A SURVEY ON THE INCIDENCE AND BLOOD PICTURE CHANGES IN SHEEP AND GOATS WITH GASTRO-INTESTINAL NEMATODE INFECTION

Dissertation Submitted for the Degree of Master of Philosophy in ZOOLOGY

BY

MASUD AHMAD

DEPARTMENT OF ZOOLOGY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1986
Dedicated
To
My Parents
This is to certify that the dissertation for M. Phil. in Zoology has been completed by Mr. Masud Ahmad under my supervision. It is original in nature and I have permitted the candidate to submit it for the award of M. Phil. degree in partial fulfilment of the M. Phil. requirements in Zoology.

Jamil A. Ansari

(Lr. Jamil A. Ansari)
CONTENTS

ACKNOWLEDGEMENTS

I. INTRODUCTION ... ... ... 01

II. LITERATURE REVIEW ... ... ... 04

III. MATERIALS AND METHODS ... ... ... 25

IV. RESULT AND DISCUSSION ... ... ... ...
   1. Incidence ... ... ... ... 40
   2. Serum Protein Changes ... ... ... 52
   3. Serum Alkaline and Acid Phosphatase Activity ... ... ... 57
   4. Serum Inorganic Phosphorus and Glutamic Pyruvic Transaminase ... ... 62

V. SUMMARY ... ... ... ... 67

VI. REFERENCES ... ... ... ... 72

- oOo -
ACKNOWLEDGEMENTS

I wish to express my sincere gratitudes to Dr. Jamil A. Ansari for his invaluable guidance, constant encouragement and selfless help during the progress of this work.

I am highly thankful to Prof. Ather H. Siddiqi, Chairman, Department of Zoology, for providing laboratory and other facilities.

My heartfelt thanks are to my lab. colleagues, Dr. Wajihullah, Faiz Ahmad Khan, Akbar Ali and other friends for their cooperation and constant encouragement. I am also thankful to Mr. Mohd. Rashid for typing the dissertation.

Last but not the least, I am thankful to the Council of Scientific and Industrial Research, for financial assistance.

Masud Ahmad

( Masud Ahmad )
I. INTRODUCTION

Being an agricultural country India's livestock, which account for nearly ten per cent of the world's livestock population and twenty five per cent of the cattle and buffalo, play an important role in national economy. Livestock products are an important source of foreign exchange in the country. Export of these commodities during the past few years have steadily gone up from Rs. 40 Crores, during 1962-63 to Rs. 177 Crores in 1974-75. The average wool yield of the sheep in our country is 800 to 900 gm./head/anum with an estimated total at 3.6 Crores Kg., whereas in other countries, sheep like Merinos, Ramboillet, produce about 4 to 5 Kg. per year. Foregoing comparison indicates our lack of approach and mismanagement towards the livestock and its products in the country. Goats, also play very significant role for villagers. It is a hardy animal and cheap to maintain and provides a variety of commercially useful articles. Goat's milk is wholesome, nourishing and is considered especially suitable for infants.

Although attention has been paid for the improvement and maintenance of livestock, but these appear to be quite insufficient, because animal diseases in general and parasitic diseases in particular are at alarming level. Among parasitic diseases, helminthiasis especially nematodiasis, is one of the major cause of the economic loss to our dairy industry and
livestock. Among nematodes a number of species such as Ilaemonchus contortus, Oesophagostomum columbianum, Bunostomum trigonocephalum and Tricharis ovis belonging to three major groups namely, the Trichostrongyloidea, Strongyloidea and Trichuroidea commonly parasitise the gastro-intestinal tract of herbivore animals in our country. Among these, trichostrongyle infection is much more important as a veterinary problem and causes pathological conditions like anaemia, black rush, weight loss, poor wool and milk production, bottle jaw. Oesophagostomum columbianum produces pathological conditions like diarrhoea, loss of appetite, emaciation, weight loss and nodule formation, while infection of Bunostomum trigonocephalum is associated mainly with anaemia and weight loss. Trichuris ovis, has been reported to be less pathogenic but anaemia, haemorrhagic necrosis, oedema of caecal mucosa and diarrhoea has been reported in severe infection.

Work related to biochemical pathology of these worms is scanty and scattered. Any systematic approach for investigation of pathological alterations has not been adopted. Most of reports are based on the experimental infection of the host, which are usually kept under controlled conditions. The effect of the factor like starvation, malnutrition and repeated exposure to different parasitic infections, which are generally faced by the animals of a farmer has not been screened and their effect on the establishment of parasite and ultimately
on the pathological alteration has not been worked out. At the same time any defined correlation could not be established between the experimental and natural infection. Further, biochemical pathology of the host in relation to these common gastro-intestinal nematodes appear to be most wanting. The results will be helpful for comparing the findings of natural and experimental infection.

Since, eradication and control programmes can not successfully be put to operation unless a clear data on the disease is available. Evaluation of seasonal variation in the incidence of infection and consequent blood picture changes will help to provide a better understanding of the host-parasite relations as well as adoption of control measures to achieve best possible success.

Government of India is paying special attention to provide better health care for improvement of the livestock and dairy industry. The Ministry of Agriculture has established five regional disease diagnostic laboratories. Prophylactic measures against common diseases and ailments are being attended by these institutions. The piece of work is expected to contribute immensely in National Programme of livestock improvement and may provide speedy diagnosis and expert consultation in matter of obscure and emerging diseases of animals.
II. LITERATURE REVIEW

The data available on incidence and seasonal changes of gastro-intestinal nematodes are quite meagre and very much scattered. Reports on the blood picture changes of the infected host are mostly based on the work done under induced infection. Informations about the natural infection of the host in totally exposed conditions are very few. Hence a generalized account of gastro-intestinal nematodes and blood picture changes of host are given here.

Bessonov (1958) reported the infection of Haemonchus in cattle, which rose rapidly exhibiting a single peak in August in West Kazakstan. Havorka and Dedina (1961) examined 1586 sheep in the Carpathian region of Czekoslovakia and found that Bunostomum trigonocephalum was present throughout the year. The incidence attained a first peak in November, with the highest degree of infection (i.e. the number of parasite per host) in December. He also observed that in sheep over one year old, the incidence had its first peak in spring and second in autumn. It is assumed that the life span of parasite within the host is four to six months. The incidence (i.e. number of host infected) and degree of infection increased until the age of five years, after this the incidence continued to increase whilst the degree of infection decreased. Savinkova (1963) noted the incidence of Bunostomum
trigonocephalum 86.1% in steppe area of Chita region and 63.1% in forest steppe area and 30.8% in mountain-taiga area of the same region. The infection reached a peak in April-May; in sheep aged 2 years and over and in all cases a gradual fall of infection was recorded by January. Narain (1965) also discussed the survival of first stage and infective larvae of Bunostomum trigonocephalum. The author observed that Bunostomum trigonocephalum, being originally a parasite of temperate region is better adopted to a cooler environment. Zhidkov (1966) studied the relationship between the number of larvae from faecal culture and number of Bunostomum trigonocephalum adult recovered. He found that for each adult Bunostomum trigonocephalum there were 0.9 (±0.4) larvae per gram of faeces in summer, 0.4 (±0.1) in the winter and 0.1 (±0.3) in the spring. Manuel and Madriaga (1966) reported 75% infection of Trichuris ovis, 100% infection of Oesophagostomum columbianum and 85% infection of Haemonchus contortus, by examining 20 adult goats at Manila in Philippines. Qadir (1967) reported high incidence of Oesophagostomum columbianum and Haemonchus contortus, throughout the year in East Pakistan. He observed no significant seasonal variation in the incidence of infection. Tripathi (1970) found highest number of infective larvae of Haemonchus spp. towards the end of south-west monsoon and lowest in hot season, by culturing faecal samples in Uttar Pradesh. Narain (1971) reported from Lucknow, India
that female *Oesophagostomum trigonocephalum*, were always more numerous than male in sheep. The observation was made by worm counts on eight sheep killed daily throughout the year. They found two peaks of infection of this worm, first during May-July and second during January-February. Sinha and Sahai (1973) reported 14.4% infection of *Oesophagostomum columbianum*, 48.1% of *Trichuris ovis*, 67.2% of *Haemonchus contortus* by examination of 172 goats in Bihar. Patnaik et al. (1973) reported through observation of 442 sheep from Rajasthan that *Haemonchus contortus* infection was predominant in Ramboillet sheep. They also observed the maximum infection of *Haemonchus contortus* after monsoon, while *Oesophagostomum columbianum* nodules were found throughout the year. *Trichuris ovis* infection was common to sheep of all age group, whereas *Oesophagostomum trigonocephalum* was present in the adult sheep only. Pucilowska and Borowik (1974) reported 51% infection of *Trichuris ovis*, 37% of *Oesophagostomum venulosum* and 33% of *Haemonchus contortus*, by post-mortem examination of 57 sheep, from Gola state of Poland. Qadir (1974) reported 19% infection of *Trichuris ovis*, 58% of *Oesophagostomum*, by examination of 190 pieces of goat's large intestine in Bangladesh. Guimaraes et al. (1976) examined 75 crossbreed sheep in Brazil and found 85.3% infection of *Haemonchus contortus*, 78.7% of *Oesophagostomum columbianum*, 68% of *Oesophagostomum trigonocephalum* and 4% of *Trichuris discolor*. 
The sheep were allowed to graze on the same pasture which were used by cattle earlier. Conteras et al. (1976) found 90% infection of gastro-intestinal nematodes after examination of 2700 goats in Venezuela. Infection of *Haemonchus contortus* was found 69%. They also noted that *Haemonchus contortus* causes 10 to 30% mortality particularly at faecal egg count between 650 and 4100 e.p.g. Infected goats showed considerable reduction in haemoglobin, packed cell volume and total protein.

Ghosh et al. (1976) reported death of 119 goats from a goat farm in Mizoram, India and found all the 25 random samples of stool were positive for *Haemonchus contortus* eggs and the number was also very high. Adult *Haemonchus contortus* were also found in the abomasum of goats. The abomasae were found to be highly congested and filled with chocolate coloured contents along with adult round worms. Accumulation of fluid was found in the pericardium and gelatinous mass in the heart muscle. The authors stressed on haemonchosis to be one of the main cause of mortality among ruminants. It was further observed that such animals become the victim of secondary infections because their vitalities are lowered due to heavy worm burden. Pneumonia was found to be another common manifestation with lungs highly congested, haemorrhagic and consolidated at places and pathological examination of lung
tissue revealed typical changes of purulent bronchopneumonia. Bali and Singh (1977) reported that 80% sheep and 63.4% goats were infected with *Haemonchus contortus* in Hissar. In sheep, incidence was 100% during July to November and 100% in goats during February and March. Pelinski (1980) reported 95.1% infection of abomasum by eight species of nematodes in Poland. *Haemonchus contortus* was found to be responsible for 23.7% infection with an April peak and a large peak of abundance in August. Prasad and Singh (1981) reported 74.67% infection of *Haemonchus contortus* in goats by autopsy examination of 1295 abomasa. They noted highest infection (100 per cent) during September and October and it was found lowest during April, 13.33%. The peak prevalence was found from July to December and fall in infection rate from February to April. The authors observed that heavy rain in July with R.H. 30-35 and environmental temperature 18-36°C was optimum condition for infection. The infection attained a peak from July to November only in areas where there is a good summer rain fall, which is uncommon in semi-arid regions. Biggs and Anthonissen (1982) reported that *Haemonchus contortus*, was present from March to early July in Karakul sheep of South-West Africa. The proportion of 4th stage larvae gradually increased from 49.7 to 96.3, and a 'spring rise' in faecal egg counts of sheep from the flock was seen in October. Prasad and Singh (1982) reported 74.7% infection of *Haemonchus contortus* by
examining 1295 abomasa of slaughtered goats between March 1980 and February 1981 in Hissar, India. The prevalence rose to a peak of 100% in September-October. It was found lowest (about 20%) in March-April (1980) and January-February (1981).

Jordan and Stair (1984) gave a very interesting result although not about incidence but about the significant fluctuations or differences which were found between the necropsy and endoparasitic counts. They reported that in Oklahoma, U.S.A., of 49 cattle, 32 were diagnosed by antemortem for endoparasitism. Post mortem worm count confirmed the diagnosis in only 19 cattle. It shows that diagnostic tools for these endoparasites are still not sufficient and in many cases the diagnosis is not reliable. Chiejina and Fakae (1984) made significant contribution by studying pasture survival of larval stages of gastro-intestinal nematodes. A survey was carried out from September 1981 to 1982 at Nsukka, Eastern Nigeria, which reflected that the dry season (November to March) was generally unfavourable for parasite development. The small amount of rainfall in last two months was found to be sufficient for significant development and migration of infective larvae. It was confirmed by tracer study that only those paddocks contaminated late in the dry season were infective at the start of the rains.
Little work has been done on the biochemical pathology of these parasites and no systematic approach has been made particularly to find out the effect of parasite on the blood of the host. Blood is an important factor and plays significant role in maintenance of homeostasis. It is a good source where manifestation of many parasitic diseases are found. Most of the report of blood picture changes are from the host under experimental condition. The effects were analysed using the animals kept under control conditions which do not have any chance of reinfection, and even the results with experimental infections show variations.

Shumrad and Eveleth (1955) reported a drop in haemoglobin and haematocrit levels and over 50% fall in inorganic phosphorus of blood after infecting the lambs with, 100,000, 40,000 and 10,000 larvae of *Trichostrongylus colubriformis*, *Haemonchus contortus* and *Nematodirus spathiger*, simultaneously. A decrease in serum globulin and corresponding increase in albumin was found while total serum protein varied from time to time. Bremner (1959) studied the influence of mixed helminth infection and *Bunostomum trigonocephalum* infection on blood and liver copper levels of dairy calves. The calves infected with *Haemonchus placei*, *Bunostomum phlebotomum*, *Oesophagostomum* and *Cooperia* spp. showed drop in liver copper levels from 324 p.p.m. to 139 p.p.m. after nine weeks. He
noticed that the degree of depression appeared to be related to the degree of infection. Mould and Silverman (1959) found that successive subcutaneous injection of larvae of *Haemonchus contortus* induced a pronounced increase in the β-globulin of sheep sera. Danilyavichus and Matusyavichus (1968) estimated alkaline phosphatase level in 20 pigs given per os 50, 500, 5000 and 50,000 larvae of *Oesophagostomum dentatum*. They reported a rise in faecal alkaline phosphatase level from day 3 to day 24 post infection and thereafter decline to normal levels one to two months post-infection. The level of alkaline phosphatase was particularly high in pigs given per os 5000 or 50,000 larvae. Bremner (1970) estimated average blood loss between 3 and 12 weeks, equivalent to 8.5 litres of jugular whole blood. The author concluded that intestinal haemorrhage is the prime cause of anaemia associated with *Oesophagostomum radiatum* infection in cattle. Kumar and Deo (1970) reported that 3 to 4 month-old goats become more susceptible to *Haemonchus* spp. infection by deficiencies in vitamin A and calcium but protein and phosphorus deficiencies had no effect. Barowicz and Petryszak (1970) studied haematological changes in thirty 100-day old lambs infected with 20,000 larvae of gastro-intestinal nematodes or 10,000 larvae of *Haemonchus contortus*. They found that haemoglobin level gradually declined from 30 to 120 days after infection. Greater changes in the blood picture were evoked by *Haemonchus*
contortus infection than by mixed gastro-intestinal nematodes. Graham and Charleston (1971) studied the pathogenicity of Bunostomum trigonocephalum by infecting 14 worm free sheep with 4,000 larvae. They found that levels of anaemia and hypoalbuminaemia were approximately proportional to the number of worms recovered at autopsy. Shastry and Ahluwalia (1972) reported that total serum protein and albumin level in goats fell over a 10 week period after infection with 5,000 to 40,000 3rd stage Haemonchus contortus larvae. Increase in serum globulin and decrease in albumin globulin ratio was recorded. Yujic et al. (1972) reported that chronic Oesophagostomum infection causes emaciation in sheep on sandy pastures around Bela Crkva, Yugoslavia. The condition was aggravated by poor diet and other intestinal and lung parasites.

Rajasekaraiah and Venkatarathnam (1973) experimentally infected lambs with 15,000 Bunostomum trigonocephalum larvae each and two other lambs were maintained as controls. The pre-patent periods were 75 and 78 days in lambs. The low worm burden of 150 and 175 were attributed to the adequate protein diet on which the animals were maintained. They found no significant change in the total R.B.C., Hb, total W.B.C. in the leucocyte, blood glucose or total serum protein. Blood samples from sheep naturally infected with Bunostomum trigonocephalum, showed reduction in serum globulin, but the total serum protein and blood glucose was unchanged. Steel (1974)
studied consequences of gastro-intestinal nematodes in relation to the nitrogen economy of the host and effect on animal productivity. He suggested that although there may be an apparent reduction in the next availability of nitrogen from the gut of infected animals, this is probably due to increased secretion of endogenous nitrogen rather than decreased digestion and absorption of dietary nitrogen.

Increased leakage of plasma protein into the gut is a feature common to many nematode infections of ruminants and is considered an important source of increased endogenous nitrogen secretion. The leakage also results in an increased albumin turnover, which in laboratory animals is associated with an elevated protein synthesis. Together with inappetance, these changes were probably responsible for the decreased synthesis of meat and wool protein through their influence on availability of amino nitrogen at the tissue level. Bremner and Fridemanis (1974) studied intestinal blood loss in calves infected with 7,500 to 50,000 larvae of *Oesophagostomum radiatum*, using cromium (Cr⁵¹) labelled erythrocytes. They observed that emergence of histotrophic 4th-stage larvae from the submucosal cyst was associated with intestinal dose of larvae. The bleeding was sufficient to cause anaemia and the depressed serum protein was also observed in animals. At the lower infection rate the haemorrhage caused by larvae was considerably less than those produced by ensuing adult
population. Correa et al. (1974) studied the influence of larval stage of *Haemonchus contortus* on the blood level of glucose, creatinine and cholesterol but it caused a gradual increase of blood urea from about 60 to 95 mg/100 ml. Georgieva and Vladimirova (1975) studied changes in serum protein fractions. A rise in the globulin and a drop in albumin fraction were observed. Dragie and Allonby (1975) studied the effects of single and challenge infection of *Haemonchus contortus* on red cell kinetics and self cure phenomenon. They observed that Merino sheep infected with 10,000 larvae of *Haemonchus contortus* and reininfected 7-8 weeks later, show three stages in the development of anaemia. The first stage occurs during the initial three weeks and characterized by progressive fall in P.C.V. accompanied by continuous abomasal haemorrhage and marked stimulation of erythropoiesis achieved at the expense of the animals iron store. The final stage is characterized by a dramatic reduction in P.C.V. and serum iron concentration. It shows exhaustion of the host's synthetic machinery due to deficiency of iron and possibly available protein. Oliveira (1976) studied eosinophilia in calves aged 4 to 7 months infected with approximately 1700 larvae of *Cooperia* sp., 1600 of *Oesophagostomum* sp. and 720 of *Haemonchus*. He observed no significant correlation between the ova in the faeces on one hand, and the number of eosinophils or leucocytes in the blood on the other.
Zajicek et al. (1976) studied values of Ca, P, Mg and buffer capacity in the blood serum of lambs experimentally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*, and changes after treatment with Nilverm. Six lambs were given mixed infection with approximately six thousand larvae of each and three lambs maintained as control. They found that Mg level remained with the range of healthy sheep while Ca level decreased and P level increased. Treatment with Nilverm was followed by an increase in Ca level and a decrease in P level which, however, were not due to the successful treatment. The buffer capacity of blood was not affected by the infection. No significant change in these values were observed following natural infection in summer and autumn. The experimental results show some variation between individual animals. Kerboeuf (1977) studied changes in pepsinogen, proteins and lipids in the serum during experimental haemonchosis in sheep. He found that development of larvae even in small numbers, caused lesions in abomasum, in two groups of 5 lambs aged 10 weeks. He noticed same rise in pepsinogen level comparable to those of ostertagiasis. The rise was related to number of larvae given and the level was elevated for a long period. Decrease occured in total serum protein, albumin and total globulin levels. The results were in agreement with other authors. Peregudov et al. (1977) reported the pathology of *Bunostomum trigenocephalum* and found
severe damage around the site of attachment. Mahanta and Roychoudhary (1978) reported that 12 healthy goats infected with about 5,000 infective Haemonchus contortus larvae, showed a fall in serum iron level from the initial level of 167 μg/100 ml. to 139 μg/100 ml. 30 days after infection.

Bhatnagar et al. (1978) made a study of histopathology of Oesophagostomum columbianum nodules in experimentally infected lambs. They observed that lambs orally infected with 2,000 Oesophagostomum columbianum larvae were having varying sized nodules in the small and large intestine, enteric lymph nodes, liver, kidneys, pancreas and uterus, 56 days after infection. Nodules consisted of central necrotic mass surrounded by macrophages, lymphocytes, epitheloid cells and eosinophils. Worm tracts were sometimes observed in mesenteric lymph nodes, in the large intestine, kidneys and liver. Olivera and Penha (1978) studied effect of mixed infection on the blood protein level of calves experimentally infected with 1720 larvae of Cooperia sp., 1560 of Oesophagostomum sp. and 720 of Haemonchus sp. The examination was made at 6 days interval upto 54 days. The total serum protein fell from 6.3 g/100 ml. to 4.7, and the albumin from 2.1 gm/100 ml. to 1.4. The albumin/globulin ratio was not changed. Ogunsusi (1978) studied blood changes in two group of sheep, one suffering from acute and other from chronic helminthiasis (due primarily to Haemonchus and Trichostrongylus spp.). In the
first group there was a rapid increase in helminth egg output coupled with rapid decline in the value of P.C.V., haemoglobin and R.B.C. Fluctuations were also observed in the value of W.B.C. In the group with chronic infection, a slow and unsteady increase in helminth egg output was accompanied by a slow and steady fall in the values of P.C.V., Hb. and R.B.C. Uppal and Rai (1978) reported a fall in total serum protein from 5 gm./100 ml. on day 6 to 3.35 gm./100 ml. on day 36 following infection with 10,000 infective larvae of *Haemonchus contortus* in Mandya sheep. A drop in albumin/globulin ratio was recorded from 0.963 to 0.536, on day 36 and afterwards. Silva et al. (1978) studied effect of diet and intestinal worms on copper, iron and zink levels in the blood serum of sheep. The infected sheep showed a low level of serum iron while those treated with anthelmintics and uninfected showed a high level of copper, zink and iron in their blood serum. Bone meal in the diet raised the level of serum iron.

Halviya et al. (1979) measured blood loss caused by *Haemonchus contortus* infection in sheep, by labelling red cells with Cr$^{51}$. The faecal egg counts varied between 5900 to 23500 e.p.g. and mean daily blood loss in faeces was 59 ml. and mean blood loss per worm was 0.07 ml. A correlation was found between the intensity of infection, daily blood loss and
packed cell volume. Denev and Georgiev (1979) studied the effect of experimental infection with 5,000 to 10,000 larvae of *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Nematodirus* and *Cooperia* on the serum protein of sheep. They observed increase in serum protein on day 75. The albumin fraction increased and α-globulin fraction decreased and attained a maximum and minimum simultaneously on day 75. Kamenov (1979) reported that in sheep and goats given experimental infection of *Bunostomum trigonocephalum*, decrease in total serum protein and albumin were recorded after 50 days and onward. μ and γ-globulin were found to be increased at this time. Maximum changes were recorded after the appearance of clinical symptoms and albumin-globulin coefficient decreased from 1.23 ± 0.07 to 0.32 ± 0.17 in lambs and from 0.73 ± 0.02 to 0.36 ± 0.04 in sheep. Malviya et al. (1979) studied histamine-liberating action of the saline extract of *Haemonchus contortus* larvae and worms. They found that saline extracts increased cutaneous-capillary permeability in uninfected sheep and rabbit and sheep infected with *Haemonchus contortus*. The extract was supposed to exert a histamine liberator like action.

Kamenov (1980) studied changes in the activity of some serum enzymes in ewes and lambs infected with 20,000 3rd stage larvae of *Bunostomum trigonocephalum*. Author recorded increase in lactate dehydrogenase activity, 179% in ewes and 83% in lambs, aspartate aminotransferase by 146 and 100%
respectively, malate dehydrogenase by 288 and 73%, sorbitol dehydrogenase by 380 and 312%. Increase was also recorded in the activity of leucine-aminopeptidase and alanine-aminopeptidase. Alkaline phosphatase activity dropped by 67% in lambs and 50% in ewes. Kamenov (1980) reported new isoenzymes of malate dehydrogenase in the serum of sheep experimentally infected with 20,000 larvae of *Bunostomum trigonocephalum*, after 10 days. Kerboeuf (1980) observed increase in the level of pepsinogen among sheep after experimental infection with *Haemonchus contortus*. Experiments with naturally infected sheep also showed a direct relationship between the number of worms in the abomasum and level of pepsinogen. Herlich et al. (1981) reported development of cambendazole resistance in *Haemonchus contortus* after repeated exposure to anthelminthic. The resistance was now found to be reversible after letting the generation unexposed for many progeny. Hamid et al. (1981) studied effect of haemonchosis in sheep infected with 1,000 3rd stage larvae, at three levels of infection, on serum calcium, phosphorus, iron and glucose. Phosphorus, calcium, glucose and iron levels at beginning of infection, peak of infection and after treatment were 7.39 ± 0.69,
12.13 ± 1.11 and 6.57 ± 0.94; 9.58 ± 0.69, 6.44 ± 0.34 and 7.52 ± 0.49; 53.94 ± 6.59, 22.89 ± 1.89 and 54.39 ± 4.01; 182.26 ± 26.39, 75.83 ± 3.74 and 107.64 ± 9.41 respectively.
Adam (1981) reported that experimental infection of sheep with 4500 larvae of *Haemonchus contortus* depleted leucocytes and produced leucopenia a mild lymphopenia. Thymus atrophy and decreased size of spleen and enlargement of adrenal gland occurred concomitantly suggesting that they were caused by the stress of infection. The overwhelming change in bone marrow was a 4 fold increase in erythroid series cells. Primary immunization with rat erythrocytes produced similar haemagglutination antibody response in infected sheep. Blastogenic response of blood lymphocytes from infected sheep to larval antigen correlated with faecal egg counts but not with haematocrit or leucocyte values. The results were considered suggesting unresponsiveness of lymphocytes to worm antigen which occur during infection with *Haemonchus contortus*, was more closely related to contact with the parasite than to the pathological effect of infection. Dakkak et al. (1981) infected 4 lambs intraruminantly with 25,000, L$_3$ of *Haemonchus contortus*, twice in 39 days. Serum pepsinogen increased 600% after 7 to 8 days of the first dose. The abomasal pepsin increased from 7210 to 9220 mU of the tyrosine and significant increase in abomesal pH and Na$^+$ was found. The second inoculation was associated with similar abomasal changes but for shorter duration. They considered that second inoculation stimulated the egg laying of the adult of first infection. Animal
killed 21 days after first infection had only $139 \pm 41$ worms in the abomasum. Thomas and Ali (1983) studied effect of experimental *Haemonchus contortus* infection on the pregnant and lactating ewes. They found that the effect was not significant on pregnant ewes, but lactating ewes showed a marked weight loss and milk yield was 23% less, despite a fall in faecal egg counts and the same feed intake as the controls. The haemoglobin and albumin was found to be decreased. Penikova and Kamenov (1983) studied effect of *Bunostomum trigonocephalum* infection on the Ca, P, K and Na levels of serum and sugar level of blood in lambs, experimentally infected with 20,000 3rd stage larvae. They observed that with the development of disease, the concentration of Ca, K, Na declined whilst the concentration of P rose in lambs, on day 50 and onwards. In lambs the sugar level dropped 37.6% as the infection became clinical. Obasaju and Holmes (1983) studied nitrogen balance and digestibility in response to ovine haemonchosis. Two groups of six sheep were fed with different levels of nitrogen (14.9 and 8.6% crude protein); six were infected (1st) with 350 infective larvae of *Haemonchus contortus* per Kg. body weight. It was found that infected animals fed lower protein diet, had higher losses of nitrogen due mainly to a higher urinary nitrogen out put. Infected animals retained slightly more water than control.
Salman and Duncan (1984) studied abomasal histology of sheep experimentally infected with 350 *Haemonchus contortus* per Kg. body weight. They observed that in terms of worm burden the ewes were relatively resistant to reinfection compared with the lambs and the histological changes in the abomasum at most stages after infection were more marked in the adult animals. Barger and Cox (1984) studied the effect of *Haemonchus contortus* infection on the wool production of sheep. They established a chronic infection in a group of twenty 18-months old non-reproductive Merino ewes by oral administration of 3,000 infective *H. contortus* larvae twice weekly after 12 weeks. Comparison of live-weights and wool production was made with 20 uninfected ewes grazing the same pasture. In the infected sheep, faecal egg counts increased over a period of 9 weeks to reach a mean of 5,000 e.p.g., accompanied by small, but significant effects on packed cell volume and live-weight. The effect of infection on wool growth were also small, and not statistically significant, although there was evidence of a faster seasonal decline in wool-growth in infected sheep. It was concluded that mortality induced by acute infection is the most important feature of this parasite which puts an unbearable impact on the economy.

Abbott et al. (1984) studied pathophysiology of chronic ovine haemonchosis in Merino and Scottish Black face lambs.
and observed that a low level of nutrition caused the development of a normochromic, normocytic anaemia in abomasal blood loss and slightly elevated erythropoiesis in both breed relative to the controls. Further, Abbott et al. (1985) studied influence of dietary proteins on parasite establishment and pathogenesis in Finn Dorset and Scottish Black face lambs given a single moderate infection of *Haemonchus contortus*. They observed that in Scottish Black face lambs dietary protein did not significantly affect the establishment of *H. contortus*. However, in Finn Dorset lambs dietary proteins may have influenced parasite establishment since lambs on low protein diet had a higher faecal egg output 4 weeks after infection, and more severe clinical signs than infected lambs of the same breed on a high protein diet. Abbott et al. (1985) further studied the effect of dietary protein on pathophysiology of ovine haemonchosis in Finn Dorset and Scottish Black face lambs, using Cr\(^{51}\) red cells, Fr\(^{59}\) transferrin and I\(^{12}\) albumin. They observed that in normal control animals there was no significant effect of diet or breed on any of the erythrokinetic metabolic or nutritional parameters. In infected animals these parameters were affected by breed and diet. The severest effect was found in infected Finn Dorset lambs given a low protein diet. The animals had a higher level of abomasal blood loss and this was associated with low red cell volumes and high levels of both albumin catabolism and plasma
iron turn-over. These animals were also in negative nitrogen balance which was partly accounted by a significantly greater urinary nitrogen loss compared with their pair fed control and partly due to poorer digestibility of the crude protein fraction of the diet. Rosl et al. (1985) studied pathogenicity of *Bunostomum* infection in an out break of disease in a herd of 140 beef cattle and weaned calves, in Austria. They infected further seven calves aged, 3 to 4 months with 20,000 to 30,000 larvae. They found that chronic haemorrhagic anaemia is principally responsible for the pathogenicity of bunostomiasis. Erythrocyte levels were severely reduced, already 35 days after infection of weaned calves, with an almost simultaneous drop in haemoglobin and haematocrit values and in serum levels of alkaline phosphatase, γ-glutamyl transferase and cholesterol. Aspartate aminotransferase levels tended to remain unchanged. The animals most at risk were those aged 5 months.
III. MATERIALS AND METHODS

1. Collection:

Collection of gastro-intestinal nematodes of sheep and goats was made twice in a week from local abattoir of Aligarh. For this purpose, complete gastro-intestinal tracts of slaughtered animals were obtained. These were brought to the laboratory for collection of parasites. All possible care was taken during transportation to avoid any leakage of gastro-intestinal contents and it was brought in separate bags.

Stomach, rumen, abomasum and omasum were first detached from intestine and then carefully autopsied to record the number of parasites. The contents of different portions were diluted in saline and thoroughly checked. Each part was cut open and washed under tap water and mucus membrane was carefully rubbed with fingers to remove worms adhering to it. The worms were washed in physiological saline repeatedly and successive transfer in fresh saline was made. These worms were identified and counted. Those selected for examination were checked in lactophenol and whole mount in glycerine was prepared for detailed study.

Most of the animals (sheep and goats) were between one to two years age. The slaughtered animals were mainly from the suburb of Aligarh and few from neighbouring states, as Rajasthan
and Haryana. Random collection was made in terms of sexes of hosts, but most of sheep were ewes, while goats were both he and she.

For studying blood picture changes, the samples were collected from the hosts, both sheep and goats, and hosts of approximately one year old were selected for this purpose. Blood samples from each host were labelled and gastro-intestinal tract of respective animals were kept together. Liver and lungs were also checked for infection of other parasites. In case any of these organs were found infected, the sample was discarded. Small portion of each sample was separately collected in bottles containing anticoagulants. This blood was also thoroughly checked to detect infection of possible blood parasites. Gastro-intestinal tracts of the host were scrutinized and in case of mixed infections with other parasites, the sample was discarded. Those animals which were free of helmintic infections were selected as controls. Thus by continuous sampling and checking only those samples were selected which were obtained from the hosts infected with *Haemonchus contortus* only. Bottles containing blood, were brought to laboratory in ice boxes, to check any enzymatic activity. Hemolysis of blood was avoided by using completely dry and sterile bottles for collection and other possible care was taken.
Blood was allowed to clot at room temperature for ten to fifteen minutes and serum thus retracted, was collected in sterile and dry bottles. The blood was slightly rimmed with an sterile needle and then transferred to the refrigerator for further retraction of the serum. The serum was centrifuged at 2500 r.p.m. for five minutes to remove any trace of the erythrocytes. Serum was immediately separated from the clot as the later renders many determinations like potassium and alkaline phosphatase useless. This is due to disintegration of some cells of the clot. Analysis of serum was carried out soon afterwards.

2(a). Total serum protein:

Procedure: Quantitative determination of serum protein was made by Lowry's Method.

Reagents:

(i) 2% sodium carbonate in 0.1 N sodium hydroxide. (A)
(ii) 0.5% copper sulphate in 1% sodium potassium tartrate. (B)
(iii) Mixed 50 ml. of reagent A with 1 ml. of reagent B. This is reagent (C).
(iv) Folin Phenol reagent.

0.5 ml. of serum was taken. Protein was precipitated by adding 3 ml. of F.C.A. (10%). The solution was centrifuged
and aliquot was taken for analysis. The aliquot was washed with 5 ml. of 0.1 M potassium acetate prepared in absolute alcohol. Centrifugation and washing was repeated with potassium acetate. The aliquot was then washed and centrifuged with absolute alcohol. The same process was repeated with solvent ether. In all cases washing and centrifugation was done twice with 5 ml. portions of solvents. Lastly some ether was left with the precipitate. It was evaporated gently and precipitate was dissolved in 5 ml. of 0.1 N sodium hydroxide. The 0.1 ml. of aliquot was taken and protein was estimated as follows.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Blank (ml.)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliquot</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Water</td>
<td>1</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Reagent C</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Folin Phenol Reagent</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Final volume of each sample was made 10 ml. with double distilled water and after 10 minutes, the per cent transmission of the solution was read in spectrophotometer at 540 μm.

Standard curve for protein was already plotted using bovine serum albumin. Results of different infected samples
and uninfected controls have been compared in table 2.

2(b). **Albumin and Globulin:**

**Procedure:** Albumin and globulin were estimated quantitatively by Greenburg method. Globulin was precipitated from serum by sodium sulphate solution. Albumin was determined colorimetrically in the filtrate by means of phenol reagent. Globulin was determined by subtracting albumin from total protein. For confirmation and to avoid error globulin was also determined from the precipitate.

**Reagents:**

(i) 22.5% sodium sulphate solution: - 22.5 gm. of reagent grade sodium sulphate was transferred in a volumetric flask (100 ml.) and solution was prepared. To keep all the phosphate in solution the reagent was kept in an incubator at 37°C.

(ii) 5 N sodium hydroxide solution.

(iii) Standard tyrosine solution: - 20 mg. of tyrosine was dissolved in 100 ml. of 0.1 N hydrochloric acid in a volumetric flask.

(iv) Folin phenol reagent.

0.5 ml. of serum was taken in a test tube. 9.5 ml. of 22.5% sodium sulphate was added. The solution was agitated thoroughly and kept in a water bath at 37°C for 2 hours to
allow coagulation of globulin. After two hours the content was filtered using a very retentive filter paper (Whatman No. 1). The filtrate was used for the analysis of albumin.

For the determination of albumin a 5 ml. aliquot of the filtrate was taken into a 50 ml. volumetric flask and about 25 ml. water was added. The other reagents added were as follows:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Blank (ml.)</th>
<th>Samples in ml.</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Aliquot</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5 N NaOH</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Folin Phenol Reagent</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

All the flasks were filled to 50 ml. mark with distilled water. The colour was read in photometer after 5-10 minutes at 540 μμ.

Precipitate of the above experiment was analysed for globulin determination. The precipitate was washed twice with 3 ml. each of sodium sulphate. The funnel along with filter paper containing the precipitate was transferred to a 50 ml. volumetric flask. A hole was punctured in filter paper and precipitate was washed with a stream of approximately 0.01 N sodium hydroxide from a wash bottle. The washing was completed with distilled water until the flask is half filled. In the
flask were added following reagents:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Blank (ml.)</th>
<th>Samples in ml.</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5 N NaOH</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Water</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Folin Phenol Reagent</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

All the flasks were filled to 50 ml. mark with distilled water. They were kept for 5-10 minutes for colour development. The colour was read in photometer setting the photometer to zero with blank at 540 μm. Standard curve was plotted using tyrosine solution and the same reagents as earlier. Results of different infected samples and controls have been compared in table 2.

3(a). Alkaline phosphatase:

Procedure: Alkaline as well as acid phosphatase was determined by Bondansky method with a little modification of Fisk and Subbarow (1925).

Reagents:

(i) Alkaline phosphatase substrate:— Into a 100 ml. volumetric flask introduced successively 3 ml. of petroleum ether (B.P. 20⁰ to 40⁰C), about 80 ml. of distilled water,
0.5 gm. of sodium \( p \)-glycerophosphate, 0.424 gm. of sodium diethylbarbiturate and water to 100 ml. mark. Transferred into a 100 ml. glass stoppered pyrex bottle containing an inch layer of petroleum ether. Kept in refrigerator.

(ii) 30% T.C.A.

(iii) Standard phosphate solution:— Placed 6.25 ml. of standard phosphate solution (containing 0.5 mg. of phosphorus) in a 100 ml. volumetric flask. 16.7 ml. of 30% T.C.A. was added. Now the solution was diluted to the mark with distilled water, and mixed. This solution contained 0.04 mg. of phosphorus in 8 ml. in 5% T.C.A. It was stored in cold.

(iv) Aminonaphtholsulfonic acid reagent:— 195 ml. of 15 per cent sodium bisulfate solution was placed in a glass stoppered cylinder, and then 0.5 gm. of 1,2,4- aminonaphtholsulfonic acid was added. Placed 5 ml. of 20% sodium sulfite. Stoppered and shaked until the powder is dissolved. If solution is not complete, more sodium sulfite, 1 ml. at a time was added with shaking. Transferred the solution to a brown-glass bottle and stored in cold. This solution was used only for two weeks.

Measured 8 ml. of alkaline phosphatase substrate into a glass stoppered tube and placed in an incubator or water bath
at 37°C until the tube had attained the incubator temperature. 1 ml. of serum was added and after recording the time it was incubated for exactly one hour. Tube was removed and cooled in ice water for several minutes and 2 ml. of 30% T.C.A. was added. Mixed and let stand for few minutes and filtered through low ash filter paper.

At or near incubation period, a control sample was prepared. 9 ml. of substrate was measured into a glass stoppered tube and added 2 ml. of 30% T.C.A., with mixing 1 ml. of serum was added. Stoppered, shaked and filtered.

When both of these filtrates were ready, 8 ml. of these filtrates were transferred to each graduated tubes. In a similar tube 8 ml. of standard phosphate solution was placed, containing 0.4 mg. of phosphorus. For photometric measurements blank was prepared by taking 8 ml. of 30% T.C.A. alone. When all the tubes were ready added to each 1 ml. of Molybdate II reagent and mixed. 0.4 ml. of ANSA was added to each tube. Diluted immediately to 10 ml. mark with water and mixed. Allowed to stand for 5 minutes for colour development. The colour was read in a photometer, setting the photometer to zero density with blank at 660 μm.

3(b). Acid phosphatase:

Procedure: The procedure was exactly the same as for alkaline phosphatase. The unit of acid phosphatase activity being
defined as equivalent to the liberation of 1 mg. per cent of inorganic phosphate during 1 hour incubation at pH 5.0.

Reagents: All reagents were the same as for alkaline phosphatase except the substrate.

(i) Acid phosphatase substrate: This was also identical with the alkaline phosphatase substrate already described except that sufficient acetic acid was incorporated to bring the pH to 5.0. Into a 100 ml. volumetric flask, introduced successively 3 ml. of petroleum ether, about 80 ml. of water, 0.5 gm. of sodium p-glycerophosphate, 0.424 gm. of sodium diethylbarbiturate and 5 ml. of 1 N acetic acid. Dissolved by mixing and water was added to make the volume 100 ml. The pH of final solution should be checked and should be 5.0, failing which adjustment was done by adding dilute acid or alkali as necessary.

Calculation:

The phosphatase activity is the difference between the inorganic phosphate content of the incubated and control samples, expressed in mg. of phosphorus per 100 ml.

The unknown and standard solutions were read in a photometer which was set to zero density with blank.

\[
\frac{\text{Density of Unknown}}{\text{Density of Standard}} \times 0.04 \times \frac{3}{2} \times 100 = \text{mg. inorganic phosphate per 100 ml. of serum (control or incubated}).
\]
Results of infected samples and control are presented in table 3.

4(a). **Inorganic phosphate:**

Inorganic phosphate was determined by the method of Fiske and Subbarow (1925).

**Reagents:**

(i) 10% T.C.A.

(ii) 10 N sulfuric acid: 450 ml. of concentrated sulfuric acid was mixed with 1300 ml. of water.

(iii) Molybdate I: 25 gm. of reagent grade ammonium molybdate was dissolved in about 200 ml. of water. In 1000 ml. volumetric flask, was placed 500 ml. of 10 N sulfuric acid. Molybdate solution was added and diluted with washing to 1 litre with water.

(iv) Molybdate II: 25 gm. of reagent grade ammonium molybdate was dissolved in about 200 ml. of water. In 1 litre volumetric flask placed 300 ml. of 10 N sulfuric acid and added molybdate solution in it. Diluted with washing to 1 litre with water.

(v) ANSA reagent: As in previous experiment.

(vi) 15% sodium bisulfite solution: 15 gm. of sodium bisulfite was dissolved in 100 ml. of water. If turbid, allowed to stand for several days and filtered. Kept well stoppered.
(vii) 20\% sodium sulfite solution:— Dissolved 20 gm. of sodium sulfite in 100 ml. of water. Filtered if necessary, and kept well stoppered.

(viii) Standard phosphate solution:— Exactly 0.351 gm. of pure dry monopotassium phosphate was dissolved in water and transferred quantitatively to one litre volumetric flask. 10 ml. of sulfuric acid was added, diluted to the mark with water and mixed. This solution contained 0.4 mg. of phosphorus in 5 ml.

To 8 ml. of 10\% T.C.A. solution was added slowly with mixing 2 ml. of whole blood, stoppered, shaked and filtered. Filterate was used for the analysis of inorganic phosphorus. The following reagents were added:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Blank (ml.)</th>
<th>Standard (ml.)</th>
<th>Samples in ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Aliquot</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Std. phosphate</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>T.C.A.</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Molybdate II</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ANSA</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

All tubes were diluted to the 10 ml. mark with distilled water. Allowed to stand for 5 minutes and read in a photometer at 720 m\(\mu\).
Results of different infected samples and controls have been presented in table 4.

4(b). **Glutamic-pyruvic transaminase:**

**Procedure:** Glutamic-pyruvic transaminase was determined by the method of Reitman and Frankel (1957).

**Reagents:**

(i) Phosphate buffer:— 0.1 M, pH 7.4. Mixed 420 ml. of 0.1 M disodium phosphate and 50 ml. of 0.1 M potassium dihydrogen phosphate.

(ii) Pyruvate:— 2 m M/litre (for standard curve). Dissolved 22 mg. of sodium pyruvate in 100 ml. of phosphate buffer.

(iii) α-ketoglutarate solution:— 2 m M/litre (substrate for GPT). Placed 29.2 mg. of α-ketoglutaric acid and 1.78 gm. of dl-alanine in a small beaker. 1 N sodium hydroxide was added until the solution was completed. Solution was adjusted to a pH of 7.4 with sodium hydroxide. Transfer the solution to a 100 ml. volumetric flask and dilute to the mark with buffer solution.

(iv) 2,4-dinitrophenylhydrazine:— 1 m M/litre. Dissolve 19.8 mg. of 2,4-dinitrophenylhydrazine in 100 ml. 1 N NaOH.

(v) 0.4 N NaOH:— 1.6 gm. of sodium hydroxide was dissolved in 100 ml. of water.
1.0 ml. of substrate (α-ketoglutarate for S.G.P.T.) was pipetted into a tube and placed in a water bath at constant temperature of 40°C for 10 minutes. Upon the addition of 0.2 ml. of serum the contents were mixed. After an incubation period of exactly 30 minutes the tube was removed from the water bath. 1 ml. of 2,4-dinitrophenylhydrazine reagent was mixed immediately thereby stopping the reaction. The tube was permitted to stand at room temperature for a minimum of 20 minutes. Then 10 ml. of 0.4 N NaOH was added. The contents were mixed by inversion. At the end of exactly 30 minutes the optical density of the solution was measured at 505 μμ using the blank for setting zero.

While the specimens were incubating, a blank for each sample may be prepared. 1 ml. of substrate, 0.2 ml. of water and 1.0 ml. of 2,4-dinitrophenylhydrazine reagent are mixed in test tube. After a minimum of 20 minutes, 10 ml. of 0.4 N NaOH is added. It is used as blank for setting zero.

Time for development of colour:— After addition of NaOH there is a rapid change in colour for approximately 20 minutes owing to the fact that colour continues to change slowly, all specimens should be read at exactly 30 minutes. The rate at which colour changes is not significantly influenced by temperature between 20° and 30°C.
With the help of calibration or standard curve already drawn with the increasing quantity of sodium pyruvate, the activity of the glutamic-pyruvic transaminase was calculated. It was expressed as the amount (in µ gm.) of pyruvic acid liberated per ml. of serum.

Results of infected and uninfected animals have been compared in table 4.
IV. RESULTS AND DISCUSSIONS

1. Incidence:

Incidence of gastro-intestinal nematodes was recorded regularly, twice in a week. Result (table-1) shows incidence of infection of the common worms throughout the year with fluctuation in the intensity here and there. Although, the cumulative data on the incidence level remained almost constant throughout the year, the infection rate, as a whole, showed slight decrease from February to April and the lowest incidence was found in April and from May to December. The incidence attained the peak with slight variations from month to month showing highest record of 95.16% in October. This was followed by a slight decrease in the degree of infection during the months of November and December.

Incidence of the two years 1984 and 1985 was recorded and tabulated separately and then average of the two years for every month was calculated for the mean incidence of all the four reported gastro-intestinal nematodes. This was done to minimise the possible errors of sampling and observations. Since the climatic conditions did not show any marked shift during the two years in Aligarh region so a generalized account was made to show monthly incidence.
Incidence of *Haemonchus contortus* was found to be highest among all the four gastro-intestinal nematodes recovered from sheep and goats of Aligarh region. From January to April, the incidence ranged between 34 to 40 per cent which showed an increase from May, reaching on 60.62 per cent. In June the incidence was found to be moderately high at 76.94 per cent. In July and August further rise was recorded as 83.87 and 89.89 per cent respectively, attaining the peak 92 per cent by October. A fall was recorded towards November, 82.54 per cent and further decline in December, as 50.54 per cent.

The results of the present study are very much in agreement with the findings of Sinha and Sahai (1973) who reported 74.2 per cent infection of this worm by examining 162 goats in Bihar, India. Approximately similar report was given by Bali and Singh (1977) from Hissar, which may be taken as an added support of the present observations. They recorded 80 per cent infection of *Haemonchus contortus* in sheep and 63.4 per cent in goats. In sheep incidence of infection was found 100 per cent during July to November and 100 per cent in goats during February and March. Although, data has not been separately analysed for sheep and goats in the present study but the pattern and degree of infection was found to be almost identical in the two hosts. The report of Prasad and Singh (1981) from Hissar (India) also revealed identical
pattern of incidence of *Haemonchus contortus*. They recorded 74.61 per cent infection from the goat, with a peak level of infection 100 per cent during September and October and 13.33 per cent during April. Prasad and Singh (1982) further reported 74.4 per cent infection of this worm from Hissar with almost similar peak. The authors attributed the peak of infection to heavy rain in July, and RH of 30-35 and environmental temperature of 18 to 36°C during the year. The present results are in agreement with the findings of Prasad and Singh with certain deviations. The incidence of infection showed an upward trend during May and peak was recorded from August to October, showing highest infection, 92.22% in the later month. The peak recorded one month earlier than the findings of Prasad and Singh (1981; 1982), may only be due to different geographical situations and variation in rain fall of the two places, Hissar and Aligarh. The incidence of *Haemonchus contortus* infection may be correlated with the seasonal pattern of the region, where the study has been made. In Aligarh region well defined summer, spring, winter and autumn are found. The summer rainfall (May) provides an opportunity for the parasite to infest new hosts successfully. Present findings may adequately be explained showing its correlation with climatic conditions. Free living development of *Haemonchus contortus* is directly related to temperature and moisture which act as a limiting factor.
for hatching and development. The rain fall of the early summer (May) in Aligarh region accounts for the crest of infection in June in the present study. Since the rain fall is very occasional and not severe in this month, so that the incidence was found moderate. The usual rainy season which approaches from early June provides optimum condition for the herbage growth and required moisture is maintained on the grass blade. At this time the temperature is also moderate to facilitate the activity of infective larvae. As a consequence incidence starts showing an upward trend in the following months, and a crest of infection was found from June to November, with slight fluctuations. Since no records have been maintained about the rain fall and temperature of Aligarh region, so any exact comment is not possible. In general the peak was followed by the rainy season, and optimum temperature as well as humidity appears to be the main factor influencing the degree of incidence. This statement is well evident by the sudden fall in the month of December, as shown in table - 1. Since temperature starts decreasing from the month of October as winter season approaches, so that infective larvae of *Haemonchus contortus* could not survive for long time and their development would have been slowed down, and all these factors collectively resulted in the fall of incidence during the month of December. At the same time rainy season terminates by the month of September
and absence of sufficient film of water on the leaf blade may also be a factor for decrease in the incidence. From January to April, further decrease in incidence was observed which may be due to little humidity available during these months which acts as a barrier for the infective larvae to reach the host. Since survival of *Haemonchus contortus* larvae is very little during winter, though a few may do so, as a result the incidence was found very low from January to April.

Besides, the factors discussed for the decrease of *Haemonchus contortus* infection, one of the most important and well established phenomena 'self cure' can not be over ruled, Stewart (1953); Soulsby (1960); Dineen (1966), reported and discussed the phenomenon to be responsible for the regulation of worm burden by a loss and replacement of worms. This process may also be partly responsible for the decrease of infection during the months from January to April. The parasites would have been expelled out from the host regularly and availability of massive infective larvae during rainy season appear to be responsible for high level of infection from July to November. After the rainy season, the phenomenon of self cure might have been continued and less availability of infective larvae may be a reason for the decrease in infection from December to April.
The incidence of *Oesophagostomum columbianum* infection which is found in the colon region remained almost constant throughout the year. The highest infection rate 68 per cent was recorded in June and second highest 65.65 per cent in November. Incidence of this parasite does not reveal any definite seasonal pattern and from January to December, random decrease and increase was recorded. In January, the infection was recorded 64.08 per cent followed by a gradual decrease by the month of April, reaching 50%. From May to November, the infection was recorded as: 54.99, 68.28, 59.30, 55.35, 61.57, 60.41 and 64.65 per cent respectively showing a peak in June and second in November. In December there was again a fall in infection attaining 52.77 per cent.

The present data shows slight variation from the findings of Qadir (1967), who reported from East Pakistan (now Bangladesh) a maximum infection 80 per cent during August and minimum during April as 60 per cent. The change appear totally due to the difference in the climatic conditions of the two places. Findings of Sinha and Sahai (1973), who observed 50% infection of this worm are almost similar and agree with present study. The results are also in agreement with Patnaik *et al.* (1973) and Guimeraes *et al.* (1976). The moderate incidence of infection of *Oesophagostomum columbianum* may be due to low survival of its infective larvae. The eggs and hatched larvae require a temperature above 9°C for
development. Extremely low temperature of December and January in Aligarh region appears to be a factor for checking the development of eggs and larvae on pasture, which ultimately results in reduction of infection during March and April. Environmental conditions naturally account for the intensity of infection but at the same time under heavy infection some of the larvae (L₃) may remain in the mucosa of small and large intestine of the host for 1 to 3 months and in mature animals for larger periods. However, bulk of juveniles that do not migrate out early, later become encapsulated and destroyed (Banner and Philip, 1949). The process of encapsulation also appears to be a factor, responsible for the moderate incidence of Oesophagostomum columbianum infection. After the rainy season, growth of herbage is promoted by the optimum environmental condition and infective larvae also get suitable condition for development. During this period infection is propagated among the grazing hosts frequently but does not attain a high degree because of the third stage larvae. After rainy season, in winter most of the larvae die on pasture due to very low temperature of this region but the larvae which return to the lumen of the alimentary tract appear to be responsible for maintaining a moderate incidence of this parasite during the months from March to May. Extremely long life span of some of the Oesophagostomum columbianum, as long as 21 months, may also
be a reason for the persistence of incidence throughout the year. Actually the process of encapsulation which is due to host's reaction, offers a resistance against the establishment of this parasite. The larvae may stay in these nodules for about 3 months, and when contents caseate and calcify, the parasites either die or leave the nodules. Since most of the larvae die within the nodules, so the phenomenon appears to be the main cause for the maintenance of a moderate incidence of the *Oesophagostomum columbianum* throughout the year as well as for the presence of few adult worms in the colon region. It appears that during the extreme environmental conditions which is not at all suitable for the development of egg and larvae, the population of this parasite is maintained in the host not only by the transmission of infective larvae from the pasture but by the emergence of encapsulated larvae from the wall of the small and large intestine.

The incidence of *Bunostomum trigonocephalum* was found to be lowest among all the four reported worms in the present study. The incidence of *Bunostomum trigonocephalum* ranged from 37.94 to 46.56% from January to April. In May and June the infection remained stable at 46% and in July slight decrease was noted attaining the infection rate 40.68 per cent. From July to December gradual decrease was
observed and minimum level of infection was found to be 30.53 per cent in November.

The results agree with the reports of Srivastava (1945), Narain (1965), Patnaik et al. (1973). They observed almost constant infection of this parasite throughout the year from different part of India. The findings of Shastry and Ahluwalia (1972), also confirm the present report although they had not recorded monthly incidence, but overall infection of *Bunostomum trigonocephalum* was found to be 50 per cent. Findings of Patnaik et al. (1973), although not in good agreement with the present observations, but 27% infection as revealed by them may be due to the fact that bulk of the hosts autopsied comprised of mostly lambs. Results of Misra and Ruprah (1968), from Hissar, India, also does not correlate with the present report because they observed only 0.83 per cent infection of this worm and that too during the summer season. This variation appears to be due to selective sampling of the viscera by the authors, as they autopsied only goats and out of which 87 per cent were males. The major factor which seems to be responsible is the age of the autopsied animals. Since it is well established that infection of *Bunostomum trigonocephalum* is predominantly found only in adult sheep and goats (Patnaik et al., 1973) and all of the animals selected by the authors were young.
The pattern of incidence in the present study shows that the peak was found in the months when the temperature remains moderate, that is after the winter season. The infective as well as first stage larvae can not resist dryness and survival time is also very low at high temperatures (Narain, 1965). The moderate temperature of late winter and sufficient moisture in the form of dew provides suitable conditions for the development of larvae on the pasture. As a result peak of incidence was found during March and April, in Aligarh and nearby areas, temperature starts increasing from February and eggs get suitable condition for development to infective larvae, and irrespective of the route of entry, infection starts decreasing and the low incidence of rainy season and winter appears to be due to adverse environmental conditions of summer which inhibit the process of larval development and so also the transmission.

Incidence of *Trichuris ovis*, infection was found to be highest in June and November with slight differences and the lowest about 38.63 per cent was recorded during April. From May to November the incidence of infection was found around 42 and 52 per cent with a drop in July which was 40.68 per cent. From August to November, the infection remained almost constant and in December, it was 49.27 per cent. It appears that the incidence of this parasite remains almost constant.
throughout the year with slight fluctuations, from month to month. The results presented here agree with the reports of Sinha and Sahai (1973), Shastry and Ahluwalia (1972). Findings of Qadir (1967), Misra and Ruprah (1968), are in good agreement with this result. Qadir reported 6 per cent infection in March, 1965 and touched the zero level in April and this drop may be specific for the region of East Pakistan due to its environmental conditions. The present observations also agree with the result of Misra and Ruprah (1968), with slight deviation. They reported incidence of *Trichuris ovis* infection around 60 to 65 per cent during winter, spring and summer but in autumn it was found to be 93 per cent. The present observation does not show any distinct rise in any month and increase in June and November was also not much marked. As already mentioned, these differences may be due to the fact that the random samples were obtained both from sheep and goats and also irrespective consideration of the age of the host. Slightly different mode of transmission of *Trichuris ovis*; as instead of infective larvae, eggs, bearing infective larvae are ingested by the host, may be responsible for constant incidence of infection. Since it is not a recognized pathogenic parasite, so reports on this worm are quite lacking from any part of the India or elsewhere outside, so any comparison at the moment is not possible.
Since more than one variety of species are involved in parasitic gastro-enteritis in sheep and goats, hence it becomes quite difficult especially to make an approach to work out a relationship between climate and disease. In general, it can be concluded, that incidence of infection at any given time is influenced by the previous level of disease and disease level responds more rapidly to weather changes in sheep and goats.
Fig. 1: Prevalence of *Haemonchus contortus* infection in Aligarh
Fig. 2: Prevalence of *Oesophagostomum columbianum* infection in Aligarh
Fig. 3: Prevalence of *Bunostomum trigonocephalum* infection in Aligarh
Fig. 5: Prevalence of gastro-intestinal nematodes in Aligarh
<table>
<thead>
<tr>
<th>Name of Nematode Parasites</th>
<th>JAN.</th>
<th>FEB.</th>
<th>MAR.</th>
<th>APRIL</th>
<th>MAY</th>
<th>JUNE</th>
<th>JULY</th>
<th>AUG.</th>
<th>SEP.</th>
<th>OCT.</th>
<th>NOV.</th>
<th>DEC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemonchus contortus</td>
<td>34.99</td>
<td>22.88</td>
<td>37.64</td>
<td>40.26</td>
<td>60.62</td>
<td>76.94</td>
<td>83.87</td>
<td>89.87</td>
<td>88.44</td>
<td>92.22</td>
<td>82.45</td>
<td>50.54</td>
</tr>
<tr>
<td>Nematodiphagostomum columbianum</td>
<td>64.08</td>
<td>60.66</td>
<td>55.46</td>
<td>50.80</td>
<td>54.99</td>
<td>68.28</td>
<td>59.30</td>
<td>55.35</td>
<td>61.57</td>
<td>60.41</td>
<td>64.65</td>
<td>52.77</td>
</tr>
<tr>
<td>Bunostomum trigonocesphalum</td>
<td>37.94</td>
<td>45.49</td>
<td>54.24</td>
<td>52.41</td>
<td>46.56</td>
<td>46.10</td>
<td>40.68</td>
<td>33.33</td>
<td>32.46</td>
<td>32.03</td>
<td>30.53</td>
<td>32.84</td>
</tr>
<tr>
<td>Trichuris ovis</td>
<td>42.94</td>
<td>47.46</td>
<td>46.53</td>
<td>38.63</td>
<td>42.99</td>
<td>46.24</td>
<td>40.68</td>
<td>44.66</td>
<td>46.31</td>
<td>48.75</td>
<td>52.87</td>
<td>49.27</td>
</tr>
</tbody>
</table>

---

Total infection of gastro-intestinal nematodes | 79.41| 79.54| 76.11| 72.63| 80.31| 87.66| 88.41| 91.66| 91.87| 95.16| 91.71| 84.55
2. Serum Protein Changes:

Changes in total serum protein were estimated from the animals free of all observable infections, treated as controls; and from animals (sheep and goats) infected with Haemonchus contortus. Each analysis was replicated four times. In case of uninfected sheep, the concentration of total serum protein was found to be 6.748 and 6.438 gm/100 ml., and treated as control for comparison with infected sheep. A gradual decrease in total serum protein contents of animals infected with increasing number of Haemonchus contortus was found. The lowest concentration of protein was found as 3.162 gm/100 ml. of serum in case of sheep infected with 218 worms.

As far as goats are concerned, the control animals exhibited a slightly elevated level of total serum protein than the control animals of sheep. This variation was already standardized and it was found that goats of the same age group always show total serum protein concentration a little higher and the variation was always within the range of 1 gm./100 ml. of serum. Similar pattern of decrease in total serum protein concentration of animals infected with Haemonchus contortus was recorded in goats also. Decrease in total serum protein concentration was less pronounced in animals infected comparatively with smaller number of
parasites. Total serum protein concentration of those animals with heavy worm burden revealed a very pronounced depression as indicated by the table - 2.

The albumin and globulin fractions of uninfected animals (control) was found to be almost equal with slightly increased concentration of globulin. In case of uninfected sheep taken as control, the concentration of albumin and globulin was found as 3.205 and 3.542, while the infected animals revealed upto 1.224 and 2.367 gm./100 ml. of serum. The A/G ratio of non-infected sheep was found within the range of 0.90 while that of infected animals was from 0.63 to 0.52 in order of the severity of infection, as shown in table - 2. The albumin/globulin ratio of goats taken as control was found as 0.90 and 0.87, while the samples of infected animals revealed the ratio varying from 0.64 to 0.51 in the sequence of severity of infection.

The observations show a distinct pattern for the depression in total serum protein contents of infected animals, whether sheep or goats. Observations from the infected sheep show a distinct decrease in total serum protein from animal to animal as the worm burden increases. The albumin/globulin ratio also follow a constant pattern of decrease as indicated by the table - 2. Decrease in total serum protein concentration of infected goats also verify the generalization that decrease in total serum protein is directly proportional to
the degree of infection. Whereas, goat no. 7 and 10 show slight variation from this pattern and the concentration was found slightly elevated from the preceding samples corresponding to less number of worm burden. The variation is not very significant and may be attributed to the physiological status of different animals. The albumin/globulin ratio of these two animals was also a little elevated than the preceding one having less severe infection. These results are in good agreement with the finding of Shumrad and Eveleth (1955), Bawden (1969), Shastry and Ahluwalia (1972), Bremner and Fridemanis (1974), Georgieva and Vladimirova (1975), Olivera and Penha (1978), Thomas and Ali (1983). Of course, these workers had made their observations on experimental infection of animals and few cases included mixed gastrointestinal nematode population. The only comparable data found in the reports of all these workers is a condition of hypoproteinaemia and hypoalbuminaemia.

It appears that more than one factor is responsible for the serum protein changes. Blood sucking habit of Haemonchus contortus, leading towards haemorrhage of abomasal lining and blood loss is the basic cause and sounds reasonable because control animals, presented absolutely normal serum protein level. Hypoalbuminaemia, a factor reported by all workers may be due to reduction in synthesis of albumin and its
increased catabolism. This may be quite possible because anaemia which is a pre-existing condition of hypoproteinaemia, stresses for the increased synthesis of haemoglobin. It ultimately leads to the deficiency of amino acids in the infected animals. The explanation of amino acid deficiency for decrease albumin synthesis appears to be most reasonable because of the fact that during deficiency states, haemoglobin synthesis has priority over serum and other body proteins for available amino acids. Since, under heavy parasitic burden of *Haemonchus contortus*, anaemia will essentially be an existing condition which would ultimately lead towards increased erythropoiesis and consequently haemoglobin synthesis. From the foregoing observations, conclusion could be drawn that blood sucking habit of *Haemonchus contortus*, and blood loss from haemorrhage are basically responsible for the hypoproteinaemia. Increased leakage of plasma protein into the gut is a feature common to many nematode infection of ruminants and appear to be an important source of increased endogenous nitrogen secretion. This leakage basically results in an increased albumin turn over, which in animals is associated with an altered hepatic protein synthesis. Similar view was given by Steel (1974) and the idea of exhaustion of host's synthetic machinery by Dragie and Allonby (1975), support the present view about protein
alteration. Change in albumin/globulin ratio (table - 2) is obviously expected when there is decrease in albumin counterpart of the protein without any marked change in globulin fraction.
<table>
<thead>
<tr>
<th>No.</th>
<th>No. of worms recovered</th>
<th>Total Serum Protein (gm./100 ml.) Mean ± S.E.</th>
<th>Serum Albumin (gm./100 ml.) Mean ± S.E.</th>
<th>Serum Globulin (gm./100 ml.) Mean ± S.E.</th>
<th>A/G Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SHEEP (Control)</td>
<td>NIL</td>
<td>6.748 ± 0.000</td>
<td>3.205 ± 0.029</td>
<td>3.542 ± 0.029</td>
</tr>
<tr>
<td>2</td>
<td>SHEEP (Control)</td>
<td>NIL</td>
<td>6.438 ± 0.086</td>
<td>3.063 ± 0.044</td>
<td>3.371 ± 0.053</td>
</tr>
<tr>
<td>3</td>
<td>SHEEP</td>
<td>49</td>
<td>5.726 ± 0.062*</td>
<td>2.225 ± 0.039</td>
<td>3.501 ± 0.025</td>
</tr>
<tr>
<td>4</td>
<td>SHEEP</td>
<td>52</td>
<td>5.386 ± 0.070*</td>
<td>2.240 ± 0.026*</td>
<td>3.146 ± 0.049</td>
</tr>
<tr>
<td>5</td>
<td>SHEEP</td>
<td>144</td>
<td>4.816 ± 0.033*</td>
<td>1.946 ± 0.022*</td>
<td>2.870 ± 0.018</td>
</tr>
<tr>
<td>6</td>
<td>SHEEP</td>
<td>177</td>
<td>3.640 ± 0.045*</td>
<td>1.228 ± 0.016*</td>
<td>2.412 ± 0.033</td>
</tr>
<tr>
<td>7</td>
<td>SHEEP</td>
<td>218</td>
<td>3.612 ± 0.028*</td>
<td>1.224 ± 0.008*</td>
<td>2.367 ± 0.019</td>
</tr>
<tr>
<td>1</td>
<td>GOAT (Control)</td>
<td>NIL</td>
<td>7.067 ± 0.038</td>
<td>3.360 ± 0.037</td>
<td>3.716 ± 0.041</td>
</tr>
<tr>
<td>2</td>
<td>GOAT (Control)</td>
<td>NIL</td>
<td>7.077 ± 0.049</td>
<td>3.295 ± 0.000</td>
<td>3.782 ± 0.049</td>
</tr>
<tr>
<td>3</td>
<td>GOAT</td>
<td>45</td>
<td>5.687 ± 0.073*</td>
<td>2.240 ± 0.026**</td>
<td>3.447 ± 0.052</td>
</tr>
<tr>
<td>4</td>
<td>GOAT</td>
<td>46</td>
<td>5.278 ± 0.057*</td>
<td>2.101 ± 0.028**</td>
<td>3.178 ± 0.036</td>
</tr>
<tr>
<td>5</td>
<td>GOAT</td>
<td>129</td>
<td>4.963 ± 0.067*</td>
<td>1.974 ± 0.048*</td>
<td>2.988 ± 0.031</td>
</tr>
<tr>
<td>6</td>
<td>GOAT</td>
<td>154</td>
<td>4.434 ± 0.013*</td>
<td>1.720 ± 0.013**</td>
<td>2.714 ± 0.041</td>
</tr>
<tr>
<td>7</td>
<td>GOAT</td>
<td>159</td>
<td>4.592 ± 0.051*</td>
<td>1.617 ± 0.021**</td>
<td>2.974 ± 0.035</td>
</tr>
<tr>
<td>8</td>
<td>GOAT</td>
<td>183</td>
<td>4.466 ± 0.051*</td>
<td>1.696 ± 0.018**</td>
<td>2.769 ± 0.032</td>
</tr>
<tr>
<td>9</td>
<td>GOAT</td>
<td>235</td>
<td>3.752 ± 0.045*</td>
<td>1.272 ± 0.023**</td>
<td>2.479 ± 0.024</td>
</tr>
<tr>
<td>10</td>
<td>GOAT</td>
<td>265</td>
<td>3.892 ± 0.059*</td>
<td>1.365 ± 0.026**</td>
<td>2.464 ± 0.032</td>
</tr>
<tr>
<td>11</td>
<td>GOAT</td>
<td>284</td>
<td>3.675 ± 0.077*</td>
<td>1.282 ± 0.040**</td>
<td>2.393 ± 0.037</td>
</tr>
<tr>
<td>12</td>
<td>GOAT</td>
<td>324</td>
<td>3.528 ± 0.045**</td>
<td>1.194 ± 0.020**</td>
<td>2.333 ± 0.025</td>
</tr>
</tbody>
</table>

* = Haeemonchus contortus,  * = Significant at P<.05,  ** = Very significant at P<.001
3. **Serum Alkaline and Acid Phosphatase Activity:**

Alkaline and acid phosphatase activity from the serum of both uninfected and infected animals including sheep and goats, was estimated using the technique mentioned earlier. Each analysis was replicated four times. Analysis was separately made for sheep and goats. The uninfected sheep taken as control, exhibited serum alkaline phosphatase activity in the range of 2.5 Bodansky unit/100 ml. of serum as shown in table - 3. The sheep infected with relatively less number of parasites as 49 and 52 worms, revealed the activity higher than 3.2 Bodansky unit/100 ml. of serum. Those infected with larger number of parasites as 144 and 177 worms, showed comparatively higher degree of activity as 3.992 and 3.999, Bodansky unit respectively. The highest activity was recorded as 4.2 Bodansky unit from a sheep infected with 218 worms. The changes were found to be significant at P<.005 and very significant at P<.001.

Almost similar activities of serum alkaline phosphatase were recorded from uninfected goats, as 2.39 and 2.37 Bodansky unit/100 ml. of serum. Increase in serum alkaline phosphatase activity was found to respond more distinctly to the intensity of infection in case of goats than in sheep. Goats, infected with 45 and 46 *Haemonchus contortus* exhibited enzyme activity as 3.17 and 4.50 Bodansky unit respectively.
The enzyme activity remained below 5 Bodansky unit/100 ml. upto worm burden of 235 nematodes. Infected goat harbouring 265 parasites exhibited the serum enzyme activity as 5.077 Bodansky unit and the highest activity was recorded as 5.9 Bodansky unit from a goat with parasite burden of 324 worms.

Serum acid phosphatase activity from the uninfected sheep was recorded as 1.39 and 1.37 Bodansky unit/100 ml. of serum. The table - 3 shows that reduction in activity of acid phosphatase enzyme was found in all the infected sheep, when compared with controls. The minimum activity among the infected sheep was recorded to be 0.791 Bodansky unit, corresponding to sheep harbouring 218 worms. In uninfected goats, acid phosphatase activity was found to be slightly higher than sheep. It was recorded as 1.51 unit for both uninfected goats. It is evident from the table that successive fall in the serum acid phosphatase activity was observed from animal to animal (both sheep and goats) with increasing worm burden. Serum of goat infected with 45 worms had shown the activity at 0.94 Bodansky unit while the serum activity of enzyme was found lowest as 0.58 Bodansky unit in animal infected with 324 worms.

It may be quite worthwhile to elaborate, a little, the mode of action of phosphatase enzymes before discussing its
possible metabolic significance in the present study. The enzyme belongs to phosphomonoesterases which is an important group of non-specific hydrolytic enzymes and catalyze the liberation of inorganic phosphorus from phosphate esters through the general reaction

\[
\begin{align*}
R-O-P &= O + HOH \\
R-OH + OH-P &= O
\end{align*}
\]

The two phosphomonoesterases, alkaline and acid phosphatases, were classified on the basis of their pH optima and later termed as phosphomonoesterase I (pH 9.2 - 9.6) and phosphomonoesterase II (pH 5.0 - 6.0) by Roche (1950). These enzymes have now been classified and named by the Enzyme Commission as orthophosphoric monoester phosphohydrolase with E.C. No. 3.1.3.1. for alkaline phosphatase and E.C. No. 3.1.3.2. for acid phosphatase (Florkin and Stotz, 1964). The phosphatases are intimately associated with phosphate cycles, tissue transformation, growth, protein formation and nerve action etc., (Roche, 1950; Stadtman, 1961).

As indicated by the table, increase in serum alkaline phosphatase activity was noticed in all infected animals, whether sheep or goats. Some studies have been carried out on this enzyme in relation to gastro-intestinal parasitism of
ruminants, but any report on the serum enzyme changes in response to *Haemonchus contortus* infection is not available and so deprives any possible comparison here. Kamenov (1980) reported a drop in serum alkaline phosphatase activity of lambs infected with *Bunostomum trigonocephalum*. Danilyavichus and Matusyavichyus (1968) observed an increased activity of alkaline phosphatase in the faecal matter of pigs infected with *Oesophagostomum dentatum* and attributed it to a disturbance in the intestinal phosphatase activity. Prosl et al. (1985) noticed a fall in the serum alkaline phosphatase activity of cattle and weaned calves infected with *Bunostomum*. Present findings can be justified according to Roche (1950), and Stadtman (1961), who reported the enzyme to be intimately associated with tissue transformation and protein formation. The anaemic state of the animals infected with *Haemonchus contortus*, appears to stress the synthetic machinery of the animal for increased synthesis of blood corpuscles as well as protein. As a consequence, it seems quite likely that increased consumption of energy rich molecule for active protein synthesis built up, from phosphorylated intermediates ultimately leads to the increased glycolysis. Since phosphatases, accelerate the breakdown of glucose-1-phosphate into glucose, so that increased availability of glucose will enable the animals for increased biosynthesis of
essential proteins and corpuscles. Therefore, physiological stress on the infected animal for the synthesis, appears to be the main factor responsible for the increased activity of alkaline phosphatase. In this way increased enzyme level may be interpreted as an adoption to an active glycolytic mechanism, involving a dephosphorylative process in the alkaline range.

A conclusion from the observations being made may be drawn based on the generalization that an increase in the intensity of infection is directly proportional to the increase in the activity of serum alkaline phosphatase and decrease in acid phosphatase activity. However, the results as sheep No. 4 and goat No. 8 which showed deviation from the above basis may possibly be due to the differences in physiological state of animals.
TABLE - 3

<table>
<thead>
<tr>
<th>No.</th>
<th>HOSTS</th>
<th>No. of worms(^*) recovered</th>
<th>Serum Alkaline Phosphatase activity. Bodansky unit/100 ml. Mean ± S.E.</th>
<th>Serum Acid Phosphatase activity. Bodansky unit/100 ml. Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SHEEP (Control)</td>
<td>NIL</td>
<td>2.544 ± 0.020</td>
<td>1.393 ± 0.008</td>
</tr>
<tr>
<td>2</td>
<td>SHEEP (Control)</td>
<td>NIL</td>
<td>2.418 ± 0.014</td>
<td>1.375 ± 0.020</td>
</tr>
<tr>
<td>3</td>
<td>SHEEP</td>
<td>49</td>
<td>3.408 ± 0.125(^*)</td>
<td>0.879 ± 0.011(^*)</td>
</tr>
<tr>
<td>4</td>
<td>SHEEP</td>
<td>52</td>
<td>3.275 ± 0.011(^*)</td>
<td>0.850 ± 0.009(^*)</td>
</tr>
<tr>
<td>5</td>
<td>SHEEP</td>
<td>144</td>
<td>3.992 ± 0.021(^**)</td>
<td>0.873 ± 0.047(^*)</td>
</tr>
<tr>
<td>6</td>
<td>SHEEP</td>
<td>177</td>
<td>3.999 ± 0.026(^**)</td>
<td>0.791 ± 0.012(^*)</td>
</tr>
<tr>
<td>7</td>
<td>SHEEP</td>
<td>218</td>
<td>4.205 ± 0.085(^*)</td>
<td>0.791 ± 0.007(^*)</td>
</tr>
<tr>
<td>1</td>
<td>GOAT (Control)</td>
<td>NIL</td>
<td>2.395 ± 0.031</td>
<td>1.516 ± 0.018</td>
</tr>
<tr>
<td>2</td>
<td>GOAT (Control)</td>
<td>NIL</td>
<td>2.378 ± 0.033</td>
<td>1.516 ± 0.003</td>
</tr>
<tr>
<td>3</td>
<td>GOAT</td>
<td>45</td>
<td>3.175 ± 0.041(^*)</td>
<td>0.948 ± 0.008(^*)</td>
</tr>
<tr>
<td>4</td>
<td>GOAT</td>
<td>46</td>
<td>4.501 ± 0.030(^*)</td>
<td>0.933 ± 0.001(^*)</td>
</tr>
<tr>
<td>5</td>
<td>GOAT</td>
<td>129</td>
<td>4.540 ± 0.034(^*)</td>
<td>0.877 ± 0.017(^*)</td>
</tr>
<tr>
<td>6</td>
<td>GOAT</td>
<td>154</td>
<td>4.806 ± 0.050(^*)</td>
<td>0.859 ± 0.001(^*)</td>
</tr>
<tr>
<td>7</td>
<td>GOAT</td>
<td>159</td>
<td>4.932 ± 0.014(^**)</td>
<td>0.807 ± 0.046(^*)</td>
</tr>
<tr>
<td>8</td>
<td>GOAT</td>
<td>183</td>
<td>4.897 ± 0.022(^**)</td>
<td>0.798 ± 0.005(^*)</td>
</tr>
<tr>
<td>9</td>
<td>GOAT</td>
<td>235</td>
<td>4.974 ± 0.033(^**)</td>
<td>0.779 ± 0.015(^*)</td>
</tr>
<tr>
<td>10</td>
<td>GOAT</td>
<td>265</td>
<td>5.077 ± 0.039(^**)</td>
<td>0.684 ± 0.007(^*)</td>
</tr>
<tr>
<td>11</td>
<td>GOAT</td>
<td>284</td>
<td>5.214 ± 0.024(^**)</td>
<td>0.623 ± 0.006(^**)</td>
</tr>
<tr>
<td>12</td>
<td>GOAT</td>
<td>324</td>
<td>5.903 ± 0.042(^**)</td>
<td>0.582 ± 0.005(^**)</td>
</tr>
</tbody>
</table>

\(^*\) = *Haeomonchus contortus*, \(^*\) = Significant at \(P<.05\), \(^**\) = Very significant at \(P<.001\)
4. Serum Inorganic Phosphorus and Glutamic Pyruvic Transaminase:

Inorganic phosphorus was estimated from the serum of uninfected and infected animals, and comparison was made to find out changes due to *Haemonchus contortus*, infection. Each analysis was replicated four times. The level of inorganic phosphorus in uninfected sheep was found to be 7.368 and 7.571 mg./100 ml. of serum, respectively. In infected sheep level of inorganic phosphorus showed an upward trend. Sheep infected with 49 worms exhibited level of inorganic phosphorus as 9.978 mg./100 ml. and the highest concentration was found as 12.285 mg./100 ml. of serum in two sheep, former having a worm burden of 117 nematodes and the later, 218 nematodes. As indicated by the table – 4, these values were found to be significant P<.05 and very significant at P<.001 when compared with uninfected sheep.

Similarly, inorganic phosphorus was estimated from uninfected and infected goats. In uninfected goats the level of inorganic phosphorus was found as 7.730 and 7.573 mg./100 ml. of serum. The level of inorganic phosphorus as evident in infected sheep, presented similar pattern in infected goats also. The minimum increased level was found in goat, as 8.924 mg./100 ml. harbouring 45 worms while the maximum increase was recorded in goats with 324 worms, as 11.551 mg.
per 100 ml. Except goat No. 8, the differences in the level of uninfected and infected goats was found to be significant at P<.05 and very significant at P<.001.

It is quite apparent from the table – 4 that elevation in serum inorganic phosphorus level is in direct correlation with severity of infection. Results of sheep No. 7 and goat No. 8 show slight deviation from the generalized pattern between the increase in serum inorganic phosphorus and number of worms present in gastro-intestinal tract. Sheep No. 7 has indicated similar concentration of inorganic phosphorus as No. 6, while there is considerable difference in the number of worms present in gut. This difference and slightly decreased level of inorganic phosphorus of goat No. 8 in comparison to No. 7 with comparatively less number of parasites, may be due to differences in individual physiological state of animals. In general, the elevated level of inorganic phosphorus of serum may be due to disturbances in mineral metabolism of host, which appears to be a manifestation of gastro-intestinal parasitic infection. Effect of parasitism on mineral metabolic disturbance of host may be mediated by the disfunctioning of parathyroid gland. Since the condition of hyperphosphaemia as observed in the present study is associated with the increased renal tubular phosphorus recovery and which appears to be a clear indication of
hyperparathyroidism. Studies on the effect of gastro-intestinal parasitism on the host's endocrine system are quite lacking, so any evidence in support of disfunctioning of parathyroid is not possible. Present results are in agreement with the findings of Zajeck et al. 1976; Hamid et al. 1981, who reported increased level of inorganic phosphorus in sheep experimentally infected with Haemonchus contortus. Present results indicate a correlation between the findings of experimental infection and natural infection of hosts; sheep and goats. It is evident that the effect of Haemonchus contortus infection follows almost similar pathological mode in both experimental as well as natural infections and a comparision of the findings of animals in the above mentioned condition is quite possible. As already mentioned in such studies, possibility of other infections should be checked as much as possible.

Serum glutamic-pyruic transaminase activity was determined from the serum of infected and uninfected animals and comparision between the two values was made. Glutamic-pyruic transaminase catalyses the following reaction:

\[ \alpha\text{-}ketoglutaric \text{ acid} + \text{alanine} \rightleftharpoons \text{Pyruic acid} + \text{glutamic acid}. \]

The process is defined as transfer of an amino group from an amino acid to a keto acid with subsequent formation of
<table>
<thead>
<tr>
<th>No.</th>
<th>HOSTS</th>
<th>No. of worms† recovered</th>
<th>Inorganic Phosphorus (mg./100 ml.) Mean ± S.E.</th>
<th>G.P.T. activity µg.m. of P.A. liberated/ml. of serum Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SHEEP (Control)</td>
<td>NIL</td>
<td>7.368 ± 0.000</td>
<td>2.790 ± 0.033</td>
</tr>
<tr>
<td>2</td>
<td>SHEEP (Control)</td>
<td>NIL</td>
<td>7.571 ± 0.002</td>
<td>2.792 ± 0.060</td>
</tr>
<tr>
<td>3</td>
<td>SHEEP</td>
<td>49</td>
<td>9.978 ± 0.005**</td>
<td>2.931 ± 0.050</td>
</tr>
<tr>
<td>4</td>
<td>SHEEP</td>
<td>52</td>
<td>11.551 ± 0.022**</td>
<td>3.809 ± 0.066*</td>
</tr>
<tr>
<td>5</td>
<td>SHEEP</td>
<td>144</td>
<td>11.819 ± 0.109**</td>
<td>4.023 ± 0.051*</td>
</tr>
<tr>
<td>6</td>
<td>SHEEP</td>
<td>177</td>
<td>12.285 ± 0.031**</td>
<td>4.338 ± 0.047*</td>
</tr>
<tr>
<td>7</td>
<td>SHEEP</td>
<td>218</td>
<td>7.730 ± 0.050**</td>
<td>3.102 ± 0.054</td>
</tr>
<tr>
<td>1</td>
<td>GOAT (Control)</td>
<td>NIL</td>
<td>7.573 ± 0.002</td>
<td>2.463 ± 0.035</td>
</tr>
<tr>
<td>2</td>
<td>GOAT (Control)</td>
<td>NIL</td>
<td>8.924 ± 0.002</td>
<td>2.931 ± 0.060</td>
</tr>
<tr>
<td>3</td>
<td>GOAT</td>
<td>45</td>
<td>9.434 ± 0.004*</td>
<td>3.132 ± 0.072</td>
</tr>
<tr>
<td>4</td>
<td>GOAT</td>
<td>46</td>
<td>9.434 ± 0.004*</td>
<td>4.428 ± 0.075*</td>
</tr>
<tr>
<td>5</td>
<td>GOAT</td>
<td>129</td>
<td>9.702 ± 0.003*</td>
<td>2.997 ± 0.079</td>
</tr>
<tr>
<td>6</td>
<td>GOAT</td>
<td>154</td>
<td>10.342 ± 0.073*</td>
<td>3.125 ± 0.080</td>
</tr>
<tr>
<td>7</td>
<td>GOAT</td>
<td>159</td>
<td>10.206 ± 0.369*</td>
<td>3.123 ± 0.063</td>
</tr>
<tr>
<td>8</td>
<td>GOAT</td>
<td>183</td>
<td>10.566 ± 0.010*</td>
<td>3.127 ± 0.129</td>
</tr>
<tr>
<td>9</td>
<td>GOAT</td>
<td>235</td>
<td>10.566 ± 0.566**</td>
<td>3.487 ± 0.065</td>
</tr>
<tr>
<td>10</td>
<td>GOAT</td>
<td>265</td>
<td>10.566 ± 0.010**</td>
<td>3.168 ± 0.058</td>
</tr>
<tr>
<td>11</td>
<td>GOAT</td>
<td>284</td>
<td>11.212 ± 0.018**</td>
<td>3.732 ± 0.065*</td>
</tr>
<tr>
<td>12</td>
<td>GOAT</td>
<td>324</td>
<td>11.551 ± 0.022**</td>
<td>3.510 ± 0.045*</td>
</tr>
</tbody>
</table>

† = *Haemonchus contortus*,  * = Significant at P<.05,  ** = Very significant at P<.001
different amino and keto acids. The activity of glutamic-
pyruvic transaminase was measured by the amount of pyruvic acid
liberated per ml. of serum. The activity was found as 2.790
and 2.792 µgm. pyruvic acid/ml. of serum, in case of uninfec-
ted sheep. In infected animals increased activity was re-
corded (table - 4) but a distinct correlation was not observed
between the number of parasite and increased activity of
enzyme. The highest activity was recorded in sheep infected
with 177 worms. Sheep infected with highest number of para-
site as 218, has shown comparatively low activity, although
the change in activity of sheep Nos. 4, 5 and 6 was signifi-
cant at P<.05. The uninfected goats exhibited enzymatic
activity as 2.463 and 2.931 µgm. of pyruvic acid/ml. of serum,
respectively. Comparision of infected animals, was made with
the control goat No. 2 showing comparatively higher activity
of enzyme. It was found that all infected goats showed com-
paratively increased enzymatic activity. However, statistical
comparision revealed that values of goat Nos. 4, 9, 11 and 12
were only significant at P<.05 and rest of the animals indica-
ted normal enzymatic activity.

Results indicate that the enzymatic activity in infected
animals may either be distinct or indistinct but can not be
correlated with the degree of infection. A gradual increase
in the enzymatic activity could not be established with
increasing worm burden in the host. The variation may be due
to fluctuating level of enzyme in the host at different period of infection. Since random collection and sampling was made, and any information regarding the longevity of infection was not possible so it can not be firmly attributed to the different stages of infection.

Increased level of enzyme may be due to reduction in level of total serum protein. Since under the condition of hypoproteinaemia, host synthetic machinery is always found in deficient state for amino acids because the condition of hypoproteinaemia in *Haemonchus contortus* infection is always followed by anaemia. Consequently, haemopoiesis is always at increased level and comparatively increased level of amino acids is required. In this way increased transaminase activity reasonably appears to be responsible for the formation of amino acids required to maintain homeostatis of the host by the synthesis of essential proteins. Since work on the serum transaminase activity of the host with *Haemonchus contortus* infection is quite lacking, both in experimental as well as natural infection, so no comparision is possible with the findings of other workers. Sinson *et al.* (1970/1972) during the study of *Fasciola* infection in bovine, reported increase in glutamic-oxalacetic transaminase activity, but observed glutamic-pyruvic activity of less diagnostic value. The later part could not be put to comparision with the present result because of the different host and parasite.
V. SUMMARY

A survey on the incidence of gastro-intestinal nematodes infection was carried out in and around Aligarh. Collection of worms from slaughtered animals (sheep and goats) was made twice in a week. The infection in general, included Haemonchus contortus, Oesophagostomum columbianum, Bunostomum trigonocephalum and Trichuris ovis. The survey being conducted was spread over the whole year of 1984 and replicated in 1985. The data was tabulated and monthly average per cent infection for each parasite was calculated.

Overall infection of gastro-intestinal nematodes was found almost constant throughout the year showing highest infection, 95.16 per cent in October, and lowest 72.63 per cent in April. Among the parasites collected, Haemonchus contortus exhibited highest degree of infection. Peak of infection was found from June to November and during rest of the periods it remained moderate showing a minimum value in February. Peak of infection correlated with the prevailing climatic condition of the region. Moderate temperature and sufficient moisture happened to be the contributing factor for the increase in infection as it permits better herbage growth as well as successful development of infective larvae. Infection of Oesophagostomum columbianum, was found almost constant throughout the year with two indistinct peaks in
June and November and lowest infection in April. Incidence of *Bunostomum trigonocephalum* was found lowest among all the four gastro-intestinal parasites. Its infection was found highest in March and April and lowest in November. The peak infection period was associated with the moderate temperature and moisture conditions of the preceding months, which permitted successful development and transfer of the infective larvae to the hosts. High temperature of summer season adversely affects the growth and development of the free living stages and is the factor responsible for the decrease of infection during winter because first stage as well as infective larvae of this worm can not resist dryness and high temperature. *Trichuris ovis*, showed a pattern very similar to *Oesophagostomum columbianum*, with almost constant level of infection throughout the year, interrupted by two indistinct peaks, one in June and the other in November. Since transfer of infective larvae of this worm is different from other parasites reported, that is, instead of infective larvae, eggs, bearing infective larvae are ingested by the host. This factor indicates that infection may take place even through moist herbage as well as water, obviously, constant rate of infection was encountered throughout the year.

Blood samples were obtained from infected as well as uninfected, control animals (sheep and goats) slaughtered in
local abattoir. Different organs, as liver, lungs and gastro-intestinal tract of corresponding animals were autopsied to select only those samples, with *Haemonchus contortus* infection. Blood samples were brought to laboratory in ice boxes to check any enzymatic or metabolic activity.

Total serum protein was estimated from uninfected control and infected animals. Decreased total serum protein was observed in both sheep and goats, infected with varying number of parasites, in comparison to uninfected controls. A direct correlation was found between the decrease in total serum protein and severity of infection. Similarly, marked decrease was evident in serum albumin fraction of infected host while the change in globulin fraction was not very distinct. Albumin/Globulin ratio of infected animals was found to be considerably decreased, in comparison to uninfected controls. Blood sucking habit of the parasite was attributed for decrease in total serum protein and that led to the anaemic condition of the host and consequent stress on haemopoiesis. Marked decrease in albumin and indistinct fall in globulin fraction brought considerable decrease in albumin/globulin ratio. Slight deviation from the correlation between decrease in total serum protein and degree of infection was considered due to differences in individual physiological status of hosts.
Acid and alkaline phosphatase activity of serum was estimated from uninfected control and infected hosts. A decrease in serum acid phosphatase activity of infected animals and an increase in activity of alkaline phosphatase was recorded. Increased alkaline phosphatase activity was interpreted as a consequence of increased consumption of energy rich molecules for active protein synthesis built up from phosphorylated intermediates, leading to increased glycolysis. Reduced activity of acid phosphatase was explained by the established fact that in any organ, higher activity of one is accompanied by the decrease of other. A correlation in increase of alkaline and decrease of acid phosphatase activity; with severity of infection was observed.

Serum inorganic phosphorus was estimated from uninfected control and infected animals. Marked increase in level of inorganic phosphorus of infected hosts was evident in comparison to uninfected controls. Glutamic-pyruvic transaminase activity of serum has shown an increase in infected animals but the elevation was irregular from animal to animal, consequently any correlation with the degree of infection could not be established.

Besides, contributing to biochemical pathology of sheep and goats due to *Haemonchus contortus* infection, present findings provide a base for the correlation between the
findings of controlled experimental infection and natural infection of hosts which are exposed to innumerable environmental factors. Data obtained from animals naturally infected by these worms and pathological basis, will contribute enormously to design integrated control programmes.
VI. REFERENCES


