NEMATODES OF BIRDS WITH SPECIAL REFERENCE TO TETRAMERES INFECTION IN DOMESTIC FOWLS

THESIS SUBMITTED TO THE
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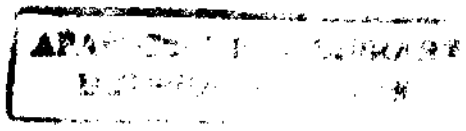

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CERTIFICATE

SRI K. RADHAKRISHNA REDDY has satisfactorily prosecuted the course of research and that the thesis entitled "NEMATODES OF BIRDS WITH SPECIAL REFERENCE TO TETRAMERES INFECTION IN DOMESTIC FOWLS" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

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CERTIFICATE

This is to certify that the thesis entitled "NEMATODES OF BIRDS WITH SPECIAL REFERENCE TO TETRAMERES INFECTION IN DOMESTIC FOWLS" submitted in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Veterinary Science of the Andhra Pradesh Agricultural University, Tirupati, is a record of the bonafide research work carried out by Sri K. Radhakrishna Reddy under my guidance and supervision the subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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ABSTRACT

Studies were undertaken to record the incidence of nematode parasites in poultry with special reference to Tetrameres mohtedai. Experiments were conducted to study the biological development and pathogenic effects of T. mohtedai. Experiments were also conducted to study the effects of irradiation, to standardise the dose of irradiation for optimum attenuation of T. mohtedai and to study the immunogeneeity to evolve immunoprophylactic measures to control the infection. Drug trials were also made to evolve a suitable chemotherapeutic agent.

A total of 6 nematodes was recorded from 3000 birds out of which 1000 were Desi birds. T. mehtedai was found in Desi birds alone with an incidence of 51 per cent. In captive zoo birds Capillaria sp. Ascaridia galli, Ascaris sp. were the common. In Myna Splendidofilaria osmaniae and in Magpie-blood microfilaria were the rare findings. An outbreak terminating in heavy mortality in pigeons due to Ascaridia columbae was recorded.

18 species of grasshoppers, and one species of beetle were found to be suitable intermediate hosts. Among the grasshoppers 6 species were found to be efficient intermediate hosts. 12 species of grasshoppers and the beetle were found to serve as intermediate hosts for the first time in India.

Infective larvae developed in the intermediate hosts in about 17 to 18 days and the pre-patient period in chick was found to be 45 to 60 days. The worms developed with difficulty in pigeon as the prepatent period was prolonged.

Histopathological changes included granulocytic reactions, lymphoid hyperplasia, plasma cell reaction and inter glandular oedema with evidence of pressure atrophy of the proventricular glands due to the female mature worms invading the proventricular glands.

The infective larvae irradiated at 2 to 8 Kr level did not show any conspicuous morphological changes. But at 10 Kr level the larvae was stunted in growth and degenerative changes took place in the gonads. Larvae irradiated beyond 10 Kr level did not develop at all.

The larvae irradiated at 10 Kr level did not produce marked pathogenecity but at the same time stimulated immune response undergoing full biological development.

Electrophoretic pattern of sera of the chicks infected with irradiated larvae of T. mehtedai at 10 Kr level showed that band No.9 was found to be conspicuous.

The circum larval precipitation test, revealed precipitates around oral, and anal region in the infected larvae incubated in the serum of chick infected with irradiated larva at 10 Kr level.

Intradermal test did not give any reliable results.

Infective larvae irradiated at 10 Kr level did not allow subsequent challenge infection to develop.

Among the anthelmintic drugs, caricide proved to be choicest showing 80 per cent efficacy.

Introduction

Andhra Pradesh has been recognised as one of the important poultry rearing areas in India. The rural and urban population have taken to poultry farming for over two decades to augment their family income. Millions of eggs are being exported to other states like Maharastra, Madras, Calcutta annually, in addition to the shipping to the middle east. Lack of protein in the diet of growing children has been recognised as responsible for mental retardation in them. This protein hunger can be adequately met by both poultry eggs and meat. This necessitated the Andhra Pradesh Government to constitute a poultry marketing board.

According to 1977 statistics (poultry guide), it is estimated that India possesses approximately 180 million poultry and ranks one among the World's fifteen leading countries in egg production accounting for 10,500 million eggs per year. Twenty years ago, the production of eggs was around 2,000 million. By 1981, it is expected to exceed 12,000 million eggs. Seventy per cent of the production of these eggs come from the genetically improved stock, indicating that much of the improvement had occurred in the commercial poultry sector.

Profitable poultry rearing calls for better husbandry measures including timely attention to the control

of diseases, while the losses due to viral and bacterial diseases are spectacular, the losses due to insidious diseases such as those caused by parasitic helminths are not apparently felt. The morbidity, loss in egg production or poor weight gain appear to account for greater losses than acute infections resulting in sudden mortality.

No systematic efforts had been made to determine the ill-effects due to commonly occurring parasitic helminths such as the Spirurid worms. The main reason why such studies had not been attempted is due to complicated life cycles, and the range of intermediate hosts involved.

Studies on life cycles conducted at Kerala, have thrown new light on the biology of all Spirurid worms frequently met within poultry so that it is now possible to critically determine the role of these parasites in assessing the losses to poultry industry. A detailed study on the pathogenecity of the parasites in the fowl would also help in the early diagnosis of this disease condition and to institute timely measures of control and the susceptibility of the worms to chemotherapy and prophylactic measures which are essential for formulation of proper control.

Effect of irradiation on the development and the immunogenecity of the larval forms will also help to keep the infection at a low level. Irradiated vaccines against certain parasites, are being marketed commercially in some countries while in India the irradiated lung worm vaccine in sheep is already in use.

Immunoprophylaxis may prove more economical than chemotherapy in the long run, as such the work on irradiated vaccines particularly against poultry worms should attract immediate attention.

In the present study attempts were made to survey (1) the incidence of nematodes affecting domestic fowls as well as other birds in Andhra Pradesh with special reference to the incidence of Tetrameres sp. (2) to study the host specificity of adult and larvae (3) to study the ability of different grasshoppers, and other arthropods, to act as intermediate hosts, and the dynamics of the infection on the domestic fowl (4) attempts were also made to evolve an irradiated larval vaccine against Tetrameres mohtedai in chicks and to assess host immune response (5) To study the efficacy of some of the commercially available anthelmintics against T. mohtedai on experimentally infected chicks.

Review of Literature

I. Incidence of *Tetrameres mehtedai*:

Srivastava (1939) studied the helminth parasites of poultry and reported on two spirurid nematodes. He was the first to record the occurrence of the genus Tetrameres.

Bhalerao and Krishna Rao (1944) mentioned *Acuaria* (*Cheilospirura*) *hamulosa*, *Acuaria* (*Dispharynx*) *spiralis* and *Tetrameres mehtedai* n. sp. from fowls. They described in detail the morphological characters of *T. mehtedai*, which they had received from Hyderabad.

Qureshi (1950), who studied in detail the incidence of helminth infections in desi fowls and improved stock in U.P. reported the percentage of *Ascaridia galli* infection was 50% in desi birds.

Dee (1964) mentioned that the life cycle of *T. mehtedai* was not known and described other nematode parasites of chicken, ducks, geese, guinea fowls, pigeons, pea-fowls etc.

Rao *et al*. (1972) studied the histopathology of spirurid nematodes in Sarus crane kept at Nangan Zoo in Orissa.

Chauhan et al. (1973) reported the common parasitic infections among zoo birds at Lucknow and Delhi zoos. They recorded only Ascarids, Capillarids commonly and Spirurids rarely.

Sastry et al. (1974) reported the incidence of various nematodes in fowls in Maharastra, though no Tetrameres was recorded.

Bhaskar Rao (1977) investigated into the incidence of helminth parasites of ducks in and around Kolleru lake in Andhra Pradesh. He encountered Echinuria uncinata (14%), Amidostomum skrjabini (79%), Capillaria contorta (10%), Capillaria gleboacudata (20%) and Tetrameres anatis (59%).

The earliest record of the genus Tetrameres in India was by Maplestone (1931), who obtained only a few male worms from a Pochard or Dunbird (Nyroca (Aythya) ferina) and also from an unidentified duck at the zoological gardens, Calcutta. He described the worm as Echinuria spinosa, Wher (1933) transferred the above species to the genus Tetrameres based on the presence of body spines and dorsal and ventral thickening on the lateral alae at the head end. The first report on the occurrence

of Tetrameres in fowl in India was made by Srivastava (1939). He subsequently reported that in a number of ducks received from Peshawar (now in Pakistan). Tetrameres were recorded but he did not furnish the description of the parasite encountered by him. He only stated that the worm conformed to Cram's description of Tetrameres fissispina. Worms obtained from a fowl which died at Hyderabad (Deccan), Bhalerao and Rao (1944) described Tetrameres mehtedai. Mudaliar and Alwar (1947) in the check list of parasites at the Department of Parasitology, Madras Veterinary College had listed Tetrameres spinosa, as occurring in fowls in Madras State.

Anantaraman and Nair (1955) recorded the occurrence of Tetrameres mehtedai in fowls at Srivilliputtur, Madras State and studied the pathology in detail. Koshy (1961) and Sriramulu (1961) had experimentally demonstrated grasshoppers, Atractomorpha crenulata and Pyrgomorpha sp. as intermediate hosts in Madras. Sundaram et al. (1963) reported that the species Tetrameres mehtedai was of common occurrence in desi birds of Kerala State and further they elucidated the life cycle of the parasite under laboratory conditions using grasshoppers as intermediate hosts. Purohit et al. (1965) from Maharastra and

Mukhopadhyay and Varma (1965) from Bihar reported the incidence of Tetrameres mehtedai. Gangadhar Rao and Anantaraman (1968-69) reported an incidence of Tetrameres mehtedai in fowls at Madras as 78.1%. They also recorded the presence of other nematodes like Ascaridia galli, Heterakis gallinarum, Acuŕia hamulosa. In Kerala 48.6% of the parasites recorded in fowls was Tetrameres mehtedai in addition to other nematodes like Ascaridia galli, Heterakis gallinarum, Acuŕia spiralis, Acuŕia hamulosa, Capillaria annulata, Syngamus trachea etc (Verghese and Peter, 1970). Similarly in Bihar there was high incidence of nematodes like Ascaridia galli, Heterakis gallinarum and Acuria sp. in fowls, but Tetrameres could not be found (Kumar and Sahai, 1975).

2. Experimental studies with Tetrameres mehtedai:

a) Host specificity

Koshy (1961) was the first to do experimental infection in grasshoppers and successfully infected Atractomorpha crenulata and Pyrgomorpha sp. as intermedia hosts.

Sundaram et al. (1963) had experimentally infected the grasshoppers Spathosternum prasiniferum, Oxya nitidula, Atractomorpha crenulata, Oedaleus abruptus, Conocephalus maculatus and Duestra japonica as intermediate host for

Tetrameres mohtedai. Subsequently Mukerjee and Sinha (1965) found that two species of beetles Listronus sinistus and Sphaeridium quinquemaculatus could be infected with eggs of Tetrameres mohtedai.

Sundaram (1971) attempted experimental infection in chickens, ducks and pigeons with Tetrameres mohtedai. He was successful only in chicken, but not in other birds used.

Lim (1975) found that a Lepidopteran larva (Setomorpha rutella) acted as intermediate host for Tetrameres mohtedai in Singapore.

Ramaswamy and Sundaram (1979) have reported the successful completion of the life cycle of T. mohtedai using the Isoped, Porcellio laevis as the experimental intermediate host.

b) Histopathology

Almost all the earlier works pertaining to the pathogenicity of T. mohtedai were based on the natural infections in fowls and more or less confined to the histopathology of the affected proventriculus (Anantaraman and Nair, 1955; Sriramulu, 1961; Purohit et al. (1965) and Joshi and Kamalapur, 1971).

Ramaswamy (1977) recorded the pathogenecity of Tetrameres mehtedai under controlled conditions. He observed that the gross pathological changes of the affected proventricululi included, petechical haemorrages in the mucous membrane on the 4th day post-infection, intense congestion with focal areas of erosion of the mucosa on the 12th day, dilated compound glands with immature female worms on the 25th day and greatly reduced lumen of the organ on the 55th day post-infection. The microscopic changes observed were severe granulocytic reaction on the 4th to 7th day post-infection, lymphoid hyperplasia from 9th to 12th day, plasma cell reaction on the 15th day, interglandular oedema and fibroplastic changes on the 18th to 25th day and pressure atrophy and cystic dilatation on the 32nd to 39th day post-infections. The reparative process found on the 55th day post-infection indicated the development of a host parasite balance.

The decrease in the live body weight and reduced rate of egg production seen in monospecific T. mehtedai infection therefore clearly indicate that the parasite was of economically important.

3. Immunological studies

a) Irradiated vaccines:

The principle involved in the use of radiation attenuated vaccine is now well appreciated. Like any other living organisms, helminths are also damaged by ionizing radiation. If infected stages are treated with a suitable dose of radiation and administered to the host, this damage is manifested by morphological and physiological disturbances in these helminths. A reduction in the number of parasites which develop to maturity and an impairment of their reproductive capacity are also noticed. The pathogenic effect of the parasite is usually reduced. An immunogenicity is however still exerted if sufficient number of parasites persist in the host for long. The magnitude of the changes outlined above is clearly related to the dose of radiation used and therefore the preparation of a vaccine depends on being able to choose a dose of radiation for the treatment of the infective stage which will reduce the pathogenic effect of the parasite to an acceptable level without significant reduction of immunogenicity. Radiation attenuated infective larvae had been successfully used as vaccines in the control of certain helmintic infections(Clegg and Smith, 1978).

Jarret et al. (1960) studied the immunological response produced by the administration of irradiated larvae of Dictyocaulus viviparus, subsequently Sokolic et al.(1965) reported successful vaccination with irradiated larvae of Dictyocaulus filaria.

Tewari et al. (1972) conducted studies on radiation attenuated Dictyocaulus filaria larvae in sheep in India. These workers claimed successful results against vaccination of yearling with the irradiated larvae of sheep lung worm D. filaria.

Doe and Gangadhar Rao (1969) conducted studies of chicken against Ascaridia galli.

Subramanian and Suresh Singh (1971) worked on immunological response in chicken against Ascaridia galli immunized with irradiated eggs.

Miller (1964, 1965, 1966, 1967) had made considerable efforts to evoke an irradiated vaccine against Ancylostomiasis in dogs and cats. The attenuated larvae was shown to develop an immune response of greater magnitude than to a normal larvae. Double dose of vaccine with one month

of age was found to be more effective. Prenatal infection of pups could be prevented by vaccination of bitches as early as possible.

Varga (1964, 1965, 1966, 1968) conducted extensive studies with irradiated larvae of Syngamus trachea to induce immunity against further infections with this gape worm infections in chicken.

Millar (1971) gave an extensive review regarding vaccination. After field trial Miller (1978) have given the difficulties in production and continuing the patronage this vaccine by the Veterinarians.

Gregg et al. (1976) studied the effect of gamma radiation on the development of infective larvae of Trichostrongylus colubriformis in guinea pigs and sheep. These authors try to explain the depressed egg production in females might be due to absence of males which are very radiosensitive to the gamma radiation.

Good protection of an adult sheep against experimental challenge Haemonchous contortus could be achieved by the administration of radiation attenuated larvae, however no practical vaccine could emerge from this work because of complete failure of immunising young lambs (Mulligan, 1975).

b) Host response

Clegg and Smith (1978) stated that all the helminthic infections induce specific antibody production which could easily be detected and measured by immunodiagnostic tests. Only rarely, the immune response lead to complete elimination of parasites. Several mechanism permitting parasite survive in the immunised host had been recognised including antigenic variation and disguise, production of soluble blocking antigens, location of parasites and inhibition of various host defence mechanism. Unlike bacterial and viral antigens helminthic antigens vary greatly during different stages of development of the parasite. Each parasite has mosaic of antigens. This is one of the important drawbacks in the production of good immunity against helminthic diseases. Antigens are not only shared among different helminth but also by unrelated species. This gives cross protection and also non-specific immune reactions in the field of immunodiagnosis. Antigens can be classified as particulate and soluble.

Particulate antigens:

Eggs, different larval stages of parasites and adult worms act as particulate antigens (Cohen and

Sadun, 1976). However, the antigenicity of such antigens varies greatly and produce varying degrees of protection in the host. In general, injection of homogenates of non-living parasites does not provoke any immunity. This has inhibited progress towards identifying the antigenic stimulus to immunity and the understanding of the stimulation of immunity has been confined to the living parasite. Irradiated third stage larvae acted as good antigen particularly in Dictyocaulus viviparous (Jarett et al., 1960) in cattle D. filaria (Mulligan, 1975) in sheep. Angylostoma caninum (Miller, 1965) in dogs and Haemonchus contortus (Urquhart et al., 1966) in sheep. Williams (1971) isolated 13 fractions of antigens from Angylostoma caninum.

Soluble antigens

Most of the helminth parasites contained release of many antigenic substances which evoke a variety of immunological response. Most of these responses are not deleterious to the worms which suggests that most nematode antigens which stimulate an antiworm response are termed as 'functional' antigens (Wakelin, 1978). Therefore is a clear evidence that functional antigens of most of the gastrointestinal nematodes come

from the glands associated with their excretion and secretion and they are probably enzymes (Ogilvie and Jones, 1973).

In Trichenella spiralis and Trichuris sp. antigens originate from the multicellular strichosome which comprises mostly of the anterior end of the worms and consists of many cells filled with a secretory granules (Wakelin, 1978). The source of antigens from Ascaris and filarid worms is considered to be from glandular oesophagus (Soulsby, 1975).

In vitro serological tests

Gel diffusion test:

This test was first introduced by Oudin (1946) which was later modified by Ouchterlony (1948). Its application in diagnosis of parasitic disease has been reviewed (Capron, 1970).

Immunodiffusion test has also been used for the identification of the soluble antigens for Haemonchus and to study the antigens in excretions and secretions of various parasites (Sommerville, 1957) and Rogers and Sommerville (1960). Immunodiffusion test has been used for the identification and characterisation of helminth

antigens (Kent, 1963) and in the study of Schistosomes (Capron *et al.*, 1968). The application of this test in the diagnostic of parasitic infection have been reviewed by Soulsby (1973).

In vitro antibody precipitation test with living parasities.

An *in vitro* antibody precipitation test was first described in Schistasomiasis (CHR test) by Vogel and Minning (1949) which was modified as an agglutination test using Cercaria of *Schistasoma mansoi* as an antigen (Liu and Bang, 1950). Senterfit (1953) modified this test as a meracidal immobilisation test in human Schistasomasis and Soulsby (1957) used this test with miracidia of *Fasciola hepatica* in sheep. Oliver-Gonsales (1954) first described the circum oval precipitation test with living Schistasome eggs and homologus serum from infected human or monkeys. Later the test was found to be species specific, precipitates appearing around the eggs of homologous serum Gonsales *et al.*, (1955a). On further studies it was demonstrated that this test is of value in the diagnosis to chronic Schistasomiasis but not in acute cases. It was also found to be useful in assessing the success of chemotheraphy in Schistasomiasis Gonsales *et al.*(1955b).

The application of these *in vitro* tests has been reviewed by Soulsby (1975).

Intradermal test:

The allergic manifestation with Ascaris antigen was demonstrated in guinea pigs several years ago (Sprent, 1949) later both early and delayed skin reaction were demonstrated in *Trichonella spirilis* infection in pigs (Soulsby, 1962) passive cutaneous anaphylaxis was also used for the demonstration of reagan like antibodies against helminthic parasites (Ogilvie, 1964). He has further demonstrated reagan type of antibodies in rats with intestinal parasites viz., *Nippostrongylus brasiliensis* where the skin reaction was seen only in natural infection but not by immunisation (Oglivie, 1967). In mice allergic response was seen gainst *Trichanella spiralis* infection but not with Schistasome (Saduan and Gore, 1970).

The application of intradermal test against parasitic disease has been reviewed by Soulsby (1975).

Drug trials:

Within recent years, a number of compounds have 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 antihelmintics against the common nematode of chicks with special reference to *Tetrameres mohtedai*.

The antihelmintic efficacy of Tetramisole (R 8299) was first studied by Thieupont *et al.* (1966) and since then the drug was extensively used in animals and birds. Zajicek *et al.* (1975) studied the efficacy of this drug in amidostomiasis of geese at different dose rates. Chandrasekharan (1977) tried this drug in the domestic ducks against *Tetrameres anatis* and found to be more effective.

Materials and Methods

Collection of worms

Worms collected from naturally infected domestic fowls (*Gallus gallus domesticus*) procured from different parts of Andhra Pradesh were the initial source for the nematode parasites. Thereafter, the worms were raised experimentally in 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, crop, proventriculus, gizzard, small intestines, large intestines, caecum and rectum were kept in a separate petridishes and examined separately. The oesophagus and crop after opening longitudinally, were stretched with fingers and examined against light for the presence of adult Capillaria sp. The mucous membrane of the oesophagus and crop was also teased under the steroscopic microscope for the recovery of Juveniles.

The external surface of proventriculus was examined for the presence of females of Tetrameres sp. Nodular swellings were teased with blunt needles and worms, if any, were collected in normal saline. Presence of females of 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 over the dark spots. The organ was then opened longitudinally and the mucous membrane scrapped with a blunt scalpel or with a slide to recover worms present deep in the lumen. Finally the proventriculus were squeezed repeatedly in small quantities of normal saline to express the male worms of Tetrameres and juvenile stages of the worm from the glandular lumina. The materials obtained by scrapping or squeezing were examined under the binocular dissection microscope for the recovery of parasites. In the case of experimental birds, after scraping the mucous membrane several longitudinal incisions were made on the serous surface of the proventriculus with a sharp razor blade, and the organ was squeezed and teased in normal saline for extracting the female worms.

The gizzard was partially split open by an incision connecting the duodenal and proventricular opening and the organ was everted inside out. The contents of the gut 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 steroscopic microscope.

The intestines 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 materials were sedimented in several changes of normal saline to obtain the worms free of debris.

The other birds such as ducks, pigeons, sparrows, parrots were procured from the local bazaar and birds naturally died at the zoo were obtained through the authorities of the zoo. All were examined as per procedure described above.

Examination of the 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 the specimens fixed in 70% alcohol as well as in 10% formalin. Finer details of morphology of specimens were studied from materials cleared in lactophenol. Juvenile stages collected from controls, 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 for after immobilising and straightened them by

applying gentle heat or with Lugol's iodine. Both adult and larval forms were preserved in 70% alcohol or 10% formalin.

To study the histopathological changes produced by different developmental stages of Tetrameres mohtedai in the proventriculous of the host (experimentally infected chicks), chicks were infected with known number of juveniles (either irradiated or normal sacrificed at different days). A portion of the proventricular glands were fixed in 10% formalin for histopathological studies.

II. Host specificity of the adult worm and infective larvae infection of host other than fowl

Ducks

Infection was given to ten, 17 day old Khaki Campbell ducklings with 100 to 200 infective larvae of Tetrameres mohtedai. The ducklings were sacrificed on 2nd, 4th, 12th, 15th, 17th, 24th, 30th, 40th, 50th and at 60th day after infection were killed. The proventriculie were examined for the Juveniles as well as adult worms.

Pigeons

Similarly ten young pigeons were infected with the infective larvae of Tetrameres mohtedai. The pigeons were

sacrificed after 20, 30, 40, 60, 70, 80, 90, 105 and 110 days post-infection and the worms if any were always recovered in the proventriculus.

Sparrows

Three house sparrows were experimentally infected with Tetrameres mohtedai obtained from the intermediate hosts.

Myna (Acridothorae distes)

Three Mynas were infected with infective larvae of Tetrameres mohtedai.

III. Intermediate hosts

a) Collection and rearing of grasshoppers:

Different varieties of grasshoppers were collected from Tirupati and Hyderabad regions from the land, fodder, vegetable gardens, paddy fields etc where the poultry were not frequently visiting. They were caught by means of net and tubed in a plastic container with holes in the lid. They were transferred into a cage (with complete wire netting)(Figs.1, 2 & Table I). Common varieties of garden grass was placed on the floor of the cages and also fresh water in a petridish was placed before transferring

the grasshoppers. Every day the grass and water were changed. The grasshoppers were identified by the Entomology Department of A.P. Agricultural University, Hyderabad.

b) Method of infecting the grasshoppers

The grasshopper is secured with the thumb and index finger so as to direct the ventral surface towards the operator, one fresh female worms of the Tetrameres mohtedai was fed with a means of forceps. Some of the grasshoppers readily took the worm without much difficulty.

c) Method of obtaining infective larvae from the grasshopper

It is observed that the infected grasshoppers died within one to four days post-infection. The surviving grasshoppers looked healthy without showing any symptoms. After 18 days the grasshoppers were caught, the wings and legs were separated, the heads were removed, the abdomen and the thoracic portions were opened in a straight line, the whole digestive tract is removed and kept in the normal saline solution mostly the infective larvae were seen mostly at the posterior portion of the gut. After half an hour, most of the larvae came out from the fat bodies. The bits of digestive tract, the

head, thorax and abdomen portions were kept in a tea stainer, then it is transferred to a petridish which contains normal saline, after sometime most of the larvae will migrate into the saline of the petridish.

The larvae were counted and transferred to fresh physiological saline solution and given to the chicks within two hours after the dissection of grasshoppers.

d) Other intermediate hosts

Other than grasshoppers, beetles, earth worms, garden millipeds, Cockroaches and house fly larvae and adult houseflies were fed with the eggs of Tetrameres mohtedai.

IV. Immunological studies

a) Method of irradiation of grasshoppers

The infective grasshoppers were kept in plastic tubes (Fig.3) with both the ends opened, they were closed with wooden lids. 5 to 6 large sized grasshoppers can be kept in each tube, they were irradiated with 2 Kr, 3 Kr, 4 Kr, 5 Kr, 6 Kr, 7 Kr, 8 Kr, 10 Kr, 12 Kr, 15 Kr, 20 Kr and 25 Kr with the cobalt 60 shine at the Genetics Department of the Osmania University, Hyderabad.

b) Method of identifying chicks

Picric acid or ink pad ink were applied to different parts of the chicks for easy identification (like head region, left wing, right wing, whole body etc).

c) Preparation of antigen

Adult female worms weighing 2-3 grams were defatted by grinding with 20 ml of chilled ether in a glass morter kept in ice. Ether soluble portion was removed with a pasture pipette. The residue was treated again with 10 ml of chilled ether and other soluble portion was removed and discorded. The remaining residue was ground with 5 ml of phosphate buffered saline (PBS, P.H 7.2). The mixture was centrifuged. The clean supernatant portion was removed and sediment was again ground with 3 ml of PBS and centrifuged to obtain a second supernatant portion which was added to the first. This pooled extract was stored at -20°C till used.

d) Immunization of Rabbit (Herbert, 1973)

Three rabbits weighing about 1.5 kg each was immunized with antigen prepared from the female Tetrameres mohtedai. Antigen-adjuvant mixture was made by mixing equal amount of antigen and Freund's adjuvant (0.5 ml

antigen + 0.5 ml of adjuvant). The mixture was injected intradermally on every 4th day and 4 such injections were given. A booster dose was given on 14th day of last injection and the animal was bled after 10th day. Serum was separated and stored at -20°C by adding Gentamycin.

POLYACRYLAMIDE GEL ELECTROPHORESIS

Apparatus - Pipettes, Test tubes, Test tube stands, Electrophoretic chamber, beakers, measuring cylinders etc.

Reagents - Acrylamide, Bis, Sucrose, Tris buffer, Ammonium per sulphate, Bromophenol blue and Amido Scwartz (black) stainer).

Procedure

Working solutions needed for the electrophoresis were prepared 1 ml of the small pore solution (7% acrylamide, 0.07% APS, 4.6% Tris, 0.184% Bis and 1 ml of ammonium per sulphate was pipetted out into a different test tube, these solutions were mixed and the tubes were filled with this solution in which the electrophoresis was carried at a thin water layer was layered on this and the tubes were left for solidification for 1 hour.

After soldification the water layer at the top was removed by means of a syring. The large pore solution (Acrylamide 1.25%, Bis 0.3125%, riboflavin 1%) was added into the electrophoresis tube. Again water was layered on the solution serum (sample) 0.5 ml was mixed with 0.5 ml of 40% sucrose and Bromophenol blue. Out of this 0.1 ml (115 μg) of the solution was taken and layered over the gel. Bromophenol blue helps in visualising the rapid separation of the proteins. The sucrose used to help in keeping the sample from getting diffused into the reservoir buffer. Tris buffer was layered on this, and the same was used in the electrophoretic chamber. The tubes were placed in an electrophoretic chamber and electric current of proper amperage and 200 volts was applied for 1½ hour (Electrophoresis was run from cathode to anode). After the Electrophoresis the tubes were removed from the electrophoretic chamber and the gel was removed from the tubes by injecting water slowly through the sides of the tube by means of a syringe. The gel comes out without breaking and the gel was stained for visibility with amido schwartz (0.5%). The proteins present in the gel staine blue with amido schwartz. The whole gel first turns blue and the excess of stain was removed by adding 7% acetic acid.

The presence of proteins in the gel was indicated by blue bands and rest of the gel was unstained.

Gel diffusion (Immunodiffusion test)

Procedure for Immunodiffusion test:

The procedure followed here was essentially that of Witter (1962) with slight modification, which was developed by Kumar and Mallick (1972) as given below:

Diffusion medium

Sodium chloride	-	8 g
Agar (Difco)	-	1 g
Distilled water	-	100 ml
Methiolate	-	1: 10,000

The medium was buffered at pH 7.2 and melted in a steamer and filtered through Whatsman No.1 filter paper and again steamed for 15 minutes.

Preparation of Gel plates

Melted agar in 3.5 ml quantities (Difco purified) was poured on a clean sterile 2" x 1½" glass slide and allowed to solidify, then kept in the refrigerator at 4°C placing the plates over a moistened absorbent cotton in a petridish for 24 hours to set the agar.

Five wells of 4 mm diameter, one central and four peripheral were punched in the medium by means of cork borer. The distance between the central well and the peripheral was 4 mm. The punched agar slab was removed by a section and the bottom of the wells sealed with a drop of molten agar.

Screening of sera samples employing immunodiffusion reaction:

The central well was charged with antigen and the peripheral wells were filled with hyperimmune serum raised in rabbits and also with normal rabbit serum as control.

The slides were kept in petridishes leaving water soaked layer of absorbent cotton and were incubated at 37°C for 5 days. Observations were made every 12 hours upto 5 days and if by that time no clear cut precipitation lines were detected, they were discorded.

Intradermal test (Delayed type hypersensitivity):

Test Procedure

0.1 ml of antigen was injected intradermally at the foot pad as well as at the wing region in certain birds. At an adjacent area the same amount of PBS was

also injected. Increase in thickness of the skin alongwith other gross pathological changes in the former was compared with the latter. The skin reaction appearing after 24 to 48 hours and persisted for few days are considered positive.

Circum larval precipitation test (CLPT)

Test Procedure

One drop of antigen suspension (100 larval) and 0.5 ml inactivated antiserum were mixed with an applicator stick in a cavity slide with a coverslip and sealed with paraffin, it was incubated in a moist chamber at 37°C and examined at 2 hours interval for 6 hours for the appearance of granular precipitates at the natural orifices of the larvae.

Chick Mash

	kg
Maize	38 parts
Jowar	10 "
Groundnut cake	27 "
Fish meal	7 "
Wheat bran	10 "
Molasses	5 "
Mineral mixture	2.5 "
Salt	0.5
Total:	100
and AB_2D_3 Vitablend	25 g
TM 50	10 g

Pathological studies

Histopathology:

To study the lesions produced in different stages, the birds were sacrificed and infected proventriculus was fixed in 10% formalin. The fixed tissues were processed, embedded in paraffin and sections of 4 to 5 microns thickness were cut and stained by haematoxylin and eosin.

Weight gain of birds

Weights of control and experimental groups were also recorded to note the effect of parasitism on the weight of the infected chicks. Birds were selected from the same hatch for this study. The chick mash was fed uniformly to all the chicks whether infected or controls.

Anthelmintic trials

The chicks were given medication in certain drugs orally and in one parentrally.

Eight drugs were tried viz., Heltac, Dewormies, Mendazole, Caricide, Ancylol, Banminth II, Tetramisole, Mintezol as per the body weight of the chicks.

The birds were sacrificed 1 to 5 days interval and studied the action of drugs on the worm. The worms recovered from the proventriculus were kept in the normal saline for their movement etc.

Results

A. INCIDENCE OF NEMATODES OF BIRDS IN INDIA

The present investigation was conducted from March 1978 to February 1981 (3 years) for the incidence of nematodes of birds in Andhra Pradesh. A total number of 3000 fowls comprising of 1000 desi birds, 2000 exotic birds were examined. Thirty captive birds died in the Zoo were also available for study and some rare nematodes were recorded in them.

The nematode parasites of fowl with their percentage incidence had been presented in Table I.

A total of six types of nematodes were recorded with an incidence varying from 1% to 51%. The highest incidence was found to be that of Tetrameres mohtedai in desi birds examined. This was followed by Ascaridia galli, the large round worm of poultry, accounting for 45 per cent, mostly in exotic birds, the incidence of caecal worm, Heterakis gallinarum was next in order to the extent of 42%, the other 3 nematodes, Acuria hamulosa, Acuria spiralis and Capillaria annulata had an incidence of 6%, 5.5% and 1% respectively.

The incidence of nematodes mentioned in the captive birds are summarized in Table II. The Myna

(Acridotheres tristes) harboured heart worms Splendidofilaria osmaniae, the worm load consisted of 2 males and 4 females in one Myna. The black partidge (Francolinus sp.) had Ascaridia sp. whereas Guinea fowl (Numida meleagris) and great horn bill (Buceros bicornis) both harboured Ascaridia galli infection. The most interesting finding was microfilariae in blood of Magpie (Cissa flavirostris) (Fig. 4). American Rhea (Rhea americana) had Capillaria sp. in the intestines. The author had some across an outbreak in the Ornithology Department of the University, and the main reason for mortality was observed to be due to heavy infection of Ascaridia columbae.

B. EXPERIMENTAL STUDIES AND BIOLOGY OF TETRAMERES MOHTEDAI

1. Intermediate host-specificity

In Andhra Pradesh, different types of grasshoppers were found to be susceptible and 18 species (Fig.2) were successfully infected and the relative larval burden was given in Table IV and also in Histogram IVa.

As indicated in the histogram, 6 species of grasshoppers developed more than 2000 larvae when one full adult gravid female was fed. The grasshoppers readily devoured the worm. When the saliva was collected from them

and a few eggs of T. mohtedai (Fig.5) were added and incubated for about half an hour, the larvae were found to hatch out from the eggs (Fig.6).

Another 8 species of grasshoppers harboured more than 1500 larvae were found to be seen when fed by one female T. mohtedai, while four other species had less than 5000 larvae developing in them. One single grasshopper which was fed with one gravid female a maximum of 2726 infective larvae were harvested after 18 days of infection.

It was observed that the grasshoppers infected had shown considerable mortality after they were fed with the females of T. mohtedai. The grasshoppers started dying from 1 to 3 days and approximately 20% of casualities were observed among young grasshoppers. Once they survived the critical period, 3 days after infection, they continued to live. The load of infective larval burden was observed to make the grasshoppers less active.

In addition to the 18 species of grasshoppers successfully infected, one species of beetle (Coccinella septempo-netuta), Cockroaches 2 species (Periplanata americana), Blatta orientalis, local earth worms, garden millipedes (Anoplodesmus tanjorious), houseflies (Musca vicina)and its

larvae and dung beetles were tried to find out whether they serve as intermediate host for Tetrameres mohtedai. All were found to be refractory except the beetle (C. septemponetuta). These larvae were fed to the experimental chicks and normal adults were recovered . The development in the larvae in the beetle as well as the development of adult worm in the chick were similar to those observed in case of grasshoppers (Table VI).

II. LIFE CYCLE STUDIES

The eggs of Tetrameres mohtedai measured 50-53x30-31 microns (Fig.5). The egg can be distinguished by the presence of a small knob at one pole of the egg shell containing larvae in them (Ovoviviparous).

The larvae reached infective stage in 17 to 18 days post-infection. The third stage larvae of T. mohtedai possess a rosette of 12 similar spines on the tail end. It was observed that all the 18 species of grasshoppers behaved almost similar in developing infective larvae in 17 to 18 days (L3). However, it was observed that beyond 18th day the larvae developed slight golden brown pigmentation in the gut region.

The location of the larvae was usually in the fat bodies of the infective host. The encysting larvae

preferred the fat bodies just under the tergal plates of the abdomen. They were also found in significant number in fat bodies under the sternal and pleural plates of the abdomen and in heavier infections in fat bodies over all parts of body including those found in the head. The infective larvae survived for nearly three months in the normal saline solution when stored at 4°C. The infective larvae measured on an average from 20.5 x 12-15 microns.

On the second day post-infection the third stage larvae was still found morphologically the same, except increase in length and on an average males measured 1.637 and females measured 1.920 microns. On the fourth day the 4th stage larvae had been recovered and apparently the moulting took place in the proventriculus itself. On the 12th day, late 4th stage larvae had been observed and significant morphological changes were not seen except in increase of length, the male gaining a length of 2.88 microns while the female measured 3.2 microns. Early 5th stage larvae were recovered after 12th day and this stage the migration took place and the females were tending to be globular and were located in the proventriculus glands while the males were still located in the lumen.

On 17th day the morphological changes were more conspicuous particularly in females. On 24th day, sexual maturity was observed in both male and females, they attained a longth of 5.48 and 4.78 millimeters respectively. The males became longer, whereas female did not growth long but starts becoming globular.

Observations on the 39th day indicated that the length of male and females were nearly equal (Table VI) and they stop growing. Embroynated eggs were observed in the uterus of the females and they looked blood red in colour. The teased out female worms after experimentally infected birds was shown in Fig.7. The incubation period was observed to vary from 45 to 60 days. The pateney of infection was observed more than an year.

III. DEFINITIVE HOST SPECIFICITY

A wide range of domestic and wild birds were tried to understand their susceptibility for T. mohtedai infection. 100 to 200 larvae were administered orally to 10 ducklings, 10 pigeons, 4 sparrows and 4 mynas. They were examined 3 days after the infection (Table V).

In ducks, when examined on 2nd and 4th day, there were few larvae in the proventriculus. In other ducklings that were destroyed after 4th day did not harbour any worms, either in the lumen of proventriculus or in the gland.

In sparrows, the larvae could not establish and in Myna the birds did not live longer and died four days after infection. A few larvae could be seen, still in infective stage (L3) in the lumen of the proventriculus.

Pigeons

In each pigeon 10 to 12 adult worms could be recovered (Fig.8). Large majority of them were only males, remaining in the lumen of the proventriculus. The worms took unusually long time to mature and also to become gravid. Cellular reaction in the gland was scanty except atrophy of the neighbouring glands (Fig.9).

IV. HISTOPATHOLOGICAL CHANGES

To study the changes produced by various developmental stages of T. mohtedai in the proventriculus, chicks were infected with 100 juveniles and were sacrificed on 4th, 7th, 9th, 12th, 15th, 25th, 30th, 40th and 60th days (corresponding to their developmental changes) post-infection.

Gross lesions

No appreciable changes were noticed grossely. However, when the organ was cut open the lumen was filled with copious mucous secretion. Numerous petechieal haemorrages were seen on the mucous membrane especially around

the glandular openings. Under steeomicroscope large number of juveniles were found embedded in the mucus in these areas.

The proventriculus was enlarged nearly one and half times the normal size and was hard. After 12th day and upto 25th day the proventricular wall was thickened with corresponding reduction of the lumen which contained straw coloured exudate. The mucous membrane was intensely congested with focal areas of erosin of the epithelial lining.

From 25th to 40th day, pin head sized black spots were observed on the serosal surface representing the glands infected with the female worms. Mucopurulent exudate was seen blocking the glandular opening and the infected glands were well raised above the surface of the mucosa.

From 50th day post-infection, the serous surface of the proventriculus appeared mottled and the size of the organ was larger than the normal. The lumen of the organ when cut opened was found to be very much reduced due to the thickened wall. The glandular openings were very prominant in the mucosa. Cheesy semisolid exudate along with engorged female worms were observed within the compound glands.

Microscopic changes

The microscopic changes observed were severe granulocytic reaction. On the 4th to 7th day post-infection, lymphoid hyperplasia from 9th to 12th day, plasma cell reaction on the 15th day, intraglandular oedema and fibroplastic changes on 20th to 25th days and pressure atrophy and cystic dialatation on the 30th to 40th day post-infections. The reperative process was observed from 55th to 60th day post-infection. In some glands the adults completely replaced the glandular epithelium. Red blood corpuscles were seen in the cut sections of the female worms (Fig.10).

C) IMMUNOLOGICAL STUDIES ON TETRAMERES MOHTEDAI

1) Effects of irradiation on the infective larvae:

The grasshoppers which carried the infective larvae of T. mohtedai were exposed at different radiation dose levels from 2 Kr to 25 Kr (2, 3, 4, 5, 6, 8, 10, 12, 15, 20 and 25 Kr).

At 2 to 6 Kr levels nearly normal pathogenecity was observed and the larvae reached sexual maturity. When they were challenged with non-irradiated infective larvae the challenged worm, developed along those with earlier infection and no appreciable changes were noticed.

At 10 Kr level the larvae developed and the worms nearly grew, but the reproductive organs were not functional (Fig.11) but on challenge with non-irradiated larvae, the challenged infective larvae could not establish in the host.

From 12 Kr and above doses the worms did not develop in the host.

The worms developing from the irradiated larvae were found to be stunted in growth and functionally sterile at 10 Kr level. In the male worm, effect of irradiation was conspicuous in that the size of the genital organs were not normal(Table VII & Fig.12).

The developing male worms from the larvae measured 4.1 to 4.88 mm (average 4.37 mm) long and 0.1 mm in maximum width. The female worms measured on an average 3.3 mm long and 1.68 mm in maximum width. The normal male worms resulting from the non-irradiated larvae measured on an average 5.48 mm long with maximum width of 0.15 mm. The normal female worms developing from the non-irradiated larvae measured 4.77 mm long and 1.98 mm in maximum width. In addition, there were morphological abnormalities in their reproductive organs and failure of normal development of eggs as well as the uterine coils (Fig.13) while it is normal in the non-irradiated female worms.

ii) Host response to irradiated larvae:

The irradiated larvae at 10 Kr level failed to bring about the normal pathogenicity of the parasite. However, some of them underwent full life cycle and stimulated immune response and the production of antibodies in the host. This was markedly evident by detecting the presence of precipitates near the mouth and anus of the infective larvae.

Clinical manifestations were evident in experimental infections. These included emaciation, loss of body weight, droopiness, weak and unthriftiness followed by poor feed intake and anemic appearance. But in birds receiving larvae irradiated at 10 Kr level did not show these symptoms vividly.

Histopathological observations revealed that the larvae irradiated at 10 Kr induced local tissue reactions characterised by focal aggregation of lymphoid cells (Figs.14,15 & 16) in the mucosa.

There were certain physiological changes observed by the help of ideograms of Electrophoretic patterns of the sera from chicks infected with irradiated larvae of T. mohtedai as shown in Figs.17 and 18. The total number of bands in each serum sample varied from 4 to 6.

Ideogrammes were made use of in differentiating between the bands common to all the serum samples as well as those proteins that were specific to a particular shape (Table VIII & IX). From the table it is evident that band numbers 2, 3 and 12 are common to 7 samples and these can be considered as the most common bands.

Protein band No.4 and No.14 were observed to be common to 5 and 4 samples respectively. Band No.9 was found to be specific for serum sample irradiated at 10 Kr.

From the electrophoretic pattern it was clear that most of the bands were common to various serum samples and the sample treated at 10 Kr alone had the unique band No.9 lacking in rest of the sample.

On the basis of the number of protein bands observed in a sample and the number of bands it had in common with other sample, a per cent protein similarity was calculated using Ellison's (1962) paired affinity test. From the paired affinity values (P.A.values), it was observed that no significant variations existed among the sera with regard to the distribution of protein bands. However, it was observed that band No.9 specific to the serum sample irradiated at 10 Kr had a significant role

in explaining the protective abilities observed in chickens infected with larvae irradiated at 10 Kr.

The minor variations in the electrophoretic patterns reported here might represent the possible physiological changes caused by the invading larvae.

It had been reported that a serum factor is required for the parasite to bind to the host cells. Thus the sera might show both qualitative as well as quantitative variations in view of the fact the parasites utilised some of these proteins during the course of adherence and replication.

Bands specific to some of the sample had also been observed. Since these protein bands had not been characterised using known proteins like albumin, globulin etc, it is difficult to explain the chemical nature of the antigens.

A maximum P.A value of 50% was observed among these samples, which indicated significant qualitative as well as quantitative variations caused by the parasite.

It will be interesting to carry out further studies using high resolution SDS (Sodium Dodecyle Sulphate), Polyacrylamide gel electrophoresis to confirm the extent of variations reported here as well as to characterise the various protein bands.

The purpose of these experiments were to investigate the possible physiological changes induced by the parasite in the host which may be helpful for diagnostic purposes.

III. IMMUNODIAGNOSTIC STUDIES

1. Hyper immunisation of rabbits and raising anti sera

a) Agar gel double diffusion test

Three precipitation lines appeared, which were considered to be specific as no lines appeared with normal rabbit serum. Faint lines were seen by 24 hours and by 60 hours they were very sharp and clear. All the three were identical lines (Fig.19). The precipitate lines were observed nearer to antigen well.

b) Circum larval precipitation test (CLPT):

This test was employed to demonstrate that the larvae had antigenic fractions which reacted against the antibodies produced in the experimental chicks. The presence of granular precipitates were seen at the natural orfices of the larvae from 2 hours onwards till 6 hours. When examined on the next day it was seen that the granular precipitations were loosened and floating in the serum. No precipitates were detected in the sera from healthy birds.

c) Intradermal test (Delayed type hypersensitivity):

Though the test was sensitive but no reaction could be seen. Hence antigen improvement perhaps was needed. The observations were not consistent and hence results obtained were inconclusive.

IV. IMMUNOPROPHYLACTIC STUDIES

Immunoprophylactic studies included vaccination with irradiated larvae (10 Kr) and it was observed when 10 Kr irradiated larvae were vaccinated, there was significant decrease in worm burden when challenged with double the number of normal larvae. The resultant worm burdens were examined and it was interesting to note that no worms developed in birds given larvae irradiated at 10 Kr and subsequently challenged 25 days later with 200 larvae.

In an other experiment 21 days old chicks were given 100 infective larvae irradiated at 10 Kr and challenged with 200 normal larvae. On the 6th day of challenge, 3 birds were sacrificed and no worms could be detected. Subsequently on 15th, 25th, 39th and 45th day the remaining birds were destroyed similarly and no worms could be detected.

In a third experiment 21 day old chicks were given normal infective larvae and subsequently challenged with

200 larvae. On challenge the birds were sacrificed at regular intervals and the results were compared with earlier experiment. They showed significantly higher worm burden as compared to respective replicate vaccinated with the irradiated larvae and then challenged with normal. The proventriculus was heavily infected with the worms.

At irradiation level of 10 Kr only sterile female population developed. The worms developed from the irradiated larvae were stunted in growth, shrunken in appearance and exhibited, morphological abnormalities. The reproductive organs of female worms were not properly developed as described earlier.

Vaccination and subsequent challenge worms were distinguished on the basis of incomplete development and immature nature of the worm from the challenge infection respectively.

In 10 Kr infection and challenge, it was observed that there was considerable changes viz., hyperplasia of the surface epithelium and patchy squaamous metaplasia along with moderate and diffuse lymphocytic infiltration, cut section of the deeper glands were surrounded by diffuse lymphocytic infiltration (Fig.20). Development of challenged worms were not noticed. These changes were

clearly suggestive that the previous infection with larvae irradiated at 10 Kr, prevented the development of the challenged worms.

d) Antihelmintic trials on experimental infection of Tetrameres mohtedai:

To study the antihelmintic efficacy on the T. mohtedai and experimental chicks the following eight antihelmintics were tried and their results have been summarised in the Tables X and XI.

Heltac (Parabendazole), Dewormies, Mendazole, Caricide, Ancylol, Banminth II, Tetramisole (Decaris) and Mintezol (Thiabendazole).

Heltac:

This was tried at a dose rate of 30 mg per kg body weight, administered orally 20 days after the infection. The dosed birds were destroyed 4 days after the medication. All the male worms were expelled, however, the females were not affected except that they became slightly brownish.

Dewormis:

This antihelminthic was tried at a dose rate of 0.75 mg per kg body weight. The birds were destroyed 4 days after medication. There was no appreciable action of this drug either on females or males.

Ancylol:

The drug was tried at a dose rate of 0.22 ml/kg body weight, after 2 days the female worms could not be detected, but the drug had no effect on the males. At higher doses the drug was found to be toxic to the birds.

Mendazole:

Mendazole was used at 50 mg/kg body weight as well as 75 mg/kg body weight orally at 20, 40, 41 days after infection. It was found that the drug had no effect on *T. mohtedai*.

Tetramisole:

The drug was tried at a dose rate of 50 mg/kg body weight orally after 20 days after infection. When they were killed after 4 days after treatment, no worms could be detected in the proventriculus.

Once again the drug was tried with a dose rate of 50 mg/kg body weight in birds infected 37 days earlier. No worms were seen when the birds were sacrificed 2 days after dosing. When the birds were treated at a dose rate of 100 mg/kg body weight orally and sacrificed on the next day no male worms could be detected, but female worms were observed.

When caricide was tried at a dose rate of 100 mg/kg body weight 41 days after infection, male and female worms were dull, when sacrificed 5 days after medication.

Banminth II:

The drug was given at a dose rate of 40 mg/kg, 50 mg/kg and 200 mg/kg body weights were tried after 33rd day, 40th day and 41 days after infection. The birds were sacrificed on 2nd, 2nd and 5th day after giving the medication. The worms were found to be dull and inactive.

Mintezol :

Mintezol was tried at a dose rate of 200 mg/kg body weight orally, showed no effect on the worms, when the birds were sacrificed 2 days after infection.

Sexual maturity and Egg laying:

The age at first egg was taken as the time of attainment of sexual maturity. The experimental groups showed delay in attainment of sexual maturity compared to the controls which laid its first egg on the 145th day whereas in infected group they took 170 to 180 days for laying first egg.

The rate of egg production was very poor with lack of persistency and long phase. During the observed period of 90 days, the total number of eggs produced by the affected birds was on an average 41 eggs whereas the egg production by the control birds was 80.

Thus the maintenance of Tetrameres infected birds throughout the laying birds may result in considerable loss of income by reduction in the egg yield.Further it is casually observed during the study, the affected birds were unthrifty and had poor feed efficiency though no data was collected on this aspect. However, the effect of Tetramers infection on weight gain and feed efficiency has already been reported by Ramaswamy (1977) and these observation in confirmation by the earlier findings.

By vaccination, lesser egg production by the worms reduce contamination of the soil in limiting the spread of disease.

Discussion

A study of the incidence in nematodes of fowls conducted in Andhra Pradesh, six species were encountered. Out of the 3000 birds examined, 1000 were desi birds. The desi birds showed extraordinary susceptibility (51%) to T. mohtedai. These observations corresponded with those of Rao and Anantaraman (1969) who recorded higher incidence (78.18%) of this nematode. Varghese and Peter (1970) reported an incidence of 42.6% in Kerala. However, Joshi and Kamalapur (1968) encountered only 1.10% of the fowls in Madhya Pradesh. Only the last observation is not in agreement with the present study.

The incidence of Ascaridia galli varied from 28.09% in Madras to 80% in Bihar while Heterakis gallinae had an uniform incidence of 48.18% at Madras, 52.8% at Maharastra and 42% during the present study. However, Bihar had the highest incidence of 86%. In Kerala State the maximum number of nematodes of poultry were recorded with as many as 12 species. Varghese and Peter (1970) recorded rare nematodes like Syngamus trachea, Oxyspirura mansoni, Gangylonema ingluvicola, Capillaria annulata, Capillaria anatis, Strongyloides avium which had not been recorded in Andhra Pradesh, Madras or Bihar.

In so far as the nematodes encountered among the zoo birds in the country are concerned, not many investigations had been conducted except a few (Patnaik et al., 1980; Chauhan, et al.,1972).

The interesting feature in the present study was the occurrence of Splendidofilaria osmaniae in Myna (Acridotheres tristes) and microfilariae in the blood of magpie (Cissa fivirostris).

As far as the author could go through the published records, microfilariae in the blood of in wild and captive birds had not been recorded in India, except the microfilariae of Bhalfilaria badami in domestic fowl by Bhalerao and Rao in 1944 from Hyderabad.

Tetrameres mohtedai was first described by Bhale Rao and Rao (1944) and subsequently, Anantaraman and Nair (1955) made a critical study of T. mohtedai at Madras and described the pathology in detail. The life cycle of this parasite was not known at that time, but these authors had anticipated grasshoppers, cockroches and earthworms to be the intermediate hosts. Koshy (1961) successfully infected the grasshoppers Atractomorpha crenulata and Pyragomorpha sp. However, he could not raise the adults in fowl. Sundaram et al. (1963) had experimentally infected

six species of grasshoppers as intermediate hosts for T. mohtedai. Subsequently Mukherjee and Sinha (1965) infected two species of beetles (Liatongus cinctus and Sphaeridium quiquemaculatus) with eggs of T. mohtedai. Lim (1975) found that a Lepidopteran viz., Setomorpha rutiella acted as an intermediate host for T. mohtedai in Singapore. Ramaswamy and Sundaram (1979) successfully completed the life cycle of T. mohtedai by using the Isopod viz., Porocello livis (Table III).

Out of these 18 species of grasshoppers infected in the present study 12 species had been experimentally infected for the first time in India with T. mohtedai. One species of beetle Coccinella septempunctuta were shown to serve as an intermediate host apart from 12 species of grasshoppers which were infected for the first time in India. Among the 18 species of intermediate hosts, susceptibility to T. mohtedai varied considerably. Six of the grasshoppers as described in Table IV and histogram IVa were found highly susceptible harbouring 2000 and over larvae when fed with a single gravid female. 8 species of grasshoppers had slightly lower susceptibility and harboured over 1500 larvae while 4 species of grasshoppers had shown some resistance and had low susceptibility having only 1000 larvae.

The local earthworms, cockroaches, dungbeetles, houseflies and its larvae, garden millipedes could not be infected. A gravid female worm fed to the grasshopper Oxy nitidula resulted in the production of 2726 larvae during the present study while Ramaswamy (1977) recorded the maximum number of over 4000 infective larvae in a single grasshopper. He, however, did not mention how many eggs were fed to the grasshopper (Table IV).

From these observations, it is apparent that there are several susceptible grasshoppers serving as intermediate hosts to T. mohtedai. In view of this, the birds growing in free range system have more chances of acquiring T. mohtedai infection. This might probably account for high incidence of infection in desi birds which are reared in the backyard with full free range facility.

The larvae reached infective stage in 17 to 18 days in the grasshoppers and the older larvae developed golden brownish pigmentation in the gut region. Sundaram et al. (1963) and Ramaswamy (1977) made such similar observation but did not notice the pigmentation developed in the aged larvae. The prepatent period was observed to be from 45 to 60 days. The patency of infection was recorded to last more than an year.

The infective larvae survived for nearly 3 months at 4°C when stored in normal saline. There was no record of investigation of the survival time and hence, the present data could not be compared with any other published work. The male and females attained equal lengths in juveniles obtained around 39th day and subsequently the males grew longer while the females tended to be globular.

To study the definitive-host susceptibility, ducks, pigeons, sparrows and Myna had been infected with 100 to 200 larvae each. The pigeons developed the adult worms, but the prepatent period was longer when compared with the chicken. There were more males than females. The histopathology revealed minimum amount of host reaction and the worms were easily coming out of the proventricular glands unlike those in the fowl. All the males encountered were in the lumen of the proventriculus. The results could not be compared with those of Sundaram (1971) and Ramaswamy (1977) since both these authors could not infect the pigeons. However, the establishment of large number of males contrary to those in fowls could not be explained. The high male/female ratio probably indicates that the pigeon is not a suitable host for the ready propagation of the species.

In case of ducks 3 day old juveniles were obtained but in those ducks that were autopsied later, the infection was not detected. Obviously, the fourth stage larvae could not establish themselves. This observation was in agreement with that of Sundaram (1971).

Attempts to infect Myna for the first time could not be continued beyond 4 days due to heavy mortality. All the four experimental birds died on the 4th day. But there was evidence of the larvae trying to establish themselves since few jumeniles could be collected from the proventriculus.

Sparrows however did not take up the infection.

In experimentally infected birds, proventriculus was enlarged. Between 12th and 25th day there was thickening of the proventricular wall with corresponding reduction of the lumen. Histopathological changes included granulocytic reactions, lymphoid hyperplasia followed by plasma cell reaction, inter glandular oedema and fibroplastic changes. There was evidence of pressure atrophy. The reparative processes, were seen much later almost 8 weeks post-infection. Antaraman and Nair (1955) observed in natural infections, thickening of the proventriculus with corresponding reduction of the lumen,

haemorrhagic petichae, there were enlargement of proventricular glands to the extent of 2 or 3 times the normal size. There was glandular atrophy with complete absence of secretions. The present observations also corresponded with those of Ramaswamy (1977) who noticed granulocytic changes in the early stages of infection followed by lymphoid hyperplasia and later plasma cell reaction and glandular atrophy and presence of red blood corpusles in the cut sections of the female worm.

The present observations were also similar to those of Anantaraman and Nair (1955) with regard to male worms. The male worms merely remained in the lumen of the proventriculus and did not provoke much host response inspite of two rows of spines on the cuticle.

The larvae irradiated at lower doses from 2 Kr to 8 Kr established themselves well and did not show any apparent morphological differences. However, at 10 Kr level considerable changes were observed and the worms developing from them were predominantly females and were also stunted. The females were mostly sterile and there was considerable reduction in number of worms. The observations of Varga (1968) lend support to the present findings that a poultry nematode Syngamus trachea behaved similarly when its larvae were irradiated

at 1 to 3 Kr level with undeveloped state of reproductive organs. Similar observations on stunted growth of irradiated larvae were made by Miller (1964) in respect of Ancylostoma caninum and Sokolic (1963) in the case of Dictyocaulus filaria. Cuticular thickening of the vulva, Vagina and atrophy of the uterus were also observed by Ansari and Singh (1978) in case of Gaigeria pachyscelis. Gibbs et al. (1961) found similar observations in the tissue nematode T. spiralis in rats. The larvae exposed to 9 Kr exhibited appreciable immaturity and inhibition of the reproductive potential resulting in inability of its offspring to invade the host tissues.

Gould et al. (1957) in the case Trichinella spiralis found irradiated with 60_{Co} and x-rays brought about changes in morphology and reproduction. The pathogenic effects of the parasite was usually reduced but immunogenic effect still exerted if a sufficient number of parasites persisted in the host for long enough at the appropriate stages of development (Mulligan, 1975).

The larvae of T. mohtedai irradiated at 10 Kr did not bring about normal pathogenicity as observed in experimental infections. At 10 Kr level although the adult nematodes developed in the proventricular glands, there were degenerative changes in the reproductive

organs and the eggs were poorly developed. With higher doses of irradiation there was complete absence of worms. Therefore 10 Kr dose was considered to be the optimum dose wherein although the parasites developed, their reproductive capacity was damaged. The tissue reaction characterised by focal aggregation of lymphoid cells in the mucosa and the deeper glands in 10 Kr infection suggested local immune response.

The larvae damaged by radiation may remain in the tissues without normal migration and may stay a longer period of time and stimulate local immunity. Similar findings were recorded by Alicata (1951) and Gould et al. (1957) in case of T. spiralis and by Clordia and Bizzell(1960) in case of T. axei and Ansari & Singh (1978) in case of Gaigeria pachyscelis.

Immunodiagnosis studies on Tetrameres infection has been attempted and three tests were planned ether extract of ground female Tetrameres mohtedai was used as antigen from the immunization of rabbits. The test was standardised with antigera raised against rabbits and agar-gel double diffusion (Ouchterlony, 1948) was conducted. Within 24 hours there were 3 faint lines which become sharp later by 60 hours. The sera from the experimental infected birds were used to detect antibodies with an antigen described above although the rabbit sera produced

precipitin lines, the sera from the experimental infected birds did not give any precipitin lines. The usefulness of gel diffusion test as a tool to analyse the various antigens of the parasite has been discussed in detail though it has the limitation of requiring higher antibody content and to achieve this repeated injection were needed (Kagan, 1967). This point has been reemphasize by Solubsy (1973) who has stated that to obtain satisfactory precipitation sera, it was often necessary to immunize animals repeatedly with antigens since, the level of precipitating antibodies in natural infections were of low order. Thus it is presumed that the concentration of antibody were quite low in experimental birds and hence did not react. Further concentration of antibodies was contemplated to understand the immunological response due to this parasitic invasion.

Circum larvae precipitation test (CLPT) was done with the sera collected from infected birds incubating with the infected larvae raised in the grasshoppers. The precipitates were seen at the natural orifices of the larvae from 2 to 6 hours of incubation. However, the precipitates detached from the larvae within 24 hours of incubation, a similar invitro precipitation test against eggs, Cercaria were also conducted in different parasites. Some of the antigens used was crude and not purified,

However, the antigen could detect antibodies while no precipitates could detect from the sera of healthy birds.

Circum oval precipitation test:

The formation of precipitates around the live parasites like schistesomes and nematodes when incubated with the homologous antiserum have been demonstrated to be due to soluble and secretary antigens (Smither, 1967).

Similar observations have been reported with living Schistosome eggs (Oliver-Ganzalez, 1954). While these reactions have been shown to be antigen antibody reactions, the protective value of these antibodies against the parasitic infections could not be concluded (Smither, 1967).

In the case of circum oval precipitation test the antibodies appeared to be induced by the eggs which are deposited in the liver and venules of the infected animals and against the antigens diffusing through the egg shell, thus the test is of diagnostic value in chronic parasitic infections (Soulsby, 1973).

In the present study the specific circum oval antibodies could be demonstrated within 2 to 6 hours of incubation and the reactions found to be specific. It is

possible that in the present study since the larva do not migrate through the reticulo endothelial system the antibody response might be poor and the circum oval reaction was weak, this observation further substantiate one earlier observation the gel diffusion test.

Intradermal test:

In the present study the intradermal test was found to be of no value as there was no reaction in birds which were infected with T. mohtedai (the correlation between the skin and the presence of parasitic infection has been discussed by Soulsby, 1973). The possible reason may be that the Tetrameres infection being a localised in the proventriculus and does not pass through the visceral organs of the body it does not delayed or immediate type of hypersensitivity reaction. Further the birds were tested between 45 to 60 days of post-infection. The cell mediated immune response might not have been sufficient enough to give this reaction. Further studies are warranted to try different types of antigens of T. mohtedai in chronically infected birds.

In irradiated larvae there were certain behaviourial changes. In the Ancylostome larvae when given subcutaneously was found to be more efficient than the oral administration in immunogenecity (Miller,1956). Larvae damaged by radiation in some locations during migration may evoke

better immunity than normal larvae. Greater immunogenicity of irradiated larvae compared with normal infective larvae was also recorded in case Uncinera stenocephela in dogs (Dovet et al., 1959) and Haemonchus contartus in sheep (Jarrett et al., 1959). Hence irradiated larvae were preferred for bringing about better immunologic response in the host than the normal larvae.

In the present study T. mohtedai larvae attenuated by irradiation were used to bring about immunological response in the host. It was observed that larvae attenuated at 10 Kr level evoked better response than the irradiated larvae at other doses as evidenced by immunological response studied by preceptin bands of the disc electrophoresis (Ideogram) IXa as well as histopathological changes particularly the cellular changes encountered.

The optimum response was at 10 Kr level and beyond that the radiation damage was probably extensive and the larvae could not migrate resulting in absence of developing worms. So there was sufficient evidence that the birds vaccinated with 10 Kr had given adequate protection to stand a challenge. Further and critical intensive studies may have to be undertaken to understand both humoral and cellular components that might play a role in the protection against challenge.

It is of interest to note that T. mohtedai could withstand 10 Kr irradiation dose while in other nematodes the dose of irradiation is usually much lower ranging from 2 Kr to 6 Kr.

The reason for the high radio resistance by this spirurid worm warrant further investigation.

The present study is only a preliminary investigation to show that sufficient immunological response can be induced in chicken vaccinated with T. mohtedai larvae irradiated at 10 Kr to withstand the subsequent challenge.

The drug trials gave an indication that Caricide at the dose rate of 100 mg/kg body weight at 41 days post-infection was found to be 80% efficacious against T. mohtedai infection. Most of the drugs that had been used in these experiments had been utilised against poultry nematodes by number of workers. Chandrasekharan (1977) used Banminth 11 against Amidostomiasis in ducks and found the same to be less of ficacious. Tetramisole is widely used by Thieupont et al. (1966) in animals and birds against nematode infections with advantage. However Chandrasekharan (1977) found the efficacy of Tetramisole as 79.23% when used against Tetrameres anatis. McGregor (1963), Horton-Smith, Long and Rowell (1963) carried extensive drug trials on Syngamus trachea infection in poultry with Mintezol and found it useful.

Zajicek *et al*. (1973) tried Heltac against Amidostomiasis and found it efficacious. Chandrasekharan (1977) tried Heltac in ducks against *T. anatis* infection and found it 45.7% efficacious.

Very few studies had been made on drug trials against *T. mohtedai* in chicks. However, Chandrasekaran (1977) tried Mintezol and found it 41% efficacious against *T. anatis* in ducks.

Summary

A study on the incidence of nematodes parasiting poultry was conducted in 3000 birds, out of which 1000 birds were desi and the rest were exotic birds. A total of six nematodes Tetrameres mohtedai, Ascaridia galli, Heterakis gallinae, Acuria hamulosa, Acuria spiralis and Capillaria annulata were recorded. Among them T. mohtedai ranked first in desi birds with an incidence of 51%, while A. galli was recorded with the highest incidence of 45% in exotic birds.

The interesting findings in some of the captive birds at the Zoo available for study were Splendidofilariae osmaniae, the heart worms in Myna (Acridotheres tristes) and microfilaria in Magpie (Cissa fivirostris). The other findings were Ascaridia sps. in black patridge (Francolinus), A. galli in guinae fowl (Numida meleagris) and great horn bill (Buchos bicornis) and Capillaria sp. in American Rhea (Rhea Americana). Heavy mortality in pigeon colony maintained at the Ornithology section of this University occurred due to unusual worm load of A.columbae.

Eighteen species of grasshoppers were screened to study the biological development of T. mohtedai. Out of which the following species of grasshoppers acted as efficient vectors: Atractomorpha crenulata, Heirogeuphus

bonian, Oxyvelox chinensis, Oxy nitidula, Oedaleus abraptus, and Spathosternum prasiniferum. Among these the following twelve grasshoppers Hoirogeuphus bonian, Oxyvelox chinensis, Acrida turreta, Acrida zyyancea, Cyrtacanthacris ranacea, Conocephalus indicus, Crotogonus oxyplerus, Phyllocerorcia ramakrishnae, Catantops sp., Colemania sphenerioida, Palanger reinacea, Satrophyllia rugosa were found to serve as intermediate hosts for the first time in India along with the beetle (Coccinella septempunctuta). Cockroaches, earthworms, garden millipedes, houseflies, larvae of houseflies and dung beetles had been tried and were not found to take infection.

Infective larvae developed in 17-18 days within the grasshoppers. The prepatent period in chick varied from 45-60 days and the patency of infection lasted for more than an year.

The host susceptibility was studied in ducks, pigeons, Myna and sparrows. The pigeons developed the adult worms, but the prepatent period was longer. They developed large number of males when compared to females. It was presumed that the worms developed with difficulty and therefore the pigeon could not be considered as a normal host.

The grass pathological findings in general, in experimental birds showed thickening of the proventriculus with the reduction in the lumen of the organ. Histopathological changes included granulocytic reactions, lymphoid hyperplasia, plasma cell reaction and interglandular oedema with evidence of pressure atrophy of the glands.

The effect of irradiation at 2 to 8 Kr level on infective larvae was not conspicuous but at 10 Kr level the resultant worms were stunted, and degenerative changes took place in the gonads. Morphological changes in males as well as in females were conspicuous. The resultant population was predominant with female worms with degeneration of uterine coils. There was characteristic tissue reaction with focal aggregation of lymphoid cells in the mucosa.

Electrophoretic patterns showed distinct bands in sera samples from chicks infected with larvae irradiated at 10 Kr. The unique band No.9 was not seen in other samples.

The larvae irradiated at 12 Kr and beyond did not develop at all.

Among the immunodiagnostic tests conducted to detect infection of T. mohtedai in chickens only circum

larval test gave encouraging results, with formation of precipitins at natural orifices of the infective larvae when incubated with test sera.

Vaccination trials with irradiated larvae at 10 Kr level gave optimum immune response in the host in that the challenge dose of larvae given 20 days after infection with irradiated larvae at 10 Kr level, did not result in recovery of the adults. There is hyperplasia of the surface epithelium and patchy squamous metaplasia along with diffuse lymphocytic infiltration.

The study of anthelmintic efficacy on experimentally infected chicks with T. mohtedai revealed that caricide at dose rate of 100 mg per kg body weight at 41 days post-infection was found to be 80% efficacious, among the 8 drugs screened.

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Fig.1 : Equipment for collection of grasshoppers and their maintenance in the laboratory.

Fig.2 : Different grasshoppers used for experimental infection of *Tetramores mohtedai*.


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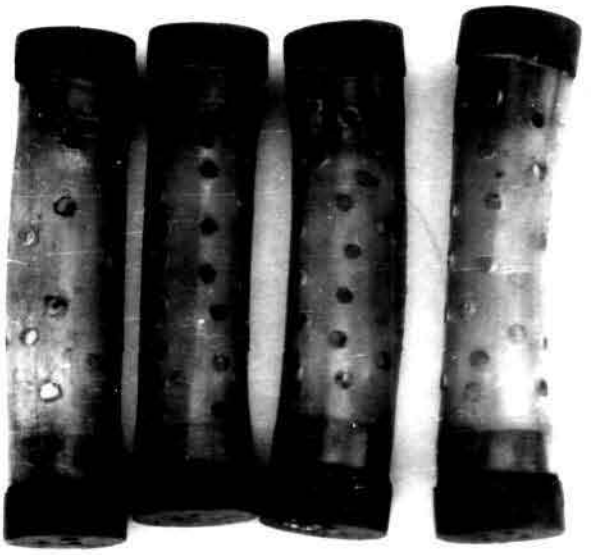
Fig. 3 : Plastic tubes used for irradiating the infective grasshoppers

Fig. 4 : Note the Microfilaria in the blood smear of Magpie Leishman stain. x800



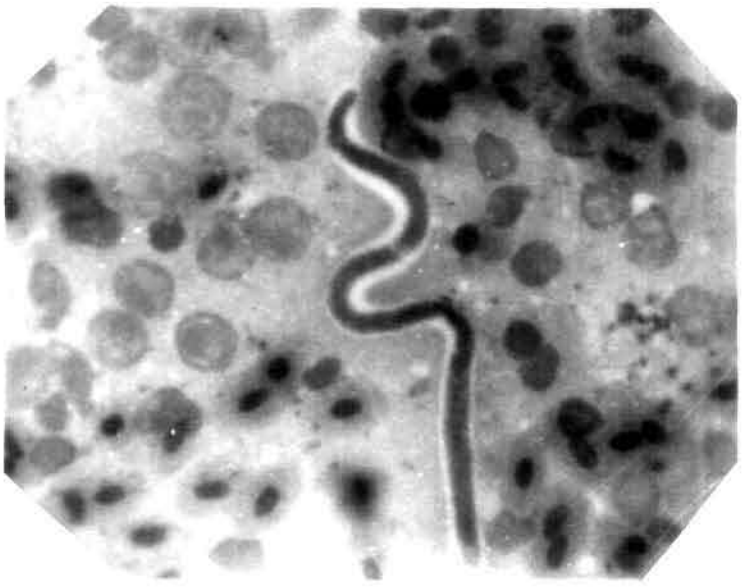
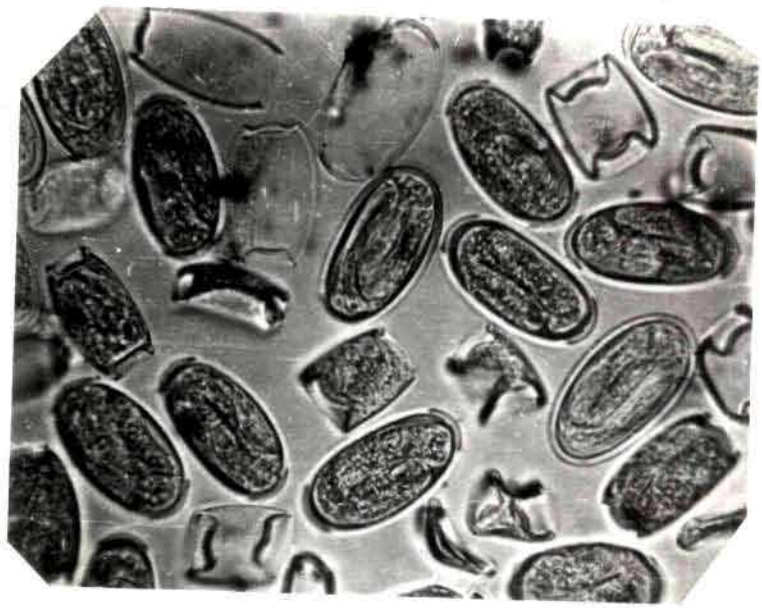


Fig. 5 : Eggs of T. mohtedai containing fully developed larvae. x350

Fig. 6 : Larva emerging of the egg when incubated with the saliva from grasshoppers. x 350



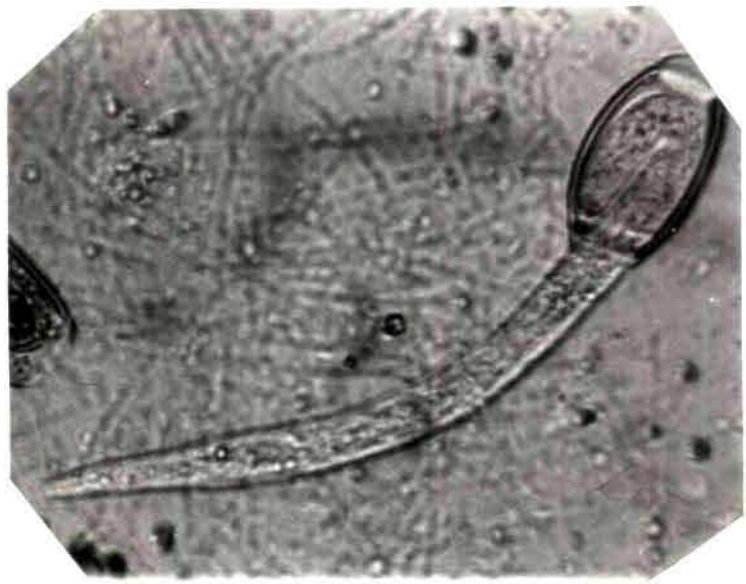


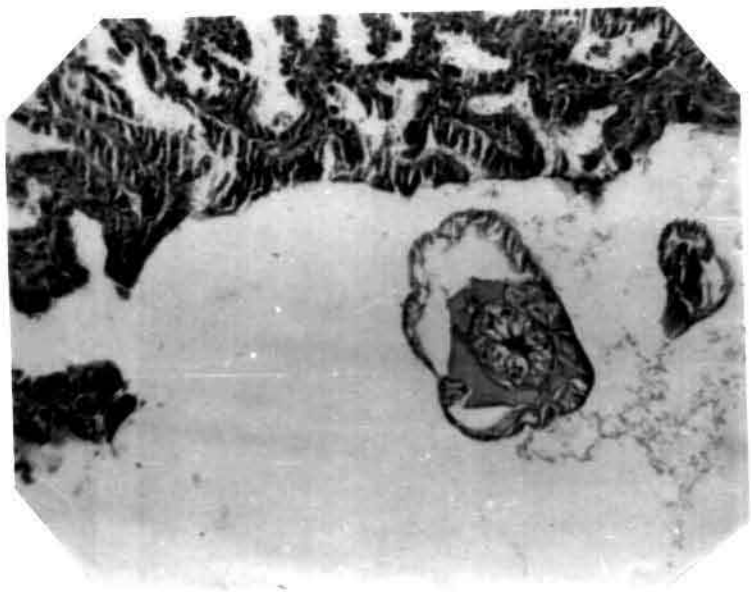
Fig. 7 : Female T. mohtedai from experimental chicks

Fig. 8 : Proventriculus - Pigeon
Note the black pin head size of female T. mohtedai

Fig. 9 : Proventriculus - Pigeon
Note: The section of T. mohtedai in the proventricular gland
H.E x100

Fig. 10 : Proventriculus - Chick - 40 days
Note: a) The cut section of gravid female worm with eggs in the uterine coils

b) Pressure atrophy of the glandular epithelium
H.E x75



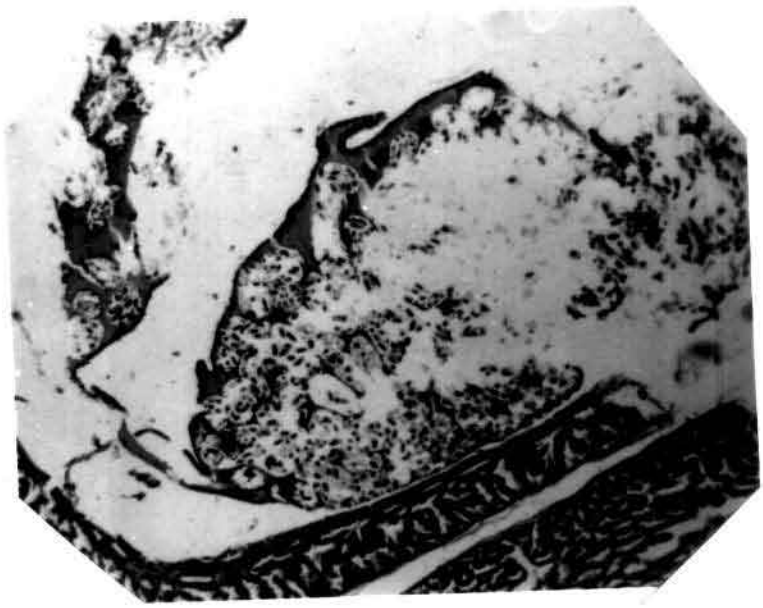
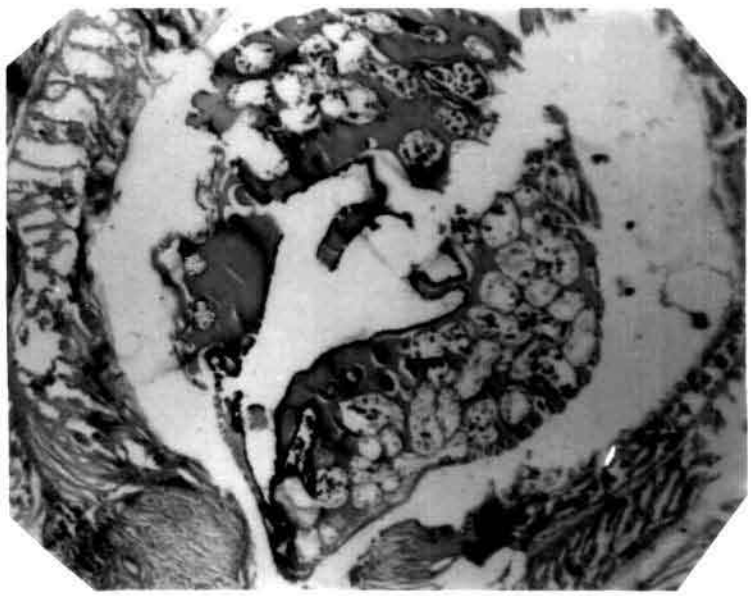


Fig. 11 : Proventriculus - Chick - 10 Kr
40 days
Note: The cut section of female
T. mehtedai showing dege-
nerative changes in the
reproductive organs
H.E x75

Fig. 12 : Male caudal end - 10 Kr - 40 days
Note : The improper development
of spicules. x 350



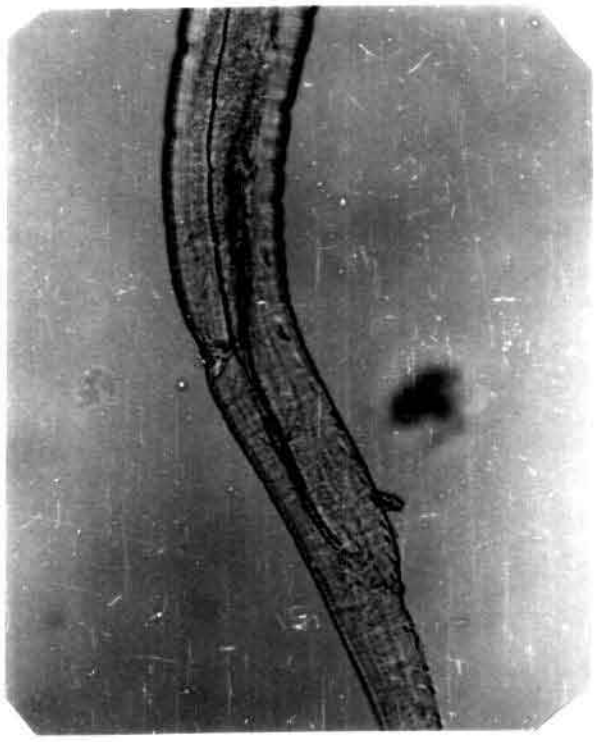
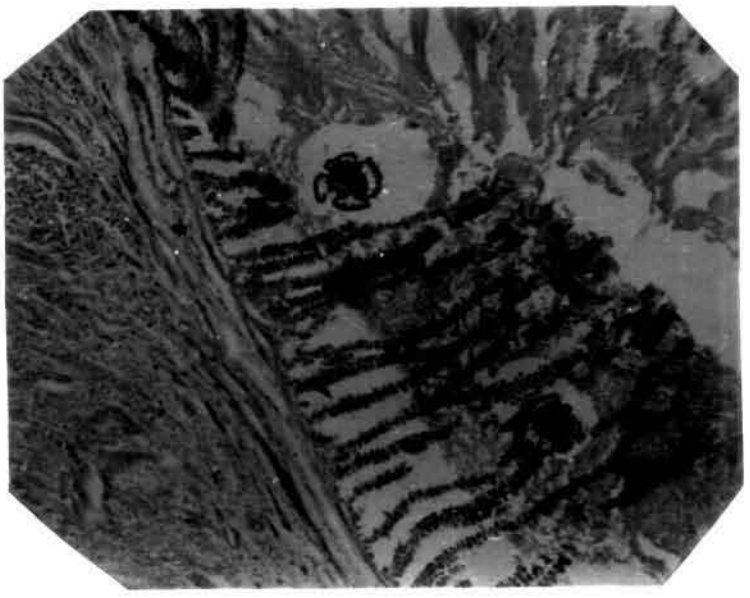


Fig. 13 : Section showing the irradiation effect by 10 Kr. Cut section showing the parasite with other histopathological changes
H.E x75

Fig. 14 : Adult T. mohtedai - 10 Kr - 40 days
Note: The retarded development of various organs and degenerative changes of the worm .
H.E x75



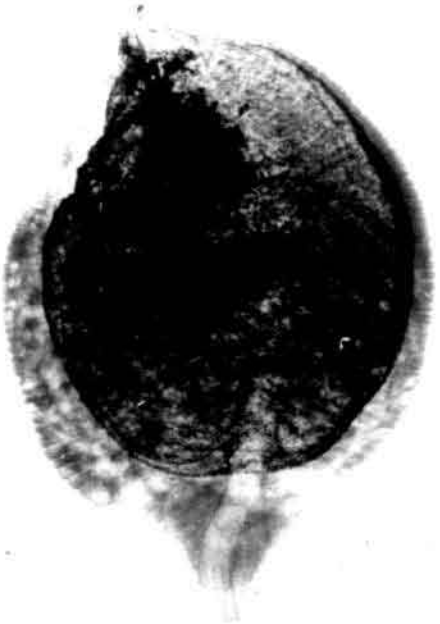
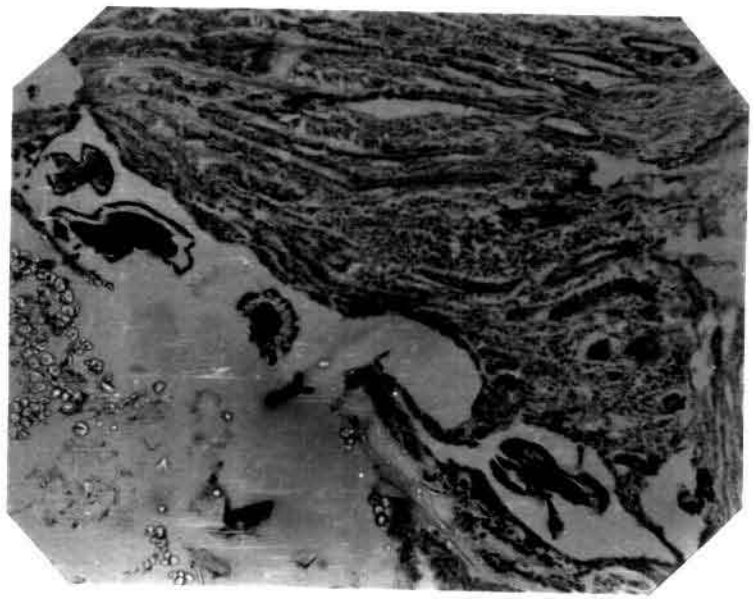
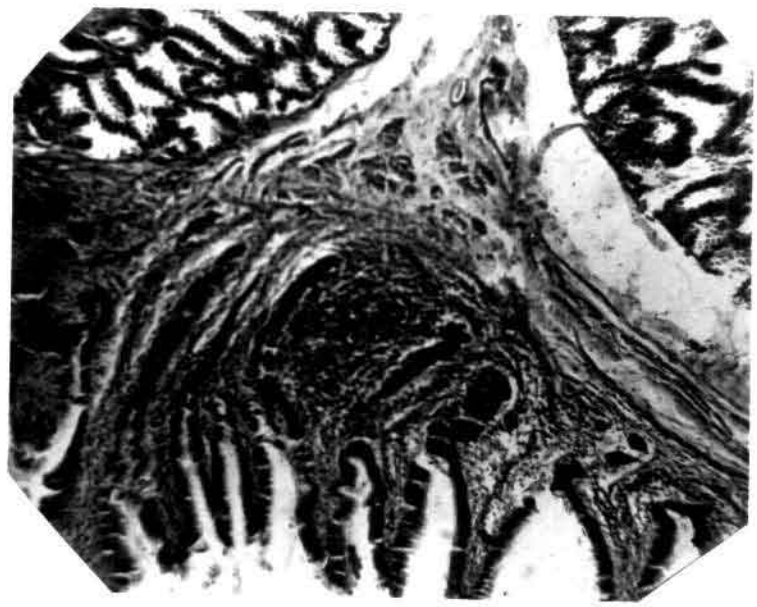


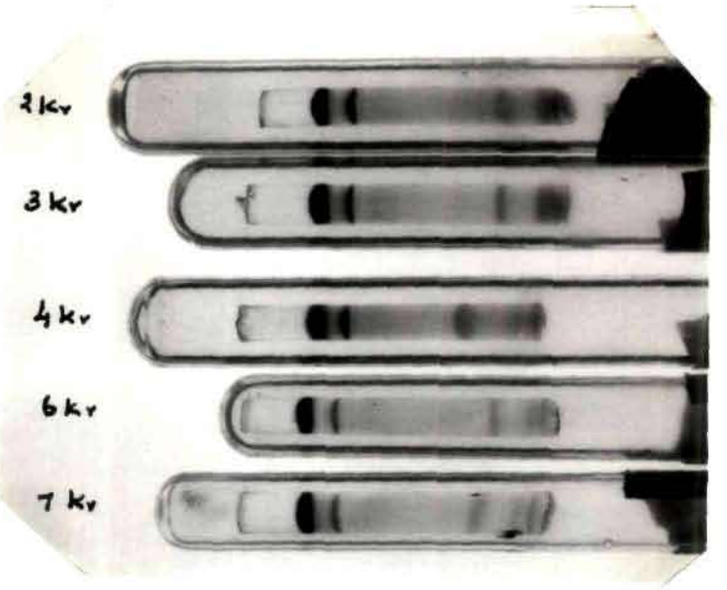
Fig. 15 : Proventriculus - 10 Kr - 40 days
Note: The focal aggregates of lymphocytes
H.E x75

Fig. 16 : Proventriculus - 10 Kr - 40 days
Note: The worms entering the mucosa and invoking mild lymphoid infiltration
H.E x75





Figs. 17 & 18 : Protein bands from the serum samples of the chick - infected with 10 Kr infective larvae of T. mohtedai (with different dose rates: 2, 3, 4, 6, 7, 8, 10, 15, 20 and 25 Kr)


2kv
3kv
4kv
6kv
7kv

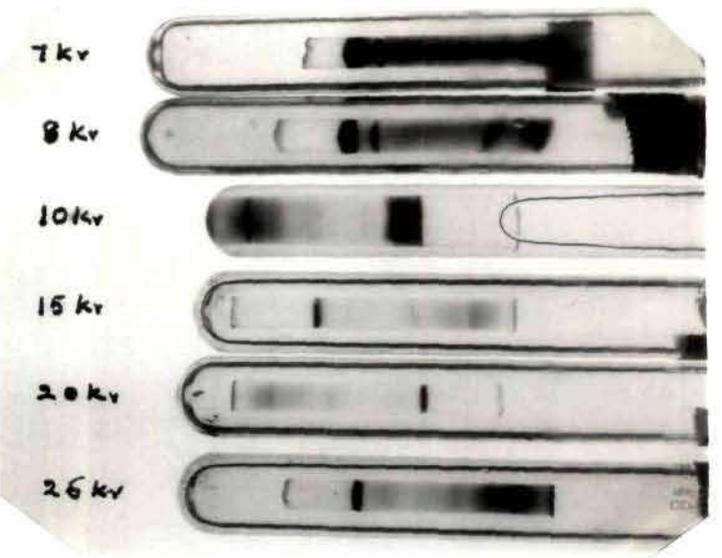
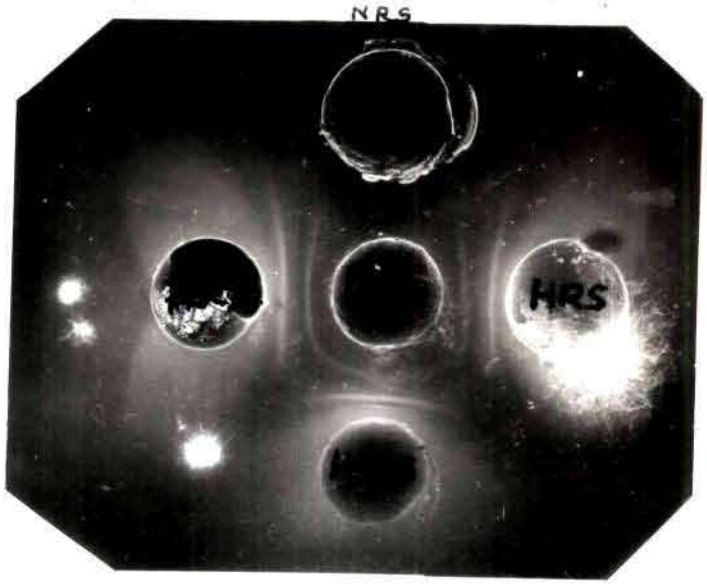

7kv
8kv
10kv
15kv
20kv
26kv

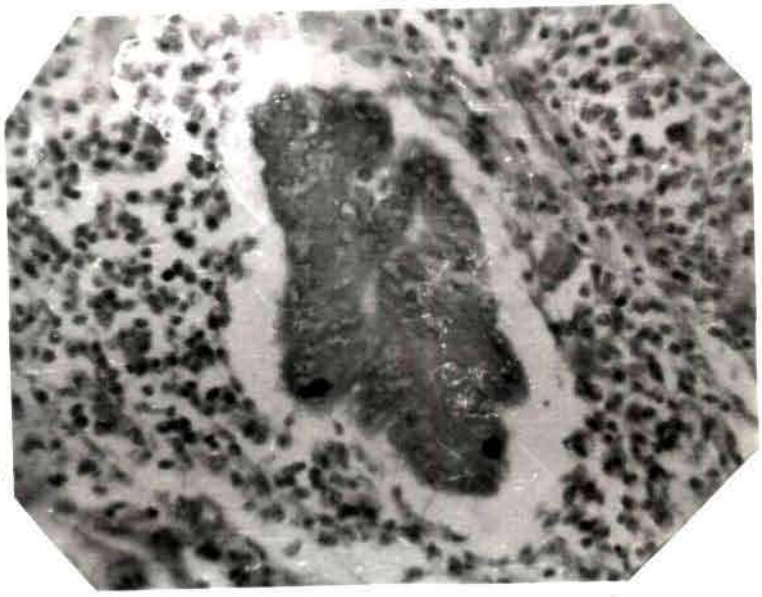
Fig. 19 : Gel-diffusion test

Note: The three precipitation lines with the antiserum raised against with T. mehtedai antigen, when normal serum of rabbit was used as control

Fig. 20 : Proventriculus - 10 Kr - 40 days

Note: The cut section of the worm in the gland, surrounded by mononuclear infiltration H.E x75


NRS
HRS



Tables

LIST OF TABLES

TABLE I

PERCENTAGE OF INCIDENCE OF COMMON NEMATODES IN THE DOMESTIC FOWLS RECORDED IN ANDHRA PRADESH AND IN OTHER STATES IN INDIA

S. No.	Name of the Nematode	Andhra Pradesh (Present study)	Madras	Madhya Pradesh	Kerala	Maha-rastra	Bihar
1	Ascaridia galli	45	28.09	-	51.7	56.6	80
2	Heterakis gallinae	42	48.18	-	+	52.8	86
3	Acuria hamulosa	6	4.85	-	+	18.8	28
4	Acuria spiralis	5.5	NR	-	0.3	5.6	4
5	Tetrameres mohtedai	51 (Desi birds only)	78.18 (Desi fowls)	1.10 (Desi fowls)	42.6 (Desi fowls)	NR	NR
6	Capillaria annulata	1	NR	-	+	3.75	NR
7	Sublura brumpti	NR	NR	-	+	26.7	NR
8	Strongyloides avium	NR	NR	-	+	NR	NR
9	Capillaria anatis	NR	NR	-	+	NR	NR
10	Gongylonema ingluvicola	NR	NR	-	+	NR	NR
11	Oxypirura mansoni	NR	NR	-	+	NR	NR
12	Syngamus trachea	NR	NR	-	+	NR	NR
			Rao & Anantaraman (1969)	Joshi & Kamalapur (1968)	Varghese & Peter (1973)	Sashtri et al. (1974)	Kumar & Sahai (1975)

NR : Not recorded

+ : Recorded but percentage not mentioned

TABLE II contd..

S. No.	Name of the host	Types of Nematodes at different Zoo's			
		Andhra Pradesh (Present study)	Orissa	Lucknow	Delhi
31	Black paartridge (E. francolinus)	-	-	-	Ascarid, Capillarid
32	Red-checked bulbul (Pycononotus jocosus)	-	-	-	Ascarid, Capillarid
33	Ring dove (Streptopelia decaoto)	-	-	-	Capillarid
34	Shah Baz (Spizaetus cirrhatus)	-	-	-	Capillarid
35	Green parakeet (Psttacula eupatria)	-	-	-	Strongyloides sp.
36	Black crow (Corvus splendens)	-	-	-	Ascarid
37	Laughing thrush (Garrulax albogularis)	-	-	-	Ascarid
38	Bagula (Babulcus ibis)	-	-	-	Ascarid
39	Painted strok (Ibis (leucocephalus)	-	-	-	Capillarid
40	Manchurian crane (Grus japonensis)	-	-	-	Ascarid, Capillarid
		Present study (1981)	Patnaik, Acharjyo & Rao(1980)	Chauhan, Bhatia, Arora, Agrawal and Ahluwalia (1972)	

* - Indicates not recorded

TABLE VII

EFFECT OF GAMMA RADIATION ON THE DEVELOPMENT OF TETRAMERES MOHTEDAI INFECTIVE LARVAE IN CHICKS AT DIFFERENT DOSE LEVELS

S. No.	Type of infective material: Infective larvae in grasshoppers irradiated at		No. of chicken	Challenge if given	Average number of worms recovered		Remarks
					Male	Female	
1	2 Kr		25	200	1	8	The challenge was given on 20th day after initial infection.
2	3 Kr		25	200	-	9	
3	4 Kr		25	200	2	6	
4	5 Kr		25	200	-	8	
5	6 Kr		25	200	-	7	
6	7 Kr		25	200	-	4	
7	8 Kr		25	200	-	4	
8	10 Kr		25	200	-	3	
9	12 Kr		25	200	-	-	
10	15 Kr		25	200	-	-	
11	20 Kr		25	200	-	-	
12	25 Kr		25	200	-	-	
13	Non-irradiated	Controls	20	-	2	20	
14	Uninfected		10	-	-	-	

TABLE VIII

PER CENT PROTEIN SIMILARITY AMONG THE SERUM SAMPLE FROM FOWLS INFECTED WITH IRRADIATED INFECTIVE LARVAE OF TETRAMERES MOHTEDAI (PA values : PA = Paired Affinity)

Dose	2 Kr	3 Kr	4 Kr	6 Kr	7 Kr	8 Kr	10 Kr	15Kr	20 Kr	25 Kr
2 Kr	100	36.36	33.33	45.45	36.36	30.00	27.27	20.00	22.22	10.00
3 Kr	36.36	100	40	36.36	25.00	18.18	25.00	18.18	20.00	18.18
4 Kr	33.33	40	100	33.33	20.00	22.22	30.00	11.11	12.50	-
6 Kr	50.00	27.27	33.33	100	36.36	30.00	27.27	30.00	22.22	10.00
7 Kr	40.00	25.00	20.00	36.36	100	25.45	25.00	36.36	20.00	27.27
8 Kr	30.00	18.18	11.11	27.27	25.45	100	18.18	20.00	-	20.00
10 Kr	27.27	25.00	30.00	27.27	25.00	16.16	100	18.18	10	9.09
15 Kr	20.00	18.18	11.11	30.00	27.27	10.00	18.18	100	44.44	20.00
20 Kr	22.27	20.00	12.50	22.22	20.00	22.22	10.00	44.44	100	11.11
25 Kr	10.00	18.18	-	10.00	27.27	30.00	9.09	20.00	11.11	100

TABLE IX

DIFFERENT GRASSHOPPERS SUCCESSFULLY INFECTED DURING PRESENT STUDY INDICATING THEIR COMPARATIVE SUSCEPTIBILITY TO *T. mohtedai* INFECTION

S. No.	Name of the Grasshopper	Number of infective larvae recovered	Remarks
1	Atractomorpha crenulata (T & H)	+ + + +	
2	Hoirogeuphus bonian (T & H)	+ + + +	F
3	Oxyvelox chinensis (T & H)	+ + + +	F
4	Oxy nitidula (H)	+ + + +	
5	Oedaleus abraptus (H)	+ + + +	
6	Spathosternum prasiniferum (T)	+ + + +	
7	Acrida turreta (T & H)	+ + +	F
8	Acrida xyyancea (T & H)	+ + +	F
9	Cyrtacanthacris ranacea (T & H)	+ + +	F
10	Conocephalus indicus (T & H)	+ + +	F
11	Conocephalus maculatus (H)	+ + +	
12	Crotogonus oxyplerus (H)	+ + +	F
13	Ducetia Japanica (H)	+ + +	
14	Phyllocerorcia ramakrishnae (H)	+ + +	F
15	Catantops sp.	+ +	F
16	Colemania sphenerioida (T & H)	+ +	F
17	Palanger reinacea	+ +	F
18	Satrophyllia rugosa (H)	+ +	F

"+" indicates more than 500 larvae liberated from one single mature female Tetrameres taken by one grasshopper

"T" Collected from Tirupati "H" Collected from Hyderabad

"F" First time successfully infected

IVa

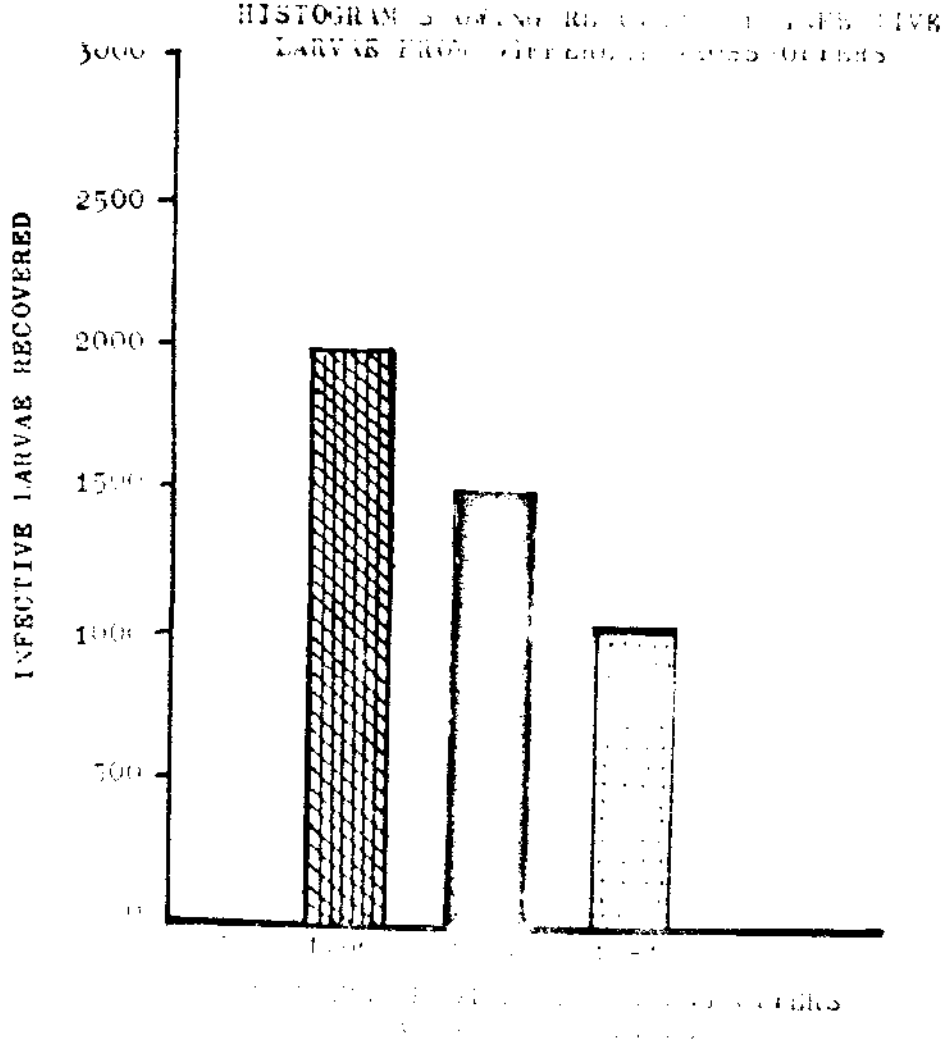


TABLE V

DIFFERENT BIRDS INFECTED TO STUDY THE DEFINITIVE HOST-SPECIFICITY OF *T. mohtedai*

	Different birds tried	Number given infection	Number of larvae administered	Number of worms recovered	Remarks
1.	Ducks (*Khaki campbell*)	10 ducklings (17 days old)	100 to 200 each	Very few juvenils	Failed to recover the worms three days after infection
2.	Pigeons	10(6 to 8 months)	100 to 200 each	10 to 12 adult worms	Large majority of them are male worms
3.	Sparrows	4	100 to 200 each	No worms recovered	-
4.	Myna	4	100 to 200 each	Very few juvenils	All the experimental birds died within four days after infection

TABLE IV

MEASUREMENTS OF DIFFERENT DEVELOPMENTAL STAGES OF Tetrameres mohtedai RECOVERED FROM THE CHICKS

Particulars of the Nematode	3rd stage (2nd day)		4th stage (4th day		Late 4th stage (12th day)		Early 5th stage 15th day 17th day		Sexually mature 24th day	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Length	1.637	1.920	2.113	2.146	2.88	3.20	3.55	3.7	5.48	4.78
Breadth	0.07	0.083	0.074	0.084	0.1	0.2	0.12	1.0	0.15	1.99
Buccal capsule	0.011	0.014	0.026	0.0204	0.026	0.033	0.027	0.025	0.03	0.024
Nerve ring	0.137	0.143	0.164	0.16	0.202	0.197	0.229	0.226	0.225	0.23
Excretory pore	0.159	0.160	0.185	0.18	0.225	0.22	0.24	0.275	0.24	0.236
Glandular oesophagus	0.45	0.530	0.477	0.493	0.818	0.969	0.927	0.25	0.96	1.29
Intestine	0.795	0.954	1.139	1.134	1.457	1.53	1.961	1.75	3.8	2.8
Anus from tail tip	0.153	0.185	0.206	0.238	0.265	0.34	0.266	0.4	0.31	0.3
Gental primodium (from tail end)	0.506	0.135	0.37	0.19	0.36	0.245	-	-	-	-
Vulva (from tail end)	-	-	-	-	-	-	-	0.35	-	0.356
Short spicule	-	-	-	-	-	-	-	-	0.15	-
Long spicule	-	-	-	-	-	-	-	-	0.35	-
Tail papillae	0.0745	0.08	0.12	-	0.133	0.16	0.19	-	0.13	-

*All measurements are in millimeters

IX a

IDEOGRAMS OF THE [illegible]
SAMPLES FROM [illegible]
LARVAE OF [illegible]

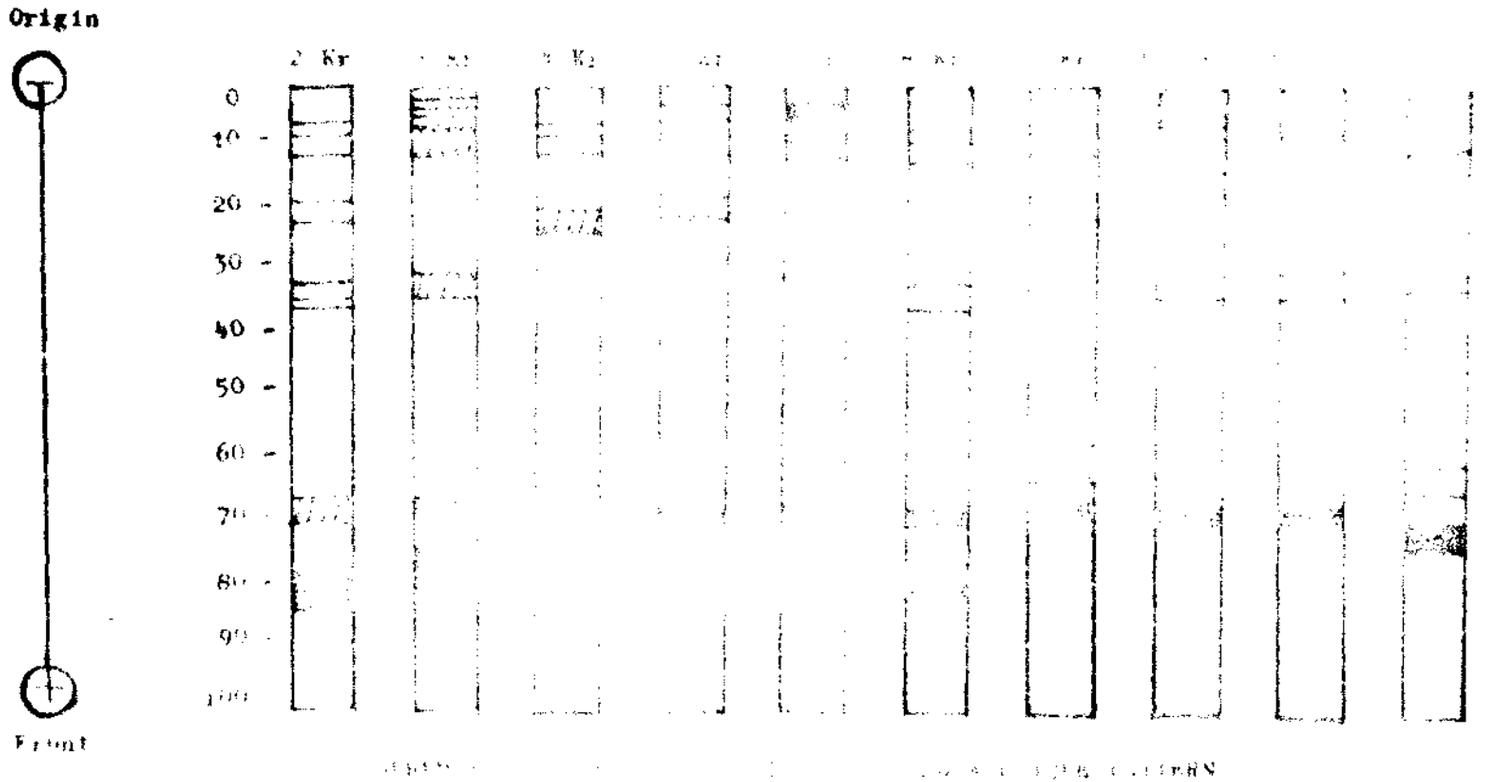


TABLE IX

PROTEIN COMPONENTS OF SERUM SAMPLES OF FOWL INFECTED WITH IRRADIATED INFECTIVE LARVAE OF TETRAMERUS MOHTEDAI BAND NUMBERS*1

Dose	1 2%	2 4.5%	3 8%	4 10%	5 15%	6 20.5%	7 21.5%	8 34%	9 60%	10 62%	11 65.5%	12 70%	13 76.5%	14 79.5%	15 81%
2 Kr	-	+	+	-	-	+	-	-	-	-	-	+	-	+	-
3 Kr	+	+	+	-	-	+	-	-	-	-	-	+	+	-	-
4 Kr	-	+	+	-	-	+	-	-	-	-	+	-	+	-	-
6 Kr	-	+	+	-	-	+	-	-	-	-	-	+	-	+	-
7 Kr	-	+	+	+	-	-	+	-	-	-	-	+	-	+	-
8 Kr	-	+	-	+	-	-	+	-	-	-	-	+	-	+	-
10 Kr	-	+	+	+	-	+	-	-	+	-	+	-	-	-	-
15 Kr	-	-	+	+	+	-	-	+	-	-	-	+	-	-	-
20 Kr	-	-	+	-	+	-	-	+	-	-	-	+	-	-	-
25 Kr	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-