

STUDIES ON
HELMINTH PARASITES OF BUFFALOES
WITH
SPECIAL REFERENCES TO SIGANTOCOTYLIDAE
AND SETABIASIS.

B. N. SHARMA.

Studies On
Helminth Parasites of Buffaloes
WITH
Special References to Gigantocotylasis
And Setariasis.

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WITH
SPECIAL REFERENCES TO GIGANTOCOTYLIASIS AND SETARIASIS

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March 14, 1971

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Helminth parasites of *Leishmania* with special reference to
Charadriidae

THESIS

Submitted to the Rajendra Agricultural
University, Bihar, Patna in Partial
fulfilment of the requirements for the
degree of Master of Science (Vet.) in
PARASITOLOGY.

1971

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Certified that the thesis entitled, "Studies on helminth parasites of buffaloes with special reference to Gigantocotylasis and Setariasis" embodies the results of work actually carried out by Dr. B.N. Sharma, under my supervision and guidance for the award of the degree of Master of Science (Veterinary - Parasitology) of the Rajendra Agricultural University, Bihar.


(B. N. SAHAI)

The work embodied in this thesis was carried out in the Department of Parasitology Bihar Veterinary College, Patna under a Junior Fellowship awarded by the Rajendra Agricultural University, Bihar, Patna. The author is grateful to the Vice-Chancellor for the financial help.

CONTENTS

			Page
Acknowledgements.	
Introduction	1
Materials and Methods	4
Incidence and Nature of helminthic infection in buffaloes.	8
Redescription of <u>Testifrondosa cristata</u> (Bhalerao, 1924) from a new host- <u>Bos bubalis</u>	29
Histochemistry and pathology of liver in <u>Gigantocotyle explanatum</u> infection in buffaloes....			32
A: Histochemistry	32
B: Pathology	38
Studies on Setariasis	43
A: Implantation of rabbit with living worms	47
B: Histopathology & histochemistry of liver of rabbits, having erratically migrated <u>Setaria cervi</u>	53
C: Histopathology of intestine of rabbit, having erratically migrated <u>Setaria digitata</u>	59
D: Morphology of microfilariae of <u>S. digitata</u> and <u>S. cervi</u>	61
Summary	67
References	70
Key to lettering	83
Explanation of plates	84

ACKNOWLEDGEMENTS

Ever since this work was started, it progressed smoothly throughout the period only due to valuable guidance and supervision of my Professor Dr. B.N. Sahai, M.V.Sc., Ph.D., R.F.G.A.E.S. (Giessen/Germany), F.H.S.I., Professor in Charge and Head, Post-graduate Department of Parasitology, Bihar Veterinary College, Patna (R.A.U.). I feel immense pleasure in expressing my most sincere indebtedness and gratitude for his keen interest, constructive criticism, exhaustive and competent review of the manuscript without which it would have been extremely difficult to submit the thesis in the present form.

I am deeply grateful to Dr. R.C.P. Yadava, M.S., Ph.D. (Mich.), Principal of the College for keen interest in the work and also for providing facilities.

Thanks are due to the staff of the Department of Parasitology and Surgery for their whole-hearted cooperation during the course of research.

I am thankful to Dr. G.J. Jha, M.V.Sc., Ph.D., Department of Pathology and to Sri S.P. Singh, Lecturer, Zoology, for their time to time help.

The author expresses his heart touching feelings for affectionate assistance of his father through all means.

Author will be failing from his duties, if he forgets to thank from his softest corner of heart Mrs. Lalita Sharma, his caretaker, whose long patience and love proved a potential source of inspiration during the whole period of study.

INTRODUCTION

In tropical countries like India, the impact of helminth parasites has always been known to be sufficiently detrimental to livestock health and consequently animal production. This is true in many species of the livestock and also for buffaloes which have selectively tropical habitat.

Of buffalo diseases including those of virus, microbial, constitutional or of other origin - those caused by the helminth parasites are most common and important. Although, the exact data are not available with regard to actual loss suffered as a result of worm infestation in India, it is much larger in buffalo than in any other domestic animals. The worms in buffalo take a heavy toll and insidiously undermine their health without giving rise to spectacular symptoms.

Bhalerao (1935) has enlisted in Indian buffaloes, Camynerius gregarius Looss, 1896; Fischoederius elongatus Poirier, 1883; Gastrothylax crumenifer Creplin, 1847 from stomach; Paramphistomum explanatum Creplin, 1847; Fasciola gigantica Cobbold, 1855 from gall-bladder, bile ducts, liver, stomach and duodenum amongst trematodes; Diplophallus polymorphus, Cysticercus of Taenia hydatigena Pallas, 1766 and Taenia Sp. Southwell, 1912 from liver and abdominal cavity amongst cestodes; Gongylonema pulchrum Molin, 1857 from lining of oesophagus and rumen; Mecistocirrus digitatus (V.Linstow, 1906) Railliet & Henery, 1912 from stomach; Oesophagotomum

radiatum Railliet, 1898 from large intestine and caecum, Onchocerca armillata Railliet & Henery, 1909 from lining of aorta, Onchocerca indica Sweet, 1915 from subcutaneous tissues, Onchocerca Sp. Sweet, 1915 from subcutaneous nodules, Setaria cervi Rudolphi, 1819 from peritoneal cavity, Synsamus laryngeus Railliet, 1890 from larynx and rarely pharynx and Thelazia rhodesii (Desmarest, 1827) de Balinville, 1828 from lachrymal ducts amongst nematodes. Except for few systematic investigations on helminth parasites of buffaloes by Srivastava (1945), Thapar (1956) and Sharma (1961) reports about other helminths harboured by this animal species is not consistent and reports are rather scattered.

No studies on helminth parasites of buffaloes in Bihar are available, except Varma (1957) and Sinha (1962). They reported 57.1% and 37.5% infection of Gigantocotyle explanatum (Syn. Paramphistomum explanatum) in buffaloes in Bihar, respectively. Again, Varma et.al (1971) have described Setaria cervi, a common filarial worm of buffaloes in Bihar. Under the circumstances, comprehensive studies on helminth parasites of buffaloes was undertaken. The studies included histochemistry and histopathology of liver of buffaloes infected with Gigantocotyle explanatum, a common conical fluke infecting gall-bladder, bile duct, liver, duodenum and stomach of buffaloes, rarely in cattle, sheep and goats. Rabbits were implanted by Setaria digitata and Setaria cervi, recovered from buffaloes to study morphology of their microfilariae.

In view of the importance of these helminth parasites, investigations as mentioned above have been carried out in the Department of Parasitology, Bihar Veterinary College, Rajendra Agricultural University, Bihar, Patna. The results of investigations incorporated in the thesis deal with the incidence of helminth parasites of buffaloes in Bihar, histochemical and histopathological changes in buffalo's liver in G. explanatum infection, and transplantation of Setaria digitata and Setaria cervi in rabbits and studies on their larval stages.

MATERIALS AND METHODS

SOURCE AND COLLECTION OF MATERIALS.

The materials for present studies were collected from buffaloes slaughtered in the different slaughter houses of Patna, Phulwarisharif and Dinapur and Department of Surgery, Bihar Veterinary College, Patna from time to time. For this purpose all the organs and tissues were carefully examined for the presence of helminths. Trematodes cestodes and nematodes were isolated and preserved immediately after collection. The methods adopted for collection were the same as described by Sahai (1960, 1967).

FIXATION AND PRESERVATION.

Representative specimens of the trematodes and cestodes were flattened and fixed for whole mounts. The parasites were fixed in hot solution of 10% formalin or steaming 70% alcohol. After a couple of hours the parasites were transferred to fresh solution of fixative for preservation.

STAINING AND MOUNTING.

The Acetic alum carmine and Borax carmine stains were used in normal way for whole mount preparations. The trematodes, cestodes and sections were mounted in D.P.X. or Canada balsam.

CLEARING FOR MICROSCOPICAL EXAMINATION.

For microscopical examination of the preserved

nematodes, it was necessary to clear them and the following media were used for the purpose of clearing.

(a) Lactophenol :- The clearing agent was prepared as given by Taylor (1935)

Carbolic acid	- One part
Lactic acid	- One part
Glycerine	- Two parts
Distilled water	- One part

This agent has got the property of softening the cuticle and counteracting the shrinkage. The parasites were transferred to this chemical directly from the preservatives, alcohol or formalin. It was found that there was violent shrinkage in the beginning but after sometimes the specimens have taken natural size. Mostly medium sized nematodes were cleared in lactophenol with satisfactory results.

(b) Glycerine-alcohol :- The mixture was prepared by mixing glycerine and 70% alcohol in the ratio 1 : 19. The specimens preserved in 70% alcohol were transferred to this clearing agent and the alcohol was allowed to evaporate. It took about one week to make the specimen transparent for microscopical examination. This clearing agent was used only for small nematodes with good results.

HISTOCHEMICAL TECHNIQUES:

For histochemical study, liver of buffaloes

infected with Gigantocotyle explanatum and liver of rabbits having erratically migrated Setaria cervi, post transplantation of these worms in the peritoneal cavity of two rabbits, were selected. The standard methods of fixation and various histochemical staining procedure described by Culling (1963) and Pearse (1968) were employed. The following histochemical staining procedure were used :

A. Protein : Mercury-Bromphenol blue method

B. Glycogen: Periodic acid schiff technique (PAS)

HISTOPATHOLOGICAL TECHNIQUES.

For histopathological studies liver of buffaloes infected with G. explanatum and the liver and intestine of rabbits having erratically migrated S. cervi and S. digitata, post-transplantation of these worms in the peritoneal cavity of two different rabbits, were selected. Routine procedures of fixation, dehydration, clearing, paraffin embedding, section cutting and staining were used. Sections were cut 4-6 microns and stained with haematoxylin and eosin (H & E) technique.

TRANSPLANTATION.

After identification, mature specimens of males and females of S. digitata and S. cervi were sorted out. These worms were transplanted into the peritoneal cavity of the rabbits, separately, within 2-3 hours of collection by the methods described by previous workers (Williams, 1955,

Victor, 1958, Nelson, 1962, Lakra and Singh, 1963, Sahai et.al 1966, Varma et. al. 1971).

Six rabbits were thus transplanted three with S. cervi and three with S. digitata and smears from their peripheral blood were examined daily from the next day of transplantation until sacrificed or dead.

PREPARATION AND EXAMINATION OF STAINED BLOOD FILM.

Thick blood smears were prepared from ear vein of the rabbits transplanted with S. cervi and S. digitata. Smears were dehaemoglobinised, fixed in methyl alcohol and stained with haemotoxylin-eosin (Varma et.al. 1971). This was first located under low power (10X) and then examined under high powers (40X & Oil immersion) for the detailed study of microfilariae.

MEASUREMENTS.

The measurements where ever needed were taken by the help of eye-piece micrometer. The measurements given refer to the range shown by 20-25 specimens and averages are given in parentheses, except in case of Testifrons cristata (Bhalerao, 1925) where only four specimens were available for study.

DIAGRAMS.

The diagrams were prepared with the help of Camera lucida. Photomicrographs of sections showing histopathological and histochemical changes were taken as usual.

INCIDENCE AND NATURE OF HELMINTHIC INFECTIONS
IN BUFFALOES

INCIDENCE AND NATURE OF HELMINTHIC INFECTIONS IN BUFFALOES

GENERAL.

Considering the importance and utility of Indian buffaloes, the literature on the helminth parasites of this animal in India is rather meager. Bhalerao (1935) while enumerating helminth parasites from his own collection and from the earlier valid records of previous workers has described the following trematodes, cestodes and nematodes. The trematodes are : Carmyerius gregarius Looss, 1896, Fischocoederius elongatus Poirier, 1883, Gastrothylax crumenifer Creplin, 1847, Paramphistomum explanatum Creplin, 1847 and Fasciola gigantica Cobbold, 1855. The Cestodes are : Diplophallus polymorphus, Cysticercus of Taenia hydatigena Pallas, 1766 (Cysticercus tenuicollis Rudolphi, 1810) and Taenia sp. Southwell, 1912. The nematodes are : Gongylonema pulchrum Molin, 1857, Mecistocirrus digitatus (V.Linstow, 1906) Railliet & Henery (1912), Oesophagotomum radiatum Railliet, 1898, Onchocerca armillata Railliet & Henery, 1909, Onchocerca indica Sweet, 1915, Setaria labiatopapillosa, Railliet, 1890, and Thelazia rhodesii (Desmarest, 1827) de Blainville, 1828. Baylis (1936) has supplemented the list by adding two more nematodes viz. Ascaris vitulorum Goeze, 1782 and Onchocerca gibsoni (Cleland and Johnston, 1910) Cleland and Johnston, 1911.

During studies on Verminous pneumonia in Indian buffaloes, Srivastava (1940) described lung worm, which was different in some respects from Protostrongylus rufescens Leucart, 1865. Sarwar (1944) reported the nematodes : Cooperia punctata (V. Linstow, 1907), Ransom, 1907, Paracooperia nodulosa (Schwartz, 1928) Travassos, 1937, Bunostomum phlebotomum (Railliet, 1900) Railliet, 1902, and Capillaria sp. from Indian buffaloes.

/// Srivastava (1945), during his survey on the incidence of helminth parasites of ruminants in India, recorded for the first time Cotylophoron cotylophorum Fischöder, 1901 and Haemonchus contortus (Rudolphi, 1803) Cobbold, 1898 from buffaloes. Thapar & Sinha (1945) described a new genus Olveria with a new species Olveria indica from the rumen of buffaloes in Uttar Pradesh. //

Srivastava (1947) reported Gastrothylax crumenifer as most common parasite of Indian buffaloes. Gopalkrishnan (1949) has described earsore in 20 to 50% of buffaloes examined in Assam and attributed its causative agent Stephanofilaria sp., identical with Stephanofilaria assamensis Pande, 1935, which was later named as S. Zaheeri Singh (1958) on the basis of materials collected from earsore of buffaloes in Andhra Pradesh. Vaidyanathan (1949) has described prenatal infection of Ascaris vitulorum in buffaloes.

Tandon (1951) has described another species of the

genus Olveria, namely, Olveria bosi from buffaloes in Lucknow (Uttar Pradesh). Roy (1954) reported 50 to 75% of buffaloes slaughtered at Kalimpong Municipal Abattoir (Assam) had Fasciola gigantica infection. Tandon (1955) have added one more new parasite to the genus Paramphistomum viz. Paramphistomum spinicephalus from buffaloes in Uttar Pradesh.

A survey of helminth parasites of buffaloes in Uttar Pradesh, Bihar, Bengal, Assam and Orissa was carried out by Thapar (1956), who reported the occurrence of 14 trematodes, 4 cestodes and 11 nematodes:

Carmyerius spatiosus, (Brandes, 1898), Cotyllophoron cotyllophorum, Gastrothylax crumenifer, Fischocoederius cobboldi, Fischocoederius elongatus, Olveria indica Thapar and Sinha, 1945, Olveria bosi Tandon, 1951, Paramphistomum explanatum, Paramphistomum gotoi Fukui, 1926, Fasciola gigantica, Homalostomum poloniae Poirier, 1883, Paramphistomum cervi Schrank, 1790, Paramphistomum orthocoelium Maplestone, 1923, and Schistosoma indicum Montgomery, 1906 amongst trematodes, Moniezia benedeni (Moniez, 1879) Blanchard, 1891, M. expansa (Rudolphi, 1810), Hydatid cysts and Cysticercus bovis (beef measles) amongst Cestodes; Ascaris lumbricoides Linnaeus, 1758, Bunostomum trigonocephalum (Rudolphi, 1808) Railliet, 1902, Haemonchus contortus, Mecistocirrus digitatus, Oesophagostomum columbianum (Curtice, 1890), O. radiatum, Setaria labiata - papillosa, Gongylonema pulchrum, Thelazia rhodesii, Trichuris ovis Abildgaard, 1795 and Ascaris vitulorum amongst nematodes.

Out of these parasites only five trematodes; Cotylophoron cotylophorum, Gastrothylax crumenifer, Paramphistomum cervi, Paramphistomum explanatum and Fasciola gigantica, One Cestode (Hydatid Cyst) and four nematodes; Ascaris vitulorum, Mecistocirrus digitatus, Setaria labiato-papillosa and Thelazia rhodesii have been reported from Bihar.

Varma (1957) had made an extensive survey on the nature, incidence and geographical distribution of amphistomes in Bihar (India). During this study he examined 21 buffaloes and obtained 57.1% of them harbouring Gigantocotyle explanatum in the bile ducts. Bhatia (1960) has reported Onchocerca armillata from the aorta of Indian buffaloes in Uttar Pradesh. Ahmad (1961) has reported Stephanofilaria Zaheeri from ear-sore of the buffaloes in Andhra Pradesh.

Sharma (1961) examined 103 buff-calves, used for operative surgery in the Department of Surgery, U.P. College of Veterinary Science and Animal Husbandry, Uttar Pradesh, Mathura, with special reference to gastro-intestinal tracts and reported seventeen nematodes, two cestodes and two trematodes. He also encountered several amphistomatous parasites from the rumen and their developmental stages from the duodenum. The nematodes recovered were:-

- Paracooperia nodulosa, Cooperia punctata,
C. laterouniformis Chen, 1937, Trichostrongylus colubriformis
 (Giles, 1892) Ransom, 1911, T. longispicularis Gordon, 1933,

Haemonchus contortus, Bunostomum phlebotomum, Gaigeria pachysalis Railliet and Henery, 1910, Bosicola radiatus (Rudolphi, 1803), Strongyloides papillosus (Wedl, 1856) Ransom, 1911, Trichuris ovis (Abildgaard, 1795) Smith, 1908, Ascaris vitulorum, Gongylonema pulchrum, Onchocerca armillata, Artionema africana Yeh, 1959 and Thelazia rhodesii. The cestodes were: Moniezia benedeni and Avitellina lahorea Woodland, 1927. The trematodes were :- Schistosoma spindalis Montgomery, 1906 and Sindicum.

Sinha (1962) examined eight buffaloes at Patna, Bihar and reported G. explanatum in the liver of three buffaloes. At one occasion this parasite was found to be associated with Fasciola indica Varma, 1953. Srivastava (1963) attributed death in buff-calves due to Neoscaris vitulorum (Goeze, 1782) Travassos, 1907, infection. Patnaik & Pande (1963) have studied the helminth parasites, in order of frequency and relative pathogenecity during their survey of helminth in buff-calves, as follows : Neoscaris vitulorum, Strongyloides papillosus, Paracooperia nodulosa, Cooperia laterouniformis, Bunostomum phlebotomum and Setaria labiato-papillosa.

Mukherjee (1966) has reported Calicophoron cauliorchis (Stiles and Goldberger, 1910) Näsmark, 1937, an amphistome-parasite, from Indian buffalo. Again, the same year he has described life history of Fischoederius elongatus, an amphistome of cattle and buffalo in India. Ahluwalia et.al (1966) has reported Onchocerca sp. , Ostertagia sp. ,

Paracooperia sp. , Oesophagotomum sp. , Bunostomum sp. ,
Gigeria sp. , Strongyloides sp. & Setaria sp., Fasciola
gigantica, Schistosoma spindalis and Gigantocotyle explanatum
 from buffaloes slaughtered at Gorakhpur, Rampur and Mathura.

Varma et.al (1971) had studied the incidence and
 morphology of Setaria cervi and Setaria digitata from
 buffaloes and cattle in Bihar. During a survey of helminth
 parasites of buff-calves in Mhow, Madhya Pradesh, Bhopale et.al
 (1971) have reported the occurrence of Avitellina gouhi
 Woodland, 1927 for the first time in India.

In addition, check lists of helminth parasites of
 buffaloes in India, have been published by Gaiger (1910,1915),
 Maplestone (1923), Bhalerao (1935), Mudaliar & Alwar (1947),
 Ramanujachari & Alwar (1954), Alwar & Lalitha (1961) and Singh
 (1961, 1962). Besides these surveys and check lists,
 occurrence of helminths in buffaloes have been reported from
 time to time.

MATERIALS AND METHODS

In an examination of 52 buffaloes and 14 cows, 46 buffaloes and 13 cows were found to be infected with one or the other groups of helminths. Although the number of helminths recovered was not large, it was sufficient to indicate the high rate of parasitism in buffaloes in Bihar. The collection represented a total number of 10 species of helminths, out of which there were seven species of trichostrongylids, four species of nematodes and five species of cestodes, listed below:

RESULTS AND DISCUSSION

1. *Trichostrongylus axei* Cobbold, 1908
2. *Trichostrongylus colubriformis* (Stankiewicz, 1923) Fülleborn, 1937
3. *Trichostrongylus axei* Cobbold, 1908 and *Trichostrongylus axei* (Cobbold, 1908) Fülleborn, 1937
4. *Trichostrongylus axei* Cobbold, 1908 and *Trichostrongylus axei* (Cobbold, 1908) Fülleborn, 1937
5. *Trichostrongylus axei* Cobbold, 1908 and *Trichostrongylus axei* (Cobbold, 1908) Fülleborn, 1937
6. *Trichostrongylus axei* Cobbold, 1908 and *Trichostrongylus axei* (Cobbold, 1908) Fülleborn, 1937
7. *Trichostrongylus axei* Cobbold, 1908 and *Trichostrongylus axei* (Cobbold, 1908) Fülleborn, 1937

REFERENCES

1. *Trichostrongylus axei* Cobbold, 1908
2. *Trichostrongylus colubriformis* (Stankiewicz, 1923) Fülleborn, 1937
3. *Trichostrongylus axei* Cobbold, 1908 and *Trichostrongylus axei* (Cobbold, 1908) Fülleborn, 1937
4. *Trichostrongylus axei* Cobbold, 1908 and *Trichostrongylus axei* (Cobbold, 1908) Fülleborn, 1937

RESULTS AND DISCUSSION

In an examination of 52 buffaloes and 14 buff-calves, 48 buffaloes and 13 buff calves were found to be infected with one or the other groups of helminths. Although the number of hosts examined was not large, it was sufficient to indicate the high rate of parasitism in buffaloes in Bihar.

The collection represents a total number of sixteen species of helminths, out of which there were seven species of trematodes, four species of cestodes and five species of nematodes, listed below:

T R E M A T O D A

1. Fasciola gigantica Cobbold, 1855
2. Cotylophoron cotylophorum (Fischöeder, 1901) Näsmark, 1937
- *3. Calicophoron cauliorchis (Stiles and Goldberger, 1910)
Näsmark, 1937
4. Gigantocotyle explanatum (Creplin, 1847) Näsmark, 1937
- **5. Fischöderius elongatus Poirier, 1863
6. Castrothylax crumenifer Creplin, 1847
- **7. Testifrondosa cristata Bhalerao, 1924

C E S T O D A

- *1. Moniezia expansa Rudolphi, 1810
- *2. M. benedeni (Moniez, 1879) Blanchard, 1891
- **3. M. denticulata Rudolphi, 1810
- **4. Stilesia hepatica Wolff hügel, 1903

N E M A T O D A

- *1. Haemonchus contortus Rudolphi, 1803
- *2. Oesophagotomum radiatum Rudolphi, 1803
3. Mecistocirrus digitatus (V.Linstow, 1906)
4. Setaria digitata (V.Linstow, 1906)
5. Setaria cervi Rudolphi, 1819.

The results of the incidence has been presented in Tables I, II, III and IV. Table I and II represents the incidence of trematodes, cestodes and nematodes in adults and in buff calves, respectively. Table III represents the percentage of helminthic infection in buffaloes and buff calves. Table IV shows percentage of incidence in three groups of helminths, viz. trematodes, cestodes and nematodes.

* First record from this host in Bihar.

** First record in buffaloes.

ARTIST

Showing Incidence of Helminthic infection in Buffaloes

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TABLE I (Continued)

Buff. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
33	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-
34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
36	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
37	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
38	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
39	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
40	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-
41	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+
42	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
43	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
47	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
48	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+
49	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+

- 1 = F. gigantea
- 2 = C. cotylophorum
- 3 = C. caulicorhis
- 4 = G. explanatum
- 5 = F. elongatus
- 6 = G. crumenifer
- 7 = T. cristata
- 8 = M. expansa
- 9 = M. benedeni
- 10 = M. denticulata
- 11 = S. hepatica
- 12 = H. contortus
- 13 = M. digitatus
- 14 = O. radiatum
- 15 = S. digitata
- 16 = C. cervi

TABLE II

Showing incidence of helminthic infection in Buff-calves
(6 months to 1 year)

Buff Calves No.	TREMATODA		CESTODA		NEMATODA	
	C. Cotylophorum	G. explanatum	M. expansa	M. benedeni	M. denticulata	S. Cervi
1	+	-	+	-	-	-
2	-	+	-	-	-	-
3	-	-	-	+	-	-
4	-	+	-	-	-	-
5	-	+	+	-	-	+
6	-	-	+	-	-	-
7	-	-	+	-	-	-
8	+	-	-	-	-	-
9	-	+	-	-	-	+
10	-	+	-	-	-	+
11	-	-	+	-	-	-
12	-	-	-	-	-	-
13	-	-	-	-	+	-
14	-	-	+	-	-	-

TABLE III
Showing percentage of helminthic infection in buffaloes
(Adults & Calves)

Sl. No.	Name of Parasites	Adult buffaloes (No. of animals examined)	% of infection	Buff.-calves between six months and one year (No. of animals examined)	% of infection
1	<u>P. asantica</u>	52	6	14	Nil
2	<u>C. cotylophorum</u>	52	13	14	14.2
3	<u>C. cauliorchis</u>	52	1	14	Nil
4	<u>G. explanatum</u>	52	35	14	35.7
5	<u>F. elongatus</u>	52	10	14	Nil
6	<u>G. crumenifer</u>	52	6	14	Nil
7	<u>T. cristata</u>	52	1	14	Nil
8	<u>M. expansa</u>	52	Nil	14	42.8
9	<u>M. benedeni</u>	52	Nil	14	7.1
10	<u>M. denticulata</u>	52	Nil	14	7.1
11	<u>S. hepatica</u>	52	2	14	Nil
12	<u>H. contortus</u>	52	12	14	Nil
13	<u>M. digitatus</u>	52	1	14	Nil
14	<u>O. radiatum</u>	52	2	14	Nil
15	<u>S. digitata</u>	52	9	14	Nil
16	<u>S. cervi</u>	52	19	14	21.4

Showing percentage of infection of trematodes, cestodes and nematodes in buffaloes (Adults & Calves)

TABIE IV

Buffaloes over two years		Buff-calves between six months and one year age group	
No. of animal examined		No. of animals examined	
No. of animals infected with trematodes		No. of animals infected with trematodes	
% of trematodal infection.		% of trematodal infection	
No. of animals infected with cestodes		No. of animals infected with cestodes	
% of cestodal infection		% of cestodal infection	
No. of animals infected with hematodes		No. of animals infected with nematodes	
% of nematodal infection		% of nematodal infection.	

52 45 86.5 2 3.8 32 61.5 14 7 50 8 57.1 3 21.4

T R E M A T O D A

The following seven species of trematodes were encountered during the survey.

Fasciola gigantica Cobbold, 1855

The history of liverfluke is traceable as early as 1379, but Gaiger (1910) was the first who mentioned the incidence of F. gigantica in ruminants in Uttar Pradesh, Punjab and Himalian regions. Later on it was reported and described from different localities of India by several workers (Baylis, 1929; Bhalerao, 1935; Roy, 1954; Thapar, 1956; Ahluwalia et.al 1966).

In the present investigations only 6 buffaloes (11.5%) were found to harbour the parasite, the usual site being the liver and the bile ducts. Buff-calves, however, did not show any trace of infection. At two occasions the parasite was found to be associated with G. explanatum infection and at one occasion with S. hepatica infection. Roy (1954) had reported F. gigantica in 50% to 75% of buffaloes examined in Assam. Thapar (1956) had reported this parasite in 53.3% to 100% of buffaloes examined in different localities of India.

Cotylophoron cotylophorum (Fischöder, 1901) Näsmark, 1937.

In India, Srivastava (1945) reported C. cotylophorum from buffaloes for the first time. Thapar (1956) reported its occurrence in Bihar in buffaloes. The present examination revealed the infection in 13 out of 52 buffaloes (25%) and

2 out 14 buff-calves (14.28%). Thapar had reported C.cotylophorum in 5.2% to 65% of buffaloes examined in different localities of India.

Calicophoron cauliorchis (Stiles & Goldberger, 1910) Näsmark, 1937 (Syn. Calicophoron calicophorum (Fischöder, 1901) Näsmark, 1937).

Varma (1957) had reported its occurrence for the first time from Indian sheep and goat. Mukherjee (1966) described the occurrence of C.cauliorchis in buffaloes in India. He opined that the C.calicophorum described by Varma (1957) is synonym of C.cauliorchis. In the present investigation only one buffalo (1.9%) was found to harbour this parasite. It appears to be the first report from buffalo in Bihar.

Gigantocotyle explanatum (Creplin, 1847) Näsmark, 1937
(Syn. Paramphistomum explanatum)

G. explanatum is known to be one of the commonest parasite of buffaloes in Bihar. Its occurrence has been reported from different localities of India by various workers (Baylis, 1929; Bhalerao, 1935; Thapar, 1956; Varma, 1957; Sinha, 1962; Ahluwalia et.al. 1966). In the present investigation 35 buffaloes (67.3%) and 5 buff-calves (35.7%) were found to harbour this parasite in their livers, bile ducts and gall-bladders. Earlier Varma (1957) and Sinha (1962) have reported the parasite in 57.1% and 37.5% of buffaloes respectively, examined by them in Bihar. Thapar (1956) found

the parasite in 5% to 40% of buffaloes in different localities of India.

Fischoederius elongatus Poirier, 1883.

F. elongatus was for the first time described in Indian buffaloes by Bhalerao (1935). Later its occurrence was reported by Thapar (1956) and Mukherjee (1966) from Indian buffaloes in different localities. In the present investigation 10 buffaloes (19.2%) were found to harbour the parasite which appears to be the first report in Bihar. No parasite was recovered from the buff-calves examined in the present survey. Thapar (1956) found the parasite in 5% to 10% of buffaloes in different localities of India.

Gastrothylax crumenifer Creplin, 1847.

G. crumenifer is one of the commonest helminth parasite of cattle and buffaloes in Indian sub-continent. (Gaiger, 1910, 1915; Maplestone, 1923; Baylis, 1929; Bhalerao, 1935; Srivastava, 1947; Thapar, 1956). During the present survey the infection was found in 6 (11.5%) buffaloes. No parasite was recovered from buff-calves examined in the present survey. Thapar (1956) had found the parasite in 86.7% to 100% of buffaloes in different localities of India.

Testifroncosa cristata Bhalerao, 1924.

T. cristata an intestinal fluke has been originally described by Bhalerao (1924) from pigs in Rangoon and later mentioned by Baylis (1929) and Yamaguti (1958). But its

occurrence in only one buffalo is being reported for the first time.

C E S T O D A

These species of Cestodes, encountered in this investigation, are reported below :

Moniezia expansa Rudolphi, 1810.

Although the occurrence of M. expansa had already been described extensively by several workers in sheep, goat and cattle, Thapar (1956) was the first man who reported its incidence in buffaloes. The present report appears to be the first one from buffaloes in Bihar, when only 6 buff-calves (42.8%) were found to harbour the parasite.

Moniezia benedeni (Moniez, 1879) Blanchard, 1891.

In India Thapar (1956) reported its occurrence for the first time from buffaloes. Later its occurrence was reported by Sharma (1961). The present study revealed the infection only in 1 buff-calf (7.1%). The present report appears to be the first report from buffaloes in Bihar.

Moniezia denticulata Rudolphi, 1810.

M. denticulata has not been reported from buffaloes in India, though Southwell (1922) and Saxena & Deo (1964) have reported its occurrence from intestine of sheep in different localities of India. The present study revealed the infection only in 1 buff-calf (7.1%).

Stilesia hepatica Wolffhügel, 1903.

S. hepatica has not been reported from buffaloes in India. In the present investigation 3.8% of the buffaloes (2) were found to harbour the parasite in their liver. At one occasion the parasite was associated with F. gigantea and on other occasion with G. explanatum.

N E M A T O D A

The following five species of the nematodes have been encountered in the present investigation.

Haemonchus contortus Rudolphi, 1803.

In Indian buffaloes H. contortus was first reported by Srivastava (1945). Then Thapar (1956) and Sharma (1961) had reported its occurrence from different localities in India. The present study revealed the infection in 12 (23.07%) of buffaloes examined in Bihar. It appears to be the first report in the buffaloes in Bihar.

Sahai (1966) examined 238 buffaloes for the presence of Haemonchus sp. in Bareilly, Uttar Pradesh, but none had any infection.

Oesophagotomum radiatum Rudolphi, 1803.

In India, O. radiatum was first reported by Bhalerao (1935), Ahluwalia et.al. (1966) also reported its occurrence in buffaloes of Uttar Pradesh. In the present

investigation only 2 buffaloes (3.8%) were found to harbour the parasite, the usual site being the large intestine.

Mecistocirrus digitatus (V.Linstow, 1906).

In India, Bhalerao (1935) had reported the occurrence of the parasite in buffaloes for the first time. Later on its occurrence has been mentioned by Thapar (1956) in Bihar (India). The present study revealed the infection only in 1.9% of buffaloes examined in Bihar. Thapar had reported M. digitatus in 5% to 21% of buffaloes examined in different localities in India.

Setaria digitata (V.Linstow, 1906).

In India S. digitata was reported for the first time by Sarwar (1946) in buffaloes. Varma et.al (1971) had studied incidence and morphology of S. digitata and S. cervi in buffaloes in Bihar and were of the opinion that the incidence of S. digitata is rather lower than S. cervi in buffaloes. In the present investigation 9 (17.3%) buffaloes were found to harbour the parasite, the usual site being the peritoneal cavity.

Setaria cervi Rudolphi, 1819.

S. cervi is one of the common parasite of buffaloes in Bihar as described by Varma et.al (1971). In the present investigation 19 buffaloes (36.5%) and 3 buff-calves (21.4%) were found to harbour the parasite, the usual site being the peritoneal cavity.

RE-DESCRIPTION OF TESTIPRONDOSA CRISTATA BHALERAO,
1924 - FROM A NEW HOST. BOS BUBALIS (BUFFALO)

Buffalo (Bos bubalis) - A new host for the
trematode Testifrondosa cristata Bhalerao, 1924

PSILOSTOMIDAE Odhner, 1913

TESTIFRONDOSA Bhalerao, 1924

Testifrondosa cristata Bhalerao, 1924

Bhalerao (1924) created a new genus Testifrondosa with type species T. cristata. He recovered these parasites from the intestine of pigs in Burma (Rangoon). Baylis (1929) has described seven genera under the family Psilostomidae, commonly infecting small intestine of aquatic birds, except one species T. cristata which occurs in the intestine of mammals. Yamaguti (1958) has mentioned a number of genera under the family Psilostomidae infecting mammals. He also maintained the validity of T. cristata.

During the present investigation four specimens of T. cristata from the intestine of a buffalo (Bos bubalis) slaughtered at Phulwarisharif, Patna could be obtained. This parasite is being recorded for the first time from this new host in India. The main features and metric data of this worm are given below :

TABLE V

Showing the measurements of *T. cristata* (in mm.)

	Specimen 1	Specimen 2	Specimen 3	Specimen 4	Range	Mean
Body Length	11.0	9.5	7.5	9.5	7.5 - 11.0	9.25 ± 0.71
Body width	3.0	2.5	2.0	3.0	2.0 - 3.0	2.50 ± 0.24
Oral Sucker	0.24	0.18	0.19	0.22	0.18 - 0.24	0.20 ± 0.14
Pharynx	-	0.26X0.18	0.18X0.02	0.22X0.19	0.18 - 0.26X 0.18 - 0.2	0.22 ± 0.04 X 0.19 ± 0.005
Oesophagus	0.19	0.4	0.98	0.58	0.19 - 0.98	0.58 ± 0.16
Ventral Sucker	1.09X0.92	0.88X0.88	0.86X0.75	1.08X0.86	0.86 - 1.09X 0.75 - 0.92	0.97 ± 0.06 X 0.83 ± 0.03
Cirrus Sac	1.02X0.48	0.7X0.32	-	0.8X0.44	0.7 - 1.02X 0.32 - 0.48	0.86 ± 0.09 X 0.40 ± 0.04
Ovary	0.56X0.64	0.48X0.44	0.24X0.32	0.48X0.64	0.24 - 0.56X 0.32 - 0.64	0.40 ± 0.06 X 0.48 ± 0.07
Anterior Testis	0.8 X 2	1.6 X 1.6	0.64X1.2	1.2X1.6	0.64 - 1.6X 1.2 - 2.0	1.12 ± 0.2 X 1.6 ± 0.33
Posterior Testis	1.92X2	1.4X 1.6	0.48X0.8	1.8X1.52	0.48 - 1.92X 0.8 - 2.0	1.2 ± 0.32 X 1.4 ± 0.25

Body is dorso-ventrally flattened measuring 9.25X 2.5 mm. The cuticle of anterior portion is covered with scales. Oral sucker is 0.2 mm. in diameter and smaller than ventral sucker. The pharynx is usually small, distinct and measures 0.22 mm. X 0.19 mm. The Oesophagus is 0.58 mm. long. The intestinal caeca usually extends upto the posterior end of the body. Ventral sucker is large, drawn out posteriorly, and measures 0.97 mm. X 0.83 mm. The cirrus sac is elongate and extends posteriorly behind the ventral sucker. It measures 0.86 mm. X 0.40 mm. Genital pore is present just behind the intestinal bifurcation. The two testes, are tandem i.e. one behind another and placed in the posterior half of the body. They are usually branched. The anterior testis measures 1.12 mm. X 1.6 mm. while the posterior testis is 1.2 mm. X 1.4 mm. Ovary is nearly oval and present on the left side of the median plane in front of the testis. It measures 0.4 mm. X 0.48 mm. Uterine coils are usually very much overlapping and coiled usually remains between the Genital pore just posterior to intestinal bifurcation and ovary and contain a number of ova. (Plate 1; Fig.1).

On the above observations the present specimen conformed with the description of T. cristata, earlier described by Bhalerao (1924) from pig in Rangoon. It appears to be the first record from a buffalo, in India. The main features and metric data have been given for diagnostic purposes. (Table V)

Host : Buffalo.

Location: Intestine.

Locality: Bihar (India).

HISTOCHEMISTRY AND PATHOLOGY OF LIVER IN GIGANTOCOTYLE EXPLANATUM INFECTION IN BUFFALOES.

A. - HISTOCHEMISTRY

HISTOCHEMISTRY OF LIVER

REVIEW .

According to Pearse (1968) the beginning of histo-chemistry as science can be said, the year 1830 to 1835. It was originally adopted in the field of Botany. Though the science of histochemistry was unknown till 1829, isolated reports of the investigation of morphological structure in tissue preparation were available. The histochemical study in parasitic infestations was very much neglected till recent years. The histochemical studies of liver in the Gigantocotyle infection was not done, though some work had been done by previous workers in the other parasitic infestations.

Lewart & Lee (1953) during the histochemical study of the liver of rat and cat infected with Taenia taeniaformis and Amphimerus pseudofeliscus, observed changes in glycogen, glycoprotein and phosphatase.

Mercado and von Brand (1954) observed that there was a progressive decrease in the glycogen of the liver of white rats in Plasmodium berghei infection. Mercado and von Brand (1957) marked that the cell lying towards the centre of the hepatic lobules lose glycogen earlier than other cells, however, certain cells of infected liver stored more glycogen than others. The same worker in 1960 mentioned that every infection produced its own characteristic changes in the

distribution of glycogen and lipid.

Sawada et.al. (1956) found an increase in glycogen and fat contents of the cytoplasm of the hepatic cells during their cytochemical studies on the hepatic tissue of mice having Schistosoma japonicum infection.

Münnich (1960) marked a decrease in glycogen and increase in glycoprotein in the focus of inflammation caused by the larvae of Ascaris lumbricoides.

Kadziolka (1962) opined that during early cirrhosis there was a slight reduction in glycogen content while in advance cases there was a marked glycogen depletion in fascioliasis. Dhar & Singh (1963) found complete depletion of glycogen of liver in dicroceliasis. In the same year Roneus (1963) mentioned the loss of glycogen from the localised area of hepatic cells around Toxocara cati in the liver of pig.

During the histochemical study of liver in opisthorchiasis, Ansari (1968) observed a marked depletion of glycogen in the hepatic cells. Glycogen was found in the parenchyma of the parasite. He also described an increase in mucin secretion by the lining epithelial cells and those of newly formed glandular tissue. At the same time he also marked a slight increase in protein contents of the infected liver.

OBSERVATIONS.

1. GLYCOGEN.

Periodic acid-schiff reaction (PAS)

Normal liver.

The normal section of the liver showed usual pattern of glycogen content in the cytoplasm of the hepatic cells, connective tissue of the portal tracts, wall of blood vessels and epithelial cells lining the bile-ducts, gave PAS positive reaction of moderate to strong intensity (Plate II, Fig. 1).

Infected liver.

On PAS staining it was found that glycogen content in the cytoplasm of hepatic cells around the bile - ducts containing the parasites was markedly reduced, though normal glycogen content could be observed in the cytoplasm of hepatic cells located away from the parasites. (Plate II; Fig. 2).

2. PROTEIN.

Mercury Bromphenol Blue method.

Although, there appeared to be slight depletion of protein in the liver cells around the bile-ducts containing the parasites (Plate III; Fig. 1), no significant alteration, in other liver cells of buffalo infected with G. explanatum, could be seen, when compared with non-infected control.

DISCUSSION.

GLYCOGEN.

In the present study marked reduction in the glycogen reserve was noticed in the hepatic cells surrounding the bile-ducts containing the parasites. Quite in agreement with present observations the glycogen content of the liver has been reported to decrease in many parasitic infections. The depletion in liver glycogen was observed in T. taeniaformis infection in rat and cat (Lewart & Lee, 1953), in P. berghei infection in rat (Mercado and von Brand, 1954, 1957, 1960), in early stage of experimental clonorchiasis in rabbit (Kuwamura, 1958), in A. lumbricoides infection in mouse (Münich, 1960, Kumar 1967), in early cases of fascioliasis (Kadziolka, 1962), in dicrocoeliasis of cattle and goat (Dhar & Singh, 1963) in Toxocara cati infection in pig (Roneus, 1963) and in opisthorchiasis of dogs (Ansari, 1968). However, contrary to this Swada et.al (1956) observed a marked increase in liver glycogen of mouse infected with S. japonicum.

Many workers have discussed about the factors responsible for decrease in liver glycogen in different parasitic infestations from time to time. Mercado & von Brand (1954), however, did not explain the exact mechanism involved, but opined that the possibility of liver disfunction and semi-starvation by the host as the probable cause of glycogen

depletion in P. berghei infection in rats. A similar reason was also attributed for glycogen depletion by Sinton & Hughes (1924), von Brand & Regendanz (1931) and von Brand & Mercado (1956) in cases of human malaria, trypanosomes and P. berghei infection in rat, respectively. Later, Mercado & von Brandh (1957, 1960) suggested the adrenal disfunction as one of the cause for reduction of glycogen content in the liver of rats in P. berghei infection.

Cheng (1963), observed that glycogen molecule was first broken down to glucose molecules which were then absorbed by the parasite and resynthesised as glycogen within their body. These workers, however, have not explained the exact mechanism involved in this.

Besides this, Macgraith (1956) and Ansari (1968) suggested the possibility of a factor originating from the parasite itself which interferes with the oxidative function of the cells, possibly by inhibiting the cytochrome systems. The possibility of glycogen depletion in present studies may also be considered to be due to this factor.

P R O T E I N .

During the present study a slight but non - significant depletion of protein reserve of the liver cells, surrounding the bile-ducts, having parasites, was revealed.

Quite in agreement with present observation, Kuwamura (1958) observed a decrease in the protein content of

the liver in early stage of C. sinensis infection in rabbits. A decrease in amino acids of liver in case of Plasmodium gallinaceum infection in chicken and A. lumbricoides infection in mouse was reported by Ramaswamy (1956) and Ramarao & Sirsi (1958), respectively (cited by Ansari, 1968).

However, contrary to these observations, Ansari (1968) reported a slight increase in the basic protein content of the liver of the dog in opisthorchiasis.

Kumamura (1958) hypothesized that a continuous hepatic disturbance by the infection may be responsible for these changes. In my opinion, the explanation of Kuwamura may be true.

REVIEW OF LITERATURE

INTRODUCTION

Mulastri & Senaviratne (1956) studied the gross pathology and histopathology of the liver of buffalo infected with *S. pyraumatum*. Most of the paramphistomes were in the bile-ducts, though some were found in the liver substance and occasionally, in the portal veins. Invasion of the bile-ducts appeared to be uniform throughout the liver but the gall-bladder, although thickened and fibrosed, never had parasites. There was marked acinar hypertrophy and

B.- PATHOLOGY.

ret-endothelial proliferation of vessels, particularly the hepatic arteries, this is attributed to the hepatic hypertension caused by substance produced by the parasite or the widespread granulomatous lesions of the portal tract. The cirrhosis was the nodular type. The route of infection was intra-peritoneal. These authors have considered *S. pyraumatum* and *Explanatus pyraumatus* synonymous to *S. pyraumatum*.

Varma (1957) had described *S. pyraumatum* from the bile-ducts of the buffalo in Bihar, but he has not studied the pathogenesis.

Prasad (1954) and Singh (1958) have studied the life cycle of this parasite. Singh (1958) opined that the parasite was either non-pathogenic or slightly pathogenic in

HISTOPATHOLOGY OF LIVER

REVIEW.

Kulasiri & Seneviratne (1956) studied the gross pathology and histopathology of the liver of buffalo infected with G. explanatum. Most of the paramphistomes were in the bile-ducts, though some were found in the liver substance and occasionally, in the portal veins. Invasion of the bile-ducts appeared to be uniform throughout the liver but the gall-bladder, although thickened and fibrosed, never had parasites. There was extra-ordinary muscular hypertrophy and sub-endothelial proliferation of vessels, particularly the hepatic arteries, this is attributed to the hepatic hypertension caused by substance produced by the parasites or the widespread granulomatus lesions of the portal tract. The cirrhosis was the monolobular type. The route of infection was trans-peritoneal. These authors have considered G. bathycotyle and Explanatum explanatum synonym to G. explanatum.

Varma (1957) had described G. explanatum from the bile-ducts of the buffaloes in Bihar, but he has not studied its pathogenesis.

Srivastava (1944) and Singh (1958) have studied the life cycle of this parasite. Singh (1958) opined that this parasite was either non-pathogenic or slightly pathogenic in

adult forms.

Sinha (1962) noticed slight enlargement of the liver though he did not observe any major change. In the section of liver he had seen many amphistomes in different developmental stages in bile-ducts. He also confirmed the findings of Kulasiri & Seneviratne (1956) that the invasion of the liver by the parasites were uniform. The bile-duct was thickened and enlarged. The gall-bladder was also thickened and fibrosed. The gall-bladder contained large number of amphistomes. He observed increased connective tissue in the portal areas, in bile-ducts with hyperplasia of glandular epithelium and infiltration of mononuclear cells.

Singh & Kuppuswamy (1969) observed that the liver was enlarged, hard, pale with haemorrhagic spots on the surface with mottled appearance. The parasite were either lying free in the lumen or attached to the mucosa of the bile-ducts. The wall of gall-bladder was thickened containing the amphistomes. They also observed that the haemorrhagic tracts were surrounded by fibrous tissue. Portal areas also showed increase in fibrous tissue. The parasite was attached to the mucous membrane of the bile-duct by the help of acetabulum. Desquamation and hyper-trophy of the billiary and hyperplasia of the mucous gland of the lamina propria were also noticed. The lymphocytic and mononuclear infiltration in the portal area as well as in the hepatic lobules was described.

OBSERVATIONS.

Gross Pathology

The liver was enlarged, hard and pale. The capsule of the liver was thick with haemorrhagic spots on the surface. The amphistomes were firmly attached to the mucosa of bile-ducts by the help of acetabulum, though some of them were lying free in the lumen of the bile-ducts. The bile-duct and the gall-bladder were thickened and very much distended. The parasite at one occasion were seen free in the gall-bladder.

Histopathology.

The histopathological study of liver revealed that the changes were very much severe in the periportal tracts although the other parts of the liver were also affected. The parasite was seen inside the lumen of the bile-duct (Plate IV; Fig.1). The epithelial lining cells of the bile-ducts showed severe degree of hyperplasia and hypertrophy. The connective tissue around the bile-duct also showed severe hyperplasia with well formed collagen fibrils (Plate IV; Fig.2). The liver cells in general showed varying degree of retrogressive changes. The retrogressive changes in the hepatic cells were cloudy swelling and fatty degeneration. At places the degenerative changes in the hepatic cells were so severe that frank coagulative necrosis and fibrosis could also be noticed (Plate V; Fig.1). The blood-vessels in general appeared to be dilated. Periportal veins, central veins and sinusoids showed

appreciable degree of dilatation. In some of the portal tracts there were infiltration of mononuclear cells - macrophages and lymphocytes.

DISCUSSION.

During the present gross study, the liver was enlarged, hard and pale. The capsule of the liver was thick with haemorrhagic spots on the surface and the parasites were found to be attached firmly to the mucosa of bile-ducts by the help of acetabulum. Quite in agreement with the present observations the liver has been reported to be enlarged, hard, pale and the parasites were found firmly attached to the mucosa of the bile-ducts by Kulasiri & Seneviratne (1956), Sinha (1962) and Singh & Kuppuswamy (1969).

During present investigations at one occasion the parasite were seen free in the gall-bladder. These observations are in agreement with Sinha (1962) and Singh & Kuppuswamy (1969), who mentioned that the parasite might have migrated into the gall-bladder after death of the host when the sphincter muscle at the mouth of gall-bladder had relaxed. Contrary to this, in the present studies the parasites were recovered from a gall-bladder, before the relaxation of sphincter muscle, in a surgical case. This suggests that the relaxation of sphincter muscle did not play any role in the migration of parasite in the gall-bladder.

Microscopically the main lesions were observed in and around the bile-ducts in periportal tracts, because large majority of parasites were found in it. The epithelial lining cells of the bile-ducts showed severe degree of hyperplasia and hypertrophy. These observations are in agreement with Kulasiri & Seneviratne (1956), Sinha (1962), Singh & Kuppuswamy (1969) and Sahai & Srivastava (1971).

During present investigations the liver cells in general showed varying degree of retrogressive and degenerative changes and at places the degenerative changes were so severe that frank coagulative necrosis and fibrosis were also noticed. Similar changes were observed by Kulasiri & Seneviratne (1956) and Sinha (1962).

In some of the portal tracts the infiltration of mononuclear cells - macrophages and lymphocytes were also revealed during present studies. Similar type of infiltrations were also observed by Sinha (1962) and Singh & Kuppuswamy (1969).

STUDIES ON SETARIASIS

R E V I E W.

GENERAL.

The classic work of Yeh (1959) on the nematode genus Setaria Viborg, 1795, has no doubt elicited interesting additional informations to settle many disputed issues, particularly the long standing controversy on the validity and separate identity of von Linstow's (1906) species S. digitata. He proposed division of the genus with creation of two additional genera viz. Hyraconema and Artionema, besides Setaria with slight morphological differentiations to hold the forms parasitic in hyracoides, artiodactyls and perissodactyle, respectively. Yamaguti (1961), although mentioned about the said division in foot note, maintained only the old known single genus Setaria to hold all the species irrespective of the Zoological status of their hosts. Nelson (1962) was also reluctant to accept the proposed division of the genus. But, Dutt (1963) recognized the new genus Artionema and had added a new species A. indica from an Indian sheep. Thereafter most of the workers have recognized only well known genus Setaria.

Patnaik & Pande (1963) described S. labiato - papillosa (Syn. S. cervi) from the peritoneal cavity of one month old buff-calves. Varma et.al (1971) have established clear distinction between two species of the genus Setaria viz. S. digitata and S. cervi. But, no work is available on the

taxonomy of microfilariae of S. digitata and S. cervi, except Varma et. al (1971), who gave only measurement and diagrams of S. digitata.

IMPLANTATION.

Williams (1955) had implanted live specimens of S. cervi in experimental animals successfully and recovered microfilariae in the peripheral blood. As a prelude to the study of life history of S. digitata, Anantaraman & Victor (1957) and Victor (1958) attempted to transplant adult male and female worms recovered from bovines, into the abdominal cavity of two goats and a rabbit on the lines adopted by Williams (1955). But, they could not succeed in their attempt to recover the microfilariae. Iakra & Singh (1963) were successful in implanting both S. cervi and S. digitata in three out of four rabbits and got the microfilariae in the peripheral circulation in 48 hours to 13th day post implantation. Thereafter, several workers have implanted live worms of S. digitata in the peritoneal cavity of rabbits, but did not find microfilariae in the peripheral circulation of the implanted rabbits. They have noticed the migration of these worms in different visceral organs - (Iakra, Singh & Srivastava, 1964, Sahai et. al., 1966; Sahai & Singh, 1968).

Again Varma et. al. (1971) successfully transplanted these worms in 16 rabbits and recovered micro-filariae in 13 of them. They have completed the insect phase of

life cycle of S. digitata from these donor rabbits by using laboratory bred mosquito Aedes vittatus and Armigeres obturbans.

Erratic migration and histopathology of affected organ.

Species belonging to the genus Setaria have been reported to occur erratically in several other locations. Erratic migration of S. digitata into the facial attachment of right kidney, mesentry and lobe of the liver (Lakra & Singh, 1963), in the diaphragm, liver omentum and urinary bladder (Lakra et.al. 1964), in the liver (Sahai et.al., 1966) and in the lung (Sahai & Singh, 1968) was observed in cases of implantation of these worms into the peritoneal cavity of rabbits. Again, Varma et.al. (1971) reported its occurrence in the kidney of a cow in Bihar (India) for the first time. Similarly S. cervi has been reported to occur ectopically in the liver (Vasudev, 1955), in the liver diaphragm and heart (Williams, 1955) and in the small intestine, eyes and pericardium (Dutt, 1963) of normal and abnormal hosts. S. equina has also been ectopically reported from the bladder (Ohbayashi, 1953), brain (Vogelsang & Warvarz, 1956), thoracic cavity, lungs, pleural sac, liver, testicle, stomach, intestine and eye (Dutt, 1963) of normal and abnormal hosts.

Sarwar (1947) studied on the pathogenecity of S. cervi in buff-calves and described main pathological alterations as congestion of the mucosa of small intestine,

presence of endothelial cells and fibrous tissue formation.

Sahai et.al. (1966) studied pathological changes in the liver parenchyma of rabbit due to erratic migration of S. digitata. The changes were mostly centred round the parasite and were necrosis, marked leucocytic infiltration around the necrosed lobules, extensive haemorrhages and blood vessels engorged. The hepatic cells showed characteristic changes of cloudy swelling, fatty degeneration and condensation of chromatin material on the nuclear membrane, karyorrhexis and/or Pyknosis. They have also observed proliferation of bile-ducts and increase in connective tissue elements.

IMPLANTATION OF PARASITES

WITH LIVING WORMS

INTRODUCTION

After identification, mature specimens of *S. digitata* and *S. gylis* both males and females (proved) were implanted into the peritoneal cavity, separately, each in three rabbits, within two hours of collection. For *S. digitata* rabbit nos. 1, 2 and 3 and for *S. gylis* rabbit nos. 4, 5 and 6 were used. Rabbit no. 1 received 12 females and 4 males; no. 2 received 12 females and 7 males while no. 3 received 12 females and 3 males of *S. digitata*. In the case of *S. gylis* rabbit nos. 4, 5 and 6 received 12 females and 5 males and 6 females and 2 males of *S. gylis* were implanted in rabbit nos. 4, 5 and 6. The blood smears were regularly examined every morning and evening from next day of implantation till they survived.

A. IMPLANTATION OF RABBITS

WITH LIVING WORMS.

Rabbit no. 3, implanted with *S. digitata*, revealed the presence of microfilariae in their peripheral circulation from 10th day of implantation and the microfilariae continued to be present in circulation only upto 6th day post-implantation. Thereafter the microfilariae were not visible in circulating blood and so the rabbit was sacrificed on 20th day of implantation. Even on thorough examination no worm could be detected except a little specimen in the peritoneal cavity. This

IMPLANTATION OF RABBITS

WITH LIVING WORMS

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OBSERVATIONS.

After identification, mature specimens of S. digitata and S. cervi, both males and females (gravid) were implanted into the peritoneal cavity, separately, each in three rabbits, within two hours of collection. For S. digitata rabbit nos. 1, 2 and 3 and for S. cervi rabbit nos. 4, 5 and 6 were used. Rabbit no. 1 received 14 females and 4 males; no. 2 received 12 females and 7 males while no. 3 received 4 females and 3 males of S. digitata. On the other hand 18 females and 7 males; 14 females and 5 males and 6 females and 2 males of S. cervi were implanted in rabbit nos. 4, 5 and 6. The blood smears were regularly examined every morning and evening from next day of implantation till they survived.

Rabbit no. 3, implanted with S. digitata, revealed the presence of microfilariae in their peripheral circulation from 10th day of implantation and the microfilariae continued to be present in circulation only upto 6th day post-appearance. Thereafter the microfilariae were not visible in circulating blood and so the rabbit was sacrificed on 20th day of implantation. Even on thorough examination no worm could be detected except a little caseation in the peritoneal cavity. While

other two rabbits (Nos.1 and 2), implanted with S. digitata, did not reveal any microfilariae in the peripheral circulation. The rabbit no.1 died on 6th day of implantation. On post-mortem examination 12 specimens of living S. digitata was recovered from their peritoneal cavity. The rabbit no.2 died 2nd day of implantation and on post-mortem examination 6 live S. digitata were recovered from peritoneal cavity. It is interesting to mention that one dead worm was found in the small intestine of this rabbit.

The blood smear of rabbit no.6, implanted with S. cervi, showed the presence of microfilariae in circulation after 16th day of implantation which continued only for 2 days. Thereafter, the blood examination revealed negative results. Again, this rabbit was sacrificed on 22nd day of implantation and thorough search revealed no worms but only caseation in the peritoneal cavity. Whereas other two rabbits (Nos.4 and 5), implanted with S. cervi, did not reveal presence of any microfilariae in blood circulation. The rabbit no.4 died on 5th day and rabbit no.5 on 2nd day of implantation. Their post-mortem examination revealed 10 live S. cervi in the peritoneal cavity of rabbit no.4, whereas both of them had erratically migrated worms in their livers.

The results of the present investigations have been presented in tables VI and VII.

TABIE VI

Showing details of implantation of S. digitata in rabbits.

Rabbit no.	No. of S. digitata implanted	Sex	Date of implantation	Date of Ist. appearance		Remarks
				Days from implantation	Days from Ist. appearance	
1	14	4	16.10.71	-	-	Microfilariae did not appears in the peripheral circulation. Died 6th day post implantation. 12 worms were recovered from peritoneal cavity.
2	12	7	24.10.71	-	-	Microfilariae did not appears in the peripheral blood. Died on 2nd day of implantation. 6 worms were present in peritoneal cavity and one in the intestine.
3	4	3	1.12.71.	10	18.12.71.	Sacrificed after 20th days of implantation. No worm was recovered, but cessation in peritoneal cavity was noticed.

TABLE VII

Showing details of implantation of *S. cervi* in rabbits.

Rabbit no.	No. of <i>S. cervi</i> implanted.	Sex	Date of Ist. appearance.		Date of dis- appearance.		Remarks.
			Date	Days from implantation.	Date	Days from Ist. appearance.	
4	18	7	16.10.71	-	-	-	Microfilariae did not appear in the peripheral blood. Died after 5 days of implantation. 10 live worms were present in peritoneal cavity and two in liver.
5	14	5	24.10.71	-	-	-	Microfilariae did not appear in the peripheral circulation. Died on 2nd day of implantation. One worm was found to be embedded in the liver.
6	6	2	6.12.71	22.12.71	24.12.71	2	Sacrificed after 22nd days of implantation. No worm was recovered, but caseation in peritoneal cavity was noticed.

DISCUSSION

As apparent from description and tables VI and VII that the worms have established themselves only in two out of six rabbits. The microfilariae could be recovered from the peripheral blood of these two rabbits, one implanted with S. cervi and other with S. digitata. The present findings are quite in agreement with those of Williams (1955); Lakra and Singh (1963) and Varma et.al (1971). They had also implanted these worms in rabbits but did not observe microfilariae in the peripheral blood of all the implanted rabbits.

Contrary to these Anantaraman & Victor (1957) and Victor (1958) failed in their attempts to recover the microfilariae in the peripheral blood of two goats and one rabbit after implanting these worms in the peritoneal cavity of these animals. Thereafter, Lakra, Singh and Srivastava (1964), Sahai et.al. (1966), Sahai and Singh (1968), also failed in their attempts to recover the microfilariae in the peripheral blood of rabbits post implantation.

During present study also the erratic migration of S. cervi has been observed in the liver of two implanted rabbits (Nos. 4 & 5). These observations are in conformation with that of Vasudev (1955) and Williams (1955), who had noticed the erratic migration of S. cervi in liver and Sahai et.al. (1966) who also noticed erratic migration of S. digitata in the liver of rabbit.

S. digitata in the present investigation was found to migrate erratically in the intestine (rabbit no.2) which appears to be the first record in abnormal host.

It is interesting to record that during present studies, the rabbits could not survive the heavy worms burden (wherever number of worms implanted were more) and died. However, Varma et.al.(1971) could implant even upto 25 worms without any adverse effect.

B. MIGRATION AND HISTOPATHOLOGY OF LIVER
IN RABBIT, HEAVY PARASITIC BURDEN
INTESTINAL SERVI

HISTOPATHOLOGY AND HISTOCHEMISTRY OF LIVER OF RABBIT. HAVING ERRATICALLY MIGRATED SETARIA CERVI.

OBSERVATIONS

HISTOPATHOLOGY.

Although the severe changes were seen in the liver tissue adjacent to the parasite, but slight alterations were evident throughout the liver parenchyma. The cut surface of parasite (*S. cervi*) was seen around which there was a large amount of hemorrhage and serofibrinous exudate. The liver

B. HISTOPATHOLOGY AND HISTOCHEMISTRY OF LIVER OF RABBIT, HAVING ERRATICALLY MIGRATED

SETARIA CERVI

revealed varying degrees of pathological changes like cloudy swelling, fatty degeneration and accumulative necrosis. (Plate VI; Fig. 1).

The bile ducts in the periportal region showed hyperplasia of the lining epithelium. Many new bile ducts formation could also be seen (Plate VI; Fig. 2). There were many hemorrhagic tracts in the liver and blood vessels (portal veins, sinusoids and hepatic veins), were dilated and engorged with erythrocytes.

However, cellular infiltration was not a feature in this case.

HISTOPATHOLOGY AND HISTOCHEMISTRY OF LIVER OF RABBIT,
HAVING ERRATICALLY MIGRATED SETARIA CERVI.

OBSERVATIONS

HISTOPATHOLOGY.

Although the severe changes were seen in the liver tissue adjacent to the parasite, but slight alterations were evident throughout the liver parenchyma. The cut section of parasite (S. cervi) was seen around which there was a large amount of haemorrhage and serofibrinous exudate. The liver cells around the parasite showed complete necrosis with little amount of connective tissue proliferation. The liver cells revealed varying degree of degenerative changes like cloudy swelling, fatty degeneration and coagulative necrosis.

(Plate VI; Fig.1).

The bile ducts in the periportal region showed hyperplasia of the lining epithelium. Many new bile ducts formation could also be seen (Plate VI; Fig.2). There were many haemorrhagic tracts in the liver and blood vessels (Portal veins, sinusoides and hepatic veins), were dilated and engorged with erythrocytes.

However, cellular infiltration was not a feature in this case.

HISTOCHEMISTRY.A: PROTEIN.Mercury Brom-phenol Blue method.Normal liver.

The normal section of liver showed usual pattern of protein content in the hepatocytes in all parts of the liver. (Plate VII; Fig.1).

Infected liver.

The liver cells adjacent to the parasite showed almost complete depletion of stainable protein while the cells situated away from the parasite revealed good amount of protein. (Plate VII; Fig.2).

B: GLYCOGEN.Periodic Acid-schiff reaction. (PAS technique)Normal liver.

The glycogen content of the normal liver of rabbit revealed the usual pattern of glycogen distribution, viz. the cytoplasm, the connective tissue of the portal tracts, wall of the blood vessels and the epithelial cells lining the bile ducts gave PAS positive reactions of moderate to strong intensity.

Infected liver.

There was a complete disappearance of glycogen in the hepatocytes around the cut sections of parasite. Though

normal glycogen content could be seen in the hepatocytes situated away from the parasites.

DISCUSSION

HISTOPATHOLOGY.

During present investigations, the severe changes were seen in the liver tissue adjacent to the parasite, with mild alterations in the other parts of the liver parenchyma. However, contrary to these observations, Sahai et.al. (1966) observed that the changes were mostly confined around the parasite. Present observations suggests that the parasite causes either mechanical or chemical irritation and since, the irritation remains milder towards periphery than centre, it can be well explained the reason of mild alterations towards periphery.

A large amount of haemorrhage and serofibrinous exudate around the cut section of parasites were noticed. The liver cells around the parasite showed complete necrosis with a little amount of connective tissue proliferation. Similar changes were also described by Sahai et.al. (1966).

Sahai et.al. (1966) had described about the proliferation of bile-ducts and noticed a marked leucocytic infiltration around necrosed lobules of hepatic cells. Quite in

agreement with his findings a slight proliferation of bile - ducts with mild hyperplasia of epithelium was observed during present study, however, the cellular infiltration was not a feature in this case. Probably, the time was too short to produce these changes.

Since, the severe pathological changes were noticed around the parasite, it is presumed that the parasite entered the liver through parenchyma. Similar view was also mentioned by Sahai et.al. (1966). They did not notice any alteration in the liver parenchyma away from the parasite, but in the present study it has been noticed to be mild alterations in the hepatic parenchyma away from the parasite. These alterations can be attributed due to mild irritations caused by the parasites. It is just possible that the parasite may be liberating metabolites which acts as mild toxin.

HISTOCHEMISTRY.

GLYCOGEN.

In the present study there was a complete disappearance of glycogen content in the hepatic cells around the cut sections of the parasite. Similar depletion in glycogen content have been reported in various parasitic infections by several workers. The depletion of liver glycogen was observed in T. taeniformis infection in rat and cat (Lewart & Lee, 1953) in P. berghei infection in rat (Mercardo and von Brand, 1954, 1957, 1960); in early stage of experimental clonorchiasis in

rabbit (Kuwamura, 1958); in A. lumbricoides infection in mouse (Münich, 1960, Kumar, 1967); in early cases of fascioliasis (Kadziolka, 1962); in microcoeliasis of cattle and goat (Dhar and Singh, 1963); in T. cati infection of pig (Roneus, 1963) and in opisthorchiasis of dogs (Ansari, 1968).

However, contrary to this Swada et.al. (1956) observed a marked increase in liver glycogen of mouse infected with S. japonicum.

Many workers have discussed about the factors responsible to reduce the liver glycogen in different parasitic infestation from time to time. The possibility of glycogen depletion in the present study may be considered to be due to the factors originating from the parasite itself which interferes with the oxidative function of the cells, possibly by inhibiting the cytochrome system. Similar hypothesis have also been described by Maegraith (1956) and Ansari (1968).

PROTEIN.

In the present study the liver cells adjacent to the parasite showed almost complete depletion of stainable protein. Quite in agreement with the present observation, Kuwamura (1958) observed decrease in the protein content of the liver in early stage of C. sinensis in rabbits. However, contrary to these observations, Ansari (1968) reported a slight increase in the basic protein content of the liver of

dog in opisthorchiasis.

Kuwamura (1958) hypothesized that a continuous hepatic disturbance by the infection may be responsible for these changes. I also agree with views of Kuwamura (1958).

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HISTOPATHOLOGY OF INTESTINE OF RABBITS, HAVING ERRATICALLY MIGRATED SETARIA DIGITATA

INTRODUCTION

The cut sections of parasite (*S. digitata*) were seen lying in the lumen of intestine. There were haemorrhage and cellular infiltration around the parasite. (Plate VIII, Fig. 1). Lymphocytes and macrophages were forming the bulk of cellular infiltration. At places there was also an attempt to form multinuclear foreign body giant cells. (Plate VIII, Fig. 2).

C.- HISTOPATHOLOGY OF INTESTINE OF RABBITS, HAVING ERRATICALLY MIGRATED SETARIA DIGITATA.

The histopathological changes in the intestine showed little proliferation with infiltration of lymphocytes. The epithelial cells of the intestine showed hyperplasia and hypertrophy. At places there was desquamation of epithelial cells resulting in a thinner layer of mucus.

Histological appearance of the intestine suggested that probably both the processes proliferation and desquamation of intestinal epithelial were enhanced.

DISCUSSION

During present investigations it was noticed that probably both the processes proliferation and desquamation of the intestinal epithelium were enhanced. Lymphocytes and macrophages were forming the bulk of

HISTOPATHOLOGY OF INTESTINE OF RABBITS HAVING
ERRATICALLY MIGRATED SETARIA DIGITATA

OBSERVATIONS.

The cut sections of parasite (S. digitata) were seen lying in the serous of intestine. There were haemorrhage and cellular infiltration around the parasite. (Plate VIII; Fig.1). Lymphocytes and macrophages were forming the bulk of cellular infiltration. At places there was also an attempt to form multinuclear foreign body giant cells. (Plate VIII, Fig.2).

The connective tissue in lamina propria showed little proliferation with infiltration of lymphocytes. The epithelial cells of the intestinal mucosa showed hyperplasia and hypertrophy. At places there was desquamation of epithelial cells resulting in a thinner layer of mucosa.

Histological appearance of the intestine suggested that probably both the processes proliferation and desquamation of intestinal epithelial were enhanced.

DISCUSSION

During present investigations it was noticed that probably both the processes proliferation and desquamation of the intestinal epithelium were enhanced. Lymphocytes and macrophages were forming the bulk of

cellular infiltration around the parasite and at places multinuclear foreign body giant cells were also seen.

Contrary to this Sarwar (1947) observed only congestion, presence of endothelial cells and fibrous tissue formation in the intestine during his study on the pathological reaction to the parasite (S. cervi) in the small intestine of buffalo.

The erratic migration of S. digitata in the intestine of rabbit appears to be the first report in India.

MORPHOLOGY OF MICROFILARIAE OF SETARIA DIGITATA AND SETARIA CERVI

Setaria digitata (Van Linster, 1906) and *Setaria Cervi* (Rudolphi, 1819) are common filarial worms in India. The natural hosts of these worms are cattle and buffaloes, in which they inhabit the peritoneum without producing any spectacular pathological lesions (Green, 1960 and Pillai, 1959) though, Cameron (1951) described a small peritoneal nodule produced by this parasite in cattle.

D.- MORPHOLOGY OF MICROFILARIAE OF

SETARIA DIGITATA AND SETARIA CERVI

Although *S. digitata* inhabits the peritoneal cavity, Warner (1947) and Pillai (1959) reported the occurrence of *S. cervi* in the small intestine of buffalo and *S. digitata* in the kidney of a cow, respectively.

Although adult worms are non-pathogenic the larvae of *S. digitata* have been reported to cause cerebral, spinal meningitis and lumber paralysis in sheep and goats (Warner Research Commission, 1939 - 1944), Warner (1947) and in goats (Pillai, 1951).

Still recently there was controversy on the morphological differences on the separate specific identities of *S. digitata* and *S. cervi*, which have been established by several workers (Van, 1959, Choudh and Jain, 1960, Varma et al., 1971). Still, no account is available on any successful attempt to differentiate the microfilariae

MORPHOLOGY OF MICROFILARIAE OF
SETARIA DIGITATA AND SETARIA CERVI

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Setaria digitata (Von Linstow, 1906) and Setaria Cervi (Rudolphi, 1819) are common filarial worms in India. The natural hosts of these worms are cattle and buffaloes, in which they inhabit the peritoneum without producing any spectacular pathological lesions (Innes, Shoho and Pillai, 1952) though, Cameron (1951) depicted a small peritoneal nodule produced by this parasite in cattle. Although these are the parasite of peritoneal cavity. Sarwar (1947) and Varma et.al (1971) reported the occurrence of S. cervi in the small intestine of buffalo and S. digitata in the kidney of a cow, respectively.

Although adult worms are non-pathogenic the larvae of S. digitata have been reported to cause cerebro - spinal nematodiasis and lumbar paralysis in sheep and goats (Korean Research Commission, 1939 - 1944), Horses (Itagaki et.al., 1947) and in Goats (Ishii, 1951).

Till recently there was controversy on the morphological differences on the separate specific identities of S. digitata and S. cervi, which have been established by several workers (Yeh, 1959, Shoho and Nair, 1960, Varma et.al. 1971). Still, no account is available on any successful attempt to differentiate the microfilariae

of S. digitata and S. cervi. Therefore, the present study was undertaken to find out, morphological differences if any, between the microfilariae of these two species, recovered from the rabbits, implanted with these worms.

DESCRIPTIONS

Microfilariae of Setaria digitata.

They were sheathed and measured 281 ± 26.45 microns in length with sheath and 209 ± 2.76 microns without sheath. Their width was 11 ± 0.37 microns with sheath and 7 ± 0.58 microns without sheath. The anterior end was somewhat tapering. The cephalic space was 5 ± 0.24 microns. The nerving, excretory and anal pores were at a distance of 43 ± 1.5 microns; 94 ± 1.58 microns and 135 ± 7.06 microns from the anterior end, respectively. The nuclei in the nuclear column of the body were considerably overlapping and intermingled at the anterior and than the posterior end. The Caudal space was present which measured 10 ± 0.7 microns. The sheath at the anterior end was observed invariably long and bent (Plate IX,B).

Microfilariae of Setaria cervi.

They were also sheathed. The sheathed microfilariae measured 249 ± 13.79 microns in length while the measurement without sheath was 182 ± 6.07 microns. Their width was 10 ± 0.57 microns with sheath and 5 ± 0.13 microns

without sheath. The anterior end was rounded and the posterior end was somewhat tapering. The cephalic space was measured 5 ± 0.11 microns. The nerve ring, excretory and anal pores were at a distance of 37 ± 1.41 ; 96 ± 3.32 and 130 ± 4.65 microns, respectively, from the anterior end. The nuclei in the nuclear column of the body were much more overlapping and intermingled than that of S. digitata. No Caudal space was seen. The sheath was not seen as long as in S. digitata, anterior to the cephalic end. (Plate IX, A).

A comparative study of corresponding measurement reveals the following distinctive features between these species.

7. Caudal space	10 ± 0.70	Absent
8. Nuclei of the nuclear column of the body	Considerably overlapping and intermingled at the anterior end than the posterior.	Much more overlapping and intermingled.
9. Sheath anterior to Cephalic end.	Long and invariably bent.	Short

TABLE VIII

Showing measurements and differences of microfilariae
of S. digitata and S. cervi (in microns)

Differential points.	<u>S. digitata</u>	<u>S. cervi</u>
1. Length with sheath	281 \pm 26.45	249 \pm 13.79
2. Length without sheath	209 \pm 2.76	182 \pm 6.07
3. Breadth with sheath	11 \pm 0.37	10 \pm 0.57
4. Breadth without sheath	7 \pm 0.58	5 \pm 0.13
5. Cephalic space	5 \pm 0.24	5 \pm 0.11
6. Distance from anterior end to (a) Nerve ring	43 \pm 1.50	37 \pm 1.41
(b) Excretory pore	94 \pm 1.58	96 \pm 3.32
(c) Anal pore	135 \pm 7.06	130 \pm 4.65
7. Caudal space	10 \pm 0.70	Absent
8. Nuclei of the nuclear column of the body	Considerably overlapping and intermingled at the anterior end than the posterior.	Much more overlapping and intermingled.
9. Sheath anterior to Cephalic end.	Long and invariably bent.	Short

DISCUSSION

From the literature it will be seen that the morphology of microfilariae of Setaria species viz. S. digitata and S. cervi, have not been studied, except that a brief note on microfilariae of S. digitata by Varma et. al. (1971). They recovered the microfilariae from the abdomen of mosquitoes, immediately after their blood meal and found them similar to that of peripheral blood. In the present study, the comparative morphology of microfilariae of S. digitata and S. cervi has been studied for the first time.

In S. digitata the sheath at anterior end was so long that it was seen invariably bent while in S. cervi the sheath was not so long. Although, Varma et. al. (1971) have not given any description of microfilariae of S. digitata, but from their figure it is evident that they have also noticed quite long sheath anterior to cephalic end of the body.

The Caudal space in the present observation in S. digitata was invariably seen whereas in S. cervi no Caudal space was visible. Again, Varma et. al. (1971) have drawn a distinct caudal space in S. digitata. These two criterion of the projection of anterior sheath and the presence of caudal space in S. digitata is quite distinct to differentiate the two species on the basis of taxonomy of microfilariae.

The measurements of microfilariae of both species

obtained from peripheral circulation are compared in Table VIII. Of the measurements made only that of body length was of some value in morphological differentiation. The body lengths of microfilariae of both species were found to be highly significant and they could also be used with a reasonable degree of accuracy for differentiation. The body length of the microfilariae of S. digitata was 281 ± 26.45 (with sheath) microns and 209 ± 2.76 (without sheath) microns, whereas of S. cervi was 249 ± 13.79 (without sheath) microns and 182 ± 6.07 (without sheath) microns.

Apart from the body length, there were also slight variation in the other measurements, except that of cephalic space which appeared to be the same in both the species.

The microfilariae of S. digitata in present study can be compared with those of Varma et.al. (1971), except that the present specimens were a little smaller than that of Varma et.al. (1971). As the specimens studied by Varma et.al. (1971) were recovered from the abdomen of mosquitoes, it is presumed that they might have developed in the abdomen of mosquitoes.

S U M M A R Y

S U M M A R Y

INCIDENCE AND NATURE OF HELMINTHIC INFECTION IN BUFFALOES

A survey of the incidence of helminthic infection in buffaloes in Bihar was carried out. 52 buffaloes and 14 buff-calves were examined during this period and 48 (92.3%) buffaloes and 13 (92.8%) buff-calves were found to be positive for one or other helminth. The collection represents a total number of sixteen species of helminths, out of which there were seven species of trematodes: Fasciola gigantica, Cotylophoron cotylophorum, Calicophoron cauliorchis, Gigantocotyle explanatum, Fischöderius elongatus, Gastrothylax crumenifer, Testifrondosa cristata; four species of cestodes, : Moniezia expansa, M. benedeni, M. denticulata, Stilesia hepatica; and five species of nematodes, : Haemonchus contortus, Oesophagotomum radiatum, Mecistocirrus digitatus, Setaria digitata and Setaria cervi. The percentage of infection of each parasite has been presented in Table III. Testifrondosa cristata from a new host, Bos-bubalis (Buffalo) has re-described.

HISTO- PATHOLOGY AND CHEMISTRY OF GIGANTOCOTYLE EXPLANATUM INFECTION

Histochemistry of the liver in Gigantocotyle explanatum infection in buffaloes.

The histochemical studies revealed a marked

depletion of glycogen in the hepatic cells and its presence in the parenchyma of the parasite.

A slight depletion of protein in the liver cells around the bile ducts containing the parasite was also evident.

Pathology of the liver in *G. explanatum* infection in buffaloes.

The liver was enlarged, hard and pale with haemorrhagic spots on the surface.

Histopathological changes were very much confined to the periportal tracts characterised by hyperplasia and hypertrophy of the epithelial lining cells of bile ducts. The connective tissue around the bile duct also showed severe hyperplasia. The liver cells showed varying degree of retrogressive and degenerative changes with cellular infiltrations.

STUDIES ON SETARIASIS

Morphological differentiations between the microfilariae of *S. cervi* and *S. digitata* are furnished.

Implantation of the adult worms of *S. digitata* and *S. cervi* in 6 rabbits through laparotomy were done. 2 rabbits (one each implanted with *S. cervi* and *S. digitata* respectively) subsequently showed microfilariae in their peripheral circulation.

Ectopic occurrence of mature S. digitata in the intestine and S. cervi in the liver of rabbit has been noticed. The histopathological changes of liver and intestine affected with these worms were furnished.

The histochemical observation of the liver infected with S. cervi revealed a complete disappearance of glycogen and stainable protein in the hepatic cells around the cut sections of the parasites.

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KEY TO LETTERING.

AT	-	Anterior Testis
CU	-	Curtain and
EG	-	Egg
GP	-	Genital pore
IT	-	Intestine
O	-	Ovary
OP	-	Oral sucker
OS	-	Oesophagus
P	-	Pharynx
PT	-	Posterior Testis
SC	-	Sperm
VS	-	Ventral sucker.

KEY TO LETTERING

KEY TO LETTERING.

AT	-	Anterior Testis
CS	-	Cirrus sac
E	-	Egg
GP	-	Genital pore
INT	-	Intestine
O	-	Ovary
OS	-	Oral sucker
OE	-	Oesophagus
P	-	Pharynx
PT	-	Posterior Testis
UT	-	Uterus
Vit	-	Vitellineduct
VS	-	Ventral sucker.

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EXPLANATION OF PLATES

