CHD/ST

STUDIES ON THE BIOLOGY, PATHOGENICITY AND TREATMENT OF IMPORTANT NEMATODES OF DOMESTIC DUCK

By

K. CHANDRASEKHARAN

THESIS

submitted in partial fulfilment of the requirement for the degree

DOCTOR OF PHILOSOPHY

Faculty of Veterinary and Animal Sciences

Kerala Agricultural University

Department of Parasitology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Mannuthy : : Trichur

1977

DECLARATION

I hereby declare that this thesis entitled "Studies n the biology, pathogenicity and treatment of important ematodes of domestic duck" is a bona fide record of esearch work done by me during the course of research nd that the thesis has not previously formed the basis or the award to me of any degree, diploma, associateship, ellowship, or other similar title, of any other University r Society.

K. Chandrasekharan.

31/3/77

annuthy,
1-3-1977.

CERTIFICATE

Certified that this thesis, entitled "Studies on the biology, pathogenicity and treatment of important nematodes of domestic duck" is a record of research work done independently by Shri K. Chandrasekharan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

31/3/77

Dr. R. Kalyanasundaram
(Guide and Chairman of the Advisory Board)
Professor of Parasitology,
College of Veterinary and Animal Sciences,
Mannuthy.

Mannuthy,
31-3-1977.

ACKNOWLEDGEMENT

I place on record my most sincere gratitude to Dr. R. Kalyanasundaram, G.V.Sc., D.V.M., M.S., Ph.D., Professor of Parasitology, College of Veterinary and Animal Sciences, Mannuthy, under whose guidance, this work was carried out.

I am indebtedly grateful to Dr. C.T. Peter, B.Sc., G.M.V.C., B.V.Sc., M.Sc., Ph.D., Retired Dean and to Dr. P.G. Nair, B.V.Sc., M.Sc., Ph.D., Dean, College of Veterinary and Animal Sciences for rendering all facilities during the work, to Dr. P.U. Surendran, Professor of Statistics and to Dr. T.G. Rajagopalan, Associate Professor of Animal Management for the help rendered in statistical analysis and to Dr. K.M.N. Namboothiripad, Associate Professor of Botany, College of Horticulture, Mannuthy for helping in translating articles from Russian Language.

Grateful acknowledgement are also due to the Director of Commonwealth Institute of Helmintholegy, London for supplying photocopies of articles and to the staff of the Parasitology Department for all the encouragement and assistante rendered.

I wish to record my deep sense of gratitude to the Indian Council of Agricultural Research, New Delhi for the award of Senior Research Fellowship in Helmintholegy.

Lastly but not the least, I record my indebtedness to the Kerala Agricultural University for deputing me to prosecute this study.

....

C O N T E N T S

(contd.....)

LIST OF TABLES

LIST OF FIGURES

Fig. 14 Echinuria uncinata - development within Daphnia - first stage juveniles in the body cavity with distinct intestinal primordium and rectal cells - 2 days after infection.

Fig. 15 Echinuria uncinata - development within Daphnia - first stage juveniles under moulting and second stage in the body cavity - 3 days after infection.

Fig. 16 Echinuria uncinata - development within Daphnia - first stage juvenile under moulting - loose cuticle at both ends.

Fig. 17 Echinuria uncinata - development within Daphnia - first stage juvenile under moulting - loose cuticle at head end.

Fig. 18 Echinuria uncinata - development within Daphnia - first stage juvenile under moulting - loose cuticle at tail end.

Fig. 19 Echinuria uncinata - development within Daphnia - second stage juvenile.

Fig. 20 Echinuria uncinata - development within Daphnia - second stage juvenile - head end.

Fig. 21 Echinuria uncinata - development within Daphnia - second stage juvenile - tail end.

Fig. 22 Echinuria uncinata - development within Daphnia - second stage juvenile in the body cavity - 4 days after infection.

Fig. 23 Echinuria uncinata - development within Daphnia - second stage juvenile under moulting - 5 days after infection.

Fig. 24 Echinuria uncinata - development within Daphnia - second stage juvenile under moulting - loose cuticle at head end.

Fig. 25 Echinuria uncinata - development within Daphnia - second stage juvenile under moulting - loose cuticle at tail end.

Fig. 26 Echinuria uncinata - development within Daphnia - third stage juvenile - 5 days after infection.

g. 27 Echinuria uncinata - development within Daphnia - third stage juvenile in the body cavity - 6 days after infection.

g. 28 Echinuria uncinata - development within Daphnia - third stage juvenile in the body cavity - 6 days after infection.

g. 29 Echinuria uncinata - development within Daphnia - third stage juvenile in the antennae.

g. 30 Echinuria uncinata - development within Macrothrix sp.- first stage juveniles in the body cavity - 2 days after infection.

g. 31 Echinuria uncinata - development within Macrothrix sp.- first stage juveniles under moulting in the body cavity - 10 days after infection.

g. 32 Echinuria uncinata - development within Sida sp. - first stage juvenile in the posterior abdomen - 24 hours after infection.

g. 33 Echinuria uncinata - development within Scapholeberis sp. - first stage juveniles in the body cavity - 24 hours after infection.

g. 34 Echinuria uncinata - development within Moina sp. - first stage juveniles in the body cavity - 2 days after infection.

g. 35 Echinuria uncinata - development within duck - third stage juvenile - head end - 24 hours after infection.

g. 36 Echinuria uncinata - development within duck - third stage juvenile - tail end - 24 hours after infection.

g. 37 Echinuria uncinata - development within duck - third stage juvenile under moulting - head end - 3 days after infection.

g. 38 Echinuria uncinata - development within duck - third stage juvenile under moulting - tail end - 3 days after infection.

g. 39 Echinuria uncinata - development within duck - fourth stage juvenile - 3 days after infection.

. 40 Echinuria uncinata - development within duck - fourth stage juvenile - head end with distinct cordons and spines - 3 days after infection.

. 41 Echinuria uncinata - development within duck - fourth stage male juvenile - tail end with distinct spicular primordium - 6 days after infection.

. 42 Echinuria uncinata - development within duck - young adult - head end with distinct cordons and spines - 7 days after infection.

. 43 Echinuria uncinata - development within duck - young female - hind portion with distinct ovijector and uterine coils - 7 days after infection.

. 44 Echinuria uncinata - development within duck - young female - tail end with distinct ovijector and uterine branches - 7 days after infection.

. 45 Echinuria uncinata - development within duck - young male - posterior portion with distinct testis and spicular primordia - 7 days after infection.

. 46 Echinuria uncinata - development within duck - young female - tail end with distinct ovijector and uterine coils - 15 days after infection.

. 47 Echinuria uncinata - development within ducks - young female vulvar region with distinct genital opening and ovijector - 15 days after infection.

. 48 Echinuria uncinata - development within duck - young male - caudal end with well developed spicules and papillae - 15 days after infection.

. 49 Echinuria uncinata - development within duck - young female - head end with distinct cordons and spines - 20 days after infection.

. 50 Echinuria uncinata - development within duck - mature male - tail end with distinct spicules and caudal papillae - 20 days after infection.

. 51 Echinuria uncinata - development within duck - nodule containing worms on the proventriculus - 30 days after infection.

Fig. 52 Echinuria uncinata - development within duck - openings of the nodules present on the proventriculus - 30 days after infection.

Fig. 53 Echinuria uncinata - development within duck - fully matured males - head ends - 30 days after infection.

Fig. 54 Echinuria uncinata - development within duck - fully matured female - head end - 30 days after infection.

Fig. 55 Echinuria uncinata - development within duck - fully matured female - tail end - 30 days after infection.

Fig. 56 Tetrameres anatis - development within Daphnia pulex - first stage larva in the body cavity - 24 hours after infection.

Fig. 57 Tetrameres anatis - in vitro hatching - larva emerging with head end.

Fig. 58 Tetrameres anatis - in vitro hatching - larva emerging with tail end.

Fig. 59 Tetrameres anatis - in vitro hatching - empty egg shells and unhatched eggs.

Fig. 60 Tetrameres anatis - in vitro hatching - first stage larvae with distinct intestinal primordium.

Fig. 61 Tetrameres anatis - development within grasshopper - first stage juvenile - 8 minutes after infection.

Fig. 62 Tetrameres anatis - development within grasshopper - first stage juvenile under moulting - head end with loose cuticle - 3 days after infection.

Fig. 63 Tetrameres anatis - development within grasshopper - first stage juvenile under moulting - tail end with loose cuticle.

Fig. 64 Tetrameres anatis - development within grasshopper - second stage juvenile with distinct oesophagus and intestine - 3 days after infection.

Fig. 65 Tetrameres anatis - development within grasshopper - second stage juvenile - tail end with faint spines of third stage - 4 days after infection.

g. 66 Tetrameres anatis - development within grasshopper - second stage juvenile under moulting - head end with loose cuticle - 5 days after infection.

g. 67 Tetrameres anatis - development within grasshopper - second stage juvenile under moulting - tail end loose cuticle - 5 days after infection.

g. 68 Tetrameres anatis - development within grasshopper - third stage juvenile - tail end with spines - 5 days after infection.

g. 69 Tetrameres anatis - development within grasshopper - third stage juvenile - tail end with distinct tail papillae - 5 days after infection.

g. 70 Tetrameres anatis - development within grasshopper - third stage juvenile - head end with distinct cervical papillae - 5 days after infection.

g. 71 Tetrameres anatis - development within grasshopper - encysted third stage juveniles - 6 days after infection.

g. 72 Tetrameres anatis - development within duck - third stage juvenile - female tail end with distinct genital primordium - 24 hours after infection.

g. 73 Tetrameres anatis - development within duck - third stage juvenile under moulting - head end with loose cuticle - 2 days after infection.

g. 74 Tetrameres anatis - development within ducks - third stage juvenile under moulting - tail end with loose cuticle - 2 days after infection.

g. 75 Tetrameres anatis - development within duck - fourth stage juvenile - tail end with terminal spine and tail papillae - 3 days after infection.

g. 76 Tetrameres anatis - development within duck - fourth stage female juvenile - head and tail end - 3 days after infection.

g. 77 Tetrameres anatis - development within duck - fourth stage juveniles - head ends of male and female with cylindrical buccap capsule - 3 days after infection.

g. 78 Tetrameres anatis - development within duck -

Fig. 79 Tetrameres anatis - development within duck - fourth stage female juvenile - tail end with vacuole like vagina - 6 days after infection.

Fig. 80 Tetrameres anatis - development within duck - fourth stage female juvenile - distinct anterior limbs of ovarian tubules - 6 days after infection.

Fig. 81 Tetrameres anatis - development within duck - fourth stage male juvenile - hind end with granular testis - 6 days after infection.

Fig. 82 Tetrameres anatis - development within duck - fourth stage male and female juveniles - 8 days after infection.

Fig. 83 Tetrameres anatis - development within duck - fourth stage female juvenile - hind end with well distinct vagina and developing ovijector - 8 days after infection.

Fig. 84 Tetrameres anatis - development within duck - cross sections of fourth stage male and female juveniles within the mucous layer of proventriculus - 9 days after infection.

Fig. 85 Tetrameres anatis - development within duck - cross section of young male within the mucous layer of proventriculus - 9 days after infection.

Fig. 86 Tetrameres anatis - development within duck - fourth stage male and female juveniles - 9 days after infection.

Fig. 87 Tetrameres anatis - development within duck - fourth stage female juvenile - vulvar region with prominent ovijector and vagina.

Fig. 88 Tetrameres anatis - development within duck - fourth stage male juvenile under moulting - head end - 9 days after infection.

Fig. 89 Tetrameres anatis - development within duck - fourth stage male juvenile under moulting - loose cuticle at extremities - 9 days after infection.

Fig. 90 Tetrameres anatis - development within duck - fourth stage female, just moulted male and discarded cuticle - 9 days after infection.

Fig. 91 Tetrameres anatis - development within duck - young male - head end with pseudolabial buttress and body spines - 9 days after infection.

Fig. 92 Tetrameres anatis - development within duck - young male - tail end with caudal spines - 9 days after infection.

Fig. 93 Tetrameres anatis - development within duck - young male - tail end with spicules and spines - 9 days after infection.

Fig. 94 Tetrameres anatis - development within duck - young male - tail end with terminal spine - 9 days after infection.

Fig. 95 Tetrameres anatis - development within duck - cross sections of male worms in the mucous layer of proventriculus - 11 days after infection.

Fig. 96 Tetrameres anatis - development within duck - cross section - female worm in the proventricular gland and male in mucous layer - 11 days after infection.

Fig. 97 Tetrameres anatis - development within duck - male and female worms - 11 days after infection.

Fig. 98 Tetrameres anatis - development within duck - head end of female worm with goblet shaped buccal capsule - 11 days after infection.

Fig. 99 Tetrameres anatis - development within duck - young female with distinct muscle fibres and ovario-uterine junctions - 11 days after infection.

Fig.100 Tetrameres anatis - development within duck - young male - head end with pseudolabial buttress and spines - 11 days after infection.

Fig.101 Tetrameres anatis - development within duck - young female - hind portion with distinct copulatory receptaculum - 14 days after infection.

Fig.102 Tetrameres anatis infection in duck - female worms in the proventricular glands.

Fig.103 Tetrameres anatis infection in duck - cross section of female worm in the proventricular gland with marked desquamation of epithelia - 11 days after infection.

g. 104 Tetrameres anatis infection in ducks - cross section of female worm within the proventricular gland and males in the lumen of the proventriculus- 11 days after infection.

g. 105 Tetrameres anatis infection in ducks - cross section of worms in the proventricular gland - 28 days after infection.

g. 106 Tetrameres anatis infection in duck - cross section of worm in the proventricular gland - 28 days after infection.

g. 107 Tetrameres anatis infection in duck - cross sections of female worms within proventricular glands - marked pressure atrophy and necrosis of glands - 43 days after infection.

g. 108 Tetrameres anatis infection in duck - cross sections of female worms within proventricular glands - pressure atrophy and necrosis of glands more evident - 43 days after infection.

g. 109 Tetrameres anatis infection in duck - cross sections of 2 female worms in a proventricular gland - pressure atrophy and necrosis of gland more evident - 43 days after infection.

g. 110 Tetrameres anatis infection in duck - cross sections of female worms with the proventricular gland - marked pressure atrophy and necrosis of gland - 43 days after infection.

g. 111 Amidostomum skrjabini infection in duck - cross sections of worms in the mucosa and superficial epithelium of gizzard - marked desquamation and degeneration of superficial layer - 15 days after infection.

g. 112 Amidostomum skrjabini infection in duck - cross section of worm in the mucosa of gizzard - marked desquamation and degeneration - 15 days after infection.

g. 113 Amidostomum skrjabini infection in duck - cross sections of worms in the mucosa and superficial epithelium of gizzard - areas of erosion and ulcers in the keratinised superficial layer - 30 days after infection.

Fig. 114 Eponidiostomum uncinatum infection in duck - raised crateriform ulcer on the cornified lining of gizzard - 36 days after infection.

Fig. 115 Eponidiostomum uncinatum infection in duck - raised crateriform ulcer on the cornified lining of gizzard - 36 days after infection.

Fig. 116 Eponidiostomum uncinatum infection in duck - cross sections of worms in the keratinised superficial layer of gizzard - necrosis and ulceration - 21 days after infection.

Fig. 117 Eponidiostomum uncinatum infection in duck - cross section of worm in the keratinised superficial layer of gizzard - necrosis and ulceration - 21 days after infection.

Fig. 118 Eponidiostomum uncinatum infection in duck - cross sections of worms in the superficial keratinised layer of gizzard - marked haemorrhages, necrosis and ulceration - 36 days after infection.

Fig. 119 Eponidiostomum uncinatum infection in duck - cross sections of worms in the superficial keratinised layer of gizzard - marked haemorrhages, necrosis and ulceration - 36 days after infection.

Fig. 120 Eponidiostomum uncinatum infection in duck - cross section of worms in the superficial keratinised layer of gizzard - marked haemorrhages, necrosis and ulceration - 36 days after infection.

INTRODUCTION

INTRODUCTION

Kerala has been recognised as one of the important duck rearing regions within the country. The topography and climatic conditions are idealy suited for the rearing of ducks. Ducks are commonly reared by small farmers, as a means of side income, taking advantage of the vast areas of back waters, lowlying inundated areas and harvested paddy fields. Since confined rearing is not practised by the farmers, ducks become easily infected with various kinds of helminths, especially with those having aquatic fauna as intermediate hosts. Not less than 17 species of Trematodes and 7 species of Nematodes have already been reported from ducks in Kerala. Field observations indicate that birds which are of under-weight and which are unthrifty, invariably harbour many varieties of helminths. Helminthoses can, therefore, be identified as an important problem adversely affecting the economy of duck industry, and as a solution, it becomes necessary to formulate proper control measures against the parasites involved.

Though enough details are available on the incidence of helminths occurring in domestic ducks, there is paucity of information on their biology, pathogenicity and susceptibility to various chemotherapeutic agents, which are essential for the formulation of proper control measures.

OBJECT OF THE PRESENT STUDY

As an aid in the formulation of control measures against the common Nematodes affecting domestic ducks in Kerala, the present investigations were undertaken with an immediate object of elucidating the complete life histories of Echinuria uncinata and Tetrameres anatis and studying the histopathological and haematological changes caused by Tetrameres anatis, Epomidiostomum uncinatum and Amidostomum skrjabini, under controlled experimental conditions and treatment trials with nine chemotherapeutic agents against pure experimental infections of domestic ducks with Tetramer anatis, Epomidiostomum uncinatum and Amidostomum skrjabini.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of worms

Naturally infected domestic ducks (*Anas platyrhynchos domesticus*) purchased from different parts of Kerala and also from Vellore District of Tamil Nadu were the initial sources for the nematode parasites. Thereafter, the worms needed for study were obtained from experimentally infected birds maintained in the laboratory. In the case of naturally infected birds the entire alimentary tract and other internal organs were subjected to detailed examination for the presence of adult and immature nematodes. Different parts of the alimentary tract *viz*., Oesophagus and crop, proventriculus, gizzard, small intestine, large intestine, caecum and rectum were kept in separate petridishes and examined separately. The oesophagus and crop after being opened longitudinally, were stretched with fingers and examined against light for the presence of adult *Capillaria contorta*. The located worms were recovered with the help of hooked teasing needles. The mucous membrane of the oesophagus and crop was also teased under a binocular dissection microscope for the recovery of juveniles.

The external surface of proventriculus was examined for

the presence of nodules containing Eustrongylides sp. and Echinuria sp. and for the presence of female Tetrameres sp. Nodular swellings were teased with blunt needles and worms if any were collected in normal saline. Presence of female Tetrameres sp. appeared as dark spots within the wall of the proventriculus and the worms were recovered with a blunt and hooked needle after making a small incision near to the dark spots. The organ was then opened longitudinally and the mucous membrane scraped with a blunt scalpel or with a slide to recover worms embedded in the mucus. Finally the proventriculi were squeezed repeatedly in small quantities of normal saline to express the males of Tetrameres and juvenile stages of the worm from the glandular lumina. The materials obtained by scraping or squeezing were examined under binocula dissection microscope for the recovery of parasites. In the case of experimental birds, after scraping the mucous membran several longitudinal incisions were made on the serous surfac of the proventriculus with a sharp razor blade, and the organ was squeezed and teased in normal saline.

The gizzard was partially split open by an incision connecting the duodenal and proventricular openings and the organ was everted inside out. The contained grit and coarse food materials were washed out gently to expose the cornified

layer. The cornified layer of the organ is then peeled off and placed in a petridish containing normal saline. The worms were collected by teasing with dissecting needles under a binocular dissection microscope.

The intestine and caeca were cut longitudinally, washed separately in a small basin of water and the mucous membrane scraped deeply to recover the parasites embedded in the mucosa. The washings and scraped material were sedimented in several changes of water to obtain the worms free of debris.

Examination of adult and larval nematodes

The worms were washed in several changes of normal saline to remove adherent mucus and dirt. Studies on the morphological details of adult worms were made both on live worms and on specimens fixed in 10% formalin. Finer details of morphology of thick specimens were studied from materials cleared in lactophenol. Juvenile stages collected from cultures, intermediate hosts, or from experimental birds were studied, as far as possible, under live conditions. They were mounted on a slide in a drop of normal saline and were either examined as such or after immobilising and straightening them by applying gentle heat or with lugol's iodine. Both adult and larval forms were preserved in 10% formalin or 70% alcohol, for reference study.

Intermediate hosts

Five species of grasshoppers Spathosternum prasiniferum, Oxya nitudala, Oedaleus abruptus, Conocephalus maculatus, Ducetia japonica and Atratomorpha crenulata, six cladocerans, Daphnia magna, Daphnia pulex, Macrothrix sp., Sida sp., Moina sp. and Scapholeberis sp., Cyclops and isopoda Porcellio laevis were exposed to artificial infections.

Grasshoppers were collected from fodder and paddy fields where ducks and poultry were not frequented. They were reared and multiplied in the laboratory in cages provided with wire net. Potted common variety of garden grass was placed on the floor of the cage before transferring the grasshoppers, as a source of feed for grasshoppers. Fresh grass slips were also supplied as and when it was required. The laboratory raised grasshoppers were utilised to study the day to day development of Tetrameres anatis and to collect infective juveniles for setting experimental infection in ducklings. When smaller number of grasshoppers were needed, museum jars covered with perforated paper lids were used as rearing containers.

Daphnia pulex and Daphnia magna were collected from a shallow pond at Arany, Tamil Nadu. They were reared and multiplied in small basins or beakers containing aquarium

water and green algae. Only the laboratory raised waterfleas (Daphnia pulex and Daphnia magna) were used for experimental infection with Echinuria uncinata and Tetrameres anatis. Other crustaceans, Macrothrix sp., Sida sp., Moina sp. and Scapholeberis sp. and cyclops were collected from paddy fields in and around Trichur and reared and multiplied in the laboratory in small beakers. They were exposed to infection with eggs of Echinuria uncinata. Attempts were also made to infect cyclops with eggs of Tetrameres anatis. Porcellio laevis was reared and multiplied in cultures set up in polythene buckets containing goat pellets. Isopodes (Porcelli laevis) which were exposed to infection with eggs of Echinuria uncinata were kept in small glass vials and maintained on powdered casein.

In vitro hatching of eggs

In order to observe the process of hatching of eggs and to study the emerged out first stage larvae, attempts were made to hatch the eggs of Echinuria uncinata and Tetrameres anatis, in vitro.

Echinuria uncinata

Two laboratory raised Daphnia magna were dissected in a drop of aquarium water on a slide to liberate the digestive

fluids. Gross pieces of dissected out material were removed with a fine needle so that the fluid contained only the liberated tissue and digestive fluids. Freshly collected viable eggs of Echinuria uncinata were mixed with the fluid and a cover slip was applied. The edges of the preparation were then sealed with vaseline to prevent drying. Studies on the manner of in vitro hatching and the morphology of the hatched out juveniles were made under a phase contrast microscope. (Besides the body fluids of Daphnia spontaneous in vitro hatching was also evident in normal saline solution or aquarium water. But in these fluids the hatching took longer time than with the body fluids of Daphnia).

Tetrameres anatis

The digestive canal of a grasshopper (Spathosternum prasiniferum) was dissected out and placed on a slide containing a drop of normal saline solution. The digestive enzymes were liberated into the drop of saline by dissecting with a pair of dissection needles. Freshly harvested eggs from female worm were added to the digestive enzyme preparation. A cover slip was placed and the edges were sealed with vaseline to facilitate prolonged examination upto an hour or more.

Experimental infection of intermediate hosts

Waterfleas

containing aquarium water. Eggs collected from the uterus of adult worms were added into the beaker by means of a glass pipette, taking care to distribute the eggs uniformly at the bottom of the beaker. The water was aerated periodically to maintain the oxygen tension. After 30 minutes of exposure al the Daphnia were transferred to a larger container. To find out the time taken for hatching of eggs of Echinuria uncinata and Tetrameres anatis, the exposed Daphnia were examined at one or two minutes interval until hatching was evident. Cavity blocks (Embryo cups) were found to be more convenient for this purpose, as infected Daphnia could be easily picked up from them than from beakers. Other waterfleas and cyclops were also examined in a similar manner to note whether the eggs hatch out in vitro or not.

Grasshopper

Laboratory raised grasshoppers were secured individuall in a manner so as to direct the ventral surface towards the operator. Uterine pieces of Tetrameres anatis containing mature and viable eggs were picked up by means of a fine mounted needle and fed directly to grasshoppers. The infecte grasshoppers were then transferred to museum jars containing grass leaves. Time needed for in vitro hatching and the details of migration by first stage juveniles were studied by dissecting the infected grasshoppers at very frequent interva

Porcellio laevis was exposed to infection with eggs of Echinuria uncinata and Tetrameres anatis by keeping them in small specimen tubes. For this purpose, fresh eggs harvested from live worms were mixed with small quantity of casein and the mixture was sprinkled evenly at the bottom of the tubes. The isopodes were dissected and examined under a microscope, at very frequent intervals for the presence of juveniles.

Egg culture

Egg cultures, to obtain infective juveniles of Amidostomum skrjabini, Epomidiostomum uncinatum and Ascaridia galli were set up in the following manner. Mature and viable eggs were obtained by dissecting several gravid female worms in a small quantity of water. The eggs were then washed in several changes of clean water and transferred to culture dishes containing well aerated pond water. The preparations were incubated at room temperature (25° to 30°c).

Infection of experimental birds

Day old ducklings and chicks hatched out at the University Poultry Farm were employed for the study. The experimental birds were maintained under confined rearing in battery brooders to preclude the possibilities of acquiring natural infection. The experimental birds were

given standard mash and water ad libitum.

The ducklings were infected when they attained 5 to 15 days of age. Known number of infective juveniles as specified below were administered with the help of a long narrow glass pipette directly into the crop of individual ducklings.

Echinuria uncinata

25 numbers of third stage juveniles of varying age viz., 5 days, 6 days, 7 days, 8 days and 10 days from experimentally infected Daphnia were given to each of 25 ducklings aged below 15 days. At periodical intervals, as specified in the lifecycle studies, 18 infected birds were sacrificed and were subjected to detailed examination for the presence of developing stages of the worm. On completion of the fourth moulting, daily faecal examination of the remaining 7 ducklings was done to determine the prepatent period.

Tetrameres anatis

A total of 25 ducklings aged 10 days were infected with 50 numbers of 6 day old encysted third stage larvae, collected from artificially infected grasshoppers. 14 birds were sacrificed at periodic intervals to study the different developmental stages of the worm until the period of maturity of the parasite. Prepatent period was determined by finding

the first eggs of the parasite in the droppings of 11 infected ducklings.

Measurements

The measurements were taken either with the aid of an eyepiece micrometer or from camera lucida drawings. Unless otherwise stated the average measurements of adults and larvae were based on 50 specimens and in the case of eggs, 100 numbers

Photomicrographs

Photomicrographs of juveniles and adults were taken as far as possible while the parasites were alive.

Pathological studies

Histopathology

For studying the histopathological lesions produced by adult worms (Tetrameres anatis, Epomidiostomum uncinatum and Amidostomum skrjabini) 200 infective larvae of each parasite were administered to a total of 15 ducklings and the birds wer sacrificed, soon after the prepatent periods were over. Sections stained with haematoxylin eosin and Heidenheims Azan stains were studied for noting the pathological changes.

Haematology

Blood studies were conducted in ducklings infected with

Tetrameres anatis, Eponidiostomum uncinatum and Amidostomum skrjabini. For this purpose 200 infective juveniles of each worm were given separately to a total of 24 clean healthy ducklings so as to obtain pure and unmixed parasitic infection with each species of worm. Estimations of total red blood corpuscles, white blood corpuscles, haemoglobin concentration, erythrocyte sedimentation rate, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of infected and non infected control birds were made by adopting standard methods. The haematological values were taken on two occasions, the first when the worms attained full maturity which was determine by the presence of eggs in the droppings, and the second, 15 days after the prepatent period. Accordingly blood values in Tetrameres anatis infection were determined on the 28th and 43rd day following infection, whereas in Eponidiostomum uncinatum and Amidostomum skrjabini infections these periods were 21st and 36th day and 15th and 30th day respectively. After the second values were taken all the infected birds were sacrificed and the number of worms present in each bird was enumerated. The data obtained was analysed statistically.

Weight gain

Along with the haematological values the weights of

possible effect of parasitism on the weight gain in infected ducklings. Thus data on two occasions (immediately after the prepatent period was over and again on the 15th day of patency were available for comparison with the non infected control groups.

Treatment trials

Treatment trials were undertaken with ten drugs viz., Carbon tetrachloride at 2 ml/kg body weight, Tetramisole hydrochloride (Nilverm 3% liquid - Imperial Chemical Industrie at 50 mg/kg, Parbendazole (Helatac 4% premix - Smith Kline and French) at 100 mg/kg, Thiabendazole (Thibendol 75% water dispersible powder - Merck Sharp and Dohme) at 200 mg/kg, Morantel tartrate (Banminth II 4% liquid - Pfizer) at 40 mg/kg Phenothiazine (Phenovis - Imperial Chemical Industries) at 500 mg/kg, Disophenol (Ancylol 4.5% liquid - Cyanamid) at 10 mg/kg, Methyridine (Promintic 90% liquid - Imperial Chemical Industries) at 200 mg/kg, Cashewnut shell oil at 10 g/kg and Ramotin (Bayer) at 200 mg/kg. All the above drugs were used against experimentally set up tetrameriasis, while in trials against eponidiostomiasis and amidostomiasis, Ramotin was excluded. Carbon tetrachloride, Parbendazole, Thiabendazole, Phenothiazine and Ramotin were administered in gelatin capsules. Long and narrow graduated glass pipette

was used to administer Tetramisole hydrochloride, Morantel tartrate and Cashewnut shell oil directly into the crop. The cashewnut shell oil was diluted with equal parts of liquid paraffin before administration. Methyridine and Disophenol were injected subcutaneously.

For the purpose of treatment trials 280 parasite free ducklings were infected each with 50 numbers of third stage larvae of Tetrameres anatis, Epomidiostomum uncinatum or Amidostomum skrjabini, as the case may be, to obtain pure infections with these parasites. After the birds became positive for infections, they were separated at random into two groups viz., untreated positive control and treatment group. The treatment group received the drugs under trial on the days specified below: against Tetramerisis - on the 27th day; against Amidostomiasis - on the 15th day and against Epomidiostomiasis on the 23rd day.

Evaluation of anthelmintic efficacy

All the birds, i.e. both experimental and controls were sacrificed on the 5th day following medication and the worms if present were enumerated. The percentage of efficacy was calculated by comparison with the mean number of worms established in positive control groups (vide formula noted below):

The percentage of efficacy = $\frac{x-y}{x} \times 100$

x = Mean number of worms recovered in non-medicated positive control group.

y = Mean number of worms recovered in infected group.

Cross infection trial with Ascaridia galli

In order to find out whether Ascaridia galli of chicken is transmissible to domestic ducks a trial was undertaken to infect 10 ducklings with 500 embryonated eggs of the parasite. As a positive control 10 chicks were also infected simultaneously with the same number and batch of eggs. One duckling and one chick were sacrificed at intervals of 24 hours, 8 days, 17 days, 25 days, 35 days, 42 days, 47 days and the remainder as soon as the prepatent period was over in ducklings. The worms obtained were enumerated to note the percentage of establishment in each case.

INCIDENCE OF NEMATODE INFECTION IN DUCKS

INCIDENCE OF NEMATODE INFECTION IN DUCKS

As per the available literature a total of 20 species of nematodes belonging to 11 genera were reported from the domestic ducks in India. They are as follows:

Species	Recorder	Locality
1. *Amidostomum anseris*	Lalitha & Alwar, 1960	Madras
2. *Amidostomum fuligulae*	Maplestone, 1930	Calcutta
3. *Amidostomum skrjabini*	Dubey & Pande, 1965	Madhya Prade
4. *Amidostomum nodulosum*	Srivastava, 1939	Uttar Pradesh
5. *Ascaridia galli*	Mudaliar & Alwar, 1947	Madras
6. *Capillaria anatis*	Lalitha & Alwar, 1960	Madras
7. *Capillaria* sp.	Lalitha & Alwar, 1960	Madras
8. *Capillaria contorta*	Chandrasekharan, 1967	Kerala
9. *Capillaria globocaudata*	Chandrasekharan, 1967	Kerala
10. *Contracaecum microcephalum*	Cram, 1927 (Reported by Deo, 1964)	Uttar Pradesh
11. *Echinuria uncinata*	Lalitha & Alwar, 1960	Madras
12. *Epomidiostomum uncinatum*	Chandrasekharan, 1967	Kerala
13. *Heterakis gallinae*	Lalitha & Alwar, 1960	Madras
14. *Porrocaecum crassum*	Deo, 1964	Uttar Pradesh
15. *Strongyloides avium*	Lalitha & Alwar, 1960	Madras
16. *Tetrameres* sp.	Lalitha & Alwar, 1960	Madras
17. *Tetrameres fissispina*	Srivastava, 1939	Uttar Pradesh

18.	Tetrameres mohtedai	Dubey, 1964	Madhya Prades
19.	Tetrameres anatis	Chandrasekharan, 1967	Kerala
20.	Trichostrongylus tenuis	Lalitha & Alwar, 1960	Madras

In the course of the present investigations (January, 197 to March 1976) a total of 340 domestic ducks were examined, out of which 231 birds or 67.94 % had harboured one or more species of nematodes. In all, eight species of nematodes belonging to 7 genera were encountered. The species and their location within the host are as follows:

Species	Location
1. Amidostomum skrjabini	Gizzard
2. Capillaria contorta	Oesophagus and crop
3. Capillaria globocaudata	Caecum
4. Echinuria uncinata	Nodules on the wall of proventriculus
5. Epomidiostomum uncinatum	Gizzard
6. Eustrongylides papillosum	Nodules on the wall of proventriculus
7. Strongyloides avium	Small intestine
8. Tetrameres anatis	Proventriculus

Incidence of Amidostomum skrjabini

Out of the total of 340 birds examined, Amidostomum skrjabini was encounted in 111 birds or 32.65 % of the total.

mixed infection with other species of nematodes. The number of worms recovered from a single bird varied from 3 (females 2 & Male 1) to 45 (females 26 & males 19). The worms were encountered throughout the year.

Incidence of Capillaria contorta

Capillaria contorta was seen in 37 ducks or 11.47 % of the total number of birds examined. Out of these 37 birds 8 had pure infection with the parasite. The maximum number of worms recovered from a single bird was 13 (females 8 & males 5) while the minimum was 1 (one female). No seasonal variation in the incidence was observed.

Incidence of Capillaria globocaudata

Capillaria globocaudata was recovered from the caeca of 38 ducks (11.19 %) of which eight birds showed pure infection. Worms per bird varied from 1 (female one) to 14 (females 8 & males 6). No significant seasonal influence on the incidence was noticed.

Incidence of Echinuria uncinata

34 ducks (10 %), mostly brought from Tamil Nadu, were found to be infected with Echinuria uncinata of which 11 had pure infection with the species. The maximum and minimum number of worms encountered in a single duck was 58 (females 39 & males 19) and 3 (females 2 & male 1) respectively.

Incidence of Eponidiostomum uncinatum

Out of 340 gizzards examined 140 were found to be positive for Eponidiostomum uncinatum, giving a rate of infection of 41.18%. Among the positive birds 34 had pure infection with this parasite. The number of worms present in a single gizzard varied from 3 (females 2 & male 1) to 33 (females 19 & males 14). No seasonal variation was noticed in the incidence.

Incidence of Eustrongylides papillosus

Eustrongylides papillosus was seen in proventriculi of 3 ducks (0.88%) only. All these 3 birds had mixed infection with other nematodes. The maximum number obtained from a bird was 2 (female 1 & male 1) and the minimum one (female one).

Incidence of Strongyloides avium

Out of the total number of birds examined, 2 ducks (0.59%) were found infected with Strongyloides avium. Only female worms were detected. The maximum and minimum number of worms obtained were 7 and 4 respectively.

Incidence of Tetrameres anatis

Tetrameres anatis was recovered from 31 proventriculi (9.12%) of ducks, of which 6 had pure infection with this

parasite. A maximum of 21 (females 16 & males 5) and minimum of 2 (females 2) worms could be collected from a single proventriculus. Influence of season in the incidence of Tetrameres anatis was not significant.

Thus the rate of infection with various species of nematodes were Epomidiostomum uncinatum 41.18%; Amidostomum skrjabini 32.65%; Capillaria contorta 11.47%; Capillaria globocaudata 11.18%; Echinuria uncinata 10%; Tetrameres anatis 9.12%; Eustrongylides papillosus 0.88% and Strongyloides avium 0.59%.

In an earlier record of nematode parasites in domestic ducks within the Kerala State, Chandrasekharan (1967) reported the following: Amidostomum skrjabini (54.23%); Capillaria contorta (18.49%); Capillaria globocaudata (5.33%); Echinuria uncinata (2.5%); Epomidiostomum uncinatum (49.90%); Strongyloid avium (2.51%) and Tetrameres anatis (8.77%). No seasonal influence on the incidence on nematode infection was also observed by him. The present observations more or less confirm the previous findings, except in the case of Amidostomu skrjabini, Echinuria uncinata and Strongyloides avium.

The details of incidence are presented in Table 1.

Table 1. Incidence of nematode infection in domestic ducks

. of ks amin-	C. contorta		E. papillosus		E. uncinata		T. gnatis		A. skrjabini		E. uncinatum		S. avium		C. glo
	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive
13	1	7.69	-	-	2	15.38	5	38.46	5	38.46	4	30.76	-	-	2
12	3	25.00	-	-	-	-	7	58.33	6	50.00	4	33.33	-	-	3
54	7	12.96	-	-	2	3.70	2	3.70	12	22.22	27	50.00	-	-	6
21	1	4.76	3	14.29	2	9.52	1	4.76	12	57.14	17	80.95	-	-	4
34	2	5.88	-	-	2	5.88	1	2.94	5	14.70	6	17.65	-	-	-
43	5	11.63	-	-	3	6.99	1	2.32	6	13.98	10	23.26	-	-	4
80	7	8.75	-	-	3	3.75	2	2.50	32	40.00	39	48.75	2	2.50	12
4	-	-	-	-	3	75.00	-	-	3	75.00	3	75.00	-	-	-
2	-	-	-	-	2	100.00	-	-	1	50.00	-	-	-	-	-
13	2	15.37	-	-	3	23.08	2	15.37	7	53.84	10	76.92	-	-	2
22	4	18.18	-	-	6	27.27	5	22.73	6	27.27	1	4.54	-	-	1
2	1	50.00	-	-	2	100.00	1	50.00	2	100.00	-	-	-	-	-
37	6	16.21	-	-	4	10.81	4	10.81	14	37.84	17	45.95	-	-	2
2	-	-	-	-	-	-	-	-	-	-	1	50.00	-	-	1
1	-	-	-	-	-	-	-	-	-	-	1	100.00	-	-	1

Table 1. Incidence of nematode infection in domestic ducks

. of ks amin-	C. contorta		E. papillosus		E. uncinata		T. gnatis		A. skrjabini		E. uncinatum		S. avium		C. glo
	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive	%	Posi-tive
13	1	7.69	-	-	2	15.38	5	38.46	5	38.46	4	30.76	-	-	2
12	3	25.00	-	-	-	-	7	58.33	6	50.00	4	33.33	-	-	3
54	7	12.96	-	-	2	3.70	2	3.70	12	22.22	27	50.00	-	-	6
21	1	4.76	3	14.29	2	9.52	1	4.76	12	57.14	17	80.95	-	-	4
34	2	5.88	-	-	2	5.88	1	2.94	5	14.70	6	17.65	-	-	-
43	5	11.63	-	-	3	6.99	1	2.32	6	13.98	10	23.26	-	-	4
80	7	8.75	-	-	3	3.75	2	2.50	32	40.00	39	48.75	2	2.50	12
4	-	-	-	-	3	75.00	-	-	3	75.00	3	75.00	-	-	-
2	-	-	-	-	2	100.00	-	-	1	50.00	-	-	-	-	-
13	2	15.37	-	-	3	23.08	2	15.37	7	53.84	10	76.92	-	-	2
22	4	18.18	-	-	6	27.27	5	22.73	6	27.27	1	4.54	-	-	1
2	1	50.00	-	-	2	100.00	1	50.00	2	100.00	-	-	-	-	-
37	6	16.21	-	-	4	10.81	4	10.81	14	37.84	17	45.95	-	-	2
2	-	-	-	-	-	-	-	-	-	-	1	50.00	-	-	1
1	-	-	-	-	-	-	-	-	-	-	1	100.00	-	-	1

LIFECYCLE OF *Echinuria uncinata*

LIFECYCLE OF ECHINURIA UNCINATA

The earliest reference to the life history of Echinuria uncinata was made by Hamann (1893) who encountered the larvae of Echinuria uncinata in naturally infected Daphnia pulex. Akhtar (1936) obtained larvae of Echinuria uncinata by pressure hatching in aqueous mounts. Romanova (1938, 1947 & 1948) studied the developmental stages of Echinuria uncinata both in the intermediate hosts Daphnia pulex and Daphnia magna and in the final host, ducks. Radin (1959) also reported the role of Daphnia in the transmission of echinuriasis. Garkavi (1960) employed Daphnia as intermediate host and described the development of the parasite in ducks. Kotelnikov (1961) succeeded in infecting Ceriodaphnia, Asellus aquaticus, Gammarus sp. and an unidentified cypid ostracod with the eggs of Echinuria uncinata and elucidated the lifecycle in ducks. Later, the developmental phases of the parasite were studied by Srilkov (1963) in Daphnia and Misiura (1970) in Daphnia pulex, Daphnia magna and Heterocypris incongruens. Guilhon et al. (1971) studied the development of the worm both in the Daphnia and in ducklings. Austin and Welch (1972) succeeded in infecting 11 species of crustaceans and described the biology of the parasite in Delta mallard ducks.

During the present study, the biology of Echinuria uncinata was worked out in full, using the waterfleas,

Daphnia pulex and Daphnia magna as experimental intermediate hosts and domestic ducklings as final host.

In vitro hatching

Observations were made on the in vitro hatching of eggs using body fluid of Daphnia, normal saline solution and aquarium water on the media. The manner of hatching was as noted below:

Body fluid of Daphnia

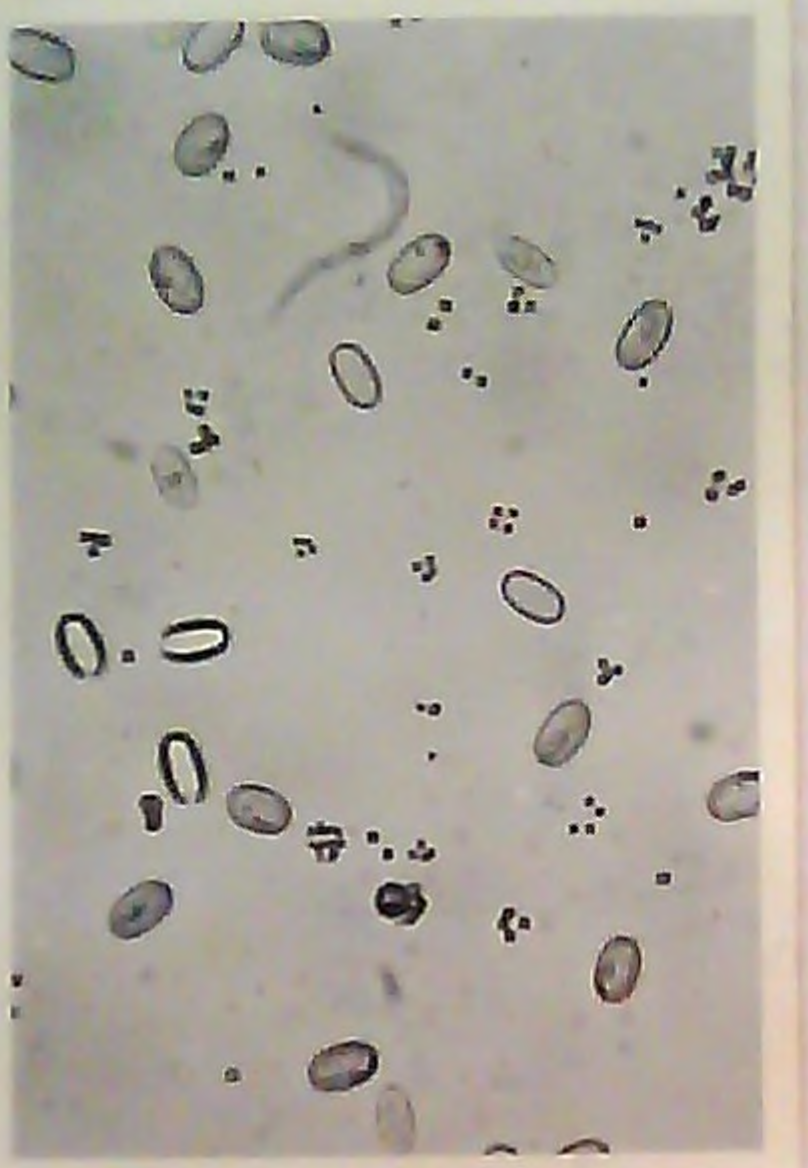
On mixing the eggs with the material, the motility of the larva within the eggs became very much pronounced, and in approximately one minute a bulging was formed at one pole of some of the mature eggs. Two minutes after mixing the content, the anterior extremity of the larva emerged through a narrow opening formed at the bulged out area. This marked the commencing point of actual hatching. The larva extricated itself out of the shell by wreathing or twisting movements, in a further period of 3 minutes. Thus the complete process took only five minutes in the case of eggs that commenced to hatch early. In approximately 10 minutes 70% of the matured eggs hatched. Fig. 1 shows a hatched out larva. While a majority of larvae came out of the egg with their anterior end a few larvae hatched out by extricating their tail end first (Figs. 2 & 3). The empty shell did not contain any inclusions after the exiɸt of the larva. The opening through which the larva had emerged out was seen at a slightly subterminal portion of the egg shell (Fig. 4).

Fig. 1 Echinuria uncinata - in vitro hatching - hatched out larvae and empty egg shells.
200x

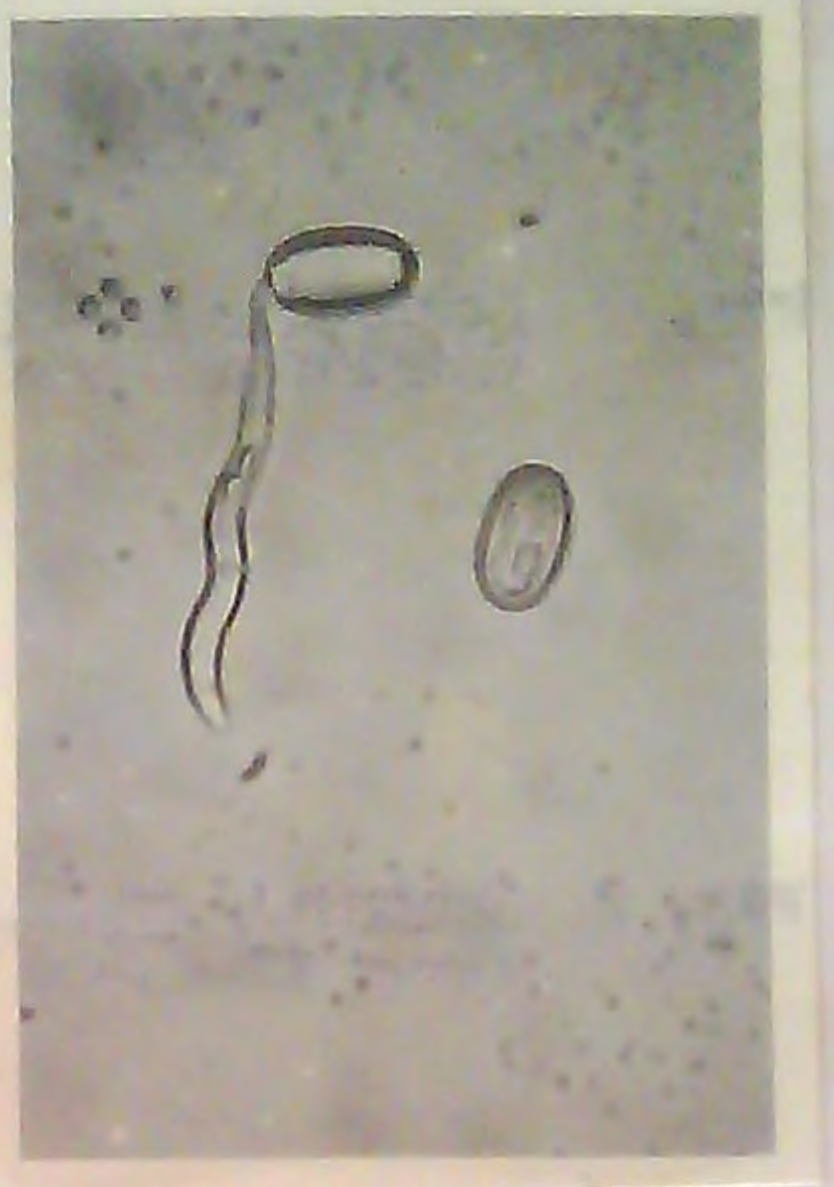
Fig. 2 Echinuria uncinata - in vitro hatching - larva emerging with head end.
400x

Fig. 3 Echinuria uncinata - in vitro hatching - larva emerging with tail end.
400x

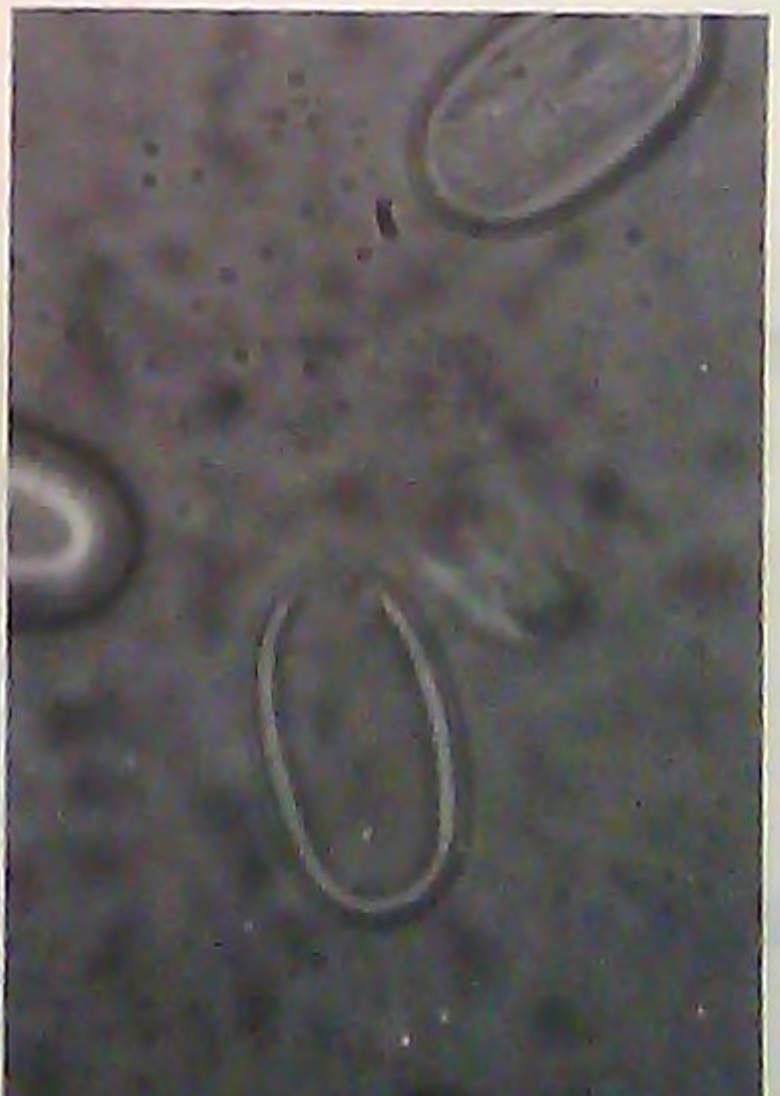
Fig. 4 Echinuria uncinata - in vitro hatching - empty egg shell.
900x



1



2



Normal saline and Aquarium water

Eggs in cover slip preparations with normal saline or aquarium water, commenced hatching after 10 minutes and completed in about 15 minutes. Only a few number of eggs hatched and the larvae were found to be less active, than those hatched out with body fluids of Daphnia. Some of the eggs present within the uteri of female worms were also found to hatch in 24 hours after placing the dissected female worms in normal saline (Fig. 5).

First stage larvae hatched out in vitro

The first stage larvae of Echinuria uncinata obtained by in vitro hatching measured 0.1518-0.1744 mm long and 0.0099mm broad. The head of the larva moved mostly on dorso-ventral plane. Intestinal primordium was noticed as an elongated hyaline area in the second half of the larva. It measured on an average 0.030 mm in length and was located at 0.080-0.085mm from the head end. This structure was also clearly visible in fully developed larva within the egg as a cresent shaped structure (Fig. 2). Other morphological details were not clear in the freshly hatched first stage juvenile.

DEVELOPMENT WITHIN DAPHNIA

Laboratory raised Daphnia were exposed to infection with Echinuria uncinata and at periodical intervals commencing from

ne minute after exposure to 15 minutes one Daphnia each was xamined for the presence of eggs or larvae within their body.

irst minute after exposure

A few eggs with vigorously motile larvae were observed in th nterior part of the gut of the daphnia.

econd minute

More number of eggs were present in the anterior part of ut indicating that the Daphnia were feeding actively. A few of he eggs were observed to have commenced hatching.

hird minute

In 3 minutes after exposure, a few hatched out larvae coul e seen in the gut of Daphnia, and correspondingly, empty shells imilar in morphology to those obtained by in vitro hatching wer lso seen among the eggs. Though some of the mature eggs hatche 3 minutes time, a large number of unhatched eggs were also en (Fig. 6).

ourth minute

Hatched out larvae and eggs were noticed in the mid gut r the crustacean.

ifth minute

Viable and motile larvae, unhatched eggs and empty egg

shells were present in the hind gut of infected Daphnia (Fig. 7

Sixth minute

By the end of 6 minutes majority of the eggs had hatched as evidenced by the presence of a large number of first stage juveniles at the posterior part of abdomen of the intermediate host (Fig. 8).

Ninth minute

By the end of 9 minutes all viable eggs of the worm had hatched and the entire gut of the daphnia contained large number of larvae.

Twelfth minute

At the end of 12 minutes after exposure to the eggs many of the hatched out larvae had penetrated through the gut wall of Daphnia and were found in all parts of body cavity (Figs. 9 & 10).

In approximately 14 minutes the larvae were to be found only in the body cavity and the gut contained unhatched eggs and empty shells.

First stage juveniles, 15 minutes after infection

The early first stage juveniles collected 15th minute after exposure to infection were identical in morphology with

ig. 5 Echinuria uncinata - in vitro hatching - hatching of eggs in utero.

70x

ig. 6 Echinuria uncinata - development within Daphnia - hatched out larvae, empty shells and unhatched eggs within the body cavity - 3 minutes after infection.

200x

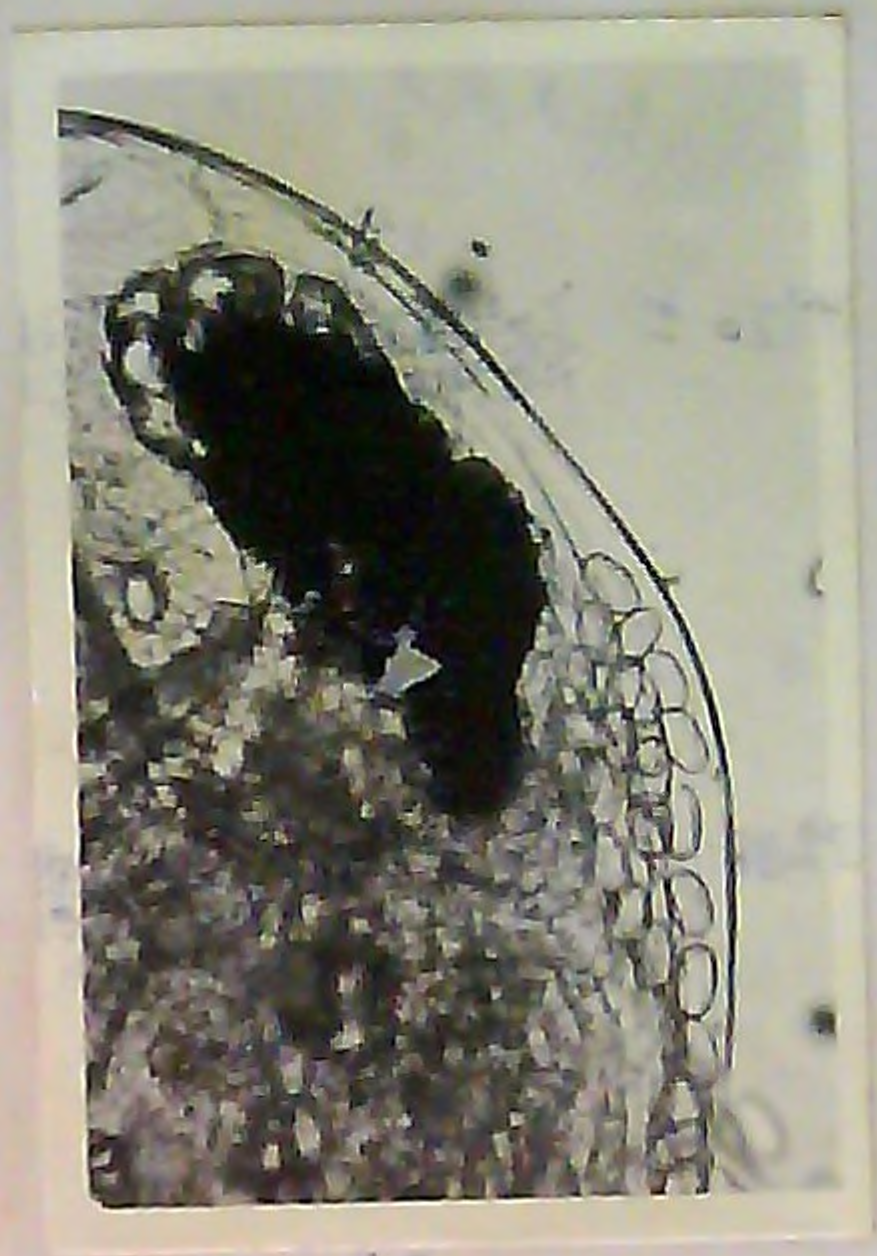
ig. 7 Echinuria uncinata - development within Daphnia - first stage larvae, empty shells and unhatched eggs - 5 minutes after infection.

200x

ig. 8 Echinuria uncinata - development within Daphnia - first stage larvae in the hind portion - 6 minutes after infection.

400x

5



6

7

8

0.1625-0.1775 mm and 0.01 mm respectively. The excretory pore opened at a distance of 0.05-0.0575 mm from head end. The intestinal primordium measured 0.03 mm in length and was located at 0.075-0.0925 mm from head end. The head end of larva was narrow and rounded while the tail end was more pointed with blunt tip (Fig. 11).

Twentyfour hours after infection

Larvae were mostly seen in the posterior part of the Daphnia (Fig. 12) and were more or less similar to the freshly hatched out larvae excepting that the nerve ring and oesophagus had become distinct. The former appeared as a translucent band surrounding the oesophagus. The outline of intestinal cells were more defined.

The measurements of the larvae were as follows: length 0.16-0.1775 mm; breadth 0.0125-0.0175 mm; nerve ring and excretory pore 0.04-0.045 mm and 0.05-0.0625 mm respectively from head end; oesophagus 0.07-0.9 mm in length and intestinal primordium 0.0325-0.035 mm in length.

2 days after infection

Larvae obtained on the second day after infection were distinctly stouter (Fig. 13) with better differentiation of internal structures. Thus a well defined cylindrical buccal capsule was evident and the anus was more clear with distinct

Fig. 9 Echinuria uncinata - development within Daphnia - first stage juveniles in the body cavity - 12 minutes after infection.

90x

Fig.10 Echinuria uncinata - development within Daphnia - First stage juveniles at the head region - 12 minutes after infection.

90x

ig.11 Echinuria uncinata - development within Daphnia - First stage juveniles in the body cavity - 15 minutes after infection.

400x

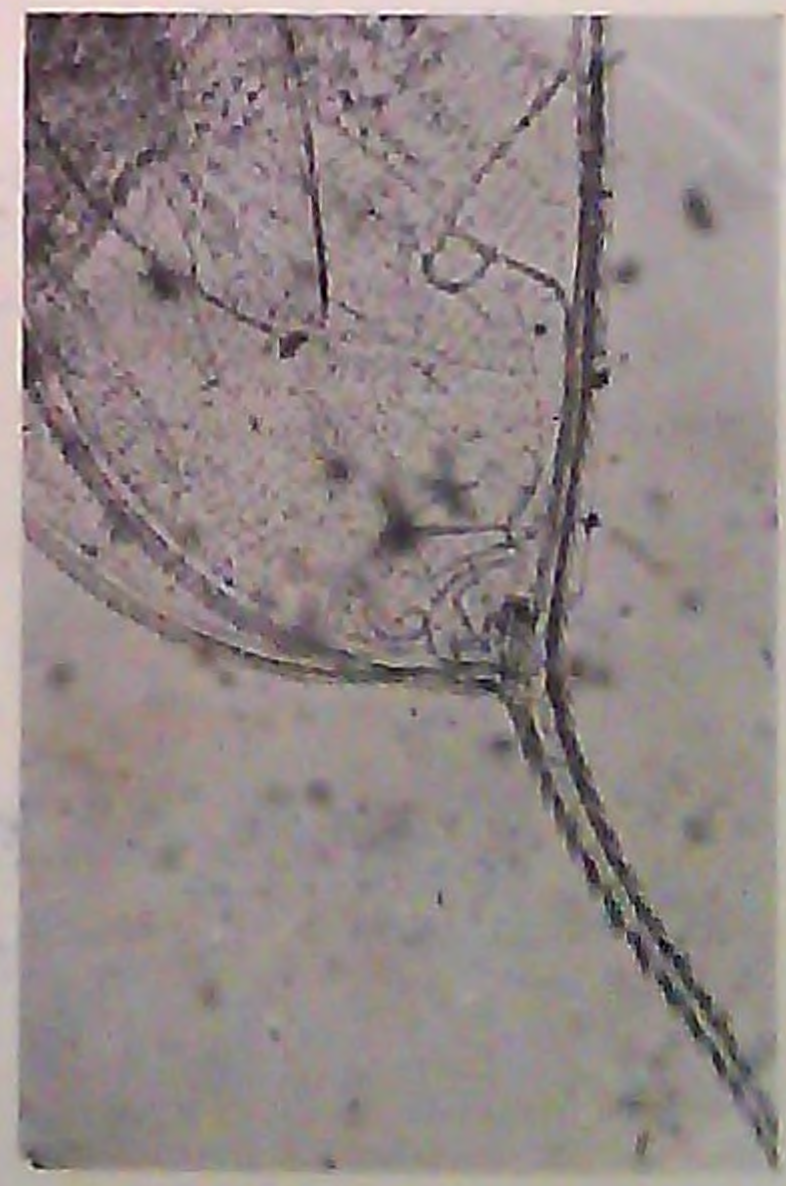
g.12 Echinuria uncinata - development within Daphnia - First stage larvae in the hind portion - 24 hours after infection.

90x

9

10

11



12

larvae measured 0.15-0.205 mm in length and 0.0175-0.029mm in width. The buccal capsule was 0.015 mm deep. The nerve ring and excretory pore were located at 0.040-0.050 mm and 0.0475-0.055 mm respectively from the head. The intestinal primordium was 0.0525-0.065 mm long. The tail was 0.025-0.0375 mm in length.

3 days after infection (First larval moult)

Infected Daphnia contained both moulting first stage juveniles and second stage larvae (Fig. 15). Larvae undergoing moulting were more fragile and sluggish in movements and were provided with loose cuticle at extremities (Figs. 16, 17 & 18). The moulting larvae measured 0.375-0.400 mm in length and 0.03-0.035 mm in width. The buccal capsule was more distinct and measured 0.025-0.030 mm. The nerve ring and excretory pore were located at 0.045-0.060 mm and 0.065-0.08 mm respectively from head end. The oesophagus was demarcated into two distinct parts viz., an anterior muscular part (measuring 0.0625-0.065 mm) and a posterior glandular part (measuring 0.075-0.08 mm in length). The intestinal cells were more conspicuous. Rectal cells lining the rectal chamber were also more prominent. The rectal chamber was found to be filled with a transparent plug like material which protruded like a bubble through the anus. The tail was 0.0525-0.055 mm in length.

Second stage juveniles

Fig. 13 Rahinuria uncinata - development within Daphnia - first stage juveniles in the body cavity - 2 days after infection.

90x

Fig. 14 Rahinuria uncinata - development within Daphnia - first stage juveniles in the body cavity with distinct intestinal primordium and rectal cells - 2 days after infection.

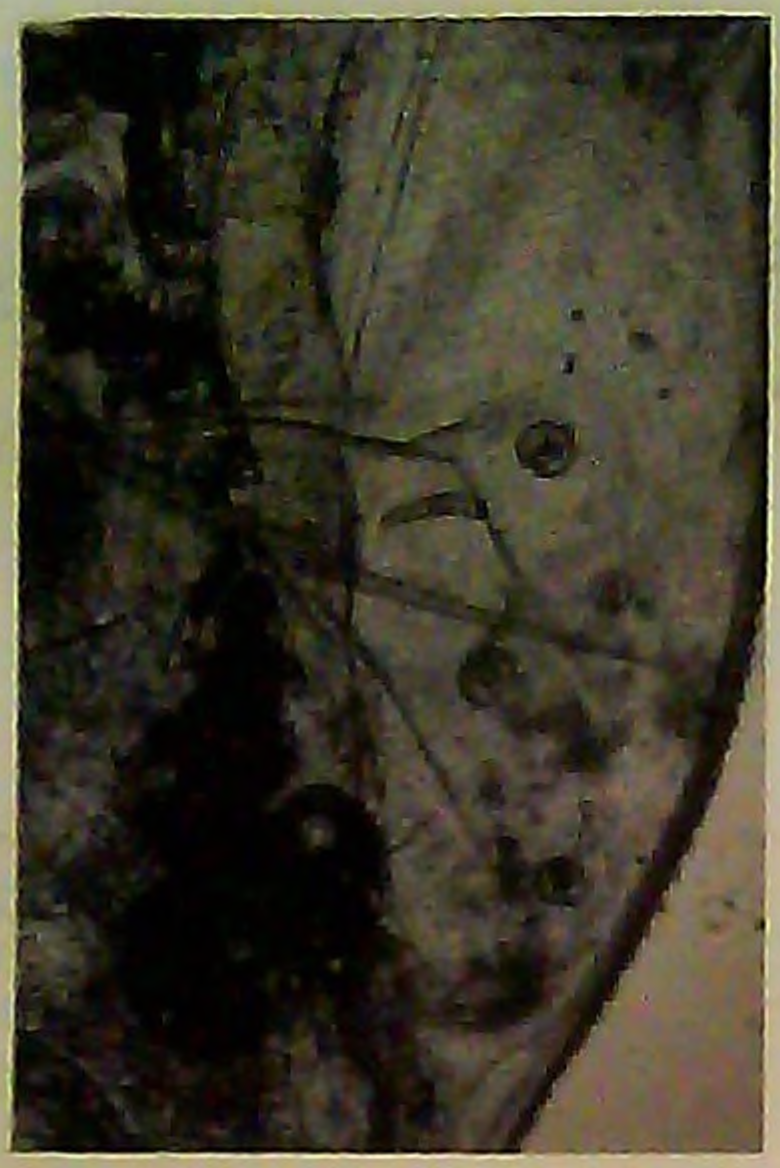
400x

Fig. 15 Rahinuria uncinata - development within Daphnia - first stage juveniles under moulting and second stage juveniles in the body cavity - 3 days after infection.

90x

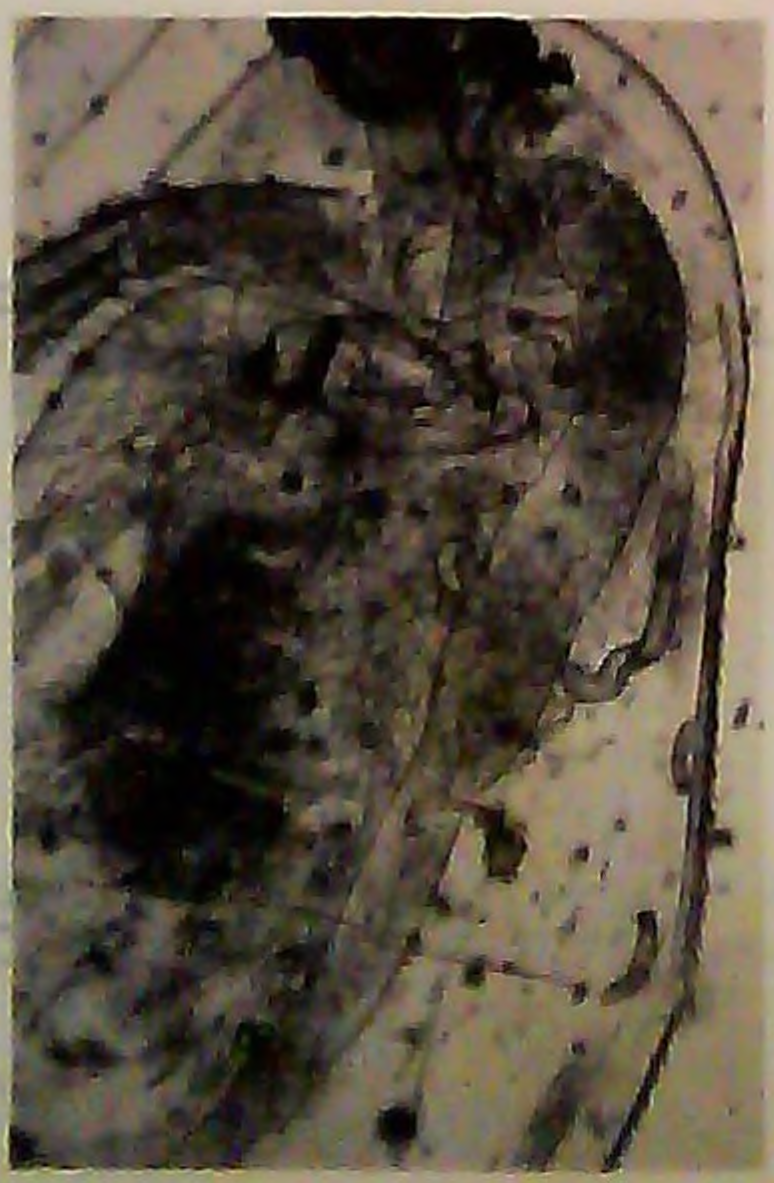
Fig. 16 Rahinuria uncinata - development within Daphnia - first stage juvenile under moulting loose cuticle at both ends.

200x



13

14



active than the first stage (Figs. 19, 20 & 21). The head was slightly rounded and the tail was narrow and bent dorsally. The larvae measured 0.4625-0.7125 mm in length and 0.030-0.0475 mm in breadth. The buccal capsule was deep and more chitinised at its base and measured 0.030-0.045 mm. The nerve ring and excretory pore were located at 0.060-0.0975 mm and 0.075-0.1125 mm respectively from head end. The muscular and glandular parts of oesophagus measured 0.075-0.100 mm and 0.1125-0.150 mm respectively in length. The genital primordium comprising of a mass of hazy cells was noticed at 0.105-0.125 mm anterior to the tip of tail. The anus was located at 0.055-0.075 mm from tail tip.

4 days after infection

Infected Daphnia contained only second stage larvae (Fig. 22). No additional features were discernible in the morphology of second stage larvae on the fourth day after infection excepting that the genital primordium was more distinct as a band shaped structure. The measurements of the larvae were as follows: length 0.750-0.850 mm; breadth 0.035-0.0475 mm; buccal capsule 0.050 mm; nerve ring 0.075-0.085 mm; excretory pore 0.105-0.115 mm; muscular portion of oesophagus 0.075-0.0975 mm; glandular portion of oesophagus 0.1125-0.150mm; genital primordium 0.110-0.1375 mm from hind end and tail 0.075-0.0975 mm in length.

Five days after infection (Second larval moult)

Fig. 17 Bohinuria unciata - development within Daphnia - First stage juvenile under moulting - loose cuticle at head end.

900x

Fig. 18 Bohinuria unciata - development within Daphnia - First stage juvenile under moulting - loose cuticle at tail end.

900x

Fig. 19 Bohinuria unciata - development within Daphnia - second stage juvenile.

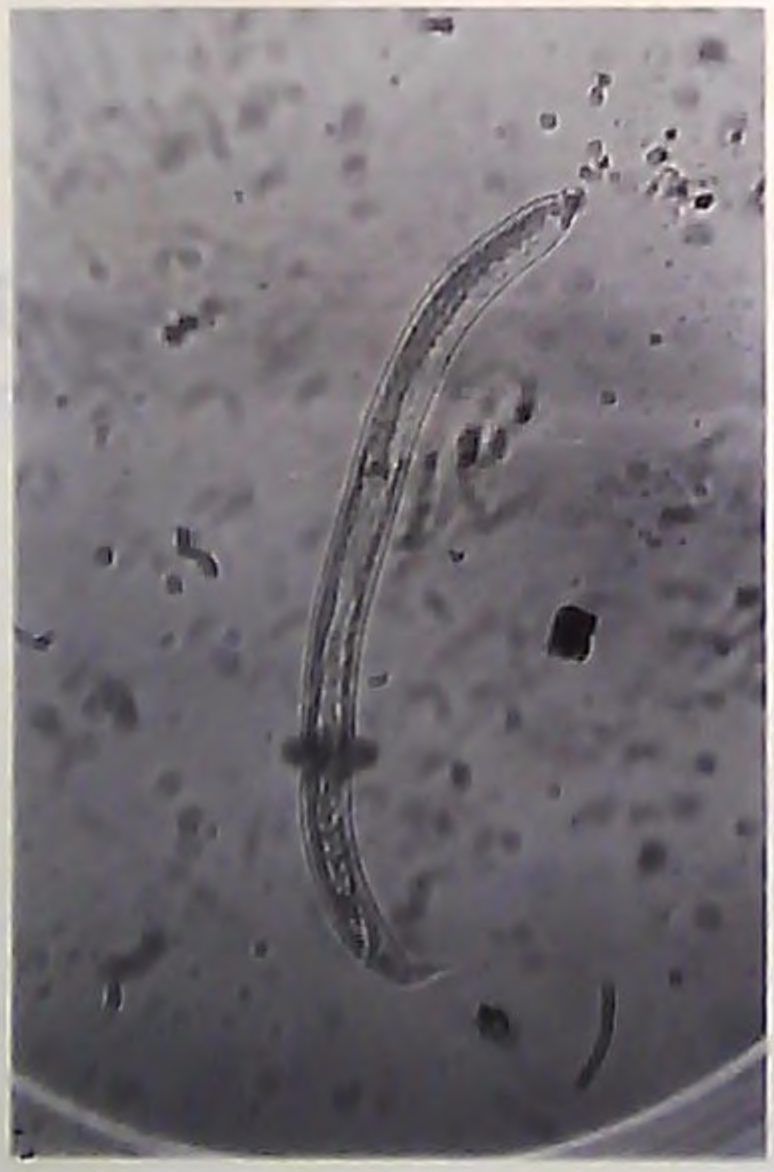
200x

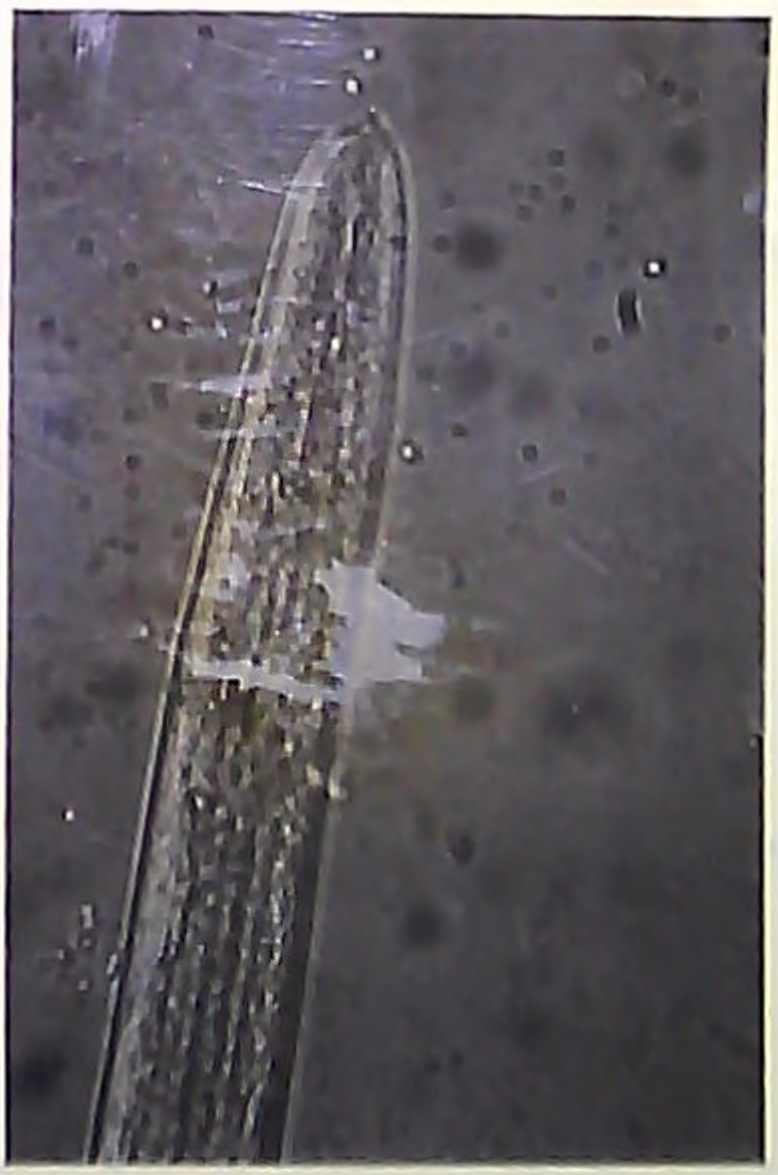
Fig. 20 Bohinuria unciata - development within Daphnia - second stage juvenile - head end.

400x

17

18





larval moult was seen (Figs. 23, 24 & 25). The moulting larvae were lethargic and had the following measurements: length 0.725-1.0375 mm; breadth 0.0375-0.0425 mm; buccal capsule 0.040-0.055 mm; nerve ring 0.070-0.0875 mm; excretory pore 0.085-0.1375 mm; muscular oesophagus 0.130-0.1625 mm; glandular oesophagus 0.165-0.2075 mm; genital primordium 0.125-0.1375 mm from tail tip and tail 0.070-0.100 mm in length.

Third stage juveniles

The third stage juveniles were longer than the second stage and were more active. The tail ended in a knob and was bent dorsad (Fig. 26). The sex of the larvae could be easily detected from the relative position of genital primordia. In the case of male larvae the genital primordium which is attached diffusely over the intestine is found to be located far anteriorly from the tail tip than that of the female ones. The measurement of third stage larvae were as follows: length 1.15-1.2375 mm; breadth 0.035-0.04 mm; buccal capsule 0.0475-0.0525 mm; nerve ring 0.0775-0.100 mm; excretory pore 0.1125-0.1375 mm; muscular oesophagus 0.1625-0.200 mm; glandular oesophagus 0.2975-0.325 mm; genital primordium of males 0.185-0.215 mm; genital primordium of females 0.130-0.145 mm from tail tip and tail 0.065-0.100 mm in length.

5 days after infection

Only third stage larvae could be detected on the 6th day

Fig. 21 Rabiuria uncinata - development within Daphnia - second stage juvenile - tail end.

400x

Fig. 22 Rabiuria uncinata - development within Daphnia - second stage juvenile in the body cavity - 4 days after infection.

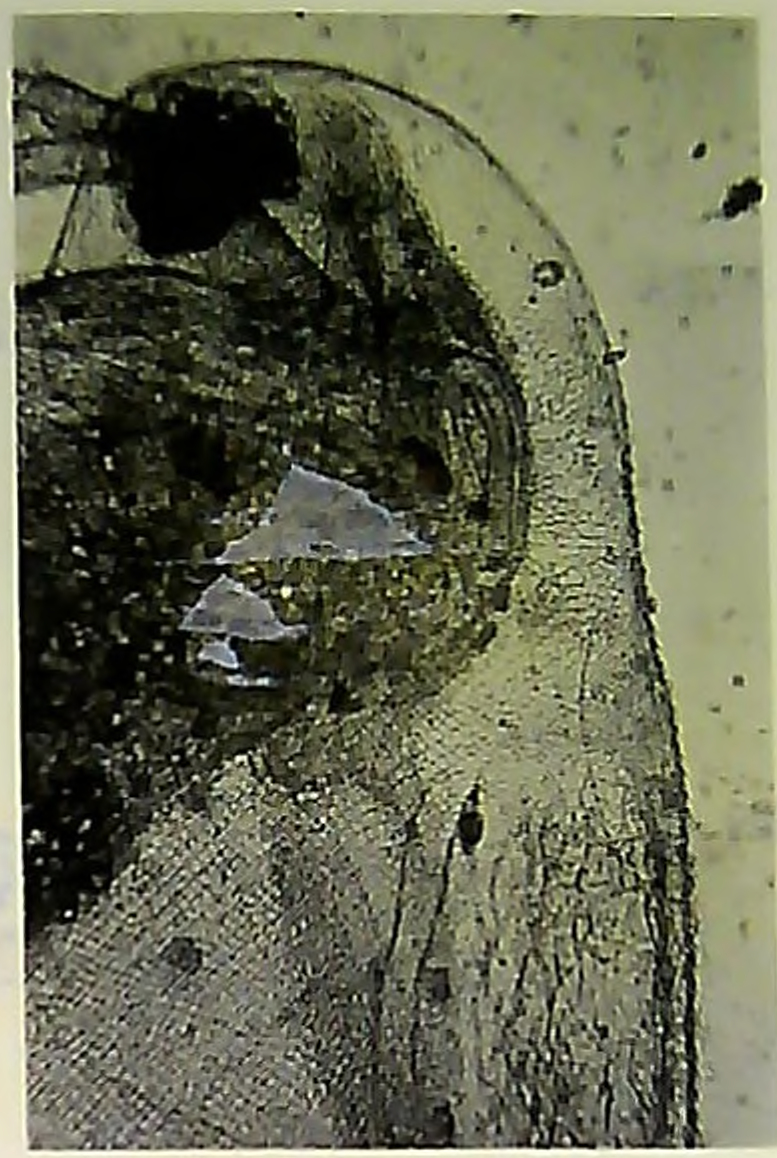
100x

Fig. 23 Rabiuria uncinata - development within Daphnia - second stage juvenile under moulting - 5 days after infection.

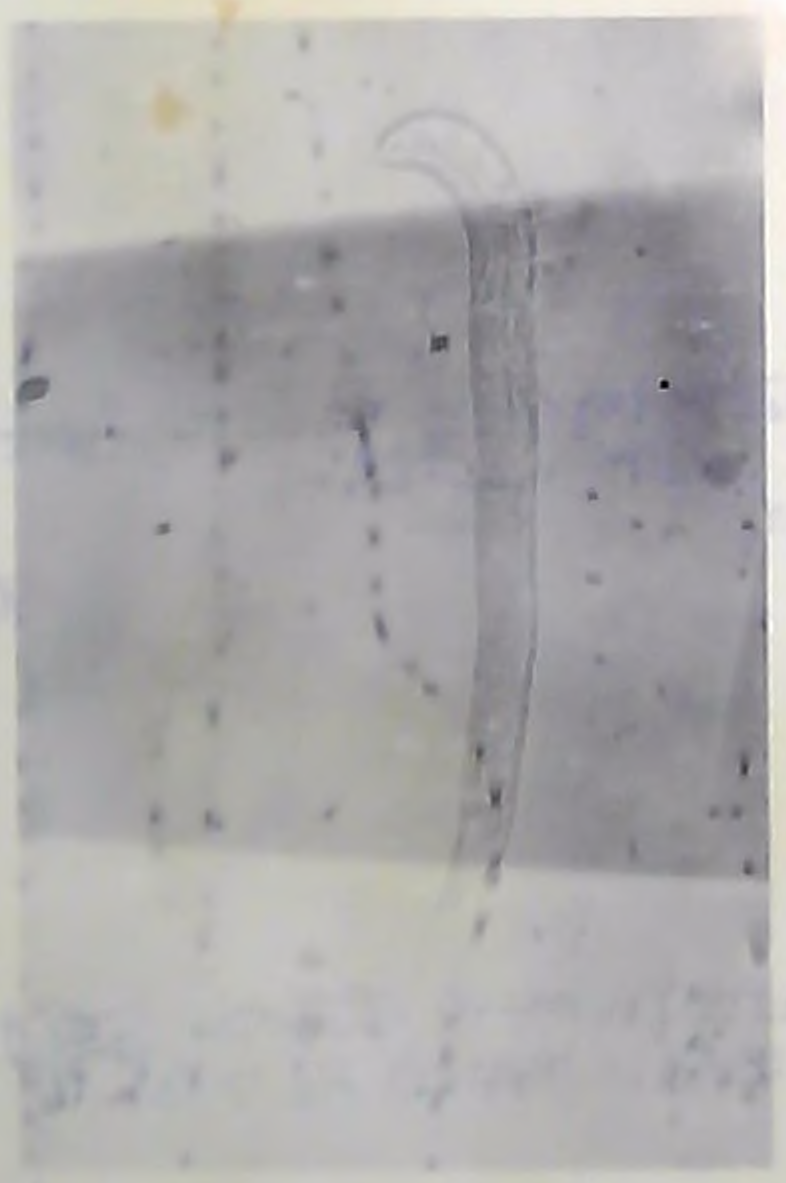
100x

Fig. 24 Rabiuria uncinata - development within Daphnia - second stage juvenile under moulting - loose cuticule at head end.

21



22



23

24

Fig. 25 Echinuria uncinata - development within Daphnia - second stage juvenile under moulting - loose cuticle at tail end.

450x

Fig. 26 Echinuria uncinata - development within Daphnia - third stage juvenile - 5 days after infection.

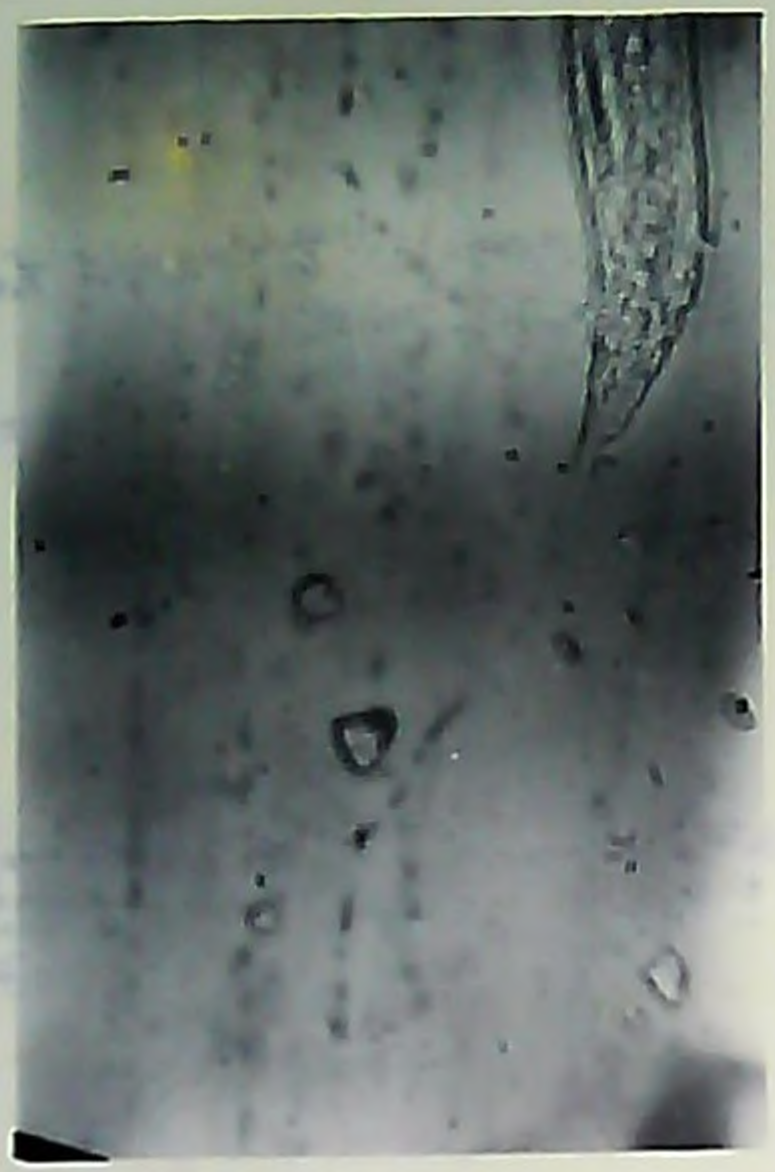
100x

Fig. 27 Echinuria uncinata - development within Daphnia - third stage juvenile in the body cavity - 5 days after infection.

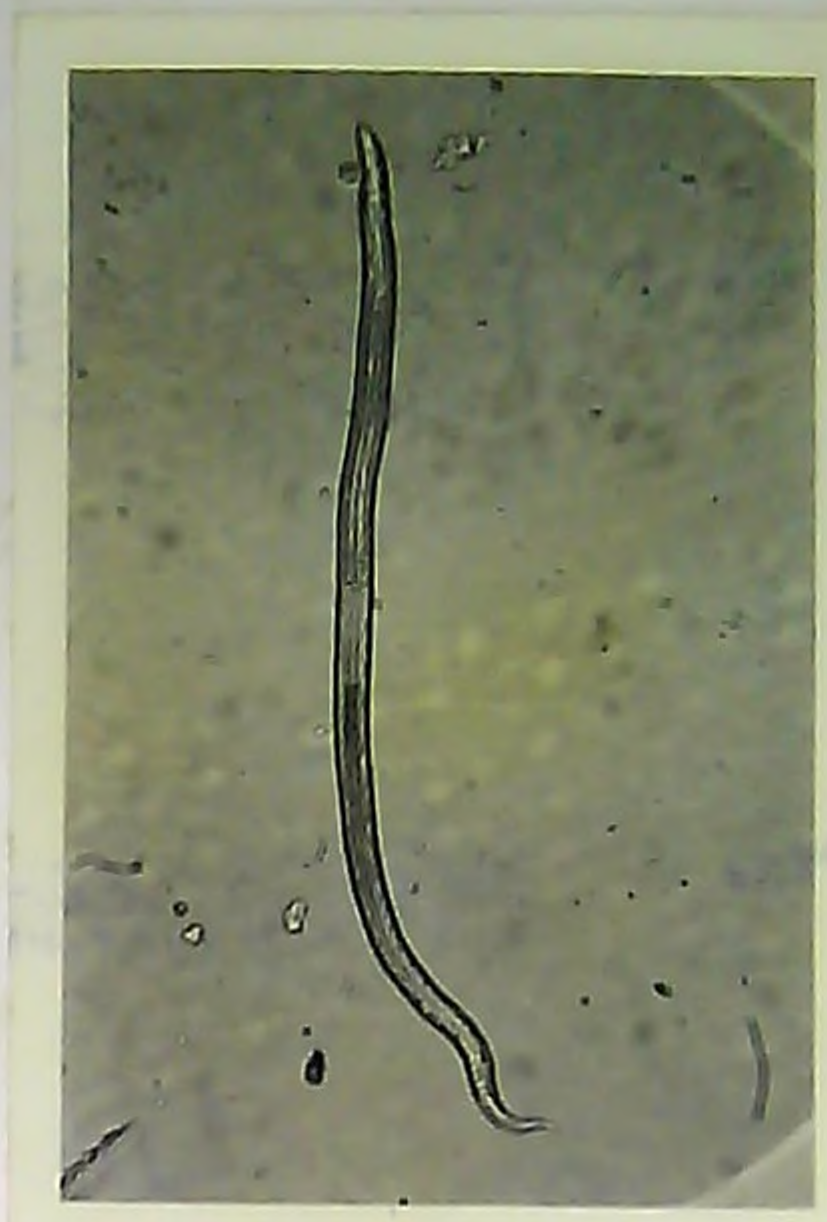
40x

Fig. 28 Echinuria uncinata - development within Daphnia - third stage juvenile in the body cavity - 5 days after infection.

40x



25



26

27

28

juveniles were actively motile in haemocoele of the intermediate host.

The female larvae measured 1.25-1.425 mm in length and 0.040-0.0425 mm in breadth. Other measurements were as follows buccal capsule 0.055-0.0675 mm; nerve ring 0.080 mm; excretory pore 0.125-0.1375 mm; muscular oesophagus 0.170-0.195 mm; glandular oesophagus 0.295-0.325 mm; genital primordium 0.140-0.150 mm from tail tip and tail 0.0875-0.100 mm.

Measurements of male larvae were: length 1.175-1.25 mm; breadth 0.0375-0.04 mm; buccal capsule 0.050-0.0525 mm; nerve ring 0.080-0.0825 mm; excretory pore 0.1175-0.130 mm; muscular oesophagus 0.1825-0.1875 mm; glandular oesophagus 0.30-0.3275mm genital primordium 0.190-0.230 mm from tail tip and tail 0.075-0.0875mm.

7 days after infection

The third stage larvae recovered from infected Daphnia on the 7th day after infection showed no additional features except that the intestinal contents were deep brown coloured. The larvae were found in all locations within the body of Daphnia including the antennae (Fig. 29). The female and male larvae measured 1.425-1.545 mm and 1.275-1.375 mm respectively in length and 0.050-0.0525 mm and 0.0425-0.050 mm respectively in breadth.

Fig. 29 Echinuria uncinata - development within Daphnia - third stage juvenile in the antennae.

100x

Fig. 29 Dahinuria uncinata - development within Daphnia - third stage juvenile in the antennae.

100x

to 10 days after infection

No additional morphological changes were noticed in the hird stage larvae collected during the period from 9 to 10 ays after infection. The female and male juveniles, on tenth ay, had attained a total length of 1.625-1.75 mm and 1.48-.52 mm and a breadth of 0.050-0.0575 mm and 0.050-0.0525 mm espectively.

ime taken to reach infective stage

In order to find out when the third stage juveniles of chinuria uncinata reach the infective stage to ducklings, a otal of 10 ducklings aged below 15 days were given 25 larvae ach of 5 to 8 and 10 days development within Daphnia. All the arvae had reached the third stage as mentioned supra. The ucklings which received larvae of 5 days and 6 days of develop-ent within Daphnia failed to become infected as proved by ecal examination and at autopsy, whereas ducklings that eceived larvae aged 7, 8 and 10 days of development, showed ggs in their droppings and different developmental stages of he parasite could also be recovered from the proventriculi of ucks. It is, therefore, evident that the infective stage of chinuria uncinata is reached within Daphnia in 7 days after xposure to infection.

The salient morphological features of different develop-ental stages of Echinuria uncinata within the intermediate osts, Daphnia pulex and Daphnia magna are presented in the

Table 2. Details of developmental stages of Echinuria uncinata in Daphnia

Stage of larvae	Body length	Body breadth	Buccal capsule	Nerve ring	Excretory pore	Oesophagus		Genital primordium
						Muscular	Glandular	
Early first stage	0.1625-0.1775	0.01	-	-	-	-	-	-
First stage	0.1600-0.1775	0.0125-0.0175	-	0.040-0.045	0.050-0.0625	0.070 to	0.090	-
-do-	0.156-0.205	0.025-0.0375	0.015	0.040-0.050	0.0475-0.055	0.0625 to	0.0875	-
First moulting	0.375-0.400	0.030-0.035	0.025-0.030	0.045-0.060	0.065-0.080	0.0625-0.065	0.075-0.080	-
Second stage	0.4625-0.7125	0.030-0.0475	0.030-0.045	0.060-0.0875	0.075-0.1125	0.075-0.100	0.120-0.150	0.105-0.125
Second stage	0.750-0.850	0.035-0.0475	0.050	0.075-0.080	0.105-0.115	0.075-0.0875	0.1125-0.0150	0.110-0.1375
Second moulting	0.725-1.0375	0.0375-0.0425	0.040-0.055	0.070-0.0875	0.085-0.1375	0.130-0.1625	0.165-0.2075	0.125-0.1375
Third stage Female	1.175-1.2375	0.0375-0.040	0.047-0.050	0.080-0.100	0.1125-0.1375	0.1625-0.200	0.3025-0.3125	0.130-0.145
Male	1.150-1.175	0.035-0.040	0.0475-0.0525	0.0775-0.0875	0.1125-0.125	0.180-0.1875	0.2875-0.325	0.185-0.215
Third stage Female	1.250-1.425	0.040-0.0425	0.055-0.0675	0.080	0.125-0.1375	0.170-0.195	0.295-0.325	0.140-0.150
Male	1.175-1.250	0.0375-0.040	0.050-0.0525	0.080-0.1825	0.1175-0.130	0.1825-0.1875	0.300-0.3275	0.190-0.230
Third stage	1.425-	0.050-	0.0625-	0.095-	0.135-	0.1875-	0.3325-	0.140-

Stage of larvae	Body length	Body breadth	Buccal capsule	Nerve ring	Excretory pore	Oesophagus		Genital primor-dium	Ta
						Muscular	Glandular		
Third stage Female	1.575-1.590	0.045-0.0525	0.0625-0.070	0.095-0.100	0.1325-0.140	0.1925-0.230	0.325-0.380	0.140-0.155	0. 0.
Male	1.325-1.455	0.0375-0.050	0.065-0.070	0.075-0.095	0.1075-0.1375	0.150-0.195	0.320-0.3575	0.205-0.240	0. 0.
Third stage Female	1.625-1.705	0.045-0.050	0.065-0.0675	0.100	0.140-0.1525	0.210-0.2425	0.3625-0.3875	0.145-0.155	0. 0.
Male	1.450-1.545	0.0425-0.050	0.067-0.070	0.080-0.095	0.115-0.1375	0.175-0.1975	0.330-0.365	0.220-0.240	0. 0.
Third stage Female	1.625-1.755	0.050-0.0575	0.0625-0.065	0.100	0.140-0.1525	0.200-0.245	0.375-0.3975	0.1475-0.160	0. 0.
Male	1.485-1.585	0.050-0.0525	0.065-0.070	0.085-0.090	0.1175-0.1375	0.195-0.200	0.345-0.365	0.230-0.2425	0. 0.

ments are in millimetre.

Effect of Echinuria uncinata infection on Daphnia

Infection with Echinuria uncinata larvae, appears to adversely affect Daphnia particularly when the larvae reach the third stage as evidenced from the fact that many of the infected Daphnia died as soon as the second larval moult was over.

INFECTION OF OTHER INTERMEDIATE HOSTS

During the present studies, attempts were also made to infect, 4 other waterfleas, Macrothrix sp., Sida sp., Moina sp and Scapholeberis sp., one copepoda, cyclops sp., one isopoda, Porcellio laevis and one variety of grasshopper Spathosternum prasiniferum. The results of these attempts are given below:

Macrothrix sp.

The body cavity of exposed Macrothrix sp. was found to contain first stage larvae after 24 hours. The larvae were active and were identical in morphology with those collected from infected Daphnia. The larvae measured 0.1725-0.175 mm in length and 0.010 mm in breadth. The nerve ring and excretory pore were located at 0.040 and 0.060 mm respectively from head end. The oesophagus extended upto 0.0925-0.0975 mm from anterior end.

During the period from 2 days to 9 days after infection of the Macrothrix sp., only first stage larvae could be seen

the first moulting on the tenth day (Fig. 31). The corresponding stage was reached by the parasite in Daphnia as early as, on the third day (Vide supra).

The first stage moulting larvae measured 0.225 mm in length and 0.030 mm in width. Other measurements were: buccal capsule 0.025 mm; nerve ring 0.055 mm; excretory pore 0.085mm; oesophagus 0.1075 mm and tail 0.030 mm.

The measurements of second stage larvae were as follows: length 0.483-0.600 mm; breadth 0.030-0.0315 mm; buccal capsule 0.025 mm; nerve ring 0.063-0.0875 mm; excretory pore 0.091-0.125 mm; muscular oesophagus 0.070-0.080 mm; glandular oesophagus 0.105-0.120 mm and tail 0.0455-0.0625 mm.

Further careful observations of the infected Macrothrix sp. indicated that though the first moulting took place within the haemocoele, the second stage larvae perished and failed to establish themselves. Only rare second stage juveniles were encounted upto the eleventh day and thereafter none of the exposed Macrothrix sp. showed any developing larvae, thus proving that this species is not a suitable intermediate host.

Sida sp.

First stage larvae were recovered from the body cavity of Sida sp. (Fig. 32) on first and second day following infection They were identical in morphology with those collected from Daphnia and measured 0.1775-0.1925 mm in length and 0.010 mm in

breadth. The nerve ring and excretory pore were located at 0.045-0.0475 mm and 0.0625 mm respectively from head end. The oesophagus was 0.057 mm and the tail 0.025-0.0275 mm. These first stage larvae failed to establish themselves in the Sida sp. tried, as evidenced by the fact that from the third day onwards, no larvae could be detected in the exposed water-fleas.

Scapholeberis sp.

First stage larvae similar to those obtained from Daphnia could be recovered from exposed Scapholeberis sp. (Fig. 33) upto a period of 4 days. The measurements of these first stage larvae were as follows: length 0.1675-0.170 mm; breadth 0.020 mm; nerve ring 0.035 mm; excretory pore 0.050 mm; oesophagus 0.0775 mm and tail 0.0275 mm.

As with Sida sp., the first stage juveniles failed to develop further in Scapholeberis sp. also, since from the fifth day onwards no developing larvae could seen in exposed waterfleas.

Moina sp.

Infected Moina sp. revealed first stage larvae in their body cavity (Fig. 34) upto 4 days only. The larvae collected on the fourth day measured 0.170-0.1774 mm in length and 0.0125-0.0175 mm in breadth. Other measurements were as follows: nerve ring 0.030-0.0525 mm; excretory pore 0.060-

Fig. [illegible] Echinuria uncinata - development within Haemothrix sp. - first stage juveniles in the body cavity - 2 days after infection.

70x

Fig. 31 Echinuria uncinata - development within Haemothrix sp. - first stage juveniles under moulting in the body cavity - 10 days after infection.

140x

Fig. 32 Echinuria uncinata - development within Sida sp. - first stage juvenile in the posterior abdomen - 24 hours after infection.

100x

Fig. 33 Echinuria uncinata - development within Scapholeberis sp. - first stage juveniles in the body cavity - 24 hours after infection.

70x

30

31

32

33

The worms failed to establish in this waterflea also, as no larvae could be detected from the fifth day onwards.

Cyclops sp., Porcellio laevis and Spathosternum prasiniferum

All the above were found to be refractory since even hatching of Echinuria uncinata egg was not noticed in them.

DEVELOPMENT WITHIN DUCK

Development of the parasite within the final host viz., domestic ducks was studied by artificially infecting 15 ducklings of 15 days of age with 25 infective larvae each of 8 days development within Daphnia.

24 hours after infection

On sacrificing an infected duckling, third stage larvae were found attached to the mucous membrane of the proventriculu These larvae were identical in morphology with those collected from infected Daphnia (Fig. 35). The tail end of the larvae were bent dorsad in the form of a hook (Fig. 36). The female larvae measured 1.65-1.75 mm in length and 0.045-0.055 mm in breadth. Buccal capsule was 0.060-0.065 mm deep. Nerve ring and excretory pore were located at 0.095-0.105 mm and 0.135-0.145 mm respectively from head end. Muscular oesophagus extended 0.175-0.225 mm and the glandular part 0.345-0.365 mm. The genital primordium was located at 0.165-0.175 mm from the

Fig. 34 Echinuria uncinata - development within Moina sp. - First stage juveniles in the body cavity - 2 days after infection.

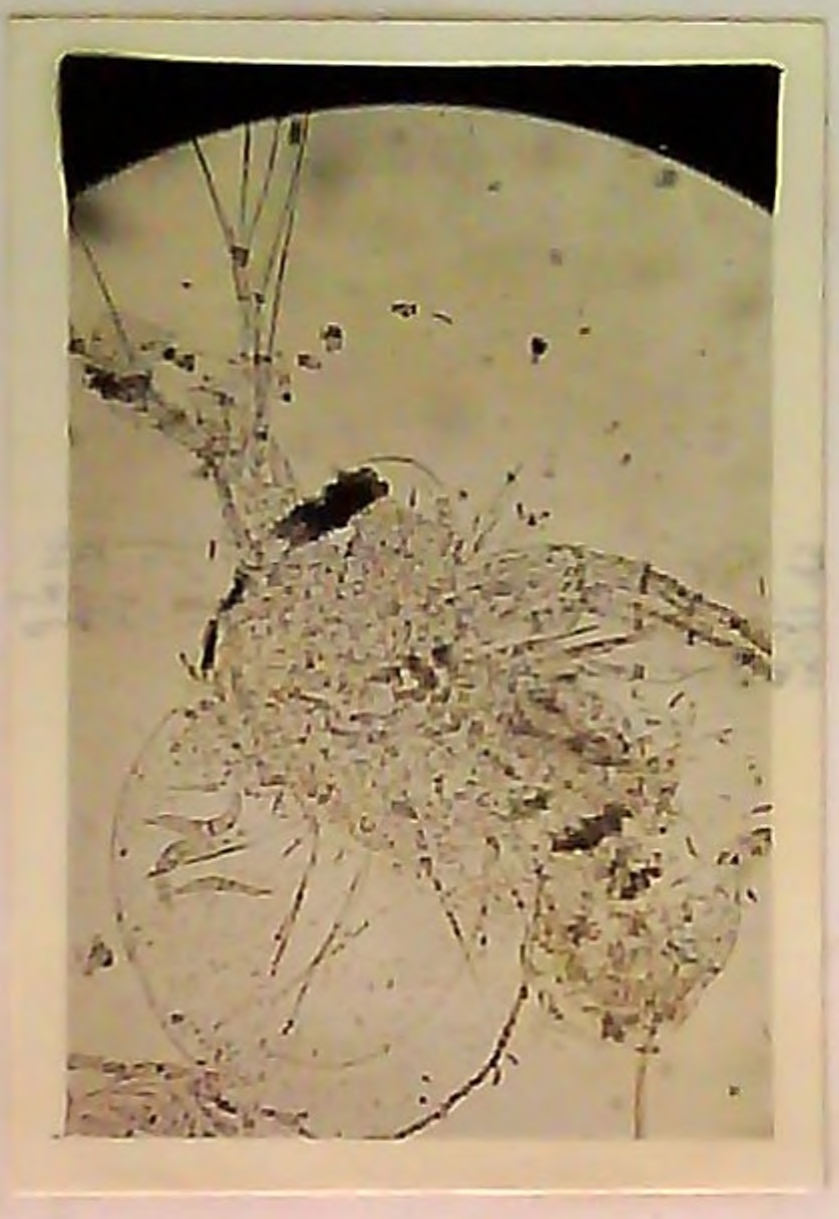
70x

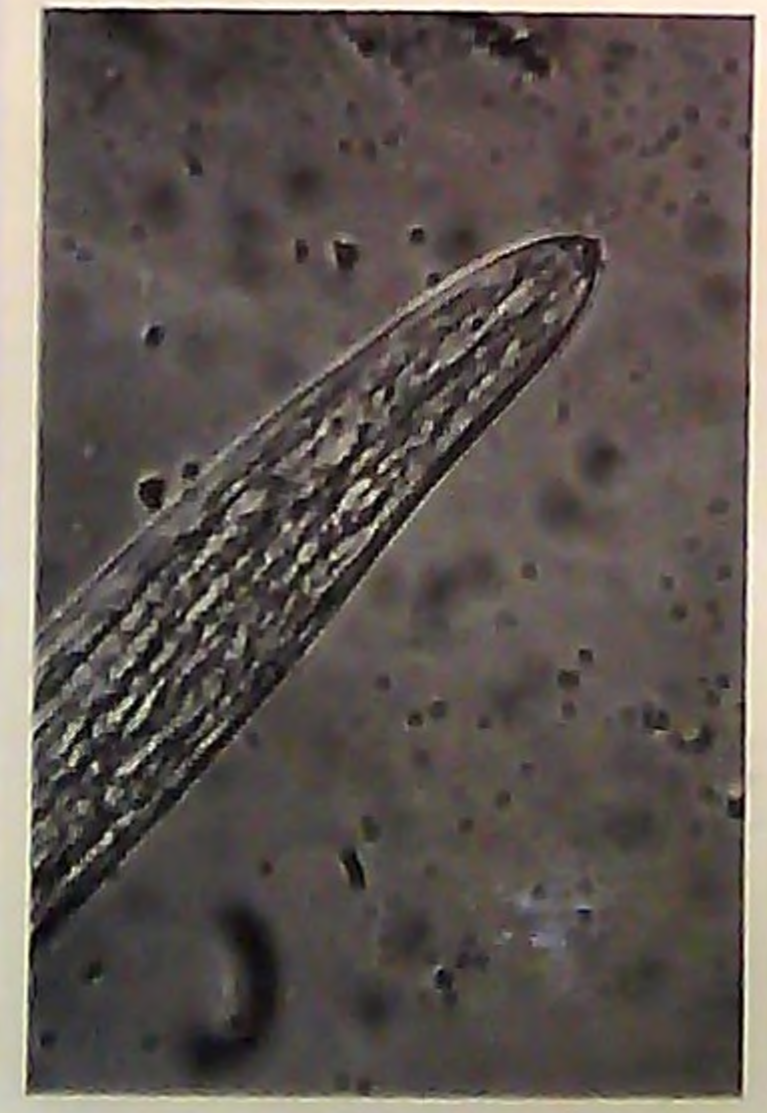
Fig. 35 Echinuria uncinata - development within duck - third stage juvenile - head end - 24 hours after infection.

300x

Fig. 36 Echinuria uncinata - development within duck - third stage juvenile - tail end - 24 hours after infection.

300x





The measurements of male larvae were as follows: length 1.55-1.65 mm; breadth 0.050-0.0525 mm; buccal capsule 0.0575-0.065 mm; nerve ring 0.090-0.0975 mm; excretory pore 0.120-0.135 mm; muscular oesophagus 0.170-0.205 mm; glandular oesophag 0.3400-0.3575 mm; genital primordium 0.375-0.390 mm from tail ti and tail 0.0975-0.105 mm.

2 days after infection

Within 2 days after infection of the duckling the larvae penetrated into the wall of the proventriculus with the result the scraping from mucous membrane of the organ yielded only very rare larvae, on the second day. The larvae recovered from the duckling were similar in appearance to those obtained on the previous day except that there was slight increase in the body size with further differentiation of genital primordium. The male larva measured as follows: length 1.775 mm; breadth 0.055 mm; buccal capsule 0.065 mm; nerve ring 0.100 mm; excretory pore 0.1325 mm; muscular oesophagus 0.225 mm; glandular oesophagus 0.385 mm; and tail 0.110 mm. The testicula primordium which was visible in the form of a band-shaped cluster of germinal cells in the ventral aspect of the body was located at a distance of 0.4975 mm from the tip of tail.

3 days after infection (Third larval moult)

On the third day, the larvae were seen only within the

larvae were undergoing the third ecdysis while a few had alread reached the fourth stage.

Moulting third stage juveniles

In the moulting third stage juveniles a set of faint anastamosing cordons were visible under the loose cuticle (Figs. 37 & 38). In female larvae primordia of vagina, uteru and ovary were more prominent, and in the case of male juveni the testicular primordium was more conspicuous and granular than in the young third stage.

The measurements of the moulting larvae were as follows length 1.815-1.95 mm; breadth 0.060-0.0625 mm; buccal capsule 0.0625-0.065 mm; nerve ring 0.100-0.105 mm; excretory pore 0.140-0.145 mm; muscular oesophagus 0.215-0.245 mm; glandular oesophagus 0.390-0.420 mm; vulvar primordium 0.250-0.285 mm from tail tip and tail 0.115-0.1175 mm.

Male larvae measured: length 1.75-1.935 mm; breadth 0.0575-0.60 mm; buccal capsule 0.060-0.065 mm; nerve ring 0.100-0.1025 mm; excretory pore 0.130-0.135 mm; muscular oesophagus 0.210-0.240 mm; glandular oesophagus 0.395-0.410 m and tail 0.110-0.115 mm. The testis extended upto 0.650-0.715 mm from the tail tip.

Fourth stage larvae

The fourth stage larvae were distinct from the previous stage by the presence of well formed cuticular cordons and bo

spines (Figs. 39 & 40). A central vacuolation appeared at the vaginal primordium of females. In the case of male larvae the testis extended upto the oesophago-intestinal junction.

Measurements of fourth stage female larvae were as follow length 1.95-2.15 mm; breadth 0.065-0.070 mm; buccal capsule 0.065-0.0675 mm; nerve ring 0.1075-0.110 mm; excretory pore 0.145-0.1475 mm; muscular oesophagus 0.220-0.255 mm; glandular oesophagus 0.460-0.515 mm; vulva 0.335-0.365 mm from tail tip and tail 0.120-0.125 mm.

The male larvae measured 1.85-1.975 mm in length and 0.060-0.065 mm in breadth and were, therefore, slightly smaller in size. Other measurements of the male larvae were as follows buccal capsule 0.065-0.0675 mm; nerve ring 0.105-0.1075 mm; excretory pore 0.140-0.145 mm; muscular oesophagus 0.220-0.250m glandular oesophagus 0.470-0.500 mm and tail 0.060-0.065 mm.

6 days after infection

Larvae collected on the sixth day were at the late fourth stage and had the following morphology.

Female larvae: The female larvae were comparatively larger and thicker than the males and measured 2.17-2.195 mm in length and 0.030-0.035 mm in breadth. They could be easily identified by the large number of coils and flexures of the developing uterus and ovary. The anterior limb of ovary reached upto the basal

Fig. 37 Echinuria uncinata - development within duck - third stage juvenile under moulting - head end - 3 days after infection.

400x

Fig. 38 Echinuria uncinata - development within duck - third stage juvenile under moulting - tail end - 3 days after infection.

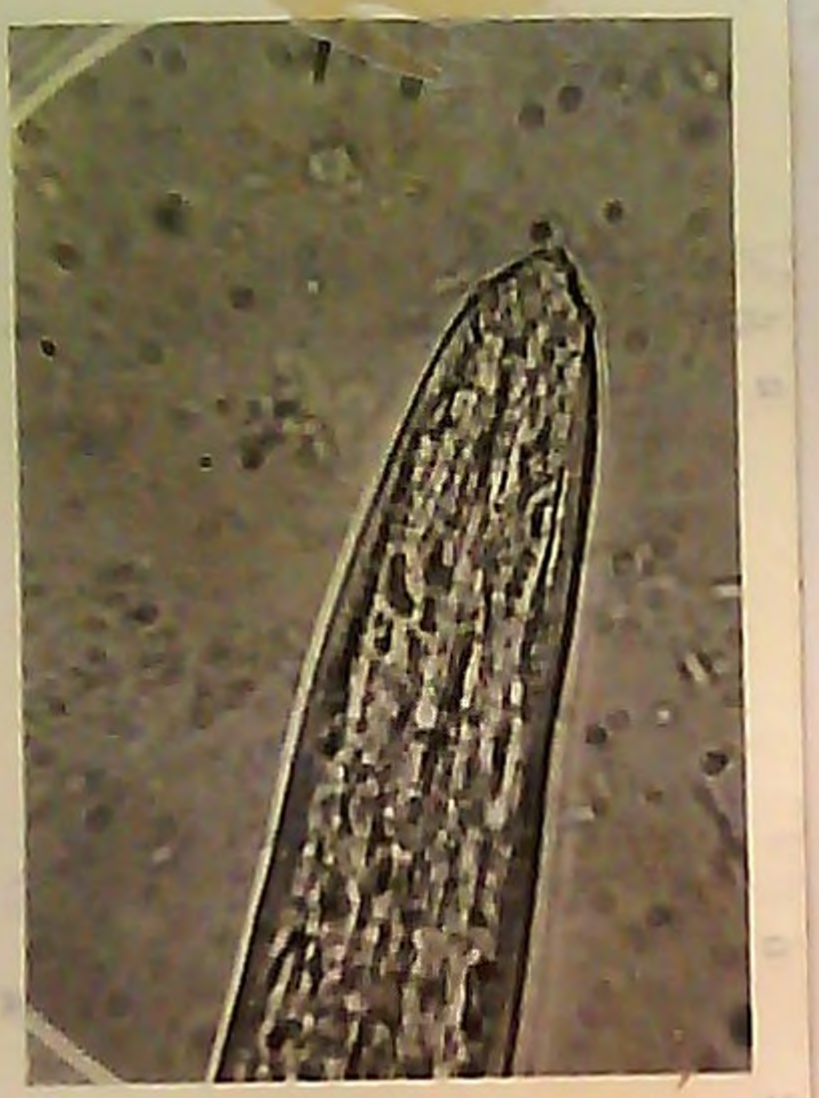
400x

Fig. 39 Echinuria uncinata - development within duck - third stage juvenile under moulting - tail end - 3 days after infection.

70x

Echinuria uncinata - development within duck - fourth stage juvenile - head end with distinct cordons and spines - 3 days after infection.

200x



37

38

as follows: buccal capsule 0.0675-0.075 mm; nerve ring 0.115-0.130 mm; excretory pore 0.165-0.1675 mm; muscular oesophagus 0.280-0.290 mm; glandular oesophagus 0.550-0.570 mm; vulva 0.375-0.3875 mm from the tail tip and tail 0.125-0.130 mm.

Male larvae: The male larvae were comparatively thinner and measured 2.05-2.175 mm in length and 0.070-0.075 mm in breadth. The spicular primordia were easily detectable (Fig. 41). The other measurements were as follows: buccal capsule 0.0675-0.070 mm; nerve ring 0.110-0.115 mm; excretory pore 0.1625-0.165 mm; muscular oesophagus 0.290-0.295 mm; glandular oesophagus 0.525-0.540 mm and tail 0.070-0.075 mm.

7 days after infection (Fourth and final larval moult)

On the seventh day after infection of ducklings, small nodular lesions with central openings were seen on the mucous membrane of proventriculus, especially in areas adjacent to the gizzard. These patent nodules were found to contain moulting fourth stage and young adult worms.

Fourth stage moulting larvae

The general morphology of fourth stage female and male larvae was similar to those recovered on the sixth day. The female and male larvae measured 2.25 and 2.10 mm in length and 0.085 and 0.075 mm in breadth respectively.

distinctly larger. The head end of both sexes presented the typical adult characters with well developed cordons and rows of body spines (Fig. 42). In female worms the outline of ovijectors was clear (Figs. 43 & 44) and in the case of male worms the flexures of the testis were very conspicuous (Fig.4) Additional features that could be noticed in the case of male worms were spicular primordia and caudal papillae. The latter consisted of four pairs of pre-cloacal and 4 pairs of post-cloacal papillae. The tail was comparatively longer than in females.

The measurements of female worms were: length 2.4375-3.6375 mm; breadth 0.117-0.125 mm; buccal capsule 0.085-0.105 mm; cordon 0.075-0.080 mm; nerve ring 0.125-0.1275 mm; excretory pore 0.190-0.215 mm; muscular oesophagus 0.460-0.5125 mm; glandular oesophagus 0.7875-0.915 mm; ovijector 0.150-0.1675 mm in length, vulva 0.4825-0.575 mm from tail tip and tail 0.1375-0.150 mm.

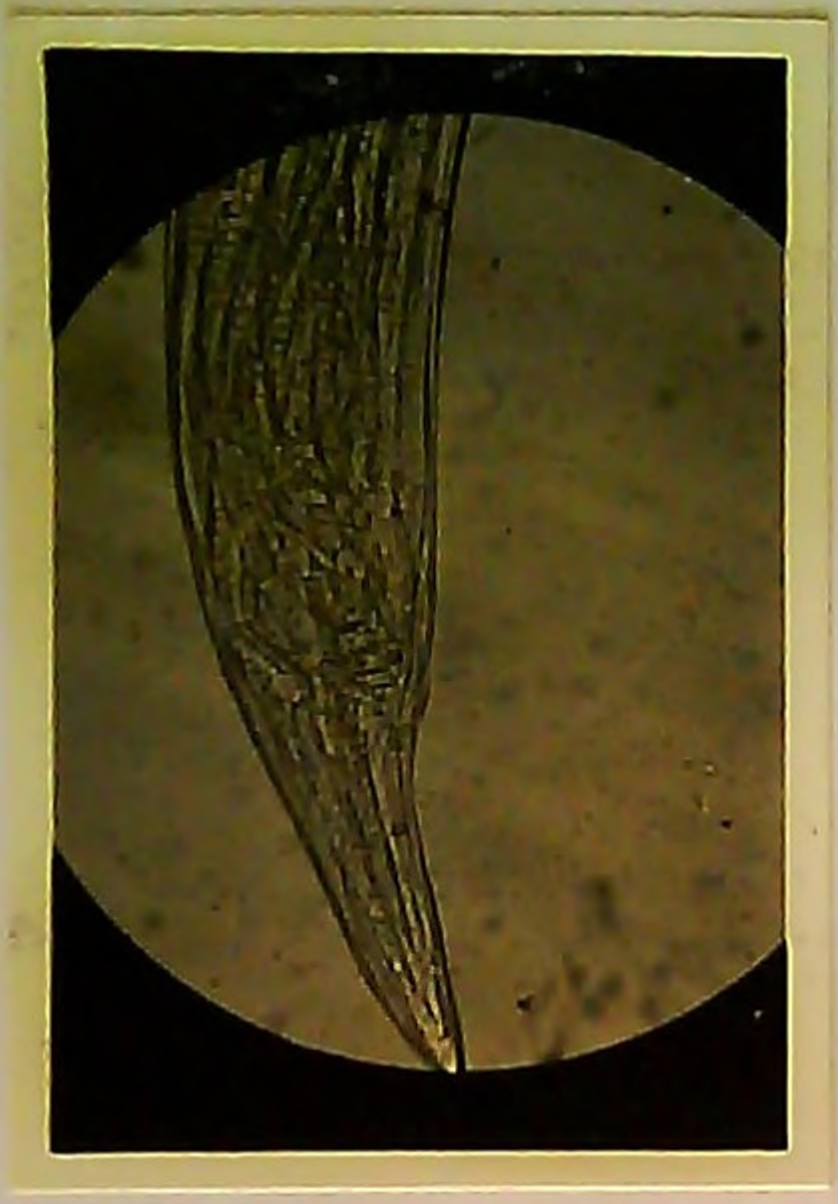
Measurements of male worms were as follows: length 2.2775-2.45 mm; breadth 0.100-0.120 mm; buccap capsule 0.075-0.080 mm; cordons 0.1025-0.1125 mm; nerve ring 0.1125-0.125mm excretory pore 0.165-0.200 mm; muscular oesophagus 0.325-0.340 mm; glandular oesophagus 0.665-0.7625 mm; tail 0.195-0.225 mm and testis reflexed backward at a distance of 0.2625-0.290 mm posterior to the junction of oesophagus and intestine.

Fig. 41 Echinuria uncinata - development within duck - fourth st[age] male juvenile - tail end with distinct spicular [pouch] and gubernaculum - 6 days after infection.
225x

Fig. 42 Echinuria uncinata - development within duck - young adult - head end with distinct cordons and spines - 7 days after infection.
450x

Fig. 43 Echinuria uncinata - development within duck - young female - hind portion with distinct ovijector and uterine coils - 7 days after infection.
100x

Fig. 44 Echinuria uncinata - development within duck - young female - tail end with distinct ovijector and uterine branches - 7 days after infection.
200x



41

42

43

44

considerable size and the contained young adults were significantly longer in size. In the case of females more numbers of coils and flexures of the uterus were visible in the perienteric space (Fig. 46) and the female genital opening was quite conspicuous (Fig. 47). The measurements of 15 day old females were as follows: length 4.5625-5.3625 mm; breadth 0.1625-0.170 mm; buccal capsule 0.105-0.125 mm; nerve ring 0.165-0.180 mm; excretory pore 0.275-0.345 mm; muscular oesophagus 0.5375-0.6575 mm; glandular oesophagus 1.25-1.375mm; vulva 0.650-0.750 mm from tail tip, ovijector 0.1625-0.175 mm and tail 0.1625-0.195 mm.

In males the caudal end was coiled and the spicules were more chitinised with characteristic shape and size (Fig. 48). The measurements of 15 day old males were as follows: length 4.425-4.725 mm; breadth 0.150-0.1625 mm; buccal capsule 0.100 mm; nerve ring 0.150-0.170 mm; excretory pore 0.265-0.320 mm; muscular oesophagus 0.525-0.5875 mm; glandular oesophagus 1.175-1.250 mm; tail 0.225-0.230 mm. The left spicule was long, slender and alate with a cup like widening at its distal end and measured 0.600-0.615 mm in length. The right spicule was thick and short in shape and measured 0.2125-0.215 mm. Caudal alae straight with pedunculated caudal papillae.

<u>20 days after infection</u>

On the twentieth day, the presence of nodules on the wall

45

46

47

48

of proventriculus could be easily detected, even without opening the organ, as they were raised, over the serous surface of the organ also. Worms dissected out of the nodules were almost double the size than those obtained on the fifteenth day and formation of eggs could be made out within the uterine coils of the female worms (Fig. 49). The worms could therefore be considered to have become gravid.

The measurements of female worms were: length 8.1875-8.4565 mm; breadth 0.375-0.400 mm; buccal capsule 0.125 mm; nerve ring 0.275-0.2825 mm; excretory pore 0.625-0.635 mm; muscular oesophagus 0.725-0.735 mm; glandular oesophagus 1.41-1.6375 mm; ovijector 0.240-0.280 mm; tail 0.2875-0.300 mm and vulva 1.25-1.3125 mm from tail tip.

The male worms were also fully mature with the presence of a large number of sperms in the seminal vesicle which gave a dark appearance to the organ (Fig. 50). The measurements of males on the twentieth day post infection of ducklings were as follows: length 6.9375-7.85 mm; breadth 0.225-0.2375 mm; buccal capsule 0.105-0.1125 mm; nerve ring 0.250-0.2525 mm; excretory pore 0.515-0.570 mm; muscular oesophagus 0.6125-0.650 mm; glandular oesophagus 1.5-1.6575 mm; left spicule 0.6625-0.6975 mm; right spicule 0.213-0.225 mm; seminal vesicle 1.5625-1.75 mm and tail 0.310-0.3225 mm.

30 days after infection

size (Figs. 51 & 52) and contained adult males and fully gravid females (Figs. 53, 54 & 55). The measurements of the worms were as follows:

Females: length 13.55-16.3625 mm; breadth 0.515-0.600 mm. The cuticular cordons extended upto 0.750-0.8125 mm over the body and were 0.025-0.030 mm thick. Buccal capsule 0.1575-0.1625 x 0.010 mm; nerve ring and excretory pore 0.250-0.275mm and 0.690-0.715 mm respectively from head end, muscular and glandular portion of oesophagus measured 0.950-1.065 mm and 2.55-2.75 mm respectively. Maximum breadth of oesophagus 0.1825-0.200 mm; ovijector 0.315-0.375 mm in length. Vulva in the posterior third of the body 1.35-1.425 mm from the tail end, tail 0.285-0.300 mm in length, eggs embryonated elliptical in shape thick shelled and measuring 0.030-0.035mm by 0.020 mm.

Males: length 7.750-8.125 mm; breadth 0.325-0.375 mm; buccal capsule 0.120-0.1375 mm; cordons 0.525-0.610 mm; nerve ring 0.250-0.2875 mm; excretory pore 0.585-0.610 mm; muscular oesophagus 0.8125-0.850 mm; glandular oesophagus 0.1625-1.7875 x 0.1625-0.175 mm; tail 0.350-0.3875 mm; left spicule 0.650-0.675 mm and right spicule 0.2125-0.2225 mm.

Details of the development of Echinuria uncinata within the final host are presented in Table 3.

Prepatent period

49

50

ble 3. Salient morphological details of developing Echinuria uncinata within ducklings

Third stage (24 hours)		Third stage moulting (3 days)		Early 4th stage (3 days)		Fourth stage moulting (7 days)		Young adults (7 days)		Adults (30 days)	
Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
1.65-1.75	1.55-1.65	1.815-1.85	1.75-1.835	1.95-2.15	1.85-1.975	2.25	2.10	2.4375-3.6375	2.45-2.775	13.55-16.3625	7.750-8.125
0.045-0.055	0.050-0.0525	0.060-0.0625	0.0575-0.060	0.065-0.070	0.060-0.065	0.085	0.0725	0.0117-0.0125	0.100-0.120	0.515-0.660	0.325-0.375
0.060-0.065	0.0575-0.065	0.0625-0.065	0.060-0.065	0.065-0.0675	0.065-0.0675	0.0725	0.0675	0.085-0.105	0.175-0.080	0.1575-0.1625	0.120-0.1375
0.095-0.105	0.090-0.0975	0.100-0.105	0.100-0.1025	0.1075-0.110	0.105-0.1075	0.120	0.115	0.125-0.1275	0.1125-0.125	0.250-0.275	0.250-0.2875
0.135-0.145	0.120-0.135	0.140-0.145	0.130-0.135	0.145-0.1475	0.140-0.145	0.165	0.165	0.190-0.215	0.165-0.200	0.690-0.715	0.585-0.610
0.175-0.225	0.170-0.205	0.215-0.245	0.210-0.240	0.220-0.255	0.220-0.250	0.395	0.295	0.460-0.5125	0.325-0.340	0.950-1.065	0.8125-0.850
0.345-0.365	0.340-0.3575	0.390-0.420	0.395-0.410	0.460-0.515	0.470-0.500	0.565	0.530	0.7875-0.915	0.665-0.7625	2.55-2.75	1.625-1.7875
0.030-0.035	0.030-0.0325	0.0375-0.040	0.035-0.0375	0.0425-0.045	0.040-0.0425	0.055	0.050	0.065-0.080	0.065-0.070	0.1825-0.200	0.1625-0.175
0.165-0.175	0.375-0.390	0.250-0.285	0.650-0.715	0.335-0.365	0.875-0.950	0.435	-	0.4825-0.575	-	1.350-1.425	-
0.095-0.105	0.0975-0.105	0.115-0.1175	0.110-0.115	0.120-0.125	0.120-0.125	0.1275	0.125	0.1375-0.150	0.195-0.225	0.285-0.300	0.350-0.3875
-	-	-	-	-	-	-	-	0.150-0.1675	-	0.315-0.375	-

infection were collected from a total of seven birds which received experimental infection with 8 days old third stage larvae collected from Daphnia pulex.

All the birds became positive for infection in from 33 to 36 days.

DISCUSSION

Spontaneous hatching of Echinuria uncinata eggs was observed by Akhtar (loc. cit.) in 15 minutes time after transferring the eggs into distilled water. Guilhon et al. (loc. cit.) found that the slightest pressure on the egg shell, as produced while gently moving the coverslip of aqueous mount was sufficient to induce the eggs to hatch. In the present studies in vitro hatching could be obtained within 2-3 minutes by placing the eggs of Echinuria uncinata in a few drops of normal saline solution containing the body fluids of Daphnia. Spontaneous in vitro hatching was also observed when the dissected uterine pieces were left in normal saline for 24 hours.

Natural infection of Daphnia pulex with larvae of Echinuria uncinata was recorded first by Hamann (loc. cit.). Subsequently Romanova (1938, 1947 & 1948), Radin (loc. cit.), Garkavi (loc. cit.), Srilkov (loc. cit.), Misiura (loc. cit.), Guilhon et al. (loc. cit.) and Austin and Welch (loc. cit.) have recovered the infective larvae of Echinuria uncinata by successfully infecting Daphnia pulex and Daphnia magna with

the eggs of the parasite. Kotelnikov (loc. cit.) exposed Ceriodaphnia, Gammarus sp., Asellus aquaticus and an unidentified cyprid ostracod to infective eggs of Echinuria uncinata and recovered the third stage juvenile from Ceriodaphnia and only first stage larvae from the rest of the waterfleas. Misiura (loc. cit.) obtained third stage larvae from Heterocypris incongruens in addition to Daphnia pulex and Daphnia magna. Austin and Welch (loc. cit.) also reported development upto the third stage juveniles in Simocephalus vetulus, Daphnia magna, Daphnia pulex and conchostraca Lynceus brachyurus, upto second stage juveniles in Gammarus lacustris, Hyalella azteca and first stage juveniles in Moina macrocopa, Ceriodaphnia reticulata, Ceriodaphnia acanthina, Eurycercus lamellatus and anotracan Chirocephalopsis bundyi. The following aquatic fauna could not be infected by him: viz., Alona sp., Scapholeberis sp., ostracods, copepods, odonatan, hemipteran nymphs and larvae of culicid, chironomid and coleopteran.

In the present study, development of the parasite could be seen upto the infective third stage larvae in Daphnia pulex and Daphnia magna; upto early second stage larvae in Macrothrix sp. and upto first stage larvae in Moina sp., Scapholeberis sp. and Sida sp. under experimental infection. The parasite failed to develop in cyclops, Porcellio laevis and grasshopper Spathosternum prasiniferum.

Romanova (1947) observed the first moulting of Echinuria

after experimental infection at a temperature ranging from 17 to 24°c. The same author (1948) while confirming her earlier findings, found that at higher temperatures (25-29°c) the infective third stage would be reached in 12 days time. Garkavi (loc. cit.) observed third stage larvae within Daphnia in 5 days at 26-30°c and in 6 days at 16-20°c. Kotelnikov (loc. cit.) obtained infective third stage larvae in Ceriodaphnia on the eleventh day at 18-20°c. Misiura (loc. cit. observed the first moulting of the parasite within Daphnia pulex and Daphnia magna in 9-14 days and second moulting in 14-21 days at 19-25°c. Guilhon et al. (loc. cit.) reported first stage in Daphnia pulex in 24 hours, second stage in 8-9 days and third stage in 14-15 days at 18°c. In cultures maintained at 12°c, they could not observe third stage larvae within 19 days after infection. Austin and Welch (loc. cit.) observed unhatched and hatching eggs and juveniles of Echinuria uncinata in the intestine of Daphnia in 15 minutes following exposure to eggs of parasites at 20-24°c. They also noticed the first moulting of the larvae within 100 hours of infection, larvae with loosened cuticle of second moult in 7 days and third stage larvae in 10 days, following infection when the waterfleas were maintained at a temperature of 20-24°c. The time taken to develop into third stage was found to be longer at lower temperatures as evidenced by their findings, since it took 30 days to reach this stage at 15°c.

of Echinuria uncinata within Daphnia viz., hatching in 2-3 minutes, second stage in 3 days and third stage in 5 days have taken place at a laboratory temperature ranging from 25-30°c. These findings agree with a more rapid rate of development of the parasite within Daphnia as reported by Garkavi (loc. cit.). It is clear from the account given above that the incubation temperature has a direct bearing on the development of the parasite within the intermediate hosts.

Guilhon et al. (loc. cit.) reported that the third and fourth moult of Echinuria uncinata in ducks took place in 2 days and 6 days respectively and the eggs of the parasite appeared in droppings on the thirtieth day following infection. Austin and Welch (loc. cit.) studied the development of Echinuria uncinata in mallard duck and observed the third moulting on the third day, fourth moulting on the twentieth day, sexually matured males on the thirtyfirst day and eggs in the droppings on the fortieth day after infection. Romanova (1938, 1947 and 1948) and Radin (loc. cit.) stated that Echinuria uncinata needed 51-52 days and 48 days respectively to attain maturity in ducks. Garkavi (loc. cit.) found fully matured female worms with unembryonated eggs in 34 days after infection. Kotelnikov (loc. cit.) observed matured female worms containing unembryonated eggs in 31 days and fully embryonated eggs in the droppings of ducks in 40 days after infection. The observations made during the present study in

respect of the development of Echinuria uncinata in domestic ducklings (maintained at room temperature of 25-30°c) viz., the third moulting as occurring on the third day, the fourth moulting on the seventh day, sexually matured male on the twentieth day, sexually matured female with embryonated eggs on the thirtieth day and first appearance of eggs in droppings on the thirtythird day agree with the data given by Guilhon et al. (loc. cit.).

LIFECYCLE OF *Tetrameres anatis*

LIFECYCLE OF TETRAMERES ANATIS

Tetrameres anatis was first reported and described by Chandrasekharan (1967) as occurring in the proventriculus of domestic ducks in Kerala. He had also worked out the life-cycle of this parasite using the grasshopper species Spathosternum prasiniferum and Oedaleus abruptus as experimental intermediate hosts. He separated the species from Tetrameres mohtedai of fowls mainly on the basis of cross transmission experiments. It was proved by him that Tetrameres anatis is not transmissible to fowls and Sundaram (1971) had similarly found that Tetrameres mohtedai is not transmissible to duck and pigeon. In his lifecycle studies on Tetrameres mohtedai Sundaram (loc. cit.) also used grass-hoppers as experimental intermediate hosts. During the course of routine parasitological investigations Tetrameres grusi was encountered in an Indian crane. Eggs harvested from this worm yielded encysted third stage juveniles when fed to the same species of grasshoppers. Further, Cram (1931 & 1933) had also found development of Tetrameres americana and Tetrameres pattersoni upto infective stage within grasshoppers. Thus, grasshoppers have been successfully used as intermediate hosts for Tetrameres mohtedai, Tetrameres anatis, Tetrameres americana, Tetrameres pattersoni and Tetrameres grusi. It, only, appears that grasshoppers are convenient experimental intermediate hosts for studying the lifecycle of Tetrameres species under laboratory conditions. Hence it is felt that

mere development of Tetrameres anatis in grasshoppers and variations in the timings of larval moults and development may not be sufficient grounds to establish it as a new species unless other proven intermediate hosts for other duck Tetrameres species were also used to observe the variations in developmental details.

Among the Tetrameres species Tetrameres anatis is difficult to be distinguished from Tetrameres fissispina purely on morphological grounds, since from time to time additional details have been given to Tetrameres fissispina by many workers like Tubangui (1926), Czaplinki (1962) and Arambulo et al. (1970), and as a result the body measurements of Tetrameres anatis fall within the consolidated range of measurements given by many authors to Tetrameres fissispina (Vide Table 4). Sundaram (loc. cit.) also felt the same difficulty in separating Tetrameres mohtedai from Tetrameres fissispina and relied more on biological criteria in distinguishing between the two species. In the case of Tetrameres anatis, too, biological criteria have to be necessarily applied in order to establish it as a distinct species. Attempts were therefore made to infect Daphnia pulex and Daphnia magna the proven intermediate hosts of Tetrameres fissispina with the eggs of Tetrameres anatis and the following results were obtained.

Table 4. Morphological features of Tetrameres fissispina and Tetrameres anatis

Morphological features	Tetrameres fissispina Dies, 1861	Tetrameres fissispina Tubangui, 1926	Tetrameres fissispina Czaplinki, 1962	Tetrameres fissispina Arambulo et al. 1970	Tetrameres anatis Chandrasekharan, 1967	Tetrameres anatis Present study
Male						
Body length	3.00-6.00	3.48-5.00	3.30-4.90	3.50-5.00	4.11-4.86	4.41-4.90
Maximum width of body	-	0.090-0.20	0.103-0.134	0.160	0.110-0.125	0.120-0.125
Buccal capsule	0.008 x 0.003	Short	0.019-0.025x0.023 x 0.009-0.013 0.011		0.020-0.025 x 0.0075-0.010	0.0225-0.025 0.010
Cervical papillae from head end	0.15	0.180	0.155-0.189	0.160	0.1625-0.210	0.175-0.1875
Nerve ring from head end	-	0.22	0.190-0.245	0.210	0.1875-0.225	0.230-0.2375
Excretory pore from head end	-	-	0.225-0.270	-	0.2025-0.2375	0.255-0.275
Oesophagus - length	0.78	1.296	1.50-1.85	1.30	1.10-1.390	1.53-1.71
Long spicule - length	0.280	0.310-0.320	0.290-0.405	0.330	0.345-0.3975	0.355-0.390
Short spicule - length	0.082-0.150	0.115-0.140	0.108-0.174	0.115	0.1425-0.1625	0.150-0.160
Tail - length	0.130-0.250	0.130-0.250	0.210-0.265	0.250	0.2025-0.270	0.250-0.2675
First cephalic spine from head end	-	-	Close to the base of buccal capsule	-	0.020-0.030	0.02285-0.030
Pseudolabial buttress length	-	-	0.048-0.069	-	0.050-0.0525	0.060-0.0625
Total no. of spines	-	-	S.V. 65-73 S.D. 31-35	-	-	S.V. 53-57 S.D. 30-33

ological features	Tetrameres fissispina Dies, 1961	Tetrameres fissispina Tubangui, 1926	Tetrameres fissispina Czaplinki, 1962	Tetrameres fissispina Arambulo et al. 1970	Tetrameres anatis Chandrasekharan, 1967	Tetrameres anatis Present study
e						
length	1.67-6.00	1.67-6.00	1.50-3.92	1.50-5.50	2.85-4.05	3.48-4.12
um width of body	-	1.08-3.50	0.96-3.10	1.50-3.00	1.54-2.22	1.45-1.995
l capsule	0.021x0.010	-	0.018-0.023x 0.008-0.015	0.017x0.012	0.0175-0.020x 0.010	0.020x0.010
cal papillae from head end	-	-	Front of nerve ring	-	0.1125-0.145	0.1375-0.1425
ring from head end	-	0.400	0.130-0.185	0.520	0.155-0.190	0.185-0.195
tory pore from head end	-	-	Behind nerve ring	-	0.180-0.2175	0.205-0.220
hagus - length	1.460	-	1.530-2.475	1.130	1.25-1.63	1.48-1.667
to tail tip	0.130	-	0.23-0.41	0.560	0.400-0.580	0.440-0.4625
- length	0.071	0.070-0.20	0.120-0.177	0.260	0.1375-0.165	0.155-0.160
	0.045-0.056x 0.026-0.030	0.048-0.056x 0.030	0.046-0.0557x 0.026-0.034	0.055x0.029	0.0475-0.050x 0.025	0.0475-0.05x 0.025
	Duck	Chicken	Duck	Chicken	Duck	Duck

easurements are in millimetres.

DEVELOPMENT WITHIN DAPHNIA

24 hours after infection

The first stage larvae were recovered from the haemocoele of Daphnia pulex and Daphnia magna (Fig. 56) and the larvae were very active. They measured 0.195-0.210 mm in length and 0.01-0.0125 mm in breadth. The excretory pore was located at 0.0525-0.055 mm from the head end. The internal organs of the first stage juveniles were at primordial stage and therefore no further details could be noticed in these larvae.

48 hours after infection

On the second day after infection of the waterfleas, no larvae could be detected within the haemocoele proving that Daphnia pulex and Daphnia magna were not suitable intermediate hosts.

The experiments were repeated several times using different lots of Daphnia, with identical results.

In a separate series of experiments attempts were made to infect Daphnia pulex and Daphnia magna with eggs of Tetrameres mohtedai but the waterfleas proved to be totally refractory to infection as even hatching of eggs did not occur.

As positive controls, the development of the parasite was simultaneously observed in grasshopper species Spathosternum prasiniferum in which the development of the parasite could be

studied upto the infective third stage juveniles and upto sexually mature stage within ducks. More details on the various larval stages within the intermediate hosts and final host could be gathered during the present investigation than reported earlier by Chandrasekharan (loc. cit.). The results of the present studies on the lifecycle of Tetrameres anatis using grasshoppers as experimental intermediate hosts are furnished below.

In vitro hatching

Adopting Sundaram's technique (Sundaram loc. cit.) for in vitro hatching of poultry spirurid eggs, successful in vitro hatching of Tetrameres anatis eggs could be obtained by using stomach contents of grasshoppers (Figs. 57, 58 & 59). The manner of hatching was similar to that described for Tetrameres mohtedai by Sundaram (loc. cit.).

In vitro hatched first stage juveniles

The freshly hatched out first stage juveniles were active The anterior end was rounded, and possessed an anterior boring apparatus as described for Tetrameres mohtedai. Sixteen transverse rows of minute spines were observed on the anterior portion of the body which extended upto a distance of 0.0125-0.0135 mm from the head end. The mouth opening was simple and difficult to be located. The larvae measured 0.1825-0.1875 mm in length and 0.011-0.0125 mm in breadth. The excretory pore was located at 0.0525-0.055 mm from the head end. The intestin

56

57

58

59

primordium appeared as a hyaline structure with a length of 0.0225-0.030 mm and situated at a distance of 0.1075-0.1375 mm from the head end (Fig. 60). The primordium of rectum was located at 0.025-0.0275 mm anterior to the tail tip. The tail tip was rounded and provided with seven small refringent points, of which six were arranged along the periphery of tail tip and one at the centre. Other structures like nerve ring, oesophagus and genital primordium were not discernible in the larvae.

DEVELOPMENT WITHIN GRASSHOPPER

Laboratory raised grasshoppers of the species Spathosternum prasiniferum were fed with eggs of Tetrameres anatis and they were dissected at regular intervals to note the development of the parasite. Empty egg shells and larvae at various stages of hatching were noticed in the stomach of the infected grasshoppers within five minutes after infection. Actively motile first stage larvae could be collected from the haemocoele of grasshopper in eight minutes.

The early first stage larvae collected from the haemocoele of infected grasshopper at eight minutes after feeding the eggs were identical in morphology with those obtained by in vitro hatching (Fig. 61). They measured 0.1925-0.195 mm in length and 0.0125 mm in breadth.

24 hours after infection

Infected grasshopper yielded first stage larvae. The larv

exhibited active movement and measured 0.200-0.215 mm in length and 0.0125-0.015 mm in breadth. The buccal capsule was indistinct. The oesophagus was faintly visible and measured 0.067-0.075 mm. The nerve ring and excretory pore were located at 0.040-0.045 mm and 0.050-0.0525 mm respectively from the head end. The intestinal primordium was better delineated. The anus opened at 0.0375-0.040 mm from the tail tip.

2 days after infection

The larvae recovered from the haemocoele of infected grasshoppers on the second day following infection were still in first stage and had identical anatomical features to those of the previous day. They were, however, comparatively longer and thicker and measured 0.210-0.2475 mm in length and 0.020-0.025 mm in breadth. The nerve ring and excretory pore were located at 0.045-0.0475 mm and 0.050-0.0575 mm respectively from the head end. The oesophagus extended upto 0.075-0.085mm from the head end. The genital primordium was more distinct and was located at 0.060-0.095 mm from the tip of tail. The tail measured 0.040-0.0425 mm in length.

3 days after infection (First larval moult)

The infected grasshopper yielded both first stage moulting and second stage larvae.

The first stage larvae which were under the process of moulting, were longer and thicker and were provided with loose

cuticle at the head and tail ends (Figs. 62 & 63). They measured 0.365-0.400 mm in length and 0.0275-0.0325 mm in breadth. The buccal capsule was distinct and measured 0.010 mm deep. Muscular and glandular portions of oesophagus could be easily made out and they measured 0.040-0.0475 mm and 0.095-0.1125 mm respectively. Other measurements of the larvae were as follows: nerve ring 0.077-0.0375 mm; excretory pore 0.1025-0.110 mm; genital primordium 0.0725-0.110 mm from tail tip and tail 0.0425-0.055 mm.

The second stage larva was characterised by the blunt tail tip with a bulb like transparent cuticular dome (Fig. 64). The internal structures were more distinct than in the previous stage. The second stage juveniles measured 0.375-0.425 mm in length and 0.0525-0.0575 mm in breadth. Other body measurements were as follows: buccal capsule 0.010 mm; nerve ring 0.085-0.125 mm; excretory pore 0.105-0.1425 mm; muscular oesophagus 0.060-0.070 mm; glandular oesophagus 0.105-0.1375 mm; oesophageal bulb 0.020-0.0225 mm and tail 0.050-0.055 mm. The sex of the larvae could be differentiated by the relative position of genital primordia. In female it was located at 0.1125-0.120 mm anterior to tail tip whereas in male it was at 0.1475-0.1575 mm.

<u>4 days after infection</u>

On dissection of infected grasshoppers only second stage larvae could be recovered. They were identical in appearance

60

61

62

63

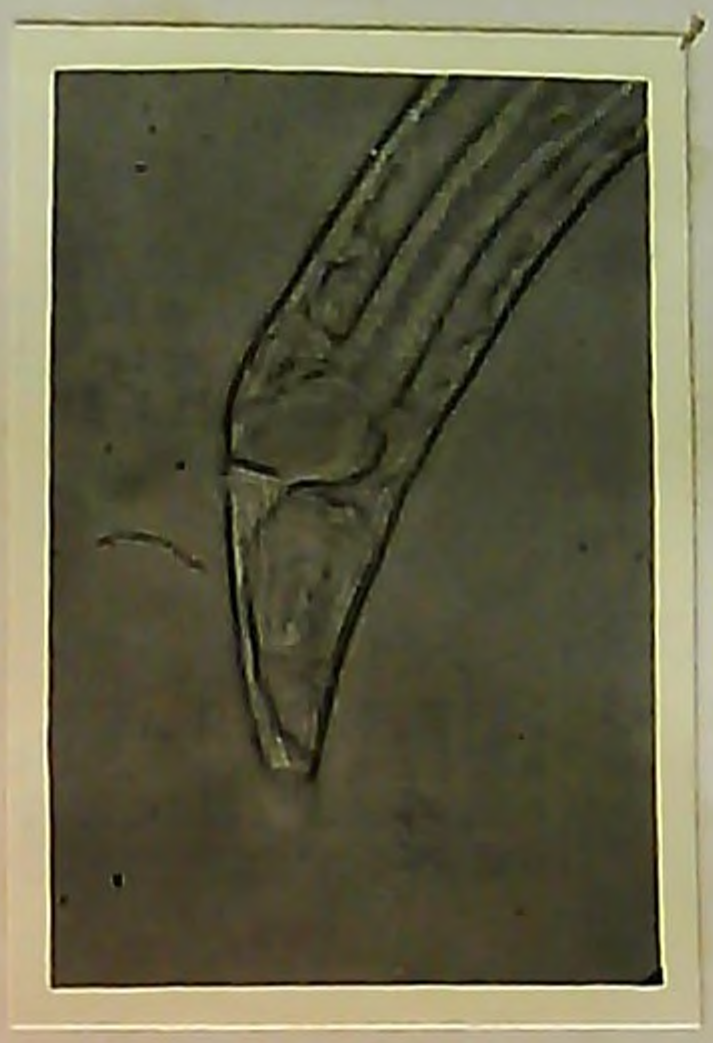
with that of the previous day excepting for a slight increase in length. The tail spines of the third stage larvae were discernible underneath the faint cuticle (Fig. 65). The body measurements of the larvae were as follows: length 0.475-0.760 mm; breadth 0.0525-0.055 mm; buccal capsule 0.010-0.0125 mm; nerve ring 0.0875-0.115 mm; excretory pore 0.105-0.1525 mm; muscular oesophagus 0.075-0.0875 mm; glandular oesophagus 0.115-0.1675mm; oesophageal bulb 0.015-0.0175 mm; tail 0.0675-0.08 mm; genital primordium in female 0.1475-0.1525 mm anterior to tail tip and in male 0.180-0.205 mm.

5 days after infection (Second larval moult)

Infected grasshopper contained larvae of two stages viz., second stage moulting and third stage juveniles.

The larvae undergoing second moulting were similar in appearance to those collected on the fourth day but with loose cuticle at either ends (Figs. 66 & 67). The tail spines of the third stage larvae were noticed underneath the loose cuticle at the tail tip as described earlier. The moulting second stage larvae measured 0.750-1.025 mm in length and 0.05-0.055 mm in breadth. Other body measurements of the larvae were as follows: buccal capsule 0.010-0.0125 x 0.005 mm; nerve ring 0.100-0.115 mm; excretory pore 0.1375-0.145 mm; muscular oesophagus 0.1425-0.1575 mm; glandular oesophagus 0.175-0.225 mm; oesophageal bulb 0.0175-0.020 mm; tail 0.100-0.1125 mm; genital primordium in female 0.1575-0.1825 mm and in male 0.2125-0.250 mm.

64



65

68

67

The freshly moulted third stage larvae were distinct from the previous stage since they possessed a rosette of 8 tail spines (Fig. 68), a pair of lateral tail papillae (Fig. 69) and a pair of cervical papillae (Fig. 70). The larvae measured 1.215-1.5 mm in length and 0.050-0.0525 in breadth. The buccal capsule was 0.0125 x 0.005 mm. Two distinct cervical papillae were observed at 0.105-0.1125 mm from head end. The nerve ring and excretory pore were situated at 0.125-0.1375 mm and 0.145-0.1575 mm respectively from the head end. The muscular and glandular parts of oesophagus measured 0.210-0.2325 mm and 0.370-0.3875 mm respectively in length with a maximum thickness of 0.020 mm at the posterior bulb. Genital primordium in female was located at 0.1725-0.195 mm from tail tip while that of male at 0.245-0.2925 mm. Anus opened at 0.110-0.125 mm and the tail papillae were located at 0.050-0525 mm respectively from tail tip.

6 days after infection (Encystation of 3rd stage juveniles)

The third stage larvae were found to have encysted in the fat bodies under the tergal plates of abdomen, pleura and sternum of the grasshopper. The cyst containing larvae measured 0.525-0.585 mm in length and 0.315-0.415 mm in breadth. The cyst wall was very thin and transparent. The larvae within the cysts showed serpentine movements and quickly emerged out from the cysts on the application of

even very slight pressure. The number of larvae present in a single cyst was found to range from 1 to 23 (Fig. 71). The measurements of the encysted third stage larvae were as follows: length 1.27-1.52 mm; breadth 0.050-0.0575 mm; buccal capsule 0.0125 x 0.005 mm; nerve ring 0.135-0.175 mm; excretory pore 0.150-0.160 mm; cervical papillae 0.105-0.115mm; muscular oesophagus 0.2175-0.235 mm; glandular oesophagus 0.3775-0.3925 mm; oesophageal bulb 0.020 mm; tail 0.1175-0.1275 mm; tail papillae 0.050-0.0525 mm and genital primordium in female 0.175-0.195 mm and genital primordium of male 0.250-0.300 mm from tail tip.

7 days after infection

Fat bodies of infected grasshoppers revealed large number of cysts containing third stage juveniles. Further changes in the morphology of larvae were not apparent. The body measurements were as follows: length 1.415-1.525 mm; breadth 0.050-0.0575 mm; buccal capsule 0.0125 x 0.005 mm; cervical papillae 0.1075-0.115 mm; nerve ring 0.135-0.140mm; excretory pore 0.1525-0.165 mm; muscular oesophagus 0.220-0.2425 mm; glandular oesophagus 0.380-0.3925 mm; oesophageal bulb 0.020 mm; tail 0.120-0.1275 mm; tail papillae 0.050-0.0575 mm; genital primordium in females 0.195-0.200 mm and in males 0.255-0.310 mm from tail tip.

No appreciable changes in the development of third stage larvae within the grasshoppers were noticed during the period

68

69

70

71

from 8 to 15 days. The third stage larvae collected on the fifteenth day measured 1.3875-1.555 mm in length and 0.055-0.0575 mm in breadth.

The salient morphological features of the developing stages of Tetrameres anatis within the grasshoppers are presented in the Table 5.

INFECTION OF OTHER INTERMEDIATE HOSTS

In addition to Daphnia pulex and Daphnia magna, as already mentioned, a variety of cladocerans, Macrothrix sp., Sida sp., Moina sp., and Scapholeberis sp. and copepoda Cyclops sp. were exposed to the viable eggs of Tetrameres anatis in small volumes of water. Though eggs of Tetrameres were found in large numbers within the gut of the cladocera and copepods no hatching or development of the larvae were noticed.

Infectivity of third stage larvae

In order to find out the time at which the third stage larvae of Tetrameres anatis is capable of infecting the ducklings, 50 larvae each of different age, ranging from 3 days to 13 days, were fed to parasite free ducklings aged below 15 days. The ducklings which received larvae of sixth day development onwards became positive for infection as revealed by eggs in their droppings and on autopsy adult parasites could be recovered from the proventriculus. The

Table 3. Salient morphological features of [illegible] within grasshopper

rphological features	Freshly hatched larvae	24 hours after infection first stage	2 days after infection first stage	3 days after infection		4 days after infection	5 days after infection		6 days after infection	7 days after infection
				First stage moulting	Second stage	second stage	Second stage moulting	Third stage	third stage	third stage
gth	0.1825-0.185	0.200-0.215	0.210-0.2475	0.365-0.400	0.375-0.425	0.475-0.760	0.750-1.025	1.215-1.50	1.27-1.52	1.415-1.525
adth	0.0125	0.0125-0.015	0.020-0.025	0.0275-0.0325	0.040-0.050	0.0525-0.055	0.050-0.055	0.050-0.055	0.50-0.0575	0.050-0.0575
cal capsule	-	-	-	0.010	0.010	0.010-0.0125	0.010-0.0125	0.0125	0.0125	0.0125
vical papillae from head end	-	-	-	-	-	-	-	0.105-0.1125	0.105-0.118	0.1075-0.115
ve ring from head end	-	0.040-0.045	0.045-0.0475	0.077-0.0875	0.085-0.1025	0.0875-0.115	0.100-0.115	0.125-0.1375	0.135-0.1375	0.135-0.140
retory pore from head end	0.050-0.055	0.050-0.0525	0.050-0.0575	0.1025-0.110	0.105-0.1425	0.105-0.1525	0.1375-0.145	0.145-0.1575	0.150-0.160	0.1525-0.165
cular oesophagus length	-	0.0675-0.075	0.075-0.085	0.040-0.0475	0.060-0.070	0.075-0.0875	0.1425-0.1575	0.210-0.2325	0.2175-0.235	0.220-0.2425
ndular oesophagus length	-			0.095-0.1125	0.105-0.1375	0.115-0.1675	0.175-0.225	0.370-0.3875	0.375-0.3925	0.380-0.3925
ophageal bulb	-	-	-	0.010-0.0125	0.015-0.0175	0.015-0.0175	0.0175-0.020	0.020	0.020	0.020
ital primordium from tail tip	-	-	0.060-0.095	0.0725-0.110	0.1125-0.120	0.1475-0.1525	0.1575-0.1825	0.1725-0.195	0.175-0.195	0.195-0.200
l length	0.025-0.0275	0.0375-0.040	0.040-0.0425	0.0425-0.055	0.050-0.055	0.0675-0.085	0.100-0.1125	0.110-0.125	0.1175-0.1225	0.120-0.1275
l papillae from tail tip	-	-	-	-	-	-	-	0.050-0.0525	0.050-0.0575	0.050-0.0575

ducklings which received larvae of 3 to 5 days of development within the grasshopper failed to show eggs in droppings or the worms on autopsy. It is therefore evident that the infective stage of Tetrameres anatis is reached within grasshoppers in 6 days after the grasshoppers were fed with eggs of the parasite. The rate of development of the parasite is found to be high (75%) when the ducklings were infected with 9 day old larvae and low (52%) with larvae of 6 days of development.

DEVELOPMENT WITHIN DUCK

To study the different developmental stages of Tetrameres anatis within the final host viz., ducks, 25 ducklings aged 10 days were fed each with 50 nos. of infective larvae of 6 days development within grasshoppers. The experimental birds were sacrificed at regular intervals and all the developing juveniles recovered from the proventriculus were studied in detail.

24 hours post infection of ducklings (3rd stage juveniles within ducklings)

The mucus scrapings of proventriculus revealed third stage larvae of Tetrameres anatis. The larvae were very active in movements and were identical in appearance to those recovered from grasshoppers. The sex of the larvae were identifiable by the location of genital primordium. The body measurements of female and male larvae were as follows:

Female: length 1.5-1.625 mm; breadth 0.060-0.0675 mm; buccal capsule 0.0125 x 0.005 mm; cervical papillae 0.1125-0.115 mm

from head end; nerve ring 0.135-0.140 mm; excretory pore 0.1575-0.160 mm; muscular oesophagus 0.225-0.235 mm; glandular oesophagus 0.415-0.4375 mm; oesophageal bulb 0.025-0.0275 mm in breadth; tail 0.115-0.125 mm; tail papillae 0.0525-0.055 mm from tail tip. The genital primordium was elongated (Fig. 72) and measured upto 0.050-0.0675 mm and was located at 0.175-0.195 mm from the tip of tail.

Male: length 1.45-1.55 mm; breadth 0.060-0.065 mm; buccal capsule 0.0125 x 0.005 mm; cervical papillae 0.110-0.115 mm; nerve ring 0.135-0.140 mm; excretory pore 0.155-0.1575 mm; muscular oesophagus 0.225-0.2375mm; glandular oesophagus 0.400-0.4275 mm; oesophageal bulb 0.025-0.0275 mm; tail 0.1175-0.1275 mm and genital primordium 0.300-0.325 mm from tail tip.

2 days after infection (3rd larval moult)

Late third stage and larvae under the process of third moultings were recovered from the mucus scrapings of proventriculus.

Late third stage

They are similar in appearance to those recovered on the previous day with more distinct internal structures. The female and male juveniles measured 1.75-1.85 mm and 1.65-1.80mm respectively in length and 0.0625-0.0675 mm and 0.060-0.065 mm respectively in breadth.

Third stage moulting juveniles

The third stage moulting juveniles were identified by the presence of loose cuticle at the head and tail end (Figs. 73 & 74). They were comparatively sluggish in movements. The female juveniles measured 1.83-1.875 mm in length and 0.0675-0.0725mm in breadth. Other measurements were as follows: buccal capsule 0.0125 x 0.0075 mm; cervical papillae 0.115-0.1225 mm; muscular oesophagus 0.235-0.250 mm; glandular oesophagus 0.475-0.495 mm; oesophageal bulb 0.0325-0.035 mm; genital primordium 0.200-0.205 mm from tail tip, tail 0.145-0.150 mm and tail papillae 0.055-0.060 mm from hind end.

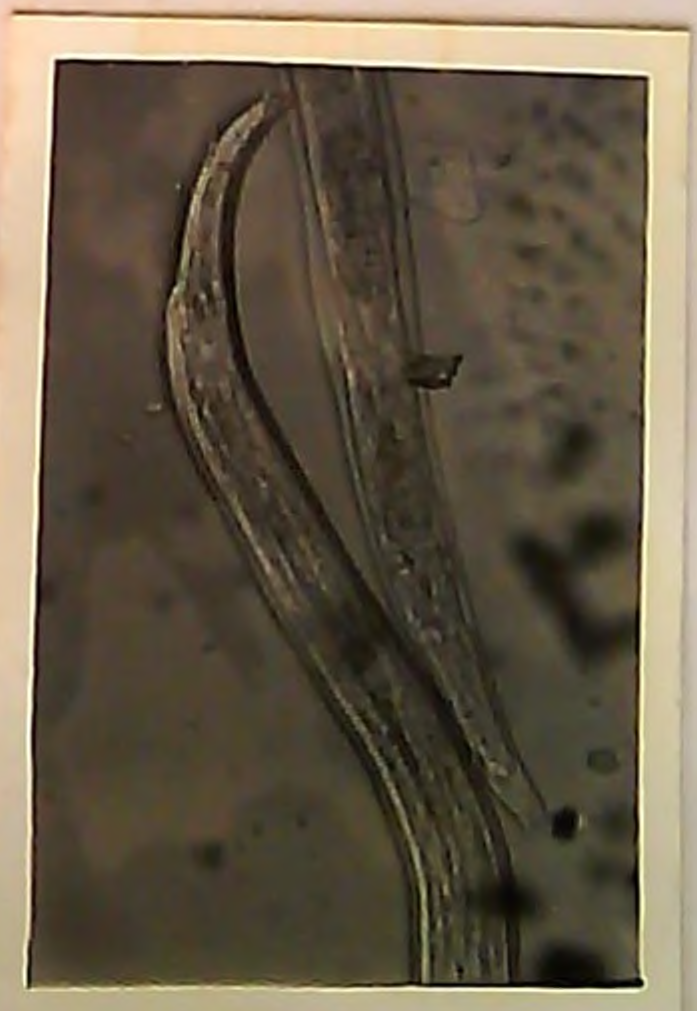
The body measurements of third stage moulting male juveniles were as follows: length 1.65-1.775 mm; breadth 0.065-0.070 mm; buccal capsule 0.0125 x 0.0075 mm; cervical papillae 0.115-0.1225 mm; nerve ring 0.140-0.145 mm; excretory pore 0.160-0.165 mm; muscular oesophagus 0.230-0.245 mm; glandular oesophagus 0.465-0.4875 mm; oesophageal bulb 0.0325-0.035 mm; tail 0.140-0.150 mm; tail papillae 0.055-0.0625 mm from caudal end. The primordium of testis was elongated further and reached 0.330-0.335 mm from tail tip.

3 days after infection (Fourth stage juveniles)

All the juveniles recovered from the mucus scrapings of proventriculus were in the early fourth stage. They were comparatively larger and stouter than those of the previous stage. The tail tip of the larva possessed a simple terminal

72

73



74

spine which was characteristic of the fourth stage (Fig. 75). The genital rudiments of both sexes have commenced to differentiate further. In female juveniles the rudiments of vagina and uterus were distinct (Fig. 76). In males the testicular primordium reached more anteriorly than in the moulting larvae. The buccal capsule was deep and cylindrical in both sexes (Fig. 77).

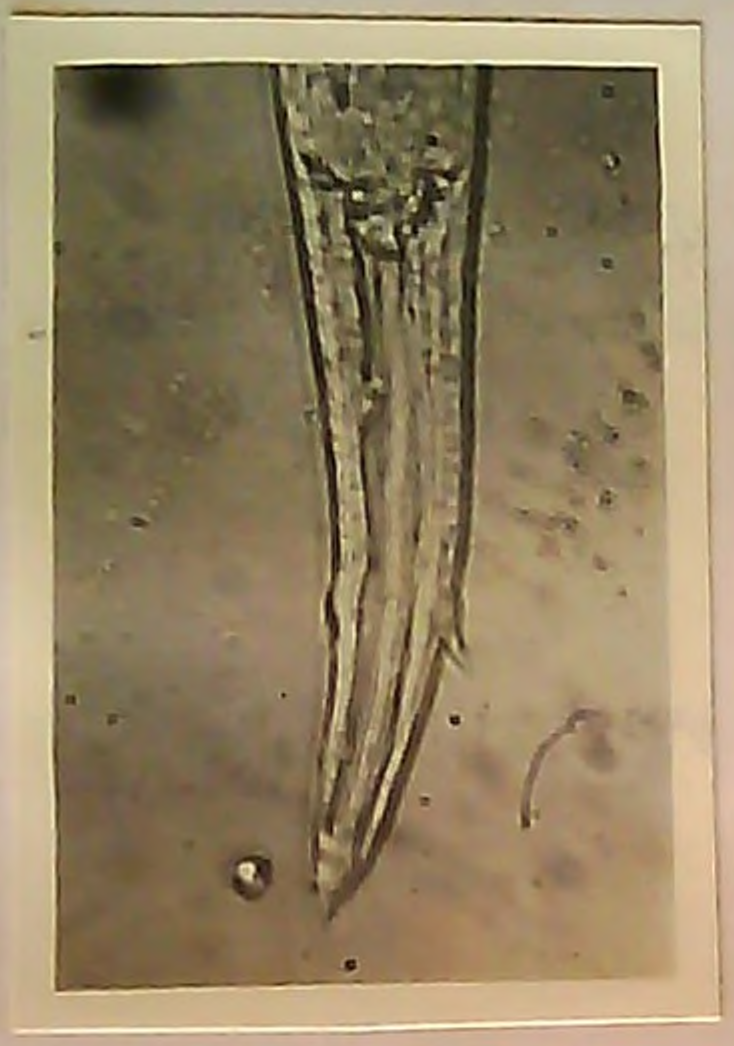
The fourth stage female juvenile measured 2.125-2.25 mm in length and 0.065-0.075 mm in breadth. The cylindrical buccal capsule measured 0.015 mm deep and 0.0075 mm in diameter. Cervical papillae were located at 0.120-0.1225 mm from the head end. The nerve ring encircled the oesophagus at a distance of 0.155-0.1575 mm from the anterior extremity. The excretory pore opened at 0.1755-0.1975 mm from head end. The muscular and glandular oesophagus measured 0.255-0.300 mm and 0.5125-0.675mm respectively in length and 0.035-0.0375 mm in breadth. The rudiments of vulva and vagina were located at 0.250-0.260 mm from the tip of tail. Anus opened at 0.1375-0.1525 mm from tail tip. Tail papillae were located at 0.055-0.0775 mm from hind extremity.

The fourth stage male juveniles measured 1.9875-2.05 mm in length and 0.060-0.070 mm in breadth. Other measurements were as follows: buccal capsule 0.015 x 0.0075 mm; cervical papillae 0.120-0.1225 mm; nerve ring 0.1575-0.1775 mm; excretory pore 0.175-0.1975 mm; muscular oesophagus 0.240-0.290 mm;

Fig. 75 Tetrameres anatis - development within duck - fourth stage juvenile - tail end with terminal spine and tail papillae - 3 days after infection.
450x

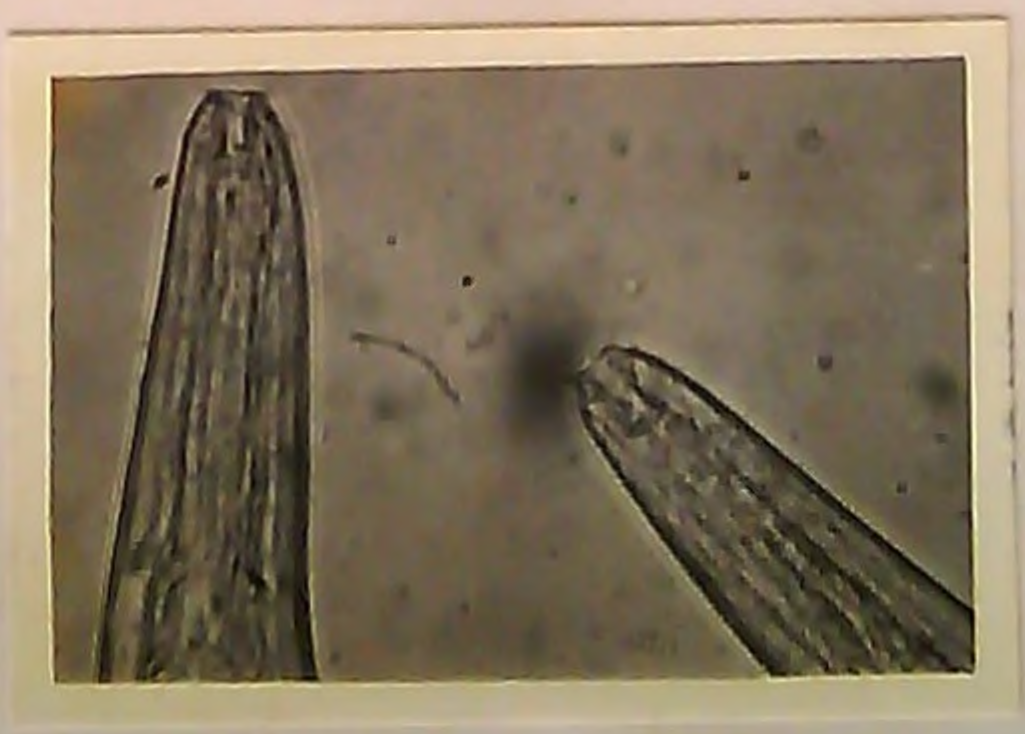
Fig. 76 Tetrameres anatis - development within duck - fourth stage female juvenile - head and tail end - 3 days after infection.
100x

Fig. 77 Tetrameres anatis - development within duck - fourth stage juveniles - head ends of male and female with cylindrical buccal capsule - 3 days after infection.
450x



75

76



77

glandular oesophagus 0.500-0.605 mm; oesophageal bulb 0.035-0.0375 mm; tail 0.1375-0.1475 mm; tail papillae 0.055-0.065 mm from tail tip. Testicular primordium was located at 0.415-0.505 mm from tail tip and extended forward to a further distance of 0.140-0.155 mm.

4 days after infection

The fourth stage juveniles were recoverable from the mucous membrane of proventriculus only. The larvae were very active and identical to those obtained on the previous day.

The body measurements of female larvae of 4 days development were: length 2.65-3.00 mm; breadth 0.075-0.0825mm; buccal capsule 0.015 x 0.0075 mm; cervical papillae 0.120-0.1325 mm; nerve ring 0.175-0.200 mm; excretory pore 0.185-0.2375 mm; muscular oesophagus 0.2725-0.325 mm; glandular oesophagus 0.5875-0.6875 mm; oesophageal bulb 0.0375 mm; vulva 0.300-0.310 mm from tail tip; tail 0.155-0.1875 mm and tail papillae 0.065-0.0775 mm.

Male juveniles measured 2.55-2.9875 mm in length and 0.075-0.0775 mm in breadth. Other body measurements were as follows: buccal capsule 0.015 x 0.0075 mm; cervical papillae 0.120-0.1325 mm; nerve ring 0.175-0.200 mm; excretory pore 0.190-0.235 mm; muscular oesophagus 0.235-0.325 mm; glandular oesophagus 0.575-0.675 mm; oesophageal bulb 0.0375 mm; tail 0.165-0.195 mm. The testis was 0.1725-0.190 mm long and its anterior tip was located 0.520-0.580 mm posterior to oesophago-intestinal junction.

6 days after infection

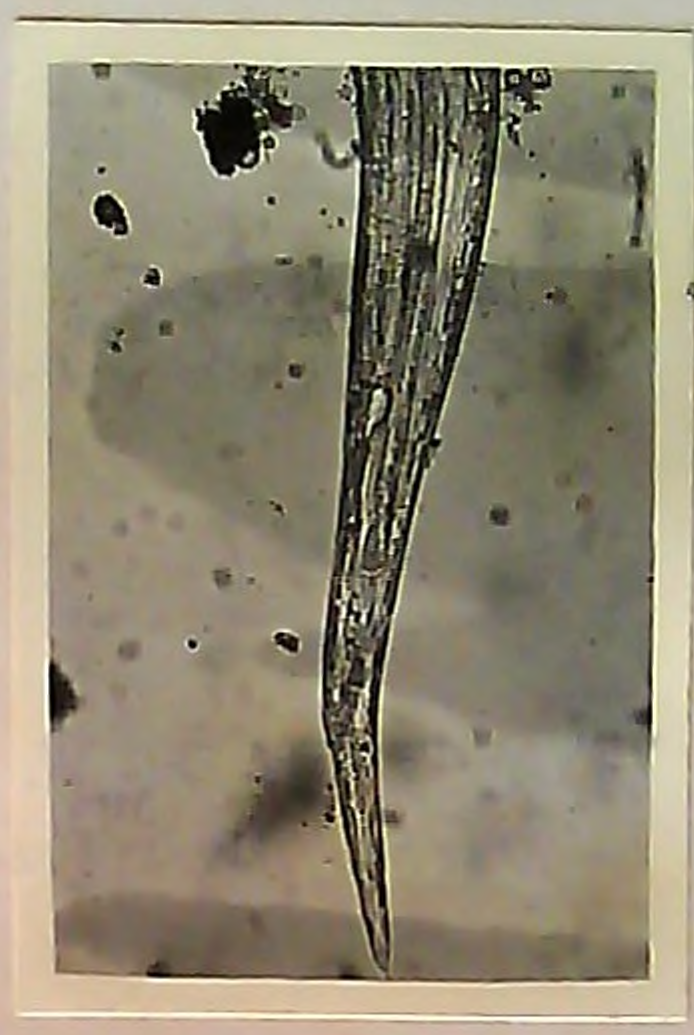
Both female and male juveniles were found only on the mucous membrane of the infected proventriculus. The worms collected on the sixth day showed appreciable sexual dimorphism in that the female juveniles were more stouter than males (Fig. 78). The genital primordium of female juveniles was differentiated into a vacuole like vagina (Fig. 79) and a bifurcated uterus connected anteriorly with ovarian tubules. The ovarian tubules reached upto the middle portion of the body (Fig. 80). In males, the testis was a granular and elongated organ. It extended from a distance of 0.195-0.215mm posterior to oesophago-intestinal junction and occupied the ventral aspect of the intestine (Fig. 81). The spicular primordium was discernible at the caudal end.

The body measurements of female and male juveniles were as follows:

Female: length 2.925-3.0725 mm; breadth 0.145-0.1875 mm; buccal capsule 0.020 x 0.0075 mm; cervical papillae 0.1375-0.150 mm; nerve ring 0.175-0.200 mm; excretory pore 0.200-0.240 mm; muscular oesophagus 0.350-0.400 mm; glandular oesophagus 0.7625-0.9125 mm; oesophageal bulb 0.0375-0.050 mm; vulva 0.4375-0.5725 mm from tail tip; tail 0.1875-0.2125 mm and tail papillae 0.0875-0.1025 mm from hind extremity.

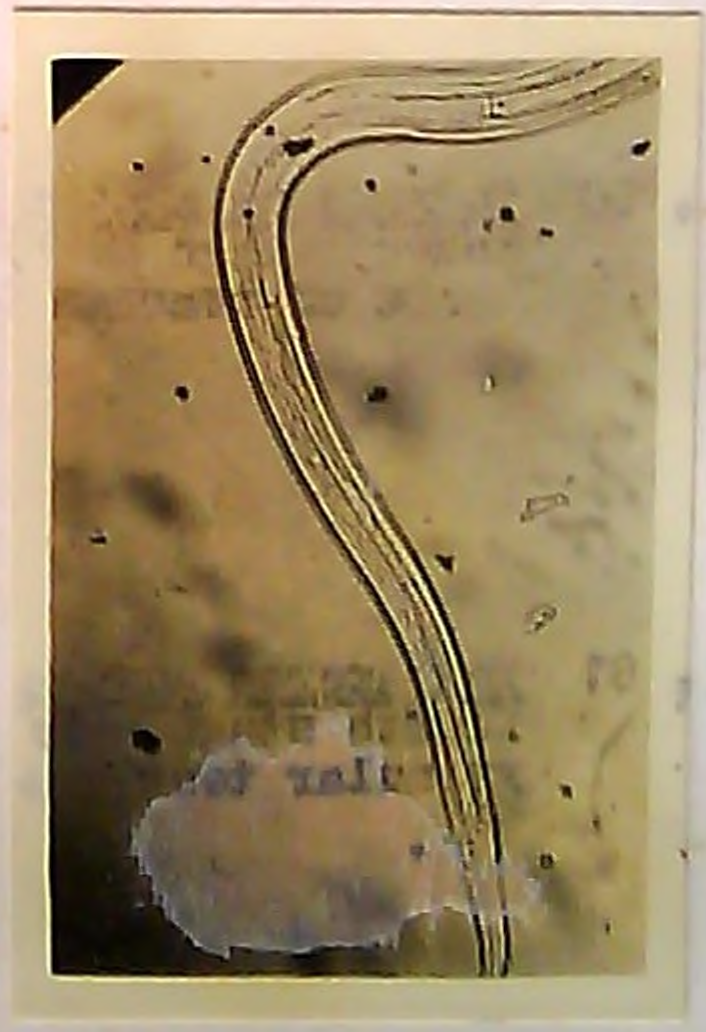
Males: length 2.65-2.6975 mm; breadth 0.1025-0.105 mm; buccal capsule 0.020 x 0.0075 mm; cervical papillae 0.125-0.130 mm;

78



79

80



81

nerve ring 0.175-0.190 mm; excretory pore 0.205-0.2125 mm; muscular oesophagus 0.300-0.3575 mm; glandular oesophagus 0.6875-0.700 mm; oesophageal bulb 0.0375-0.0425 mm; tail 0.1625-0.175 mm and tail papillae 0.0775-0.080 mm from tail end.

8 days after infection

The developing fourth stage juveniles were still found on the mucous membrane of proventriculus. The female juveniles were more stouter than males (Fig. 82), and measured 2.4375-3.0625 mm in length and 0.105-0.155 mm in breadth. The buccal capsule was cylindrical and measured 0.020 in depth and 0.0075mm in diameter. The cervical papillae were located at 0.1125-0.1375 mm from anterior end. The nerve ring and excretory pore were situated at 0.1625-0.1875 mm and 0.240-0.255 mm respectively from head end. The muscular and glandular oesophagus measured 0.3125-0.350 mm and 0.625-0.7125 mm respectively in length and 0.040-0.050 mm in maximum breadth. The vagina was more conspicuous and the ovijector was prominent (Fig. 83). The ovarian coils extended more anteriorly and upto the base of oesophago-intestinal junction. The vulval opening was located at 0.4375-0.4875 mm from the tail tip. The tail measured 0.1875-0.200 mm in length. The tail papillae were located at 0.075-0.090 mm from the hind end.

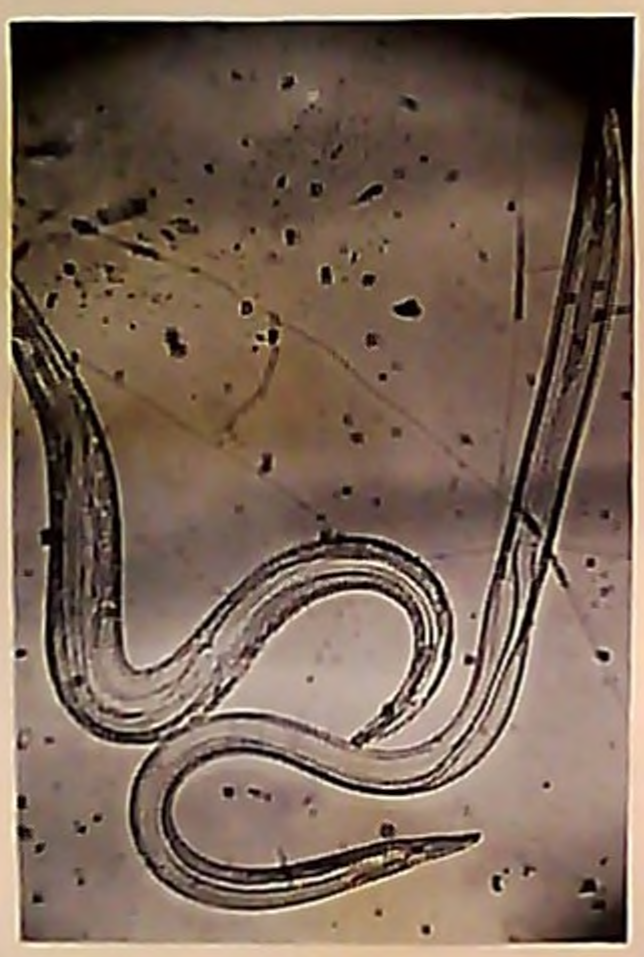
In males the testis extended anteriorly upto the base of the oesophagus and at the tail end feebly chitinised spicules

Fig. 92 Tetrameres anatis - development within duck - fourth stage male and female juveniles - 9 days after infection.

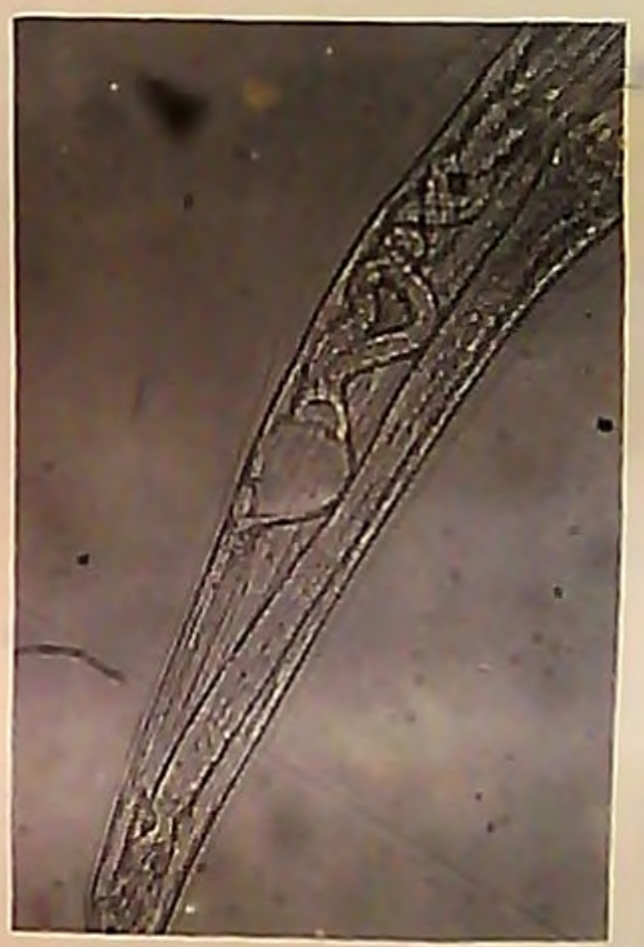
40x

Fig. 93 Tetrameres anatis - development within duck - fourth stage female juvenile - hind end with well distinct vagina and developing ovijector - 9 days after infection.

250x



82



83

were evident for the first time. The male juveniles measured 3.0375-3.125 mm in length and 0.100-0.105 mm in breadth. Other measurements were as follows: buccal capsule 0.020 x 0.0075 mm; cervical papillae 0.1275-0.1375 mm; nerve ring 0.165-0.175 mm; excretory pore 0.215-0.245 mm; muscular oesophagus 0.3125-0.3375 mm; glandular oesophagus 0.655-0.6875 mm; oesophageal bulb 0.035-0.0375 mm; tail 0.165-0.175 mm and tail papillae 0.0725-0.075 mm from caudal end.

9 days after infection (Fourth larval moulting of males)

Infected ducklings showed three types of juveniles viz., late fourth stage females, males in the process of fourth moulting and male young adults. All the juveniles were located in the mucous layer of proventriculus (Figs. 84 & 85) and none within the proventricular glands.

Sexual diamorphism was more evident than in the previous stage since the late fourth stage female juveniles were two times thicker than the males (Fig. 86). The body of females was maximally thick at the level of the proximal part of the intestine. The length and breadth of females were 2.87-3.125mm and 0.175-0.1875 mm respectively. The ovijector was well developed with heavy musculature (Fig. 87). The developing ovarian limbs almost reached upto the junction of oesophagus and intestine. The other body measurements were as follows: buccal capsule 0.020 x 0.0075 mm; cervical papillae 0.1375-0.1425 mm; nerve ring 0.175-0.1875 mm; excretory pore 0.250-0.2675 mm; muscular oesophagus 0.3025-0.3125 mm; glandular

84

85

86

87

oesophagus 0.700-0.755 mm; oesophageal bulb 0.0475-0.050 mm; vulval opening 0.450-0.475 mm from tail tip; ovijector 0.080-0.085 mm in length; tail 0.2125-0.2375 mm and tail papillae 0.0825-0.085 mm from hindend.

Fourth stage moulting male juveniles

The moulting male larvae were recognised by the presence of loose cuticle at the extremities, which was more conspicuous at the tail end (Figs. 88 & 89). In a slide preparation the shedding of cuticle was found to be completed in one hour (Fig. 90). The moulting larvae measured 3.16-3.3875 mm in length (excluding sheath) and 0.0925-0.105 mm in breadth.

Male-young-adults

The young adults were easily identifiable by the presence of body spines, 2 subventral rows of 53-57 spines and 2 subdorsal rows of 30-33 spines characteristic of the worm; pseudolabial buttress at the head end (Fig. 91) and well formed spicules. The post-cloacal spines were arranged in four rows, 2 laterals of 3 each and 2 medians of 5 each (Fig. 92). The spicules were characteristic in shape but unequal and dissimilar (Fig. 93). Testes had already developed into a massive gland extending anteriorly upto the base of the glandular oesophagus where it was reflexed on its own axis.

The body measurements of the male-young-adults were as follows: length 3.125-3.5625 mm; breadth 0.0975-0.1125 mm; buccal capsule 0.020 x 0.0075 mm; cervical papillae 0.1375-0.150mm;

ig. 88 Tetrameres spatia - development within duck - fourth stage male juvenile under moulting - head end - 9 days after infection.

450x

ig. 89 Tetrameres spatia - development within duck - fourth stage male juvenile under moulting - loose cuticle at extremities - 9 days after infection.

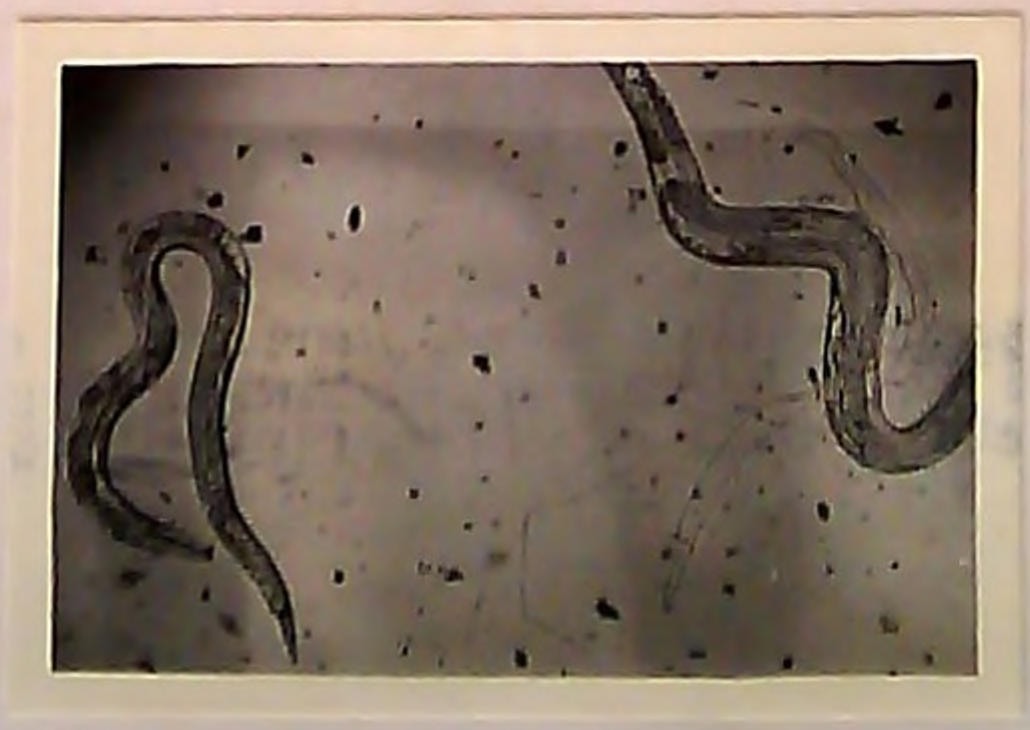
40x

ig. 90 Tetrameres spatia - development within duck - fourth stage female just moulted male and discarded cuticle - 9 days after infection.

40x

88

89



90

nerve ring 0.185-0.1875 mm; excretory pore 0.220-0.2475 mm; muscular oesophagus 0.3125-0.3375 mm; glandular oesophagus 0.750-0.775 mm; oesophageal bulb 0.040-0.0475 mm; tail 0.175-0.195 mm; right and left spicule 0.120-0.130 and 0.295-0.3875 mm respectively in length. The tail ended in a terminal spine (Fig. 94).

10 days after infection (Fourth larval moult of females)

On the tenth day after infection the fourth larval moult in respect of female worms was observed. The female worms were just becoming spindle shaped with loose cuticle at extremities. The uterine and ovarian coils filled almost three-fourth the portion of the perienteric space.

The body measurements of fourth stage moulting female juveniles were as follows: length 2.67-2.7375 mm; breadth 0.815-0.925 mm; buccal capsule 0.020 x 0.010 mm; cervical papillae 0.1275-0.1375 mm; nerve ring 0.165-0.1925 mm; excretory pore 0.1975-0.205 mm; muscular oesophagus 0.300-0.3125 mm; glandular oesophagus 0.750-0.815 mm; oesophageal bulb 0.060-0.065 mm; vulva 0.415-0.475 mm from tail tip, ovijector 0.080-0.0875 mm in length and tail 0.165-0.185 mm.

The male-young-adults recovered on the tenth day had the following measurements: length 3.54-3.775 mm; breadth 0.105-0.1125 mm; buccal capsule 0.020 x 0.0075 mm; pseudolabial buttress 0.0625-0.065 mm; cervical papillae 0.1375-0.145 mm;

91

92

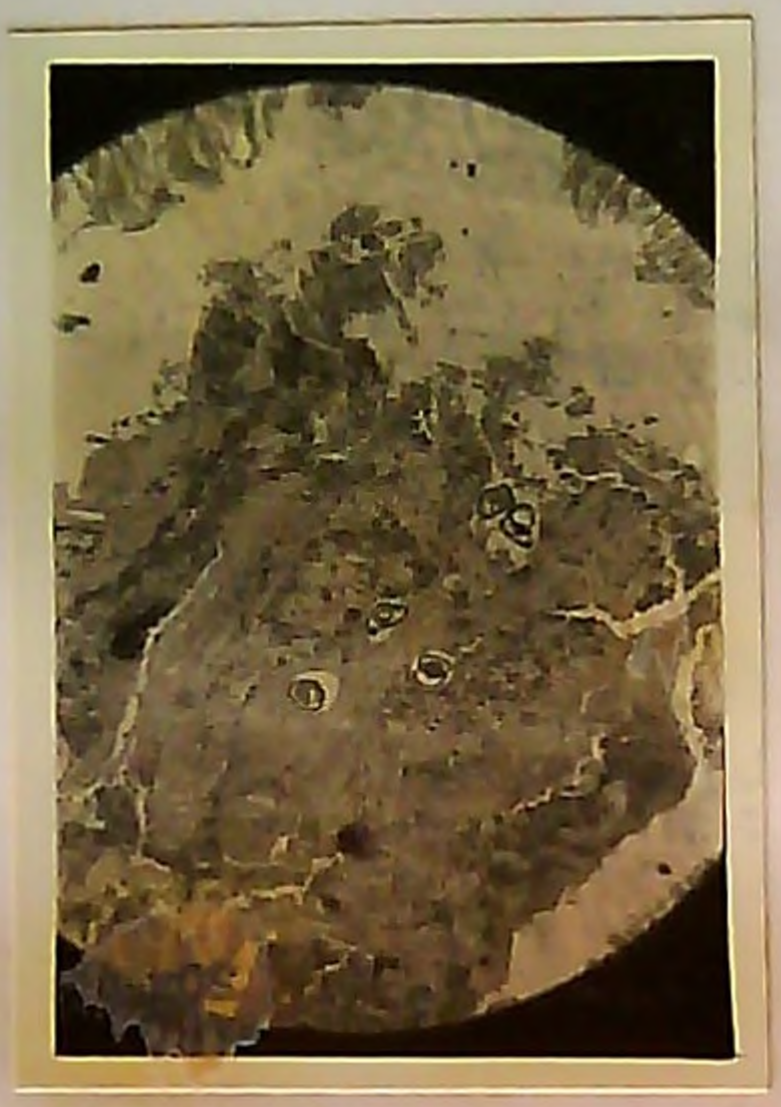
93

94

nerve ring 0.1875-0.190 mm; excretory pore 0.205-0.2125 mm; muscular oesophagus 0.295-0.340 mm; glandular oesophagus 0.750-0.800 mm; oesophageal bulb 0.0475-0.050 mm; right spicule 0.140-0.150 mm; left spicule 0.345-0.375 mm and tail 0.180-0.1975 mm.

11 days after infection

Mucus scrapings of proventriculus revealed only male worms (Fig. 95). The young female worms therefore had migrated into the proventricular glands (Fig. 96). The young females are more or less pear shaped with projections at both ends (Fig. 97) representing their anterior and posterior extremities. They measured 3.100-3.25 mm in length and 1.45-1.55 mm in breadth. The buccal capsule was changed to the shape of a goblet (Fig.98) and measured 0.020 x 0.010 mm. The body of young females was found to be divided into four equal quadrants by dorsoventral and lateral fields. On the inner aspect of cuticle, over the area of intestine about 40-45 transversely parallel muscle fibres were present. These muscle fibres were arranged very widely at the middle portion of the body and closely at the extremities. Cervical papillae were located at 0.135-0.145mm from anterior end. The nerve ring and excretory pore were situated at a distance of 0.180-0.1975 mm and 0.190-0.210 mm respectively. The muscular and glandular oesophagus measured 0.310-0.315 mm and 0.950-1.025 mm respectively in length and with a maximum breadth of 0.0675-0.075 mm at the posterior portion. The intestine was saccular and pigmented. Its anterior



95

96

97

98

portion was reflexed forward and encased the oesophageal bulb. The vulva was located at 0.400-0.425 mm anterior to tail tip. The ovijector measured 0.0825-0.0875 mm in length. The uterine flexures and ovarian coils filled up more or less the entire perienteric space of the body. The ovarian and uterine junctions were sharp and provided with sphincter like apparatus (Fig. 99). The anus opened at 0.140-0.155 mm from the tail tip.

Male worms

The male worms measured 3.95-4.150 mm in length and 0.115-0.120 mm in breadth. Other body measurements were as follows: pseudolabial buttress was distinct and extended to a distance of 0.0625-0.065 mm over the alae (Fig. 100); buccal capsule 0.020 x 0.0075 mm; cervical papillae 0.1525-0.165 mm from head end; nerve ring 0.205-0.210 mm; excretory pore 0.2125-0.2225 mm muscular oesophagus 0.3125-0.320 mm; glandular oesophagus 0.800-0.825 mm; oesophageal bulb 0.0525-0.055 mm; right spicule 0.140-0.145 mm; left spicule 0.350-0.360 mm; tail 0.195-0.2025 mm and tail papillae 0.080-0.085 mm from caudal end.

14 days after infection

The females and males were recovered from proventricular glands and mucus scrapings respectively. The females were red in colour and globular in shape. The copulatory receptaculum was well formed (Fig. 101), and the muscular bands were more distinct. Males were found to have attained full maturity with

99

100

101

a distinct seminal vesicle turgid with sperms and connected to the testis by an isthmus.

The measurements of male worms were as follows: length 4.35-4.8125 mm; breadth 0.120-0.125 mm; buccal capsule 0.025 x 0.0125 mm; pseudolabial buttress 0.060-0.065 mm; cervical papillae 0.1625-0.175 mm; nerve ring 0.215-0.2325 mm; excretory pore 0.250-0.275 mm; muscular oesophagus 0.400-0.4125 mm; glandular oesophagus 0.985-1.025 mm; oesophageal bulb 0.045-0.050 mm; right spicule 0.140-0.145 mm; left spicule 0.350-0.3525 mm; tail 0.215-0.225 mm and tail papillae 0.080-0.0825mm from tail tip.

The female worms measured 3.15-3.3 mm in length and 1.575-1.75 mm in breadth. Other body measurements were as follows: buccal capsule 0.020 x 0.010 mm; cervical papillae 0.135-0.150 mm; nerve ring 0.185-0.1925 mm; excretory pore 0.210-0.2175 mm; muscular oesophagus 0.300-0.325 mm; glandular oesophagus 0.975-1.15 mm; oesophageal bulb 0.0725-0.075 mm; vulva 0.415-0.445 mm; ovijector 0.085-0.095 mm; tail 0.1475-0.160 mm and copulatory receptaculum 0.180-0.215 x 0.025-0.0325 mm.

17 days after infection

On the seventeenth day after infection of ducklings, the females became fully globular with the entire perienteric space filled with uterine and ovarian coils. The uterus and oviducts contained a large number of oocysts. The female and

male worms measured 3.05-3.68 mm and 4.4-4.78 mm in length and 1.65-1.85 mm and 0.115-0.125 mm in breadth respectively.

20 days after infection

Female worms recovered on the twentieth day had reached almost the gravid adult size i.e. 3.65-3.95 mm long by 1.55-1.975 mm broad. Their uteri contained a large number of immature eggs.

The males measured 4.05-4.85 mm in length and 0.120-0.125 mm in breadth.

23 days after infection

On the twentythird day the females were fully gravid with a large number of embryonated eggs in their uteri. The eggs collected from live worms were hatched *in vitro* and were infectious to grasshoppers. Females measured 3.2-4.10 mm in length and 1.55-1.985 mm in breadth. Other measurements were as follows: buccal capsule 0.020 x 0.010 mm; cervical papillae 0.135-0.140 mm; nerve ring 0.170-0.195 mm; excretory pore 0.210-0.220 mm from head end; muscular oesophagus 0.3075-0.3475 mm; glandular oesophagus 1.17-1.30 mm; oesophageal bulb 0.080-0.095 mm; vulva 0.450-0.460 mm from hind end; tail 0.145-0.160mm; copulatory receptaculum 0.350-0.450 x 0.15-0.195 mm and the eggs 0.0475-0.050 x 0.025 mm.

The body measurements of male worms were as follows: length 4.10-4.85 mm; breadth 0.120-0.125 mm; buccal capsule

0.0225-0.025 x 0.015 mm; pseudolabial buttress 0.060-0.0625mm; cervical papillae 0.170-0.1875 mm; nerve ring 0.225-0.2375 mm; excretory pore 0.260-0.275 mm; muscular oesophagus 0.415-0.430 mm; glandular oesophagus 1.09-1.22 mm; oesophageal bulb 0.0475-0.050 mm; right spicule 0.145-0.160 mm; left spicule 0.350-0.390 mm and tail 0.250-0.265 mm.

24 days after infection

The first eggs of the parasite were seen in droppings of infected ducklings on this day and at autopsy. The female and male worms were recovered from the glands and mucus scrapings of proventriculus respectively. They measured as follows:

Females: length 3.48-4.12 mm; breadth 1.45-1.995 mm.
Males: length 4.31-4.9 mm; breadth 0.120-0.125 mm.

Further studies on the development of the parasite within the ducklings were not made since the females have commenced to lay eggs.

Prepatent period

The prepatent period of Tetrameres anatis infection in ducks was determined by observing the first egg of the parasite in the droppings of the infected birds. The first eggs were seen in from 24 to 28 days after infection in all the infected birds.

Salient morphological features of the different developmental stages of Tetrameres anatis within the ducks are presented in the Table 6.

DISCUSSION

Though Tetrameres anatis resembled morphologically to Tetrameres fissispina, the parasite could be differentiated from the latter on the basis of the following biological characters. Tetrameres fissispina was recorded from ducks and fowls by many workers, whereas attempts to infect fowls with infective larvae of Tetrameres anatis was futile. Moreover, Daphnia pulex one of the proven intermediate hosts of Tetrameres fissispina failed to become infected with Tetrameres anatis eggs. While studying the lifecycle of Tetrameres fissispina using Gammarus lacustris as intermediate host, Garkavi (1949) found the first and second moulting in from 9 to 11 and 12 to 14 days respectively after infection and encystment of infective larvae on the eighteenth day in the muscles and gills at a temperature ranging from 16-20°c and on the eighth day at 26°c. During the present study, juveniles of Tetrameres anatis underwent the first and second moulting on the third and fifth day respectively and the third stage infective larvae encysted in the fat bodies of grasshopper species Spathosternum prasiniferum on the sixth day at a room temperature ranging from 25-30°c. Garkavi (loc. cit.) observed third and fourth moulting of Tetrameres fissispina within ducklings, in 4-5

Within the ducks

al features	One day after infection		2 days after infection				3 days after infection	
	Female third stage	Male third stage	Female third stage	Male third stage	Female third moulting	Male third moulting	Female fourth stage	Male fourth stage
	1.50-1.625	1.45-1.55	1.75-1.95	1.65-1.80	1.85-1.975	1.65-1.775	2.125-2.225	1.9875-2.05
	0.060-0.0675	0.060-0.065	0.0625-0.0675	0.060-0.065	0.0675-0.0725	0.065-0.075	0.065-0.075	0.060-0.070
ule	0.0125x 0.005	0.0125x 0.005	0.0125x 0.0075	0.0125x 0.0075	0.0125x 0.0075	0.0125 x 0.0075	0.015 x 0.0075	0.015 x 0.0075
pillae d end	0.1125-0.115	0.110-0.115	0.115-0.120	0.1125-0.1225	0.115-0.1225	0.115-0.1225	0.120-0.1225	0.120-0.1225
from	0.135-0.140	0.135-0.140	0.135-0.140	0.1375-0.140	0.140-0.145	0.140-0.145	0.155-0.1875	0.1575-0.1775
ore from	0.1575-0.160	0.155-0.1575	0.155-0.160	0.155-0.160	0.1625-0.165	0.160-0.165	0.1775-0.1975	0.175-0.1975
sophagus	0.225-0.235	0.225-0.2375	0.225-0.245	0.225-0.235	0.235-0.250	0.230-0.245	0.255-0.300	0.240-0.290
esophagus	0.415-0.4375	0.400-0.4275	0.425-0.450	0.420-0.430	0.475-0.495	0.465-0.4875	0.5125-0.675	0.500-0.605
bulb -	0.025-0.0275	0.025-0.0275	0.0325	0.0325	0.0325-0.035	0.0325-0.035	0.035-0.0375	0.035-0.0375
ordium/ from	0.175-0.195	0.300-0.325	0.185-0.1975	0.315-0.335	0.200-0.205	0.330-0.355	0.250-0.200	0.415-0.505
h	0.115-0.125	0.1175-0.1275	0.135-0.145	0.140-0.145	0.145-0.150	0.140-0.150	0.1375-0.1525	0.1375-0.1475
e from	0.0525-0.055	0.050-0.0525	0.0525-0.055	0.0525-0.055	0.055-0.060	0.055-0.0625	0.055-0.0775	0.055-0.065
ght-length	-	-	-	-	-	-	-	-

orphological features	4 days after infection		5 days after infection		8 days after infection		9 days after infection			10 days infec
	Female fourth stage	Male fourth stage	Female fourth stage	Male fourth stage	Female fourth stage	Male fourth stage	Female fourth stage	Male fourth moult-ing	Male fifth stage	Female fourth moult-ing
ength	2.65-3.00	2.55-2.9875	2.925-3.0725	2.65-2.6875	2.4375-3.0625	3.0375-3.125	2.875-3.125	3.16-3.3875	3.125-3.5625	2.67-2.7375
readth	0.075-0.0825	0.075-0.0775	0.145-0.1875	0.1025-0.105	0.105-0.155	0.100-0.105	0.175-0.1875	0.0925-0.105	0.0975-0.1125	0.815-0.925
uccal capsule	0.015x0.0075	0.015x0.0075	0.020x0.0075	0.020x0.0075	0.020x0.0075	0.020x0.0075	0.020x0.0075	0.020x0.0075	0.020x0.0075	0.020x0.010
ervical papillae from head end	0.120-0.1325	0.120-0.1325	0.1375-0.150	0.125-0.130	0.1125-0.1375	0.127-0.1375	0.1375-0.1425	0.1375-0.150	0.1375-0.150	0.1275-0.1375
erve ring from head end	0.175-0.200	0.175-0.200	0.175-0.200	0.175-0.190	0.1625-0.1875	0.165-0.175	0.175-0.1875	0.175-0.1775	0.185-0.1875	0.165-0.1825
xcretory pore from head end	0.185-0.2375	0.190-0.235	0.200-0.240	0.205-0.2125	0.240-0.255	0.215-0.245	0.250-0.2675	0.225-0.255	0.220-0.2475	0.1975-0.205
uscular oesophagus - length	0.2725-0.325	0.285-0.325	0.350-0.400	0.300-0.3575	0.3125-0.350	0.3125-0.3575	0.3025-0.3125	0.3125-0.3275	0.3125-0.3575	0.300-0.3125
landular oesophagus - length	0.5875-0.6875	0.575-0.675	0.7625-0.8125	0.6875-0.700	0.625-0.7125	0.655-0.6875	0.700-0.750	0.675-0.6875	0.750-0.775	0.750-0.815
sophageal bulb - breadth	0.0375	0.0375	0.0375-0.050	0.0375-0.0425	0.040-0.050	0.035-0.0375	0.0475-0.050	0.035-0.0375	0.040-0.0475	0.060-0.065
nital primordium/ lva/testis from il tip	0.300-0.310	-	0.4375-0.5125	-	0.4375-0.4875	-	0.450-0.475	-	-	0.415-0.4575
il - length	0.155-0.1875	0.165-0.185	0.1875-0.2125	0.1625-0.175	0.1875-0.205	0.165-0.175	0.2125-0.2575	0.175-0.1875	0.175-0.195	0.165-0.185
il papillae from tail tip	0.065-0.0775	0.065-0.0775	0.0875-0.1025	0.0775-0.090	0.075-0.090	0.0725-0.075	0.0825-0.085	0.075-0.0775	-	-
icule - right - length	-	-	-	-	-	-	-	-	0.120-0.130	-

cal features	11 days after infection		14 days after infection		17 days after infection		20 days after infection		23 days after infection	
	Female fifth stage	Male fifth stage	Female	Male	Female	Male	Female	Male	Female	Male
	3.100-3.25	3.95-4.15	3.15-3.30	4.35-4.8125	3.05-3.68	3.40-4.78	3.65-3.95	4.05-4.85	3.20-4.10	4.10-4.95
	1.45-1.55	0.115-0.120	1.575-1.75	0.120-0.125	1.65-1.85	0.115-0.125	1.55-1.975	0.120-0.125	1.550-1.985	0.120-0.125
sule	0.020x 0.010	0.020x 0.0075	0.020x 0.010	0.025x 0.0125	0.020x 0.0125	0.025x 0.0125	0.020x 0.010	0.025x 0.015	0.020x 0.010	0.025x 0.015
apillae ad end	0.135-0.145	0.1525-0.165	0.135-0.150	0.1625-0.175	0.130-0.150	0.165-0.185	0.135-0.140	0.160-0.1875	0.135-0.140	0.170-0.1875
from l	0.180-0.1875	0.205-0.210	0.185-0.1925	0.215-0.2325	0.180-0.195	0.220-0.235	0.175-0.195	0.220-0.235	0.170-0.195	0.225-0.2375
pore from l	0.195-0.210	0.2125-0.2225	0.210-0.2175	0.250-0.275	0.200-0.220	0.260-0.275	0.210-0.220	0.250-0.275	0.210-0.220	0.260-0.275
esophagus -	0.310-0.315	0.3125-0.320	0.300-0.325	0.400-0.4125	0.325-0.340	0.415-0.425	0.310-0.345	0.4175-0.4275	0.3075-0.3475	0.415-0.430
esophagus -	0.950-1.025	0.800-0.825	0.975-1.15	0.985-1.025	1.05-1.25	0.995-0.1025	1.15-1.29	1.06-1.12	1.17-1.30	1.09-1.22
bulb -	0.0675-0.075	0.0525-0.055	0.0725-0.075	0.045-0.050	0.080-0.085	0.045-0.050	0.080-0.085	0.0475-0.050	0.080-0.085	0.0475-0.050
tail tip	0.400-0.425	-	0.415-0.445	-	0.450-0.460	-	0.445-0.460	-	0.450-0.460	-
	0.140-0.155	0.195-0.2025	0.1475-0.160	0.215-0.225	0.140-0.160	0.240-0.260	0.135-0.160	0.245-0.265	0.145-0.160	0.250-0.265
ight - length	-	0.140-0.145	-	0.140-0.145	-	0.140-0.160	-	0.140-0.160	-	0.145-0.160
eft - length	-	0.350-0.360	-	0.350-0.3525	-	0.340-0.390	-	0.340-0.390	-	0.350-0.390
length	0.0825-0.0875	-	0.090-0.0975	-	0.095-0.105	-	0.1025-0.1075	-	0.105-0.110	-

and 10 days respectively and the first eggs in the droppings of the birds on the eighteenth day after infection. The third and fourth moulting of Tetrameres anatis as observed during the current study, took place in ducklings on the second day and ninth to tenth day respectively. The first eggs in the droppings was observed on the twentyfourth day following infection. Thus the details of development given for Tetrameres fissispina by Garkavi (loc. cit.) are widely different from the details of development of Tetrameres anatis.

The observations on the development of the parasite within the intermediate and as well as the final host were in agreement with the previous findings of Chandrasekharan (loc. cit excepting that the fourth moult in respect of males was observed one day earlier in the present work than that reported by Chandrasekharan (loc. cit.).

The development stages viz., moulting times, infective phase, prepatent period of other Tetrameres species of ducks and fowls were also found to be different from those of Tetrameres anatis. The above observations, therefore, confirm the validity of Tetrameres anatis as a distinct species occurring in domestic ducks of India.

PATHOGENESIS OF *Tetrameres anatis*

PATHOGENESIS OF TETRAMERES ANATIS

Tetramerosis in ducks and other birds is fairly common in many countries. The pathological manifestations produced by different species of Tetrameres in different species of birds were described by many workers. The male worms were regarded to be less harmful as they live in the mucosa and mucus exudate and apparently are not active blood suckers. On the contrary female worms cause serious damage during invasion of the proventricular gland and subsequently by feeding on blood and cellular exudates.

Histopathological lesions produced by Tetrameres fissispina in ducks were reported by Popov (1953); Willomitzer and Gilka (1959); Zajicek (1959); Gerasimova (1960); Tsvetaeva (1960); Zabello (1964); Shevtsov and Zabello (1965); in pigeon by Timon Devid (1932) and in fowl by Srivastava (1939). Swales (1936) described the histopathological changes produced by Tetrameres crami in the proventriculus of domestic ducks. The lesions produced by Tetrameres americana in the poultry were studied by Barber (1915), Cram (1926) and Todd (1947). Pande et al. (1960) observed some pathological lesions caused by Tetrameres spinosa in wild aquatic birds. While describing the lifecycle of Tetrameres confusa, Nery and Dubois (1960) mentioned the lesions produced by the parasite in the proventriculus of poultry. Pathogenicity of Tetrameres mohtedai in poultry was elucidated by Anantharaman

and Chandrasekharan Nair (1955), Purohit et al. (1964) and Joshi and Kamalapur (1971). Histopathological lesions found in the proventriculus caused by tetramerosis in domestic ducks were reported by Bhattacharya and Doss (1967). While describing a new species, Tetrameres anatis from domestic ducks Chandrasekharan (1967) briefly mentioned the histopathology of the parasite.

During the present study, the clinical manifestations, effect on the weight gain, histopathology of lesions and haematology in ducklings which were subjected to artificial infection with 200 infective larvae of Tetrameres anatis are reported.

Clinical symptoms

The infected birds were found to be apparently healthy and normal upto a period of two weeks after infection. Thereafter the infected ducklings began to exhibit anaemia, dullness and signs of weakness. The severity of weakness and anaemia were found to increase progressively in accordance with the development and blood sucking habit of the parasite within the proventriculus. In about 28 to 43 days time the anaemic changes became more marked. The birds appeared to be reluctant to move and showed inappetance. The breast muscle was found to be wasted and the droppings diarrhoeic. No further observations were made on clinical symptoms beyond

43 days after infection since the birds had to be autopsied for studying the histopathology of infection.

Effect in weight gain

A significant reduction in weight in infected ducklings was observed on the twentyeighth day (i.e. when the parasites were attaining sexual maturity) and fortythird day following infection. The mean weight gain of eight infected birds were 202.785 ± 9.827 gm and 324.250 ± 2.453 gm respectively and in eight uninfected control birds the same were 322.50 ± 7.636 gm and 526.0 ± 14.189 gm respectively. The reduction in weight gain noted in infected group was found to be highly significant (P /0.01).

The data on the weight gain in infected and uninfected groups are presented in the Table 7 along with analysis of variance.

Gross lesions

The proventricular glands containing the female worms were detectable even on a cursory examination of the serous coat of the organ since the worms *in situ* appeared as dark spots over the organ (Fig. 102). On opening the proventriculus, the wall of the organ was found to be thickened and the lumen reduced considerably. The gastric papillae were prominent with petechial haemorrhage over mucosa. The mucus coat was also thickened and covered with thick tenacceous mucus exudate. Male worms were observed in this mucous layer.

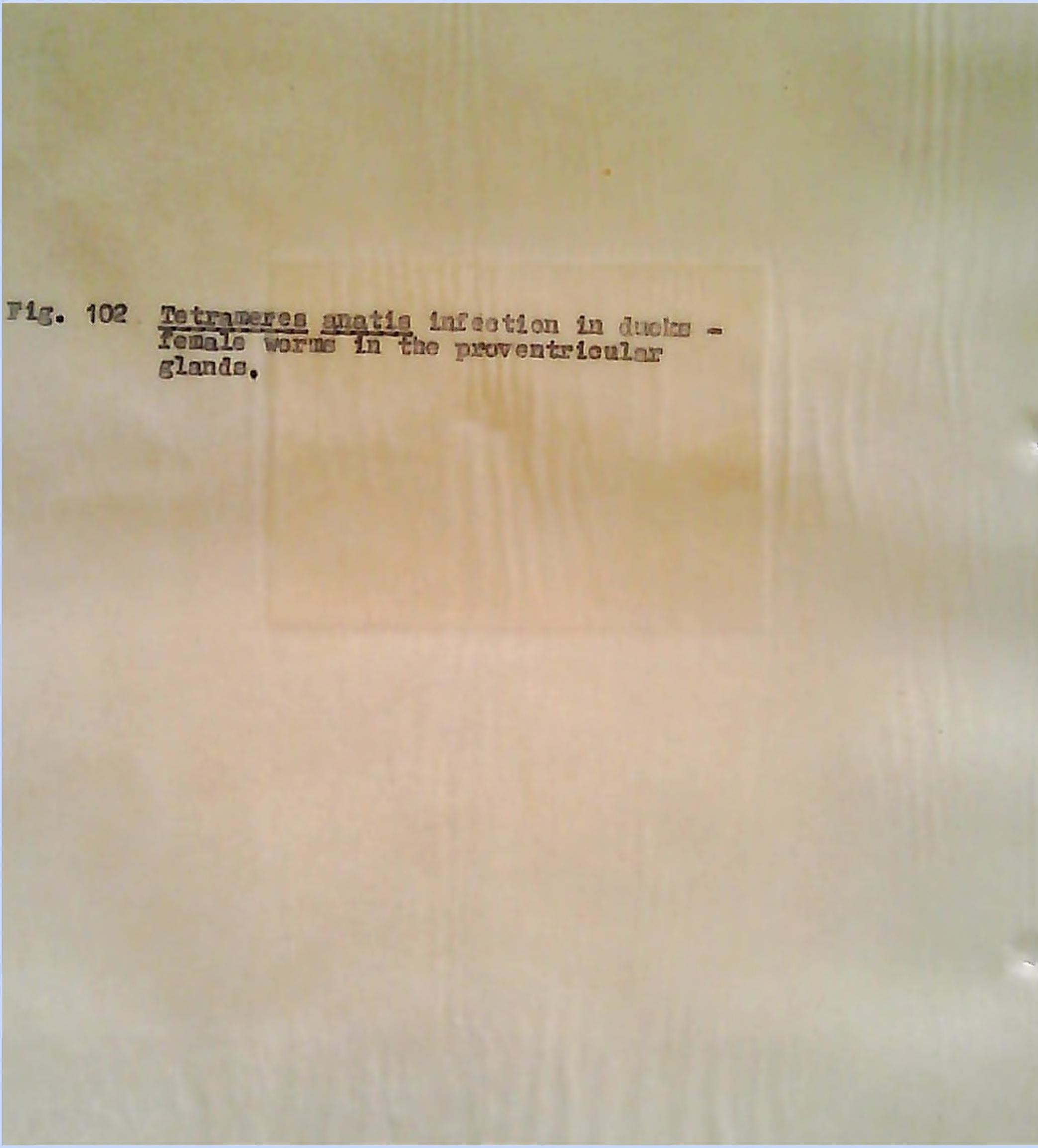


Fig. 102 Tetrameres spatia infection in ducks - female worms in the proventricular glands.

Table 7. Difference in weight gain between ducklings infected with 200 larvae of *Tetrameres anatis* and uninfected controls.

Group	Day on which weight recorded	Weight gained (gm)									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	207	220	230	224	161	196	190	195	202.875	9.827
	43rd day	323	323	319	324	316	325	332	332	324.25	2.453
Control	28th day	311	363	309	314	320	328	315	320	322.50	7.636
	43rd day	496	573	572	499	520	536	491	521	526.0	14.189

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	431140.344	61591.477	111.93**
Error	24	13206.375	550.265	
Total	31	444346.719		

** Highly significant.

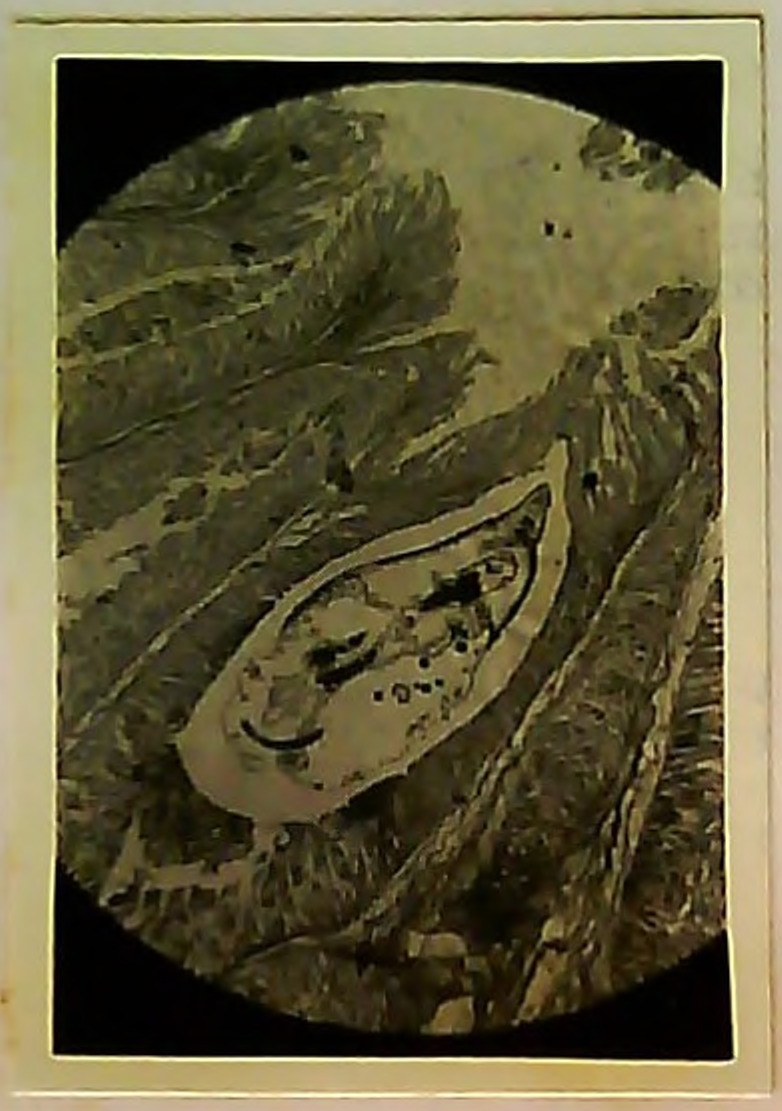
HISTOPATHOLOGY

11 days after infection

The infected glands showed acute catarrhal inflammation with marked desquamation of the glandular epithelia (Fig.103). There was also evidence of pressure atrophy particularly in areas near the orifices of the glands. The lamina propria showed focal areas of cellular infiltration chiefly comprising of mononuclears and heterophiles. The lumen of the organ was filled with catarrhal exudate and mucous and contained male worms (Fig. 104).

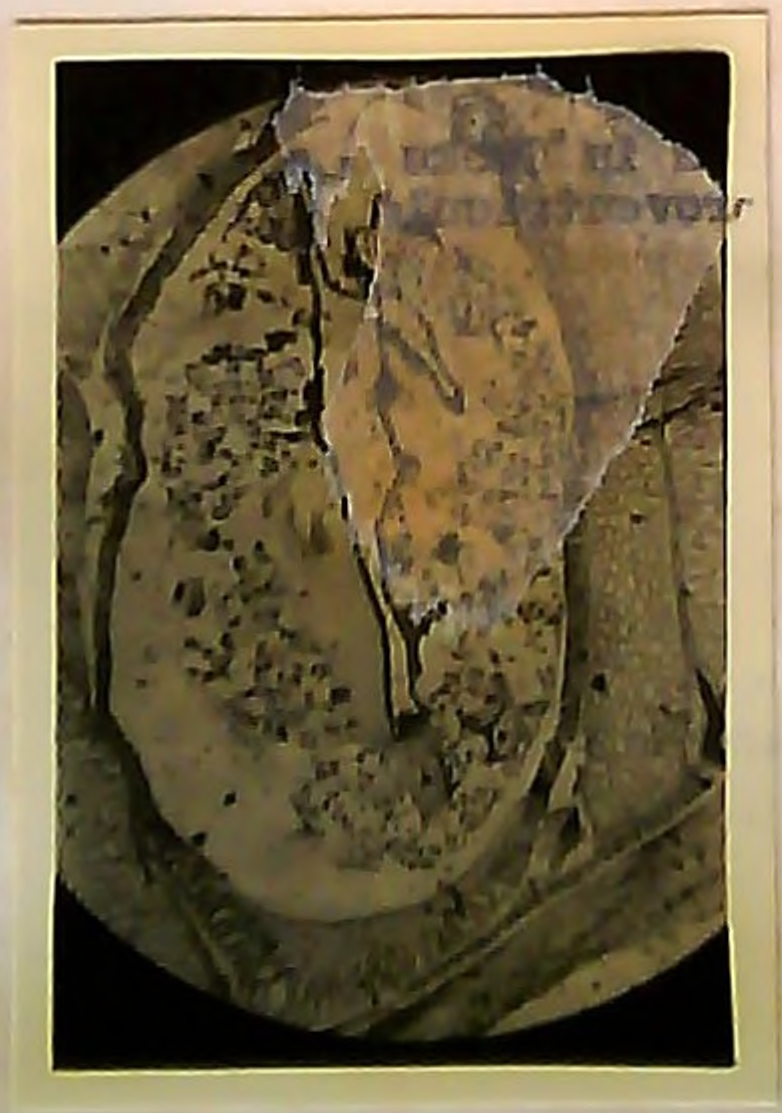
28 days after infection

Histopathological lesions of infected proventriculi were more pronounced on 28th day than those observed on the eleventh day. The infected glands containing the female worms showed an extreme degree of pressure atrophy with a marked zone of necrosis immediately surrounding the worms. There was heavy cellular infiltration around the parasite. The superficial mucous membrane of the proventriculus was completely denuded of epithelium and ulcerated over the affected glands. Reparative changes in the form of keratinised metaplasia was also evident side by side. The acute catarrhal change noticed on the eleventh day, is therefore, giving place to a chronic inflammation originating from the infected proventricular glands (Figs. 105 & 106).



103

104



105

106

43 days after infection

Histopathological lesions on the fortythird day of infection were essentially similar to those seen on the twentyeighth day, but the degree of pressure atrophy and necrosis in infected glands were more severe. There were several areas of diphtheritic proventriculitis particularly in the vicinity of infected glands. There was no evidence of regeneration of superficial epithelium and only keratinised metaplacia was seen over the affected area. The cellular infiltration was more or less diffuse over the entire organ, suggesting that infection had led to a chronic diphtheritic proventriculitis with very little reparative change (Figs. 107, 108, 109 & 110). The above changes clearly indicate that from about the eleventh day after infection i.e. when the female worms commence to invade the proventricular glands, an acute catarrhal inflammation of the glands sets in with marked superficial necrosis and desquamation and these changes gradually pass on to a chronic diphtheritic proventriculitis involving practically the whole organ, as the worms settle in the glandular lumina. Pressure atrophy and a marked zone of necrosis around the worms are more or less a constant feature.

CHANGES IN HAEMATOLOGICAL VALUES

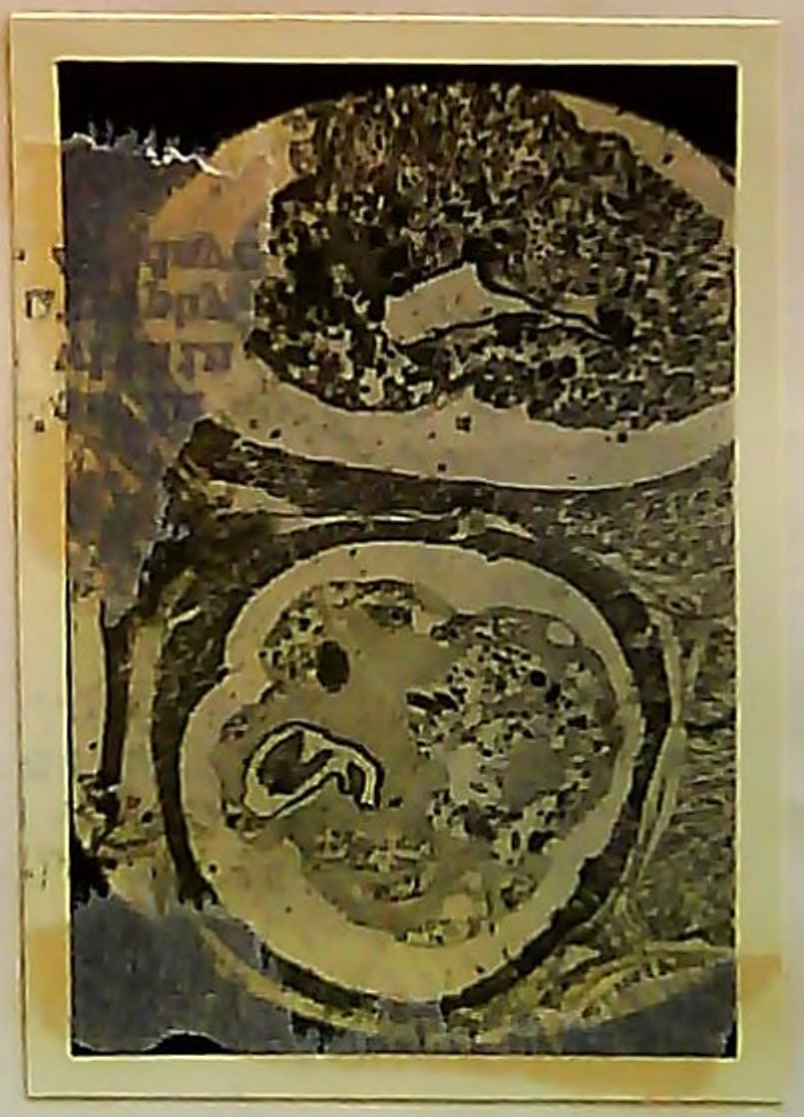
Haemoglobin

The haemoglobin concentration on the twentyeighth day after infection in duckling was found be reduced from

109

110

107



108

10.337 ± 0.138 gm/100 ml in the control group to 7.87 ± 0.210 gm/100 ml in the experimental group. Similarly haemoglobin value was also found to be reduced on the fortythird day after infection, from 10.368 ± 0.180 to 6.927 ± 0.206gm/100ml.

The reduction in haemoglobin concentration noted in the present study was found to be highly significant (P < 0.01).

The data are presented in the Table 8 along with analysis of variance.

Red blood corpuscles

A significant reduction in the number of red blood corpuscles in infected birds was observed as compared to uninfected control birds (P < 0.01). The mean values of red blood corpuscles in infected ducklings recorded on twentyeighth and fortythird day were 2.231 ± 0.0043 million/C.mm and 2.266 ± 0.0061 million/C.mm respectively. Whereas in the control birds the values were 2.366 ± 0.0145 and 2.393 ± 0.041 million/C.mm respectively.

The data are presented in the Table 9 along with analysis of variance.

Packed cell volume

The packed cell volume in infected birds after twenty-eight days and fortythree days of infection was also reduced

Table 8. Difference in haemoglobin concentration between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Group	Day on which haemoglobin determined	Haemoglobin concentration in gm/100 ml									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	8.18	7.27	7.27	7.73	8.64	8.18	8.18	7.73	7.897	0.210
	43rd day	7.27	6.36	6.36	6.81	7.73	6.81	7.27	6.81	6.927	0.206
Control	28th day	10.00	10.45	10.45	10.00	10.90	10.45	10.45	10.00	10.337	0.138
	43rd day	10.45	10.45	11.45	10.45	11.45	10.90	10.90	10.90	10.868	0.180

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	86.334	12.333	58.174**
Error	24	5.098	0.212	
Total	31	91.432		

** Highly significant.

Table 9. Difference in red blood corpuscles between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Group	Day on which R.B.C. determined	Red blood corpuscles in million/C.mm									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	2.29	2.28	2.25	2.28	2.30	2.29	2.30	2.26	2.281	0.0043
	43rd day	2.28	2.26	2.24	2.26	2.28	2.28	2.28	2.25	2.266	0.0061
Control	28th day	2.45	2.36	2.40	2.36	2.35	2.36	2.32	2.33	2.366	0.0145
	43rd day	2.49	2.38	2.46	2.35	2.41	2.36	2.38	2.30	2.393	0.041

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	0.100	0.0142	17.75**
Error	24	0.021	0.0008	
Total	31	0.121		

** Highly significant.

significantly (P ⟨ 0.01). The mean values of packed cell volume of infected and non-infected groups were 23.575 ± 0.66%; 21.0 ± 0.761%; 31.212 ± 0.433% and 33.225 ± 0.806% respectively.

The data pertaining to packed cell volume are presented in Table 10 along with analysis of variance.

White blood corpuscles

The mean values of white blood corpuscles of infected birds were found to be increased from 29.515 ± 0.339 thousand/C.mm to 31.656 ± 0.394 thousand/C.mm on the twentyeighth day and from 29.492 ± 0.374 thousand/C.mm to 34.986 ± 0.698 thousand/C.mm on the fortythird day after infection.

The increase in the white blood corpuscles recorded was found to be highly significant (P ⟨ 0.01).

The data are presented in the Table 11 along with analysis of variance.

Erythrocyte sedimentation rate

The values of erythrocyte sedimentation rate of infected ducklings were found to be increased significantly as compared to control group (P ⟨ 0.01). The erythrocyte sedimentation rate values of infected and non-infected birds were 3.725 ± 0.14 mm/hr. and 4.05 ± 0.078 mm/hr. respectively on the twentyeighth day

Table 10. Difference in packed cell volume between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Group	Day on which P.C.V. determined	Packed cell volume (%)									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	24.6	22.0	21.8	22.0	25.8	24.6	24.4	25.4	23.575	0.66
	43rd day	21.8	19.4	19.2	20.6	23.4	20.4	23.8	20.2	21.100	0.761
Control	28th day	30.0	31.6	31.4	30.2	32.8	31.6	31.9	30.2	31.212	0.433
	43rd day	31.4	31.8	34.4	31.4	34.6	32.8	36.6	32.8	33.225	0.806

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	821.816	117.402	41.720**
Error	24	67.559	2.814	
Total	31	889.375		

** Highly significant.

Table 11. Difference in white blood corpuscles between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Group	Day on which W.B.C. determined	White blood corpuscles in 1000/C.mm									S.E
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	32.2	31.66	32.09	31.23	31.23	30.30	31.21	33.33	31.656	0.3
	43rd day	34.92	34.63	37.04	34.11	31.75	36.11	35.56	35.77	34.986	0.6
Control	28th day	30.10	29.63	28.34	30.36	29.88	29.88	29.63	28.30	29.515	0.3
	43rd day	30.37	29.63	29.63	30.00	29.89	28.67	29.98	27.77	29.492	0.3

Analysis of variance

Source of variance	df	SS	MSS	F
Between groups	7	160.944	22.992	16.831**
Error	24	32.794	1.366	
Total	31	193.738		

** Highly significant.

following infection and 2.90 ± 0.070 mm/hr. and 2.925 ± 0.009mm/hr. respectively on the fortythird day after infection of ducklings.

The data are presented in the Table 12 along with analysis of variance.

Mean corpuscular volume

The mean corpuscular volume of infected ducklings on twenty-eighth and fortythird day following infection were 103.31 ± 2.64 C. microns and 93.07 ± 0.756 C. microns respectively. Whereas the same values in control birds were found to be 131.69 ± 2.28 C. microns and 136.455 ± 2.33 C. microns respectively. The reduction in the mean corpuscular volume recorded in the infected birds was also found to be highly significant (P / 0.01).

The data are presented in the Table 13 along with analysis of variance.

Mean corpuscular haemoglobin

It may be seen from the Table 14 the values of mean corpuscular haemoglobin on the twentyeighth day after infection were found to be reduced from 43.942 ± 0.711 micro microgram in the control group to 34.606 ± 0.651 micro microgram in infected birds. Similarly the values were also reduced on the fortythird day following infection from 45.317 ± 0.776 micro microgram in the control birds to 30.655 ± 0.834 micro microgram in infected

Table 12. Difference in erythrocytic sedimentation rate between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Groups	Day on which E.S.R. determined	Erythrocyte sedimentation rate mm/hour									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	3.8	3.4	3.6	3.8	3.2	3.8	4.2	4.0	3.725	0.14
	43rd day	4.0	4.0	4.4	4.0	3.8	4.0	4.0	4.2	4.05	0.078
Control	28th day	3.0	2.8	2.9	2.7	3.0	2.6	2.8	2.6	2.80	0.070
	43rd day	3.0	2.8	3.0	2.8	3.2	2.9	2.8	2.9	2.925	0.009

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	8.890	1.270	24.423**
Error	24	1.250	0.052	
Total	31	10.140		

** Highly significant.

Table 13. Difference in mean corpuscular volume between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Group	Day on which M.C.V. determined	Mean corpuscular volume in C. microns									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	107.42	96.49	96.88	96.49	112.17	107.42	106.08	103.53	103.310	2.64
	43rd day	95.61	85.84	85.71	91.55	102.63	89.47	104.38	89.77	93.07	0.756
Control	28th day	122.45	133.89	130.82	127.15	139.57	133.89	135.34	129.61	131.69	2.280
	43rd day	126.11	133.61	139.83	133.61	143.55	138.98	136.97	138.98	136.455	2.330

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	10810.682	1544.383	55.925**
Error	24	662.765	29.615	
Total	31	11473.447		

** Highly significant.

Table 14. Difference in mean corpuscular haemoglobin between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Group	Day on which M.C.H. determined	Mean corpuscular haemoglobin in micro microgram									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	35.72	31.88	32.31	33.90	35.56	35.72	35.56	34.20	34.606	0.65
	43rd day	31.88	28.14	28.39	30.39	33.90	30.13	31.88	30.53	30.655	0.83
Control	28th day	40.82	44.28	43.54	44.28	46.38	44.28	45.04	42.92	43.942	0.71
	43rd day	41.97	43.91	46.54	44.46	47.51	46.18	45.79	46.18	45.517	0.77

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	1221.892	174.556	45.935**
Error	24	91.211	3.80	
Total	31	1313.103		

** Highly significant.

group. The reduction of mean corpuscular haemoglobin values determined during the present study was found to be highly significant (P / 0.01).

Mean corpuscular haemoglobin concentration

No significant difference in mean corpuscular haemoglobin concentration between infected and non-infected control birds was observed on the twentyeighth and fortythird day after infection. The mean corpuscular haemoglobin concentration values of both infected and non-infected groups were found to be 33.75 ± 0.347%; 32.86 ± 0.346% and 33.12 ± 0.061% and 33.21 ± 0.059% respectively.

The data are presented in the Table 15 along with analysis of variance.

DISCUSSION

The first available report on tetramerosis is that of Barber (loc.cit.) who described a marked catarrhal condition in chicks parasitised with 47 worms in their proventriculus. Cram (loc. cit.) observed emaciation and droopiness, petechial haemorrhages, congestion and thickenings in the proventricular glands of fowls harbouring Tetrameres americana. Timon Devid (loc. cit.) observed destruction of mucosa and complete digestion of most of the glandular tissue of proventriculus of pigeon infected with Tetrameres fissispina. Great distension

Table 15. Difference in mean corpuscular haemoglobin concentration between ducklings infected with 200 larvae of Tetrameres anatis and non-infected controls.

Group	Day on which M.C.H.C. determined	Mean corpuscular haemoglobin concentration (%)									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	28th day	33.25	33.04	33.34	35.13	33.48	33.25	35.52	33.03	33.75	0.347
	43rd day	33.34	32.78	33.12	33.05	33.03	33.38	30.54	33.71	32.86	0.346
Control	28th day	33.33	33.07	33.28	33.11	33.23	33.67	32.76	33.11	33.12	0.061
	43rd day	33.28	32.86	33.29	33.28	33.09	33.23	33.43	33.23	33.21	0.059

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	3.346	0.478	0.824
Error	24	13.925	0.580	Not significant
Total	31	17.271		

of crypts of Lieberkuhn of the proventriculus of ducks infected with Tetrameres crami was reported by Swales (loc. cit.). Anaemia and emaciation were mentioned in infected birds by Sugimoto and Nishiyama (1937). Srivastava (loc. cit.) noticed anaemia and emaciation, inflammatory changes and thickening of stomach wall of fowls infected with Tetrameres fissispina. In ducks, parasitised with the same parasite Popov (loc. cit.) reported catarrhal inflammation and enlargement of proventricular glands. Clinical symptoms like droopiness, abstinence from food, emaciation, anaemia and diarrhoea and histopathological changes as chronic catarrhal inflammation of the mucosa, occasional desquamation of superficial epithelium, lymphocytic infiltration in the tunica propria and distension and atrophy of proventricular glands were observed by Anantharaman and Chandrasekharan (loc. cit.) in fowls, infected with Tetrameres mohtedai. Zajicek (loc. cit.) found epithelial changes with secondary proliferative inflammation in the proventriculus of ducks parasitised with Tetrameres fissispina. While studying the pathological lesions produced by Tetrameres spinosa in the proventriculus of wild ducks, Pande et al. (loc. cit.) observed increase in inter-glandular connective tissue, delimitted fibrous capsules around the parasites and infiltration of eosinophils and lymphocytes. Tsvetaeva (loc. cit.) observed dilation and desquamation of cells of proventricular glands in 5 days following infection and degeneration and atrophy of glandular tissue, transformation of glands into cysts lined with flattened atrophic epithelium, periglandular oedema,

of crypts of Lieberkuhn of the proventriculus of ducks infected with Tetrameres crami was reported by Swales (loc. cit.). Anaemia and emaciation were mentioned in infected birds by Sugimoto and Nishiyama (1937). Srivastava (loc. cit.) noticed anaemia and emaciation, inflammatory changes and thickening of stomach wall of fowls infected with Tetrameres fissispina. In ducks, parasitised with the same parasite Popov (loc. cit.) reported catarrhal inflammation and enlargement of proventricular glands. Clinical symptoms like droopiness, abstinence from food, emaciation, anaemia and diarrhoea and histopathological changes as chronic catarrhal inflammation of the mucosa, occasional desquamation of superficial epithelium, lymphocytic infiltration in the tunica propria and distension and atrophy of proventricular glands were observed by Anantharaman and Chandrasekharan Nair (loc. cit.) in fowls, infected with Tetrameres mohtedai. Zajicek (loc. cit.) found epithelial changes with secondary proliferative inflammation in the proventriculus of ducks parasitised with Tetrameres fissispina. While studying the pathological lesions produced by Tetrameres spinosa in the proventriculus of wild ducks, Pande et al. (loc. cit.) observed increase in inter-glandular connective tissue, delimited fibrous capsules around the parasites and infiltration of eosinophils and lymphocytes. Tsvetaeva (loc. cit.) observed dilation and desquamation of cells of proventricular glands in 5 days following infection and degeneration and atrophy of glandular tissue, transformation of glands into cysts lined with flattened atrophic epithelium, periglandular oedema,

infiltration of lymphocytes, eosinophils, histiocytes, proliferative adenomatous reaction with epithelial desquamation and production of excessive mucus in the crypts during the period from 10 to 18 days in Tetrameres fissispina infection in ducks. In his opinion, the upsets of digestion and resulting emaciation in ducks were due to the presence of the parasites in the fundal glands of proventriculus.

Bhattacharjee and Doss (loc. cit.) reported dullness, emaciation, loss of weight and anaemia as clinical symptoms, congested and thickened proventricular wall, slightly obliterated lumen, increased secretion of mucus, and loss of the normal lusture of mucosa as gross lesions and desquamation of epithelium, cellular infiltration between the tubules, highly thickened muscular coat, change of epithelial cells of superficial glands into cuboidal epithelium, thinned and quite indistinct muscularis mucosa between the superficial and deep layer of glands as histopathological lesions of tetramerosis of ducks. Dullness, droopiness, emaciation and anaemia in ducks infected with Tetrameres anatis and histopathological changes like degeneration of the superficial parts of glandular epithelium, pressure atrophy of glandular tissue, and focal leucocytic infiltration of proventricular epithelium were observed by Chandrasekharan (loc. cit.). Soulsby (1968) opined that in tetramerosis, though the adult worms suck blood, great damage was done when the juveniles migrate into the wall of the proventriculus causing marked irritation and

inflammation. Joshi and Kamalapur (loc. cit.) observed swelling and haemorrhages in gastric papillae, necrosis of glandular acini, proliferation of connective tissue in the lobular septum around the worms, chronic catarrhal inflammation with necrosis and desquamation of the superficial epithelium of the mucous membrane, periglandular oedema and infiltration of lymphocytes, eosinophils and macrophages and pressure atrophy of glandular tissue in the proventriculus of fowls infected with Tetrameres mohtedai.

The reduction in weight gain, clinical symptoms and the gross and histopathological lesions observed during the present study are in agreement with the findings made by previous workers in case of other species of Tetrameres infection. Proliferative adenomatous reaction in the crypts of proventricular gland reported by Tsvetaeva (loc. cit.) was not seen in any of the infected ducklings, during the current observations.

During the present study significant reductions in haemoglobin concentration, red blood corpuscles, packed cell volume, mean corpuscular haemoglobin and mean corpuscular volume of infected birds were observed. These findings corroborate the clinical symptoms of microcytic hypochromic anaemia in the infected birds owing to probably the blood sucking habits of the parasites. The increased leucocytosis and erythrocytic sedimentation rate also indicate a chronic type of parasitism.

PATHOGENESIS OF *Amidostomum skrjabini*

PATHOGENESIS OF AMIDOSTOMUM SKRJABINI

Three species of Amidostomum viz., Amidostomum anatinum Amidostomum skrjabini and Amidostomum anseris were found to occur in domestic ducks (Lapage, 1961). Akhmedova (1954) considered Amidostomum anseris to be the most pathogenic species in geese. Amidostomiasis in geese was studied in detail by Cram (1926); Ullrich (1932); Walter (1935); Jestard (1936 & 1937); Vsevolodov (1938); Srivastava (1939); Schmid (1943); Cassamgnaghi (1946); McCraw (1952); Oliver (1952); Herman and Wehr (1953); Czaplinki (1954); Czaplinki et al. (1956); Kobulej (1959); Negru et al. (1960); Zajicek (1960); Visachi (1961); Georgiev (1962); Tsvetaeva (1965) and Soulsby (1965). Though sufficient information on amidostomiasis of geese is available, information on amidostomiasis in domestic ducks is meagre in literature. Some aspects of pathogenesis of Amidostomum skrjabini in domestic ducks were elucidated by Dubey and Pande (1965) and Chandrasekharan (1967).

During the present study, detailed observations were made on the clinical symptoms, effect on body weight, histopathological and haemotological changes in ducklings which were artificially infected with 200 infective larvae of Amidostomum skrjabini.

Clinical symptoms

The infected ducklings showed listlessness, dullness,

loss of condition and inappetence from a period of 15 days post infection (i.e. when the parasites were attaining sexual maturity). As the disease advanced, there was anaemia, emaciation, incordination of movements and diarrhoea. No mortality was noticed within the observed period of 30 days.

Effect in weight gain

The body weights of both infected and non-infected birds were made on 15th and 30th day after infection. The mean body weight gain was found to vary from 221.75 ± 8.85 gm to 240.25 ± 9.028 gm in infected birds and 301.25 ± 5.213 gm to 396.75 ± 5.735 gm in control group. The reduction in body weight gain observed in infected ducklings, was found to be highly significant ($P < 0.01$).

The data are presented in the Table 16 along with analysis of variance.

Gross lesions

Gross pathological lesions of amidostomiasis in ducks were found to be confined to softer part of the cornified lining particularly at the junction of gizzard and proventriculus or gizzard and duodenum. Worms were seen partially embedded within the softer part of the mucous membrane just below the cornified layer. Marked inflammation and haemorrhages were seen underneath the horny lining. Dirty diphtheritic

Table 16. Difference in weight gain between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which weight recorded	Weight gain in gm									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	216	240	240	258	211	202	179	228	221.75	8.85
	30th day	244	261	263	274	218	224	199	239	240.25	9.028
Control	15th day	297	288	328	304	294	286	295	318	301.25	5.213
	30th day	385	393	383	380	427	401	392	413	396.75	5.735

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	150203.5	21457.643	45.411**
Error	24	11340.5	472.521	
Total	31	161554.0		

deposits could also be seen around the worms. The cornified layer could be pealed off easily from the underlying tissue from places where large number of worms were adhering.

HISTOPATHOLOGY

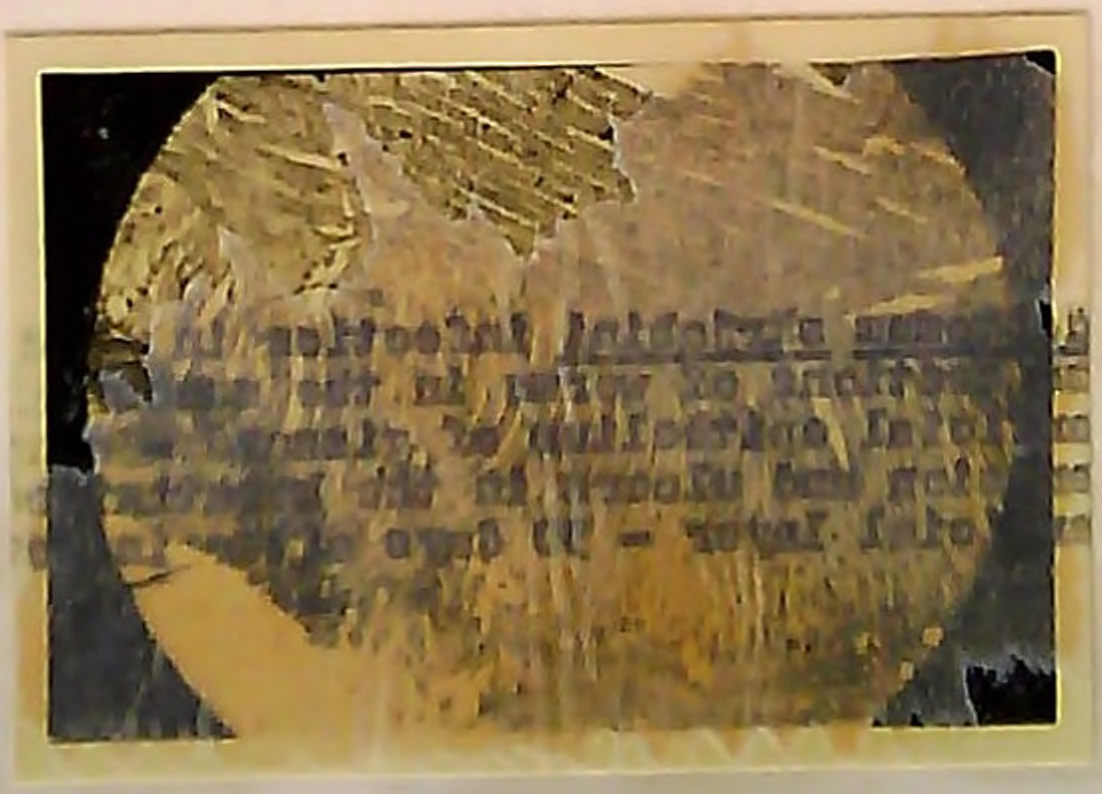
The microscopical changes were confined to the superficial epithelium of the gizzard. On the fifteenth day following infection large number of worms werefound to be deeply embedded into the mucosa of the organ. There were degeneration and desquamation of the cells of the superficial layer (Figs. 111 & 112). Inflammatory exudate contained a large number of desquamated cells and lymphocytes. Blood vessels of submucosa were found congested. On the thirtieth day, the lesions were found to be more marked. Focal areas showed hyperkeratosis and there were areas of erosion and ulcers in the keratinised superficial layer (Fig. 113). Focal areas of haemorrhages were also evident. The glandular and muscular coat showed scattered foci of heterophilic infiltration. The over all histopathological changes presented the characters of dephtheritic ulcers on the mucous membrane of gizzard.

CHANGES IN HAEMATOLOGICAL VALUES

Haemoglobin concentration, red blood corpuscles, white blood corpuscles, erythrocyte sedimentation rate, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were determined

111

112



113

on the fifteenth and thirtieth day following infection and the values compared with non-infected control birds of identical age group maintained along with the infected birds.

Haemoglobin concentration

The mean value of haemoglobin concentration of infected birds on the fifteenth and thirtieth day following infection were 8.33 ± 0.180 gm/100 ml and 7.80 ± 0.177 gm/100 ml while in the control groups these values were 10.30 ± 0.124 gm/100 ml and 10.66 ± 0.123 gm/100 ml. The reduction in the concentration of haemoglobin of the infected ducklings as compared to controls was highly significant (P < 0.01).

The data are presented in the Table 17 along with analysis of variance.

Red blood corpuscles

The mean number of red blood corpuscles in infected ducklings on the fifteenth day following infection was found to be reduced from 2.34 ± 0.02 million/C.mm in the control group to 2.28 ± 0.009 million/C.mm in the experimental group. Similarly the value was also found to be decreased on the thirtieth day, from 2.38 ± 0.019 million/C.mm to 2.34 ± 0.02 million/C.mm.

The reduction in the number of red blood corpuscles observed in the present study was highly significant (P <0.01).

Table 17. Difference in the haemoglobin concentration between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which haemoglobin determined	Haemoglobin concentration in gm/100 ml									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	8.09	9.05	8.57	7.62	8.09	8.09	8.09	9.05	8.33	0.180
	30th day	7.72	8.57	7.62	7.14	7.62	7.62	7.62	8.57	7.80	0.177
Control	15th day	10.48	10.48	10.95	10.00	10.00	10.48	10.00	10.00	10.30	0.124
	30th day	10.95	10.95	10.95	10.48	10.00	10.95	10.48	10.48	10.66	0.123

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	48.209	6.887	31.022**
Error	24	5.357	0.222	
Total	31	53.559		

** Highly significant.

The data are presented in the Table 18 along with analysis of variance.

Packed cell volume

A significant reduction in the mean values of packed cell volume of infected birds was noticed as compared to control birds ($P < 0.01$). The mean values of packed cell volume determined on the fifteenth and thirtieth day of infected group were 25.07 ± 0.630% and 23.87 ± 0.698% respectively and in control birds were 31.05 ± 0.438% and 32.20 ± 0.458% respectively.

The data are presented in the Table 19 along with analysis of variance.

White blood corpuscles

The mean number of white blood corpuscles of infected ducklings on fifteenth and thirtieth day following infection were found to be 31.49 ± 0.256 thousand/C.mm and 31.91 ± 0.436 thousand/C.mm respectively, whereas in controls the values were 28.94 ± 0.476 thousand/C.mm and 30.77 ± 0.350 thousand/C.mm respectively. The increase in the mean number of white blood corpuscles observed in the infected duckling was also highly significant ($P < 0.01$).

The data are presented in the Table 20 along with analysis of variance.

Table 18. Difference in red blood corpuscles between ducklings infected with 200 larvae of *Amidostomum skrjabini* and non-infected controls.

Group	Day on which R.B.C. determined	Red blood corpuscles in million/C.mm									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	2.29	2.35	2.28	2.27	2.29	2.26	2.24	2.25	2.28	0.009
	30th day	2.28	2.30	2.26	2.24	2.28	2.23	2.25	2.20	2.25	0.008
Control	15th day	2.30	2.40	2.40	2.31	2.28	2.39	2.26	2.30	2.34	0.02
	30th day	2.41	2.46	2.38	2.37	2.29	2.44	2.31	2.38	2.38	0.019

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	0.075	0.0107	5.0952**
Error	24	0.051	0.0021	
Total	31	0.126		

Table 19. Difference in packed cell volume between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which P.C.V. determined	1	2	3	4	5	6	7	8	Mean	S.E.
Infected	15th day	24.6	27.4	25.4	22.8	24.4	24.6	24.2	27.2	25.07	0.680
	30th day	23.0	26.2	23.2	22.4	23.4	22.8	23.4	26.6	23.87	0.698
Control	15th day	31.8	31.6	32.8	30.2	30.4	31.4	30.0	30.2	31.05	0.438
	30th day	33.2	32.8	33.0	31.8	30.2	33.2	31.6	31.8	32.20	0.458

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	420.03	60.004	29.156**
Error	24	49.41	2.058	
Total	31	469.44		

Table 20. Difference in the white blood corpuscles between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which W.B.C. determined	White blood corpuscles in 1000/C.mm									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	31.77	31.10	31.91	30.30	30.69	32.22	31.58	32.32	31.49	0.256
	30th day	31.11	31.44	31.01	31.44	31.74	31.59	33.62	33.33	31.91	0.436
Control	15th day	29.63	29.88	30.36	29.63	28.14	28.34	27.98	27.58	28.94	0.476
	30th day	31.14	31.42	32.16	30.39	30.82	30.09	30.47	29.63	30.77	0.350

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	41.195	5.885	6.208**
Error	24	22.764	0.948	
Total	31	63.959		

Erythrocyte sedimentation rate

The values of erythrocyte sedimentation rate of infected birds on fifteenth and thirtieth day after infection were found to have increased very significantly as compared to controls ($P < 0.01$). The erythrocyte sedimentation rate of infected ducklings were 3.61 ± 0.107 mm/hr. and 3.92 ± 0.123 mm/hr. respectively, and that of control group were 2.9 ± 0.045 mm/hr and 2.86 ± 0.039 mm/hr. respectively.

The details are presented in the Table 21 along with analysis of variance.

Mean corpuscular volume

The mean corpuscular volume of infected birds on the fifteenth and thirtieth day following infection was reduced significantly ($P < 0.01$) when compared with controls. The mean corpuscular volume of infected birds were 110.13 ± 2.283 C. microns and 105.90 ± 3.276 C. microns respectively, while that of control group were 133.28 ± 3.328 C. microns and 134.53 ± 1.068 C. microns respectively.

The data are presented in Table 22 along with analysis of variance.

Mean corpuscular haemoglobin

The mean corpuscular haemoglobin determined on the fifteenth and thirtieth day following infection in infected

Table 21. Difference in erythrocytic sedimentation rate between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which E.S.R. determined	Erythrocyte sedimentation rate in mm/hr.									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	3.4	3.3	3.7	3.5	3.4	3.8	4.0	3.8	3.61	0.107
	30th day	3.6	3.6	4.0	3.8	3.8	4.0	4.4	4.2	3.92	0.123
Control	15th day	3.0	2.9	2.8	2.9	3.0	2.9	3.0	2.7	2.9	0.045
	30th day	2.9	2.9	2.8	2.8	2.9	2.9	3.0	2.7	2.86	0.030

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	6.697	0.956	20.782**
Error	24	1.123	0.046	

** Highly significant.

Table 22. Difference in mean corpuscular volume between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which M.C.V. determined	Mean corpuscular volume in C. microns									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	107.42	116.59	112.28	100.44	106.55	108.84	108.03	120.88	110.13	2.283
	30th day	100.97	113.91	101.75	100.00	102.63	102.24	104.93	120.90	105.90	3.276
Control	15th day	138.26	131.66	136.66	130.73	133.33	131.38	132.74	131.30	133.28	3.328
	30th day	137.75	133.33	138.65	134.17	131.87	136.06	136.79	133.61	134.53	1.068

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	5601.032	800.147	24.775**
Error	24	775.119	32.296	
Total	31	6376.151		

** Highly significant.

ducklings were 36.55 ± 0.923 micro microgram and 34.57 ± 1.055 micro microgram respectively, while that of control group were 44.19 ± 1.052 micro microgram and 44.77 ± 0.941 micro microgram respectively. The reduction in the mean corpuscular haemoglobin in infected birds was found to be highly significant (P / 0.01).

The data are presented in the Table 23 along with analysis of variance.

Mean corpuscular haemoglobin concentration

No significant difference in the mean corpuscular haemoglobin concentration between the infected and non-infected ducklings was noticed. The mean corpuscular haemoglobin concentration values determined from infected birds were 33.19 ± 0.105% and 32.69 ± 0.345% respectively and in that of control groups were 33.16 ± 0.08% and 33.08 ± 0.064% respectively.

The data are presented in the Table 24 along with analysis of variance.

DISCUSSION

While studying the pathological changes produced by Amidostomum anseris Jestard (1937) observed erosion of the keratinoid layer of gizzard of water fowls. Srivastava (loc. cit.) noticed diarrhoea, incoordination of movement, inappetence,

Table 23. Difference in mean corpuscular haemoglobin between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which M.C.H. determined	Mean corpuscular haemoglobin in micro microgram									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	35.32	38.51	37.58	33.56	35.32	35.79	36.11	40.22	36.55	0.923
	30th day	33.42	37.26	33.71	31.45	33.42	34.17	34.17	38.95	34.57	1.055
Control	15th day	45.56	43.66	45.62	43.29	43.85	43.94	44.24	43.47	44.19	1.052
	30th day	45.43	44.51	46.06	44.21	43.66	44.87	45.36	44.03	44.77	0.941

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	653.396	93.342	27.293**
Error	24	82.088	3.420	
Total	31	735.484		

Table 24. Difference in mean corpuscular haemoglobin concentration between ducklings infected with 200 larvae of Amidostomum skrjabini and non-infected controls.

Group	Day on which M.C.H.C. determined	Mean corpuscular haemoglobin concentration in %									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	15th day	32.88	33.02	33.47	33.42	33.13	32.88	33.42	33.27	33.19	0.105
	30th day	33.13	32.70	32.84	31.97	32.56	33.42	32.56	32.21	32.67	0.345
Control	15th day	32.95	33.16	33.38	33.11	32.89	33.37	33.33	33.11	33.16	0.08
	30th day	32.98	33.38	33.18	32.95	33.11	32.98	33.16	32.94	33.08	0.064

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	1.455	0.207	0.869 Not significant
Error	24	5.730	0.238	
Total	31	7.185		

emaciation and anaemia in goose infected with Amidostomum nodulosa. McGraw (loc. cit.) and Czeplinki et al. (loc. cit.) recorded marked necrosis of mucosa of gizzard and reduction in weight gain respectively in goslings parasitised with Amidostomum anseris. Dubey and Pande (loc. cit.) while studying the pathogenicity of Amidostomum skrjabini in domestic ducks, observed erosion of horny lining, absence of eosinophilia, hyper activity of lymphoid follicles and necrosis in the gizzard. In ducks infected with Amidostomum skrjabini Chandrasekharan (loc. cit.) noticed anaemia, weakness, inappetence and listlessness. He also reported petechial haemorrhage within cornified layer, degeneration and desquamation of cells of superficial layer, inflammatory exudate containing lymphocytes and desquamated cells and congestion of blood vessels in the submucosa in the lesions of gizzard. He could also observe a slight reduction in haemoglobin concentration and total number of red blood corpuscles in ducklings experimentally infected with 100 infective larvae of the parasite.

During the present study, the clinical symptoms in the infected ducklings and the microscopic changes observed in the parasitised gizzard are in agreement with the previous findings. The significant reduction noted in the weight gain of infected birds agrees with the observation made by Czaplinki et al. (loc. cit.). The clinical evidence of anaemia in infected birds was therefore supported by the

observation on the haematological values such as haemoglobin concentration, number of red blood corpuscles, packed cell volume, mean corpuscular volume and mean corpuscular haemoglobin.

PATHOGENESIS OF *Epomidiostomum uncinatum*

PATHOGENESIS OF EPOMIDIOSTOMUM UNCINATUM

Though epomidiostomiasis is considered to be a serious disease in domestic ducks, very limited work seems to have been undertaken. Kurochkin (1954) made some observations on Epomidiostomum anatinum infection in ducks. Chandrasekharan (1967) studied the lesions produced by Epomidiostomum uncinatum in domestic ducks.

The present study embodies the observations made on the effect on weight gain, clinical manifestations, macroscopical and microscopical lesions and changes in haematological values of ducklings artificially infected with 200 larvae as compared with non-infected ducklings.

Clinical symptoms

The infected birds exhibited dullness, inappetence, weakness, anaemia, emaciation, incordination of movements and semi-solid droppings from about the twentieth day after infection i.e. when the worms were reaching sexual maturity. The severity of symptoms became aggrevated as the disease advanced. But no mortality was observed during the observed period of 36 days following infection.

Effect on weight gain

A significant reduction in weight gain in infected birds on twentyfirst and thirtysixth day following infection was

recorded as compared to non-infected control birds (P < 0.01). The mean weight gain determined in infected ducklings were 239.75 ± 11.379 gm and 341.75 ± 11.321 gm respectively and that of control groups were 363.37 ± 7.663 gm and 521.875 ± 7.720 gm respectively.

The details of weight gain are presented in the Table 25 along with analysis of variance.

Gross lesions

The parasitised gizzards could be easily detected by the presence of raised crateriform ulcers having ragged edges on the cornified lining (Figs. 114 & 115). A loose dark coloured sequestrum was found to be covering the ulcerated areas. The horny lining of the organ was softened and broken in many places due to liquifaction necrosis. Deep seated ulcers extending upto the muscular coat were also found. The worms were usually seen to be lodged in galleries formed at the infected area.

HISTOPATHOLOGY

On the twentyfirst day following infection, necrosis and aplasia of the mucous membrane extending upto the muscular layer of the gizzard were evident where the worms have settled. The keratinised superficial layer was ulcerated. The floor of the ulcer consisted of fibro-vascular granulation tissue. Cross sections of worms were found at the kerato-glandular

Table 25. Difference in weight gain between ducklings infected with 200 larvae of Epomidiostomum uncinatum and non-infected control.

Group	Day on which weight recorded	Weight gained in gm									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	21st day	215	225	207	269	245	290	264	203	239.75	11.379
	36th day	350	335	325	330	321	395	380	298	341.75	11.321
Control	21st day	365	374	362	397	345	358	380	326	363.37	7.663
	36th day	535	528	542	555	505	510	504	490	521.875	7.720

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	331032.25	47290.321	53.841**
Error	24	21079.75	878.322	
Total	31	352112.00		

** Highly significant.

junction of the ulcerated area (Figs. 116 & 117). Moderate degree of fibrosis and heterophilic infiltration were seen in the glandular zone extending from the keratinised layer of the mucosa.

Extensive lesions were more evident on the thirtysixth day after infection, with severe haemorrhage and necrosis of superficial mucosa and glandular zone. The glands were replaced by a mass of homogenous necrotic tissue which contained large number of degenerating mononuclear leucocytes and several eggs and cut sections of the worms (Figs. 118, 119 & 120). The interstitial tissue revealed moderate fibrosis and heterophilic infiltrations. The blood vessels in the submucosa were highly congested. At many places, there was evidence of extension of necrotic process to the adjoining areas in which eggs of the parasites could be seen indicating that the worms burrow deep into the tissues and deposit eggs and cause deep seated ulcers.

CHANGES IN HAEMATOLOGICAL VALUES

During the present study the changes in haemoglobin concentration, number of red blood corpuscles, number of white blood corpuscles, values of packed cell volume, erythrocyte sedimentation rate, mean corpuscular concentration, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in infected and non-infected birds were recorded on the twentyfirst and thirtysixth day after infection.

" 114

" 115

116

" 117

118

119

120

Haemoglobin concentration

The mean haemoglobin concentration of infected birds on twentyfirst and thirtysixth day were 8.869 ± 0.124 gm/100 ml and 8.541 ± 0.112 gm/100 ml respectively and that of control birds were 10.716 ± 0.125 gm/100 ml and 11.038 ± 0.110gm/100ml respectively. The reduction in the values of haemoglobin concentration of infected birds was highly significant as compared to control birds ($P < 0.01$).

The data are presented in the Table 26 along with analysis of variance.

Red blood corpuscles

There was significant reduction in the mean number of red blood corpuscles in infected birds as evidenced by the fact that the values on the twentyfirst and thirtysixth day were 2.268 ± 0.010 million/C.mm and 2.253 ± 0.005 million/C.mm as against 2.390 ± 0.032 million/C.mm and 2.420 ± 0.030 million/C.mm in control birds. The reduction in the total count of red blood corpuscles was therefore highly significant ($P < 0.01$).

The data are presented in the Table 27 dong with analysis of variance.

Packed cell volume

In infected birds the mean values of packed cell volume

Table 26. Difference in haemoglobin concentration between ducklings infected with 200 larvae of Echinostomum uncinatum and non-infected controls.

Group	Day on which Hb. determined	Haemoglobin concentration in gm/100 ml									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	21st day	9.05	8.57	9.05	8.57	9.05	9.52	8.57	8.57	8.868	0.124
	36th day	8.75	8.33	8.33	8.33	8.75	9.18	8.33	8.33	8.541	0.112
Control	21st day	10.95	10.48	10.48	11.43	10.48	10.48	10.95	10.48	10.716	0.125
	36th day	11.25	10.83	10.83	11.66	10.83	10.83	10.25	10.83	11.038	0.110

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	38.601	5.514	41.772**
Error	24	3.170	0.132	
Total	31	41.771		

** Highly significant.

Table 27. Difference in red blood corpuscles between ducklings infected with 200 larvae of Echmidiostomum uncinatum and non-infected controls.

Group	Day on which R.B.C. determined	Red blood corpuscles in million/C.mm									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	21st day	2.26	2.27	2.29	2.25	2.23	2.30	2.20	2.30	2.268	0.010
	36th day	2.25	2.25	2.26	2.24	2.26	2.28	2.19	2.28	2.253	0.005
Control	21st day	2.45	2.32	2.30	2.58	2.32	2.35	2.40	2.40	2.39	0.032
	36th day	2.48	2.37	2.34	2.6	2.36	2.38	2.48	2.42	2.428	0.030

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	0.233	0.033	16.50**
Error	24	0.067	0.002	
Total	31	0.300		

** Highly significant.

were found to be 26.675 ± 0.415% and 25.57 ± 0.322% on the twentyfirst and thirtysixth day after infection respectively whereas in the control birds the same were 32.255 ± 0.402% and 33.95 ± 0.33%. The reduction in packed cell volume was therefore highly significant (P < 0.01).

The data are presented in the Table 28 along with analysis of variance.

Erythrocytic sedimentation rate

Blood of infected ducklings revealed a high mean erythrocytic sedimentation rate of 3.575 ± 0.122 mm/hour and 3.50 ± 0.091 mm/hour on the twentyfirst and thirtysixth day respectively as against 2.875 ± 0.036 mm/hour and 2.950 ± 0.107mm/hr. respectively in control birds. The increased erythrocytic sedimentation rate in infected group was also highly significant (P < 0.01).

The data are presented in the Table 29 along with analysis of variance.

White blood corpuscles

Infected birds showed an increase in the mean number of white blood corpuscles. The values determined on the twenty-first and thirtysixth day after infection in infected birds, were 35.77 ± 0.544 thousand/C.mm and 36.72 ± 0.522 thousand/C.mm respectively and that of control birds were 29.38 ± 0.372 thousand/C.mm and 29.99 ± 0.371 thousand/C.mm respectively. This increase

Table 28. Difference in packed cell volume between ducklings infected with 200 larvae of Epomidiostomum uncinatum and non-infected controls.

Group	Day on which P.C.V. determined	Packed cell volume in %									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	21st day	27.4	25.8	27.6	25.6	27.2	28.6	25.4	25.8	26.675	0.415
	36th day	26.4	25.2	25.4	25.0	24.6	27.4	25.0	25.4	25.57	0.322
Control	21st day	33.0	31.6	31.4	34.4	31.4	31.6	33.2	31.4	32.255	0.402
	36th day	33.8	32.6	32.8	35.0	32.4	32.6	33.8	32.4	33.175	0.33

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	356.965	50.995	39.971**
Error	24	30.71	1.279	
Total	31	387.675		

** Highly significant.

Table 29. Difference in erythrocyte sedimentation rate between ducklings infected with 200 larvae of Epomidiostomum uncinatum and non-infected controls.

Group	Day on which E.S.R. determined	Erythrocytic sedimentation rate in mm/hr. 1	2	3	4	5	6	7	8	Mean	S.E.
Infected	21st day	3.6	3.4	3.8	3.8	3.0	3.8	3.2	4.0	3.575	0.122
	36th day	3.4	3.4	3.6	3.6	3.2	3.6	3.2	4.0	3.50	0.091
Control	21st day	2.9	2.7	3.0	2.8	3.0	2.9	2.8	2.9	2.875	0.036
	36th day	3.0	3.0	2.9	3.1	3.0	2.9	2.8	3.0	2.95	0.107

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	3.17	0.4528	5.327**
Error	24	2.04	0.085	
Total	31	5.21		

** Highly significant.

in the white blood corpuscles was also significant.(P / 0.01).

The details are presented in the Table 30 along with analysis of variance.

Mean corpuscular volume

The mean corpuscular volume of infected birds were lower than that of control birds. The values in the infected birds on twentyfirst and thirtysixth day after infection were found to be 117.552 ± 1.548 C. microns and 113.48 ± 1.133 C. microns respectively and that of control birds were 134.96 ± 0.796 C. microns and 136.91 ± 0.714 C. microns respectively. The reduction noted in the mean corpuscular volume was highly significant (P / 0.01).

The data are presented in the Table 31 along with analysis of variance.

Mean corpuscular haemoglobin

A significant reduction in the mean corpuscular haemoglobin of infected ducklings was evident, as compared to controls (P / 0.01). The mean corpuscular haemoglobin in the infected birds were 39.08 ± 0.479 micro microgram and 37.92 ± 0.446 micro microgram respectively and that of control birds were 44.97 ± 0.248 micro microgram and 45.5 ± 0.195 micro microgram respectively on the twentyfirst and thirtysixth day following infection.

ble 30. Difference in white blood corpuscles between ducklings infected with 200 larvae of Echinostomum uncinatum and non-infected controls.

oup	Day on which W.B.C. determined	White blood corpuscles in 1000/C.mm									S.E.
		1	2	3	4	5	6	7	8	Mean	
fected	21st day	37.22	32.40	35.70	36.23	36.71	36.59	36.55	34.78	35.77	0.547
	36th day	38.16	35.04	37.69	35.56	37.37	39.02	34.86	36.06	36.72	0.522
ntrol	21st day	30.36	28.14	28.34	30.36	27.98	29.63	30.36	29.88	29.38	0.372
	36th day	29.63	30.37	31.41	27.99	30.95	29.89	29.36	30.37	29.99	0.371

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	349.11	49.87	24.44**
Error	24	4912	2.04	
Total	31	398.23		

** Highly significant.

Table 31. Difference in mean corpuscular volume between ducklings infected with 200 larvae of Epomidiostomum uncinatum and non-infected controls.

Group	Day on which M.C.V. determined	Mean corpuscular volume in C.microns									S.E
		1	2	3	4	5	6	7	8	Mean	
Infected	21st day	121.23	113.65	120.52	113.77	119.29	124.34	115.45	112.17	117.552	1.54
	36th day	117.33	112.00	112.38	111.60	108.94	120.17	114.15	111.40	113.96	1.1
Control	21st day	134.69	136.20	136.52	133.33	135.34	134.46	138.33	130.93	134.96	0.7
	36th day	136.29	137.55	140.17	134.61	137.28	136.97	139.52	133.88	136.91	0.7

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	3416.365	488.052	40.212**
Error	24	291.280	12.137	
Total	31	3707.659		

** Highly significant.

The data are presented in the Table 32 along with analysis of variance.

Mean corpuscular haemoglobin concentration

The mean corpuscular haemoglobin concentration in infected birds were found to be 33.25 ± 0.099% and 33.18 ± 0.427% as against 33.23 ± 0.047% and 33.27 ± 0.044% in controls on the twentyfirst and thirtysixth day after infection. No significant difference between the values of infected and non-infected birds was noticed.

The details are presented in the Table 33 along with analysis of variance.

DISCUSSION

Kurochkin (loc. cit.) reported foliation of keratinised layer, inflammatory changes and ulceration in the gizzard of ducks infected with Epomidiostomum anatinum. In the domestic ducks infected with Epomidiostomum uncinatum Chandrasekharan (loc. cit.) observed raised crateriform ulcers, complete necrosis of mucous membrane, congestion of blood vessels and replacement of epithelium of glands into a homogenous necrotic tissue. He could not observe significant changes in the blood picture of ducklings infected with 100 larvae.

The clinical symptoms and histopathological changes in infected birds, observed during the present study were more or

Table 32. Difference in the mean corpuscular haemoglobin between ducklings infected with 200 larvae of Epomidiostomum uncinatum and non-infected controls.

Group	Day on which M.C.H. determined	Mean corpuscular haemoglobin in micro microgram									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	21st day	40.04	37.75	39.51	38.08	39.69	41.49	38.95	37.26	39.08	0.479
	36th day	38.88	37.02	36.85	37.18	38.91	40.20	38.01	36.53	37.92	0.446
Control	21st day	44.69	45.17	45.56	44.30	45.17	45.59	45.62	43.66	44.97	0.248
	36th day	45.36	45.69	46.28	44.94	45.88	45.50	46.10	44.75	45.5	0.195

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	371.982	53.140	42.924**
Error	24	29.716	1.238	
Total	31	401.698		

** Highly significant.

Table 33. Difference in mean corpuscular haemoglobin concentration between ducklings infected with 200 larvae of Epomidiostomum uncinatum and non-infected controls.

Group	Day on which M.C.H.C. determined	Mean corpuscular haemoglobin concentration in %									S.E.
		1	2	3	4	5	6	7	8	Mean	
Infected	21st day	33.02	33.21	32.78	33.47	33.27	33.28	33.74	33.21	33.25	0.099
	36th day	31.14	33.05	32.79	33.32	35.56	33.45	33.32	32.79	33.18	0.427
Control	21st day	33.18	33.16	33.37	33.22	33.37	33.16	32.98	33.37	33.23	0.047
	36th day	33.28	33.22	33.01	33.31	33.42	33.22	33.28	33.42	33.27	0.044

Analysis of variance

Source of variation	df	SS	MSS	F
Between groups	7	0.047	0.0067	0.0159 Not significant
Error	24	10.055	0.4189	
Total	31	11.102		

less similar to the previous findings. Significant reduction in weight gain, haemoglobin concentration, red blood corpuscles, packed cell volume, mean corpuscular haemoglobin and increase in the erythrocytic sedimentation rate and white blood corpuscles can be attributed to the effect of the parasite on its host.

TREATMENT OF *Tetrameres anatis*, *Amidostomum skrjabini* AND *Epomidiostomum uncinatum*

TREATMENT OF TETRAMERES ANATIS, AMIDOSTOMUM SKRJABINI AND EPOMIDIOSTOMUM UNCINATUM

Within recent years, a number of compounds has been tested as anthelmintics, but most of them are far from being ideal for poultry on account of their toxic or other side effects. Very little information is available on the efficacy of recently introduced anthelmintics against the common nematodes of ducks. During the present investigation, controlled trials were conducted in ducklings, infected experimentally with Tetrameres anatis, Amidostomum skrjabini and Epomidiostomum uncinatum with ten anthelmintics viz., Tetramisole hydrochloride, Parbendazole, Thiabendazole, Morantel tartrate, Carbon tetrachloride, Methyridine, Disophenol, Phenothiazine, Cashewnut shell oil and Rametin.

The anthelmintic efficacy of Tetramisole hydrochloride or dl 2, 3, 5 tetrahydro-6-phenyl-imidazo (2, 1-6) thiazole hydrochloride (Nilverm 3% - Imperial Chemical Industries) was first studied by Thienpont et al. (1966) and since then the drug was found to be used very extensively in animals and birds. Enigk and Hazra (1967); Swietlikowski et al. (1968); Enigk and Hazra (1971); Pumpr et al. (1973); Zajicek et al. (1973) and Khaziev et al. (1975) studied the efficacy of this drug in amidostomiasis of geese at different dose rates. Actor et al. (1967) introduced a new broad spectrum anthelmintic, Parbendazole or Methyl-5-butyl-2-benzimidazole carbamate (Helatac 4%

premix powder - Smith Kline and French) against nematodes of cattle, sheep and goat and it was also found useful in amidostomiasis in geese by Zajicek et al. (loc. cit.). The anthelmintic efficacy of Thiabendazole or 2-(4 thiazolyl) benzimidazole (Thibendol 75% powder - Merck Sharp and Dohme) was first reported by Brown et al. (1961) against nematodes of animals and birds. The drug was tried against amidostomiasis of geese by Swietlikowski et al. (loc. cit.); Emigk et al. (1971) and Stoican et al. (1971) and against tetramerosis of duckling by Garkavi and Luzhkov (1972). Against nematodiasis of sheep, Cornwell and Jones (1970) released a new broad spectrum anthelmintic, Morantel tartrate or Trans-2 (2-3 (3-Methyl-2-Thienyl) Vinyl) 1-methyl-1, 4, 5, 6 tetrahydropyrimidine tartrate (Banminth II 4% liquid - Pfizer). Though it was found to be useful in animals no data on the efficacy of the drug in anseriform birds are available. Carbon tetrachloride, an age old anthelmintic was widely used in tetramerosis of ducks by Litvishko (1959); Vorontsov (1963); Garkavi (1964); Naumenko (1965); Mohiuddin and Lone (1967) and Petrov and Mukhamedshin (1969). It was also found useful against amidostomiasis in geese by Doroshko (1948); Nickel (1952); Labatut et al. (1958); Negru et al. (1960) and Swietlikowski et al. (loc. cit.). Methyridine or 2-C-B-methoxy-ethylpyridine (Promintic 90% liquid - Imperial Chemical Industries) was first introduced by Walley (1961) as a potent anthelmintic for parasites of sheep and goats. The efficacy

of this drug was evaluated by Swietlikowski et al. (loc. cit.) against amidostomiasis in geese. Disophenol or 2, 6-diiodo-4-nitrophenol (Ancylol 4.5% liquid - Cyanamid) was introduced by Wood et al. (1961) against hookworms of dogs and cats. Later, the anthelmintic efficacy of the drug was determined against amidostomiasis in geese by Enigk and Hazra (1967 & 1971). Phenothiazine (Phenovis - Imperial Chemical Industries) was found to be useful against tetramerosis of ducks by Bouneau (1948); Litvishkov (loc. cit.); Garkavi (loc. cit.); Naumenko (loc. cit.) and Romanovski (1969). Ozerskaya (1950); Selivanova-Yartseva (1955) and Stoican et al. (1960) also evaluated the efficacy of the drug against amidostomiasis of geese. George Varghese et al. (1971) reported for the first time the high anthelmintic activity of cashewnut shell oil in eliminating Ascaridia galli of chicken. Further trials with the drug is not seen undertaken in animals and birds. Anthelmintic activity of Rametin or O-O-diethyl-O-naphthal-oxemide phosphate (Bayer) was reported by Federmann (1962) and since then it was found to be used in animals.

In the present investigation anthelmintic efficacy of 10 drugs against adult worms of Tetrameres anatis, and of nine drugs (except Rametin) against adult worms of Amidostomum skrjabini and Epomidiostomum uncinatum was evaluated under controlled experimental infections in ducklings. The details of the experimental trials are presented in the Tables 34, 35 and 36 along with analysis of variance.

4. Details of drugs, dose rate, number of worms recovered at necropsy and percentage of efficacy of drugs against Tetrameres anatis infection in ducklings as compared to non-treated positive control birds.

Name of drugs	Dose rate	Route of administration	Mean no. of worms recovered at necropsy	S.E.	Percentage of efficacy
sole hydrochloride	50 mg/kg	Orally	9.37	0.595	79.23
azole	100 mg/kg	Orally	24.50	0.845	45.70
dazole	200 mg/kg	Orally	26.63	1.051	41.00
l tartrate	40 mg/kg	Orally	33.00	0.906	26.85
tetrachloride	2 ml/kg	Orally	18.37	0.754	59.28
dine	200 mg/kg	S/C	27.37	1.322	35.94
nol	10 mg/kg	S/C	36.25	0.795	28.87
iazine	500 mg/kg	Ora	13.78	0.679	70.36
ut shell oil	10 g/kg	Orally	31.13	0.989	31.02
	200 mg/kg	Orally	35.12	0.666	22.16 *
ated positive control birds	-	-	45.12	0.718	-

e medicated birds died within 24 hours.

Analysis of variance

Source of variation	df	SS	MSS	F

35. Details of drugs, dose rate, number of worms recovered at necropsy and percentage of efficacy of drugs against Amidostomum skrjabini infection in ducklings as compared to non-treated positive control birds.

Name of drugs	Dose rate	Route of administration	Mean no.of worms recovered at necropsy	S.E.	Percentage of efficacy
lsole hydrochloride	50 mg/kg	Orally	6.00	0.626	82.15
dazole	100 mg/kg	Orally	11.00	0.422	67.29
ndazole	200 mg/kg	Orally	12.13	0.638	63.93
ated positive control birds	-	-	33.63	0.822	-
l tartrate	40 mg/kg	Orally	9.25	0.839	74.80
tetrachloride	2 ml/kg	Orally	12.75	0.860	61.06
ated positive control birds	-		32.75	1.305	-
dine	200 mg/kg	S/C	23.50	0.981	30.12
nol	10 mg/kg	S/C	26.88	0.811	20.07
iasine	500 mg/kg	Orally	16.00	0.755	52.42
ut shell oil	10 g/kg	Orally	20.05	0.755	39.04
ated positive control birds	-	-	29.63	3.560	-

Analysis of Variance

6. Details of drugs, dose rate, number of worms recovered at necropsy and percentage of efficacy of drugs against Epomidiostomum uncinatum infection in ducklings as compared to non-treated positive control birds.

Name of drugs	Dose rate	Route of administration	Mean no. of worms recovered at necropsy	S.E.	Percentage of efficacy
ole hydrochloride	50 mg/kg	Orally	5.13	0.515	79.88
zole	100 mg/kg	Orally	10.75	0.452	57.84
azole	200 mg/kg	Orally	14.50	0.422	43.13
ted positive control birds	-	-	25.50	0.755	-
tartrate	40 mg/kg	Orally	8.88	0.776	65.68
etrachloride	2 ml/kg	Orally	12.25	0.958	52.66
ted positive control birds	-	-	25.88	1.108	-
ine	200 mg/kg	S/C	24.00	0.807	13.13
ol	10 mg/kg	S/C	24.50	0.500	11.32
azine	500 mg/kg	Orally	13.75	0.587	50.23
t shell oil	10 g/kg	Orally	24.13	0.610	12.66
ted positive control birds	-	-	27.63	0.730	-

Analysis of variance

Tetramisole hydrochloride

Tetramisole hydrochloride at a dosage of 50 mg/kg body weight, administered orally, was proved to be 79.23% efficient in expelling adult worms of Tetrameres anatis infection. In the case of Amidostomum skrjabini and Epomidiostomum uncinatum the drug showed 82.15% and 79.88% efficacy respectively. The drug was found to be well tolerated by the ducklings.

Parbendazole

At an oral dose rate of 100 mg/kg body weight administered in gelatin capsule Parbendazole was found to be 45.70% efficient against Tetrameres anatis, 67.29% against Amidostomum skrjabini and 57.98% against Epomidiostomum uncinatum without causing any toxic symptoms in medicated birds.

Thiabendazole

Thiabendazole at a dose rate of 200 mg/kg body weight, administered orally in gelatin capsule showed an anthelmintic efficacy of 41% against Tetrameres anatis, 63.93% against Amidostomum skrjabini and 43.13% against Epomidiostomum uncinatum. The medicated birds showed no toxic symptoms at the dosage level tried.

Morantel tartrate

During the trial 26.95% clearance of adult worms of

Tetrameres anatis, 74.80% of clearance of Amidostomum skrjabini and 65.86% Epomidiostomum uncinatum were obtained when Morantel tartrate was administered orally at a dose rate of 40 mg/kg body weight. The drug was also found to be well tolerated by the birds.

Carbon tetrachloride

Carbon tetrachloride at a dose rate of 2 ml/kg body weight was 59.28% effective against Tetrameres anatis, 61.06% against Amidostomum skrjabini and 52.66% against Epomidiostomum uncinatum without causing any deleterious effects in the medicated ducklings.

Methyridine

Methyridine at a dosage rate of 200 mg/kg body weight, administered subcutaneously, showed anthelmintic efficacy of 35.94% against Tetrameres anatis, 30.12% against Amidostomum skrjabini and 13.13% against Epomidiostomum uncinatum. No toxic symptoms in medicated groups were noticed.

Disophenol

During the trial 29.97% clearance of worms in respect of Tetrameres anatis, 20.07% in respect of Amidostomum skrjabini and 11.32% in respect of Epomidiostomum uncinatum infection were observed at a dosage of 10 mg/kg body weight. The medicated birds did not exhibit any toxic symptoms.

Phenothiazine

The percentage of anthelmintic efficacy evaluated at a dose rate of 500 mg/kg body weight against adult worms of Tetrameres anatis, Amidostomum skrjabini and Epomidiostomum uncinatum were 70.36; 52.42 and 50.23 respectively. The drug was found to be well tolerated by the birds.

Cashewnut shell oil

Cashewnut shell liquid was administered orally be means of a long pipette at 10 g/kg body weight after mixing with equal parts of liquid paraffin. It was proved to be 31.02% effective in eradicating Tetrameres anatis, 39.04% against Amidostomum skrjabini and 12.66% against Epomidiostomum uncinatum. No harmful effects were observed in the medicated birds.

Rametin

The drug at a dose rate of 200 mg/kg body weight was tried against tetramerosis but assessment of its efficacy could not be made as the drug was found to be highly toxic and all the treated birds died of its toxicity within 24 hours. Further trial with this drug against duck nematodes were not undertaken.

The results obtained in the treatment trials were analysed statistically and found to be highly significant ($P < 0.01$).

DISCUSSION

During the present investigation, a single oral dose of tetramisole hydrochloride at 50 mg/kg body weight was found to be superior than all other drugs employed with an anthelmintic efficiency of 79.23% against adult worms of Tetrameres anatis, 82.15% against Amidostomum skrjabini and 79.23% against Epomidiostomum uncinatum infections in ducklings. The result obtained on amidostomiasis is comparable with those of Swietlikowski et al. (loc. cit.) and Enigk and Hazra (1971) where the former authors reported 78% efficacy at 50 mg/kg body weight and the latter authors 83.5% efficacy at 40 mg/kg body weight respectively against amidostomiasis in geese. Zajicek et al. (loc. cit.) reported that at 80 mg/kg body weight the drug was 100% effective a against amidostomiasis in geese. Cent per cent efficacy against amidostomiasis has also been reported by Kaziev et al. (loc. cit.) when the drug was mixed in feed at the rate of 200-300 mg/kg.

The next drug in order of efficiency was found to be Morantel tartrate, which was 74.8% effective against Amidostomum skrjabini, 65.63% against Epomidiostomum uncinatum and 26.85% against Tetrameres anatis at a single dose of 40 mg/kg body weight. The present report on the efficacy of Morantel tartrate against nematodes of ducks appears to be the first.

Phenothiazine at a dose rate of 500 mg/kg body weight is another drug of choice as the percentage of removal of worms were as follows: Tetrameres anatis 70.30, Amidostomum skrjabini 52.42, Epomidiostomum uncinatum 50.23. Compared to the findings of Romanovski (loc. cit.) who reported 94.4% of clearance of Tetrameres at a dosage rate of 250 mg/kg and that of Stoican et al. (1960) who found an 82% clearance of Amidostomum at 1 g/kg, the present findings point to a lesser efficacy for this drug. However, the efficacy rate worked out during the present trials is higher than those reported by Vorantsov (loc. cit.) and Naumenko (loc. cit.) who found only an efficacy percentage of 50 and 23.6 respectively when the drug is administered at 500 mg/kg against tetramerosis.

The anthelmintic efficacy of 59.28% for Carbon tetrachloride at a single dose of 2 ml/kg body weight observed during the present trial against Tetrameres anatis was in conformity with those reported by Litvishkov (loc. cit.) and Vorontsov (loc. cit.) and higher than that of Naumenkov (loc. cit.) who could get only 44.1% removal of worms. On the other hand, Garkavi (loc. cit.) reported a higher efficacy of 97-98% against tetramerosis of ducks with this drug. In the case of amidostomiasis of geese Labatut et al. (loc. cit.); Negru et al. (loc. cit.) and Olteanu et al. (1965) recorded complete removal of worms at a dose rate of 1.25-1.5 ml/kg body weight and Swietlikowski et al. (loc.cit.)

reported 96.4% clearance at 3 ml per bird. The 61.06% efficacy of the drug at a dosage rate of 2 ml/kg body weight was thus lower than the reports of the above two authors.

The percentage of efficacy of 67.29% recorded during the present study for single dose administration of Parbendazole at 100 mg/kg against Amidostomum skrjabini was found to be higher than that of Zajicek (loc. cit.) who got only 29.17% efficacy in amidostomiasis of geese at the same dose rate.

Garkavi (loc. cit.) did not obtain satisfactory results with thiabendazole at a dosage of 160 mg per bird administered daily for a period of 15 days against tetramerosis of ducks. On the contrary during the present trial, a 41% efficacy could be observed when the drug was administered at a single dose of 200 mg/kg body weight. In the case of Amidostomum skrjabini infection, the efficacy of 63.93% was comparable with that reported by Enigk and Hasra (loc. cit.) who recorded a 60.1% efficacy at 400 mg/kg body weight in amidostomiasis of geese. Stoican et al. (1971) reported complete removal of worms with 300 mg/kg body weight administered continuously for 3 days, which indicates that the efficacy of the drug increases if the treatment is repeated for 3 days.

While treating gooslings against amidostomiasis, Swietlikowski et al. (loc. cit.) could not observe convincing efficacy with methyridine at a dose rate of 500 mg per bird, whereas during the present study the drug at 200 mg/kg body weight exerted an anthelmintic efficacy of 30.12% against Amidostomum skrjabini, in ducklings.

Disophenol at a dose rate of 10 mg/kg body weight as a single dose removed 28.87% of Tetrameres anatis, 20.07% of Amidostomum skrjabini and 11.32% of Epomidiostomum uncinatum. Whereas Enigk and Hazra (1971) reported a higher efficacy of 73% against amidostomiasis in goose at 10 mg/kg body weight for this drug.

The results obtained on the anthelmintic efficacy of Morantel tartrate and cashewnut shell oil, against tetramerosis, amidostomiasis and epomidiostomiasis and that of Methyridine, Disophenol, Tetramisole hydrochloride and Parbendazole, against tetramerosis and epomidiostomiasis in the current treatment trials appear to be the first attempt in enlarging the range of chemotherapeutic agents against duck nematodes.

CROSS INFECTION TRIAL WITH *Ascaridia galli*

CROSS INFECTION TRIAL WITH ASCARIDIA GALLI

Studies on cross infections of nematodes between fowls and ducks were made by many workers like Akhmedova (1954); Leiby and Olson (1965); Chandrasekharan (1967); Klimes and Stejskal (1968) and Sundaram (1971). In order to find out the host specificity of Ascaridia galli of fowl origin attempts were made to infect ducklings and chicks and the results were furnished below.

Development of Ascaridia galli in ducklings and chicks

Ten ducklings and 10 chicks, aged 10 days were artificially infected with 500 embryonated eggs of Ascaridia galli of fowl origin. The infected birds were sacrificed at periodical intervals in order to find out the fate of the embryonated eggs.

24 hours after infection

At 24 hours following infection, 125 and 165 numbers of first stage juveniles of Ascaridia galli were recovered from the intestine of a duckling and a chick respectively. The juveniles from both the hosts were morphologically identical with each other. The first stage juvenile from duck measured 0.325-0.3375 mm in length and 0.0175 mm in breadth while that from chick were 0.350-0.3575 mm and 0.020 mm respectively.

8 days after infection

On sacrificing a chick and a duckling on the eighth day following infection 115 juveniles and 51 juveniles respectively could be obtained from their intestines. All the juveniles were at the second stage. The larvae collected from duckling measured 1.625-1.75 mm in length and 0.040-0.0425 mm in breadth and were similar in morphology with those collected from chicks. The body measurements of larvae recovered from chick were 1.85-1.975 mm in length and 0.0525-0.055 mm in breadth.

17 days after infection

On the seventeenth day after infection 47 juveniles from a duckling and 111 juveniles from a chick were recovered. All the juveniles had reached the third stage and were similar in morphology. The body measurements of female and male worms recovered from duckling were 12.75-14.375 x 0.275-0.2875 mm and 5.925-7.5625 x 0.1375-0.175 mm respectively. The young females from chick measured 13.65-15.25 mm in length and 0.2775-0.2875 mm in breadth while the males measured 9.95-10.15 mm x 0.1975-0.205 mm.

25 days after infection

On the twentyfifth day after infection both duckling and chick yielded 35 and 117 young adults respectively. The

worms recovered from duckling were comparatively smaller in size and the female and males measured 15.825-19.9 x 0.3125-0.450 mm and 9.25-13.025 x 0.2875-0.350 mm respectively. The uterine coils of the female worms contained large number of oocysts.

The female and male worms from chicks measured 22.15-26.5 x 0.314-0.365 mm and 21.25-22.5 x 0.310-0.355 mm respectively. Immature eggs were noticed in the uteri of female worms.

35 days after infection

The droppings of all the remaining six chicks showed eggs when examined on the thirtyfifth day following infection and on autopsy of a chick 104 adult worms could be recovered. The measurements of which were as follows:

Female: length 41.15-42.45 mm; breadth 0.535-0.5775 mm.

Male: length 40.5-41.25 mm; breadth 0.475-0.485 mm.

In the case of the duckling sacrificed on the thirty-fifth day only 30 young adults could be recovered having the following range of measurements.

Females: length 21.25-25.75 mm; breadth 0.375-0.415 mm.

Male: length 11.55-15.65mm; breadth 0.365-0.395 mm.

The uteri of female worms contained only a few number of immature eggs.

42 days after infection

On the fortysecond day after infection, the worms were found to be still at the incubation stage as no eggs were found in the droppings of ducklings, whereas all the chicks continued to avoid the eggs of the parasite. On autopsy of a duckling and a chick 14 and 75 worms respectively were obtained from their intestines. The worms recovered from the duckling were distinctly smaller and less developed. The body measurements were as follows:

Female: length 27.65-35.45 mm; breadth 0.475-0.525 mm.
Male: length 28.35-32.45 mm; breadth 0.485-0.520 mm.

The worms recovered from chicks were comparatively larger and the females were fully gravid. The body measurements of the worms were as follows:

Female: length 52.75-55.5 mm; breadth 0.715-0.875 mm.
Male: length 41.5-55.25 mm; breadth 0.525-0.565 mm.

47 days after infection

On the fortyseventh day, one of the ducklings became positive for infection and when this bird was sacrificed 9 adult worms (3 males and 6 females) could be recovered from its intestine. The body measurements were as follows:

Female: length 32.05-37.55 mm; breadth 0.515-0.565 mm.
Male: length 31.75-37.35 mm; breadth 0.475-0.535 mm.

On sacrificing a chick, 91 adult worms could be recovered from its intestine, with the following measurements:

Female: length 57.5-65.75 mm; breadth 0.875-0.915 mm.
Male: length 45.75-60.5 mm; breadth 0.555-0.595 mm.

53 days after infection

Fully matured eggs of the parasite were detected in the droppings of all the remaining three ducklings. The remaining three chicks also continued to show the eggs of the parasite in their droppings. On autopsy a total of 13 adult worms from ducklings and 158 adult worms from chicks were recovered from their intestine. The collected worms were studied in detail to note any difference in the body size or morphology. It was found that the parasites collected from ducklings were markedly smaller than those obtained from chicks eventhough no appreciable morphological peculiarities could be detected. The measurements of the worms obtained from chicks were as follows:

Female: length 63.5-75.5 mm; breadth 0.950-0.975 mm.
Male: length 50.75-62.35 mm; breadth 0.560-0.600 mm.

The worms recovered from ducklings had the following range of measurements:

Female: length 36.95-45.15 mm; breadth 0.555-0.675 mm.
Male: length 35.65-42.15 mm; breadth 0.605-0.635 mm.

The details of worms recovered from infected ducklings and chicks are presented in the Table 37.

Table 37. Details of worms recovered from ducklings and chicks artificially infected with 500 embryonated eggs of *Ascaridia galli*.

Day on which birds autopsied	Ducklings					Chicks				
	No. of birds autopsied	Worms recovered			Percentage of establishment of infection	No. of birds autopsied	Worms recovered			Percentage of establishment of infection
		Female	Male	Total			Female	Male	Total	
24 hours after infection	1	-	-	125	25.0	1	-	-	165	33.0
8 days after infection	1	-	-	51	10.2	1	-	-	115	23.0
17 days "	1	32	15	47	9.4	1	60	51	111	22.2
25 days "	1	18	17	35	7.0	1	65	52	117	23.4
35 days "	1	21	9	30	6.0	1	60	44	104	20.8
42 days "	1	8	6	14	2.8	1	49	26	75	15.0
47 days "	1	6	3	9	1.8	1	49	42	91	18.2
53 days "	3	8	5	13	0.87	3	88	70	158	10.53
Total	10	93	55	324	6.48	10	371	285	936	18.72

DISCUSSION

In a cross transmission experiment involving Ascaridia galli of fowl origin, Klimes and Stejskal (loc. cit.) noted that only 72% of the infected ducks (18 out of 25) became positive for infection, whereas 100% of the chicks could be infected with eggs of Ascaridia galli (25 out of 25). They also recovered a total of 2109 worms from chicks and 646 worms from ducks during a period ranging from 18 to 55 days after infection giving 16.84% of establishment in chicks and 7.18% of establishment of infection in ducks.

During the present study all the ducklings and chicks exposed to the infection became ultimately positive for infection. A total of 324 worms from ducklings and 936 worms from chicks were recovered during the period ranging from 24 hours to 53 days following infection giving 6.48% of establishment of infection in ducklings and 18.72% of establishment of infection in chicks. This observation more or less agrees with that of Klimes and Stejskal (loc. cit.). Ducks being unnatural hosts, there was lesser percentage of establishment of the parasite than in chicken and the parasites were also distinctly smaller than the worms obtained from chicks.

SUMMARY

SUMMARY

1. The incidence of nematode parasites in 340 ducks purchased locally and from Tamil Nadu was recorded. Out of these, 231 birds or 67.94% had harboured one or more species of nematodes. In all, eight species of nematodes *viz.*, *Amidostomum skrjabini*, *Capillaria contorta*, *Capillaria globocaudata*, *Echinuria uncinata*, *Epomidiostomum uncinatum*, *Eustrongylides papillosus*, *Strongyloides avium* and *Tetrameres anatis*, were encountered. *Epomidiostomum uncinatum* was found to be the commonest worm with a rate of infection of 41.8% and *Strongyloides avium* to be the least common with a rate of infection of 0.59%. The occurrence of *Eustrongylides papillosus* reported during the present investigation constitutes a new host record in India.

2. The lifecycle of *Echinuria uncinata* was elucidated in full with detailed description of various development stages in the intermediate host, *Daphnia pulex* and *Daphnia magna* and in the final host, *viz.*, the duck. The juveniles of *Echinuria uncinata* underwent the first and second moulting on the third day and on the fifth day respectively in the *Daphnia pulex* and attained infective stage on the seventh day following infection at a room temperature ranging from 25 to 30°c. In ducklings the juveniles completed third and fourth moulting on the third day and on the seventh day

respectively and the first eggs were passed in the droppings on the thirtythird day following infection. Other waterfleas like Macrothrix sp., Sida sp., Scapholeberis sp. and Moina sp., Cyclops sp. (Copepoda), Porcellio laevis (Isopoda) and grasshopper Spathosternum prasiniferum, were not infectible with the eggs of the parasite. The body fluid of Daphnia and normal saline were found to induce in vitro hatching of the eggs of Echinuria uncinata.

Six species of grasshoppers viz., Spathosternum prasiniferum, Oxya nitidula, Oedaleus abruptus, Conocephalus maculatus, Ducetia japonica and Atractomorpha crenulata were found to act as experimental intermediate hosts of Tetrameres anatis. Daphnia pulex and Daphnia magna, the proven intermediate hosts of Tetrameres fissispina, could not be infected with the eggs of Tetrameres anatis, thus proving that Tetrameres anatis is distinct from Tetrameres fissispina. Attempts to infect Macrothrix sp., Sida sp., Scapholeberis sp., Moina sp. and Cyclops sp. did not also yield successful results. In vitro hatching of eggs of Tetrameres anatis could be induced by the addition of gastric juice of grasshoppers. Within the grasshopper, Spathosternum prasiniferum, the juveniles of Tetrameres anatis reached the second and third stage on the third and fifth day respectively following infection. The third stage larvae encysted in the fat bodies and attained infective stage in 6 days at a room temperature

ranging from 25 to 30°c. In ducks, the juveniles of the parasite underwent the third and fourth moulting on the second day and nineth to tenth day respectively within the lumen of the proventriculus. The female juveniles just after the fourth moulting, entered into the glands of the proventriculus and attained full maturity in 24 days time following infection.

3. The pathology of Tetrameres anatis, Amidostomum skrjabini and Echinuria uncinata infections was studied by infecting 3 groups of parasite-free ducklings with 200 infective larvae of each species of worm.

All the 3 groups of ducklings (Amidostomum skrjabini group, Tetrameres anatis group, Echinuria uncinata group) showed dullness, weakness, incordination of movements, inappetence, emaciation and diarrhoea especially from the time the worms became sexually mature. A significant reduction in the weight gain, haemoglobin concentration, number of red blood corpuscles, packed cell volume, mean corpuscular haemoglobin and mean corpuscular volume and an increase in number of white blood corpuscles and erythrocytic sedimentation rate were noticed in all the birds infected with Tetrameres anatis, Amidostomum skrjabini and Epomidiostomum uncinatum. Microscopical examination of lesions produced by Tetrameres anatis revealed catarrhal inflammation, desquamation of glandular epithelium, pressure atrophy, focal areas of cellular infiltration, necrosis and ulceration of glandular structures of proventriculus.

Marked inflammation, haemorrhage underneath the horny lining, degeneration and desquamation of cells of superficial layer, focal areas of hyperkeratosis and erosion and diphtheretic ulcers on the mucous membrane of gizzard were evident in amidostomiasis.

Deep crateriform ulcers, moderate degree of fibrosis and heterophilic infiltration were noticed in epomidiostomiasis.

4. The anthelmintic efficacy of 9 drugs was evaluated in controlled trials against adult worms of Tetrameres anatis, Amidostomum skrjabini and Epomidiostomum uncinatum. Tetramisole hydrochloride at 50 mg/kg body weight, Parbendazole at 100 mg/kg, Thiabendazole at 200 mg/kg, Morantel tartrate at 40 mg/kg, Carbontetrachloride at 2 ml/kg, Phenothiazine at 500 mg/kg and Cashewnut shell oil at 10 g/kg were administered orally, while Disophenol at 10 mg/kg and Methyridine at 200 mg/kg were given as subcutaneous injections. All the drugs were well tolerated by the birds. The drug Rametin, which was tried against Tetrameres anatis was found to be toxic at a dosage of 200 mg/kg body weight as all the medicated birds died. All the treated and infected positive controls were sacrificed on the fifth day following the administration of drugs, to enumerate the parasites for assessing the percentage of efficacy. Tetramisole hydrochloride was found to be superior to all other drugs employed, with an anthelmintic efficacy of 79.23% against Tetrameres anatis, 82.15%

against Amidostomum skrjabini and 79.88% against Epomidiostomum uncinatum. The other drugs in order of efficacy against tetramerosis were Phenothiazine (70.36%), Carbontetrachloride (59.28%), Parbendazole (45.70%), Thiabendazole (41.0%), Methyridine (35.94%), Cashewnut shell oil (31.02%), Disophenol (28.87%) and Morantel tartrate (26.35%). Against Amidostomum skrjabini, Morantel tartrate came next in the order of efficacy with an elimination percentage of 74.8. The other drugs had the following percentage of efficacy: Parbendazole (67.29), Thiabendazole (63.93), Carbontetrachloride (61.06), Phenothiazine (52.42), Cashewnut shell oil (39.04), Methyridine (30.12) and Disophenol (20.07).

In the case of epomidiostomiasis also, Morantel tartrate was found to be the second choice with an anthelmintic efficacy of 65.68%. The percentage of efficacy of other drugs were as follows: Parbendazole (57.84), Carbontetrachloride (52.66), Phenothiazine (50.23), Thiabendazole (43.13), Methyridine (13.13), Cashewnut shell oil (12.66) and Disophenol (11.32).

5. In a cross infection trial, Ascaridia galli of fowl was successfully transmitted to a group of 10 ducklings of which 7 ducklings were destroyed for studying the comparative features of the developmental stages within the hosts. The remaining 3 birds became positive for infection in from 47 to 53 days while the prepatent period in chicken was only 35 days.

In general the larval stages and sexually mature worms from ducklings were comparatively smaller in size and lesser in number than those recovered from control chickens.

ABSTRACT

ABSTRACT

Studies on the incidence of nematode infections in 340 domestic ducks indicated that 67.94% of the birds harboured one or more species of the following nematodes viz., Amidostomum skrjabini, Capillaria contorta, Capillaria globocaudata, Echinuria uncinata, Epomidiostomum uncinatum, Eustrongylides papillosus, Strongyloides avium and Tetrameres anatis. The rate of infection was highest in the case of Epomidiostomum uncinatum (41.86%) and lowest in the case of Strongyloides avium (0.59%). The lifecycles of Echinuria uncinata and Tetrameres anatis were worked out in detail. It was established that Tetrameres anatis was distinct from Tetrameres fissispina. The complete details of the morphology of juveniles both within the intermediate and final hosts were given. The juveniles of Echinuria uncinata completed the first and second moultings on the third and fifth day respectively and the third stage juveniles reached infective stage on the seventh day following infection in Daphnia pulex at a room temperature ranging from 25 to 30°c. In ducklings the juveniles underwent the third and fourth moultings on the third and seventh day respectively and the eggs of the parasite were first found in the droppings on the thirty-third day. The juveniles of Tetrameres anatis completed first moulting on the third day, the second moulting on the fifth day and the third stage encysted in fat bodies of the grasshopper, Spathosternum prasiniferum. The third stage

juveniles attained infective stage on the sixth day following infection. Within the ducklings, the parasite underwent the third moulting on the third day. The fourth moulting in respect of males was seen on the nineth day and in the case of females on the tenth day. The prepatent period of the parasite was found to be 24 days. Development of the juveniles was also noticed in five other species of grass-hoppers viz., Oxya nitidula, Oedaleus abruptus, Conocephalus maculatus, Ducetia japonica and Atractomorpha crenulata. No development of Tetrameres anatis beyond the first stage was observed in Daphnia pulex and Daphnia magna on experimental infection.

Anaemic changes with significant reduction in weight gain and haematological values were observed in experimental infections of Tetrameres anatis, Amidostomum skrjabini and Epomidiostomum uncinatum. Histopathological changes like haemorrhage, desquamation of cells, catarrhal inflammation and necrosis were seen in experimental tetramerosis. Ulceration and atrophy were also evident in amidostomiasis and epomidiostomiasis.

The anthelmintic efficacy of 9 drugs against adult worms of Tetrameres anatis, Amidostomum skrjabini and Epomidiostomum uncinatum under experimental infections were determined. The efficacies against tetramerosis, amidostomiasis and epomidiostomiasis were 79.23%, 82.15% and 79.89% with Tetramisole hydrochloride at 50 mg/kg body weight; 70.36%,

52.42% and 50.23% with Phenothiazine at 500 mg/kg; 26.85%, 74.8% and 65.68% with Morantel tartrate at 40 mg/kg; 45.70%, 67.29% and 57.84% with Parbendazole at 100 mg/kg; 41.0%, 63.93% and 43.13% with Thiabendazole at 200 mg/kg; 59.28%, 61.06% and 52.66% with Carbontetrachloride at 2 ml/kg; 35.94%, 30.12% and 13.13% with Methyridine at 200 mg/kg; 28.87%, 20.17% and 11.32% with Disophenol at 10 mg/kg and 31.02%, 39.04% and 12.66% with Cashewnut shell oil at 10 g/kg body weight respectively. The drug Rametin at 200 mg/kg was found to be fatal to ducklings.

In a cross infection trial delayed development and lesser percentage of establishment of Ascaridia galli of fowl origin were observed in ducklings as compared to chicks.

REFERENCES

REFERENCES

Actor, P., Anderson, E.L., Dicuollo, C.J., Ferlauto, T.J., Hoover, J.R.E., Payano, J.F., Ravin, L.R., Scheidy, S.F., Stedman, R.J., and Theordorides, V.J. (1967). New broadspectrum anthelmintic, methyl 5 (6)-butyl-2-benzimidazole carbamate. Nature 215 : 321-322.

Akhmedova, S.I. (1954). (The influence of the host on the morphological and biological characters of some nematodes of birds). Trudy gelmint. Lab. 7 : 380-387*

Akhtar, S.A. (1936). Notes on helminth parasites from Afghanistan. Rec. Indian Mus. 38 (3) : 373-375.

Anantharaman, M. and Chandrasekharan Nair, K.P. (1955). Tetrameres infection of fowls in India. Zool. Anz. 154 (7/8) : 182-193.

Arambulo, P.V., Sarmiento, R.V., Borjal, A.C. and Hermandez, V.C. (1970). The incidence of Tetrameres fissispina in Philippine chickens with a redescription of the local species. Philipp. J. Sci. 97 (4) : 349-359.

Austin, F.G. and Welch, H.E. (1972). The occurrence, life-cycle and pathogenicity of Echinuria uncinata (Rudolphi, 1819) Soloviev, 1912 (Spirurida, Nematoda) in water fowl at Delta, Manitoba. Can. J. Zool. 50 (4) : 385-393.

Barber, L.B. (1915). Livestock disease investigation. Ann. Rep. Guam. Agr. Expt. Sta. 25 : 1915.*

Bhattacharjee, M.L. and Doss, D.D. (1967). Tetrameriasis in ducks in Assam. Indian Vet. J. 44 (1) : 81-82.

Bouneau, P. (1948). Observation de tetramerose dans un troupeau de canards traitement par la phenothiazine. Revue Med. vet. 99 : 275-277.*

Brown, H.D., Matzuk, A.R., Ilves, I.R., Peterson, L.H., Harris, S.A., Sareth, L.H., Egerton, J.R., Yakatis, J.J., Campbell, W.C. and Cuckler, A.C. (1961). Antiparasitic drugs IV, 2-(4 thiazolyl) benzimidazole, a new anthelmintic. J. Am. Chem. Soc. 83 : 1764-1765.

Cassamagnaghi, Jr. A. (1946). Amidostomum anseris en Anser anser domesticus. Boln mens. Dir. Ganad. Montev. 29 : 618-623.*

Chandrasekharan, K. (1967). Studies on the common nematodes encountered in domestic ducks. M.Sc. Thesis submitted to the University of Kerala.

Cornwell, R.L. and Jones, R.M. (1970). Controlled laboratory trials in sheep with anthelmintic Morantel. Br. vet. J. 126 : 142.

Cram, E.B. (1926). A parasitic nematode as the cause of loss among domestic geese. North Am. vet. J. 7 : 27-29.

Cram, E.B. (1931). Developmental stages of some nematodes of spiruroides parasitic in poultry and game birds. U.S. Dept. Agric. Techn. Bull. No. 227 : 1-27.

Cram, E.B. (1933). Observation on the life history of Tetrameres pattersoni. J. Parasit. 20 : 97.

Czaplinki, B. (1954). Incidence of Amidostomum anseris in geese of Poland. Acta. parasit. pol. 2 (7/14) : 275-298.

Czaplinki, B., Malczewski, A. and Swietlikowski, M. (1956). Amidostomum anseris: effect of weight gain. Wiad. parazyt. 2 (5) : 187-188.*

Czaplinki, B. (1962). Nematodes and acanthocephalans of domestic and wild anseriformes in Poland. Acta. parasit. pol. 10 : 125-319.

Deo, P.G. (1964). Round worms of poultry. Indian Council of Agricultural Research, New Delhi. 1st Ed. pp. 146.

Doroshko, I.N. (1948). (A new method of treating ducks with carbontetrachloride). Veterinariya. 25 (4) : 27-29.*

Dubey, J.P. (1964). On some helminth parasitic in Indian domestic ducks (Anas platyrhynchos domesticus). Indian J. Helminth. 16 (16) : 33-43.

Dubey, J.P. and Pande, B.P. (1965). On some of the helminthic lesions in the Indian domestic duck (Anas platyrhynchos domesticus). Indian J. vet. Sci. 35 (3) : 190-196.

Enigk, K. and Hazra, A.D. (1967). Zur Behandlung des Amidostomum - Befalles der Gans. Dt. tierarztl. Wschr. 74 (20) : 524-530.

Enigk, K. and Hazra, A.D. (1971). (Therapy of common nematode infections in domestic poultry). Dt. tierarztl. Wschr. 78 (6) : 178-181.

Federmann, M. (1962). (Maretin (O,O-diethyl-O (naphthaloximida) phosphate a new anthelmintic - Experimental studies in sheep and goat). Dt. tierarztl. Wschr. 71 : 62-67.*

Garkavi, B.L. (1949). (The lifecycle of development of Tetrameres fissispina parasite of domestic and wild duck). Dokl. Akad. Nauk. SSSR. 66 (6) : 1215-1218.

Garkavi, B.L. (1960). (Observations on the biology of Echinuria uncinata and epizootology of the infection in the Krasnodar territory). Tezisi. Dokl. nauch. knof. vses. obshch. Gelmit. 28-29.*

Garkavi, B.L. (1964). (Therapeutic experiments on domestic ducks experimentally infected with Tetrameres). Mater. nauch. knof. vses. obshch. Gelmit. (1) : 63-67.

Garkavi, B.L., and Luzhkov, A.D. (1972). (Trials of hygromycin B, piperazine dithiocarbamate and thiabendazole against experimental Tetrameres fissispina infection in ducks). Nauch. Trudy. Krasnodarskogo Nauchno Issledovatelskoi Veterinarnoi Stantsii. 45 (10) : 5391.*

George Varghese, C., David Jacob, P., Georgekutty, P.T. and Peter, C.T. (1971). Use of cashew (Anacardium occidentale) nut shell oil as an anthelmintic against ascaridiasis in the domestic fowl. Kerala J. vet. Sci. 2 (1) : 5-13.

Georgiev, B. (1962). (Epizootology and pathogenesis of amidostomiasis in geese in the Plovdiv area). Izvestiya. na. Tsentralniya Veterinaren Institute Za. Zavazni Parazitni. (4) : 171-184.*

Gerasimova, G.N. (1960). (Study of the pathology and morphology of Tetrameres and Polymorphes infection in ducks). Tezisi. Dokl. Nauch. knof. vses. obshch. Gelmit. 29-31.*

Guilhon, J., Baratsiotis, A., Jolivet, G. (1971). Experimental echinuriasis in ducks. Recl. Med. vet. 147 (1) : 1-21.

Hamann, O. (1893). Die Filarienseuche der Enten und der Zwischenwirt. von Filaria uncinata. Centrabl. Bacteriol. 14 : 555-557.*

Herman, C.M. and Wehr. E.E. (1953). Occurrence of Amidostomum in Canada geese. J. Parasit. 39 (Suppl.) : 34.

Jestard, A.C. (1936). The gizzard worm Amidostomum anseris of geese in Western Washington. J. Am. vet. med. Ass. 90 : 318-320.

Jestard, A.C. (1937). Further records of the gizzard worm Amidostomum anseris in the State of Washington. Report of cases in wild water fowls. J. Am. vet. med. Ass. 90 (6) : 785-786.

Joshi, S.C. and Kamalapur, S.K. (1971). Histopathological study of the lesions in Tetrameres mohtedai infection in domestic fowls. Indian vet. J. 48 (11) : 1116-1118.

Khaziev, G.Z., Samigullin, R.N., Ionov, M.P. and Sultanaeva, E.A. (1975). (Use of tetramisole granules for nematode infections of poultry - fowl and geese). Veterinariya Moscow 12 : 67-68.

Klimes, B. and Stejskal, J. (1968). Contribution to the problem of specificity of Ascaridia for hens, turkeys and ducks. Acta Univer. Agric. 37 : 221-226.

Kobulej, T. (1959). Uber die parasitische Phase der postembryonalen Entwick lung von Amidostomum anseris (Zeder, 1800). Acta vet. hung. 9 : 243-260.*

Kotelnikov, G.A. (1961). (Biology of Echinuria uncinata from ducks). Sbornik Nauchnotekhnicheskoi Informatsii Vsesoyuznigo Instituta Gelmintologii (7/8) : 30-33.

Kurochkin, Yu. V. (1954). (On the biological cycle of nematodes pathogenic for epomidiostomiasis of duck). Dokl. Akad. Nauk. SSSR. 98 (3) : 509-511.

Labatut, R.I., Camguilhem, R. and Cantau (1958). Tentative treatment of amidostomiasis and coccidiosis in geese. Rev. med. vet. 21 : 730-737.*

Lalitha, C.M. and Alwar, V.S. (1960). Parasites of domestic ducks (Anasboschas domesticus) in Madras. Indian vet. J. 37 (4) : 179-181.

Lapage, G. (1961). A list of parasitic protozoa, helminths and arthropods recorded from species of family Anatidae (ducks, geese and swan). Parasitology 51 : 1-609.

Litvishko, N.T. (1959). (Experimental treatment of ducks against Tetrameres infection). Veterinariya 36 (11): 29.

Maplestone, P.A. (1930). Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens. Rec. Indian Mus. 32 : 385-412.

McCraw, B.M. (1952). Gizzard worm (Amidostomum) in geese. Can. J. Comp. Med. 16 (9) : 342.

Misiura, M. (1970). Development of Echinuria uncinata (Rud, 1819) larvae (Nematoda) in Cladocera and Ostracoda. Acta parasit. pol. 17 (27) : 247-251.

Mohiuddin, G.H. and Lone, M.M. (1967). Incidence and control of endoparasites in ducks. Indian vet. J. 44 (6) : 493-495.

Mudaliar, S.V. and Alwar, V.S. (1947). A check list of parasite (Class Nematoda) in the department of Parasitology Madras Veterinary College Laboratory. Indian vet. J. 24 (2) : 77-94.

Naumenko, A.N. (1965). (Tetramerosis of ducks). Ptitsevodstvo 15 (11) : 30-31.

Negru, D., Marica, D., May, I. and Jurchita, I. (1960). Consideratii epizootiologice si terapeutice in amidostomoza gistelov. Probl. Parazitol. Vet. 8 : 46-59.*

Nery, J. and Dubois, R. (1960). Contribuicao as estudo do Tetrameres confusa, Travassos, 1917 na Bahia. An. Escola Med. Vet. Bahia. 42-46.*

Nickel, E.A. (1952). Ein Beitrag Zur wirksamkeit und Vertraglichkeit des Tetrachlorkohlenstoffes bei Magenwurmbefallder Ganse. Tierarztl. Umsch. 7 (19/20) : 382-384.*

Oliver, W.T. (1952). Amidostomiasis in domestic goose. Can. J. Comp. Med. 16 : 235-237.

Olteanu, G., Negru, D. and Coman, E. (1965). Amidostomosa la ratele domestice din bazinul inferior al prulului si nordul Dobrogei. Lucr. Inst. Cerc. vet. bioprep. Pasteur. 2 (2) : 257-269.

Ozerskaya, V.N. (1950). (Experiments with Phenothiazine against Amidostomum anseris in geese). Trudy vses. Inst. Gelmit. 4 : 136-137.

Pande, B.P., Rai, P. and Srivastava, J.S. (1960). A note on some pathogenic effects observed in certain nematode infection of wild aquatic birds with remarks on its significance. Poult. Sci. 39 : 1121-1125.

Petrov, Yu.E. and Mukhamedshin, R.A. (1969). (Single dehelminthisation of ducks infected with tetramerosis). Veterinariya Moskva. 48 (8) : 49-50.

Popov, N.P. (1953). (Pathological and morphological changes in the proventriculus of ducks caused by natural Tetrameres infection). Izdatelstvo Akademii nauk. SSSR. 547-551.*

Pumpr, V., Marjankova, K., Zajicek, J., Budejovice, C., Vondrika, K., Hradec, J. and Pisek, J.N. (1973). Overeni ucinku tetramizolu proti Amidostomum anseris v podminkach velkochovu. Veterinarstvi 23 (6) : 267-268.

Purohit, B.L., Sardeshpande, P.D. and Deodhar, N.S. (1964). Incidence of Tetrameres in poultry in Maharastra State. Bombay vet. Coll. Mag. 15 : 25-26.

Radin, I.D. (1959). (On the biology of Echinuria uncinata of aquatic birds). Sb. nauch. Rab. vet. Akad. 4 : 165-169.*

Romanova, N.P. (1938). (Biology of Echinuria uncinata, causative agent of the helminthic disease of the gizzards of aquatic birds). Sov. Ptitsov. 8 (9): 51-55.

Romanova, N.P. (1947). (A study on the development cycle of Echinuria uncinata Rud, 1819, a nematode parasitic of the stomach of Natatores). Dokl. Akad. Nauk. SSSR. 55 (4) : 371-372.

Romanova, N.P. (1948). (The lifecycle of Echinuria uncinata in aquatic birds). Trudy gelmit. Lab. 1 : 189-190.

Romanovski, A.B. (1969). (Tetrameres infection of duck - Treatment and prophylaxis). Veterinariya Mosk. 46 (12) : 48-49.

Schmid, F. (1943). (Die Magenwurmseuche der Ganse und ihne Bekampfung). Dt. tierarztl. Wschr. 51 (13/14): 122.*

Selivanova-Yartseva, A.S. (1955). (Phenothiazine as a phrophylactic for Amidostomum disease in geese). Veterinariya 32 (4) : 42.*

Shevtsov, A.A. and Zabello, I.M. (1965). (On studying epizootology and pathology of Tetrameres and Echinuria of ducks in the Ukraine). Visin. sil. hospod. Nauky. 8 (2) : 107-112.

Soulsby, E.J.L. (1965). Text Book of Veterinary Clinical Parasitology Vol. I. Helminths. Oxford, Blackwell Scientific Publications. pp. 1120.

Soulsby, E.J.L. (1968). Helminths, Arthropods and Protozoa of domestic animals (6th Ed. Monnig's Veterinary Helminthology and Entomology). The English Language Book Society and Bailliere, Tindall and Cassel Ltd. London. pp. 824.

Srilkov, A.I. (1963). (Daphnia as principle intermediate host of Echinuria uncinata in Chelyabink region). Mater. nauch. knof. vses. obshch. Gelmit. Part 2: 90-92.*

Srivastava, H.D. (1939). Studies on the helminth parasites of Indian poultry. Part III. The occurrence of two spirurid stomach worms in fowls. Indian J. vet. Sci. 9 : 225-227.

Srivastava, H.D. (1939b). The important helminth parasite of poultry and their treatment and control. Indian J. vet. Sci. 9 : 393-409.

Stoican, E., Margu, M., Pop, M. and Lepadatu, G. (1960). (Studies on the prophylactic anthelmintic effect of Phenothiazine on the treatment of parasitism in ducks and turkeys). Lucr. stiint. Int. Patol. Ig. anim. 30 : 1575.

Stoican, E., Paunescu, G. and Stoican, V. (1971). (Action of thiabendazole in amidostomiasis in geese). Lucr. Inst. Cerc. vet. biopren. Pasteur. 7 (1) : 275-283.

Sugimoto, M. and Nizhiyama, S. (1937). On the nematode Tropisurus fissispina (Diesing, 1961) and its transmission to chicken in Formosa. J. Jap. Soc. vet. Sci. 16: 305.

Sundaram, R.K. (1971). Studies on spirurids of fowl (Gallus gallus domesticus). Ph.D. Thesis submitted to University of Kerala.

Swales, W.E. (1936). Tetrameres crami Swales, 1933, a nematode parasite of ducks in Canada - Morphological and Biological studies. Can. J. Res. 14 (10): 151-164.

Swietlikowski, M., Zebrowskaplata, D. and Malczewski, A. (1969). (The need for amidostomiasis treatment of fattening geese). Medycyna wet. 26 (12) : 727-730.

Thienpont, D., Vanparijs, O.F.J., Raeymaekers, A.H.M., Vadenberk, J., Demoen, P.J.A., Allewiju, F.T.N., Marsboom, R.P.M., Niemegers, C.J.E., Schellenkens, K.H.L. and Janssen, P.A.J. (1966). Tetramisole (R. 9299) a new potent broad spectrum anthelmintic. Nature 209 : 1084-1086.

Timon-David, J. (1932). Leskystes a Tetrameres du pigeon - Annls Parasit. hum. Comp10(5) : 425-430.

Todd, A.C. (1947). Helminth infection in chicken from Tennessee. Poult. Sci. 26 : 469-474.

Tsvetaeva, N.P. (1960). (Lesions in the proventriculus of ducks experimentally infected with Tetrameres fissispina). Helminthologia. 2 (2) : 143-150.

Tubangui, M.A. (1926). Worm parasites of Philippine chickens. Philipp. agric. Rev. 19 : 1-43.*

Ullrich, K. (1932). Die Magenwurmseuchede Ganse. Prag. Arch. Tiermed. 12 (3) : 61-68.*

Visachi, V. (1961). Prilog poznavanju lokalizacije patoanatomskih promena kod amidostomioze. Vet. Glasn. 15 (12) : 1043-1044.*

Vorontsov, S.A. (1963). (Treatment of Tetrameres in ducks). Mater. nauch. knof. vses. obshch. Gelmit. Part I: 63-64.

Vsevolodov, B.P. (1938). Pathological anatomy and histology of amidostomiasis in geese. Trudy vses. Inst. Gelmit. 3 : 59-63.*

Walley, J.K. (1961). Promintic a new anthelmintic for sheep and cattle. Vet. Rec. 73 (1) : 20-21.

Willomitzer, J. and Gilka, F. (1959). Prispevek k vyskytu Llistice Tetramere fissispina u Kacheu. Sb. esl. Akad. Zemed. Ved. 30 (11) : 825-828.*

Walter, E. (1935). Beitrag Zur Biologie des Gansemagenwurms Amidostomum anseris (Zeder, 1800). Berlin Inaugural Dissertation. pp. 35.*

Wood, I.B., Pankavich, J.A., Wallace, W.S., Thorson, R.E., Burkhart, R.L. and Waletsky, E. (1961). Disophenol, an injectable anthelmintic for canine hook worms. J. Am. vet. med. Ass. 139 : 1101-1105.

Zabello, E.M. (1964). (Characteristic pathology of polymorphosis, tetramerosis and echinuriasis of ducks). Trudy vses knof. Patl. Anat. 210-214*

Zajicek, D. (1959). (Stomach worms in ducks). Sb. esl. Akad. Zemed. Ved. 4 : 133-140.

Zajicek, D. (1960). (Incidence and pathogenicity of Amidostomum sp. in domestic and wild aquatic birds). Sb. esl. Akad. Zemed. Ved. 5 : 775-788.*

Zajicek, D., Hybasek, J. and Pavlicek, J. (1973). (Anthelmintic efficacy of Nilverm - ICI - Pyrantel tartrate - SPOFA and Helatac - SKF - on nematodes of geese). Veterinarstvi 23 (6) : 269-271.

* Original not consulted

---oOo---

Popov, N.P. (1953). (Pathological and morphological changes in the proventriculus of ducks caused by natural Tetrameres infection). Izdatelstvo Akademii nauk. SSSR. 547-551.*

Pumpr, V., Marjankova, K., Zajicek, J., Budejovice, C., Vondrika, K., Hradec, J. and Pisek, J.N. (1973). Overeni ucinku tetramizolu proti Amidostomum anseris v podminkach velkochovu. Veterinarstvi 23 (6) : 267-268.

Purohit, B.L., Sardeshpande, P.D. and Deodhar, N.S. (1964). Incidence of Tetrameres in poultry in Maharastra State. Bombay vet. Coll. Mag. 15 : 25-26.

Radin, I.D. (1959). (On the biology of Echinuria uncinata of aquatic birds). Sb. nauch. Rab. vet. Akad. 4 : 165-169.*

Romanova, N.P. (1938). (Biology of Echinuria uncinata, causative agent of the helminthic disease of the gizzards of aquatic birds). Sov. Ptitsov. 8 (9): 51-55.

Romanova, N.P. (1947). (A study on the development cycle of Echinuria uncinata Rud, 1819, a nematode parasitic of the stomach of Natatores). Dokl. Akad. Nauk. SSSR. 55 (4) : 371-372.

Romanova, N.P. (1948). (The lifecycle of Echinuria uncinata in aquatic birds). Trudy gelmit. Lab. 1 : 189-190.

Romanovski, A.B. (1969). (Tetrameres infection of duck - Treatment and prophylaxis). Veterinariya Mosk. 46 (12) : 48-49.

Schmid, F. (1943). (Die Magenwurmseuche der Ganse und ihne Bekampfung). Dt. tierarztl. Wschr. 51 (13/14): 122.*

Selivanova-Yartseva, A.S. (1955). (Phenothiazine as a phrophylactic for Amidostomum disease in geese). Veterinariya 32 (4) : 42.*

Shevtsov, A.A. and Zabello, I.M. (1965). (On studying epizootology and pathology of Tetrameres and Echinuria of ducks in the Ukraine). Visin. sil. hospod. Nauky. 8 (2) : 107-112.

Soulsby, E.J.L. (1965). Text Book of Veterinary Clinical Parasitology Vol. I. Helminths. Oxford, Blackwell Scientific Publications. pp. 1120.

Soulsby, E.J.L. (1968). Helminths, Arthropods and Protozoa of domestic animals (6th Ed. Monnig's Veterinary Helminthology and Entomology). The English Language Book Society and Bailliere, Tindall and Cassel Ltd. London. pp. 824.

Srilkov, A.I. (1963). (Daphnia as principle intermediate host of Echinuria uncinata in Chelyabink region). Mater. nauch. knof. vses. obshch. Gelmit. Part 2: 90-92.*

Srivastava, H.D. (1939). Studies on the helminth parasites of Indian poultry. Part III. The occurrence of two spirurid stomach worms in fowls. Indian J. vet. Sci. 9 : 225-227.

Srivastava, H.D. (1939b). The important helminth parasite of poultry and their treatment and control. Indian J. vet. Sci. 9 : 393-409.

Stoican, E., Margu, M., Pop, M. and Lepadatu, G. (1960). (Studies on the prophylactic anthelmintic effect of Phenothiazine on the treatment of parasitism in ducks and turkeys). Lucr. stiint. Int. Patol. Ig. anim. 30 : 1575.

Stoican, E., Paunescu, G. and Stoican, V. (1971). (Action of thiabendazole in amidostomiasis in geese). Lucr. Inst. Cerc. vet. biopren. Pasteur. 7 (1) : 275-283.

Sugimoto, M. and Nizhiyama, S. (1937). On the nematode Tropisurus fissispina (Diesing, 1961) and its transmission to chicken in Formosa. J. Jap. Soc. vet. Sci. 16: 305.

Sundaram, R.K. (1971). Studies on spirurids of fowl (Gallus gallus domesticus). Ph.D. Thesis submitted to University of Kerala.

Swales, W.E. (1936). Tetrameres crami Swales, 1933, a nematode parasite of ducks in Canada - Morphological and Biological studies. Can. J. Res. 14 (10): 151-164.

Swietlikowski, M., Zebrowskaplata, D. and Malczewski, A. (1969). (The need for amidostomiasis treatment of fattening geese). Medycyna wet. 26 (12) : 727-730.

Thienpont, D., Vanparijs, O.F.J., Raeymaekers, A.H.M., Vadenberk, J., Demoen, P.J.A., Allewiju, F.T.N., Marsboom, R.P.M., Niemegers, C.J.E., Schellenkens, K.H.L. and Janssen, P.A.J. (1966). Tetramisole (R. 9299) a new potent broad spectrum anthelmintic. Nature 209 : 1084-1086.

Timon-David, J. (1932). Leskystes a Tetrameres du pigeon - Annls Parasit. hum. Comp10(5) : 425-430.

Todd, A.C. (1947). Helminth infection in chicken from Tennessee. Poult. Sci. 26 : 469-474.

Tsvetaeva, N.P. (1960). (Lesions in the proventriculus of ducks experimentally infected with Tetrameres fissispina). Helminthologia. 2 (2) : 143-150.

Tubangui, M.A. (1926). Worm parasites of Philippine chickens. Philipp. agric. Rev. 19 : 1-43.*

Ullrich, K. (1932). Die Magenwurmseuchede Ganse. Prag. Arch. Tiermed. 12 (3) : 61-68.*

Visachi, V. (1961). Prilog poznavanju lokalizacije patoanatomskih promena kod amidostomioze. Vet. Glasn. 15 (12) : 1043-1044.*

Vorontsov, S.A. (1963). (Treatment of Tetrameres in ducks). Mater. nauch. knof. vses. obshch. Gelmit. Part I: 63-64.

Vsevolodov, B.P. (1938). Pathological anatomy and histology of amidostomiasis in geese. Trudy vses. Inst. Gelmit. 3 : 59-63.*

Walley, J.K. (1961). Promintic a new anthelmintic for sheep and cattle. Vet. Rec. 73 (1) : 20-21.

Willomitzer, J. and Gilka, F. (1959). Prispevek k vyskytu Llistice Tetramere fissispina u Kacheu. Sb. esl. Akad. Zemed. Ved. 30 (11) : 825-828.*

Walter, E. (1935). Beitrag Zur Biologie des Gansemagenwurms Amidostomum anseris (Zeder, 1800). Berlin Inaugural Dissertation. pp. 35.*

Wood, I.B., Pankavich, J.A., Wallace, W.S., Thorson, R.E., Burkhart, R.L. and Waletsky, E. (1961). Disophenol, an injectable anthelmintic for canine hook worms. J. Am. vet. med. Ass. 139 : 1101-1105.

Zabello, E.M. (1964). (Characteristic pathology of polymorphosis, tetramerosis and echinuriasis of ducks). Trudy vses knof. Patl. Anat. 210-214*

Zajicek, D. (1959). (Stomach worms in ducks). Sb. esl. Akad. Zemed. Ved. 4 : 133-140.

Zajicek, D. (1960). (Incidence and pathogenicity of Amidostomum sp. in domestic and wild aquatic birds). Sb. esl. Akad. Zemed. Ved. 5 : 775-788.*

Zajicek, D., Hybasek, J. and Pavlicek, J. (1975). (Anthelmintic efficacy of Nilverm - ICI - Pyrantel tartrate - SPOFA and Helatac - SKF - on nematodes of geese). Veterinarstvi 23 (6) : 269-271.

* Original not consulted

---oOo---