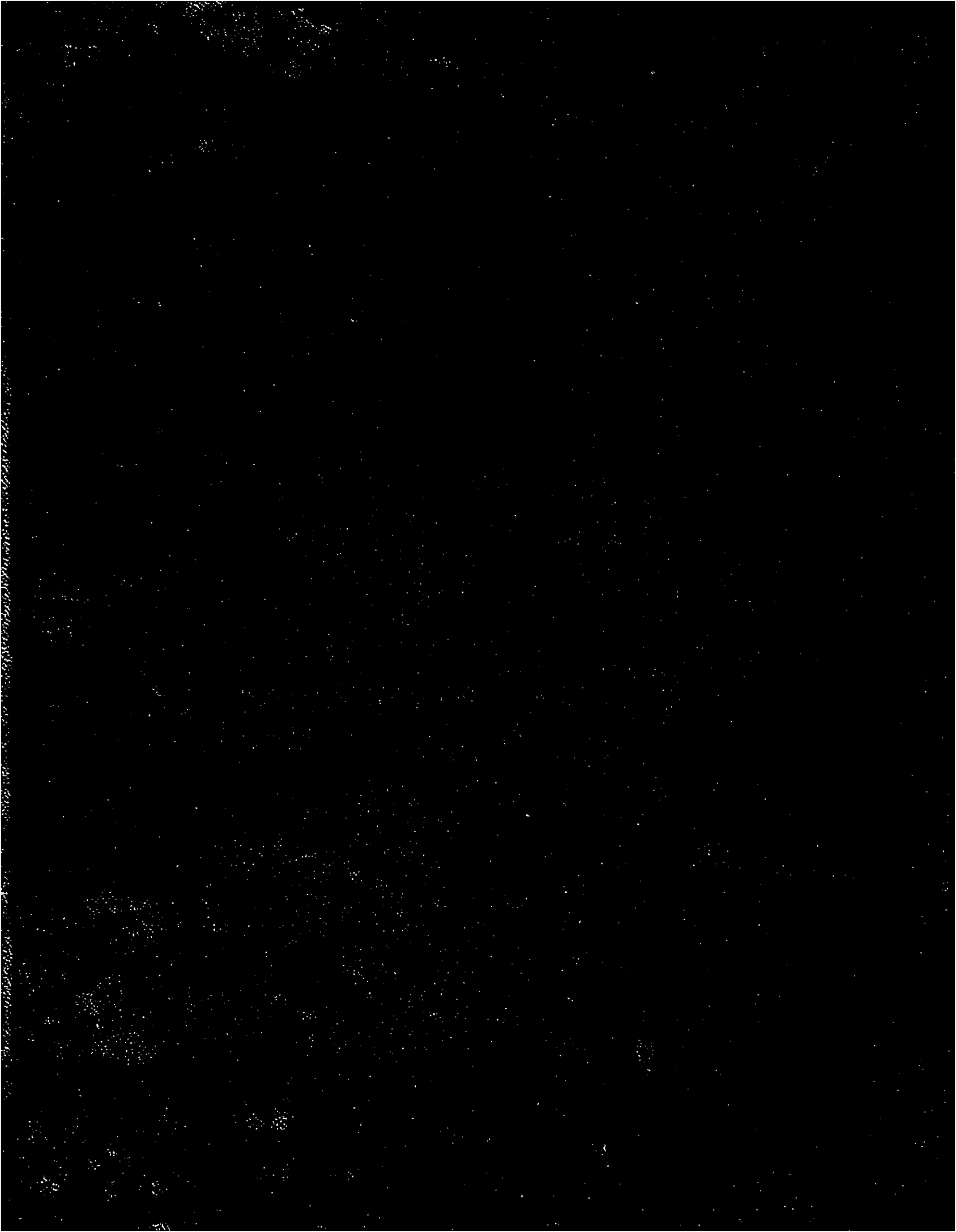

# Studies On Helminth & Protozoan Parasites of Sheep ( *Ovis aries* )



Harbans Singh Bali

**October, 1963.**

# S T U D I E S  O N
# H E L M I N T H  &  P R O T O Z O A N  P A R A S I T E S
# O F
# S H E E P ( OVIS ARIES)

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T H E S I S

SUBMITTED TO THE UNIVERSITY OF MAGADH IN THE FACULTY OF VETERINARY SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE ( VETERINARY ) DEGREE.

O C T O B E R , 1963.

POSTGRADUATE DEPARTMENT OF PARASITOLOGY,
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PATNA.

Dr. A.K.Varma,Ph.D.(London),
Professor & Head of the Department &
Director,
Livestock Research Station.

The 23rd October,1963.

Certified that the work described in this Thesis entitled "STUDIES ON HELMINTH & PROTOZOAN PARASITES OF SHEEP (Ovis aries)" is the bonafide work of HARBANS SINGH BALI, carried out under my guidance and supervision.

A. Varma 23.10.63

( A. K. VARMA )

# ACKNOWLEDGEMENT

The author is highly grateful to Dr.A.K.Varma, Ph.D.(London),Professor & Head of the Postgraduate Department of Parasitology, Bihar Veterinary College, and Director,Livestock Research Station ,Bihar,Patna, under whose guidance and supervision this study has been pursued.

I also gratefully acknowledged the generous assistance and guidance given me by Dr.H.Prasad,Ph.D. (London),Research Officer (Poultry) and Deputy Director, Key Village Scheme,Bihar,Patna in the studies on coccidia and coccidiosis of sheep included in this work.

The author is thankful to Sri R.K.Choudry,D.T.V.M. (Edin.), of the Postgraduate Department of Parasitology and also to Sri P.Lakra,Assistant Professor,Parasitology, B.V.College for their assistance in my day to day routine work.

I thankfully acknowledge the assistance rendered by Shri Raghubans Pd. Artist and his other staff particularly Sri Mohan Pd., for their kind help in preparing drawings,photographs & microphotographs.

Lastly my thanks are also due to Sri J.N.Pd.,R.O. (Statistics),L.R.S.,Patna for his help in statistical analysis of some of the data incorporated in this work and to Principal,B.V.College for kindly rendering facilities for this study.

# A B S T R A C T

Title: " STUDIES ON HELMINTH & PROTOZOAN PARASITES OF SHEEP ( Ovis aries )."

This thesis deals with the helminth and protozoan parasites collected from Government Sheep Breeding Farms at Gauriakarma (Hazaribagh) & Takuna (Gaya). Most of the helminths were recovered at autopsy from an out-break of amphistomiasis at Gauriakarma Farm.

In addition, the helminths were also collected from abattoirs at Patna and Rajabazar. These helminths were morphologically studied and systematically described. Twenty one species of helminths were encountered during this investigation. Out of these, Schistosoma indicum, Hydatid cysts, Coenurus cerebralis and Gaigeria pachyscelis were recorded for the first time in Bihar. The incidence of infection with all these species was worked out.

Histopathological studies of Schistosoma indicum in liver of sheep have been done and important changes described with illustrations. Characteristic eggs of the worms were recovered from the liver by tissue-digestion method.

Studies on the biology of Trichuris spp. eggs have been carried out, as the faeces of about 40.6 % of sheep revealed infection with Trichuris spp. at Takuna Farm.

Haematology of amphistomiasis was studied and compared with apparently healthy group of sheep. This study was based on examination of eight sheep from each group. The data, thus obtained, were statistically analysed to find out the significant changes. The significant changes were increase in white cell count and decrease in haemoglobin content and red cell count of the infected group.

Faecal examination of 425 sheep was carried out from different localities of the State. Twelve types of eggs were encountered, which have been drawn with the help of Camera lucida. Cases of multiple infection of coccidia were also encountered in this survey. Five species of coccidia (E.arloingi, E.parva, E.ninakohlyakimovae, E.crandalis, E.intricata) have been found to infect the sheep in this State.

During the course of faecal examination about 1.1% of the sheep were found positive for Ascaris spp. infection at Gauriakarma. This gave an indication of the presence of Ascariasis in sheep which was not recorded in the State by earlier workers. Bhalerao (1935) records the presence of Ascaris lumbricoides infection in sheep for the first time in India. None of the Ascaris spp. were encountered at autopsy.

An experiment was carried out to study the developmental stages of Coenurus cerebralis in pups

Adverse effect of refrigeration on the viability and development of Coenurus in pups is recorded. Haematological, pathological and histopathological studies of the pups under experimental infection were carried out. Haematological studies revealed decrease in haemoglobin content and increase in eosinophilic count of the infected pups. Scolices of the tape worms were found buried deep down in the intestinal glands of the small intestine without any evidence of cellular infiltration in the histopathological sections. Mature, gravid proglottids and large hooks of the mature tape worm recovered from pup, were studied.

Larvae of the most common infections, viz; Haemonchus contortus, Bunostomum trigonocephalum, Trichostrongylus colubriformis, Strongyloides papillosus and Oesophagostomum columbianum were recovered by culturing eggs of these species in the laboratory at room temperature. Their morphological and biological studies were made.

Coccidian oocysts were the only protozoan parasites recovered during the course of this work. The oocysts were separated and used after proper sporulation in cross infection experiment in kids and vice versa to throw light on the host-specificity of these Eimeria spp., and it was observed that there was

no host-specificity of these Eimeria in these phylogenetically related hosts. Observations on the biology and staining methods of coccidia were also made.

Endogenous stages of Eimeria arloingi in naturally infected cases were studied. Different stages of development were encountered in histopathological sections.

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# C O N T E N T S

TABLEs- XIV
PLATEs- XVIII

# INTRODUCTION

Good healthy livestock forms part of the wealth of a country. The sheep which ranks next in importance to cattle and stands 4th in population in India (40.2 million) according to the livestock census of India, 1961, constitutes valuable wealth of our country.

Sheep seems to have been domesticated since early times. The origin of the Ovis aries is, however, not traceable. Possibly several races of wild sheep were the fore-runners of our present day domesticated sheep. Today sheep inhabit practically every corner of the globe and is considered to be probably the foremost animal domesticated by man (Duch & Jayaraman, 1954).

India has a very long history about its sheep and wool. In "RIGVEDA", there are HYMNS to Pasham, the God of shepherds, and there are references to the bleaching and spinning of wool. In the Economic dictionary of India, Vol.6 Part II, it is stated that wool was known to the earliest writers of India and that the injunctions of the "Institutes of Manu" assign wool to be used for the sacred thread of Vaisya.

The role played by sheep towards our national economy is many-fold. There is hardly any part of the body of the sheep, dead or alive, which is not of use

to man. Thousands of people are supported by this animal after its demise. Big tannaries are working day in and day out, giving work to many workers. The total wool production of the country was estimated at 54.33 million pounds in the year 1954. The average annual production of sheep skin in the country is estimated at 142.26 lacs and most of these skins are exported which help in earning foreign currency. Throughout the world sheep have been very important to man as sources of wool, meat and in some areas their milk is also used extensively for human consumption.In the hills they are also used as pack animals.

Like all other living forms the sheep also suffer from a number of ailments. Of all the sheep diseases, those caused by parasitic worms are most common and important. Although the exact data are not available with regard to actual losses suffered as a result of worm infections in India, it is much larger in sheep than in any other domestic animal. The worms in sheep and goat take a heavy toll and insidiously undermine their health and productivity.

There is a proverb that " a sick sheep is a dead sheep". Also, the sheep says and rightly so that "keep me free from parasites and I shall take care of myself". The sickness among the sheep is mostly due to parasites.

There is hardly any sheep which is free from one or other kind of parasites.

To what extent the losses in animal wealth of any country is accountable to parasitic infections and diseases may be realised from the following statement of Lord John Boydon, an ex-Director General of the Food and Agricultural Organization of the United Nations. " If it was possible to control Fascioliasis alone amongst the ruminants of a particular country, the resultant increase in the stock of meat and milk products would suffice to feed the population for a number of years" (Presidential address by P.G.Pandae in 46th Indian Science Congress Delhi,1959). That may be an eye opener to any one-scientists, planners or administrators - concerned with food production drives in the country.

Man with all intelligence and civilization is yet to claim a success in the battle against diseases, though victory in some cases is outstanding. For a Veterinary Parasitologist the battle is just in the beginning, involving many species of domesticated animals exposed in extensive unsatisfactory living conditions and to many varied parasites. But, unfortunately a problem of such great magnitude has received only scant attention.

Meagre informations are available about the parasitic fauna of sheep in Bihar State. Sarcar(1955), whose's survey was confined to Gaya and Patna Farms,gives

very scant information about the sheep parasites encountered in the State. Till to-date perhaps no survey work for the incidence of Protozoan parasites of sheep has been taken up.

In view of the above circumstances the present study, extended to more wider areas of the State, was undertaken to fill up the gap. The study has been based on postmortem, blood and faecal examinations of the sheep.

Incidence of different coccidian parasites has also been recorded. An experiment was carried out to establish the cross infectivity of sheep coccidia to goat and vice versa to confirm the statement that sheep and goats share the same types of coccidia. Also, morphology, biology and endogenous stages of coccidia in sheep have been studied.

In addition, the effect of refrigeration on the viability and development of Coenurus recovered from a sheep in pups was studied. Simultaneously, pathological, haematological and histopathological studies of the Taenia multiceps infection in pups were also made.

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# C H A P T E R - I

## "INCIDENCE OF HELMINTH PARASITES IN SHEEP IN BIHAR."

The helminth diseases of sheep, specifically recorded so far in India, are immature paramphistomiasis reported by Haji (1935), Srivastava (1938;1948), Bawa (1939), Bhalerao (1944), Kuppuswamy (1948), Iyer (1949), D'Souza (1949) and Varma (1961). Also, Pandey (1935) and Mudaliar (1945) described similar infections from cattle and goats. Monieziasis, ascariasis and strongylosis were reported by Rahim-ud-Din (1937), and Cymbiformiasis by Katiyar (1956). From other countries, trichostrongylosis (black scour disease), cooperiasis, nematodiriasis, ancylostomiasis (hook worm disease), strongyloidosis and cestodiasis have also been described.

In India many species of parasites of sheep have been reported by different authors from time to time.

Amongst the trematodes, parasitising the Indian sheep, the species (in biliary system) reported by Bhalerao (1935) are; *Fasciola hepatica* Linnaeus, 1758; *F. gigantica* Cobbold, 1955; *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899; *P. cervi* (Zeder, 1790); *Schistosoma indicum* Montgomery, 1906 and *S. spindale* Montgomery, 1906.

The incidence of amphistomiasis due to *Cotylophoron cotylophorum*, *Gastrothylax crumenifer*, *Fischoederius cobboldi*, *Calicophoron calicophorum*, *Gigantocotyle explanatum*, *Gastrodiscoides hominis* and *G. hominis* var *suis* has been reviewed by Varma (1957).

Stomach worms that have so far been reported from Indian sheep by different workers are; H.contortus (Rudolphi,1803) Cobb,1898; H.longistines Railliet and Henry,1909; Trichostrongylus colubriformis (Giles,1892) Ransom,1911; Ostertagia circumcincta (Stadelmann,1894) Ransom,1907; O. orientalis Bhalerao,1932 (Syn.Marshallagia orientalis (Bhalerao,1932) Travassos,1937); O.trifurcata Ransom,1907; O.pinnata Daubney,1933 and O.mentulata Railliet and Henry,1909 (Syn.camelostrongylus mentulata (Railliet & Henry,1909) Orless,1933).

Of the large number of helminths parasitising the small intestines of sheep, the species recorded in the Indian sheep are reported by Bhalerao (1935;1942; 1942a), Baylis (1936), Sarwar (1945), Thapar (1956), Gupta (1958) and Yamaguti (1958).

Bhatia (1960) in his survey encountered the following helminths in the small intestines of sheep:- Trichostrongylus colubriformis (Giles,1892) Ransom,(1911); Cooperia punctata (V.Linstow,1907);Bunostomum trigonocephalum (Rudolphi,1808) Railliet,1902; Gaigeria pachyscelis Railliet & Henry,1910; Strongyloides papillosus (Wedl, 1856) Ransom,1911 and Hematodirrus fillicolis (Rudolphi, 1809) Ransom,1907; Moniezia expansa Rudolphi,~~1809~~ 1810; M.benedeni Moniez, 1879; Avitellina spp. and Stilesia spp. and Ogmocotyle indica (Bhalerao,1942) Ruiz,1946 (Syn. Cymbiforma indica Bhalerao,1942) and also a variety of

immature amphistomes as mentioned above.

The helminth parasites which have so far been recorded from large intestines in Indian sheep number twelve only. Bhalerao (1934;1935) and Baylis (1936;1939) have listed Schistosoma indicum Montgomery,1906; S. spindalis Montgomery, 1906; S. Bovis Sousino,1876 in portal veins; Oesophagostomum columbianum (Courtice,1890) Stossich, 1899; O. venulosum (Rudolphi,1809) Railliet, 1885; Chabertia ovina (Gemelin, 1790) Railliet & Henry, 1909; Trichuris ovis (Abildgaad,1795) Smith,1908 and T. globulosa (V.Linstow,1901) Ransom,1911 (Syn.T.alcocki V.Linstow,1906). Skrjabinema ovis (Skrjabin,1915) Wereschtschagin, 1925 was first recorded from a sheep from the Punjab by Abdulssalaam (1938). Later, Sarwar (1945; 1945a) established the first record of T.parvispiculum Ortlepp (1937) and described a new species, T.ovina. Some of these species have also been reported in the survey conducted by Mudaliar (1942), Moghe(1945), Srivastava (1945), Mudaliar & Alwar (1947;1948) and Thapar (1946). Thapar (1956) also recorded the occurrence of O. columbianum, O.venulosum, O.asperum, O.vitulorum and T.ovis for the first time from certain areas of the country (Bihar,Bengal,U.P.,Orissa and Assam).

Of the filariids parasitising the aorta and other blood vessels in sheep, the two species that have been recorded are:- Elaeophora schneideri Wehr and Dickman 1934, from the posterior aorta, carotid, mesentric and

iliac arteries from new Mexico (North America) and Onchocerca armillata Railliet & Henry, 1909, found in the anterior part of the thoracic aorta first reported from India by Bhatia & Sood (1959) and Bhatia (1960).

Sarwar (1946) has recorded the occurrence of Setaria cervi for the first time in India in sheep at Izatnagar. Shoho (1956) has found a male S. digitata in a goat in Ceylone.

Bhalerao (1935) and Baylis (1936) have reported the occurrence of Protostrongylus rufescens (Leuckart, 1865) Kamensky, 1905 (Syn. Synthetocaulus rufescens Railliet and Henry, 1907), in India (Punjab). Bhalerao (1932) had also recorded Varestrongylus pneumonicus in India for the first time in the bronchi of hill sheep. Mohan (1948) reported for the first time the occurrence of Dictyocaulus filaria infection in sheep in Bengal. Subsequently, Sarwar (1953) reported a new lung worm, Pneumostrongylus ovis from sheep in Pakistan.

Sarcar (1956) reports on the occurrence of stomach worms (H. contortus, Macistocirrus, Trichostrongylus), Whip worms, Strongyloides spp., Intestinal amphistomes Moniezia, coccidia in sheep population of the Bihar State. He also mentions of the occurrence of other helminthic disease of sheep in the State, like nasal schistosomiasis, fascioliasis, oesophagostomiasis, hook worm infections, and monieziasis etc. But he was unable to give a detailed

account of the species encountered in the State and his work was confined to the survey of the two Govt. Sheep Farms (Gaya and Patna) only.

In the present study occurrence of 21 species in sheep in different areas of Bihar is reported.

## MATERIALS AND METHODS

The helminth specimens were collected from Government Sheep Breeding Farms at Gauriakarma and Gaya. Some of them were also collected from abattoirs at Patna (recognised) and the suburban Rajabazar (Private). All the organs of the carcases were autopsied and thoroughly searched for the helminth parasites.

The protozoan parasites were recovered mostly from Rajabazar and Patliputra Colony in Patna from local and private flocks. Only coccidian parasites were encountered during the survey in these localities.

The helminth collections were kept in separate Petri dishes (depending upon the class they belonged to) containing normal saline solution. The methods adopted for their collection were the same as are commonly employed. At times, it was found that some mucus was attached to the worms. To free the worms from mucus one percent sol. of caustic soda was added to the normal saline which dissolved the mucus (Schmid, 1935). The helminths were washed three or four times in normal saline, to get rid of the attached debris, mucus etc.

After collecting the parasites, the nematodes were fixed in steaming 70% alcohol, hot 5% formaline and in some cases in steaming glycerine - alcohol made up in the ratio of 1:19. All these three media gave good results.

After fixing the worms they were transferred to the respective same preservative -medium in properly labelled phials.

The tape worms were fixed by constantly dipping the worms in 5% lukewarm formol-saline, by holding the tail end of the worm with a forcep and then drawing it along the edge of the beaker. By repeating the process 10-15 times the worms became completely flattened and extended. The thick worms were gently pressed in between the two slides, tying the latter lightly with a fine cotton thread. After the process was over, the worms were preserved in fresh formol-saline in labelled phials.

The trematodes were fixed by pressing them gently with a cover slip or between two slides and immersing them in 5% formol-saline, in which they were later preserved.

The trematodes were stained with Borax-carmine, Aceto-carmine, Aceto-acetic acid-carmine. Permanent slides were made for detailed studies and identification.

The tape worms were stained with Acetic-acid alum-carmine, Borax-carmine and also Aceto-carmine. Permanent slides of these were also prepared for detailed studies and specific identification.

For microscopical examination of the preserved nematode specimens, it was essential to clear them. The

following three media were used for clearing them.

1. Beechwood creosote:- gave satisfactory results in case of large and medium sized nematodes, which were examined after 2 to 3 hours of this treatment. It does not give good results with small-sized worms, because its refractive index is high and the small specimens become too transparent.

2. Lacto-phenol:- This clearing agent was freshly prepared as given below (Taylor, 1935) and used for medium-sized nematodes which were examined after 12 hours.

Carbolic acid - 1 part
Lactic acid - 1 "
Glycerine - 2 "
Distilled water - 1 "

3. Glycerine-alcohol :- It was found to give satisfactory results in case of thin nematodes. The mixture was prepared by mixing glycerine and 70% alcohol in the ratio of 1:19. The specimens became transparent within a period of 5-6 days and then examined after the lapse of this period. Though it is slow in action, it has been found to give good results in case of thin, small and larval stages of the nematodes. Unlike the aforesaid clearing media it does not damage the specimens even on prolonged treatment. Another advantage with this medium is that the specimens after examination are

straightway put back in the same preservative without any other treatment.

For the study of morphology of the nematode larval stages 1% iodine solution was used to stain them. after mounting the larvae on a slide in glycerine, a cover slip was put over them. One drop of 1% iodin sol. was allowed to percolate under the cover slip from one side . From the opposite side of the cover slip, the glycerine was slowly taken out by means of a filter paper and the larvae were thus left in the iodine sol. They were seen to take the tinge of iodine. This method helped to differentiate the internal structures of the larvae.

The larvae were ~~xxx~~ recovered from the eggs which were incubated at laboratory temperature in charcoal-faeces culture. This culture was prepared in the laboratory by adding 3 parts of sterilised powdered faeces and 2 parts of bone-charcoal powder. The culture medium was moistened by adding just sufficient quantity of water so that the medium remained sufficiently humid for the development of the larvae. The development of the eggs was observed regularly and the larvae thus obtained were separated by Baerman's method of separation of larvae.

The methods employed for faecal examination in course of survey work were the usual ones, the sedimentation and floatation methods.

All the eggs encountered in the survey have been outlined with the help of camera lucida and identified on morphological grounds.

Tissues for histopathological studies were preserved in 10% formol-saline and the sectionsprepared from them for detailed histopathological studies and stained with Haemo-toxyline eosin stain.

Measurements were taken with the help of ocular and stage micrometers.

## TABLE No.II(a)

Table showing the percentage of infection with different Trematodes at Government Sheep Farm, Gauriakarma.

| Sl.No. | Name of the parasite | Total No. examined. | No. found infected | Percentage of infection. |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Cotylophoron cotylophorum | 44 | 31 | 70.45 |

## TABLE No.II (b)

Table showing the percentage of infection with different Cestodes in sheep at Gauriakarma Sheep Farm.

| Sl.No. | Name of the parasite | Total No. examined | No.found infected | Percentage of infection. |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Echinococcus granulosus cyst. | 44 | 7 | 15.90 |
| 2. | Cysticercus tenuicollis | 44 | 21 | 47.72 |
| 3. | Moniezia expansa | 44 | 3 | 6.81 |
| 4. | Moniezia benedeni | 44 | 1 | 2.27 |
| 5. | Moniezia denticulata | 44 | 1 | 2.27 |
| 6. | Avitellina centripunctata | 44 | 1 | 2.27 |
| 7. | Stilesia globipunctata | 44 | 1 | 2.27 |

## TABLE No. II(c)

Table showing the percentage of infection with different Nematodes in sheep at Gauriakarma Sheep Farm.

| Sl.No. | Name of the parasite | Total No. examined | No.found infected | Percentage of infection. |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Trichuris ovis | 44 | 12 | 27.27 |
| 2. | Oesophagostomum columbianum | 44 | 11 | 25 |
| 3. | O. venulosum | 44 | 1 | 2.27 |
| 4. | Haemonchus contortus | 44 | 4 | 9.09 |

## TABLE No.III

Table showing the percentage of multiple infection among sheep at Gauriakarma Farm.

| Sl. No. | Nature of multiple infection | Total No. examined | No. infected | Percentage of infection. |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Cotylophoron cotylophorum<br>Cysticercus tenuicollis<br>Trichuris ovis<br>Oesophagostomum columbianum | 44 | 1 | 2.27 |
| 2. | C. cotylophorum<br>T. ovis<br>Cysticercus tenuicollis<br>Hydatid cyst | " | " | " |
| 3. | C. cotylophorum<br>T. ovis<br>O. columbianum<br>Moniezia expansa | " | " | " |
| 4. | C. cotylophorum<br>T. ovis<br>Cysticercus tenuicollis | " | " | " |
| 5. | C. cotylophorum<br>T. ovis<br>O. columbianum | " | 2 | 4.54 |

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 6. | T. ovis<br>Hydatid cyst<br>Haemochus contortus | 44 | 1 | 2.27 |
| 7. | Cysticercus tenuicollis<br>M. expansa<br>M. benedeni | " | " | " |
| 8. | Cysticercus tenuicollis<br>H. contortus<br>O. venulosum | " | " | " |
| 9. | C. cotylophorum<br>C. tenuicollis<br>H. contortus | " | " | " |
| 10. | C. cotylophorum<br>C. tenuicollis<br>O. columbianum | " | 2 | 4.54 |
| 11. | C. tenuicollis<br>T. ovis<br>O. columbianum | " | " | " |
| 12. | C. cotylophorum<br>C. tenuicollis<br>Hydatid cyst | " | 1 | 2.27 |
| 13. | Hydatid cyst<br>C. tenuicollis | " | " | " |

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 14. | *Schistosoma indicum* | 44 | 1 | 2.27 |
| | *C. tenuicollis* | | | |
| 15. | *C. tenuicollis* | " | " | " |
| | *O. columbianum* | | | |
| 16. | *Hydatid cyst* | " | " | " |
| | *O. columbianum* | | | |
| 17. | *C. cotylophorum* | " | " | " |
| | *T. ovis* | | | |
| 18. | *C. cotylophorum* | " | " | " |
| | *H. contortus* | | | |
| 19. | *C. cotylophorum* | " | 5 | 11.36 |
| | *C. tenuicollis* | | | |
| 20. | *C. tenuicollis* | " | 2 | 4.54 |
| | *T. ovis* | | | |
| 21. | *C. cotylophorum* | " | 1 | 2.27 |
| | *O. columbianum* | | | |
| 22. | *C. cotylophorum* | " | 2 | 4.54 |
| | *Hydatid cyst* | | | |

## TABLE NO.IV(a)

Table showing the percentage of infection with Trematodes in sheep at slaughter houses at Patna & Rajabazar.

| Sl.No. | Name of the parasite | Total No. examined | No. found infected. | Percentage of infection |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Gastrothylax crumenifer | 34 | 3 | 8.82 |
| 2. | Cotylophoron cotylophorum | 34 | 2 | 5.88 |
| 3. | Fischoederius elongatus | 34 | 1 | 2.94 |

## TABLE No.IV (b)

Table showing the percentage of infection with Cestodes in sheep at Slaughter houses at Patna & Rajabazar.

| Sl.No. | Name of the parasite | Total No. examined | No.found infected | Percentage of infection. |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Multiceps multiceps | 34 | 2 | 5.88 |
| 2. | Cysticercus tenuicollis | 34 | 1 | 2.94 |
| 3. | Moniezia expansa | 34 | 5 | 14.70 |
| 4. | Moniezia benedeni | 34 | 1 | 2.94 |
| 5. | Avitellina centripunctata | 34 | 4 | 11.76 |
| 6. | Stilesia globipunctata | 34 | 3 | 8.82 |

TABLE No.IV(c)

Table showing the percentage of infection with Nematodes in sheep at slaughter houses, Patna & Rajabazar.

| Sl.No. | Name of the parasite | Total No. examined | No.found infected | Percentage of infection |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Strongyloides papillosus | 34 | 1 | 2.96 |
| 2. | Oesophagostomum columbianum | 34 | 8 | 23.52 |
| 3. | Haemonchus contortus | 34 | 7 | 20.58 |
| 4. | Bunostomum trigonocephalum | 34 | 5 | 14.70 |
| 5. | Gaigeria pachyscelis | 34 | 1 | 2.94 |
| 6. | Trichostrongylus colubriformis | 34 | 7 | 20.58 |
| 7. | Trichuris ovis | 34 | 4 | 11.76 |
| 8. | Trichuris globulosa | 34 | 1 | 2.94 |

## TABLE No.V

Table showing the percentage of common multiple infections among sheep at Patna and Rajabazar.

| Sl.No. | Nature of multiple infection | Total No. examined | Total No. infected | Percentage of infection |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Trichuris ovis<br>O. columbianum<br>H. contortus<br>T. globulosa | 34 | 1 | 2.94 |
| 2. | T. ovis<br>B. trigonocephalum<br>G. pachyscelis | " | " | " |
| 3. | T. ovis<br>B. trigonocephalum<br>S. papillosus | " | " | " |
| 4. | Gastrothylax crumenifer<br>C. tenuicollis<br>H. contortus | " | " | " |
| 5. | T. ovis<br>H. contortus | " | " | " |
| 6. | M. expansa<br>A. centripunctata | " | 3 | 8.82 |
| 7. | M. expansa<br>O. columbianum | " | 1 | 2.94 |

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 8. | G. crumenifer | 34 | 1 | 2.94 |
| | H. contortus | " | | |
| 9. | Coenurus Cerebralis | " | " | " |
| | S. globipunctata | | | |
| 10. | O. columbianum | " | " | " |
| | B. trigonocephalum | | | |
| 11. | T. colubriformis | " | 2 | 5.88 |
| | B. trigonocephalum | | | |
| 12. | O. columbianum | " | 1 | 2.94 |
| | T. colůbriformis | | | |
| 13. | C. cotylophorum | " | " | " |
| | Fischoederius elongatus | | | |

## TABLE No.VI

Table showing the incidence of Helminth infections among the sheep as revealed by faecal examination in Bihar.

| Place of examination | No. examined | Group of helminth | Trematodes | | | Cestodes | | | Nematodes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Light | Med. | Heavy | Light | Med. | Heavy | Light | Med. | Hea-vy. |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Takuna Farm | 187 | Amphistome sp. | - | 19 | - | - | - | - | - | - | - |
| | 187 | Moniezia sp. | - | - | - | 3 | 2 | - | - | - | - |
| | 187 | Trichuris sp. | - | - | - | - | - | - | 50 | 25 | 1 |
| | 187 | Bursate worm | - | - | - | - | - | - | 5 | 5 | - |
| | 187 | Strongyloides sp. | - | - | - | - | - | - | 4 | - | - |
| Gauriakarma Farm | 180 | Amphistome sp. | 4 | - | - | - | - | - | - | - | - |
| | 180 | Moniezia sp. | - | - | - | 1 | - | - | - | - | - |
| | 180 | Trichuris sp. | - | - | - | - | - | - | 13 | 3 | - |
| | 180 | Ascaris sp. | - | - | - | - | - | - | - | 2 | - |
| | 180 | Bursate worm | - | - | - | - | - | - | 34 | 19 | 4 |
| | 180 | Strongyloides sp. | - | - | - | - | - | - | 4 | - | - |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Patliputra | 45 | Moniezia sp. | - | - | - | 1 | 2 | - | - | - | - |
| | 45 | Trichuris sp. | - | - | - | - | - | - | 7 | 3 | - |
| | 45 | Bursate worm sp. | - | - | - | - | - | - | 13 | 9 | 2 |
| | 45 | Strongyloides sp. | - | - | - | - | - | - | 13 | 9 | - |
| Rajabazar | 13 | Bursate worm sp. | - | - | - | - | - | - | 2 | 3 | - |
| | 13 | Trichuris sp. | - | - | - | - | - | - | 1 | 2 | - |

## TABLE No.VI(a)

Table showing the percentage of infection with different Helminth eggs in sheep as revealed by faecal examination.

| Place of examination | Kind of parasite | Total No. examined | No.found infected | Percentage of infection |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| Sheep Farm, Tekuna (Gaya) | Amphistome sp. | 187 | 19 | 10.16 |
| " | Moniezia sp. | 187 | 5 | 2.6 |
| " | Trichuris sp. | 187 | 76 | 40.6 |
| " | Bursate worm sp. | 187 | 10 | 5.3 |
| " | Strongyloides sp. | 187 | 4 | 2.1 |
| Gauriakarma Farm (Hazaribagh) | Amphistome sp. | 180 | 4 | 2.2 |
| " | Moniezia sp. | 180 | 1 | 0.55 |
| " | Ascaris sp. | 180 | 2 | 1.1 |
| " | Trichuris sp. | 180 | 16 | 8.8 |
| " | Bursate worms | 180 | 57 | 31.6 |
| " | Strongyloides sp. | 180 | 4 | 2.2 |

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Patliputra Colony (Patna) | Amphistome sp. | 45 | - | - |
| | Moniezia sp. | 45 | 3 | 6.6 |
| | Trichuris sp. | 45 | 10 | 22.2 |
| | Bursate worm sp. | 45 | 24 | 53.3 |
| | Strongyloides sp. | 45 | 22 | 48.8 |
| Rajabazar (Patna) | Amphistome sp. | 13 | - | - |
| | Moniezia sp. | 13 | - | - |
| | Trichuris sp. | 13 | 3 | 23.0 |
| | Bursate worm sp. | 13 | 5 | 38.4 |

From the foregoing tabular statements the extent of sheep parasites and parasitosis encountered in different geographical zones of the State of Bihar is well understood. It does not only reveals the incidence, percentage, and instances of multiple infections with different helminths but also gives a clue of certain infections which were unknown and not encountered by the pervious workers. No sheep was found free from one or the other parasite.

The following are the important observations which may have direct bearing in one way or the other upon improving the sheep industry, particularly in this State. These will be helpful in designing effective control measures and studying more details about the different aspects of unknown parasites.

1. Twenty one species of helminths have been recorded in the present study. Out of these species there have never been any record of the occurrence of Schistosoma indicum, Echinococcus granulosus cyst, Coenurus, & Gaigeria pachyscelis in sheep in Bihar.

2. The percentage of infection at Government Sheep Farm,Gauriakarma situated in Chotanagpur plateau (Hazaribagh district) with cestodes,trematodes and nematodes was found to the tune of 75.55, 70.45 & 65.90% respectively. Infestations with Cotylophoron cotylophorum was the highest (70.45%) and with Cysticercus tenuicollis was 47.7%.

As indicated in table No.I two helminths, Schistosoma indicum (recorded for the first time) and Cysticercus tenuicollis were recovered from only one sheep at Gaya Farm, available for postmortem examination.

3. The faecal examinations carried out at Gauriakarma and Gaya Farms revealed that the maximum number of sheep were infected with bursate worms (31.6%) at Gauriakarma and 40.6% with Trichuris sp. at Takuna (Gaya). In addition 1.1% of sheep were found infected with Ascaris sp. at Gauriakarma. The eggs closely resembled that of Ascaris vitulorum. However, none of the species of Ascaris group was encountered at P.M.examination.

4. At autopsy the percentage of infection was in the sequence of 100%, 47.0%, 20.58% with nematodes, cestodes and trematodes at abattoirs at Patna and Rajabazar. The animals slaughtered at these abattoirs,hailed from Arrah,Palamau,Muzaffarpur,Santhal Parganas and Ranchi districts. Highest percentage was found infected with Oesophagostomum columbianum (23.52%) and H.contortus ranked next in order (20.58%).

5. Highest percentage of infection with bursate worms based on faecal examination at Patliputra,and Rajabazar stood to the tune of 53.3% and 38.4% respectively.

6. Cases of multiple infections were very common among the sheep at autopsy. Out of 44 cases examined

30 were found infected with multiple infection and 14 with single infection. Multiple infections with four helminths, three helminths and two helminths stood to the tune of 6.66%, 26.66% and 36.36%. This gives an indication of the intensity about the relative preponderence of different helminths in sheep.

7. Cases of multiple infection were also encountered at autopsy at slaughter houses Patna and Rajabazar. Out of 34 cases examined, 16 were found infected with multiple infection with helminths of different groups. The maximum number of helminths recovered were four in one case, three in three cases and two helminths in twelve cases; which stood in terms of percentage to the tune of 2.94, 8.8,&35.2 respectively.

# C E S T O D A

I. Family:- Anoplocephalidae Cholodkovsky, 1902.

Genus :- MONIEZIA R. Blanchard, 1891.

1. Species:- M. expansa (Rudolphi, 1810).

Host :- Sheep (Ovis aries).

Location:-Small intestines.

Locality:-Patna, Hazaribagh.

The genus Moniezia was established by Blanchard in the year 1891. Stiles and Hassal, devided the genus into three groups, namely planissima group in which the inter-proglottidal glands form a line across the segment posteriorly; expansa group in which these glands are grouped into the form of rings and denticulate (alba) group in which the glands are lacking.

In India three species of this genus have so far been recorded in ruminants in different parts of the country. These are M. expansa (Rudolphi, 1810), M. benedeni (Moniez, 1879) and M. denticulata (Rudolphi, 1910). Bhalerao (1935) recorded the occurrence of all the three species in certain localities of India. These are distinguished by the position and the presence or absence of inter-proglottidal glands, which are saccular in M. expansa, linear in M. benedeni, and absent in M. denticulata. They are found more commonly in young

stock than in adults. Out of three species, M.expansa appears to be the commonest species in India. In the present study the percentage of these three species stood M. expansa 6.81%, M. benedeni 2.27% and M.denticulata 2.27% at Gauriakarma. At Patna the percentage was 14.70 and 2.94 with M. expansa and M.benedeni respectively.

M. expansa is 4-5 meter in length. It may some times measure upto 600 cm. The maximum width found is 1.5 cm. The segments are broader than length. Each segment has double set of reporductive organs. The scolex is 0.5 to 0.7 mm. wide. Suchers which are four in each scolex are unarmed. The genital pore opens in middle of each segment. The testes which usually number 200-400 occupy posterior 2/3rd of each segment but some times the position of these testes is variable. They are small rounded bodies and may be distributed throughout the central field or concentrated towards the sides. Stiles and Hassal (1893) proposed a new species M. trigonophora on the basis of the arrangement of testes. In this new species the testes were arranged in the form of two triangles on either side and did not meet in the mid line. Theiler (1924) doubted its validity on this character, which he did not considered enough to give the species an independent name and thus he proposed dismissal of this species. Baer (1927)insisted

on its validity and in his "Monographie des Anoplocephalidae" maintained this species. Taylor (1928) after examining lot of specimens came to a conclusion that there is variation in this character even in the segments of the same worm. Hence he too supported dismissal of M. trigonophora. Varma's (1956) observations on M. expansa from a specimen of sheep from Bihar is also in full agreement with Taylor's observation.

The cirrus sac is a pyriform or fusiform organ. The vasdeferens is thrown into one or two loose coils. The ovaries are bilobed and are median in position to the excretory canal. The lobes present a fan shaped appearance. Immediately behind the ovary, there are conspicuous vitelline glands; and ovaries and vitelline glands form a ring on either side of the segment. The uterus is single & reticulate. The saccular interproglottidal glands are arranged in a row posterior to each segment. The glands are grouped, thereby giving rise to an appearance like rosette. The gravid segments consists of reticular net work of two uteri with eggs having well developed pyriform apparatus.

There is great diversity, as to the shape and outline of Moniezia eggs amongest the different workers. Baylis (1929) has described the eggs as

spherical in shape and measure 0.05 to 0.27 mm. in diameter. Bhalerao (1935) gives their measurement as 0.050 to 0.090 mm. and his text figure shows that they are spherical. But, Monning(1956) states that eggs are somewhat triangular in shape with a well developed pyriform apparatus and according to his findings measure 56-67 microns in diameter. Varma (loc.cit.) after examining a series of specimens recovered from different faecal samples has come to the conclusion that the eggs of M. expansa are neither spherical or triangular in shape but instead rhomboidal in out line. He supports his findings with camera lucida diagrames. Eggs from faeces were separated from the debris by suger floatation methods. He gave their measurement as 50-56 microns (diameter). He further points out that examination of ova in utero in stained preparations does not reveal the true picture of ova as they get shrivelled up and present irregularities in their shape. The ova in fresh faecal samples appear rhomboidal in shape.

The number of tapeworms encountered varies from 1 to 7. On account of their large size they were found almost to pack the lumen of small intestines in few lambs. The measurements of the ova were in complete agreement with Varma's (loc.cit.) findings.

2. Species :- M. benedeni (Moniez, 1879), Blanchard, 1891.

Host :- Sheep.

Location :- Small intestines.

Locality :- Patna and Hazaribagh.

Wardle and MCloeod (1952) states it to be cosmopolitan in distribution, common in all stock raising areas, but much commoner in calves than in sheep. Its incidence in the present study was found to be 2.5%. It has been recorded by Bhalerao (1935) in Punjab, Bombay, U.P. and Madras from sheep. This worm has also been recorded by Thapar (1956) in other ruminants of Bihar, Bengal and U.P.

It closely resembles M. expansa in appearance and in its morphological characters. The distinction between the two species lies in the arrangement of inter-proglottidal glands which are arranged in short, transverse row (linear arrangement) in case of M.benedeni posteriorly in each segment.

(iii). Genus :- AVITELLINA Gough, 1911.

Species:- A. centripunctata Rivolta, 1874.

Host :- Sheep.

Location:- Intestines.

Locality:- Patna & Hazaribagh.

It was to the credit of Rivolta in 1874 who first described A. centripunctata from Italy. His description was inadequate. In South Africa Gough(1911) gave detailed morphological characters of this species which he recovered from a sheep. Upto 1927 till the publication of Wood-land this parasite was the only known species of the Genus. Wood-land in 1927 examined more specimens of this Genus from Italy and redescribed A. centripunctata, which he believed to be Rivolta's species and also concluded that Gough's specimens from South Africa were certainly different from A.centripunctata as originally described by Rivolta and thus referred Gough's specimens to what he named as A.goughi Wood-land (loc.cit.) also described a new species from India viz., A.lahorea and also added three more species - sudanea, chalmersi and goughi making the list of species of Genus Avitellina to five. Further additions were made by Wood-land (1928), Southwell (1929) and Wood-land(1935).

Bhalerao (1935) did not attach importance to the depth of testes and stated "That depth of the outer

column of testes emphasised by Wood-land (1927), as a distinction between A. centripunctata and A.lahorea is not a constant feature". According to him in one and the same strobilus, the outer column of testes was one testis deep in places and several testes deep in other. Bhalerao (1936) while reviewing the Genus recognised ten species, viz., A. tatia, A. woodlandi, A. pinitneri, A. aegyptica, A. sudanea, A. lahorea, A. goughi, A. southwelli, and A. centripunctata. These species are based upon the characters of parutrine organs and depth and arrangement of testes. Bhalerao (loc.cit.) records the incidence of A. centripunctata, A. loherea, A.goughi, in certain parts of India.

Monning (1956) states that several species of this Genus have been described but the question of their identity is unsettled. Thapar (1956) records the A. centripunctata is the commonest species found in different parts of India. He also recorded the occurrence of A. loherea, and A.tatia in sheep at Lucknow. In the present study out of the 78 sheep examined,5 were found infected with this parasite.

This tapeworm is 3 meter long and 2.6 mm. wide. Segmentation is indistinct except in the ripe ones which are cylindrical. It has four unarmed suckers and one set of reproductive organ in each segment. The genital pores open near middle of the lateral margin of the segment and

are irregularly alternating and ventral in position in each segment. Testes are arranged in 4 rows and 12-20 in number. The ovary is single in each segment and is situated not far from the middle in the segment. Vagina lies posterior to the cirrus sac on both sides. Uterus is single in each segment. In ripe segments uterus is replaced by Paruterine organ. This paruterine organ is pear shaped and arranged in the centre of each segment. Each such organ may contain 20.30 eggs.

II. Family :- Taeniidae Ludwig, 1886.
(i). Genus :- TAENIA Linnaeus, 1758.
1. Species :- T. hydatigena Pallas, 1766.
Larval stage :- Cysticercus tenuicollis.
Host :- Sheep.
Location :- Mesentery, omentum, free in the abdominal cavity or attached to the liver.
Locality :- Patna, Hazaribagh & Gaya.

This is the intermediate stage (larval stage) of the dog-tapeworm Taenia hydatigena and is cosmopolitan in its distribution. In the present investigation the percentage of infection with this larval tapeworm was found to be 47.72 % at Gauriakarma Farm & 2.94% at Patna.

This larval stage is sac or bladder-like filled with clear fluid in appearance. It is on this account, that it is known as "bladder worm". The bladder has a thin neck and it has got white opaque spot which marks the location of the scolex. From few bladder worms, the scolex was pressed out between two slides to make the structures of the scolex visible. This was fixed in formol-saline and stained with Borax-carmine. The scolex showed 26-40 rostellar hooks and four suckers, as in case of adult worm.

The large rostellar hooks measure 160-200 microns and smaller hooks 100-150 microns, and are

arranged in two rows. The bladder worm measures 2 cm. to several inches in length. The bladder worm has got a covering which forms the wall of the cyst. More than one cyst was recovered from the sheep in all cases, with a maximum of seven cysts in a few sheep.

2. Species :- T.multiceps Leske, 1780.
Larval stage:- Coenurus cerebralis.
Host :- Sheep.
Location :- Brain surface.
Locality :- Patna (Rajabazar).

Multiceps multiceps is the larval stage of T.multiceps, a dog-tapeworm. This is a cosmopolitan parasite of dogs, fox, jackals. Coenurus has been recorded from the brain of man, horse, cattle and sheep. It has also been recorded from goats, pigs and rabbits. It occurs infrequently in other regions of the body. The percentage of infection with this species was found to be 5.88%.

Multiceps multiceps is of wide geographical and biological distribution and may contain several different strains which establish themselves in different hosts, where they adopt an individual form and behave in their own characteristic way.

In sheep the cyst may occur in brain or in the lumber region of the spinal cord. If they are present in numbers in the brain, the victim shows symptoms of blindness and acute encephalitis; if the parasite is located as a single or double egg sized cyst in the cerebral hemisphere of one side, the animal shows the syndrome termed "Gid" (English), "Wirbling" (Germany) or "Tournoiement" (French), the animal walking in circles, staggering and showing giddiness. Similar cases have been

recorded though rarely in man.

The disease caused by this larval parasite is known as coenuriasis or Gid and has been recognised in Europe for several generations. The classical description in the United States is that of Hall in 1910. At that time it was wide spread in Moutana and was believed to have occurred in New York,Illinois, Nevada, Kansas, Iowa,Michigan,Ohio,Missouri and Indian territory. Judging by the paucity of subsequent reports the malady has become rare.

Coenurus has the form of a cyst filled with a transparent liquid in its internal (germinal) layer. There are scolices 2-3 mm. in diameter, numbering 100-250. Externally the cyst ranges from a pea size to that of a hen's egg and is covered with a fine chitinous memberane.

(ii). Genus :- ECHINOCOCCUS Rudolphi, 1801.

Species:- E. granulosus Batsch, 1786.

Larval stage:- Hydatid cyst.

Host :- Sheep.

Location:-Lungs, & liver.

Locality:- Gauriakarma.

This is the intermediate stage of the dog tapeworm Echinococcus granulosus. In the present study the percentage of the larval stage in sheep was found to be 15.9%. This indicates the importance of this parasite for detailed study and erradication in Bihar which is of zoonotic importance.

The intermediatehosts develop the hydatids by ingesting eggs from the faeces of dogs. The hydatid occasionally attains the size of a football. One of the records reported is that the cyst attained a diameter of 20 inches and contained 3.5 gallons of fluid. The internal germinal layer of the cyst wall produces numerous small vesicles or brood capsules, about 5-6 months after infection, and scolices are formed in these and also some times on the germinal layer directly.These scolices are attached to the walls by their stalks. Some times the brood capsules are detached from the wall of the cyst and float freely in the vesicular fluid. These floating brood capsules are known as "Hydatid sands".The final host acquires the infection by ingesting fertile

hydatids.Some times the scolices are absent in the cyst and such cysts are known to be sterile ones and are harmless. These may be found in any organ but they are usually common in liver and lungs.

The cyst is spherical in shape,but its shape usually depends upon the organ in which it grows,because it is moulded by resistant tissues, for instance by bile ducts in the liver.

The cyst may burst into a cavity, e.g. thoracic- and the librated scolices, brood capsules and germinal layer can form new bladders. It is because of this reason which prevents one to puncture the cyst for withdrawing the fluid in it.

Scolices and brood capsules recovered from a cyst fx from the lungs of a sheep were stained and permanent slides prepared. About 0.5 lb. of the fluid was recovered from one cyst.

Gaiger (1915); Hutyra and Marek(1926);Aggarwala (1925); Bhalerao (1935) and Thapar (1956) have reported the occurrence of Hydatid cyst in different animals including sheep in India from the different localities.

# TREMATODA

I. Family :- Paramphistomidae Fischoeder, 1901.

(i) Genus :- GASTROTHYLAX Poirier, 1883.

Species :- G. crumenifer (Creplin, 1847).

Host :- Sheep.

Habitate :- Intestines and abomasum (immature forms) Adults in rumen.

Locality :- Rajabazar & Patna.

This species is included in the sub-family Gastrothylocinae which is characterised by the possession of a ventral pouch. This is the commonest, and cosmopolitan in distribution. The percentage of infection is 8.82 at Patna.

This trematode species is dark red in colour when alive. The powerful acetabulum is terminal in position. The ventral pouch extends over the entire ventral surface upto the acetabulum. The acetabulum measures 1.2- 1.3 mm. The ratio of the acetabulum to its length is 1:4. The intestinal caeca are simple and end at the anterior border of the testes. The testes are horizontal in position and lobed in outline. The testes measure 1.2-1.5 mm. in length and 0.8-0.92 mm. in breadth. The ovary is small and is situated between the testes. The uterus crosses from one side to the other in the middle of the body and passes between the two testes. This character differenciates it from other allied genera.

The genital pore opens into the pouch anteriorly in between the pharynx and the intestinal bifurcation. The vitelline follicles are large and situated laterally.

Pathology:-

One sheep during collection work of helminths was found suffering from pure infection of Gastrothylax crumenifer almost covering about 2/3rd of the total surface area of rumen. All of them were in adult mature stage. About 100 of the worms were removed and gross lesions found were nothing but pin point processes with which these worms have fixed their posterior suckers. No other gross lesion was encountered. Histopathological sections of the affected area did not show any remarkable change. This gave an indication of the fact that the adult parasites are non-pathogenic even if present in large numbers. There was only proliferation of the epithelial cells and no other cellular change was visible.

(ii). Genus :- FISCHOEDERIUS Stiles & Goldbergar, 1910.

Species :- F.elongatus Poirier, 1883.

Host :- Sheep.

Location:- Rumen.

Locality:- Patna.

In India Gaiger (1915) was the first to report the presence of this trematode in stomach of cattle and buffaloes. Simultaneously Baylis (1929) and Bhalerao (1935) reported its presence in cattle in India. Thapar (1956) also found it to occur in sheep and goats at Orissa and U.P. Varma (loc.cit.) found it in sheep for the first time in State of Bihar.

Body of this species is cylindrical, ventral pouch is present and oesophagus is without bulb. Testes are obliquely dorsoventral and lobed. Intestinal caeca are short ending at about middle of the body. Length varies from 6-20 mm. and thickness from 2-3 mm. Laurer's canal opens anterior to excretory pore. Ovary is somewhat between the testes.

(iii) Genus :- COTYLOPHORON Stiles and Goldberger, 1910.

Species :- C. cotylophorum (Fischoeder, 1901).

Host :- Sheep.

Location:- Rumen.

Locality:- Gauriakarma & Patna.

The genus Cotylophoron was proposed by Stiles and Goldberger(1910) to contain C.cotylophorum (Fischoeder,1901) and C.indicum Stiles and Goldberger, 1910. Further additions have been made to the list of species of this genus. These are C.ovatum, C.orientalis and C.elongatum by Harshey (1934); C.okapi by Leiper (1935); C.congolense by Beer (1936); C.jacksoni and C.filleborni by Nasmark (1937). Two more new additions have been made from American sheep by Price & MCintosh (1953). These are C.noverboracensis and C.panamensis. Price and MCintosh (loc.cit.) have excluded C.indicum from this genus and have proposed that C.indicum should be transferred to the genus paramphistomum and renamed as Paramphistomum thapari. C.cotylophorum is frequently found in sheep and is cosmopolitan in its distribution. It has since been recorded, by different workers from different localities in India.

In the present study it was found to be the only amphistome encountered in an outbreak of amphistomesis at Government Sheep Breeding Farm, Gauriakarma. Some of

these parasites were also collected from abattoir at Patna.

In living condition these worms are purple in colour. Body is conical, poterior part being wider than the anterior, with prominent acetabulum. In formaline fixation, colour changes to pale. In the present study the most of the specimens collected were in immature forms, mostly attached and embedded in duodenum and abomasum. Only few had attained maturity which were found lodged in rumen. The characteristic genital sucker is demonstrable even in very immature specimens after flattening the worms in between two slides. Formaline fixed specimens which were not fully developed, measure 3.1-5.3 mm. The pharynx measures 0.6 mm. This is followed by a short oesophagus. The intestinal caeca are simple and they extend up to the acetabulum. The testes are lobed and one is situated behind the other. The genital pore is posterior to the intestinal bifurcation and it is surrounded by a genital sucker which is the distinguishing feature of this genus. The ovary is small, oval and is post-testicular in position. Vitelline follicles are large and extracaecal in situation and extend from near the oesophagus to the anterior margin of the acetabulum.

Haematological studies in an out break of immature amphistomiasis due to Cotylophoron cotylophorum:-

During the course of an out break of amphistomiasis at Gauriakarmasheep farm; an attempt has been made to study and compare the blood picture of the ailing sheep with those of the apparently local healthy sheep. This study was taken up to know the extent of the blood changes occurring in amphistomiasis on comparative basis, from those of the healthy sheep. The results of the observations have been tabulated in table No.VII. In this study only eight sheep were examined from each group. The figures thus obtained were statistically analysed and it revealed a significant increase in white cell count, decrease in Red cell count and haemoglobin content of the infected group. The main increase was in neutrophils and Eosinophils.

## TABLE No.VII

Table showing the haematological studies of amphistomiasis in sheep carried out at Gauriakarma Sheep Farm.

| Group exained | Sheep No. | Total count R.B.C. | Total count W.B.C. | Differential count (in %) Neutro. | Lympho. | Eosino. | Mono. | Baso. | Haemo-globin in gm. |
|---|---|---|---|---|---|---|---|---|---|
| Infec-ted. | 10 | 7810000 | 23440 | 56 | 37 | 3 | 3 | 1 | 10.2 |
| | 152 | 4450000 | 15140 | 51 | 38 | 5 | 5 | 1 | 7.6 |
| | 29 | 3820000 | 9840 | 26 | 37 | 12 | 4 | 1 | 6.4 |
| | 168 | 7630000 | 9240 | 61 | 32 | 4 | 3 | - | 8.4 |
| | 200 | 5770000 | 13640 | 62 | 31 | 2 | 4 | 1 | 10.0 |
| | 157 | 6150000 | 10200 | 54 | 35 | 8 | 2 | 1 | 10.0 |
| | 141 | 4920000 | 14240 | 42 | 50 | 5 | 3 | - | 6.4 |
| | 146 | 4550000 | 9940 | 43 | 48 | 7 | 2 | - | 7.0 |
| Apparent-ly healthy. | 1 | 11210000 | 7040 | 40 | 52 | 5.5 | 2 | 0.5 | 11.0 |
| | 2 | 7200000 | 7740 | 42 | 50 | 5 | 3 | - | 9.0 |
| | 3 | 9520000 | 7340 | 40 | 52 | 5 | 3 | - | 10.6 |
| | 4 | 8970000 | 8900 | 43 | 50 | 5 | 2 | - | 9.0 |
| | 5 | 9930000 | 5440 | 41 | 50 | 5 | 4 | - | 10.0 |
| | 6 | 6320000 | 8100 | 43 | 49 | 5 | 2 | 1 | 7.2 |
| | 7 | 8850000 | 8640 | 40 | 51 | 6 | 3 | - | 7.2 |
| | 8 | 11220000 | 10300 | 39 | 51 | 6 | 3 | 1 | 10.0 |

II.Family :- Schistosomatidae Looss,1899.

Genus :- SCHISTOSOMA Weinland,1858.

Species :- S.indicum Montgomery 1906.

Host :- Sheep.

Location:- Portal vein in liver.

Locality:- Sheep Farm,Takuna (Gaya).

Schistosoma indicum has been recorded to parasitise equines, camels,sheep,goat,cattle and rarely buffaloes (Srivastava,1960) in India. It was to the credit of Montgomery (1906) who has elaboratly dealt with five species of the Genus Schistosoma Weinland for the first time in India. Dutt (1951; 1955) gave an account of the life history. Rao (1947) states it to be a common infection in sheep and goats of Marwar (Rajputana). He also described briefly the cirrhotic and degenerative changes in liver due to heavy infections.

In the present work only one sheep was found infective with this species, out of the few animals available at autopsy. It is first record of its occurrence in sheep in this State.

Adult worms were recovered from portal veins in liver tissue. Because of the heavy infection and being an advanced case, two types of lesions were encountered macroscopically. The liver had either (1) assumed a dark greyish colour without the development of cirrhotic nodules on its surface due to heavy infection or (2) the

liver had undergone a varying degree of nodulation, being white and pin head size, without calcification.

Detailed morphological studies were made. Males were 8.35 -19 mm. long and 0.35-0.5 mm. thick. They were stout and their anterior end narrow than posterior. Testes are 8-9. Body is tuberculate except the anterior portion. Some males were found to carry females in their gynaecophoric canals.

Females were elongated, thread like, longer and considerably thinner than males. No tuberculation is present. They measure 10-28 mm. in length and 0.12-0.26 mm. in thickness. The cuticle is free from tubercles & spines except inside the suckers and the posterior extermity. The ovary is in the middle of body. The uterus occupies most of the area between the ventral sucker and the posterior end, and contains oval eggs, with a terminal spine.

The ova of S.indicum were recovered from the liver tissue by Ferguson's(1911) digestion method, by digesting the selected portions in 3 or 4% caustic potash solution. The tissue was sliced with the help of scissors to smallest possible pieces and then caustic potash solution added. The material was kept in this solution for 3-5 hours and then examined for the presence of the eggs. Lot of eggs were found on examination under low power of microscope. Microphotographs of these eggs were taken. The eggs are oval with a terminal spine, and measure 0.09 to 0.14 x 0.042 to 0.072 mm.

Histopathology:- Dutt (1933) gave a detailed histological description of the lesions. Raghawan (1958) has described the nodular cirrhosis of liver in equines which was practically on the same line as that of Dutt (loc.cit.). Rai (1959) has also made some interesting observations on histopathological aspects in natural infections in equines with this species. Bhatia(1960), too has studied the histopathological aspects of S. indicum in sheep.

In the present study, which mainly supports the findings of the above authors; the following observations have been made.

Different degrees of tissue reactions around the eggs, were noticed in sections. The eggs containing fully developed miracidia, exhibited a marked cellular infiltration around them. The epitheliod cells in large numbers were seen prominently in most of reactions. A constant feature of all the sections under examination, were the presence of small black engulfed particles of the haematin pigment distributed in the liver tissue, as has also been reported by Faust in S.Japonicum infection. The parasite inside the interlobular vein in the liver tissue is surrounded by mononuclear and polynuclear leucocytes around it. In few sections males and females were found in copula in portal veins.

# N E M A T O D A

I. Family :- Rhabditidae.

Genus :- STRONGYLOIDES, Grassi, 1879.

Species :- S. papillosus (Wedl, 1856).

Host :- Sheep.

Location:- Small intestines.

Locality:- Patna.

Wedl (1856) discovered and named the common ovine species (S. papillosus); Pasgenstecher (1865) described a strongyloides from pigs before Bavay(1876) described the human species of strongyloides(S.stercoralis). Species of the genus strongyloides have since been described from many groups of vertebrates -- birds and several species of herbivorous,carnivorous and ommivorous mammals.

In India Ware (1923) appears to be the only worker who has recorded a strongyloides infection in a fatal case of Strongyloides stercoralis in a dog.

S. papillosus was recorded by Vaidyanathan(1942) for the first time in India from cattle and subsequently by Sarwar (1945) in sheep and goats from Madras,was encountered both in the local and the hill sheep.

Turner (1955), studied the experimental strongyloidosis in lambs that have died and described the pathological changes mostly produced in the duodenum and to some extent in jejunum. Later Turner &

Wilson (1958) during an outbreak of the disease in lambs, reported few deaths on pasture and Garkavi (1956), after describing the lesions and symptoms of disease concluded that this species, pathogenic in young lambs, produced acute symptoms and death.

In the present study only few female worms were encountered in one autopsy cases only. The parasitic female is a long and filiform, like all the parasitic adults of the genus. The cuticle bears fine striations which are resolvable only under an oil immersion objective. Situated around the mouth are six papillae. The oesophagus is simple and almost cylindrical. The tail is bluntly rounded and finger shaped. The vulva is transverse slit, with rather salient lips, is situated posterior to the middle of the body which it divided approximately in the proportion of ~~xhm~~ five to two. The gonad is double and is composed of an anterior and a posterior loop. In S.papillosus both the anterior and posterior loops of the ovary are twisted round the intestine which serves as the axis. It is 3.4-6.4 mm. long. Vulva to tail end distance is 1.6 mm.

II. Family :- Trichostrongylidae Leiper, 1912.

(i). Genus :- TRICHOSTRONGYLUS Looss, 1905.

Species :- T. colubriformis (Giles, 1892), Ransom, 1911.

Host :- Sheep.

Location :- Small intestines.

Locality :- Patna.

This species is known to occur in men, monkeys, squirrels and in domestic and wild ruminants. Giles (1892) first described this Parasite and his description was based on the material obtained from sheep in Assam and Punjab. Bhalerao (1941) described the species in details. The percentage of infection in the present study was to the tune of 20.58%.

The males measure 5.8 - 6.5 mm. in length and 1.08 mm. in maximum thickness. The spicules measure left 0.147-0.173 mm., right 0.136-0.162 mm. and the gubernaculum measures 0.070-0.083 mm.

The female measures 7.67-7.85 mm. in length. The vulva is situated 1.4-1.53 mm. from the posterior end. Post-anal portion of body in female is short and has a pair of small caudal papillae near the tip. Spicules are short, twisted and spoon or spatula shaped. The proximal end is thickened like a knob or disc like process; towards the distal end, a more or less prominent angular projection is usually present.

(ii). Genus :- HAEMONCHUS Cobb,1898.

Species :- H. contortus(Rudolphi,1803).

Host :- Sheep.

Location:- Abomasum.

Locality:- Patna.

According to Baylis (1936) this species was first recorded by Gaiger (1910) from ox and sheep in Punjab. Subsequently it has been reported from different localities of India by Bhalerao (1935), Srivastava (1945), Rathore et al.(1955), and Thapar (1956), from sheep,goat,cattle and buffaloes. The percentage of infection in the present studies was 9.09% at Gauriakarma and 20.58% at Patna.

This worm is popularly known as "Stomach worm" or "Red worm" of ruminants. In living condition the ovaries of the female coil round almost the whole length of the intestines and this gives the appearence of a"barbers pole," hence the name "barbers pole worm".

The male of this parasite measures 8 to 16 mm. and female 15 to 26 mm. in length. Diameter of the male is 0.38 mm. and of female is 0.48 mm. The buccal cavity is small and contains a dorsal lancet. The oesophagus is club-shaped and measures 1.1 mm. to 1.5 mm. in length. The two cervical papillae are situated at a distance of 0.34 - 0.39 mm. from the anterior extermity. The intestine is simple and measures

11.2 - 17.5 mm. in length. The rectum is simple. The cloaca is an elongated vacity in which the anterior end is larger than the posterior end. It receives the opening of the rectum,ejaculatory duct, and of spicular canal.

The male possesses copulatory bursa with large lateral lobes and a small asymmetrical dorsal lobe. There are two spicules situated dorsally to the posterior end of the intestines and the rectum. They are equal and appear 'X' shaped, measuring 0.398 - 0.447 mm. in length. They are slightly twisted and brown in colour. The tip of each spicule possesses a small knob. Each spicule has a small barb at the posterior end. The gubernaculum is spindle shaped. It measures 0.119 to 0.25 mm. in length.

The vulva in female is situated at a distance of 3 to 3.54 mm. from the posterior end. It is guarded by a large chitinous vulvar flap, the shape of which varies from a knob to tongue shaped flap. The anus is situated at a distance of 0.3-0.4 mm. from the posterior extermity. The tail is pointed. The uterus contains eggs in different stages of development.

In heavy infections, the mucus membrane of abomasum showed pinpoint haemorrhagic lesions with desquamation of mucus membrane. The parasites are blood suckers. This worm is responsible for acute type of abomasitis in sheep and causes heavy economic loss.

III. Family :- Strongylidae Baird.

Genus :- OESOPHAGOSTOMUM Molin,1861.

1. Species :- O. columbianum(Curtice,1890), Stossich, 1899.

Host :- Sheep.

Location:- Stomach and intestines.

Locality:- Patna & Gauriakarma.

According to Gaiger(1910), it is common in sheep and cattle in Punjab. It has almost been recorded from all parts of India from time to time. The percentage of infection is to the tune of 25 % at Gauriakarma and 23.52% at Patna.

Male is 12 - 16 mm. long. The anterior end of the worm is frequently curved into a hook. The mouth collar is in the form of a truncate cone and its posterior edge is rather prominent. There is no cephalic inflation. Lateral alae extends from the groove through out the length of body. The cervical papillae are just behind the cervical groove and the nerve ring is behind them. The external and internal leaf crowns contain 20-24 & 40-48 elements respectively. Spicules measure 0.75-0.86 mm.in length and have an accessory piece.

The female is 14- 18.5 mm. long. The tail of the female is tapering. The vulva is 1 -1.4 mm. from the posterior extermity. The anus is situated posterior to vulva and its distance from vulva varies from 0.68 to 0.78 mm. in length.

2. Species :- O.venulosum (Rudolphi,1809).
Host :- Sheep.
Location:- Intestine.
Locality:- Gauriakarma.

The percentage of infection with this helminth is 2.2% at Gauriakarma. It has been recorded by Gaiger, (1910) from sheep in India and by Bhalerao (1935) from the hill goats.

The male measures 10 - 16 mm. in length. The mouth collar is marked off, by a distinct groove and is truncate cone shaped. The cephalic inflationis fairly well developed. Narrow lateral alae extend throughout the length of body. External leaf crown has 18 elements and the internal 36 elements. The oesophagus is 0.9 mm. long. Nerve ring is at the level of cervical groove. Cervical papillae are situated just behind the posterior end of the oesophagus. Spicules are 1.1-1.5 mm. long. Accessory piece is shovel-shaped with a short handle.

The female measures 13 - 25 mm. long. The tail of female is 0.15-0.2 mm. long,straight and sharply pointed. Vulva is situated at 0.34-0.5 mm. from the posterior extermity.

IV. Family :- Ancylostomidae (Looss, 1905) Lane, 1917.

(i) Genus :- BUNOSTOMUM Railliet, 1902.

Species :- B. trigonocephalum, Rudolphi, 1808.

Host :- Sheep.

Location :- Small intestines.

Locality :- Patna.

This species was recorded by L-ane (1917a) from the sheep in Darjeeling district of Bengal. The percentage of infection in the present study was to the tune of 14.70%.

When fresh it is greyish or reddish in colour. Male measures 11-17 mm. in length and females are 19.26 mm. long. The anterior end is bent dorsally. Buccal capsule is large funnel shaped and has a large dorsal tooth and two short ventral buccal teeth. Dorsal tooth is relatively long and its dorsal border is longer than the distance from its tip to the mouth opening. The oesophagus is 0.8 to 1.45 mm. long. The nerve ring is situated at about 0.6 mm. from the anterior end. The spicules measure 0.6-0.75 mm. in length, slightly twisted and possess striated alae.

The tail of the female measures 0.3-0.4 mm. long and bears a pair of papillae near rounded tip. The vulva is situated at 5-8 mm. from anterior end.

(ii) Genus :- GAIGERIA Railliet & Henry, 1910.

Species :- G. pachyscellis Railliet & Henry, 1910.

Host :- Sheep.

Location :- Intestines (Abomasum).

Locality :- Patna.

It has been recorded from sheep, goats and cattle from different parts of India by various workers (Railliet, Baylis, Gaiger, Lane, Ransom, and Cameron), from time to time. In the present study the percentage of infection was 2.94%. Anterior end of the worm is bent dorsally. The mouth opening is oval and guarded by a pair of ventral cutting plates. Buccal capsule is large and cup-shaped. At the base of capsule there is a freely projecting cone carrying the dorsal gutter and a pair of subventral lancets.

Male measures 11-18.5 mm. in length. The oesophagus is slender and measures about 2-3 mm. in length. The excretory pore is situated a little infront of the middle of oesophagus. Bursa has a large dorsal lobe and two smaller lateral lobes which are joined ventrally. Spicules with slender, recurved terminations are without barbs, Spicules measure 1.1-1.4 mm. in length and have a fine flexible point.

Female measures 15-24 mm. in length. The tail of the female is narrowed suddenly behind the anus and has a blunt tip. Vulva is just anterior to the middle of body.

V. Family :- Trichinellidae Stiles & Crane, 1910.

Genus :- TRICHURIS Roederer, 1761.

1. Species :- T. Ovis (Abildgaad, 1795), Smith, 1908.

Host :- Sheep.

Location :- Caecum and small intestines.

Locality :- Gauriakarma and Patna.

Commonly known as whip worm of the sheep. The percentage of infection with this worm was to the tune of 27.27% at Gauriakarma and 11.76% at Patna.

This is the commonest and most widely distributed whip worm of sheep. It has also been recorded from goats, ox, camel and cattle from different parts of India. It was Gaiger in 1910 who first recorded this species in sheep in Punjab. They are 50-60 mm. in length, but female is thicker than male. The posterior portion of the body contains the reproductive organs and intestine is stout. Posterior end of the male is coiled dorsally and possesses a single spicule which is contained in a protrusible, prepuce like sheath. Spicule is of uniform thickness through out except pointed distally. It measure 5-7 mm. in length. The spicular sheath is covered with numerous small spines and has a bulbous distal enlargement when evaginated. The vulva in the female is near the junction of two portions of the body. Posterior end in female is rounded and not curved as in male. There is no expansion of egg chamber as in Trichuris globulosa.

Biology of eggs:- In view of the heavy infection rate of sheep with Trichuris spp. (Table No.VI) and also meagre information available in literature on the biology of the eggs in nature, it was considered essential to study the biology of eggs of T. ovis in nature, under different environmental stresses. About twenty T. ovis females were disected and eggs were recovered from them. These eggs were thoroughly washed and made free of debris and then utilized for the study. These eggs were divided in two equal portions and cultured in two different media (a) aerated distilled water and (b) clay soil. Natural conditions were provided by keeping the eggs at 30°C in the laboratory. Care was taken to keep both the cultures moist during the course of the experiment. Daily observations were recorded and maintained in a tabular from given below:-

| Date | Culture No.(a) | Culture No.(b) |
|---|---|---|
| 28.6.63 to 1.7.63. | Unsegmented eggs | Unsegmented eggs. |
| 2.7.63 | Segmented 2 cells | 2 cells stage. |
| 3.7.63 | --do-- | --do-- |
| 4.7.63 | 4 cells stage in 50%eggs | 4 cells in 60%eggs. |
| 5.7.63 | 25% in Morula stage & rest 4 & 8 cells stage | 30% in Morula stage,rest in 8 cells |
| 6.7.63 | 90% in Morula stage | 99% in Morula stage. |
| 7.7.63 | Gastrula stage in 50% eggs. | Gastrula stage in 60%. |
| 8.7.63 | Gastrula stage | Gastrula stage. |
| 9.7.63 | Late Gastrula stage | Late Gastrula stage. |

| | | |
|---|---|---|
| 9.7.63 | Late Gastrula stage | Late Gastrula stage |
| 10.7.63 | 10% Vermiform stage | 25% Vermiform stage |
| 11.7.63 | 80% Vermiform stage | 95% Vermiform stage |
| 12.7.63 | Few eggs embryonated | 20% embryonated |
| 13.7.63 | 50% embryonated | 80% embryonated |
| 15.7.63 | 95% embryonated | All eggs containing mortile larvae. |

The above observations reveal that the eggs embryonated fully between 15-18 days in both the medias. In soil media the rate of growth was slightly rapid comparably.

An attempt has been made on extra-corporeal hatching of the embryonated eggs by placing them in artificially prepared gastric juice (Prepared by dissolving 0.8 gm. of pepsin in 1.3 cc. of conc. HC1-1000 cc. distilled water). The eggs were examined after the lapse of 10-20 hours. Only few eggs were hatched and rest remained intact.

2. Species :- T. globulosa V. Linst, 1901.

Host :- Sheep.

Location :- Caecum and colon.

Locality :- Patna and Gauriakarma.

This species, as quoted by Baylis (1929) was originally recovered from a dromedary; but is known to occur in cattle, sheep, and goats. Baylis is of opinion that this species is probably as common as T. ovis in domestic ruminants and he assumes that it is not improbable that the two species have been confused together. The percentage of infection is 2.94% in this State.

It is differenciated from the other species on the grounds that it has the everted spicular sheath in the male and the vagina leading into the egg chamber in the female.

The male measures 40.75 mm. and female 42-60 mm. The length of oesophagus is about 2/3rd of the whole length in male while in female about 3/4th of the whole length. Spicule is 4.1-4.8 mm. in length. Spicular sheath is spiny with large globular swelling distally. Spines on the swelling are larger than elsewhere.

# D I S C U S S I O N

The sheep industry of Bihar lacks in many respects especially so far the knowledge of their parasites, parasitosis and their control measures is concerned.

In face of the increasing sheep population of the State ( as can be viewed from the livestock census of 1961, when the ovine population has risen to 11.5 lacs as against only 10 lacs in 1951) and many gaps left to investigate the parasitic fauna of sheep, as also the meagre information available in this connection, it becomes imperative to discuss certain aspects.

Minette (1950) during his survey to find the cause of heavy mortality among the Indian sheep ascribed it to be mostly due to parasitic infestations. The death rate in Bihar at Monghyr Farm (now at Gaya) was to the tune of 66.8% during the period 1942-46. This farm then ranked 2nd in India in mortality; the first being Hissar where mortality was upto 76 % for the year 1937-1946. He has not given any account of the parasites responsible for such heavy losses, because his work was mostly based on the reports of the different States. To an open eye, it was sufficient hint to attack the problem from different angles. But this problem of such importance was neglected, so much so that this industry did not show any appreciable progress.

Sarcar (1956) took up this work after six years of Minette's survey. He stated that the infection with gastro-intestinal nematodes, particularly the stomach worms (Haemonchus, Mecistocirrus and Trichostrongylus spp.), Strongyloides and also coccidia was fairly common. His work was confined to only two farms, Gaya and Patna. He also mentions of the occurrence of other helminth diseases of sheep in this State like nasal schistosomiasis, fascioliasis, oesophagostomiasis, hook worms and monieziasis etc. His studies and findings were lacking in many respects. He did not mention of the incidence and identity of the individual parasites, their intensity, and percentage of infection in sheep.

Varma (1957) gave a detailed account of amphistomiasis in sheep in Bihar. This malady was creating havoc by taking heavy toll of sheep and goats every year. He states that the disease commonly called "Gillar & Pitto" (Cherrah) is caused by immature amphistomes. He identified Cotylophoron cotylophorum the main amphistome responsible for the malady in sheep and goats, which was previously confused with P. cervi by Kuppuswamy (1948). Varma (loc.cit.) also reports on the occurrence of Fischoederius elongatus, F. cobboldi, Gastrothylax crumenifer and Calicophoron calicophorum spp. associated the dreadly malady.

In the present investigation the incidence, intensity and regional distribution of the helminth parasites of sheep have been explored. Some new additions, which add to our existing knowledge on the incidence of sheep parasites in this State are the prevalence of the helminths, Schistosoma indicum, Echinococal cysts (Hydatid), Coenurus (C.cerebralis) and Gaigeria pachyscelis. The presence of the Hydatid in sheep is of special consideration in view of its zoonotic importance. The percentage of infection with this dreadly zoonotic disease was found to the tune of 15.9% at Gauriakarma Farm.

This work also revealed the presence of Ascaris eggs to the tune of 1.1% in faeces examined at Gauriakarma Farm. This has never been encountered by the previous workers.

Cases of multiple infections were encountered in the present studies. The percentage of these multiple infections was 71.1% at Gauriakarma and 44.1% at Patna.

# CHAPTER -II

## OBSERVATIONS ON THE BIOLOGY OF SOME COMMON SHEEP NEMATODES.

The presence of gastro-intestinal parasites can be determined at ante-mortem by demonstrating the ova or some stage of the larval forms in faecal samples.

The eggs of flukes,tapeworms and whip worms can be picked out fairly easily,but sheep and goats generally harbour such worms, particularly those belonging to strongyloides which produce eggs of apparently identical morphology. In such cases the examination of the eggs is of little value for identifying the species and for this specific identification examination of the infective larvae raised by culturing these eggs may have to be resorted to.

Standards for comparison of eggs have been given by Wood (1931), Sharb (1939;1940),Kates and Sharb (1943), Kates (1947),Tetley (1949),and Cunliff and Crofton (1953). Measurements of hookworm eggs are given by Schwartz (1924), Conrodi and Barnette(1908) and Sprent (1946); and of nodular worm eggs by Andrews and Maldonado (1941). Sharb (1939;1940) gives an excellent account of the eggs of nematode parasites of all ruminants and Krug & Mayhew (1949) for eggs of bovine infections.

Methods for the recovery of the infective larvae of Strongyloides and Trichostrongyloides from faecal cultures, based on their migratory habits, have been described by Looss (1911),Darling (1911),Fullborn(1921)

and Africa (1931). Veglia (1923 & 1928) advocates the use of tall fruit jars to make faecal cultures. These jars after being tightly screwed are stored in a dark room at room temperature in summer and placed in an incubator (25°C) in winter. The mature larvae,which migrate up the walls of the culture jar, are collected by means of a blotting paper lining in the interior. This is then washed in a few cubic centimeters of water. Monning (1938) proposed for collection of these migratory larvae with the help of point of a needle or a fine camel brush.

White (1927) described a simple apparatus which trapped migratory larvae in water and a modification of this method was used by Vaidyanathan (1943).

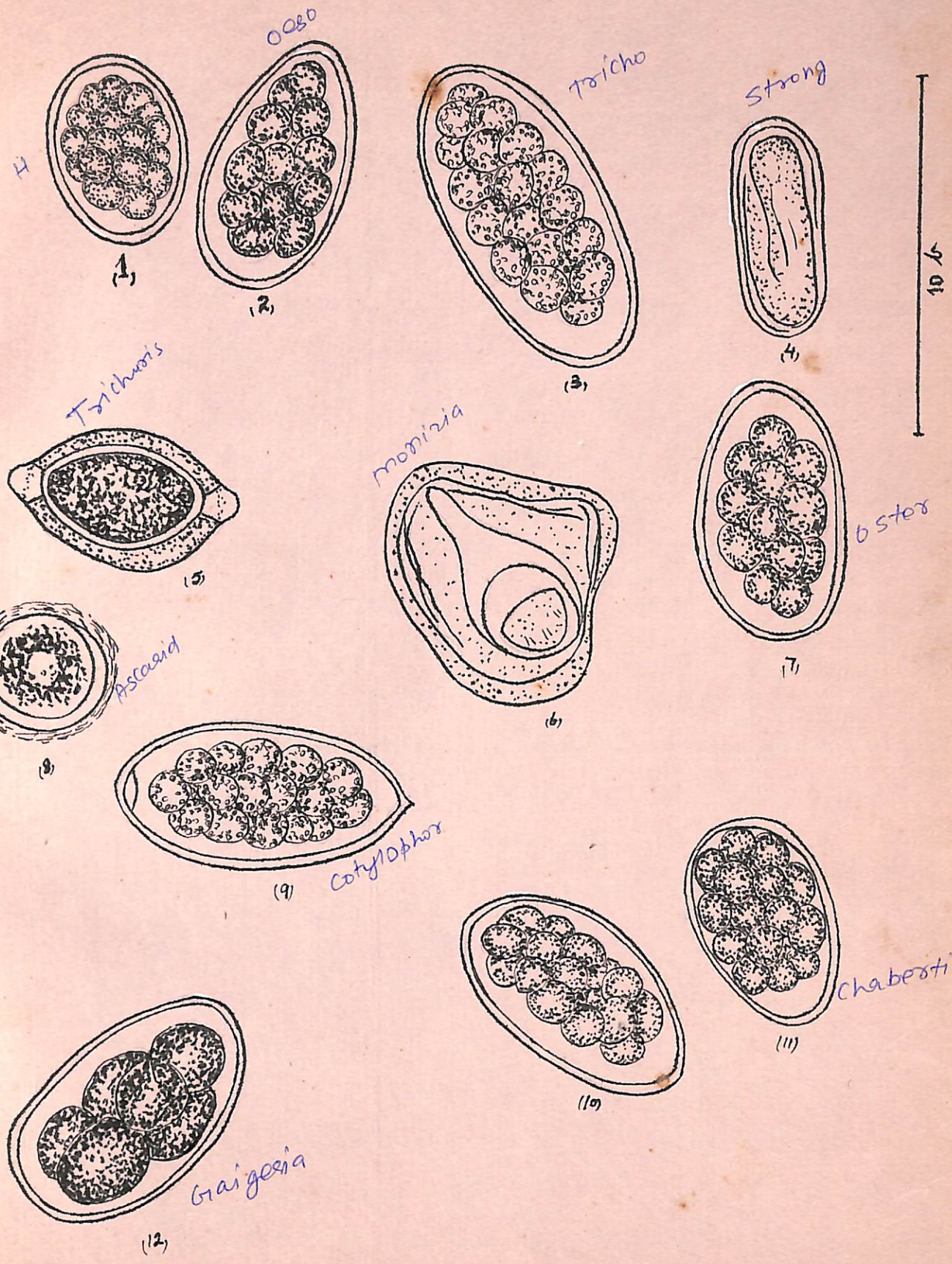
Deschiens (1939) described a method which avoids breaking and contamination of the faecal mass and yet yields a large number of larvae in a suspension free from faecal matter.

Vaidyanathan (loc.cit.) also considered it to be quite satisfactory for all larvae exhibiting positive hydro-tropism.

Whitelock (1950 & 1959) has described a method of culturing sheep faeces for the recovery of the infective larvae. He utilised the migratory habits of the infective larvae to trap them in water surrounding an inner tube in which the faeces are cultured. Regulation of the

moisture content of the culture is achieved by the saturated atmosphere in the jar surrounding the culture.

In the present study an attempt was made to find out still simple and easy method that can be adopted in routine work for specific diagnosis of verminous infestations. Larval studies of the most common nematode parasites of sheep prevalent in Bihar have been carried out for specific diagnosis.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## MATERIALS AND METHODS

The eggs were obtained from different groups of helminths by teasing out female worms. These were washed several times with normal saline solution to get rid of the attached debris.

These were thus cultured at room temperature (25 to 27°C) in sterilised sheep faeces, mixed with animal charcoal and sterilised sand in equal proportion. Cultures were kept in series of sterilised Petri dishes. While preparing the culture, watery or even semisolid consistency was avoided, as the surface tension prevents the larvae from leaving the medium. Monning's (1930) method of preparation of the faecal cultures was used. This method advocates that the consistency of the cultures should be like normal, fresh sheep pellets, broken down into a loose mass, especially to resemble in consistency and moisture content.

Three Petri dishes, two of equal diameter and one of half the diameter of the first two are required for each culture. Cultures with the infective material are spread to fill, half of the small Petri dish. This Petri dish is then transferred to the centre of one of the large Petri dishes, so that a trench of uniform width forms between the two. Distilled water is run into the trench to a height, quarter inch below the brim

of the Petri dish. This belt of the water will trap any larvae migrating from the culture. The 3rd Petri dish is inverted over the culture, so that the opposing brim of the two large Petri dishes are adjusted to flush against each other and thus incubated as such.

Five such cultures infected with eggs of different nematodes were incubated (culture nos.I,II, III,IV,V, containing eggs of Strongyloides papillosus, H. contortus, T.colubriformis, B.trigonocephalum, and O.columbianum species respectively).

Examination of the distilled water surrounding the culture No.I revealed larvae of Strongyloides papillosus on 3rd day. In rest of the cultures larvae were recovered after the lapse of 7 to 10 days.

Larvae of T.colubriformis (Culture No.III) and O.columbianum (Culture No.V) were mostly recovered by Baermann's technique, as the larvae of these parasites were only a few in the surrounding water around their respective cultures.

The larvae thus recovered, were placed on a slide and killed by gentle heat. The slide was then examined and the length of the tail of each larvae was measured excepting,larvae which had other distinguishing characters. Measurements were taken by ocular and stage micrometers.

## OBSERVATIONS

Cultural method described above, which is mainly a simple modification of Whites (1927) and Vaidyanathan's (1943) method of culturing the eggs, has proved best and simplest. In addition Baremann's method of separation of larvae has proved best in case Trichostrongylus and Oesophagostomum larvae.

Larval characters:- The characteristic larval characters of the species under study are as under. Total larvae examined in each group were ten only.

Strongyloides papillosus:- The total length of the larvae is 210-220 /u. The oesophagus of the infective larvae is about half the length of the larvae. The tail of the larvae also showed a bifid appearance in some specimens on close examination at higher magnification.

Haemonchus contortus:- The larvae have a long tail but slightly shorter and not so slender as that in case of Bunostomum trigonocephalum, on comparative basis. The tail measures 140-160 /u and the larval length varies from 680-720 /u.

Bunostomum trigonocephalum :- The larvae have a very slender and longer tail than Haemonchus contortus. The tail measures 130-150 /u. The larvae measure from 560-670 /u in length.

Trichostrongylus colubriformis:- The larvae measure 351-356 /u in length, and 18.0 to 18.2 /u in

maximum breadth. Tail is short and confuseable to that of H.contortus and measures 85-105 /u.

Oesophagostomum columbianum :- Larvae are broad and have a long whip like tail measuring 204-230 /u. The length oflarvae varies from 780-820 /u. Another characteristic feature is presence of a peculiar structure in the anterior end of the oesophagus. The lumen of the oesophagus at this point swells out until it becomes almost as wide as the oesophagus. The process of this nature is of taxonomical interest and must be studied thoroughly and considered. Morgon (1930) remarks that this structure helps to separate the larvae of large intestine parasites, from those of the larvae of parasites found in other parts of the alimentary canal.

Presence of this structure has also been described by Goodey (1924) in the infective larvae of Oesophagostomum dentatum and also by Cameron (1926) in that of Chabertia ovina.

## DISCUSSIONS

It is possible to diagnose the presence of most of the common helminth parasites found in sheep by the method described above.

This procedure does not involve any costly equipment and can very easily be employed, as a routine in the diagnosis of parasitic infestations at far off places, where other sufficient facilities are not available for the purpose.

This study however, is not enough and it is probable that further detailed studies will reveal possibly many other morphological differences of the larvae. The possibility of using biological differences in separating the larvae must not be disregarded.

Species diagnosis as a result of studying the larvae has the disadvantage of being time consuming because of culturing the eggs and the necessary careful microscopic examination of the larvae.

However, for ante-mortem specific diagnosis of verminous infestations the author considers this method to be comparatively simpler and the cheaper than other known methods.

Measurements of the larvae in the present study are comparable to Monning's(1931a) measurements but the measurement of S.papillosus is in close agreement with that given by Whitelock (1939).

# CHAPTER - III

## ON THE DEVELOPMENT OF *TAENIA MULTICEPS* IN PUPS, FED WITH A COENURUS RECOVERED FROM A SHEEP.

Cobbold (1884) described a coenurus, which he recovered from lemur in Madagascar. He considered it to be a new coenurus and thought that coenurus described by Ross (1883), was not cerebralis, although Ross at that time concluded it not to be a new parasite. But however, latter workers also claim the coenurus recovered by Ross from rabbit was undoubtedly coenurus serialis and not the C. cerebralis.

Gaiger (1907) recovered the Coenurus gaigeri from goats, from their connective tissue, at Lahore but to this at that time he considered to be Multiceps serialis. He states to have seen daughter cysts in his materials.

Tylor and Boynton (1909) found an out break in a flock of sheep from Ithaca, New York. This was the Ist authentic instance of "Gid" in Eastern United States.

Dey (1909) reported C.gaigeri from goats from their brain, intermuscular connective tissue, subcutaneous tissue, mesentry, abdominal wall and the serous coverings on the viscera.

Southwell(1912) reported Coenurus serialis from goats and Taenia serialis from dogs in Ceylon.

Coenurus cerebralis, however, does not appear to be as prolific as C.gaigeri. According to Southwell (1930) C.cerebralis is a vesicle as large as a golf ball, ~~fixxx~~ filled and containing about 150 heads. In Coenurus serialis according to Monning (1938) internal as well as

external daughter bladders may be formed and these are also to produce scolices.

Hall (1920) considered the intermediate stages of M. gaigeri tobe morphologically more closely related to M. multiceps than to M. serialis. He did not find any daughter cyst on examination of Gaiger's materials.

Bhalerao (1939) described Coenurus of M.gaigeri from the mucus membrane of the eye of a she goat,which had shown scolices both on inner and outer surfaces. He could not find daughter cysts in the cyst.

Rahim-uddin (1941) reported the occurrence of a Coenurii in the eye and the base of ear of sheep, which was not previously reported by any other workers in this host. He could not however, get the cyst identified.

Clapham (1942) has given a detailed account of the identity of the Multiceps spp. His identity of the species was mainly based on the large hook measurements. The main features of this measurement were total length of the hook and the blade length, both from dorsal and ventral sides.

Sarwar (1953) states that out of 39 cysts, only in 3 cases few floating scolices were found. He also comments that in Hall's material the free floating scolices were due to frequent handling and long preservation.

Rao et al.(1957) reported the occurrence of Coenurus gaigeri from the connected tissue of ewe in

Bombay State. He observed a greater tendency of development of the scolices on the outer walls of the cyst. He believed that the normal method of development of daughter cysts is not from the inner wall but directly from scolices.

The coenurus recovered in the present study, is from a sheep suffering from "Gid". To confirm the identity of the parasite, the cyst has been fed to young pups and the adult tapeworm, thus recovered identified. In addition an attempt has been made, to study the period of development, in final host (pup), the pathology and blood picture of the host and histopathology of the host's intestine effected with adult tapeworm. Simultaneously an observation has been made to find out, the effect of refrigeration on the viability of the coenurus.

## MATERIALS AND METHODS

A cyst (*coenurus*) was removed from a sheep showing the symptoms of "Gid" at Rajabazar. The cyst was strongly adherent to the surrounding structures and despite, careful dissection, could not be removed whole.

Three pups (few days old) were procured and kept under observation for one week and found free of infections.

Blood for haematological studies was collected from Sphenous veins & examined in the usual way.

Materials for histopathological examination were preserved in 5% formol-saline. Sections were cut (5-7 $\mu$ thickness), stained and permanent slides made in the usual way. Portion of the *coenurus* was also preserved in 5% formol-saline.

The pups were fed on cow's milk (pasturised) and bread and were kept in a clean kennel.

*Coenurus* was divided into three equal portions. One of them was fed immediately to pup no. I and second portion was fed to the pup no.II, but after two days refrigeration (4°C), to know the effect of refrigeration on the development of the worm and its viability.

Measurements of the hooks were taken with the help of stage and ocular micrometers.

## OBSERVATIONS

### (A) Morphology:

Larval stage (coenurus): Described on page 49.

Adult stage (T.multiceps): This tapeworm parasitises, the small intestines of carnivores and is 40-80 cm. long, consisting of 200-250 proglottids. The Scolex is equiped with four suckers and a double crown of 22-32 small and large hooklets. Width of the segment is 5 mm. The large hook measures 120-170 /u in length and the small hooklets are 90-130 /u. The large hooks have a tapering handle with a sinuous out line while, smaller ones have a long curving handle having a blunt distal extermity. Mature segments are longer than broader and the lateral margin of each segment is smooth and not scalloped.

The posterior margin of each segment projects slightly. Genital pore is posterior to the middle of the lateral margine of each segment. Vagina has a reflexed curve near the lateral excretory canal. There are about 200 testes in each segment and they do not extend to the ovaries or between the vitellaria and the ovaries. The uterus in gravid segment has 9-26 lateral branches.

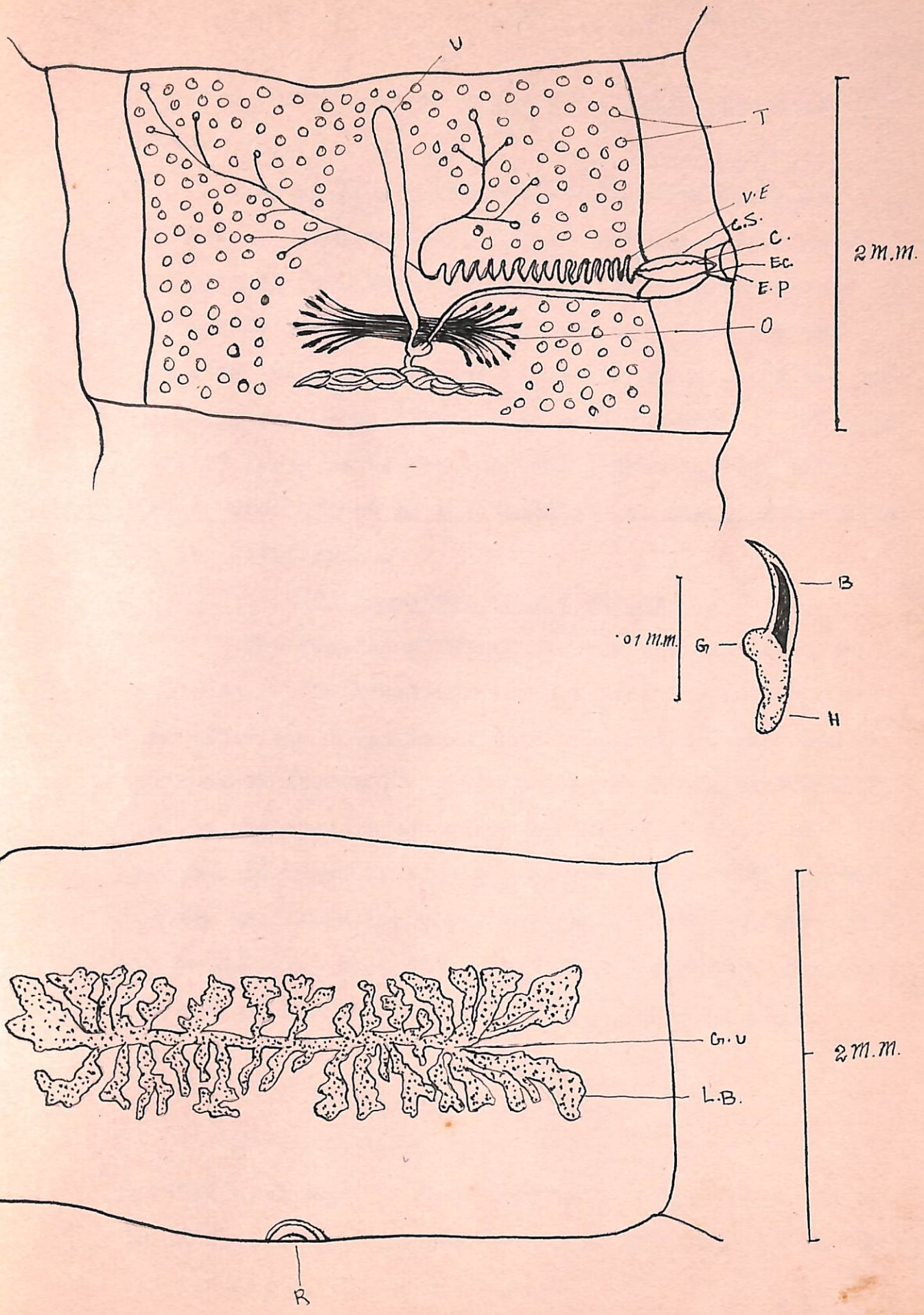
### (B) Development & effect of refrigeration on coenurus:

Three,few days old pups were procured,pup no.1 was fed with some fresh material from the coenurus.

Pup no.II was fed with an equal quantity of coenurus after two days refrigeration (4°C). And pup no.III was kept as control. All the three pups were kept together through out the experiment.

Daily faecal examination of the pups was carried out and also temperature record was maintained. Pup no.I, after the lapse of 24 days started to librate cestode segments in the faeces but pup no.II & III remained negative through out. Pup no.I started straining and showed symptoms of uneasiness and disinterest towards food after a lapse of 2 weeks of infection. These symptoms continued for few days but again it returned to normal, without showing any untoward symptom. None of the other two pups showed any type of syndrome during the course of the observations.

Dog no.I(Pup) was sacrificed, after the lapse of 36 days when it librated plenty of tapeworm segments in faeces. On autopsy, whole of the small intestines were found packed with the bunches of the parasite of uniform appearance. These tape worms were studied in the usual manner and were preserved as such in 5% formol-saline. The intestines were thickened approximately four times than normal. Parasitic heads were found embedded deep into the intestines. Button shaped ulcers were very common, around the border of which parasitic heads were also attached. Pieces of effected intestines were cut and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preserved in 5% formol-saline for histopathological study.

Pup no.II was sacrificed after the lapse of 39 days in order to find,if there was any development of coenurus fed. Whole of the intestines were searched, no cestode segment could be traced out. This pup was fed with the same material, in equal quantity of the same coenurus as pup no.I but after refrigeration of two days. Thus it leads us to the conclusion that refrigeration has adverse effect on the viability of coenurus and thus on its development.

(C) Haematological studies:

The results of these studies are tabulated in table no.I. The blood picture was examined both before and after the infection in Dog no.I and II, and also of pup no.III (control). There is decrease of packed-cell volume and also in haemoglobin content of blood in pup no. I, There is also increase of eosinophilic count in pup no.I than the other two pups. These finding carry us to believe, that there is definitely change in blood picture of the dogs suffering from Taenia multicep infections.

(D) Histopathological studies:

Histopathological sections showed tapeworms heads burried deep down in the intestinal glands. No cellular infiltration was encountered except that hypertrophy of the intestinal glands was marked.

## DISCUSSIONS

Morphological studies of the coenurus and adult Taenia multiceps were in agreement with the descriptions given by other authors.

There appears to be no published record of the effect of refrigeration on the viability and development of coenurus . In the present studies the author has encountered an interesting finding, that the refrigeration has an adverse effect on the viability and development of coenurus in the final host.

There also appears to be no authentic record of haematological picture of pups suffering from T. multiceps infection. These studies in the present work revealed, decrease in packed-cell volume and haemoglobin and also increase in eosinophilic count of infective final host (pups).

Histopathological studies did not show any cellular infiltration in the intestine except the hypertrophy of the surrounding intestinal glands. The scolices of the worm were burried deep down in the intestinal glands.

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## TABLE No.VIII

Table showing the blood picture of pups before and after infection with T.multiceps and faecal examination.

| No.of pup with kind of infective material | Date of examin. | Date of infection | Total count R.B.C. in million | Total count W.B.C. In thousand | Differential count Neut. % | Lymph. % | Mono. % | Eosino % | Baso. % | Hb. % | P.C.V | Faecal examin. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No.1 Fed with fresh coenurus | 24.3.63 | - | 6.475 | 10.8 | 59 | 25 | 14 | 1 | 1 | 13 | 36 | - |
| | 26.3.63 | 26.3.63 | 6.167 | 11.2 | 62 | 30 | 6 | 2 | - | 14 | 40 | - |
| | 11.4.63 | - | 5.105 | 10.5 | 56 | 25 | 7 | 1 | 1 | 10 | 32 | - |
| | 30.4.63 | Sacrificed | 3.040 | 10.3 | 63 | 20 | 9 | 7 | 1 | 7.2 | 32 | +ve for taenia seg. from 18.4.63 |
| No.2 Fed with refrige-rated coenurus (2 days) | 24.3.63 | - | 7.350 | 10.7 | 61 | 22 | 14 | 2 | 1 | 14 | 42 | - |
| | 29.3.63 | 29.3.63 | 6.232 | 10.5 | 61 | 26 | 10 | 3 | - | 13 | 40 | - |
| | 11.4.63 | - | 6.030 | 11.6 | 63 | 24 | 10 | 2 | 1 | 12.5 | 39 | - |
| | 6.5.63 | Sacrificed | 5.122 | 9.8 | 60 | 26 | 11 | 3 | - | 13 | 38 | - |
| No.3 (Control) | 24.3.63 | - | 6.221 | 10.6 | 63 | 24 | 10 | 2 | 1 | 12.5 | 40.2 | - |
| | 1. 4.63 | - | 6.125 | 9.8 | 63 | 25 | 29 | 2 | - | 13 | 39.6 | - |
| | 16.4.63 | - | 7.202 | 10.5 | 59 | 31 | 9 | 1 | - | 14 | 41.2 | - |

# C H A P T E R -IV

## "COCCIDIA AND COCCIDIOSIS OF THE SHEEP"

Leukart (1878) first mentioned the presence of coccidia in a sheep but it was to the credit of Marotel(1905) to describe the ovine Eimerian species Eimeria arloingi. Later on accurate description of this species was given by several authors (Becker 1934, Yakimoff 1929 and 1931). Moussou and Marotel described the second species E.faurei from the sheep (Becker 1939). This species was further studied by Thompson and Hall (1931). Spigel (1925) described the 3rd species E. intricata in sheep from Germany. The 4th species E.parva was described by Kotlan, Mocsy and Vadja (1929). The following year Yakimoff and Rastegaieff (1930) added two more species E.ninakohl-yakimovi and E.galonzoi: the latter species has been regarded as synonym of the former (Becker 1934). Besides the above,the following species of Eimeria have also been described from the sheep. E.arkhari yakimoff and Matik-schoulsky,1937 from wild sheep; E.pellida Christensen,1938 from domestic sheep; E.ah-sata Honess,1942 and E.crandalis Honess,1942 also from domestic sheep.

A survey of the Coccidial fauna of sheep and goats in India was carried out at the Indian Veterinary Research Institute,Mukteshwar,and the occurrence of E.arloingi, E.faurei, E.ninakohl-yakimovi,and E.crandalis in sheep and goats was reported upon ~~in~~ in the annual report of the institute for the year 1949-50. In a few cases diarrhoea due to E.arloingi was also noted though there was no mortality.

In faecal samples of sheep and goats from Hyderabad, West Bengal, Bihar, Orissa, Ajmer and Marwar, E. arloingi, E. faurei, E. ninakohl-yakimovi appeared to be common, while in those from Hyderabad E. intricata was also present.

Sharma (1953-54) recorded the incidence of E. arloingi, E. pallida, E. parva, E. faurei, E. granulosa and E. ninakohl-yakimovi in goats of the livestock farm, Hissar.

Rao & Hiregandar (1953-54) and Munjrekar (1954) recorded the occurrence of E. faurei, E. arloingi, E. ninakohl-yakimovi, E. intricata and E. parva in sheep and goats in Bombay State from where E. pallida, E. granulosa and E. crandalis had been reported earlier (loc. cit.).

Ray (1952) described a new species of Coccidium, E. hawkensi Ray, 1952 from sheep and goats which has not so far been recorded from outside India.

Clinical symptoms characterised by dysentry have been ascribed to coccidia. Apparently healthy adult sheep commonly harbour these parasites. Symptoms occur in association with other parasites and it is difficult in all such cases to decide whether the coccidia alone are the specific cause of the disease. Coccidiosis on the other hand is definitely a serious disease in young lambs. It has been studied by Newsom and Cross (1931), Deem and

Thorp (1939 & 1940) and Christensen (1940).

In India information on the coccidia and coccidiosis of sheep is meagre. According to Ray H.N. (1945) sheep and goats generally harbour E. arloingi and E.faurei but no definite data regarding the extent of harm done by these protozoa is available.

A subacute form of coccidiosis of sheep and goats in the Punjab had been described by Baldrey(1906). He found that disease locally known as Fuvee (i.e.the condition associated with infestation with lice) of sheep and goats characterised by diarrhoea,excessive anemia and death was actually a subacute type of coccidiosis. He found their lower small intestine and whole of large intestine studded with white spots about 1/32 inch in diameter. In badly affected cases,these white spots were distinctly seen through the peritoneal surface. Scrapings from these spots when examined under the microscope showed endogenous coccidial stages.

Gill and Katiyar (1960) recorded an acute out break of coccidiosis due to E.arloingi Marotel,1905 in kids of Orai Sheep Breeding Farm.

The present study was taken up to determine the incidence of coccidia in sheep in the State of Bihar (Table No.IX) in different localities. Out of 180 samples examined at Gauriakarma Farm, 63 sheep (35%) were found positive for coccidia. Similarly at Takuna Farm out of 187

samples examined, 21 sheep (11.2% were found infected with coccidiosis. Also, out of 58 samples examined at Pataliputa and Rajabazar, 25 sheep (55.5%) were harbouring coccidia. Effect of variable temperatures on the sporulation of oocysts has been done and also staining of oocysts with Ziehl-Neelsens method has been tried with encouraging results.

An attempt was also made to study the host-specificity of the coccidia of sheep and goats.

Endogenous stages have also been studied in naturally affected sheep with pure infestation of E. arloingi species of coccidia.

*@*@*

gation floatation technique.

Blood for haematological studies was collected from the Jugular vein of the kids and lambs.

Both the kids & lambs were infected with the materials by feeding oocysts after floating them in Sodium chloride sol. immediately before administration. This was followed by plain water to make sure that all the oocysts were fed. The whole procedure of administration was done

with Pasture pipette. The number of oocysts administered was ascertained by the haemocytometer method (Long & Rawel, 1958).

The endogenous stages have been studied by sectioning the affected portion of the intestines by the usual methods.

## OBSERVATIONS

### (A) Morphology:

Out of the 9 species so far known in Indian sheep, only 5 species of coccidia were encountered in Bihar State. The specific identification of the coccidia encountered was based on the following characters mainly.

1. Shape of the oocysts
2. Size
3. Micropyle and polarcap, presence or absence
4. Oocyst residual body
5. Sporocystic residual body, presence or absence
6. Length-width ratio of oocysts.

The species encountered in the State are tabulated in table no.II and their individual description detailed in the following paras.

1. Eimeria arloingi (Marotel, 1905) Martin, 1909.

E.arloingi was the most widely ranged and common species of coccidia encountered. The variation in size is so considerable that one is some times led to doubt whether he is not actually dealing with more than one species. But the polarcap and micropyle are constant features and are quite distinctive of this species.

The oocysts measured were 17-40 /u x 13-27 /u in dimensions and their length-width ratio was 1.1-1.8. Total oocysts measured were 20. The oocysts were elongated ellipsoidal in shape but few of them were found with one

side curved. Micropyle and Micropylarcap were present and quite prominent. The polar cap was crescent shaped. Oocyst residual body was not visible but sporocystic residual body was present in most of the specimens studied. The outer wall the oocyst when examined in day light was of light orange colour.

Sporulation time :- At room temperature the oocysts kept in potassium dichromate 2.5% solution sporulated in 3 to 4 days (95%).

Endogenous stages of E.arloingi infection in sheep naturally infected cases:- During the collection work of the Helminth parasites, white colonies about 1/16-1/32 inch were encountered on the mucus membrane of the small and large intestines of the sheep. These colonies were both round and irregular in shape. Scrapings from these colonies were examined under microscope and were finally diagnosed to be colonies of E.arloingi infection. The portions of the intestine were cut and preserved in 5% formol-saline. Serial sections (5 to 7 /u) were cut after the usual procedures,for the preparation of the histopathological sections. The sections were stained with Haemotoxyline-Eosin and finally mounted and studied for endogenous stages of coccidia and the pathological changes caused thereby.

In the present study the microscopic studies revealed that the warty growth of the mucus membrane were

villi greatly hypertrophied due to the infection. The epithelial layer of the villi had almost peeled off but the intact lining of the intestinal glands was densely parasitised . Most of the cells were harbouring gametocytes at various stages of development. They ranged from the smallest gametocytes to mature macro and microgametes and also oocysts, all of which corresponded to those of E.arloingi in shape and size. The sexual stages were confined to the epithelial layer. Asexual stages were not encountered in the sections. Blood vessels were dilated. No leucocytic infiltration was encountered in the sections.

However, at large it is concluded that macroscopic lesions (enlarged villi) were similar to those of, produced experimentally by Lotze (1953) by feeding E.arloingi oocysts to the sheep. The enlarged villi had hypertrophied due to heavy parasitisation.

Lotze has shown that E. arloingi develops its schizogonous stages in lacteals of crypts of Lieberkuhn which attain large size and rupture the epithelial covering of the villi and crypts. He also showed that sexual stages develop and are confined to the epithelial layer and unlike schizonts do not penetrate deeper into lamnia propria.

From the present studies it is clear that:

(1) Macroscopic lesion (white spots) are the hypertrophied villi heavily parasitized with coccidial stages.

(2) There is almost loss of epithelial covering of the villi and most of the villi, are denuded.

(3) The sexual stages develop in and are restricted to the epithelial layer.

(4) No Asexual stage was encountered in the sections examined.

(5) Denuded villi indicated that the parasites during their development preceding the sexual stage had extensively damaged the epithelial layer. Such a damage is known to be caused by coccidial stages developing deeply.

2. E. ninakohl-yakimovi,Yakimoff & Rastegaieff, 1930:- This species measures from 16-26 /u in length and 13-22 /u in breadth; with length-width ratio as 1.1-1.5. These measurements were based on 20 oocysts. The shape was usually ellipsoidal and in few ovoid. The oocysts had a single dark refraction line between the membranes and the inside of the wall. When examined in day light oocysts were pale brownish yellow in colour with a thin and transparent wall. Micropyle & polarcap were absent. In sporulated oocysts no oocystic and sporocystic residual bodies were detectable.

3. E.parva Kotlan,Mocsy and Vajda (1929):-The oocysts were subspherical in shape and also few of them were spherical. They measure 12-22 x 10-18 /u in

dimension. Their length-width ratio ranged from 1.0 -1.5. Micropyle and polarcap were absent. No sporocystic or oocystic residual bodies were seen in sporulated specimens. In day light the oocyst wall appeared pinkish in colour. It was the smallest of all the species found in Bihar.

4. E. crandalis,Honess,1942:- The dimensions of the oocysts ranged from 17-23 x 17-22 /u. The length-width ratio was 1.0-1.35. The shape of the oocysts studied were ellipsoidal, as well as subspherical. Micropyle and polarcap were both present and resembles that of E.arloingi.There were two dark refraction lines on either side of the oocystic membranem and the inner surface of the wall,giving oocyst a characteristic double contoured appearance. This character differenciates it from E.arloingi.In sporulated oocysts,oocystic and sporocystic residual bodies were not traceable.

5. E.intricata Spiegl, 1925:- The oocysts measure 39 to 54 x 27 to 36 /u, with length-width ratio of 1.3- 1.8. Micropyle and polarcap were both present. The polarcap was crescent in shape. The shape was ellipsoidal with very little variation in individual specimens. The body was relatively slender and narrow at the micropylar end. The outer wall of the

oocyst was very thick and stout,deep-brown in colour,transversely striated and opaque in appearance. The general contour of the external coat was yellowish brown which extended up to the neck of the polarcap. In sporulated oocysts,oocystic ~~k~~ residual body was not traceable but sporocystic residual body was seen when viewed from different angles. Sporocysts were elongate and ovoid in shape. Sporozoites were wedge shaped with several refractile globules.

TABLE No.

Table showing the incidence of coccidial infection in the sheep based on faecal examination.

| Place of examination | No.of samples examined | No. positive | Percentage of infection. | Nature of infection with percentage | |
|---|---|---|---|---|---|
| | | | | Single | Multiple |
| Takuna Farm | 187 | 21 | 11.2 | 5.3 | 5.4 |
| Gauriakarma Farm | 180 | 63 | 35 | 10 | 25 |
| Patliputra | 45 | 35 | 77.7 | - | 77.7 |

## TABLE- X

Table showing observations in tabular form of coccidia of sheep in Bihar.

| Sl.No. | Species | Shape of oocyst | Size in $\mu$ | Micropyle and polar-cap. | Oocystic residual body | Sporocystic residual body | Length-width ratio |
|---|---|---|---|---|---|---|---|
| 1. | E.arloingi | Elongated, ellipsoidal, Few with one side curved. | 17-40 x 30-27. | Micropyle present(cone shaped or crescent shaped). | Absent | Present | 1.1-1.8 |
| 2. | E.ninakohl-vakimovi | Ellipsoidal. | 16-26 x 13-22 | Both present | Absent | Present | 1.1-1.5 |
| 3. | E. parva | Subspherical & also spherical. | 12-20 x 10-18. | Absent | Absent | Not seen | 1.0-1.5 |
| 4. | E.crandalis | Ellipsoidal and also subspherical. | 17-23 x 17-22. | Both present | Not traceable | Not seen | 1.0-1.3 |
| 5. | E.intricata | Ellipsoidal. | 39-54 x 27-36 | Both present Polarcap crescent shaped. | - | Present sporocyst elongate & ovoid, sporozoites wedge shaped with several refractile globules. | 1.3-1.8 |

(B) Effect of varied temperatures on sporulation of coccidial oocysts, placed in different cultures:- Observations in the present study reveal that the oocysts of sheep in Bihar sporulate best in 2.5% potassium dichromate sol. at 32°C , as compared to the other observations made. These are tabulated in table no. XI The oocysts fail to sporulate in either of the solutions used at 40°C and also at refrigerator temperature (4°C). Thus it leads us to the conclusion that sporulation of the oocysts is dependent upon the atmospheric temperature and moisture which is variable in our country in different geographical zones and States.

(C) Demonstrations of coccidial oocysts by Ziehl Neelsen staining method:- In the present study an attempt has been made to demonstrate the Eimeria oocysts in smear of faeces, by staining the smears with carbol fuchsine and methylene blue staining technique. The method is quite reliable for demonstration of the oocysts but does not help at all in identification of the oocysts. Sporulated oocysts were also stained but the stain did not proved good for staining the internal structures.

This method was also tried to demonstrate coccidial oocysts in crushed preparation of the organs infected with coccidia (suspected). The oocysts were not demonstrable.

(D) Host specificity of sheep and goat coccidia:-

It is assumed that the goats and sheep share the same species of coccidia butno authentic literature is vailable to support the fact on experimental grounds by cross infection from one species to the other. In the present studies an attempt has been made to infect kids with the species of Eimeria collected from lambs, and that collected from lambs to kids.

The result of observation made, with the dosage of oocysts (mostly E.arloingi type), administered to the experimental animals have been tabulated (Table no. XII ). A control was kept in each group of animals to check up the results thus obtained during the course of experiment.

From the above table it is quite evident that the kids infected with oocysts collected from lambs took the infection in 17 to 18 days and it was checked up by the control kid no.3, which remained negative in the same environmental conditions and of the same age group. Similarly the lambs which were infected with kid coccidia also picked up the infection in 17 to 20 days. This group too was having one control lamb, which remained negative through out the experimental period.

Kid nos.1,2 and lamb no.3 were sacrificed for studying the microscopic changes in histopathological sections of the intestinal canal. No gross lesions except slight congestion in certain regions of the small

intestines were observed. The suspected portions were studied, in duly prepared sections cut at 5-7 microns thickness after staining with Haemotoxylene Eosin stain. No endogenous stage could be traceable during the examination, probably due to certain defects in selection of the pieces of the intestinal wall or any other experimental error or light infection.

Haematological studies which included total count, differential count, Haemoglobin content and packed cell volume were done before and after the infection with the oocysts. This was done with an idea to know whether if there is any gross change in blood picture. The results thus obtained are tabulated (Table no. XIII & XIV), which indicates only slight change in haemoglobin content of blood after infection, which may be responsible for the clinical anemic syndrome in infected animals.

Thus in view of the present findings it is concluded that sheep and goat share the coccidia of the same type and each one of them can be infected by cross transmission in both the cases from each other. It shows that the host specificity in case of coccidia of genus Eimeria in sheep and goats is relaxable.

## TABLE No.XIII

Table showing the changes in blood picture of kids in cross transmission experiment of coccidia of ovines.

| No.of kid | Date of examin. | Date of infection | Total count | | Differential count | | | | | Haemo. % | P.C.V. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | R.B.C. in million. | W.B.C.in thousand | Neut. % | Lymph. % | Mono. % | Eosino. % | Baso. % | | |
| 1 | 22.4.'63 | - | 15.56 | 8.5 | 33 | 53 | 10 | 1 | 3 | 9.4 | 37 |
| | - | 26.4.'63 | 14.45 | 6.6 | 38 | 52 | 7 | 1 | 2 | 10.0 | 39 |
| | - | 11.5.'63 | 12.48 | 6.7 | 30 | 56 | 10 | 1 | 4 | 9.0 | 36 |
| 2 | 23.4.63 | - | 14.28 | 8.5 | 33 | 53 | 9 | 2 | 3 | 9.2 | 37 |
| | - | 26.4.'63 | 12.92 | 6.6 | 33 | 51 | 10 | 2 | 4 | 10.2 | 38 |
| | - | 11.5.'63 | 12.20 | 7.8 | 35 | 52 | 10 | 1 | 2 | 8.6 | 37 |
| 3 (Control) | 22.4.63 | ~~xxxx~~ | 14.67 | 6.3 | 34 | 58 | 4 | 3 | 1 | 10.6 | 39 |
| | 29.4.63 | - | 13.50 | 8.5 | 34 | 59 | 3 | 4 | - | 10.8 | 37 |
| | 5.5.63 | - | 13.32 | 7.3 | 31 | 61 | 2 | 2 | 1 | 11.0 | 39 |
| | 11.5.63 | - | 12.97 | 7.1 | 38 | 56 | 4 | 2 | - | 11.2 | 38 |

## TABLE No.XIV

Table showing the changes in blood picture of lambs in cross transmission experiment of coccidia of caprines.

| No. of lamb. | Date of examin. | Date of infection | Total count | | Differential count | | | | | Haemo. % | P.C.V. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | R.B.C. in million. | W.B.C. in thousand | Neut. % | Lymph. % | Mono. % | Eosino. % | Baso. % | | |
| 1 | 6.7.63 | - | 9.04 | 10.8 | 22 | 66 | 4 | 2 | 6 | 14.0 | 45 |
| | 8.7.63 | 8.7.'63 | 10.15 | 10.2 | 30 | 61 | 5 | 1 | 3 | 13.0 | 40 |
| | 15.7.63 | - | 11.02 | 5.7 | 31 | 60 | 4 | 1 | 4 | 14.2 | 43 |
| 2 | 6.7.'63 | - | 13.56 | 7.6 | 29 | 58 | 6 | 2 | 5 | 10.2 | 44 |
| | 8.7.'63 | 8.7.'63 | 11.25 | 6.4 | 30 | 63 | 3 | 1 | 3 | 13.2 | 43 |
| | 15.7.63 | - | 12.86 | 6.2 | 25 | 65 | 3 | 2 | 5 | 14.0 | 40 |
| 3 | 6.7.'63 | - | 12.47 | 5.4 | 55 | 38 | 4 | 1 | 2 | 13.6 | 43 |
| | 8.7.'63 | 8.7.'63 | 13.25 | 6.2 | 45 | 45 | 5 | 2 | 5 | 13.8 | 44 |
| | 15.7.'63 | - | 11.82 | 5.2 | 42 | 52 | 3 | 1 | 2 | 12.8 | 45 |
| 4 (Control) | 6.7.'63 | - | 10.39 | 4.7 | 34 | 60 | 3 | 2 | 1 | 9.8 | 44 |
| | 8.7.'63 | - | 10.94 | 5.3 | 33 | 61 | 3 | 3 | - | 9.8 | 45 |
| | 15.7.63 | - | 10.32 | 6.2 | 35 | 59 | 4 | 2 | - | 10.4 | 43 |

TABLE NO.XII

Table showing the observations made in cross infection experiment of genus Eimeria in ovines and caprines.

| Nature of infection | No. & kind of animal | Dose of oocysts | Date of admission | Faeces positive for coccidia on. | Date of sacrifice for studying the micro & macroscopic changed. | Daily temperature record. |
|---|---|---|---|---|---|---|
| Coccidia of lambs to kids. | Kid No.1 | 100000 | 26.4.63 | 11.5.'63 | 11.5.'63 | No gross change was observed. |
| | Kid No.2 | 60000 | -do- | 12.5.'63 | 23.5.'63 | -do- |
| | Kid No.3 (control) | - | - | Negative | - | -do- |
| Coccidia of kids to lambs. | Lamb No.1 | 4000 | 8.7.63 | 25.7.'63 | - | -do- |
| | Lamb No.2 | 6000 | -do- | 28.7.'63 | - | -do- |
| | Lamb No.3 | 20000 | -do- | 27.7.63 | - | -do- |
| | Lamb No.4 (control) | - | - | -ve | - | -do- |

## DISCUSSIONS

The present study brings out the fact that the coccidial infection is the rule with normal sheep, and that even apparently healthy sheep harbour oocysts.

An examination of the literature on different species of the genus Eimeria indicates the controversy prevailing in identifying the species. Moussu and Marotel (1902) described and figured oocysts in faeces of sheep which showed a distinct micropyle but no micropylar cap and for this they gave the specific name E. faurei. Three years latter Marotel (1905) described oocysts in a goat which showed prominent micropylar caps which he named E. arloingi. Certain authors considered that E.faurei of the sheep and E.arloingi of the goats are identical specifically and are transmissible to the sheep and goats but this specific identification is evidently not universally accepted. Both these species (E.arloingi & E.faurei), have simultaneously been recorded from sheep and goats in India. So there should exist no controversy in the validity of these species in our country. Ray's (1949) work is quite enough in this connection to support their validity.

Out of the above two species only E.arloingi has been encountered by the author with certain variations in size, as if more than one species is being examined. Though there was also difficulty to disting-

distinguish the Micropyle and polarcap of E.arloingi, E. crandalis and E. intricata, being closely in resemblence to one another in unsporulated oocysts. But these difficulties were overcome by critically studying, the other morphological characters of the oocysts. The polarcap was present in E.arloingi, E.crandalis and E.intricata and absent in rest. On close examination it varied in general structure from species to species. In E.arloingi and E.intricata it was conspicuous and of proportionate size, while in E.crandalis it was rounded and very small or almost evanescent.

Owing to the difference of opinion and the varied morphological descriptions given by observers in very widely scattered parts of the world, it is quite impossible to decide definitely whether the forms described are variations in the oocysts of the same species or definitely different species. It would appear that the general openion of many competent authors isthat the difference in the caps is simply a slight variation in oocysts of the same species.

The mx naked eye lesions and microscopic changes encountered in naturally infected cases of sheep with coccidia (E.arloingi) are in complete agreement with that of the lesions produced by Lotz (1953) in lambs experimentally infected with E.arloingi.

The observations made by Ray(1951) on relation of temperature to the sporulation of coccidian oocysts of Indian sheep and goats revealed that at 38.5°C after 41 hours, 79% sporulated and at 32°C after 25 hours, 88% of oocysts sporulated. So also the studies made by Christensen (1939) showed that at 32°C the oocysts developed abnormally while at 40°C they seized to sporulate. The author found that the oocysts when exposed at 32°C in potassium dichromate sol.(2.5%), 40% sporulated on 3rd day and 85% on 5th day. But the same material exposed to 40°C and 4°C (refrigerator temperature) in the same solution; they almost seized to sporulate upto two weeks except only 1.5% sporulated at 40°C. These findings lead one to the conclusions that the sporulation of oocysts is dependent upon the atmospheric temperature, moisture and the environmental conditions of a particular locality which differs from place to place and thus is variable.

Schwaz & Hohner (1960), could demonstrate oocysts in faecal smears and crushed tissue smears of the suspected portions easily by Ziehl-Neelsen's staining technique. During an attempt made in this study it has been found only possible to demonstrate the presence of oocysts in faecal sample smears only but not in suspected tissue smears. This does not help

in species identification, as it does not stain the internal structures of the oocysts. This method is the simplest and quickest for the demonstration of the oocysts in faecal smears.

Considerable literature has been devoted to prove the host specificity of coccidia particularly those belonging to the Genus Eimeria in Pylogenitically related vertebrates. Work done on this line before many species of the coccidia were recognised is open to much criticism. Lot of the cross infection attempts of the various workers(Dieben 1924; Johnson 1923; Andrew 1927; Becker 1927 and 1934; Tyzzer 1929;Boughton 1929;Henry 1931; Lee 1932; Balozet 1933;Jankiewicz 1941;Honess 1942; Carvallho 1943;Gill 1954;Steward 1947; Pellerdy 1956b), which were authentically accepted have been published by these authors from time to time. Their success was mainly due to the fact that their experiments were designed to know the host specificity in closely related animals.

A cross infection experiment was carried out to know the host specificity of coccidia in sheep and goats. The results have lead to the conclusion, that the host specificity in these hosts with regard to Eimeria spp. is relaxable , but an extensive experimental evidence to establish the authenticity of this work seems to be necessary.

# S U M M A R Y

1. Fortyfive autopsy cases were examined at two Government Sheep Farms, Gauriakarma and Takuna. In addition 34 autopsy cases were examined from abattoirs at Patna and Rajabazar. 21 species of helminths were recovered from them. The percentage of infection with different helminth parasites is recorded as follows.

At Government Farms

C.cotylophorum 70.45%, Hydatid cyst 15.9%, Cysticercus tenuicollis 47.72%, M.expansa 6.81%, M. benedeni 2.27%, M.denticulata 2.27%, A.centripunctata 2.27%, Stilesia globipunctata 2.27%, T. ovis 27.27%, O. columbianum 25%, O. venulosum 2.27%, H. contortus 9.09%.

At abattoirs (Patna & Rajabazar)

C. cotylophorum 5.88%, F. elongatus 2.94%, G. crumenifer 8.82%, S. globipunctata 8.82%, A. centripunctata 11.76%, Cysticercus tenuicollis 2.94%, Multiceps multiceps 5.88%, M.expansa 14.70%, M.benedeni 2.94%, T.ovis 11.76%, B. trigonocephalum 14.70%, G. pachyscelis 2.94%, T. colubriformis 20.58%, H.contortus 20.58%, O. columbianum 23.52%, T. globulosa 2.94% and S. papillosus 2.94% .

2. Incidence of multiple infections was found to be more than single infection. Helminths belonging to all the three classes (Cestoda, Trematoda and Nematoda)

were found. Maximum number of helminths present were 4 in four cases, 3 in fourteen cases, and 2 in twentyeight cases, out of 78 cases examined (autopsy).

3. Schistosoma indicum, Coenurus cerebralis, Hydatid cyst, Gaigeria pachyscelis are recorded for the first time in Bihar.

4. Detailed histopathological changes of S. indicum infection in liver of sheep are described.

5. Biology of Trichuris spp. eggs, which were recovered from 40.6% of sheep at Takuna Farm, was studied under different environmental stresses. 15-18 days are required for their complete development in aerated distilled watar and 15 days in soil. The development of the eggs in soil was much more rapid than in distilled water.

6. Faecal examination of 425 sheep from different localities of the Bihar State has been carried out to find out the percentage of different verminous infestations in different geographical zones. The percentage of verminous infection based on the faecal examination is recorded as follows:-

At Takuna Farm:

Amphistome spp. 10.16 %, Moniezia spp. 2.6% Trichuris spp. 40.6%, Bursate worm spp. 5.3%, Strongyloid spp. 2.1%.

At Gauriakarma Farm:

Amphistome spp. 2.2%, Moniezia spp. 0.55%, Ascarid spp. 1.1%, Trichuris spp. 8.8%, Bursate worms spp. 31.6%, Strongyloides spp. 2.2%.

At Patliputra Colony:

Moniezia spp. 6.6%, Trichuris spp. 22.2%, Bursate worms spp. 53.3%, Strongyloides spp. 48.8%.

At Rajabazar:

Trichuris spp. 23.0%, Bursate worm spp. 38.4%.

The presence of infection with Ascaris spp. is of special interest, being the first record of its kind in Bihar, though no Ascaris spp. was encountered at autopsy in any case. In all 12 types of eggs have been met with and these are illustrated in this thesis.

7. Haematology of amphistomiasis has been studied and compared with apparently healthy group of sheep. Statistically significant changes like increase in white cell count, decrease in haemoglobin content and also in red cell count were observed in the infected group.

8. Larvae of most common nematode parasites, encountered in this work, were raised in the laboratory. These larvae viz., H. contortus, B. trigonocephalum,

S. papillosus, O. columbianum and T. colubriformis have been studied and their morphological differences recorded with microphotograph illustrations.

9. Study on the development of Coenurus cerebralis in pups recovered from a sheep was carried out. Adverse effect of refrigeration on the viability and development of coenurus was observed. Haematological and histopathological studies of the experimentally infected pups were made. Haematological studies revealed decrease in haemoglobin content and increase in Eosinophilic count of the infected pups. Scolices of the tape worm were found buried deep down in the intestinal glands of the small intestine without any evidence of cellular changes. Macroscopically button-shaped ulcers and intense thickenning ( 4 times more than the normal) of the small intestine were found in the infected pup.

10. Five spp. of coccidia have been encountered in sheep in the State of Bihar. They are E.arloingi, E. parva, E.ninakohl-yakimovi, E.crandalis, E.intricata. These have been studied and systematically described.

11. The percentage of infection with coccidian parasites was 11.2% at Takuna , 35% at Gauriakarma and 77.7% at Patliputra Colony. Mostly cases of multiple infection were encountered.

12. Staining of the oocysts in faecal smears with Ziehl-Neelsen's method has been demonstrated.

This method has been found quickest and simplest of all the methods known for demonstration of the oocysts in faeces. But, this method does not help in species identification.

13. Effect of variable temperature on the sporulation of oocysts have been carried out and important findings noted. The oocysts sporulated best at 32°c in 2.5% potassium dichromate solution. They failed to sporulate at 4°C and 40°C in the same sol. except 1.5% sporulated at 40°C after two weeks.

14. Endogenus stages of Eimeria arloingi in naturally infected cases have been studied. In the endogenus stages no asexual stage of coccidia was encountered in histopathological sections.

15. An attempt was made to find out the host-specificity of Eimeria spp. in phylogenitically related hosts like sheep and goats, and no host-specificity was observed.

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