Studies on Helminth Parasites of Dogs With Special Reference to Histochemistry & Histopathology in Opisthorchiasis noverca

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Studies On
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BY
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Certified that the thesis entitled, "STUDIES ON HELMINTH PARASITES OF DOGS WITH SPECIAL REFERENCE TO HISTOCHEMISTRY AND HISTOPATHOLOGY IN OPISTHORCHIASIS NOVERCA" embodies the results of work actually carried out by Dr. Gajendra Mohan Kumar, B. Sc., B. V. Sc. & A. H. 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)

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CONTENTS

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INTRODUCTION

INTRODUCTION

Care about helminth parasites of dogs is important not only because of their own well being but also because of its zoonotic importance to human beings.

A number of helminthic diseases in relation to dogs listed to be of zoonotic importance by WHO/FAO Expert Committee on Zoonosis (1959) are: Bilharziasis, Clonorchiasis, Echinostomiasis, Fasciolopsiasis, Heterophyiasis, Metagonimiasis, Opisthorchiasis, Paragonimiasis, Diphyllobothriasis, Dipylidiasis, Hydatidosis, Ancylostomiasis and cutaneous larva migrans ("Creeping eruption"), Strongyloidiasis and Toxocariasis (visceral larva migrans). It is possible that changing climatic conditions and improvement upon experimental techniques applied might enlist many more new helminth parasites which may not only be detrimental to the health of dogs but might be of interest also from public health point of view.

This necessitates an exhaustive study on the helminth parasites infesting dogs. Elucidative work on certain aspects of the helminthic infections of dogs in India are available (Rao, 1958; Gupta, 1961; Sahai, 1967). Ansari (1968) have also described histopathological and histochemical changes of liver of dogs in opisthoschiasis, but no descriptive study on histopathological and histochemical changes of pancreas of dogs in Opisthorchis noverca infection is documented. However, a little

pathological changes of pancreas of pigs in opisthorchiasis noverca has been reported by Sahai and Srivastava (1971). As no studies on helminth parasites of dogs in Bihar are available, except incidence of helminthic infection on the basis of faecal examination. Under the circumstances, comprehensive studies on helminth parasites of dogs was undertaken. The studies included histochemistry and histopathology of pancreas and liver of dogs infected with O. noverca, an important and common parasite of dogs, cats, pigs and also man (Bhalerao, 1935). In addition histopathological changes of intestine of dogs in Dipylidium caninum infection was also studied and also a new indigenous compound, "WOPELL" was tested for its anthelmintic value against most commonly encountered Ancylostoma caninum infection in dogs.

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 deals with the incidence of helminth parasites of dogs in Bihar, histochemical and histopathological changes of the pancreas and liver in Opisthorchiasis noverca and of the intestine in Dipylidium caninum infection in dogs; and anthelmintic activity of "WOPELL" against Ancylostimiasis caninum in dogs.

MATERIALS AND METHODS

Source and Collection:

For the purpose of the survey of helminth parasites of dogs, stray dogs from the Veterinary Hospital, Bankipur, the campus of Bihar Veterinary College, Patna, the villages nearby the college and dogs from College Hospital and Post mortem room were obtained from time to time. The living dogs were sacrificed by injecting saturated solution of Magnesium sulphate either intravenously or intracardially. In case of anaemic and debilitated dogs, chloroform was used to destroy them.

All their visceral organs and tissues were carefully examined for helminth parasites, special attention was paid to examine muscles of diaphragm for Trichinella cyst. A thick blood smear was made and examined for the presence of microfilariae, if any, just before the animal was sacrificed.

Certain large worms, like hook worms and cestodes were isolated and preserved immediately after collection, but the smaller worms were collected over a number of days from the intestinal contents preserved and stored for subsequent examination.

The method adopted for collection were the same as described by Sahai (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 couple of hours the parasites were transferred to fresh solution of fixatives. Smaller trematodes, like Haplorchis, Echinochasmus and Pharyngiostomoides etc. have been fixed and preserved in 5 % formalin.

Neutral formalin, buffered formalin saline and Zenker's fluid were used as fixatives and preservatives for the tissues intended for histopathological and histochemical studies.

Staining and Mounting

The stains used for whole mount preparations were Acetic alum carmine (aqueous), Borax carmine (Alcoholic), Gower's carmine (alcoholic), Haemalum (aquecous) and Trypan blue (aqueous). The common stains - Acetic alum carmine Borax carmine and Gower's carmine - were prepared and used in usual manners, except that clearing of parasites were done either in clove oil or methyl salicylate. The parasites were mounted either in DPX or Canada balsam. The later gave better results for cestodes and opisthorchid worms.

For staining the parasites in Trypan blue a 0.142 % (0.142 mg in 100 c.c.) solution was prepared in distilled water. After washing the parasites as usual for removing the fixative it was left overnight in the stain. The parasites were stained in Trypan blue to such an extent that no differentiation was required and were directly dehydrated in usual way. They were cleared in clove oil.

In case of Haemalum 5 % aqueous solution was prepared. It was exposed to sunlight for a day, after which it was heated to boiling. It was cooled and then filtered. Minimum staining hour was 4 to 5 hours, but over night staining was found to be convenient. Worms were differentiated in acid water, and were treated with tap water until the organs became bluish. After which usual dehydration and mounting was done. The 70 % alcohol in over night deepened the stain too much which was not seen in other stains used. The parasites were cleared in clove oil and mounted in DPX 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 suggested by Taylor (1935).

Carbolic acid	5 ml
Lactic acid	5 ml
Glycerine	10 ml
Distilled water	5 ml

The medium sized nematodes were cleared in this agent with satisfactory results.

(b) Glycerine alcohol The mixture was prepared by mixing glycerine and 70 % alcohol in the ratio of 1:19. The specimens preserved in 70 % alcohol were transferred to this clearing agent.

Glycerine-alcohol can be used for smaller as well as medium sized nematodes.

Histochemical Techniques

For histochemical studies liver and pancreas of dogs infected with Opisthorchis noverca were selected. The standard methods of fixation (fixatives have been given earlier) and various histochemical staining procedures described by Lillie (1954), Culling (1957) and Pearse (1968) were adopted.

The following histochemical staining procedures were used:

A. Protein:- Mercury - Bromphenol blue method.

B. Carbohydrate and carbohydrate containing tissue elements:-

(i) Periodic Acid Schiff technique (PAS)

(ii) Best's caramine for glycogen

(iii) Alcian blue method for acid muco-polysaccharides.

C. Calcium:- von Kossa method

D. Fibrin:- Weigert method

E. Connective tissue:- von-Gieson's method

Histopathological Techniques

Histopathological studies of liver of dogs infected with O. noverca and intestines of dogs infected with D. caninum were made. Fixation, dehydration, clearing paraffin embedding, section cutting and staining were done by conventional method. Sections were cut at 6 - 8 μ and stained with haematoxylin and eosin (H & E).

Preparation and Examination of Stained Blood Film

Thick blood smears were prepared from ear vein of dogs, before sacrificed for collection of parasites. Smears were dehaemoglobinised, fixed in methyl alcohol and stained with haematoxylin and eosin (Verma et al., 1971). Some fresh blood drop were also examined.

Measurements and Diagrams

The measurements, whenever needed were taken by the aid of an ocular micrometer. Photomicrographs of sections showing histopathological and histochemical changes were taken as usual.

Anthelmintic and Dose

Wopell, an indigenous compound manufactured by Indian Herbs Research and Supply Company, Saharanpur, Uttar Pradesh, was used in following dose schedule.

The five dogs used for this experiment were divided into two groups with one dog as control. Dog No. 2, 3 (Group I) were treated with 1 gm of Wopell, No. 4, 5 (Group II) were treated with 2 gms of Wopell and dog No. 1 was kept as control. The drug was administered orally. Faecal samples were examined regularly till the dogs were autopsied. In the first round only dog No. 2 (from Group I) and dog No. 4 (from Group II) were sacrificed. The worms were collected from these dogs. A second dose of 1 gm of the drug was administered to dog No. 3 (Group I) and 5 (Group II) which were not sacrificed. The egg count of these two dogs (No. 3 & 5) were made pre- and post-medication, i.e. after second

dose. These dogs were also sacrificed after an interval of a day.

Faecal Examination

The faecal samples of the dog were examined every day pre- and post-medication in chemotherapeutic trials by the method described by Sheather (1923) later adopted by Bhalerao (1935). Quantitative examinations as and when required were done by Stoll's egg count technique as modified by Faust (1949).

Criteria of Therapeutic Activity

The presence or absence of eggs and also their number in faeces before and after treatment indicated the therapeutic activity of the drug. Secondly the activity of the drug was assessed according to the degree of damage done by it to the worms, seen after autopsy of the treated and untreated control dogs.

INCIDENCE AND NATURE OF HELMINTHIC INFECTIONS IN DOGS

INCIDENCE AND NATURE OF HELMINTHIC INFECTIONS IN DOGS

GENERAL

The literature on the incidence of helminths and helminthiasis of dogs in India is rather meagre even though these worms are much important in view of public health.

For the first time in India, Gaiger (1911) examined 50 pariah dogs and reported 6 helminth parasites: Spiroptera sanguinolenta (36 %), Ascaris marginata (38 %), Taenia (90 %), Ancylostomes (38 %) and Opisthorchis noverca ? (38 %). In a survey of helminths of pariah dogs, Sondhi (1923) reported 9 cestodes from these animals in Lahore. They are Dipylidium walkeri n. sp., Dipylidium sexoronatrum von Ratz, 1900, D. oerlevi von Ratz 1900, Multiceps multiceps (Leske, 1780) Goeze, 1782, Multiceps gaigeri Hall, 1916, Multiceps serialis Gervais, 1847, Taenia hydatigena. Pallas, 1766, Taenia ovis Cobbold, 1869, and Echinococcus granulosus (Batsch, 1786) Rudolphi, 1801.

Acharya (1933) examined 50 pariah dogs in Lucknow, Uttar Pradesh and found 49 harbouring one or other helminth parasites. A total of seven species of helminths were reported, viz., Ancylostoma caninum (Ercolani, 1859), Hall, 1913, Dipylidium caninum (Linnaeus, 1758) Leuckert, 1863, Taenia serrata, Taenia marginata, Taenia coenurus, Toxocara canis Werner, 1782, Spirocerca sanguinolenta. Again, he examined 200 dogs from the same locality in 1939 and recovered 10 species of helminths: A. caninum, T. canis, Spirocerca lupi (Rudolphi, 1809),

Railliet and Henry, 1911, Chlamydonema sp., D. canium, T. hydatigena, T. pisiformis Bloch, 1780, E. granulosus, M. gaigeri and Opisthorchis noverca Braun, 1902.

During a survey of hydatid disease in Punjab, Sami (1938) reported the helminth parasites from 87 out of 156 dogs examined. They were T. hydatigena, Toxocara limbata Railliet and Henry, 1911, Opisthorchis caninus Barker, 1911 and D. caninum.

Maplestone and Bhaduri (1940) examined 200 street dogs from Calcutta and recovered 21 species of helminths. They had also given a complete list of 49 species of helminths so far recorded from Indian dogs. They have mentioned Alaria alata (Goeze, 1782) Schrank, 1788, Echinochasmus perfoliatus (Ratz, 1908) Dietz, 1909, Echinochasmus sp. Heterophyes heterophyes (Siebold, 1853) Stiles and Hassal, 1900, Opisthorchis felineus (Rivolta, 1884) Blanchard, 1895, Paramphistomum sp., Paryphostomum sufrartyfex Lane, 1915, Platynosoma sp., Troglotrematidae (new genus) among trematodes; Diphyllobothrium mansoni Cobbold, 1882, D. caninum, E. granulosus, T. gaigeri, T. hydatigena among cestodes; Ancylostoma braziliense, A. caninum, Gnathostoma spinigerum, Rictularia cahirensis, Toxocara canis and Trichostrongylus colubriformis (Giles, 1892) Looss 1905 among nematodes. They have come across only one acanthocephalan species Echinorhyncus sp.

An exhaustive survey of helminth parasites of domesticated animals in Uttar Pradesh, Bihar, Bengal and Orissa was carried out by Thapar (1956). He reported only eight helminths

from dogs, viz., Cestodes - E. granulosus, D. caninum, T. pisiformis; Nematodes - A. caninum, Dirofilaria immitis (Leidy, 1856) Railliet and Henry 1911, Dirofilaria sp. (female), Spirocera sanguinolenta and T. canis. Rao (1958) examined 26 dogs in South India and reported 19 helminths from them : six trematodes - Episthmium (Echinochasmus) caninum (Verma, 1935), Chatterjee, 1954, Heterophid sp., Haplorchis yokogawai Katsuta, 1932, Galactosomum sp., Stictodera manilensis Africa and Gracia, 1935 and Heterophyes heterophyes; five cestodes - M. gaigeri, E. granulosus, Taenia sp., T. hydatigena, and D. caninum and two nematodes - A. caninum, T. canis. Patnaik (1959) in a survey of trematodes of dogs at Bhubaneshwar, Orissa, examined 15 dogs and recovered helminths from them. Eleven dogs had O. felineus and one Schistosoma suis Rao and Ayyar, 1933 (Syn. S. incognitum) in livers while three harboured Paragonimus westermanii (Kerbert, 1878) Braun, 1899, and 9 had Echinochasmus perfoliatus.

A survey of parasites of carnivorous mammals in Uttar pradesh was carried out by Gupta (1961) who reported the occurrence of 13 helminths from dogs : Trematoda - Opisthorchis caninus (Lewis et Cunnigham, 1872) Barker 1911, Haplorchis yokogawai Katsuta, 1932, Echinochasmus caninum Verma, 1935; Cestodes - D. caninum, Joyeuxiella pasquelei, Diamara, 1893, Taenia hydatigena, M. multiceps, E. granulosus; Nematodes - Spirocerca lupi (Rudolphi, 1809), Railliet and Henry, 1911, physaloptera sp., Rictularia cahirensis Jaegerskiold, 1904, A. caninum, Syphacea sp. and Toxocara canis.

Sinha (1961), during routine faecal examination of dogs suspected for parasitic diarrhoea at Bihar Veterinary College Hospital, Patna, reported 42.3 % nematode infection, viz., 35.2 % Ancylostoma, 3.55 % Ascaris and 0.59 % Spirocerca. During his studies on certain helminth parasites of domesticated mammals of Bihar, Sinha (1962), has reported two trematodes, viz., Opisthorchis noverca and Echinochasmus perfoliatus. In a routine faecal examination in Bihar, Karan and Mandal (1963) examined 250 samples from dogs and reported 41.6 % helminthic infection in them, viz., Ancylostoma 35 %, Ascaris 4.5 %, Toxocara 1.2 %, Tapeworms 1.2 %, Spirocerca 2.5 % and Trichuris 2.5 %. Sharma and Chitkara (1963) made an extensive studies on hydatid infection in Amritsar, Punjab. During this study they had examined 460 dogs and reported in them 8.04 % infection of E. granulosus.

Malaki (1966) made a survey of gastrointestinal parasites of 61 dogs in Bangalore and reported - D. caninum, T. hydatigena, T. ovis, S. lupi, A. caninum, T. canis, Physaloptera canis and Acanthocephala. An interesting observation made by him was the presence of ova of Toxocara canis in the buccal capsule of Ancylostoma caninum.

Sahai (1967, 1969) made a systematic survey of helminthic fauna of 51 stray dogs in Uttar Pradesh. He reported 12 species of helminths : Trematodes - O. noverca (60.78 %), E. perfotiatus (41.17 %), H. taichui (25.49 %), T. hydatigena (31.37 %), E. granulosus (5.88 %); Nematodes - A. caninum (70.58 %), S. lupi (19.60 %), Toxocara canis (7.84 %), Physaloptera canis (1.96 %)

and Acanthocephala (Echinorhyncus sp.) (1.96 %). He recorded O. noverca from the pancreas of dogs for the first time. P. canis a nematode and H. taichui a trematode parasites were recorded by him for the first time from Indian dogs. Thereafter H. taichui has also been reported from Madras (Rao and Anantaraman, 1967) and Madhya Pradesh (Sahasrabudhe et al., 1969).

Sahasrabudhe et al. (1969) examined 74 homeless dogs for evidence of helminthic infection in Madhya Pradesh and noticed the presence of 16 species of helminths : Trematodes - Euparyphium inerme (1.35 %), Echinochasmus corvus (13.51 %), Haplorchis taichui (14.86 %), Artyfechinostomum sufrartyfex (1.35 %) and Opisthorchis caninus (31.08 %); Cestodes - Spirometra ranarum (1.35 %), Spirometra erinaceieuropaei (1.35%), D. caninum (47.29 %), T. hydatigena (22.97 %), M. gaigeri (2.70%), E. granulosus (10.81 %) and Cysticercus cellulosae (1.35 %) and nematodes - Toxocara canis (2.70 %), A. caninum (89.18 %), Spirocerca lupi (54.05 %) and Physaloptera praeputalis (1.35 %) were recorded.

In a routine diagnostic examination in Tripura, Majumdar (1969) examined 385 faecal samples from dogs and reported 60.2 % nematodes and 1.9 % cestodes infection in them. He did not get any trematode infection. Again in similar faecal examination of 3842 samples from dogs in Kerala, Rajmohan and Paily (1971) have reported infection of Toxocara (20.8 %), Trichiuris (0.05%); Ancylostomes (60 %), Spirocerca (0.8 %) and Spirurid (0.05 %), Cestodes (1.9 %).

Besides the above mentioned works, check lists of helminth parasites of dogs have been published by Gaiger (1910, 1915), Bhalerao (1935), Mudaliar and Alwar (1947), Ramanujachari and Alwar (1954), Lalitha and Alwar (1960), Alwar and Lalitha (1961) and Singh (1961, 1962).

Apart from these check lists and surveys occurrence of helminths in dogs have been reported from time to time.

RESULTS AND DISCUSSION

In an examination of 36 dogs, 35 were found to be infected with one or the other helminth parasites. The number of dogs examined was not large though it was sufficient to indicate the high rate of parasitism in dogs of Bihar.

The collection represents a total number of 12 species of helminths, out of which 6 of trematodes, 4 of cestodes and 2 of nematodes as listed below:

TREMATODA

1. *Opisthorchis noverca* Braun, 1902
*2. *Haplorchis taichui* (Nishigori, 1924) Chen, 1936
3. *Echinochasmus perfoliatus* (Ratz, 1908) Dietz, 1909
*4. *Echinochasmus corvus* Bhalerao, 1926
5. *Schistosoma incognitum* Chandler, 1926
**6. *Pharyngostomoides* sp.

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* First record in Bihar
** First record in India.

CESTODA

1. Dipylidium caninum (Linnaeus, 1785) Leuckart, 1863
2. Echinococcus granulosus (Batsch, 1786) Rudolphi, 1801
*3. Taenia hydatigena Pallas, 1766
*4. Multiceps multiceps (Leske, 1780) Goeze, 1782.

NEMATODA

1. Ancylostoma caninum (Ercolani, 1859) Hall, 1913
2. Spirocerca lupi (Rudolphi, 1809) Railliet and Henry, 1911.

TREMATODA

Opisthorchis noverca Braun, 1902

It was originally described by Braun (1902) from the liver of Indian dogs. Thereafter this parasite was reported by different workers from different localities (Gaiger, 1911; Acharya, 1939; Mudaliar, 1943; Mudaliar and Alwar, 1947; Sahai, 1969; Sahai and Srivastava, 1971). Bhalerao (1931) recorded it from pig at Calcutta and Sinha (1968) from Bihar.

In the present investigation 16 dogs (44.44 %) were found to harbour the parasite. The usual location being bile ducts and pancreatic ducts, in three cases the parasites were also recovered from duodenum and gall bladder. Parasites recovered from pancreatic duct, however, were not mature which is supported by the data obtained from comparative measurements of the parasites from two different location. It is presumed that the pancreatic duct is not the usual site of predeliction for Opisthorchis though in this case 9 out of 16 dogs showed the presence of parasite in pancreas.

* First recorded in Bihar.

Acharya (1939) had found 13.5 % of dogs infected in Lucknow and Sahai (1969) reported 60.78 % of dogs infected with O. noverca in Bareilly, Uttar Pradesh. In Madhya Pradesh, Sahasrabudhe, Dubey and Srivastava (1969) observed 31.08 % dogs infected with O. caninus, which is synonym with O. noverca (Sahai and Srivastava, 1972).

Echinochasmus perfoliatus (von Ratz., 1908) Dietz, 1909

In India, Chandler (1925) reported E. perfoliatus for the first time from dog and cat in Calcutta. This parasite was later reported by Bhalerao (1936), Patnaik (1959), Sahai (1969). Sinha (1962) reported it for the first time in Bihar.

The present investigation revealed the infection in 11 out of 36 dogs (36.55 %) examined. Maplestone and Bhaduri (1940) had reported Echinochasmus species in 26.5 % of dogs in Calcutta. Patnaik (1959) reported 60 % infection with E. perfoliatus in Orissa, while Sahai (1969) observed 41.17 % of dogs examined in Bareilly, Uttar Pradesh having this infection.

Echinochasmus corvus Bhalerao, 1926 (Syn. E. caninum Verma, 1935)

Rao (1958) reported from South India the presence of E. caninum. Gupta (1961) considered E. corvus as valid species with E. caninum as its synonym and he reported the occurrence of E. corvus from Uttar Pradesh.

In the present study it was observed in 13.88 % of dogs examined in Bihar. Sahasrabudhe et al. (1969) reported the infection of E. corvus in 13.51 % of dogs examined in Madhya Pradesh.

Haplorchis taichui (Nishigori, 1924) Chen, 1936

Bhalerao (1936) had reported its occurrence from India for the first time in cats. Sinha and Despande (1964) recovered this parasites for the first time in fox in India. Sahai (1967) for the first time reported the occurrence of this parasite from Indian dogs in Uttar Pradesh. Later, Rao and Anantaraman (1967) reported its occurrence from dogs of Madras. Sahasrabudhe et al. (1969)have also reported its occurrence from dogs of Madhya Pradesh.

The present investigation revealed that 13.88 % dogs examined in Bihar had this infection. Sahai (1967, 1969) reported 25.49 % of the dogs had this infection. However, contrary to this Rao and Anantaraman (1967) observed 1.4 % infection in dogs. Sahasrabudhe et al. (1969) found the parasites in 14.86 % of dogs examined.

Schistosoma incognitum Chandler, 1926 (Syn. S. suis Rao & Ayyar, 1933)

S. incognitum is known to be a common parasite of dogs in India and has been reported from different localities by several workers (Swaminathan, 1934; Rao, 1937, 1943; Srivastava, 1942; Patnaik, 1959). Sinha and Srivastava (1956) recovered it from pig and experimentally infected pig, dog, cat, sheep, goat, cattle, rabbit and guinea pig with this parasite. Rai and Ahluwalia (1958) recovered it from pigs at Aligarh.

In the present investigation the incidence of infection was 11.11 %. Patnaik (1959) reported its occurrence in 6.6 %

of dogs from Orissa and Sahai (1967, 1969) reported in 7.84 % of dogs examined in Bareilly, Uttar Pradesh

Pharyngostomoides sp.

Parasite of this genus had not been reported from carnivorous mammals in India so far. In the present investigation only 3 dogs were found to harbour this parasite in its intestine (8.33 %). This appears to be the first record of this trematode in India. A detail study is still to be completed for specific identification.

CESTODA

Dipylidium caninum (Linnaeus, 1785) Leuckart, 1863.

It is one of the common tapeworms of dogs in India and has been reported by several authors (Gaiger, 1915; Sondhi, 1923; Chandler, 1925; Southwell, 1930; Sami, 1938; Mapelstone and Bhaduri, 1940; Thapar, 1956; Rao, 1958; Gupta, 1961; Malaki, 1966; Sahai, 1967, 1969).

In the present investigation 9 dogs (25 %) were found to harbour this parasite. Sami (1938) found the parasite in 4 %, Maplestone and Bhaduri (1940) in 60.5 %, Rao (1958) in 3 % and Sahai (1969) in 39.91 % of dogs examined.

Echinococcus granulosus (Batsch, 1786) Rudolphi, 1801

The incidence of E. granulosus is common and its occurrence has been reported by several authors (Gaiger, 1915;

Sondhi, 1923; Sami, 1938; Acharya, 1939; Maplestone and Bhaduri, 1940; Mudaliar and Alwar, 1947; Thapar, 1956).

In the present investigation only one dog (2.77 %) were found to harbour the parasite. Sondhi (1923) also reported the incidence of this parasite occasional. On the other hand, Sami (1938) and Maplestone and Bhaduri (1940) found the parasite in 30 % and 10 % of dogs examined by them. Sahai (1969) observed 5.88 % infection in dogs in Bareilly, Uttar Pradesh.

Taenia hydatigena Pallas, 1766

It is a common parasite of dogs in India and has been reported by several authors (Gaiger, 1915; Sondhi, 1923; Southwell, 1930; Sami, 1938; Acharya, 1939; Malestone and Bhaduri, 1940; Rao, 1958).

In the present investigation (19.44 %) of dogs were found to harbour T. hydatigena. Sami (1938) had reported the parasite in 20 %, Maplestone and Bhaduri (1940) in 17 %; Rao (1958) in 8 % and Sahai (1967) in 31.37 % of dogs examined.

Multiceps multiceps (Leske, 1780) Goeze, 1782

In India, Gaiger (1911) recorded this parasite for the first time. Thereafter, it has been reported by many workers.

In the present investigation only two dogs (5.55 %) were found to harbour the parasite. Gupta (1961) also recorded it from dogs in Uttar Pradesh.

NEMATODA

Ancylostoma caninum (Ercolani, 1859) Hall, 1913

This nematode is the most common parasite of dogs in India; and has been reported by several authors (Lane, 1913; Baylis and Daubney, 1922, 1923; Chandler, 1925; Acharya, 1939; Maplestone and Bhaduri, 1940; Mudaliar and Alwar, 1947; Thapar, 1956; Rao, 1958).

In the present investigation out of 36, 26 dogs (72.22%) were found to harbour this parasite in the intestine. Acharya (1939) reported it from 87.5 %, Maplestone and Bhaduri (1940) from 95 %, Rao (1959) from 88 % and Sahai (1967, 1969) from 70.58 % of the dogs examined by them.

Spirocerca lupi (Rudolphi, 1809) Railliet and Henry, 1911

It is also a common nematode of dogs in India. It has been reported by several workers (Acharya, 1939; Decroos, 1941; Mudaliar and Alwar, 1947; Ranganathan, 1950; Vaidyanathan, 1952; Menon, 1953; Chandrasekhariah, 1953; Parmanand Rao, 1953; Thapar, 1956; Hadis, 1956; Chandrasekharan, Sastry and Menon, 1958; Gupta and Pandey, 1963; Malaki, 1966; Sahai, 1967, 1969; Sahasrabudhe et al., 1969).

In the present investigation 10 out of 36 dogs (27.77 %) were found to harbour this parasite in the Oesophageal nodules. Acharya (1939) studied the incidence of the adult and juvenile parasites in dogs and reported the infection in 35.5 % and 6.5 % respectively. Chandrasekharan et al. (1958) examined 910 dogs

and found 23.5 % and Sahai (1967, 1969) observed 19.60 % of dogs infected with this parasite.

The results of the incidence has been presented in Tables 1, 2 and 3. Table 1 is showing the incidence of trematodes, cestodes and nematodes. Table 2 is showing the percentages of different helminths, whereas Table 3 indicates percentage of infection in three different groups of helminths.

Table 1

Showing incidence of helminthic infection in dogs

Sl. No.	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	-	-	-	+	-	-	-	-	-	-
2	-	+	-	-	-	-	+	-	-	-	+	-
3	-	-	-	-	-	-	+	-	-	-	+	+
4	-	-	-	-	+	-	-	-	+	+	+	-
5	+	-	+	-	-	-	-	-	-	-	+	+
6	-	-	-	+	-	-	+	-	-	-	+	-
7	+	-	-	-	+	-	-	-	-	-	+	-
8	+	-	-	+	-	-	-	-	-	-	-	-
9	+	-	-	-	-	-	+	-	-	-	+	-
10	-	-	-	+	-	-	-	-	-	+	-	+
11	-	-	-	+	-	-	-	-	-	+	+	-
12	+	+	+	-	-	-	+	-	-	-	+	-
13	-	-	-	-	-	-	-	-	-	-	+	-
14	+	-	-	+	-	-	-	-	-	+	-	+
15	+	-	+	-	-	-	-	-	-	+	+	-

Contd.

Table 1 (Contd.)

Sl. No.	1	2	3	4	5	6	7	8	9	10	11	12
16	-	-	-	+	-	-	-	-	-	-	+	-
17	+	-	-	+	-	-	+	-	-	-	+	-
18	-	-	-	-	-	-	-	-	+	-	-	+
19	+	-	+	-	-	-	-	-	-	-	+	-
20	-	-	-	+	-	-	-	-	-	-	+	+
21	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	+	-
23	+	-	-	-	-	-	+	-	-	+	+	-
24	-	+	-	+	-	-	-	-	-	-	+	-
25	-	-	-	+	-	+	-	-	-	-	+	-
26	+	-	-	-	-	+	-	-	-	-	-	+
27	-	-	+	-	-	-	-	-	-	-	+	-
28	-	-	-	-	+	-	-	-	-	-	+	-
29	+	-	-	+	-	-	+	-	-	-	-	-
30	+	-	-	-	-	-	-	-	-	-	+	-
31	-	+	-	-	-	+	-	-	-	-	-	-
32	+	+	-	-	-	-	-	-	-	-	+	-
33	+	-	-	-	+	-	-	-	-	-	-	+
34	-	-	-	-	-	-	+	-	-	+	+	+
35	-	-	-	-	-	-	-	+	-	-	+	-
36	+	-	-	-	-	-	-	-	-	-	+	-

1. O. noverca
2. H. taichui
3. E. corvus
4. E. perfoliatus
5. S. incognitum
6. Pharyngostomoides sp.
7. D. caninum
8. E. granulosus
9. M. multiceps
10. T. hydatigena
11. A. caninum
12. S. lupi

Table 2

Showing percentage of helminthic infection in dogs

Sl. No.	Name of parasite	No. of animals examined	No. of animals infected	Percentage of infection
1	Opisthorchis noverca	36	16	44.44
2	Haplorchis taichui	36	5	13.88
3	Echinochasmus corvus	36	5	13.88
4	Echinochasmus perfoliatus	36	11	36.55
5	Schistosoma incognitum	36	4	11.11
6	Pharyngostomoides sp.	36	3	8.33
7	Dipylidium caninum	36	9	25.00
8	Echinococcus granulosus	36	1	2.77
9	Multiceps multiceps	36	2	5.55
10	Taenia hydatigena	36	7	19.44
11	Ancylostoma caninum	36	26	72.22
12	Spirocerca lupi	36	10	27.77

Table 3

Showing percentage of trematodes, cestodes and nematodes infection in dogs

Total No.	No. of animals infected with trematodes	Percentage	No. of animals infected with cestodes	Percentage	No. of animals infected with nematodes	Percentage
36	27	75	21	58.33	31	86.11

HISTOCHEMISTRY AND PATHOLOGY OF LIVER AND PANCREAS IN OPISTHORCHIASIS NOVERCA IN DOGS

HISTOCHEMISTRY OF LIVER AND PANCREAS

HISTOCHEMISTRY AND PATHOLOGY OF OPISTHORCHIASIS NOVERCA IN DOGS

Liver and pancreas are vital organs of the body which may be parasitized by Opisthorchis besides some other parasites. In the present investigation liver and pancreas of dogs were found to be infected with O. noverca and studies on histochemical and histopathological alterations of the infected organs have been made.

HISTOCHEMISTRY OF LIVER IN OPISTHORCHIASIS NOVERCA

Review of Literature

The alterations in the distribution of glycogen, glycoprotein and phosphatase in the liver of rat infected with T. taeniaeformis and of cat infected with Amphimerus pseudofelineus was observed by Lewert and Lee (1953). The changes were particularly evident in the areas close to hyperplastic bile duct epithelium. Mercado and von Brand (1953) described progressively decreasing glycogen content in the liver of female white rats infected with Plasmodium berghei. They opined that the reduction of liver glycogen was due to liver dysfunction and a reduction in the capacity of liver to synthesize glycogen from orally administered carbohydrates.

Sawada et al. (1956) studied histochemistry of the liver of mice infected with Schistosoma and described marked increase in glycogen and fat and a decrease of ribonucleic acid in the cytoplasm of hepatic cells associated with excessive

accumulation of acid mucopolysaccharide in the connective tissue of schistosomal granulomata and wall of portal vessels in the liver. Haemosiderin like pigment present in the von Kupffer's cells of liver was shown to be very much similar to melanin. While working on glycogenesis in the liver of rat infected with P. berghei, von Brand and Mercado (1956) showed that control animals can synthesize glycogen more efficiently than infected ones. This might be due to deficient liver function rather than faulty absorption. Loss of glycogen was visible earlier in those cells lying towards the centre of the hepatic lobules, however, certain cells of infected liver stored more glycogen than others.

von Brand and Mercado (1958), during their studies on rat liver infected with P. berghei, reported that the lipid content of infected liver was higher than that of control ones. Rats having high degree of parasitaemia showed a centrilobular distribution of lipids. Kuwamura (1958), in his experimental infection to rabbits with O. sinensis, studied the histochemical changes of the liver. He considered that in the earliest stages of infection (10 - 15 days) a depression in the function of the hepatic cells took place. The nucleic acids, proteins and polysaccharides content decreased but the positive reaction for alkaline phosphatase was intense. One of the remarkable feature was the appearance of polysaccharide granules in the epithelium of the proliferated bile ducts which was free before. Normal condition existed between 60th and 90th day of infection but after 100th day of infection when cirrhosis developed the

contents of all the essential cell constituents markedly decreased. Vershinin (1958) reported deposition of calcium salts in the necrotic areas of interlobular connective tissue and intima of the blood vessels of the liver infected with Dicrocoelium.

Mercado and von Brand (1960) studied the distribution of liver glycogen and lipids associated with some of the parasitic infestations and observed that every infection produced its own characteristic changes in the distribution of lipid and glycogen. They suggested the possibility of a specific factor originating from the parasite itself which may be responsible for all these changes. Munnich (1960) conducted detailed histochemical studies on the intestine, liver and lung of albino mice infected with Ascaris lumbricoides. They have seen a marked decrease in glycogen, fat, ribonucleic acid and amino-acids of liver and an increase in glycoprotein in the focus of inflammation. The RNA increased in the parenchyma after the 4th day of infection. In his opinion the function of the liver as a whole was not dislocated.

Campbell (1960) considered the nature of pigment deposited in the tissue with Fascioloides magna infection to be iron porphyrin. Kublitskene (1962) observed increased DNA content in the nuclei and decreased glycogen content of hepatic cells in acute fascioliasis of guinea pigs. Kadziolka (1962) described in fascioliasis that during early cirrhosis there was slight reduction in glycogen content but in advance cirrhosis there was a marked glycogen depletion.

During the study on the histochemical changes of the liver of hill bulls, naturally infected with Dicrocoelium. Dhar and Singh (1963) observed complete depletion of glycogen, a higher lipid content and increased lipofuscin deposition in the hepatic cells. The chemical irritation caused by the parasite resulted in excessive mucin production. They suggested that glycogen in the parenchyma of the parasite may have been derived from the hepatic cells. Roneus (1963) observed loss of glycogen from the hepatic cells surrounding the white spots in the liver of pigs with Toxocara cati infection. The pigment deposition has been considered to be of the nature of haemosiderin in O. caninus infection by Gupta and Pande (1963).

Ansari (1968) was the first man to study a detailed histochemical studies of the liver in Opisthorchiasis noverca in dogs. He observed a marked depletion of glycogen in the hepatic cells, its presence in the parenchyma of the parasite and increased mucin secretion by the lining epithelial cells and those of newly formed glandular tissue. A slight increase in lipid and protein contents of infected liver was also observed. The infected liver revealed deposition of pigment granules of haemosiderin and bile. The pigment granules of bile were present in the cytoplasm of the hepatic cells and von Kupffer's cells lining the lumen of the hepatic sinusoids. The collagen fibres formed the main bulk of connective tissue in the area of fibrosis. A decrease in cytoplasmic RNA content of the hepatic cells was observed. No deposition of calcium salts and amyloid material was observed by him.

Banerjee and Singh (1969) observed marked depletion of glycogen in the hepatic cells of rat in cysticercosis. A strongly PAS positive reaction was shown by the outer cyst membranes and contiguous hepatic cells. Various other cells of the hepatic parenchyma gave a less intense positive reaction.

OBSERVATIONS

A. Periodic Acid-Schiff Reaction (Regular PAS)

Normal Liver:- The cytoplasm of the hepatic cells, luminal border of the tubular glands and of the epithelial cells lining the duct gave PAS positive reaction of moderate to strong intensity.

Infected Liver:- The hepatic cells, luminal border of the tubular glands and luminal border of the epithelial cells lining the duct gave very mild reaction, however, they can be given one plus since the outline of the cell was clearly visible even without counterstaining. The lining cells of the tubular glands gave positive reaction but it was very mild except in the basal zone where it gave negative reaction. The cuticle of the parasite gave more intense reaction than its parenchyma (Table 4). The amorphous material in the lumen of the glands showed comparatively stronger reaction than its border area of the cell.

B. Alcian Blue Methods for Mucopolysaccharides

Normal Liver:- The hepatic cells gave negative reaction. Luminal border of tubular glands and the lining

Table 4

Showing histochemical reaction in liver tissue due to Opisthorchiasis noverca

Test	Normal liver			Infected liver			Parasite	
	Hepatic cells	Luminal border of tubular glands	Epithelial lining of bile duct	Hepatic cells	Luminal border of tubular glands	Epithelial lining of bile duct	Cuticle	Parenchyma
1. Regular PAS	++	++	++	+	+	+	++	+
2. Alcian blue	-	+	+	-	+++	+++	-	+++
3. Regular Best's carmine	+++	++	+++	+	+	+	++	++
4. Mercury Bromphenol blue	+++	++	++	+	+	+	++	+
5. von Kossa	-	-	-	-	-	-	-	-
6. Perl's method	-	-	-	-	-	-	-	-

- Negative for the test

\+, ++, +++ Indicate intensity of the staining reaction.

epithelial cells gave very mild reaction, whereas the amorphous material within the glands were not observed as clear lumen was observed in almost all the glands.

Infected Liver:- The hepatic cells of the infected liver also showed negative reaction. The luminal border of the tubular glands and the lining epithelial cells gave strong reaction (Plate I, Figs. 1 & 2). The amorphous material in the lumen of the gland also gave the reaction of the same intensity. The area where there was denudation of the epithelial cells there was negative reaction, but in the same section inspite of the presence of the parasite there was no denudation of the epithelium, still it gave positive reaction. The cuticular border of the parasite gave negative reaction and in contrast to this the parenchyma gave intense positive reaction (Table 4).

C. Regular Best's Carmine Test for Glycogen

Normal Liver:- The hepatic cells gave very intense reaction for glycogen (Plate II, Fig. 1). The luminal border and the basal zone of the glands gave positive reaction. The lining epithelial cells also gave positive reaction. The lining epithelial cells also gave the same intense reaction.

Infected Liver:- The hepatic cells surrounding the involved bile ducts gave less stronger reaction than the normal liver (Plate II, Fig. 2) and similarly the epithelial cells lining the duct and glandular cells gave less intense reaction than normal. The cuticle and the parenchyma of the parasite gave positive reaction (Table 4).

D. Mercury Bromphenol Blue Method for Proteins

Normal Liver:- The cytoplasm of the hepatic cells gave intense reaction for proteins, whereas the free border of the glandular cells and the lining epithelial cells gave mild reaction (Plate III, Fig. 1).

Infected Liver:- The free border of the glands, and the lining epithelial cells of bile duct gave faint reaction (Plate III, Fig. 2). The hepatocytes, around the bile ducts which had cut sections of parasite, were poorly stained with Bromophenol blue indicating the depletion of protein in these cells. However, the liver cells at other places gave deep blue reaction. The cuticle of the parasite gave intense reaction, while reaction in its parenchyma was not so intense (Table 4).

E. von Kossa Method for Calcium

This test did not reveal any deposition of calcium salts either in the infected liver or in the normal livers.

F. Perl's Method for Haemosiderin

The deep ~~blue~~ coloured iron containing haemosiderin pigments could not be observed either in the hepatic cells or epithelial linings in the infected liver as well as the normal ones.

DISCUSSION

Glycogen:- A marked depletion in glycogen content of the hepatic cells around the involved bile ducts of the

infected liver was observed in the present investigation which is in confirmity with previous workers who also noticed depletion of liver glycogen in P. berghei infection in rats (Mercado and von Brand, 1953, 1960; von Brand and Mercado, 1956), in T. taeniaeformis infection of rat (Lewert and Lee, 1953); in early stage of experimental clonorchiasis in rabbit (Kuwamura, 1958); in Ascaris lubbricoides infection of mouse (Munnich, 1960); in dicrocoeliasis of cattle and goat (Dhar and Singh, 1963); in Toxocara cati infection of pig (Roneus, 1963); in cysticercosis of rat (Banerjee and Singh 1969) and in opisthorchiasis in dogs (Ansari, 1968). Contrary to these observations, Sawada et al. (1956) observed a marked increase in liver glycogen of mouse in S. japonicum infection and also von Brand and Mercado (1956) noticed increase in glycogen in certain cells of liver of rat in P. berghei infection.

The various factors which may be associated with depletion in liver glycogen in parasitic infections have been discussed by many scientists from time to time. Mercado and von Brand (1953) thought that the possibility of liver disfunction and semi-starvation by the host as probable cause of glycogen reduction in P. berghei infection of rat, though they failed to explain exact mechanism involved in this. Similar explanation for reduction in glycogen reserve of liver was described in human malaria (Sinton and Hughes, 1924), in trypanosome infected animals (von Brand and Regendanz, 1931), in P. berghei infection of rat (von Brand and Mercado, 1956). Later, Mercado and von Brand (1957, 1960) suggested that adrenal

dysfunction may result in depletion of liver glycogen and faulty carbohydrate metabolism in P. berghei infection. As no study could be done on adrenal involvement in glycogen content during the present study as also not by Ansari (1968) it is not possible at the moment to give any such explanation in O. noverca infection.

Sinton and Hughes (1924) gave anoxia as one of the probability for decrease in glycogen contents of liver in human malaria. It may be true in malarial infection where excessive destruction of R.B.C. or trypanosomal infections where the parasite agglutinate in the smaller blood vessel of heart and lung resulting in localized tissue anoxia and improper aeration of blood (Andrews et al., 1930). But it may not be correct in opisthorchiasis as neither there is an excessive R.B.C. destruction nor do the liver flukes agglutinate in smaller blood vessel since they inhabit the bile ducts. Ansari (1968) also agreed with the present view.

Hou (1955) found that C. sinensis feeds on glucose and protein, the former being derived from blood, support the view that the large amount of glycogen present in the parenchyma of the parasite is derived from the host. Besides this Maegraith (1956) suggested the possibility of a factor, which originates from the parasites and interferes with the oxidative function of the cells, possibly by inhibiting the cytochrome system. Gupta and Pande (1963) have observed the presence of degenerated blood cells in the intestine of O. caninus which confirms the view that the parasites feed on blood. The possibility of glycogen

depletion in the present investigation may be corelated to the fact that the parasites probably feed on blood, as degenerated blood cells were seen in the intestine of parasites. So the liver parenchyma is deprived of the glycogen present in the blood. Secondly, there is some possibility of liberation of some chemicals from O. noverca which interferes with the oxidative function of the cells, as was suggested by Maegraith (1956) and Ansari (1968).

Mucopolysaccharides:- Mucopolysaccharides includes all those substances which resemble carbohydrates in their nature, namely, mucin, mucoid, pseudomucin paramucin (Meyer, 1938). The mucin, a glycoprotein, is secreted by highly specialised epithelial cells of mucous membrane, called "goblet cells".

In the present studies free border of the tubular glands and the lining epithelial cells showed excessive production of mucin. The amorphous material in the gland also showed the same reaction.

Kuwamura (1958) in experimental clonorchiasis and Dhar and Singh (1963) in dicrococliasis have also observed increased amount of polysaccharide granules and excessive mucin production in epithelial cells of the affected bile ducts. Sawada et al. (1956) also reported an excessive accumulation of acid mucopolysaccharides in the wall of portal vessels of the hepatic tissue of mouse in S. japonicum infection. Ansari (1968) found similar observations in the liver of dog infected with O. noverca.

To find out the exact cause of increased mucin production in the bile ducts, due to parasites, is difficult. But the explanation given for this, by Dhar and Singh (1963) in dicrocoeliasis in goat and cattle and by Ansari (1968) in opisthorchiasis in dogs are of considerable importance. The presence of worms in the bile ducts may cause mechanical irritation due to its movement or due to the suckers, while holding the mucosa in the form of a plug. The metabolic products of the living worms as well as the waste products of the dead and disintegrating worms may produce chemical irritation.

Although increased mucin production is easy to demonstrate but difficult to find out the exact mechanism or the factors which is responsible for this. It is considered that irritation either mechanical or chemical in nature are responsible for excessive mucin production. It is also in confirmity with Florey (1954) who described the main function of mucus to dilute the irritant and evacuate it.

Protein:- In the present investigation there was a marked decrease in the protein content of hepatic cells around the parasite. Kuwamura (1958) also observed a decrease in protein content of the liver in early stage of Clonorchiasis sinensis in rabbits. However, contrary to this Ansari (1968) recorded a slight increase in the protein content of infected liver. Kuwamura (1958) stated that a continuous hepatic disturbance existed between 10th and 30th day of infection which may be responsible for these changes.

However, the exact mechanism of decrease protein synthesis in parasitic infection like O. noverca is not clear. Liver cells possesses abundant rough endoplastic reticulum where protein is synthesised (Racela et al., 1966). It has been observed by Ramalingswami et al. (1954) that in protein deficiency also, R.N.A. content of periportal hepatocytes is decreased which subsequently show fatty infiltration. In the present study also the liver cells which showed marked decrease in stainable protein, revealed fatty infiltration on histological examination. Thus, the basic mechanism of fatty liver of O. noverca infection may be the same as that of protein malnutrition which causes failure of secretion of triglyceride from the liver to serum consequent upon depressed synthesis of lipoprotein due to shortage of protein (Truswell et al., 1969). In protein malnutrition the decrease in R.N.A. content of periportal hepatocytes appeared much earlier than fatty infiltration (Ramalingswami, et al.,1954). The same may also be true for O. noverca infection. It appears that the first reaction of the hepatic cells to the parasitic infection is to reduce the production of protein and the other changes follow next.

Calcium:- In the present investigation no stainable calcium salts was observed either in the bile duct or interlobular connective tissue of the infected liver. Similarly Dhar and Singh (1963) in advanced cases of dicrocoeliasis and Ansari (1968) in opisthorchiasis could not find any deposition of calcium salts.

However, contrary to these observation deposition of

calcium salts, in liver was reported by Vershinin (1958) in dicrocoeliasis by Smith and Jones (1963) in fascioliasis and by Hou (1955) in clonorchiasis.

HISTOCHEMISTRY OF PANCREAS IN OPISTHORCHIASIS NOVERCA

Sahai (1969) recorded the occurrence of O. noverca for the first time in the pancreas of dog, though earlier Bhalerao (1931) and Sinha (1968) had recorded its occurence from the pancreas of pigs. During the present investigation 25 % of dogs were found to inhabit this parasite in the pancreas. Keeping in view the high incidence of this parasite in pancreatic duct, an endeavour was made to study the histochemical changes of pancreas of dogs having O. noverca infection, specially when no information is available in the literature regarding chemical changes of the pancreas during this infection.

OBSERVATIONS AND DISCUSSION

A. Periodic Acid-Schiff's Reaction (Regular PAS)

Normal Pancreas:- The normal parenchyma of parasite did not show PAS positive result, but the collagen fibres present in the interlobular connective tissue and surrounding the alveoli were weakly positive. The lining epithelial cells of the pancreatic duct were weakly positive at their luminal ends. Mucosal glands showed somewhat better reaction at the luminal ends of lining cell. Amorphous material present in the lumen of some of the mucous glands were also PAS positive. The collagen fibres constituting the framework of the duct were also positive.

Infected Pancreas:- The infected pancreas revealed variable results with PAS. The amorphous material in the lumen of the mucosal glands gave comparatively intense reaction. However, no remarkable differences in regards to the gland parenchyma and also in the luminal ends of the lining cells of the ducts were seen. The collagen fibres of these pancreatic ducts gave more intense reaction. The cuticle and the parenchyma of the parasite gave more or less similar intense reaction.

Similar chemical changes were also noticed in the liver of dogs infected with O. noverca during present studies as well by Ansari (1968).

B. Alcian Blue for Mucopolysaccharides

Normal Pancreas:- The normal parenchyma of pancreas gave a negative reaction for mucopolysaccharides. The lining epithelial cells of the duct and that of the glands gave a feeble reaction. The collagenous material were also negative.

Infected Pancreas:- Luminal ends of the pancreatic duct and the lining cells of mucosal glands were positive (Plate IV, Fig. 1) but collagen fibres gave almost negative reaction in the infected pancreas. The parenchyma of pancreas and the collagen fibres of the parenchyma were also negative for mucopolysaccharides. Luminal ends of the lining cells of the pancreatic ducts gave more intense reaction surrounding the parasites. On the other hand, the luminal ends of the lining cells of the duct away from the parasite gave less intense reaction which was almost similar to that of normal pancreatic duct.

Similar observations were made in the liver of dogs having O. noverca infection by me and Ansari (1968).

C. Regular Best's Caramine Test for Glycogen

Normal Pancreas:- The mucosal glands and the lining epithelial cells of the pancreatic duct gave positive reaction for Best's caramine test.

Infected Pancreas:- In the infected pancreas, the lining epithelial cells of the duct gave comparatively weaker reaction than the normal ones. Mucosal glands also gave similar intense reaction. Best caramine also revealed the fact that the pathogenicity due to the infection of O. noverca was localised which was evident due to more intense reaction in the lining epithelial cells and the mucosal glands of the duct located away from the parasite.

Similar observations were observed in case of liver in O. noverca infection in the present investigation as also by Ansari (1968).

D. Mercury Bromphenol Blue Methods for Protein

Normal Pancreas:- The normal pancreas gave deep blue reaction in the collagen fibres of the duct and the interlobular and interalveolar septa of the gland. The lining epithelial cells and the mucosal glands of the duct also gave deep blue colouration at their luminal ends and in the basal zone. The rest part of the lining cells of the mucosal glands and parenchyma of pancreas gave blue colouration.

Infected Pancreas:- In the pancreas infected with O. novercs, the stainable protein showed a mild reaction in the free borders of the glands. The lining epithelial cells of the glands gave comparatively intense reaction, that of the glandular cells. The parenchyma of the infected pancreas was also showing depletion of proteins than the normal ones (Plate IV, Fig. 2). The cuticle of the parasite was comparatively intense than that of its parenchyma.

Similar histochemical changes were observed in the liver due to opisthorchiasis in the present investigation.

PATHOLOGY OF LIVER AND PANCREAS

PATHOLOGY OF LIVER

Opisthorchiasis

Essex and Bollman (1930) studied the pathological changes of the liver of a cat infected with O. pseudofelineus and Metorchis complexus. He observed extensive hyperplasia of the biliary duct system, and fibrous connective tissue surrounding bile ducts increases two to three times the normal. Zheltikoff (1932) observed liver lesions which included a circumscribed fibrosis of the bile ducts with some epithelial proliferation in a fatal human case of opisthorchiasis. Bol and Yakovleff (1932) described catarrhal inflammation of the bile duct, accompanied by epithelial proliferation and sometimes cystic dilatation in cat's liver infected with O. felineus. Hoogland (1932) described hepatic tumour of adenocarcinomatous type in a dog having O. felineus infection. KondratyeFF (1932) reported liver dysfunction in eight cases of human opisthorchiasis. Plotnikov (1939) observed cholecystitis and angiocholecystitis in human opisthorchiasis. Turk (1949) observed marked dyspnoea and severe jaundice in a cat infected with O. pseudoflineus.

Adenocarcinoma of the liver in two Thailanders due to heavy infection of O. viverrini in the bile ducts have been reported by Viranuvatti and Mattiyawongse (1953), while Botti (1954) and Salutini (1954) have observed adenocarcinomatous growths in the liver, lung, bronchial lymph glands of dogs with O. felineus infection. Sadun (1955) reported cirrhosis of the liver and carcinoma of the bile ducts in O. viverrini

infections in cat, dog and man. Altukhova (1956) and Kraft (1956) described in a man characteristic haemopoiesis and biliary peritonitis in opisthorchiasis. Levine, Beamer and Maksie (1956) described hepatitis in a cat associated with O. pseudofelineus infection. The microscopic studies of the liver revealed the distension of bile ducts containing parasites, peripheral cirrhosis, proliferation of the bile ducts with centrilobular fatty changes and chronic passive congestion of the liver. Bhatia, Sood and Pande (1959) studied the pathology of the liver of a cat and a dog infected with Paropisthorchis caninus. They described a marked cirrhosis, dilatation of bile ducts and proliferative changes in the cells around smaller bile ducts with severe eosinophilic infiltration. Harinasuta and Vajrasthira (1960) described symptoms of painful and enlarged liver with jaundice, eosinophilia, hepatic cirrhosis and carcinoma in advance cases of O. viverrini infection.

Rothenbacker and Lindquist (1963) also described cirrhosis of the liver in a cat infected with O. pseudofelineus. Rampichini, Nardi and Anantonio (1963) recovered two litres fluid from the abdominal cavity of a dog which died due to O. felineus infection and also observed damaged liver. Koshukhov (1963) recorded the various pathological changes in the liver and bile ducts of 120 white mice and 8 Kittens following experimental opisthorchiasis.

Gupta and Pande (1963) described the pathological changes in the liver of dogs having O. caninus infection. They observed a coagulative necrosis of the biliary epithelium with

desquamation and proliferation associated with crypt formation. In milder cases they observed a few lymphocytes and connective tissue cells in the walls of bile ducts, while in severe infection extensive damage to the epithelial lining with marked leucocytic infiltration and periportal connective tissue hyperplasia were found. At some places eosinophils and lymphocytes replaced the liver parenchyma. Haemosiderin deposits in the sinusoids and congestion of vessels at places were other features observed by them.

Teoh (1963) reported 24 cases of gall stones of parasitic origin from the common bile duct, hepatic and intrahepatic bile ducts associated with recurrent pyogenic cholangitis out of which O. sinensis was responsible for 8 cases. Al-Dabagh et al. (1964) described the pathological changes in the liver of dog and cat associated with moderate and heavy infection with O. tenuicollis in Iraq. Hepatocellular carcinomas and cholangiomas were important features mentioned in heavy infestation. Large area of necrosis, haemorrhage and extensive proliferation of biliary epithelium were also noticed. The parenchyma revealed areas of degeneration, infiltration with lymphocyte and neutrophils and fatty degeneration specially at the periphery of the lobules.

Sahai (1967) and Sahai and Srivastava (1971) studied the pathological changes of liver of dogs infected with O. noverca. The essential changes were mostly confined to the bile ducts, though changes in the parenchyma were also noticed. They observed varying degree of cholangitis, cellular infiltration,

biliary cirrhosis and increase in periportal connective tissue. In the hepatic parenchyma, early portal cirrhosis, degenerative changes in hepatic cells hypertrophy and hyperplasia of von Kupffer's cells were observed.

Sastry and Patnaik (1968) noticed the pathological changes mostly in the left lobe of liver, however, in heavy infection it was found in both. Extensive proliferation of bile duct epithelium and formation of fibrosis in adjoining areas with consequent pressure atrophy of surrounding liver tissue were common features. Ansari (1968) also found the essential changes confined mainly to the bile ducts which were characterised by the presence of varying degree of non-suppurative cholangitis with mild degree of peribiliary cirrhosis and cellular infiltration. He also noticed excessive mucin secretion and extensive area of haemorrhage in the liver parenchyma. Mongeau (1961) recorded infectious canine hepatitis in dogs due to massive infection of Metorchis conjuctus. Srivastava (1972) described O. noverca, a common and pathogenic liverfluk of dogs and pigs in India.

Clonorchiasis

Hoeppli (1933) during postmortem examination of 66 Chinese attributed the cause of death due to clonorchiasis. Microscopic changes revealed dilatation of the wall of the bile ducts, thickening of the wall and formation of new glandular tissue in livers. Kouri et al. (1936) described cancer of cholangio-cellular type and observed that clonorchiasis is the

primary cause. Helwing and Brown (1946) observed dilatation of bile ducts, periductal fibrosis, hyperplasia of the biliary epithelium, formation of new bile ducts and cholangitis in C. sinensis infection in man. Ling and Taur (1949) reported the death in a man due to C. sinensis infection.

Hou (1955) described the pathological changes in the liver due to C. sinensis infection and observed that the seat of main pathological changes in acute cases were localised to the walls of bile ducts, which were mainly due to abundant adenomatous tissue formation with little connective tissue. In chronic cases there was more increase in the connective tissue. In his opinion clonorchiasis was a chronic disease which may or may not be complicated with bacterial infection. The most serious consequences of this disease was malignant change in adenomatous tissue of the bile ducts. Hou considered the changes to be due to the obstruction of bile ducts.

Again, Hou (1956) incriminated C. sinensis as the casual parasite in the genesis of carcinoma of liver. He opined that C. sinensis caused stimulus which provoked persistent hyperplasia of the epithelial cells which finally lead to malignancy. He thought that the mechanical irritation caused by the parasite or chemical substances liberated might work as stimulant. He postulated that the chemical may be either extrinsic, originating from the metabolic or degenerating products of the parasites or intrinsic derived from the bile, since carcinogenic hydrocarbons are chemically related to bile acids.

Chin, Lei and Wang (1955) and Lingard, Huestis and

MacLean (1958) also recorded carcinoma associated with clonorchiasis. Yamagata and Yaegashi (1964) gave a number of references to the incidence of tumors and cancers associated with clonorchiasis and suggested that not only the parasite but also the resulting cirrhosis should be considered as the aetiological factor.

OBSERVATIONS

Gross Pathology

In early stages of infection the liver did not reveal any gross changes externally except for slight induration and discoloration. The gall bladder was slightly enlarged, and the cut surfaces of liver showed slight congestion with slight thickening of the bile ducts containing a few parasites in it.

In cases of heavy infection or advanced stages of infection the liver was much enlarged, fragile and showed a soft consistency. The liver parenchyma showed discolouration with multiple haemorrhagic spots on it. The gall bladder was very much distended in some of the advanced cases of infection with dark or deep green bile and parasites in it. The cut surfaces of liver showed greatly thickened and dilated bile ducts and when pressed the parasites were discharged in large numbers.

Histopathology

The pathological changes varied in early and advanced cases of infection.

In mild or early stages of infection the changes were not well marked and were localised only to the epithelial lining of the bile ducts. There was mild degree of proliferative changes in the walls of bile ducts with desquamation of the lining epithelial cells. The bile duct showed a few cut sections of the parasite with desquamated epithelial cells and mucus (Plate V, Fig. 1). The hepatic cells did not reveal any marked alterations.

In moderate to severe infection the extensive changes were seen in and around the bile ducts and the liver parenchyma, however, the alterations were mainly confined to periportal region. The affected bile ducts appeared to be dilated and their wall thickened. In the lumen of these bile ducts there were one or more cut sections of the parasites. The lining epithelial cells of the bile duct showed varying degree of hyperplasia and hypertrophy. In some cases, the lining epithelial cells were desquamated and were lying free in the lumen of the bile duct. Desquamation of lining epithelial cells were also observed in which the parasites were absent. The connective tissue showed proliferation around the bile ducts (Plate VI, Fig.1). In some cases due to hyperplastic changes of the lining epithelial of bile ducts, it gave the multilayered appearance. There was also infiltration of a few mononuclear cells in the peribiliary region. There was an increase in the amount of peribiliary connective tissue. The nature of connective tissue deposited were collagen like which was confirmed by von Gieson's staining.

In the periportal region the blood vessel appeared to be dilated.

In addition to the bile ducts the liver parenchyma also showed changes. Liver parenchyma adjacent to the bile ducts showed varying degree of degenerative changes. These were pyknosis, karyorrhexis and/or karyolysis of the nuclei of the hepatic cells. The cytoplasm of the hepatic cells showed cloudy swelling and fatty degeneration (Plate VI, Fig. 2). At places the hepatic cells showed frank necrosis. Hypertrophy of von Kupffer's cells were also evident.

Focal area of infiltration with mononuclear cells were also observed in the liver parenchyma and these mononuclear cells mainly consisted of lymphocytes macrophages and a few plasma cells (Plate VII, Fig. 1).

Briefly, the histopathological changes in the liver of dog due to opisthorchiasis were confined in and around the bile ducts with varying degree of non-suppurative cholangitis, associated with biliary cirrhosis, hyperplastic changes in the lining epithelium, slight increase in the peribiliary connective tissue and mononuclear cell infiltration. The hepatic parenchyma showed varying degree of degenerative changes with focal area of cellular infiltration and hypertrophy of von Kupffer's cells (Plate VII, Fig. 2).

DISCUSSION

O. noverca infection in the liver of dogs was characterised grossly, by irregularly thickened or greatly dilated bile ducts with an enlarged and fragile liver. These are in agreement with those described by Sahai (1967), Ansari (1968),

Sahai and Srivastava (1971) in O. noverca infections in dogs. Harinasuta and Vajrasthira (1960) also described painful and enlargement of liver in human beings due to O. viverrini infection and Sastry and Patnaik (1968) observed dilatation of the bile ducts with a pale and friable liver in O. felineus infection.

The present study revealed discoloration of the liver with haemorrhagic spots in case of heavy infection. Similar observation have also been reported in P. caninus infection in cats (Bhatia, Sood and Pande, 1959) but in addition they have also observed seed like structures on the surface of the liver which could not be seen in present study. Sahai (1967), Ansari (1968) and Sahai and Srivastava (1971) also described similar lesions in O. noverca infection in dogs.

The main histopathological lesions were non-suppurative cholangitis associated with biliary cirrhosis as was also reported by previous workers (Sahai, 1967; Ansari, 1968; Sahai and Srivastava, 1971). The cholangitis in O. noverca infection was, however, of non-suppurative type in contradiction to the suppurative cholangitis in C. sinensis infection in the liver of man reported by Hou, 1955. The absence of neutrophilic infiltration in the histological sections of liver is in agreement with those of O. felineus in cat (Bol and Yakovleff, 1932), of C. sinensis in man (Hoeppli, 1933) and of O. noverca infection in dogs (Sahai, 1967; Ansari, 1968; Sahai and Srivastava, 1971).

In the present study the alterations were mostly observed in and around the bile ducts, mainly in the larger bile ducts because a number of parasites were found in it. The pronounced

changes in the bile ducts observed were hypertrophy and hyperplasia of duct epithelium, degeneration and desquamation of lining cells. Similar changes were also observed in cats infected with O. pseudofelineus and Metorchis complexus (Essex and Bollman, 1930), O. felineus (Bol and Yakovleff, 1932), O. pseudofelineus (Levine, Beamer and Maksie, 1956; Rothenbacker and Lindquist, 1963), P. caninus (Bhatia, Sood and Pande, 1959) and in dogs by O. caninus (Gupta and Pande, 1963), O. tenuicollis (Al-Dabagh et al 1964), O. noverca (Sahai, 1967; Ansari, 1968; Sahai and Srivastava, 1971) and O. felineus (Sastry and Patnaik, 1968). Similar observation have been also made in clonorchiasis in man (Hoeppli, 1933) and in cats (Hou, 1965).

There was infiltration of a few mononuclear cells in the subepithelial layer of the bile duct. Contrary to this Sahai (1967) and Sahai and Srivastava (1971) have observed heavy infiltration of mononuclear cells with a few eosinophils and lymphocytes in the lamina propria of the affected bile ducts in O. noverca infection. Bhatia, Sood and Pande (1959) described an intense eosinophilic infiltration in P. caninus infection in the lining cells around the smaller bile ducts. Ansari (1968) also described cellular infiltration in the subepithelial layer in O. noverca infection.

Focal infiltration of mononuclear cells consisting mainly of lymphocytes, macrophages and plasma cells have been observed in liver parenchyma during the present investigation. Gupta and Pande (1963) also observed focal accumulation of cells, mostly lymphocytes along with a few eosinophils, replacing liver

parenchyma. Sastry and Patnaik (1968) also noticed infiltration of eosinophils, mononuclear and plasma cells in between the acini in O. felineus infection.

In the present study frank necrosis of liver parenchyma adjacent to the bile duct was also observed with other degenerative changes of varying degrees. The degenerative changes in the hepatic parenchyma has also been reported in dog infected with O. caninum (Gupta and Pande , 1963), O. tenuicollis (Al-Dabagh et al., 1964) and O. noverca (Sahai, 1967; Ansari, 1968; Sahai and Srivastava, 1971).

A similar account has been given in O. felineus infection in rabbits (Otto, 1937), which was supported by the work of Olafson (cited by Sahai and Srivastava, 1971), who succeeded in producing experimentally almost similar lesions in the liver of cattle with chemical agent. Plowright, Burdin and Thorold (1952) produced hyperplasia of bile ductules associated with retrogressive changes in the hepatic cells by chemical agents like Dimidium bromide. This is further supported by the findings of Botti (1954), who reported the occurrence of metastatic lessions in the lungs of dogs having infection of O. felineus.

The metabolites of the parasites are considered to be responsible for hyperplastic changes in the bile ducts and other parenchymatous degeneration also in other liver flukes of animals - in fascioliasis in sheep (Sogoyan, 1955), in dicrocoeliasis in sheep (Sogoyan, 1960), in goat and cattle (Dhar and Singh, 1963), in opisthorchiasis in dogs (Sahai, 1967; Ansari, 1968; Sahai and Srivastava, 1971) and in Opisthorchiasis felineus

in dogs (Sastry and Patnaik, 1968). The hypertrophy of von Kupffer's cells is also suggestive of the presence of some foreign protein, as was described by Sahai (1967) and Sahai and Srivastava (1971).

During the present study, there was an increase in mucin secretion as determined by Alcian Blue methods of staining and so possibility of excessive mucin production, a factor to bring about certain hyperplastic changes, as was reported by Hou (1955) cannot be ruled out.

Thus, the hyperplastic changes of bile ducts and liver parenchyma in this also is either due to one or all of the following factors as reported by Hou (1955).

(i) due to mechanical obstruction by the worms in confirmity with Cameron and Prasad (1960). This may be true for the bile ducts having heavy infection;
or (ii) due to a mild toxin, supposed to be liberated by the worms, as was reported by Otto (1937), Sahai (1967), Ansari (1968), Sastry and Patnaik (1968), Sahai and Srivastava (1971). This may be true for the bile ducts showing alterations but did not contain any worm in them or (iii) due to excessive production of mucin as evident by histochemical technique, as was reported by Hou (1955).

The above mentioned hyperplastic alteration in bile ducts and liver parenchyma are suggestive of some chemical or toxic substances, liberated by the parasites. During his studies on the pathology of Clonorchis sinensis infection, Hou (1955)

described three factors responsible to bring about adenomatous tissue formation (a) Mechanical irritation, (b) chemical irritation and (c) functional requirements (due to excessive mucin production). The absence of sharp spines and other appendages in O. noverca preclude the possibility of mechanical irritation. The suckers of the parasite may cause some degree of irritation to the mucosa of the bile ducts, specially during the feeding activities but this may not be sufficient mechanical stimulus to bring about such changes. Still there is a possibility that mechanical obstruction by these worms may produce similar lesions, as was reported by Cameron and Prasad (1960). But the presence of aforesaid pathological alterations in those bile ducts also where parasite was absent, strongly suggests that some chemical or toxic irritant either extrinsic (derived from metabolic products) or intrinsic (originating from the bile) in nature may be involved to bring about these changes.

PATHOLOGY OF PANCREAS

Review of Literature

For the first time Plotnikov (1939) observed chronic pancreatitis in man caused by opisthorchiasis. Pancreatitis in cats due to O. felineus infection was observed by Ramgnoli (1949). Morgan and Hawkin (1963) described thickening of the walls of pancreatic ducts due to fibrosis in O. pseudofelineus infections in dog and cat. Rothenbacker and Lindquist (1963) observed pancreatitis in a cat infected with A. pseudofelineus. They observed chronic inflammation, epithelial proliferation in larger pancreatic ducts having flukes.

Sahai (1967) and Sahai and Srivastava (1971) studied the pathology of pancreas of pigs in opisthorchiasis noverca. The lining epithelial cells of the duct showed hyperplastic changes. The hyperplasia was more severe where a papillomatous pattern of epithelium was observed. Marked infiltration of mononuclear cells in hyperplastic epithelium were also observed. There was increase of connective tissue elements in the walls of the pancreatic ducts and blood vessels were slightly congested.

In a brief note, Sinha (1968) described pathological changes due to O. noverca in pigs (Sus scrofa domestica). The most conspicuous changes were extensive proliferation of ductal epithelium with the formation of complex folds or crypts projecting in the lumen. In majority of cases the epithelium showed marked increase in secreting cells and more or less distended with their secretion, cellular infiltration in lamina propria were mainly lymphocytes and few eosinophils. Thus, a picture of chronic pancreatitis was observed by him. In some of the early cases of infections he observed little proliferative changes in epithelial lining cells and less periductal fibrosis but marked cellular infiltration in tunica propria.

OBSERVATIONS

Gross Pathology

No specific macroscopic change was observed in the pancreas of dogs infected with O. noverca. The parasite was found to be present in the lumen of the pancreatic duct. In heavy infections, the lumen of the duct was found occluded with the parasites.

Histopathology

The cut section of the parasite were lying in the lumen of the dilated pancreatic acinar duct or dilated exocrine pancreatic duct (Plate VIII, Fig. 1). There was a very severe degree of hyperplasia and hypertrophy of the epithelial cells lining the pancreatic duct. Severe degree of proliferation of the supporting connective tissue could also be seen. There was also a good amount of proliferation of the periductal connective tissue with well developed collagen fibres (Plate IX, Fig. 1). The acinar cells adjacent to the duct showed atrophy. There was also loss of zymogen granules. At places the nuclei were lying naked (Plate IX, Fig. 2).

In some of the advanced cases pancreatic duct was almost completely replaced by connective tissue (Plate IX, Fig.1). Atrophy of the acinar cells were much more severe with proliferation of connective tissue in these cases (Plate X, Fig. 2). Although cellular infiltration was not a feature in early cases but in advanced cases cellular infiltration was invariably seen. No apparent histopathological changes in the endocrine pancreas was observed.

DISCUSSION

The chief lesions in O. noverca infection in the pancreas of dogs were varying degree of proliferation of the supporting connective tissue and periductal connective tissue associated with pancreatitis. Plotnikov (1939) observed 36 % cases of chronic pancreatitis in human opisthorchiasis.

Ramagnoli (1949), Rothenbacker and Lindquist (1963) also observed pancreatitis in cat. Similar results were also observed by Sahai (1967), Sinha (1968) and Sahai and Srivastava (1971) in O. noverca infection of pigs.

Cellular infiltration in advanced cases was invariably seen, as was reported by Sahai (1967), Sinha (1968) and Sahai and Srivastava (1971).

The proliferation of the periductal connective tissue associated with well developed collagen fibres were also noticed as were reported by Morgan and Hawkin (1953) in O. pseudofelineus infection in dog and cat.

Similar pathological changes in the pancreatic duct which did not contain any parasite were observed. Therefore, these hyperplastic changes in pancreatic duct during the present investigation also is considered to be associated with some metabolites of the parasites as was discussed in the pathology of liver due to O. noverca infection.

PATHOLOGY OF INTESTINE IN DIPYLIDIUM CANINUM INFECTION IN DOGS

PATHOLOGY OF INTESTINE IN DIPYLIDIUM CANINUM INFECTION

Review of Literature

Jubb and Kennedy (1963) have described D. caninum as the most ubiquitous of the canine tapeworms which inhabits the small intestine. According to Smith and Jones (1963) the adult tapeworms apparently produces little serious effect upon the host except in very heavy infections in which they interfere with digestion or cause partial obstruction. Nath and Pande (1963) made histological study of the intestine of domestic fowl infected with Amoebotaenia sphenoides. They also observed the damage done by the parasite to be of lesser degree. The solices of the parasite were burrowed deeply into the mucosa, which in some cases also reached the muscularis mucosae. The surface epithelium was damaged from disintegration and the adjoining glands completely destroyed. The suckers containing mucosal patches and with certain degree of haemorrhage in the vicinity were noticed by them. At places where the rostellar hooks were deeply burrowed cellular infiltrations mainly of lymphocytes were observed.

Lapage (1968) remarked that cestodes, in general, are not very harmful to dogs and cats. Rees (1967) described the damage due to the tapeworms infestation as the exception rather than a rule. The mechanism by which tapeworm causes damage are passive obstruction of the lumen of the intestine traumatic action, migration to unusual sites, pyogenic action following

trauma, spoliative and toxic action reported by Rees (1967).

Although in recent years, Malaki (1966) and Sahai (1969) reported quite heavy infection of D. caninum in dogs of Bangalore and Bareilley, still no work on its pathogenicity has been reported. So pathology of intestine in D. caninum infection has been studied and reported hereunder.

OBSERVATIONS

Gross Pathology

The parasite D. caninum was recovered from the small intestine containing semi-solid materials. The parasite was firmly attached to the mucous coat of the intestine with its scolex embedded in the deeper layer of mucosa and the rest portion lying free in the lumen. The mucosa was covered with excessive white secretion and small intestine was slightly inflammed. Thus, this infection was characterised by a mild hyperaemia of the mucous coat of the small intestine.

Histopathology

The histological sections revealed the cross section of the tapeworms embedded in the mucosa of the small intestine with rostellum extended and suckers were closely attached to the villi in some cases (Plate XI, Fig. 1). There was a slight degree of tissue destruction near the adjoining areas of suckers. The cavities of suckers contained some mucus and some tissue debries which were probably due to the pulling action of the parasite.

There was a marked hypertrophy of the epithelial cells. The desquamation of the epithelial cells in large amount in some cases were also observed. Infiltration of substantia propria with a few mononuclear cells was also seen. Proliferation of the connective tissue of the substantia propria was also marked. Thus the D. caninum infection was characterised by proliferation and desquamation of epithelial cells of the intestine.

DISCUSSION

In the present study the suckers of the parasite causing tissue destruction and cavity of the suckers containing tissue debris were seen. Similar changes were also reported by Nath and Pande (1963) in case of A. sphenoides infection in poultry. Since the suckers in case of D. caninum is also unarmed, and moreover it only works as an organ of attachment probably it may not cause appreciable degree of damage to the intestine of host.

As this parasite possesses a protrusible rostellum armed with hooks, the changes encountered in this study might be due to the irritation caused by these hooks. In the present study proliferation, desquamation of epithelial cells and infiltration of mononuclear cells were observed which was also observed in case of A. sphenoides by Nath and Pande (1963).

The cestodes have no alimentary system like trematodes and nematodes and that is why they inhabit in alimentary canal of the host except in a few cases. They absorb their food

from the nutrients available in the surrounding host tissue through the small pore of their cuticle. Thus, robbing of the nutrients and practically causing very little or no pathological changes to the host tissue, can be explained in D. caninum infection also.

CHEMOTHERAPY OF HOOK WORM INFECTION IN DOGS

CHEMOTHERAPY OF HOOK WORM INFECTION IN DOGS

General

The Indian Herbs Research and Supply Company, Saharanpur, Uttar Pradesh has produced an anthelmintic "Wopell" from indigenous products. The powder contains several known anthelmintics. All of them were added in equal parts as follow: Kamala Powder, Pulv. Babarang, Pul Palas Papra, Pulv. Arecanuts, Pulv. Malefern. The firm has recommended its use as an anthelmintic against common roundworms and tapeworms of domestic animals besides dogs.

On persual of literature references are available for the individual ingredients of Wopell. Birdwood (1936) reported Embelia ribes (Babarang) and Mallotus philippinensis (Kamala) as a remedy for tapeworm infection and he further prescribed Pulv. arecanut as a good vermifuge. Mukerji and Bhaduri (1947) tried Butea frondosa (Palas), Embelia ribes and Mallotus philippinensis in hookworm, Ascaris, Taenia and Hymenolepis infestations of man. They observed that the drug has no effect on hookworms and tapeworms, however, they found Butea and Embelia better than santonin and equally good to that of oil of chenopodium in the treatment of ascariasis.

Garg and Mehta (1958) tested anthelmintic efficacy of the decoction of seeds of Butea frondosa and Embelia ribes against Bunostomum trigonocephalum and Haemonchus similis in vitro. They observed Butea superior and Embelia inferior to that of copper sulphate solution against these worms. They have

used normal saline as control. Arecanut, Kamala and Malefern have been suggested for the treatment of tapeworms in the horse, but none of these substances have been critically examined (Gibson, 1965).

Since this product is new one, not much experimental trials are available in literature. However, Nanda (1971 - Personal Communication) during his experimental chemotherapeutic trials in 15 calves, 2 buffaloes and one bullock suffering from ascariasis has found wopell as a good vermicide.

EXPERIMENTAL AND OBSERVATIONS

Five adult, medium sized pariah dogs suffering from natural infection of hookworm were procured and maintained for the entire experimental period. Infection of hookworm (A. caninum) was confirmed by finding the eggs of the parasite in their faeces. Table 5 shows the result of faecal examination by direct and flotation techniques.

Table 5

Showing result of faecal examination

No. of dogs	Observation					
	Direct Technique			Flotation Technique		
	1st	2nd	3rd	1st	2nd	3rd
1	+	-	+	+	+	+
2	-	+	+	+	+	-
3	-	+	+	+	+	+
4	+	-	-	+	-	+
5	-	+	+	+	+	+

In order to find out the intensity of infection faecal egg counts were made and 2650, 900, 1630, 730 and 3160 egg/gm of faeces were found in dog Nos. 1 to 5. Intensity of infection was found to vary from mild (730 eggs/gm) to severe ones (3160 eggs/gm). Dogs having severe infection showed comparatively emaciated and anaemic condition with loose stool. These dogs were divided into two groups with one dog as control. Dog Nos. 2, 3 (Group I) were treated with 1 gm of Wopell, No. 4,5 (Group II) were treated with 2 gms of Wopell and dog No. 1 was kept as control. The drug was administered orally. Faecal samples were examined regularly till the dogs were autopsied. In the first round only dog No. 2 (Group I) and dog No. 4 (Group II) were sacrificed. The worms collected from these dogs Nos. 2 and 4, only 47 % and 56 % respectively were found to be dead. A second dose of 1 gm of the drug was administered to dog No. 3 (Group I) and 5 (Group II) which were not sacrificed. The egg count of these two dogs (No. 3 and 5) were made pre- and post-medication, i.e. after second dose. Dog No. 5 had 100 egg/gm as compared to dog No. 3 which showed 230 egg/gm. The number of dead worms recovered after antopsy were 58 % and 61 % in dogs Nos. 3 and 5. The control dog continued to pass the eggs in faeces in similar intensity. The results are presented in Table 6.

DISCUSSION

In the present experimental chemotherapeutic trials, Wopell was found to be effective against ancylostomiasis in dogs. Complete cure was not achieved as both dead and living hookworms could be recovered from the intestine of the treated

Dog nos.
*1
2
3
4
5

of the present anthelmintic "Wopell". Thus, it proves that individual ingredient may not be effective as an anthelmintic but their combined action had satisfactory anthelmintic activity.

SUMMARY

SUMMARY

INCIDENCE AND NATURE OF HELMINTHIC INFECTIONS IN DOGS

A survey of the helminth parasites on 36 pariah dogs was conducted. 97.22 % of the dogs were found to be infected with one or more parasites. The percentage of infection of trematodes, cestodes and nematodes were 75 %, 58.33 % and 86.11 %, respectively.

Six species of trematodes were found in the present collection: Opisthorchis noverca (44.44 %), Echinochasmus perfoliatus (36.55 %), Echinochasmus corvus (13.88 %), Haplorchis taichui (13.88 %), Schistosoma incognitum (11.11 %) and Pharyngostomoides sp. (8.33 %). Four species of cestodes have been observed: Dipylidium caninum (25.00 %), Taenia hydatigena (19.44 %), Multiceps multiceps (5.55 %) and Echinococcus granulosus (2.77 %). In case of nematodes only two species, viz., Ancylostoma caninum (72.22 %) and Spirocerca lupi (27.77 %) were encountered.

HISTOCHEMISTRY AND PATHOLOGY OF OPISTHORCHIASIS NOVERCA IN DOGS

HISTOCHEMISTRY

Liver

The histochemical studies of liver revealed a marked depletion in glycogen content of the hepatic cells around the involved bile ducts, its presence in the cuticle and parenchyma of the parasite and increased mucin secretion by the luminal border of the tubular glands and the lining epithelial cells.

The amorphous material in the lumen of the gland also gave same intense reaction for mucin but the hepatic cells were negative for this. There was a depletion of protein in the hepatic cells only around the infected bile ducts. The parenchyma of the parasite was also not positive for protein. The deposition of calcium salts or haemosiderin pigment could not be observed.

Pancreas

The histochemical changes of pancreas gave more or less similar reaction as in the case of liver.

HISTOPATHOLOGY

Liver

The essential histopathological changes in liver were confined in and around the involved bile ducts with varying degree of non-suppurative cholangitis associated with biliary cirrhosis, hyperplastic changes in the lining epithelium, slight increase in the peribiliary connective tissue and mononuclear cell infiltration. The hepatic parenchyma showed varying degree of degenerative changes with focal area of cellular infiltration and hypertrophy of von Kupffer's cells.

Pancreas

The chief lesions in case of O. noverca infection in the pancreas was varying degree of proliferative changes of the supporting connective tissue and periductal connective tissue associated with well developed collagen fibres and pancreatitis. Cellular infiltration in advanced cases was invariably seen.

PATHOLOGY OF INTESTINE IN DIPYLIDIUM CANINUM INFECTION

The study of pathogenicity in D. caninum infection of dogs revealed that they do not cause appreciable degree of pathological changes, however, the infection was characterised by proliferation and desquamation of epithelial cells of the intestine with a few mononuclear cell infiltration in the substantia propria.

CHEMOTHERAPY OF HOOKWORM INFECTION IN DOGS

'Wopell' a new indigenous drug which was tried against hookworm infection proved to be satisfactory, but its efficacy was observed only when given in repeated and graded doses.

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* References marked, thus, have not been seen in original. Either they were cited by other workers or their abstracts were seen.

EXPLANATION OF PLATES

PLATE I

Figure - 1

Figure - 2

PLATE I

Figure - 1

Figure - 2

PLATE I

Figure - 1

Figure - 2

PLATE II

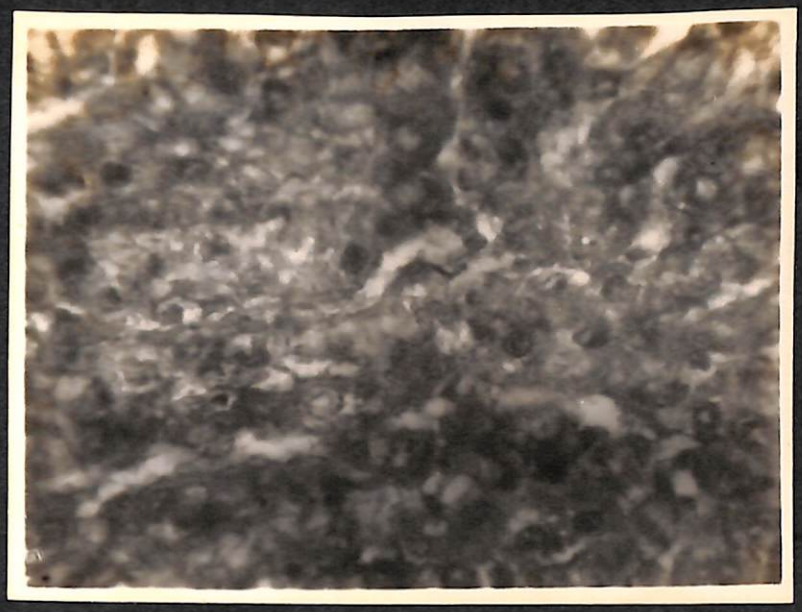

Figure - 1

Figure - 2

PLATE II

Figure - 1

Figure - 2

PLATE III

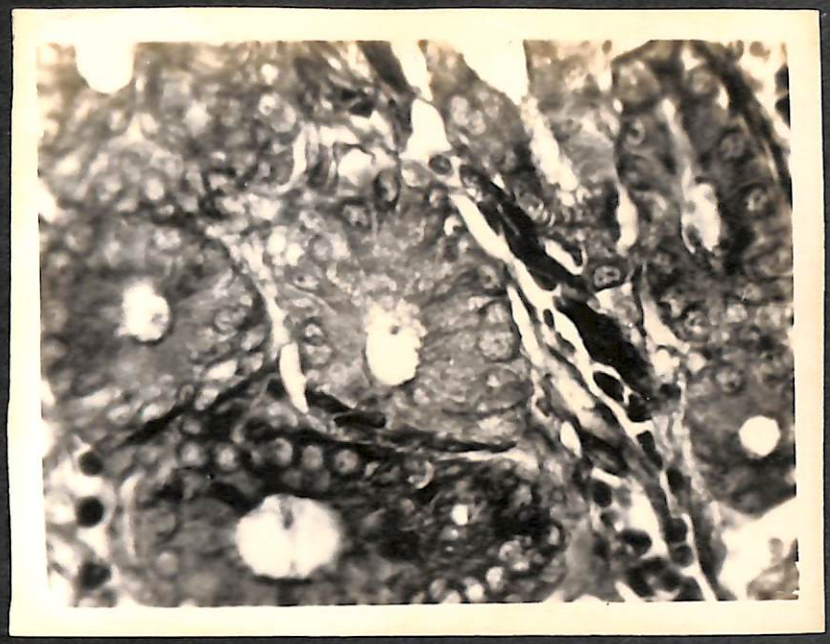

Figure - 1

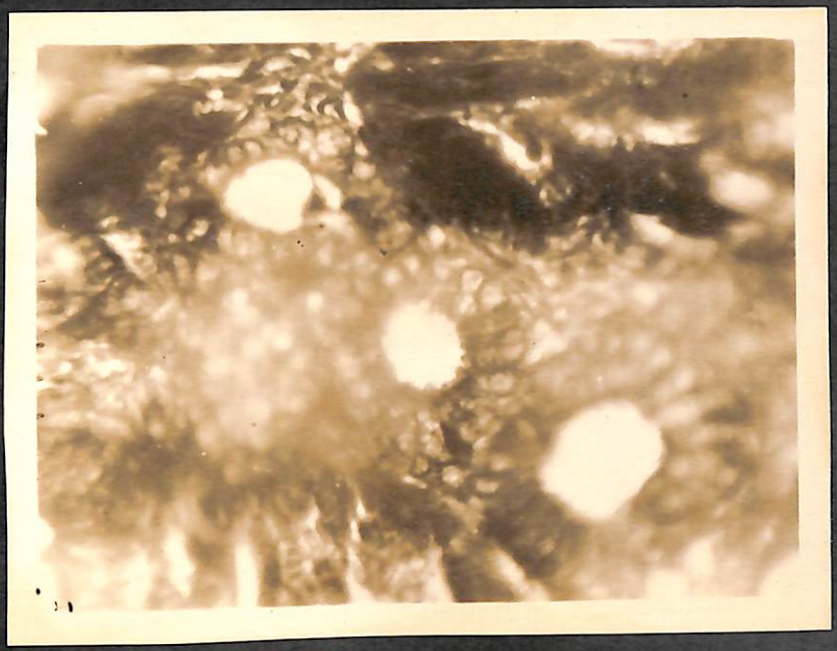

Figure - 2

PLATE III

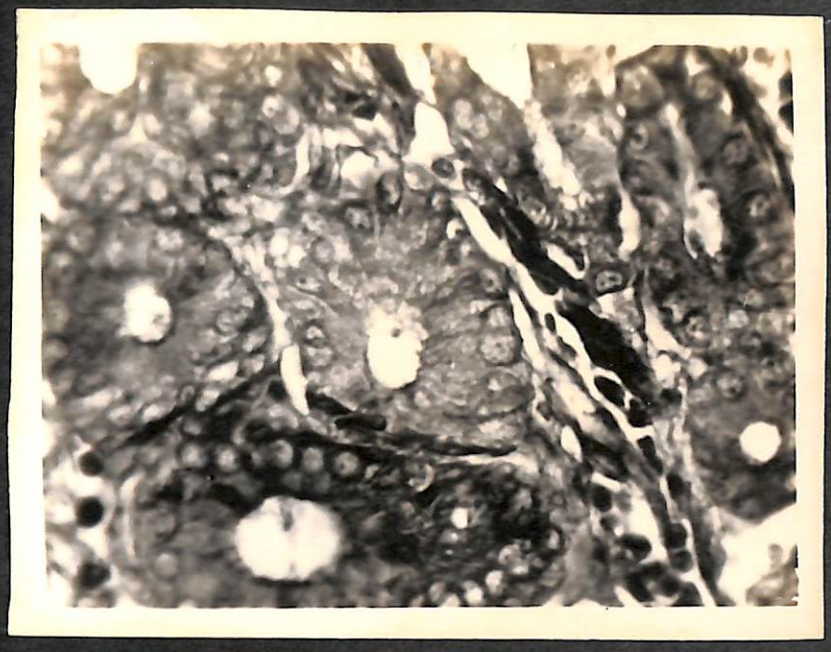

Figure - 1

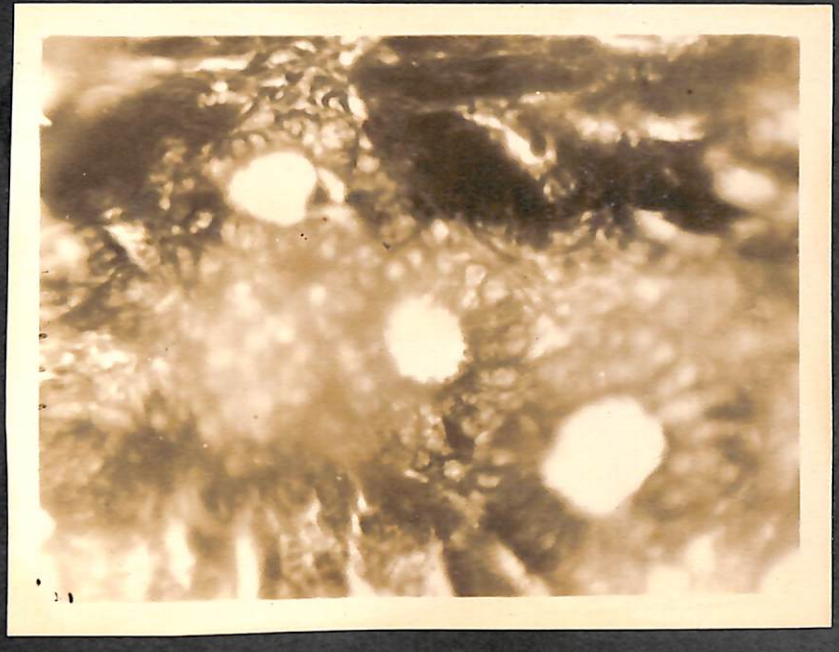

Figure - 2