SOME EPIDEMIOLOGICAL ASPECTS OF GASTROINTESTINAL STRONGYLES (NEMATODES : STRONGYLOIDEA) OF SHEEP IN THE SUB- TROPICAL ZONE, OF PAKISTAN

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CERTIFICATE

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DEDICATED TO MY DEAREST
MOTHER AND LATE FATHER

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LIST OF ABBREVIATIONS

RPM	:	Revolution per minute.
EPG	:	Egg per gram.
%	:	Percentage.
NaCl	:	Sodium chloride.
Mu	:	Milli micron.
Ml	:	Milli litre.
Mg	:	Milli gram.
^{0}C	:	Centigrade.
MM	:	Millimeter.
>	:	More than.
<	:	Less than.
Gm	:	Gram.
HCl	:	Hydrochloric acid.
CC	:	Cubic centimeter.
$Kg^{-1}Dm$	:	Per kilogram of dry matter.
KI	:	Potassium Iodide.
PPR	:	Periparturient rise

ABSTRACT

An epidemiological study of gastrointestinal nematode parasites of 1000 sheep was carried out at different abattoirs of sub-tropical (Barani or rainfed) region of northern Punjab, Pakistan. The overall prevalence of nematodes infection was recorded to be 92 per cent. The following nematode parasites were recovered and their incidence is given in parentheses: *Haemonchus contortus* (83.6 per cent), *Trichostrongylus colubriformis* (72.7 per cent), *Oesophagostomum columbianum* (69.6 per cent), *Trichostrongylus axei* (69.2 per cent), *Ostertagia trifurcata* (42.6 per cent), *Trichuris ovis* (39.6 per cent), *Strongyloides papillosus* (32.4 per cent), *Trichuris globulosa* (10.2 per cent), *Nematodirus spathiger* (9.3 per cent), *Bunostomum trigonocephalum* (7.2 per cent) and *Oesophagostomum venulosum* (6.5 per cent). Peak mean values of pepsinogen level were recorded from March-April (700-800 mu tyrosine) and from July to October with peak 1380 mu tyrosine occurring in September. The highest overall egg per gram of faeces was observed from August to November (2700-6500 epg). Larval inhibition at early fourth-stage (L_4) of *Trichostrongylus axei*, *Ostertagia* spp. and *Haemonchus contortus* were also recorded in the abomasal mucosa during hot dry summer season (May-June). Sheep having less than one year of age were heavily infected as compared to 1-2 and > 3 years of age groups. The overall infection was highest in sub-humid climate, followed by semi-arid and arid climates. Heavy infection of *Haemonchus contortus* was noted during July to September. Similarly, pastures were found heavily contaminated with infective larval stages (L_3) in July to October. *Trichostrongylus axei* and *Trichostrongylus colubriformis* were also recovered throughout the year and their respective peaks of occurrence was recorded in April and September, respectively. Although *Oesophagostomum columbianum* was recovered throughout the year with minor fluctuations in different months. Similarly, in *Ostertagia* spp. the rate of recovery of this genus rose slowly to a peak in March while remained at low level upto June. *Nematodirus*

spathiger was recovered in 18 lambs only and no significant seasonal trends was noted. The faecal egg count was not found to be a reliable measure of the size of the trichostrongyle worms burden. Observations of egg out-put showed that periparurient rise occurred in 92 per cent ewes during the lambing season. Lactating suckled ewes (LSE) acquired high worms burden than that of non-suckled lactating ewes (NLSE) and barren ewes (BE). The development of *Trichostrongylus colubriformis* eggs into infective larvae was completed throughout the year under wide range of weather conditions. Low temperature has marked effect on the development of the free-living stages as no development occurred below > 10 ^{0}C. While the infective larvae of this specie were highly resistant to desiccation. As far as *Haemonchus contortus* was concerned from March to October the majority of the eggs developed into third-stage infective larvae, but their proportion was varied depending upon moisture content of the faeces. At 10^{0}C only 5 per cent eggs developed as compared to 25^{0}C. At 40^{0}C majority of the eggs died due to desiccation. The present study has provided some excellent understandings of the epidemiology of gastrointestinal trichostrongylosis of sheep in sub-tropical areas of Pakistan, and might help in designing control measures in future.

CHAPTER 1

GENERAL INTRODUCTION

Sheep are important to the national economy of Pakistan, and are mostly reared by farmers as a subsidiary occupation to augment their income through the sale of wool and surplus animals. According to the Agricultural Census Organization of Pakistan, the population of sheep in 1992 was 29 million (Durrani and Bhatti, 1992). The Punjab, province has by far the largest proportion of sheep (48 per cent). The overall population of sheep in Punjab, is 6.3 million heads.

Sheep provide annually over 225,000 tonnes of meat about 18.4 per cent of the total meat produced in the country and 53,162 tonnes of wool of which about 7000 tonnes of raw carpet wool worth Rs.100 million is exported. With the remaining wool a thriving carpet manufacturing industry has been established with an annual export of products worth Rs. 2000 million. This industry provides jobs for thousands of families in the remote regions of the country. Small ruminants are in fact the major source of livelihood for over a million farmers (GOP, 1984).

In Pakistan, there are twenty-eight breeds of sheep distributed in different geographical regions on the basis of their body size and other morphological characteristics (Hasnain, 1985). Pakistani breeds of sheep are slow maturing reaching slaughter weight at advanced ages, whereas in developed countries the required slaughter weight is attained at a very young age. In Punjab, the estimated death rate was 10 per cent for stock older than one year

and 16 per cent for stock under one year. One of the major causes of death i.e. 13-29 per cent is gastroenteritis (FAO, 1974). Presently there is strong pressure on the livestock sector to increase its output as demand for meat, milk and wool is rising rapidly.

The production of mutton, milk and wool has not kept pace with phenomenal expansion, which has characterized to other sector of agricultural and industrial economy of Pakistan. The lag in production can be attributed to seasonal fluctuations of both quantity and quality of the pastures, mainly as the result of climatic vagaries. The efficient conversion of pasture herbage by sheep into products of agriculture value is a matter of prime importance. One of the most important factors militating against efficient feed conversion is trichostrongyle infection .

The wide-spread prevalence of gastrointestinal trichostrongylosis in tropical and sub-tropical areas has plagued the production potential of many livestock development programmes by causing countless deaths and insidious economic losses (Al-Quaisy *et al.*, 1987). Sheep are rarely parasitized by only a single nematode species. Nematodosis in sheep is usually the result of a combined assaults of numerous genera occurring in varying numbers, each contributing to the disease syndrome. Moderate numbers of parasites adversely affect growth and productivity; heavier worm burdens cause marked clinical symptoms or even death of the host. Outbreak of such diseases in domestic livestock is limited by environment hostile to the development of the free-living stages of the nematode parasites. When environment becomes favourable in the

absence of the controlling influence of anthelmintic treatment, gastroenteritis will occur (Grant, 1981). It is well known that nematode parasites have profound effects on the rate of growth of lambs and on milk and wool production in adult sheep. Recent reviews are available (Barger, 1982; Steel and Symons, 1982; Sykes, 1982; Holmes, 1986; Sykes, 1994) on different aspects viz., reduction in appetite, loss of body weight, hypoproteinemia, impaired digestive efficiency and pathogenic effects. Though the reasons of these effects are obscure, they may be due to the possible secretion of toxins by the parasites which cause pathological condition. Removal of blood by blood sucking parasites cause severe anaemia as in case of acute haemonchosis and the production of enzymes inhibitory substance which reduce digestive efficiency and induce malnutrition (Reveron and Topps, 1970).

Keeping in view the severity of the losses in livestock sector, different workers in different agro-ecological zones of the world have conducted epidemiological studies for the assessment of parasitic populations by designing various experimental studies, techniques and methods. In attempts to establish the epidemiology, the seasonal prevalence of parasites has been studied by collection faecal samples at regular intervals and by doing differential worm counts (Crofton, 1957; Rossiter, 1964; Donald, 1968; Yazwinski and Featherstone, 1979; Vercruysse, 1983). Numerous studies have also been performed by monitoring the free-living stage of trichostrongyles on seasonal basis (Rose, 1963; Rose and Small, 1984; Chiejina *et al.*, 1989; Berbigier *et al.*, 1990; Besier and Dunsmore, 1993 a,b; Fernandez *et al.*, 1994). Several authors

have demonstrated that the studies of the epidemiology are best be carried out by slaughtering tracer lambs at regular intervals, counting and identifying the worms and post-mortem on a more critical basis (Barrow, 1964; Gray and Kennedy, 1981; Grant, 1981; Gupta *et al.*, 1987, Charles, 1989; Reinecke and Louw, 1989; Uriarte and Valderrabano, 1989). However, the epidemiology of gastrointestinal trichostrongyle infections has rarely been studied in tropical areas (Nari, 1984; Anderson, 1985).

Although the prevalence of gastrointestinal parasitism was known to occur in different parts of Pakistan (Sarwar, 1962; Siddiqi and Ashraf, 1980; Shah *et al.*, 1980; Durrani *et al.*, 1981; Mohiuddin *et al.*, 1984; Marwat *et al.*, 1988; Khan *et al.*, 1989) but these studies were performed either on morphology, incidence or worm populations originating from large areas not always identified exactly from desired area. Moreover, comprehensive study of their seasonal availability and relationship to disease outbreaks has not been reported. Seasonal prevalence of all the common gastrointestinal nematodes of sheep is not well-defined in any section of the country. Availability of research data from other countries is helpful in understanding problems of disease control but wide differences in animal husbandry practices and the broad range of climatic conditions in Pakistan, in comparison with western countries or other areas limits the value of this information. Therefore, it has been decided to conduct a comprehensive study in greater detail to depict the epidemiological scenario of gastrointestinal nematodes in sub-tropical (Barani region) area of northern Punjab.

The objectives of these studies are as follows:-

i. To identify gastrointestinal nematode parasites prevalent in the different areas of Barani region (sub-tropical) of northern Punjab.

ii. To assess the seasonality of different trichostrongyles in different seasons of a year from autopsied "tracer lambs" and the availability of infective larvae (L_3) on naturally contaminated permanent pastures.

iii. To work out the possible interaction between host-parasite relationship, particularly before and after parturition also called spring rise phenomenon.

iv. To quantify larval development and their survival in ovine faecal pellets deposited on pasture at different time of the year and to evaluate the effects of different temperature and humidity regimes on the free-living stages of two major trichostrongyles (*Haemonchus contortus* and *Trichostrongylus colubriformis*).

Such types of studies will provide the basic information for the design of control measures that aim to reduce pasture infectivity and to plan the future strategic anthelmintic dosing for trichostrongyle infections.

CHAPTER 2

REVIEW OF LITERATURE

Gastroenteritis is a serious problem caused by nematode parasites to our livestock sector. The effects of gastrointestinal nematodosis in sheep vary widely, at one extreme, animals may die and represent a total economic loss and at the other, the effects may be so slight that they cannot be detected. Sheep usually harbour mixed infections of gastrointestinal nematode parasites. The degree of damage inflicted by these infections is influenced to a large extent by the type and the numbers of nematodes present. The nematodes which occur only in the gastrointestinal tract are reviewed in this chapter.

2.1 General survey studies:

Boag and Thomas (1975) studied faecal egg counts and pasture larval contamination by *Nematodirus* spp. in sheep and it was found that *N. battus* takes short period in spring for hatching. While *N. filicollis* showed extended period of hatching beginning in autumn, steadily increased in winter and finally attained peak in the late winter.

Southcott *et al.* (1976) described the seasonal pasture contamination and the availability of nematodes for grazing sheep. They pointed out that *Haemonchus contortus* and *Trichostrongylus* spp., follow the similar development pattern in summer, while *Ostertagia* spp., in autumn resulted in peak contamination in winter.

Balbo *et al.* (1977) studied the guts of 87 sheep and 12 goats in the alpine region of Piemonta and Valla d' Aosta in Italy for gastrointestinal nematodes. They reported *Nematodirus helvetianus*, *Ostertagia lyrata*, *Skrjabinema ovis* and *Trichuris skrjabini* in sheep. While *Nematodirus helvetianus* and *Ostertagia ostertagi* were recovered for the first time in goats. *Ostertagia circumcincta* found to be more prevalent in both sheep and goats. Other nematodes recovered in sheep were *Nematodirus filicollis*, *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Bunostomum trigonocephalum*. Similarly in goats the nematodes recovered were *Trichostrongylus colubriformis*, *Ostertagia trifurcata*, *Haemonchus contortus*, *Bunostomum trigonocephalum*, *Nematodirus filicollis*, *Trichostrongylus axei* and *Trichostrongylus vitrinus*.

Eslami *et al.* (1979) examined 250 wild sheep (*Ovis orientalis*) from different national parks and protected regions of Iran. The predominant nematode species were *Marshallagia marshalli*, *Ostertagia* spp., *Nematodirus* spp., and *Skrjabinema ovis*.

Sahai and Sinha (1979) studied gravid *Haemonchus contortus* females collected from sheep and goats in India and separated them into two types on the basis of their valvular flaps. They observed that *Haemonchus bisipinosus* had a knob-like flap, while it was linguiform in the case of *Haemonchus contortus*. Eggs from each type were cultured separately and passed each into a separate kids. They were found to breed true and it was concluded that these are two different species.

Hubert *et al.* (1979) conducted epidemiological study in the Limousin area in France and found that the parasitic level was high during July-August. Among the nematodes encountered were *Ostertagia circumcincta* and *Haemonchus contortus*. The blood pepsinogen level measurements was useful in establishing the level of infection in the infected sheep flock.

Shah *et al.* (1980) examined 375 guts of sheep and recovered the following species of nematodes *Haemonchus contortus*, *Oesophagostomum venulosum*, *Oesophagostomum columbianum*, *Ostertagia circumcincta*, *Skrjabinema ovis*, *Bunostomum trigonocephalum*, *Trichostrongylus axei*, *Trichostrongylus vitrinus* and *Chabertia ovina*. The overall incidence of infections was found to be 75 per cent.

Grant (1981) conducted a survey for gastrointestinal nematodes of sheep for a period of one year. *Haemonchus contortus* and *Oesophagostomum columbianum* were found to be of major importance. The incidence of *Haemonchus contortus* rose to a peak and remained at a high level throughout the winter. The incidence of *Oesophagostomum columbianum* remained at a relatively high level from March until October. The other genera recovered were *Trichostrongylus* spp., *Cooperia* spp., *Strongyloides papillosus* and *Trichuris ovis*.

Soota and Deysarkar (1981) conducted a survey from December, 1976 to January, 1977 in Himachel Pradesh, (India). The new species recorded was *Rhabdochona bariliusi* from the intestine of sheep. While *Trichuris globulosa*, *Bunostomum trigonocephalum*, *Gaigeria pachyscelis* and *Oesophagostomum columbianum* were the other

parasites found in the intestines of goats.

Mckenna (1981) worked out the relationship between the strongyle egg counts and the total strongyle worms burden. He described the total pathogenic index of the worm burden in sheep of different age groups and found that there was a definable relationship between the total worms burden and the worm egg counts.

Horak (1981) determined the seasonality of helminths infection in sheep, cattle, impala and blesbok in South Africa. The following nematodes were considered to be of major importance: *Haemonchus* spp. and *Trichostrongylus* spp., in all the above mentioned hosts, while *Ostertagia* spp., were the most prevalent nematode in sheep.

Ansari and Singh (1981) reported the incidence of *Gaigeria pachyscelis* in sheep (18.8 per cent) and goats (9.4 per cent) and found that average worm burden per host was 15 and 13, respectively. The incidence was lower in the pre-monsoon months, moderate in the monsoon period and highest in the post-monsoon and winter months.

Beveridge and Ford (1982) reported the species of trichostrongyle nematodes present in 376 sheep from different agriculture regions of the South Australia. The most common nematodes encountered in the study were *Haemonchus contortus, Trichostrongylus colubriformis, Nematodirus filicollis, Nematodirus spathiger* and *Nematodirus abnormalis*. *Cooperia* spp., were uncommon and its three species recovered were *Cooperia oncophora, Cooperia surnabada* and *Cooperia pectinata*.

Dhar *et al.* (1982) examined 62 gastrointestinal tracts of

sheep at Handwarah, (Kashmir). Sheep (85 per cent) were found to be infected with *Haemonchus contortus, Trichostrongylus axei, Chabertia ovina, Bunostomum trigonocephalum, Nematodirus spathiger* and *Trichuris ovis*.

Tarazona *et al.* (1982) recorded four different species of genus *Trichostrongylus* from various parts of the gastrointestinal tracts of sheep and goats. *Trichostrongylus axei, Trichostrongylus capricola, Trichostrongylus vitrinus* and *Trichostrongylus colubriformis*. *Trichostrongylus capricola* was predominantly found in goats as compared to sheep.

Roberts and Swan (1981) described the quantitative studies of ovine haemonchosis level and the distribution of egg counts of *Haemonchus contortus* within extensively managed flock of Merino sheep in South-east Queensland.

Specht (1982) studied the seasonal fluctuations in faecal worm egg counts over a year in sheep and goats grazed extensively near Maputo, Mozambique. Larval differentiation from faecal culture showed that *Haemonchus contortus* and *Oesophagostomum columbianum* were the most prevalent species. *Trichostrongylus colubriformis, Cooperia spp., Strongyloides papillosus* and *Trichuris* spp. were the minor occurred nematodes . After the onset of heavy rain in October, the mean total egg counts increased due to a higher egg-output of *Haemonchus contortus*, reaching peak value in January. With the beginning of the drier season the mean egg counts fell to a low level.

Coadwell and Ward (1982) studied the Clun forest sheep and gave a single dose of 20,000 infective larvae of *Haemonchus*

contortus. The total number of eggs/day in the faeces was recorded after 21 day of post infection period. Data on the population size, sex ratio and individual worms were also collected from 76 sheep. The relation between increase in worm size and uteri egg content was linear. The number of eggs present in the uteri was found to be an accurate measure of egg passed. It was shown that the daily egg output is related to total parasite weight and is not a measure of the number of individuals present.

Cabaret (1983) examined the large intestines of 105 adult non-pregnant ewes for the period of three years at the abattoirs of Moulay-Bouazza (Morocco). The encountered nematodes species were, *Oesophagostomum venulosum*, *Chabertia ovina*, *Trichuris ovis* and *Trichuris globulosa*.

Vercruysse (1983) conducted a survey study in domestic sheep and goats of the Sahelian zone,(Senegal). During this survey *Haemonchus contortus* and *Oesophagostomum columbianum* were the most important nematodes encountered in sheep and goats.

Mohiuddin *et al.* (1984) examined a total of 345 gastrointestinal tracts, 306 goats and 89 sheep and recorded *Haemonchus contortus*, *Oesophagostomum venulosum*, *Gaigeria pachyscelis*, *Strongyloides papillosus* and *Skrjabinema ovis*. The incidence and seasonal variation was studied by monthly examination of the host. *Skrjabinema ovis* was recorded for the first time in Sind province (Pakistan). The incidence of infection was generally higher in sheep than goats.

Darmona (1984) reported the incidence of haemonchosis among the sheep slaughtered at Bogor (Indonesia) during March, 1981 to

February, 1982. *Haemonchus contortus* was found in the abomasum of 85 per cent of the 142 slaughtered sheep, ranging 29-43 nematodes per head. The 2:2 ratio of male to female nematodes was observed.

Jackson and Christie (1984) reported that increase in 10 fold infective larvae of *Ostertagia circumcincta* by lambs resulted with almost no increase in faecal egg counts. They also described the relationship between larval intake and faecal egg out put and also described the relationship between larval intake and faecal eggs out put.

Gruner and Cabarat (1985) described the different parameters to assess the parasitic worms population viz., egg per gram and serum pepsinogen level. They concluded that these parameters are still of great importance for the assessment of parasitic worm population in any ruminant.

Vercruysse (1985) examined 1024 abomasa of sheep and 75 per cent were found to be infected with *Haemonchus contortus*.

Ahmed and Ansari (1987) examined the gastrointestinal tracts of 479 goats, and 392 sheep and recovered the following nematodes species: *Haemonchus contortus, Oesophagostomum columbianum, Bunostomum trigonocephalum* and *Trichuris ovis*. The prevalence of *Haemonchus contortus* infection was found to be highest from July to November. This was followed by *Oesophagostomum columbianum* and its incidence remained moderate throughout the year. Other trichostrongyles showed no seasonal fluctuation. The goats were found to be heavily infected with *Trichuris ovis* while sheep with *Bunostomum trigonocephalum*.

Ikeme *et al.* (1987) studied the seasonal changes in the

prevalence of *Haemonchus contortus* and *Trichostrongylus* spp. hypobiotic larvae in tracer goats in Malaysia. Tracer goats were grazed with naturally infected adults goats for one month and necropsied for worm count. No hypobiotic larvae of *Trichostrongylus* spp., were recovered, while those of *Haemonchus contortus* were recovered only in small proportion in each month of the year.

Asanji and Williams (1987a) carried out an investigation for gastrointestinal parasites of sheep and goats and pointed out that the nematode number fluctuated with seasonal, age and sex of hosts. Older animals harboured less nematode species than young animals. All the nematode species showed a dry season rise from August to January, the highest and lowest relative densities being recorded in October and July respectively. The nematodes recovered were: *Haemonchus contortus*, *Oesophagostomum columbianum* and *Oesophagostomum venulosum*. Female hosts harboured significantly higher worm load than males.

Asanji and Williams (1987b) conducted a survey for a period of two years from 1973 to 1975, in which they examined 34,110 sheep and goats. They recovered and identified 24 helminths species. No single host carried all the recovered species of helminths and each host harboured one to six species of helminth parasites. The combined mean annual prevalence of infection for goats and sheep was 70.0 per cent, 64.6 per cent and 5.9 per cent for *Haemonchus contortus*, *Oesophagostomum columbianum* and *Oesophagostomum venulosum*, respectively.

Gupta *et al.* (1987) discussed the epidemiology of some gastrointestinal nematodes of sheep and goats in Karnal, Ambala and

Rohtak districts of Haryana (India). The results revealed that *Haemonchus contortus* and *Trichostrongylus* spp. were responsible for parasitic gastroenteritis in these hosts. The adult parasites persisted in the host throughout the year and there was no indication of hypobiosis.

Guimaraes and Walter (1987) described the results of 83 goats of undefined breed coming from different regions of the state of Minas Gerais, (Brazil). Post-mortem examination revealed the following parasites: *Haemonchus contortus* (82. 01 per cent), *Trichostrongylus colubriformis* (80 per cent), *Trichostrongylus axei* (39 per cent), *Trichostrongylus longispicularis* (2 per cent), *Strongyloides papillosus* (36 per cent), *Cooperia curticei* (30 per cent), *Cooperia punctata* (6 per cent), *Cooperia pectinata* (6 per cent). *Bunostomum trigonocephalum* (7.0 per cent), *Oesophagostomum columbianum*, (42 per cent), *Oesophagostomum radiatum* (2 per cent), *Oesophagostomum asperum* (5 per cent) and *Trichuris ovis* (22 per cent).

Njau (1987) conducted a survey study on faecal egg counts of tracer lambs from July 1976 to August 1977 and showed that the animals passed out small and large number of trichostrongyles eggs during dry season (September-February) and wet seasons (March-August), respectively. The following nematode species were recovered at necropsy: *Haemonchus contortus*, *Oesophagostomum columbianum*, *Trichostrongylus colubriformis* and *Trichuris ovis*.

Khan *et al.* (1988) studied 68 flocks of sheep in Kalat, Pishin, Loralai and Zhob districts of Baluchistan, (Pakistan). Two most common sheep breeds Baluchi and Harnai were included in this

survey study. The incidence of nematode infections recorded in different districts was Kalat (94 per cent), Zhob (80 per cent), Lorali (71 per cent) and Pishin (70 per cent). The gastrointestinal parasites recovered were *Nematodirus* spp. (54 per cent), *Marshallagia marshalli* (25 per cent), *Dictyocaulus filaria* (21 per cent), *Strongyloides papillosus* (13 per cent), *Trichostrongylus* spp. (13 per cent), *Oesophagostomum* spp., (12 per cent) and *Haemonchus contortus* (11.75 per cent).

Taylor and Hunt (1988) proposed the worm control strategies on a commercial farm in South-East England. They monitored the contamination rate during the grazing season and recorded three peaks of larval infection in June, late August and late October in grazing ewes and lambs.

Gupta *et al.* (1988) used tracer lambs to find out the pasture contamination with infective stages of helminth parasites. Post-mortem examination of gastrointestinal tract indicated low infections of *Haemonchus contortus* occurred throughout the year except in June. *Trichostrongylus columbriformis* infection was detected throughout the year and about 150 worms per lamb were recorded during January to May and in August. Low infection with *Oesophagostomum columbianum* was recorded in this study.

Pullman *et al.* (1988) investigated trichostrongyloid nematode infections of weaner sheep at the Tarretified (South Australia) over a three-year period (1982-1985). Acquisition of nematode larvae from pasture occurred during the winter months. On the other hand, the faecal egg counts were elevated during summer, but declined to negligible level during winter months.

Khan *et al.* (1989) examined 500 gastrointestinal tracts of sheep and goats for gastrointestinal parasites. The prevalence of helminth was 58.4 per cent and 54.0 per cent in sheep and goats, respectively. The prevalence of nematode infections in sheep was 41.4 per cent and in goats 21.6 per cent. The nematodes recovered were *Haemonchus contortus, Oesophagostomum venulosum, Bunostomum trigonocephalum, Chabertia ovina, Cooperia curticei, Trichuris ovis, Ostertagia circumcincta* and *Osteragia ostertagi*.

Charles (1989) conducted a post-mortem examination of gastrointestinal tract of goats from April, 1979 to March, 1982. He noted that each goat was parasitized by more than one species of nematodes and recovered *Haemonchus contortus, Strongyloides papillosus* and *Oesophagostomum columbianum*. The total worms burden present in animal was highest during late rainy and early dry season and lowest in mid-rainy season. The acquisition of nematodes by tracer goats occurred mainly from mid-rainy to early dry season.

Reinecke and Louw (1989) recovered the total differential worm counts in slaughtered sheep during one year period of May 1987-1988, at Boontjieskraal Estate (South Africa). They found that winter born lambs were infected with *Nematodirus spathiger* at 5 to 7 weeks of age. At weaning, this specie was superseded by *Teladorsagia circumcincta* and *Trichostrongylus axei*. Small number of *Trichuris skrjabini* and *Oesophagostomum venulosum* were also reported. Infective larvae aestivate in the faeces or in the soil of the lucerne pastures in the dry, hot summer month and migrated on to the herbage during the cool wet autumn.

Uriarte and Valderrabano (1989) described the epidemiology of

parasitic gastroenteritis under an intensive grazing system on irrigated land in northeast of Spain. Two types of parasite generations were identified in the study. One of them derived from the eggs deposited in the previous March and April and were found respsonsible for the first parasitism in the lambs. *Ostertagia* spp., *Nematodirus* spp., and *Trichostrongylus* spp., were found in this population. The second generation, which appeared during May, was derived from the eggs of the previous generation and gave rise to an outbreak of parasitism in the lambs at the beginning of May and middle of June. *Haemonchus* spp.and *Chabertia ovina* were present in this population.

Van Aken *et al.* (1990) investigated the pattern of nematode infection on a goat farm in north-western Sri Lanka. The nematode species present were *Haemonchus contortus* and *Oesophagostomum columbianum*. In adult animals, the faecal egg output was not influenced by season and no significant periparturient rise and hypobiosis was observed.

Pandey *et al.* (1990) studied the prevalence of helminths in Morocco and encountered the following nematode genera, *Haemonchus*, *Trichostongylus*, *Nematodirus*, *Cooperia*, and *Oesophagostomum*. The faecal egg count of ewes showed two peaks; the first in March due to acquisition of larvae during the rainy season and exhibited periparturient rise and the second in October probably due to maturation of inhibited larvae.

Louw and Reinecke (1991) found that nematode parasite burden of ewes grazing on grass pasture, increased 58-fold after the first autumn in the southern cape province (South Africa). Lambs were

infected before the age of 8 weeks and harboured large burden of nematode parasites before the age of 14 weeks.

Jacquiet *et al.* (1992) studied the pattern of changes in faecal egg during and after the rainy season at three different sites in South-west Mauritania. *Haemonchus contortus* was the most prevalent worm followed by *Strongyloides papillosus*. Sheep were found heavily infected than goats.

Garcia Romero *et al.* (1993) investigated the parasitic fauna in Oropesa, (Spain) and recovered the following species of nematodes in order of their prevalence: *Ostertagia circumcincta, O. trifurcata, O. ostertagi, Marshallagia marshalli, Trichostrongylus axei, T. capricola,T. vitrinus, T. colubriformis, Haemonchus contortus, Nematodirus battus, N.helvetinus* and *N. filicollis*. Tracer lambs showed two peaks of infection, one in January-April and the second in October-December.

Pandey *et al.* (1994) investigated the pattern of nematodes infection in goats from the Highveld of Zimbabwe. The four dominant species: *Haemonchus contortus, Trichostrongylus axei, Trichostrogylus colubriformis* and *Oesophagostomum columbianum* were present in 88-97 per cent of the animals. Three other nematodes *Strongyloides papillosus, Bunostomum trigonocephalum* and *Trichuris* spp. occurred respectively in 9, 3 and 21 per cents of the goats. The total worm burden was least at the end of dry season and increased gradually through the rainy season to reach a peak at the end of the rainy season.

Stafford *et al.* (1994) monitored the rate of contamination of worm egg count by ewes from 1990-1991. The overall mean faecal egg

counts data was lowest during January (82 epg) and rose to a peak during October (539 epg). He suggested that the role of ewes as a source of pasture contamination should be considered when developing parasite control regimes on sheep farms.

Nishikawa *et al.* (1995) conducted survey for nematode infections in Awassi sheep from 13 provinces of Syria. Faecal egg counts and larval nematode outputs were higher in rainy areas as compared to dry areas.

Jacquiet *et al.* (1995) made observations from 647 faecal egg counts and 53 necropsies from sheep and goats originating from three sites of a Sahelian region of Mauritania over a period of two years. *Haemonchus contortus* and *Oesophagostomum columbianum* were the most prevalent species. The seasonal pattern was noted by long survival of adults and high percentages of arrested fourth-stage larvae in the dry season.

Ndao *et al.* (1995) carried out epidemiological survey on gastrointestinal helminthiasis in 51 sheep and 55 goats in the tree-cropping pasture region in Senegal. The most important parasite in sheep was *Haemonchus contortus* while *Trichostrongylus colubriformis* predominated in goats. The worm burden in sheep was significantly higher that in goats ($P< 0.001$). Larvae of *Haemonchus contortus* were found in 85-87 per cent of the small ruminants. There was a negative correlation between hematocrit, number of worms and egg per gram of the faeces during the rainy season. They concluded that nematode burden was high during the dry season nutritional problems are aggravated by adults worms and residual larvae.

Dorny *et al.* (1995) studied the pattern of infection in West Malaysia. *Haemonchus contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. were the most prevalent species in goats. Faecal egg counts showed that the larvae were available throughout the year and were not influenced by the small climatic variations. Mean faecal egg counts decrease from the age of 8 months onwards. Moreover, periparturient rise in trichostrongyle counts was also noted.

2.2 Hypobiosis:

Larvae may become arrested in development within the host as a manifestation of acquired immunity or may also arrest in development as a result of prior experience of certain adverse environmental conditions. They resume their development and attain sexual maturity when external environmental conditions become favourable.

Michel (1963) originally believed that host resistance was of major importance in the induction of larval inhibition.

Andersen *et al.* (1965b) described that hypobiosis phenomenon could be due to hormonal changes occurred within the host.

Soulsby (1965) suggested that the exact aetiology of inhibition in larval nematodes was obscure but there had been considerable speculation on its mechanism. Initially, hypobiosis was thought to be associated with the acquisition of immunity

Armour *et al.* (1969 a,b) suggested that the inherent developmental changes in the infective larval stages, either genetically or environmentally induced, were responsible for the onset of inhibition.

Stockdale *et al.* (1970) investigated that the ageing of the infective larvae might be responsible for larval inhibition inside the host.

Blitz and Gibbs (1972a) studied the possibility of induce inhibitions of larval development by conditioning the infective larvae under different conditions of storage before their inoculation in the susceptible animals and suggested the importance of environmental conditions influencing the metabolism of the free-living stages.

McKenna (1973) revealed that marked inhibition of *Haemonchus contortus* at an early fourth larval stage occurred during the winter season in New Zealand. However, he also indicated less marked inhibition in *Ostertagia* spp. while there was no evidence of inhibition in *Trichostrongylus axei*.

McKenna (1974a,b) studied the persistence and fate of inhibition-prone larvae in tracer lambs and found that although some inhibited larvae of this parasite are capable of persisting for a considerable period in sheep and eventually resuming their development. Most of them were lost within 10 weeks of their establishment without attaining sexual maturity.

Michel *et al.* (1974) investigated that the hypobiosis in northern temperate zones of the world is due to be chilling or falling temperature.

Michel *et al.* (1974, 1975) suggested that host resistance may play only a minor role in bringing about inhibition, while environmental or climatic factors may be more important

Waller and Thomas (1975) studied the inhibition of *Haemonchus contortus* under field condition of north-east England. They found that the percentage of inhibition increased to 57 per cent in July, 75 per cent in August and virtually 100 per cent in September and concluded that neither autumn climatic effects nor host immunity were responsible for inhibition in this strain of *Haemonchus contortus*.

Ogunsusi and Eysker (1979) suggested that in dry or arid zone of the world, inhibition might occurred due to desiccation and high atmospheric temperature that acts as a stimulus for inducting inhibition

Barger and Le Jambre (1979) concluded that inhibited *Haemonchus contortus* larvae are capable of producing sufficient eggs when they resume their development and initiate an outbreak of haemonchosis in susceptible sheep.

Michel *et al.* (1979) demonstrated that host factors, such as age and previous experience of infection play a significant role in causing arrested development. They suggested the possibility of interaction between environmental and host factor, in the phenomenon of larval inhibition.

Altaif and Issa (1983) observed that the proportion of inhibited larvae of *Ostertagia* spp. was markedly high during the dry summer months. It appears that seasonal inhibition of *Ostertagia* spp. in Iraq, was brought about by an environmental stimulus acting upon pre-parasitic larval stages.

Giangaspero *et al.* (1992) studied the inhibition in trichostrongylids in Awassi sheep in north-west Syria. The

percentage of inhibition was lowest in January (5 per cent), it increased during spring upto 76.7 per cent in April and 84.6 per cent in June. Their percentage of inhibition was decreased during autumn season. *Teladorsagia circumcincta* was the main specie undergoing inhibition as compared to *Marshallagia marshalli*.

El-Azazy (1995) investigated that *Haemonchus contortus* and *Marshallagia marshalli* were the most important parasites that undergo inhibition during hot dry month in Saudi Arabia. However the inhibition was less pronounced in *Haemonchus contortus* as compared to *M. marshalli*.

2.3 Spring rise or peri-parturient or post-parturient rise:

Morgan *et al*. (1951) found that the nematode faecal egg counts of ewes are higher if they are subject to excessive stress such as extremes of weather and poor nutrition.

Crofton (1958) demonstrated that increased eggs per gram also occurred in lactating ewes from autumn-lambing flocks. He suggested that the increase was associated with parturition and lactation rather than season.

Armour (1967) investigated that the levels of cortisone are known to increase during periods of stress and the experimental administration of cortisone to sheep, and cattle with nematode infections results in an elevated nematode faecal egg count. .

O'Sullivan and Donald (1970) found that if lambs were removed from ewes with 12 hours of birth then ewes did not develop resistance and thus showed increased susceptibility to nematodes

Brunsdon and Vlassoff (1971) studied the relative generic composition of post-parturient strongyle egg counts for lactating

and non-lactating ewes. Mean egg counts were similar until after the conclusion of lambing, when the egg counts for the non-lactating ewes declined rapidly to a negligible level. While egg counts for the lactating ewes rose to a normal post-parturient peak. In lactating ewes, *Haemonchus contortus* and *Ostertagia* spp. were the major contributors to the egg output but only negligible numbers of eggs of these genera were passed by non-lactating ewes.

Wedderburn (1970) suggested that in many parts of the world, parturition of grazing animals is synchronized to occur with the climate favourable to pasture growth and also suitable for development and survival of free-living stages of most helminths.

Connan (1972) demonstrated that the host factors were responsible for immunological impairment around parturition and thus resulted in periparturient eggs rise.

Jansen (1973a) viewed that spring rise phenomena can start already before and during lambing time. He also monitored the numbers of eggs of *Haemonchus contortus* and *Ostertagia* spp., in one to two years old ewes.

Jansen (1973b) attempted to suppress the spring rise in sheep using thiabendazole in the day after parturition and during the spring rise period. It was found that in spite of a suppression of the egg output after a treatment or by repeated treatments with thiabendazole, the course of the weight, the production of lambs and the wool production of the ewes were not influenced.

Jansen (1973c) investigated the relationship between the spring rise and the lactation in sheep. He speculated that the combination of the immune and the endocrinal state of the host is

considered to be responsible for the appearance of the spring rise.

Kelly and Dineen (1973) demonstrated an association, either direct or indirect with circulating levels of the lactogenic hormone prolactin.

Yazwinski and Featherstone (1979) noted that maturation of hypobiotic larval forms were responsible for post-parturient rise. *Haemonchus contortus*, *Trichostrongylus* and *Ostertagia* genera were the major eggs contributor during the spring rise phenomenon.

Courtney *et al.* (1984) studied the comparison of the periparturient rise in faecal egg counts of exotic and domestic ewes and have noticed that three exotic breeds (Florida Native, Barbados Blackbelly and St.Croix) showed no periparturient rise in faecal egg counts. While domestic breed ewes (Rambouillet and Finn-Dorset) showed a pronounced periparturient rise after 6-7 weeks of post-lambing period.

Gibbs and Barger (1986) monitored the level of faecal egg counts in pregnant and dry ewes. Peak egg counts were seen in pregnant ewes just before lambing. Moreover, lactating ewes acquired greater burdens of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* than did dry ewes. They suggested that the impairment of immunity to helminth infections was responsible for this rise.

Courtney *et al.* (1986) found that significant ($P < 0.05$) differences in the magnitude of the periparturient rise in faecal egg counts occurred in ewes of three strains of Florida Native sheep. Maxcy and Backlinie strain ewes showed a pronounced periparturient rise, none occurred in non-lambing ewes and the

slight rise in faecal egg counts was observed in university ewes was not significantly different from that of the non-lambing ewes. No difference occurred in faecal egg counts of non-lambing ewes regardless of strain. Moreover, six weeks after weaning faecal egg counts of all ewes were uniformly low regardless of strain or prior lactation status.

Lyons *et al.* (1987) investigated the egg per gram and number of helminths in ewes that increased progressively during the parturition period and subsequently responsible for building of infectious stages on pastures. These infectious stages have become the major source of nematodes acquired by the lambs after weaning.

Jansen (1987) monitored the level of trichostronglid and trichostrongylid egg output in normal and late lambing ewes. He concluded that ewes normally lambed, showed egg per gram rise was related to parturition. Similar trend was observed in late lambing ewes and in this case host factor was also responsible for egg counts rise.

Reinecke and Louw (1988) suggested the association of lactation with an increased susceptibility to nematode infection resulted in rise in faecal egg counts.

Lyons *et al.* (1992) investigated that in ewes large increase in egg per gram counts began after parturition. The numbers of helminths in lambs increased progressively two months after weaning.

Rehman and Collins (1992) studied faecal egg counts and serum prolactin concentration in pregnant and non-pregnant Angora goats over a period of 20 weeks. The mean weekly egg counts of pregnant

goats were significantly higher ($P < 0.01$) than those of non-pregnant goats. There was a positive linear regression between prolactin levels and faecal egg counts.

Fleming (1993) successfully propagated a strain of *Haemonchus contortus* that exhibited periparturient egg rise in sheep after 10 generations. He further noted that ewes inoculated with the periparturient rise strain had significantly higher faecal egg counts. Similar results were noted when same strain was inoculated in lambs.

Fleming (1993) observed that increases in endogenous circulating prolactin during late pregnancy and lactation in ewes might contribute to periparturient egg rise irrespective of the developmental stage of the parasite when the hormone exposure occurred.

2.4 Development of free-living stages:

Silangwa and Todd (1964) have suggested that, because of microclimate differences, nematode larvae of ruminants and equids tend to remain near the base of the herbage.

Anderson *et al.* (1965b) have shown over a 2 years period in West Scotland, the population of *Ostertagia ostertagi* and *Cooperia oncophora* worms in calves showed marked increase in the month August, September and October.

Anderson and Levine (1968) have reported that dry sheep faecal pellets containing *Trichostrongylus columbriformis* egg which have been exposed to maximum daily field temperatures at ground level as high as 61 ^{0}C, yield small numbers of infective larvae.

Salisbury and Arundel (1970) showed that ewes contamination

was of substantially greater importance for *Ostertagia* spp., infections in lambs than residual larvae on pasture but for *Trichostrongylus* spp., the effects of the two sources were equivalent and for *Nematodirus* spp., residual larval populations were more important.

Boag and Thomas (1971) and Thomas and Boag (1972) have suggested that there are probably not more two parasite generations in lambs during the grazing season in Britain.

Waller and Donald (1972) reported that in the presence of high evaporation rates, embryonated eggs of *Trichostrongylus colubriformis* were capable of surviving high temperature, while on the otherhand, it was lethal to other stages of development.

Vlassoff (1973) fairly suggested a regular annual pattern of trichostrongyles with a peak of larvae in spring and a larger peak in the autumn. More common nematode larvae occurred in both spring and autumn peak. Many of the nematodes genera overwintering on the pasture each year.

Thomas (1974) has pointed out that bioclimatographs from laboratory determinations of the parasite's temperature-humidity constraints may be unreliable because the microclimate experienced by larvae in the soil-herbage zone is difficult to measure.

Pandey (1976) conducted study under controlled laboratory conditions rather than in the field to monitor larval movement in relation to air temperature and relative humidity.

Anderson (1983) suggested that under hot and dry season *Ostertagia* spp. and *Trichostrongylus* spp., larvae were difficult to

develop, but their availability enhanced in the presence of rain.

Callinan and Westcott (1986) argued that climate especially temperature and humidity, profoundly influenced the movement of nematode larvae on herbage.

Chiejina *et al.* (1988) reported that faecal reservoirs of L^3 were the most important means of carry over of infection from the end of one wet season to the beginning of another incubated under optimum conditions of temperature and moisture.

Chiejina *et al.* (1989) reported that gastrointestinal larvae could develop and survive on open pasture during the dry season in the Nigerian derived savanna zone.

Krecek *et al.* (1990) showed that larval movement of third - stage *Ostertagia ostertagi* on herbage was regulated primarily by temperature.

2.5 General climatic factors:

Silverman and Campbell (1959) demonstrated that the time taken for development of the free-stage was regulated by temperature.

Silverman and Patterson (1960) found that the development of parasite stages depended on the age and immune status of the host.

Reinecke (1960) suggested that many other factors would affect development and survival within faeces, e.g., consistency, disintegration, and husbandry operation such as harrowing.

Waller and Donald (1970) considered that under New South Wales conditions any eggs deposited at a dry time would not develop as there was too little moisture in sheep faecal pellets to prevent desiccation of *Haemonchus contortus* eggs.

Waller and Donald (1970) showed that *Haemonchus contortus* eggs

differed structurally from *Trichostrongylus colubriformis* which were more resistant to desiccation.

Le Jambre and Whitlock (1973) and Mckenna (1974) investigated that low temperatures caused prolonged development of the free-living stages and higher temperatures shortened their development but it was likely that various geographically distributed phenotypes or strains might have varying responses to temperature changes .

Armour (1980) studied that several environmental factors which affect the microhabitats and microclimate in which the free-living nematodes exists were responsible for fluctuations in the process of translation.

Berbigier *et al.* (1990) studied that the development of free-living of nematodes and it was found that presence of adequate moisture in the soil was main factor influenced their development.

SURVEY STUDY

CHAPTER 3

3.1 INTRODUCTION

Indigenous sheep are wide-spread in Pakistan, and play an important role in the culture and economies of particularly the indigent farming communities. These sheep are seldom subject to anthelmintic treatments and therefore parasitic gastroenteritis is a common health hazard among them (Pal and Qayyum, 1996).

The prevalence of the gastrointestinal nematodes of sheep is dependent on a number of factors, but the burden of all worms species depend on the intake of infective larvae (L_3). The availability of infective larvae (L_3) is influenced by various factors viz., climate, season (Ollerenshaw *et al.*, 1978), pasture management and grazing rotations (Armour, 1980; Morley and Donald, 1980) and general weather conditions (Thomas and Boag, 1972,73; Starr, 1981; Thomas and Starr, 1978). Therefore, an increase in stocking rate and mixed grazing sheep and cattle results in less herbage availability and thus concentration of larval availability on the herbage may rise (Southcott, 1971).

Parasite populations may also be modulated by the host characteristics such as breed, age and nutritional condition which have considerable influence on the parasites and their capacity to invade and inflict damage on the host (Rivera *et al.*, 1983). Although the intensity of transmission of nematode depends on the grazing behavior of the sheep viz., quality and quantity of herbage ingested and also on their susceptibility (Gruner and Cabaret, 1985). The concept of areas at risk has broad outline in terms of

their management practices, botanical composition, attractiveness of various pasture zones and grazing behaviour of sheep.

It is thought that due to transactions and subsequent movements of animals from region to region, it is impossible to trace their exact geographical origin. However, necropsies of sheep from abattoir can be of interest, particularly in developing countries (Pandey *et al.*, 1980). Epidemiological investigations of nematode parasitism in small ruminants have never been conducted over the wide array of climatic and geographical regions of Pakistan.

In Pakistan, previous studies were based on the results obtained from survey of one slaughter house originating from large areas. This obviously depicts incomplete nature of correct records of parasites and therefore, further prompted a more detailed study of the nematode parasites prevalent in sub-tropical zone (Barani region) of northern Punjab. Furthermore, sub-tropical zone of northern Punjab, comprises a large region that has not yet been exploited to its full potential as far as epidemiology of gastrointestinal trichostrongyles is concerned.

In that line, the present comprehensive study has been designed to obtain better knowledge of parasitic fauna from the micro-region in order to assess the most important parasitic infections.

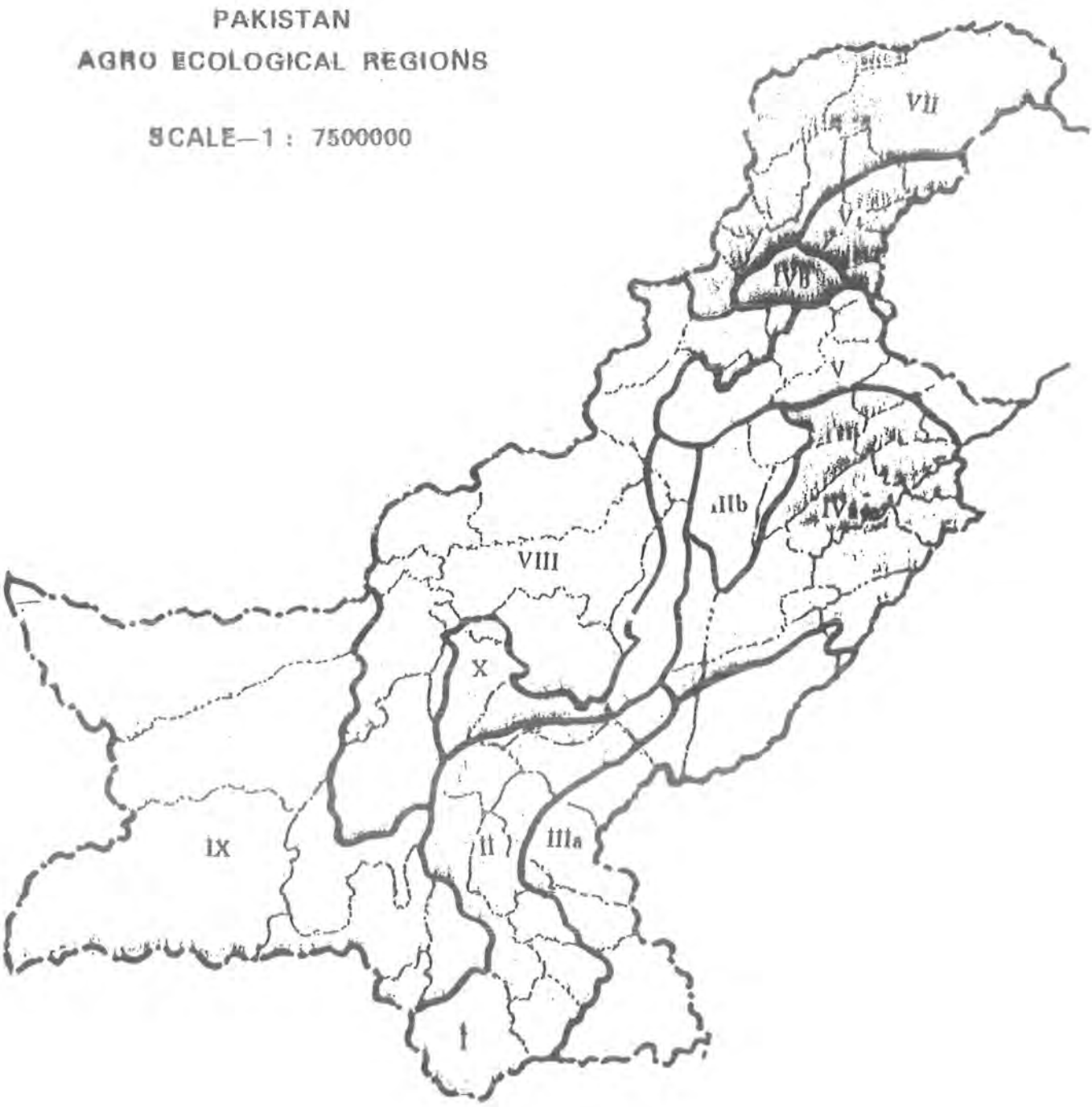


Figure 1. Different agro-ecological regions of Pakistan.

Figure 2. Different districts located in sub-tropical (Barani or rainfed) region of northern Punjab, Pakistan.

3.2 MATERIALS AND METHODS

3.3 STUDY AREAS:

Pakistan, is divided into ten different agro-ecological zones on the basis of climate, rainfall, temperature and potential land use (Fig.1). One of its zones, selected for present study is commonly known as " Barani or rainfed area" (Fig.2). This zone comprises the districts of Attock, Jhelum, Rawalpindi, Gujrat, Chakwal and Mainwali (Fig.1). The zone is spread over the salt range, Potohar plateau is generally open and undulatingly developed mainly on sand stones. The land form in the salt range is eroded with well-developed scarp and slopes intervening between hill range and narrow valleys that are filled with silty and loamy material.

Climatically, the Barani zone is sub-tropical and further divided into three sub-zones. The first one is arid, comprising the district of Mainwali. It receives an annual rainfall of 150-300 mm. While the second is semi-arid (300-500 mm), covering the districts Attock, Gujrat, Chakwal and Jhelum. The last one is sub-humid laying along the foot of the Margala hills and cover the district of Rawalpindi, this sub-zone receives a total annual rainfall of 500-1000 mm.

Out of the total rainfall in these areas, about 70 per cent is received only during monsoon (July-September). The remaining rainfall is received mostly during February to March. This rainfed tract also experiences great extremes of temperature. The temperature is highest in June before the onset of monsoon season. The daily maximum temperature exceeds 40°C and seldom it

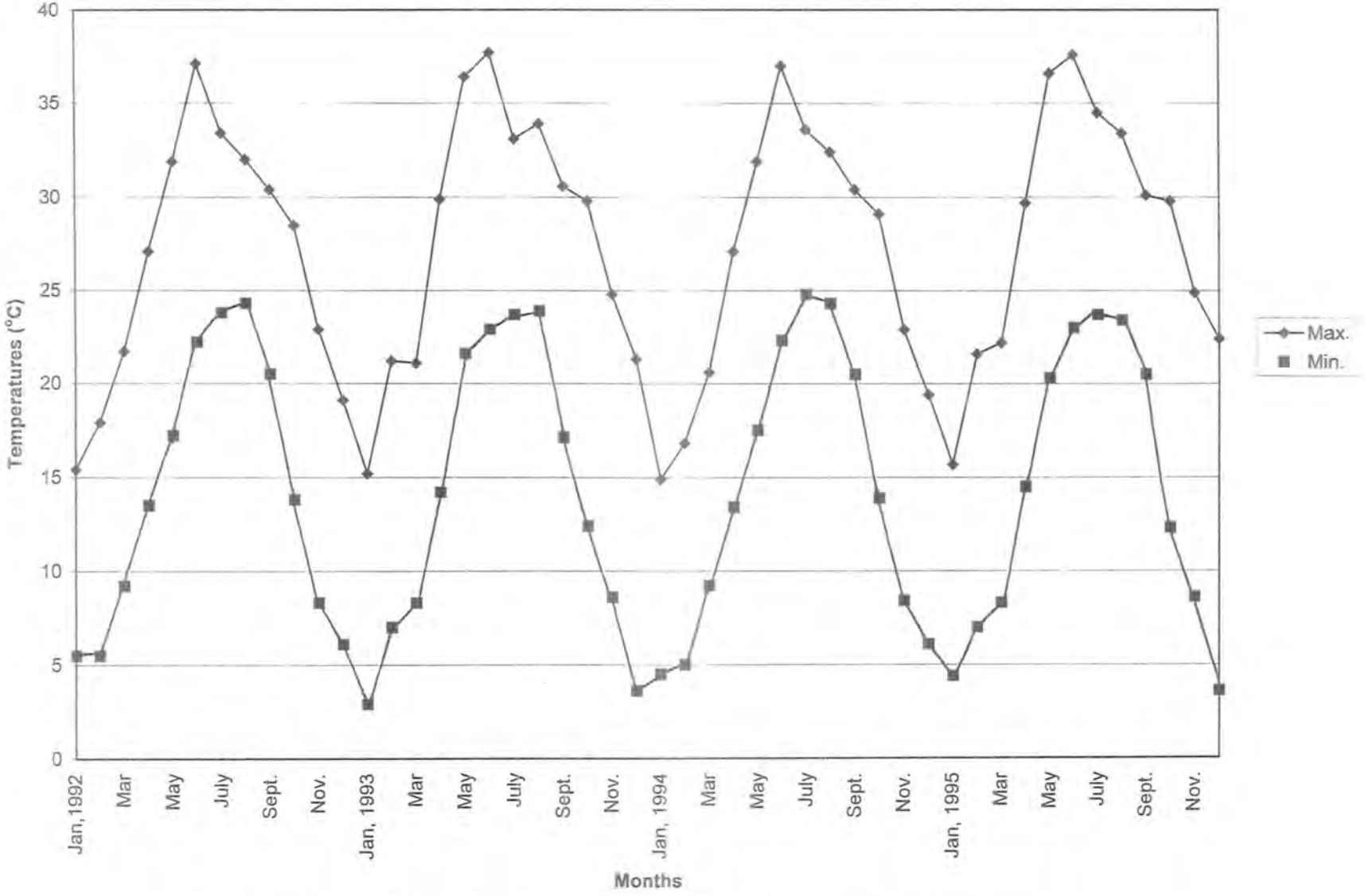


Figure 3. Mean monthly minimum and maximum temperatures (°C) recorded during 1992-95.

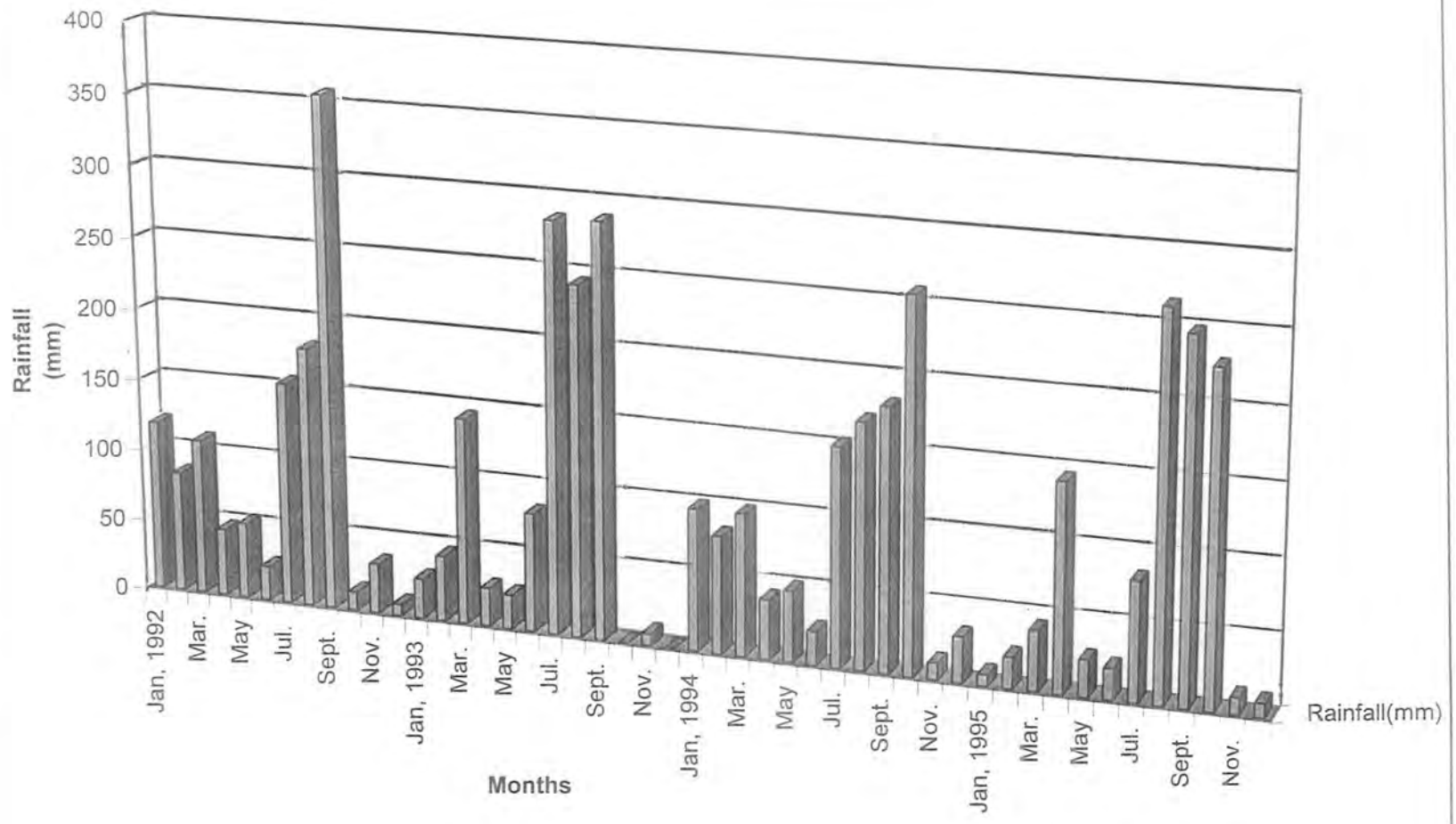


Figure 4. Mean monthly rainfall (mm) recorded during 1992-95.

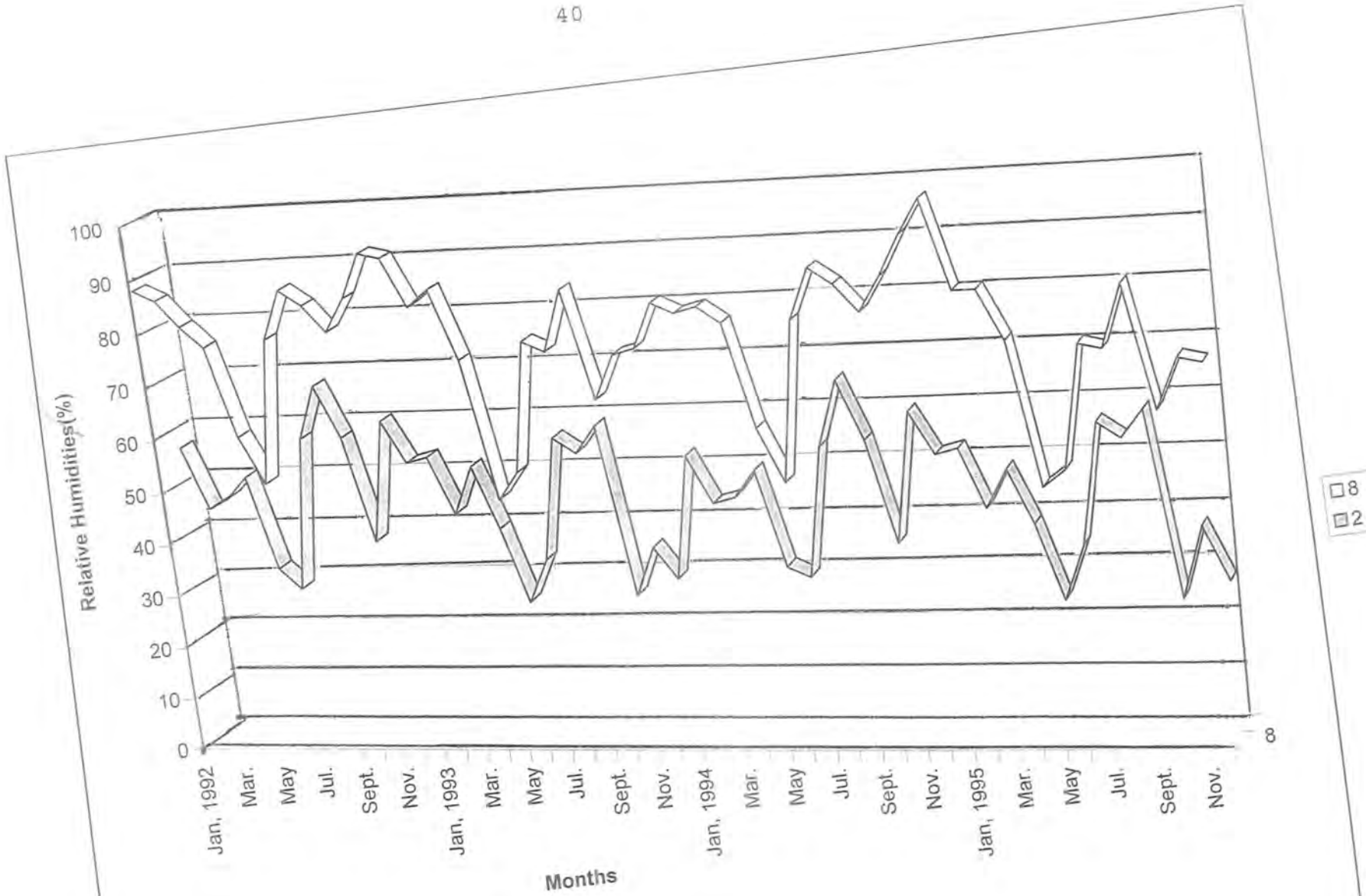


Figure 5. Mean monthly relative humidities recorded during 1992-95.

goes below 24°C. Relative humidity is lowest during May-June and rises during the monsoon season. One year cycle is divided into three seasons viz. Winter (November-February), Summer (March-June) and Rainy (July-October). The native vegetation comprises the following species: *Heteropogon contortus, Chrysopon anontanus, Cymebopogon schenanthus, Artistida depressa* and *Sorghum halepense*.

3.3.1 *Animal management:*

In this sub-tropical (Barani region) sheep farmers lead a settled life in villages. They and their flocks live in mud or brick or thatched and mud-plastered houses in winter. In the field during summer, thornbush enclosures provide protection against wild animals for flock. The flocks are taken out in morning for grazing on canal banks, roadsides, crop stubbles, fallow and common lands and brought back to the holding by sunset. Lambing season are autumn and spring. Lambs are allowed to accompany their dams to pasture as soon as they able to walk. Culling is commonly practiced in male lambs when money is needed or before the start of the breeding season.

3.3.2 *Weather data:*

The weather data for Barani areas was obtained through the courtesy of the Director, Water Resources Research Institute (WRRI), NARC, Islamabad. The figures 3,4 and 5 summarizes the overall mean meteorological data recorded during 1992-1995.

3.4 COLLECTION OF MATERIALS:

This survey covers the period from 1st December, 1992 to 1st November, 1993 during which a total of 1000 complete gastrointestinal tracts of sheep were obtained once a week from the

local abattoirs of districts (Attock, Rawalpindi, Mainwali, Gujrat, Chakwal and Jhelum) of Barani (sub-tropical) areas. The number of gastrointestinal tracts collected at each locality depended on their availability. At necropsy, each gastrointestinal tracts was removed and transported to the laboratory for immediate examination. The animals included in this study were brought from suburbs of these districts. Random samplings were made irrespect of age and sex of the host(s). The age of the sheep was determined by presence of incisors in the lower jaw, as given by Khan (1969). Their ages were categorized into less than 1 year, 1-2 years and 3-4 years. While breed of the sheep were identified according to descriptions and figures described by Hasnain (1985). The indigenous breed of sheep included in this study was "Latti" commonly known as Salt Range. The data of each gastrointestinal tract was noted on a prescribed questionnaire (Annexure I).

3.5 PARASITOLOGICAL PROTOCOLS:

3.5.1 *Nematodes parasitizing the abomasum, small and large intestines:*

Sampling of nematodes from the abomasum, small and large intestines was carried out within four hours after the slaughter of animals. Abomasum, small and large intestines were ligated at omasal-abomasal, abomasal-duodenal and ileo-caecal junctions to prevent worms spilling from one location to another. From abomasal, small and large intestines samples of worms were collected and counted according to the technique described by Charles and Baker (1988). In laboratory, each part of gastrointestinal tracts was opened separately and the mucosa was washed in water to remove all

the parasites. Abomasal and small intestinal contents were washed through wire mesh of 71 µm aperture. While those of the large intestine were washed through wire mesh size of 500 µm aperture. The material retained on the wire mesh was preserved in 10 per cent formalin and were stored for later examination. The abomasal and small intestinal mucosa were soaked separately in saline at 37 ^{0}C for 1 hour. After soaking, the saline solution was set aside and the abomasal and small intestines mucosa were washed separately with water which then was passed through a wire mesh of 71 µm aperture. The retained material was fixed in 10 per cent formalin. Total worm counts in aliquot of abomasal and small intestinal contents were estimated after washing on a 71 µm aperture sieve. The material retained on the sieve was collected and was brought to volume of 1 liter with constant stirring. Two 50 ml aliquots were taken and all nematodes in each aliquots were counted and identified. When more than 100 worm were counted, additional aliquot were taken until entire 1 liter was examined. Worms in the material obtained from the soaking of the abomasal and small intestine mucosa were counted in the same manner as those in the abomasal and small intestinal contents. Contents of the large intestine were emptied into a bucket. The entire contents were washed in small amount on a wire screen with the aperture 500 um. After all the contents were washed, the material that remained on the screen was fixed in 10 per cent formalin. The entire contents were examined and worms were counted under Stereomicroscope (Wild, Heerbrugg, Germany).

3.5.2 *Enzymatic digestion of the abomasal mucosa for the recovery of immature larval stages:*

Worms inhabiting the lumen of the abomasum were isolated by the method described above. The conventional (Sedimentation) procedure is not suitable for demonstrating immature (larvae) parasitizing in the wall of the abomasum. First they have to be released from the tissue by enzymatic digestion method. The method described in a British methodological publication (MAFF, 1979) was chosen for the purpose. After careful removal of their contents by washing, the abomasa were weighed to determine the volume of digestion solution required. Subsequently, the entire abomasa were digested in a digestive solution pre-heated at 37 ^{0}C. The composition of the digestive solution was as follows:

940 ml distilled water, 8 gm pepsin, 20 ml of concentrated HCl and 23 ml saturated NaCl solution. One liter of digestive solution was sufficient for the digestion of 500 gms of abomasum. Each abomasum was digested at 37 ^{0}C for 8 hours by shaking at a speed of 20-40 rpm in an incubator. Undigested tissues were removed and the solution containing the digested mucosa was filtered through two sieves. The pore size of the upper and lower wire mesh was 120 µm and 71 µm, respectively. The upper sieve retained undigested tissues, fat and some time few mature worms, while on lower sieve immature worms inhabiting the abomasal wall and colloidal fat were left over. The material retained by the lower sieve was washed with physiological saline, made volume up to 100 ml and preserved in 10 per cent formalin. One tenth of the decanted and digested samples were examined. From the aliquot the total

worms and larval counts were estimated.

3.5.3 *Pepsinogen analysis:*

Out of 1000 sheep 642 sheep were randomly selected for pepsinogen analysis. Blood was withdrawn from the jugular vein into evacuated 10 ml plain glass tubes (Venoject, Terumo) and allowed to clot. Serum was separated and stored at - 20 °C for pepsinogen test. Pepsinogen analysis was determined by the method of Hirschowitz (1955) as modified by Koroto'ko and Islyamova (1963). The principle of the test is that the sample of serum (1.5 ml) is acidified to pH 2.0, thus activating the inactive pepsinogen. This activated pepsin is then allowed to react with a protein substrate (Bovine serum albumin) and the enzyme concentration in international units (μ mole tyrosine released per 100 ml serum per minute). The tyrosine liberated from the protein substrate by the pepsin is estimated by the blue colour which is formed when phenolic compounds react with Folin-Ciocalteu's reagent.

3.5.4 *Faecal examination:*

To determine the prevalence and intensity of infection, faecal samples were collected from the same autopsied animals. The faeces collected directly from the rectum were preserved in 10 per cent formalin, stored at 4°C and examined within 72 hours. The modified McMaster technique with saturated Sodium Chloride was used as described by Hatch and Larkin (1988). A correction factor was used according to the consistency of faeces. X 1 for normal pellets, X 1.5 for soft formed faeces, X 2 for soft faeces and X 3 in case of diarrhoea (Skerman and Hillard, 1966).

3.5.5 *McMaster technique:*

1. 3 gms of faeces was weighed out and put in the bottle and 42 ml of distilled water was added.
2. The faeces were homogenized with help of homogenizer (Kallenkapen).
3. The mixture was poured through a wire mesh screen with an aperture of 0.15 mm to strain solution caught in a bowl. The debris left on the screen was discarded.
4. The strained fluid was stirred and its sample was poured into a centrifuge tube within 1 cm of the top. The tube was centrifuged for 2 minutes at 1500 rpm and the supernatant was poured off and discarded.
5. The tube was agitated until the sediment was loosened and formed a homogeneous sludge at the bottom of the tube. The tube was filled with saturated salt solution to the same level as before.
6. The contents of the tube were thoroughly mixed by shaking it five to six times with the thumb over the end, sufficient fluid was immediately with-drawn with a pasture pipette and carefully allowed to run into one chamber of the counting slide. After further mixing, a second and third sample was withdrawn and run into the other chambers.
7. Bringing the lines on the counting chamber into sharp focus under the low power of microscope (Nikon) using subdued light, the number eggs in each chamber were counted and multiplied each sum by 50 to give the results in eggs per gram. Main interest was centered on trichostrongyle eggs, but other worm eggs were also differentiated and counted.

3.5.6 *Parasitological data:*

The mean intensity (total number of worms recovered/ number of infected sheep) and the prevalence of infection (number of infected hosts/number of surveyed sheep) were calculated. Additionally, for each genus the intensity i.e. the number of worms per infected sheep, and the frequency (number of worms of one particular genus/ total number of worms recovered) were recorded.

3.6 PRESERVATION:

The nematodes were recovered and washed in physiological saline (0.89 gm/100ml distilled water) and were fixed in 70 per cent alcohol for 24 hours.

3.6.1 *Preparation of whole mount:*

After treatment with 70 per cent alcohol, the nematodes were transferred to a vial containing a hot mixture of 70 per cent ethyl alcohol (Merck) and glycerol (Merck) 50 parts each. The worms were kept in this vial partly covered until all ethyl alcohol was evaporated and worms left in pure glycerol. The worms were cleared in lactophenol and then placed on microscope slide and mounted in pure glycerol. Excess of glycerol was removed with the help of filter paper and the edges of the cover slip were sealed with slide sealer.

3.6.2 *Identification of the nematodes:*

Majority of the trichostrongyles were identified by the use of low power microscope (Nikon) and identified according to the keys and morphological characteristics discussed by Yamaguti (1961), MAFF (1979) and Soulsby (1982).

3.6.3 *Preparation of solutions:*

Lactophenol was prepared according to procedure described by Morgan and Hawkins (1960).

Phenol	1 Part
Glycerol (Chemically pure)	2 Parts
Distilled water	1 Part
Lactic acid	1 part

Saturated salt solution was prepared according to procedure described by Hatch and Larkin (1988) i.e. dissolving 360 gm of Sodium Chloride (NaCl) in 1 liter of distilled water.

3.7 RESULTS

In this investigation, eleven different species of nematodes from eight different genera were recovered from a total of one thousand (1000) necropsies. Nine hundred and two gastrointestinal tracts were found positive (92 per cent) for mixed parasitic infection throughout the areas surveyed for the year 1992-1993. Majority of the sheep (76 per cent) examined, harboured more than one species of nematode parasites, having minimum two and maximum seven species of nematode parasites in each host. Figures 6,7,8 and 9 give the different combinations of parasites recovered during the present study indicating their relative abundance in sheep of Barani areas. Their overall prevalences, range, host sites and the mean worm burdens are summarized in the Table 1. The following species of nematode parasites were identified:

Haemonchus contortus (Rudolphi, 1803), *Oesophagostomum columbianum* (Curtice, 1890) *Oesophagostomum venulosum* (Curtice, 1890), *Ostertagia trifurcata* (Stiles, 1892), *Strongyloides papillosus* (Stadelman, 1894), *Trichostrongylus axei* (Cabbold, 1879), *Trichostrongylus colubriformis* (Giles, 1892), *Trichuris ovis* (Abildgaard, 1795), *Trichuris globulosa* (Linstow, 1901), *Bunostomum trigonocephalum* (Rudolphi, 1808), and *Nematodirus spathiger* (Raillet,1896).

The overall prevalence of nematode infections was recorded to be 92.0 per cent. It was observed that *Haemonchus contortus*, *Trichostongylus* spp., and *Oesophagostomum columbianum* were the major nematodes, while *Trichuris* spp., *Ostertagia* spp., *Oesophagostomum venulosum* and *Bunostomum trigonocephalum* were the

minor nematodes encountered during the present investigation. *Haemonchus contortus* was by far the predominant parasite recovered during the whole study period. The level of infection was noted to be 83.6 per cent. *Trichostrongylus colubriformis* was second in predominance, its incidence was noted to be 72.7 per cent. This was followed by *Trichostrongylus axei* (69.2 per cent), *Oesophagostomum columbianum* (69.6 per cent), *Trichuris* spp. (49.8 per cent), *Ostertagia trifurcata* (42.6 per cent), *Strongyloides papillosus* (32.4 per cent), *Nematodirus spathiger* (9.3 per cent), *Bunostomum trigonocephalum* (7.2 per cent), and *Oesophagostomum venulosum* (6.5 per cent). Two species of *Trichuris* were recorded during this study period. Among these two species *Trichuris ovis* (39.6 per cent) was predominant followed by *Trichuris globulosa* (10.2 per cent).

3.7.1 ***Pepsinogen level:***

Mean pepsinogen values didn't reach clinically high level from December to February and were then substantially higher during March-April (700-840 mu Tyrosine). Even though mean values were below the clinically significant level through May-June. Peak mean values were observed from July to October with highest value (1380 mu Tyrosine) occurring in September (Fig.10).

3.7.2 ***Faecal egg counts:***

The seasonal pattern of the egg output are shown in Table 2. The highest overall egg per gram (EPG) was recorded from August to November (2700-6500). It has been observed that the overall mean of per gram (EPG) rose steadily from July (750) to a peak in November (6500). The overall egg counts was lowest in May-June (100-300).

3.7.3 *Fourth stage larval worm counts:*

The intensity of immature stages (L_4) in sheep are illustrated in Table 3. The proportion of the worm population occurring as inhibited fourth-stage larvae was highest in arid sub-zone followed by semi-arid and sub-humid sub-zones. In majority of necropsies, arrested (L_4) larval stages of *Trichostrongylus axei* and *Ostertagia* spp., were recorded under the mucosa during the dry summer season (May-June), which then disappeared in August onwards. Similarly low level of inhibition of *Haemonchus contortus* also occurred in dry season (May -June). After which no incidence of arrested larvae (L_4) was noted during the remaining period of investigation (Fig.11).

3.7.4 *Age-wise distribution of worms burden:*

Sheep were categorized into three age groups, their percentage of infection with different nematode parasites is presented in Figure 12. It was found that sheep having less than one year of age were heavily infected as compared to sheep having 1-2 years and more than 3 year of age groups. It was further observed that ewes harboured higher worm burdens as compared to males. As far as different species were concerned males harboured a variety of nematode parasites than that of ewes.

3.7.5 *Area wise distribution of parasites:*

The overall infection rate was highest in sub-humid climate, followed by semi-arid and arid sub-zones. The incidence of parasitic infection was found to be highest in Rawalpindi district, where 94.66 per cent sheep were infected with different nematode parasites (sub-humid). This was followed by Gujrat (semi-arid),

where 87.54 per cent sheep were positive for nematode parasites. Other levels of infections were in the following order: Jhelum, 82.56 per cent; Attock, 86.76 per cent; Chakwal, 79.33 per cent and Mainwali (Arid), 70.78 per cent.

Haemonchus contortus was the most prevalent trichostrongyle in all districts surveyed except in Chakwal, where lowest infection was recorded (Fig.13a). Infection with *Oesophagostomum columbianum* was also very high in the sheep of districts Gujrat, Jhelum and Rawalpindi. But moderate infection was noted in district Attock, while the sheep belong to district Mainwali showed the lowest incidence (Fig.13b).

Trichostrongylus axei was the most predominant trichostrongyle of Rawalpindi district, while lowest infection of about 4-2 per cent was observed in Mainwali district. Its incidence remained to be at moderate level in the rest of the districts surveyed where its incidence varied from 15.9 to 46.2 per cent (Fig.13c). As far as *Trichostronglus colubriformis* is concerned 86.2 per cent sheep of Rawalpindi were found positive (Fig.13d). This was followed by districts of Gujrat and Jhelum, where prevalent rate was noted to be 61.2 per cent to 47.6 per cent while lowest level of infection of this parasite was found in Mainwali district (10.4 per cent).

In case of *Ostertagia* spp., although the percentage was lower among rest of the nematode species recorded in present investigation. Higher incidence rate was noted in district Rawalpindi (40.7 per cent), while on the other hand 21.5 per cent sheep of district Mainwali was found positive (Fig.13e).

Trichuris spp. were present with moderate rate in all the

district survey but sheep of districts Gujrat and Jhelum showed 49.2 per cent and 42.0 per cent infection, respectively (Fig.13f). Similarly in case of *Strongyloides papillosus* again the incidence of infection was about 47.0 and 42.9 per cent in districts Gujrat and Jhelum, respectively (Fig.13g).

Nematodirus spathiger was found in four districts out of six sites surveyed in the present investigation, but its prevalence rate of about 20 per cent was observed in Rawalpindi (Fig.13h). *Oesophagostomum venulosum* was recovered from the sheep of district Rawalpindi, Jhelum, and Gujrat (Fig.13i).

Table 1. Prevalence of gastrointestinal nematodes found in 1000 sheep of Barani areas surveyed during, 1992-93.

				worms burden	
Organs	Parasites	No. Infected	Per. Infect. (%)	Range	Mean
Abomasum	*Haemonchus contortus*	836	83.6	25-501	351
	Trichostrongylus axei	692	69.2	48-723	489
	Ostertagia trifurcata	42.6	42.6	19-241	105
Small Intestine	*Trichostrongylus columbriformis*	727	72.7	184-892	512
	Strongyloides papillosus	324	32.4	'4-22	10
	Bunostomum trigonocephalum	72	7.2	'2-19	7
	Nematodirus spathiger	93	9.3	5-49	23
Large Intestine	*Oesophagostomum columbianum*	696	69.6	17-290	98
	Oesophagostomum venulosum	65	6.5	'2-33	13
	Trichuris ovis	396	39.6	26-69	31
	Trichuris globulosa	102	10.2	4-11	6

Table 2. Monthly level of eggs per gram (EPG) of gastrointestinal nematodes of sheep surveyed during, 1992-93.

		Sheep					
Months		*Strongylids*			*Strongyloides $*		
	No. Examined	No. Posit. (%)	EPG Mean[a]	EPG Range[b]	No. Posit. (%)	EPG Mean[a]	EPG Range[b]
Dec.1992	73	68	510	350-850	8	250	100-300
Jan.1993	80	78	300	175-550	7	100	50-200
Feb.	81	76	250	150-450	15	50	25-150
Mar.	72	65	425	200-650	23	100	75-250
Apr.	70	66	450	200-600	4	200	100-300
May	72	63	250	150-300	0	0	0
Jun.	75	61	200	100-250	31	50	25-150
Jul.	83	77	400	350-750	49	100	75-150
Aug.	80	85	2050	1750-2701	58	250	250-400
Sep.	79	75	2550	2000-3500	36	200	200-350
Oct.	80	73	3500	2500-4800	45	300	250-550
Nov.	83	72	4500	3100-6500	21	250	200-450

$: Embryonated eggs.

Table 3. The incidence of immature larval stages in the abomasum of sheep.

Moisture/Status/Climate	Annual rain fall (mm)	Districts/towns	No. examined	Percentage of inhibition (%)
Very low/arid/very hot	150-300	Mianwali	115	56(48.69)
Low/semi-arid/hot	300-500	Attock, Chakwal	291	123(31.27)
Medium/sub-humid/moderate	500-1000	Rawalpindi	206	42(7.76)

Figure 11. Monthly profile of abomasal larval inhibition recorded during 1992-93.

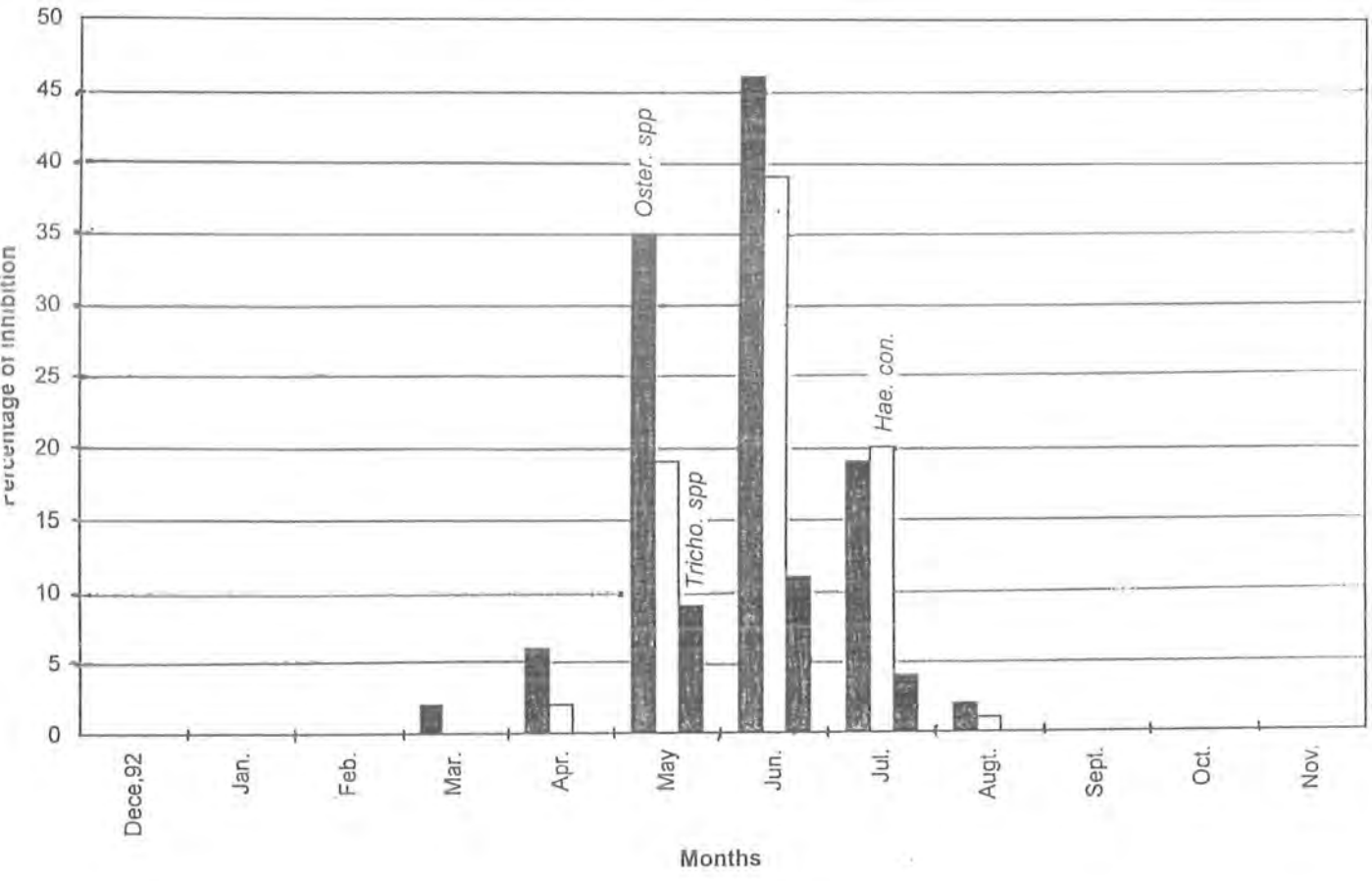


Figure 6. Showed different combinations of two parasites.

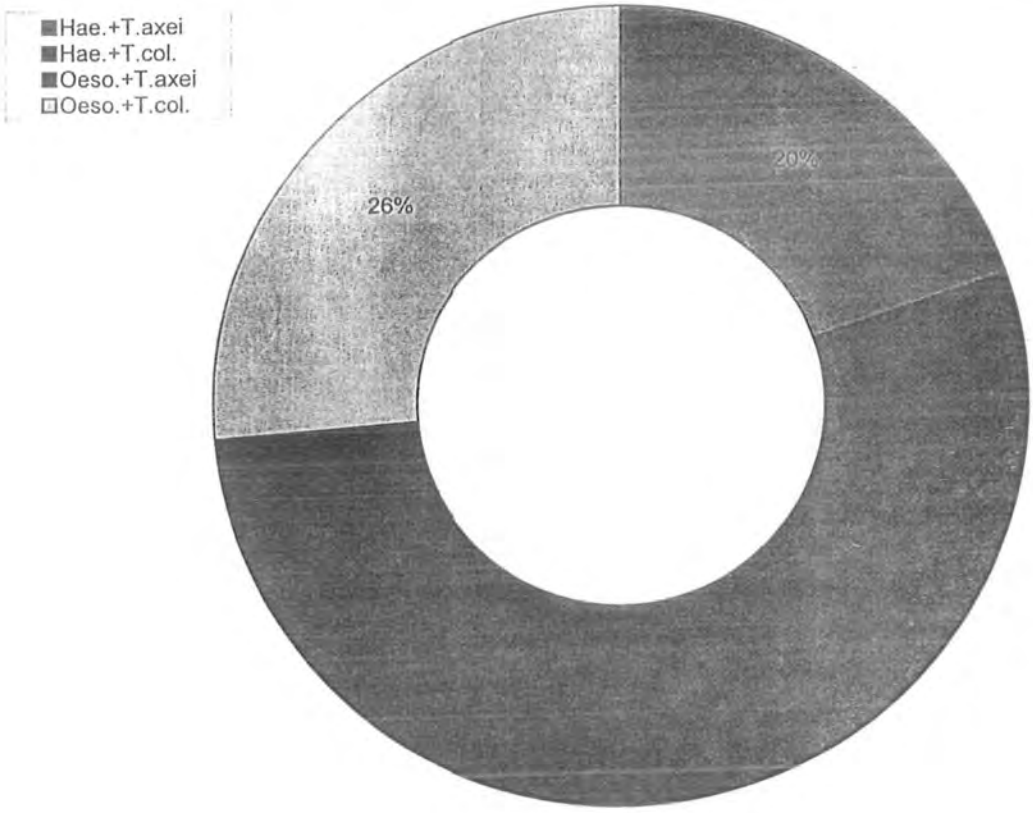


Figure 7. Showed different combinations of three parasites.

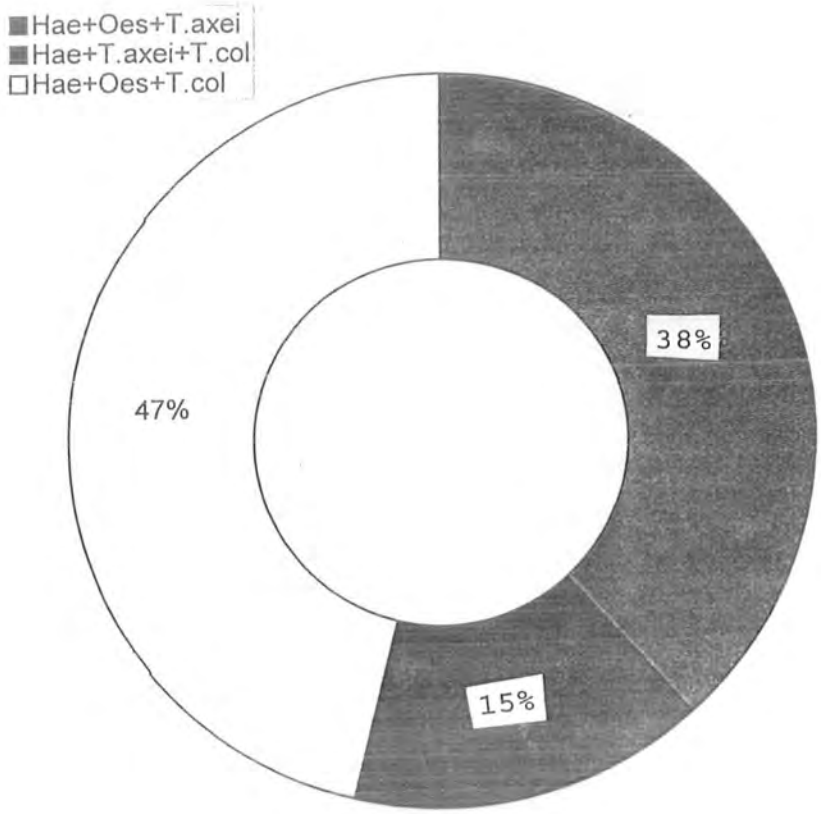


Figure 8. Showed different combinations of four parasites.

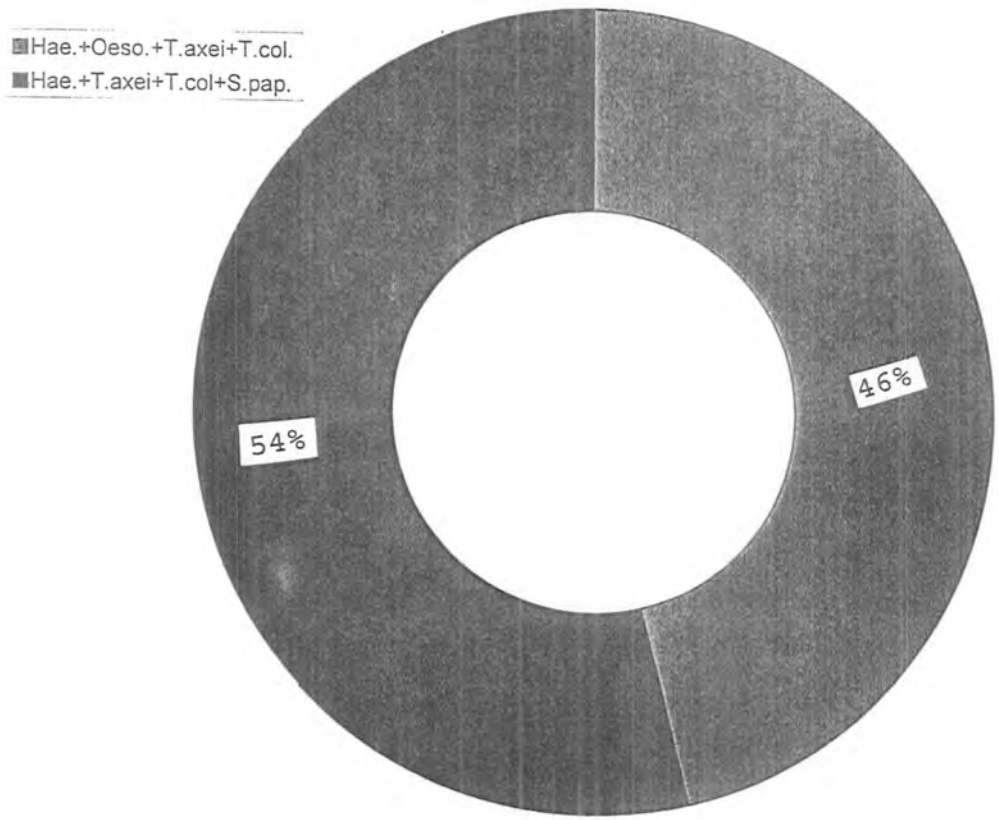
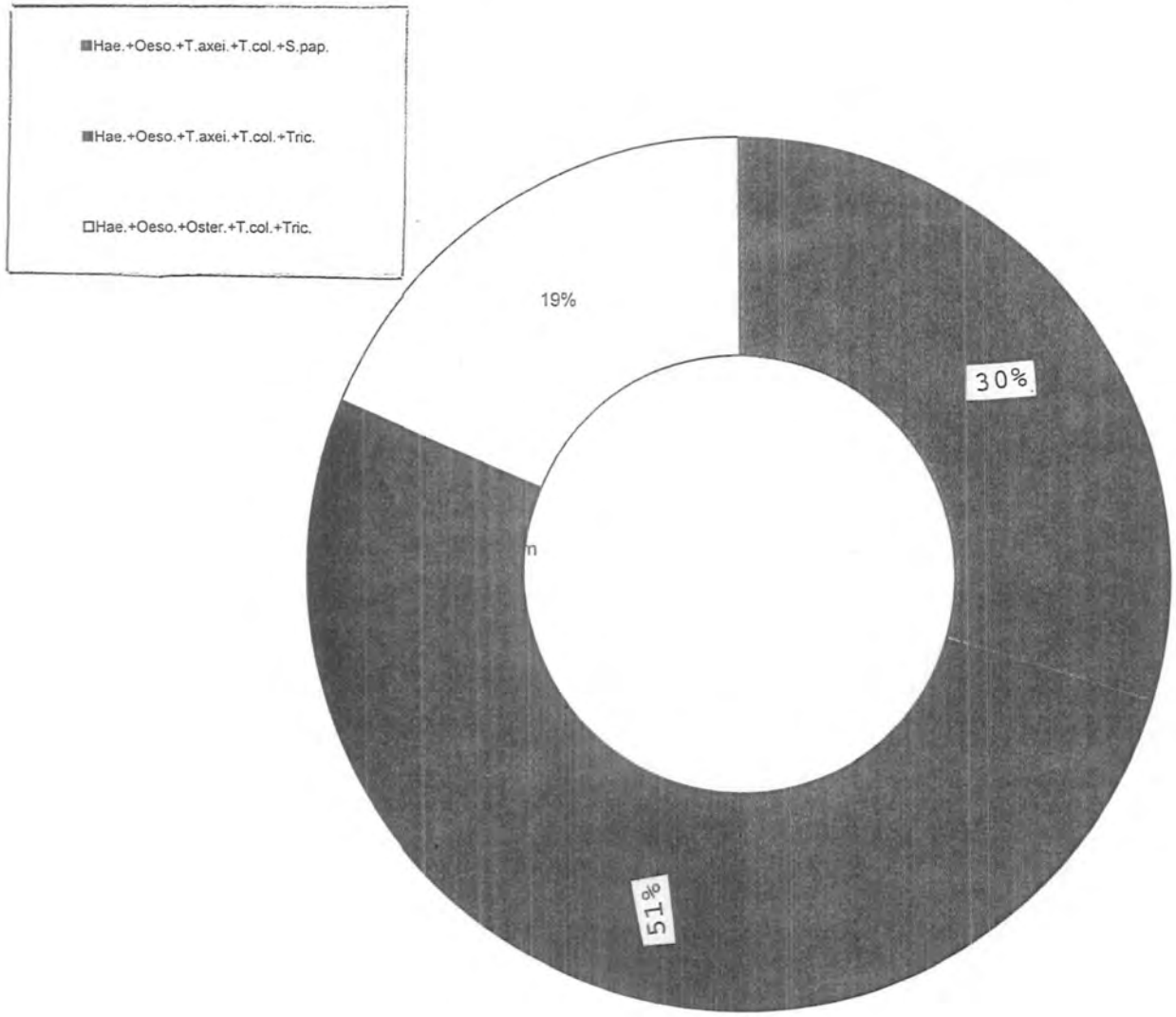
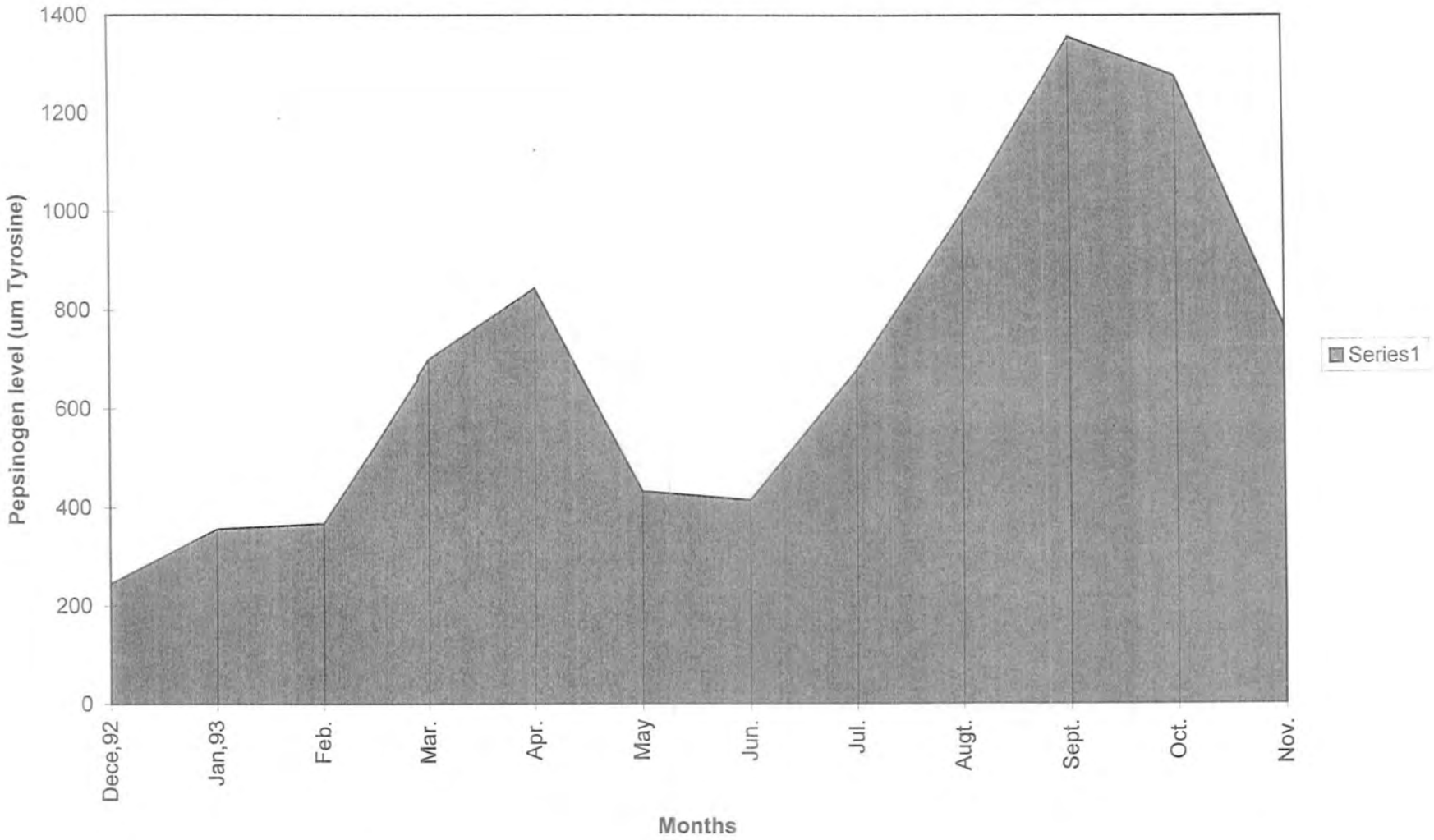


Figure 9. Showed different combinations of five parasites.




1400
1200
1000
800
600
400
200
0
Pepsinogen level (um Tyrosine)
Dece,92
Jan,93
Feb.
Mar.
Apr.
May
Jun.
Jul.
Augt.
Sept.
Oct.
Nov.
Months
Series1

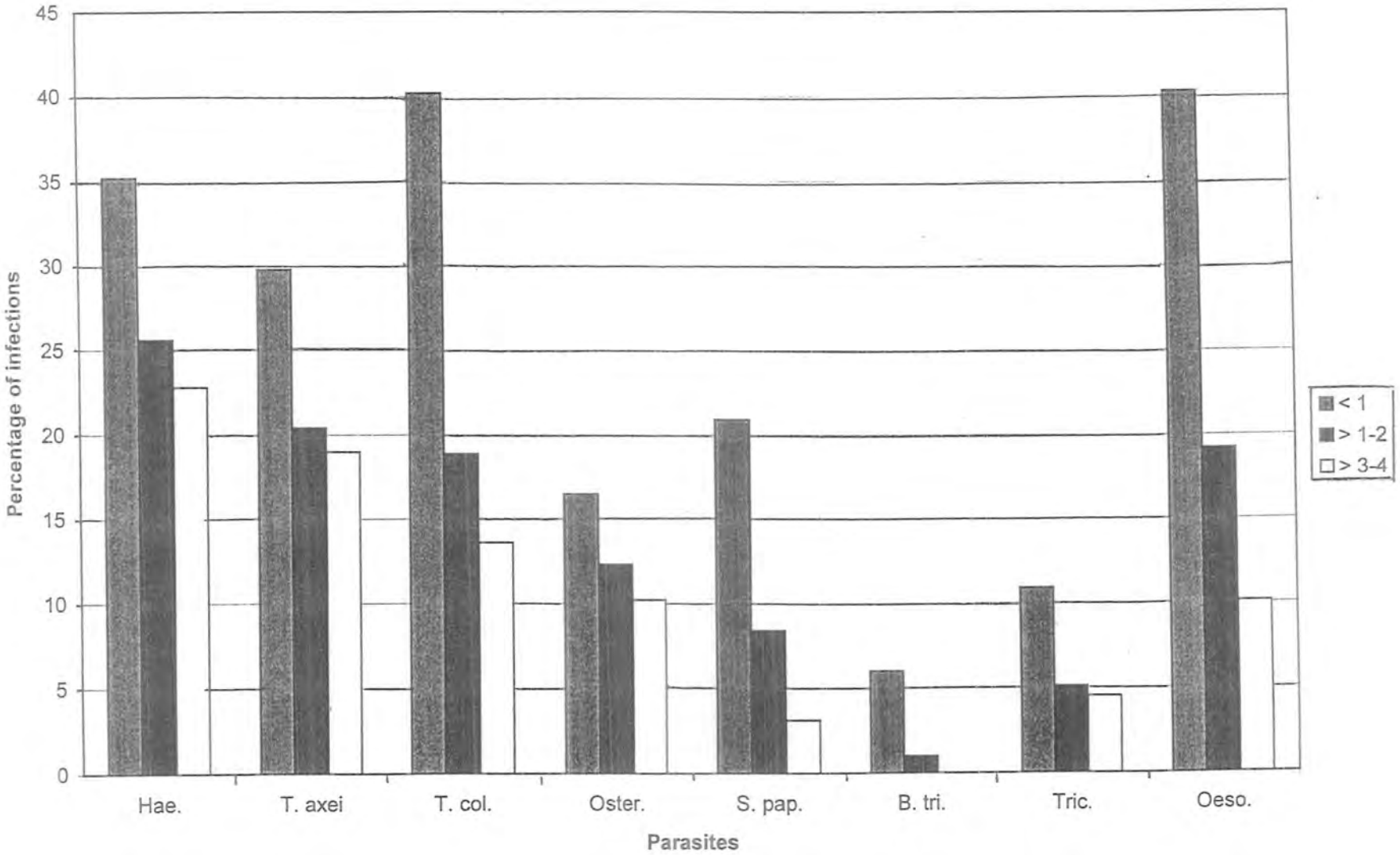
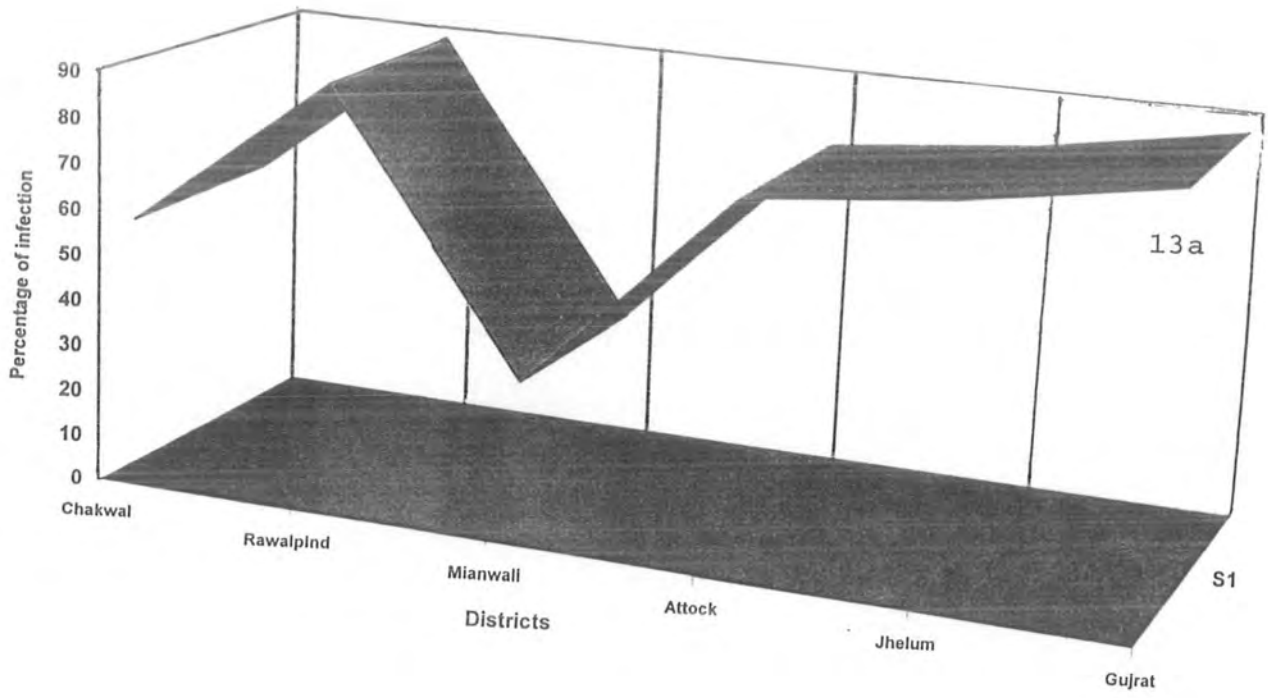
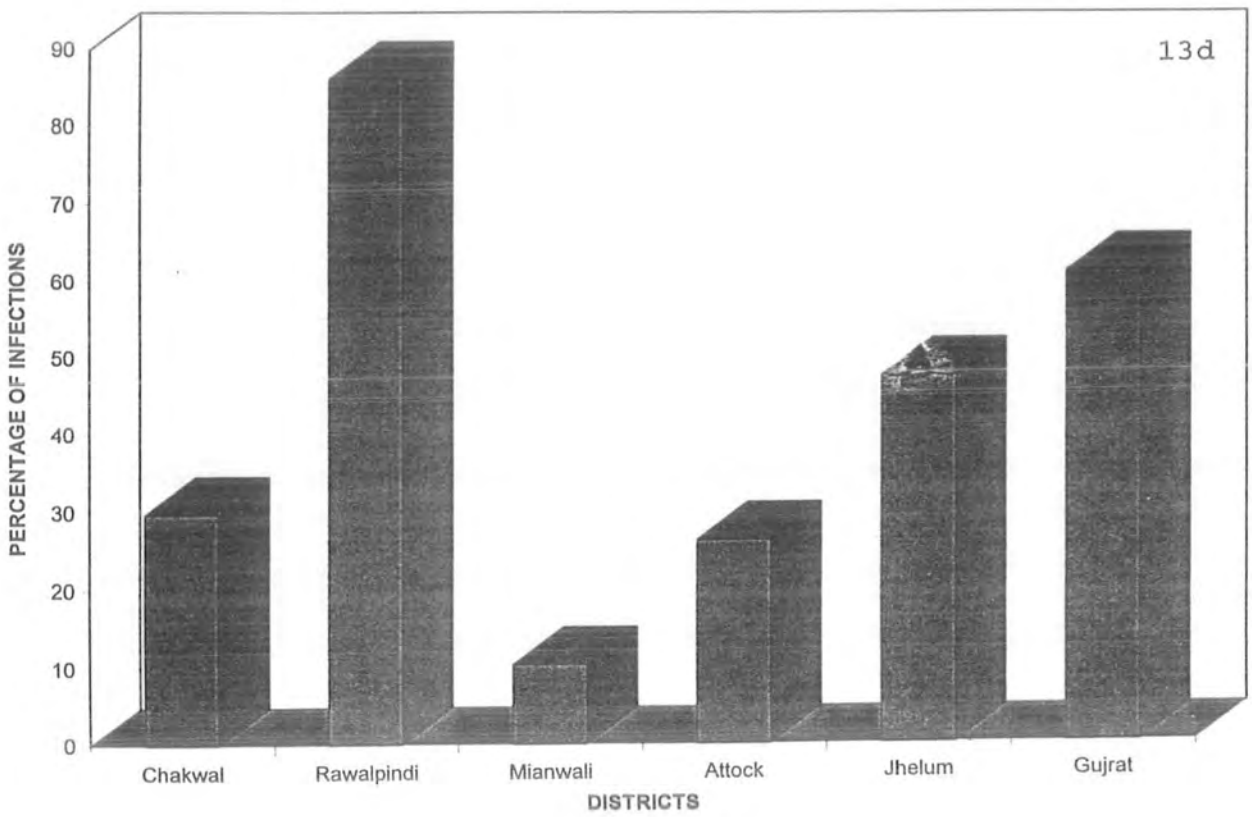


Figure 12. Distribution of parasites in different age groups of sheep.

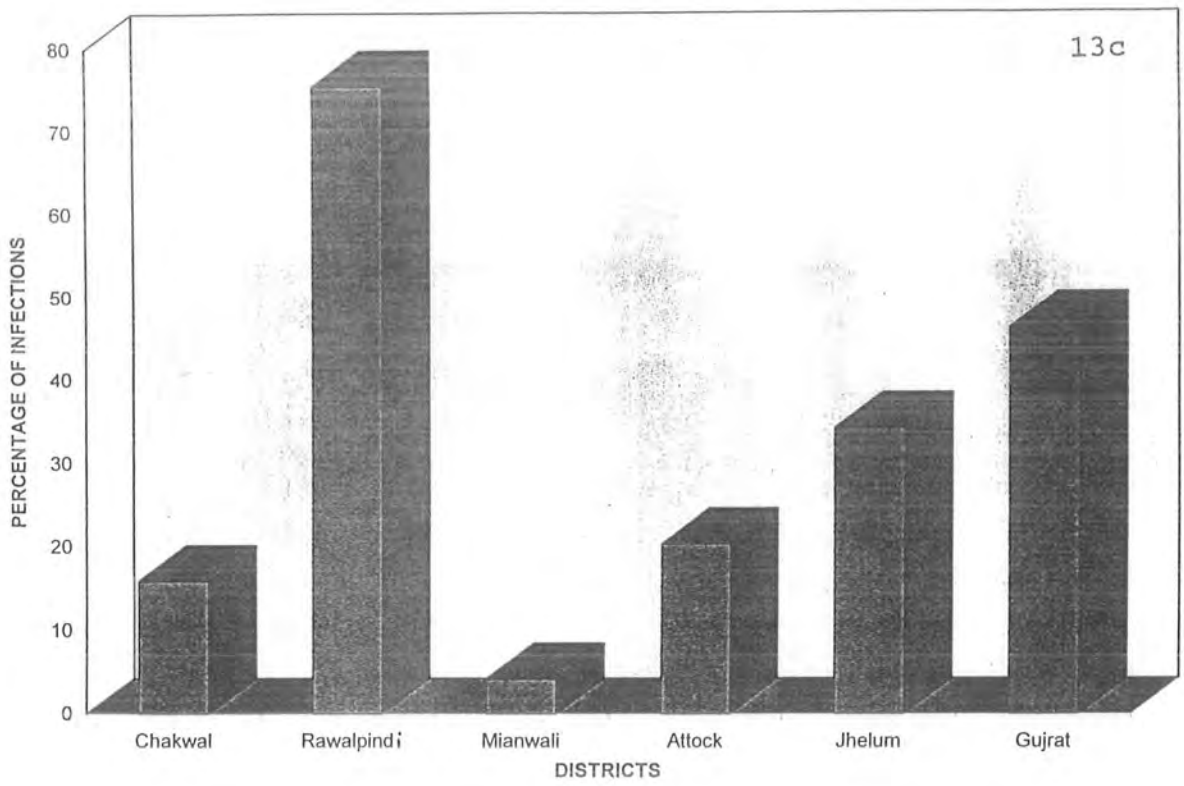
Haemonchus contortus



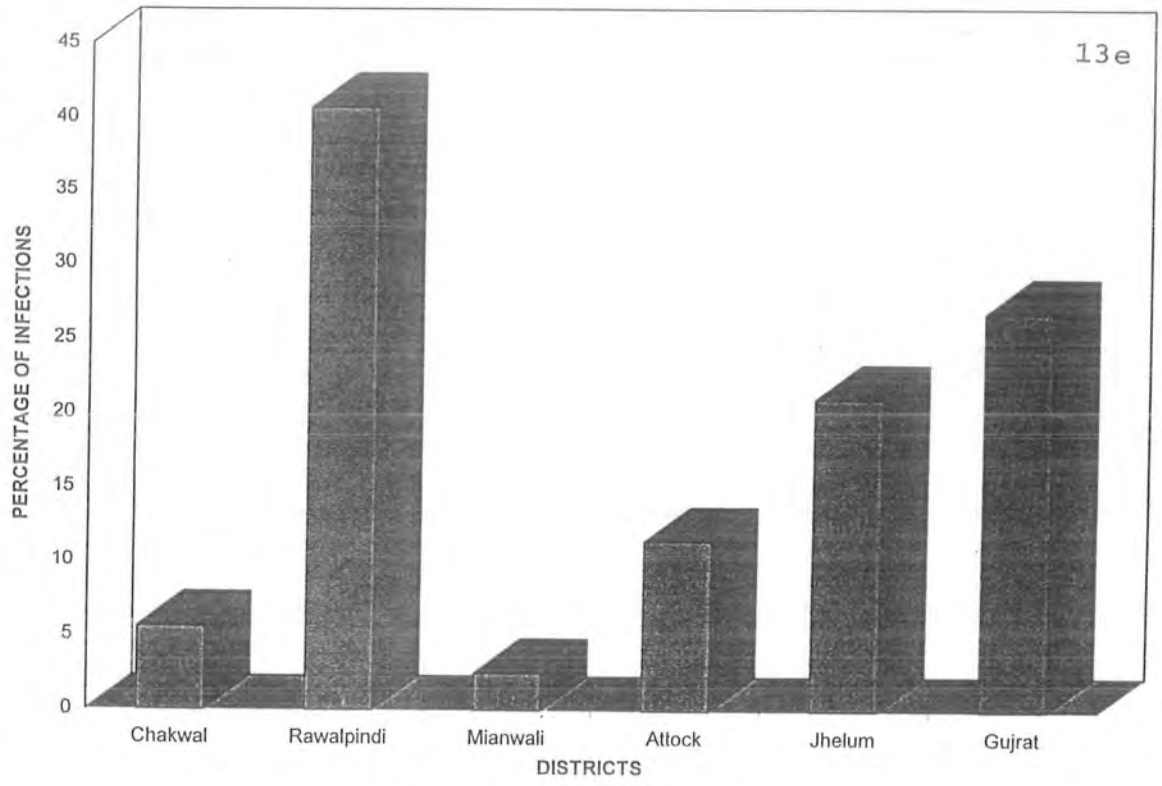
Trichostrongylus columbriformis



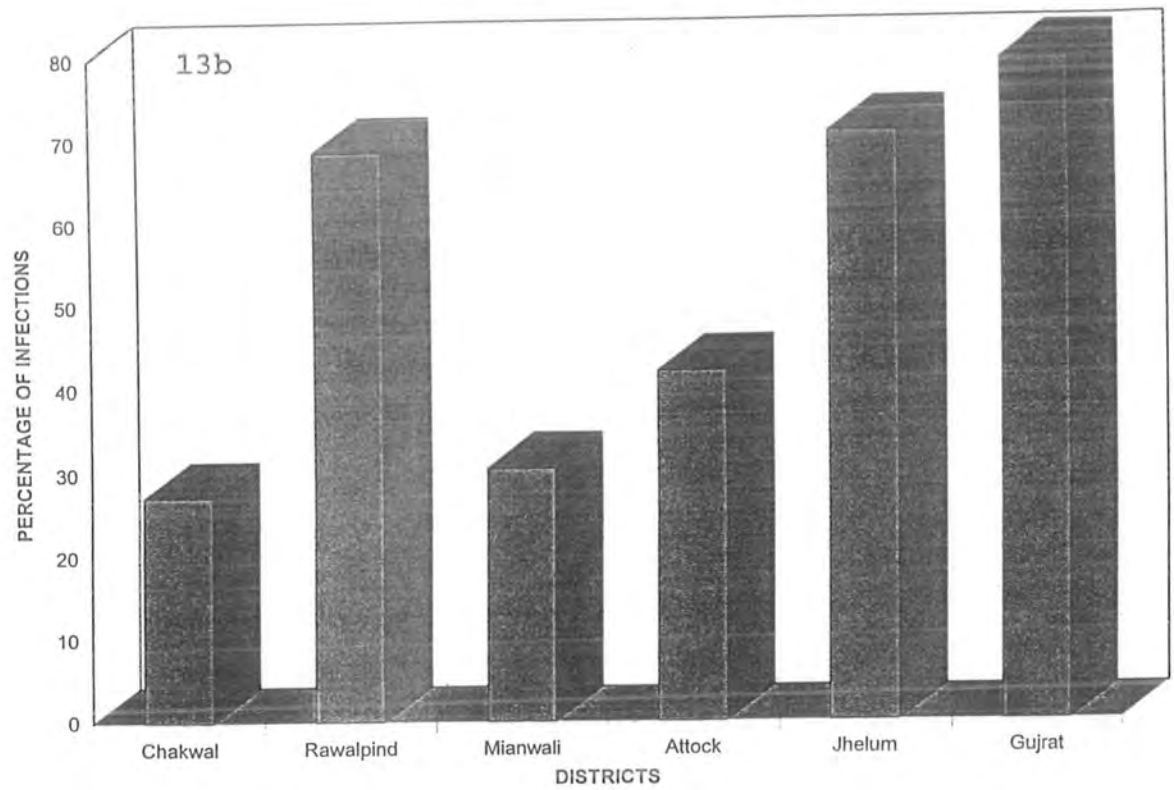
Trichostrongylus axei



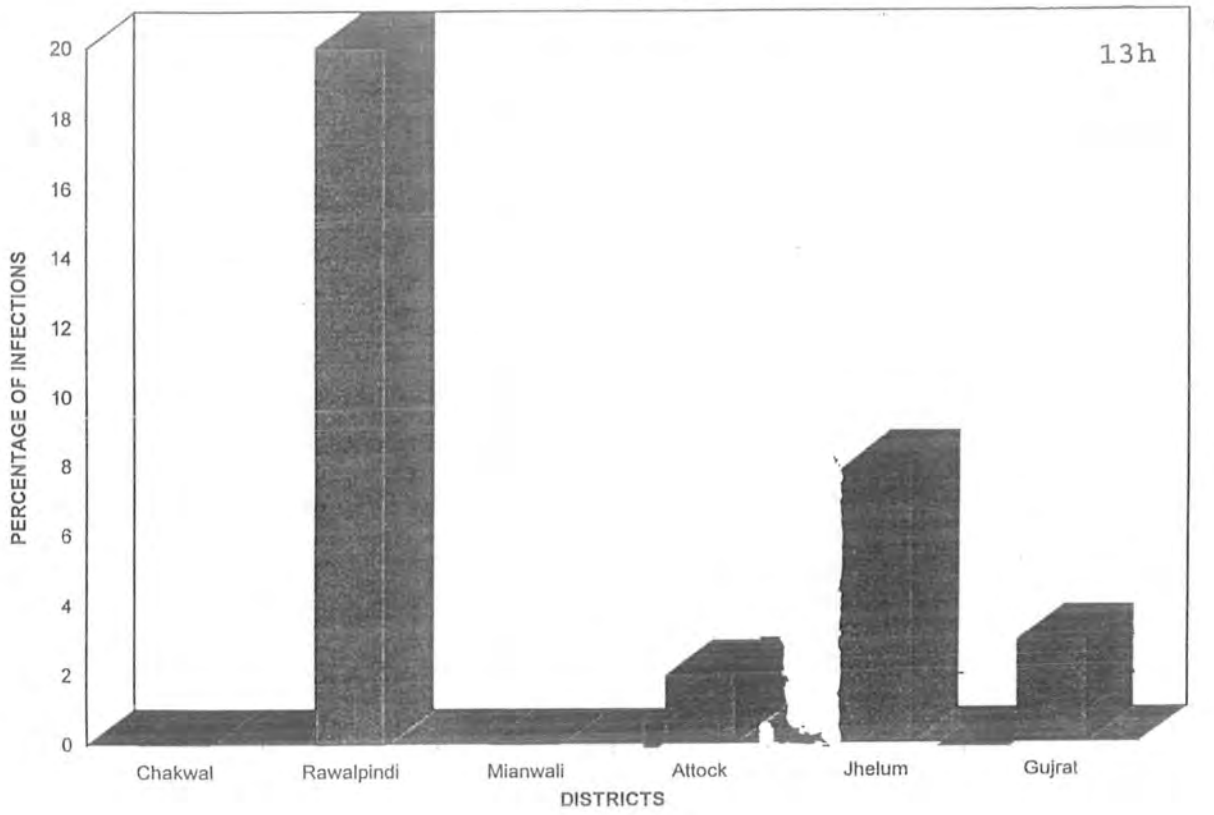
Ostertagia spp.



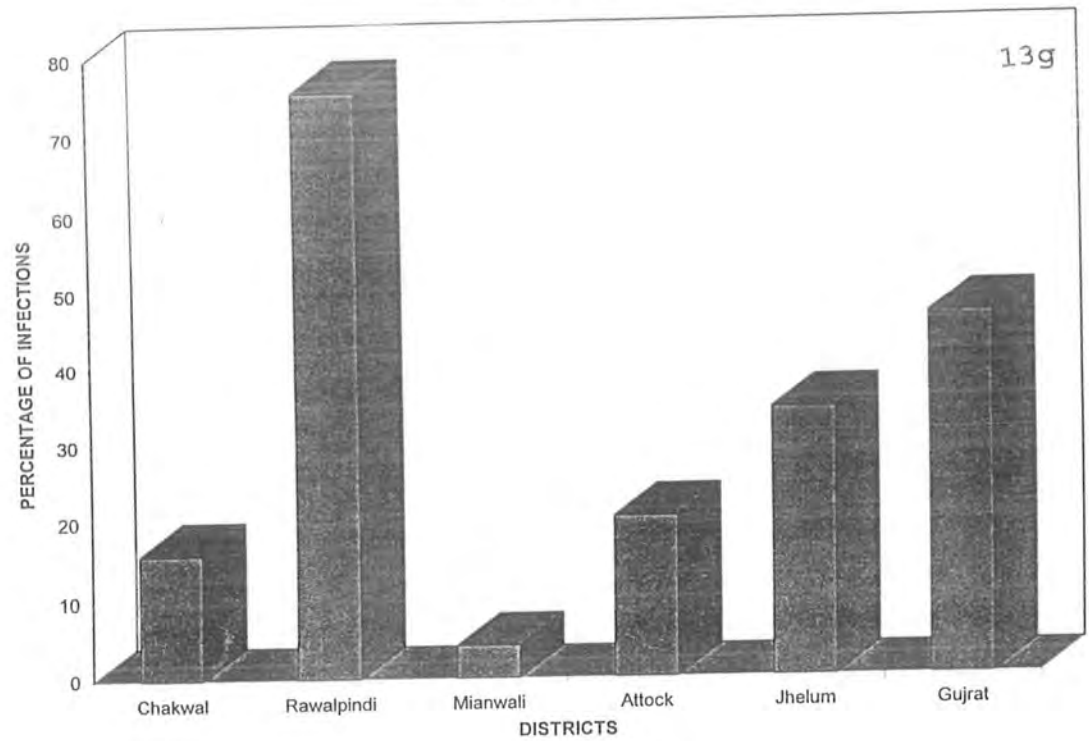
Oesophagstomum columbianum



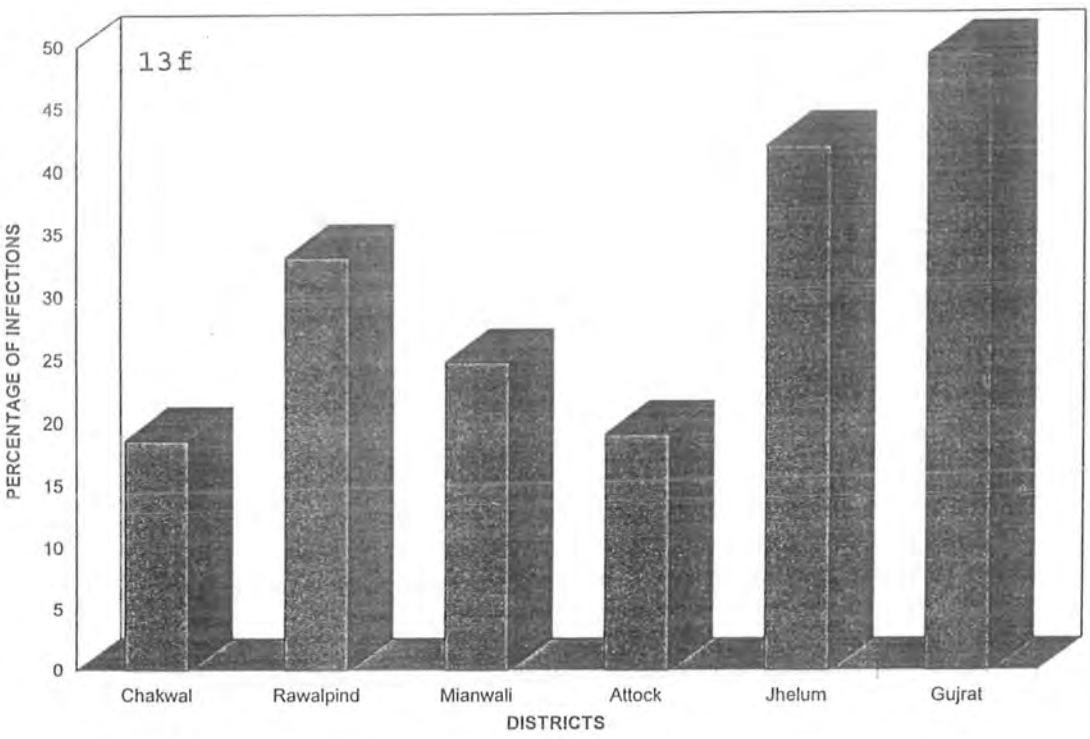
Nematodirus spp.

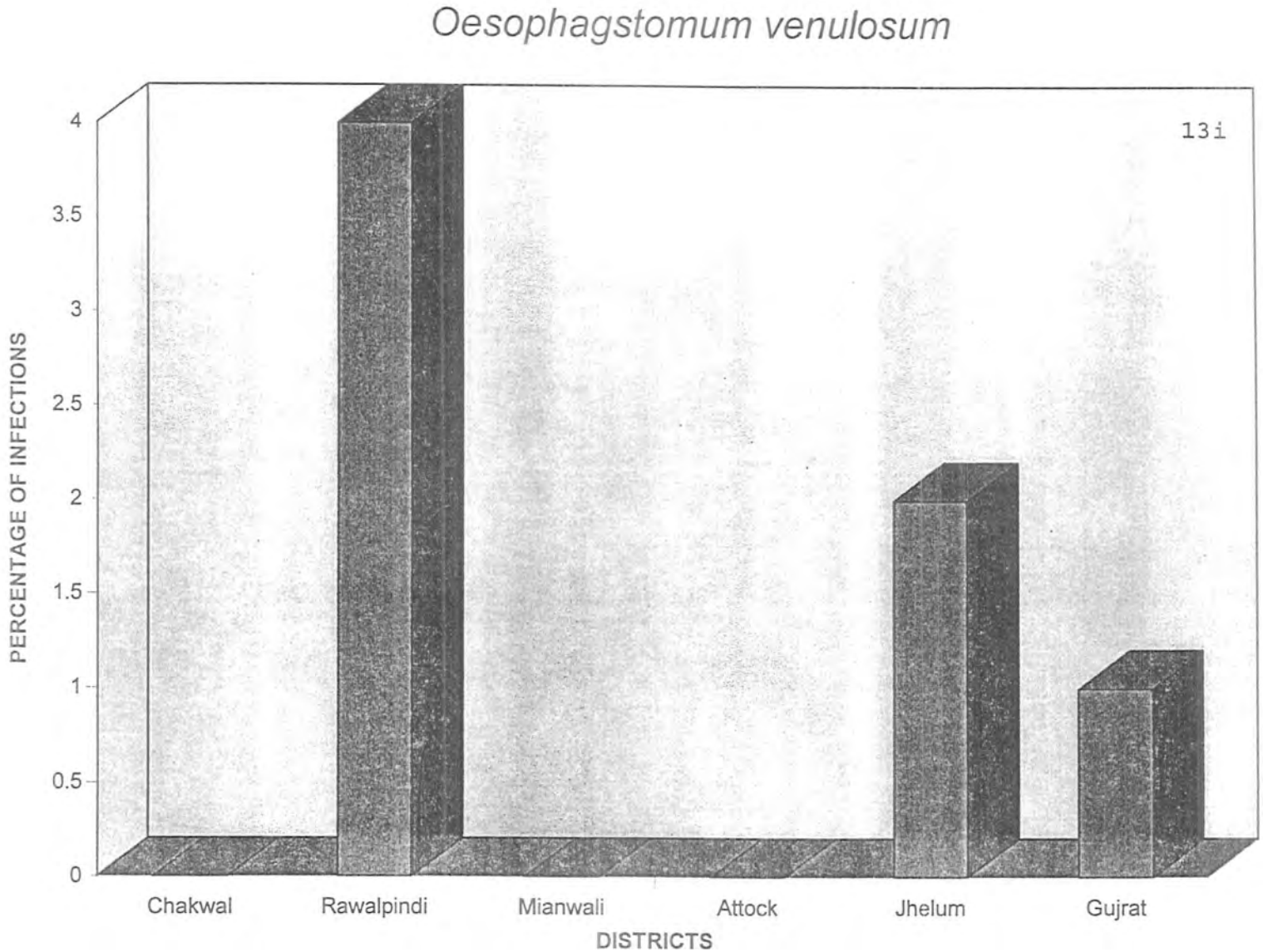


Strongyloides papillosus



Trichuris spp.




Oesophagstomum venulosum
13i
PERCENTAGE OF INFECTIONS
4
3.5
3
2.5
2
1.5
1
0.5
0
Chakwal
Rawalpindi
Mianwali
Attock
Jhelum
Gujrat
DISTRICTS

3.8 DISCUSSION

The nematode parasites recovered during this study has previously been reported by Siddiqi and Arhraf (1980), Shah *et al.* (1980), Mohiuddin *et al.* (1984) and Khan *et al.* (1989) from different areas of Pakistan and by Specht (1982); Vercruysse (1983); Hunter and Heath (1984); Gupta *et al.* (1987); Charles (1989); Van Aken *et al.* (1990); Louw and Reinecke (1991); Pandey (1994); Jacquiet *et al.* (1995) and Dorny *et al.*(1995) in different parts of the world. In Pakistan, in addition to these species the following species are also reported viz., *Chabertia ovina* by Siddiqi and Ashraf (1980) and Shah *et al.* (1980) at Peshawer (NWFP) and Lahore (Central Punjab), respectively. *Gaigeria pachyscelis* by Mohuddin *et al.* (1984) at Hyderabad (Sind). *Marshallagia marshalli* by Khan *et al.*(1988) at Quetta (Baluchistan). The above mention nematodes which are not recorded during this study could be due to the fact that different nematode species have different geographical distribution and required different climatic conditions for the development of their free-living stages.

The most prevalent nematode recovered in this study is *Haemonchus contortus*. This is in the agreement with findings of Bali and Singh (1977), Grant (1981), Ahmed and Ansari (1987), Gupta *et al.* (1987). They also observed that *Haemonchus contortus* was the most prevalent strongyle in small ruminants of their respective study areas. The higher prevalence could be due to the fact that this nematode has a relatively short generation interval and its ability to take the advantage of favourable environmental conditions (Grant, 1981). According to Gordon's (1953) criteria

mean monthly maximum temperatures of 18 °C or above and total monthly rainfalls of 50 mm or more are conducive for translation and transmission of *Haemonchus contortus*. These conditions are found in almost all the areas surveyed especially during July-October (Figs. 3 and 4). In this study, low infection of *Haemonchus contortus* was recorded especially during winter season (January-February). This seems contrary to the work of Grant (1981), Vercruysse (1983) and Ahmed and Ansari (1987). It could be due to the low temperature that retards the development of free-living stages and even at 9 °C no development takes place (Soulsby, 1982). Besides weather conditions, self cure phenomena (Dineen *et al.*, 1965) may also be the reason for the decrease in the incidence from January to February.

The incidence of *Oesophagostomum* spp., is in agreement with the reports of Patnaik *et al.*(1973), Ahmed and Ansari (1987), Khan *et al.* (1989). Moderate infection of *Oesophagostomum* spp., may be due to the fact the warm, moist summer experienced in this study area, is well suited to the development and survival of the free-living stages of this parasite (Grant, 1981). The incidence of *Oesophagostomum* spp., however in not in conformity with the results of Misra and Ruprah (1988), Khan *et al.* (1988) and Gupta *et al.* (1988). They reported that low incidence of this species in different geographical regions could be due to the adverse semi-arid climatic conditions in these areas, because of the low resistance of the free-living stages of this parasite to weather conditions (Kates, 1950).

These results show that *Trichostrongylus* spp., is recorded

throughout this study period with relatively higher prevalence during winter months (November-February), which is not consistent with that described by Gupta *et al.* (1987). *Trichostongylus* spp., are generally cool-season parasites (Southcott *et al.*, 1976) and thrive best at low temperatures.

The highest numbers of *Ostertagia* spp. was recorded during October-November. The high incidence of *Ostertagia* spp. could be attributed to the fact that the larvae of this nematode are abundant on the pasture from October-January when mean maximum temperature was below 15 ^{0}C and mean relative humidity at 1500h was above 60 per cent (Anderson, 1972). Over the same time period in these Barani areas where temperature during autumn-winter seasons was almost consistent with that of described by Anderson (1972) in Australia, for the development of free-living stages of *Ostertagia* spp.

Moderate prevalence of *Trichuris* spp., is in close agreement with the findings of Cabaret (1983). However, its prevalence is related to soil moisture (Smith and Archibald, 1965) and temperatures'(6-20 ^{0}C). These conditions are satisfied during the rainy and winter seasons (Figs.3 and 4) in the study area.

The intensity of the infection of *Bunostomum trigonocephalum* in the slaughtered animals is low and doesn't show any seasonal trend. This could be due to the susceptibility of the free-living stages of this parasite to winter and summer conditions (Shorb, 1940, Kates, 1947) resulting in their shorter survival time on the pasture under most of the climatic conditions. Similar studies have also been reported by Griffiths (1937) and Swales (1940).

Low infection of *Nematodirus spathiger* was consistent with the result of Smith and Archibald (1965), which could be due to the fact that this specie is a poor egg producer.

Pepsinogen level was comparatively higher in March-April and from July to October. This coincided with a higher worm burdens and other clinical signs such as diarrhoea. Moderate rainfall in March-April and heavy monsoon rainfall in July-September appears to extend the spring and summer acquisition of infection by the sheep. Moreover, these times correspond when the free-living stages of different nematodes is maximum on the herbage and serve to indicate the rapid onset of pathological effects due to accumulation of parasites in the host.

It is observed that higher number of trichostrongyles egg are shed in the faeces from July to November. This finding is consistent with those of Misra *et al.* (1974), Reinecke (1961), Vercruysse (1983) and Gupta *et al.* (1987), They also had reported that the egg production was generally highest during the summer, when conditions for gastrointestinal infections are more conducive. The higher faecal egg counts during this season may be largely due to highest prevalence of *Haemonchus contortus* from July-November, as this parasite is considered more prolific egg producer then *Trichostrongylus* spp., (Grant, 1981; Hunter and Heath, 1984).

The numbers of *Ostertagia* spp., *Trichostrongylus axei* and *Haemonchus contortus* occurring as adults in July to onwards were higher then May-June, indicating that a number of inhibited larvae develop into adults. Our results are in conformity by those of Altaif and Issa (1982), El-Azazy (1995), they demonstrated these

summer inhibition of this species in lambs in Iraq and Saudi Arabia, which may be due to high temperature and desiccation. Therefore in Barani areas, adverse environmental conditions such as hot dry climate appears to be the most important factor stimulating the inhibition. Thus maximum inhibition occurred during the hottest period of the year when strong desiccating winds blow and the atmospheric temperature on some days goes as high as 42 °C. The results of a recent study conducted by El-Azazy (1995) in Saudi Arabia, where dry season extends from May to June, also indicated the occurrence of a high level of inhibition.

Higher worm burden in young animals are in general agreement with findings of Kerboeuf (1978). The reason could be due to the fact that the establishment of immunocompetence in lambs is usually not achieved until 10 months of age (Urquhart *et al.*, 1960). This finding has also supported the results of (Manton *et al.*, 1962; Gibson and Parfitt, 1972), that the resistance to establishment of nematodes and the ability to expel established infections increase with age so that the number of worms found in older animals is reduced.

The incidence of nematode parasites in an area is directly related to the ability of the free-living stages to withstand the environmental conditions (Gupta *et al.*, 1987). According to Blood *et al.* (1983), variations in infection intensity could be due to difference in the micro and macro climate of the environment, volume and height of the herbage. In arid sub-zone, both temperature and lack of rainfall probably accounts for the decline in available infections. Larvae were probably trapped in pellets

which become hard during the hot summer months. Similarly in semi-arid sub-zone, only temperature is main factor for the development and survival of most of the nematode species. However, in sub-humid climate both temperature and rainfall are optimum for the year round survival of free-living stages of nematode species. Apart from prevailing agro-climatic conditions of the areas surveyed, other factors such as overstocking of animals, grazing young and adult animals together with poorly drained land can provide an ideal conditions for the transmission and translation of gastrointestinal nematodes, particularly *Haemonchus contortus*, *Trichostrongylus* spp., and *Oesophagostomum* spp. to build up clinical infections in the hosts. Higher prevalence of *Ostertagia* spp., and *Trichostrongylus* spp in Rawalpindi could be attributed to the fact that climatic conditions are generally suitable in Rawalpindi district and its adjoining areas. The lowest incidence of various nematodes in Chakwal and Mainwali districts could be due to prevailing hot summer conditions (high temperature and rapid evaporation rate) which are deleterious for the translation and transmission of infective stages (L_3). Moreover, poor water facilities in the surveyed areas, especially in Chakwal, Attock, and Mainwali districts could hardly have created favourable conditions for the hatching of nematodes larvae and their release from pellets.

An important conclusion to be drawn from the results of this study is that *Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichostrongylus* spp., were the most prevalent nematode parasites of sub-tropical (Barani areas). Moreover, the livestock management

factors, such as high stocking rate, age of the animal, heavily grazed pastures and mixed grazing of small and large ruminants further enhanced the risk of gastrointestinal infections. Before recommending any parasitic control strategy, it must be emphasized that experiments on farms should be designed to monitor the seasonality of important trichostrongyle parasites.

TRACER LAMBS STUDY

CHAPTER 4

4.1 INTRODUCTION

In Pakistan, sheep are usually loaded with mixed infections of gastrointestinal nematodes consisting largely of trichostrongyle worms and are rarely free of infection (Pal and Qayyum, 1996). Increased understanding of the biology and epidemiology of gastrointestinal parasites of cattle and sheep has led to improved in control measures and a decrease in population losses (Michel, 1985). Numerous studies have been carried out to detect accurately and investigate the ecology and epidemiology of nematode parasites (Brunsdon, 1970; Donald and Waller, 1973; Boag and Thomas, 1977; Waller and Thomas, 1978; Grant, 1981; Waller *et al.*, 1981; Gupta *et al.*, 1987; Charles, 1989; Pandey *et al.*, 1990; Uriarte and Valderrabano, 1989; Van Aken *et al.*, 1990; Garcia Romero *et al.*, 1993; Pandey *et al.*, 1994 ; Jacquiet *et al.*, 1995), in devising strategic anthelmintic programmes to when advise farmers (Callinan *et al.*, 1982; Paton *et al.*, 1984; Thomas *et al.*, 1986; Paton and Boag, 1989).

Knowledge of the bionomics of free-living stages of parasites in a local area is an important pre-requisite to understanding the epidemiology of parasites (Hutchinson *et al.*, 1989). In this regard, several studies on the availability of infective stages of sheep trichostrongyles have been conducted in cold continent Canada, (Smith and Archibald, 1965; Smith and Fulton, 1989), cool temperate areas of U. S. A. (Smith, 1988; Smith, 1989; Coyne *et al.*, 1991) desert areas of Africa (McCulloch and Kasimbala, 1970;

Cabaret, 1976; Jacquiet *et al.*, 1992; 1995) and to a lesser extent in tropical and sub-tropical areas of Asia (Gupta *et al.*, 1988; Van Aken *et al.*, 1990).

It was recently reported that the development of free-living stages of trichostrongyles in sub-tropical areas of Malaysia, occurred throughout the year (Dorny *et al.*, 1995). In Pakistan, there are two distinct rainy seasons, one is in late winter (March-April) and second is from mid of July to end of September, during which the pasture availability to ruminants is very high. Therefore, estimates of the abundance of infective larval stages on the herbage are integral part of the study of the epidemiology.

Currently, there are two techniques frequently used for the larval estimation, one involves the direct regular pasture sampling from which infective larvae (L_3) were recovered (Taylor, 1939; Donald and Waller, 1973; Vlassoff, 1973; Waller *et al.*, 1981) and in other studies worm free lambs commonly known as " tracer " were allowed to graze for short intervals before slaughter and worm recovery (Donald *et al.*, 1978; Smith and Fulton, 1989; Charles, 1989; Uriarte and Valderrabano, 1989).

The purpose of the present study was to examine changes in the composition of worms burden in tracer lambs during one year grazing season in a semi-arid sub-zone, of northern Punjab. The results will be used to outline the seasonal pattern of transmission of trichostrongyle parasites upon which a future control strategy will be recommended.

4.2 MATERIALS AND METHODS

4.3 STUDY AREA:

4.3.1 *Location:*

The study was carried out, from June 1993 to July, 1994, on privately owned small ruminant farm located in Chakwal district, about 98 Km south-west of Rawalpindi city, Punjab province (Pakistan).

4.3.2 *Climate and grazing conditions:*

The farm is located in sub-arid sub-zone (sub-tropical) of Pakistan, and receive rainfall mostly during the summer months (July-September) and in winter (March-April). The grazing land is heavily grazed during summer growing season.

The native vegetation is a mixture of grasses and shrubs like *Heteropogon contortus, Chrysopogon anontanus, Bothrio Chloanpertusa, xanthium strumarium, Sageretia brandrethiana, Sorghum halepense, cenchrus ciliaris* and *Artistida depressa.*

4.3.3 *Animals:*

Local indigenous sheep and lambs used in the study were Latti type and are commonly known as "Salt Range ". They were maintained under a traditional animal husbandry management system. They were grazed on roadsides, crop stubbles, fallow and common lands during the day and were kept in an open fenced enclosure or in a simple thatched roof accommodation during the night. The permanent flock used in the present trial did not receive any anthelmintic treatment prior to start of this trial. The management of a flock during the whole year is summarized in figure 14.

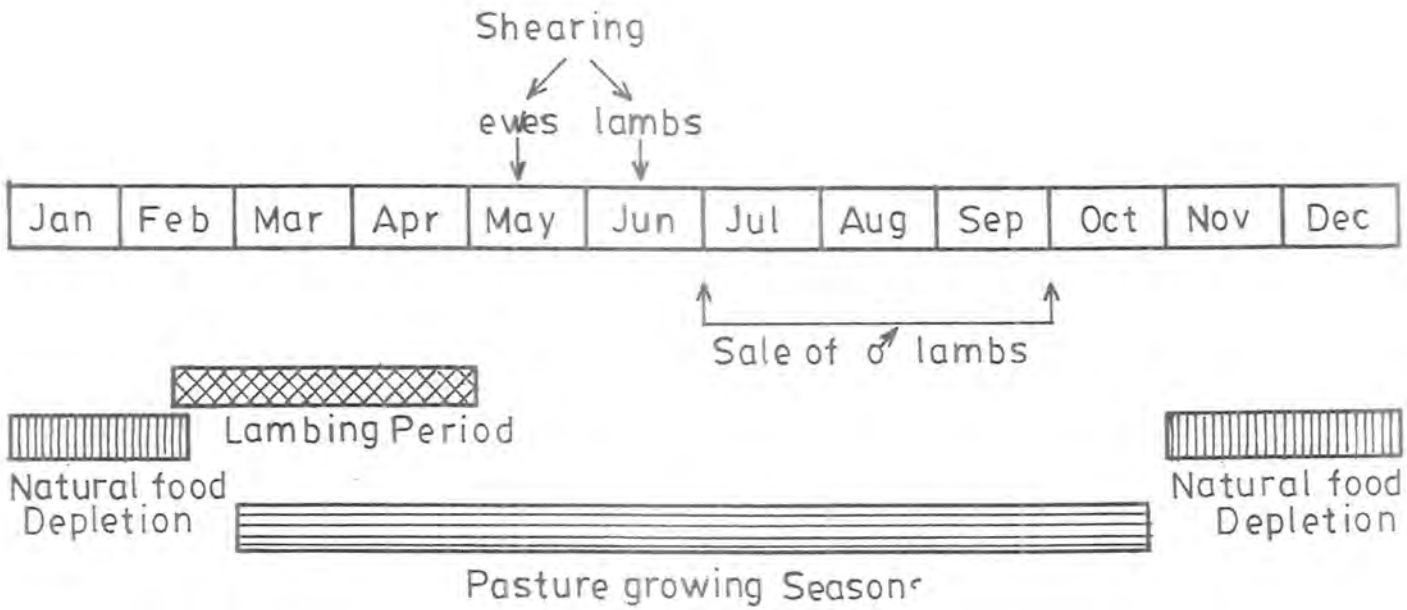


Figure 14. Summarized animal husbandry management observed in northern Punjab.

4.4 EXPERIMENTAL DESIGN:

4.4.1 *Permanent flock:*

Eighty ewes naturally infected with strongyles were allowed to graze on the permanent pastures (0.4 hac) throughout the year. Prior to start of this trial ewes faecal samples were examined for gastrointestinal strongyles and all were found positive. Thus pasture land had received continuous contamination from this permanent flock. Lambing occurred from the end of February to the start of April but the vast majority of ewes lambed in March, 1993. Fifty five ear-tagged ewes were selected on the basis of equal faecal egg counts and on the previous reproductive performance so as to get synchronize lambing. Rectum faecal samples from these ewes were collected every month to detect strongyles infection level.

4.4.2 *Tracer Lamb:*

Thirty-six tracer lambs of comparable age 2-3 month old were selected on the basis of equal weights. They were housed indoors under worm-free conditions for at least one month, before the start of study. After weaning in June, they were divided into 12 batches, each having 3 lambs of comparable age. Between June, 1993 and July, 1994 batch of 3 lambs were introduced each month on to the pasture where they grazed (8.00 a.m to 5.00 p.m) for one month with the permanent flock. After one months of grazing they were housed indoors for one month to get mature worms and then slaughtered. Prior to release on to pasture they were dosed with Fenbendazole at the rate of 15 mg kg^{-1} body weight. Figure 15 depicts the protocol of the study.

Figure 15. Depicted the trial protocol of the tracer lambs grazed during 1994-95.

Tracer No.	Age of Lamb (days)	Date of Grazzing	Date of Housing	Days Kept Indoors	Days of Slaughter
BATCH-I					
1	58	28/06/94	29/07/94	30	29/08/94
2	60	28/06/94	29/07/94	30	29/08/94
$3	65	28/06/94	29/07/94	30	29/08/94
BATCH-II					
4	58	29/07/94	28/08/94	30	26/09/94
5	65	29/07/94	28/08/94	30	26/09/94
$6	63	29/07/94	28/08/94	30	26/09/94
BATCH-III					
7	68	29/08/94	28/09/94	30	28/10/94
8	63	29/08/94	28/09/94	30	28/10/94
$9	64	29/08/94	28/09/94	30	28/10/94
BATCH-IV					
10	70	29/09/94	28/10/94	30	28/11/94
11	67	29/09/94	28/10/94	30	28/11/94
$12	73	29/09/94	28/10/94	30	28/11/94
BATCH-V					
13	72	29/10/94	28/11/94	30	29/12/04
14	69	29/10/94	28/11/94	30	29/12/04
$15	74	29/10/94	28/11/94	30	29/12/04
BATCH-VI					
16	75	29/11/94	29/12/94	30	28/01/95
17	73	29/11/94	29/12/94	30	28/01/95
$18	70	29/11/94	29/12/94	30	28/01/95
BATCH-VII					
19	76	30/12/94	29/01/95	30	28/02/95
20	74	30/12/94	29/01/95	30	28/02/95
$21	71	30/12/94	29/01/95	30	28/02/95
BATCH-VIII					
25	78	30/01/95	29/01/95	30	30/03/95
26	74	30/01/95	29/01/95	30	30/03/95
$27	70	30/01/95	29/01/95	30	30/03/95
BATCH-IX					
25	77	1/3/95	31/03/95	30	30/04/95
26	75	1/3/95	31/03/95	30	30/04/95
$27	73	1/3/95	31/03/95	30	30/04/95
BATCH-X					
28	78	1/4/95	1/5/95	30	31/05/95
29	77	1/4/95	1/5/95	30	31/05/95
$30	74	1/4/95	1/5/95	30	31/05/95
BATCH-XI					
31	79	2/5/95	1/6/95	30	1/7/95
32	80	2/5/95	1/6/95	30	1/7/95
$33	76	2/5/95	1/6/95	30	1/7/95
BATCH-XII					
33	81	2/6/95	2/7/95	30	2/8/95
34	79	2/6/95	2/7/95	30	2/8/95
$35	74	2/6/95	2/7/95	30	2/8/95

$: Slaughtered on bi-monthly basis.

4.5 PARASITOLOGICAL TECHNIQUES:

4.5.1 *Coproscopical tests:*

To determine the intensity of infection, faecal samples were collected directly from the rectum, preserved in 10 per cent formalin, stored at 4 °C and examined within 72 hours. The modified McMaster technique with saturated Sodium Chloride was used according to the consistency of faeces, X 1 for normal pellets, X 1.5 for soft formed faeces, 2 X for soft faeces and X 3 in case of diarrhoea (Skerman and Hillard, 1966).

4.5.2 *Parasite counts:*

Sampling of nematodes from abomasum, small and large intestines was carried out within 4 hours after the slaughter of animal. Abomasum, small and large intestines were ligated at omasal-abomasal, abomasal-duodenal and ileo-cecal junctions to prevent worms spilling from one location to another. Counting of round worms is based on technique described by Charles (1989) with slight modification. According to which immediately after arrival in laboratory, each organ was opened separately and the mucosa washed in water to remove all parasites. Abomasal and intestinal contents were washed through wire mesh of 71 µm aperture while contents of the large intestines were washed through wire mesh of 105 µm aperture. The remaining material was preserved in 10 per cent formalin and examined at a later stage. For counts of worms recovered from the abomasa and small intestines the total volume were first diluted to a volume of 2 liter and an initial aliquot of 10 per cent of the total volume was counted. If this initial aliquot contained <100 worms, another 10 per cent aliquot was

examined. Samples which contained few worms were completely counted under Stereomicroscope (Wild, Heerbrugg). Total counts were made for each large intestine examined. Every nematode recovered was cleared in lactophenol and mounted on microscope slide for identification with the help of keys and descriptions given in MAFF (1979).

4.5.3 *Enzymatic digestion of abomasa:*

The entire mucosa of abomasum was scraped off and digested by incubating it overnight at 37^{0}C in 8 gm pepsin (Merck) and 20 ml concentrated HCl solution and 940 ml distilled water (MAFF, 1979).

4.5.4 *Collection of the herbage samples:*

The distribution of larvae on the pasture is highly discontinuous, therefore, the size of the pasture and the physical properties of the herbage should be taken into consideration (Michel, 1976). In each sampling two herbage samples at (8.00 a.m and 12.00 noon) were collected from the study area. The herbage over the soil to be sampled was cut at ground level with scissors. Starting from the corner, the field was traversed in a predetermined W shaped pattern, preferably two per field. The samples were collected by walking the W route at regular intervals and cut the grass as close to the ground as possible. At each stop, four sample were taken, one each in front of toe, one straight ahead and one between the feet. The modification conducted by Ludwig and Johnstone (1984) was that barring from collecting the sample in W shaped pattern, another sampling was done by collecting grass from near to and far off the faecal pellets on the pasture.

Care was taken not to have excessive soil or tall, thick forage. In each sampling approximately 250 gm grass sample was collected in a 15 liter bucket.

4.5.5 *Processing of the samples:*

When the herbage was wet because of rain or dew, it was sun-dried for a while before the weight was taken. The herbage was then immersed in a bucket of water, washed and allowed to remain as such overnight. In water few drops of detergent was added to reduce surface tension. Next day, the grass samples was transferred to a second bucket. The water in the first bucket was screened through double cheese cloth. The grass in the second bucket was re-soaked in water for overnight. Next day the grass was removed, thrown off and the water was added to the first bucket after screening. The water was allowed to settle for at least four hours. The supernatant was poured off. From the well mixed sediment 60 ml was transferred to a 100 ml beaker and allowed to settle for at least 4 hours. The supernatant was then decanted and the sediment was poured in a 15 ml centrifuge tube and centrifuged at 2000 rpm for 3 minutes. The supernatant was syphoned off and saturated solution of NaCI was added till a positive meniscus was formed. Then 2 mm square cover slip was placed on the tube taking care that no bubbles were trapped underneath. The coverslip was removed carefully by lifting it off the tube vertically and placed on a microscope slide where one drop of Lugol's iodine was added. It was then examined under the microscope X100 (Nikon, Japan).

4.5.6 *Microscopic examination:*

Under the microscope a mixture of parasites as well

as free nematodes could be seen. They could be distinguished by the fact that free specimens absorb iodine and are orange coloured where as parasitic ones remained colourless (Ludwig and Johnstone, 1984). Further, parasitic nematode larvae have wavy cuticle. The parasitic nematode larva were identified with the keys described by MAFF (1979).

4.5.7 *Interpretation:*

The total of parasitic larvae were counted, multiplied by 1000 and divided by weight of initial grass samples. This gives the number of larvae per kilogram of herbage (Larvae kg^{-1} of herbage).

4.6 RESULTS

Nine different species of nematodes from eight genera were recorded from the 36 lambs necropsies. The following were the major nematode parasites: *Haemonchus contortus, Trichostrongylus axei, Trichostrongylus colubriformis, Oesophagostomum columbianum, Trichuris* spp. and *Ostertagia* spp. While *Strongyloides papillosus, Nematodirus spathiger* and *Oesophagostomum venulosum* were the minor nematodes found during this study.

Haemonchus contortus was one of the predominant trichostrongyle parasite recovered in the study. The distribution pattern is given in figure 16. The prevalence of *H.contortus* rose steadily to a peak in October, was relatively low during the winter months of December to February and then rose again to moderate level in March-April. After this its number declined to a low level in the preceding months of May-June.

Trichostrongylus axei was recovered in fairly large numbers mainly between December and April (Fig.17). During this period individual lambs harboured heavy worm burden,the largest number recovered being 2,600 adult specimens from a lamb slaughtered in March. From June onwards upto December, a gradual incline was noted in the number of worms which then fell to low level in May-June.

As far as *Trichostrongylus colubriformis* was concerned, the worm burden rose fairly rapidly to culminate in a major peak in October, after which it fell suddenly to low level in December and then rose again to a second peak level in March. The maximum worm burden of 2,000 was found in a lamb necropsied in November (Fig.18).

Oesophagostomum columbianum was recovered throughout the year with minor fluctuations in different months. Prior to December, relatively small number of adult specimens were seen (June-November). But their number inclined steadily to a peak in March and then dropped again to low level in April-May. In the month of June it was entirely absent (Fig.19)

Ostertagia spp: numerically *Ostertagia circumcincta* dominated the *Ostertagia trifurcata* during this investigation. No mixed infection among these two species were found during this study. The rate of recovery of this genus rose slowly to a peak in March and again declined to a low level in April and remained at this level upto June (Fig.20). A maximum number of worms 2400, were recovered in a lamb slaughtered in March.

Nematodirus spathiger was recovered in 18 lambs only and no significant trends were noted. Although from October to January tracer lambs harboured significantly greater numbers of adult worms. *Oesophagostomum venulosum* was recovered from four lambs, their numbers varied from 9 to 15 adult worms. Although *Strongyloides papillosus* was recovered throughout the year with minor fluctuations it was entirely absent in December and February. *Trichuris* spp. was consistently present in all the tracer lambs and showed no obvious seasonal variations (Tab.4).

4.6.1 ***Worm burden on bi-monthly basis:***

The bi-monthly worm burden of tracer lambs is shown in Tables 5 to 11. The nematode parasites viz., *Haemonchus contortus* *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Oesophagostomum columbianum* and *Ostertagia* spp. were consistently

present throughout the year. While *Nematodirus spathiger, Trichuris* spp., *Oesophagostomum venulosum* and *Strongyloides papillosus* were observed irregularly and in low number (Table 12). Throughout the study, the nematode burden of the abomasum were higher than those of the small intestine. The worm burden of the large intestine were comparatively low. On the average 70.5 per cent of nematodes was localized in the abomasum, 24.7 per cent in small intestine and 4.8 per cent in large intestine. Heavy worms burden (16,200) was noted during the winter season, followed by rainy season (14,900) and 7600 worms in summer season. *Haemonchus contortus* was the frequent strongyles (1100) recovered during rainy season while *Trichostrongylus* spp., were the predominant species during winter (November-February). On the other hand 780 worms of *Oesophagostomum* spp., were found in summer season. The low percentage of inhibition at 4th larval stage (L_4) of *Ostertagia* spp, and *Trichostrongylus axei* was observed in lambs slaughtered during May-June but it was entirely absent from rest of the study period.

4.6.2 *Pasture larval counts:*

Infective larvae (L_3) of three genera of trichostrongyles nematodes viz., *Trichostrongylus* spp., *Haemonchus contortus* and *Oesophagostomum* spp. were recovered during the 12 month period of monitoring (Fig.21). The seasonal distribution of larval availability on pasture is shown in Figure. *Trichostrongylus* spp., was the predominant species and there was significantly greater numbers of larvae recovered per kilogram of dry matter (Kg^{-1} DM) from the pasture. Peaks of *Trichostrongylus* spp. larval availability on pasture occurred in late February to mid May (6,000

Kg^{-1} DM), and rose again in July to December with intermittent peaks.

The second predominant trichostrongyle nematode was *Haemonchus contortus* which was recovered throughout the year Its seasonal availability rose steadily from the start of spring season in March and reached a peak (4000 Kg^{-1} DM) in mid of rainy season in August but dropped to between (250 Kg^{-1} DM to 3000 Kg^{-1} DM) in the winter season (November-December).

As far as *Oesophagostomum* spp., was concerned, the infective stage (L_3) of this genus was detected from January to April and then fell to undetectable level in May and June. This was followed by a graded incline reaching the highest level (2000 larvae Kg^{-1} DM) in between August to October, the rainy season. The overall mean level counts were observed in the morning hours (8.00 a.m-10.00 p.m) as compared to mid day hours (12.00 noon-2.00 p.m) during the whole study period.

4.6.3 ***Comparison of trichostrongyles worms burden and egg per gram (EPG):***

The comparative distribution of trichostrongyles worms burden and egg per gram (EPG) is plotted in Figure 22. The results showed that heavy worm burden of all the species was recorded in March, when *Trichostrongylus* spp., were the major worms contributor. However, different strongyle species showed different peaks depending upon their availability. The worm burden of *Haemonchus contortus* was highest during July-October (460-890). As far as *Trichostrongylus* spp. were concerned, the availability of *T. axei* was highest during December-April, while the worms burden of

T. colubriformis was highest from October to March (860-1980). Similarly the recovery of the *Oesophagostomum columbianum* was the highest during February-March (180-200). Although low worms burden of *Ostertagia* spp., was recorded in the present study, but their high worm burden was noted from January-March (230-400). The comparative results of worm burden and egg per gram (EPG) showed that there was a close relationship between these two parameters. However, it was found that worms burden rose progressively from June, to a peak in October which also caused a corresponding increase in egg counts. But from November to May reverse trend was noted and no definable relationship between these two parameter was observed.

Table: 4 Mean worm distribution in tracer lambs slaughtered after one month period during 1994-95.

Parasites	Months											
	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Trichuris spp.	-	-	12	34	32	46	9	-	22	17	26	11
Nematodirus spatiger	-	-	-	-	-	2	15	27	33	21	-	-
Oesophagostomum venulosum	-	-	3	8	-	-	-	-	-	-	-	-
Strongyloides papillosus	-	23	45	53	76	85	100	-	-	4	19	13

Table: 5 Composition of nematodes infection in lambs slaughtered after two months periods (June, 1994 - August, 1994).

	Age: 188 days Grazing time : 8:00 a.m - 5:00 p.m.			
Parasites	Adults	Stage 5[$]	Stage L_4	Total
Haemonchus contortus	893	16	-	909
Trichostrongylus axei	381	-	6	387
Trichostrongylus columbriformis	668	-	-	668
Ostertagia spp.	23	-	2	25
Oesophagostomum columbianum	63	-	-	63
Nematodirus spathiger	12	-	-	12
Trichuris spp.	46	-	-	46

$: Adolescent immature not gravid females.

Table: 6 Composition of nematodes infection in lambs slaughtered after two months periods (August, 1994 - October, 1994).

	Age: 218 days Grazing time : 8:00 a.m - 5:00 p.m.			
Parasites	Adults	Stage 5	Stage L_4	Total
Haemonchus contortus	1680	212	-	1892
Trichostrongylus axei	1263	-	-	1263
Trichostrongylus columbriformis	3064	-	-	3064
Ostertagia spp.	109	-	-	109
Oesophagostomum columbianum	212	-	-	212
Nematodirus spathiger	24	-	-	24
Trichuris spp.	109	-	-	109

Table: 7 Composition of nematodes infection in lambs slaughtered after two months periods (October, 1994 - December, 1994).

	Age: 248 days Grazing time : 8:00 a.m - 5:00 p.m.			
Parasites	Adults	Stage 5	Stage L_4	Total
Haemonchus contortus	1191	2	-	1193
Trichostrongylus axei	3382	-	-	3382
Trichostrongylus columbriformis	734	-	-	734
Ostertagia spp.	251	13	-	263
Oesophagostomum columbianum	196	-	-	196
Nematodirus spathiger	143	-	-	143
Trichuris spp.	69	-	-	69

Table: 8 Composition of nematodes infection in lambs slaughtered after two months periods (December, 1994 - February, 1995).

	Age: 278 days Grazing time : 8:00 a.m - 5:00 p.m.			
Parasites	Adults	Stage 5	Stage L_4	Total
Haemonchus contortus	918	-	-	918
Trichostrongylus axei	1471	-	-	1471
Trichostrongylus columbriformis	2067	-	-	2067
Ostertagia spp.	432	-	-	432
Oesophagostomum columbianum	227	2#	-	227
Nematodirus spathiger	102	-	-	102
Trichuris spp.	41	-	-	41

: Numbers of nodules present on caecum wall

Table: 9 Composition of nematodes infection in lambs slaughtered after two months periods (February, 1995 - April, 1995).

	Age: 308 days Grazing time : 8:00 a.m - 5:00 p.m.			
Parasites	Adults	Stage 5	Stage L_4	Total
Haemonchus contortus	438	30	-	468
Trichostrongylus axei	2762	79	-	2841
Trichostrongylus columbriformis	1957	-	-	1957
Ostertagia spp.	413	-	-	413
Oesophagostomum columbianum	261	14#	-	261
Nematodirus spathiger	59	-	-	59
Trichuris spp.	32	-	-	32

: Numbers of nodules present on caecum wall

Table: 10 Composition of nematodes infection in lambs slaughtered after two months periods (April, 1995 - June, 1995).

	Age: 338 days Grazing time : 8:00 a.m - 5:00 p.m			
Parasites	Adults	Stage 5	Stage L_4	Total
Haemonchus contortus	309	33	-	342
Trichostrongylus axei	1651	23	181	1855
Trichostrongylus columbriformis	673	36	-	709
Ostertagia spp.	244	14	53	311
Oesophagostomum columbianum	202	11#	-	213
Nematodirus spathiger	49	-	-	49
Trichuris spp.	89	-	-	89

: Numbers of nodules present on caecum wall

Table: 11 Composition of nematodes infection in lambs slaughtered after two months periods (June, 1995 - August 1995).

	Age: 368 days Grazing time : 8:00 a.m - 5:00 p.m.			
Parasites	Adults	Stage 5	Stage L_4	Total
Haemonchus contortus	361	10	-	371
Trichostrongylus axei	405	-	145	550
Trichostrongylus columbriformis	603	-	-	603
Ostertagia spp.	35	-	13	48
Oesophagostomum columbianum	46	5#	-	46
Nematodirus spathiger	-	-	-	-
Trichuris spp.	15	-	-	15

: Numbers of nodules present on caecum wall

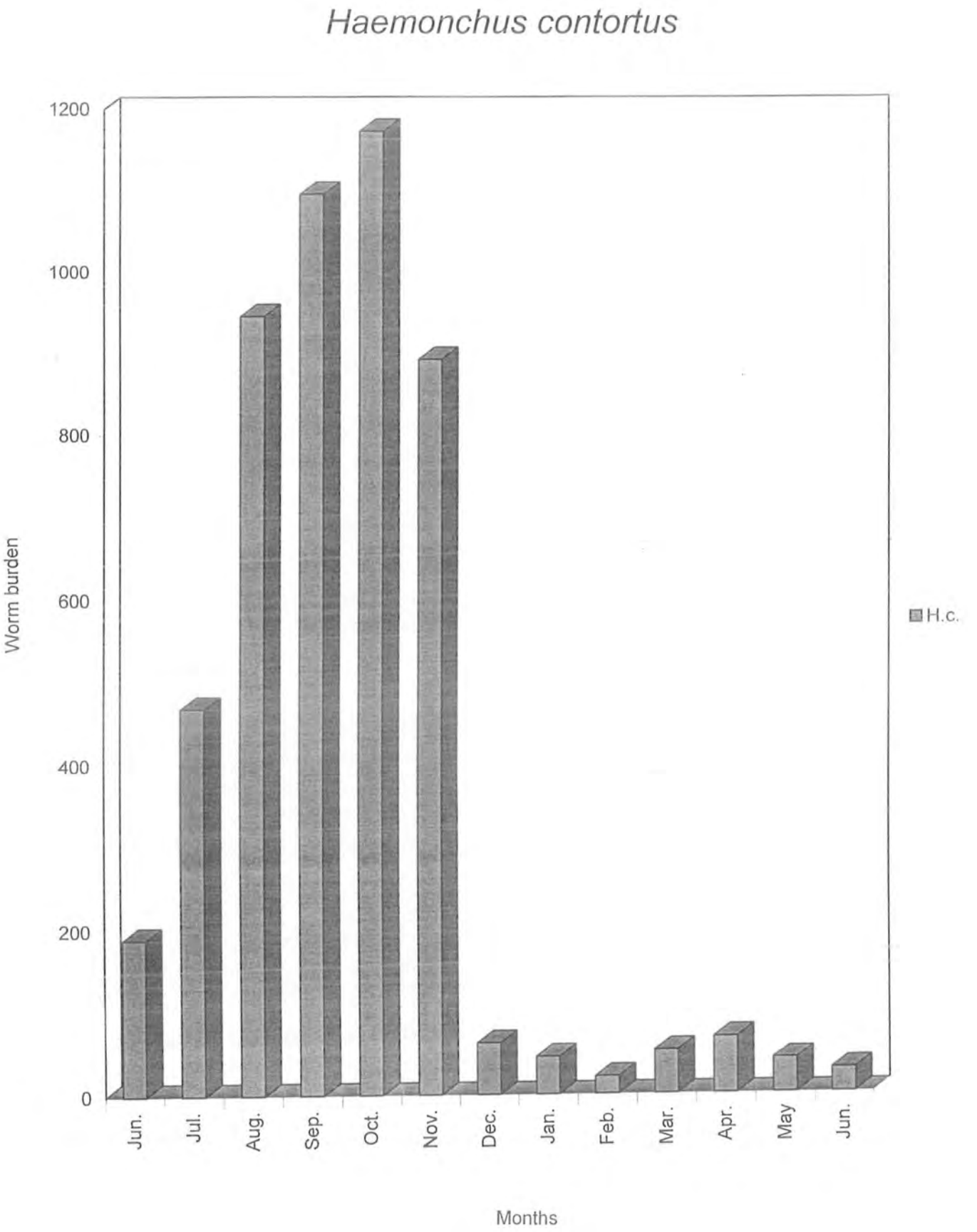


Figure 16. Monthly distribution of *Haemonchus contortus* recovered from slaughtered lamb during 1994-95 period.

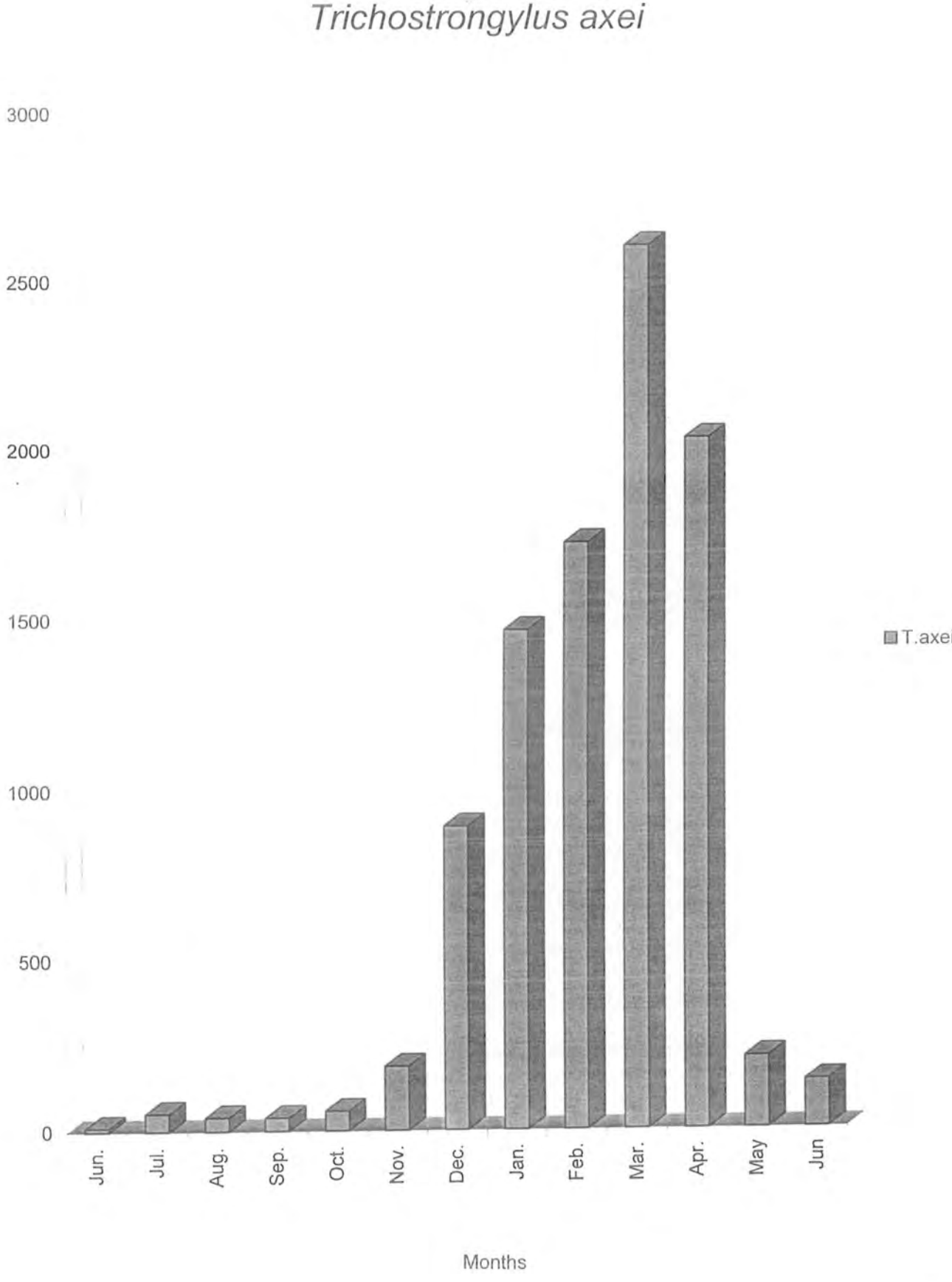


Figure 17. Monthly distribution of *Trichostrongylus axei* recovered from slaughtered lamb during 1994-95 period.

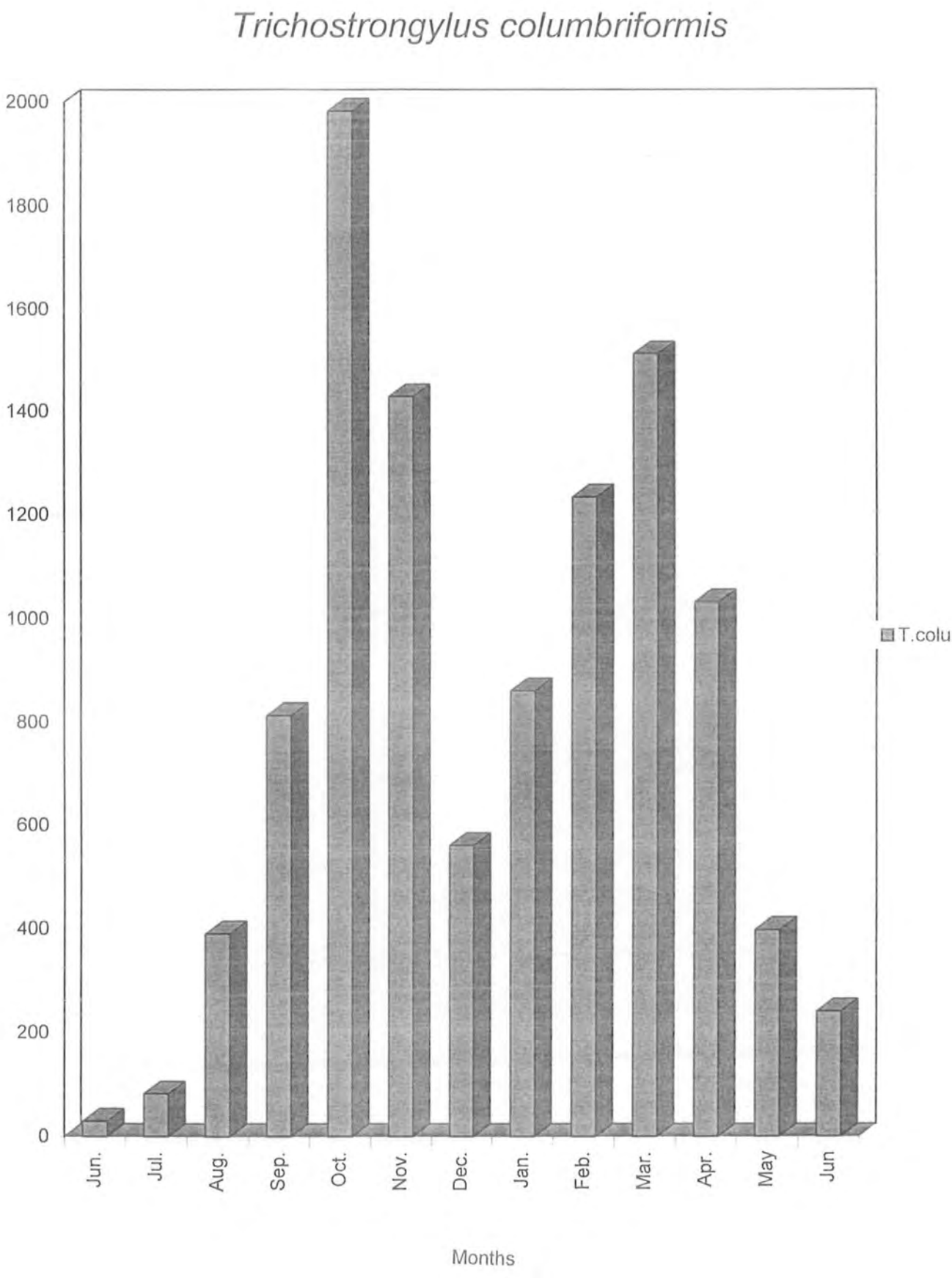


Figure 18. Monthly distribution of *Trichostrongylus colubriformis* recovered from slaughtered lamb during 1994-95 period.

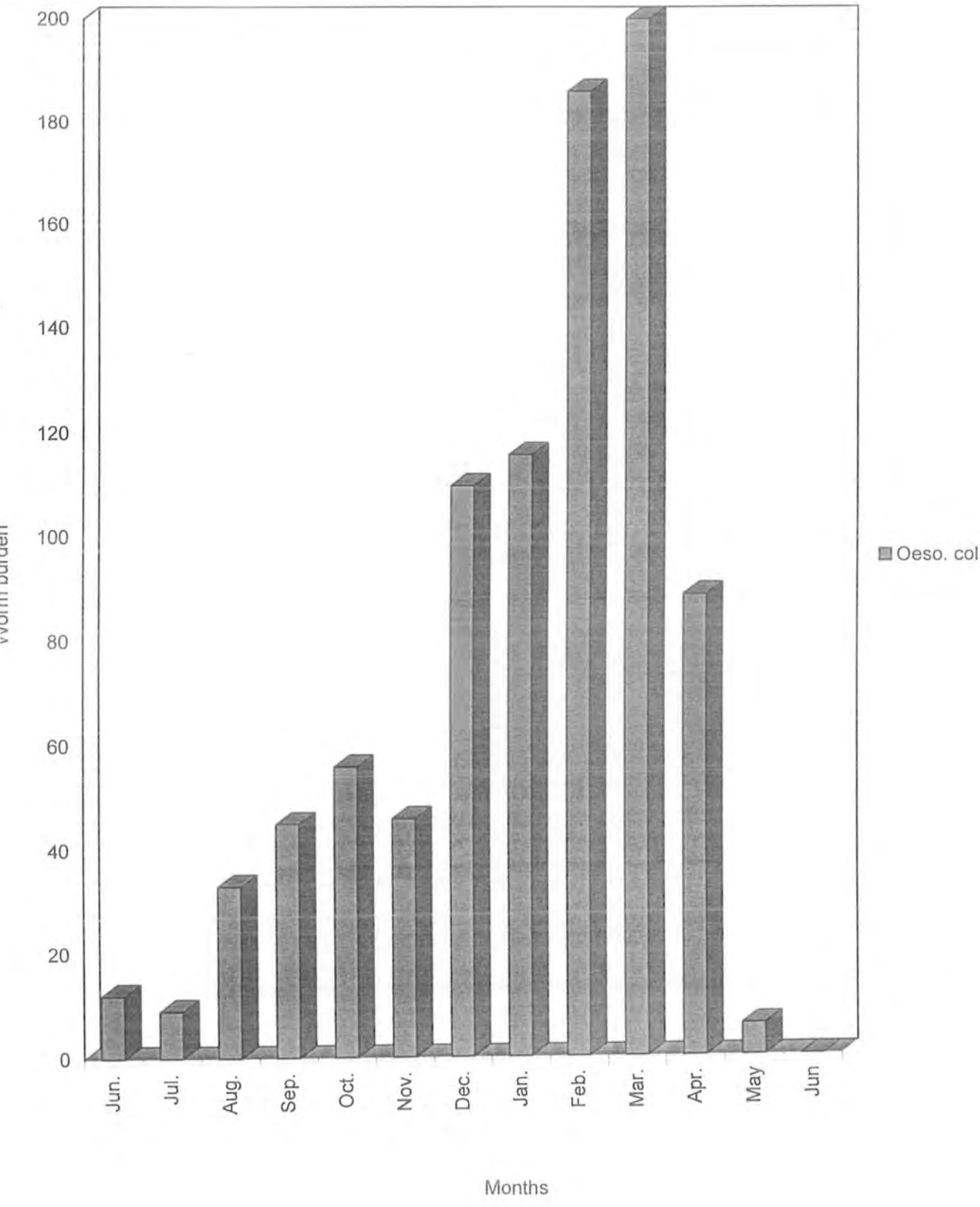


Figure 19. Monthly distribution of *Oesophagostomum columbianum* recovered from slaughtered lamb during 1994-95 period.

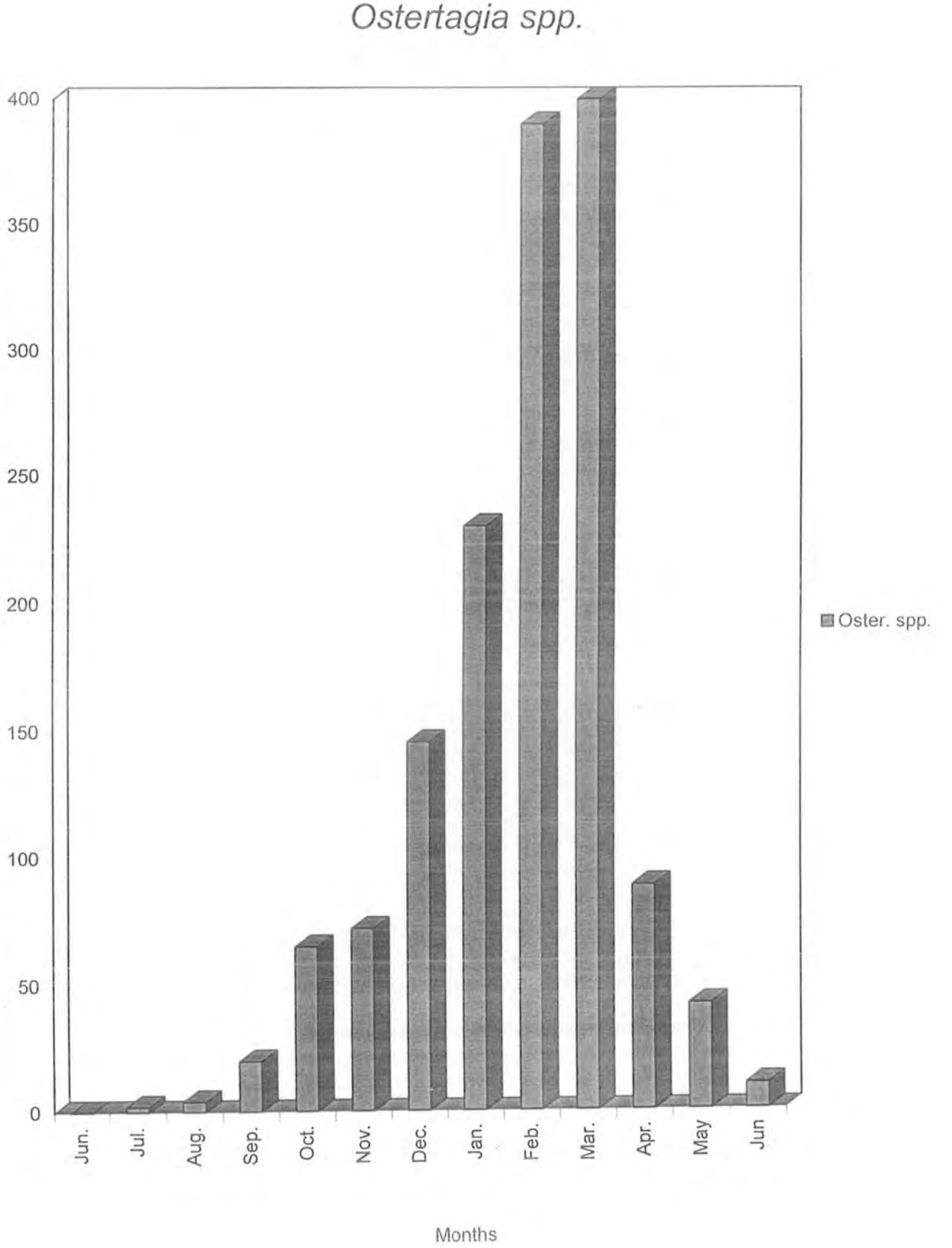


Figure 20. Monthly distribution of *Ostertagia* spp. recovered from slaughtered lamb during 1994-95 period.

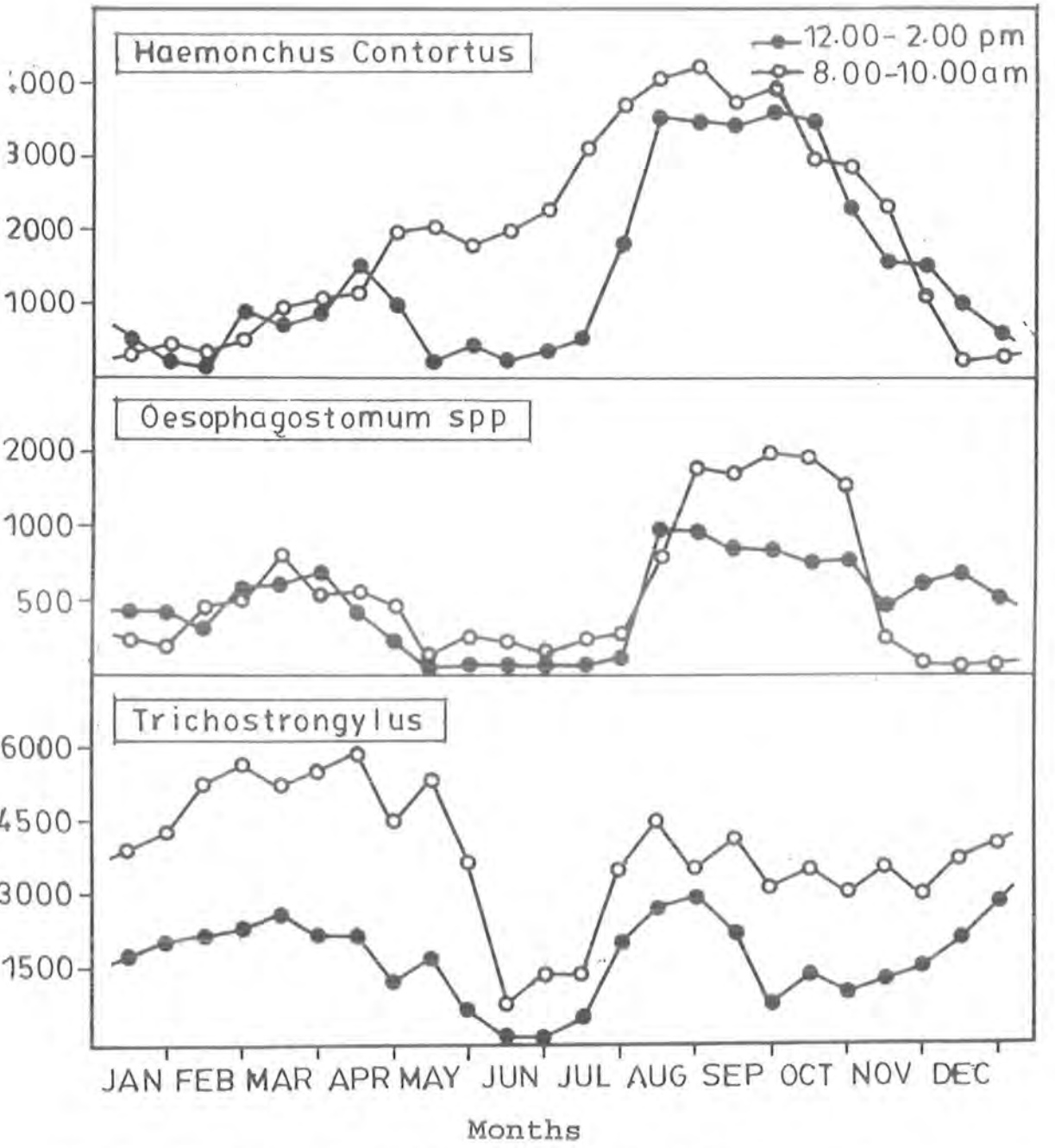


Figure 21. Mean fortnightly pasture larval counts (L_3) of three genera of trichostongyle nematodes recorded during Jan.1993-Dece.193.

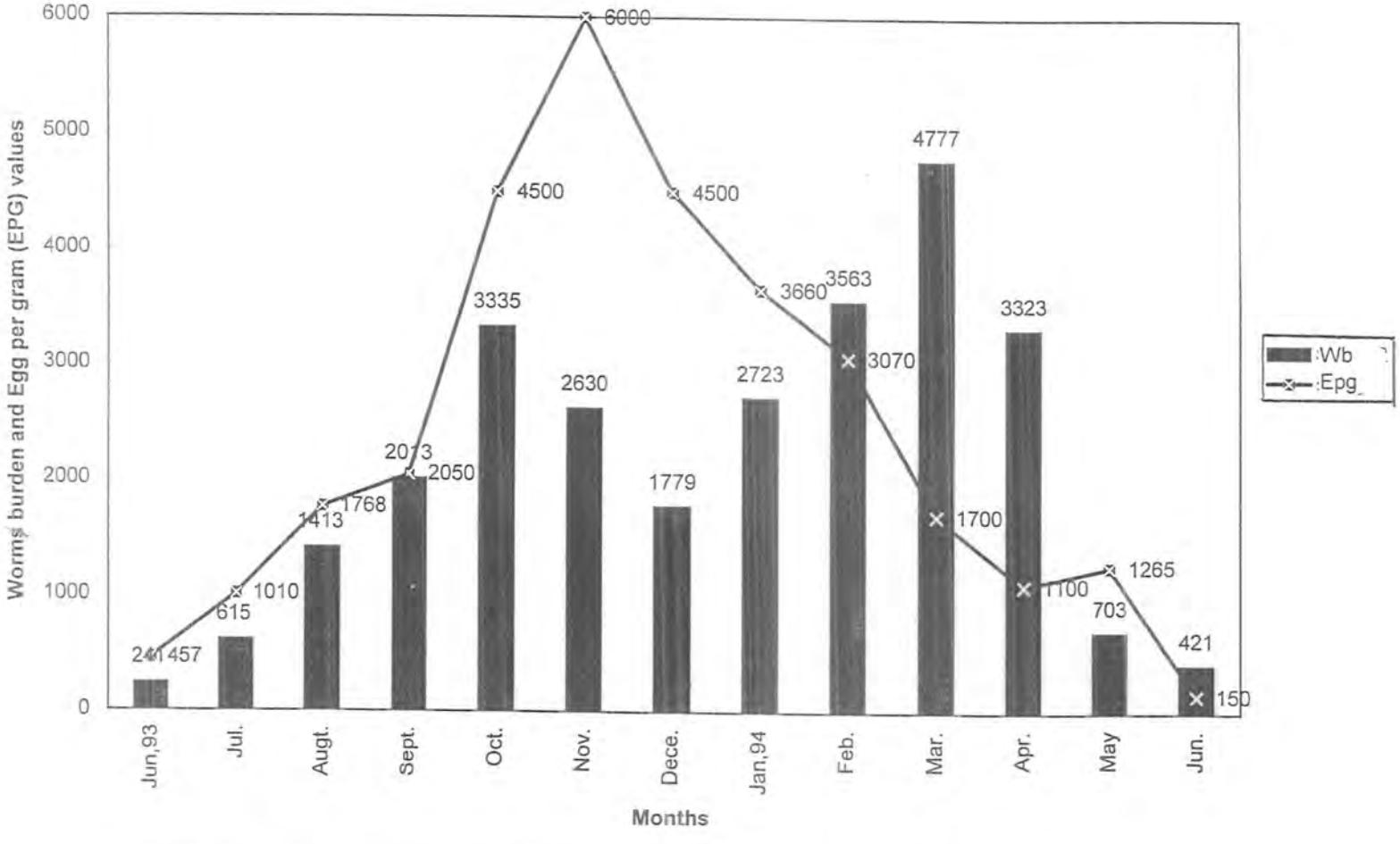


Figure 22. Showed comparative distribution of trichostrongyle worms burden and egg per gram in tracer lambs.

4.7 DISCUSSION

The results of this study indicate the seasonal differences of larvae survival on pastures under semi-arid climatic conditions. The gastrointestinal nematodes found in tracer lambs in this study generally are similar to those reported previously in south-east Asia (Ahmed and Ansari, 1987; Gupta *et al.*,1988; Van Aken *et al.*, 1990). *Haemonchus contortus, Oesophagostomum columbianum, Trichostrongylus* spp., and *Ostertagia* spp., are the most common gastrointestinal trichostrongyles encountered during present study.

As all the nematode parasites have direct life-cycle, the development and survival of the free-living stage are vitally affected by agro-climatic conditions (Armour, 1980). The large variety of nematode species recorded in this study complicates the study of the epidemiology and for the sake of convenience this discussion is dealt with under the separate headings of each trichostrongyle species.

Heavy infection of *Haemonchus contortus* is noted during the rainy season (July-September), following the well distributed rains during this period. Generally conditions are favourable for the development and translation of the free-living stages of this species, when mean maximum temperatures exceed 18 ^{0}C (Dinaburg, 1944) diurnal fluctuation is between 23.3 ^{0}C and 11.6 ^{0}C (Dinik and Dinik, 1958) and mean monthly rainfall exceeds 50 mm (Gordon, 1950, Grant, 1981). These conditions are satisfied in this part of Pakistan, between July-September, and outbreaks of haemonchosis may occur during at least four months of the year. From November

onwards, the degree of pasture infection fells off rapidly to reach its lowest level in December, rising gradually to a moderate level in February and thereafter, increasing markedly in April (Fig.16).

Similarly, pastures are found heavily contaminated with infective stages (L_3) in July-October. But larval counts (L_3) falling from November-January due to some reasons i.e., high morality of these free-living stages as the result of the depletion of energy reserves associated with low temperature and inadequate rain in winter season (Muller, 1968). Similar observations on the availability and abundance of the *Haemonchus contortus* during rainy season have been made previously by Gupta *et al.*(1988), Van Aken *et al.* (1990) and Dorny *et al.*(1995).

Trichostrongylus axei and *T. colubriformis* are also observed throughout the year and show peaks in their occurrence. The peak of intensity of *Trichostrongylus axei* is observed in April, six months earlier than *T. colubriformis*. *Trichostrongylus* spp. These are generally cool season parasites thriving best when mean monthly temperatures ranges from 2.8 ^{0}C-18.3 ^{0}C. They disappear when temperature exceeds 20 ^{0}C (Gordon, 1953). These nematodes seem to be of considerable importance in winter rainfall of this region (Barani region) of Pakistan. It has been noted that during the winter months (November-February) the infective larvae (L_3) are very common on pasture and their level drop to lowest level in hot summer months (May-June). Higher recovery of *Trichostrongylus* spp., from November-March confirm the high prevalence of this specie during November to February (Figs.17 and 18).

Oesophagostomum columbianum has been recovered in relatively

small numbers during the whole study period although some individuals had a worm burden (Fig.19). It is considered high enough to be of pathogenic significance which is in consistent with the finding of Gordon (1950). It was found that the occurrence of *Oesophagostomum columbianum* is highest in tracer lambs necropsied on bi-monthly basis as compared to data obtained on monthly basis. The reason may be due to the fact that the larvae of this parasite show a delayed development in the third to fourth stages when they are encysted in nodules. Previously similar results have been reported by Vercruysse (1983). Presence of this species in December-February pose a serious threat not to only the lambs but also to adult animals. The average worm burden of 105 - 153 adult worms per lamb in 6-12 month old weaners was from March-April, while it has been indicated that 200 worms in adults and 80-90 in young sheep would constitute a sever infection (Gordon, 1950). The warm, moist summers experienced in this region (July-October) are well suited to the development and survival of the free-living stages of this species (Figs.3 and 4). *Oesophagostomum* spp. show little resistance to desiccation and would be unable to survive long dry winter (Kates, 1950; Crofton, 1963). These observations are in conformity with present findings because from January to June relatively small number of infective larvae (L_3) were recovered from the herbage (Fig.20).

As far as *Ostertagia* spp. are concerned the temperature and moisture requirement of *Ostertagia* spp. are similar to those of *Trichostrongylus* spp., since the highest numbers were recovered during cooler, moist months from autumn to spring (October-April).

As soon as the mean temperature drops below 20 ^{0}C in winter, the recoveries increase rapidly. The observations that the free-living stages of this genus thrive in cool moist conditions has been shown by the other workers also (Kates, 1950; Gordon, 1953; 1958; Crofton, 1963; Levine, 1963).

Strongyloides papillosus is of little pathogenic importance, but moderate number of adult worms were recovered throughout the study, having maximum intensity during rainy season (July-September). This finding agrees with the suggestion of Grant (1981) that *Strongyloides papillosus* shows little resistance to desiccation due to absence of sheath. Moreover, it penetrates the host through skin and reach the small intestine via the blood stream, lungs and trachea. Higher number in rainy season may be due to the fact that lambs get this infection when they are housed in thorn made enclosures and have close contact with contaminated soil.

Trichuris spp. were observed infrequently, therefore, seasonal patterns could not be observed. Although the recovery of this parasite is higher during the rainy season it is to be pointed out that *Trichuris* spp., has no free-living larvae. The host is infected by swallowing the eggs in which the first-stage larvae has developed (Crofton, 1963). The importance of this nematode is reported to be very slight. This finding is in general agreement with the observations of Specht (1982).

Nematodirus spathiger follows a similar trend in the winter as that of other *Trichostrongylus* spp. The moderate level of this specie occur in those months when mean monthly temperature is less

then 20 ^{0}C. It is in conformity with the findings of Levine (1963). According to him maximum number of *Nematodirus* spp. are found at 6 ^{0}C to 20 ^{0}C.

The number of eggs present at a given time is directly linked to the length of stay of a host animal in an area. The population of infective larvae which eventually results depends on the ways in which environmental conditions affect the development of these eggs. It is likely to be mentioned that time period allowed to the lamb in present study was 30 days. As a result lamb got maximum exposure to free-living stages. Our findings are not in conformity with that of Gupta *et al.* (1987) who used tracer lambs for a short time period.

In tracer lambs the maximum number of *Ostertagia* spp. and *Trichostrongylus* spp. was recovered during November-July, while that of inhibited larvae of both species were encountered during May-June. This inhibition occurred during hot summer days (May-June), when the atmospheric temperature on some days goes as high as 42 ^{0}C, suggests that inhibition of *Ostertagia* spp., and *Trichostrongylus* spp., larvae in the sheep of this country is not associated with the onset of winter. These findings are in contrast to the reports from the northern temperate zones of the world, where the trigger to naturally occurring inhibited larval development is believed to be chilling or falling temperatures (Armour, 1970; Michel *et al.*, 1974). It seems that maturation of nematode larvae doesn't take place during the summer months, (May -June) in this part of Pakistan. The results of present study are consistent with the findings of Ogunsusi and Eysker (1979) and

Altaif and Issa (1983) in North Nigeria and Iraq, respectively, where inhibited larvae of *Ostertagia* spp., are markedly high during the summer months. According to them, the inhibition of these species is brought about by a environmental stimulus acting upon free-living larval stages baring hot dry summer months. The most abrupt reductions in larval numbers on natural pasture occurs during the hottest months and coincides with the shortest length of survival of larvae. It is believed that rain was necessary for development of eggs and larvae to the infective stage in pellets. Moreover, rain may also be required for movement and translation of infective larvae on to herbage where they are available to sheep.

In this study it is observed that higher number of trichostrongyle eggs are shed in the faeces from July-October, which is consistent with the finding of Gupta *et al.* (1987). The higher faecal egg counts during these months may be largely due to highest prevalence of *Haemonchus contortus* from July to October, as this parasites is consider more prolific egg producer than *Trichostrongylus* spp (Grant, 1981). Similar results are reported by Vercruysse (1983) who reported that heavy infection (2000-3000 adult worms) was common in the rainy season and further reported that the *Haemonchus contortus* together with *Oesophagostomum columbianum* contribute to elevate the level of eggs in the faeces. From the present results the faecal egg counts is not found to be a reliable measure of the size of the trichostrongyle worms burden. These results are in agreement with the findings of Rubin (1967), Smeal *et al.* (1973), Rose and Small (1980) and Ndao *et al.* (1995). According to them faecal egg counts may be influenced by variables

such as faecal out put, composition of infection and host resistance. However, the discrepancy of egg per gram and worms burden in an individual animal is likely to be reduced when egg counts of a large number of animals are available (Stampa and Linde, 1972; Roberts and Swan,1981). Similarly, McKenna (1981) viewed that when eggs and worm counts are categorized according to the concept of low, moderate and high then the association between them was found to be almost equal consistent in all age classes of sheep. It is to be pointed out that the present data about these two parameters was obtained from tracer lambs of comparable age groups. It is thought that major drawbacks of egg per gram cannot provide the information about the composition of infection, as the egg of various trichostrongyle genera cannot be differentiated. Moreover, the egg counting provides little evidence about the occurrence of immature worms.

It can be concluded from the above discussion that heavy monsoon rainfall occur from the month of July to September. The number of rainy days in these months are 9, 15, and 20, respectively. The period between November-December and May-June constitutes the drier season of the year having 2-1 and 3-2 rainy days, respectively. The number of rainy days from January-April varies from 8-11. The profile of the graph of worm counts data parallels that of total rainfall and, therefore, it is reasonable to assume that worm count peaks occurred when the availability of moisture was optimum (Fig.4) Similarly, temperature is also optimum during most part of the year. The overall picture indicates that the semi-arid (sub-tropical) environment of this part of Pakistan

is favourable for the development of various trichostrongyles species of nematodes, namely *Haemonchus contortus*, *Trichostrongylus* spp., and *Oesophagostomum columbianum*.

From the foregoing discussion it seems that sheep in the sub-tropical (Barani) areas have a high probability of acquiring infection from pasture during and immediately following the wet seasons. Therefore, it would be appropriate to treat sheep before the onset of wet season to reduce the pasture contamination.

HOST-PARASITE RELATIONSHIP STUDY

CHAPTER 5

5.1 INTRODUCTION

In the view of the epidemiology of trichostrongylosis, it is of importance to know the spring rise is a yearly feature in a flock of sheep by which the transference of the nematode population of the adult animals in the spring born lambs is made possible (Uriarte and Valderrabano, 1989; Lyons *et al.*, 1992; Fleming, 1993). This phenomenon of egg-rise during the peri-parturient period has previously been described (Brunsdon, 1964a; Dunsmore, 1965; Tetley and Langford, 1965; Southcott *et al.*, 1972; Gibbs, 1977; Yazwinski and Featherstone, 1979; Jansen, 1987; Lyons *et al.*, 1987; Waller *et al.*, 1987; Jackson *et al.*, 1988).

An increase in nematode faecal egg counts of sheep during spring was first described by Zawadowsky and Zvjagvintzav (1933) in Russia, but only achieved global recognition following the studies by Scottish workers, commencing with those of Morgan and Sloan (1947). Crofton (1957) introduced the name post-parturient rise for the phenomenon because he found that the rise in egg-counts was seen mainly in sheep after parturition. Similarly, Jacobs (1966) introduced the term peri-parturient rise commences already before parturition which is, however, also the case in sheep. The phenomenon of the peri-parturient rise appears to be of considerable importance in nematode transmission in sheep, goat and swine, but absent or moderate in horses and cattle (Michel, 1974).

Increased egg production in lactating ewes compared with non-lactating ewes may result from several factors including the

resumptions in development of larvae previously arrested in their development, increased fecundity of existing adult female worms, the development to maturity of new infections and possibly the failure to eliminate existing infections (Fleming *et al.*, 1988).

The source of the peri-parturient rise (PPR) varies in different geographical regions. In arid or semi-arid zones where survival of infective stages as negligible during the dry season, the development of arrested larvae makes the major contribution (Armour, 1980). In temperate zones where larvae on pasture survive the winter in reasonable numbers, the acquisition of fresh infections and the maturation and maintaintance of the resulting gravid populations also make a significant contribution to the peri-parturient rise (Reid and Armour, 1972).

Although the prevalence of gastrointestinal nematodes of goats and sheep in Pakistan is documented (Shah *et al.*, 1980; Khan *et al.*, 1989), no information is available regarding the role of peri-parturient rise (PPR) on the epidemiology. It was found that the sub-humid, tropical environments is very favourable for year-round development and survival of pre-parasitic stages of trichostrongyles on pasture (Ikeme *et al.*, 1987).

Under these conditions it is important to know as to what extent the parasitological factors which contribute pasture contamination in sub-tropical area of Pakistan. The purpose in the present study is to demonstrate the possible occurrence of this phenomenon in naturally infected sheep in Barani region (sub-tropical) to identify the principal nematode species involved.

5.2 MATERIALS AND METHODS

5.3 STUDY AREA:

The study was carried out, in 1992-1993, at small ruminants farm, located in the jurisdisication of districts Rawalpindi-Islamabad of northern part of Punjab Province (33^{0} N 42, 73^{0} E 08').

The climate of the region is of sub-humid type and characterized by annual rainfall of 737 mm and a marked rainy season (July-September). There is a wide variation in minimum (3-15 ^{0}C) and maximum temperature (15-36 ^{0}C). The average relative humidity is 64 per cent over the entire year. The pasture is consisted of shrubs, herbs and perennial grasses typical of rainfed area.

5.3.1 *Animal Management:*

Local indigenous ewes and lambs used in the study were Latti type and commonly known as "Salt Range". In the morning the animals grazed extensively on roadsides, crop stubbles, fallow and common lands and brought back to the holdings by sunset. At the beginning of the study there were approximately 250 sheep in the herd. In winter, they were housed at night in a variety of stone and mud made houses and since summer 1993, they were kept in specially made enclosures made from thorn wood and straw. During the day they grazed over areas of naturally growing grasses regenerating shrubs and herbs. Animals drink from wells practically throughout the year except in the rainy season when they drink from temporary ponds. The animals selected for this study had not received anthelmintic during the one and half year prior to the

start of study till it is completed. Moreover, these animals did not receive complementary feeding during the whole year.

5.4 DESCRIPTION OF THE TRIAL-I

Initially, three herds in three villages of Fetah Jang area, were selected for a this trial (July, 1992-October, 1993). However, as the success of the regular sampling depended on the goodwill of the farmers, two herds was quickly abandoned. Therefore, only one herd could be studied over a one year period without any interruption. Out of 250 ewes in the study 200 were ear-tagged.

A total of 200 ewes and their lambs were randomly selected. All ewes had maximum exposure to pasture in the pervious grazing season. Lambing commenced on 28 February , 1993 and was completed by 4 April, 1993 with mean lambing date of 19 March, 1993 and majority of the lambing completed by 3 April, 1993.

DATE	CHARATERISTICS
TRIAL-I	
20 September-1 October, 1992	One flock of 200 ewes were exposed to teaser rams.
20 October-5 November, 1992	Ewes mated.
1 January, 1993	41 barren were selected as a control (BE).
2 January, 1993	37 pregnant ewes selected as (LSE).
28 January, 1993	30 pregnant ewes were selected, as (NLSE).

28 February-4 April, 1993	Lambing period.
15 May, 1993	Two ewes were died of some disease.
TRIAL-II	
16 May, 1993	92 ewes and their lambs were randomly selected per experiments.
17 May-30 September, 1993	The lambs were allowed to run with mothers until weaned at June-July.

From July, 1993 to August, 1993, 11 ewes were died due to some disease. But their gastrointestinal tract was regularly examined for the any trichostrongyle infection.

5.5 DESCRIPTION OF TRIAL-II

In this trial 92 ewes and their lambs were randomly selected. All these ewes lambed inbetween 20th February, 1993 to 4 April, 1993. Moreover they did not receive any anthelmintic treatment and all of them showed faecal egg counts rise during parturient period and therefore serve as source of pasture contamination. During this period their lambs were kept on supplementary feed and on their dam milk. After weaning in June, 1993 these lambs were allowed to run with their dams on naturally contaminated pasture. After two weeks of grazing the faecal samples were taken from these lambs in order to detect the level of trichostrongle infection while the composition of nematodes were determined after the autopsy of lambs

on monthly basis.

5.6 PARASITOLOGICAL PROTOCOLS:

5.6.1 *Faecal collection and faecal worm egg counts:*

Rectal faecal samples were collected weekly from each ewe and lamb randomly selected beginning from four week prior to parturition and continued for 12 weeks after each ewe lambed. The faecal samples was collected directly from the rectum of the ewes by following procedure described by Swan (1970). Faecal egg counts were done by the modified McMaster technique (Skerman and Hillard, 1966). The eggs were identified as *Strongyloides*, *Nematodirus* spp., and trichostrongyle, while the eggs of *Trichuris* spp. and *Moniezia* were seen on occasion.

5.6.2 *Faecal culture of infective larvae:*

Faecal cultures were made from the fortnightly specimens used for the egg counts by using the method of Roberts and O'Sullivan (1950). Third stage larvae (L_3) were identified as characterized by MAFF (1979) and Jackson *et al.* (1986). The first 100 or 200 larvae were used to derive the population composition, and where the larval harvest was lows, at least 25 larvae were used. The larvae were recovered from culture by Baermannization process (MAFF, 1979).

5.6.3 *Necropsy worm counts:*

With ligations made at the pyloric and fundic regions of the abomasum, at the ileocolic junction and at the end of the rectum, the gastrointestinal tract was brought to the laboratory. The worms were collected by sieving through a series of standard screens (71, 100 and 200 um mesh sizes). One tenth of the ingesta

was then fixed in 10 per cent formalin until the worms were counted. The abomasal mucosa was scraped off and digested in 1 per cent pepsin-2 per cent HCl digested at 37°C for six to eight hours. Larvae present were collected by sieving (400 um mesh screen), counted and identified. The worms recovered were recorded as either adults or immature, the latter representing dormant larvae. The identification of the immature was done according to Douvres (1957).

5.7 RESULTS

In the first trial the mean fortnightly trichostrongyles egg output of the three groups viz., lactating suckled ewes (LSE), lactating non-suckled ewes (NLSE) and the barren ewes (BE) are plotted in Figure 23. While the frequencies of trichostrongyle genera is given in Table 12. From October, 1992 to January, 1993, the egg counts of lactating suckled ewes (LSE) remained at low level, from 250 to 1100 epg until the last week of February. But reached a peak of over 6300 epg by the mid of April, when the ewes and their lambs were run on pasture. Observations of individual egg output showed that periparturient rise occurred in 92 per cent of the ewes following lambing. However, individual peak counts ranged from 850-14500 epg which occurred at times varying from four to twelve weeks after the lambing. Figure 23 depicts that the majority of the ewes showed maximum epg counts during the mid and last week of April. This spring rise was dropped to 3,500 epg and continued to decline until it reached a low level of 2,800 in last week of June. The epg count increased again during the following few weeks and another peak of 5,000 epg was attained in the last week of August. Further decline in epg counts and subsequent peak was seen in the last week of September.

As far as non-suckled lactating ewes (NSLE) were concerned, the egg counts started declining from mid of October to mid of December, then it rose steadily from the first week of January to a maximum peak in the last week of April. But no significant peak was observed as it was found in suckled-lactating ewes (SLE). A similar trend was observed in barren ewes (BE) where the level of

epg counts was relatively lower than that of non-suckled lactating ewes (NSLE). In these ewes intermittent fluctuations were observed from the start of the trial until it was completed.

5.7.1 *Worm counts in necropsied ewes:*

Haemonchus contortus, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Nematodirus spathiger*, *Ostertagia spp.* and *Oesophagostomum columbianum* were recovered at necropsy. The data on the genera and species of these nematodes are presented in Table 13. Individual ewes harboured upto five mixed trichostrongyle species and worm burdens varied between group died at different times of the trial. The lowest average counts of *Haemonchus contortus* occurred in the winter months of this trial. The majority of the worms of this parasite were recovered from ewes died during March-May.

Ostertagia spp. were recovered in all the ewes died during the months of November-May. *Trichostrongylus axei* was more numerous during the winter months than either *Haemonchus contortus* or *Ostertagia spp.* It occurred in all sheep in substantial number varying from 617-2,100 in almost all adults from November to May. As far as *Trichostrongylus colubriformis* is concerned substantial numbers of worm of this species were recovered during March-May.

Oesophagostomum columbianum were frequently recovered from large intestine. In spite of the fact that there was a large number of nodules found in ewes during November-December, a large number of adult worms were recovered during March-May.

5.7.2 *Larvae on pasture:*

The distribution of trichostrongyle larvae of each

species is presented in Fig.24. Examination of individual counts revealed that the infective larvae of *Haemonchus contortus* were the predominant species from March to onward and attained peak on September 27. *Trichostrongylus axei* reached peaked numbers of abundance between October-April, having the maximum numbers of larvae in the last week of January. While *Ostertagia* spp., were the major source of infection between November and April. On the other hand the infective larvae of *Trichostrongylus colubriformis* occurred from February to July attaining a peak in the last week of May. Similarly *Oesophagostomum columbianum* was the most numerous nematode between October to May. Table 14 presented the generic composition of trichostrongyle nematodes.

5.7.3 ***Egg counts in the lambs:***

Egg and worms counts data for the lambs indicated a gradual increase from first week of July (Fig.25). From mid July through the end of the trial, worm counts increased more rapidly with the highest number being found in lambs examined in August. The nematodes present in high numbers were *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Oesophagostomum columbianum*. These three parasites increased in numbers throughout the study with highest worms recovered in lambs examined in August. Worm counts and egg counts data had general correlation, although egg per gram counts for the lambs examined on July 15 were much lower than be worm count data. The composition of trichostrongyle infection is presented in Table 15.

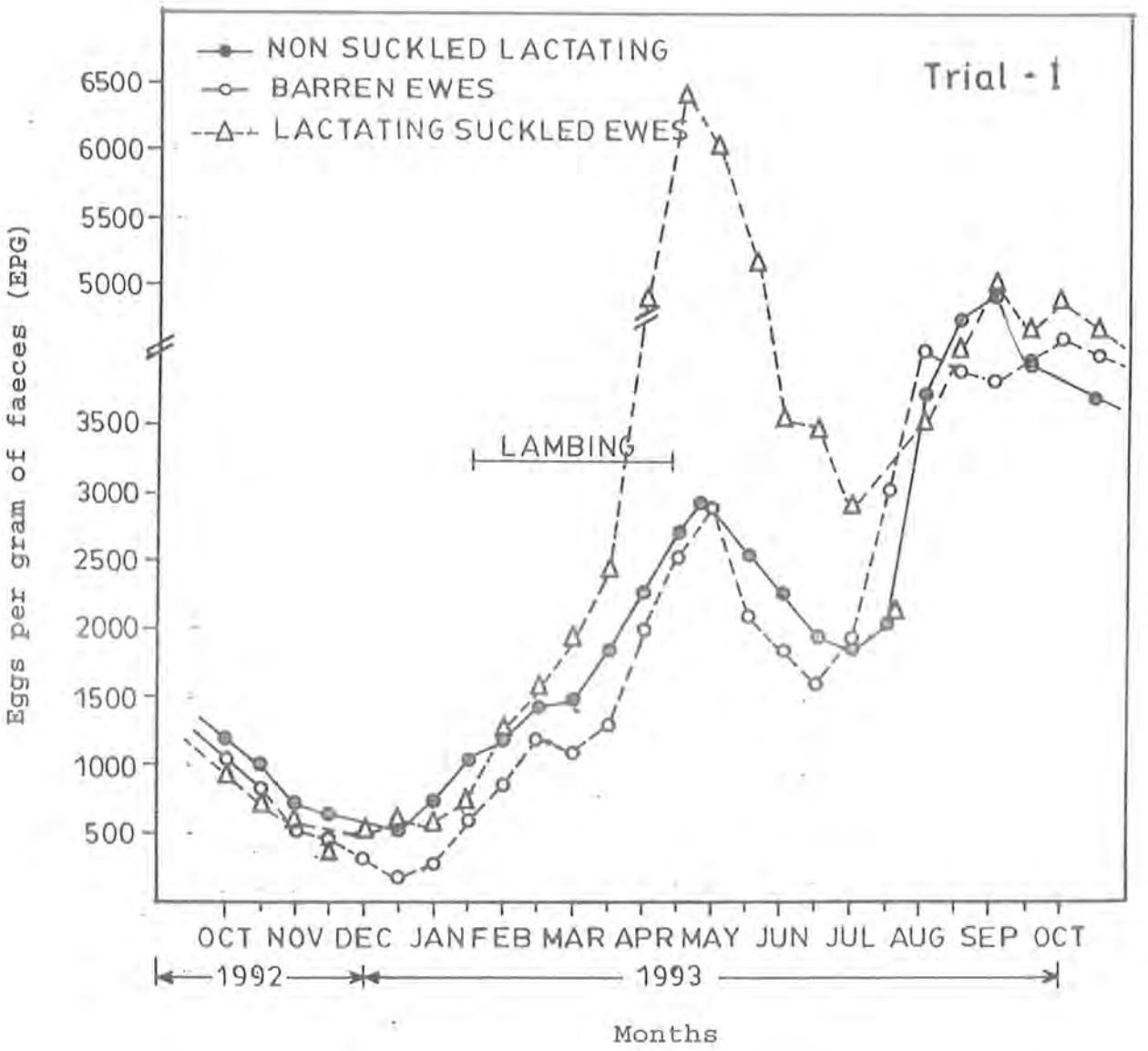


Figure 23. Mean fortnightly trichostrongyles egg out put of lactating suckled ewes (LSE), lactating non-suckled ewes (NLSE) and barren ewes (BE) recorded during lambing season.

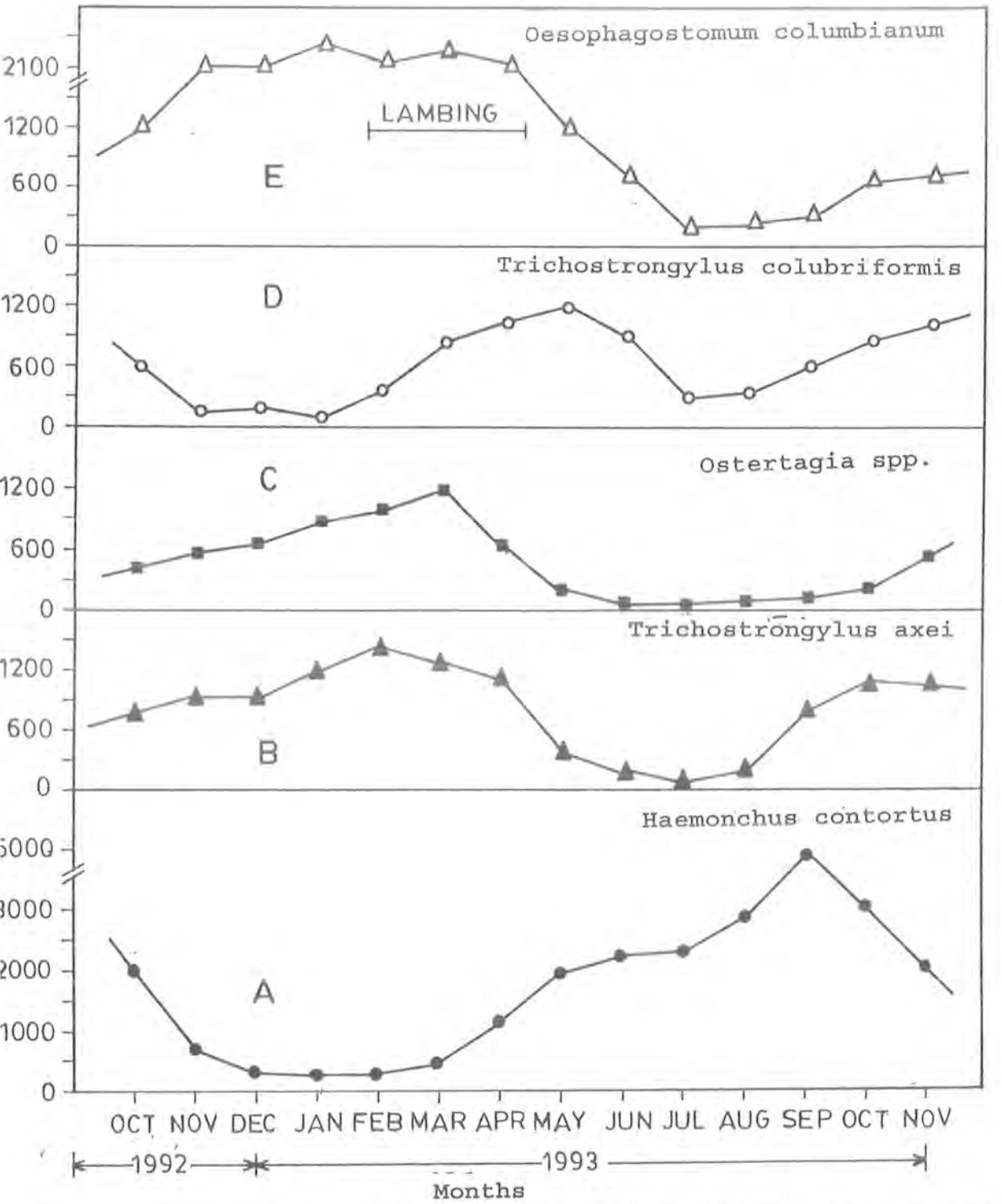


Figure 24. Mean fortnightly distribution of different trichostrongyle larvae (L_3) recorded during 1992-93.

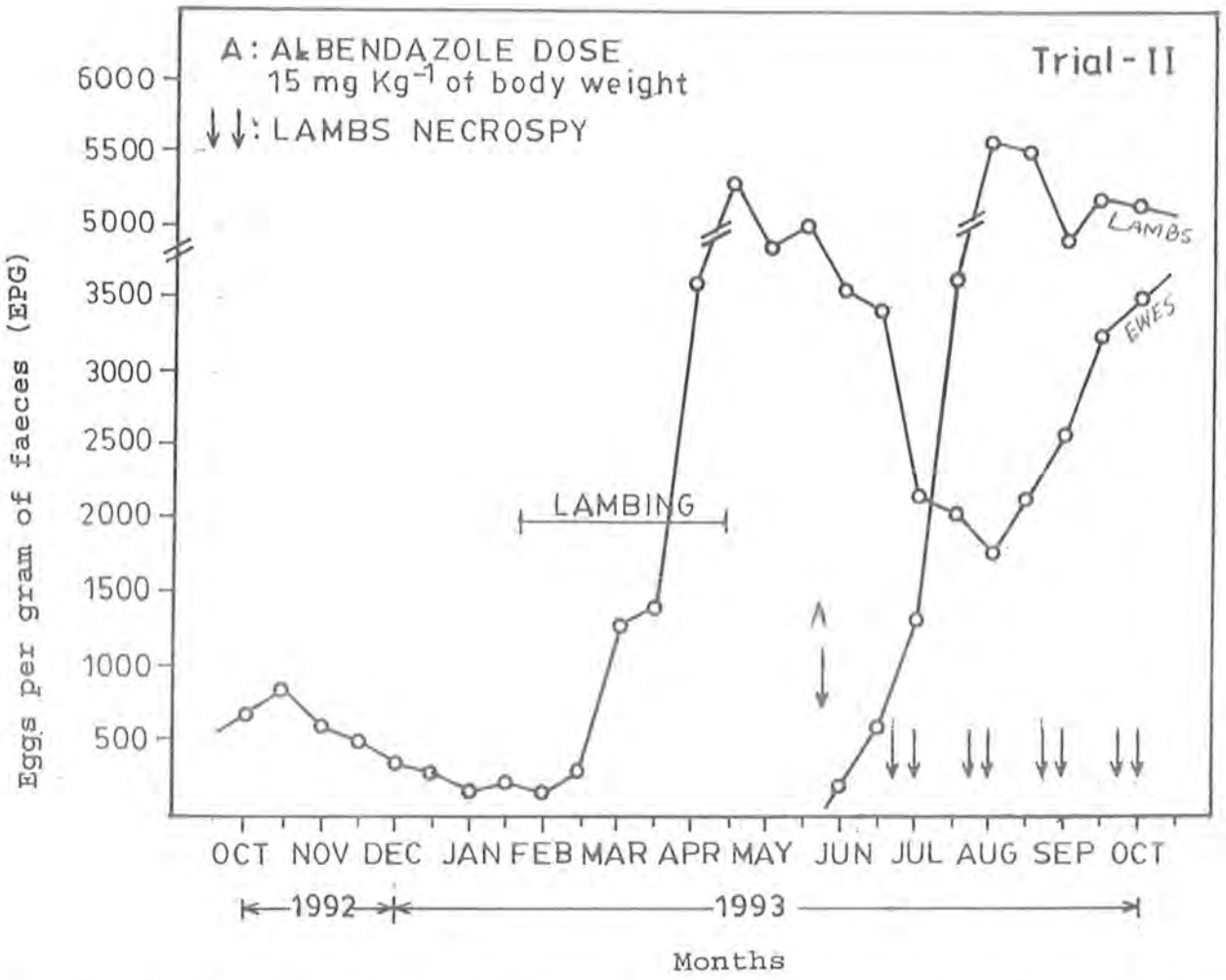


Figure 25. Mean periparturient egg rise in ewes and subsequent eggs per gram rise in lambs.

Table 12. The frequencies of trichostrongyles found during lambing period (February, 1993-April, 1993).

Parasites	WORM BURDEN OF strongyles		
	Barren ewes(BE)	Lactating ewes with suckled lambs (LSE)	Lactating ewes with non-suckled lambs (LNSE)
Haemonchus contortus	32	35	37
Trichostrongylus spp.	17	28	32
Ostertagia spp.	20	26	23
Oesophagostomum columbianum	31	34	26

Table 13. Trichostrongyles worm counts for the set of ewes died due to some disease during the trial-I.

		Sites of predilation					
		Abomasum			Small intestine		Large intestine
Date of death	Tag No.	*Haemonchus contortus*	*T. axei*	*Ostertagia spp. ($)*	*T. columbriformis*	*Nematodirus spp,.*	*Oesophagostomum columbianum*
Group-I (LSE)							
25-11-92	175	45	617	0	30	0	3
09-01-93	81	26	939	20	70	0	81
03-03-93	105	60	1000	57	800	13	105
29-04-93	73	80	1100	60	1100	16	99
Group-II (LNSE)							
01-10-92	117	36	703	80	20	0	0
06-12-92	190	21	590	150	0	2	40
26-02-93	45	80	1100	250	100	0	76
17-03-93	135	100	1400	200	1900	10	144
04-05-93	120	150	2100	0	2300	25	30
Group-III (BE)							
02-11-92	99	26	365	135	276	20	29
12-01-93	155	16	370	92	340	8	146

$: Ostertagia circumcincta & O. trifurcata.

Table 14. Percentage of generic composition of trichostrongyles recovered from the cultures of bulked faecal sample from each group.

Parasites	Percentage of strongyles (%)		
	Barren ewes(BE) N=41	Lactating ewes with suckled lambs (LSE) N=37	Lactating ewes with non-suckled lambs (LNSE) N=30
Haemonchus contortus	38	45	52
Trichostrongylus axei	13	12	8
Trichostrongylus columbriformis	16	8	21
Ostertagia spp.	4	2	1
Oesophagostomum columbianum	28	33	17
Nematodirus spp.	1	0	1

Table 15. The composition of trichostrongyles infection of weaners at necropsy.

Group No.	Date of necrospy	No. of animals	Organs	Parasites	No. infected (%)	Worm load Range	Worm load Mean
1	15/08/93	2	Abomasum	*H. contortus*	2(100)	46-67	56
				T. axei	2(100)	30-168	99
				Ostertagia spp.	1(50)	0-85	85
			Small intestine	*T. columbriformis*	2(100)	246-620	433
				N. spathiger	1(50)	0-18	18
			Large intestine	*O. columbianum*	2(100)	4-13	8
2	20/09/93	2	Abomasum	*H. contortus*	2(100)	139-340	239
				T. axei	2(100)	10-98	54
				Ostertagia spp.	2(100)	46-188	117
			Small intestine	*T. columbriformis*	2(100)	145-385	265
				N. spathiger	2(100)	10-30	20
			Large intestine	*O. columbianum*	2(100)	29-44	36
				Trichuris spp.	1(50)	4-16	10
3	29/10/93	2	Abomasum	*H. contortus*	2(100)	250-871	560
				T. axei	2(100)	200-608	404
			Small intestine	*T. columbriformis*	2(100)	330-579	554
			Large intestine	*O. columbianum*	2(100)	29-140	84
				Trichuris spp.	1(50)	0-10	10

5.8 DISCUSSION

The periparturient rise in faecal counts of ewes in the present investigation is in consistent to that reported in other parts of the world (Brunsdon and Vlassoff, 1971; Blitz and Gibbs, 1972b; Ayalew and Gibbs, 1973; Yazwinski and Featherstone, 1979; Herd *et al.*, 1983; Gibbs and Barger, 1986; Courtney *et al.*, 1986; Jansen, 1987; Lyons *et al.*, 1987; 1992). while the results further confirm that *Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichostrongylus* spp., are the major contributor to the periparturient rise in this study area. Crofton (1954) stated that peak egg counts are closely associated to time of parturition and occur six to eight weeks after lambing. This is not in conformity with present findings in which the peak faecal egg output are spread between four to twelve weeks after lambing i.e. from mid of February to end of May.

Moreover in this investigation, lactating suckled ewes (LSE) acquire high parasite burdens than that of non-suckled lactating ewes (NLSE) and barren ewes (BE), which is in agreement with the results of Fleming *et al.*(1988). This phenomenon may be regulated by the immunhormonal physiology of pregnancy and lactation (O'Sullivan and Donald, 1970; Stites and Siiteri, 1983; Rehman and Collins, 1992; Gessert, 1995). Another cause of a periparturient rise in egg counts has been attributed to the resumptions of development of arrested larvae within host (Brunsdon, 1967; Gibbs, 1967; Blitz and Gibbs, 1972a; Armour, 1980). Previously no data on arrested development in sheep strongyles is available. It is generally accepted that hypobiosis is associated with climatic

changes that are unfavourable for the continued development of the pre-parasitic stages of the trichostrongyles in the external environment (Armour *et al.*, 1969a; Michel *et al.*, 1970; Armour and Bruce, 1974). These can be extreme cold in temperate areas or extreme heat and dryness in tropical or sub-tropical areas.

Examinations of Figures 24 and 25 reveals that results are consistent with the contention that periparturient rise is due to continuous acquired infection from pasture as the environmental conditions are conducive for the rapid development of free-living stages of *Haemonchus contortus* and *Trichostrongylus* spp. Apart from this Brunsdon (1971) claimed that the principal factors governing the magnitude of the rise are most likely to be the degree and specificity of the relative loss of host immunity which occurred at the time of lambing. Brunsdon (1971) hypothesis is further strengthened by other workers (O'Sullivan and Donald, 1970; Michel *et al.*, 1979; Donald *et al.*, 1982). After passing dry spell in winter (November-December) the period of highest rise in acquiring infection is February-April, when the rainfall is moderate (Fig.4). Ewes are either in late gestation or in lactation. In this area which is charactertically sub-tropical and sub-humid is highly contaminated with infective larvae of strongyle species. But from July-October pastures are heavily grazed by both sheep and goats resulting in reduced pasture availability in the forth coming months (November-January).

It has been observed that March to April is a pasture growing season when the plenty of pasture is available to lactating ewes. From these results it is apparent that lactation and season were

the stimuli for enhanced nematode fecundity, a finding which has been reported previously (Crofton, 1958, Gibbs, 1967). It is also interesting to note, that greater the percentage of ova of *Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichostrongylus* spp., the higher the total egg per gram (EPG) level. These species are accounted for the elevated ova levels in the spring and also, during lactation. The contribution of *Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichostrongylus* spp., to the periparturient rise, however might be explained by an increased fecundity of these newly acquired trichostrongyle population which are in good agreement with previous estimates (Gordon, 1967; Coyne *et al.*, 1991).

One week from the time that ewes and lambs of second trial were run on pasture, in the ewes the periparturient egg per gram (EPG) fell drastically and continued to decline for the next few weeks (Fig.25). Although *Haemonchus contortus* and *Trichostrongylus spp.* eggs first appeared in the faeces of lambs on June 15, the proportion of these eggs was a 2 per cent of the mean trichostrongyle egg count of about 50 eggs per gram (EPG). Figures 24 and 25 suggest that until July 16 the lambs eggs per gram did not reflect the majority of eggs deposited by ewes on pasture during the periparturient rise which is in consistent with the results of Michel (1974) and Herd *et al.* (1983) that the periparturient rise may be more important source of infection for lambs than residual pasture contamination. Therefore, assuming the prepatent period of 20 days for *Haemonchus contortus* and *Trichostrongylus* spp., it would seem that translation of eggs

deposited in June is delayed for four to six weeks. This might partially be explained by high temperature and low rainfall conditions prevailing in June (Figs.3 and 4). The logarithmic increases in the egg outputs of lambs in the end of July 31 may well be attributable to infective larvae ingested by July 2 to July 10. The great majority of these infective stages would therefore be referable to the ewes periparturient rise, since until June 15, the lambs are not passing egg in appreciable proportion. It would appear that there is an interval of six-eight weeks before the majority of *Haemonchus contortus* and *Trichostrongylus* spp., eggs derived from the periparturient rise complete one generation when it reachs peak by the end of July, it is responsible for the disastrous clinical parasitism in lambs. The results therefore lend support to the findings of Hunter and Health (1984), Michel (1969a), Boag and Thomas (1971) and Ayalew and Gibbs (1973) that eggs derived from the periparturient rise provide harmful pasture contamination for lambs per grazing season. From end of July to onward upto end of September, it would appear to be the most favourable period when the maximum of infective larvae of these trichostrongyle species are acquired from pasture in the area (Fig.24). Moreover, more than one generation of these trichostrongyles species may occur in between July-October, because from July to October the optimum conditions exist for development and translation of infective larvae of the species.

As a result, the contamination of the pasture takes place throughout the year. The sheep are the principal source at the beginning of the season (March-April) but the role of the lambs

become more important from the second month following entry on to grazing pasture. Their maximum contribution is in July-September when the greatest numbers of eggs per gram is found in the lambs.

From the above discussion it can be concluded that periparturient rise in worm egg output in sheep does occur in sub-tropical, Barani area of Pakistan, influencing early infection of lambs. Thus the animal are exposed to trichostrongyle infections from their early life. Furthermore, from these results it can be concluded that the lambs at a very young age need special attention because of highly susceptibility to gastrointestinal trichostrongyle and their clinical parasitism at this age. It is suggested that young lambs be included in deworming programs. Finally, in order to avoid the negative effects of the periparturient rise control measures should be undertaken by treating ewes before and after lambing period.

LABORATORY AND FIELD EXPERIMENTS STUDY

CHAPTER 6

6.1 INTRODUCTION

The comparative field ecological study on the free-living stages of *Haemonchus contortus* and *Trichostrongylus colubriformis* suggested that a substantial difference has been found between these two species in the capacity for survival of their free-living stages (Donald, 1968).

Several agro-climatic factors which affect the microclimate and macroclimate in which the free-living stages of trichostrongyles exist are responsible for fluctuations during the course of translation on herbage (Gibson and Everett, 1971; Besier and Dunsmore, 1993 a,b). In this regard, moderate temperature and high humidity are particularly important for most of the trichostronglids eggs and larvae (Armour, 1980). The microclimate humidity depends not only on rainfall but on elements influencing the amount of moisture which remains in the soil viz. the soil type, native vegetation and drainage system of a particular area.

Many other factors have also effect the development and the survival of free-living stages of these trichostrongyles viz. consistency of faeces, its disintegration and husbandry practices. Moreover, the activity of certain soil invertebrate species have also been shown to distribute the numbers of gastrointestinal larvae on pasture (Reinecke, 1960; Bryan, 1972).

Outdoor observations carried into various aspects of the biology of the free-living stages of trichostrongylids have been well documented in different areas of the world (Kates, 1963; Rose,

1964; Smith and Archibald, 1969; McKenna, 1973; Cabaret, 1979; Chiejina *et al.*, 1989; Gruner *et al.*, 1989; Aumont and Gruner, 1989; Berbigier *et al.*, 1990; Krecek *et al.*, 1991; Gruner and Suryahadi, 1993; Besier and Dunsmore, 1993 a,b; Rossangio *et al.*, 1994; Fernandez *et al.*, 1994) for *Haemonchus contortus* and for *Trichostrongylus colubriformis* (Waller and Donald, 1970; 1972; Beveridge *et al.*, 1989; Rehman and Collins, 1990a). These studies emphasized knowledge of seasonality of free-living stages of individual species of parasites infecting sheep.

It is to be pointed out that the results of work done overseas under climatic conditions different from Pakistan, would be non-applicable, especially in view of the lack of agreement between the findings of different workers. There is clearly a need for ecological studies to be made under sub-tropical (Barani) climatic conditions.

In this regard, outdoor observations and laboratory experiments are carried out in sub-tropical (Barani) area into various aspects of the biology of the free-living stages of *Haemonchus contortus* and *Trichostrongylus colubriformis* which would help in controlling parasitic gastroenteritis in sheep. The outdoor observations deal with the relationship between environmental factors and the development and survival of the free-living stages, while the laboratory experiments deal with the influence of temperature and humidity on their development and survival.

Previously in Pakistan, no such knowledge was generated in any of its agro-ecological zones. Therefore, present study will help to know the epidemiologic trends of these two trichostrongyles in sub-

tropical area of Pakistan.

6.2 MATERIALS AND METHODS

TRICHOSTRONGYLUS COLUBRIFORMIS

6.3 INFECTIVE LARVAE:

The female worms of *Trichostrongylus colubriformis* were collected at necropsy, in luke-warm normal saline and washed in several changes of saline. The posterior ends of fully gravid females were cut, the uteri pulled out, and eggs squeezed out of the uteri by gentle pressure using the blunt end of a pair of forceps. The eggs were collected with a Pasteur pipette and transferred to wells of 24-well castor tissue culture plates (Linbro, Division, Flow Laboratories Inc., Hamden. CT, U.S.A) in approximately 200 ul saline. These eggs were incubated at room temperature ($24\pm2^{0}C$) for two week in order to obtain infective larvae (L_3). These infective larvae were stored at $4^{0}C$ for experimental animals.

6.3.1 *Experimental animals:*

Two Salt Range (Latti) lambs raised under worm-free condition on concrete-floored pens were used. These lambs were six months old at the beginning of the experiment and were maintained throughout on a diet of pelleted concentrate (PARC, Feed Technology Brand) at rate of 0.54 Kg^{-1} animal day^{-1} and green fodder at libitum. To obtain sufficient *Trichostrongylus colubriformis* eggs for the experiment, two a helminth free lambs, were orally injected with *Trichostrongylus colubriformis* strains at dose of 20,000 infective larvae per lamb (L_3).

6.3.2 *Contaminating inoculum:*

To detect the presence of *Trichostrongylus colubriformis*

eggs in the faeces, weekly samples were examined by using McMaster technique (Hatch and Larkin, 1988). After confirmation of contaminating inoculum of *Trichostrongylus colubriformis* they were prepared for regular deposition on pasture plots.

6.4 OUT-DOOR EXPERIMENTS DESIGN:

6.4.1 *The effect of temperature and humidity on the development of Trichostrongylus colubriformis*:

Experiments were started at the beginning of each month from October, 1992 until September, 1993. Two hundred and fifty gram of freshly collected faecal pellets containing *Trichostrongylus colubriformis* eggs was spread on experimental plots each having size approximately 30 cm square. On one plot **A** the herbage was about 3 cm high at the outset and was maintained at this height; on the second plot **B**, the herbage was 6 cm at the outset and was maintained at same height while on the third plot **C** the herbage was 12 cm high at outset and was allowed to grow. Eggs and larvae were separated from the faeces and examined microscopically in order to monitor the rate of development. The numbers of the different free-living stages were counted in one third of the sample and the percentages of eggs which had developed into infective larvae (L_3) were calculated.

6.4.2 *Larval recovery and identification:*

The larval recovery technique was essentially that described by Taylor (1939) with modifications of the larval separation process (Martin *et al.*, 1990). Each sample was placed in a 45 litre plastic container and enough water added to cover it (> 10 litre). A non-ionic detergent was added (1 gm per 2 liter).

and the herbage was allowed to soak for a minimum of 4 hour. The herbage was then removed and rinsed manually in two, 4 litre volumes of water each and then washed. After centrifugation, the supernatant was poured into a 50 ml conical centrifuge tube and the sides of the first tube rinse with a fine jet of water. The volume was then made up to 50 ml with water, mixed thoroughly and again centrifuged for 2 minute at 2000 rpm. The supernatant was drawn off leaving > 0.5 ml containing the larvae and a small amount of cellular debris. This was adjusted to a final volume of 2 ml with saturated solution of Potassium Iodide (KI). After mixing, four chambers of McMaster egg counting slide (total volume, 1.2 ml) were filled rapidly using a wide-bore pipette (Pasteur pipette). The infective larvae were then identified and counted and the number of larvae per Kg of dry pasture was calculated using the following formula :

$$N = \frac{\text{Final volume (2.0ml)}}{\text{Volume examined (1.2ml)}} \times \frac{AC}{BD} \times 1000 = 1670 \frac{AC}{BD}$$

Where N = Number of larvae Kg^{-1}, A = total of sediment examined (ml); C =number of larvae counted in chamber; D = dry weight of herbage sample (g).

The trichostrongyle larvae were examined under a light microscope and worm were counted and differentiated at the stage of development according to the description given by Veglia (1915) and Douvres (1951).

6.5 LABORATORY EXPERIMENTS DESIGNS:

6.5.1 *Recovery of eggs:*

The faeces were well mixed with cold distilled water and passed through screens of increasing finer mesh to remove large pieces of debris. The washings were left in a refrigerator for 1 to 2 hours for sedimentation, the supernatant fluid was siphoned off and discarded, more tap water was added and this process was repeated until the supernatant fluid become clear. The sediments were then well mixed with saturated Sodium chloride solution and the eggs were collected by the cover slip flotation technique. They were washed several time with cold distilled water to remove traces of salt solution. Afterwards they were concentrated by sedimentation.

6.5.2 *Helminthological sterile faecal solution:*

A helminthological sterile faecal was prepared from fresh faeces. The faeces were diluted with water and after thorough mixing sieved through a tea strainer. The fluid was placed in a tall measuring cylinder and refrigerated overnight at 4 ^{0}C. The following morning the upper fluid was collected without disturbing the sediment. This fluid was found to contain bacteria and ciliates, but helminthologically sterile.

6.5.3 *Development of egg in the helminthologically sterile solution:*

About 200 egg in 3 ml helminthologically sterile solution were put in each well of culture flask (Linbro, Division, Flow Laboratories Inc., Hamden. CT, U.S.A). One such flask was held in each of the constant temperature incubators (accuracy $\pm$ 0.5^{0}C),

ranging from 5 to 40 °C. At different time intervals the well of culture flask were examined under inverted microscope (NiKon) to monitor the different developmental larval stages and individual stages were counted.

6.5.4 *Development of infective larvae (L_3) in faeces:*

For study of development of eggs to infective (L_3) stages faeces-turf cultures were used. Fresh faeces were well mixed by hand so that distribution of egg could be as even as possible. The eggs counts were made on four 4 gram samples by the McMaster technique (Hatch and Larkin, 1988) and the average egg count per gram was calculated in weighed faeces and sterilized turf were mixed and distributed in equal weighed amount in 6 cm plastic petri discs. Tap water was added to make a crumbling consistency of this mixture. Filter papers were placed inside petri discs lids, which were occasionally moistened in the order to keep humidity at 100 per cent. These cultures were held at temperatures ranging from 5 to 40°C. From the 2nd day onwards, each day 3 cultures were removed from each of the temperature cabinets and baermannized for 12 hour. The larvae were collected as previously described.

6.5.5 *Effect of desiccation on the survival of the infective larvae (L_3):*

Different relative humidity (RH) gradients were obtained by the use of super salt saturated solutions or Sulphuric acid (Winston and Bates, 1960). Humidity chamber (GalenKappan) having temperature control facility was used in these experiments.

The infective larvae of *Trichostrongylus colubriformis* were obtained by coproculture of the faeces from the lambs carrying a

pure experimental mono-specific infection. Only freshly harvested larvae were used. The concentration of infective larvae was adjusted in such a way that one drop of water contain approximately 100 larvae. Two drops of this suspension were put on 50 clean glass slides and left to dry over-night at room temperature (Approximately 24°C). Next day the slides were transferred to different temperature cabinets or RH chambers. At specified time intervals, test slides were removed from each of the temperature cabinets or RH chambers; a few drops of water were added to cover the area occupied by the dried larvae and kept overnight at 25 °C, after which the living larvae were counted. Those showing even a slight movement were considered alive.

In order to examined the effects of repeated desiccation and rehydration, 200 infective larvae were accurately counted, put in small petri discs in 1 ml of water and left open to dry over-night at room temperature. Next morning they were transferred to different RH chambers. After 1 day storage they were removed from these chambers and 1 ml of water was added. They were kept at room temperature for 1 day, after which living larvae were counted. After counting they were again transferred to RH chambers. This process of keeping the larvae 1 day in Rh chambers and 1 day at room temperature in a rehydrated state was continued up to sixth treatment. In subsequent treatments the period was 2 days in RH chamber and 1 day of room temperature alternatively until all the larvae were dead in a particular set of experiment.

In order to obtain information on the longevity of rehydrated larvae they were desiccated in petri discs and stored for 3 days at

-10, -5, 4, 20, 30, 35 and 40 ^{0}C. After 3 days they were removed from these temperatures and 3 ml of water was added. They were then stored at 25^{0}C. At intervals of 1, 4 and 7 days and 2,4, weeks the numbers of living larvae were determined. The petri discs were regularly observed and water was added, if necessary, to prevent drying.

Relative chemical humidity to make (RH) per cent solution		Amount of chemical required for 100 ml of the solution
95	98% sulphuric acid	11.02 gm
86	Potassium chloride	56 gm
75	Sodium chloride	39.8 gm
65	98% Sulfuric acid	35.8 gm
55	Calcium nitrate	506 gm
42	Magnesium chloride	367 gm
31	Potassium acetate	Dry Salt put in desiccator to decompose.

Winston and Bates (1960)

6.6 RESULTS

6.6.1 *Development of eggs into infective larvae (L_3):*

Eggs developed into infective larvae (L_3) in faecal pellets deposited on the small grass plots at all the time of the year. The minimum and maximum time taken for development to the different larval stages is shown in Table 16. There were marked individual variations in the rates of development to the infective stages, as can be seen from the difference between the minimum and maximum recorded. Maximum time was not recorded for some of the plots as the faecal pellets had disintegrated before a point was reached when only infective larvae were present.

The number of the eggs in the faecal pellets deposited on the small plots in December-February showed delayed development rate as compared to rest of the year. A large proportion of these larvae died in December-February and also died during May-June. During reminder of the year, more eggs developed into infective larvae, but, even so, mortality was still heavy in May-June on the A and B plots with short herbage. Except in May-June, mortality was less heavy on the plot C with well grown herbage, especially in July and October, when more than 50 per cent eggs developed to infective larvae. The faecal pellets on the plots A and B were alternatively wet and dry, depending upon prevailing weather conditions, whereas the well grown herbage on the plot C remained moist for a larger period, except in May-June, when there was no rainfall in the region.

The out-door observations suggested that development completed throughout the year, over a wide range of temperatures. The rate

development enhanced as the temperatures rose (Table 17). On the plots, however, the rate of development was slowed down when the faecal pellet dried out rapidly, this occurred on the plot A and B in May-June and on plot C in May. Mortality of the developed stage was high at temperature 4°C (December-January) only 4 per cent of egg becoming infective larvae, but development was high when temperature approached about 20°C and at that time more than 35 per cent eggs developed into infective larvae. While maximum number (58 per cent) of infective larvae were recovered from plot C in August.

6.6.2 *The effect of temperature on hatching of eggs:*

Table 18 showed the time taken for hatching of the eggs at different temperatures. At 40°C the eggs never developed beyond the gastrula stage the same happened at 5°C. At 15°C small number of them hatched only after an incubation of 36 hours and about 70-100 per cent after 4 days. Between 20 °C to 35 °C, the hatching of eggs enhanced (40-90 per cent) as the temperature rose.

6.6.3 *Effect of temperature on the development of infective larval stages (L_3):*

The eggs after hatching developed into second stage larvae and finally to third stage infective larvae. Table 19 presented the percentages of eggs thus developed into infective larvae at different temperatures. Occasionally, a culture showed very poor development. The abnormally low results have been excluded in calculating the percentage development. No development to infective stage occurred at 5°C and 40°C. However, at 10 °C to 15 °C the time taken to reach infective larvae were 19 to 8 days. Upto 25°C large number of infective larvae were recovered with increase

in temperature. Above 25°C their number decreased gradually, but the speed of development was more rapid.

6.6.4 *Effect of RH on survival of desiccated infective larvae:*

Percentage survival of desiccated larvae at relative humidities (RH) ranging from 31 to 95 per cent at room temperature is presented in Table 20. Control larvae, kept in water, were all alive till the end of the experiments. A greater percentage of desiccated larvae survived over a longer time when held at the lowest RH 31 per cent. At RH of 86 and 95 percent the minimum survival was for 3 to 4 weeks. At other ranges of RH (42-75 %) a moderate number of larvae was survived (51-92 per cent).

6.6.5 *Effect of repeated desiccation and rehydration on survival of infective larvae:*

Table 21 showed that the larvae died more quickly when alternatively desiccated and rehydrated than kept permanently desiccated at different RH. The lower humidities were more lethal than higher ones.

6.6.6 *Longevity of rehydrated infective larvae at 25 °C:*

Table 22 showed that the holding of dried larvae at lower (-5°C) and higher temperatures (40°C) for 3 days was more deleterious. The number of larvae surviving after rehydration and storage for 1 day at 20°C was inversely proportional to the original holding temperature. Therefore, their survival capacity decrease gradually. The higher percentage of larvae survived upto eight week at other temperatures (20-35°C).

6.6.7 *Effect of constant temperature on survival of*

desiccated infective larvae:

The survival of the desiccated larvae at various constant temperature ranging from -10 to 40 ^{0}C is shown in Table 23. The survival was maximum at 20-25^{0}C and minimum at -10 ^{0}C. From 20 ^{0}C to 35 ^{0}C were favorable for survival of larvae. After two week of storage, 55 per cent of the larvae were alive at 20 ^{0}C, 35 per cent at 30 ^{0}C and 40 per cent at 35 ^{0}C. At 3 to 4 weeks maximum numbers of larvae were alive at temperature 20-30^{0}C.

Table: 16 The rate of development of the free-living stages of *Trichostrongylus colubriformis* deposited on grass plot.

Time of development (Days)												
Plots	A				B				C			
	Min.			Max.	Min.			Max.	Min.			Max.
Months	L1	L2	L3	L3	L1	L2	L3	L3	L1	L2	L3	L3
1992												
Oct.	2	6	15	21	3	6	17	19	4	7	16	21
Nov.	4	8	9	-	4	9	11	-	3	-	-	-
Dec.	8	12	18	29	7	14	21	29	3	-	-	30
1993												
Jan	10	14	21	31	9	16	25	21	6	14	20	21
Feb.	4	8	20	27	6	11	24	26	4	6	20	26
Mar.	2	6	15	-	4	5	15	-	3	7	17	-
Apr.	2	6	17	-	3	4	-	-	3	5	14	-
May	5	9	29	35	3	8	31	32	4	8	20	37
June	6	11	30	42	4	12	35	38	3	12	25	34
July	2	6	19	-	3	7	16	-	4	-	-	-
Aug.	2	5	-	-	2	-	-	-	2	-	-	-
Sep.	2	6	15	-	2	-	-	-	4	-	-	-

- Indicates that faeces has disintegrated completely before was reached when only L3 were present

Table: 17 The percentage of eggs in faeces on grass plots which devloped into infective larvae (L_3).

	Percentage of eggs developed into infective larvae		
	Plots		
Months	*A*	*B*	*C*
October, 1992	14	16	35
November	6	10	20
December	4	9	11
January, 1993	2	4	14
February	2	5	18
March	21	24	32
April	27	30	37
May	8	10	13
June	6	9	10
July	35	32	39
August	29	40	58
September	35	20	45

Table: 18 Effect of temperature on hatching of *Trichostrongylus colubriformis* eggs.

Temperatures	Percentage of eggs hatched after							
	Hour				Day			
^{0}C	6	12	24	36	2	3	4	5
40	0	0	0	0	0	0	0	0
35	0	0	0	30	40	0	_	_
30	0	30	75	88	87	0	_	_
25	0	35	81	92	96	98	_	_
20	0	40	88	94	82	90	_	_
15	0	0	0	10	70	75	100	_
10	0	0	0	6	10	0	_	_
5	0	0	0	1	0	0	0	0

Table: 19 Effect of temperature on percentage development of infective larvae (L_3) of *Trichostrongylus colubriformis.*

Temperature C^0	Percentage of recovery of infective larvae on days											
	3	4	5	6	7	8	9	10	19	20	21	22
40	-	-	-	-	-	-	-	-	-	-	-	-
35	21	36	38	-	-	-	-	-	-	-	-	-
30	13	27	49	50	-	-	-	-	-	-	-	-
25	5	15	29	45	91	-	-	-	-	-	-	-
20	3	8	16	45	51	70	-	-	-	-	-	-
15	-	-	-	-	-	8	20	30	-	-	-	-
10	-	-	-	-	-	-	-	-	5	8	15	20
5	-	-	-	-	-	-	-	-	-	-	-	-

Table: 20 Percentage survival of desiccated infective larvae of *T. colubriformis* at various relative humidities at room temperature.

	Percentage (%) survival at relative humidity					
	31	42	55	75	86	95
Days						
1	86	92	93	89	93	94
2	88	80	90	83	90	89
4	70	77	59	51	86	75
Weeks						
2	40	35	31	23	20	25
3	28	34	21	6	0	0
4	25	21	9	7	0	2

Table: 21 Percentage survival of dried infective larvae of *T. colubriformis* after repeated desiccation and rehydration at different (R. H.)

No. of Treatment	Time 65	55	42	31	Remarks
1	96	87	72	8	Alternatively 1 day in RH
2	92	83	56	2	chamber and 1 day in room
3	82	75	40	0	temperature, in replicated state
4	60	52	6	-	Alternatively 1 day in RH
5	82	45	3	-	chamber and 1 day in room
6	43	39	-	-	temperature, in replicated state

Table: 22 Longevity of infective larvae of *T. colubriformis*, desiccated for 3 days at different temperature and 'rehyderated and kept at 25 °C.

Percentage survival of rehyderated infective larvae at 25°C						
	Days			week		
Temperatures °C	1	3	6	2	6	8
-10	0	0	0	0	0	0
-5	0	0	0	0	0	0
5	0	10	17	0	0	0
20	89	85	75	49	28	11
30	60	50	35	35	16	12
35	40	25	20	15	8	2
40	0	0	0	0	0	0

Table: 23 Percentage survived of desiccated infective larvae of *Trichostrongylus colubriformis* of constant temperature.

	Temperature °C							
	-10	-5	5	20	25	30	35	40
Days								
1	0	0	0	85	98	87	82	0
2	0	0	10	87	86	67	65	0
4	0	0	0	88	84	70	57	10
Weeks								
2	0	0	20	55	40	35	40	0
3	0	0	20	28	17	15	11	1
4	0	0	0	6	5	5	2	0

6.7 DISCUSSION

From the results of our observations it is apparent that the development of *Trichostrongylus colubriformis* eggs into infective larvae was completed throughout the year under wide range of weather conditions. However, heavy mortality during the month of May-June may be due to lethal effect of high temperature, generally experience in this region (>40°C) on the free-living stages of this specie. Which is consistence with the finding of Waller and Donald (1972) they indicated that a higher mortality at high temperature could be due to direct lethal effect of temperature at 30°C on the developing embryo, or due to an increased rate of water loss which may change the permeability of the egg envelope. During rest of the period from February to April and July to November, the weather conditions are optimum for development and survival of free-living stages of this specie.

Moreover, from December to January, when mortality of the infective stages was again high, the low temperature prevailing is the most important limiting factor for development. These findings on *T. colubriformis* agree closely with those of Andersen and Levine (1968). Heavy death of the pre-infective stages is probably due to the indirect effect of temperature affecting their metabolism and limiting the availability of bacteria on which they feed (Rose and Small, 1984). Moreover, very cold weather during December and January, when ground temperatures was at freezing point for a short time period which is in conformity with the findings of Rose and Small (1984), but they worked on *Trichostrongylus vitrinus*.

The effect of low temperature on the development of the free-

living stages is most marked with *Trichostrongylus colubriformis*. This indicates that at temperature below > 10 °C there is no development of this specie. This agree with the reports of various workers (Gibson and Everett, 1969, Levine and Andersen, 1973; Southcott *et al.*, 1976; Callinan, 1979; Beveridge *et al.*, 1989). They conducted their studies under field condition and therefore, the laboratory experiments described in the present study agrees with their observations. The highest limit for hatching and development for this specie is 35 °C, and at 40 °C there is complete absence of development beyond the gastrula stage. The similar findings were reported by Wang (1967) and Pandey (1972) who worked on *Ostertagia ostertagi* in cattle.

The optimum temperature for development is found to be 25°C which agrees with the view of Wallace (1961), who suggested that 20-30 °C is the range for optimum development of many zooparasitic nematodes. It seems that the low recovery of infective larvae at high and low temperatures is due to unfavorable effects of temperature on the pre-infective larvae of this specie. Generally these lethal climatic days are met between December-January for low temperature and May-June for high temperature in this region. The ideal conditions for development are between 20°C to 30 °C. This range of temperatures is met in most part of sub-tropical climate during the grazing season. Consequently, the heavy herbage infection by the infective larvae may be build up and trichostrongylosis is likely to become a serious problem in sheep on the pasture.

The present study indicates that the infective larvae of

Trichostrongylus colubriformis were highly resistance to desiccation. Andersen and Levine (1968) showed the effects of desiccation on infective larvae of this species and found that at temperatures below freezing and 35°C to 40°C, desiccated larvae survived better than the non-desiccated ones. They further suggested that the first-stage larva after hatching was extremely vulnerable to desiccation the egg envelope must exert an important controlling influence on the water content of this stage prior to hatching. While water loss below a critical level is likely itself to be lethal, it is also possible that some dehydration of the prehatch larva is necessary for the protection of important life systems from high temperature damage. Similar results is obtained by Pandey (1972) who worked on *Ostertagia ostertagi*.

Poole (1954) found that the infective larvae of *Trichostrongylus retertaeformis* survive less than 7 weeks when rehydrated after desiccation. Andersen and Levine (1968) showed that a good number of *Trichostrongylus colubriformis* infective larvae were alive up to 2 week after rehydration. In the present experiments, survival of *Trichostrongylus colubriformis* infective larvae which had been desiccated and then rehydrated was alive upto 4 weeks.

The ability of infective larvae to resist desiccation seems to be an important aid in the maintenance and spread of infection on pasture. Once the larvae have reached the grass blades, they probably become desiccated and remain there at that state until rehydrated by rain or dew or until eaten by the grazing sheep. After ingestion they will rehydrated again in the digestive tract

of sheep, exsheath and continue their parasitic phase of life. Therefore, desiccation may be looked as one of the favourable factor in maintenance of infection on pasture thus helping in the perpetuation of the species.

Therefore, attempts are made in this study to relate development and survival of the free-living stage of *Trichostrongylus colubriformis* to weather conditions. This will help in establishing relationships between the elements of climate and development of *Trichostrongylus colubriformis* in local climatic conditions which may prove more fruitful in the construction of future predictive models.

6.8 MATERIALS AND METHODS

HAEMONCHUS CONTORTUS

6.9 INFECTIVE LARVAE:

The female worms of *Haemonchus contortus* were collected at necropsy, in luke-warm normal saline and washed in several changes of saline. The posterior ends of fully gravid females were cut, the uteri pulled out, and eggs squeezed out of the uteri by gentle pressure using the blunt end of a pair of forceps. The eggs were collected with a Pasteur pipette and transferred to wells of 24-well castor tissue culture plates (Linbro, Division, Flow Laboratories Inc., Hamden. CT, U.S.A) in approximately 200 ul saline. These eggs were incubated at room temperature ($24\pm2^{0}C$) for two week in order to obtain infective larvae (L_3). These infective larvae were stored of $4^{0}C$ for experimental animals.

6.9.1 *Experimental animals:*

Two Salt Range (Latti) lambs raised under worm-free condition on concrete-floored pens were used. These lambs were six months old at the beginning of the experiment and were maintained throughout on a diet of pelleted concentrate (PARC, Feed Technology Brand) at rate of 0.54 Kg^{-1} animal day^{-1} and green fodder at libitum. To obtain sufficient *Haemonchus contortus* eggs for the experiment, two a helminth free lambs, were orally injected with an *Haemonchus contortus* at dose of 10,000 infective larvae (L_3).

6.9.2 *Contaminating inoculum:*

To detect the presence of *Haemonchus contortus* eggs in the faeces, weekly samples were examined by using McMaster technique (Hatch and Larkin, 1988). After confirmation of

contaminating inoculum of *Haemonchus contortus* they were prepared for regular deposition on pasture plots.

6.10 OUT-DOOR EXPERIMENTS DESIGN:

6.10.1 *The effect of climatic conditions on the survival of the infective larvae on grass plots:*

One Kilogram (Kg) of freshly collected pellets containing *Haemonchus contortus* eggs was scattered over a plots of grassland having 30 cm^2 size, in each month from October, 1992 to December, 1993. The herbage in these plots were maintain at 3 cm height at outset. It was maintained at this height throughout to stimulate a pasture grazed by sheep. The cuttings remained on the plots, so that no infective larvae were removed. Forty (40) samples of herbage were plucked from each plot at random at fortnightly intervals, and 3 gram sample of faeces were taken at the same time until the faeces had disintegrated. Larvae were separated from herbage and faeces and counted. The herbage collected was equivalent to the amount covering an area of 30 cm^2.

6.10.2 *Larval recovery and identification:*

The larval recovery technique was essentially that described by Taylor (1939) with modifications of the larval separation process (Martin *et al.*, 1990). Each sample was placed in a 45 litre plastic container and enough water added to cover it (> 10 litre). A non-ionic detergent was added (1 gm per 2 liter). and the herbage was allowed to soak for a minimum of 4 hour. The herbage was then removed and rinsed manually in two, 4 litre volumes of water each and then washed. After centrifugation, the supernatant was poured into a 50 ml conical centrifuge tube and the

sides of the first tube rinse with a fine jet of water. The volume was then made up to 50 ml with water, mixed thoroughly and again centrifuged for 2 minute at 2000 rpm. The supernatant was drawn off leaving > 0.5 ml containing the larvae and a small amount of cellular debris. This was adjusted to a final volume of 2 ml with saturated solution of Potassium Iodide (KI). After mixing, four chambers of McMaster egg counting slide (total volume, 1.2 ml) were filled rapidly using a wide-bore pipette (Pasteur pipette). The infective larvae were then identified and counted and the number of larvae per Kg of dry pasture was calculated using the following formula :

$$N = \frac{\text{Final volume (2.0ml)}}{\text{Volume examined (1.2ml)}} \times \frac{AC}{BD} \times 1000 = 1670 \frac{AC}{BD}$$

Where N = Number of larvae Kg^{-1}, A = total of sediment examined (ml); C =number of larvae counted in chamber; D = dry weight of herbage sample (g).

The trichostrongyle larvae were examined under a light microscope and worm were counted and differentiated at the stage of development according to the description given by Veglia (1915) and Douvres (1951).

6.11 LABORATORY EXPERIMENTS DESIGNS:

6.11.1 Recovery of eggs:

The faeces were well mixed with cold distilled water and passed through screens of increasing finer mesh to remove large pieces of debris. The washings were left in a refrigerator for 1 to

2 hours for sedimentation, the supernatant fluid was siphoned off and discarded, more tap water was added and this process was repeated until the supernatant fluid become clear. The sediments were then well mixed with saturated Sodium chloride solution and the eggs were collected by the cover slip flotation technique. They were washed several time with cold distilled water to remove traces of salt solution. Afterwards they were concentrated by sedimentation.

6.11.2 *Development of infective larvae (L_3) in faeces:*

For study of development of eggs to infective (L_3) stages faeces-turf cultures were used. Fresh faeces were well mixed by hand so that distribution of egg could be as even as possible. The eggs counts were made on four 4 gram samples by the McMaster technique (Hatch ana Larkin, 1988) and the average egg count per gram was calculated in weighed faeces and sterilized turf were mixed and distributed in equal weighed amount in 6 cm plastic petri discs. Tap water was added to make a crumbling consistency of this mixture. Filter papers were placed inside petri discs lids, which were occasionally moistened in the order to keep humidity at 100 per cent. These cultures were held at temperatures ranging from 5 to 40^{0}C. From the 2nd day onwards, each day 3 cultures were removed from each of the temperature cabinets and baermannized for 12 hour. The larvae were collected as previously described.

6.11.3 *Effect of desiccation and continous freezing in the longevity of different stages of Haemonchus contortus:*

Different relative humidity (RH) gradients were obtained by the use of super salt saturated solutions or sulphuric acid

(Winston and Bates, 1960). Humidity chamber (GalenKappan) having temperature control facility was used in these experiments.

The infective larvae of *Haemonchus contortus* were obtained by coproculture of the faeces from the lambs carrying a pure experimental mono-specific infection. Only freshly harvested larvae were used. The concentration of infective larvae was adjusted in such a way that one drop of water contain approximately 100 larvae. Two drops of this suspension were put on 50 clean glass slides and left to dry over-night at room temperature (Approximately 24^{0}C). Next day the slides were transferred to different RH chambers. At specified time intervals, test slides were removed from each of the RH chambers; a few drops of water were added to cover the area occupied by the dried larvae and kept overnight at 25 ^{0}C, after which the living larvae were counted. Those showing even a slight movement were considered alive.

In order to obtain information on the longevity of continuous freezing on the third-stage, embryonated eggs and non-embryonated eggs they were desiccated in petri discs and stored for 3 days at -4^{0}C to -6^{0}C. After 3 days they were removed from these temperature and 3 ml of water was added. They were then stored at 25^{0}C. At intervals of 1 to 12 days the numbers of living larvae were determined. The petri discs were regularly observed and water was added, if necessary, to prevent drying. The test, in all the experiments were run in triplicate.

6.12 RESULTS

From March to October the majority of the eggs of *Haemonchus contortus* developed into third stage larvae (L_3), but their proportion were varied depending upon moisture content of the faeces. In May-June, climatic conditions were hot thus faeces become dried out soon after being deposited on the grass plot, and only 5 per cent of the eggs developed into infective stages (Fig.25). In July, August, September and October, however, the climatic conditions were conducive to the faeces remaining moist for a longer period of time, and majority of the egg developed into infective larvae. From November to onwards upto February, the majority of the eggs in embryonated state were died, therefore few first-stage larvae were recovered for few weeks, but failed to developed to the second stage larvae.

The development of eggs into infective larvae was successfully completed and take less time when faeces were deposited out of doors from October to November, and then from March-April and finally from July to September. However, from December to February they have taken longer period than was required for the completion of infective larval stages as can be seen by refereeing to Table 24. Similarly, in May-June the examination of faeces demonstrated that a large proportion of eggs was dead within 48 hours of the faeces being spread over the plots. These eggs which survive succeeded in hatchery, but only few of them were survived and reached infective stage.

6.12.1 *Larval migration:*

Majority of the infective larvae migrated to the herbage

at 20-25°C. Maximum infective larvae (L_3) were recovered from the inner area of the grass plot, that they didn't migrated more then 2 inch from the after edge of the faecal pellets. The greatest mean percentage recovery of infective larvae in soil was occurred at 35°C (Table 25). While least number of larvae was recovered at temperature 40°C.

6.12.2 ***Effect of desiccation and temperature:***

The effect of two factors, namely desiccation and freezing is clearly demonstrated in the Figures 26 and 27. The failure of so many of the eggs to developed into infective larvae (L_3) was not due to any lack of egg fertility but, resulted from unfavorable effect of temperature and desiccation. At 30 % RH only 2.97 per cent egg survived as compared to 90 % RH, at which 26.8 % egg developed into infective stage. Similarly, at temperature -4 to -6 °C third stage (L_3) were survived for a longer period i.e upto 12 day while non-embryonated, first and second stage larvae died more quickly at these temperatures. However, embryonated egg were survived in appreciable number upto 4 days when kept at -4 to -6 °C.

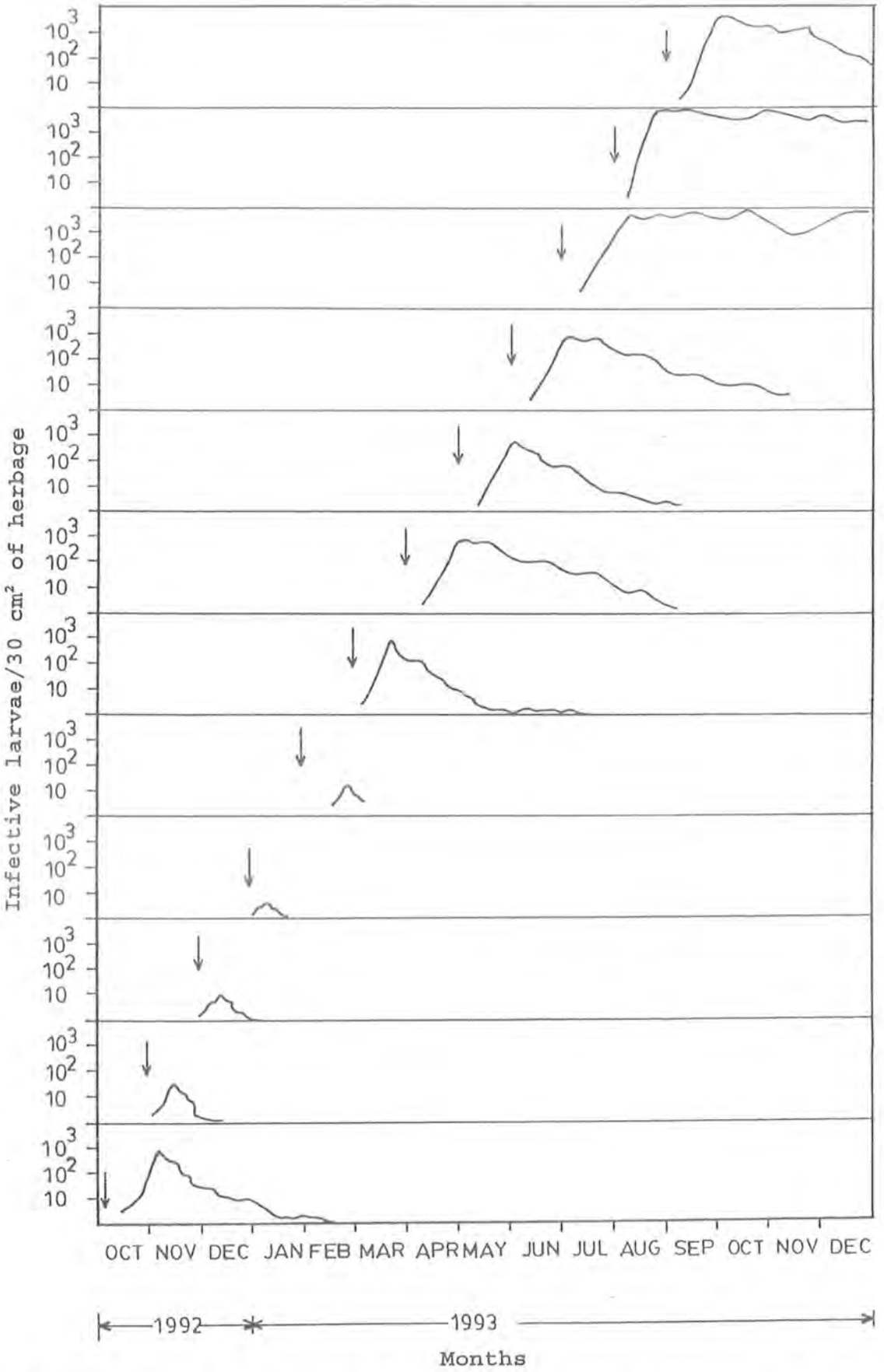


Figure 26. The longevity of the infective larvae (L_3) of *Trichostrongylus colubriformis* recovered from the herbage of plots equivalent to an area of 30 cm². Arrow indicate the time of when faeces were deposited on the plots.

Table 24. The rate of development of the free-living stages of *Haemonchus contortus* during October, 1992 to September, 1993.

Months	Faeces deposited (250 gm)	Time taken	
		Minimum day	Maximum day
Oct. 1992		7	10
Nov.		9	13
Dec.		#14	15
Jan. 1993		#14	20
Feb.		#13	18
Mar.		8	12
Apr.		8	12
May		$9	13
Jun.		$10	13
July		5	8
Aug.		5	8
Sep.		5	8

#: Very few larvae reached to L_3 stage (10%).
$: Majority of the eggs dead (45 %).

Table 25. Mean percentage of *Haemonchus contortus* infective larvae (L_3) found in soil and herbage.

Variable range	Percentage L_3 on herbage (%)	Percentage of L_3 on soil (%)
Temperature^{0}C		
10	5	35.2
15	7.71	27.5
20	20.13	13.7
25	25	18
30	14	31.9
35	10.5	39.3
40	4.7	6.7

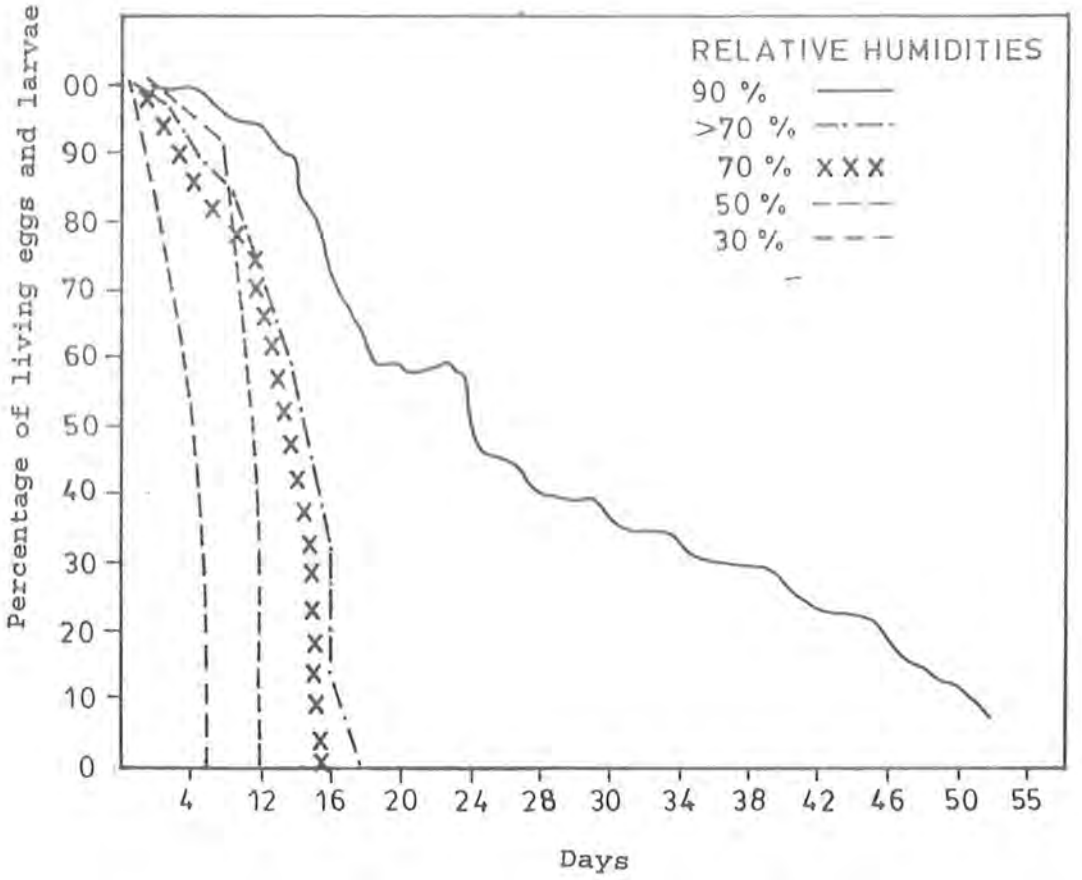


Figure 27. The effect of different relative humidities on the infective larvae of *Haemonchus contortus*.

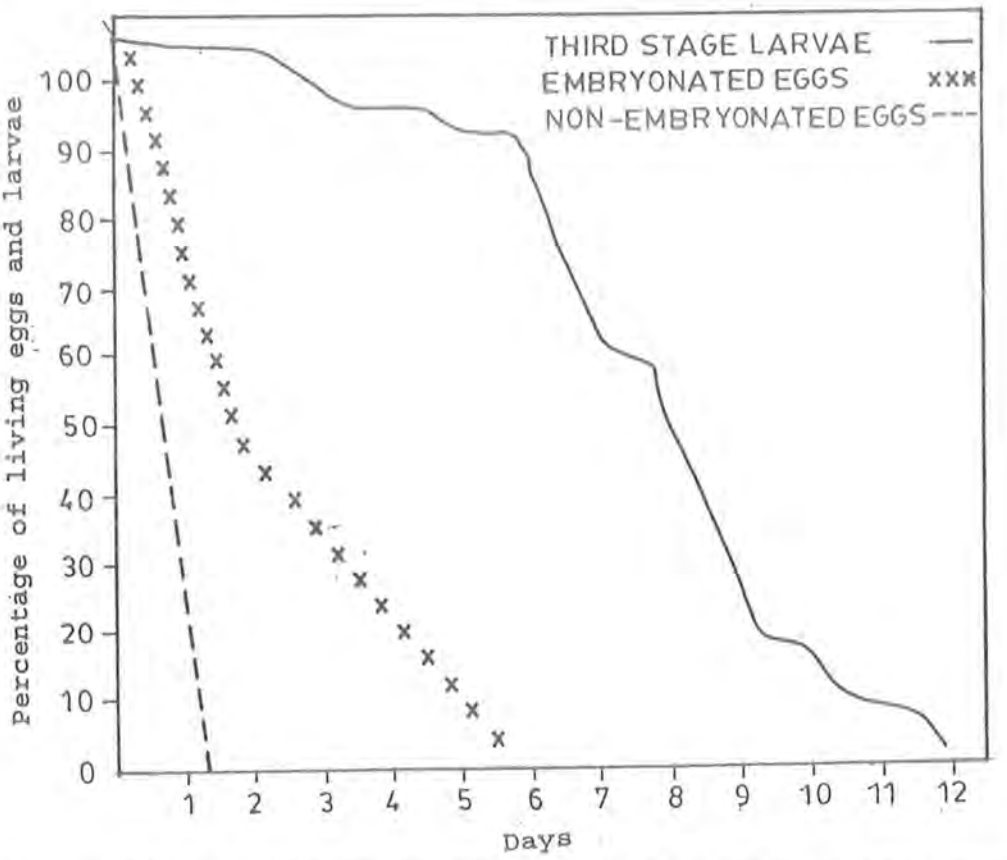


Figure 28. The effect of continuous freezing on the longevity of eggs and larvae of *Haemonchus contortus* suspended in water and kept at -4°C to -6°C.

6.15 DISCUSSION

In outdoor experiments majority of the eggs at embryonated and first stage were died, particularly the during the winter months (December-February) and also in hot summer days (May-June). This mortality can be attributed to due to adverse effect of climatic factors. On the basis of these experiments, the heavy mortality of eggs in faeces deposited out-of-doors during the winter can be explained as due to the effects of the low temperatures then prevailing. It can be inferred that they may not develop into infective larvae and therefore, will not act as a source of infection for sheep from December to February.

The heavy mortality of eggs in dried faeces observed during the hot dry summer could be explain partly due to the effect of high temperatures. Our observations is supported by the work of Rose (1963). Similar findings are reported by Agrwal (1966) and Premvati and Lal (1961) they reported that the embryonated eggs died after 8 hour and non-embryonated survived after 4 hour. The heavy death rate of eggs in faeces which become dry within a short time of being deposited out-door may also have resulted from the effect of high temperature. According to Silverman and Campbell (1959) egg at the pre-hatch stage have a certain amount of resistance to desiccation. They found that the rapid desiccation of faecal pellets under laboratory conditions resulted in the destruction of the certain eggs if they were at an early stage of development, but egg at an advanced stage of development survived for up to six weeks in dry faeces. Similarly Veglia (1915) had reported that some *Haemonchus contortus* eggs were still viable

after exposing at 50°C, but that they all dead after 4 hours. Moreover, Todd *et al.* (1976) estimated that 1 per cent of non-embryonated eggs verses 4 per cent of embryonated eggs survive at a temperature 45°C for one hour which is in conformity to our observations.

The most favourable conditions for development of the pre-infective stage are moderate high temperatures to ensure a rapid rate of development and adequate moisture to ensure that faeces don't become desiccated before the infective stage has been reached. Fulfillment of the moisture requirements will depend to considerable extend on the amount and frequency of rainfall, but as the rate at which faeces dry out is influenced by their form and by the nature of the surrounding environment these factors also may be important in this respect. In general, however, the most favourable condition for development of egg into infective larvae will occur during a warm wet summer (July-September). Although mortality of egg is heavy in hot dry summer (May-June). Nevertheless, it is still possible for heavy herbage contaminations to be built up due to the prolific eggs laying capacity of female *Haemonchus contortus* (Grant, 1981). According to Gordon (1948), one female can lay upto 10,000 egg per day. There is, however, adequate number of infective larvae of *Haemonchus contortus* that can survive in the winter months (December-February) retains there until the following spring and thus cause the periparturient egg rise in the lactating ewes. It is probable that such a pasture could serve as a source of infection for spring born lambs. Therefore, overwintering larval stages are all-important in perpetuating infection from one year to

the next year which is in agreement with the finding of Thomas and Stevens (1960).

The present observations demonstrate that the availability of soil moisture could be significant for the development of *Haemonchus contortus* eggs into infective larvae. Herbage height can also have some effect in this respect. These factors operate by influencing the humidity of the microclimate around the developing eggs and pre-infective larvae. Moreover, the microclimate is influenced by the macroclimate, so the precise significance of soil moisture and herbage height in relation to development will vary with changes in the macroclimate conditions.

In May-June macroclimatic conditions are such that the soil moisture and herbage height have a profound part the influencing the humidity of the microclimate. As a result the faecal pellets dried out rapidly at this time of year. In July to onwards upto October, the warm wet weather is particularly conducive to the successful development of infective larvae. The humidity of the microclimate is high on all of the plots and many infective larvae were recovered irrespective of the nature of the environment. Moreover, herbage height and availability of soil moisture appears to further facilities the influence the development of eggs to infective larval stages. Callinan and Westcott (1986) claimed that the soil acted as a reservoir of L_3 which could survive in soil for long periods, contaminating herbage when environmental conditions were suitable. Our results confirm that the soil is important reservoir of L_3

The difference in vertical distribution of larvae between the

short and the tall herbage can be attributed on the basis of variations in the microclimate at different levels of the herbage, especially differences in light intensity (Rogers, 1940; Crofton, 1948; Rees, 1950; Rose, 1964).

The significance of the verticle distribution of infective larvae on the herbage in relation to host grazing behaviour could be significant. It would seem that larvae on well-grown herbage are more favorable positioned for ingestion than those on short herbage, at least by cattle which don't graze so close to the soil surface as do sheep (Rose, 1964). A tentative conclusion regarding the relationship between the type of pasture grazed and infection of the final host can be drown from the present observation, it is clear that further field experiments are necessary before the significance of different types of pasture in relation to the epidemiology of *Haemonchus contortus* infection can be properly assessed.

CHAPTER 7

GENERAL DISCUSSION

In the present study trichostrongyloidea, were the most prevalent and abundant nematode parasites of sheep, particularly adaptated to summer conditions (Anderson and Levine, 1968; Waller and Donald, 1972) and is also characterized in sub-tropical (Barani) region of Pakistan. Results in the present studies have indicated that gastrointestinal trichostrongylosis can be a serious threat in all seasons, depending on the prevailing agro-climatic conditions.

The twelve species of nematode parasites reported in this study have previously been recorded in south-east Asia (Gupta *et al.*, 1987; Ahmed and Ansari, 1987; Van Aken *et al.*, 1990) also indicates that species and their frequencies in this region is not essentially like those reported in other parts of the sub-continent. This is probably due to the fact that those studies were carried out in different agro-ecological zones. Moreover, environmental conditions, especially relative humidity and temperature have a profound effect on the global distribution of parasite species (Hinz, 1986) but the composition of infection is similar which is because of frequent movements of animals from one region to another. It is to be pointed out that nomadic and transhumance are the common animals husbandry practices followed in various regions of Pakistan. The major objective of the both husbandry practices is utilization of the valuable permanent grassland. Transhumance are frequently related to changes caused by

rainy and dry seasons (Eckert and Hertzberg, 1994). The most important factor influencing the transmission of parasitic disease is human behavior. The significance of human behavior in influencing the rate and pattern of parasitic transmission varies with cultural factors. Nomadic pastoralist have the closest possible association with their own ecosystem (Macpherson, 1994). Their low population density, mobility and intimate association with their animals in the arid environment and their subsequent movements to new pasturelands, have profound influence on the distribution and transmission of parasites.

The pattern of larval availability on the herbage and the broad climatic pattern suggest that there are two peaks of larval availability, the first one is observed during winter rainfall period giving rise to the spring peak and the second in the rainy season, giving rise to the autumn peak. This could be attributed to the fact that in winter rainfall the environmental conditions become conducive for most readily distribution of larvae on the herbage whereas the possibility of heavy infection occurring in rainy season would also be likely as the instances of heavy rainfall and high stocking rate on permanent pastures. Excessively high temperatures, high evaporation rates in mid-summer (May-June) appears unfavorable for larval translation on herbage. Similarly in winter (January-February) temperature is too low for development of most of the eggs deposited in previous rainy season.

Ostertagia spp., and *Trichostrongylus* spp., the main genera recovered have similar patterns of availability on herbage with major peaks occurring during winter months and smaller fluctuating

peaks from April to onwards upto October. These peaks coincides with period of higher rainfall which facilitates the migration of infective larvae onto herbage. It is in agreement with the findings of Brunsdon (1963b) and Vlassoff (1973) in New Zealand but different from Pakistan, in having difference with respect to onset of seasons.

It is interested to note that *Haemonchus contortus* and *Oesophagostomum columbianum* are also common gastrointestinal nematodes prevalent in this region. According to Cabaret (1979) haemonchosis is usually associated with ruminants in tropical or sub-tropical rather arid environments. Similar is the case with oesophagostomosis which is common health hazard in south-east Asia (Ahmed and Ansari, 1987). The spring peak is probably due to overwintering of larvae of *Haemonchus contortus*, *Trichostrongylus* spp., and *Ostertagia* spp. While that of *Oesophagostomum columbianum* long prepatent period of this nematode may be the reason for high infection in spring. The similar results has previously been reported in different region of sub-continent (Gupta *et al.*, 1987; Ahmed and Ansari, 1987; Van AKen *et al.*, 1990). Similar seasonal peak in autumn, may be attributed to the fact that heavy rainfall, mainly in monsoon, will account of this rise in nematode numbers in sheep. Our observations are in agreement with Gupta *et al.* (1987) in India, Van Aken (1990) in Sri Lanka, Rehman (1992) and Dorny *et al.* (1995) in Malaysia. The period from March-April is the only time in Pakistan, in general and in this region in particular, when temperatures are between 15 ^{0}C to 20 ^{0} C and with adequate rainfall provide the optimum conditions for translation and migration for

most of the trichostrongyles larvae (Figs. 3 and 4).

Throughout the study, a clear seasonal pattern in egg counts was observed. In late winter (February-March), sheep harboured heavy worms burden result in an increase in egg counts with peaks between March-April. The decline in egg counts during May-June may be attributed to the lack of worms burden in sheep. In July to onwards upto September, heavy monsoon rainfall may results in the subsequent increase in potential infection rates in August-September. Subsequently high faecal egg counts was observed during July-November.

Moreover, periparturient eggs rise was observed in the parturient ewes which is attributed due to increase in the worms burden. It is evident from the present observations that the infective larvae (L_3) of trichostrongyles become accumulated onto the herbage during the lambing period (March-April) resulting in heavily contaminated pasture for this period of the year and is unlikely to render them free from infection. Prior to this there is no evidence of larval (L_4) inhibition in the abomasal mucosa throughout winter season (November-February). Our observations are in consistent with the findings of Parnell *et al.* (1954) and Reid and Armour (1975) in Scotland. However, the climate of Scotland, is almost completely opposite to that of Pakistan, and is characterized by wet severe winter and less wet summer. In Pakistan, there is a moderate rainfall for the period of two month in late winter (February-March), followed by dry (May-June) and wet summer (July-September). Therefore, the larval availability has marked influence on the time of onset of periparturient eggs rise.

Furthermore, nutritional level also have profound effect on this egg rise phenomena, because there are two growing season in this region March-April and July-September, when a plenty of lush green vegetation is available together with conducive environment. Prior to March there is a nutritional stress particular in this region (Barani region), when non-availability of pasture is noted as a result immunity in sheep in general and in ewes particular become lowered. Present results have confirmed previous observations of Soulsby (1957) who gave the reason that an inverse relationship exist between season worm egg counts and antibody titre. It also agrees with Crofton's (1954) who postulates on the point that the falling immune status will pass through various stages which will allow invading larvae to establish themselves. Soulsby (1957) also considers the principal mechanism causing low resistance is due to lack of adequate stimulation of immune apparatus, with secondary factors viz., malnutrition, sever climatic conditions and pregnancy operating within this frame work.

The periparturient rise is responsible for increasing the output of eggs to a sufficiently high number so that significant levels of pasture infection can occur. It thus serves a useful purpose as a mechanism in ensuring persistence of parasitic infection from one grazing season to another.

Apart from above mentioned findings hypobiotic were larvae also recovered in the present study. It is observed that *Ostertagia* spp., *Trichostrongylus axei* and *Haemonchus contortus* is inhibited during dry and hot months of a year and indicates that these nematodes survive the dry summer season as inhibited larvae. This

agrees with observations of Altaif and Issa (1983) in Iraq, Vercruysse (1985) in Senegal, El-Azazy (1995) in Saudi Arabia. The reasons of larval inhibition of these species could be attributed to the environmental factors on the free-living stages in dry season, as described for other tropical and sub-tropical countries where inhibition of many trichostronglids occurs (Ogunsusi and Eysker, 1979; Chiejina *et al.*, 1989; Kaufman and Pfister, 1990). The tracer lambs used were raised virtually worm-free, and their age at the time of testing was standardized as far as possible. It is believed that environmental factor acting on the free-living stages of the nematode rather than immunological responses are responsible for the pattern of inhibition. Although in sub-humid zone the numbers of inhibited larvae (L_4) was to small to induce any clinical signs of gastroenteritis after resumptions of development. However, the frequencies of inhibition in semi-arid and arid zones have much importance because as monsoon approaches in July then they may manifest clinical gastroenteritis. In the light of inhibition the life cycle of both *Ostertagia* spp., *Trichostrongylus axei* and *Haemonchus contortus* it can be summarized as follows: during the spring the larvae ingested by sheep, being inhibition-prone, are arrested in development and final shelter in the mucosa of abomasum. During the summer, the adults represent the residue of the parasite burdens acquire the previous winter and spring. In summer rainy season the inhibited larvae start to mature and start lying eggs. From these eggs, a new generation of worms develop during the following late winter and early spring.

In young animals a significant worm count increase was noted.

This increase in worm counts suggests a host immune reaction against trichostrongyle infections which has not been established in early age of lambs (Dorny *et al.*, 1995). Therefore, our observations indicates that young animals are the important source of pasture contamination under normal management conditions where lambs are not dewormed regularly. It is also highlighted that trichostrongylosis is more probably a cause of chronic diarrhoea in lambs aged less than 10 months.

Seasonal variations in length of the survival and rate of development of two major trichostrongyles viz., *Trichostrongylus colubriformis* and *Haemonchus contortus* were demonstrated in laboratory experiments. It is observed that infective larvae were recovered from pasture samples in each month of the year and thus indicating potential for infection under sub-tropical climatic conditions.

From the epidemiological point of view it is important to note that mixed grazing systems are common between young and old animals species mainly cattle and sheep. These husbandry practices are of relevance regarding their control. It is thought that mixed grazing of sheep and cattle has been shown to produce better pasture utilization and better weight gains than single-host grazing in Australia (Johnstone, 1979), Ireland (Nolon and Connolly, 1977), northern Europe (Berlin, 1979) and United States (Jordan *et al.*, 1988). This is probably due to lack of cross infection between cattle and sheep. Moreover, cattle and sheep select different forages for consumption throughout the grazing season and therefore increase the utilization of the available forage without competing

with one another (Jordan *et al.*, 1988). It is to be pointed out that these studies had been carried out in well-established pasture management system in these parts of the world. As far as Pakistan, is concern the mixed grazing is being carried out over centuries. Therefore, it is very difficult to conclude that mixed grazing have produced some better results and thus merits further investigation on this important aspect.

The present study can provide some excellent understandings of the epidemiology of gastrointestinal trichostrongylosis of sheep in Barani (rainfed) areas of Pakistan. Some observations might help in designing control measures. There is no simple answer to these problems, and under such conditions, every caution to minimize infection must be exercised viz., use of management systems which provide for sufficient rest of pastures in critical period, avoidance of overstocking and placing young stock on pastures previously grazed by uninfected animals, judicious use of anthelmintic and use of improved pasture or supplemented feeding on permanent pastures. Therefore, anthelmintic treatments during the wettest months are advisable. Infection of lambs at a very young age needs special attention because of high susceptibility to the effects of parasites at this age. It is recommenced that young lambs be included in deworming programmes. However, a better approach would be to prevent this age group from becoming infected by keeping then penned until weaning age. Moreover, grazing of young and adult animals together on poorly drained land, overstocking and forced grazing be discouraged. Finally, the ewes are usually a more important source of infection of the newly born

young animals then residual pastures contamination and consequently anthelmintic treatment of the ewes before or after lambing time in conjunction with provision of uncontaminated pasture is recommended.

As far as parasitic control in nomadic and transhumance is concerned such control programmes in nomadic communities are hindered by lack of adequate macroepidemiological data on parasitic disease. No attention has been paid to generate relevant information on the ecological aspects in any part of Pakistan. Under such conditions rapid field diagnostic test should be developed such technologies should be used in collaboration between farmers, scientists and veterinarians to determine the importance and incidence of parasitic infections in different agro-ecological zones of the country. New demographic and epidemiological methods exist, enhancing acquisition of infection required to develop appropriate veterinary and health services polices. One important dimension in the development of veterinary and health services policies, in developing institutional capacity to process and use such information. Nomadic health units are expensive to run then fixed units and has no greater impact in health delivery (Aliou, 1992; Omar, 1992). However, fixed units have certain limitations but there advantages include providing a sense of permanency for the local population and they are increasingly being developed (Aliou, 1992). Mobile and seasonally flexible primary health care and veterinary services matching the needs of specific nomadic population should be developed.

Relevant information, education, involvement of the local

people and training programmes should be generated which will help in control attempts when gastroenteritis outbreak will occur. However, a good knowledge of the ecology of the free-living stages of other main trichostrongyle genera viz., *Oesophagostomum* spp. *Trichostrongylus axei* and *Ostertagia* spp., is necessary in order to adapt any control strategy. Similarly the epidemiological studies in other geographical zones should also be carried at comprehensive level. On the basis of these data(s) in future mathematical predicting model of trichostrongyle infections can be designed which will then help in control measures.

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ANNEXURE-I

PROFORMA FOR THE RECOVERY OF GASTROINTESTINAL NEMATODES

Serial _____ Date_____

Locality _____ Sheep breed _____

Zone : Arid/Semi-arid/Sub-humid Age of animal _____

Physical status : Healthy/ Weak/Very weak/Diarrohea/Non-Diarrohea

Nematodes	Genus	Species	Site of predilection	Worm burden
				-
				-
				-
				-
				-
				-
				-
				-

Faecal egg counts (EPG):

Numer of egg in McMaster chammber

i _____ ii _____ iii _____

Mean egg per gram _____

Serum pepsinogen level (Tyrosine um):

_____

ANNEXURE-II_

PROFORMA FOR THE RECOVERY NEMATODES LARVAE FROM SOIL AND HERBAGE SAMPLES

Positive/Negative Dated:

1. Locality _____ 2.Vegetation type_____

3. Weight of fresh samlple_____ gms. 4.Dry matter_____

5. Soil type_____ 6. Height of herbage_____

7. Depth of soil_____ cm. 8.Time of sample_____ am/pm.

9. Min.temp___^{0}C Max..Temp.^{0}C_____ 10.Humidity._____%

11. SEASONS (Win.Spri.Summ.Rainy.Autum)_____

12. Duration of sunshine_____ 13.Tim.sun set & rise_____

14 Total number of larvar recovered from soil_____

15 Total number of larvae recovered from herbage_____

GENERA AND SPECIES WISE DISTRIBUTION OF LARVAE

GENIUS	SPECIES	MEASUREMENTS	NUMBER
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Nematodes eggs Positive/negative_____

Egg per gram(EPG)_____ Larvar per gram (LPG)_____

Misscell._____

ANNEXURE-III

PROFORMA FOR LABORATORY CULTURE OF TRICHOSTRONGYLES LARVAE

Serial no._____ Date._____

1.Nematodes spp_____ 2.Culture season_____

3.Culture starting date _____ 4.Culture termination___

5.Maxi.Temp._____ 6.Mini.Tempt._____7. Hum.(%)_____

8.Stage of hatching. Embry./Non-Embry./Ist/2nd/3rd stage
9.Incubation duration._____ 10.Culture material_____

11.Number of larvae obtained(LPG)_____

12 Egg per gram(EPG)_____ 13.Mon.bi.tri.tetra. infection

14.Comments_____

_____

_____

_____