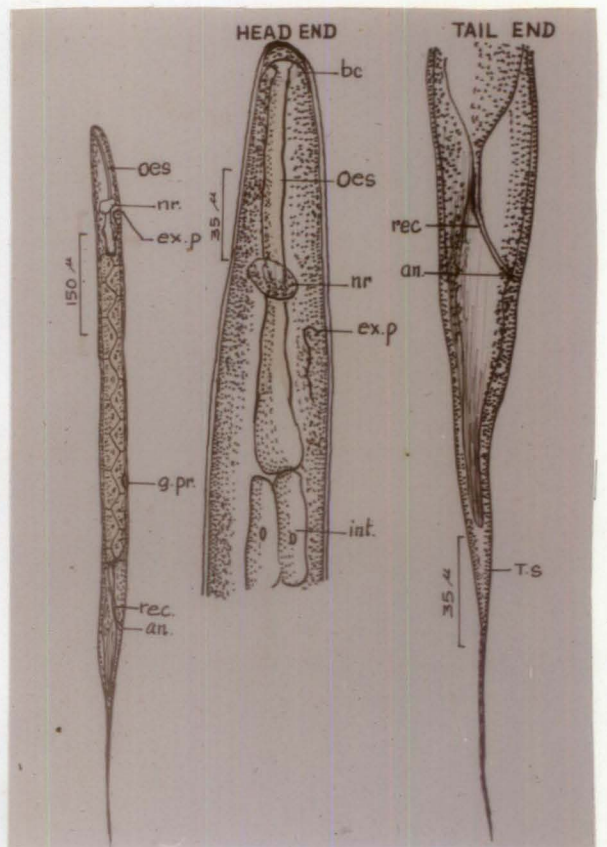


Fig. 4

PLATE III

- Fig.1. Infective larva of Cooperia punctata (x 160)  
Fig.2. Head end (x 400)  
Fig.3. Tail end (x 400)  
Fig.4. Camera lucida drawings

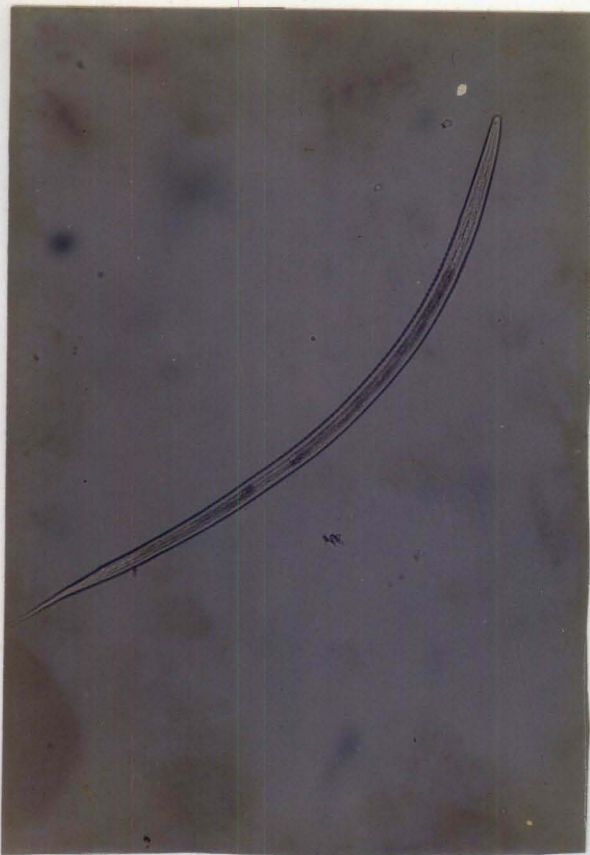


Fig. 1



Fig. 2



Fig. 3

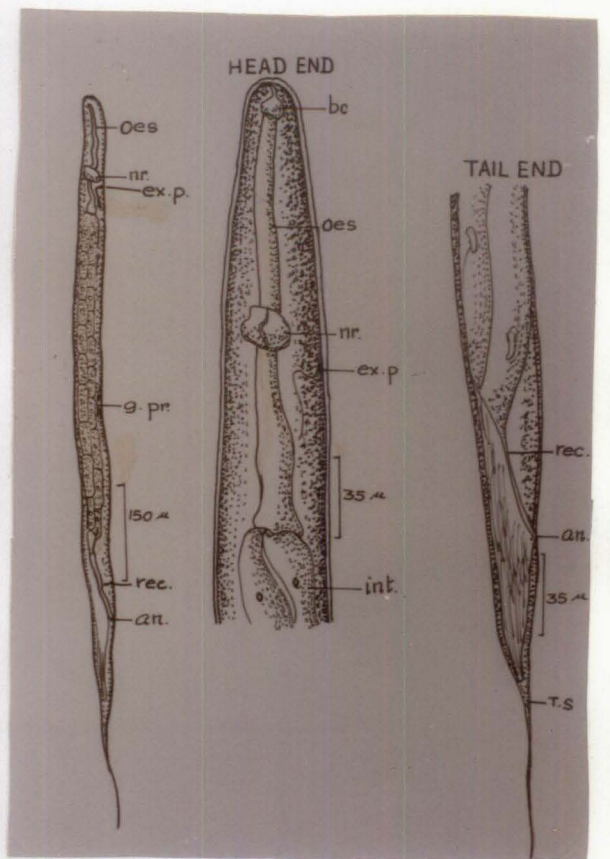


Fig. 4

PLATE IV

- Fig.1. Infective larva of Trichostrongylus colubriformis (x 160)
- Fig.2. Head end (x 400)
- Fig.3. Tail end (x 400)
- Fig.4. Camera lucida drawings



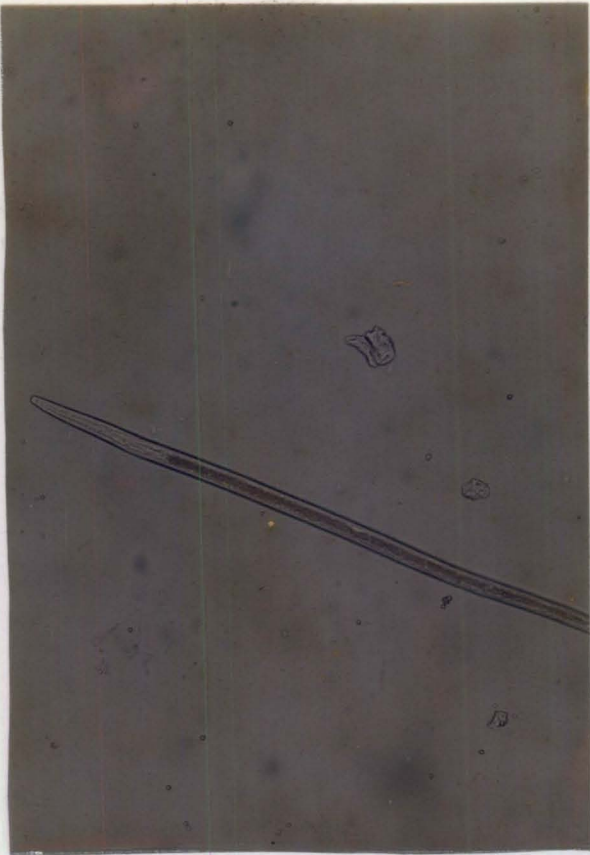


Fig.1



Fig.2



Fig.3

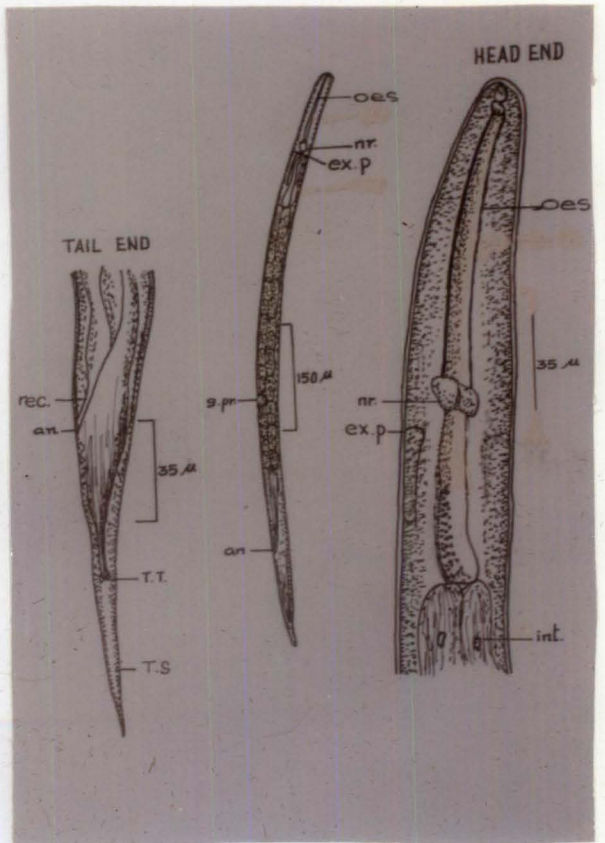


Fig.4

PLATE V

- Fig.1. Infective larva of Trichostrongylus  
axei (x 160)
- Fig.2. Head end (x 250)
- Fig.3. Tail end (x 250)
- Fig.4. Camera lucida drawings

PLATE VI

- Fig.1. Infective larva of Bunostomum  
phlebotomum (x 160)
- Fig.2. Head end and tail end (x 400)
- Fig.3. Camera' lucida drawings

PLATE VI



Fig.1

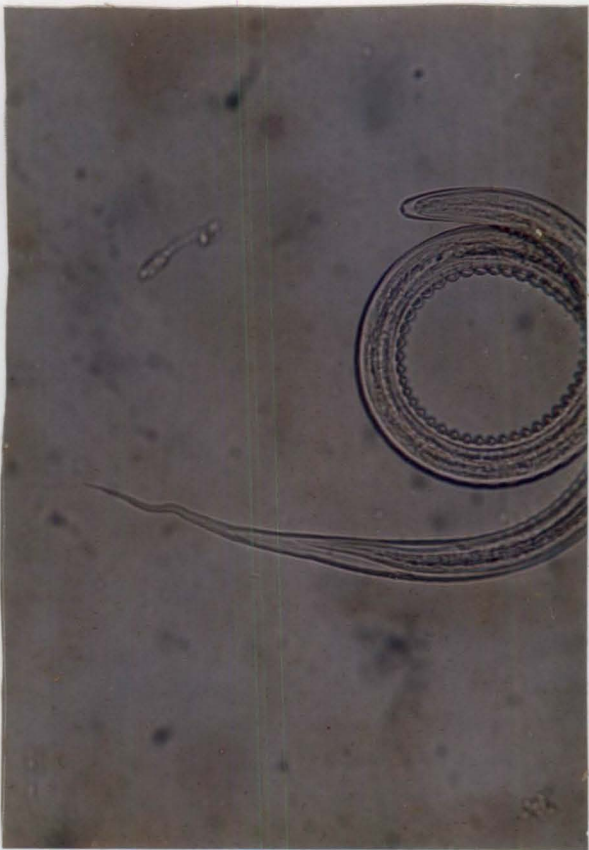


Fig.2

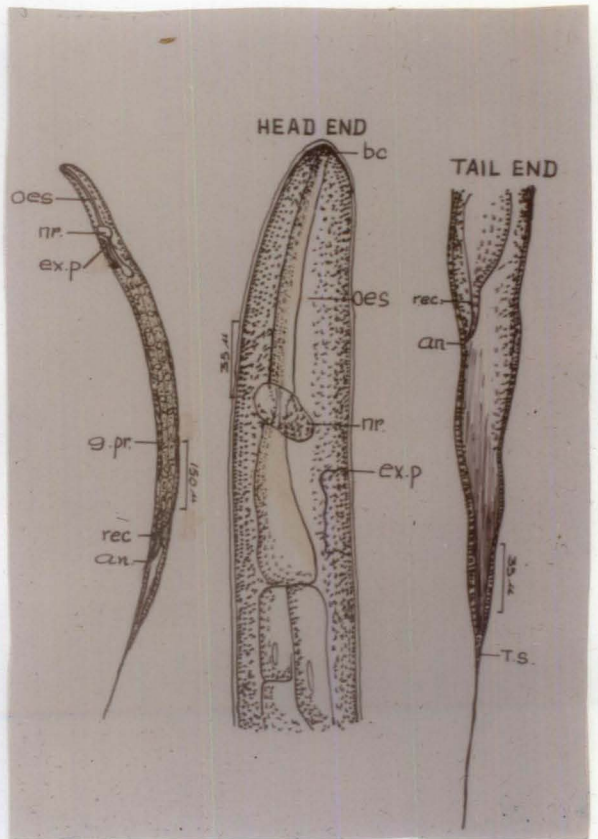


Fig.3

PLATE VII

- Fig.1. Infective larva of Strongyloides papillosus (x 160)
- Fig.2. Head end (x 250)
- Fig.3. Tail end (x 250)
- Fig.4. Camera lucida drawings



Fig.1



Fig.2

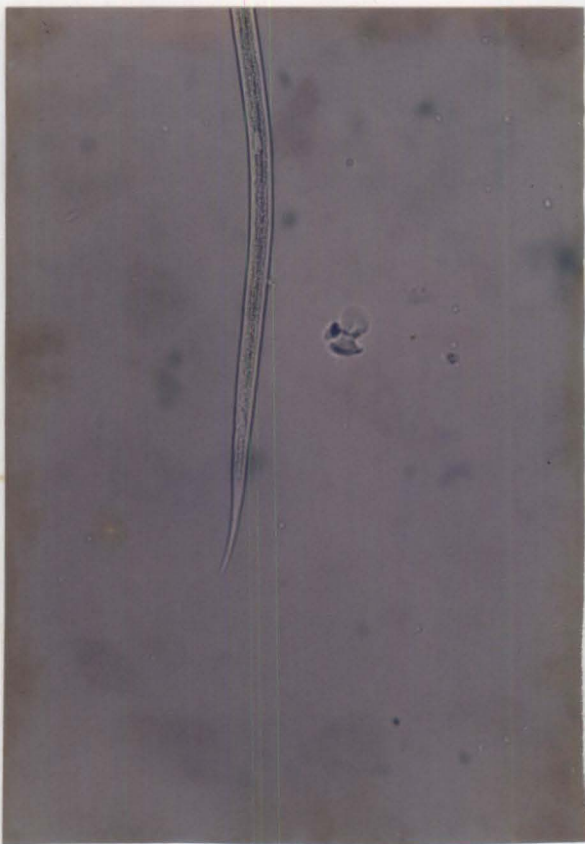


Fig.3

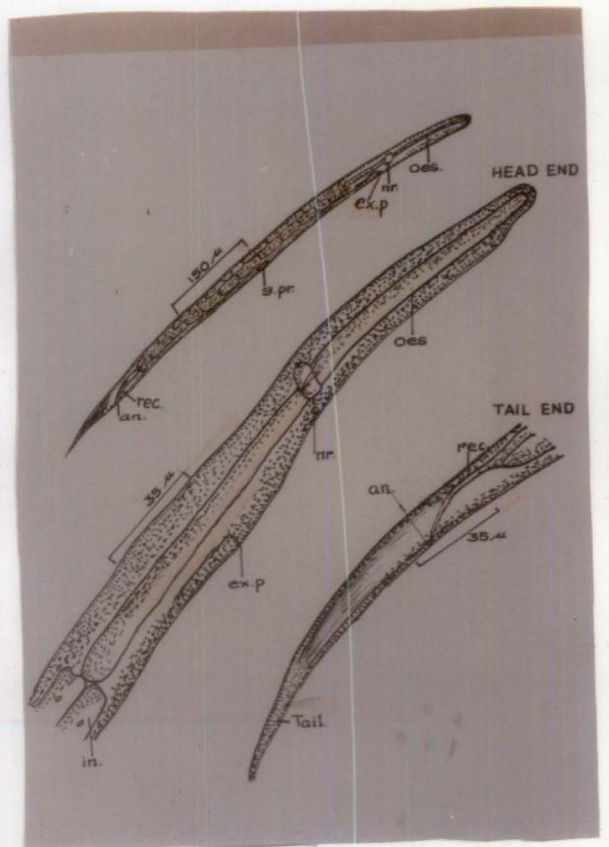


Fig.4

PLATE VIII

- Fig.1. Infective larva of Oesophagostomum columbianum (x 160)
- Fig.2. Head end (x 400)
- Fig.3. Tail end (x 400)
- Fig.4. Camera lucida drawings

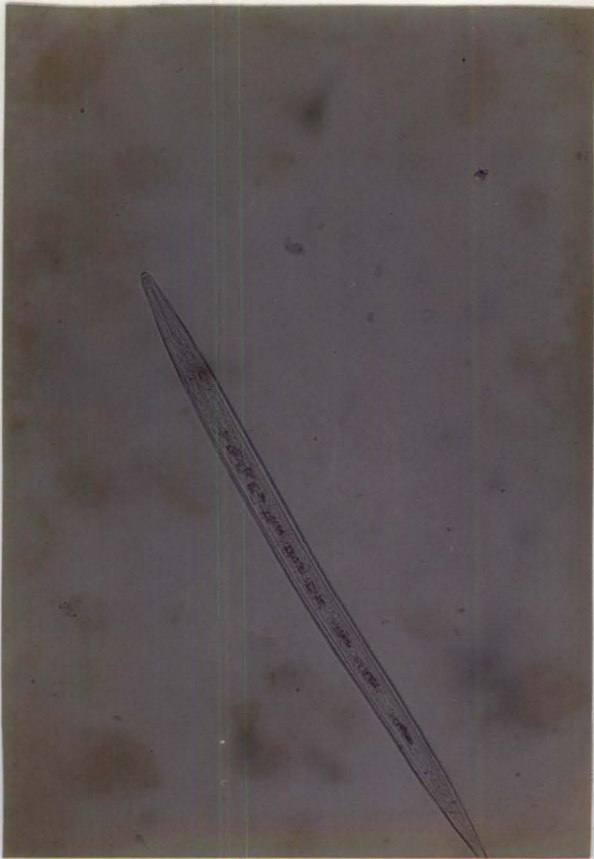


Fig.1

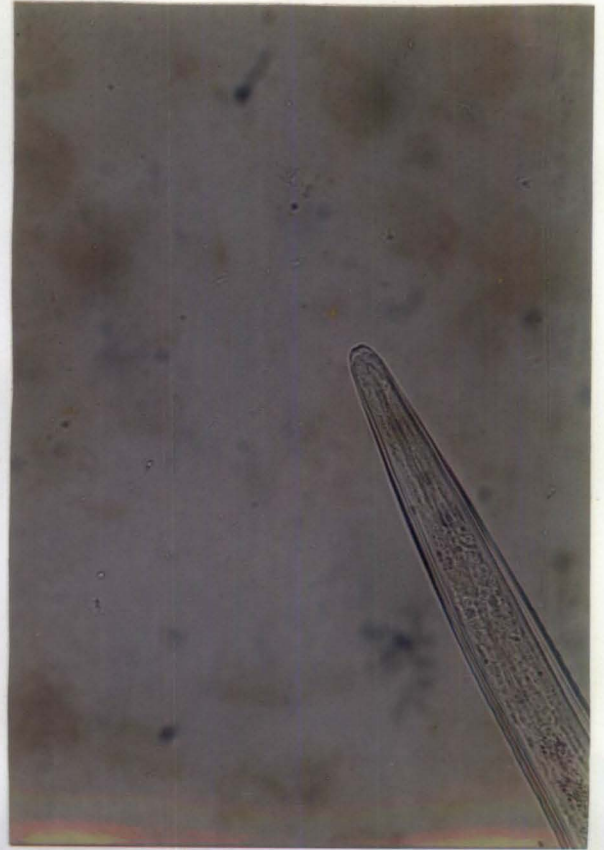


Fig.2



Fig.3

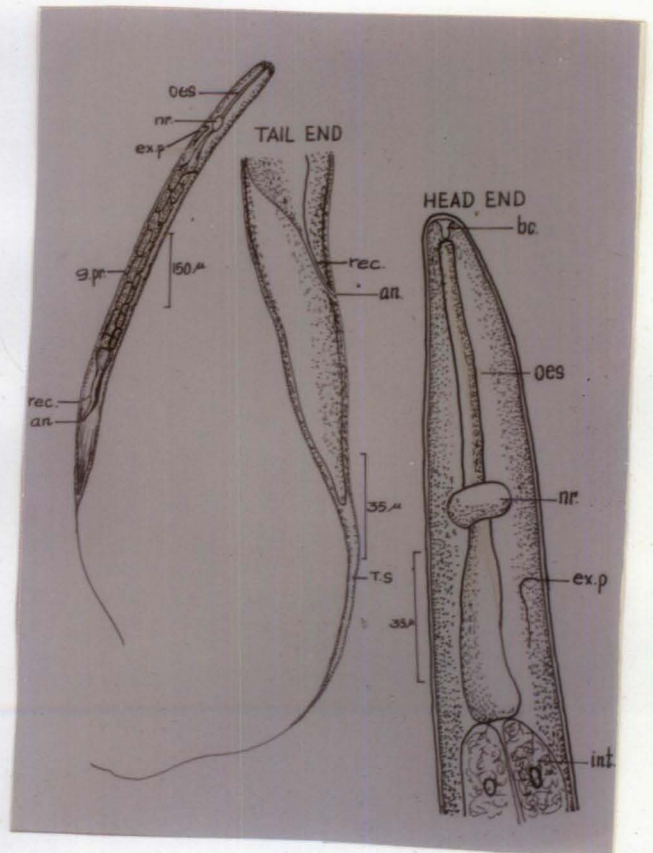


Fig.4



PLATE IX

- Fig.1. Infective larva of Oesophagostomum  
asperum (x 160)
- Fig.2. Head end (x 250)
- Fig.3. Tail end (x 250)
- Fig.4. Camera lucida drawings

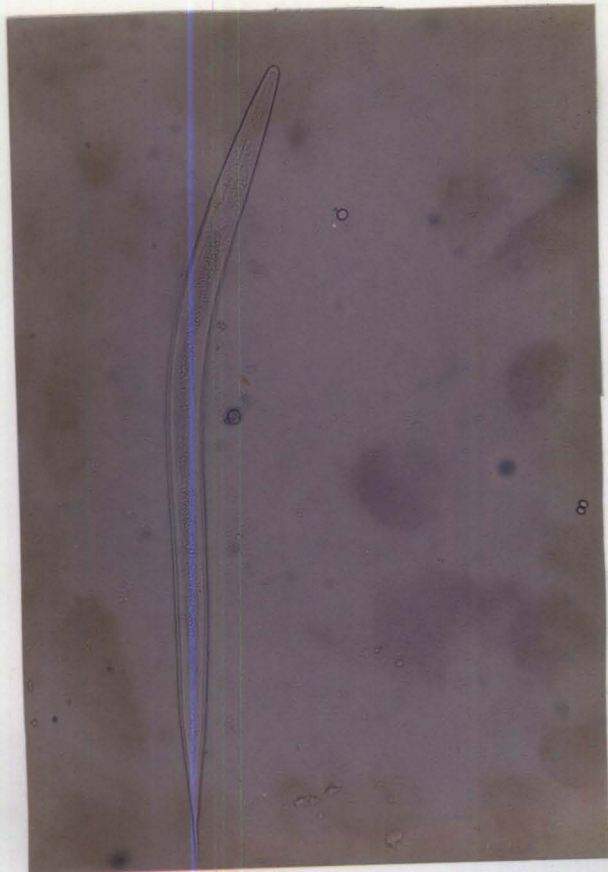


Fig.1



Fig.2

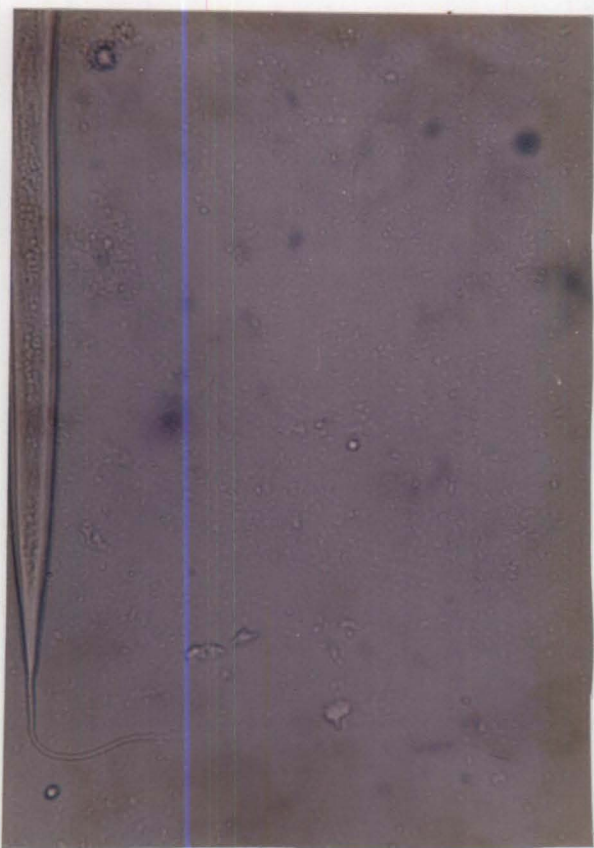


Fig.3

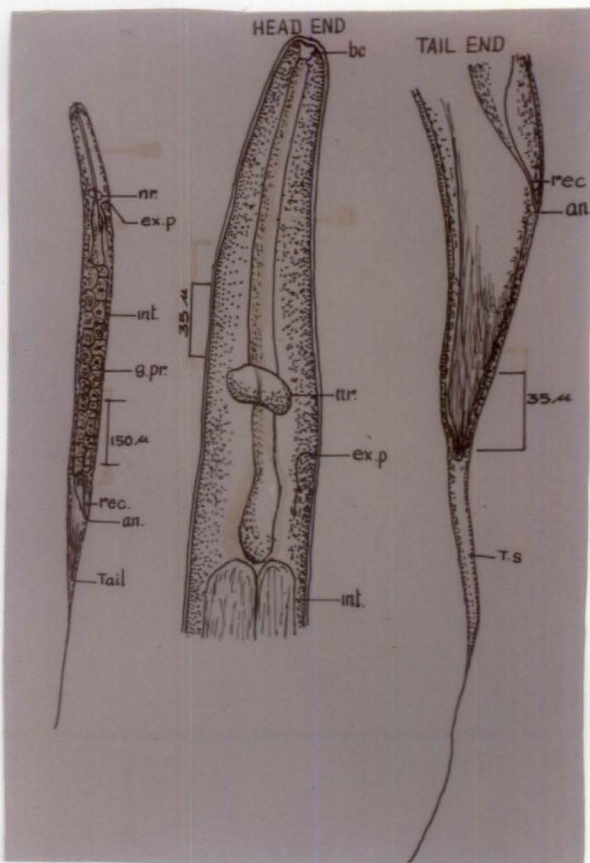


Fig.4

PLATE X

- Fig.1. Infective larva of Bunostomum  
trigonocephalum (x 160)
- Fig.2. Head end (x 250)
- Fig.3. Tail end (x 250)
- Fig.4. Camera lucida drawings



Fig.1

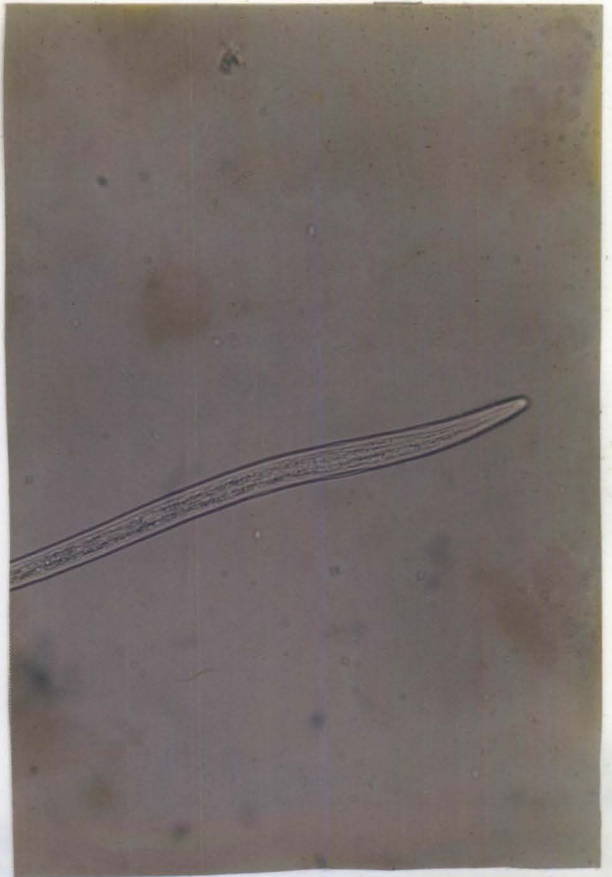


Fig.2

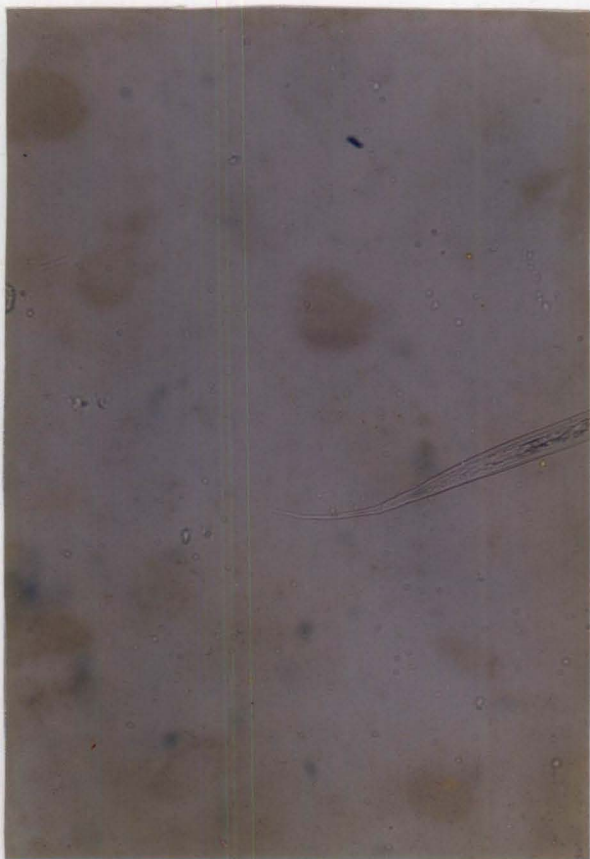


Fig.3

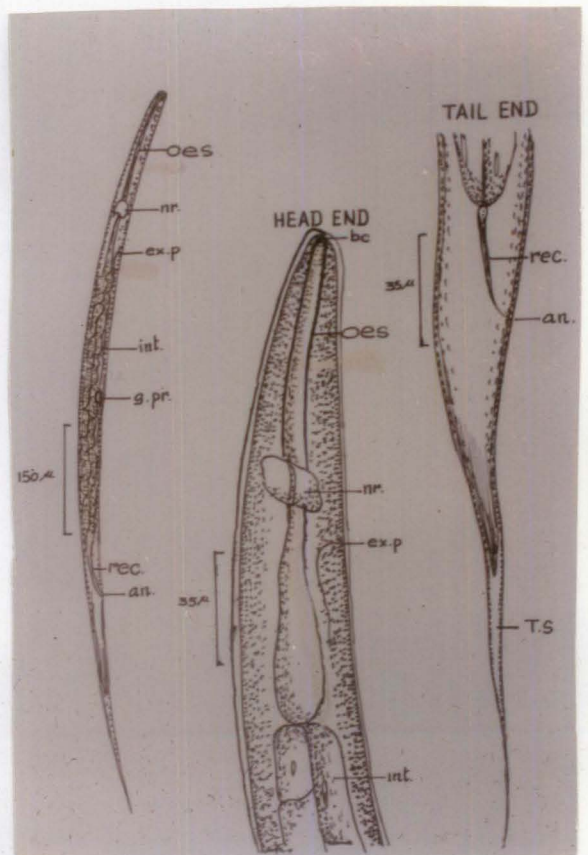


Fig.4

PLATE XI

- Fig.1.        Infeetive larva of Strongyloides  
              papillosus - Head end
- Fig.2.        Middle portion
- Fig.3.        Tail end

PLATE XI

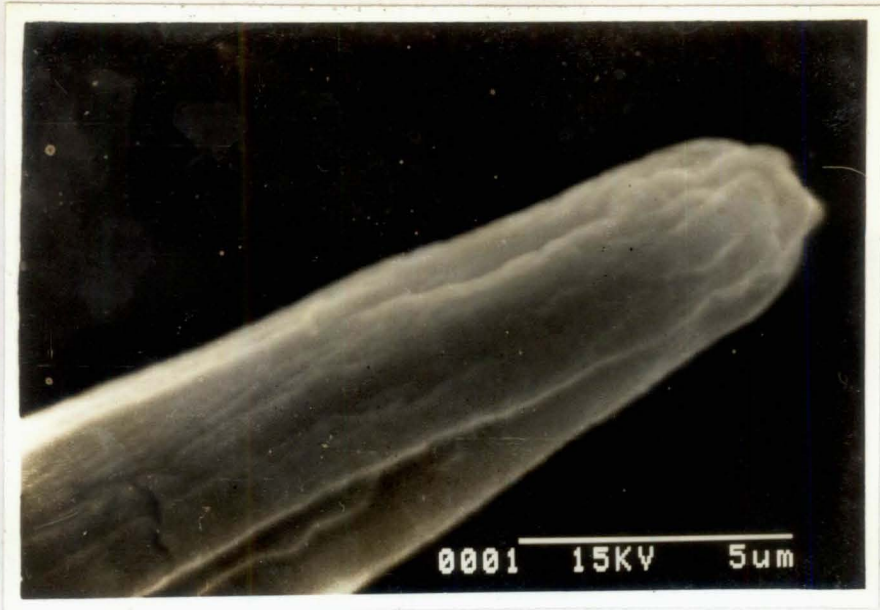


Fig.1

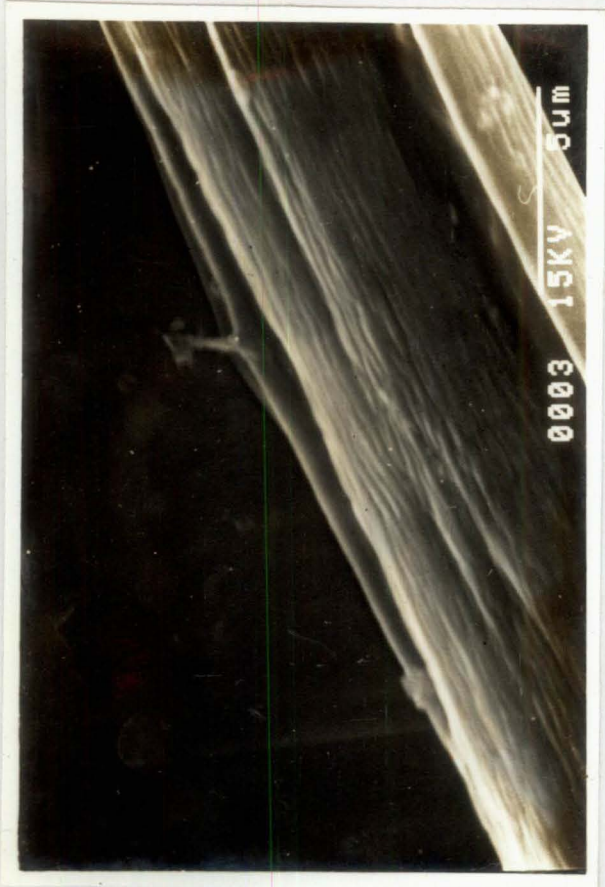


Fig.2

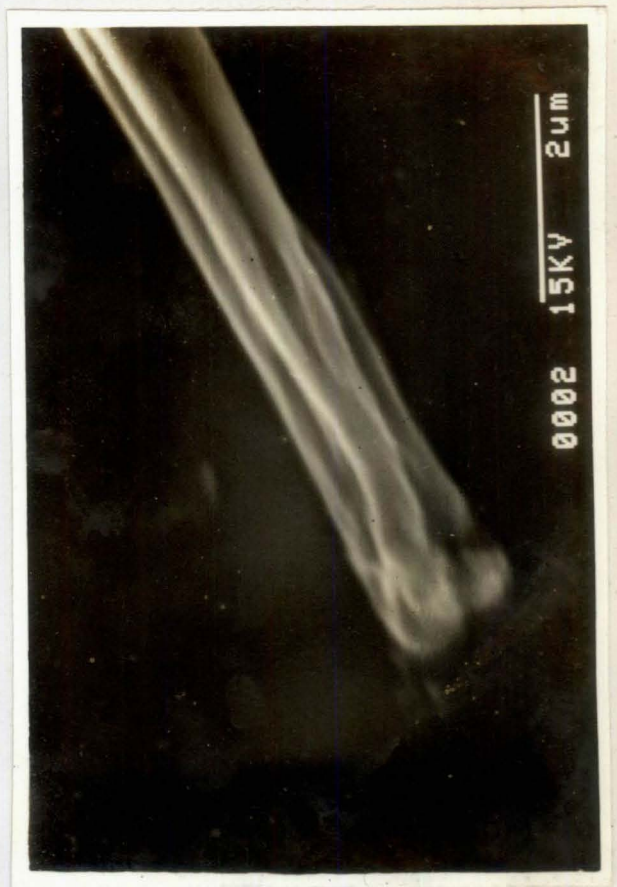


Fig.3

PLATE XII

- Fig.1. Infective larva of Cooperia  
punctata - Head end
- Fig.2. Tail end

PLATE XII

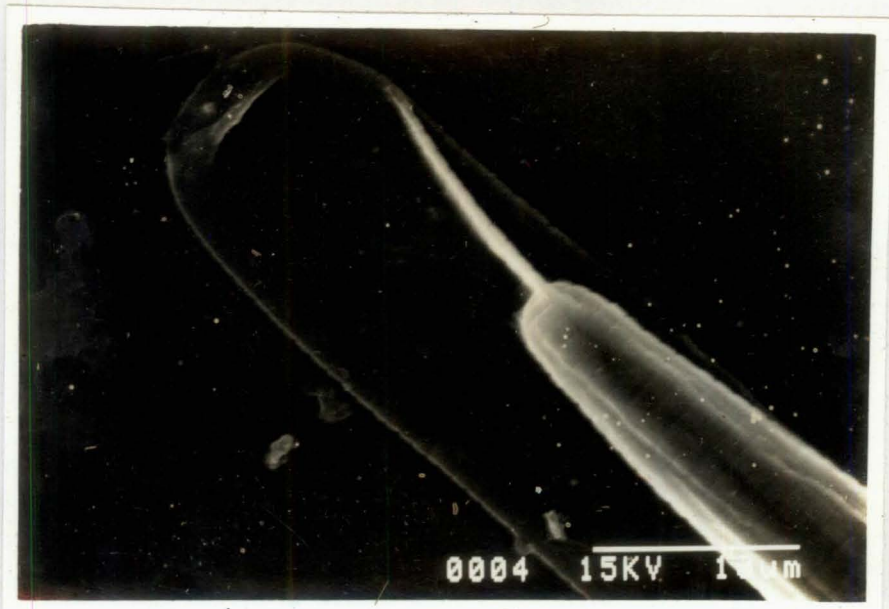


Fig.1



Fig.2



PLATE XIII

- Fig.1. Infective larva of Trichostrongylus  
axei - Head end
- Fig.2. Tail end

PLATE XIII

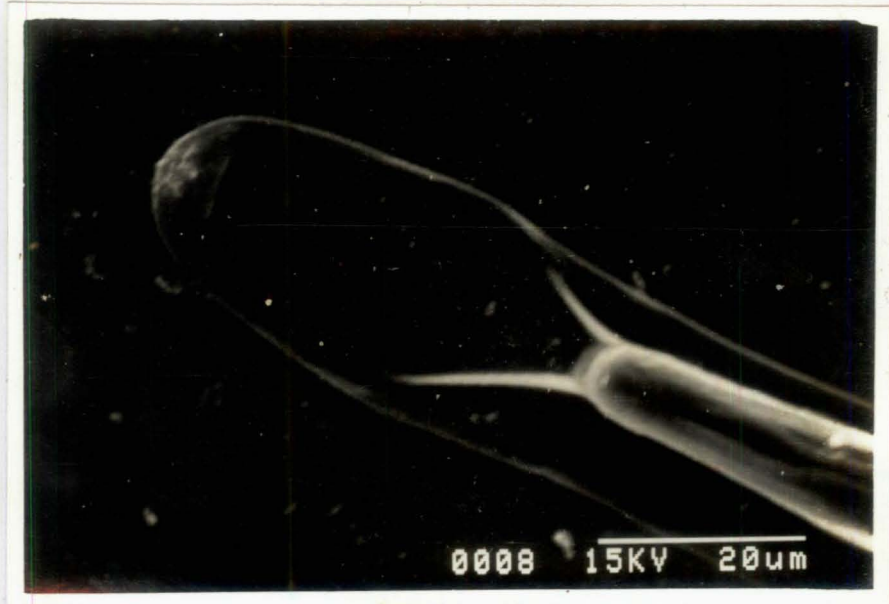


Fig.1

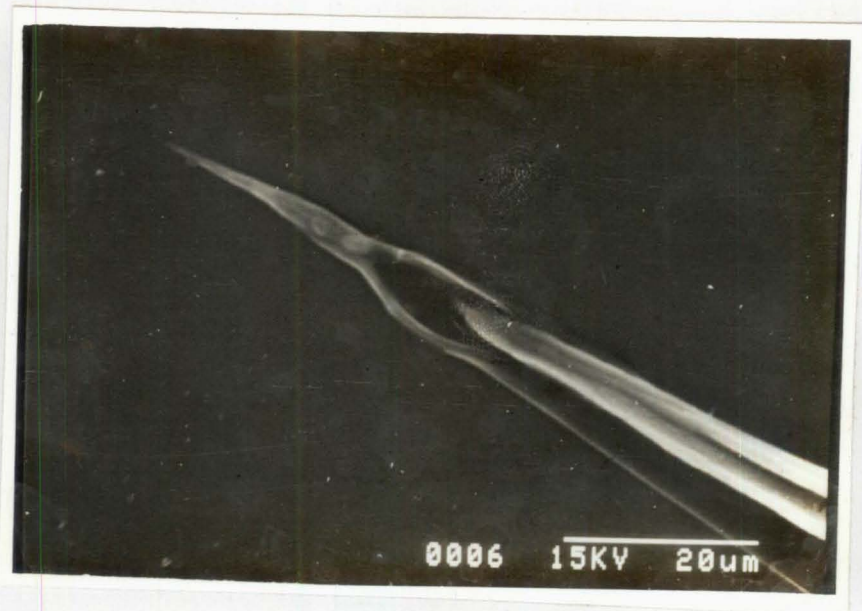


Fig.2

PLATE XIV

- Fig.1. Infective larva of Oesophagostomum radiatum
- Fig.2. Head end
- Fig.3. Tail end

PLATE XIV

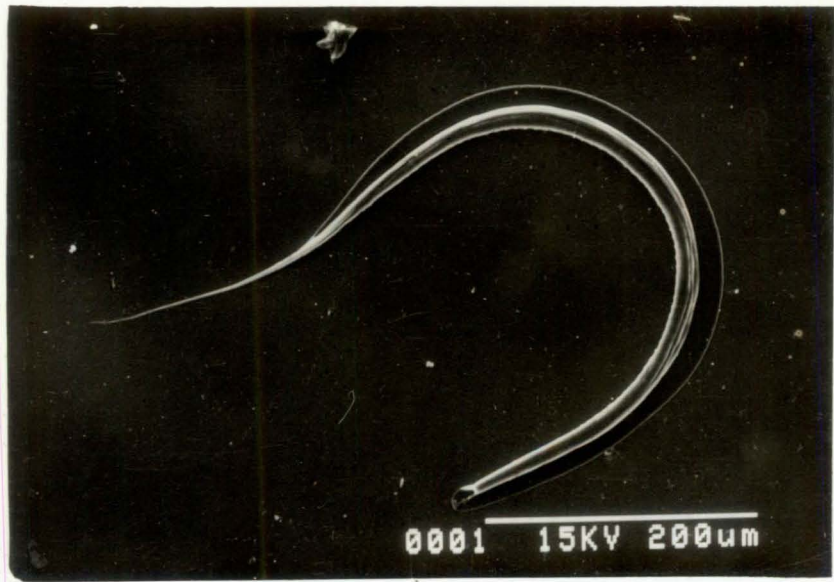


Fig.1

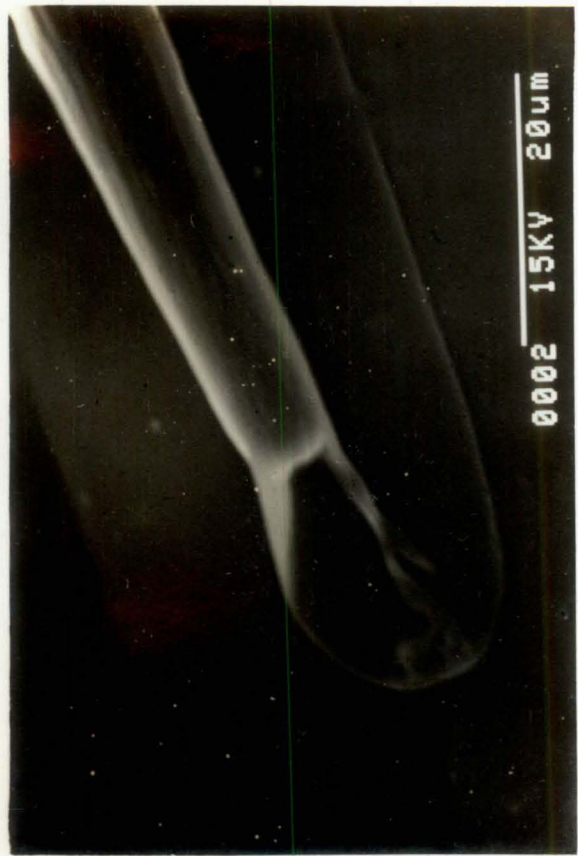


Fig.2

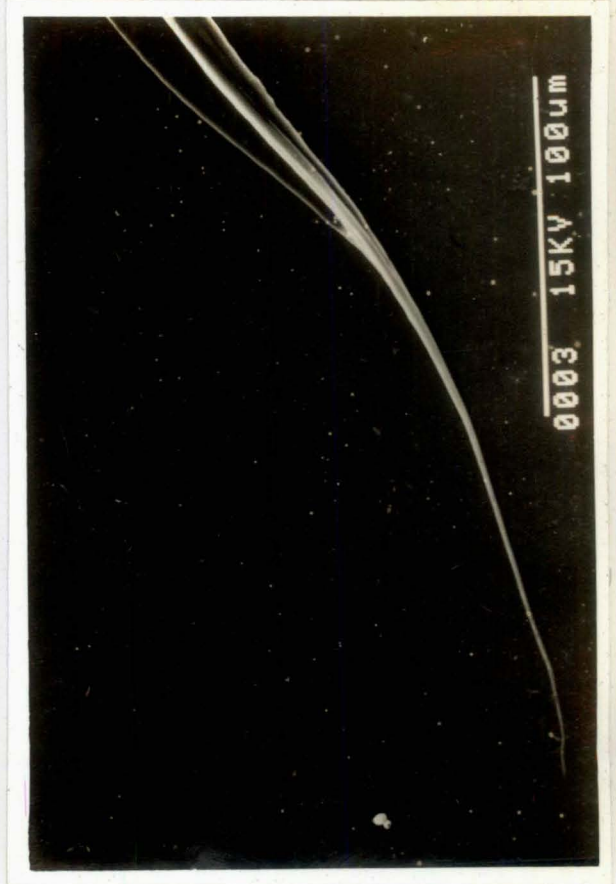


Fig.3

PLATE XV

- Fig.1. Infective larva of Haemonchus  
contortus - Head end
- Fig.2. Oesophageal region
- Fig.3. Tail end

PLATE XV



Fig.1

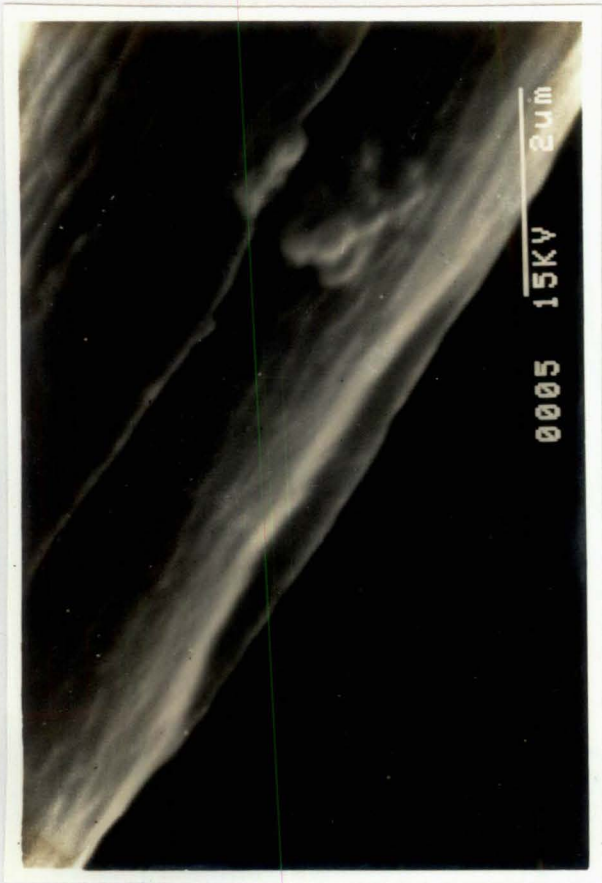


Fig.2

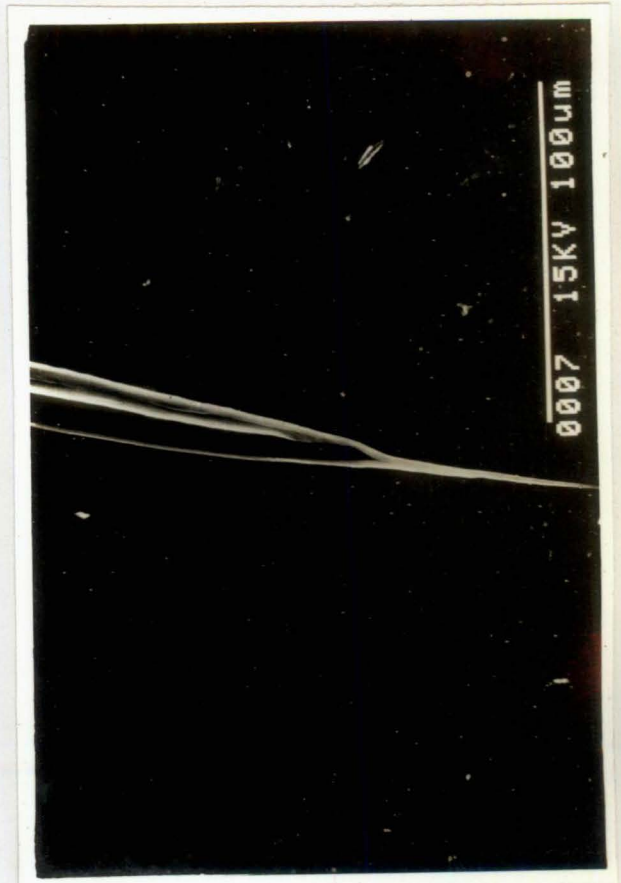


Fig.3

## *Discussion*

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## DISCUSSION

### Prevalence of common nematodes of domestic ruminants

The prevalence of common nematode parasites of domestic ruminants such as cattle, buffalo and goat was determined by faecal examination for eggs and by faecal culture for larvae.

#### Cattle

The prevalence of common nematodes in cattle in the present study was 38.26 per cent as against 34 per cent reported by Borkakoty et al. (1984). Among the nematodes the incidence of strongyles was 30.86 per cent which is almost equal to 29.8 per cent reported by Rajamohan and Paily (1971) and slightly lower than 33.2 per cent reported by Raote et al. (1991). As reported by the latter the incidence of strongyles was noticed throughout the year. The incidence of strongyloides and Ascarids was found to be more in calves in the present studies which is in agreement with the findings of Vaidyanathan (1942), Sarwar (1945), Sukumarapillai (1980) and Mathur et al. (1994). Haemonchus contortus was found to be the commonest species (57.95%) of nematodes occurring in cattle in the present investigation, which finding is in agreement with that of Borkakoty et al. (1984), Muraleedharan et al. (1990), Borthakur



and Das (1994), Dixit and Sahasrabudhe (1994) and Mathur et al. (1994).

### Buffalo

In the present investigation the prevalence of common nematodes in buffaloes was found to be 31.94% and the species of nematodes involved were Haemonchus sp., Oesophagostomum sp., Trichostrongylus sp., Strongylides sp and Ascarid sp. as reported by Patnaik and Pande (1963), Sharma and Pande (1963), Bhopale et al. (1971), Baruah et al. (1981), Gupta and Chhabra (1990), Muraleedharan et al. (1990), Agnihotri (1993), Dixit and Sahasrabudhe (1994) and Rasool Saheb and Md. Hafeez (1994). Among the five species, Haemonchus was found to be the commonest species of nematodes occurring in adult buffaloes which finding is also in agreement with that of Muraleedharan et al. (1990) and Dixit and Sahasrabudhe (1994). But in calves Ascarid and strongyloides were more common as reported by Patnaik and Pande (1963), Gupta and Chhabra (1990), Agnihotri (1993) and Dixit and Shasrabudhe (1994).

### Goat

According to Sathianesan and Peter (1971) the incidence of nematodes was very high in goats, 97.8 per cent in indigenous goats and 89 per cent in farm bred goats. Similarly in the present investigation also it was found to be very high in goats,

77.5 per cent when compared with that in cattle and buffaloes. The prevalence of strongyles was also found to be higher (62%) in goats which finding was almost comparable with that (68%) of Rajamohan and Paily (1971).

The prevalence of Haemonchus contortus was found to be the highest (72.25%) in the present study as reported by Tripathi (1970), Sathianesan and Peter (1971), Misra (1972), Shastry and Ahluwalia (1972), Sinha and Sahai (1973), Bali and Singh (1977), Chellappa and Gopalakrishnan (1977), Masud Ahamed and Ansari (1987) and Upadhyay and Bhatia (1987). But ~~Sandhu et al~~ (1990) reported Oesophagostomum columbianum as the predominant species occurring in goats. No seasonal variation in the incidence of gastro intestinal nematodes in ruminants was noticed in the present study also as observed by Sathianesan and Peter (1971).

### **Identification of infective larvae**

#### **Infective larvæ of Haemonchus controtus**

The infective larvae of H. contortus of cattle, buffalo and goat origin were almost identical in morphology though there were slight variations in measurements. Since the details of the present larva were almost matching with the details of H. contortus infective larvae furnished by the previous workers like Dikmans and Andrews (1933), Keith (1953), Hansen and

Shivnani (1956), Donald (1963), Sahai (1966), Sathianesan and Peter (1977), Tripathi (1968), Padmavathi et al. (1971) and Chauhan et al. (1973), it was identified to be the H. contortus infective larva.

#### **Infective larvae of Oesophagostomum radiatum**

The oesophagostomum infective larvae of cattle and buffalo origin were found to be identical in morphology and measurements in the present study. The present larvae were almost identical with the infective larvae of O. radiatum described by the previous workers like Anantharaman (1942), Keith (1953), Kharichkova (1953), Hansen and Shivnani (1956) and Levine (1978). So they were identified as O. radiatum infective larvae.

#### **Infective larvae of O. columbianum**

The present larvae of goat origin were identical in morphology and measurements with the infective larvae of O. columbianum furnished by the previous workers like Dikmans and Andrews (1933), Sathianesan (1968) and Tripathi (1968). So they were identified as that of O. columbianum.

#### **Infective larvae of O. asperum**

There are only two publications on the infective larvae of O. asperum and they are of Sathianesan and Peter (1976) and

Hariantha Rao and Venkataratnam (1977). The details of the present larvae were closely matching with that of Q. asperum described by Sathianesan and Peter (1976). So it is identified as infective larva of Q. asperum.

#### Infective larvae of Trichostrongylus colubriformis

The infective larvae of T. colubriformis of cattle, buffalo and goat were almost identical except in certain minor differences in measurements. The present larvae were almost identical with the infective larvae of T. colubriformis described by Dikmans and Andrews (1933), Keith (1953), Hansen and Shivnani (1956), Sathianesan and Peter (1979), Tripathi (1968) and Chauhan et al. (1973). So the present species is described as the infective larva of T. columbiformis.

#### Infective larvae of T. axei

In the present study T. axei was obtained only from cattle and goats. When the details of the present larva were compared with that of infective larvae of T. axei described by Keith (1953), Hansen and Shivnani (1956), Sathianesan (1968) and Levine (1978) they were found closely matching with each other. So the present larva was described as infective larva of T. axei.

### Infective larvae of Bunostomum phlebotomum

Since the morphology and measurements of the present larvae matched with that of B. phlebotomum described by Krug and Mayhew (1946), Sprent (1946), Keith (1953), Hansen and Shivnani (1956), Supperer (1958), Chauhan et al. (1973) and Levine (1978) the present larvae were described as infective larvae of B. phlebotomum.

### Infective larvae of B. trigonocephalum

The larvae obtained in the present study were identical in all respect with that of B. trigonocephalum described by Dikmans and Andrews (1933), Ortlepp (1939), Sathianesan (1968), Tripathi (1968) and Nascimento et al. (1984). So the present larvae were identified as B. trigonocephalum infective larvae.

### Infective larvae of Cooperia punctata

The morphology and measurements of the infective larvae of C. punctata described by Dikmans and Andrews (1933), Andrews (1935), Keith (1953), Hansen and Shivnani (1956) and Levine (1978) were almost similar to that of the present species. So the present larvae obtained from cattle was identified as C. punctata.

### Infective larvae of Strongyloides papillosus

The morphology and measurements of Strongyloides papillosus infective larvae obtained from different domestic ruminants by different workers like Basir (1950), Keith (1953), Hansen and Shivnani (1956), Zeletzki (1959), Sathianesan (1968), Tripathi (1968) and Chauhan et al. (1973) were identical with that of the present larvae. So they were identified as S. papillosus. infective larvae.

### Scanning electron microscopic study of infective larvae

The infective larvae that were subjected to SEM study by the present worker were of Strongyloides papillosus, Cooperia punctata, Tricoststrongylus axei, Oesophagostomum radiatum and Haemonchus contortus. SEM study of none of the above larvae other than H. contortus was done earlier by any workers. In the case of H. contortus Lichtenfels et al. (1990) were the only authors who could make some studies of the larvae under SEM. But the present observations and their observations differ. They could see amphids, and cephalic papillae at the head end which the present worker could not notice. In the case of S. papillosus, the tail end of the larva was found to be trifid under SEM which under light microscope was found to bifid by the present worker as well as by previous workers except Alicata (1935) who found it to be trifid under light microscope. Since there are no previous SEM studies of the larvae of C. punctata,

T. axei, Q. radiatum and S. papillosus, the present study is the first SEM study of these larvae.

### **Bionomics of infective larvae**

#### **Phototropism**

In the present study none of the infective larvae showed phototropism, concurring with the findings of Stewart and Douglas (1938), Silangwa and Todd (1964), Tripathi (1969a), Voznyi (1978) and Mittal (1987). But Rogers (1940), Rees (1950), Deodhar (1966) and Misra and Ruprah (1972) noticed a favourable influence of light on the migration of larvae on pasture. The present observation is in total disagreement with that of Sprent (1946), Soliman (1953) and Soulsby (1965) who observed a positively phototropic behaviour of infective larvae.

Another interesting observation made in the present study is that the larvae were seen migrated to the water droplets formed on the sides of the culture bottles above the faecal pad indicating that the moisture is more attractive to infective larvae than the light. It also indicates that the infective larvae prefer more of cooler and moist areas than the hotter and drier areas as occurring in faecal pad.

## Viability

In the present study infective larvae H. contortus of cattle, buffalo and goat origin were found to remain viable for 68-102 days in water under room temperature. This finding agrees with that of previous workers like Sathianesan (1968), Tripathi (1977), Misra (1978), Boag and Thomas (1985) and Harbinder Singh et al. (1994) who also found the larvae to be viable for more or less the same period. But it disagrees with observations of Daskalov (1965), Todd et al. (1976) and Tripathi (1969) who found the larvae to be viable for a shorter period of 40-64 days and with Jehan and Gupta (1974) and Sood and Charanjit Kaur (1975) who observed a longer period of viability for the larvae (110-120 days).

Viability of infective larvae of Oesophagostomum radiatum was found to be 65-97 days in present study which was in confirmity with the maximum period of 3 months as observed by Anantaraman (1942). But the period of 26 weeks reported by Delgado (1983) in Cuba was too long.

Oesophagostomum columbianum infective larvae in the present study remained alive for 91-114 days. Almost the same period of viability was observed by Premwati and Lal (1961), Agarwal (1966) and Sathianesan (1968). But shorter periods of 55 days and 2 months were observed by Chhabra and Singh (1965) and Tripathi (1969b) respectively and longer periods



of 220 days and 12 months were noticed by Daskalov (1965) and Zhidkov (1965) respectively. The longevity of infective larvae of Q. asperum was found to be 62-97 days in the present study. But a slightly longer period of 12-15 weeks was the observation made by Sathianesan (1968). But Hariantha Rao and Venkataratnam (1977) noticed only a very short period of 45 days.

The viability of infective larvae of Trichostrongylus axei and T. colubriformis were 60-80 days & 56-86 days respectively in the present study. The period reported by Sathianesan (1968), Tripathi (1969) and Boag and Thomas (1985) was also more or less the same. But the period of 7 months reported by Altaev (1967) was too long probably due to the difference in the climatic conditions prevailing in Russia and also due to the change of species which being T. skrjabini. Rose and Small (1984) reported a still longer period of 16 months for T. vitrinus in grass plots. Here also the conditions prevailed and species involved were different.

The infective larvae of Cooperia punctata found to thrive in water for 54-73 days in the present findings. Ahluwalia (1974), Boag and Thomas (1985) also found the larvae to thrive in water for 52 days and 64 days respectively. But Delgado (1983) got a longer period of longevity, 22 weeks with which the present observation differs.

In the present study, infective larvae of Bunostomum phlebotomum and B. trigonocephalum were found to survive 28-43 days and 30-46 days respectively in water under room temperature. This observation is in agreement with that of Narain (1965) and Sathianesan (1968) who found that infective larvae of B. trigonocephalum survived for 38 days and 4-5 weeks. But Zhidkov (1965) and Delgado (1983) reported a longer period of 4 months and 10 weeks respectively and Daskalov (1965) reported a shorter period of 15 days with all of which the present observation is in disagreement.

Infective larvae of Strongyloides papillosus survived for 10-20 days in water under room temperature in the present study. This observation is in agreement with Nath (1977) and Verma et al. (1986) who also found the larvae to be viable for 12 days and 17 days respectively.

### **Control of infective larvae**

Assessment of ovicidal and larvicidal property of common fertilizers viz. urea, ammonium sulphate quicklime and ash

Ovicidal property

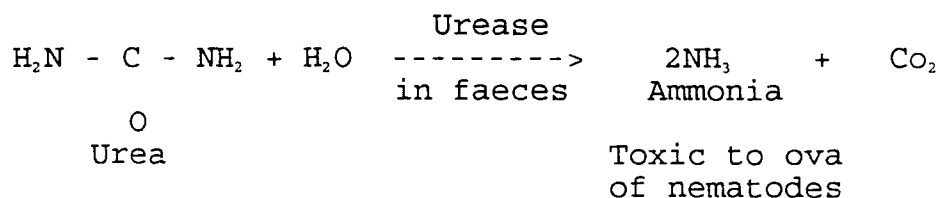
Urea

In the present study urea was found to be 100 per cent ovicidal at the strength of 1, 2.5 and 5 per cent in faecal

cultures. This was evident by the absence of larvae in the treated cultures and presence of larvae in the control cultures. Sarimsakov and Nikolski (1961), Abdul Qadir (1971), Helle et al. (1989) and Agarwal et al. (1991) also got the same results. But Abdul Qadir (1976b) got only a lower efficacy of 82.4 per cent and 88.9 per cent against the ova of gastrointestinal nematodes of goats and calves respectively in pasture land.

In egg cultures in the present study urea was found to be effective only at 5 per cent strength and ineffective at lower strengths.

The inhibitory effect of urea on the development of eggs in faecal culture, according to Helle et al. (1989) was mainly caused by toxicity of ammonia produced when urea undergoes hydrolysis in the presence of an enzyme urease (microbial origin) which is abundantly present in faeces of ruminants. The reaction according to him may be as follows:

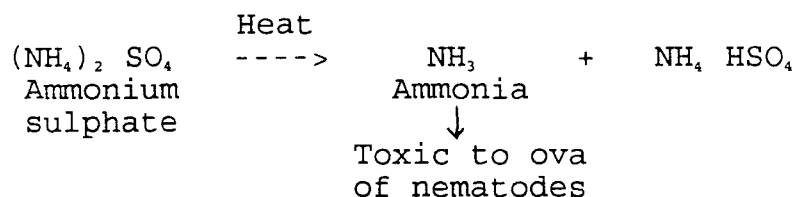


But in egg cultures, there is no release of ammonia from the urea due to absence of urease. So urea cannot prevent the development of egg to larvae at 1 per cent and 2.5 per cent level. But its inhibitory effect on the development of eggs at

5 per cent level may probably due to its innate capacity to do so at higher strengths.

### Ammonium sulphate

No difference was noticed in the ovicidal property of ammonium sulphate and urea in the present study and the former gave the same ovicidal effect as that of urea in all the three strengths tried in the present study. Edirisinghe and Ihalamulla (1976) observed that ammonium sulphate only depress the decortication of eggs, larval motility and hatchability and not exactly ovicidal in his experiments with ascarid eggs which are thick shelled. But the present study was with strongyles and strongyloides eggs which are thin shelled. The ovicidal property of ammonium sulphate in faecal culture may also due to the release of ammonia when it is heated and its reaction with urease is not well studied. The release of ammonia may be as follows:



So it is suggested that heat is produced by the fermentation process which is taking place inside the faecal culture which in turn activate the ammonium sulphate to release ammonia. The latter then inhibits the development of eggs.

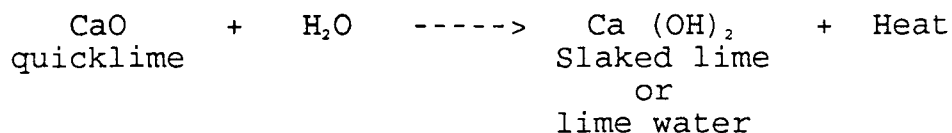
In egg cultures, just like urea, ammonium sulphate was effective only at 5 per cent strength and ineffective at lower strengths.

#### Quicklime (Calcium oxide)

The ovicidal property of quicklime was found to be 32.35 per cent, 56.02 per cent and 77.72 per cent at the strengths of 1, 2.5 and 5 per cent in the present study. Persson (1973) also noticed the ovicidal property of quicklime and explained that this property may be due to the lethal effect of high pH and high temperature of quicklime solution. Chongrak Polprasert and Gamboa Valencia (1981) also noticed the ovicidal property of quicklime and it was only 26.5 per cent at 1.9 per cent strength. This lower percentage of efficacy may be due to the higher thickness of the shell of Ascarid ova with which he was experimenting.

In egg cultures in the present study, quicklime was found to be 100 per cent ovicidal even at the lowest strength of 1 per cent.

The mechanism of inhibition of development of eggs of nematodes by quicklime is obscure. But it may probably be in the following way. When quicklime comes in contact with water, it is transformed into slaked lime ( $(\text{CaOH})_2$ ). As a result more heat is produced during this process.



The pH is also increased when the concentration of quicklime becomes more. So the increase in temperature and pH may be responsible for the ovicidal effect of quicklime. This was the view expressed by Persson (1973) also.

The higher percentage of ovicidal effect of quicklime in egg culture than that in faecal culture may be due to the increased contact surface and the availability of sufficient water in the egg culture

#### **Ash**

Ash in the present study showed 5.62, 21.38 and 52.40 per cent ovicidal property at the strengths of 1, 2.5 and 5 per cent respectively in faecal cultures and in egg culture the ovicidal property of ash could not be studied, because of turbid nature of solution. The mechanism of action of ash in faecal culture is obscure.

#### **Comparative ovicidal property of the four fertilizers**

On a comparative basis in the present study urea and ammonium sulphate were found to be superior over quicklime and ash in their ovicidal property with no difference between the

former two. But between quicklime and ash the latter was found to be the least effective.

### Larvicidal property

#### Urea

Urea did not show any larvicidal property at 0.1, 0.5 and 1 per cent strengths against infective larvaè of common nematodes in present study. Boloanta et al. (1968~~a~~<sup>b</sup>) also did not find larvicidal property of urea against lung worm and strongyle larvae, contrary to this Abdul Qadir (1976a) and Ramajomartin (1981) found a fairly high percentage of larvicidal efficacy (68-72.7%) at one per cent of urea in soil against infective larvae of Trichostrongylide and Ostertagia spp. Hamdy et al. (1984) observed the reduction of longevity of Ancylostoma duodenale larvae by urea. But in the present study in a separate experiment urea at 10 per cent strength was found to have 100 per cent larvicidal property.

#### Ammonium sulphate

Ammonium sulphate was also with no larvicidal property just like urea at 0.1, 0.5 and 1 per cent strengths as observed in the present investigation. But Mango (1971) found some amount of larvicidal action of ammonium sulphate against larvae of hook worms of dog. Similarly Hamdy et al. (1984) also

observed reduction of longevity of Ancylostoma duodenale larvae by ammonium sulphate.

### Quicklime

Quicklime showed 100 per cent larvicidal property at 1 per cent level with no effect at 0.1 and 0.5 per cent strengths. Similar result was obtained by Persson (1973) also. But according to Ramajo Martin (1981) lime did not have larvicidal property.

### Ash

Ash did not show larvicidal property at 0.1, 0.5 and 1 per cent strengths. It was also difficult to observe the larvae because of turbidity particularly at higher strengths.

### Comparative larvicidal property of four fertilizers

As a larvicide quicklime was found to be the choicest fertilizer among the four fertilizers tried with 100 per cent efficacy at 1 per cent strength. The others being totally ineffective at that strength.

From the above experiments it is concluded that mixing of fresh dung with urea or ammonium sulphate at a strength of 1 per cent before it is used as a manure will definitely control the incidence of gastrointestinal nematode infection in domestic



ruminants to a great extent, simultaneously increasing the manurial value of the dung for better yield from the land. Spraying of pasture land with quicklime at 1 per cent strength is also recommended to keep the larval burden in the pasture under control.

# *Summary*

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## SUMMARY

An investigation into the occurrence of infective larvae of common nematodes of domestic ruminants (cattle, buffaloes and goats) their specific identify, study on their bionomics and assessment of ovicidal and larvicidal property of certain fertilizers for their control were undertaken in the present study.

1. Occurrence or prevalence was investigated by coprological examination for eggs and coproculture for larvae. The prevalence was highest (77.50%) in goats out of all animals examined and it was highest (62%) for strongyles out of all species of nematodes encountered and that was also in goat. From copro-culture the incidence of Haemonchus contortus was noticed to be highest in all animals. It was lowest for Bunostomes in cattle and goats and for Trichostrongylus columbriformis in buffaloes. No seasonal variation was noticed.
2. The specific identity of the infective larvae of the strongyle and strongyloides species of cattle, buffalo and goat has been made by the study of detailed morphology and measurements of each infective larvae and also by comparing these details with the details of infective larvae

- described by previous workers. A key for identification of infective larvae was also furnished.
3. A total of seven species of infective larvae of common nematodes of cattle, viz., Haemonchus contortus, Oesophagostomum radiatum, Cooperia punctata, Trichostrongylus axei, T. colubriformis, Bunostomum phlebotomum and Strongyloides papillosus, four species of infective larvae of buffaloes, viz. H. contortus; O. radiatum, T. colubriformis and S. papillosus and seven species of infective larvae of goats viz., H. contortus, O. columbianum, O. asperum, T. colubriformis, T. axei, B. trigonocephalum and S. papillosus have been identified.
  4. The SEM study of infective larvae of Haemonchus contortus, Trichostrongylus axei, Cooperia punctata, Oesophagostomum radiatum and Strongyloides papillosus was carried out. Under SEM the tail end of infective larvae of S. papillosus was found to be tripartite which under light microscope was bipartite. Horn like structures were seen on the head end of infective larvae of C. punctata, T. axei O. radiatum.
  5. Bionomics of infective larvae such as phototropism and viability were studied and none of the larvae were found to show phototropism.

6. The viability of infective larvae of all the species of nematodes in all the domestic ruminants viz., cattles buffaloes and goat in all the seasons was assessed under room temperature in water. The longest viability was noticed in winter. The longest viable larvae were that of Oesophagostomum species closely followed by Haemonchus. The shortest viability was noticed for the nude Strongyloides papillosus larvae irrespective of the season. For the percentage of viability also the same trend as for the period of viability was noticed and the percentage of viability was highest for Oesophagostomum and lowest for strongyloides.
7. The ovicidal property of four fertilizers such as urea, ammonium sulphate, quick lime and ash at 1 per cent, 2.5 per cent and 5 per cent strengths was studied in faecal culture. In faecal culture, urea and ammonium sulphate showed 100 per cent ovicidal property even at the minimum strength of 1 per cent quick lime had 36.52, 58.60 and 78.02 per cent ovicidal property at 1, 2.5 and 5 per cent strengths respectively. Ash showed the lowest efficacy of 5.62, 21.38 and 52.40 per cent at the above strength respectively.
8. The larvicidal property of the above fertilizers was also assessed in larval suspension at the strengths of 0.1, 0.5 and 1 per cent. Urea, ammonium sulphate and ash did not

show any larvicidal effect even at the highest strength of 1 per cent, but quick lime was 100 per cent larvicidal at 1 per cent strength below which it was ineffective.

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**IDENTIFICATION, BIONOMICS AND CONTROL OF  
INFECTIVE LARVAE OF COMMON NEMATODES  
OF DOMESTIC RUMINANTS**

By

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**ABSTRACT OF A THESIS**

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## ABSTRACT

An investigation by coprological examination and copro-culture into the occurrence of common nematodes of cattle buffaloes and goats, specific identity and bionomics of their infective larvae and assessment of ovicidal and larvicidal properties of common fertilizers like urea, ammonium sulphate, quick lime and ash for their control were carried out.

The prevalence was highest in goats with strongyle having highest percentage and Haemonchus being the commonest nematode involved.

As per the specific identity Haemonchus contortus, Oesophagostomum radiatum, Cooperia punctata, Trichostrongylus axei, T. colubriformis, Bunostomum phlebotomum and Strongyloides papillosus, H. contortus, O. radiatum, T. colubriformis and S. papillosus and H. contortus, O. columbianum, O. asperum, T. colubriformis, T. axei, B. trigonocephalum and S. papillosus were the species of nematodes encountered in cattle, buffaloes and goats respectively.

Regarding bionomics none of the larvae showed phototripism and the highest and shortest viability was for Oesophagostomum species and Strongyloides species respectively.



Urea and ammonium sulphate (1%) were found to have the highest ovicidal property (100%) with ash having the lowest, when mixed with faeces. The larvicidal property was 100% for quicklime (1%) with no efficacy for other fertilizers, when treated with larval suspension.

Scanning electron microscopic study of some of the infective larvae was also carried out.