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17th National Congress of Parasitology
Dibrugarh, Assam, India
24-26 Oct., 2005

Parasite Diversity with specific reference to Nematodes

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The ways of obtaining food and fighting hunger differ from group to group and within one group from species to species. Feeding by predation and leading a parasitic way of life are by and large pre-dominant in the Animal Kingdom but both are largely insignificant to almost non-existent among plants. The mobility being an inherent ability of animals is in most probability the main reason for this difference. It seems that at some point of time in the evolutionary history certain animal groups realized that killing in reality destroyed their source of food almost instantaneously. Consequently, it would be a better strategy to keep feeding on the source as long as it was possible and sometimes permanently. This ultimately would have been the beginning of parasitism. But a quantum jump from being predaceous to parasitism could not have been a cakewalk nor was it easy to visualize. As in predation, the organisms shall have to be stronger than the prey, as the former have to kill the latter, but in parasitism it is just the reverse. So in most likelihood, parasitism should have taken a separate path, quite apart from predation. In predation there being no physiological dependence of the predator on its prey, but in parasitism an intimate physiological and metabolic compatibility is essential between the host and its parasite.

The major groups in the Animal Kingdom that have all or significantly a large number of species of parasites are Protozoa, Platyhelminthes, Acanthocephala, Nematoda, Arthropoda and a few others rather insignificant ones. Each of these groups is extremely dynamic and very interesting structurally, biologically, pathologically and in so many other ways. It would be impossible to cover all these groups in this brief presentation. I have thus chosen only one group out of those mentioned above for my present talk, namely the nematodes. This would allow me to do justice with the topic as the group happens to be the subject of my study for close to 50 years.

The nematodes, much like insects are ecologically the most diverse group that one may conceive of. They inhabit water, both marine and freshwaters of all kinds and characteristics, occur in all conceivable types of soils from the highest peaks of the snow-clad hills down to the bottom of the deepest oceans in the coldest to the hottest and most inhospitable areas of the world. They are spread over from the Arctic to the Antarctic, in all the seven continents and the seven seas. They are known to parasitize all groups of animals right from nematodes to man and all conceivable types of plants that occur on this living planet, the earth. Their diversity and adaptability is such that one is simply wonderstruck and filled with awe.

Some leading Nematologists have described nematodes in these words "*They occur in arid deserts and at the bottom of lakes and rivers, in the waters of hot springs and in the polar seas where the temperature is constantly below the freezing point of freshwater. They were thawed out alive from Antarctic ice and they occur at enormous depths in Alpine lakes and in the oceans. As parasites of fishes they traverse the seas; as parasites of birds they float across continents and over high mountain ranges. Man, without wings, flies in aeroplanes but nematodes without wings, fly in birds, bats, bees, flies, or fleas, or just catch on as these go by and sail with them. Few nemas have anything resembling feet, but here again they need not exert themselves in walking for representatives of the whole animal kingdom act as their common carriers, and even the winds may on occasions stoop to lift them and take them to their destination.*"

Regarding the numerical strength of nematodes as also

about the actual estimates of their species numbers, the zoologists, parasitologists and even the nematologists are by and large ignorant. The fact remains that unlike other group of animals such as Arthropods, the nematodes are adapted to such lifestyles that they remain largely concealed to hideous in their varied kinds of habitats which could be soils, plants or animal bodies. Apart from the aquatic habitats they can survive on land though never exposed to soil surface, always in deeper layers which hold moisture. The fact remains that nematodes in general cannot survive dehydration or can live in the absence of water or some kind of body fluid of their hosts. It is precisely for this reason that they are generally not known to be ectoparasitic. Though the soil-inhabiting nematodes that feed on roots of the plants from outside are referred to as ectoparasites, even the well-known cyst nematodes, *Heterodera* or *Globodera* also feed the same way, but it is again the soil water that actually protects their bodies. The closest that nematodes come to being called ectoparasites are some species of insect parasitic nematodes (e.g., *Acugutturus*, *Noctuidonema*) that feed from outside the body of their hosts while lying beneath the tegument of the insects which obviously protects them from desiccation and death.

While only some 4,500 species of nematodes were known in the year 1930, this number rose to about 9000 by 1950 and the present-day estimates for all types of species is approx. 25-30,000. This is in actual fact a fraction of species that are estimated to be present in this world. The zoologists as also many nematode taxonomists believe that the nematode diversity, their occurrence and the numerical strength is such that they would be close on heels to that of the insects. Taking into account, for example, the fact that every vertebrate has a minimum of two nematode species as its definitive parasite, some 100,000 species of nematodes parasitize the vertebrate hosts. Man alone has some 38 species of nematodes for which he is the definitive host. Add to this the various kinds of invertebrates, insects in particular, all of which have their own species of nematodes. Let us also not forget that the abundance and the diversity of nematode species parasitizing plants of all kinds, cultivated or wild, as also those nematodes that live in the marine and freshwaters and in the soils in huge numbers constitute tremendous and unimaginable diversity. Some say it may be 5,00,000 species, others believe it may be much higher and possibly very close to that of insect species, if not more, as all species of insects have their own specific species of nematodes. Numerically also it can be said that apart from protozoan and microbes, nearly 90% of all metazoans in the world are nothing but nematodes.

The variations in the body size of nematodes are enormous, starting from the smallest plant-parasitic nematodes, which could be as small as 150-200 μm or even less compared to the gigantic-sized, about 27ft or 8 metres long, *Placentonema gigantissima*, parasite of the placenta of sperm whales which themselves are the largest mammals of the world. There could be some correlation between the size of the host and their parasites, but this does not necessarily always hold true. Look for example at the nearly three dozen or more definitive species of nematode parasites of man which vary greatly in their body sizes. The other species of nematodes which have significantly large body sizes, about 1 feet to 2 metres long, are *Dioctophyme renale*, the giant kidney worm of dogs; *Wucheraria bancrofti*, the well-known filarial worm of man; *Dracunculus medinensis*, that causes 'Naru' disease and *Ascaris lumbricoides* which is the causative agent of ascariasis in man, etc.

It is not only the length that varies, but the shape varies too. The majority of aquatic and soil-inhabiting nematodes are elongate and cylindrical. It is due to this reason that in earlier literature they were referred to as thread-worms, eelworms or serpentine animals, and this is what they also apparently look like. A lot of those which have adapted to parasitic mode of life have also changed rather significantly, either in certain parts of their bodies such as anteriorly where their feeding and sensory structures are located, or in the middle or posteriorly where their reproductive regions are situated. Some species that are typical eelworm or serpentine-like in shape are often exceedingly narrow and excessively long, looking more a fine thread-like (*Echphydophora*, *Rhadinaphelenchus*), without much of structural differentiation or markings on their body

surfaces. A few species are whip-like (*Trichuris*). However, apart from this, there are many nematode species and genera that assume fantastic to ubiquitous shapes, a few may be spindle-shaped (*Tetrameres*), other may be globular, some swollen to subspherical (*Rotylenchulus*, *Tylenchulus*, *Nacobbus*) while still others may assume an almost real spherical shape (*Heterodera*, *Meloidogyne*).

I don't wish to go into the structural details and the functional diversity of the body surface structures of nematodes nor do I intend describing the various sensory structures that are largely associated with it because they are far too many. In the same way the variations of the stoma and esophageal structure of the free living, microphagous, phytophagous groups of nematodes are innumerable and no single person can imagine putting them into one place. Last that it was attempted was 6 to 7 decades back and now the knowledge of nematode structure and diversity has grown so much that it is beyond the comprehension of any one nematologist to do it all by himself. I thus leave it to the various specialists in the area of animal Nematology, plant and insect Nematology as also the marine Nematology to deal with it. But in spite of the differences in the structural features of various groups of nematodes, the basic plan of organization remains essentially the same.

Much like the structure of feeding part (stoma) and the oesophagus/pharynx which are jointly called the oesophagostom the gonads and the associated accessory structures show innumerable diversity in both sexes. The number, structure and position of female gonads, the location of vulva on the body are all greatly variable. Generally there may be one, two or few ovaries but *Placentonema gigantissima* has as many as 32 ovaries. Vulva, for instance opens very close to mouth in a filarid nematode, *Diplotriaena*, but in an acuarid nematode, and in *Heterodera* and *Meloidogyne*, it is almost at or near the anus or tail tip. In the same way, in some nematodes, the spicules are very long and attenuated almost 70-90% of body length, but in others all gradations in size occur until we reach a stage in *Aspicularis* in which the spicules are totally absent. Similarly, the bursa, caudal and cephalic alae, the eggs with their different shapes and sizes also show innumerable variations. The patterns of life cycles may be direct host to host (*Haemonchus*, *Ascaridia*, *Trichinella*), or indirect with one host (*W. bancrofti*, *D. medinensis*), or rarely with two hosts (*Gnathostoma spinigerum*).

Among the animal and human parasitic nematodes, a very large number of species live in the intestine, of most common occurrence being *Ascaris lumbricoides*, *Ancylostma duodenale*, *Necator americanum*, etc., causing intestinal disorders. Their juvenile stages as they pass through the lungs may result into severe discomfort. The strongylid nematode juveniles as they bore through the skin also cause severe irritation and itching. The *Ascaris* juveniles can also invade the liver which may then develop white spots. *Dioctyocaulus filariae* lives in the lung of sheep whereas *Dirofilaria immitis* lives inside the heart of dogs and while *Dioctophyme renale* inside its kidneys. *Wucheraria bancrofti*, is the most well-known nematode species that causes elephantiasis in man, and *Loa loa* and *Onchocerca volvulus* are called eyeworms because of the sites of their infection. The three species produce microfilariae which circulate in the peripheral blood either in night (*W. bancrofti*) or in the day, depending upon their intermediate hosts which may be either visiting their hosts in the day or in night. The fiery serpent, *Dracunculus medinensis*, the causative agent of 'Naru' disease in this country lives in the muscles or connective tissues of body of human beings. *Trichinella spiralis* settles down inside the voluntary or striated muscles of their hosts such as in pig, rat, fox, bear, badger, man, etc., which are essentially flesh-eating. Likewise, there are innumerable other examples of nematodes causing diseases to man, domesticated animals or to those living in wild, including fishes in the marine and freshwaters. Nematodes parasitize all groups of invertebrate animals and also cause pathogenesis and death, but very little work has come to light in this area.

The nematodes attacking plants feed either ectoparasitically, semi-endoparasitically or endoparasitically, either alone or in combination with viruses, bacteria, fungi, etc. The ectoparasitic

surfaces. A few species are whip-like (*Trichuris*). However, apart from this, there are many nematode species and genera that assume fantastic to ubiquitous shapes, a few may be spindle-shaped (*Tetrameres*), other may be globular, some swollen to subspherical (*Rotylenchulus*, *Tylenchulus*, *Nacobbus*) while still others may assume an almost real spherical shape (*Heterodera*, *Meloidogyne*).

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Much like the structure of feeding part (stoma) and the oesophagus/pharynx which are jointly called the oesophagostom the gonads and the associated accessory

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Review

Parasitic Infections of the Central Nervous System

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Many parasites are known to cause infection of the central nervous system (CNS) both in the immunocompetent and immunocompromised hosts. Neurocysticercosis, meningoencephalitis due to free-living amoeba, toxoplasmic encephalitis, cerebral malaria, and cerebral strongyloidiasis are the distinct parasitic diseases associated with high morbidity and mortality. In India, neurocysticercosis (NCC) is emerging as an important disease of the CNS, and next only to tuberculosis is the second most important cause of intracranial space occupying lesion. NCC is also emerging as one of the principal cause of epilepsy. Primary amoebic meningoencephalitis, which is invariably fatal, and caused by *Naegleria* and *Acantiamoeba* is being increasingly reported from India. Cerebral malaria, resulting from *Plasmodium falciparum* infection continues to wreak havoc on both urban and rural population with deadly intensity fn the era of AIDS, and other conditions of immune deficiency due to chemotherapy, organ transplantation, and malignancy. Encephalitis due to *Toxoplasma gondii* and systemic infection with cerebral involvement in disseminated strongyloidiasis have become major problems in patient care. Nearly, 3-4% of population who have AIDS had developed CNS toxoplasmosis depending on the series. *S. stercoralis* is known to produce hyperinfection syndrome characterized by massive invasion of the CNS. The present paper will focus on the epidemiology and laboratory diagnosis of these parasitic diseases of CNS with special reference to India.

Key words: Cerebral malaria, Amoebic meningoencephalitis, Neurocysticercosis, Strongyloidiasis, Toxoplasma encephalitis.

INTRODUCTION

Infection of the central nervous system (CNS) is life threatening. The microbia etiology of this morbid state is varied. Parasites, both protozoa and helminths have been recognized as a leading cause of CNS morbidity and mortality. The emergence of numerous immunocompromized states, the advancement of our understanding about the pathophysiology of parasitic CNS diseases, and rapid developments in the field of diagnostic technology, has enhanced the importance of neuro-parasitosis as a distinct, and prominent disease entity. The aim of this article is to present a comprehensive review of the parasitic infections of the CNS, with special reference to India. Its principal objective is to put forward the recent advances in the field of neuro-parasitology, both in epidemiology, and also in its diagnosis. The article will focus on these diseases under two distinct categories, viz. major, and minor parasitic infections of the CNS. This distinction is essential to lay appropriate emphasis on the infections that are predominantly related to India. Neurocysticercosis, *Toxoplasma* encephalitis, amoebic meningoencephalitis, and cerebral malaria will be dealt as major diseases in India, whereas

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cerebral strongyloidiasis, cerebral amoebiasis, eosinophilic meningitis, cerebral hydatid disease, cerebral paragonimiasis and *Balamuthia* encephalitis will be treated as minor.

MAJOR CNS PARASITIC INFECTIONS

NEUROCYSTICERCOSIS

Cysticercosis is caused by the larval form of the cestode *Taenia solium*. This "armed tapeworm" of man was known since the time of Hippocrates, but was distinctly differentiated from *T. saginata* only in 1782 by Goez (Parija, 1990). Neurocysticercosis (NCC), which is an infection of the CNS by the larval form of the parasite, is the most frequent parasitosis of the CNS (Maiti, 1995). It is estimated that between 5 and 20% of all cases with epilepsy in India have neurocysticercosis (Singhal and Renu, 2001). Accidental ingestion of the eggs leads to this condition. The cyst gets localized in virtually any organ, commonly in the CNS. Whereas *T. solium* intestinalis occurs in non-vegetarians through eating undercooked measly pork. Cysticercosis affects vegetarians and non-vegetarians alike and is due to ingestion of vegetables and water contaminated with the eggs (Parija, 1990) .NCC is said to be the most frequent parasitosis of the CNS with a possible frequency of 3.6% in general population as proved by autopsy (Maiti, 1995). It has been referred to as "a modern day plague" by some workers due to its worldwide distribution, high incidence and occasional fatal complication (Brown and Voge, 1985).

Epilepsy is the commonest feature of NCC. In one series 59-94% of cases of NCC were found to be associated with seizure disorder (Venkataraman, 1989). The introduction of CT scan in India in early 1980's and later on of MRI showed that most cases of epilepsy had single small enhancing lesions (SSEL) initially thoaght to be tuberculoma and treated with antitubercufous therapy. Subsequent works with biopsy studies showed that majority of these lesions represent cysticercosis in the dying form (Chandy *et* al, 1989). Epilepsy and convulsions due to NCC are increasingly reported from India and up to half of patients with seizure disorders are found to have serological evidence of NCC (Clinton, 1997). A study conducted by Gulati and colleagues demonstrated that 24% of 361 epileptic cases in Delhi had unequivocal evidence of NCC on MRI scan and additional patients had evidence of resolving lesions on their scans (Gulati *et al*, 1994).

Intracranial space occupying lesion and increased intracranial tension (pseudotumor syndrome) are slightly less common features of NCC seen in India. Majority of studies using CT scan and MRI with contrast (eg. gadolinium) to enhance the lesion shows a single small superficial ring or disc enhancing lesion of size usually less than 2cm, which, over several weeks or years either disappears, regress or heal by calcification even without specific medication. Subarachanoid cyst in some patients can enlarge to 10 cm in diameter and cause CNS mass effect (Clinton, 1997), Cysticercotic encephalitis is another form of disseminated NCC (Del Brutto, 1992). The condition has also been described from India (Kalra *et al*, 1987). Meningitic and other varieties are relatively infrequent in India.

The NCC is endemic in many parts of the world. The condition is seen in central and South Africa, Mexico and Central and South America, Pakistan, China and also in India. The disease is related to underdevelopment. Several epidemiological studies have shown a correlation between seropositive people, infected pigs and unsanitary disposal of feces.

In JIPMER, Pondicherry a total of 21 childhood neurocysticercosis was reported over five years from 1986 to 1989. Before the advent of CT/MRI era, the frequency of NCC as a cause of epilepsy in India was reported to vary from 2.2% to 9.6% (Mani *et al*, 1974). After the availability of CT/MRI imaging, NCC has been found to be the cause in 9% to 18.6% of patients with epilepsy. (Kumar, 1990). The frequency is maximal in communities that breed pigs and where standards of hygiene and sanitation are poor. In one study the stool examination of 2,50,000 hospitalized patients showed taeniasis in 0.5% to 2.0% of the cases, while in labour colonies and slums where pigs are raised the figure rose to 12-15% (Mahajan, 1982). Varied reports are available regarding the role of neurocysticercosis in intracranial space occupying lesion. While some workers report that 1% of intracranial space-occupying lesions in children in

India is accounted for by parasites (Reddy and Murthy, 1986), others show that 17.4-22% of cases of intracranial space occupying lesions and epilepsy are due to cysticercosis. In Bangalore, approximately 26% of the space-occupying sessions of the CNS were found to be caused by cysticercosis (Marti, 1995).

The clinical diagnosis of NCC is aided by imaging methods supplemented by immunodiagnostic methods. Specific diagnosis is made by demonstrating cysticerci in the biopsy tissue obtained from brain. In atypical cases where diagnosis is in doubt or if the lesion in brain enlarges under observation, steriotactically directed excision biopsy may be considered to confirm the diagnosis (Singhal *et al*, 1995).

Over the years, serological diagnostic procedures have been widely used. Indirect haemagglutination (IHA), enzyme-linked immunosorbent assay (ELISA) and enzyme linked immunoelectro transfer blot assay (EITB) are the most commonly used serologic techniques in recent times. However, these techniques vary in sensitivity and specificity, mainly due to poor reproducibility and cross reactivity of antigens used with other cestodes. The EITB assay is nearly 100% sensitive for patients with either multiple active parenchymal cysts or extra parenchymal NCC and is likely to be positive when serum samples are tested than when samples of CSF are tested (Clinton, 1997). This test is based on the detection of specific antibody to defined parasite glycoprotein antigens. Sensitivity in SSEL is seen by some workers in India as only 27% (Del Brutto, 1992). Antigens in many of these tests used are whole parasite extracts, whole porcine cyst sonicate, vesicular fluids and various components of cyst (Larralde *et al*, 1986).

Detection of antibody in the serum or CSF has its own limitations. It may indicate only exposure to infection and not necessarily the presence of established viable infection. Also, antibody may persist long after the parasite has been eliminated, resulting in false positivity and unnecessary anti-parasite therapy. Hence various assays have been developed for detection of antigen in serum or CSF with variable results. The use of Dot-ELISA and standard ELISA in one study for antigen detection in CSF showed that 59% of patients with cysticercosis had demonstrable antigens with Dot ELISA and 77% showed positive with standard ELISA (Edmundo and Giron, 1987). A monoclonal antibody based ELISA (Ag-ELISA) designed to detect the presence of excretory-secretory antigens from variable parasites showed 86%sensitivity in detecting antigens in CSF (Garcia et al, 1998).

The challenging problem in the Indian context is the aetiological diagnosis of SSEL. In the majority it represents dying cyst of NCC but in practice there is a need to distinguish between NCC and tuberculoma of the brain. Guidelines have been suggested for differentiating between NCC and tuberculoma (Rajashekhar *et al*, 1993). Atypical cases or cases worsening under observation are subjected to open "excision biopsy', under stereotaxic localization. However mere CT guided stereotaxic aspiration biopsy does not give satisfactory results (Rajashekhar, 1991). At times, it may be difficult to demonstrate the dying parasite if it is replaced by inflammatory cells and one hopes that in the near future immunohistologic tests will become available for precise diagnosis in such cases

The varied presentations of NCC warrant different therapeutic approaches. The introduction of cysticidal drugs, namely praziquantel (PZQ) and albendazole (ALB), was considered a major advance, but the use of these drugs requires careful assessment of the benefit versus risk ratio in different forms of NCC. The essential management even today consists of control of symptoms (eg. antiepileptic drugs for control of seizures), measures to control cerebral oedema and raised intracranial pressure and specific treatment by PZQ or ALB. Prior treatment with steroids to reduce vasogenic edema and inflammatory reaction is recommended.

TOXOPLASMA ENCEPHALITIS

Toxoplasmosis is a zoonotic disease caused by the obligate intracellular protozoan, *Toxoplasma gondii*. Owing to the increasing number of patients with AIDS and patients requiring immunosuppressive therapy, this disease has emerged as a potentially life threatening opportunistic pathogen (Arnold *et al*, 1997).

Toxoplasmosis is generally asymptomatic in immunocompetent adults (Parija, 1996). Most infections are either completely asymptomatic or sub clinical with mild fever and flu-like symptoms, sometimes associated with posterior cervical lymphadenopathy 7 to 20 days after infection (Arnold *et al*, 1997). On the other hand, *Toxoplasma* encephalitis (TE) is the most frequent cause of focal nervous system disorder complicating AIDS. An estimated 3-40% of HIV infected patients develop cerebral toxoplasmosis and in 10-38% of them it is the initial opportunistic infection. (Chaddha *et al*, 1999). More than 95% of TE in the AIDS patients is due to recrudescence of latent infection as a result of progressive loss of cellular immune surveillance, and infection is most often seen to occur when the CD4+ count is less than $0.1X\ 10^3$ per mm (Arnold *et al*, 1997). In bone marrow transplant patients, the disease is usually systemic and not related to the CNS. (Arnold *et al*, 1997).

There have been various reports of TE from India (Chaddha *et al*, 1999, Handa *et at*, 1996). The common presenting symptoms are fever, seizures, altered sensorium and headache (Chaddha *et al*, 1999). In one study in India, focal neurological deficit was present in 80% of cases. This is seen as the commonest clinical presentation and is of a sub acute nature. The common neurological deficit include altered sensorium, cranial nerve palsies, pyramidal tract signs, movement disorders, neuropsychiatric manifestations and cerebellar signs (Chaddha *et al*, 1999). A study conducted to see the significance of new onset seizures in patients with HIV showed that cerebral toxoplasmosis was an identified cause in 30-43% and is the commonest infection causing seizures in all series (Chaddha, 2000). Seizure has been documented as an early symptom in 15-40% of cerebral toxoplasmosis. The mean survival of patients after first episode of toxoplasmosis has been seen as 104 ± 52 days (Chaddha *et al*, 1999).

In India, seroepidemiological studies carried out by IHA, CFT, ELISA and dye tests, have shown significant levels of toxoplasma antibodies in normal population as well as in high numbers of suspected population residing in Bombay, Chandigarh and other parts of India (Parija, 1990). Seroprevalence of 57% has been reported from Uttar Pradesh using IHA (Singh and Nautiyal, 1991) and an even higher rate of 75% in adults suspected of having toxoplasmosis has been reported from a tribal area in Maharashtra. The highest seroprevalence reported from India was 77% in women in Kumaon region of Uttar Pradesh (Singh and Nautiyal, 1991). With the advent of the AIDS epidemic, there has been significant increase in the reports of toxoplasmosis reported to the national AIDS control organization between 1986 and 1997 (Lanjewar *et al*, 1998). A presentation from South India at the 4th International Congress on AIDS reported 10% TE in HIV patients (Greg, 1997). An autopsy study performed in India to study the spectrum of neuropathology of brain lesions in HIV/AIDS cases showed that 33 out of 85 adults brains showed opportunistic infections out of which 11 cases (13%) were *T. gondii* (Lanjewar *et al*, 1998).

Wright's stained peripheral blood smears may show the organism, which is 2 to 6 microns in size with light blue cytoplasm and delicate, stippled, azurophilic central band of nuclear chromatin (Arnold *et al*, 1997). Histoiogical examination of biopsy specimens after staining can be done. Bradyzoites are strongly PAS positive and *T. gondii* stain well with any of the Romanovsky stains (Parija, 1996). In TE, brain biopsy is the definite confirmatory method (Handa *et al*, 1996). CT and MRI are also helpful in the diagnosis.

Various immunodtagnostic methods are available. These include isolation of the parasite and serological methods for the detection of antigens and/or antibodies in serum and other body fluids. The classic and most specific test is the Sabin-Feldman dye test, but is used only in reference laboratories. The serological tests commonly employed are agglutination tests and IgM-ELISA (Parija, 1996). Studies have been done on stage specific antigens of *Toxoplasma* and their diagnostic potential. Use of a panel of tests (i.e. dye test, ELISA for IgG, IgM, IgA and IgE) may identify upto 70% of AIDS patients with *toxoplasmosis* (Chaddha *et al*, 1999). Occasionally antibody detection in CSF helps in confirmation of diagnosis of TE (Potasman *et al*, 1988). The lack of seroconversion in AIDS patients most likely reflects

inability to mount an immunological response. It could also be that antibodies produced may be immediately adsorbed onto the surface of the abundant circulating extra cellular tachyzoites, resulting in a false negative reaction (Arnold *et al*, 1997). Various recombinant antigens have been developed to detect *T. gondii* specific antibodies, which have greatly improved the sensitivity and specificity. In one study, *T. gondii* recombinant antigens were evaluated for their diagnostic utility in IgG and IgM recombinant ELISA (Rec-ELISAs). Relative sensitivity, specificity and agreement for IgG Rec-ELISA were 98.4%, 95.7% and 97.2% respectively and that for the IgM Rec-ELISA were 93.1%, 95.0% and 94.5% respectively (Aubert *et al*, 2000). A double sandwich ELISA with recombinant P35 antigen (p35 TgM-ELISA) has been shown to be highly useful in detecting acute infection. Currently the enzyme immunoassay for IgM is reliable. However, it is unsatisfactory for AIDS patients with latent or reactivated infections because they fail to produce an IgM response or an increasing IgG titer.

Several PCR based techniques have been developed as an alternative diagnostic measure. These make use of the most concerned gene sequences among different strains of *T. gondii* like the B1 gene repetitive sequence, P30 gene and ribosomal DNA (Ellis, 1998). RT-PCR designed for quantitative detection of *T. gondii* has been developed which detects as little as 0.05 *T.gondii* tachyzoites in one assay. It is a rapid, sensitive and quantitative way of detecting the parasite in clinical specimen (Lin ef *al*, 2000).

Sulphonamides and pyrimethamine are widely used for treatment of toxoplasmosis. In patients with AIDS, it has been suggested that a therapeutic trial in a suspected patient with *Toxoplasma* encephalitis is mandatory even in presence of atypical features, and patients should have a clinical response within 14 days of starting anti T*oxoplasma* therapy.

AMOEBIC MENINGOENCEPHALITIS

Primary amoebic meningoencephalitis (PAM) and chronic granulomatous encephalitis (CGE) are the potentially life threatening fatal Infections of the CNS caused by free-living amoeba, *Naegleria* and *Acanthamoeba* respectively. Both the conditions are seen in the normal as well as in immunocompromised individuals. Distinct from other protozoa by nature of their free-living existence, these free-living amoebae have no known insect vectors, no human carrier states of epidemiological importance, and there is little relationship of poor sanitation to the spread of infection.

Both *N.fowleri* and *Acanthamoeba* are distributed in thermally polluted streams and tolerate temperatures of 40°C to 45°C. The amoebae can also be found in coastal water, freshwater, heating and ventilation units, poorly chlorinated swimming pools, artificial lakes and warm water near the discharge outlets of power plants. The presence of N. *fowleri* in fresh water is directly related to water temperature. Warm water (>30°C) and pollution of the water with organic material are ideal for the proliferation of *N.fowleri* (Martinez and Visvesvara, 1999). Infection is transmitted to man by inhalation of dust and aspiration of water contaminated with both the cysts and trophozoites (Parija and Jayakeerthee, 1999). It occurs commonly in healthy children and young adults who usually have been recently swimming in fresh water. *Acanthamoeba* spp invade through skin lesions and spread to the brain and meninges causing insidious and prolonged disease. GAE primarily occurs in the immunocompromised and debilitated persons with AIDS.

Both RAM and GAE have been reported to occur in the central and southern USA, Australia, New Zealand, Korea, Japan, Peru, Europe, Africa, Central America and India (Parija, 1996). As of 1997, approximately 179 cases of PAM and 50 cases of GAE have been reported worldwide (Martinez and Visvesvara, 1997), with around 20 cases of PAM reported from India. The PAM and GAE caused by free-living amoebae in India are under-reported, most probably due to the inadequate information regarding their pathology or due to a very low autopsy rate in the country (Parija and Jayakeerthee, 1999).

Pan and Ghosh from Kolkatta, West Bengal reported the first two cases of PAM in India, both in children, in the year 1971 (Pan *et al*, 1971). The wet mount examination of centrifuged deposit of CSF showed motile amoebae. Both the child were treated with amphotericin B, sulfadiazine and intrathecal steroids. These children had the history of swimming. They responded to the treatment and recovered. Third case of PAM was reported by Bedi *et al* (1972) in a 45-year-old female from Udaipur, Rajasthan, in 1972. The case did not respond to treatment and she died of the disease. Since then many cases have been reported from different parts of India (Parija and Jayakeerthee, 1999) .A fatal PAM in a five month old child has been recently reported from, Mangalore India. This was the second case of PAM in an infant in the absence of the history of swimming (Shenoy *et al*, 2002). A case of GAE caused by *Acanthamoeba culbertsoni* was reported from Vellore in a 40-year-old man, who had cerebrospinal fluid rhinorrhea before the meningitis developed. The amoebae was demonstrated in and cultured from the cerebrospinal fluid. (Lalitha *et al*, 1985).

Recent history of swimming in thermal or stagnant water or of contact with fresh water, mud or dust, 2 to 6 days prior to the onset of symptoms of meningeal irritation and the age of the patient (usually children and young adults) may suggest a possible diagnosis of amoebic meningoencephalitis (Parija and Jayakeerthee, 1999).

Final diagnosis is always parasitic and depends on the detection and identification of trophozoites in the CSF or biopsied brain tissue. Motile trophozoites can be demonstrated on immediate microscopic examination of the uncentrifuged fresh CSF specimen either by light microscopy or phase contrast microscopy. Confirmation is done by culture and fluorescent antibody staining of CSF. Trophozoites can also be identified in the histologic sections of the brain biopsied tissue by immunofluorescence and immunoperoxidase method. CSF is sanguinopurulent or bloody and shows a strong neutrophilic reaction (Parija, 1996). CT can be used to demonstrate pathological changes in the cerebral hemisphere. Molecular methods like PCR (Sparagano ef *al*, 1994) and DNA probes (Sparagano, 1993) have been used.

Majority of cases of amoebic meningoencephalitis have proved to be fatal (Parija, 1996). Till date six patients are known to have survived the infection. Some of these cases responded to intravenous and intrathecal amphotericin B. Combination of intravenous and intrathecal amphotericin B, miconazole and oral rifampicin were also successful in some cases (Parija and Jayakeerthee, 1999). Avoidance of contact with stagnant or thermal water may be the only method for prevention of the disease (Parija, 1996).

CEREBRAL MALARIA

The cases of cerebral malaria are being increasingly documented from different parts of India .A prospective study of 441 adult patients of cerebral malaria from Bikaner, north-west India showed that fever and unconsciousness was common in all patients. One hundred forty five (32.87%) patients expired and mortality was highest in pregnant ladies (39.28%). The important observations of this study were stormy presentation, increased incidence of haemoglobinuria and jaundice, presence of neck rigidity, no prognostic relation to fundus abnormalities and high incidence of cerebellar ataxia and psychosis as neurological sequelae in survivors (Kochar *et al*, 2002).

Cases of cerebral malaria during pregnancy have been described. Cerebral malaria worsens the outlook of both for the mother and of the foetus in pregnancy. In a series of three pregnant patients with cerebral malaria one patient had intrauterine foetal death and died, one patient delivered a dead baby and the other had severe postpartum haemorrhage (Arya *et al*, 1989).

A study on clinical profile of falciparum malaria in a tertiary care hospital in Vellore, south India, revealed that among 86 patients with *P. falciparum* and mixed infection in Vellore, 24 (28 per cent) had cerebral malaria. Mortality of the order of 10 per cent was seen only in *P. falciparum* malaria. Out of a total of

64 patients of *P. falciparum* infections admitted to the District Hospital, Ukhrul, Manipur, 9.37% patients did not develop complication while the rest 90.63% developed one or more complications. Cerebral malaria was found to be the second most common complication after anaemia (76.56%) accounting for 59.38% of the cases (Chishti *et al*, 2000). Age and sex-wise break-up of cerebral malaria in Jabalpur, India showed that males suffered more from malaria and majority of patients belonged to 16-40 yrs age-group. Mortality was significantly higher in patients with hyperparasitaemia, hypoglycaemia and in patients with delayed diagnosis and treatment. Comatose condition was the main determinant of death (Shukla *et al*, 1995).

The incidence of severe malaria including cerebral malaria and malaria-specific mortality were investigated in a hospital, for miners and their families, at Tensa in the Sundergarh district of Orissa. Tensa lies in area where malaria (predominantly caused by *P. falciparum)* is hyper-endemic. It appears that the outcome of malaria is influenced not only by the intensity of local transmission (which affects the immunological status of the human hosts) but also by social factors such as the education and health-seeking behaviour of the local population and the health-care facilities available. The low incidence of severe malaria observed in Tensa was probably the result of patients presenting early in the course of their illness and taking antimalarial treatment, iron supplementation and supportive therapy at the appropriate times (Prusty and Das, 2001).

Recently, it was shown in the Akola district, Maharashtra State that Lambda-cyhalothrin 10% WP (ICON 10WP) spraying had a positive impact on the containment of malaria. The reduction of *P. falciparum* cases in three months post-spray period was 77% (from 47 cases to 11 cases) as compared to similar months of preceding year and overall reduction of total malaria cases was 50% during the same period. Neither cerebral malaria cases nor deaths due to malaria were recorded in the sprayed villages (Doke, 2000).

Diagnosis of malaria is established by demonstration of malaria parasites in peripheral thin and thick blood smears by microscopy. Peripheral blood should be collected before starting treatment with antimalarials. Blood is collected from earlobe or finger in older children and adults; and from the great toe in infants. It can be collected any time during the fever. Timing of collection of the blood is less important although a high density of malaria parasites appear in circulation during paroxysm of fever, in which schizonts burst and release merozoites. More important is the frequency of examination of blood smears. Smears should be examined at least twice daily until parasites are detected. The diagnosis of malaria is ruled out by obtaining negative thick blood smears on at least three different occasions. Microscopy confirms the diagnosis of malaria. A negative examination does not rule out malaria. Only 50% of cases, specially, children with malaria are smear-positive, even on repeated examination. Quantitation of parasitaemia is of prognostic value. It is carried out to determine whether parasitaemia is increasing or decreasing during antimalarial treatment. Quantitative buffy coat (QBC) is a method of microhematocrit centrifugation of the capillary blood, which is stained with a fluorescent dye such as acridine orange. QBC is a more sensitive method of detection of malaria infection. Disadvantage of the method is that it does not allow the identification of the species of *Plasmodium.*

Dipstick tests depend on the detection of histidine-rich protein-2 (PfHRP-2) antigen, a metabolic product specifically produced by *P. falciparum.* Monoclonal antibody (MAB) produced against PfHRP-2 antigen is employed in the test to detect (PfHRP-2) antigen in the serum or urine. Dipstick test is useful in detecting only *P falciparum* infections. It does not detect the other three malaria species. The test has high sensitivity and specificity. In patients with high parasitaemias, it is rarely negative. In low parasitaemias below 100 parasites/mL of blood, the test is not that effective The test is commercially available as the Parasite F test.

DNA and RNA probe, and PCR are highly sensitive and specific molecular methods for the diagnosis of falciparum malaria. PCR is highly specific. The species identification of malarial parasites present in the blood of an infected person can be made by this method. PCR is also highly sensitive .It can detect *Plasmodium* species in patients with parasitemias as low as 10 parasites/mL of blood.

The meticulous supportive cares along with intravenous administration of antimalarial drugs are the mainstay of treatment. Quinine is currently, drug of choice. Artimisinin derivatives are equally effective and can be used by intramuscular route. There is a need to search for effective malaria prevention and interventional strategies to avert high mortality and morbidity associated with cerebral malaria (Garg, 2000). Therapeutic monitoring of severe malaria should involve quantitative estimation of parasite load (Gupta *et al*, 2001).

MINOR CNS PARASITIC INFECTIONS

CEREBRAL STRONGYLOIDIASIS

Stmngyloides stercoralis, also known as the dwarf threadworm is the smallest pathogenic nematode known to cause infection in man (Parija, 1996). Infection with this nematode is potentially lethal because of its capacity to cause overwhelming autoinfectton particularly in the immunosuppressed host. The increased incidence of AIDS and the use of immunosuppressive therapy as in organ transplantation, leukemia, lymphoma has resulted in the occurrence of hyper infection by this nematode. Massive larval invasion of lungs, central nervous system and other tissue may occur with autoinfection, particularly in immunocompromised hosts (Heyworth, 1996). The disease manifestations are protean, mainly involving the respiratory, gastrointestinal, central nervous system and skin.

Over 100 million people are infected worldwide. *S. stercoralis* is worldwide in distribution however; it is more common in the tropics and sub-tropics including those of Africa, South America and Asia including India (Parija, 1996). Highly endemic areas have been identified in Southeast Asia and rural Brazil, where prevalence rates may approach 60%. Hyper infection strongyloidiasis has been described in patients with lymphomas, leukemia and lepromatous leprosy and in those treated with corticosteroids. Increasing reports of hyper infection in patients with AIDS have been documented in the last few years (Cahill and Shevchuk, 1996; Morgello *et al*, 1993 and Takayanagui *et al*, 1995). The exact prevalence of strongyloidiasis infection of the CNS in India is not known (Patil *et al*, 1999).

CEREBRAL AMOEBIASIS

Cerebral amoebiasis is a rare but serious complication of *Entamolba histolytica* infection. Brain abscess due to infection with *E. histolytica* although rare, has been reported from India. The condition is usually secondary to liver abscess, and may be associated with meningitis. Multiple intracerebral lesions may develop, however they may resolve almost completely without any neurological sequelae. The condition has been reported sporadically from different parts of India (Rao *et al*, 1988; Reddy *et al*, 1974 and Banerjee *et al*, 1983). Four cases of amoebic abscesses of the brain over a period of 18 years have been reported from Chandigarh. The patients were 2, 30, 40 and 50 year old males. The cases showed extensive, almost confluent lesions in the brain (Banerjee *et al*, 1983).

The diagnosis of amoebic brain abscess is based on a combination of clinical symptoms and signs, CT and MRI findings, a positive serologic test result, a dramatic response to anti-amoebic drugs after an ineffective therapeutic history with antibacterial drugs, and application of the PCR (Ohnishi *et al*, 1994). Oral administration of metronidazole, and parenteral dehydroemetine, in addition to drainage of the abscess is usually effective.

EOSINOPH1LIC MENINGITIS

Eosinophilic meningitis caused by *Angiostrongylus cantonensis*, was first detected in rats in Canton, China in 1933. The first human case was detected in Taiwan in 1944. Epidemic outbreaks were noted on Ponape from 1944 to 1948. The disease may present as transient meningitis or a more severe disease involving the brain, spinal chord and nerve roots, with a characteristic eosinophilia of the peripheral n

blood and CSF. Since 1961 it has been known that human infections are usually acquired by purposeful or accidental ingestion of infective larvae in terrestrial mollusks, planaria and fresh-water Crustacea. The use of mollusks and Crustacea as famine foods, favored delicacies and medicines has resulted in numerous outbreaks and isolated infections. Economic and political instability, illicit trade, unsanitary peridomestic conditions and lack of health education promote the local occurrence and insidious global expansion of parasitic eosinophilic meningitis (Ktiks and Palumbo, 1992).

In the past 50 years, *A. cantonensis*, the most common cause of eosinophilic meningitis, has spread from Southeast Asia to the South Pacific, Africa, India, the Caribbean, and recently, to Australia and North America. This disease, which is a peridomestic zoonosis though has been reported from Bombay in India, but is relatively rare (Parija, 1996).

CEREBRAL HYDATID DISEASE

Hydatid disease caused by *Echinococcus granulosus* is world wide in distribution. The presence of the cyst in the brain causing cerebral hydatid disease is relatively less frequent. Primary hydatid cyst of the brain in adults is rare, constitutes 1 %-2% of all cases of hydatid disease (Behari *et al*, 1997) and can pose various diagnostic problems.

Intracranial hydatid cyst although rare has been reported from India infrequently. In a series of five cases of cerebral hydatid disease reported from Mumbai, the mean age of presentation was 13.4 years. Four patients (80%) were in the first decade of life. All patients presented with focal neurological deficit and clinical features of raised intracranial pressure. CT, MRI and cystogram were instrumental in diagnosis. Two patients had multiple intracranial cysts. One patient had a solitary cyst in the lateral ventricle. Commonest location was in the parietal lobe (3 cases). Total excision of the cyst was done in all five cases. Recurrence was seen in two cases, probably as a result of rupture of the cyst during first surgery (Gupta *et al*, 1999). Intracerebral hydatid cyst has also been reported in a child with atrial septal defect (Patankar *et al*, 1999).

Multiple, infected, extradural, parasellar hydatid cysts in a patient constitutes an extremely rare presentation. A rare case of multiple infected extradural hydatid cysts of the parasellar region was reported from Lucknow. Two unusual histologically confirmed cases of cerebral hydatid diseases were also reported from Nagpur, These cases were exceptional because of the multiplicity of intracranial lesions and sparing of the liver and lungs (Patrikar *et al*, 1993).

BALAMUTHIA ENCEPHALITIS

Balamuthia mandrilaris is a recently described free-living amoebae known to cause sub acute-to-chronic infection of the CNS and belongs to the group *Leptomyxiidae* (*leptomyxid* amoeba). These parasites like *Acanthamoeba granulomatous* can cause amoebic encephalitis (GAE). Distinct from *Acanthamoeba*, which appears to favor the immunocompromised host, *Balamuthia* is capable of infecting both healthy and immunosuppressed hosts. It primarily affects the nasal pyramid or the skin, producing granulomatous amoebic lesions.

Although extremely uncommon, granulomatous amoebic encephalitis should be considered in the differential diagnosis of cerebral lesions. Till 1994, 30 cases of GAE due to *B. mandrillaris* have been recognized in humans, two in AIDS patients (Martinez *et al*, 1994). The condition is yet to be reported from India.

Conclusion

Parasitic infections of the CNS are being increasingly recognized as a significant cause of morbidity and mortality. Early diagnosis of these infections by rapid, near patient, bedside tests is a priority. The growth of international travel has made even non-endemic diseases fairly common in those areas of the world where these diseases were not recognized previously. It is clear that a comprehensive approach involving parasitologists, neurologists, neurosurgeons, and neuropathologists is needed in the field of patient management. This is a challenge and an opportunity.

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Host-parasite relationship : Fatty acid compositions of a trematode, *Paramphistomum cervi* and common Indian goat, *Capra hircus*

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Lipid classes and fatty acid compositions of a digenetic trematode, *Paramphistomum cervi* were analyzed by GLC. Fatty acid composition of the serum of its host, *Capra hircus* was compared with that of the parasite. The percent content of neutral lipid (39.04), glycolipid (21.23) and phospholipid (39.73) were recorded. The total percent of saturated, monoene, diene and PU in FA m both the parasite and the host were 48.41 and 33.93; 28.59 and 22.29; 13.42 and 14.87 as well as 3.67 and 9.69, respectively. Palmitic (saturated) and oleic (unsaturated) acid were the predominant fatty acids in both the parasite and the host. It is presumed that the parasite is completely dependent on the host for fatty acids, however, parasite increases the amount of some of the fatty acids by chain elongation process.

Keywords: Digenetic trematode, Goat, fatty acids, Lipid classes, *Paramphistomum cervi.*

INTRODUCTION

Adult stages of digenetic trematodes live in an anaerobic condition within the vertebrate host. It is found that parasitic helminths are unable to synthesize long chain fatty acids *de novo* (Barrett, 1981; Brouwers *et al.*, 1998a,b). However, trematodes acquire fatty acids from its host in the form of short chain acids. Platyhelminths can change the relative proportions of lipid classes (Barrett, 1981) in the membrane, probably, as a part of parasitic adaptation.

In the present study, the major lipid classes and fatty acid composition of *Paramphistomum cervi*, a digenetic trematode inhabiting the rumen and reticulum of common Indian goat, *Capra hircus* have been investigated together with the study of fatty acid compositions of host serum to decipher the host-parasite relationship.

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MATERIALS AND METHODS

Paramphistomum cervi (Schrank, 1790), a digenetic trematode parasite, were collected from rumen and reticulum of stomach of common Indian goat, *Capra hircus*, from Pandooah area, District Hooghly, West Bengal, India. The parasitic samples were pooled to achieve a wet weight of 2 gram. Worms were washed in Locke's solution for further processing. Blood serum sample (4.5 ml) of the host was also collected at the same time to make a comparative study in the fatty acid compositions of the host and parasite. Total lipids were extracted from the trematode, *P. cervi* sample and from the serum sample of the host, *C. hircus* following the procedure of Bligh and Dyer (1959) using methanol: chloroform (2:1, v/v), methanol: chloroform: water (2:1:0.8, v/v/v) and then again the first solvent system. The chloroform solution of lipid was evaporated under vacuum, redissolved in distilled hexane and kept at -20°C for future use. BHT (butylated hydroxy toluene) was added at a level of 100 mg/L to the solvent as antioxidant. A portion of total lipid of the trematode sample was subjected to

thin layer chromatography (TLC) using silica gel G (SRL) following Rouser *et al.* (1976) for identification of lipid classes. The remaining portion of total lipid of the trematode and the serum sample of the host were processed following Christie (1982) for fatty acid methyl ester (FAME) formation. These FAMEs were purified separately following Mangold (1969) and Misra *et al.* (1984) by TLC and gas liquid chromatography (GLC) which has been performed for the analyses of fatty acids separately on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID). It was done on a BPX-70 megabore capillary column of 30 mt length and 0.53 mm i.d. obtained from SGE, Australia. Oven temperature was programmed from 150°C - 240°C with a rate of 8°C per minute. Initial and final times were kept isothermal for 2 min and 20 min, respectively. Injection port and detector temperatures were 250°C and 280°C, respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.7 ml per min. Quantification was done by computer using specific Winchrom software.

RESULTS

Total lipid content of *P.cervi* is 1.23% of the wet weight of the tissue. Percentages of lipid classes of *P.cervi* are 39.04 (neutral lipid), 21.23 (glycolipid) and 39.73 (phospholipid). The fatty acid composition of both the trematode parasite and the serum of the host are represented in Table I. Twenty-two fatty acids are identified from both the parasite and host serum. The total percent of saturated fatty acid is 48.41 in the parasite, among which the percentage of palmitic acid (38.15) is highest. Among the unsaturated monoenoic (28.59%), dienoic (13.42%) and polyenoic (3.76%) fatty acids, 18:1, 18:2n-3 and 22:5n-6 fatty acids, respectively, are present as the highest amounts.

A total of 14.49% of fatty acids of the host serum remain unidentified. However, it shows 33.93% saturated fatty acids among which palmitic (16.66%) is present as the highest amount. Among the unsaturated monoenoic (22.29%), dienoic (14.87%) and polyenoic (9.69%) fatty acids, 18:1, 18:2n-3 and 20:4n-6 are present in significant amounts.

DISCUSSION

Total lipid content of trematodes varies among species (Table II). The phospholipid and neutral lipid contents of *P.cervi* are very close. In trematodes, phospholipids account for 25% of total lipids (Goil, 1964; Smith and Brooks, 1969; Oldenborg *et al.*, 1975), whereas it is much higher in *P.cervi* (39.73%). Both phospholipid and glycolipid are known to form an integral part of membrane component (both cellular and tegumental) and neutral lipid probably functions as storage form of fatty acids. In the present study, a good amount of glycolipid (21.23%) is recorded. It is to be mentioned that glycolipid was not reported from parasitic trematodes. Glycolipid is very much essential for

Table I. Fatty acid compositions (expressed as % of total fatty acids) of trematode, *Paramphistomum cervi* and blood serum of common Indian goat, *Capra hircus*, analyzed by GLC as FAME.

Fatty Acids	*Paramphistomum cervi*	*Capra hircus*
16:0	38.15	16.66
17:0	0.18	0.77
18:0	9.21	16.38
20:0	0.87	0.12
Σ Saturated	48.41	33.93
16:1	0.17	1.34
17:1	0.56	1.00
18:1	26.41	17.91
20:1n-9	0.36	0.27
22:1n-9	0.11	1.21
22:1n-11	0.57	0.22
24:1n-9	0.41	0.34
Σ Monoenes	28.59	22.29
17:2	0.18	0.74
18:2n-3	13.02	13.87
18:2n-6	0.22	0.26
Σ Dienes	13.42	14.87
18:3n-3	0.36	0.18
18:3n-6	0.91	2.41
18:4n-3	0.14	0.36
20:4n-3	0.85	0.40
20:4n-6	0.02	3.77
22:5n-3	0.07	1.34
22:5n-6	1.31	0.19
22:6n-3	0.10	1.04
Σ Polyenes	3.76	9.69
Unidentified	-	14.49

membrane structure in all categories of animal organization especially for the endoparasitic stages of trematodes where tegument is actively engaged in absorption.

Oleic acid (18:1) is the main C 1/8/ fatty acid in most parasitic helminths. However, in adult *Echinostoma trivolvis* the predominant fatty acids were saturated and most prevalent ones were palmitic (17%), stearic (16%), linoleic (11%), oleic (9%) and arachidonic (9%) acids (Fried *et al.,* 1993). Stearic acid (18:0) was the most predominant one in *F. hepatica* and helminths, in general, tend to have higher percent (40-70% of total acids) of unsaturated C_{18} acids (Barrett, 1981). Occurrence of higher percent of unsaturated C_{18} acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{18:4}$) in *P. cervi* (41.06%), thus, lies within the above mentioned range (Table I).

Fatty acid classes are common to both *P. cervi* and its host serum, however, they differ in their percent content. Earlier it was assumed that the chain elongation mechanism of fatty acids in platyhelminthes (cestodes and trematodes) might be similar to that of mammalian mitochondrial system (Barrett, 1981). Furlong (1991) reported that trematodes could modify fatty acids by chain elongation. Such mechanism has been demonstrated in *Spirometra mansonoides* for C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ to C_{20} and C_{22} fatty acids (Meyer *et al.,* 1966). It has also been reported that the cestode, *H. diminuta,* can convert palmitate and stearate into saturated fatty acid chains as long as 26 carbons and *F. hepatica* can synthesize $C_{20:1}$ and $C_{20:2}$ from $C_{18:1}$ and $C_{18:2}$ (Barrett, 1981). Trematodes in general have lost the ability to synthesize long chain fatty acids *de novo* (Meyer *et al.*, 1970; Barrett, 1981). Brouwers *et al.* (1997) mentioned that efficient conversion of oleate (18:1) to eicosanoids (20:1) took place by chain elongation in adult *S. mansoni.* Thus, *P. cervi* might rely on chain elongation of preformed or absorbed short chain fatty acids procured through the body tegument from the host. The major product of the fatty acid synthase is palmitate. Evidence also suggests that in most organisms, the fatty acid synthesis cycle stops at palmitate (C_{16}) and it acts as the precursor for both saturated and unsaturated long chain fatty acids (Barrett, 1981). Ackman (2000) commented that 16:0 is the principal fatty acid at all evolutionary and trophic levels. Therefore, it is also quite probable that palmitic acid serves as a precursor for both saturated and unsaturated acids in *P. cervi.* Barrett (1981) reported that *F. hepatica* derive short chain fatty acids (acetate, propionate, butyrate) through a mixture of diffusion and use mediated transport mechanism. Therefore, being a secondary consumer, *P.cervi,* might follow these strategies for the uptake of fatty acids present in lower percentages.

Presence of higher amounts of fatty acids such as 16:0, 18:0, 18:1 and l8:2n-3 both in the parasite as well as in host serum signify that these fatty acids play an important role in both the systems. Fatty acids are physiologically important components of phospholipid and glycolipid, lipophilic modifiers of proteins, fuel molecules and intracellular messengers in mammals. Stryer (1994) commented that arachidonic acid (20:4n-6) derived from linoleate is the major precursor for several classes of signal molecules as is found in the serum of the host, *C.hircus.* It was reported that palmitic acid and oleic acid are incorporated into the neutral lipids of *P. microbothrium* (Awharitoma *et al.*, 1990), therefore, it may be assumed that the presence of oleic and palmitic acid in higher percent in the trematode, *P. cervi* is due to efficient incorporation into neutral lipid.

Unsaturated fatty acids in mammals are derived from either palmitoleate (16:1), oleate (18:1), linoleate (18:2) or linolenate (18:3) (Stryer, 1994). Henderson (1996) reported the conversion of 18:2n-6 and 18:3n-3 fatty acids to e_{20} and e_{22} homologues in freshwater fishes. Okuyama (2000) reported that the two essential fatty acids linoleate and linolenate show a desirable balance. However, plants are the major source for linoleic and linolenic acids (Ackman *et al.*, 2002) as is reflected in the result of serum of *C.hircus.* Higher levels of these acids in the trematode, *P.cervi,* prove that these fatty acids propagate through trophic levels.

Adaptation to anaerobic conditions by trematode limits its energy budget. This might restrict the parasite for *de novo* synthesis of fatty acids. The major outcome of the host-trematode relationship is that the parasite by absorbing all its required fatty acids minimize their

Table II. Lipid compositions in various flatworms.

Flatworms	Total lipid (%)		Phospho-lipid	Neutral lipid	References
	Dry weight	Wet weight	(%)	(%)	
P. cervi	-	1.23	39.73	39.04	Present observation
P. cervi	4.7	-	49.67	50.33	Vykhrestyuk and Yarygina, 1975
C.erschowi	4.75	-	49.7	50.3	Vykhrestyuk and Yarygina, 1982
E.pancreaticum	15.73	-	24.72	75.28	Vykhrestyuk and Yarygina, 1975
S.mansoni	34.2	-	48	50	Smith and Brooks, 1969; Meyer *et al.*, 1970
F.hepatica	12-13	-	30	-	vonBrand, 1928, 1973; Smyth and Halton, 1983
P.explanatum	1.36-4.5	-	-	-	Goil, 1964
G.crumenifer	1.36-4.5	-	-	-	Goil, 1964
C.cotylophorum	27.3	-	-	-	Smyth and Halton, 1983
G.explanatum	34.4	-	-	-	Yusufi and Siddiqi, 1976
P.microbothrium	-	2.5	-	-	Hrzenjak and Ehrlich, 1975

energy budget for this purpose, which is otherwise used in their successful reproductive strategy.

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Prevalence of Visceral leishmaniasis in suspected rodent reservoirs in Azarshahr County, northwest of Iran

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A survey was conducted during 2003-2004 in Azarshahr area which is a new endemic region for visceral leishmaniasis in Iran for determination of host reserviors. 265 rodents of 7 genera/species were trapped alive. Antileishmanial antibodies were detected by the direct agglutination test (DAT), indirect fluorescent antibody test (IFAT) and parasitological method. Fourteen (5.3%) animals were seropositive and twenty-seven (10.2%) of them provided lower titres than positive titer and two hundred and twenty-four (84.5%) animals were shown to be seronegative. Amastigotes of *Leishmania* were observed in four seropositive rodents including one *Meriones persicus,* two *Cricetalus migratorius* and one *Mesocicetus auratus* after dissection and parasitological examinations. The parasites isolated from these rodents were identified as *Leishmania infantum* through PCR analysis. According to results of this study, those infected rodents are assumed to be potential host reserviors for visceral leishmaniasis in this region. This work is the first report of the detection of *L. (L.) infantum* in a naturally infected *ricetalus migratorius, Meriones persicus* and *Mesocicetus auratus* from Iran.

Keywords : **Epidemiology, Host reservior, Kala azar, Leishmaniasis, PCR**

INTRODUCTION

Visceral leishmaniasis (VL), or Kala-azar, is dispersly endemic in several provinces of Iran including East Azerbaijan, Ardabil, Fars, and Quam. In other provinces of the country, the disease has been reported in sporadic form (Nadim *et al.*, 1978 Edrissian *et al.*, 1988; and Fallah and Mohebali, 2001 and Mazlumi-Gavgani *et al.*, 2002). Azarshahr county has been also reported to be endemic for VL (Mirsamadi *et al.*, 2003 Farshchian *et al.*, 2004) Although dogs are the main reservoirs for human infection to VL (Edrissian *et al.*, 1988), wild carnivores such as jackals and foxes have been also found infected with *Leishmania* spp. These animals are assumed to be reservoirs for parasites, particulary where sporadic cases of disease have been reported. (Nadim *et al.*, 1978; Hamidi *et al.*, 1982; Mohebali *et al.*, 2005). Infection of *L. infantum* in rodents have been previously reported in multiple genera/species (Edrissian 1990) Mohebali *et al.* 1998 and Fernanda *et al.*, 2005.

The main objective of this investigation was to screen *leishmania* infection in rodents through serological and parasitological examination in Azarshahr county, an endemic part of East Azerbaijan. The second aim of this study was to assess the role of rodents in transmission of the disease to children and animals.

MATERIAL AND METHODS

Source of samples

Rodents were trapped alive in various parts of the Azarshahr county,located in East Azerbaijan.Blood samples were collected from each animal in two heparinized capillary tubes before sacraficing them.Initially.blood samples were tested by direct agglutination test (DAT) and indirect fluroscent

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antibody test (IFAT) as per the procedures described by Harith AE, 1988 and 1989.

ANTIGEN PREPARATION AND SOURCE OF ISOLATE

Briefly, to prepare the antigens, promastigotes of *Leshmania* were mass produced in RPMI 1640 (Sigma) containing fetal calf serum (FCS) followed by trypsinization of the parasites, staining with Coomassie blue and fixing with formaldehyde. The parasite source included an isolate of *Leishmania donovani* strain I-S, kindly provided by Harith to the Protozoology Unit of the School of Public Health, Trehan. The live culture was maintained in Novy-Mac Neal-Nicolle (NNN) medium enriched with liver infusion broth typtose (LIT), liquid phase (Sadigursky M and Brodeskeym CT, 1986) and used for antigen preparation. *Leshmania donovani* antigen prepared in our laboratory (Edrissian et al, 1981) was used for testing rodents serum samples through IFAT.

SAMPLE PROCESSING

The spleen and liver samples of seropositive rodents ($\geq$1:80 in DAT and IFAT) were cultured in NNN medium containing FCS and checked twice a week for six weeks. A total of 1512 smears were prepared from blood (as for thick and thin smears), spleen and liver samples (as of impression smears). The smears prepared from all animals were stained with standard giemsa stain, and examined microscopically for presence of *Leishmania* amastigotes. The promastigotes isolated from the media as well as corresponding organ samples were tested using PCR.

RESULTS

A total of 265 rodents belonging to 7 genera/species were trapped alive in various parts of the Azarshahr county. These included eight (3%) *Cricetulus migratorius* (grey hamster), one hundred and seventeen (44.2%) *Mus musculus* trapped in residential houses. Animals trapped outdoors in

Table I. Rodents trapped alive in Azarshahr county, located in north-western region of Iran during 2003-2004

Trapping Location	*Mus musculus*	*Merones persicus*	*Rattus norvegicus*	*Cricetalus migratorius* (Grey hamster)	*Mesocricetus auratus* (Golden hamster)	*Sciurus anomalus*	*Hystrix Indica*
Azarshahr-Pazikuh	5	0	0	3	0	0	2
Azarshahr-Kurdan	17	0	0	0	0	0	0
Azarshahr-Shahidbehshti	16	0	0	0	0	0	0
Azarshahr-(river, sewage stream)	0	0	60	0	0	0	0
Segayesh Village	23	27	0	5	2	1	0
Gavahir Village	8	0	0	0	0	0	0
Jaraghil Village	8	0	0	0	0	0	0
Yengieh Village	10	30	0	0	0	0	0
Germizigol Village	2	11	0	0	0	0	0
Amirdizaj Village	5	0	0	0	0	0	0
Almalx Village	3	7	0	0	0	0	0
Total	117	75	60	8	2	1	2
Percentage (%)	44.2	28.3	22.5	3	08	0.4	0.8

Table-II: Parasitological & serological (DAT) screening of 265 rodents trapped in Azarshahr county, located in north-western region of Iran, during 2003-2004.

Species	No tested	Positive on Parasito exam		Positive on Culture medium		Neg		DAT, *Leishmania* antibody titers >1:20		1:40		1;80		1:160		1:132	
		No	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Mus musculus	117	0	0	0	0	106	89.7	5	4.3	4	3.4	3	2.6	0	0	0	0
Meriones persicus	75	3	4	3	4	53	70.6	8	10.6	7	9.3	4	5.3	3	4	0	0
Rattus norvegicus	60	0	0	0	0	60	100	0	0	0	0	0	0	0	0	0	0
Cricetalus migratorius (Grey Hamster)	8	3	37.5	3	37.5	3	37.5	0	0	2	25	2	25	0	0	1	12.5
Mesocricetus auratus (golden hamster)	2	2	100	2	100	53	0	0	0	1	50	0	0	1	50	0	0
Sciurus anomalus	2	0	0	0	0	2	100	0	0	0	0	0	0	0	0	0	0
Hystrix Indica	1	0	0	0	0	54	100	0	0	0	0	0	0	0	0	0	0
Total	265	8	3.02	5	1.9	224	84.5	13	4.9	14	5.3	9	3.4	4	1.5	1	0.4

Table-III: Parasitological & serological (IFAT) screening of 265 rodents trapped in Azarshahr county, located in north-western region of Iran, during 2003-2004.

Species	No tested	Positive on Parasito exam		Positive on Culture medium		IFAT, *Leishmania* antibody titers													
		Neg		>1:10		1:20		1:140		1:180		1:160		1:320					
		No	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Mus musculus	117	0	0	0	0	106	89.6	5	4.3	2	1.7	2	1.7	2	1.7	0	0	0	0
Meriones persicus	75	3	4	3	4	50	70.6	5	6.7	6	8	7	9.3	5	6.7	2	2.7	0	0
Rattus norvegicus	60	0	0	0	0	60	100	0	0	0	0	0	0	0	0	0	0	0	0
Cricetalus migratorius (Grey Hamster)	8	3	37.5	3	37.5	3	37.5	0	0	0	0	2	25	2	25	0	0	1	12.5
Mesocricetus auratus (golden hamster)	2	2	100	2	100	0	0	0	0	0	0	1	50	0	0	1	50	0	0
Sciurus anomalus	2	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0
Hystrix Indica	1	0	0	0	0	2	100	0	0	0	0	0	0	0	0	0	0	0	0
Total	265	8	3.02	5	1.9	222	83.8	10	3.6	8	2.9	12	4.4	9	3.3	1	1.1	1	0.4

villages included seventy five (28.3%) *Meriones persicus,* two (0.8%) *Mesocricetus auratus,* sixty (22.5%) *Rattus norvegicus,* one (0.4%) *Sciurus anomalus* and two (0.8%) *Hystrix indica.* Tables I-III describes the results of the parasitological and serological tests.

Leishmania spp. were isolated from spleens of two *Mer. persicus.* one *Mes. auratus* and one *Mes. migratorius* in NNN+FCS culture media. The promastigotes isolated from *Mer. persicus*, *Mes. auratus* and *Mes. migratorius* were characterized *Leishmania infantum* by PCR technique.

DISCUSSION

Mer. persicus was reported to be naturally infected with *Leishmania* in East Azarbaijan, Iran. In the smears prepared from the cutaneous lesions of *Mer. persicus,* considerable numbers of amastigotes were seen. No amastigote was seen in microscopic examination of the the smears prepared from the internal organs and blood samples of this rodent (Edrissian *et al.,* 1975). According to data collected in this study, amastigotes were observed in 1.5% of the rodents after microscopic examination of the smears prepared from internal organs. As reported by Mohebali and coworkers from the Meshkin-Shahr county, *Leishmania* spp. were isolated from spleen of 2 *Mer. persicus* and 1 *Mes. auratus* as cultured in NNN+LIT. Amastigotes were observed in 16.5% of the rodents, and *L. donovani* LON-50 was isolated from 2 *Mer. persicus.* Meanwhile, using isoenzyme analysis, promastigotes isolated from *Mes. auratus* were identified as *L. infantum* zymodeme LON-49 (Mohebali *et al* 1998 and 1995).

Rattus rattus and *Trichomys apereoides* were the most abudant rodent species in an endemic area of visceral leishmaniasis in Brazil. Meanwhile, DNA belonging to *L. braziliensis, L. mexicana* and *L. donovani* complexes was confirmed in several individuals of *R. ratus* (Fernanda *et al.,* 2005). As reported by Edrissian (1990). *L. infantum,* a zoonotic species, was isolated from humans in Meshkin-Shahr county. Parasitological and serological tests performed in 30 wild canines showed that 10% of these animals were infected with *L. infantum* (Edrissian *et al.,* 1993). According to Mohebali and coworkers (1995, 1998 and 2005), the parasite was isolated from 2 *Mer. persicus,* 1 *Mes. auratus,* 1 *Mes. migratorius* and dogs in Meshkin-Shahr county and from dogs in Karaj vicinity located 40 km from west Tehran. Using molecular and biochemical procedures, 10 out of 11 *Leishmania* isolates obtained from dogs and wild canines were identified as *L. infantum* and one as *L. tropica.* In addition to humans and dogs, wild carnivores such as jackals and foxes have been reported to be infected with *Leishmania* in Iran (Edrissian *et al.,* 1993 and Hamidi *et al.,* 1982). *L. infantum* was isolated from *Rattus rattus* in Italy and Iraq (Desjeux , 1991).

Observing natural *Leishmania* infection in rodents trapped in a highly endemic area may provide an association of rodents, particularly those living in houses, with transmission and the spread of disease to the children. Further studies are needed to clarify the exact role of rodents as reservoirs ofkala-azar in endemic areas.

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The ABO Blood Groups and Malaria

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A total of 215 malaria patients and 180 non-malarial (control) subjects were investigated for evidence of ABO blood groups. The blood group 'A' (39.06) was found to be dominant followed by 'O' (33.95%), 'B' (23.72%) and 'AB' (3.26%) group in the malarial cases. In non-malarial healthy controls, most common blood group was 'B' (36.67%) followed by group 'O' (30.55%), group 'A' (25.00%) and group 'AB' (7.77%). Thus the blood group 'B' was found to be significantly less frequent ($p<0.05$) in material cases as compared to be control subjects whereas the blood group 'A' was found more frequently in malaria cases ($p<0.05$). The results suggest some correlation between the blood groups and malaria.

Key words : ABO blood groups, Malaria, *Plasmodium falciparum*

INTRODUCTION

Human being have been classified into four categories depending upon the presence of A or B or both the blood group antigens or their complete absence on the red blood cells (erythrocytes) after discovery of ABO blood groups (Landsteiner, 1900). The distribution of the respective blood groups greatly varies amongst different ethnic group or human races of the world (Boyd, 1939; Mourant and Kopec, 1958). A association of these blood groups with certain infectious (leprosy, tuberculosis, syphilis, hepatitis, chicken pox, small pox, diarrhoea etc.) and non-infectious (cancer, diabetes mellitus, dental carries etc.) diseases has also been observed by several workers (Chakravarti, 1986). However, the information related to malaria and ABO blood groups is relatively scanty and in conclusive. Therefore, the present study was undertaken simultaneously with the survey of haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency (G^d) in inhabitants of tribal rural areas of Aravali hilly region (Southern-Rajasthan), where malaria is also hyper-endemic, to find out any correlation between the ABO blood groups and malaria.

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MATERIALS AND METHODS

The present study was performed in the tribal rural areas of Banswara. Dungarpur and Udaipur districts of southern Rajasthan where *Plasmodium falciparum* and *P. vivax* species are widely prevalent. During the survey of red cell genetic disorders (Choubisa, 1991; Choubisa, 1997) blood samples from 586 unrelated pyrexia cases were taken. Simultaneously thin and thick blood smears were also prepared. Their staining and examination procedures for malaria parasite were as described earlier (Choubisa and Choubisa, 1992). The blood samples of infected individuals (malarial cases) were investigated further for evidence of ABO blood groups according to Dacie and Lewis (1986). Simultaneously, blood samples from 180 apparently healthy and unrelated subjects (control) were also screened for determining their blood groups. The major sources for blood samples were Primary Health Centres, Hostels and Schools located in the studied areas.

RESULTS AND DISCUSSION

Out of 586 unrelated pyrexia cases 215 (36.68%) were found to be infected with *Plasmodium falciparum*

parasites with varying degrees of parasitaemia. Amongst infected individuals 84 (39.06%) showed the evidence of blood group 'A', 73 (33.95%) of'0', 51 (23.72%) of'B' and 7 (3.26%) of 'AB' (Table). The trend ofphenotype picture ofABO blood groups in these infected malarial cases was found as A>0> B>AB.

In the control group (apparently healthy subjects) blood group 'B' was found to be predominant instead of blood group 'A' and the trend of phenotypic picture of these ABO blood groups was found as B > 0 >A > AB. These data were also analysed statistically by x^2 test and the difference in the phenotype frequency of blood groups 'A' and 'B' in malarial and non-malarial subjects was found to be significantly different ($p<0.05$).

Results of the present study suggest a possible relationship between the ABO blood groups and malaria. In the present study, blood group 'A' was found to be more frequent in malarial subjects as compared to the non-malarials. Relationship between ABO blood groups and malaria is yet not so clear and the findings are controversial. It has already been suggested that malaria like many other communicable diseases share common antigen with ABO blood groups (Gupta and Rai Choudhuri, 1980). These workers have also reported that malarial parasites share group 'A' antigen and hence are better tolerated by host's immune system. In another study, the blood group 'A' was found to be dominant and frequently present in the malarious cases as compared to the non-malarious subjects (Pal, 1976). Later on, Pant et al. (1992) have also observed a significant correlation between ABO blood groups and falciparum malaria and suggested that blood group 'B' and '0' may have an advantage against malaria parasite infection. Similar observations and suggestions have also been made previously by Athreya and Coriell (1967). However, Mollineaux and Gramiccia (1980) and Vasantha et al. (1982) did not find any correlation between the ABO blood groups and malaria in Africans and Indian tribals (of Dadra and Nagar Haveli) respectively. Neverthless, another phenotype "Duffy negative" blood group is resistant to *Plasmodium vivax* infection. Miller et al (1975, 1976 and 1978) observed that "Duffy negative" erythrocytes are resistant to invasion by *P. knowlesi* and *P. vivax.* These findings indicate that the susceptibility to *Plasmodium* infection may be associated with different blood groups. It is also evident from the present study that the blood group 'B' is less frequent in malarial cases as compared to the normal healthy subjects (controls) which suggests that blood group 'B' may have an advantage against *P. falciparum* infection in the present surveyed areas (Aravali hilly region) where malaria is hyperendemic and more common in tribal population.

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Table. Phenotype frequency of ABO blood groups in malarial and non-malarial (control) subjects

Subjects	No tested	Phenotype frequency			
		A*	B*	O	AB
Malarial	215	84 (39.06)	51 (23.72)	73 (33.95)	7 (3.26)
Non-malarial	180	45 (25.00)	66 (36.67)	55 (30.55)	14 (7.77)

Figures in parentheses indicate percentage.

$X^2 = 7.89$ ($p<0.05$) significant at the level of 5% (Malarial, non-malarial Vs A, B, O, AB).

* - > X^2 value ($p<0.05$) significant (for group A and B in Malarial Vs Non-malarial subjects.)

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Plasmodium chabaudi chabaudi AS infection in mice : role(s) of erythrophagocytosis and nitric oxide in parasite clearance

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Several lines of evidences, both from field and experimental studies, have shown that protective immunity to malaria does develop; however, the mechanism(s) of parasite clearance remain elusive. In an attempt to answer this question, using *Plasmodium chabaudi chabaudi* AS infection in BALB/c mice in which it causes a self-resolving infection, we studied the relationship between erythrophagocytosis and nitric oxide (NO) production, and parasite clearance. *Ex vivo,* splenic macrophages from infected-mice exhibited 8.6-fold augmented erythrophagocytosis, and produced enhanced (42.2-fold) NO, just before the beginning of the resolution of parasitaemia. Apparently, these preliminary data, for the first time, indicate the effective role(s) of both erythrophagocytosis and NO in augmented parasite clearance during *P. chabaudi chabaudi* AS malaria in mice, and thus suggest their plausible role(s) in the expression of protective immunity to human malarias.

Keywords: *P. chabaudi chabaudi* AS, Macrophages, Erythrophagocytosis, Nitric oxide

INTRODUCTION

Malaria, a re-emerging disease, is caused by the parasites of the genus *Plasmodium* of which only four species infect humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Though the cause of malaria was identified more than a hundred years ago, it still remains one of the leading cause of morbidity and mortality in the tropical and sub-tropical regions of the world, and is considered a humanitarian disaster (Hastings *et al.*, 2002). Approximately 40% of the world population is at the risk of malaria, and its worldwide incidence is estimated to be 300 - 500 million clinical cases and 2 - 3 million deaths (mostly children), annually (Moorthy *et al.*, 2004), representing 2.3% of the overall global disease burden (Engers and Godal, 1998). Globally, every 30 seconds a child dies of malaria (Webster, 2001), resulting in a loss of more than 2,000 lives/day. It is a death toll that far exceeds the mortality rate of AIDS. Vaccination against malaria is considered a cost-effective control strategy (Sachs, 2002). However, despite over 22 years of intensive research, a safe and effective vaccine against human malaria has not been developed (Butler *et al.*, 1997; Facer and Tanner, 1997; Kaur *et al.*, 2002; Moorthy *et al.*, 2004). Though *P. falciparum* genome has been completely sequenced (Gardner *et al.*, 2002), our understanding of the functional genomics and proteomics is still very poor. Consequently, one of the difficulties hindering the development of a successful vaccine against malaria is our incomplete knowledge of the molecular mechanism(s) of the pathogenesis and protective immunity, and of how it can be induced (Angulo and Fresno, 2002).

Early events in the host response of the innate immunity to an invading malaria pathogen are thought to be responsible for determining the development of acquired immunity, and the eventual outcome of infection. The innate immune system mainly consists of professional phagocytes of the granulocyte and

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monocyte lineage, including neutrophils, circulating monocytes and tissue-based macrophages, and several cytokines. Though several rodent (Taylor-Robinson, 1995) and simian malaria models (Deans *et al.*, 1988; Yang *et al.*, 1999) are available for understanding protective immune mechanisms leading to parasite clearance, all of them apparently have their own limitations. *P. chabaudi chabaudi* AS infection in mice that causes a biphasic parasitaemia, is a recognized model for *P. falciparum* infection in humans, and is considered a suitable model for the study of the induction of protective immune mechanisms during malaria (Taylor-Robinson *et al.*, 1993). Previous studies have shown that Th1-associated cytokines, including interleukin-12 (IL-12), interferon-γ (IFN-γ), and tumour necrosis factor-α (TNF-α), play protective roles in resistance to the acute-stage of infection during *P. chabaudi chabaudi* AS malaria in A/J mice (Stevenson *et al.*, 1995). Further, it has long been recognized that macrophages play a critical role in protection from microbial pathogens, and are the main cells responsible for the elimination of malaria parasite-infected erythrocytes from the circulation (Taliaferro and Cannon, 1936; Shear *et al.*, 1979; Playfair, 1990; Mota *et al.*, 1998). Reportedly, macrophages have the potential to phagocytose and kill the asexual erythrocytic forms of malaria parasites, *in vitro* (Kumaratilake and Ferrante, 1992). Treatment of mice with silica, a selective killer of macrophages, has been reported to result both in the rise in *P. berghei* (Singh *et al.*, 1994) and *P. chabaudi* AS parasitaemia, and the death of mice (Stevenson *et al.*, 1989). At molecular level, nitric oxide (NO) has been reported to be an effector molecule involved in the killing of intraerythrocytic stages of malaria parasites (Nussler *et al.*, 1994). Rockett *et al.* (1991; 1992) have suggested that reactive nitrogen intermediates can have a direct cytotoxic effect against the asexual stages of *P. falciparum, in vitro*. *P. chabaudi* AS blood-stage infection in resistant mice has been shown to increase NO production (Jacobs *et al.*, 1995; Su and Stevenson, 2000), that in turn has also been shown to effectively control blood-stage malaria (Taylor-Robinson *et al.*, 1993). Thus, though both erythrophagocytosis and NO, separately, have been thought to be involved in protection form rodent malarias; however, their combined action has apparently not been studied in exclusive terms of parasite clearance. We, thus, hypothesized that erythrophagocytosis and NO, together or cumulatively, may be responsible for the augmented clearance of malaria parasite. Therefore, the present study was designed specifically to investigate the combined role(s) of erythrophagocytosis and NO in augmented parasite clearance during *P. chabaudi chabaudi* AS infection in resistant BALB/c mice. The results showed that both erythrophagocytosis and NO seem to be involved in the augmented clearance of malaria parasites.

MATERIALS AND METHODS

Animals, parasites and infection: Male 20±2 g, BALB/c mice, were obtained from the Central Animal Facility of the Institute, and maintained at 22 - 24°C with standard animal feed and clean water (supplemented with 0.5% *p*-amino benzoic acid) provided *ad libitum*. All studies were carried out in accordance with the Guide for Care and Use of Animals in Scientific Research, Indian National Science Academy, New Delhi, as adapted and promulgated by the Institutional Animal Ethics Committee. *P. chabaudi chabaudi* AS clone (kindly provided by Professor D. Walliker, Edinburgh, UK) was maintained by weekly passage in mice, intraperitoneally (i/p), and cryopreservation. Mice were infected by inoculating with $1x10^6$ parasitized-erythrocytes (PE), i/p, from a donor mouse. The parasitaemia was monitored by light microscopic examination of $1x10^4$ erythrocytes in Giemsa-stained thin tail-blood films, and expressed as % PE.

Splenic macrophage culture: The adherent splenic macrophages (SM) were harvested from uninfected and *P. chabaudi chabaudi* AS-infected mice, and their number was determined as described (Singh and Singh, 2001). The SM were harvested on day 0, 2, 4, 6, 8, 10, 12 and 14 post-infection, and separate sets of experiments were run on different days. SM were >96% pure according to morphologic, phagocytic and non-esterase staining criteria, and >98% viable as judged by trypan blue exclusion.

Determination of nitrite: SM from uninfected and *P. chabaudi chabaudi* AS-infected mice were adjusted to

$1x10^5$ cells/ml in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2.0 mM L-glutamine, 0.01 M HEPES, $5x10^{-5}$ M 2-mercaptoethanol and 40 µg/ml gentamicin sulfate (CDMEM), and 1 ml of this cell suspension was then added in the wells of 24-well tissue culture plates. The SM were then exposed to $1x10^6$ PE /ml, and incubated for 1 h at 37°C in humid 5% CO_2-air atmosphere. The conditioned media (CM) were then collected and stored at -20°C until NO assay. NO was measured as nitrite in the CM by using Griess reaction (Green *et al.*, 1982).

Target cells: The PE were used as the target cells, and were prepared as described (Singh *et al.*, 1994). Briefly, blood from mice having 28-30% parasitaemia was collected in acid-citrate-dextrose saline, spun at 450 x *g* for 10 min at 4°C, and the PE-rich layer was aspirated, aseptically. The PE were washed (x3) with sterile Hank's balanced salt solution, and resuspended ($1x10^6$ cells/ml) in CDMEM.

Phagocytosis assay: The phagocytosis assays were performed as described (Su *et al.*, 2002; Kaur *et al.*, 2004). The SM in CDMEM ($1x10^5$ cells/ml; 100 µl) were layered over 12 mm round cover slips, and incubated at 37°C for 1 h in 5% CO_2-air humid atmosphere to allow the formation of monolayer. The SM monolayers were washed (x1) with warm DMEM, and then overlaid with $1x10^6$ PE in 100 µl CDMEM, and incubated at 37°C for 30 min. The assay was stopped by adding an excess of ice-cold medium, the SM were washed with phosphate-buffered saline (pH 7.2) diluted with distilled water (1:5), and stained with Giemsa to assess the ingestion. Two hundred SM on each cover slip were examined by light microscopy, and the number of PE ingested/100 SM was determined. All experiments were run in triplicate, three-times, separately. For statistical analysis, Student's *t*-test was used, and $p<0.05$ was considered significant.

RESULTS

***Ex vivo* erythrophagocytosis by SM from *P. chabaudi chabaudi* AS-infected mice:** The parasitaemia in mice became patent on day +3 (0.5±0.05 %), increased gradually and reached a maximum of 32.1±3.5 % on day +8, and then gradually resolved by day +14 (Fig.1). *Ex vivo*, SM from uninfected-mice, on day 0, showed very low level of erythrophagocytosis (2.4±0.26 %). However, SM from infected-mice showed 2.45±0.25 % erythrophagocytosis on day 0, and a significantly ($p<0.05$) enhanced erythrophagocytosis (7.5±0.79 %)

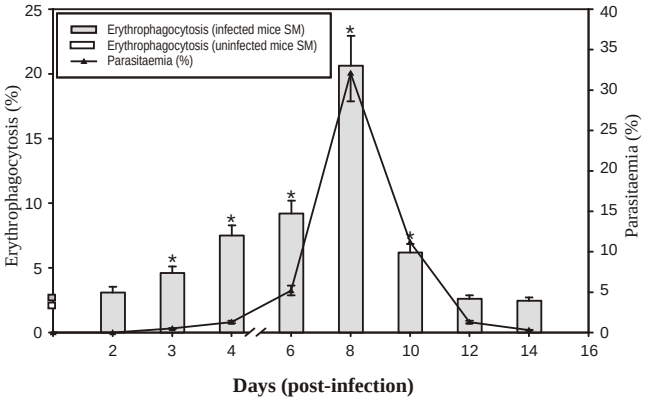

Figure 1. Course of infection and SM erythrophagocytosis in *P. chabaudi chabaudi* AS-infected BALB/c mice. PE from *P. chabaudi chabaudi* AS-infected mice (28-30% parasitaemia) were used as the target cells in the phagocytosis assays. Values are mean ± S.D. of three experiments run in triplicates, separately. Parasitaemia has been expressed as % PE. For infection, mice were inoculated with $1x10^6$ PE, i/p, on day 0. *Significantly different ($p<0.05$) from the uninfected mice.

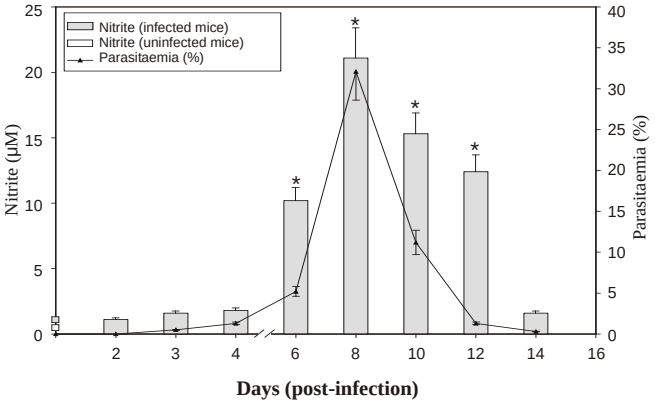

Figure 2. Course of *P. chabaudi chabaudi* AS infection in BALB/c mice, and the *ex vivo* nitrite levels (µM) in the CM of their SM. *Significantly different ($p<0.05$) from the uninfected mice. Other details as for Fig. 1.

on day +4 which reached its maximum of 20.64±2.3 % on day +8 (Fig. 1), when the parasitaemia was at its peak (32.1±3.5 %). Curiously, with the beginning of the decline in parasitaemia, from this point on, the erythrophagocytosis also started declining, and on day +12 parasitaemia was reduced to 1.3±0.15 % and the erythrophagocytosis declined to merely 2.6±0.28 %. Finally, on day +14 when the parasitaemia became subpatent, the erythrophagocytosis also returned to the background level.

Nitrite levels in CM of SM from *P. chabaudi chabaudi* AS-infected mice: Significant ($p<0.05$) nitrite levels (10.2±1.0 μM) were observed in the CM of SM on day +6, peaked (21.1±2.3 μM) on day +8, remained significantly high till day +12, and then abruptly plunged to background levels by day +14 (Fig. 2).

DISCUSSION

Our laboratory is engaged in research into the cellular and molecular basis of the induction of protective immune mechanism(s) in malaria. In this context, the objective of this study was to investigate the combined role(s) of erythrophagocytosis and NO in parasite clearance in *P. chabaudi chabaudi* AS-infected BALB/c mice. The results, apparently for the first time, clearly demonstrate that both erythrophagocytosis and NO, together, are involved in augmented parasite clearance, and thus appear to be consistent with similar but individual role of both these components in immunity to malaria.

Several research groups have used *P. chabaudi chabaudi* AS infection in mice as an animal model of *P. falciparum* infection in humans (Cox *et al.*, 1987). *P. chabaudi chabaudi* AS is similar to *P. falciparum* in that it usually infects normocytes, undergo partial sequestration, and in resistant strains of mice (BALB/c, C57/BL and NIH) recovery from primary parasitaemia is followed by one or more patent recrudescences (Cox *et al.*, 1987). It has been reported earlier that antibody-mediated immunity may not contribute to protection during the acute-phase of infection, as significant levels of parasite-specific antibodies are not produced during this time (Su and Stevenson, 2000; 2002). We, therefore, focused our present investigations to only the acute-phase of infection. Both erythrophagocytosis and NO have been thought to be involved in protection from rodent malarias, and constitute the first-line of defense. Nevertheless, apparently, there is no report wherein the combined effect of both erythrophagocytosis and NO has been studied in terms of augmented parasite clearance during rodent, simian or human malarias. The present study was, therefore, designed specifically to address this question. Our results show that when infection was at its peak and ready to decline, both the phagocytic and NO production activities of SM were also at their peak, and both of these indices later returned to the background levels with the contemporaneous fall in the parasitaemia. Therefore, these results, apparently for the first time, demonstrate that simultaneous enhanced erythrophagocytosis and NO production correlate well with augmented parasite clearance. The kinetics of erythrophagocytosis and NO production also closely coincided with the course of parasitaemia in our experiments, indicating that, apparently, both these mechanisms, in conjunction, play role(s) in parasite clearance.

The role(s) of macrophages and phagocytosis in protective immunity to malaria was proposed long ago. BanHarel and Amer (1923) have shown that there was a large increase in “large mononuclear leukocytes” in the blood of birds 36 to 40 h after primary infection with *P. gallinaceum*, which was associated with a decrease in the number of circulating, PE. Using experimental plasmodial infections in monkeys and cannaries, Taliaferro and Cannon (1936) observed that during primary infections, while the parasitaemia was rising slowly, macrophages in the spleen, and to a lesser extent in the liver and bone marrow, phagocytose the PE. Also, Taliaferro and Mulligan (1937) found increased number of macrophages and lymphocytes in the organs, especially the liver and spleen, in monkeys and man during malarial infection. In rodents, Zuckerman *et al.* (1973) reported that splenic macrophages from *P. berghei*-infected rats showed heavy phagocytosis of PE. Macrophage hyperplasia and the presence of normal erythrocytes and PE in the mononuclear phagocytes of the malarious monkeys also strongly

suggest their role in the destruction and elimination of parasites (Taliaferro and Cannon, 1936; Langhorne *et al.*, 1979; Barnwell *et al.*, 1983). Dutta *et al.* (1982) also reported heavy phagocytosis by splenic macrophages in *P. knowlesi*-infected convalescent monkeys. Brown and Greenwood (1985) have reported that recovery from malaria in children correlates with the ability of their monocytes/ macrophages to phagocytose and kill *P. falciparum*, *in vivo*. Using SCID mice model for maintaining *P. falciparum*-infected human red blood cells; researchers have confirmed that host macrophages help clear infected red blood cells in an antibody-dependent manner (WHO, 1999). All these reports appear to be in line with our observations of increased erythrophagocytic activity at the time of the resolution of infection.

Nevertheless, the above studies do not provide evidence for the exclusive role(s) of erythrophagocytosis in parasite clearance; therefore, in order to look for molecular mechanism(s) involved, we decided to study NO production. Taylor-Robinson *et al.* (1993), for the first time, reported the protective role of NO in rodent malaria. The role of NO in malaria parasite killing mechanisms is being increasingly appreciated (Jacobs *et al.*, 1995). Also, NO has been proposed as the mediator of tolerance in populations in regions where malaria is considered endemic (Clark *et al.*, 1996) on the basis that NO production in asymptomatic malaria-exposed children exceeds that of children with severe malaria (al-Yaman *et al.*, 1998; Anstey *et al.*, 2002). In our studies, we also observed that NO levels were significantly ($p<0.05$) enhanced at the time of the resolution of infection, and thus may be playing role(s) in parasite clearance along with enhanced erythrophagocytosis.

In summation, the result of this study suggest that both enhanced erythrophagocytosis and NO may be responsible for augmented parasite clearance during the acute-phase of *P. chabaudi chabaudi* AS infection in BALB/c mice. Detailed studies are warranted along these lines as several other factors besides erythrophagocytosis and NO may also be involved in parasite clearance.

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Host-parasite relationship : Fatty acid compositions of a trematode, *Paramphistomum cervi* and common Indian goat, *Capra hircus*

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Lipid classes and fatty acid compositions of a digenetic trematode, *Paramphistomum cervi* were analyzed by GLC. Fatty acid composition of the serum of its host, *Capra hircus* was compared with that of the parasite. The percent content of neutral lipid (39.04), glycolipid (21.23) and phospholipid (39.73) were recorded. The total percent of saturated, monoene, diene and PU in FA m both the parasite and the host were 48.41 and 33.93; 28.59 and 22.29; 13.42 and 14.87 as well as 3.67 and 9.69, respectively. Palmitic (saturated) and oleic (unsaturated) acid were the predominant fatty acids in both the parasite and the host. It is presumed that the parasite is completely dependent on the host for fatty acids, however, parasite increases the amount of some of the fatty acids by chain elongation process.

Keywords: Digenetic trematode, Goat, fatty acids, Lipid classes, *Paramphistomum cervi.*

INTRODUCTION

Adult stages of digenetic trematodes live in an anaerobic condition within the vertebrate host. It is found that parasitic helminths are unable to synthesize long chain fatty acids *de novo* (Barrett, 1981; Brouwers *et al.*, 1998a,b). However, trematodes acquire fatty acids from its host in the form of short chain acids. Platyhelminths can change the relative proportions of lipid classes (Barrett, 1981) in the membrane, probably, as a part of parasitic adaptation.

In the present study, the major lipid classes and fatty acid composition of *Paramphistomum cervi*, a digenetic trematode inhabiting the rumen and reticulum of common Indian goat, *Capra hircus* have been investigated together with the study of fatty acid compositions of host serum to decipher the host-parasite relationship.

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MATERIALS AND METHODS

Paramphistomum cervi (Schrank, 1790), a digenetic trematode parasite, were collected from rumen and reticulum of stomach of common Indian goat, *Capra hircus*, from Pandooah area, District Hooghly, West Bengal, India. The parasitic samples were pooled to achieve a wet weight of 2 gram. Worms were washed in Locke's solution for further processing. Blood serum sample (4.5 ml) of the host was also collected at the same time to make a comparative study in the fatty acid compositions of the host and parasite. Total lipids were extracted from the trematode, *P. cervi* sample and from the serum sample of the host, *C. hircus* following the procedure of Bligh and Dyer (1959) using methanol: chloroform (2:1, v/v), methanol: chloroform: water (2:1:0.8, v/v/v) and then again the first solvent system. The chloroform solution of lipid was evaporated under vacuum, redissolved in distilled hexane and kept at -20°C for future use. BHT (butylated hydroxy toluene) was added at a level of 100 mg/L to the solvent as antioxidant. A portion of total lipid of the trematode sample was subjected to

thin layer chromatography (TLC) using silica gel G (SRL) following Rouser *et al.* (1976) for identification of lipid classes. The remaining portion of total lipid of the trematode and the serum sample of the host were processed following Christie (1982) for fatty acid methyl ester (FAME) formation. These FAMEs were purified separately following Mangold (1969) and Misra *et al.* (1984) by TLC and gas liquid chromatography (GLC) which has been performed for the analyses of fatty acids separately on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID). It was done on a BPX-70 megabore capillary column of 30 mt length and 0.53 mm i.d. obtained from SGE, Australia. Oven temperature was programmed from 150°C - 240°C with a rate of 8°C per minute. Initial and final times were kept isothermal for 2 min and 20 min, respectively. Injection port and detector temperatures were 250°C and 280°C, respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.7 ml per min. Quantification was done by computer using specific Winchrom software.

RESULTS

Total lipid content of *P.cervi* is 1.23% of the wet weight of the tissue. Percentages of lipid classes of *P.cervi* are 39.04 (neutral lipid), 21.23 (glycolipid) and 39.73 (phospholipid). The fatty acid composition of both the trematode parasite and the serum of the host are represented in Table I. Twenty-two fatty acids are identified from both the parasite and host serum. The total percent of saturated fatty acid is 48.41 in the parasite, among which the percentage of palmitic acid (38.15) is highest. Among the unsaturated monoenoic (28.59%), dienoic (13.42%) and polyenoic (3.76%) fatty acids, 18:1, 18:2n-3 and 22:5n-6 fatty acids, respectively, are present as the highest amounts.

A total of 14.49% of fatty acids of the host serum remain unidentified. However, it shows 33.93% saturated fatty acids among which palmitic (16.66%) is present as the highest amount. Among the unsaturated monoenoic (22.29%), dienoic (14.87%) and polyenoic (9.69%) fatty acids, 18:1, 18:2n-3 and 20:4n-6 are present in significant amounts.

DISCUSSION

Total lipid content of trematodes varies among species (Table II). The phospholipid and neutral lipid contents of *P.cervi* are very close. In trematodes, phospholipids account for 25% of total lipids (Goil, 1964; Smith and Brooks, 1969; Oldenborg *et al.*, 1975), whereas it is much higher in *P.cervi* (39.73%). Both phospholipid and glycolipid are known to form an integral part of membrane component (both cellular and tegumental) and neutral lipid probably functions as storage form of fatty acids. In the present study, a good amount of glycolipid (21.23%) is recorded. It is to be mentioned that glycolipid was not reported from parasitic trematodes. Glycolipid is very much essential for

Table I. Fatty acid compositions (expressed as % of total fatty acids) of trematode, *Paramphistomum cervi* and blood serum of common Indian goat, *Capra hircus*, analyzed by GLC as FAME.

Fatty Acids	*Paramphistomum cervi*	*Capra hircus*
16:0	38.15	16.66
17:0	0.18	0.77
18:0	9.21	16.38
20:0	0.87	0.12
Σ Saturated	48.41	33.93
16:1	0.17	1.34
17:1	0.56	1.00
18:1	26.41	17.91
20:1n-9	0.36	0.27
22:1n-9	0.11	1.21
22:1n-11	0.57	0.22
24:1n-9	0.41	0.34
Σ Monoenes	28.59	22.29
17:2	0.18	0.74
18:2n-3	13.02	13.87
18:2n-6	0.22	0.26
Σ Dienes	13.42	14.87
18:3n-3	0.36	0.18
18:3n-6	0.91	2.41
18:4n-3	0.14	0.36
20:4n-3	0.85	0.40
20:4n-6	0.02	3.77
22:5n-3	0.07	1.34
22:5n-6	1.31	0.19
22:6n-3	0.10	1.04
Σ Polyenes	3.76	9.69
Unidentified	-	14.49

membrane structure in all categories of animal organization especially for the endoparasitic stages of trematodes where tegument is actively engaged in absorption.

Oleic acid (18:1) is the main C 1/8/ fatty acid in most parasitic helminths. However, in adult *Echinostoma trivolvis* the predominant fatty acids were saturated and most prevalent ones were palmitic (17%), stearic (16%), linoleic (11%), oleic (9%) and arachidonic (9%) acids (Fried *et al.,* 1993). Stearic acid (18:0) was the most predominant one in *F. hepatica* and helminths, in general, tend to have higher percent (40-70% of total acids) of unsaturated C_{18} acids (Barrett, 1981). Occurrence of higher percent of unsaturated C_{18} acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{18:4}$) in *P. cervi* (41.06%), thus, lies within the above mentioned range (Table I).

Fatty acid classes are common to both *P. cervi* and its host serum, however, they differ in their percent content. Earlier it was assumed that the chain elongation mechanism of fatty acids in platyhelminthes (cestodes and trematodes) might be similar to that of mammalian mitochondrial system (Barrett, 1981). Furlong (1991) reported that trematodes could modify fatty acids by chain elongation. Such mechanism has been demonstrated in *Spirometra mansonoides* for C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ to C_{20} and C_{22} fatty acids (Meyer *et al.,* 1966). It has also been reported that the cestode, *H. diminuta,* can convert palmitate and stearate into saturated fatty acid chains as long as 26 carbons and *F. hepatica* can synthesize $C_{20:1}$ and $C_{20:2}$ from $C_{18:1}$ and $C_{18:2}$ (Barrett, 1981). Trematodes in general have lost the ability to synthesize long chain fatty acids *de novo* (Meyer *et al.*, 1970; Barrett, 1981). Brouwers *et al.* (1997) mentioned that efficient conversion of oleate (18:1) to eicosanoids (20:1) took place by chain elongation in adult *S. mansoni.* Thus, *P. cervi* might rely on chain elongation of preformed or absorbed short chain fatty acids procured through the body tegument from the host. The major product of the fatty acid synthase is palmitate. Evidence also suggests that in most organisms, the fatty acid synthesis cycle stops at palmitate (C_{16}) and it acts as the precursor for both saturated and unsaturated long chain fatty acids (Barrett, 1981). Ackman (2000) commented that 16:0 is the principal fatty acid at all evolutionary and trophic levels. Therefore, it is also quite probable that palmitic acid serves as a precursor for both saturated and unsaturated acids in *P. cervi.* Barrett (1981) reported that *F. hepatica* derive short chain fatty acids (acetate, propionate, butyrate) through a mixture of diffusion and use mediated transport mechanism. Therefore, being a secondary consumer, *P.cervi,* might follow these strategies for the uptake of fatty acids present in lower percentages.

Presence of higher amounts of fatty acids such as 16:0, 18:0, 18:1 and l8:2n-3 both in the parasite as well as in host serum signify that these fatty acids play an important role in both the systems. Fatty acids are physiologically important components of phospholipid and glycolipid, lipophilic modifiers of proteins, fuel molecules and intracellular messengers in mammals. Stryer (1994) commented that arachidonic acid (20:4n-6) derived from linoleate is the major precursor for several classes of signal molecules as is found in the serum of the host, *C.hircus.* It was reported that palmitic acid and oleic acid are incorporated into the neutral lipids of *P. microbothrium* (Awharitoma *et al.*, 1990), therefore, it may be assumed that the presence of oleic and palmitic acid in higher percent in the trematode, *P. cervi* is due to efficient incorporation into neutral lipid.

Unsaturated fatty acids in mammals are derived from either palmitoleate (16:1), oleate (18:1), linoleate (18:2) or linolenate (18:3) (Stryer, 1994). Henderson (1996) reported the conversion of 18:2n-6 and 18:3n-3 fatty acids to e_{20} and e_{22} homologues in freshwater fishes. Okuyama (2000) reported that the two essential fatty acids linoleate and linolenate show a desirable balance. However, plants are the major source for linoleic and linolenic acids (Ackman *et al.*, 2002) as is reflected in the result of serum of *C.hircus.* Higher levels of these acids in the trematode, *P.cervi,* prove that these fatty acids propagate through trophic levels.

Adaptation to anaerobic conditions by trematode limits its energy budget. This might restrict the parasite for *de novo* synthesis of fatty acids. The major outcome of the host-trematode relationship is that the parasite by absorbing all its required fatty acids minimize their

Table II. Lipid compositions in various flatworms.

Flatworms	Total lipid (%)		Phospho-lipid	Neutral lipid	References
	Dry weight	Wet weight	(%)	(%)	
P. cervi	-	1.23	39.73	39.04	Present observation
P. cervi	4.7	-	49.67	50.33	Vykhrestyuk and Yarygina, 1975
C.erschowi	4.75	-	49.7	50.3	Vykhrestyuk and Yarygina, 1982
E.pancreaticum	15.73	-	24.72	75.28	Vykhrestyuk and Yarygina, 1975
S.mansoni	34.2	-	48	50	Smith and Brooks, 1969; Meyer *et al.*, 1970
F.hepatica	12-13	-	30	-	vonBrand, 1928, 1973; Smyth and Halton, 1983
P.explanatum	1.36-4.5	-	-	-	Goil, 1964
G.crumenifer	1.36-4.5	-	-	-	Goil, 1964
C.cotylophorum	27.3	-	-	-	Smyth and Halton, 1983
G.explanatum	34.4	-	-	-	Yusufi and Siddiqi, 1976
P.microbothrium	-	2.5	-	-	Hrzenjak and Ehrlich, 1975

energy budget for this purpose, which is otherwise used in their successful reproductive strategy.

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Relevance of the polymerase chain reaction in the diagnosis of visceral leishmaniasis

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A definitive diagnosis of VL depends on correct laboratory diagnosis. The purpose of the present study was to perform *Leishmania-PCR* for detection of *Leishmania* DNA in peripheral blood samples collected from patients with clinically suspected VL. The results were then compared with the anti-*Leishmania* antibody titer and the microscopic detection of Leishman Donovan bodies in Giemsa-stained bone marrow aspirations as well. The whole blood and serum samples were collected from the 67 patients with clinically suspected VL. The whole blood specimens were used for the detection of *leishmania* DNA by PCR. Anti-leishmania antibody titer was done by IFA on all patients' sera using standard procedure. Bone marrow samples were stained by Giemsa and investigated under light microscope. Based on clinical presentation, hematological, biochemical and serological (IFA) laboratory findings, 40.3% of the patients first time admitted with clinically suspected VL were found to be diagnosed correctly. False negative IFA results were found in 37% of patients with definite diagnosis of VL. However, IFA results have been shown 17.5% false positive with serum samples taken from patients diagnosed with disease other than VL. *Leismania* DNA, on the other hand, was detected in blood samples of 85.2% cases with VL. No *Leishmania* DNA was detected in any 40-blood samples collected from patients diagnosed with diseases other than VL. *Leishmania* amastigotes were detected in 20.5% bone marrow aspirations by Giemsa staining. The results illustrated the worth of the PCR for the diagnosis and follow-up treatment of Kala-azar patients. However, at the first admission in endemic area, preliminary findings of at least two important clinical features (i.e. splenomegaly and hepatomegaly), along with hematological profile of patient could be sufficient to perform *leishmania-PCR*, which could ultimately result in a rapid diagnosis of visceral leishmaniasis.

Keywords: Diagnosis, Polymerase chain reaction, Visceral leishmaniasis

INTRODUCTION

Visceral leishmaniasis (VL) or Kala-azar is a worldwide disseminated intracellular infection of the liver, spleen and bone marrow that is mainly caused by *Leishmania donovani*, *L. infantum* and *L. chagasi*. The disease is endemic in southwest of Iran. Mortality and morbidity of visceral leishmaniasis is about 15% even in case of treatment (WHO 1995-96). Multiple factors, including delays in diagnosis and therapy, contribute to mortality. In an endemic area the constellation of prolonged fever, progressive weight loss, hepatomegaly, splenomegaly, anemia, leukopenia, and reversed albumin / globulin ratio level are highly suggestive for VL. Clinical pictures of diseases such as malaria, typhoid fever, miliary tuberculosis, amebic liver abscess or in some cases of brucellosis are usually identical with VL. Therefore, a definitive diagnosis of VL depends on the correct laboratory diagnosis.

At present the routine diagnosis of VL is done by direct microscopy of patient material or culture. The microscopic detection *of Leishmania* amastigotes in

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Giemsa-stained spleen, bone marrow, or lymph node aspirates is relatively simple and cheap, but performance of spleen aspiration and bone marrow puncture may be dangerous. These methods are invasive and inconvenient for patients. Moreover, because of some technical problems, the sensitivity of the test is rather low (Siddig et al., 1989; Zijistra et al., 1992). Isolation of parasites by culturing is time-consuming, expensive, and difficult (Weigle et al., 1987).

Non-invasive serological methods such as enzyme-linked -immunosorbent assay (ELISA), indirect fluorescent antibody (IFA), and direct agglutination test (DAT) are rapid and readily adaptable to mass screening, but their suitability for early diagnosis is controversial (Allain & Kayan, 1975; Hommel et al., 1978; Pal et al., 1991). False positive results can be seen in patients with other infectious diseases. Following the treatment of VL, antibodies persist in serum of the patients for months, and its detection is not valuable for definite diagnosis of the disease.

Polymerase chain reaction (PCR), on the other hand, has been proved to be a rapid, sensitive, and specific method for early detection of *Leishmani* DNA in different clinical materials (Smits and Hartskeeri, 1995). Most of the previous studies established PCR for the detection of *Leishmania* DNA in specimens such as bone marrow, spleen aspirate, liver biopsy, lymph nodes and buffy coat of blood (Adhya et al., 1995; Andressen et al., 1997; Nuzum et al., 1995; Osman et al., 1997; Ravel et al., 1995; Smyth et al., 1992). However, there have been few studies establishing PCR for detection *of Leishmania* DNA in peripheral blood (Adhya et al., 1995; Osman et al., 1997).

The combination of clinical skills and the interpretation of all the investigative data as well as PCR results are all important in achieving correct diagnosis. The purpose of the present study was to investigate the significance of correlation between clinical data and detection of *Leishmania* DNA by PCR in peripheral blood from patients suspected with VL. The results were then compared with the *alati-Leishmania* antibody titer using IFA technique and the microscopic detection of leishman bodies in Giemsa-stained bone marrow aspirations as well.

MATERIALS AND METHODS

Patients : A cross-sectional study was performed on 67 whole blood and serum samples collected from the same number of patients with clinically suspected visceral leishmaniasis. All the patients were admitted to the teaching hospitals of Shiraz University of Medical Sciences (SUMS), Shiraz, Iran. The group consisted of 42 males (age between 3 months and 34 years with the average of 5.7 years) and 25 females (age between 5 months and 15 years with the average of 3.2 years). All the subjects were citizen of Southwest of Iran which is an endemic region for visceral leishmaniasis. The criteria for suspicion of visceral leishmaniasis in any patients were fever >38°C, splenomegaly, hepatomegaly, paleness and appetite loss. Hepatosplenomegally was defined when it could be palpated below the costal margin of the ribs). Any suspected diagnosis confirmed by an imaging study. Objective data including hematological, biochemical, and serological results, were obtained from patients' clinical records. Clinical data were also obtained from available clinical records. The local ethics committee granted ethical approval for the study.

SAMPLES :

Two milliliters of blood samples (1ml with and 1 ml without EDTA) was taken by vein puncture from the subjects. The sera were isolated by centrifugation. All the sera and EDTA-blood samples were frozen at -20°C until use. Thawed EDTA-treated blood specimens were used for the detection of *Leishmama-D'NA* by PCR. Negative blood samples for either anti-*leishmania* antibody or *leishmainia-D'NA* were used as a negative control and part of them spiked with *Leishmania* promastigotes and used as a positive control for PCR assay, respectively. Anti-leishmania antibody titer was determined by using IFA technique. The *Leishmania* promastigote was used as an antigen. Positive and negative control sera were also included.

Bone marrow aspiration was available only for 39 of 67 patients. Bone marrow samples were stained by Giemsa and investigated under light microscope.

DNA EXTRACTION:

Five hundred microlitters of EDTA-treated blood was added to 1 ml of cold lysis buffer [0.32 M Sucrose, 10 mM Tris-HCl (pH 7.5), 5mM MgCl2, 1% v/v Triton X-100] and vortex to suspend evenly. Then centrifuged 5 minutes in 4°C at 14,000 rpm, 1 ml of supernatant discarded and the late step repeated three times until no hemoglobin remained. Fifty microlitters of the pellet resuspended in 50 al double distilled water and incubated in 95°C in heating-block (Techne Dri-Block DB-2D) for 20 minutes. After centrifuging (14,000 rpm, 25 sec.), 10 al of supernatant solution was then used as a template for the PCR.

PCR:

Ten microliters of extracted DNA was added to 20 ul of a PCR mixture containing [Ix PCR buffer, dNTP mixture (200 uM), 22.5 pmol primer 13 A (5'-GTG GGG GAG GGG CGT TCT-3') and primer 13B (5'-ATT TTA CAC CAA CCC CCA GTT-3') each, and 1 unit of Taq DNA polymerase]. The two PCR primers amplify the DNA from a wide range of *Leishmania spp*. They amplify the 120-bp conserved region of the *Leishmania* kinetoplast minicircle (Rodgers et al., 1990; Laskay et al., 1995). The tubes were then subjected to a thermocycler (Eppendorf & Master cycler 5330), preincubated at 94°C for 5 min. for initial denaturation and 45 cycles consisting of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, and sequence-extended at 72°C for 1 min., and extra incubation at 72°C for 2 min.

Gel Electrophoresis; Ten microliters aliquots of the amplified samples were run in 2% agarose gel, then stained with ethidium bromide and examined for the bands of appropriate size with ultraviolet Trans-illumination.

Sensitivity and specificity of the PCR assay: *Leishmania infantum* was originally isolated from bone marrow sample of a patient with visceral leishmaniasis. The parasite was maintained at promastigote stage at 25°C in RPMI containing 10% of fetal calf serum.

Sensitivity of the PCR assay was performed using 10-fold serial dilutions of the precise number of *Leishmania* promastigote seeded in normal blood samples. The final concentrations of parasites tested were 10^4, 10^3, 10^2, 10 and 1 parasite per PCR tube, respectively.

For the detection of the PCR specificity test, DNA extraction was done using blood samples of the patients infected with *Plasmodium vivax, Plasmodiumfalciparum, Toxoplasma gonidii, L.infantum, L. tropica* and *L. major* previously diagnosed with standard methods (i.e. Gimsa satiating for direct detection of plasmodium or *L. tropica* and *L. major* and IFA test for the detection of *Toxoplasma* and *L. infantum* antibodies in serum samples).

For the determination of specificity of IFA, serum samples collected from patients with definite diagnosis of malaria (n=5) and toxoplasmosis (n=6) were tested against *Leishmania* antigen.

Indirect fluorescent antibody test (IFA):

Antibody titration was done by IFA on all the patients' sera using standard procedure.

Statistical analysis:

The characteristics of clinical pictures of the patients and results obtained by three different diagnostic assays were analyzed by Two-tailed Fisher's exact test and Chi-square test with Yates' correlation as appropriate.

Results

Sensitivity and specificity of PCR assay:

The limit sensitivity of the *Leishmama-PCR* using agarose gel electrophoresis and ethidium bromide staining was found to be 10 copies of *Leishmania* kinetoplast DNA per PCR tube. No cross-reaction was found between DNA extracted from blood samples of patients infected with *Plasmodium vixax* (n=5) or *Toxoplasma gondii* (n=6) and *Leishmania* primer pair.

Results of clinical samples:

Since visceral leishmaniasis is endemic in this part of our country (i.e., southwest of Iran), anti- leishmania antibody titer>l:128 considered being positive. Overall, the results of bone marrow aspiration were ta,

available for 39 of 67 patients. Based on clinical data, laboratory findings and response to anti-leishmania treatment (i.e. glucantime or amphotricin B in case of glucantime resistance individuals), 27 of 67 (40.3%) of the patients first time admitted with clinically suspected visceral leishmaniasis were found to be diagnosed correctly. Remaining (n=40, 59.7%) were finally diagnosed to have the following diseases: fever unknown origin (FUO, n=15), autoimmune diseases (n=5), leukemia and lymphoma (n=5), malaria (n=3), chronic liver disease (n=2), typhoid fever (n=2), thalasemia (n=3), miliary tuberculosis (n=l), aplastic anemia (n=2), viral infection (n=l) and parotiditis (n=l).

Anti-leishmania antibody titer was found positive (1:128) in 17 of 27 (63%) visceral leishmaniasis patients. However, 10 patients (37%) with definite diagnosis of visceral leishmaniasis had negative IFA. All 10 IFA-negative cases, were positive by PCR, in which 3 of them were under one year old, 4 between 1-2 years old and 3 between 3-5 years old. Seven of forty (17.5%) serum samples from patients diagnosed with disease other than visceral leishmaniasis were also positive with IFA method.

Leishmania-DNA, on the other hand, was detected in the blood samples of 23 out of 27 patients (85.2%) with VL. Remaining i.e. 4 out of 27 (14.8%) were found to be negative by PCR. No *Leishmania-DNA.* was detected in any 40-blood samples collected from patients diagnosed with diseases other than VL. *Leishmania* amastigote was detected in 8 out of 39 (20.5%) bone marrow aspirations by Giemsa staining.

Comparison analysis among bone marrow aspiration, IFA and PCR results has been shown in Tables Iand II. Taking discrepancies, i.e., specimens regarded as true positive into consideration, sensitivity and specificity were also calculated. Results of those three methods performed on 39 specimens showed that PCR is more sensitive and specific than IFA and bone marrow aspiration methods for diagnosis of visceral

Table I. Statistical analysis of Bone marrow, IFA and PCR results on 39 samples from patients with suspected visceral leishmaniasis

Method	Patients with VL (n=14)		Patients with other diseases (n=25)		Sensitivity (%)	Specificity (%)	Positive predictive Value (%)	Negative value value (%)
	Positive	Negative	Positive	Negative				
BM	8	6	0	25	57.1	100	100	80.6
IFA	10	4	4	21	71.4	84	71.4	84
PCR	13	1	0	25	92.8	100	100	96.1

VL = Visceral leishmaniasis, BM = Bone marrow, IFA = Indirect fluorescent antibody, PCR = Polymerase chain reaction.

Table II. Statistical analysis of IFA and PCR results on 67 samples from patients with suspected visceral leishmaniasis.

Method	Patients with VL (n=27)		Patients with other diseases (n=40)		Sensitivity (%)	Specificity (%)	Positive predictive Value (%)	Negative value value (%)
	Positive	Negative	Positive	Negative				
IFA	17	10	7	33	63	82.5	70.8	76.7
PCR	23	4	0	40	85.2	100	100	90.9

VL = Visceral leishmaniasis, BM = Bone marrow, IFA = Indirect fluorescent antibody, PCR = Polymerase chain reaction.

leishmaniasis (PCR v IFA, p=0.004; PCR *v* BM, p=0.007, respectively).

The correlation between different clinical presentations of patients with suspected visceral leishmaniasis and results obtained from the *leishmania-PCR* on the whole peripheral blood samples have been shown in Table III. As the results indicate, most clinical symptoms are not specific for definitive diagnosis of visceral leishmaniasis, except the splenomegaly (P=0.002). Despite the fact that no statistically significant correlations between hepatomegaly and laboratory outcomes were demonstrated, but it appears that there is an association between hepatomegaly and correct diagnostic assays.

NEGATIVE CONTROLS:

Cross-reactivity of each assay was checked against samples collected from normal volunteers and patients with malaria or toxoplasmosis. All controls gave negative results in PCR. How ever, false positive reaction was found in serum samples collected from patients with malaria by IFA using *Leishmania* antigen.

DISCUSSION

Failure to consider the possibility of visceral leishmaniasis can result in delayed diagnosis and inefficient treatment, with subsequently increased risks of morbidity and mortality. A highly sensitive and specific test is required to diagnose visceral leishmaniasis at a very early parasite infection stage. Over the last decade, several studies have shown PCR to be highly specific and more sensitive than classical methods for diagnosis of visceral leishmaniasis (Lachaud et al. 2000). We compared the sensitivity and specificity of the three diagnostic methods for visceral leishmaniasis (Kala-azar). As it contains many possible inhibitors, blood has been found to be particularly difficult substrate for PCR. However, there have been few established PCR studies for the optimization and standardization of the detection of *Leishmania* DNA in peripheral blood (Adhya et al., 1995; Osman et al., 1997). We introduced a simple and rapid DNA extraction procedure for its ability to remove inhibitors from blood samples and provide DNA suitable for *Leishmania-PCR* amplification from whole blood. The laboratory results of the three rapid diagnostic

Table III. Results of *Leishmania* - PCR and its relation to clinical findings of 67 patients with suspected visceral leishmaniasis

Clinical Status	PCR positive (n=23)	PCR negative (n=44)	PV
Splenomegaly	23/23 (100%)	23/44 (23.3%)	0.002
Fever	21/23 (91%)	43/44 (97.7%)	0.5
Hepatomegaly	18/23 (78.3%)	25/44 (56.8%)	0.08
Loss of appetite	10/23 (43.5%)	21/44 (47.7%)	0.3
Paleness	10/23 (43.5%)	10/44 (22.7%)	0.13
Weight loss	9/23 (39.1%)	16/44 (35.4%)	0.9
Vomiting	7/23 (30.4%)	14/44 (31.8%)	0.87
Diarrhea	6/23 (26.1%)	9/44 (20.5%)	0.82
Acitis	5/23 (21.7%)	9/44 (20.5%)	0.84
Jaundice	4/23 (17.4%)	5/44 (11.4%)	0.48

methods were then correlated with clinical status of suspected cases

Few investigators have described association between sensitivities of molecular or serological assays and presentation of clinical symptoms of visceral leishmaniasis (Adhya et al., 1995, Costa *et a*\. 1996). In one study (Nuzum et al., 1995) although patients had a somewhat smaller mean spleen size (11 cm), a comprehensive comparison of PCR positivity and clinical status has been in the study. According to our results, most clinical findings are not directly associated with visceral leishmaniasis. A significant correlation between *leishmania-PCR* positive results and splenomegaly was found in such cases (p=.002). The same result was demonstrated using IFA assay.

Based on clinical data, laboratory findings and response to anti-leishmania treatment 27 out of 67 (40.3%) patients first time admitted with clinically suspected visceral leishmaniasis were found to be diagnosed correctly. Reminders; 40 out of 67 (59.7%) patients were finally diagnosed diseases other than visceral leishmaniasis. Leishmania DNA was detected in 23 out of 27 (85.2%) patients with definite diagnosis of visceral leishmaniasis. Indirect fluorescent antibody assay was found positive in 17 out of 27 (62.9%) patients with definite diagnosis of visceral leishmaniasis. Leishmania DNA was detected in peripheral blood of 10 patients with splenomegaly who were negative by IFA method. The results indicate that lower antibody titer and less sensitivity of IFA in compare with PCR assay could be the reasons for the false to 14 days before rising in antibody titer. In one cases of visceral leishmaniasis two samples of sera and blood were collected for IFA and PCR assays in two weeks interval. The first sample was negative by IFA but positive by PCR assay. However, both serum and blood samples were positive by IFA and PCR tow weeks later. Our PCR results confirmed that circulating parasites might be common in very early infections. Same results have been reported by Piarroux et al. (1994) immunocompromised patients.

Only by using PCR assay, detection of visceral leishmaniasis in immunocompromised or immunosuppressed patients could be detected.

False positive results have been demonstrated in patients suffered with disease other than visceral leishmaniasis using IFA. In this study, 7 patients with non- visceral leishmaniasis had IFA-positive, the final clinical diagnosis of this group of patients included malaria, brocellosis, chronic liver disease, and acute lymphocyte leukemia.

To conclude, the results manifested in this study illustrated the worth of the PCR for the diagnosis and follow-up treatment of Kala-azar patients. Except for 2 important clinical features (i.e. splenomegaly and hepatomegaly), no significant correlation was established between the other clinical characteristics of visceral leishmaniasis on the one hand and PCR or IFA results on the other. PCR analysis allows the anti-parasite therapy to be performed efficiently and helps to detect the appearance of a resistant strain of the parasite. However, at the first admission, preliminary findings of at least two important clinical features mentioned above along with hematological pattern of patient's status could be sufficient to perform *leishmania-PCR*, which could ultimately result in a rapid diagnosis of visceral leishmaniasis.

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Four new species of the genus *Lytocestus* (Caryophyllidea, Lytocestidae) from Edible Catfishes in Assam and Meghalaya, India

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Four new caryophyllaeid species of the genus *Lytocestus* from catfishes, three from *Clarias batrachus* (L.) and one from *Heteropneustes fossilis* (Bloch), from Guwahati (Assam) and Shella (Meghalaya) are described. The differential characters of *L. Clarius* n. sp. are an elongated body with a short neck and undifferentiated scolex, H-shaped ovary the arms of which extend beyond the Mehlis' gland, closely lying but separate genital apertures and spiny eggs. *L. attentuatus* n. sp. has a filiform body with undifferentiated scolex and slender neck, inverted A-shaped ovary, vitellaria in two fields lateral to the testes, and smooth operculate eggs. Elongated body, narrow scolex without a terminal introvert and tapering anteriad, inverted A-shaped ovary, uterine coils not extending posterior to the ovarian isthmus and the presence of external seminal vesicle are the distinguishing features of *L. assamensis* n. sp. A short neck, undifferentiated scolex, H-shaped ovary, and smooth operculate eggs characterize *L. heteropneustii* n. sp.

Keywords **: Fish, *Lytocestus*, Trematodes**

INTRODUCTION

During an exploration of caryophyllaeids of the edible catfishes, *Clarias batrachus* and *Heteropneustes fossilis,* collected from Guwahati (Assam) and brought alive to Shillong markets for sale, and also of the same piscine hosts from Shella (East Khasi Hills, Meghalaya) eight species of *Lytocestus* were recovered- 7 from *C. batrachus* and 1 from *H. fossilis*. While 4 of these represented the already known species (Chakravarty and Tandon, 1988), the remaining 4 forms appeared new to science. It is noteworthy that these forms were strictly host specific even though *H. fossilis* and *C. batrachus* share the same habitat.

The present communication deals with the description and erection of these forms as new species of the genus *Lytocestus*.

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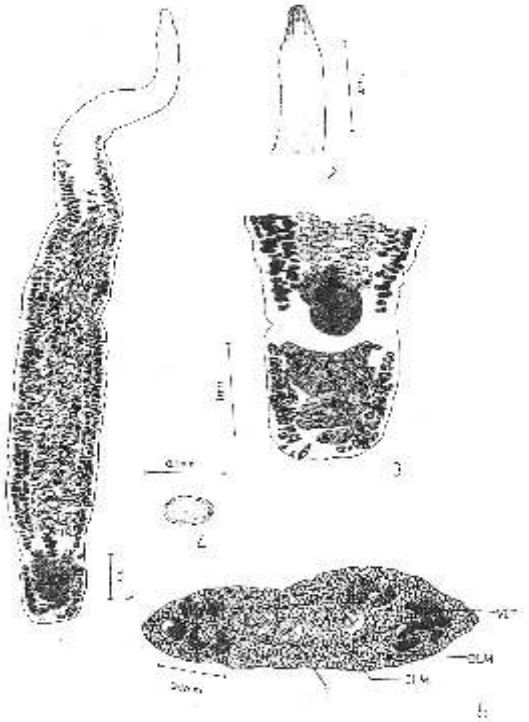
MATERIALS AND METHODS

The specimens comprising the present material were recovered from the intestine of the freshly killed fishes, *C. batrachus* and *H. fossilis,* from time to time. The intensity of infection was low in both the hosts, it being 1-2 parasites per host.

The worms, after stretching in hot water, were flattened under a coverslip. Bouin and 10% neutral buffered formaldehyde were used as fixatives and borax carmine and Meyer's carmalum were used for staining the whole mounts. For histological studies transverse and sagittally cut series of 6-7 µm thick paraffin sections, stained with haematoxylin and eosin were used.

The characters considered herein important for taxonomic purpose were also evaluated statistically. For analyzing the significance, student's t-test was applied.



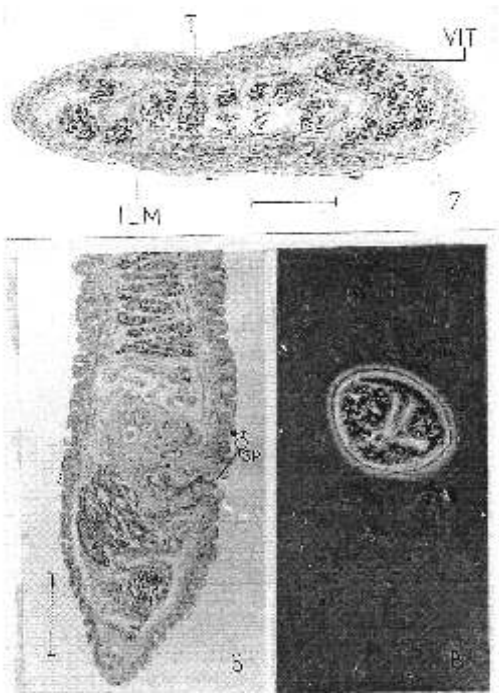


Fig. 1-8 Lytocestus clariae n.sp.
1. Full worm (whole mount). 2. Scolex end (enlarged). 3. Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system. 4. Egg. 5. Diagrammatic representation of the transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles. 6. Sagittal section through the posterior region revealing confluent genital apertures (scale bar = 0.15 mm). 7. Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles. (scale bar = 0.15 mm). 8. Operculate and spinous egg as seen under phase contrast (scale bar = 0.05 mm).

The measurements of all the forms studied herein are given in Table 1.

Family: Lytocestidae Wardle et McLeod, 1952

[Synonyms = Lytocestinae Hunter, 1927; Bovieninae Fuhrmann, 1931; Lallidae Johri, 1959]

Genus: *Lytocestus* Cohn, 1908

[Synonym = *Lucknowia* Gupta, 1961]

1. *Lytocestus clariae* n. sp.

The collections comprised 112 specimens of this form.

DESCRIPTION (Based on measurements of 10 specimens and several series of histological sections; Figs. 1-8).

Body elongate, flat with no trace of internal or external segmentation, tapering at anterior end, body proper divided into cortex and medulla by two layers of longitudinal muscles. Scolex undifferentiated, smooth, unarmed, with bluntly tapering extremity, followed by short neck devoid of reproductive organs. Testes numerous (270-495 in number), occupying medullary region, ovoid, larger than vitelline follicles, extending from just behind anterior follicles of vitellaria, posteriorly up to cirrus sac; cirrus sac compact, bulbous; ductus ejaculatorius opening close to female pore into shallow genital atrium. Ovary bilobed, H-shaped, follicular, extending posteriorly behind Mehlis' gland, lobes cortical in disposition and joined to each other by medullary ovarian isthmus anterior to Mehlis' gland; uterus glandular, extending in front of isthmus up to cirrus sac; vaginal tube joining uterus at distal end to open unitedly at shallow atrium. Vitelline follicles ovoid, commencing from short distance anterior to testes, extending up to level of cirrus sac, arranged in two rows lateral to testes; no post ovarian vitelline follicles present. Excretory pore terminal. Eggs oval, spinuous and operculate, as observed under phase contrast microscope.

Host: *Clarias batrachus* (L.)

Location: Intestine

Locality: Guwahati (Assam, 94° 16' E and 26°46' N, India), Shella (Meghalaya, 91°38' E and 25°08' N, India)

Deposition of specimens: Holotype (No. 286), 2 Paratypes (No. 287) and 1 slide of transverse sections (No. 288) in helminthological collection of Eastern Regional Station (ERS) of Zoological Survey of India (ZSI); other Paratypes and series of histological sections in helminthological collections of the Department of Zoology, North-Eastern Hill University, Shillong.

Etymology: Named after the generic name of the host.

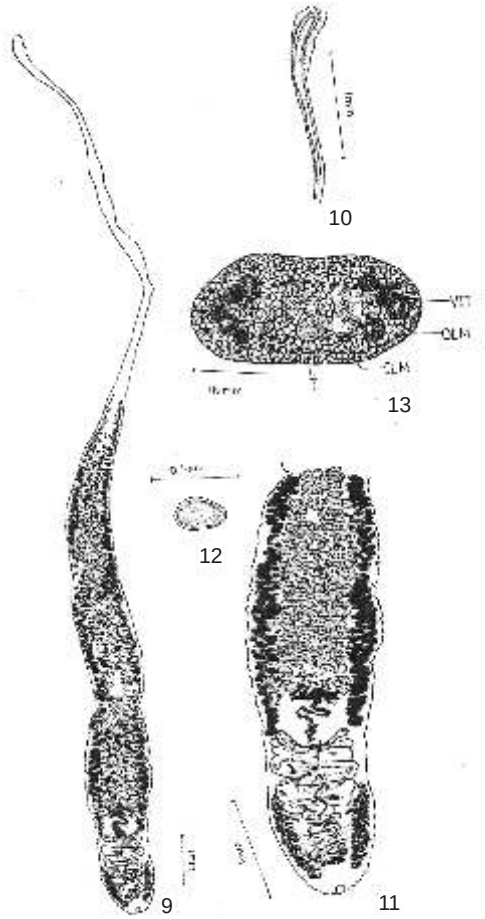
DISCUSSION

The disposition of the vitellaria in the cortex and testes in the medullary zone ascertains the inclusion of the present form in the family Lytocestidae Wardle and McLeod, 1952. Further, owing to the characters such as the presence of undifferentiated scolex, the absence of postovarian yolk glands, the uterine coils covered with a thick coat of accompanying cells and the ejaculatory duct enclosed within a compact parenchymatous bulb, the present form belongs to the genus *Lytocestus* Cohn, 1908.

The genus *Lytocestus* was erected for the cestodes from the siluroid host, *Clarias fuscus*, from Hongkong. The generic diagnosis was given as: holdfast undifferentiated and not broader than the body, parenchyma muscles in a ring around the testes, and no postovarian yolk glands present. To the type species *L. adherens* Cohn, 1908, several species have been added to date. They are *L. filiformis* (Woodland, 1923) Fuhrmann and Baer, 1925 [=*CaryophylIaeus filiformis* Woodland, 1923; *Monobothrioides filiformis* (Woodland, 1923) Woodland, 1937; *L. alestesi* Lynsdale, 1956 fide Mackiewicz. (1962)] from *Mormyrus coschive* of river Nile at Khartoum; *L. indicus* (Moghe, 1925) Woodland, 1926 [=*Monobothrioides indicus* (Moghe, 1925) according to Woodland (1937)] from *C. batrachus* in India; *L. javanicus* (Bovien, 1926) Furtado, 1963 from *C. batrachus* in Java [=*Caryocestus javanicus* (Bovien, 1926)]; *L. birmanicus* Lynsdale, 1956 [=*L. alestesi* Lynsdale, 1956, according to Johri (1959)] from *C. batrachus* from Rangoon, Burma; *L. parvulus* Furtado, 1963 from *C. batrachus* in Singapore and Malacca; *L. longicollis* Rama Devi, 1973 from *C. batrachus* in India; *L. lativitellarium* Furtado et Tan, 1973 from *C. batrachus* in Malaysia; *L. puylaerti* Khalil, 1973 from *C. liberiensis* in Sierra Leone (Africa); *L. fossilis* Singh, 1975 from *H. fossilis* from Kathmandu (Nepal); and *L. marcuseni* Troncy, 1978 from *Marcusenius harringtoni* from Chad basin in Africa. *L. fossilis* is the only species included in the genus which possesses post-ovarian vitelline follicles. Though its author placed this species under the genus *Lytocestus* the histological details for ascertaining the family or genus allocation are lacking in its account, thus raising a doubt for including the form with post-ovarian vitelline follicles in the genus. Likewise, another genus *Lucknowia* Gupta, 1961 that was erected as a new genus distinct form *Lytocestus* on the basis of the extension of vitelline glands up to the posterior end of the body (Gupta, 1961) was considered synonymous with *Lytocestus* by Mackiewicz (1994), who opined that the ovarian follicles of *Lucknowia* were mistaken for postovarian vitelline follicles (Mackiewicz, 1981). All the *Lytocestus* species, however, appear to be distributed in the Ethiopian and Oriental regions of the zoogeographical realm. Of these, three species, namely, *L. indicus*, *L. longicollis* and *L. fossilis*, are represented from the Indian Subcontinent; besides, *L. birmanicus* and *L. filiformis* have also been reported from C. *batrachus* from the northeastern region of India (Chakravarty and Tandon, 1988). The measurements of the known species of *Lytocestus* are provided herein in Tables II and III.

On comparison with the known Indo-Malaysian species of *Lytocestus* (*L. javanicus, L. parvulus, L. longicollis, L. filiformis* and *L. lativitellarium*), the present form stands close to them in possessing an undifferentiated scolex that tapers anteriad, ovarian lobes extending behind the Mehlis' gland and uterine coils up to the cirrus sac and in the anterior extent of the testes, i.e., a little posterior to the anterior follicles of vitellaria. In having a short neck and also in the extent of testes and vitellaria, the present form comes close to *L. indicus* and *L. birmanicus*. However, it differs from all of them in having a genital atrium (in which open the male and female pores) and spinous eggs. All the species mentioned above have distinctly separated genital apertures and smooth-surfaced eggs.

The present form shares the similar pattern of distribution of vitelline follicles (i.e., concentrated laterally) as in *L. lativitellarium* but stands apart from the latter species in having smaller body size and shorter neck (about one-fifth to one-sixth of the body



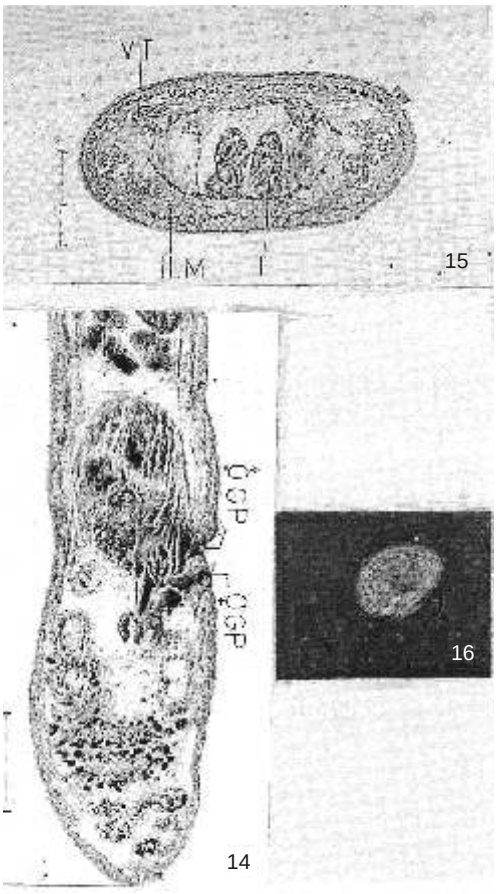


Fig. 9-16 Lytocestus attenuatus n. sp.
9. Full worm (whole mount). 10. Scolex end (enlarged). 11. posterior portion of the worm enlarged. 12. Egg. 13. Diagrammatic representation of the transverse section showing the distribution of testes and vitellaria to the longitudinal muscles. 14. Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.15 mm). 15. Transverse section showing the distribution of testes and vitellaria in relatiion to the longitudinal muscles. (scale bar=0.15 mm). 16. Operculate egg as seen under phase contrast (scale bar=0.05 mm).

length), the vitellarial distribution commencing much anteriorly, and larger spiny eggs.

In view of the above differences, the present form stands out as a species distinct from the known species of *Lytocestus* and is, therefore, considered a new species and named after the generic name of the host.

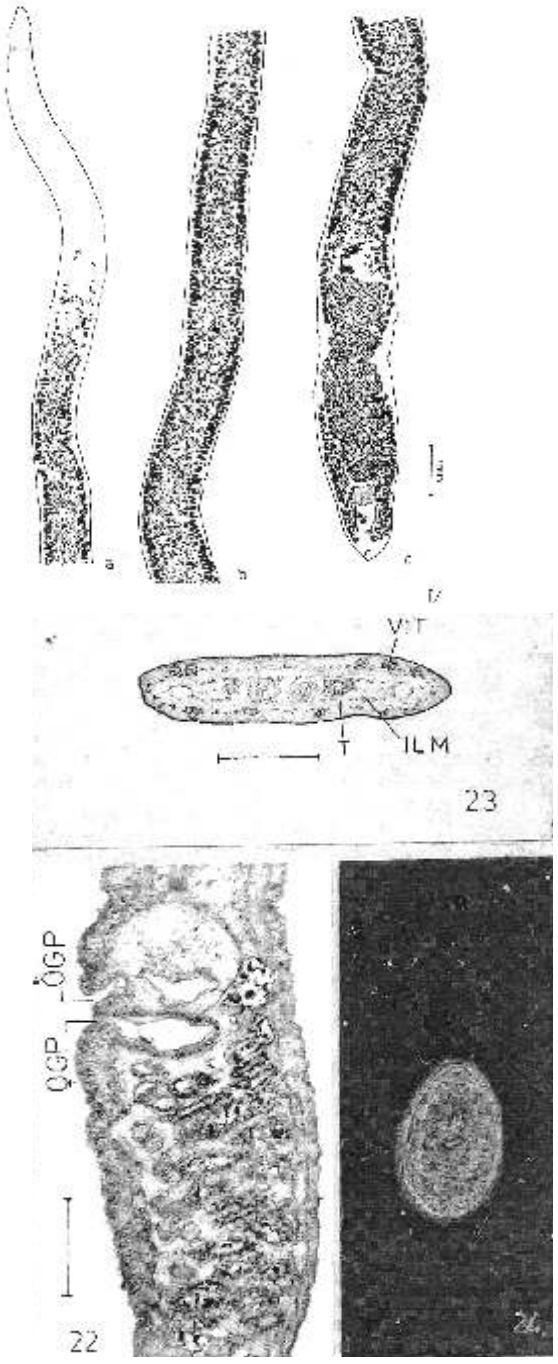
Specific Diagnosis: *Lytocestus clariae* n. sp. Elongated body, undifferentiated scolex, short neck, H-shaped ovary, with arms extending beyond the Mehlis' gland; confluent male and female apertures opening in shallow genital atrium; spiny eggs.

2. *Lytocestus attenuatus* n. sp.

A total of 98 specimens of this forms were collected.

DESCRIPTION (based on the measurements of specimens and several series of histological sections; Figs. 9-16)

Body thin, slender, elongated, flattened, posterior end broader than anterior, body proper divided into outer cortex and inner medulla by two layers of longitudinal muscles. Scolex smooth, undifferentiated, unarmed, with bluntly rounded extremity, followed by long narrow neck. Testes ovoid (155-398 in number), longer than vitelline follicles, occupying medullary region, extending from just posterior to anterior vitelline follicles caudad up to cirrus sac; cirrus sac medullary, enclosing thin winding ejaculatory duct, opening separately from and anterior to utero-vaginal pore. Ovary bilobed, follicular, inverted A-shaped, lobes extending to posterior level of Mehlis' gland and joined to each other by ovarian isthmus, ovarian lobes



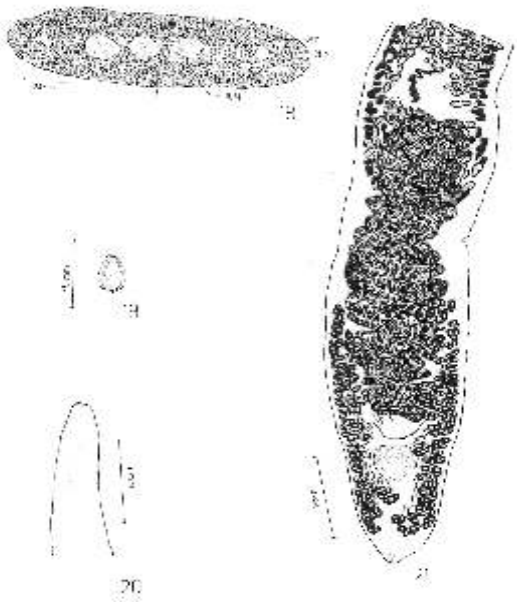


Fig. 17-24 Lytocestus assmensis n. sp.
17. Whole mount of the worm; a. anterior portion; b. middle third; c. posterior third of the body. 18. Diagrammatic representation of the transverse section showing the distribution of vitellaria and testes in relation to the longitudinal muscles. 19. Egg. 20. Scolx end (enlarged) 21. Posterior portion of the worm enlarged to show the disposition of the various components of the reproductive system. 22. Sagittal section through the posterior region revealing separate male and female genital pores (Scale bar=0.15 mm). 23. Transverse section, showing the distribution of vitellaria and testes in relation to the longitudinal muscles (scale bar = 0.15 mm) 24. Operculate egg as seen under phase contrast (scale bar = 0.05 mm).

cortical, isthmus medullary; Mehlis' gland well developed, behind ovarian isthmus; uterus glandular, extending from behind Mehlis' gland anteriad beyond lateral horns of ovary and up to cirrus pouch; vagina distinct, straight or slightly convoluted, joining terminal end of uterus to open at utero-vaginal pore. Vitelline follicles ovoid, arranged in two rows lateral to testes, extending from just anterior to testes up to cirrus sac. Excretory pore terminal. Eggs smooth, operculate.

Host: *Clarias batrachus* (L.)

Location: Intestine

Locality: Guwahati (Assam, 94° 16' E and 26° 46' N, India), Shella (Meghalaya, 91°38' E and 25°08' N, India)

Deposition of specimens: Holotype (No. 292), 2 Paratypes (No. 293) and 1 slide of transverse sections (No. 294) in Helminthological collection of ERS of ZSI; other Paratypes and series of histological sections in the Department of Zoology, North-Eastern Hill University, Shillong.

Etymology: Named after the shape of the body.

DISCUSSION

In sharing the characters such as shape of the body (which is thin, slender and elongated) and undifferentiated scolex, the present form comes close to *L. longicollis, L. parvulus, L. flliformis, L.*

Table I. Morphometric measurement (in mm or mentioned otherwise) and characters of four new species of *Lytocestus* (mean ± standard deviation)

Characters	*Lytocestus clariae* n.sp.	*Lytocestus attenuatus* n.sp.	*Lytocestus assamensis* n.sp.	*Lytocestus heteropneustii* n.sp.
Length of the body	8.58-22.44 (12.9±4.12)	11.88-35.44 (19.31±0.45)	25.54-50.82 (35.24±8.97)	9.57-19.14 (14.35±3.52)
Maximum breadth of the body (at the level of cirrus sac)	0.66-2.31 (1.32±0.67)	0.66-1.18 (0.90±0.17)	1.32-4.62 (2.45±1.24)	1.06-1.45 (1.25±0.13)
Length of the neck	1.18-6.93 (2.44±1.34)	6.14-7.06 (6.6±0.08)	4.62-15.18 (10.47±3.60)	1.98-5.41 (3.69±1.49)
Testicular follicles : Length X Breadth	0.06=0.22 (0.15±0.05)x 0.04-0.11 (1.61±0.01)	0.08=0.18 (0.12±0.04)x 0.03-0.15 (0.08±0.03)	0.10-0.53 (0.19±0.20)x 0.06-0.15 (0.09±0.05)	0.11-0.19 (0.15±0.03)x 0.03-0.08 (0.05±0.02)
Ovary shape	H-shaped	Inverted-A shaped	Inverted-A shaped	H-shaped
Ovarian lobes : Length X Breadth	0.53-1.65 (0.99±0.34)x 0.46-1.32 (0.85±0.27)	0.53-1.52 (0.85±0.32)x 0.53-0.92 (0.70±0.93)	1.52-5.08 (2.80±1.09)x 0.79-2.62 (1.58±0.57)	0.99-3.10 (2.04±0.86)x 0.92-1.32 (1.12±0.14)
Vitelline follicles : Length X Breadth	0.05-0.18 (0.10±0.04)x 0.02-0.08 (0.05±0.05)	0.05-0.17 (0.08±0.04)x 0.03-0.15 (0.04±0.03)	0.06-0.14 (0.09±0.04)x 0.04-0.08 (0.05±0.03)	0.07-0.13 (0.10±0.02)x 0.03-0.08 (0.05±0.01)
Pretestes distance	1.38-6.93 (3.13±1.54)	6.79-21.05 (10.60±4.76)	5.28-16.50 (12.47±4.04)	1.98-6.27 (4.12±1.93)
Previtelline distance	1.18-6.93 (3.13±1.54)	6.14-13.00 (6.6±0.08)	4.62-15.18 (10.47±0.01)	1.85-5.41 (3.63±1.98)
Distance between anterior extent of testes and vitellaria	0.13-1.18 (0.52±0.37)	0.46-8.05 (1.85±2.78)	0.66-0.82 (0.74±0.01)	0.12-1.12 (0.62±0.43)
Position of the genital pore from the posterior extremity	0.85-2.11 (1.48±0.38)	0.79-1.52 (1.16±0.31)	1.98-5.20 (3.43±1.07)	1.52-4.16 (2.84±0.97)
Eggs	Spinous, Operculate 30-50 (40±0.01) x 20-30 (25±0.01) µm	Smooth, Operculate 40-60 (50±0.01) x 20-30 (24±0.01) µm	Smooth, Operculate 30-50 (40±0.01) x 20-30 (25±0.001) µm	Smooth, Operculate 30-40 (35±0.006) x 20-50 (35±0.009) µm

Table II. Morphometric measurement (in mm) and some characters of some known species of *Lytocestus* (after original descriptions)

Characters	*L. filiformis* (Woodland, 1923) Fuhrmann et Baer, 1925	*L. indicus* (Moghe, 1925) Woodland 1926	*L. birmanicus* Lynsdale, 1956	*L. parvulus* Furtado, 1963	*L. lativitellarium* Furtado et Tan 1973
Length of the body	7.5-24	15-29	10-12	3.6-5.7	25-31
Maximum breadth of the body (at the level of cirrus sac)	1-2	1.82-2.73	0.9	0.24-0.90	1.35-1.95
Length of the neck				0.75-2.10	9.9-10.6
Pretestes distance					0.6-0.8
Testicular follicles (Length x Breadth)		0.095-0.119x 0.002	0.15-0.18x 0.10-0.13	0.10-0.15x 0.05-0.10	0.105-0.325x 0.030-0.090
Cirrus sac				0.12-0.15	0.225-0.238
Ovary : shape		H-shaped	H-shaped	H-shaped	H-shaped
Ovarian lobes				0.3-0.45	0.094-0.138 x 0.044-0.067
Receptaculum seminis	Absent	Absent	Absent	Absent	Absent
Genital pores (♂and ♀)	Separate	Separate	Separate	Separate	Separate
Genital atrium	Absent	Absent	Absent	Absent	Absent
Interpore distance	0.025	0.220-0.270	0.180	0.045	0.150
Previtelline distance			4.0		
Vitelline follicles (Length X Breadth)		0.077-0.088x 0.088-0.112	0.10-0.12x 0.04-0.06	-0.100x0.050	0.067-0.086 x 0.030-0.050
Distribution of vitelline follicles			Two lateral bands	5 rows encircling testes	concentrated laterally
Postovarian vitellaria	Absent	Absent	Absent	Absent	Absent
Eggs	0.062-0.070 x 0.029-0.033	0.080x0.040	0.050x0.030	0.026-0.033 x 0.023-0.045	0.019-0.023 x 0.030-0.033

Table III. Morphometric measurement (in mm) and some characters of some known species of *Lytocestus* (after original descriptions)

Characters	*L. longicollis* Rama Devi, 1973	*L. puylaerti* Khalil, 1973	*L. fossilis* Singh, 1975	*L. marcuseni* Troncy, 1978
Length of the body	10.8-20	3.06-4.12	16.0-20.5	8-11
Maximum beadth of the body (at the level of cirrus sac)	0.5-0.84	0.67-0.7	2.4-3.2	1.1
Length of the neck	5.36-7.6		1.6-1.9x0.86-1.2	
Pretestes distance		0.602-0.723		
Testicular follicles	0.10-0.16	0.058-0.14 x 0.105-0.195	0.16-0.22x0.35-0.44	0.480x0.375
Cirrus sac	0.24-0.31x0.16-0.23	0.27-2.29x0.105-0.195	0.72-0.8x0.54-0.62	
Ovary : shape	H-shaped	H-shaped	H-shaped	
Ovarian lobes	0.46-0.78	0.046-0.058 x 0.035-0.039		
Receptaculum seminis	Present	Absent	Absent	Absent
Genital pores (♂ and ♀)	Separate	Separate	Common	Separate
Genital atrium	Absent	Absent	Present	Absent
Interpore distance	0.05-0.08	Very short		0.065
Previtelline distance		0.602-0.723	3.5-4	2.5
Vitelline follicles	0.0339-0.07	0.58-0.116x 0.015-0.027	0.15-0.19x0.30x0.35	0.30-0.060x 0.010-0.020
Distribution of vitelline follicles		Annular		
Postovarian vitellaria	Absent	Absent	Present	Absent
Eggs	0.046-0.054 x 0.023-0.031	0.046-0.058 x 0.035-0.039	0.32-0.04 x 0.024-0.028	0.045-0.055 x 0.030-0.035

fossilis and *L. javanicus.* However, it differs from each of them in certain characters: from *L. longicollis* in having a still longer neck and previtellarial region and in not possessing a receptaculum seminis; from *L, parvulus* in lacking a linear arrangement of vitelline follicles in five rows; from *L. fossilis* in the absence of post-ovarian vitelline follicles; and from *L. flliformis* in having oval and large vitelline follicles as compared to the small and globular ones occurring in the latter species. *L. javanicus* differs from the present form in not having a long neck. The present form also differs from the type species in characters such as the shape and the size of the body and size of the egg. In lacking a prominent holdfast, which is a distinct feature *of L. indicus* and *L. birmanicus,* the present form can be distinguished from these species. It also stands apart from *L. clariae* n. sp. described herein, in having distantly apart genital apertures and smooth-surfaced eggs.

Therefore, considering all the above differences, it is proposed to assign to the present form the rank of a new species.

Specific Diagnosis: *Lytocestus attentuatus* n. sp. Body filliform; scolex undifferentiated; long, slender neck; ovary inverted A-shaped; testes medullary, vitellaria in two fields lateral to testes; eggs smooth, operculate.

3. *Lytocestus assamensis* n.sp.

65 specimens of this form were recovered during the study.

DESCRIPTION (based on measurements of 10 specimens and several series of histological sections, Figs. 17-24).

Body very long, slightly tapering anteriorly, body proper divided into cortex and medulla by two layers of longitudinal muscles. Scolex undifferentiated, smooth and unarmed, with bluntly tapering extremity; well developed gland cells present, with distinct zone of dense aggregation 3-4 mm from anterior extremity. [This distinct glandular region provides the only clue for distinguishing and delimiting the scolex region from the neck; limits of the latter otherwise are not well demarcated from the rest of the body proper]. Testes numerous (266-565 in number), occupying medullary region, ovoid, larger than vitelline follicles; cirrus sac prominent, opening separately from female genital pore; external seminal vesicle present. Ovary bilobed, bent inwards in shape of inverted A, ovarian wings joined by isthmus, whole ovary cortical; Mehlis' gland well developed, behind isthmus; uterus glandular, extending from in front of isthmus anteriad beyond lateral horns of ovary; vagina distinct, joining terminal end of uterus to open unitedly to exterior at utero-vaginal pore immediately posterior to male opening. Vitelline follicles cortical, mainly concentrated in lateral fields generally not spreading throughout whole peripheral medulla of testicular zone, commencing from pretesticular region, extending posteriad up to level of cirrus sac. Excretory pore at terminal hind end. Eggs smooth, operculate (as confirmed from the observations of eggs ex utero under phase contrast).

Host: *Clarias batrachus* (L.)

Location: Intestine

Locality: Guwahati (Assam, 94° 16' E and 26° 46' N, India), Shella (Meghalaya, 91 °38'E and 25°08'N, India)

Deposition of specimens: Holotype (No. 289), 2 Paratypes (No. 290) and 1 slide of transverse sections (No. 291) in Helminthological collection of ERS, ZSI; other Paratypes and series of histological sections in the Department of Zoology, North-Eastern Hill University, Shillong.

Etymology: Named after the state of Assam from where the fish hosts were first collected.

DISCUSSION

While ascertaining its specific status and on comparing it with the known forms of *Lytocestus,* the present form stands close to *L. longicollis* in sharing the characters such as the undifferentiated scolex, the inverted A-shaped ovary and the uterine coils extending beyond the anterior horns of ovary. However, it differs from the same in not possessing a receptaculum seminis, which is the characteristic feature of *L.longicollis.* Besides, the size of the body, testes and vitellaria and the distribution of vitellaria

within the testicular field in the present form further distinguish it from *L. longicollis* in which the vitellaria are confined to the lateral fields.

On comparison with *L. parvulus,* the present form distinctly differs in not possessing five rows of vitelline follicles. The size and shape of the testes and vitellaria, which are much larger and ovoid in the present form, are the characters that differentiate it from *L.filiformis.* While the present form is broad and flat occupying the full width and considerable length of the host's intestine, the body is much slender and elongated in *L. javanicus.*

On comparison with the other new forms described herein, the present form differs from *L.clariae* in possessing distinctly apart genital aperturus and non-spinous eggs, and from *L. attenuates* in the distribution of testes and vitellaria that are intermingled and not confined to the lateral fields, and in the extent of uterine coils that are limited only up to the ovarian isthmus and not beyond.

Specific Diagnosis: *Lytocestus assamensis* n. sp. Body very long; scolex undifferentiated, without terminal introvert; long neck; inverted A-shaped ovary; uterine coils not extending beyond ovarian isthmus; external seminal vesicle present; vitellaria mainly in lateral fields of testicular zone.

4. *Lytocestus heteropneustii* n.sp.

The collection comprised 22 specimens of this form.

DESCRIPTION: (based on the measurements of 6 specimens and few series of histological sections; Figs. 25-32).

Body elongate, flat, with no trace of internal or external segmentation, tapering anteriorly, broader posteriorly; body proper divided into cortex and medulla by two layers of longitudinal muscles. Scolex undifferentiated, smooth, unarmed, base conical, bluntly tapering extremity followed by short neck. Testes numerous (235-340 in number), ovoid, larger than vitelline follicles, medullary in distribution, commencing little behind anterior vitellaria, extending till near ovarian lobes; cirrus sac prominent, occupying entire thickness of medulla, opening from and just in front of utero-vaginal pore; external seminal vesicle absent. Ovary bilobed,

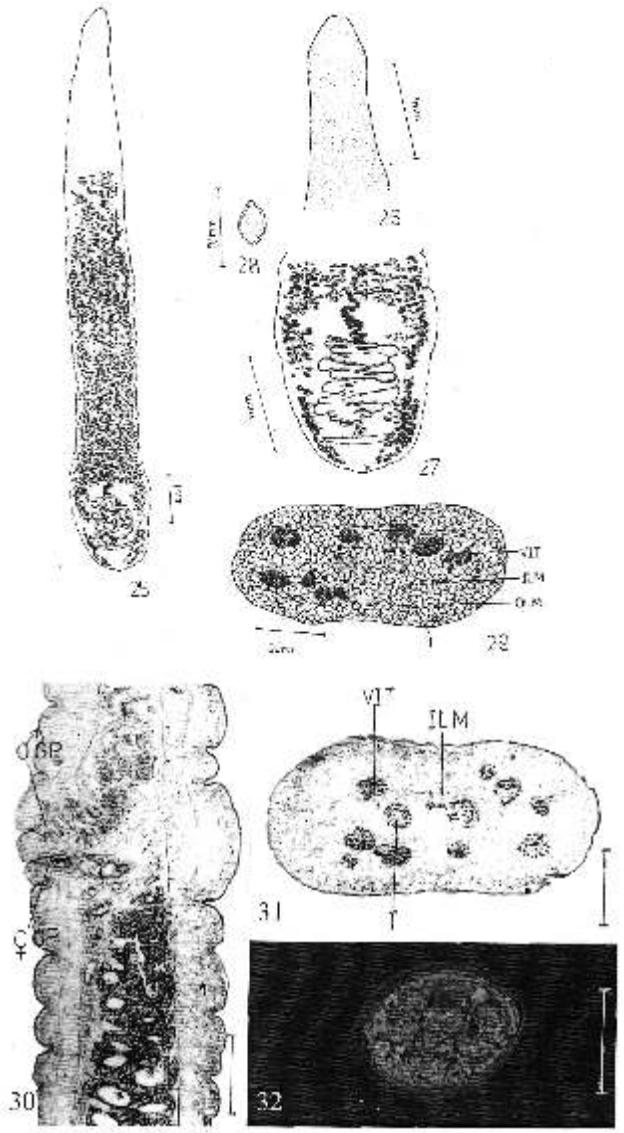


Fig. 25-32
25. Whole mount of the worm. 26. Scolex end (enlarged). 27. Posterior portion of the worm enlarged. 28. Egg. 29. Diagrammatic representation of the transverse section, showing the disposition of testes and vitellaria in relation to the longitudinal muscles. 30. Sagittal section of the posterior portion marking the separate male and female genital pores (scale bar = 0.15). 31. Transverse section, showing the distribution of vitellaria and testes in relation to the longitudinal muscles. (scale bar=0.05 mm). 32. operculate egg as seen under phase contrast (scale bar = 0.05mm)
(Abbreviations. VIT - Vitellaria; ILM - inner longitudinal muscles;♂GP- male gonopore;♀GP - female gonopores).

follicular, H-shaped, ovarian lobes joined to each other by ovarian isthmus, cortical, extending beyond Mehlis' gland posteriorly; uterus glandular, extending from in front of isthmus anteriad beyond lateral horns of ovary, no uterine coils behind ovarian

isthmus; vagina distinct, joining terminal end of uterus to open unitedly at utero-vaginal pore. Vitelline follicles ovoid or spherical, cortical in disposition, strewn in mid-field of testicular region, commencing from base of neck up to anterior horns of ovary. Excretory pore terminal. Eggs smooth, ovoid, operculate.

Host: *Heteropneustes fossilis* (Bloch)

Location: Intestine

Locality: Guwahati (Assam, 94° 16' E and 26°46' N, India), Shella (Meghalaya, 91°38' E and 25°08' N, India)

Deposition of specimens: Holotype (No. 295), 2 Paratypes (No. 296) and 1 slide of transverse sections (No. 297) in Helminthological collection of ERS, ZSI; other Paratypes and series of histological sections in the Department of Zoology, North-Eastern Hill University, Shillong.

Etymology: Named after the generic name of the host.

DISCUSSION

In having a somewhat stumpy and stout body, the present form differs from the slender and/or long-necked *L. filiformis, L. javanicus, L. lativitellarium, L. attentuatus* and *L. assamensis.* In lacking a holdfast distinct from the neck, the present form stands apart from *L. indicus* and *L. birmanicus.* In both *L. puylaerti* and *L. marcusenii* the vitellaria are arranged in an annular manner and not intermingling with the testicular follicles as in the present form. On comparison with *L. fossilis,* the only other species of the genus described from the same host, i.e., *H. fossilis,* the present form distinctly differs in the absence of postovarian yolk glands. Even if the genus *Lucknowia* is considered a valid genus and not a synonym of *Lytocestus,* the species *Lucknowia fossilis* Gupta, 1961 (described from the same host) also differs from the present form in the extension of vitellaria up to the postovarian region and posterior end.

In view of the above differences, the present form is regarded as a new species of the genus.

Specific Diagnosis: *Lytocestus heteropneustii* n. sp. Body elongate, short neck, undifferentiated scolex; H-shaped ovary, testes medullary, vitellaria strewn in mid-field of testicular zone; eggs oval, smooth-surfaced, operculate.

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Lecithochirium testelobatus n.sp (Digenea : Hemiuridae) from the lizard Fish, *Saurida undosquamis* from Andhra Pradesh Coast

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Five specimens of *Lecithochirium* n.sp were collected from the marine fish *Saurida undosquamis* and described. Body triangular in shape, ecsoma broad, thick walled and retracted into the body. Testes located in the equatorial plane of post acetabular region and show peculiar appearance. Each testis shows a lobated lateral extension. All the parasites show same type of morphological characters like triangular shape, retractile ecsoma, small suckers, lobed testes, terminal genitalia and are distinct from other members of *Lecithochirium*.

Keywords : Ecsoma, *Lecithochirium*, Terminal genitalia, Testes.

INTRODUCTION

The genus *Lecithochirium* was erected by Luhe (1901). *Lecithochirium* is a large genotype and is the most frequently encountered hemiurid in fishes of East coast. They are usually parasitic in the gut of marine teleosts. They have exclusive taxonomic characters like a well or poorly developed ecsoma and a presomatic pit or ventrocervical groove; seminal vesicle bipartite or tripartite and occasionally coiled; Vitellarium condenced and usually divided in to 6-7 oval to digitiform lobes, and with massive uterine coils. More than 100 species have been described in this genus. Gibson and Bray (1986) discussed about this genus in a detailed manner. Bray (1990) synonymised *Sterrhurus* Looss, 1907, *Ceratotrema* Jones, 1933 *Jajonetta* Jones, 1933, *Separogermiductus* Skrjabin & Guschanskaja, 1955 etc. with *Lecithochirium*. These worms are generally more morphologically complex than the hemiurids treated earlier. The ecological, physiological and environmental factors of the hosts and adaptations of parasites might have lead to morphological variations of flukes.

MATERIAL AND METHODS :

The genus *Saurida* is commonly known as lizard fish. These fishes are abundant through out the Indian coast and are very popular as food fishes. At this cost the catches of trawlers are constituted with a variety of fish, among which *Saurida* are regularly represented. These are known for their delicacy as food fish and have good quality of proteins and are widely dispersed in the World. The first fairly comprehensive description of these species known from India is that of Day (1878) who described 5 species. *Saurida tumbil, S.nebulosa,* S. *gracilis, Saurus indicus* and *Saurus myops.* Later it was reviewed by Normann (1935) who recognized some more species of genus *Saurida* which include *S. tumbil, S. Undosquainis S.pseudottumbil S.wanieso S:longimanus, S.isarankurai and S.micropectoralis,* representing from Atlantic & indopacific regions. The species available at Visakhapatnam coast are *S. tumbil, S.undosquamis and S. micropectoralis.*

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Saurida forms one of the important commercial fisheries of India. Fishing is the main occupation for people in lowest strata of society. The per capita consumption of fish by man is 3-5 Kg per annum. Recognizing the importance of fish as source of nutritional protein and also potential earning for foreign exchange, the Govt. of India gave priority to the improvement of fishing and fish processing. As a result today marine products have become a major foreign exchange in the country's economy. These fishes are usually marketed fresh, dried and salted. The increasing demand for *Saurida* for human consumption and their importance as predators of other commercially important fish and invertebrates demands a better understanding of the fish as well as it's parasitic biology.

Five trematodes were collected from the mucosa of stomach of *S. undosquamis* at two different times. They were fixed in FAA solution (formalin, acetic acid and ethyl alchohol) and stained with alum carmine. They were mounted in Canada balsum following the routine techniques. Drawings were made with the help of camera lucida. All the measurements were given in millimeters.

RESULTS AND DISCUSSION

Body nearly triangular being narrow in the anterior and widening towards the posterior region with a retracted ecsoma. Body measures 2.55-2.71x0.50-0.74. Ecsoma broad, thickwalled measuring 0.60-0.95x0.55-0.71. Oral sucker round and subterminal

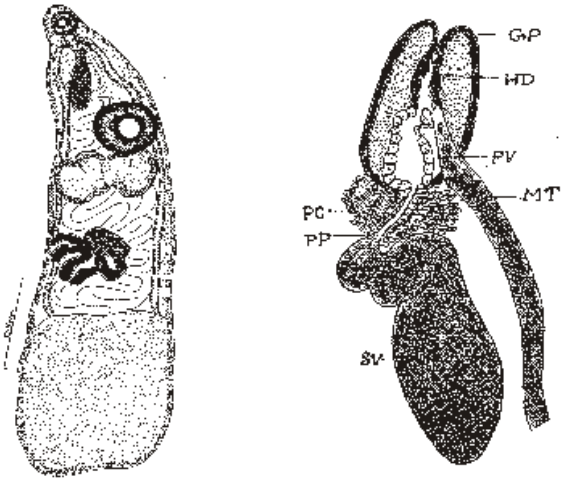

Fig. 1: *Lecithochirium testelobatus* n.sp.
Fig. 2: Terminal genitalia of *L. testelobatus* n.sp.
(SV-Seminal vesicle, HP-Hermophroditicduct, PP-Pars prostatica, PV-Prostatic vesicle, PC-Prostate gland cells, GP-Genital pore, MT-Metraterm)

Table 1. Comparison of present species with nearly resembling *Lecithochirium* species.

	***L.fusiforme* Luhe, 1901**	***L. musculus* (Looss, 1907) Nasir and Diaz, 1971**	**Present species**
Body	3.61 - 5.20 x 0.92-1.10	2.39-2.75x 0.60-0.67	2.55-2.71x 0.50-0.74
Ecsoma	Retracted 1.12-1.50x 0.85-1.03	Retracted 0.41-0.55x 0.50-0.52	Retracted 0.60-0.95x 0.55-0.71
Sucker ratio	1:2	1:1.6	1:1.5
Seminal Vesicle	Bipartite 0.31-0.554x 0.15-0.18	Bipartite 0.25-0.31x 0.11-0.15	Bipartite 0.22-0.27x 0.09-0.1
Testes	Unloaded testes	Unloaded testes	Bilobed testes
Vitellaria	7 deeply digitform lobes	7 condensed and lobed masses	6 short lobes
Eggs	0.025-0.028x 0.023-0.025	0.021-0.028x 0.019-0.023	0.022-0.025x 0.018-0.020

Note : All measurements are in millimeters.

measuring 0.13-0.17x0.11-0.13. Anterior region of the body containing acetabulum and terminal genitalia is slightly narrower. Acetablulum large, round, thickwalled and present in the anterior 1/3 of the body. It measures 0.21-0.31x0.25-0.30. The ratio of oral sucker to acetabulum is 1:1.5. In general acetabulum is quite bigger in other species of *Lecithochirium*, Pharynx bulbous measuring 0.07-0.09x0.08-0.12. Oesophagus short but not prominent. Digestive caecae narrow and terminate near the ecsoma but not extending in to the ecsoma.

Testes two in number, large and post acetabular. Testes show a peculiar appearance. Each testis gives a lateral lobated extension. In low power of microscope it appears as if there are 4 testes. They are present in the equatorial plane in the postacetabular regions. Testes lobes are symmetrical. Each lobe of left testis measures 0.21-0.25 x 0.27-0.31 and right testis measures 0.20-0.29 x 0.28-0.32 Seminal vesicle tripartite, thinwalled, much convoluted present in between ceacal bifurcation and two suckers. It measures 0.22-0.27 x 0.09x0.12 Pars prostatica elongate and surrounded by a mass of prostate gland cells. Hermophroditic duct straight. Genital atrium deep, tubular and opens in the median line, immediately posterior to the oral sucker.

Ovary round to oval, postacetabular and present in the middle region of the body. It measures 0.14-0.18 x 0.12-0.16. Vitellarium 6 lobed The lobes are short and present beneath the ovary. Uterine coils are much convoluted and are in between testes and ovary and not extend in to ecsomal cavity. Anteriorly metraterm unites with the pars prostatica following hermaphroditic duct and opens in to the genital pouch. Eggs small, oval and thin walled measuring 0.022-0.025 x 0.015-0.017.

It must be emphasized that variation of genital organs resulting in evolution of morphological structures may be a general trend. This may be achieved slowly during a long period of time which is called as micro evolution. Species variation in trematodes is mainly based on differences in genital organs. In general, trematodes possess only a pair of testes which vary in shape, location and arrangement which have taxonomic importance. Monorchism and polyorchism are also seen in very few digeneans. In the present study the parasites obtained exhibited testicular variation when compared with previously obtained species of *Lecithochirium*. It is not exactly polyorchism but it is extension of each testis into another lobe. It cannot be considered as an abnormality since both testes are showing the same features. More over such parasites were obtained at two different times. So this feature has been considered as a distinct character.

There are other species of *Lecithochirium* reported from *Saurida* They are *L. magnus* (Yamaguti, 1938) Nasir & Diaz, 1971, *L. polynemi* Chauhan, l945, *L.magnicaudatus Fischthal & Kuntz, 1963, L.fusiforme, Luhe, 1901, L.jairajpuri* Gupta & Govind, 1984, and L. *musculus* (Looss 1907) Nasir & Diaz, 1971. The present species shows some resemblances in the shape of *L. fusiforme* Luhe, 1901 and *L. musculus* (Looss 1907) Nasir & Diaz 1971. But they show variation in shape of testes and other characters like sucker ratio, seminal vesicle, longer vitelline lobes from the above species. A comparative table is given with the characters of three species. However the character of testes is unique for this species and speciation is mainly based on this character. All the parasites collected exhibited same type of morphological characters, hence they are considered as a new species and named as *L.testelobatus.*

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Short Communication

Prevalence of Setariosis in Cattle and buffaloes in Karnataka

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A study was conducted to determine the prevalence of Setariosis in cattle and buffaloes in Karnataka state with relation to age,sex and breed.Out of 500 cattle screened,27.6% were positive for worms without microfilariae in blood and 9.8% had both worms and microfilariae. Among the 200 buffaloes screened,only 5.5% were positive for worms but no microfilariae were detected. Among the cattle which were positive for worms, 56.8% had *S.digitata,* 24.13% had *S.cervi* and 18.96% had *S. labiatopapillosa.* Mixed infection with all the three species of Setaria was found in 16.04% cattle. Among buffaloes, 36.36% had *S. digitata* and remaining 63.36% had *S. cervi.*

Keywords : Buffaloes, Cattle, Setariosis, Microfilariosis, *S.digitata, S.cervi, S. labiatopapillosa*.

Setaria is one of the very commonly occurring filarid worm in the peritoneal cavity of cattle and buffaloes and the adult worms dwelling in the peritoneal cavity do not cause any significant pathogenicity. Sometimes these worms and their larval stages may occur in various organs like the anterior chamber of eye, urinary bladder, oviduct, liver and even the pericardium of the heart where they cause harmful effects in unusual locations. The microfilariae or first stage larvae produced by the adult female worms circulate in the blood and cause microfilariosis in cattle and buffaloes exhibiting a package of clinical signs or syndrome. The earlier reports on prevalence of these setarial infections have been mainly based on examination of clinical cases and postmortem studies . Therefore a systematic screening of slaughtered animals was conducted to assess the actual status of infection in Karnataka state.

The male animals screened in the study were from KMPMCL slaughter house, Bangalore. These animals were mainly from Raichur,Gulbarga, Bellary and Kolar districts of Karnataka. Samples from female animals were obtained from post mortem conducted in the Department of Pathology, Veterinary college, Bangalore and from different institutes in and around Bangalore. To observe the prevalence of microfilariosis caused by *Setaria* spp,blood samples were collected in clean and sterile glass vials with Ethylene diamine tetra acetic acid (EDTA) or sodium citrate as anticoagulants separately from cattle and buffaloes.The blood samples were examined on the same day by Wet film method, Giemsa stained blood smear, Modified Knott's method and Acid Citrate - Saponin method To record the prevalence of Setariosis, the same animals were thoroughly screened during evisceration and dressing. The entire peritoneal cavity was examined for worms which were collected in normal saline. The head and tail ends of worms were mounted separately for identification in a clearing and mounting medium, viz,Rubin's mountant and speciation was carried out based on the descriptions given by (Shoho 1958; Willard and Walker 1969; Sonin 1977; Anderson 1992). The age, sex and breed of the animal was noted and the prevalence was calculated.

A total number of 500 cattle were screened during the period of study. Of them, 138 animals (27.6%)

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harboured worms in the peritoneal cavity without any detectable microfilariae in the blood, 49(9.8%) had worms in the peritoneal cavity and microfilariae in the blood and remaining 313 (62.6%) were negative for both worms and microfilariae. Low infection was observed in 154 animals whereas 16 and 17 animals had moderate and heavy infections respectively.

Three species of Setaria *viz,S.digitata,S.cervi* and *S.labiatopapillosa* were observed in the present study. The worms were found freely in the peritoneal cavity or found attached to the intestines, mesentry, walls of the peritoneum, lungs, liver, heart, urinary bladder,uterus and fascia. Some worms were found embedded in patches of inflammatory tissue attached to the walls of the pelvic peritoneum. Out of the 187 cattle which were positive for worms, 106 (56.8%) had *S.digitata* (24.13%) had *S.cervi* and 36 (18.96%) had *S.labiatopapillosa,* 30 (16.04%) had a mixed infection with all the three species of Setaria. Of the 49 cattle which were positive for microfilariae in the blood,19 (38.77%) had low microfilaraemia (less than 100 in the whole sediment), 24 (48.97%) had a moderate infection(100-500 microfilariae in the whole sediment) and remaining 6(12.24%) had high infection (500-8000 microfilariae in the whole sediment).

Among the five different breeds of cattle consisting of 320 Hallikar cattle, 95 (31.14%) were positive for worms only and 26(8.52%) were positive for both worms and microfilariae. Among the 48 animals from Deoni breed, 15 (31.25%) were positive for worms only and 7(14.58%) were positive for both worms and microfilariae. Of the 50 non descript cattle screened, 12(24%) were positive for worms only and 1(2%) had both worms and microfilariae. Of the 42 Holstein Friesian animals,7(16.66%) were positive for worms only and 10(23.8%) were positive for both worms and microfilariae. Out of the 40 animals from Malnad Gidda breed, 8(20%) were positive for worms only and 7(12.5%) were positive for both worms and microfilariae. Worm prevalence was highest in males followed by females in 6 to 12 years age group than in younger animals and statistically significant difference was observed at 0.05 levels between age groups and not sexes,

A total number of 200 buffaloes were screened during the period of study. Of them, 11(5.5%) were positive for only worms and none was microfilaraemic. All the animals which were positive for worms were Non descript females of 9-12 years age group and all of them had a low infection. Mixed infection with *S.digitata* and *S.cervi* was observed in the affected animals.Worms were recovered only from the peritoneal cavity.

The prevalence of *S.digitata* in cattle has been found to be more common than *S.cervi* and *S.labiatopapillosa.* (Shoho 1958; Mohan 1975 , Patnaik 1989; Mohanty et al 2000) ranging from 77 to 95 %. *S.cervi* was the least common species in South India (Mohan 1975) among cattle as was observed in the present study. *S.labiatopapillosa* has been found to be less common in India among cattle. However 19.3% of the cattle in Orissa were found to be infected with this species (Patnaik 1989). In buffaloes, Scervi was more prevalent than *S.digitata* and *S.labiatopapillosa* with a 38.27% incidence (Siddiqui et al 1996). Prevalence of *S.cervi* is very common in the Tarai region of Uttar Pradesh and in Pantnagar (Kumar *et al* 1987; Sharma & Kumar 1994). In the present study also 63.36% of the infected buffaloes had *S.cervi.* In Andhra Pradesh varying prevalence rates of 5.4% and 54.54% of *S.digitata* in buffaloes respectively have been reported.(Sastry et al 1985 ; Mohan 1975). Prevalence of this species in buffaloes as observed in the present study was 36.36%. *S. labiatopapillosa* had also been observed to be more prevalent in buffaloes but the rate of infections varied from 3.59% in Mathura to 9.09% in Jabalpur (Chauhan & Pande 1980 ;Bhopale et al 1982).

The common species of Setaria causing microfilariosis in cattle was found to be *S.digitata.* A 14.7% incidence of microfilariosis in bullocks caused by *S.digitata* in Tamil Nadu and 12.5% incidence of microfilariosis among cattle in Orissa has been reported.(Manickam and Kathaperumal 1975; Mohanty et al 2000) In the present study in Karnataka,the overall incidence of microfilariosis in cattle of 9.8% was lower and is attributable to the difference in seasonal pattern, climatic condition and vector availability restricted to a lesser period in each year.

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Short Communication

Efficacy of ivermectin pour-on against natural gastrointestinal nematodes and lousiness in goats

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Ivermectin pour-on @ 0.5mg/kg body weight was effective in removing natural gastrointestinal nematode infection and *Linognathus stenopsis* infestation in goats. The percent reduction in eggs per gram (EPG) of faeces was 87.73, 96.26 and 98.54 on 7,14 and 21 days post-treatment (DPT) respectively. The reduction in lice counts was 100% on 14 DPT. The drug offered a protection for more than 21 days but less than 28 days against lousiness.

Key words: Gastrointestinal nematodes, Goats, Ivermectin pour-on, *Linognathus stenopsis*

Chemotherapy is an integral part of the parasite control strategies. One such chemotherapeutic agent is ivermectin, which is available as injectable and oral in the form of suspension, paste, tablet and controlled release bolus. All these formulations have been evaluated and found effective against both endo and ectoparasites (Bhatia and Kaur. 1999; Panda *et al*, 2003). For easy administration, pour-on formulations have now become available. The present communication reports its efficacy against natural gastrointestinal nematodes (GI) and lousiness in goats.

A total of 20 goats of either sex, aged 6 months to 2 years, weighing between 30-40 kg and naturally infected with Gl nematodes and lice infestation were selected for the study. The animals belonged to Department of Livestock Production and Management, College of Veterinary and Animal Sciences, Pantnagar. All the animals were treated with ivermectin pour-on (IPOUR, Ranbaxy Laboratories Ltd., India). The drug was poured on the back midline from shoulder to sacrum @ 0.5 mg/kg body weight. Pre and post treatment faecal egg counts were carried out on faeces collected directly from the rectum of all the goats. Eggs per gram (EPG) of faeces and coproculture were done on 0,7,14 and 21 day post-treatment (DPT) as per standard techniques. The percent efficacy (%E) was calculated using the following formula

$$\%E = \frac{\text{EPG (Pre-treament-Post-treatment)}}{\text{EPG (Pre-treament)}} \times 100$$

To evaluate the lousicidal efficacy, counting of lice was done before treatment and on 1, 3, 7, 14, 21 and 28 day post-treatment. The lice were counted in one square inch in four sample areas on each animal. The percent efficacy was calculated using the following formula

$$\%E = \frac{\text{Number of lice (Pre-treament-Post-treatment)}}{\text{Number of lice (Pre-treament)}} \times 100$$

Faecal and body surface examination revealed that animals were infected with G. 1. nematodes and lice. (Table 1 & 2). The EPG ranged from 200 to 8350 with a mean of 2405 and severity of louse infestation ranged from 28 to 43 lice with mean of 38.10. Coproculture showed infection of *Haemonchus contortus* and

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Table I. Efficacy of ivermectin pour-on against gastrointestinalnematodes on goats

Mean EPG	Day			
	0	7	14	21
Pre-treatment	2405*	-	-	-
Post-treatment	-	295	90	35
% Reduction	-	87.73	96.26	98.54

* Percent composition of L_3 in coproculture of goats : *Haemonchus contortus* 40; *Trichostrongylus* spp. 60.

Table II. Efficacy of ivermectin pour-on against *L. stenopsis* infestation in goats.

Mean number	Day						
of lice	0	1	3	7	14	21	28
Pre-treatment	38.10	-	-	-	-	-	-
Post-treatment	-	36.20	15.20	3.45	0	0	0.15
% Reduction	-	4.98	60.10	90.94	100.00	100.00	99.61

Trichostrongylus sp.

The percent reduction in EPG counts was 87.73, 96.26 and 98.54 on day 7, 14 and 21 post-treatment, respectively. The efficacy of pour-on formulation of ivermectin recorded in the present study is comparable to other formulations of ivermectin *viz.* injectable (Katoch *et al*, 2004; Rajkhowa *el al*, 2004), oral (Panda *et al*, 2003) and sustained release formulations (Gillen and Horn., 1998). The results are in consonance with those of Islam *et al* (2003) who recorded 100% efficacy of ivermectin pour-on in cattle naturally infected with Gl nematodes.

The drug was also successful in controlling the sucking louse (*L. stenopsis*) infestation in goats. By day 14, there was 100% reduction in lice counts, which continued till day 21. But reinfestation was observed in 3 animals on day 28. Bhatia and Kaur (1999) have reported a high efficacy of ivermectin against *Haematopinus eurysternus* and *L. vituli* in cattle. Titchner and Purnell (1996) have recorded a protection of more than 14 days but less than 21 days with avermectin against *L. vituli* infestation in cattle. The mode of application of ivermectin pour-on has added utility especially in small ruminants in cold inclement weather when dipping can be avoided. At the same time GI nematodes can also be controlled.

ACKNOWLEDGMENTS

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, and Director, Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar for providing the necessary facilities to conduct the study. The authors also thank M/S Ranbaxy Laboratories Ltd., India for providing free samples of IPOUR.

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Short Communication

Effect of Bleomycin Hydrochloride on the course of *Trypanosoma evansi* Infection in Swiss Albino Mice

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Bleomycin hydrochloride was used as trypanocide @ 5 mg per kg s/c against experimental *Trypanosoma evansi* (cattle strain) infection in Swiss albino mice. Thirty Swiss albino mice were randomly divided into five groups, (I-V) each consisting of six mice. The drug was applied after 24 h as single treatment (Group I), two treatments after 24 and 48h (Group II) and three treatments after 24, 48, and 72 h (Group III). The mice in-group IV and V were kept as infected and without drug treatment and uninfected controls, respectively. The effect of drug was noticed in terms of degree of parasitaemia, prepatent and survival periods. It was observed that while two treatments of the drug had trypanocidal effect for one day only. Three consecutive treatments (24, 48 and 72 h) of the drug @ 5 mg per kg s/c had trypanocidal effect for a transient period of 4 days and subsequently relapse occurred with reappearance of the trypanosomes in peripheral blood circulation. Survival period of treated mice was prolonged upto 12 days. Histopathotogical changes were suggestive of mild hepatotoxicity.

Key words: Bleomycin hydrochloride, Swiss albino mice, Trypanocidal activity, *Trypanosoma evansi*

Chemotherapy against trypanosomosis has evoked considerable interest among researchers, particularly against 'Surra' since the days of Dangerfield and coworkers (1938), who used Suramin as a chemoprophylactic agent. Of lately, the appearance of drug resistant strain of *Trypanosoma brucei* and possibly of *Trypanosoma evansi in* endemic areas and failure to treat nervous involvement of the disease at late stage have necessitated the need for research on new drugs or their combinations. Although bleomycin hydrochloride, a currently employed antitumour drug has proved to be curative against *T. brucei* and *T. brucei gambiense* either singly or in combination with DL-alpha-difluromethyle omithine. (Nathan *et al*, 1981a; Bacchi *et al* ,1982; Clarkson *et* al. 1983 and Demey, 1987). However, till date no information is available on its use against *Trypanosoma evansi*. Therefore herein we report effects of bleomycin hydrochloride on the course *of T. evansi* infection in Swiss albino mice.

Isolation and inoculation: The cattle strain of *T. evansi* originally isolated from a case of 'Surra' from cattle was maintained in mice in the laboratory by regular serial passages. The inoculum was prepared in Alsevier's solution.

Bleomycin: Bleomycin hydrochloride obtained from Khandelwal Laboratory Pvt. Ltd., Bombay, India which was originally manufactured by Nippon Kayaku Co. Ltd., Japan, was used parentally @5 mg.kg^{-1} body weight as subcutaneous injections.

Laboratory animals used: Swiss albino mice of either sex weighing 10-20 g were procured from the university animal house and maintained in the department for the conduct of experiment.

Experimental Procedure: Thirty Swiss albino mice

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were randomly divided into five groups (I,II,III,IV,V) each consisting of six mice. The mice in I-IV groups were inoculated with 2.08 $xl0^6$ trypanosomes subcutaneously. Treatments of mice consisted of subcutaneous bleomycin hydrochloride injection one at 24 h (Group I), two treatments at 24 and 48 h (Group II) and three treatments at 24, 48, and 72 h (Group III), respectively: The mice in group IV and V were kept as infected without drug and uninfected controls. The effect of drug on the course of *T. evansi* infection was analysed in terms of degree of parasitaemia, prepatent and survival periods of mice.

Histopathological changes: Neutral buffer formalin fixed and paraffin embedded tissue sections (5 µ*i* thick) stained with Haematoxylin and Eosin from different groups of mice were examined for histopathological changes.

Results: The course of infection (i.e. degree of parasitaemia, prepatent and survival periods) of *T. evansi* in different groups of mice after bleomycin treatment have been summarized in Table. It was observed that two (24, 48 h) and three (24. 48, 72 h) consecutive injections of bleomycin had trypanocidal effect for a transient period of one and four days respectively, and subsequently reappearance of the trypanosomes occurred in peripheral blood. The increase in the number of treatments reduced the parasitaemia as indicated both by intensity as well the number of trypanosomes/ml of the tail blood. The survival period in treated groups (II and III) was prolonged, up to 12 days when compared with those of untreated control group.

The findings in present study are more or less similar to those of Nathan *et al.* (198 la), Bacchi *et al.* (1982) and Demey (1987) as observed in *T. brucei* and *T. brucei gambiense* infections. These workers have also described trypanocidal activity of bleomycin against African trypanosomes singly or in combination with DL-alpha-DFMO. Demey (1987) noticed the relapse of the parasitaemia after a period of 100 days and no trypanosomes were detected in the peripheral blood circulation at 360 days post infection. Nathan and his colleagues (1981b) reported that bleomycin induced life prolongation in mice infected with *Trypanosoma brucei*. However, in the present study, *T.evansi*- mouse model, bleomycin cleared the trypanosomes from the circulation by day 10 post infection and relapse occurred on day 11 post infection in a group of mice which were given three injections (24, 48 and 72 h) of bleomycin while in a group which were given two consecutive injections (24 and 48 h) there was no parasitaemia upto day 7 post infection and relapse of parasitaemia occurred on day 8 post infection, thus indicating a dose related response of drug. Singly bleomycin has curative value in acute infections while combination of bleomycin and DMFO were effective in late stage infection of *T. brucei* (Me Cann *et al*, 1981; Clarkson *et al*, 1983). It has been reported that the curative rate of bleomycin was 75 per cent when mice

Table. Effect of bleomycin hydrochloride on the course of *Trypanosoma evansi* in Swiss albino mice.

Groups	Treatment Applied	Total Survival Days	Prepatent Period/Release (Days)	Degree of Parasitaemia (Tryps $ml^{-1}x10^6$)
I	24 h (1)	6	3/4	1^+-3^+ (3-30)
II	24,48 h (2)	12*	3/8	1^+-2^+ (0.5-10)
III	24,48,72 h (3)	12**	3/11	Rare-1^+ (0.5-5)
IV (Infected control)	No treatment	6	3	1^+-4^+ (3-70)
V (Uninfected control)	Bleocin (3)	All survived	-	Nil

1,2 and 3 in parenthesis indicate number of treatment with bleomycin hydrochloride @ 5mg/kg by S/c route.
* Disappeared for one day.
** Disappeared for four days.

with parasitaemia due to *T. evansi* were treated with bleomycin at a higher rate (30 mg.kg^{-1}) (Ono and Nakabayashi, 1980a). The early relapse, as recorded in the present study, might possibly be due to the fact that bleomycin inhibits only nuclear division and causes malformation of nucleus and disorders of the microitubule morphology as established in *T.brucei gambiense* infection or due to variation in the dose regimen of the drug employed, virulence and strain of *T. evansi* used in the experiment (Ono and Nakabayashi, 1980b).The drug appeared to have mild hepatotoxicity, as histopathologically focal congestion and vacuolar degeneration in liver were recorded.

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Short Communication

Management of Piperazine Resistant Toxocariosis with Ivermectin in Lions (*Panthera Leo*)

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The occurrence of *Toxocara* eggs was found in the faeces of all the lions, which were previously treated, with piperazine @ 220 mg. kg^{-1} orally 2 weeks earlier at M.C. Zoological Park, Chhatbir in district Patiala of Punjab. Post mortem examination of a four month old hybrid male lion cub died of toxocariosis in spite of treatment with piperazine revealed a small tear in the stomach wall, and the omentum and small intestine full of worms. On the basis of gross, light and scanning electron microscopy, the worms were identified as *Toxocara cati*. The other young and adult animals (n=22) (3 months to 2 years of age) in the Park were also found positive for *Toxocara* eggs with moderate to severe degree of infection (mean epg = 4401.82± 1156.90) inspite of deworming with piperazine@ 220mg.kg^{-1} orally two weeks earlier. Four animals were found positive for mixed *T. cati* and *Toxascaris leonina* infection Treatment of piperazine resistant *Toxocara cati* with injection of ivermectin @0.2 mg.kg^{-1} subcutaneously twice at weekly intervals was found 100 per cent effective.

Keywords: Ivermectin, Lions, Management, Morphology, Piperazine, *Toxocara*

Diseases in the wild animals may be infectious or non-infectious. Among the infectious diseases, parasitic diseases constitute one of the major managemental problems causing mortality in wild animals in captivity (Rao and Acharjyo., 1984). Due to high prevalence and zoonotic significance, toxocariosis has gained a major importance in the field of animal and public health research. In humans, this disease is the classical cause of Visceral Larva Migrans (VLM), caused by larvae of the worms (Prociv and Cross, 2001). Though data is not available in India, approximately 10,000 new cases of Visceral Larva Migrans and 700 cases of Ocular Larva Migrans (OLM) are diagnosed annually in the USA (Schantz and Stehr-Green, 1988). According to a survey of opthalmologists in Alabama, USA, at least one case of OLM is encountered in every 1,000 patients (Meatzs *et al*, 1987). Adults of *Toxocara cati* have been well documented from the human intestine, but never those of *T. canis* (Beaver *et al*, 1994). Uncontrolled defecation by different hosts infected with *T. canis* and *T. cati* results in the serious contamination of soil by *Toxocara* eggs. So the soil becomes an important source of infection to new animals and humans (Borg and Woodruff 1973; Hollan *et al* 1991; Oteifa and Moustafa, 1997; Oge and Oge, 2000). Environmenal contamination poses significant and lasting effect because of high resistance of worm eggs to unfavourable climatic conditions (Soulsby, 1978 and Urquhart *et al*, 1996). Humans acquire the infecion as a result of accidental ingestion of eggs containing 2nd stage larvae of *Toxocara*. Children are most vulnerable to infection as they frequently come in contact with soil (Schant, 1989; Hollan *et al.*, 1991). The larvae migrate to different tissues and produce different clinical effects: visceral toxocariosis or VLM and ocular toxocariosis or OLM in humans (Beaver, 1969; Schantz,1989; Arango, 1998). The introduction of ivermectin in the early 1980's brought a revolution in the control of animal parasites and is accepted as a potent anthelmintic drug (Campbell

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and Benz, 1984).

The disease occurrence at M.C. Zoological Park was high, leading to death of lions inspite of treatment with piperazine. The control of disease is important not only to reduce the worm burden in affected animals, but also to prevent excretion of large number of *Toxocara* eggs into the environment as these eggs present a serious risk to zoonotic infection. No previous report of studying the efficacy of ivermectin against piperazine resistant strain of *T. cati* in lions could by traced in litrature. Hence the present study was planned to evaluate the anthelmintic efficacy of ivermectin in piperazine resistant strain of *T. cati* in lions.

In the present study, we found the occurrence of *T. cati* eggs in the faeces of lions which were previously treated with piperazine @ 220 mg. kg^{-1} two weeks earlier at MC Zoological Park in the district Patiala of Punjab, India. Toxocariosis was suspected based on history and clinical signs of a male hybrid lion cub, aged four months which was presented with clinical manifestations of progressive enlargement of abdomen, no defecation since the last 2 days, general debility, rough body coat and inappetance. Clinical examination recorded rectal temperature 100°C, mucus membrane anaemic, moderate depression and dehydration. Respiration and heart rates were recorded to be 40 and 134 per min, respectively. There was a history of single dose deworming done with piperazine @ 220 $mg.kg^{-1}$ orally 2 weeks earlier. Abdominal palpation denied the presence of any edematous or inflammatory swelling. Luke warm soap water enema was done and small blackish-coloured faeces were recovered. The lion cub later died and the worms were collected from omentum and small intestine for gross, light and electron microscopic studies. The adult worms were cleared in lactophenol and glycerine for light microscopic studies. For scanning electron microscopy, the adult worms were processed as described by Sharma *et al* (1994). The other young and adult animals (n=22) (3 months to2 years of age) at the Park were screened for intestinal parasitism. The faecal examination was done by direct smear method and faecal floatation method using saturated salt solution. Mc master counting technique was used for quantitative estimation before and after treatment. The efficacy of the treatment was evaluated by calculating the percent reduction in egg counts.

Copro-parasitoscopic analysis (CPS) of faeces of the lion cub by direct smear method revealed severe infestation of *Toxocara* eggs (5-6 eggs per field). The quantitative estimation of eggs by Mc Master counting technique revealed 12,000 eggs per gram (e.p.g.) of the faeces. Eggs were almost spherical with a diameter of 65 to 70µ and having a thin shell with corrugated outer surfaces. Haematological examination indicated Hb 4.5 g/dl, total leukocyte count 13,300/cumm with 67% neutrophils, 27% lymphocytes, 4 % monocytes and 2% eosinophils.

The animal's condition worsened and it succumbed to death before effective anthelmintic treatment could be attempted. However, the animal was administered symptomatic treatment before the final diagnosis of toxocariosis which included Ringer's Lactate, Dextrose Normal saline, Amicacin and Hivit injections without any response. On post-mortem examination, stomach was found fully impacted with paddy straw. There was a small tear in the stomach wall and omentum was loaded with mature worms. Small intestine was also found full of worms. Petechial haemorrhages on the mucosal surface of intestine were marked. No significant lesions were seen on spleen, kidney, urinary bladder and heart except congestion of the lungs and liver. Grossly, the anterior end of the large white worm was bent ventrally (Fig. l). The mouth of the worms was having

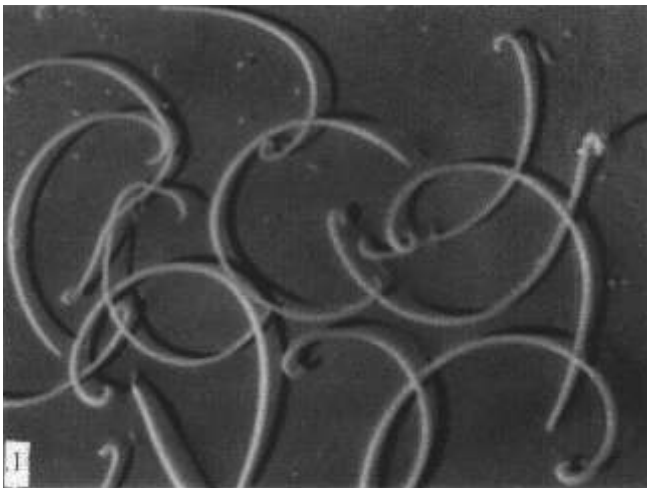

Fig. 1: *Toxocara cati* showing anterior end bent ventrally.

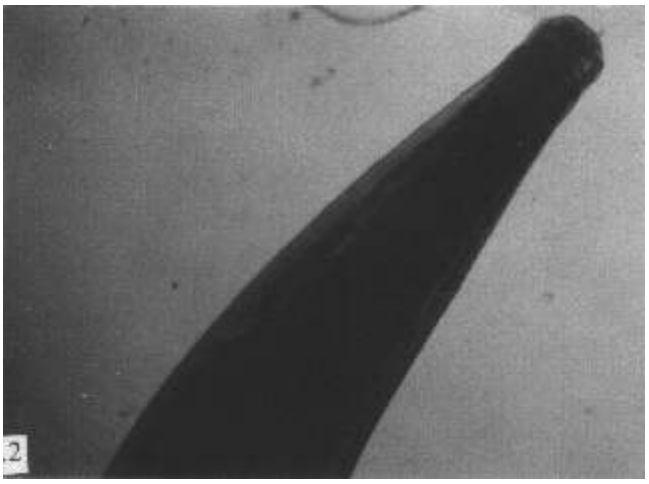

Fig. 2: Anterior end adult *Toxocara cati* (X 70)

three large lips (Fig. 2) and a glandular esophageal bulb, the ventriculus(Fig. 3) was located at the junction of the esophagus and intestine. The mouth of

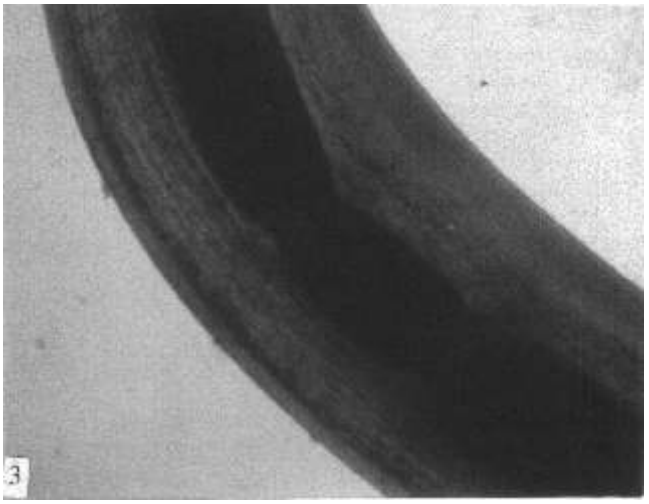

Fig. 3: *Toxocara* showing ventriculus inercalated between oesophagus and intestine (X 70)

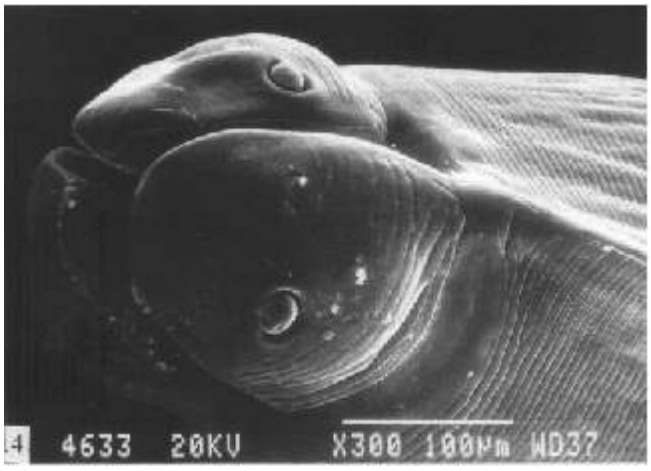

Fig. 4: *Toxocara cati* showing three lips. (SEM)

the worms was terminal and was surrounded by three large fleshy lips, one dorsal and two sub ventral (Fig.4)

The cuticular surface was ornamental with ill-defined transverse striations. Male worms suffered a marked reduction in caliber immediately caudal to the anus giving the appearance of a small finger like process on the tail (Fig.5) and the posterior end of female worms

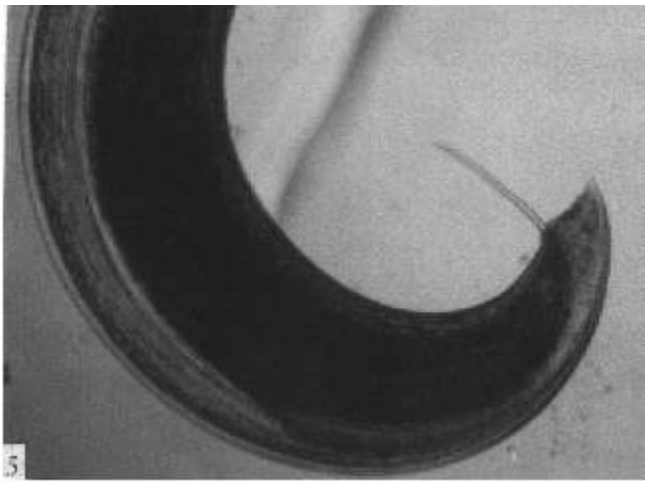

Fig. 5: Tail of male *Toxocara cati*. (X 70)

was straight (Fig.6). The uterus of the female worms

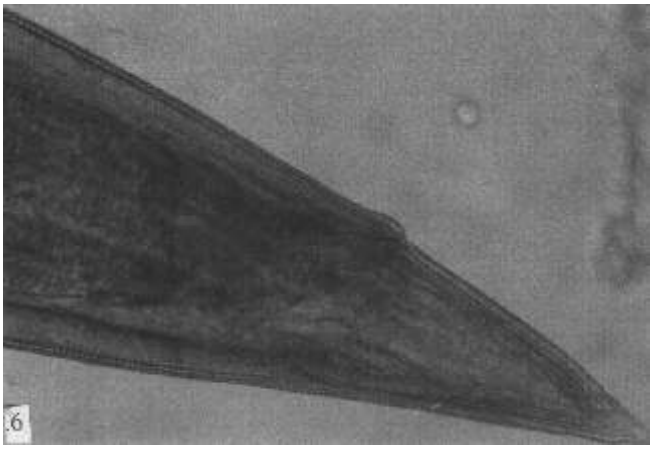

Fig. 6: Posterior end of female. (X 70)

was full of typical *Toxocara* eggs (Fig.7). Thus the worms were identified as *Toxocara cati*.

All the other animals at the Zoological Park were found positive for *Toxocara* with moderate to severe degree of infection with four lions showing mixed infection with *Toxascaris leonina*. Mc Master counting technique revealed mean e.p.g. of 4401.82±1156.90. Clinical examination also revealed somewhat similar

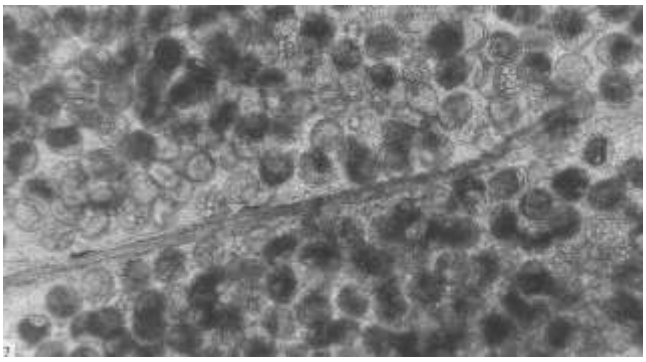

Fig. 7: Uterus filled with thick walled eggs. (X 70)

symptoms. Animals were observed to be having the habit of nibbling/eating paddy straw. Deworming of all the animals was prescribed with one injection of ivermectin @lml/50kg b.w. subcutaneously, twice at one-week interval. The diet was supplemented with Tab. Shellcal (Elder Pharmaceuticals, Mumbai) and cap. Haem-up (Cadila Pharmaceuticals, Ahmedabad).

A significant improvement in the condition of the animals was observed after ivermectin treatment. Seven days after treatment, the eggs in the faeces were reduced by 98.71 per cent (Table 1) and sixteen animals were found negative 7 days post treatment.

Table 1 : Fecal count reduction after treatment

Treatment	Day post 1st treatment	Fecal egg counts Mean ±SE (Range)	% Reduction in fecal count
Nil	0	4401.82±1156.9* (200-20,000)	--
Ivermectin	7	56.82±26.75** (50-500)	98.71
--do--	14	Zero	100

*Four lions were also found positive for *Toxascaris leonina*
**Sixteen lions negative for eggs.

Faecal examination two weeks after first deworming revealed no parasitic egg/ova in the faeces.

T. cati eggs were found in all the lions. Explanation to this may be based on the behaviour of the lions. The animals tend to defecate in the same place where the *Toxocara* eggs remain for long time. These subsequently develop to infective eggs containing second stage larvae. The lions were in the habit of continuous nibbling of bedding material leading to swallowing of embryonated eggs and thus re-infection.

Worm infestation leads to anaemia and deficiencies of iron, calcium and other trace elements resulting in symptoms of pica (Radostitis *et al*, 2000). Death of the animal in toxocariosis may result from rupture or obstruction of the intestine by ascarids. The worms after reacting to some irritant, trash out and become entangled into knots (Bowman, 1999). The infection was found to be more severe in lion cubs than in adults, reflecting age dependant prevalence of the disease (Salman & Shah, 1989; Agnihotri *et al*, 1998).

Since all the lions were infected, so the risk to humans is not negligible. However, the risk to animal handlers and the visitors at the parks to be infected with VLM has not been calculated. This risk further increases if the lion's excreta is not removed frequently. Antiparasitic treatment every three months is recommended for this situation, with simultaneous parasitological analysis of animal faeces. The eggs deposited by the infected animals in this park, may become a source of infection not only to handlers, children and visitors, but also to other mammals of canidae and felidae. So the risk to public health must be eliminated by proper treatment of infected animals (Vazquez *et al.*, 1997). Deworming of lion cubs should be carried out 7 to 8 weeks of age to prevent shedding of ova and subsequent environmental contamination.

The results indicate that ivermectin is cent percent efficacious in reducing egg counts in piperazine resistant toxocariosis in lions. Pal *et al.* (1995) observed that in dogs piperazine was far less effective both in respect of epg and worm count studies, whereas ivermectin at different dose rates revealed cent per cent efficacy. The studies indicated that anthelmintic drug may be changed with time to avoid reduced efficacy of drug and attempts should be made to identify the source of infection as the breaking of the life cycle of the parasite outside the body of the host is important.

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Short Communication

Cryptosporidiosis in Calves with other Concurrent Infections

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The present investigation was carried out by screening 651 faecal samples from non-diarrheic (374) and diarrheic (277) calves to study the prevalence of cryptosporidiosis with the presence of Cryptosporidium as the single pathogen or in combination with other bacteria and parasites. Out of 374 non -diarrheic faecal samples screened ,73 (19.52%) and of the 277 diarrheic samples, 134 (48.38%)were positive for *Cryptosporidium* oocysts. Solo infection was recorded in more number of calves (61.19%) compared to mixed infection (38.80%). Commonly isolated bacteria from cryptosporidia positive calves were *Salmonella, Proteus, E.coli and Klebsiell* species and the parasites were *Trichuris, Strongyle, Ascaris and Eimeria* species. Although concurrent infection with *Cryptosporidium* and other enteric pathogens was detected in diarrheic calves, the study revealed that Cryptosporidia could cause significant pathogenic effects as solo infectious agent.

Key words : Bacteria, Calves, Cryptosporidium, Diarrhoea, Parasite.

Cryptosporidia are regarded as opportunistic organisms and were observed in conjunction with *Escherichia coli,* rota and corona viruses in calves with severe enteric disease (Moore and Zeman 1991). The causative role of*Cryptosporidium parvum* in diarrhoea is controversial because the organism can be found even in apparently healthy animals (Radostits *et al,* 2000), but has been claimed being responsible for diarrhoea in calves by Sanford and Josephson (1982). The present study describes the occurrence of mixed infection of *Cryptosporidium* in calves along with other pathogens and delineates the pathogenic role of this organism in enteric infection of calves.

The prevalence of cryptosporidiosis was studied by screening 651 faecal samples (374 non-diarrheic and 277 diarrheic samples) from calves belonging to 5 different dairy farms and calves brought to 9 veterinary hospitals in and around Tirupati, Andhra Pradesh. Young calves (below 6 month) of Holstein Friesian, Jersey cross and non-descript breeds were included in the study.

About 5 g. of faeces, collected from rectum, was mixed with equal volume of 10% formalin, and kept for 30 min for proper fixation. Oocysts of cryptosporidia and other enteric parasites in the faeces were examined by employing Sheather's sucrose centrifugal floatation + modified acid -fast (Dimethyl sulphoxide) staining. Formal ether sedimentation + modified acid -fast (Dimethyl sulphoxide) staining methods.

Rectal swabs from *Cryptosporidium* positive calves (134) which were suffering from diarrhoea only were collected in sterile bottles and inoculated in basal media such as nutrient broth and tetrathionate broth to know the presence of other pathogenic bacteria in association with *Cryptosporidium.* The bacteria were identified on the basis of cultural characters and colony morphology on specific media; viz,, macConkey agar. Salmonella - Shigella agar, staining reaction by Giemsa method, presence of capsule,

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motility and bio-chemical tests. All the biochemical tests like indole, methyl red, voges proskeur, citrate utilization, urease and H_2S Production were carried out as per the procedures given by Krieg and Holt (1984). Enterotoxigenicity of the *E. coli* and *Salmonella* isolates was confirmed by National Salmonella and E, coli center (NSEC) Kasauli, Himachal Pradesh. Out of 374 non- diarrheic faecal samples, 73 (19.52%) and from 277 diarrheic samples, 134 (48.38%) were positive for *Cryptosporidium* oocysts. Out of 134 diarrheic faecal samples positive for *Cryptosporidium*, 82 (61.19%) had *Cryptosporidium* alone and 52 (38.80%) had mixed infection with parasites and enteric bacteria (Table). Significantly ($p<0.01$) greater number of calves had single infection with *Cryptosporidium* alone in the present study. Vilchez and Pote (1999) also found it alone in 57.50% of diarrheic calves. Acute and severe diarrhoea could be observed in 82 calves of the present study caused by *Cryptosporidium* as the primary pathogen.

In the present investigation, more calves were positive for enteric parasites *Trichuris sp.(37) Ascartis sp. (20), Strongyle sp. (14) and Eimeria sp. (9)* than enteric bacteria. The enteric bacteria isolated on culturing diarrheic feacal samples were *Salmonella sp.* (20), *Proteus sp.* (6) *Klebsiella sp.* (3). In addition, six *E.coli* isolates of entero invasive (EIEC) group comprising of five strains of O143 and one strain of O144 were isolated. Absence of regular deworming practices and lack of hygienic measures might be the causative factors for the above findings, Barragry (1994) stated cryptosporidia are frequently associated with other enteric pathogens such as *Proteus sp., E. coli, Salmonella sp.* of bacteria and *Ascaris sp.,* of parasites and *Eimeria sp.,* of protozoa. Although *Cryptosporidium* was seen as solo-pathogen (Fuente et al., 1999), mixed infections with bacteria and virus (Barragry 1994 and Radostits *et al*, 2000) and enteric parasites (Abdel salam et al., 1993) are also frequent.

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Table. Prevalence of *Cryptosporidium* in the calves.

S. No.	pathogen	Number of faecal samples		
		Examined	Positive for *Cryptosporidium (%)*	
1.	Non-diarrheic faecal samples	374	73	(19.52)
2.	Diarrheic faecal samples	277	134	(48.38)
3.	Single infection with *Cryptosporidium*	134	82**	(61.19)
4.	Mixed infection	134	52	(38.80)
a.	*Cry + Ascaris sp. + Trichuris sp. + Salmonella sp.*	52	20	(38.46)
b.	*Cry + Strongyle Sp. + Trichuris sp.*	52	14	(26.92)
c.	*Cry + Eimeria sp.*	52	9	(17.31)
d.	*Cry + Proteus sp. + E. coli*	52	6	(11.54)
e.	*Cry + Trichuris sp. + Klebsiella sp.*	52	3	(5.76)

Cry = Cryptosporidium
*** Significant at P<0.01*

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Pyrethroid insecticides, Sumicidin and Butox for the effective control of *Stomoxys calcitrans*

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Single spray of aqueous emulsion of 0.2% of pyrethroid insecticide, Sumicidin and Butox on cattle provided 100% protection to them against stable fly, *Stomoxys calcitrans* for 21 days without their application on surroundings or breeding places of flies.

Keywords : Butox , Cattle, Control, *Stomoxys calcitrans*, Sumicidin.

The stable fly, *Stomoxys calcitrans* is a temporary ectoparasite of livestock and sucks blood of the animals. The bites of the flies are highly painful and they are mechanical transmitters of anthrax, surra and some other livestock diseases. The fly menace might reduce milk yield by 25% and meat production as much as 40 to 60%. Since the flies attack the host for short periods, the application of insecticides on the host is considered as not a very effective method of control of *S. calcitrans.* In earlier days chlorinated hydrocarbons and organophosphorus compounds were used to control the flies. The information available regarding the use of synthetic pyrethroid insecticide, Sumicidin (fenvalarate-20 EC, Rallis India, Mumbai) and Butox (deltamethrin, 12.5mg/ml, Hoechst, Mumbai) against stable flies, *S. calcitrans* is scanty. Therefore, trials were conducted by spraying these insecticides on fly infested cattle and the result of the trials is reported herein.

The study was conducted on crossbred cattle including cows, heifers and calves maintained at Regional Research Station, Konehally, Tiptur. The cattle were housed in sheds and they were grazed in the fields for 7-8 hours per day. Dung and other wastes were removed from the sheds and stored at a distant place. The flooring of the sheds was scrubbed and cleaned with water every day morning. While cleaning the sheds, the animals were tied outside for a short while before they were taken for grazing. At that period, especially in winter season a large number of stable flies were found hovering near these animals and many of them attached to the skin of cattle for sucking blood. To control these flies, 0.2% of Sumicidin and Butox as aqueous emulsion were sprayed on 6 and 21 cattle respectively. At an average 1.0 to 1.5, 1.5 to 2.0 and 2.0 3.0 litres of emulsion was utilized per calf, heifer and cow respectively. Five cattle were kept as untreated controls. The flies attached to the body of cattle were counted from a distance to avoid disturbance to the flies and pre-treatment count (day 0) varied from 45 to 60 flies per animal. Both treated and untreated control cattle were taken together for grazing. Presence of flies on these cattle was watched daily during the first week and on day 14 and 21 post-treatment. The treated cattle were free from flies whereas controls had fly infestations indicating the effectiveness of treatment.

Single application of 0.2% of Sumicidin/Butox on cattle gave a full protection (100%) against stable flies for 21 days of observation. Spraying cattle with o-diethyl toluamide, crotoxyphos, methoxychlor, fenchlorophos and permethrin provides protection for 3 hours to 4 days and with other insecticides such as tetrachlorvinphos, diazinon, crotoxyphos and propoxur on fixtures reduces infestation for two weeks or longer (Radostits *et al.*, 2000). Soulsby (1982) and Bowman (1995) advocated that effort to control flies

should be primarily directed towards elimination of their breeding sites by removal of organic wastes such as moist beddings, grass cuttings, food wastes, vegetable refuses and dung from the animal environment by spraying insecticides on the resting places of flies. In the present study, single spray of Sumicidin/ Butox on cattle gave longer protection for three weeks that might be due to the residual effect of insecticides. The notable obseryation in the present study was that good control of stable flies was achieved by Sumicidin/ Butox without their application on surroundings and breeding places, reducing the cost of treatment by saving a substantial quantity of insecticides and environmental pollution.

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I, Dr. J.K. Saxena, hereby declare that the particulars given above are true to the best of my knowledge & belief.

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BOOK REVIEW

Title: Diagnostic Parasitology

Authors: Veer Singh Rathor & Yogesh Singh Sengar

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Veterinary and Medical Parasitology has come a long way as a major discipline. Several areas within the broad field have evolved and grown so much in recent decades as to become sub-disciplines justifying the need for books/handbooks exclusively devoted to each of them. As such, the initiative of the authors in coming out with a companion volume to their earlier valuable contribution on :Parasitic Zoonoses" (published early 2005) is highly welcome.

The Compilation is arranged in 14 chapters covering a broad spectrum of diagnostic materials and procedures, supported by 21 figures and 14 tables. The chapter on Examination of Faeces is comprehensive (41 pages) incorporating procedures related to even relatively emerging entities such as *Cryptosporidium* sp., apart from simple sketches of g.i. protozoa and eggs of worm parasites of humans, different livestock species, dogs and poultry. The chapter entitled "Faecal Culture" is highlighted by tabulated data on the identifying features of larvea (L_3) of mainly g.i. nematodes of different livestock species as well as a useful key for the identification of larvae in human coprocultures. However, Culture Media for protozoans such as *Leishmania* and *Trypanosoma* are incongruous under this chapter heading. The next chapter (Ch. 3), "Preparation of parasites for examination" also includes collection and preservation which is, in effect, a partial overlap of information contained in a later chapter (Ch. 8, Necropsy). A major positive feature of this chapter is a guide (key) to identify adult g.i. nematodes of ruminants. Examination of body fluids other than blood, as diagnostic material for some helminthic eggs, has found coverage in a separate small chapter (Ch. 5) following a bigger one on Examination of Blood (Ch. 4). It is hartening to find full justice done to "Diagnostic Methods in Arthropods" (Ch. 6) - an oft-neglected area, by including tables identifying features and pictorial key for manage, itch and scab mites. Pathological aspects of diagnostic parasitology have been well addressed in the chapters 8 and 9. necropsy (Ch. 8) details the post-mortem lesions and the material to be submitted in different diseases. Under the title "Preparation of tissues for Parasitic Examination" (Ch. 9), ne can find essential information on impression (organ) smears, sectioning and preservation of tissues, in addition to Histopathology and Histochemistry of commonly prevalent parasitic diseases. The separate mini-chapters on Micrometry (Ch. 10) and Scale drawing and Camera Ludica (Ch. 11) could perhaps have been better combined under the heading "Microscopy", enlarged to include something regarding phase-contrast. Chapters on Immunodiagnosis (Ch. 12), and Molecular Diagnosis (Ch. 13) incorporate the latest advances such as monoclonal antibodies, radio-immunoassays, immuno-peroxidase, immuno-blotting, nucleic acid probes and PCR. Diagnostic adjuncts (Ch. 14) should include mention of lab animal inoculation (for diagnosis of trypanosomosis) and tissue culture (for theileriosis).

There are no major flaws and even minor ones are very few (for example: Page 20, labeling of cyst and vegetative form of *Balantidium coli* are reversed. The language is lucid and precise. The handbook will be useful guide for students and diagnosticians, as well as valuable reference for teachers and researchers, both Veterinary and Medical.

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Retd. Professor of Veterinary parasitology

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