

IMMUNITY IN EXPERIMENTAL ANCYLOSTOMASIS

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UNIVERSITY OF BOMBAY**

**FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ZOOLOGY**

**SUBMITTED BY
SUSHILA MENON**

**UNDER THE GUIDANCE OF
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BOMBAY**

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Ms Sushila Menon

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HAFKINE INSTITUTE

BOMBAY

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DEDICATED TO

MY LATE PARENTS

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Statement Under University Rule No. 108

As required by University Rule No. 108, I state that the work included in this thesis has not been submitted for any degree of this or any other University on any previous occasion. All the work presented in this thesis is original.

Wherever references have been made to the work of others, these have been duly mentioned in the thesis.


Signature of the Student

Forwarded through Research Guide


Research Guide

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INTRODUCTION

INTRODUCTION

Hookworm infection has been one of the major cosmopolitan diseases of mankind and his domesticated animals especially in the warm, moist climates. This infection outranks all other worm infections of man in its production of human misery, debility and inefficiency in the tropical world. Stoll (1962) estimated that 21 per cent of the world's population carry hookworms, and therefore, on a conservative demographic estimate atleast 630 million people are infected. In more graphic terms, this infection load would cause a blood loss equivalent to that accomplished by the exsanguination of 1,500,000 people daily. Intestinal nematodes have been associated with man for atleast 10,000 years (Fry and Moore, 1970) and their distribution is dependent on human migration and movement in recent and remote historical times (Manter, 1967).

Important hookworms of man belong to the family of ancylostomidae. Hookworms that parasitise man are mainly Ancylostoma duodenale (Dubini, 1843), Necator americanus (Stiles, 1902) and Ancylostoma ceylanicum (Looss, 1911). In general, the adult human hookworm

measures: Male 5-10 mm and female 10-18 mm. Ancylostoma
ceylanicum (Male 8.0 mm female 9.5 mm in average) is
basically a canine hookworm infecting cats, dogs and
other carnivores. It was first discovered by Looss
in the year 1911 from a civet cat. Two years later,
this species of hookworm was reported as a human para-
site in prisoners (Lane, 1913). Since then there were
many reports of the presence of this parasite in cats,
dogs and man from various parts of the world. (Leiper,
1916; Chandler, 1926; Yoshida et al., 1968; Velasquez
and Cabrera, 1968; Areekul et al., 1970a). For years,
A. ceylanicum was considered a synonym of A. brasiliense
by many parasitologists. Biocca (1951) established
the validity of A. ceylanicum based on the morphological
characteristics. This was recognized later on by
Yamaguti (1961), and Rep. et al. (1968). Since there
has been a high infection rate of A. ceylanicum in dogs
and cats in some areas and also a high prevalence of
natural infection in man in various countries (Anten
and Zuidema, 1964; Velasquez and Cabrera, 1968; Yoshida
et al., 1968; 1973; Areekul et al., 1970a; Chowdhury and
Schad, 1972; Zuidema et al., 1972), this species of
hookworm is now thought to be medically important and
a significant pathogen in man.

The life cycle of all species of hookworms is direct without the involvement of any intermediate host. The adult worms are attached to the mucosa of the small intestine. The female lays a large number of eggs which are passed out with faeces. Under warm, moist and shady conditions the first stage larva develops inside the egg. When the egg hatches, the larva comes out and undergoes two moults, thus becoming a third stage filariform larva which forms the infective stage for the host. Infection to man and other animals can take place either by the ingestion of these infective larvae with contaminated food or water or by the infective larvae actively penetrating the naked skin which may come in contact with contaminated soil.

The experimental infection of A. ceylanicum in man (Areekul et al, 1970b) and in dogs (Areekul et al, 1978) proved that the infective larvae grow directly in the intestine without having any tissue migration. Similarly, when the infective larvae of dog hookworm Ancylostoma caninum were given orally to the dogs, they did not undertake any lung journey but had a straight forward development in the intestine. (Yokogawa and Oiso, 1925 a,b,c; Miwa, 1928; Foster and Cross, 1934). On the other hand, the studies on the development of

N.americana and A.duodenale in abnormal hosts like rats, guinea-pigs, hamsters and rabbits have proved that the lung migration is essential to complete the development when infected either orally or percutaneously (Nakajima, 1931; Sasada, 1936; Satomi, 1938; Kamioka, 1937; Nichols, 1956; Soh, 1958; Sen et al, 1965; Bhopale et al, 1978).

It is known that hookworm burden causes weight loss in infected persons. It affects the nutrition of the host occasioned by anorexia, intestinal blood loss and mucosal damage. Sometimes hookworm infection causes pharyngeal itching, cough, urticaria, nausea, vomiting and abdominal pain (Matsusaki, 1966) depending on the worm load and dietary iron intake. Symptoms of anaemia develop with lassitude, breathlessness, palpitations and depression. Mucous membrane and skin become pallid, face puffy and ankles swollen due to hypoproteinaemia. In certain cases haemoglobin values decrease upto 3-8 gms%. Marked microcytosis and hypochromia are also observed. The white cell count is usually normal except for a mild eosinophilia.

The experimental infection of A.ceylanicum larvae to human volunteers by cutaneous exposure had shown the same symptoms of urticaria, bulbous lesion, cough etc.

as seen in other hookworm infections of man (Wijers and Smit, 1966; Arsekul et al, 1970b). Arsekul et al, (1975) studied in detail the pathogenicity of *A. ceylanicum* infection in experimentally infected dogs. They reported a high hookworm egg load and heavy blood loss in cutaneously infected dogs when compared to those infected orally.

It is known that all types of antibodies are formed against hookworm infection in man (Banwell and Schad, 1978). IgE antibody is prominently increased and can be detected in the serum (Grove et al, 1974). Skin tests for hookworm as a diagnostic tool have been proved insensitive (Ball et al, 1971; Villarejos et al, 1978). Immune response to hookworm infections with reference to circulating antibodies in humans has been aided by the application of various immunological techniques like agar gel-diffusion, indirect haemagglutination and complement fixation (Sawada et al, 1964; Ball and Bartlett, 1969; Sood et al, 1972), immunofluorescence (Kaul and Mahajan, 1977), ELISA (Ogilvie et al, 1978) and counterimmunoelectrophoresis (Bhopale et al, 1981).

In view of the widespread distribution and severe pathogenicity, the importance of developing a laboratory adapted human hookworm strain has long been recognized.

A successful experimental infection of A.ceylanicum now considered as a human parasitic hookworm, was attempted for the first time in golden hamsters (Mesocricetus auratus) by Ray and Bhopale (1972). Various studies including host-parasite relationship and chemotherapy were made by Ray et al. (1978). They infected the hamsters orally with A.ceylanicum infective larvae obtained after coproculture methods of stool samples collected from the infected dogs and found that the larvae had a straight forward development in the intestine (Ray et al., 1972).

Considering the significance of this hookworm as a human parasite, it was thought worthwhile to study the immunological response in hamsters in which A.ceylanicum larvae develop completely and behave similarly as in the natural host.

The present study was aimed at the elucidation of humoral immunity to A.ceylanicum infection in golden hamsters (Mesocricetus auratus). With this objective, particular emphasis was laid to understand the answers to the following queries in this experimental model.

1. To study the host-parasite interactions with reference to body weight, worm load, haemoglobin level and differential leukocyte count.

2. To demonstrate the antibodies in the serum of A.cozlanicum infected hamsters at various intervals of infection period.
3. To study whether serum passive immunization has any protective role against the challenge infection of A.cozlanicum.
4. To study whether ultraviolet (UV) irradiated larvae play any role in inducing immunity enough to resist a challenge infection of A.cozlanicum.
5. Evaluation of the UV-irradiated larvae as a vaccine in hamster model.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Larval migration and development

Earlier it was believed that migration of *A. caninum* larvae through lungs was essential for further development in normal host dog (Miyagawa, 1912; Shirai, 1926; Nakajima, 1931). Miwa (1928) observed that when *A. caninum* larvae were given orally to dogs, they directly invaded the intestinal wall and reached the sub-mucosa, then returned to the intestinal lumen and developed to the adult stage. This view was supported by Yokogawa and Oiso (1925); Foster and Cross (1934) and Matsusaki (1939). Matsusaki (1950), after intensive investigations on the life cycle of *A. caninum* in dogs concluded that no migration had occurred outside the intestine of the host.

In experimental infection of *A. caninum* larvae in laboratory animal rat, Scott (1928) demonstrated that the larvae did not reach maturity and they were expelled periodically from the intestine and hence he termed rat as an abnormal host for *A. caninum*. The migration route of *A. quodanalis* and *H. americanus* in rat was studied by numerous other workers (Nakajima, 1931; Yamazaki, 1935; Sasada, 1936; Satomi, 1938; Hasebe, 1942; Yanai, 1943 and Matsusaki, 1951) and they found that some of the

inoculated larvae bored through the intestinal wall migrated via liver, heart, lungs, trachea and pharynx to the muscles, and some of them were expelled out from the host system. In rabbits, the orally infected A. caninum larvae were found to invade the wall of stomach, intestine, liver and lungs and according to Kamioka (1940) it indicated a partial development of the larvae.

The migratory behaviour of A. caninum larvae in mice was extensively studied by Yogo (1941), Nagata (1954), Nichols (1956), Soh (1958), Kono and Sawada (1961) and Banerjee *et al* (1970 a,b). Nichols (1956) stated that the migration of the larvae took place through the lungs and got accumulated in the muscles where they remained actively motile for one year. Soh (1958) studied in detail the migratory behaviour and longevity of the larvae of A. caninum, A. duodenale and H. americanus in relation to different methods of inoculation (by mouth, stomach and skin) in mice. Kono and Sawada (1961) also studied the migration of A. caninum larvae in mice after oral infection and found that migration took place through the small intestine, liver, lungs, pharynx and finally to the musculature where they gradually destroyed. Bhopale and Johri (1975 a, 1978 a) studied the migration and distribution of A. caninum larvae in the tissues of

Swiss albino mice infected orally with different doses of larvae. Migration of the larvae to the muscles particularly of the head and neck and thorax took place on the first day and it was proportional to the dose level. The distribution of the larvae was predominant in the central nervous system of mouse given single dose of *A. caninum* larval infection but was reduced on repeated infections (Bhopale and Johri, 1978 b).

The route of migration of hookworm larvae after percutaneous infection in abnormal hosts was studied by many workers. Sasada (1936) reported that when the larvae of *A. guineaensis* and *A. caninum* were put on the surface of the abdominal skin of rats, mice and guinea pigs, most of the larvae invaded the skin and remained viable in the muscles for 6 months or more. A few of them migrated through the peritoneal cavity reaching the spleen, liver, stomach and mesentery. Nichols (1956) reported that these larvae caused pneumonitis in mice, the severity and duration depending directly upon the dose of inoculum. Miller (1970) showed that mice harbouring larvae in their tissues were found to be suitable transport hosts as these larvae subsequently matured within the normal hosts.

Several attempts were made to establish the human hookworm N.americanus and A.duodenale in the laboratory animals. A partial development of N.americanus in guinea pigs was achieved by Schwartz and Alicata (1934). Migration and development of human and canine hookworms were studied by Satomi (1938). According to them the larvae after oral infection migrated via liver, heart, lungs, trachea and pharynx to the lungs. Soh (1958) and Arora et al (1976) reported the development of human hookworm upto the fourth stage in albino mice. Sen and his co-workers (1967; 1970; 1972; 1973) succeeded in developing N.americanus in hamsters with the help of cortisone. The complete development of both N.americanus and A.duodenale was made possible in pups upto the adult stage by Yoshida et al (1960), Tanabe (1962) and Nagahana and Yoshida (1965). Bhopale et al (1977) studied the migration and complete development of N.americanus in infant rabbits by cutaneous infection of N.americanus infective larvae of human origin and successfully established the infection in this host upto six generations. The third stage larvae after penetration of the skin migrated to liver and lungs within 7 days and the 4th stage larvae and preadult worms were recovered from the intestine after 3-4 weeks. The adult worms persisted in the intestine for a longer

period of 150 days or more. Bhopale and Menon (1979) infected neonatal rabbits with 3000-5000 infective larvae of A. quodanale by buccal pouch route and developing larvae recovered from the lungs and liver and the adult worms from the intestine were carefully identified. The adult worms reached the oviposition stage within 6 weeks and the worms persisted in the intestine for 7 weeks.

There are a few reports on the attempts of A. ceylanicum infection in experimental hosts. The experimental infection of dogs with different doses of A. ceylanicum by oral and percutaneous routes were studied by Rep et al (1966) and Areekul et al (1976) and they found that the mean prepatent periods were found to be 12 and 11 days by cutaneous and oral routes of infection respectively. These experimental infections only provided information regarding pathogenicity of A. ceylanicum in dog. Ray et al (1972) reported for the first time the complete development of A. ceylanicum of canine origin in the laboratory animal, hamster. The orally infected hamsters were necropsied at various intervals from 12 hours to 17 days. At necropsy, the lungs, liver, trachea, oesophagus, stomach and intestine were examined for the presence of larvae and/or adult worms. It was confirmed from the results

that the larvae did not migrate to any organs or tissues during development but completed the life cycle entirely in the lumen of the gastrointestinal tract where they developed to the 4th stage by 4th day, immature adult by 7th day and adult stage by 13th day. Fertile eggs appeared in the faeces from the 17th day onwards.

Ray *et al* (1975) developed this parasite in mice under the influence of corticosteroid. According to Areekul *et al* (1976) the experimental *A.ceylanicum* infection to rhesus monkeys by oral route showed that they were not susceptible to this species of hookworm. In other hosts, particularly laboratory animals, an attempt was made by Bhopale *et al* (1978) who reported a partial development of *A.ceylanicum* in neonatal and young mice, rats, and guinea pigs. However, no development was noted in infant and young rabbits.

During a study on the development of other hookworms, Norris (1971) reported that the infective larvae of *A.brasiliensis* and *A.tubaeforme* after oral infections in mice had a pulmonary migration and were primarily limited to the somatic musculature.

Pathogenesis

The association between anaemia and infection of

man with abdominal worms was reported approximately 3500 years ago. It has been proved that intestinal haemorrhage is the primary pathogenic mechanism in the induction of hookworm anaemia as reported by Roche *et al* (1957), Foy *et al* (1958), Clark *et al* (1961); Gilles *et al* (1964) and Miller *et al* (1966 a). There was considerable controversy and deficiency in the knowledge concerning the pathogenesis of hookworm infection in man because of the absence of suitable experimental primate host for the hookworms that infect man. However, in animals particularly in hookworm disease of dogs, there is ample information on this aspect of host parasite relationship. Miller (1965 a) in his studies with Ancylostoma caninum infection in pups reported that the haemorrhagic blood loss had commenced on the 8th day after infection and that the pups suffered from severe dysentery passing fluid faeces laden with mucous and blood. Infections of this intensity often proved lethal which was related to the age and body weight of the pups (Miller, 1965 b). According to Miller (1966 a), in pups heavily infected with A. caninum, the microhaematocrit figures were reduced by half with corresponding reductions in haemoglobin and plasma protein levels. Roche and Martinez-Torres (1960)

and later Areekul et al (1975) reported that A.caninum infection caused a blood loss of 0.037-0.12 ml/worm in dogs. Miller (1966 b) stated that anaemia was not observed to be a consequence of infection in pups and kittens with A.braziliense or untenaria stenoccephala infections but hypoproteinaemia of 10-15% was recorded in dogs and cats as a consequence of A.braziliense infection.

Many investigators (Roche et al, 1957; Foy et al, 1958; Gilles et al, 1964; Areekul et al, 1970 c) have studied the daily blood loss caused by other hookworms, Ancylostoma duodenale (0.14-0.40 ml/worm) and H.americanus (0.01-0.09 ml/worm) in man.

Miller while working on experimental infection of A.duodenale in young chimpanzees found that they were highly susceptible to this infection. The daily blood loss recorded in these infected chimpanzees was 8-9 ml between 21 and 28 days and 14-24 ml. per day by 33 days. Similar experiments in pups, subject to intestinal haemorrhage associated with A.caninum infections, suggested that infant chimpanzees were more sensitive to haemorrhagic anaemia (Miller, 1968).

During natural infection of A.sylvaticum in man, Anton and Zuidema (1964) reported a heavy infection

rate in patients with decreased haemoglobin values of 8.9 and 11.3 gms % with an average blood loss of 3.8 and 8.9 ml respectively.

Experimental infection of A. ceylanicum to human volunteers by cutaneous and oral routes was attempted successfully by Maplestone (1933); Hayden and Bearup (1963), Wijers and smit (1966), Areekul et al (1970 b) and Yashida et al (1971). They observed nodularity, urticaria, dry cough and eosinophilia in the experimentally infected volunteers. They also noted that the prepatent periods of A. ceylanicum in infected human volunteers were longer as compared to those of A. ceylanicum infected dogs.

In experimentally A. ceylanicum infected dogs, Rep (1966) and Areekul et al (1976) reported a considerable blood loss during the prepatent period of infection. The mean blood loss measured by Areekul et al (1976) was found to be 0.33 ml/worm /day and 0.38 ml/worm /day in dogs infected by cutaneous and oral routes respectively.

Areekul et al (1976) also attempted an experimental infection in Rhesus monkeys (Macaca mulatta) with filariform larvae of A. ceylanicum by oral, cutaneous and subcutaneous routes. However, the infection caused no blood loss in monkeys but the eosinophil

count showed a considerable increase.

Serology

Serological tests for the diagnosis of hookworms have been attempted by many investigators. Stumberg (1930) obtained positive precipitin and complement fixation reactions with sera of rabbits immunised with extracts of A.caninum larvae but he was unable to obtain positive results with sera from the hookworm infected dogs. Otto (1940) demonstrated precipitates around the infective larvae of A.caninum when incubated in the immunised sera of dogs. Similar results were obtained by Otto (1942) with human hookworm (N.americanus) larvae when incubated in the sera from patients, considered to be immune. Sheldon and Groover (1942) also demonstrated Sarle's precipitates in the sera of children with light infections but they were unable to obtain positive results with sera of children who were heavily infected with hookworms. Desowitz (1962) failed to detect antibody in the sera of N.americanus infected children by gel-diffusion test. He further applied the same technique to study the extract of adult A.caninum with antisera produced in guinea pigs against A.caninum larval antigen. The results revealed

that the agar gel diffusion test indicated greater antigenic relationship between identical stages of different species of hookworm (larvae of N.americanus and A.caninum) than between the different stages of the same species of hookworm (larvae and adults of A.caninum).

Precipitin test, indirect haemagglutination complement fixation and skin test were employed successfully by Savada *et al* (1964) using adult A.caninum antigen for the immunodiagnosis of hookworm infection in dogs and hookworm patients. Soulsby and Gilles (1966) used human larval hookworm antigen and demonstrated the production of haemagglutinating antibodies in children infected with hookworms after a lapse of two years. Similarly, Ball and Bartlett (1969) failed to detect haemagglutinating antibodies in patients' sera using larval antigen from human source until the infection had occurred for a considerable time. They also stated that N.americanus larvae gave rise to immediate type of antibody response which was demonstrated by fluorescent antibody technique, complement fixation test and Prausnitz Kustner reaction. Detection of haemagglutinating antibodies long time after the occurrence of infection in patients were reported later by Schad *et al* (1976). Kaul and Mahajan (1977) used fluorescent

antibody test for the serodiagnosis of hookworm infection in patients. However, the positive reaction was not related to the intensity of infection as determined by worm load.

It was reported by some investigators that the hookworms stimulate their hosts to produce IgE antibodies. Kojima *et al* (1972) observed that a low degree of infection could stimulate the production of IgE antibodies. According to Ogilvie and Worms (1976) the values of serum IgE increased in hookworm patients 20 to 30 times more than the normal values. The most sensitive test ELISA and Radio-Allergo-Sorbent Technique (RAST) were employed by Ogilvie *et al* (1978) for the detection of IgE antibodies in self-infected *N. americanus* volunteers.

The passive cutaneous anaphylaxis (PCA) test was employed by Grove *et al* (1974) for the detection of serum antibodies and found the reaction positive within four weeks post infection and the antibody titre reached the maximum after three months. The PCA antibodies were no longer detectable after one year.

Another immunodiagnostic test i.e. counterimmuno-electrophoresis (CIEP) was attempted by Bhopale *et al*

time that dogs could develop resistance to reinfection with A. caninum. This was followed by a number of reports on acquired resistance in dogs to reinfection with A. caninum (Sarles, 1929; McCoy, 1931; Foster, 1935; Otto and Kerr, 1939; Otto, 1941). The literature was extensively reviewed by Cort and Otto (1940) and later by Otto (1948) who demonstrated the establishment of resistance by the reduction in morbidity and/or mortality of a challenge infection and by the reduction in worm count from challenge dose and output of eggs from resistant dogs. Krupp (1961) studied the effects of crowding and superinfection on the egg production of A. caninum in dogs.

Resistance to reinfections with homologous hookworms in abnormal hosts like rats and mice was reported by many workers. Kerr (1936) studied the resistance in mice to repeated infections of A. caninum larvae at weekly intervals but failed to stimulate any obvious resistance. Yogo (1941) studied the effects of infection of A. caninum larvae in mice with intervals of 10 days. The larvae were found to disappear earlier from the intestine and the liver in repeatedly infected animals with reduced rate of migration to these organs. Nagata (1955) reported the development of resistance in mice, when repeatedly infected with A. caninum larvae percutaneously. Kono and Savada (1961) reported that repeated

(1961) for the detection of antibodies in hookworm infections in various host systems like rabbits experimentally infected with N.americanus and A.caninum infected mice and also hookworm patients. The test showed in general a limited application for the sero-diagnosis of hookworm infection.

Several attempts were made to investigate the hypersensitivity reaction to hookworm antigen (Stunberg and Rodriques - Molina, 1931; Noda, 1953; Yamanaka, 1960). Hutardo and Layrisse (1968) used successfully the third stage N.americanus larval antigen for intradermal test. Ball et al (1971) and subsequently Villarejos et al (1976) reported that skin test for hookworm was not reliable as an epidemiological diagnostic tool.

Acquired immunity

There is no substantial evidence that protective acquired immunity occurs in man but in the animal field particularly in hookworm disease of dogs, a wealth of information is available. Aquisition of resistance to subsequent infections with the homologous parasite A.caninum was confirmed in dogs earlier by repeated infections. Herrick (1928) demonstrated for the first

infections with A.caninum induced immunity in mice. According to Bhopale and Johri (1978a), when the mice were infected with single and repeated doses of A.caninum larvae, a considerable reduction in larval count had occurred in various organs and muscular regions of the immunised mice. The expulsion of the larvae from the gastrointestinal tract at an early period resulted in a poor larval migration to various organs and muscle regions. The larval recovery from the central nervous system of immunised mice given repeated doses of A.caninum larvae, was negligible (Bhopale and Johri, 1978b).

In hookworm infection of man and animals, serum protein changes are indicated by an increase in the globulin fraction (especially gamma globulin) accompanied by a normal or relative decrease in albumin component (Kinugasa, 1954; Mori, 1957; Ishisaka, 1959). In dog ancylostomiasis, increase in the betaglobulin and decrease in albumin (Hara, 1956 a,b) and increase in alpha-2-globulin (Tomoda, 1963) was reported. Bhopale and Johri (1978 b) in a study of serum protein pattern of mice during infection with single and repeated doses of A.caninum larvae observed significant increase in β -globulins and decrease in albumin in accordance with the increasing single dose levels but not dependent on repeated dose levels.

Passive Immunisation

There are a few reports on the attempt of passive transfer of immunity by serum to the host(s) against A.caninum infection. Kerr (1935) attempted successfully to passively transfer resistance through serum from repeatedly infected mice to fresh (uninfected) mice. Otto (1940) showed partial success in passive protection of some dogs against A.caninum larval infection. Miller (1967) passively transferred successfully the immunity of pups that had been double vaccinated with 40 Kr-X-irradiated larvae of A.caninum to susceptible pups using serum and lymphoid cells separately or a combination of both.

During the experimental studies with A.caninum in abnormal host mice, Vardhani and Johri (1978) demonstrated that the sensitized lymph node cells and peritoneal exudate cells from A.caninum infected donor mice were responsible for larval expulsion from the recipients. Transfer of thymus, bone marrow or lymphoid cells showed high protectivity in recipients as confirmed by Kolhe et al (1979) during their studies with A.caninum in mice.

Active Immunisation

Many have attempted the use of antigenically potent materials like excretions and secretions and somatic

antigens of the parasites for the stimulation of protective immunity against helminthic infections. In hookworms, Thorson (1966) succeeded in stimulating protective immunity in pups against A. caninum infection by using oesophageal extract of adult A. caninum as a source of antigen, although natural infection induced a rather high immunity.

Jain et al (1979) while working with human hookworm A. duodenale noticed that excretory-secretory antigens from the third stage larvae when injected subcutaneously to mice in single, double and triple doses at weekly intervals, no larval recovery could be made from the immunised mice although one immunised mouse was found harbouring a few larvae.

In murine hookworm studies attempts were made successfully to develop protective immunity using adult worm extract by Chandler (1932, 1936), Watt (1943), Thorson (1951, 1953), and Poulain et al (1976). Murray et al (1979) reported a 90% reduction in worm burden in rats against Hippostrongylus brasiliensis infection by using an extract antigen from adult worms.

Studies with other nematode parasites where the use of killed antigen or their metabolites for immunisation were employed and resulted in a significant

level of protection include Trichinella spiralis (McCoy, 1935; Campbell, 1955; Denham, 1967; Despontier and Muller, 1969; Larsh et al 1970; Despontier et al, 1977); Trichostrongylus colubriformis (Silverman et al, 1962; Denham, 1969; Rothwell and Love, 1974; Rothwell, 1978); Dictyocaulus viviparus (Jarrett et al, 1960; Silverman et al, 1962); Haemonchus contortus (Silverman and Paterson, 1960; Ozerol and Silverman, 1970; Scott et al, 1971); Oesophagostomum radiatum (Keith and Bremner, 1973); Nematospirides dubius (Van Zandt, 1962; Cypess, 1970); Trichiuris muris (Wakelin and Selby, 1973; Jenkins and Wakelin, 1977); Ancaria suum (Lejkina, 1963; Soulsby 1957 and 1963; Rhodes et al, 1965; Stromberg and Soulsby, 1977) and Litomosoides garinai (Macdonald and Scott, 1953). These results were obtained in a wide range of laboratory and domestic animal hosts including cattle, sheep, pigs, dogs, rabbits, cotton rats, rats, guinea pigs and mice. Techasoponmani and Sirisinha (1980) injected the rats and mice with the excretory and secretory products collected in vitro from the adult female worms of Angiostrongylus cantonensis along with Freund's complete and incomplete adjuvants separately. These animals were found protected against a lethal challenge infection of the third stage larvae.

Attenuated vaccine

The only method of attenuation which proved effective in reducing infectivity and pathogenicity without interfering their immunogenicity was to expose the larvae to ionizing radiation. Radiations of many different wavelengths have been shown to bring about radical changes in a wide range of living organisms and tissues. These changes are often associated with interference in normal physiological processes and at high doses, with cytological damage and death. Ultraviolet rays, x-rays and gamma rays are widely used to attenuate the infective stages of parasites which then serve as live vaccines. The irradiation weakens the infective stages so that they do not complete their life cycle in the host but they release antigens which stimulate protective immunity. The first evidence of ionizing radiation in hookworms was reported by Dow et al (1959, 1961). They attenuated Uncinaria stenocephala larvae with 40 kr X-rays and vaccinated pups by administering the irradiated larvae orally in single and double vaccination schedules. Miller (1964, 1965, a,b,c, 1966 c, 1971) reported that subcutaneous vaccination of 40 kr X-irradiated larvae of A. caninum gave 97% protection to pups against challenge infection and this method of vaccination proved more effective than oral admini-

stration of irradiated larvae. Recently Vinayak *et al* (1981) were successful in developing an X-irradiated *A.caninum* larval vaccine to protect pups against *A.caninum* infection.

Protective immunity in dogs by means of irradiated *A.caninum* larvae attenuated by ultraviolet rays was studied by Vieira and Rombert (1974). Vieira (1979) reported that the metopatreated larvae conferred a highly satisfactory resistance to the pathogenic effects of *A.caninum* infection in dogs when measured in terms of haematological, clinical and coprological changes.

Successful attempts were made by several workers from the beginning of this century to study the effect of ultraviolet (UV) rays on the infective stages of many nematodes and also the effect of UV-irradiated larvae on the experimental hosts (Stevens, 1909; Holf, 1932; Wright and McAllister, 1934; Shalimov, 1936; Jones *et al*, 1940; Spindler, 1940; Hollaender *et al*, 1940; Jones and Hollaender, 1942, 1944). Much of the earlier work was done with *Trichinella spiralis*. Stevens (1942) observed that exposure to ultraviolet radiation prevented the normal development of *T.spiralis* larvae and that the female worms developed after small

doses of irradiation were found sterile. Keeling (1960) attenuated the infective larvae of H.brasiliensis with various doses of UV-rays which completely inhibited their development to the adult stage. Katiyar et al. (1968) in their study with H.brasiliensis, reported that the subcutaneous injection of ultra violet irradiated larvae gave complete protection to rats against H.brasiliensis infection.

In other helminths particularly trematodes, ultra-violet irradiation was successfully attempted. Standen and Fuller (1959) demonstrated that cercariae of Schistosoma mansoni exposed to UV-rays failed to reach maturity in the mouse. However, the cercariae were able to penetrate the skin and carry out the early stages of development during the tissue phase of migration. Chandour and Webbe (1976) reported the damaging effect of S.mansoni and S.haematobium after exposure to UV-irradiation for a short while. The miracidia when exposed to UV-rays for 60 secs. were too damaged to penetrate the intermediate host snail as reported by Prah and James (1977).

The effect of X-rays and gamma rays from a variety of sources has already been tried in many helminthic infections. The earlier works were carried out on the encysted larvae of T.apicalis by Tyzzer and Honeij (1916), Schwartz (1921), Semrad (1937), Levin and Evans (1940,

1942), Evans *et al* (1941) and Alicata and Burr (1949). Gamberg and Gould (1963) reported that the infective larvae of *T. spiralis* exposed to various doses of Co⁶⁰ irradiation produced protection in rats against the challenge dose of infection.

In the veterinary field, Jarrett *et al* (1969) were the pioneer workers in the successful use of X-irradiated larvae for the immunization of calves against the cattle lung worm-*Dictyocaulus viviparus*. Subsequently Sokolic *et al* (1965) developed an X-irradiated larval vaccine against the sheep, lungworm-*Dictyocaulus filaria*. Urquhart *et al* (1966) demonstrated a high degree of protection against *Haemonchus contortus* in sheep when these were immunised with X-irradiated larvae of *H. contortus*. Similarly, Gregg and Dineen (1978) reported that irradiated larvae of *T. colubriformis* were highly effective immunogens in sheep. Ansari and Singh (1978) stated that vaccination of lambs with 20 to 80 kr gamma-irradiated infective larvae of *Geigeria pachyaeolia* stimulated the development of resistance to subsequent challenge infection as indicated by significant decrease in worm burden.

In filariasis, Wong *et al* (1969) succeeded in developing significant resistance to filarial infection in monkeys after immunising them with X-irradiated larvae

of Brugia pahangi. Later in the year 1974, they successfully immunised dogs using X-irradiated Brugia pahangi larvae. Recently, Rao and his co-workers (1980) reported that the infective larvae of rodent filaria L. carinii when exposed to gamma rays at 38-40 kr conferred high degree of protection on albino rats against filarial infection.

The method of attenuation by radiation was studied experimentally by many investigators for immunisation against cestode parasites. Urquhart et al (1963) stated that radiation attenuated eggs and oncospheres were shown to induce protection against cestode infection. This was confirmed later by Wickerhauser et al (1971) against cestode infection of Taenia saginata and Taenia hydatigena in the intermediate host. Movsesijan et al (1968) demonstrated that dogs were protected against a challenge infection of Echinococcus granulosus by immunisation with brood capsules exposed to X-irradiation.

In trematodes, cercariae and schistosomulae were successfully attenuated with gamma rays and X-rays and immunization of experimental animals with such attenuated larvae had produced various degrees of resistance to subsequent challenge (normal) cercariae. Taylor et al (1976) during their experiments with Schistosoma mathesi

had shown that immunization of sheep with gamma irradiated cercariae or schistosomulae of S. mathaei induced a high degree and duration of resistance against normal S. mathaei cercarial challenge infection. Similarly, Hussein and Bushara (1976) immunised sheep with irradiated S. bovis cercariae but Taylor et al (1979) observed that irradiated S. bovis schistosomulae gave better protection to sheep against S. bovis infection. Many investigators had immunized mice with gamma irradiated S. mansoni and found tremendous protection against infection (Vilella et al, 1961; Radke and Sudan, 1963; Perlowagora-Szumwicz and Oliver, 1963; Perlowagora-Szumwicz, 1964; Erickson and Caldwell, 1965). High levels of resistance were obtained in rhesus monkeys after vaccination with irradiated S. japonicum cercariae (Sadun et al, 1964; Hsu et al, 1965) but not in chimpanzees (Hsu, 1970).

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental animals

Healthy male hamsters of 45-50 gms body weight were obtained from the Haffkine Bio-Pharmaceutical Corporation Ltd. They were kept in groups of ten in metallic cages (40 x 27 x 24 cms), and were fed with pellet food and water ad libitum.

Anoxylostoma ceylanicum strain, obtained from Ciba-Geigy Research Centre, Bombay, was maintained in the laboratory of Haffkine Institute by serial passage in hamsters.

Culture technique

Fresh infective third stage larvae were obtained from the faecal samples of experimentally infected hamsters by petridish culture method of Sen et al (1965). Faecal sample containing hookworm eggs was made into a paste with water and then thoroughly mixed with sterilized sand in 1:3 proportion. The mixture was transferred to a clean petridish with two layers of moist filter paper. The petridish was placed in a bigger petridish containing culture water (2 gms. NaCl; 0.1 ml HCl in 1000 ml distilled water adjusted to pH 5.0). The whole apparatus was kept in dark for 6-10 days

when most of the third stage larvae had migrated into the culture water.

Preparation of inoculum

The larvae collected from the culture water were washed several times in normal saline. From this larval suspension 0.1 ml volume was pipetted out on glass slides and the larvae were counted under the dissection microscope. The suspension was adjusted to contain the required dose of larvae per 0.5 ml.

Selection of dose inoculum

Six groups of hamsters having an average body weight of 45 gms were infected orally with a dose of 50, 100, 150, 200, 500 and 1000 infective larvae of A. geylanicum. The infected animals were kept under observation for a period of 30 days. The animals infected with 50 larvae survived the infection period. Ten per cent mortality was observed in the animals which received 100 larval dose and mortality further increased to 60% at 150 larval dose and finally to 100% in the rest of the animal groups receiving 200, 500 and 1000 larval doses within a period of 20 days. The infected animals receiving a dose of 100 larvae showed a clear and better picture of pathogenicity throughout the observation period and hence this

larval dose was selected as standard inoculum for the experimental studies.

Experimental *A.ceylanicum* infection to hamsters

A group of 50 male hamsters were infected orally, each with 0.5 ml of the saline suspension containing 100 infective larvae. A separate group of 50 hamsters was kept without infection to serve as control. The animals were observed for a period of 200 days post infection. Six hamsters from infected and uninfected groups were necropsied under ether anaesthesia at intervals of 13, 20, 30, 60, 100, 150 and 200 days. Body weight of the animals was recorded prior to necropsy. Blood was collected aseptically by cardiac puncture from the necropsied hamsters for measuring the haemoglobin level in blood and serum was separated under sterile conditions for serological studies. Peripheral blood smears were also prepared at each interval for differential leukocyte count. After carefully dissecting the gastrointestinal tract in warm saline, the adult worms of *A.ceylanicum* were collected and counted from individual hamsters.

Preparation of antigen

Fresh active adult worms were recovered from

infected hamsters 17-20 days post infection. These were washed thoroughly first with normal saline and then with phosphate buffered saline pH 7.2. Worms were sonicated in an ultrasonicator in ice cold bath. This suspension was centrifuged at 3000 rpm for 30 minutes at 4°C. The supernatant after estimation of the protein content of 1.5 mg/ml (Lowry et al, 1951) was stored at -20°C until use.

Agar-gel diffusion test (AGD)

The agar-gel diffusion test was performed according to the method of Ouchterlony (1958). The test was carried out on clean glass slides (7.5 x 2.5 cms) using 1% Noble agar (Difco make) dissolved in normal saline. 3.5 ml of hot agar was poured on each slide. Wells of 2 mm diameter with 3 mm distance from each other were cut on the agar gel. These slides after filling with antigen and test sera were kept at room temperature in humid condition and were observed for one week until the precipitin reactions were seen prominently. The slides were then washed thoroughly by immersing them in normal saline overnight. Later, slides were dried and stained with 1% Amido black. The excess stain was removed by washing them with 2% acetic acid solution. The slides after drying at room temperature were preserved.

Counterimmunoelectrophoresis test (CIEP)

Counterimmunoelectrophoresis test was carried out as per the procedure followed by Bhopale et al (1980). One per cent agarose (BDH) was prepared in veronal buffer (pH 8.4). Parallel wells of 2 mm diameter spaced 3 mm apart were cut on the agar gel slide (7.5 x 2.6 cms). The wells on the anode side were filled with undiluted test sera and those on the cathode with antigen. The same veronal buffer was used in the electrophoresis tank. The electrophoresis was run at 200 volts at 10°C for one hour. These slides, after removing from the tank were kept at room temperature for about an hour and then washed with several changes of normal saline and kept in the same overnight. These were dried, stained with 1% Amido black and cleared with 2% acetic acid solution. The slides were later dried at room temperature and preserved.

Indirect haemagglutination test (IHA)

Indirect haemagglutination test was performed to detect the antibodies in the serum as per the methods described by Kagan and Norman (1974) with slight modifications. Sheep red blood cells (SRBCs) employed for the test were collected in citrated saline. 2.5% SRBCs were tanned with 1:80,000 tannic acid. All the test

sera samples collected at various intervals during an infection period of 200 days were de complemented by incubating them at 56°C in a waterbath for 30 minutes. The tanned cells were sensitized with equal volume of A.caylonicum adult worm antigen, the optimum dilution (1:20) of which was determined earlier by block titration.

The test was carried out in U-shaped microtitre plate. Serial dilutions of the inactivated sera were made in 1% normal rabbit serum. 0.025 ml of 1% suspension of antigen treated cells was added to each well containing 0.05 ml of diluted serum. Negative serum control, tanned cell control and sensitized cell control were also put up along with the test sera samples. The plate, after shaking well was incubated at 37°C for 1 hour and then at 10°C overnight. The results were read according to the pattern of the SRBCs at the bottom of the wells. A positive reaction was indicated by a mat or carpet of the SRBCs with the edges folded in case of strong reaction. The cells settled to form a compact button indicated a negative reaction. The test sera showing an antibody titre at 1:32 or above was considered as positive.

Serum passive immunisation

Healthy hamsters of 45 gms average body weight were infected subcutaneously with a dose of 100 infective larvae of A.sylvaticum at weekly intervals for three weeks. These hamsters were sacrificed after a week of the last dose of infection and the blood was drawn aseptically by cardiac puncture using 5 ml syringe. Serum was separated and pooled under sterile conditions and then stored at -20°C until use. Similarly sera samples collected from normal hamsters were pooled and stored at -20°C .

Initially a group (A) of five hamsters was injected subcutaneously with 1 ml of immune serum prepared by repeated infections of hamsters along with the control group (A_1) which received 1 ml of normal serum per hamster by the same route. Both A and A_1 groups of hamsters were challenged orally with a dose of 1000 normal infective larvae on the same day as per the method of Kwa et al (1978). Mortality upto 40% and 60% was observed in the groups A and A_1 respectively between 13 and 20 days post challenge infection. The surviving animals were sacrificed 20 days after the challenge infection.

Passive transfer of serum by intramuscular and intraperitoneal routes was also attempted. Six groups of hamsters in each were passively immunised with serum as follows: Group B was injected subcutaneously, Group C intramuscularly and Group D intraperitoneally with 1 ml immune serum per animal. The corresponding control groups B₁, C₁ and D₁ received 1 ml normal serum per hamster by subcutaneous, intramuscular and intraperitoneal routes respectively. As the mortality rate was high in the first two groups (A and A₁) due to the lethal challenge dose of 1000 larvae at the preliminary experiment, the groups B, C, D, B₁, C₁ and D₁ were challenged orally with a dose of 100 normal infective larvae. The worm count from the recipient hamsters were recorded individually on necropsy after 20 days post challenge infection.

Ultra-violet Irradiation of A.sevianicum larvae

The source of ultraviolet for the irradiation was a three feet long ultraviolet tube having a wavelength of 390 nm. The third stage larvae collected from the petridish culture methods were washed thoroughly with normal saline. The larval suspension was adjusted to contain 1000 larvae per ml of normal saline. Three ml

of larval suspension containing 3000 larvae were taken in a small clean petridish (50 x 17 mm) and were exposed to ultraviolet rays from ultraviolet tube at a distance of 30 cms. The distance of 30 cms between the larvae to be exposed and the source of irradiation was kept constant for various times of exposure. Healthy hamsters of 55 gms average body weight were used for the UV-irradiation experiments.

Four different experiments were set up to assess the efficacy of UV-irradiated A.ceylanicum larvae.

1. Effect of the time of exposure to UV-rays on the infective larvae in terms of subsequent worm establishment

Six groups of hamsters, 5 in each group were given oral infection each with a dose of 100 UV-irradiated larvae exposed for various UV-irradiation periods of 20 seconds, 40 seconds, 60 seconds, 15 minutes, 30 minutes and 60 minutes. Another group of 5 hamsters was similarly infected each with a dose of 100 normal larvae (without any exposure to UV-rays) which served as control. All the animals were kept under observation for a period of 20 days. Animals were then sacrificed by ether anaesthesia and the worm recovery was

made from the gastrointestinal tract of each hamster.

2. Immunogenic effect in hamsters of a single oral vaccination with UV-irradiated larvae followed by challenge infection

This experiment was designed to assess the immunogenic efficiency of a single dose oral vaccination with UV-irradiated larvae exposed to different time intervals following a challenge infection with normal larvae. This was assessed in terms of the establishment of adult worms in the gastrointestinal tract, when necropsied. This experiment was further divided into two categories; one with low challenge dose and the other with high challenge dose of larval infection.

(a) Four groups of hamsters without prior exposure to any infection were used for the experimental purpose. The number of animals taken is as shown in table 5. The animals were infected orally each with a dose of 100 UV-irradiated larvae. Group A received larvae exposed for 60 seconds; Group B for 15 minutes, Group C for 30 minutes and Group D for 60 minutes of UV-irradiation. Group E animals were infected each with 100 normal infective larvae on the

same day which served as control group. All the five groups of hamsters were challenged after 15 days with 100 normal larvae of A. ceylanicum. On the same day, another group (F) of 5 hamsters was also given an oral infection of 100 normal infective larvae to serve as challenge control. All the six groups of animals were necropsied on the 20th day post challenge infection and worms were counted from the individual animal.

- (b) In this category of experiment, same number of animal groups were offered UV-irradiated larvae exposed for different time intervals, i.e. Group A₁ (60 seconds), B₁ (15 minutes), C₁ (30 minutes), D₁ (60 minutes) and E₁ (non-irradiated) were infected similarly as mentioned in the previous experiment. All the five groups along with the group F₁ (challenge control) were challenged each with a lethal dose of 1000 normal infective larvae. This challenge dose was selected with a view to study the immunogenic potency of UV-irradiated larvae against a heavy challenge exposure of larval infection. Those animals that survived the

challenge infection were sacrificed under ether anaesthesia and the worm count from the gastrointestinal tract of the individual hamster was recorded. The worm recovery made from the dead animals during the observation period was also included in the results.

Based on the necropsy worm burdens from the challenge inoculation, the percent protection was calculated using the following equation:

$$\frac{\text{Mean challenge control worm burden} - \text{Mean vaccinate worm burden}}{\text{Mean challenge control worm burden}} \times 100$$

3. Effect of route of administration of UV-irradiated larval vaccine

This experiment was set up to determine the immunogenic effect of oral and subcutaneous routes of inoculation of UV-irradiated larvae (exposed for 15 minutes) along with their respective controls in terms of adult worm establishment following a challenge infection of normal larvae.

Two groups of animals A and B, five in each group, were inoculated with a dose of 100 UV-irradiated larvae (15 minutes exposure) by oral and subcutaneous routes

respectively. Groups C and D each consisting of 5 animals, were infected by similar routes respectively each with a dose of 100 normal infective larvae. All the four groups of animals were challenged orally each with 100 infective larvae after 15 days of vaccination. These animals were then sacrificed 20 days post challenge infection for the recovery of adult worms from the gastrointestinal tract.

4. Pathogenic effect of single oral dose of UV-irradiated larval vaccine

The present experiment was aimed at the study of the effect of a single oral vaccination with 15 minutes (standardised) UV-irradiated larvae on the haemoglobin level and egg load of the vaccinated animals prior to and after the challenge normal larval infection.

Two groups A and B of 5 hamsters in each were inoculated orally. Group A with a dose of 100 UV-irradiated larvae exposed for 15 minutes and Group B with a same dose of normal (non-irradiated) larvae of *A. ceylanicum*. Both the groups of vaccinated hamsters (A and B) were challenged after 15 days with a dose of 100 normal larvae along with a challenge control group (C) of hamsters which had no previous infection. The haemo-

globin levels of the UV-irradiated and control vaccinee were estimated by Sahli's method at intervals of 13, 20 and 30 days after vaccination and also the challenge infections. Similarly the egg loads in both the vaccinees calculated by Stoll's (1920) technique were recorded before and after the challenge infection. The haemoglobin level and egg count of the challenge control (Group C) were calculated after the challenge infection.

STATISTICAL ANALYSIS OF DATA

The data obtained from various experiments for measuring body weight, haemoglobin levels, worm load and protective immunity in irradiated and non-irradiated larval vaccinees against challenge controls were calculated for standard deviation (SD). To test whether the variances between two groups are equal or not, 'F' test was applied. For equal variances, unpaired 't' test (2 tailed) was used. Behrens test was applied for unequal variances (Armitage, 1977).

RESULTS

RESULTS

During the observation period of 200 days, there was 10% mortality in the infected hamsters between 13 and 60 days post infection. Six hamsters from both the *A. ceylanicum* infected and control (uninfected) groups were necropsied at various intervals of 13, 20, 30, 60, 100, 150 and 200 days. The infection in hamsters was characterized by a significant loss in body weight during the period between 13 and 60 days. The average body weight of the infected hamsters recorded on day 30 was 52.0 ± 3.63 gms. as compared to 62.2 ± 4.34 gms in the control groups. The average body weight of infected hamsters (58.3 ± 8.5 gms) was significantly low than that of the uninfected hamsters (72.0 ± 5.23 gms) on the day 60 ($p < 0.01$) (Figure 1; Table 1). Both the infected and control animals steadily gained weight during the observation period between 60 and 200 days post infection.

The experimental hamsters on necropsy during 13 - 200 days period of infection yielded adult hookworms from the gastrointestinal tract. The worm recovery from the intestine was expressed in terms of percentage of the larvae infected to the animals. The

worms thus recovered were mature, active and reddish in colour with blood inside. On the day 13, an average per cent worm load of 51.1 ± 5.25 was recovered from the infected hamsters. The average per cent worm load was ranging between 51.1 ± 5.25 and 18.0 ± 4.43 during the period between 13 and 60 days. The adult worms persisted in the intestine of infected animals till 160 days (1.0 ± 0.45). No worm recovery was made on the day 200 (Figure 2; Table 2).

The primary pathogenic symptom of *A.ceylanicum* infection was anaemia which was measured by the haemoglobin level in the blood of hamsters (Figure 3; Table 3). The haemoglobin concentration of normal hamsters prior to the infection was 18.02 ± 0.56 gms %. When these animals were infected with *A.ceylanicum* larvae, there was no remarkable change in the haemoglobin values for one week and when the larvae reached the adulthood, there was a decrease in the haemoglobin level due to the blood sucking habits of the adult worms. Thus, on the day 13 post infection, the average haemoglobin recorded in the infected hamsters was 12.09 ± 0.71 gms % as compared to 18.8 ± 0.67 gms % in the control hamsters. The haemoglobin level decreased gradually in infected animals on subsequent days and reached a minimum of

8.2 ± 0.31 gms % on the day 30 when compared with 20.4 ± 0.76 gms % of haemoglobin shown by the controls ($p < 0.01$). A gradual increase in haemoglobin was observed in the infected animals from the day 60 onwards and was within normal range between 100 and 200 days of infection period.

The differential leukocyte count in both infected and control hamsters did not reveal any significant difference during a period of 200 days post infection. ($p > 0.05$).

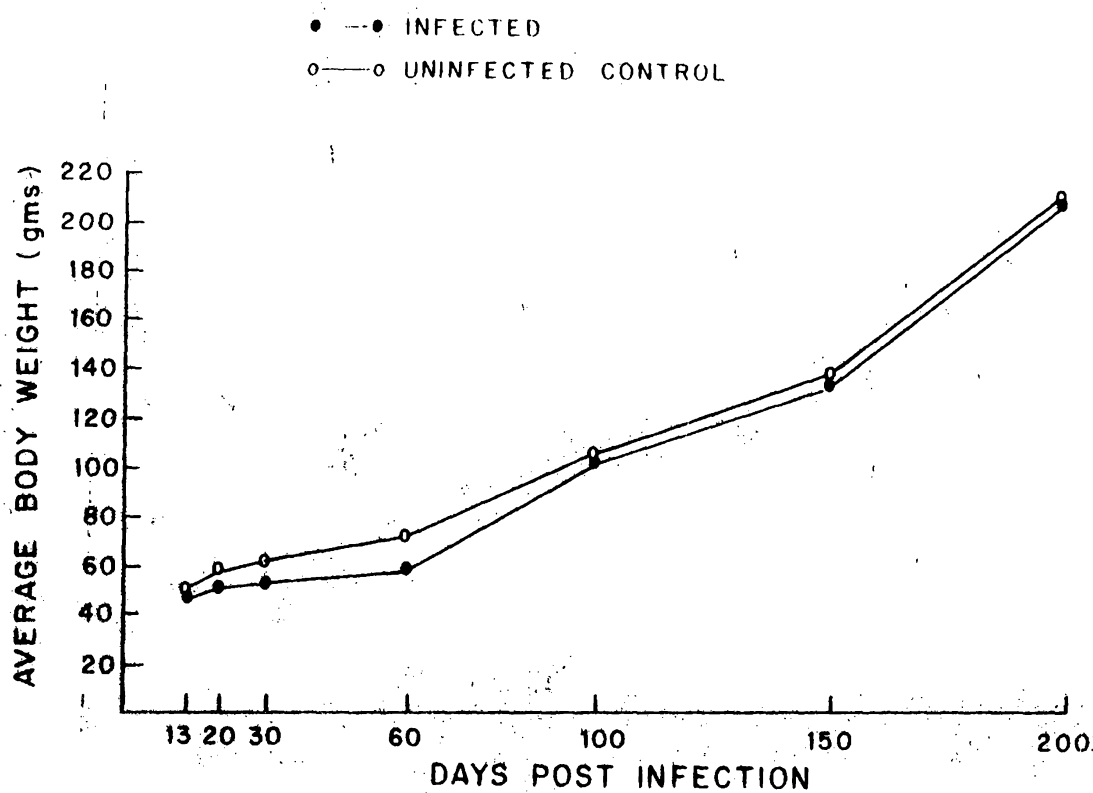


Figure 1 : Body weight of normal and A. ceylanicum infected hamsters.

Table 1

Body weight of hamsters infected with
Ancylostoma ceylanicum

Day of Infection	Body weight (gms.)	
	Infected hamsters (Average $\pm$ S.D.)	Control hamsters (Average $\pm$ S.D.)
13	47.0 $\pm$ 3.22	50.2 $\pm$ 2.37
20	51.1 $\pm$ 3.50	58.0 $\pm$ 4.52
30	52.0 $\pm$ 3.63	62.2 $\pm$ 4.34 (P < 0.01)
60	58.3 $\pm$ 8.50	72.0 $\pm$ 8.23 (P < 0.01)
100	102.2 $\pm$ 9.32	104.1 $\pm$ 6.84
150	133.0 $\pm$ 5.23	138.2 $\pm$ 9.26
200	208.0 $\pm$ 13.84	210.0 $\pm$ 10.86

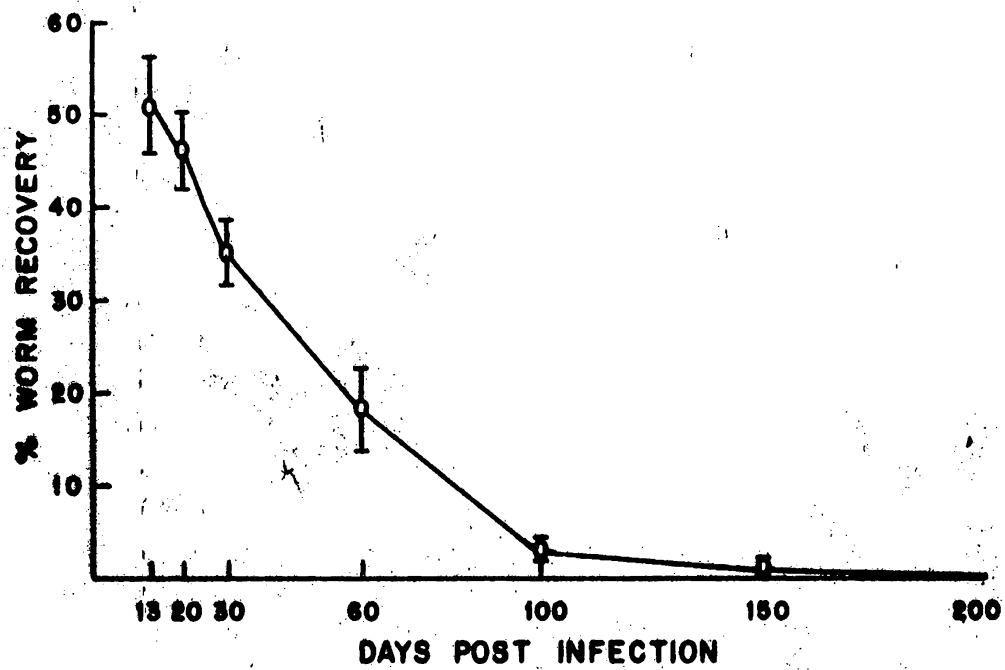


Figure 2 : Recovery of A. ceylanicum worm from infected hamsters.

Table 2

Worm load in Ancylostome ceylanicum infected
hamsters

Day of infection	No. of hamsters necropsied	Worm load (Average $\pm$ S.D.)
13	6	51.1 $\pm$ 5.25
20	6	46.0 $\pm$ 4.24
30	6	35.1 $\pm$ 3.55
60	6	18.0 $\pm$ 4.43
100	6	3.3 $\pm$ 1.26
150	6	1.0 $\pm$ 0.45
200	6	-

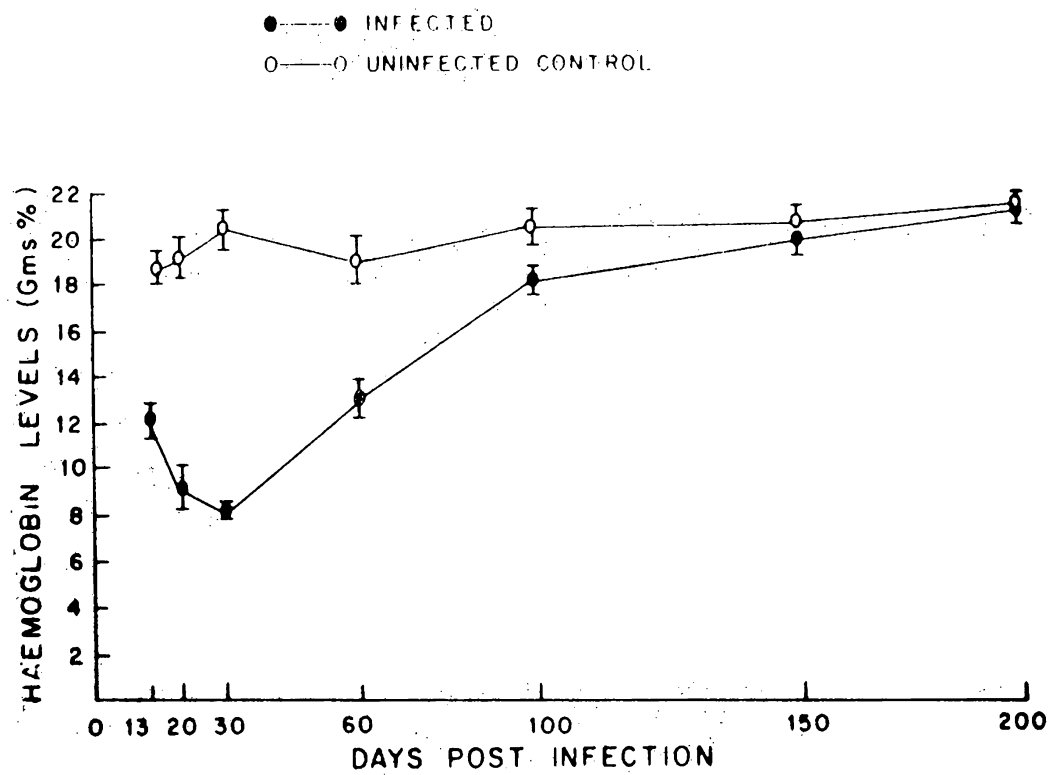


Figure 3 : Haemoglobin level (gm%) in A. ceylanicum infected and uninfected control hamsters.

Table 3

Haemoglobin level in *Ancylostoma ceylanicum*
infected and control hamsters

Day of infection	Haemoglobin (gms %)	
	Infected hamsters (Average $\pm$ S.D.)	Control hamsters (Average $\pm$ S.D.)
13	12.09 $\pm$ 0.71	18.8 $\pm$ 0.67 $P < 0.01$
20	9.20 $\pm$ 0.97	19.2 $\pm$ 0.83 $P < 0.01$
30	8.20 $\pm$ 0.31	20.4 $\pm$ 0.76 $P < 0.01$
60	13.00 $\pm$ 0.81	19.0 $\pm$ 1.04 $P < 0.01$
100	18.10 $\pm$ 0.56	20.6 $\pm$ 0.63
150	20.00 $\pm$ 0.86	20.6 $\pm$ 0.63
200	21.00 $\pm$ 0.61	21.4 $\pm$ 0.47

Agar-gel diffusion test

The sera samples collected on the 30th and 60th day post infection showed precipitin reactions by agar gel diffusion when tested against adult *A. naxianicum* antigen (Figure 4; Table 4).

Counterimmunoelectrophoresis test

The precipitating antibodies were detected by CIEP test in the sera of infected hamsters from the day 30 onwards until the day 150 post infection (Figure 4; Table 4).

Indirect haemagglutination test

The sera samples collected at various intervals of infection period of 200 days were tested by IHA to detect the presence of circulating antibodies in hamsters due to *A. naxianicum* infection. Those sera samples showed a titre of 1:32 and above were considered as positive. A positive titre of 1:32 was first obtained in the sera of infected hamsters on day 20. The titre remained steady on the 30th day and reached a maximum of 1:128 on the day 60. The antibody titre declined gradually on subsequent intervals and became undetectable from the day 100 onwards (Figure 4; Table 4).

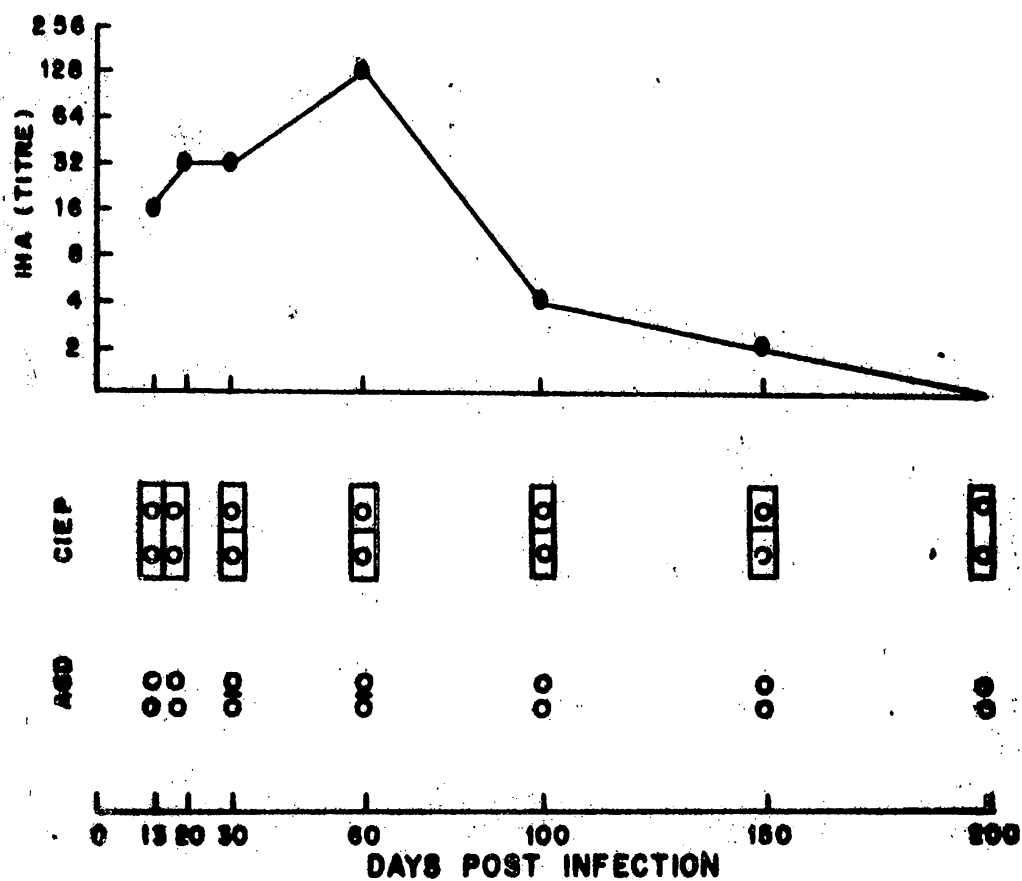
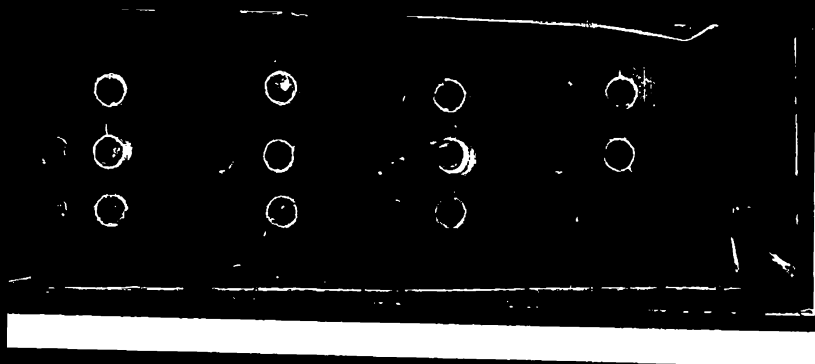


Figure 4 : Serological responses due to
A. ceylanicum infection in hamsters.

Table 4.

Worm load in Ancylostoma ceylanicum infected
hamsters and Serological responses

Day of infection	Worm load (Average $\pm$ S.D.)	AGD test	CIEP test	IHA titre
13	51.1 $\pm$ 5.25	-	-	-
20	46.0 $\pm$ 4.24	-	-	1:32
30	35.1 $\pm$ 3.55	+	+	1:32
60	18.0 $\pm$ 4.43	+	+	1:128
100	3.3 $\pm$ 1.26	-	+	1:4
150	1.0 $\pm$ 0.45	-	+	1:2
200	-	-	-	-



re showing antigen-antibody precipitin reaction
counterimmunoelectrophoresis slide.

Serum passive immunization

Results of worm recovery from the passively transferred immune serum recipient hamsters along with controls were shown in table 4. Mortality of 40% and 60% was observed in the serum recipient animals from the groups A and A₁ respectively within 20 days of post challenge infection with a dose of 1000 normal larvae. Those hamsters (Group A) that received immune serum subcutaneously, had an average worm recovery of 393.0 ± 64.84 corresponding to the control (Group A₁) with an average number of 428.0 ± 60.34 worms ($p > 0.05$). The mean worm burden in the Group B and B₁ (subcutaneously injected) after a challenge infection of 100 larvae was 46.0 ± 4.24 and 47.1 ± 4.42 respectively.

The hamsters (Group C) which received immune serum intramuscularly followed by a challenge of 100 larvae orally on the same day showed an average number of 42.0 ± 3.16 adult worm recovery on necropsy. The control animals Group C₁ immunised with the normal serum by the same route had an average worm burden of 46.4 ± 5.68 (Table 5).

The average worm count of 43.0 ± 6.67 recovered from the hamsters (Group D) passively immunized with immune serum intraperitoneally, was not significantly different from the worm recovery made from the control animals of group D₁ (47.0 ± 4.42) that received normal serum ($p > 0.05$). Thus the results confirmed that the passive transfer of immune serum by either subcutaneous or intramuscular or intraperitoneal routes did not produce any remarkable resistance in hamsters against a challenge infection of A.ceylanicum.

Table 15⁵

The effect of transfer of immune serum by different

routes on worm load

Group	No. of hamsters	Treatment	Route of serum inoculation	Challenge dose	Mean worm burden $\pm$ S.D.	
A	5	Immune serum	Subcutaneous	1000	393 $\pm$ 64.84	$P > 0.05$
A ₁	5	Normal serum	Subcutaneous	1000	428 $\pm$ 60.34	
B	5	Immune serum	Subcutaneous	100	46.0 $\pm$ 4.24	$P > 0.05$
B ₁	5	Normal serum	Subcutaneous	100	47.1 $\pm$ 4.42	
C	5	Immune serum	Intramuscular	100	42.0 $\pm$ 3.16	$P > 0.05$
C ₁	5	Normal serum	Intramuscular	100	45.4 $\pm$ 5.68	
D	5	Immune serum	Intraperitoneal	100	43.0 $\pm$ 6.67	$P > 0.05$
D ₁	5	Normal serum	Intraperitoneal	100	47.0 $\pm$ 4.42	

Ultra-violet irradiation of A.caylanicum larvae

1. Effect of the time of exposure to UV-irradiation on the infective larvae in terms of subsequent worm establishment

This experiment was designed to determine the effect of UV-irradiation on the infective larvae of A.caylanicum and the subsequent adult worm burden in the host.

The infective larvae of A.caylanicum prior to the exposure to UV-rays were actively motile but the motility was found to be reduced when they were exposed for a longer period. The motility of the larvae was not much affected when they were exposed to UV-rays for 20 and 40 seconds but they became sluggishly motile at 60 seconds exposure. After 15 minutes to UV-exposure almost all the larvae were found in coiled form with sluggish motility. All the larvae became non motile at 30 and 60 minutes UV-exposure.

Table 5 lists the average worm burdens at necropsy in the different groups of hamsters infected with A.caylanicum larvae exposed for 20 seconds, 40 seconds,

60 seconds, 15 minutes, 30 minutes and 60 minutes. The UV-irradiated larvae with an exposure for 20 seconds when inoculated orally to fresh hamsters, the average number of adult worms recovered from the intestine after 20 days was 22.0 ± 7.31 followed by 15.0 ± 3.39 and 2.8 ± 1.64 with 40 and 60 seconds exposures to UV-irradiation respectively. The average recovery made from the animals infected with larvae irradiated for 15 minutes was only 1.0 ± 0.71 . However, no adult worms were recovered from the animals after 30 and 60 minutes exposure to UV-irradiation. On the other hand, the control groups that were infected with normal larvae showed an average worm count of 41.0 ± 8.37 . The results indicated that as the time of UV-exposure of the larvae was prolonged, the infectivity as measured by adult worm establishment in the intestine of hamsters was progressively reduced. The worm count was significantly less in the UV-irradiated animals than in the controls ($p < 0.01$) (Figure 5; Table 6).

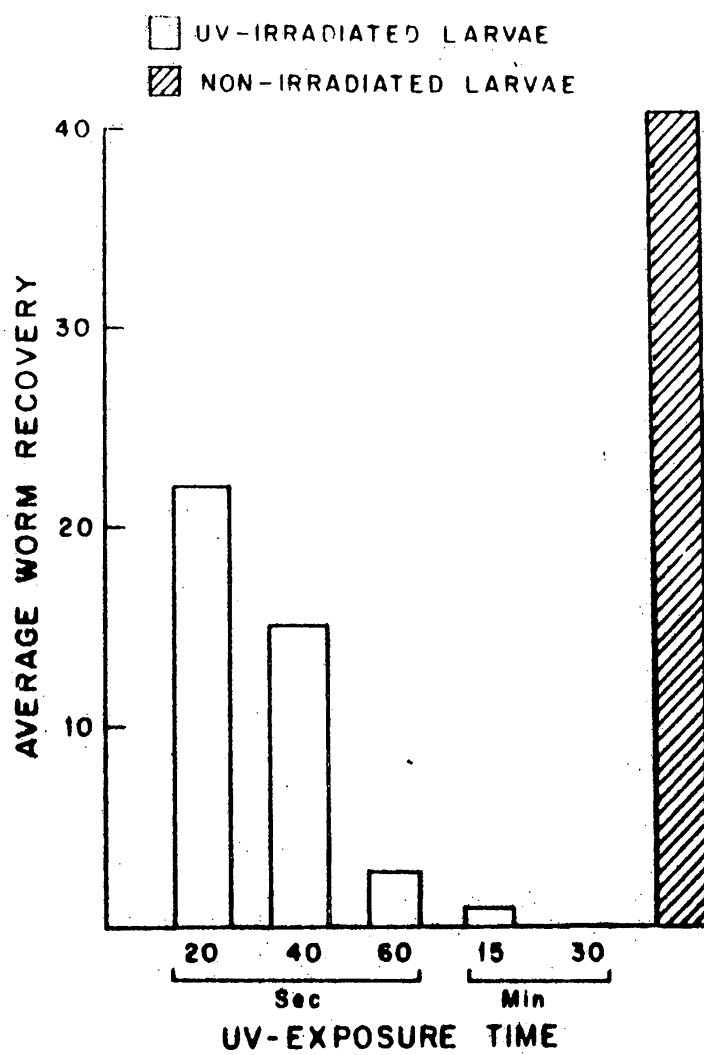


Figure 5 : The effect on the development of the UV-exposed A. ceylanicum larvae into adult worms.

Table 6

The effect on the development of the UV-exposed
A. ceylanicus larvae into adult worms

Group	No. of hamsters	UV-irradiated larvae (exposure time)	Mean worm burden $\pm$ S.D.
A	5	100 (20 secs.)	22.0 $\pm$ 7.31
B	5	100 (40 secs.)	15.0 $\pm$ 3.39(P < 0.01)
C	5	100 (60 secs.)	2.8 $\pm$ 1.64(P < 0.01)
D	5	100 (15 mins.)	1.0 $\pm$ 0.71(P < 0.01)
E	5	100 (30 mins.)	-
F	5	100 (60 mins.)	-
G (control)	5	100 (normal)	41.0 $\pm$ 8.37

2. Immunogenic effect in hamsters of a single oral vaccination with UV-irradiated larvae followed by challenge infection

The immunogenic efficiency of a single vaccination with UV-irradiated A. coylei larvae exposed to different time intervals was assessed by the adult worm establishment in the intestine after giving a challenge dose with normal larvae as shown in Figure 6.

a) Table No.7 shows the average worm burden in different groups of hamsters given infection larvae exposed for different periods to UV-rays followed by the challenge dose of the normal larvae. Group A (60 seconds UV-exposure) showed an average worm burden of 2.0 ± 1.0 followed by Group B (15 minutes UV-exposure) with only 0.6 ± 0.89 worm count thereby indicating a protection in terms of worm reduction of 99% against the challenge infection. An average number of 1.8 ± 0.84 and 5.2 ± 3.11 worms was obtained from the Groups C and D respectively. Group E animals which received normal larval infection had comparatively a high worm count (16.2 ± 5.70). The results indicated that all the UV-irradiated larval vaccinates had a significantly low worm burden when compared with the average worm load of challenge control of Group F (47.0 ± 8.49).

b) In the second category of experiment the UV-irradiated

and non-irradiated larval vaccinates were challenged with a lethal dose of 1000 infective larvae. The average recovery of adult worms was 6.3 ± 4.01 from the Group A₁ (60 seconds UV-exposure) followed by 3.2 ± 3.43 from Group B₁ (15 minutes UV-exposure). The average worm count of 4.0 ± 1.0 in Group C₁ (30 minutes UV-exposure) and 8.0 ± 4.53 in Group D₁ (60 minutes UV-exposure) was comparatively high. No mortality was observed in any of the UV-irradiated larval vaccinates during the observation period. The normal larval vaccinated animals when challenged with a dose of 1000 normal larvae had an average worm burden of 24.4 ± 9.79 with 20% mortality due to heavy infection. The challenge control (Group F₁) without any prior vaccination showed an average worm load of 68.5 ± 16.96 with 60% mortality within a period of 20 days post challenge (Table 8). The results of the two categories of experiments showed that a single oral vaccination with UV-irradiated larvae exposed for 15 minutes followed by a challenge dose of 100 normal larvae resulted in 99.0% reduction in worm count as compared to the oral vaccination with UV-irradiated larvae of other different time exposure followed by a heavy challenge infection of 1000 normal larvae.

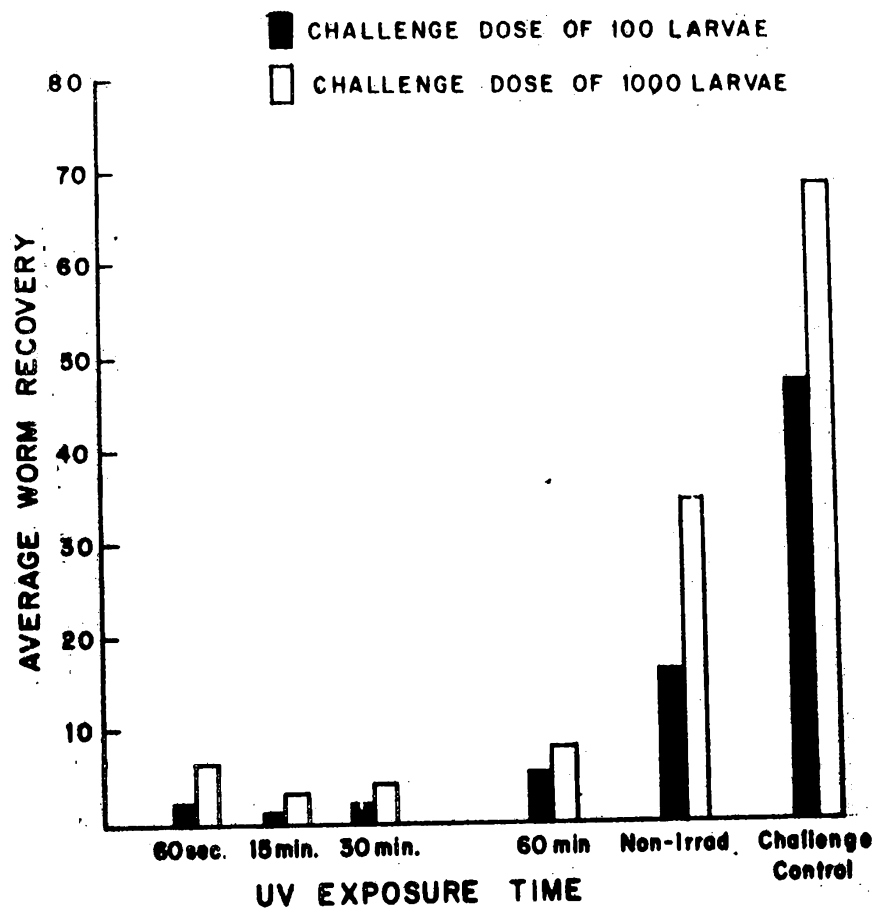


Figure 6 : The effect of orally inoculated UV-irradiated A. ceylanicum larvae following an oral challenge of 100 and 1000 larvae on the subsequent adult worm establishment in hamsters.

Table 7

The effect of orally inoculated UV-irradiated *A. ceylanicus* larvae following an oral challenge of 100 larvae on the subsequent adult worm establishment in hamsters

Group	No. of larvae inoculated (UV-exposure)	Challenge larval dose (Nos)	Mean worm burden $\pm$ S.D.	Per cent protection
A	100 (60 secs.)	100	2.0 $\pm$ 1.0	96 (P < 0.01)
B	100 (15 mins.)	100	0.6 $\pm$ 0.89	99 (P < 0.01)
C	100 (30 mins.)	100	1.8 $\pm$ 0.84	96 (P < 0.01)
D	100 (60 mins.)	100	5.2 $\pm$ 3.11	89 (P < 0.01)
E	100 (Normal)	100	16.2 $\pm$ 5.70	66
F	-	100	47.0 $\pm$ 8.49	-
(challenge control)				

Table 8

The effect of orally inoculated UV-irradiated
A. ceylanicum larvae following an oral challenge
 of 1000 larvae on the subsequent adult worm
 establishment in hamsters

Group	No. of larvae inoculated (UV-exposure)	Challenge larval dose(Nos)	Mean worm burden $\pm$ S.D.	Per cent protection
A ₁	100 (60 secs.)	1000	6.3 $\pm$ 4.01	91 (P < 0.01)
B ₁	100 (15 mins.)	1000	3.8 $\pm$ 3.43	96 (P < 0.01)
C ₁	100 (30 mins.)	1000	4.0 $\pm$ 1.0	94 (P < 0.01)
D ₁	100 (60 mins.)	1000	8.0 $\pm$ 4.53	81 (P < 0.01)
E ₁	100 (Normal)	1000	34.4 $\pm$ 9.79	12
F ₁	-	1000	68.5 $\pm$ 16.96	-
(Challenge control)				

3. Effect of route of administration of UV-irradiated larval vaccine

This experiment was set up to determine the effect of oral and subcutaneous routes of inoculation of UV-irradiated larvae of 15 minutes exposure in experimental hamster groups and was compared with normal larvae in control groups followed by a challenge infection of 100 normal larvae as shown in table 8.

Group A animals inoculated subcutaneously with UV-irradiated larvae and subsequently challenged orally with a dose of 100 normal larvae, yielded an average worm load of 2.80 ± 2.94 which was more or less same when compared with 4.4 ± 2.5 obtained from the orally inoculated and challenged animals. ($p > 0.05$). Similarly, there was no significant difference in the establishment of adult worms in those hamsters which were vaccinated with normal larvae by either subcutaneous (31.0 ± 11.87) or oral routes (32.0 ± 9.72) (Table 9).

Table 2

Immunogenic effect of UV-irradiated (15 minutes exposure) larvae by oral and subcutaneous routes of inoculation followed by an oral challenge of *A. aerlanicum* larvae in hamsters

Group	No. of hamsters	Number of larvae vaccinated	Route	Challenge larval dose (Nos.)	Mean worm burden $\pm$ S.D.
A	5	100 UV-irrad. (15 mins.)	Subcutaneous	100	2.8 $\pm$ 2.94
B	5	100 UV-irrad. (15 mins.)	Oral	100	4.4 $\pm$ 2.51
C	5	100 normal	Subcutaneous	100	31.0 $\pm$ 11.87
D	5	100 normal	Oral	100	32.0 $\pm$ 9.72

$P > 0.05$

$P > 0.05$

4. Pathogenic effect of UV-irradiated larvae (15 minutes exposure) when used as a single oral vaccine

The aim of this experiment was to study the effect of oral vaccination with UV-irradiated larvae (standardized at 15 minutes exposure) on the haemoglobin level and egg load of the vaccinated animals prior to and after the challenge larval infection.

The haemoglobin values of the UV-irradiated and normal larval vaccinated animals at various intervals before and after challenge infection were compared with those of the challenge controls. The haemoglobin values of the irradiated larval vaccinates after vaccination (before challenge) on the day 13th (14.52 ± 0.61 gms %), 20th (15.48 ± 0.69 gms %) and 30th (15.20 ± 0.87 gms %) were markedly higher than those of the normal vaccinates on the corresponding day of 13th (12.09 ± 0.71 gms %), 20th (9.2 ± 0.97 gms %) and 30th (8.2 ± 0.31). ($p < 0.001$) (Figure 7; Table 10). Besides this, there was 10% mortality in the normal vaccinates within 25 days of vaccination.

After the challenge larval infection to hamsters with normal larvae the haemoglobin values of both the irradiated and normal larval vaccinates were showing marked difference when compared with the values of challenge control ($p < 0.001$). The haemoglobin levels

of the irradiated larval vaccinates (Group A) after challenge on the day 13th, 20th and 30th were 14.44 ± 0.79 gms %, 14.80 ± 0.94 gms % and 16.04 ± 1.35 gms % respectively as compared to the values of 13.20 ± 0.76 gms %, 13.04 ± 0.65 gms % and 14.60 ± 0.55 gms % on the corresponding days in the normal vaccinates (Group B) days ($p < 0.05$). The hamsters from the challenge control (Group C) suffered severely from anaemia due to the heavy worm burden with the haemoglobin values of 11.64 ± 0.74 gms % on the 13th day, 9.24 ± 0.59 gms % on the 20th day and 8.28 ± 0.54 gms % on the 30th day. Forty per cent mortality was observed in this Group (C) within 30 days post infection. The haemoglobin values of Group C were significantly lower than those of the groups A and B ($p < 0.001$).

Hookworm eggs were not detected in the pellets of the UV-irradiated larval vaccinated hamsters at any time before the challenge infections whereas in the normal larval vaccinates, hookworm eggs were detected from the day 20 onwards (8,000 eggs per gm). This reached a maximum value of 35,000 on day 30. After the challenge infection, hookworm eggs appeared in the pellets of irradiated larval vaccinates on day 25 with a low egg count of 300 as compared to 18,000 epg as shown by the normal vaccinates and 25,000 epg by the challenge

control animals on the day 25. A maximum eggs of 40,000 per gram was recorded in the challenge control on the day 30 as compared the 800 and 30,000 eggs per gram in the UV-irradiated and normal larval vaccinates respectively (Figure 8, Table 11).

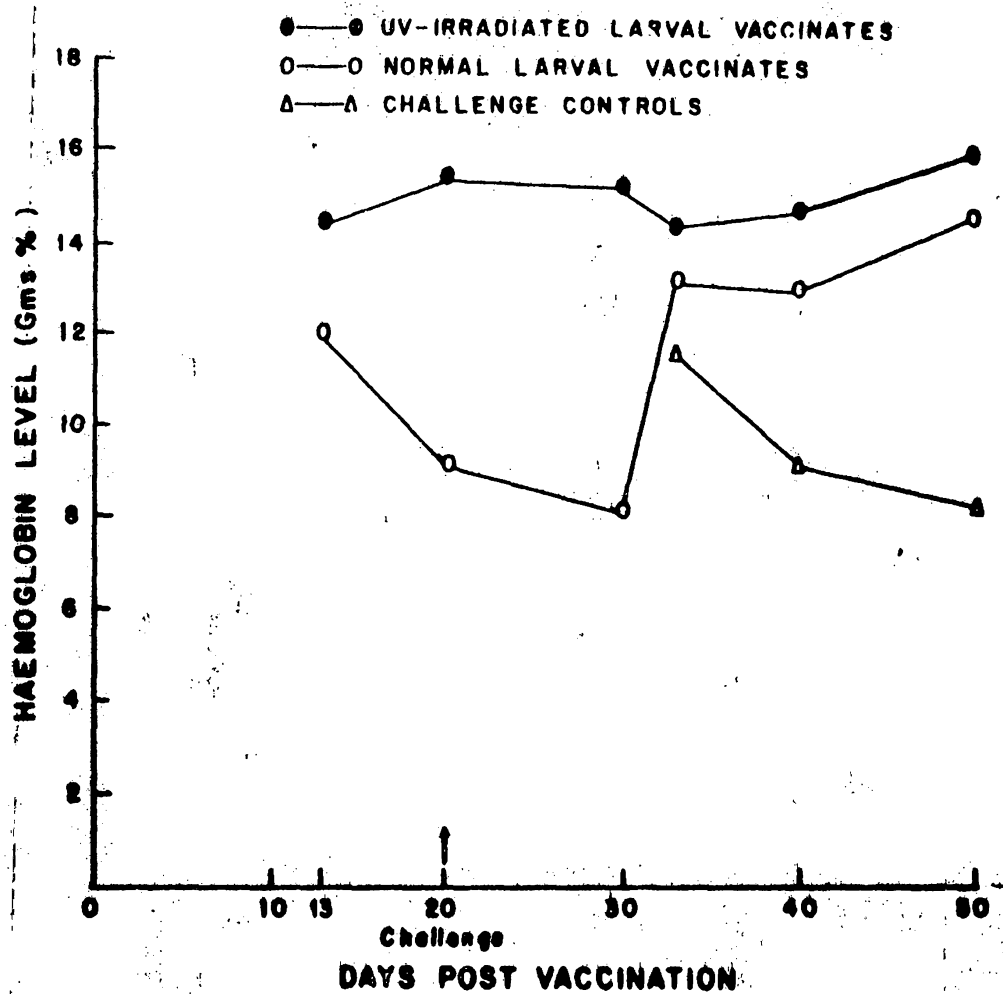


Figure 7: Haemoglobin level after single oral vaccination to hamsters with UV-irradiated A. ceylanicum larvae.

Table 10

Single oral vaccination with UV-irradiated
A.caylanicum larvae in hamsters: Haemoglobin
 levels

Haemoglobin levels (gm %)				
	UV-irradiated (15 mins. exposure) (Group A)	Normal (Group B)	Challenge controls (Group C)	
No. of hamsters	5	5	5	
<u>Day post vaccination</u>				
13	14.52 $\pm$ 0.61	12.09 $\pm$ 0.71		
20	15.48 $\pm$ 0.69	9.20 $\pm$ 0.97		
30	15.20 $\pm$ 0.87	8.20 $\pm$ 0.31		
<u>Day post challenge infection</u>				
13	14.44 $\pm$ 0.79	13.20 $\pm$ 0.76	11.64 $\pm$ 0.74	P < 0.001
20	14.80 $\pm$ 0.94	13.04 $\pm$ 0.65	9.24 $\pm$ 0.59	P < 0.001
30	16.04 $\pm$ 1.35	14.60 $\pm$ 0.55	8.28 $\pm$ 0.54	P < 0.001

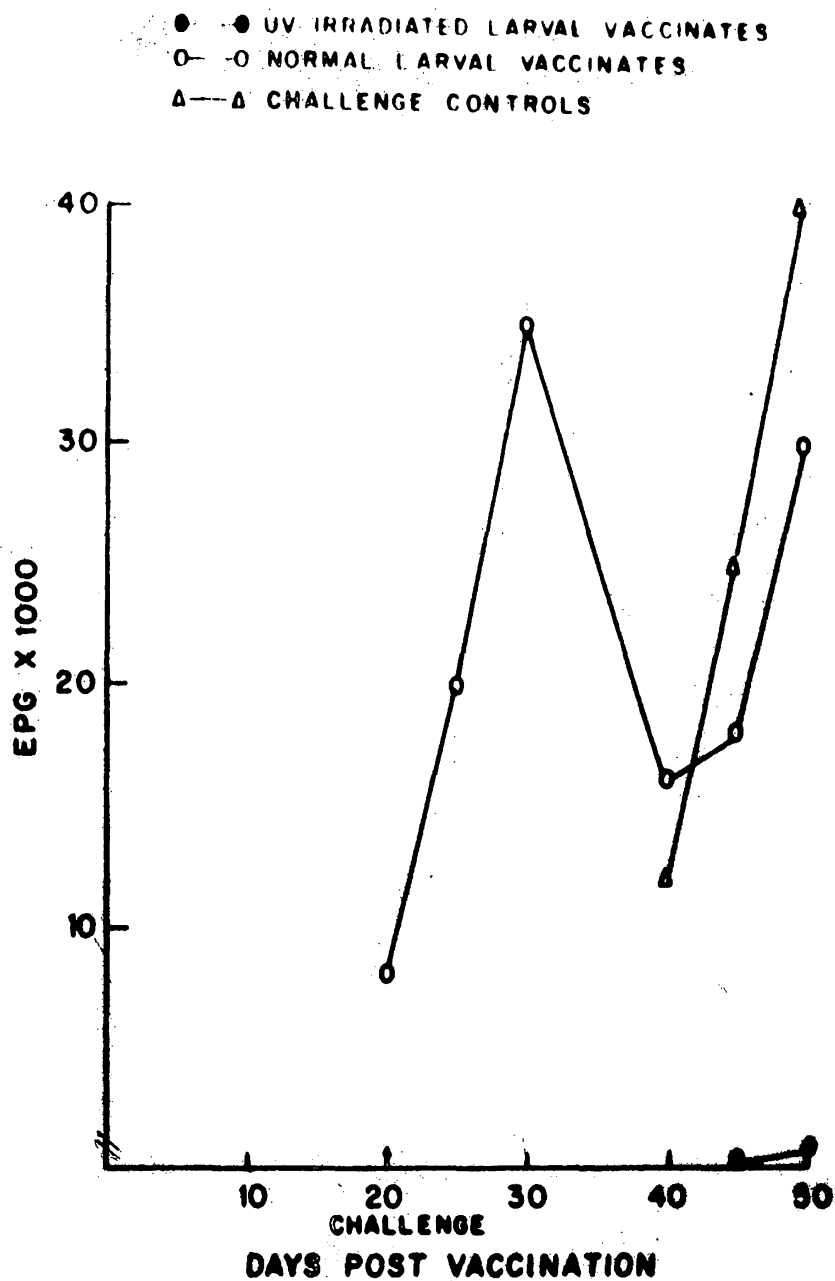


Figure 8 : EPG count after single oral vaccination to hamsters with UV-irradiated A. ceylanicum larvae.

Table 11

Single oral vaccination with UV-irradiated
A. ceylanicum larvae in hamsters: Egg load

		Eggs per gram faeces	
UV-irradiated (15 mins. exposure) (Group A)		Normal (Group B)	Challenge control (Group C)
No. of hamsters	5	5	5
<u>Days post vaccination</u>			
13	-	-	
20	-	8,000	
25	-	20,000	
30	-	35,000	
<u>Day post challenge infection</u>			
13	-	10,000	-
20	-	16,000	12,000
25	300	18,000	30,000
30	800	25,000	40,000

DISCUSSION

DISCUSSION

The results of the present study showed that A. ceylanicum infection causes a significant loss in haemoglobin concentration and body weight in the hamsters particularly during an infection period between 13 and 60 days. It is due to the fact that during 13 days period, the infective larvae attain the adult stage and become vigorous blood suckers by getting themselves attached to the intestinal wall. The adult worm count during this infection period ranges between 51.1 and 185. The average haemoglobin value varied from 8.2 to 13.0 gms % during the infection period between 13 and 60 days with the minimum level of 8.2 gms % on the 30th day. Blood loss due to A. ceylanicum infection in man as well as in dogs has been studied by other workers. In humans, a heavy infection of A. ceylanicum with worm load of 125 and 297 was reported by Anten and Zuidema (1964) in Dutch marines who were found to be anaemic with haemoglobin values of 11.3 and 8.9 gms % respectively. Rep (1966) reported a loss of 0.0137 ml blood per worm from the dogs, experimentally infected with A. ceylanicum, during prepatent period of infection. Later, Areekul et al (1975) estimated an average blood loss of 0.033 ml/worm/day (cutaneous route of infection) and 0.038 ml/

worm/day (oral route of infection) when the dogs were experimentally infected with A.caylanicum.

According to earlier reports of Roche et al (1957), Foy et al (1958), Gilles et al (1964) and Areekul et al (1970 c) the other human hookworms were found to be sucking more blood than A.caylanicum as observed from the blood loss caused by A.guadanaia (0.14 - 0.40 ml/worm/day) and N.americanus (0.01 - 0.09 ml/worm/day). Blood loss caused by A.saninum in dogs was found to be 0.037 - 0.124 ml/worm/day (Roche and Martinez - Torres, 1960 and Areekul et al, 1975). According to Miller, (1968) the haemorrhagic blood loss in pups infected with A.saninum had commenced on the 8th day after infection and the pups suffered from severe dysentery passing fluid faeces with mucous and blood. The findings of Areekul et al (1975) suggested a direct relationship between the size of hookworm species and the amount of daily blood loss caused by each species. Layrisse and Roche (1964) have demonstrated a significant relationship between hookworm infection as expressed in terms of eggs per gram faeces and the prevalence of anaemia. Even moderate hookworm loads could cause sufficient blood loss to precipitate anaemia (Woodruff, 1955; Stoll, 1961). Tasker (1961) showed that in N.americanus infections blood loss varied with

the intensity of the infection from 0.04 ml to 0.1 ml/worm/day. Miller (1968) reported that the mean faecal blood loss figures per worm were approximately ten times more with A. ceylanicum infection than those recorded with A. brasiliense. He also reported a heavy blood loss ranging from 14-24 ml per day in chimpanzees infected with A. duodenale as compared to 60 to 160 ml/day with 800-2000 A. duodenale worms and 15-40 ml with 800-1200 H. americanus worms (Foy and Nelson, 1963) in man.

Layrisse et al (1959) and Foy and Nelson stated that iron deficiency, low levels of folic acid and vitamin B₁₂ were also observed in the serum of hookworm patients and in severe hookworm infection with anaemia and oedema, the serum albumin was reduced and the oedema seemed to be directly related to the hypoproteinaemia. (Brumpt and Ho thisang, 1965). Miller (1968) also observed severe hypoproteinaemia and plasma losses in pups infected with A. caninum.

In the present study there was no remarkable change in differential leukocyte count when compared with that of healthy controls. Areekul et al (1970 b) stated that eosinophils of 58% developed in human volunteers infected cutaneously with A. ceylanicum larvae. Similarly,

when the rhesus monkeys were experimentally infected with *A. vexans*, there was a considerable increase in the eosinophil count (Areekul et al. 1975). A similar situation occurred in hookworm infection in man (Yoshida et al., 1968) and also in other helminthic diseases like *Ascaris lumbricoides*, *strongyloides stercoralis* ~~*stercoralis*~~ *Trichinella spiralis* and *Toxocara canis* where the migrating larvae in lungs caused eosinophilia in their hosts (Jaggi and Vishwanathan, 1976). Bhopale et al. (1980) observed an increase in the eosinophils in the experimentally *N. americanus* infected rabbits. This situation did not occur in hamsters because the *A. vexans* larvae had no lung migration when infected orally to hamsters.

The larvae and/or adult worms recovered from the gastrointestinal tract of infected hamsters on the 3rd and 5th days were of the 4th and early 5th stages of development. On the 7th day, 67% of immature adult worms were recovered from the intestine. The worm recovery was 51% on the day 13 and the number of worms decreased gradually as the infection period progressed. All the worms were expelled out automatically from the host after 160 days. This natural worm expulsion has been explained by several workers while studying the host-parasite relationship. Stoll (1929) attributed

the spontaneous loss of H. contortus worms from sheep to self-cure phenomenon. According to Stewart (1953, 1955) and Soulsby and Stewart (1960), the hyper-sensitivity reactions in the gut due to the allergens released from H. contortus larvae at the third moult caused the worm expulsion. The spontaneous loss of worms is due to self-cure phenomenon after developing immediate hyper sensitivity reaction in the gut (Stoll, 1929; Stewart, 1953, 1955) and thus a powerful, allergen released from larvae at the 3rd moult as in H. contortus (Soulsby and Stewart, 1960).

According to Urquhart et al (1966) Barth et al (1966), Jarrett et al (1968) and Ogilvie and Jones, (1971), the worm expulsion is governed by a two step immunological mechanism which involves both the damage of worms by the antibody and the initiation of anaphylactic inflammation in the gut by worm allergens which initiates the worm expulsion. The causative antibodies are produced entirely within IgE involved in the allergic reactions associated with mucous surfaces (Ogilvie, 1967; Ogilvie and Jones, 1971; Dobson et al, 1971; Catty and Fraser, 1972). Studies on the distribution of IgE producing cells in man and in experimentally infected

rats have revealed a predominance of mast cells at the mucosal surfaces including intestine and local regional lymph nodes (Tada and Ishizaka, 1970) and the lamina propria of small intestine contains (Mayrhofer *et al.*, 1976). The histopathological studies of the infected intestine gave support to the concept of local anaphylaxis. An infiltration of mast cells into the tissues around the site of *T.colubriformis* infection in guinea pigs was studied by Rothwell *et al.* (1974). Increase in the number of mast cells with the onset of worm expulsion was described in the case of *N.brasiliensis* infection in the rat (Wells, 1962; Whur, 1967; Murray, 1972). Local antibodies were demonstrated in the extracts and exudates of intestinal mucosa of cattle infected with *Oesophagostomum radiatum* (Douvres, 1962); Sheep infected with *O.columbianum* (Dobson, 1966, 1967) and in mice infected with *A.caninum* (Bhopale and Kamath 1979).

In the present study, the production of serum antibodies detected by agar gel-diffusion, counterimmuno-electrophoresis and indirect haemagglutination tests showed positive reaction during the patent period of infection from 30 to 60 days. However, Desowitz (1962) was unable to detect antibody by gel-diffusion test

in the sera of children heavily infected with N. americanus. But sera samples from adult patients harbouring N. americanus showed positive reactions with adult worm antigen. Sood et al (1972) during a serological study with hookworm patients failed to detect antibody in sera by circumoval precipitin and gel-diffusion tests. In the present study CIEP test showed positive reaction from the day 30 to 150 post infection. The same test was demonstrated by Hillyer (1976) in Fasciola hepatica infection in rabbits from 4 weeks to 16 weeks. Bhopale et al (1981), on the other hand, revealed a limited application of CIEP test for hookworm infection in experimental animals as well as in human patients. In other helminthiases, CIEP test was proved to be more rapid, sensitive and specific in Trichinella spiralis infection (Despommier et al, 1974), in hydatid diseases (Kelkar and Kotwal, 1978), in Dirofilaria immitis (Desowitz and Una, 1976) and in schistosomiasis (Phillips and Draper, 1978).

The haemagglutinating antibodies detected by IHA in the present study during the infection period between 20 and 60 days showed a high antibody titre of 1:128 on the 60th day post infection. Indirect haemagglutination test has been used earlier by several workers for the immunodiagnosis of hookworm infection. Soulsby

and Gilles (1965) demonstrated haemagglutinating antibodies in hookworm infected children after a lapse of two years. A similar result was obtained by Ball and Bartlett (1969) and Schad *et al* (1977). However, Ball and Bartlett (1969) stated that H. americanus larvae gave rise to immediate type antibody response which was demonstrated by fluorescent antibody test, complement fixation test and prausnitz küstner test. Soulsby (1962) reported that in helminthiases, no convincing evidence exists to show that antibodies detected by numerous serological tests play any functional part in immunity.

In the present study hamsters immunised passively by immuneserum could not be protected against the challenge infection of A. oxyuris when injected either intramuscularly or intraperitoneally or subcutaneously. A modicum of success in serum passive immunisation with A. caninum in dogs was obtained by Otto *et al* (1940). Miller (1967) failed initially in his attempt of passive immunisation of pups using 47 ml/lb serum but succeeded when the immunising dose of serum was increased to 50 ml/lb. Kagan (1970) reported that there was no conclusive evidence that immunity could be conferred by transfer of hyperimmune serum. Serum passive immunisation has been attempted by Barles and

Taliaferro (1936) and Chandler (1938) who demonstrated that rats could be passively immunised against *H. brasiliensis* with large amounts of antiserum obtained from rats given several infections of the parasite. It was reported that serum taken from rats given one infection protected as frequently and strongly as antiserum taken from repeatedly infected rats. Reaginic antibodies although were present in high levels in the antisera were not resistant to infection (Ogilvie, 1967; Ogilvie and Jones, 1968). Protective immunity in the absence of reagins has only recently been demonstrated. (Jones *et al*, 1970).

The results of the experiments on ultraviolet irradiation revealed that the exposure of infective larvae to UV-irradiation affected their motility and infectivity. As the UV-exposure time was prolonged, the motility and the infectivity as measured by the adult worm establishment was progressively reduced. The larvae which were motile at 20 and 40 seconds UV-exposure, had almost lost their motility at 15 minutes exposure to UV-irradiation, and conferred a 99.0% reduction in worm burden of challenge inoculation with a dose of 100 normal infective larvae. When the challenge dose was increased to 1000 larvae, the reduction in worm load recorded was only 95.0%. Much work on

radiation attenuation has been carried out in canine ancylostomiasis. Miller (1971) has successfully prepared a vaccine against A. caninum infection in pups by x-ray irradiation of the larvae. Miller (1965) demonstrated a high degree of resistance (97%) against A. caninum oral infection in dogs of 3 to 6 months old by double subcutaneous vaccination with 40 kr x-irradiated A. caninum larvae. Similarly, Dow et al reported earlier (1969, 1961) that double vaccination of young dogs with 40 kr x-irradiated Uncinaria stenocephala larvae conferred a highly significant resistance of 97.0% to the establishment of homologous species challenge of normal larvae. Recently, Vinayak et al (1981) have attempted a killed and an attenuated vaccine for canine hookworm. For attenuation they used x-ray irradiation and 5-fluorouracil. X-irradiated larval vaccine gave better protection to the dogs than the 5-fluorouracil treated vaccine against the challenge infection. The results of the present study are similar to those of Miller (1965) and Vinayak et al (1981) in the fact that the UV-irradiated vaccine neither eliminates nor completely destroys the larvae of the subsequent oral challenge and thus few larvae reach maturity in the host.

Another observation made in the present study was that there was no significant difference in worm recovery between oral and subcutaneous routes of vaccinations in hamsters with UV-irradiated larvae (18 minutes) followed by oral challenge with normal larvae. On the contrary, Miller (1965) in his studies with *A. caninum* in pups reported that double subcutaneous vaccination of 40 hr x-irradiated *A. caninum* larvae to pups conferred a uniform and significantly greater resistance to challenge hookworm establishment than double oral vaccination with x-irradiated larvae. However, he stated that in terms of resistance to the pathogenic effects of this experimental challenge all the routes of inoculation proved to be equally efficient.

A single oral vaccination with UV-irradiated *A. caninum* larvae in the present study showed a satisfactory resistance to the pathogenic effects of challenge infection as measured by the haemoglobin level and output of egg load in vaccinated hamsters. The haemoglobin values of the vaccinates receiving UV-irradiated larvae prior to the challenge infection were significantly higher than those of the normal larval vaccinates. In the challenge control animals, a severe depression in the haemoglobin level was noted as compared to the haemoglobin level of irradiated and normal larval vacci-

during

nated hamsters post challenge infection. A similar observation was made by Miller (1964, 1965, 1966) when he vaccinated the pups with 40 kr x-irradiated A. caninum larval vaccine. He stated that the pathogenic effects of the worm burdens in dogs exposed with x-irradiated larvae at 40 kr and 60 kr were extremely mild as shown by 10% fall in the haematocrit and haemoglobin. On the other hand, pups infected with normal larvae suffered 25 to 30% reduction in haematological values. The challenge control pups suffered severe blood loss with 50% fall in haemoglobin values. At no stage during the experiment, the pups vaccinated with x-irradiated larvae were affected by their worm burdens while the controls were severely affected with clinical ancylostomiasis. The results of the faecal examinations during the present study did not indicate the presence of hookworm eggs in the faeces of the hamsters which were vaccinated with UV-irradiated larvae prior to the challenge infection in contrast to normal vaccinates during the same period. The appearance of eggs post challenge infection was delayed in the irradiated larval vaccinated hamsters by 8 days (25th day) as compared to 17th day in the challenge controls. These evidences support the view that UV-irradiation could attenuate the A. ceylanicum larvae and such larvae when used as vaccine proved less pathogenic and highly

immunogenic. Thus, the present study confirmed that a single oral vaccination with UV-irradiated larvae of *A.caylanicum* exposed for 15 minutes irradiation conferred tremendous reduction of 99% worm establishment with satisfactory non-pathogenic effects in the host against the challenge infection. This may be due to the immunity developed by the constant supply of antigenic material by the UV-irradiated larvae. Jarrett *et al* (1968) stated that the third stage larvae stimulated little or no immunity. On the other hand Ogilvie (1964) reported that the developing larval stages were more important than adult worms as a source of protective antigens.

Ultraviolet irradiation has been successfully attempted in other nematode larvae. Stovens (1942) attenuated *I.spiralis* larvae to prevent normal development by exposing them to ultraviolet rays. Keeling (1960) attenuated the infective larvae of *H.brasiliensis* with various doses of UV-rays and found complete inhibition of the larval development to adult stage. Katiyar *et al* (1968) reported that UV-irradiated *H.brasiliensis* larval vaccine profoundly reduced the chances of maturity of the larvae and complete protection against the challenge infection. Similarly, successful attempts of UV-irradiation on Schistosome infections have been reported

by Standen and Fuller (1959), Ghandour and Webbe (1975) and Prah and James (1977). Thus, all the earlier reports together with the present study confirm that UV-irradiated larval vaccine can also be used as a means of protection against A.ceylanicum infection in hamster model.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Ancylostoma ceylanicum (Looss, 1911), a natural intestinal pathogenic blood sucking nematode parasite of cats, dogs and humans causes severe anaemia. Ray et al (1972) studied successfully for the first time the complete life cycle of A. ceylanicum in laboratory host, hamster (Mesocricetus auratus) and used this laboratory model for chemotherapeutic studies. However, studies pertaining to pathology and immunology in this model are lacking. The aim of the present investigation was to study the host-parasite relationship, role of humoral antibodies and their protective role and development of UV-irradiated larval vaccine and its evaluation in the hamster model.

Ancylostoma ceylanicum strain was maintained in hamsters using infective larvae which were obtained from copro-culture method of Ray et al (1972). Hamsters were infected with A. ceylanicum, each with a dose of 100 larvae and were sacrificed at intervals of 13, 20, 30, 60, 100, 150 and 200 days post infection. The results revealed a significant loss in body weight in the infected animals during the infection period between 30 and 60 days when the average worm recovery made was between 35.1 and 18.0. The adult worm persisted in the intes-

infections of hamsters with A.ceylanicum larvae. Healthy hamsters of 45 gms average body weight were immunised each with 1 ml immune serum subcutaneously, intramuscularly and intraperitoneally along with control hamsters which received each 1 ml normal serum by the same routes. All the animals were challenged on the same day with a dose of 100 normal larvae. The results revealed no significant difference in the worm count between the immune serum and normal serum recipient hamsters.

An attempt was made to study whether ultraviolet irradiated larval vaccine, if given orally to hamsters, could give any protection against a challenge infection of A.ceylanicum. Infective larvae of A.ceylanicum were exposed to a 3 ft. ultraviolet tube (wavelength-390 n.m.) at a distance of 30 cms for different periods of exposures. In the first experiment different batches of 3000 larvae/3 ml were exposed for 20 secs., 40 secs., 60 secs., 15 mins., 30 mins., and 60 mins. The UV-irradiated larvae were given orally to hamsters of different groups (A,B,C,D,E,F). Group G hamsters which received normal larvae served as control. After 20 days, on necropsy minimum percentage worm recovery (1.0 ± 0.71) was made from the group D which received UV-irradiated larvae exposed for 15 minutes although there was signi-

ficant reduction with 40 seconds and 60 seconds UV-exposure as compared to that of the control. No adult worms were recovered with 30 and 60 minutes exposure.

In the second experiment, the hamsters were given oral vaccination with larvae UV-irradiated for different time intervals followed by oral challenge with a dose of 100 and 1000 normal larvae separately, to find out the immunogenic potency of UV-irradiated larval vaccine in terms of adult worm establishment. A maximum protection in terms of 99% worm reduction was obtained at 15 minutes UV-exposure to larvae against 66% worm reduction in the normal larval vaccinates followed by a challenge dose of 100 larvae. At 1000 challenge, a maximum of 95% reduction in the worm count was obtained in the UV-irradiated (15 minutes exposure) larval vaccinates as compared to 12% worm reduction in the normal vaccinates.

The route of administration of similar doses of vaccine by oral or subcutaneous routes made no significant difference in worm establishment on necropsy.

The pathogenic effect of UV-irradiated larval vaccine was assessed on the basis of haemoglobin level in blood and hookworm egg load in UV-irradiated larval vaccinates. For this, hamsters were given larval vacci-

nation with a dose of 100 UV-irradiated larvae (15 minutes exposure) along with controls which received normal larvae. The haemoglobin levels in the irradiated larval vaccinates were found to be higher at different intervals after vaccination and before challenge, when compared with those of the normal larval vaccinates ($P < 0.001$). Although there was a slight fall in the haemoglobin values of UV-irradiated larval vaccinates initially after the challenge infection, the values increased gradually on subsequent days. As compared to the irradiated larval vaccinates the challenge controls had severe anaemia (Hb - 11.6 to 8.28 gms%) ($P < 0.001$).

Hookworm eggs were not detected in the faeces of UV-irradiated larval vaccinates at any time before challenge infection in contrast to that of normal larval vaccinates (8000 eggs/gm) on the day 20 post vaccination. The appearance of hookworm eggs in the pellets was observed on the day 25 in UV-irradiated larval vaccinates (300 epg) as compared to the normal larval vaccinates (18,000 eggs) and challenge controls (25000 eggs). The epg in UV-irradiated larval vaccinates reached a maximum of 800 on the day 30 which was significantly lower as compared to normal (epg 30,000) and challenge larval controls (egg 40,000).

From the results obtained, the following conclusions were drawn. A. ceylanicum larval infection in hamsters causes a significant loss in the body weight and severe anaemia with low haemoglobin values during the infection period from 13 to 60 days. The long retention (150 days) of A. ceylanicum worms was observed in the intestine of infected hamsters. The antibodies were demonstrated in the serum by the application of agar gel diffusion, counterimmunoelectrophoresis and indirect haemagglutination tests. CIEP was found to be an ideal test out of the three as positive reaction was observed for a longer period (150 days).

Passive immunization with immune serum by subcutaneous, intramuscular and intraperitoneal routes proved ineffective in terms of worm establishment after the challenge infection.

The UV-irradiated larvae (15 minutes UV exposure) on a single oral vaccination could give protection in terms of 99% reduction in the worm establishment after challenge infection of 100 normal larvae. The route of administration of vaccine made no difference in the establishment of adult worms.

The haemoglobin values of the UV-irradiated larval vaccinates were significantly higher than those of the

normal and challenge infections except for a slight fall in the haemoglobin values initially after the challenge infection.

No hookworm eggs were detected in the faeces of UV-irradiated larval vaccinates at any time before challenge infection. The appearance of eggs was delayed in the irradiated larval vaccinates as compared to the normal larval vaccinates.

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SYNOPSIS

INTRODUCTION

Ancylostoma ceylanicum is a natural parasite of cats, dogs, and other carnivores (Looss, 1911). This was first discovered in humans by Lane in the year 1913. Since then the existence of this species of hookworm in humans, cats and dogs has been reported in various parts of the world. (Velasquez and Cabrera, 1968; Anten and Zuidema, 1964; Areekul et al., 1970 a; Chowdhury and Schad, 1972; Zuidema et al., 1972). Biocca (1951) confirmed for the first time the difference in morphology between A. ceylanicum and A. braziliense.

The experimental infection conducted in human volunteers by cutaneous exposure to infective larvae of A. ceylanicum caused nodularity, urticaria, bulbous lesions, irritation of the throat and dry cough. The adult worms caused mainly anaemia, abdominal pain and general fatigue in infected individuals (Areekul et al., 1970 b).

Investigations on the immune response to hookworm infections have been aided by the application of various immunological techniques notably agar gel diffusion test, indirect haemagglutination test, complement fixation test, Sawade et al., 1954; Ball and Bartlett, 1969; Seed et al., 1972), immunofluorescence test (Kaul and Mahajan, 1977),

Counterimmunoelectrophoresis test (Bhopale et al., 1980) and ELISA (Ogilvie et al., 1978). Antibodies can be detected to antigens of third and fourth stage larvae as well as adults in patients or population groups. (Ogilvie and Worms, 1976). IgE level is also found to be increased in patients. (Grove, et al., 1974). Skin tests for hookworm as a diagnostic test or epidemiological tool have proved to be too insensitive (Ball et al., 1971; Villarejos et al., 1975). However, the sera of patients infected with hookworms showed negative reaction for precipitating antibodies. A delayed response of serum antibody to repeated infections in humans has been detected by IHA test (Soulsby and Guiles, 1965; Ball and Bartlett, 1969).

There is little direct evidence that protective immunity to hookworm develops in man. Field studies have shown that repeated infections of volunteers failed to confer resistance. Earlier reports of Otto and Kerr (1939) clearly indicated that a specific immunity was acquired by dogs when exposed to repeated infections with A. caninum larvae. The first evidence that hookworms could be attenuated by ionizing radiation was reported by Dow et al. (1959) who attenuated Uncinaria stenocephala larvae with 40 kr X-rays. In addition there is now abundant data to indicate that a canine

hookworm vaccine can provide active immunity for dogs of all ages (Miller, 1971). This was further confirmed by Vinayak et al. (1981) using dead vaccines and X-irradiated larval vaccines in pups. It was also shown earlier that immunity in pups vaccinated with gamma irradiated A.coninum larvae can be passively transferred to susceptible pups using serum and lymphoid cells (Miller, 1967).

Another method of attenuation is to expose infective larvae to ultraviolet irradiation. The effect of UV-irradiation on eggs/larvae of nematodes have been investigated by several workers during the years from 1909 to 1944. Stowens (1942) observed that exposure to UV-irradiation would prevent the normal development of Trichinella spiralis larvae and the female worms developed were sterile. Standen and Fuller (1959) have demonstrated similar results with cercariae against Schistosoma mansoni in mice. Ghandour and Wabbe (1973) reported the damaging effect of cercariae of S.mansoni and S.haematobium after exposure to UV-irradiation for a short while. Katiyar et al. (1968) reported that rats were completely protected against N.brasiliensis infection following immunization with UV-irradiated larvae. To our knowledge, such studies with reference to immune response, serology, serum passive immunization and the role of ultraviolet irradiated larvae in immunity are lacking in a laboratory model of encylosomiasis which in our present study was carried out in

hamsters infected with A.ceylanicum. This suitable model was introduced by Ray et al. (1972) who described for the first time the complete development of A.ceylanicum in hamsters. They reported that the larvae did not migrate through lungs during development. This laboratory model was also used for chemotherapeutic studies by Ray et al. (1978). Ray et al. (1975) have successfully attempted the complete development of A.ceylanicum in albino mice but only under the influence of corticosteroid. A partial development of A.ceylanicum larvae in mice and rats was reported by Bhopale et al. (1978).

The present study is aimed at the elucidation of immunity to A.ceylanicum infection in golden hamsters (Mesocricetus auratus). With this objective particular emphasis is laid to understand and establish pertinent answers to the following queries in this experimental model:

1. Studies on host-parasite interactions with reference to body weight, worm load, haemoglobin level and differential leukocyte counts during infection period.
2. Detection of antibodies in serum at various intervals during infection period.
3. To study whether serum passive immunization has any protective role against the challenge infection of

A.ceylanicum larvae in hamsters.

4. To study whether UV irradiated larvae play any important role in inducing any acquired immunity enough to resist the challenge infection of A.ceylanicum larvae in hamsters.

MATERIALS AND METHODS

Ancylostoma ceylanicum strain, procured from Ciba-Geigy Research Centre, Bombay was maintained in hamsters in the Zoonosis laboratory. Male hamsters (Obtained from Haffkine Bio-pharmaceutical Corporation Ltd.) were divided into two groups. In one group 50 hamsters were infected orally, individually with a dose of 100 infective larvae of A.ceylanicum. The other group of 50 hamsters was kept as uninfected control. The infected and uninfected animals during the observation period of 200 days were necropsied at various intervals and body weight, worm load and haemoglobin level were recorded. Blood was collected periodically for serological studies. Blood smears were also prepared for differential leukocyte counts and slides were stained with Giemsa's stain.

Antigen preparation

The somatic antigen was prepared from freshly

collected active adult worms. These worms were washed thoroughly in phosphate buffered saline (PBS-pH 7.2). After sonication of the worms in PBS, the suspension was centrifuged in cold centrifuge at 3000 rpm. for 30 minutes. The protein estimation of the supernatant (1.5 mg/ml) was determined by Lowry's method (1951) and stored at -20°C until use.

Agar gel-diffusion (AGD)

The agar gel-diffusion was performed on glass slides (7.5 x 2.5 cms) with 1% agarose (Difco) in normal saline. The slides after filling with antigen and sera samples were kept at room temperature in humid condition for a week. These slides were stained with 1% amidoblack, washed with 2% acetic acid solution and dried at room temperature.

Counterimmunoelectrophoresis (CIEP)

This This test was done as per the methods described by Bhopale et al. (1980). The electrophoresis was run at 200 volts for one hour. The slides were kept overnight in normal saline and stained with 1% amidoblack. These were washed with 2% acetic acid and dried at room temperature.

Ultraviolet irradiation

Experiment 1

The worm recovery made from the hamsters infected with a dose of 100 normal larvae was 41 ± 8.37 on the 20th day of infection. A drastic decrease in the worm load was observed when the hamsters were infected with UV-irradiated larvae exposed at various time schedules. A maximum reduction in the worm count (1 ± 0.71) was noted in the larval infection in which the larvae were exposed to UV-irradiation for 15 minutes when compared with the control animals which received normal larval infection ($P < 0.01$).

Experiment 2

Immunogenicity of UV-irradiated larvae in hamsters was assessed on the basis of worm count after challenging with a dose of 1000 larvae. It was recorded that UV-irradiated larvae (40 seconds exposure) gave 87% reduction in worm load. A maximum reduction of 95% in worm count was observed with larvae exposed for a period of 15 minutes. On the other hand, the animals infected with normal larvae (without receiving radiation treatment) showed only 12% reduction in worm load. The maximum reduction in worm recovery (99%) was observed with a challenge dose of 100

normal larvae in animals which received UV-irradiated larvae exposed for 15 minutes. The corresponding control animals infected with normal larvae followed by a challenge dose of 100 larvae showed 66% reduction in worm load.

Experiment 3

There was no significant difference in worm counts between the subcutaneously immunized (2.80 ± 2.94) and orally immunized (4.4 ± 2.51) animals after a challenge infection with 100 larvae. However, there was significant difference in worm load obtained in the corresponding control animals which received non irradiated larvae either subcutaneously (31.0 ± 11.87) or orally (32.0 ± 9.72) for immunization followed by a challenge infection ($P < 0.01$).

CONCLUSION

The results of the present investigation confirmed that A.ceylenicum infection caused anaemia in the infected hamsters which was revealed by a low haemoglobin level in blood during patent period of infection. A significant weight loss too was observed during the infection period between 30 and 60 days.

The precipitating antibodies as detected by agar gel diffusion and CIEP appeared in the serum of infected animals

four weeks after infection. CIEP test showed positive reaction as long as the worms persisted in the intestine (150 days). Haemagglutinating antibodies appeared in the serum on the day 20 onwards till the day 90.

Passive immunization by transfer of immune serum did not confirm any significant protection in immunized hamsters.

Ultraviolet irradiated A.ceylanicum larvae can induce remarkable immunity in hamsters against the challenge infection. However, a maximum protection with 99% worm reduction was obtained at 15 minutes exposure dose of irradiation against the challenge dose of 100 normal larvae.

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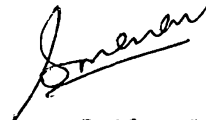
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Signature of the Guide



Signature of the Student

Indirect haemagglutination test (IHA)

The method was followed according to Kagan (1972) for indirect haemagglutination test. Sheep erythrocytes of 2.5% concentration were tanned with tannic acid (1:80,000). These tanned erythrocytes were sensitized with the optimum dilution of antigen (1:20) which was determined by block titration.

Passive immunization test

This experiment was aimed at the protection of hamsters against the challenge infection by transfer of serum from the immunised donors. Five male hamsters of 45-60 gms body weight were injected subcutaneously with 1 ml of undiluted immune serum to each recipient hamster. Another group consisting the same number of hamsters were injected similarly with equal volume of normal serum. All the animals were challenged with a dose of 1000 normal larvae. 3 hamsters died from the control group of animals receiving normal serum whereas 2 from the other group which received immunized serum. The surviving animals were necropsied after 20 days of challenge infection for the worm recovery.

Ultra violet irradiation

The third stage larvae collected from the copro-culture were transferred to a clean, sterile petridish

and this was exposed to irradiation at a distance of 30 cms from a UV tube light having wave-length of 390 nm. Male hamsters of 40-65 gms. body weight were used for this experiment.

Experiment 1

The aim of this experiment was to confirm whether there was any effect of ultraviolet radiation on larvae and the exposure can produce arrest in the growth of adult worms. Various time exposures of ultraviolet rays to the larvae were performed to select most effective ionization. This was assessed on the basis of worm recovery made from the intestine of hamsters. For this experiment 6 groups of male hamsters, 5 in each, were infected orally, each with a dose of 100 UV-irradiated larvae exposed at various times of exposure viz. 20 seconds, 40 secs., 60 secs., 15 minutes, 30 minutes and 60 minutes at a distance of 30 cms. Another group of 5 hamsters were infected similarly with a dose of 100 normal larvae without undergoing ultraviolet irradiation and this served as control. All the hamsters from both UV-irradiated and non-irradiated groups were necropsied 20 days post infection for the worm recovery.

Experiment 2

This experiment was conducted to measure the immunogenic efficiencies of UV-irradiated larvae in hamsters to resist the challenge infection. Five groups of 5 hamsters in each, were infected with a dose of 100 UV-irradiated larvae, exposed separately at various time schedules of 40 seconds, 60 seconds, 15 minutes, 30 minutes and 60 minutes. Another group of hamsters was infected similarly with a dose of 100 larvae. Hamsters from all groups were challenged with 100 normal larvae after 15 days post infection when most of the larvae or worms have already been expelled from the intestine. They were sacrificed 20 days after the challenge infection to confirm the immunogenicity of UV-irradiated larvae against the challenge larval infection in terms of worm counts. A similar experiment was set up in which a challenge lethal dose of 1000 normal larvae was given to the hamsters.

Experiment 3

The purpose of this experiment was to know whether different routes of irradiated larval infection have any significant effect in resisting a challenge infection. For this study, two groups of hamsters, 5 in each were infected subcutaneously and other group was infected orally with a dose of 100 UV-irradiated larvae (15 minutes

exposure). Other two control groups of animals were infected separately by the same routes with the same dose of normal infective larvae. All the four groups of hamsters were challenged with a dose of 100 larvae and necropsied 20 days post challenge infection for worm counts from the intestine of hamsters.

RESULTS

Mortality upto 10% was observed in the infected animals particularly during the period between 13 and 20 days post infection. Both infected and control hamsters were necropsied at various intervals of 13, 20, 30, 60, 100, 150 and 200 days post infection period.

Body weight

A significant decrease in body weight of the infected hamsters on the 30th day (52 ± 3.63) and 60th day (58.3 ± 8.50) was observed when compared with that of the uninfected control animals on respective days (62.2 ± 4.34 and 72 ± 5.23) ($P < 0.01$) during the infection period.

Worm load

All the infected animals on necropsy at various intervals were examined for the presence of worms in the intestine. The worm recovery was made in terms of percentage larval infection in animals. On the day 13, the

average worm load recovered was 51% which was reduced gradually till the day 150 when the worm load remained only 1%.

Haemoglobin level

The haemoglobin level in experimental hamsters prior to infection was 18.2 gms% which became significantly low (12.09 gms%) from the day 13 onwards post infection. This reached the minimum level of 8.2 gms% in the infected hamsters on the day 30 as compared to that of uninfected control animals (20.4 gms%) ($P < 0.01$). Haemoglobin level in the blood increased gradually after the 30th day post infection and returned to the normal level on day 100 (18.1 gms%) when compared with controls (20.6 gms%).

It was observed that the differential leukocyte count did not reveal any difference especially in the number of eosinophils during the infection period of 200 days.

Agar gel-diffusion test

The antigen-antibody reaction in gel diffusion test was performed to detect the presence of antibodies in the serum collected from infected hamsters. The sera samples showed positive reaction during the infection period from the day 30 onwards till the day 60.

Counterimmunoelectrophoresis test

The sera samples collected at various intervals during the infection period were tested by CIEP test to detect the presence of antibodies. The precipitin reaction was observed in the sera during the infection period between 30 and 150 days post infection. The sera samples collected after 150 days were found to be negative by CIEP test.

Indirect haemagglutination test

The haemagglutinating antibodies appeared in the serum of infected hamsters on the 20th day with an initial positive titre of 1:32. The antibody titre reached the maximum (1:128) on the day 60. It dropped gradually on subsequent days and disappeared completely by 90 days post infection.

Passive Immunization

The average worm yield from those animals immunized with immune sera was 393 ± 64.84 as compared to the control animals which received normal serum (428 ± 60.34). Thus there was no significant difference in worm recovery between these two groups. This confirmed that the immune serum passively transferred did not play any protective role against the challenge infection.

