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Journal of Parasitic Diseases

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Journal of Parasitic Diseases: December 2007, Vol. 31, No. 2, 77-78 ***Editorial***

Statement on publishing clinical trials in Indian biomedical journals

The registration of clinical trials will help improve reliability of data generated, help clinicians interpret research, minimize duplication of trials, and prevent exposure of volunteers to potential risks (Satyanarayana *et al.,* 2006). The Clinical Trial Registry India (CTRI) (www.ctri.in) hosted at the National Institute of Medical Statistics, Indian Council of Medical Research (ICMR), New Delhi, was formally launched on July 20, 2007. This is a free online registry of clinical trials with the aim to encourage all clinical trials conducted in India to be prospectively registered before the enrollment of the first participant and to disclose details of the 20 mandatory items of the WHO International Clinical Trials Registry Platform (ICTRP) dataset and a few additional items (http://www.ctri.in/Clinicaltrials/trials_jsp/index.jsp). Thus, the CTRI becomes a WHO's ICTRP and International Committee of Medical Journal Editors (ICMJE) compliant Primary Register for India. Clinical trial has been defined by the ICMJE (http://www.icmje.org/clin_trial07.pdf).

Within about three months of its launch the response received has been overwhelming with over 90 clinical trials already registered. But registration of trials is just a beginning. Active steps are on to sensitize researchers who actually conduct trials, funding agencies, ethics committee members, pharmaceutical companies, health professionals and medical journal editors on the need to register all trials that need registration. The WHO's ICTRP and ICMJE have drawn up clear guidelines on these issues (http://www.who.int/ictrp/en; De Angelis *et al.,* 2004; http://www.icmje.org/clin_trialup.htm). However, only prospectively registered clinical trials will be considered for publication.

While participants of clinical trials volunteer with an altruistic motive, it is too well known that still all is not well in experiments involving human subjects (Dickersin and Rennie, 2003) Clearly, there have been reports that trials have failed in their objective to carry out experiments fairly, report honestly and follow the ethical principles in India and abroad (Mudhr, 2005). There have been several instances of selective reporting or not reporting at all depending upon the outcome of the trial and when financial interests are at stake. Despite best efforts to ensure transparency and honesty, most initiatives to discourage the conduct of unethical trials have largely been unsuccessful.

Attempts to regulate clinical trials through system of record keeping at a public registry which would provide access to data on trials being carried out have not been very successful as trial registration is voluntary and there is reluctance of pharma companies to disclose data. As a step to ensure complete awareness of trial details, the ICMJE proposed comprehensive registration for clinical trials submitted for publication for the 12 member journals (*Annals of Internal Medicine, British Medical Journal, Canadian Medical Association Journal, Croatian Medical Journal, Journal of the American Medical Association, The Dutch Medical Journal (Nederlands Tijdschrift voor Geneeskunde), New England Journal of Medicine, New Zealand Medical Journal, The Lancet, The Medical Journal of Australia, Tidsskrift for Den Norske Laegeforening, Journal of the Danish Medical Association (Ugeskrift for Laeger*, http://www.icmje.org/clin_trial07.pdf). Commencing July 2005, these journals have made registration of trials in a public registry mandatory for consideration of publication. The ICMJE did not indicate a particular registry but any one that meets a set of minimum data.

With this background, the CTRI in association with the IJMR organized a meeting of editors of Indian biomedical journals to evolve a policy to be followed for publication of clinical trials in Indian biomedical journals. The meeting held at the ICMR on October 9, 2007, was attended by 12 editors of Indian biomedical journals. It was unanimously decided that the editors have the responsibility to promote the registration of all

clinical trials being conducted in India and to urge researchers to register their trials within a stipulated time, to make the clinical trial data transparent and to enable results to be published in good journals.

On behalf of all biomedical journals published from India, we urge to all those who are either conducting and/or planning to conduct clinical trials involving human subjects, to register their trials in CTRI or in any primary clinical trial register. From January 2010 onwards, we will consider publication of a trial only if it has been registered prospectively if started in or after June 2008; trials undertaken before June 2008 need to be registered, retrospectively.

K. Satyanarayana and Anju Sharma, *Indian Journal of Medical Research*

Purvish Parikh, *Indian Journal of Cancer*

V. K. Vijayan, *Indian Journal of Chest Diseases and Allied Sciences*

D. K. Sahu, *Indian Journal of Medical Sciences*

Barun K. Nayak, *Indian Journal of Ophthalmology*

R. K. Gulati, *Indian Journal of Pediatrics*

Mahendra N. Parikh, *Journal of Obstetrics and Gynecology of India*

Prati Pal Singh, *Journal of Parasitic Diseases*

S. B. Bavdekar, *Journal of Postgraduate Medicine*

U. Sreehari, *Journal of Vector Borne Diseases*

Peush Sahni, *National Medical Journal of India*

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Journal of Parasitic Diseases: December 2007, Vol. 31, No. 2, 79-91 *Invited review*

Trichomoniasis: chemotherapy, drug-resistance and new targets

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ABSTRACT. Human trichomoniasis is one of the most prevalent non-viral sexually transmitted diseases (STDs), caused by a parasitic protozoan *Trichomonas vaginalis*. Increasing global *T. vaginalis* infection rates, pregnancy complications and increased susceptibility to trichomoniasis during human immunodeficiency virus (HIV) infection and other STDs suggest the need for a definitive treatment of trichomoniasis. Molecular studies have revealed novel biochemical pathways and genome sequence of *T. vaginalis*. The metabolic machinery of *T. vaginalis* possesses some characteristics of both prokaryotes and eukaryotes. Five-nitroimidazoles, especially metronidazole (MTZ), are the most widely used drugs for the treatment of human trichomoniasis. MTZ itself is inactive, but its anaerobic reduction in hydrogenosomes by pyruvate:ferredoxin oxidoreductase results in the formation of a cytotoxic nitro radical anion, which binds transiently to trichomonal DNA, disrupts its strands and leads to cell death. Unfortunately, there is no alternative treatment for trichomoniasis. Resistance to MTZ is rampant, and cross-resistance among five-nitroimidazole drugs is on rise. A number of resistance mechanisms have been proposed, and are thought to involve reduced pyruvate:ferredoxin oxidoreductase activity, reduced amount of intracellular ferredoxin, point mutation or altered drug activation pathways in hydrogenosomes. For the treatment of resistant cases, doses of five-nitroimidazoles are given for longer duration, which often cause adverse effects. Therefore, alternative therapies to five-nitroimidazoles are urgently needed. The trichomonacidal activities of non-imidazole drugs *viz.* hamycin, paromomycin, disulfiram, nonoxynol-9, purpuromycin, alpha-difluoromethylornithine, trifuoromethionine, miltefosine and nitazoxanide have been reported. Further, several new drug targets have been proposed, which include cysteine proteinases, thioredoxin reductase, lipophosphoglycan alteration, cysteine synthase, purine nucleoside phosphorylase and kinase, thymidine kinase, polyamine transport, chitinase system, pyruvate:ferredoxin oxidoreductase and methionine-γ-lyase. Apparently, all these studies are promising in the treatment of trichomoniasis, but still a lot of research is needed to solve several unresolved questions.

Keywords: chemotherapy, drug-resistance, metronidazole, nitazoxanide, targets, trichomoniasis

INTRODUCTION

Human trichomoniasis, caused by a genital protozoan parasite *Trichomonas vaginalis*, is the most common non-viral sexually transmitted disease, with an annual global estimated 170 million incidences (WHO, 2001). In the year 2000, in a study done at a reproductive health clinic in New Delhi, India, the prevalence of trichomoniasis was found to be 10%

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among women with vaginal discharge (Thappa, 2006). Although most of the cases occur in under-developed countries, five million cases have been estimated to occur in United States, annually (Lewis, 2005). *T. vaginalis* infection is associated with adverse health consequences to both men and women (Fig. 1), including infertility, atypical pelvic inflammatory disease, and increased human immunodeficiency virus transmission (Guenthner *et al*., 2005). Trichomoniasis is also associated with preterm birth, low-birth weight infants (Cotch *et al*., 1997), predisposition to development of cervical neoplasia in women, and non-gonococcal urethritis, chronic prostatitis and prostate cancer in men (Sutcliffe *et al*., 2006).

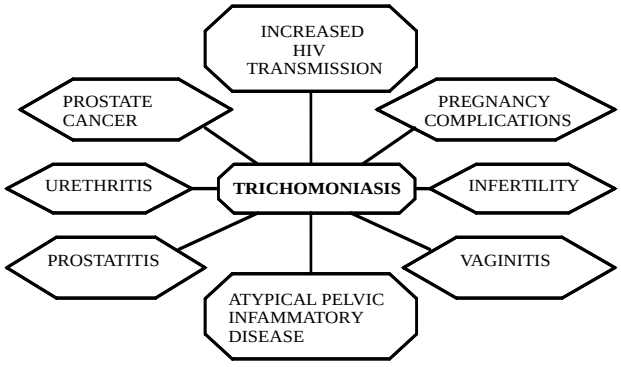


Fig. 1. Trichomoniasis and its association with other diseases.

LIFE CYCLE

In 1836, Donne for the first time described the parasite *T. vaginalis*. He examined the organism in genital secretions of both men and women. *T. vaginalis* was initially considered as non-pathogenic due to a majority of asymptomatic patients. The development of culture medium in the 1940's allowed study of the organism and its pathogenicity (Campbell, 2001).

T. vaginalis is characterized by motility due to the presence of a flagellum, and appears oval or pear shaped in axenic cultures. It exists only in trophozoite state, and humans are the only known natural host (Fig. 2). Sexual contact is the mode of transmission and the parasite multiplies by binary fission (Petrin *et al.*, 1998). In women, most common site of infection is vagina. Nearly 50% of the infected persons remain asymptomatic and continue to transmit infection. In females, signs and symptoms involve vaginal/vulvar erythema, frothy/malodorous discharge with pH > 6, vulvar itching, strawberry-shaped cervix and dyspareunia. In males, *T. vaginalis* infection is known

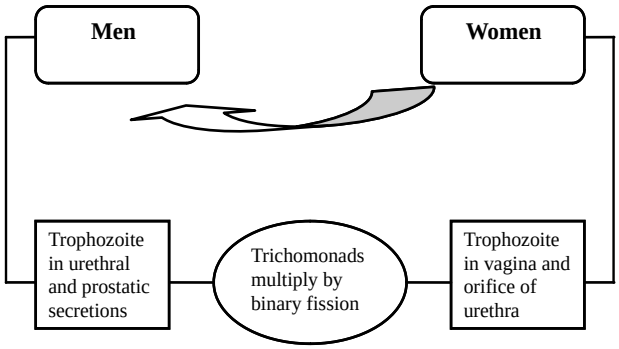


Fig. 2. The life cycle of *T. vaginalis*.

to cause abnormal urethral discharge, pruritus, semen pH > 7 and dysuria (Lewis, 2005).

PATHOGENESIS AND ITS MOLECULAR BASIS

Pathogenesis of trichomoniasis is not completely understood and a lot of factors are thought to be involved, and this topic has been last reviewed in the year 2004 (Schwebke and Burgess, 2004). Since then, a lot of research has been done in this area and the relevant findings are critically reviewed herein. For simplification, these can be described under two headings: contact-dependent and contact-independent mechanisms (Fig. 3), both of which are responsible for cytopathic effects (Krieger *et al.*, 1985).

Contact-dependent mechanisms: The proteins and glycoproteins present on the outer surface of protozoans are known to play important role(s) in the

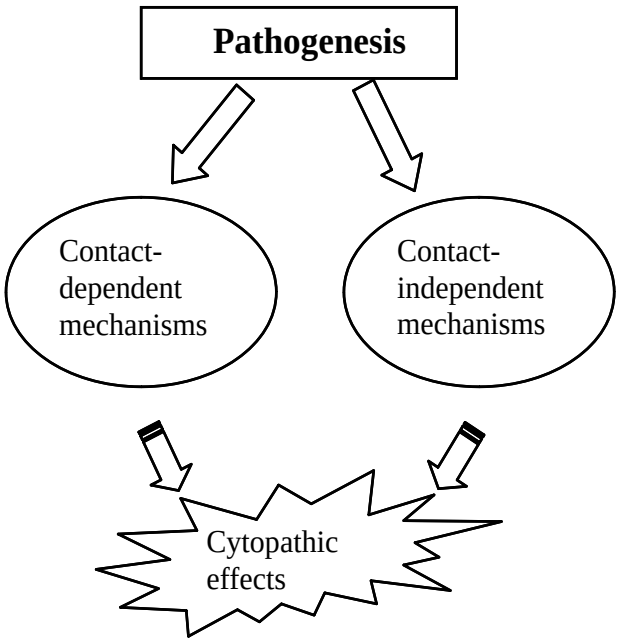


Fig. 3. Mechanisms of pathogenesis of human trichomoniasis.

establishment of contact with the host. In trichomoniasis, the first step is the adhesion of the parasite to vaginal epithelial cells, and it occurs with the aid of adhesion molecules like AP23, AP33, AP51 and AP65. Cysteine proteinases and lectin-binding carbohydrates are also required for this process (Petrin *et al.*, 1998). Presence of iron is an important factor for the regulation of the expression of adhesion proteins and cysteine proteinases. Heme iron increases AP65 adhesion molecule-mediated cytoadherence (Alderete *et al.*, 2004). Lipophosphoglycan is the main surface polysaccharide of *T. vaginalis*, and galactose and glucosamine are its most prevalent monosaccharide residues. Lipophosphoglycan seems to play a role in adherence and cytotoxicity, as it has been demonstrated that lipophosphoglycan mutants were found to be less adherent and less cytotoxic to human vaginal cells, *in vitro* (Bastida-Corcuera *et al.*, 2005). Removal of *T. vaginalis* cell-surface sugars has been reported to prevent the adherence to, and damage of, the epithelial cells (Mirhaghani and Warton, 1998). *T. vaginalis* causes hemolysis of erythrocytes to meet its requirements of fatty acids and iron. Towards this end, firstly, a *T. vaginalis* trophozoite gets attached to RBCs, forms pores in RBC membrane through the release of perforin-like proteins (cysteine proteinases) and finally, hemolysis occurs (Petrin *et al.*, 1998).

Cysteine proteinases are the lytic factors associated with *T. vaginalis* virulence, and destroy host immunoglobulins A, M and G. Studies have proven the involvement of cysteine proteinases of 30 kDa (CP30) and 65 kDa (CP65) in the cytoadherence that are important virulent factors (Solano-Gonzaleza *et al.*, 2006; Yadav *et al.*, 2007). *T. vaginalis* induces apoptosis of human neutrophils, and reactive oxygen species-dependent caspase-3 activation plays an important role in programmed cell death (Hyun-Ouk *et al.*, 2007).

Contact-independent mechanisms: Contact-independent mechanisms also play important role(s) in the pathogenicity of *T. vaginalis*, because the extracellular filtrate of *T. vaginalis* causes cytopathic effects in cell culture. A cell detaching factor (CDF) has been isolated from the extracellular filtrate of *T. vaginalis*, and production of CDF correlates well with the severity of clinical outcomes of the disease. High concentrations of estrogen, decrease CDF activity and thereby, clinical symptoms. Therefore, at the time of menses, when estrogen levels are low, symptoms of trichomoniasis get worsened (Garber *et al.*, 1991).

pH also affects the activities of *T. vaginalis*. Optimum pH for CDF activity is 6.5 and inactivity occurs below pH 4.5. This fact is important because vaginal pH is 4.5 but rises during trichomoniasis (pH > 5). This might be the reason for rise in vaginal pH during trichomoniasis (Petrin *et al.*, 1998). *T. vaginalis* also seems to phagocytose *Lactobacillus acidophilus* present in human vagina (McGrory and Garber, 1992). Infection of *T. vaginalis* causes inflammation and efflux of leukocytes in the genital tract. The mechanisms of inflammation have not been completely elucidated, but some studies have shown the involvement of toll-like receptor 4 in cellular activation (Zariffard *et al.*, 2004). *T. vaginalis* produce phospholipase A_2-like lytic factors, which are able to damage target cells and cause inflammation (Lubick and Burgess, 2004).

MOLECULAR BIOLOGY OF *T. VAGINALIS*

Recently, genome sequence of *T. vaginalis* has been elucidated (Carlton *et al.*, 2007). The estimated genome size is 160-megabase, with extraordinary homogeneity in repeat families. About two-third genome comprises of repeat sequences. Repeat sequence amplification occurred after the branching between *T. vaginalis* and its sister taxon *T. tenax*, a trichomonad of oral cavity. These findings suggest that the protozoans have undergone a very recent and bulky increase in genome size. Nearly, 60,000 protein-coding genes have been identified, which signifies that *T. vaginalis* has one of the highest coding capacities among eukaryotes. Introns were identified in 65 genes. Transfer RNAs (tRNAs) for all 20 amino acids, and ~250 ribosomal DNA (rDNA) units have been identified and are localized to one of the six *T. vaginalis* chromosomes. Inr promoter element was found to play a central role in transcription (Schumacher *et al.*, 2003). Gene expression could be possibly manipulated using RNA interference (RNAi) technology, as depicted by the existence of RNAi pathway (Carlton *et al.*, 2007).

Cases (152 nos.) of possible prokaryote-to-eukaryote lateral gene transfer (LGT) have been identified. Functionally, these genes affect various metabolic pathways, strongly influencing the evolution of *T. vaginalis* metabolome. Majority of metabolic enzymes encoded by LGT genes are involved in carbohydrate or amino acid metabolism (Carlton *et al.*, 2007). *T. vaginalis* utilizes carbohydrate as a main source of energy under both aerobic and anaerobic conditions via fermentative metabolism (Petrin *et al.*,

1998). A variety of amino acids are also utilized as energy substrates, with arginine dihydrolase metabolism being a major pathway for energy production. Pathways utilizing aminotransferases and glutamate dehydrogenase lead the parasite to synthesize glutamate, aspartate, alanine, glutamine and glycine (Lowe and Rowe, 1986; Turner and Lushbaugh, 1988). Genes required for the synthesis of proline from arginine and for threonine metabolism have been identified. A *de novo* biosynthesis pathway and genes have been identified for cysteine via cysteine synthase, an LGT candidate involved in methionine metabolism (Westrop *et al*., 2006).

T. vaginalis lacks typical mitochondria and possess hydrogenosomes, which produce adenosine triphophate (ATP) and molecular hydrogen through fermentation of metabolic intermediates. Most evidences regarding the origin of these organelles now support a common origin with mitochondria (Dyall *et al*., 2004). Genes encoding mitochondrial transporters, translocons and soluble proteins have been identified, which indicate that its hydrogenosome has undergone reductive evolution. The production of molecular hydrogen by hydrogenosome is catalyzed by a diverse group of iron-hydrogenases that possess four different sets of functional domains along with a conserved H-cluster. The pathway that generates electrons for hydrogen comprises of many proteins encoded by multiple genes. *T. vaginalis* hydrogenosomes contain complete machinery required for mitochondria-like FeS cluster formation and it suggests that hydrogenosomes may be involved in biogenesis of cytosolic FeS proteins (Sutak *et al*., 2004). Clinical resistance to MTZ is associated with decrease or loss of ferredoxin. Seven ferredoxin genes have been identified. Therefore, knockout of a single ferredoxin gene does not lead to MTZ resistance (Land *et al*., 2004). *T. vaginalis* surface molecules involved in pathogenesis have been represented by eight families containing nearly 800 proteins. BspA-like proteins are thought to be involved in cell adherence. GP63-like proteins contribute to virulence and pathogenicity of *T. vaginalis*. Genes encoding cytolytic effectors have also been identified (Carlton *et al*., 2007).

T. vaginalis is an anaerobic protozoon and thus requires redox and antioxidant systems to deal with oxidative stress. Genes encoding a range of antioxidant molecules, such as superoxide dismutases, thioredoxin reductases, peroxiredoxins and rubrerythrins have been identified. The production of various heat-shock proteins on exposure to hydrogen peroxide gets upregulated. Heat-shock proteins act as chaperons and aid in controlling the rate of protein synthesis caused by stress. P-glycoprotein, a transporter molecule is also thought to be involved in the stress response. This molecule is usually involved in multi-drug resistance, but its specific function in *T. vaginalis* has not been defined (Petrin *et al*., 1998).

BIOCHEMISTRY OF *T. VAGINALIS*

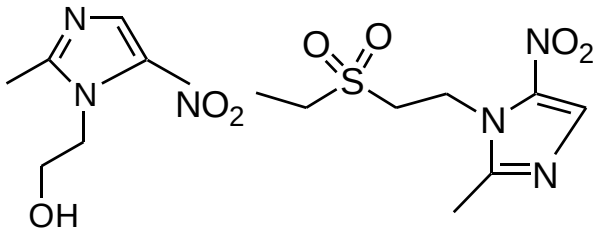
T. vaginalis is a urogenital pathogen with cytoskeleton composed of tubulin and actin fibers (Cappuccinelli *et al*., 1987). The nucleus is present in anterior portion and is surrounded by a nuclear envelope. Hydrogenosomes present in the cytoplasm are the main site of metabolic activities occurring in the protozoa, and are considered analogous to the mitochondria of higher eukaryotes (Hrdy *et al*., 2004).

During carbohydrate metabolism, fermentative oxidation of pyruvate takes place in hydrogenosomes and ATP is produced by substrate-level phosphorylation. Pyruvate:ferredoxin oxidoreductase is an enzyme that converts pyruvate to acetate. This enzyme is present in anaerobic bacteria also, but absent in mitochondria (Petrin *et al*., 1998). Acetate:succinate CoA-transferase gives rise to production of acetate and a gene encoding this enzyme has been identified (Koen *et al*., 2007). However, the ferredoxin protein present in *T. vaginalis* helps in the transfer of electrons during carbohydrate metabolism. It possesses similarity to the ferredoxins found in aerobic bacteria and in mitochondria (Hrdy *et al*., 2004). These biochemical studies have shown that its metabolic machinery possesses characteristics of both prokaryotes and eukaryotes. On the basis of sequence analysis of the 18S-like rRNA of various eukaryotes, it has been proposed that trichomonads branched off from the main stream of the eukaryotic tree before true mitochondria arose (Gunderson *et al*., 1995). Cholesterol, phosphatidylethanolamine, phosphatidylcholine and sphingomyelin are the main phospholipids of *T. vaginalis*. It lacks the ability to convert lipid precursors into phospholipids and relies on exogenous sources of lipid moieties to survive. However, *de novo* glycolipid and glycophosphosphingolipid synthesis occurs in *T. vaginalis* (Beach *et al*., 1991).

In the limited presence of carbohydrates, amino acids play an important role to sustain trichomonad survival

and growth. Arginine, threonine and leucine constitute the major amino acids used in energy production. Aminotransferases, e.g., aspartate/aromatic amino acid:2-oxoglutarate aminotransferase have been found to be involved in amino acid metabolism, but more studies are needed to elucidate their role(s) (Rowe and Lowe, 1986).

T. vaginalis utilizes salvage pathways to synthesize purines and pyrimidines. Nucleoside phosphorylases and kinases are involved in purine salvage, whereas phosphoribosyltransferases and nucleoside kinases are involved in pyrimidine synthesis (Zang *et al*., 2005). *T. vaginalis* contains two separate nucleotide transport carriers. One carrier is believed to have high affinity for adenosine and pyrimidine nucleosides, whereas the other carrier has a site for guanosine and uridine (Harris *et al*., 1988).



Metronidazole Tinidazole

Fig. 4. Chemical structures of 5-nitroimidazoles currently approved for the treatment of human trichomoniasis.

CHEMOTHERAPY: CURRENT STATUS

Five-nitroimidazoles: 5-nitroimidazoles are the only approved drugs for the treatment of human trichomoniasis (Fig. 4). Last review on this topic was published in the year 2004 (Cudmore *et al.*, 2004). Since then, a lot of research efforts have been made in this area, and a critical review of the relevant findings is presented here.

Metronidazole: Metronidazole (MTZ), a heterocyclic compound with a nitro group on the fifth position of an imidazole ring, was approved in early 1960s for the treatment of trichomoniasis. It was developed from the *Streptomyces* antibiotic, azomycin (Cudmore *et al.*, 2004). MTZ is also active against a variety of anaerobic pathogens: both gram-positive and gram-negative bacteria, and protozoans like *Entamoeba histolytica* and *Giardia lamblia*. Preparations of MTZ are available for oral, intravenous, intravaginal and topical administration. The drug is usually absorbed completely and promptly after oral intake. It penetrates well into body tissues and fluids, including vaginal secretions, seminal fluid, saliva and breast milk. Vaginal drug concentration is approximately 50% of that in serum. The plasma half-life of MTZ is about 8.7 h, compared to approximately 12 h for its hydroxy metabolite (Cudmore *et al*., 2004; Lyons and Carlton, 2004). MTZ is a prodrug. It itself is inactive, but on anaerobic reduction results in the formation of a nitro radical. This radical anion acts on nucleic acid DNA strands and disrupts them. Abstraction of atoms of the nucleic acid backbone by the nitro radical, initiates DNA degradation. Finally, cell death occurs. The redox potential of MTZ acts as a driving force for nitro radical formation in anaerobic cells and, therefore, it possesses selective toxicity against anaerobes (Meri *et al.*, 2000). The nitro radical seems to act on thymine and adenine residues of DNA and because the genome of *T. vaginalis* is rich in A+T content and, therefore, MTZ shows specificity to this parasite (Cudmore *et al.*, 2004). Activation of MTZ occurs in an organellar compartment, the hydrogenosome. Hydrogenosomes are the centers for fermentative decarboxylation of pyruvate. This pathway is linked to substrate-level phosphorylation for the production of ATP (Kulda, 1999). The key enzyme is pyruvate:ferredoxin oxidoreductase (PFOR). The electrons released in this reaction are taken up by ferredoxin. Ferredoxin is a Fe-S protein. The final products are acetate and hydrogen. MTZ competes for electrons with hydrogenase due to its lower redox potential and consequently, drug activation occurs (Fig. 5). Small molecules, like MTZ, approach the redox centre more closely in the trichomonal protein. This results in faster electron transfer reactions (Vidakovic *et al.*, 2003). An alternative pathway of MTZ activation is also found in the hydrogenosomes. In this, the source of electrons is malate instead of pyruvate. Malate is oxidatively decarboxylated to pyruvate and CO_2 by a NAD-dependent malic enzyme (Hrdy *et al.*, 2005). MTZ is the drug of choice for the treatment of trichomoniasis.

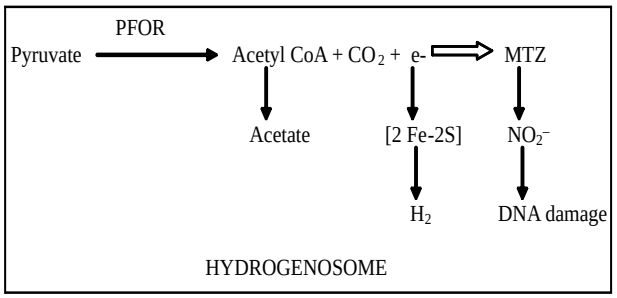


Fig. 5. Diagrammatic representation of molecular mechanism of trichomonacidal action of MTZ.

However, potential carcinogenic, teratogenic, embryogenic effects and clinical drug-resistant isolates of *T. vaginalis* have been reported (Crowell *et al.*, 2003). Common adverse reactions include metallic taste, headache, glossitis, pruritus, vertigo, nausea, dry mouth and disulfiram-like reaction with alcohol consumption. More serious side effects are rare, but include eosinophilia, leukopenia, palpitation, confusion and some central nervous system effects. Hypersensitivity reactions are also reported in some cases (Cudmore *et al.*, 2004).

Tinidazole: Tinidazole (TNZ) is a second generation synthetic nitroimidazole approved by Food and Drug Administration, USA in May 2004, for the treatment of protozoal infections, including trichomoniasis. It marked the first advancement in the treatment of trichomoniasis in over 40 years (U.S. FDA, 2004). It has a longer half-life (~12 h) than MTZ. Thus, it is suitable for single dose or once-a-day therapy (Schwebke and Burgess, 2004). It shows superior tissue distribution than MTZ. The concentrations of TNZ found in vaginal secretions are similar to that found in serum, which shows that, as compared to MTZ, it is delivered more effectively to this region of body. Minimum lethal concentration (MLC) of TNZ for *T. vaginalis* strains is lower than that of MTZ, and clinical reports have shown that TNZ is curative at lower doses than MTZ (Sobel *et al.*, 2001). Because TNZ is a nitroimidazole, its mode of action is thought to be similar to that of MTZ, and cross-resistance among nitroimidazoles is a concern. Both *in vitro* and *in vivo* assays have shown that for MTZ-resistant trichomonads, MLC of TNZ is generally significantly lower than of MTZ (Crowell *et al.*, 2003).

Ornidazole: Its activity is similar to that of MTZ, but it is slowly metabolized, has longer half-life (12–14 h), and dose and duration of regimens are similar to those for TNZ. Side effect profile is also similar (Cudmore *et al.*, 2004). In an *in vitro* study, it has been found to be more effective than MTZ in terms of minimum effective concentration (MIC) and MLC levels (Inceboz *et al.*, 2004).

Satranidazole: Satranidazole is a new 5-nitroimidazole compound having a longer plasma half-life (17-29 h) than MTZ. In an *in vitro* study, MIC of satranidazole has been found to be lower than MTZ against MTZ-resistant strain (Ray *et al.*, 1984).

Secnidazole: Its absorption after oral administration is rapid and complete, but metabolism is slower resulting in a plasma half-life of 17–29 h. A single 2 g dose has been found to yield rates of cure equal to multiple doses of MTZ and TNZ. Side effect profile is similar to MTZ and reported incidence is 2–10%. In clinical trials, secnidazole is found to be well tolerated; most adverse events were gastrointestinal in nature and did not require treatment intervention or withdrawal from therapy. Single-dose therapy and good tolerability profile make secnidazole a suitable alternative to multiple-dosage regimens with other drugs in this class (Gillis and Wiseman, 1996; Cudmore *et al.*, 2004).

Clotrimazole: Clotrimazole is an imidazole drug. It is used for topical treatment of trichomoniasis, but is not as effective as MTZ. Only little systemic absorption occurs following topical application, but approximately 5–10% drug is absorbed after vaginal use (Debbia *et al.*, 1996).

RESISTANCE TO MTZ: AN EMERGING PROBLEM

Although most of the cases of trichomoniasis can be treated with MTZ (Table I), but drug resistance to MTZ appears to be on rise (Lewis, 2005). Prevalence of MTZ-resistant cases of trichomoniasis has been found to be nearly 5% of the total cases (Schmid *et al.*, 2001). The resistance can be overcome by increasing the doses of MTZ (Lewis, 2005; Fig. 6), but there are several limitations of long-term MTZ therapy (Narcisi and Secor, 1996). Although TNZ is an alternative to treat resistant cases, but there also arises the problem of cross-resistance among nitroimidazoles, leaving no alternative drug for treatment. Mechanisms of drug resistance can be divided into two categories: aerobic and anaerobic (Dunne *et al.*, 2003; Fig. 7), and last review on this topic was published in year 2004. It has

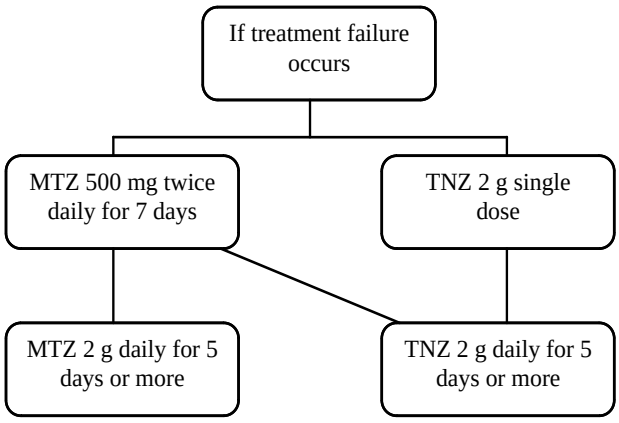


Fig. 6. Treatment regimens for human trichomoniasis MTZ-resistant cases

been suggested that drug-resistance is a multistep process. The aerobic type of resistance appears at the first stage, followed by development of anaerobic type of resistance, which is accompanied by a gradual loss of hydrogenosomal proteins involved in drug activation pathways (Rasoloson *et al.*, 2002).

Aerobic resistance: Selection of strains which manifest aerobic resistance occurs in the presence of oxygen. It is important from clinical point of view, as it is commonly present in isolates from treatment-refractory patients. Impaired oxygen-scavenging pathways appear to be responsible for aerobic resistance. Increased levels of intracellular oxygen interfere with drug activation (Rasoloson *et al.*, 2001). Decreased hydrogenase activity and thereby, decrease in hydrogen production is responsible for impaired oxygen scavenging. Also, decreased oxidase activity might be responsible for increased O_2 concentration in the hydrogenosome (Upcroft and Upcroft, 2001). Due to electronegative nature of oxygen atoms, they compete with MTZ for ferredoxin-bound electrons, resulting in impaired reduction and hence, generation of active form of MTZ. Also, in the presence of oxygen, reoxidation of reduced nitro radicals occurs. This step is known as futile cycling (Cudmore *et al.*, 2004). Alternatively, decreased ferredoxin levels have been found in aerobically-resistant strains. The defective redox properties of ferredoxin may be contributed to reduced transcription of ferredoxin gene due to a point-mutation in the 5' region. This mutation results in reduced binding affinity for a 23-kDa transcriptional protein. This results in reduced ferredoxin-gene expression and thereby, lowered reductive activation of MTZ (Rasoloson *et al.*, 2002). Selection of aerobic-resistant strain of *T. vaginalis* can be done *in vitro* by cultivation of trichomonads at sublethal concentrations of MTZ for approximately two months, and it has been demonstrated that MLC of MTZ increases with increase in O_2 concentration (Tachezy *et al.*, 1993).

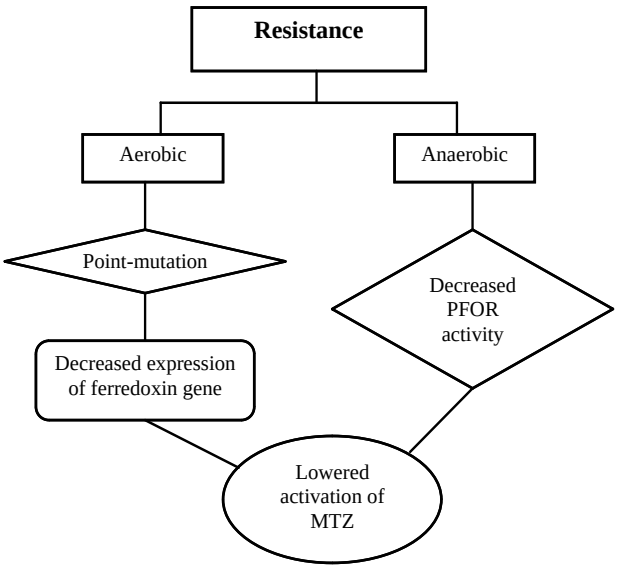


Fig. 7. Mechanisms of drug-resistance in *T. vaginalis*.

Anaerobic resistance: Although clinically resistant isolates of *T. vaginalis* usually possess aerobic resistance, but anaerobically resistant strains have also been isolated. *In vitro*, it takes more than one year to select an anaerobically-resistant strain by cultivating trichomonads at gradually increasing drug-pressure. Such strains are able to tolerate extremely high concentrations of MTZ (Kulda *et al.*, 1993). Drug activating pathways in the hydrogenosomes are usually decreased or absent in this type of resistance. The loss of pyruvate:ferredoxin oxidoreductase (PFOR) activity is important. Others include decrease in ferredoxin, malic enzyme and NAD:ferredoxin oxidoreductase levels (Kulda, 1999). Instead, 2-oxoacid oxidoreductases activity appears to increase. This alternate pathway does not donate electrons to ferredoxin and, therefore, results in the circumvention of the activation of MTZ (Upcroft and Upcroft, 2001). Also, it has been proposed that to acquire high level of drug-resistance, both pyruvate- and malate-dependent pathways of MTZ activation are eliminated from the hydrogenosomes of trichomonads (Hrdy *et al.*, 2005).

OTHER CHEMOTHERAPEUTIC AGENTS

A number of studies have been published, which demonstrate the *in vitro* and *in vivo* trichomonicidal activities of non-imidazole drugs.

Hamycin: It is an aromatic polyene related to amphotericin B. Studies have shown that hamycin at low concentrations kills both MTZ-sensitive and -resistant strains of *T. vaginalis* (Lushbaugh *et al.*, 1995). The drug is currently in use in India as a topical treatment for trichomoniasis. Unfortunately, reported side effects in patients indicate that the toxicity of hamycin may limit future clinical applications (Cudmore *et al.*, 2004).

Paromomycin (aminosidine): It is an aminoglycoside used as an oral agent to treat *E. histolytica* infection, cryptosporidiosis and giardiasis. Paromomycin formulated as a 6.25% cream has been used to treat trichomoniasis in patients who had failed

MTZ therapy or could not receive MTZ. Some cures have been reported, but vulvovaginal ulcerations and pain have complicated treatment (Nyirjesy *et al.*, 1998; Sobel *et al.*, 2001).

Disulfiram: It has been used clinically as an aid to the treatment of alcoholism. Disulfiram and its metabolite, ditiocarb, had shown activity against both MTZ-sensitive and -resistant strains of *T. vaginalis, in vitro*. The mechanism of action is not yet clear, but it inhibits alcohol dehydrogenase, and ADH activity has been recently reported in *T. vaginalis* (Bouma *et al.*, 1998).

Furazolidone: It is a nitrofuran, presently used against enteric bacteria and *G. lamblia*. This drug is metabolized to 2-hydroxyethylhydrazine, a potent monoamine oxidase inhibitor. Furazolidone is found to be effective against both MTZ-susceptible and -resistant trichomonads, *in vitro.* Clinical studies using vaginal pessary have reported excellent results (Narcisi and Secor, 1996).

Nonoxynol-9: It is a non-hormonal contraceptive with spermicidal effect. In a study conducted on female patients with MTZ-resistant vaginal trichomoniasis, it is found that topical therapy alone has a low cure rate, whereas combined therapy of oral doses of MTZ and intravaginal nonoxynol-9 treatment provides good cure rates. Thus, avoiding the adverse effects of high doses MTZ therapy (El Bassiouni and Riad, 2005).

Purpuromycin: It is an antibiotic obtained from *Actinoplanes ianthinogenes*. Purpuromycin, its semi-synthetic derivatives MDL 63, 604 and 7'-amino, 7'-methylamino, 7'-ethylamino, 7'-demethoxypurpuromycin have shown *in vitro* activity against *T. vaginalis*. They may have potential role in topical treatment. Protein synthesis appeared to be the primary target of purpuromycin, although inhibition of nucleic acid synthesis was observed at higher concentrations of the antibiotic (Goldstein *et al.*, 1995; Trani *et al.*, 1997).

Nitrothiazoles: Nitazoxanide and tizoxanide have shown activity comparable to MTZ against isolates of *T. vaginalis, in vitro.* Nitazoxanide has been approved for the treatment of *Clostridium difficile* infection, and has been proved to be having a broad spectrum of activity against anaerobes (Adagu *et al*., 2002).

Miltefosine: Hexadecylphosphocholine (miltefosine) has shown *in vitro* activity against MTZ-sensitive as well as MTZ-resistant strains of *T. vaginalis.* Treatment with miltefosine resulted in immobility, rounding up, blebbing and total lysis of the parasites, *in vitro*. It has been suggested that miltefosine inhibits phospholipid biosynthesis; however, the mode of action is under investigation (Blaha *et al.*, 2006).

NEW DRUG TARGETS

Polyamine transport: Polyamines, like putrescine, spermidine and spermine, are important for cellular growth and proliferation. *T. vaginalis* shows auxotrophy for spermidine. Spermine from the mammalian cell is taken up by protozoans via a putrescine/spermine antiporter and converted to spermidine via a spermidine/spermine N^1-acetyltransferase/polyamine oxidase coupled pathway (Reguera *et al.*, 2005). Polyamine inhibitor, alpha-difluoromethylornithine (DFMO) has been shown to have *in vitro* efficacy against *T. vaginalis.* Because there is no upregulation of polyamine transporters by DFMO in *T. vaginalis*, and polyamine transport differs significantly from other eukaryotes, further research in this area is needed (Yarlett and Bacchi, 1988).

Chitinase system: During the early stage of infection, *T. vaginalis* has to overcome the protective mucin barrier in the vagina to attach to target cells. Mucin is rich in polymer of N-acetyl-β-D-glucosamine. Chitinases of *T. vaginalis* cleave β-1,4 linkages between N-acetylglucosamine residues. Three chitinolytic systems in *T. vaginalis* have been identified: N-acetyl-β-D-hexosaminidase (NAHase), chitobiosidase and chitotriosidase. These chitinases are involved in pathogenicity and chitinase substrate, glycol chitin enhances chitinolytic activity and in turn, pathogenicity. Among these, NAHase has shown the highest level of chitinolytic activity (Sanon *et al.*, 2005). Chitinase inhibitor, allosamidine has been shown to inhibit parasite growth and hence, chitinase system could be considered as a potential target for the therapy of trichomoniasis (Loiseau *et al.*, 2002).

Thymidine kinase: *T. vaginalis* lacks *de novo* purine and pyrimidine biosynthesis, as it is unable to incorporate glycine, formate and bicarbonate into purines, aspartate, orotate and bicarbonate into pyrimidines. The parasite acquires the precursor of its nucleic acids by digesting nucleic acids of lysed host cells by nucleases and phosphatases. Nucleoside phosphorylases are involved in the conversion of

bases to nucleosides. Both ribo- and deoxyribo-purine and pyrimidine nucleosides are phosphorylated by kinases in the presence of ATP. Thymidine kinase activity is also present in *T. vaginalis* and it phosphorylates not only thymidine, but also deoxycytidine and deoxyuridine. Thymidine kinase activity is found to be inhibited by novel deoxyuridine analogues. This proves that thymidine kinase is vital for protozoal growth and viability and is a potential new target for the design of trichomonacidal agents (Strosselli *et al.*, 1998).

Purine nucleoside phosphorylase and purine nucleoside kinase: *T. vaginalis* lacks *de novo* synthesis of purine nucleotides and depends primarily on purine salvage pathway for nucleotides. It acquires exogenous adenine from the host to replenish its purine nucleotide pool. A purine nucleoside phosphorylase (PNP) in *T. vaginalis* converts adenine to adenosine, which is then converted to AMP by another enzyme in the organism, purine nucleoside kinase (PNK). The enzyme recognizes guanosine and inosine as substrates and thus, plays a key role in supplying GMP for *T. vaginalis*. PNP has been cloned and crystal structure of this enzyme has been analyzed. It is a bacterial-type hexameric PNP recognizing adenine, guanine and hypoxanthine as substrates, whereas mammalian PNP is a trimeric protein incapable of recognizing adenine as substrate. Hence, it differs from human PNP. Formycin A, an adenosine analog, is a specific inhibitor of *T. vaginalis* PNP and an inhibitor of *T. vaginalis* growth. 2-fluoro-2'-deoxyadenosine has been found out to be a subversive substrate of *T. vaginalis* PNP. It has been found to be 100-fold more potent than MTZ, *in vitro*. *T. vaginalis* PNK has guanosine as the most preferred substrate and differs from mammalian PNK. Both enzymes are *bona fide* targets for trichomoniasis chemotherapy (Zang *et al.*, 2005).

Methionine γ-lyase: It is an enzyme which catalyzes the single-step conversion of methionine to α-ketobutyrate, ammonia and methanethiol. It is highly active in many anaerobic pathogenic microorganisms but has no counterpart in mammals. This pathogen-specific enzyme can be exploited as a drug target. This enzyme has been purified and characterized, and it has been suggested that methionine catabolism is important for survival of *T. vaginalis* (Lockwood and Coombs, 1991). Prodrug, such as trifluoromethionine, is exclusively activated by it and has been shown to be highly toxic both *in vitro* and *in vivo* to anaerobic protozoan parasite *T. vaginalis* (Coombs and Mottram, 2001).

Thioredoxin reductase: *T. vaginalis* is an anaerobic, amitochondriate parasite. But it is also able to cope with the oxidative stress. Cysteine is the major antioxidant. To detoxify oxidants, it also contains thioredoxin reductase and thioredoxin peroxidase. Thioredoxin reductase and thioredoxin also reduce cystine and thereby, helps in maintaining intracellular cysteine levels. There occurs up-regulation of this antioxidant system during increased oxidative stress. Thioredoxin reductase differs in structure from its counterpart in human host and thus, may be targeted for anti-trichomoniasis drug designing and discovery (Coombs *et al.*, 2004).

Cysteine proteinases: They play an important role in the virulence of *T. vaginalis*. They are also involved in pathogenesis mechanisms: adherence, hemolysis, cytotoxicity and immune invasion by degradation of

Table 1. Treatment regimens for human trichomoniasis (Schwebke, 2002; Lewis, 2005)

Type of trichomoniasis	Recommended regimen	Alternative regimen
Symptomatic and asymptomatic cases	MTZ or TNZ 2g, orally, single dose	MTZ 500 mg, orally, twice daily for 7 days
Treatment in pregnancy	Daily intravaginal dose of 100 mg of clotrimazole for 6 days in 1st trimester followed by standard MTZ therapy in 2nd trimester	
Neonatal infections	MTZ single 50 mg/kg, orally, daily for 5-8 days	

* Stimultaneous treatment of sexual partners is recommended.

host immunoglobulins. Also, cysteine proteases are involved in apoptosis of human vaginal epithelial cells. Cysteine protease-induced programmed cell death might be involved in the pathogenesis of *T. vaginalis*. Nearly 23 proteinases have been identified in *T. vaginalis*. Compounds which can inhibit cysteine proteinases might be active as trichomonacidal agents. Some plant essential oils and extracts might act against cysteine proteinases, but more research is needed to assess their potential (Jean-Paul *et al.*, 2005). Anti-retroviral protease inhibitors have also shown activity against MTZ-resistant strains of *T. vaginalis* (Dunn *et al.*, 2007).

Cysteine synthase: Because *T. vaginalis* lacks glutathione, it relies heavily on cysteine as a major redox buffer. Cysteine is synthesized from sulfide source by the enzyme cysteine synthase. *O*-phosphoserine is utilized as a substrate for cysteine biosynthesis. Levels of cysteine synthase in *T. vaginalis* are regulated according to need. Parasites growing in an environment rich in cysteine possess low activity and *vice versa*. Humans lack this enzyme and, therefore, it could be an exploitable drug target (Westrop *et al.*, 2006).

Pyruvate oxidoreductases: Nitrothiazole benzamide derivative, nitazoxanide, is described as a noncompetitive inhibitor of PFOR of many anaerobic parasites, including *T. vaginalis*. It shares structural similarity with thiamine pyrophosphate, a cofactor involved in PFOR reaction. This agent inhibits PFOR by a novel mechanism in which the anion form interferes with the attachment of activated cofactor to PFOR, and thus, intercepts pyruvate oxidation. Thus, an important energy generating process gets inhibited, without which parasites would not be able to survive. Because nitazoxanide has been proposed to target "activated cofactor" of an enzymatic reaction rather than any substrate or enzyme, it could be beneficial in the treatment of cases where *T. vaginalis* acquires mutation-based drug resistance. But, to confirm this, further studies in this area are needed (Hoffman *et al.*, 2007).

Phospholipid membrane: Lipophosphoglycan is the main surface polysaccharide in *T. vaginalis*. It is a complex molecule present in high density on parasite surface. Galactose and glucosamine are the most prevalent monosaccharide residues. Lipophosphoglycan seems to play a role in anchoring parasite to plasma membrane, resistance to complement, host enzymes and immune evasion. Lipophosphoglycan mutants have been shown to be less adherent and cytotoxic to human vaginal ectocervical cells, *in vitro* (Bastida-Corcuera *et al*., 2005).

CONCLUSION

Increase in both the prevalence of MTZ-resistant *T. vaginalis* strains and the number of cases of cross-resistance among nitroimidazoles, indicate a need for the design, discovery and development of non-nitroimidazole drugs for the treatment of refractory trichomoniasis. More research in the areas of pathogenesis and drug-resistance, especially at molecular levels, is very much warranted. Specific points that need to be considered include: details of the life cycle of *T. vaginalis* as it would help in developing new strategies to control this disease, the exact role(s) played by immune system during *T. vaginalis* infection as nearly 50% cases remain asymptomatic and continues to transmit the parasite, how the parasite establish itself in the changing environment of vagina, the exact role(s) played by phospholipids in the host-parasite interactions and how to utilize new targets to design potential drug candidates. There appears to be a silver lining on the horizon as a number of drug candidates and new targets have been identified; however, still a lot needs to be done for providing a safe and effective drug for the treatment and control of human trichomoniasis, especially for the MTZ-resistant cases.

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Glutathione metabolism in parasitic protozoa

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ABSTRACT. This review is concerned with the current progress in unraveling biochemical and physiological roles of glutathione system in parasitic protozoa. Glutathione metabolism plays a central role in thiol-disulphide redox metabolism in many parasitic protozoa either directly or in conjugation with spermidine as in trypanosomes. Characterization of various key enzymes of metabolic pathways in parasitic protozoa has revealed that these enzymes are quite different from the host due to long sequences with large number of insertions, in having different residues at substrate binding site and in exhibiting different kinetic properties. Much of the new information about glutathione metabolism has been obtained through studies with selective inhibitors of the enzymes involved in the metabolism. These enzyme inhibitors and other compounds which inhibit *in vivo* glutathione synthesis have opened the way to selective modulation of glutathione metabolism, making several therapeutic approaches possible.

Keywords: enzymes, glutathione, methylene blue, parasitic protozoa, *Plasmodium,* trypanosomes

INTRODUCTION

Parasitic protozoa are exposed to reactive oxygen species (ROS) such as superoxide anion (O^-_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals generated by their metabolism (Sies, 1999; Grinberg *et al.,* 2005). They not only have to eliminate their toxic metabolites, but must also cope with oxidative stress generated by the host. Parasitic protozoa have evolved protective mechanisms and programmed responses to limit cellular damage in their microenvironment. The reversible thiol/disulphide reactions e.g. glutathione/glutathione disulphide (GSH/GSSG), thioredoxin/ thioredoxin disulphide [$Trx(SH)_2$/$Trx(S)_2$] are central chemical theme in biology (Holmgren, 2000). Glutathione redox system is an important thiol among parasitic protozoa (Muller *et al.,* 2003).

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GLUTATHIONE

Glutathione is the major metabolite of glutathione metabolism. The term glutathione refers to tripeptide - L-glutamyl-γ-L-cysteinylglycine in both its reduced and oxidized forms (Fig. 1). Reduced glutathione (GSH) is monomeric form chemically known as N-(N-γ-L-glutamyl-L-cysteinyl) glycine. Glutathione disulphide or oxidized glutathione (GSSG) is a dimeric form chemically known as γ-L-glutamyl-L-cysteinyl-glycine disulphide. Both the glutathiones are components of glutathione metabolism, and act as reducing agent and antioxidant. Glutathione serves as a reservoir for cysteine, participates in detoxification reactions for xenobiotics and ROS, and metabolism of numerous cellular compounds (Meister, 1983). Glutathione maintains the intracellular reducing environment e.g. thiol/disulphide ratio is usually

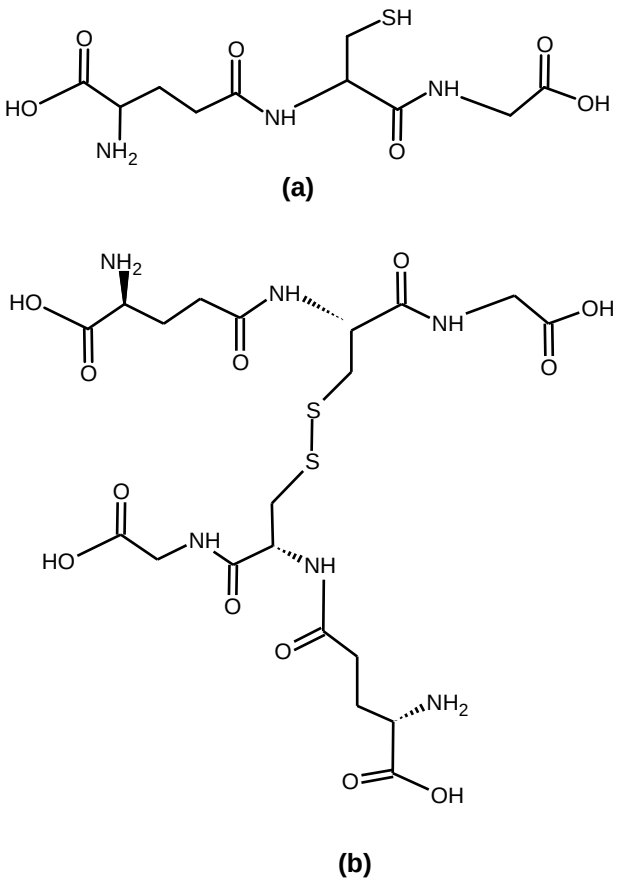

Fig. 1. Reduced (a) and oxidized (b) forms of glutathione in parasitic protozoa.

between 10 and 100 (Anderson, 1985).

Glutathione metabolism consists of γ-glutamyl cycle, glutathione reductase, glutathione peroxidase and glutathione-S-transferases (Fig. 2). GSH is synthesized intracellularly by the consecutive actions of the γ-glutamylcysteine synthetase and GSH synthetase. The GSH as well as γ-glutamyl-containing compounds including GSSG and γ-glutamylglutathione are broke down by γ-glutamyl transpeptidase which catalyzes transfer of γ-glutamyl moiety to acceptor amino acids e.g. methionine, cysteine, GSH, water, etc. The γ-glutamyl amino acids so formed are converted into corresponding amino acids and 5-oxoproline by γ-glutamyl cyclotransferase. 5-oxoproline is converted to L-glutamate by ATP-dependent reaction catalyzed by 5-oxoprolinase.

The cysteinylglycine formed in the transpeptidase is split by dipeptidase (Beutler and Dale, 1989). GSH so formed serves as substrate for glutathione peroxidase and glutathione-S-transferases, with the formation of GSSG. The latter is reduced back to GSH by glutathione reductase.

Glutathione metabolism and amitochondrial protozoa: Amitochondrial protozoan parasites such as *Entamoeba histolytica*, *Giardia* and *Trichomonas* lack glutathione metabolism (Fahey *et al.*, 1984; Brown *et al.*, 1998). Instead amitochondrial protozoans contain cysteine as their principal low molecular mass thiol (McLaughin and Aley, 1985; Brown *et al.*, 1998). Ondaza *et al.* (1997) detected trypanothione [N_1N_8bis (glutathionyl) spermidine] in *E. histolytica*, a redox metabolite thought to be unique to trypanosomatids (Fairlamb *et al.*, 1985; Fairlamb and Cermai, 1992). Later studies found trypanothione absent in this organism (Mark *et al.*, 1999).

Glutathione metabolism and apicomplexa: Apicomplexan protozoan parasites such as malaria parasite have well developed glutathione system (Atamna and Ginsburg, 1997). The *de novo* synthesis of glutathione is the dominating process in the cell. Under oxidative stress reduced glutathione (GSH) is oxidized to GSSG. GSSG is known to be toxic for cells (Rapoport *et al.*, 1968). Malaria parasite recycles a part of GSSG by glutathione reductase, while transports rest of GSSG against concentration gradient into host-cell compartment. High hexose monophosphate shunt activity in parasite compartment supplies necessary NADPH for glutathione reductase (Krauth-Siegel *et al.*, 1996). The host cell cannot supply GSH to the parasite as -

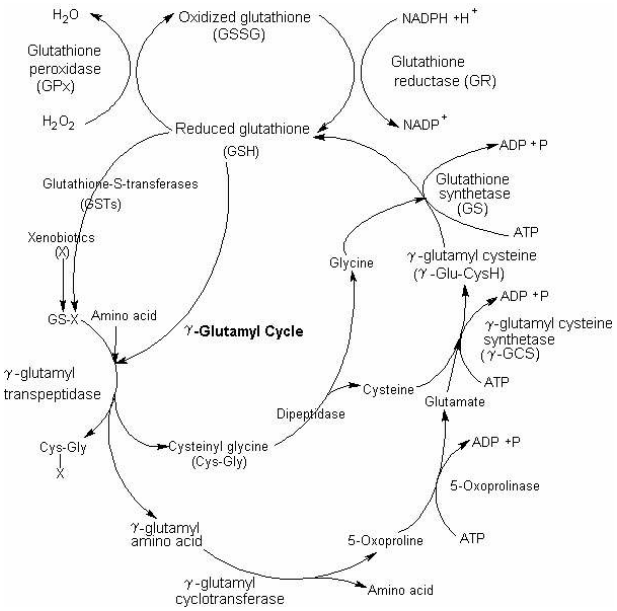

Fig. 2. Schematic diagram of glutathione metabolism.

neither Glu-Cys nor GSH can traverse the parasite membrane. This allows the parasite to efficiently engage in *de novo* glutathione synthesis without losing the intermediate or the final products and to make abundant use of the reducing capacity of GSH (Roth, 1987).

Glutathione and hemoglobin digestion: Hemoglobin is degraded by *Plasmodium* during intraerythrocytic stages and in the process haem is released (Banyal and Fitch, 1982). Haem is a toxic molecule to malaria parasite. The parasite detoxifies haem by polymerization and degradation. The parasite converts almost 30% of haem into haemozoin or malarial pigment, while rest of the haem is degraded by reduced glutathione. Glutathione degrades haem whether it is free in solution or it is bound nonspecifically to protein by conjugation. Glutathione metabolism is therefore, essential for the survival of malaria parasite (Ginsburg *et al.,* 1998; Padmanaban *et al.,* 2007).

Glutathione and drug resistance: Most of the antimalarial drugs interact with haem and form a complex, which is highly toxic to biological membranes (Fitch *et al.,* 1982). Glutathione competes with antimalarial drugs for degrading haem toxin, and is thus associated with drug resistance. Growth inhibition of *P. falciparum* chloroquine-sensitive strain (3D7) by buthionine (S,R)- sulfoximine (BSO, a specific inhibitor of -GCS) and by methylene blue, an inhibitor of GR, was significantly more pronounced than inhibition of *P. falciparum* CQ-resistant strain (Dd_2) growth by antimalarial drugs (Meierjohann *et al.,* 2002a). Also *in vivo* studies on *P. berghei* and *P. vinckei*-infected mice showed that drugs such as acetaminophen, indomethacin and disulphiram, which produce an indirect decrease in GSH, potentiate the antimalarial action of the sub-curative doses of CQ and amodiaquine (Deharo *et al.,* 2003; Zuluaga *et al.,* 2007).

Glutathione metabolism and trypanosomatids: In place of glutathione, trypanosomatids including causative agents of African sleeping sickness *(Trypanosoma brucei gambiense),* Chagas disease *(T. cruzi)* and different leishmanial parasites utilize trypanothione, a conjugate of GSH and spermidine, to maintain the redox balance of the cells (Fairlamb *et al.,* 1985). It is an unusual form of glutathione containing two molecules of glutathione joined by a spermidine (polyamine) linker (Fig. 3). Trypanothione in trypanosomatids is synthesized in four steps via the

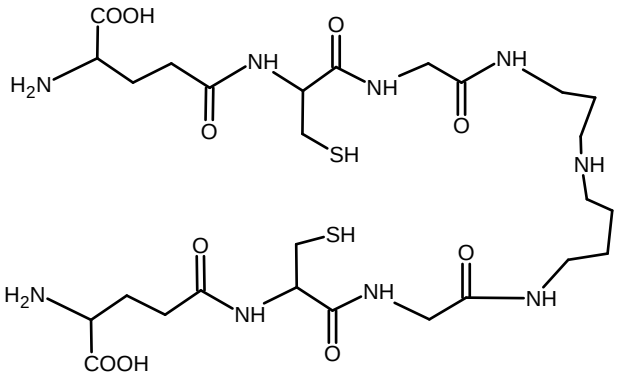

Fig. 3. Trypanothione.

synthesis of GSH and its subsequent conjugation to spermidine. GSH is synthesized by two enzymes, which are common with malarial parasite, while the conjugation of GSH to spermidine is catalyzed by two trypanothione specific enzymes (Opperdoes and Coombs, 2007). Since this thiol is absent from humans and is essential for the survival of these parasites, the enzymes that make and use this molecule are targets for the development of new drugs to treat the diseases caused by these parasitic protozoa (Schmidt and Krauth-Siegel, 2002). Trypanothione-dependent enzymes include reductases, peroxidases, glyoxalases and transferases.

ENZYMES OF GLUTATHIONE METABOLISM IN PARASITIC PROTOZOA: Although the metabolic pathway has not been investigated in any detail, γ-glutamylcysteine synthetase (Lueder and Phillips, 1996; Luersen *et al.,* 2000), glutathione synthetase (Meierjohann *et al.,* 2002a), glutathione-S-transferases (Harwaldt *et al.,* 2002) and glutathione reductase (Farber *et al.,* 1996) have been extensively studied in various protozoa, which suggests that a glutathione metabolic pathway similar to mammals is present in these parasites. Such studies may perhaps help in structure based approaches to the development of antimalarials (Brady and Cameron, 2004).

1. γ-glutamylcysteine synthetase(γ-GCS; glutamate-cysteine ligase EC 6.3.2.2): γ-GCS catalyses the first and rate limiting step in glutathione biosynthesis. The gene for γ-GCS has been isolated and characterized in various parasitic protozoa (Lueder and Phillips, 1996; Luersen *et al.,* 2000). γ-GCS activity was 3.76-fold higher in *P. berghei* as compared to normal mice erythrocytes and 1.4-fold higher compared to *P. berghei* infected erythrocytes (our unpublished data).

The *T. brucei* γ-GCS gene contains 2037 base pairs which code for 679 amino acids of a 77.4 kDa protein. Further, *T.brucei* γ-GCS is a single copy gene and the amino acid sequence shares 36–45% sequence identity with other eukaryotic enzymes (Huang *et al.*, 1995). *T. brucei* γ-GCS showed apparent Km values for the three natural substrates L-Glu, L-Cys and ATP as 0.24 mM, 0.69 mM and 0.07 mM, respectively. Unlike the mammalian enzyme L-γ-aminobutyrate is a poor substitute for L-Cys in *T.brucei* catalysed reaction. *T. brucei* γ-GCS feedback is inhibited by glutathione (apparent K_i=1.1mM) and it is inactivated by cystamine and buthionine sulphoximine (BSO; Arrick *et al.*, 1981). The kinetic properties of recombinant *T. brucei* γ-GCS suggest that substrate binding pocket and the mechanism of enzyme regulation differ from the mammalian enzyme, which provide evidence that *T. brucei* γ-GCS could be a selective chemotherapeutic target for the treatment of trypanosomiasis (Gillespie *et al.*, 2007). Genes of different *P. falciparum* strains contained 4206 bp or 4038 bp and a variable number of repeats at N-terminal (Luersen *et al.*, 1999). *P. falciparum* γ-GCS (Pfggcs) gene encodes polypeptide of 1119 and 1063 amino acids. The deduced amino acid sequences show four regions of homology (31.3-43.9%) to human and T. brucei (Gillespie *et al.*, 2007). These regions are interrupted by three large insertions between 94 and 239 amino acids, which are responsible for the different sizes of the sequences. The predicted molecular mass of the proteins from different *P. falciparum* strains ranges from 124.4 kDa to 133.2 kDa which is almost twice that of the catalytic subunit of the human host enzyme. The γ-GCS shows a stage specific transcription pattern. The transcription begins in the young trophozoite stage (12–18 h), peaks in mature trophozoites and decreases in the schizont stage (36–42 h; Perez-Rosado *et al.*, 2002). *P. berghei* contains cytosolic γ-GCS with 0.711±0.001 units/mg protein. The enzyme was observed as a heterodimer with subunits of apparent molecular weights 66 kDa and 57 kDa, and has Km of 0.75 mM for L-glutamate. As BSO acts specifically on γ-GCS, it can be used as a research tool that efficiently depletes GSH without affecting other metabolic pathways or proteins. However, the inhibitor not only acts on the parasite enzyme but also inhibits the host cell γ-GCS activity (Meierjohann *et al.*, 2002b). Therefore, it remains to be shown that it is indeed the inhibition of *Plasmodium* γ-GCS that impairs parasite survival and knock out studies of the *Plasmodium* gene are underway to address this point. BSO was also found to be an effective antileishmanial agent against *Leishmania donovani* promastigotes *in vitro* and also inhibited intracellular amastigote multiplication (Weldrick *et al.*, 1999; Kapoor *et al.*, 2000). At the concentrations used for promastigotes, the inhibitor has minimal effect on macrophages. The potent anti-leishmanial effect of this inhibitor at *in vitro* level and its selective inhibitory activity towards the parasite makes it a probable chemotherapeutic agent against kala-azar also.

2. Glutathione synthetase (GS; γ-L-glutamate-L-cysteine: glycine ligase EC 6.3.2.3): Glutathione synthetase catalyzes the final step in glutathione biosynthesis. Among parasitic protozoa, this enzyme has been extensively studied in malaria parasite (Meierjohann *et al.*, 2002a). GS activity observed in *P. berghei* was 5.52-fold higher than normal total erythrocytes and 1.95-fold more than *P. berghei* infected total erythrocytes (our unpublished observation). PfGS gene encodes a polypeptide of 655 amino acids. The sequence is much larger than GS in other organisms (Gali and Board, 1997), attributable to several insertions in the *Plasmodium* sequence. These insertions have nothing to do with stability and cofactor binding of the enzyme. Abundance of asparagine, glutamine and glutamic acid helps in circumvention of the host cell immune system (Schofield, 1991). Highly conserved region of the enzyme in *P. falciparum* Cys-517 to Cys-525 corresponds to Gly-164 to Gly-167 sequence in the eukaryotic (Gali and Board, 1997) and *E. coli* GS (Tanaka *et al.*, 1992).

PfGS protein is active as a homodimer with a subunit size of 77kDa. The Km values for γ-glutamyl-α-aminobutyrate, ATP and glycine are 0.107mM, 0.059mM and 5.04mM, respectively, and are comparable with that of mammalian enzyme (Njalsson *et al.*, 2000) but negative cooperative effect of γ-glutamyl-γ-aminobutyrate was not found. Also parasite GS has quite distinct amino acid residues at γ-glutamylcysteine binding site (Meierjohann *et al.*, 2002b). Sharma and Banyal (2007) reported *P. berghei* contained 0.443±0.001 units of GS/mg protein. Purified 70 kDa GS showed Km value of 1.33 mM, 1.17mM and 1.81mM for γ-glutamylcysteine, ATP and glycine, respectively, with noncompetitive inhibition by glutathione. *P. berghei* GS was found antigenically active and induces humoral response in

BALB/c mice model. Whether the protein is essential for the parasite survival is still to be investigated.

3. Glutathione-S-transferases (GSTs; EC 2.5.1.13): GSTs are a family of phase-II detoxification enzymes catalyzing the conjugation of glutathione (GSH) to a large variety of electrophilic substrates (Mannervik and Danielson, 1988). The less toxic and more hydrophilic products of GST-catalysed reactions can be partially metabolized and excreted (Salinas and Wong, 1999), and thus can protect cells against cytotoxic and genotoxic compounds. All the species of *Plasmodium* as well as their intraerythrocytic stages have been found to contain GST (Deponte and Becker 2005a). There was a two-fold increase in the specific activity of GST at 20% and 35% parasitaemia; however, at 55% parasitaemia, it was about 2.6-fold as compared to GST activity in total erythrocytes of normal mice (our unpublished observations). Harwaldt *et al.,* (2002) reported GST to represent >1% of the total cellular protein. The PfGST gene has been characterized and shows 37% identity with other organisms. PfGST is a homodimeric enzyme with a 26 kDa subunit that has been found to have a broad-range (16420 M) substrate specificity Km for glutathione. The PfGST was inhibited by cibacron blue, S-hexylglutathione and ferriprotoporphyrin-IX (FP-IX). FP-IX inhibits uncompetitively by binding to PfGST-GSH complex (Harwaldt *et al.,* 2002).

PfGST cannot be assigned to any of the previously known GST isoform. In contrast to other organisms, *Plasmodium* has been found to contain only one GST isoenzyme. The enzyme is highly abundant in the parasite, and was found to increase in chloroquine-resistant strains (Perbandt *et al.,* 2004). The primary as well as the three-dimensional X-ray structures of PfGST differ greatly from human GSTs. PfGST is present as a tetramer that dissociates into dimers in the presence of GSH (Hiller *et al.,* 2006). PfGST possesses a shorter C-terminal section compared to other GSTs, which results in a more solvent-accessible binding site for the hydrophobic and amphiphilic substrates (Fritz- Wolf *et al.,* 2003).

GSTs also function as glutathione peroxidases sequestering cytotoxic peroxides and ferriproto-porphyrin-IX (FP-IX) that are formed upon hemoglobin digestion (Loria *et al.,* 1999). The peroxidase activity of GSTs of these parasites is important because they lack catalase and glutathione peroxides. The inhibition of PfGST is expected to enhance peroxide level and concentration of toxic FP-IX. Thus the enzyme represents a promising target for antimalarial drug discovery (Deponte and Becker, 2005b).

Activity of GSTs has been not detected in kinetoplastida (Vickers and Fairlamb, 2004). Trypanothione is known to play a role in xenobiotic metabolism through trypanothione-S-transferase enzyme in the kinetoplastida. The enzyme activity has been reported in *L. major, L. infantum, L. tarentolae, T. brucei* and *Crithida fasciculata*, but not in *T. cruzi*. The enzyme is specific for thiols such as trypanothione and glutathionylspermidine, and only used 1-chloro-2,4-dinitrobenzene from a range of glutathione-S-transferase substrates (Liebau *et al.,* 2002 ; 2005).

Over the last many years, PfGST has been discussed as a potential target of clinically used antimalarial drugs like chloroquine, artemisinin and primaquine (Srivastava *et al.,* 1999). Hiller *et al.* (2006) reported these drugs to be very weak inhibitor of PfGST, and thus are most unlikely to lead to biologically meaningful PfGST inhibition, *in vivo.* GST plays important role in the metabolism of chloroquine and amodiaquine. But unlike these antimalarials, artemisinin and artesunate are not metabolized by PfGST (Zuluaga *et al.,* 2007).

4. Glutathione peroxidase (GPx; EC 1.11.1.9): GPx catalyses GSH-dependent reduction of hydrogen peroxide (H_2O_2). Parasitic protozoa like trypanosomes and plasmodia species, lack glutathione peroxidase (Flohe *et al.,* 1999; Muller, 2004). However, the putative glutathione peroxidase gene (Swiss Prot accession number Z68200) of *P. falciparum* was isolated and expressed in *Escherichia coli* (Gamain *et al.,* 1996). GPx gene was found to code for a thioredoxin reductase. PfGPx gene encodes a sulphur homologue of the mammalian selenoprotein, GPx that reacts with thioredoxin faster than glutathione. Hence, PfGPx is reclassified as thioredoxin peroxidase (Sztajer *et al.,* 2001). Among American typanosomes, *T. cruzi* gene coding for a 18 kDa protein shows sequence similarity with glutathione peroxidase from plants and peroxidase activity in the presence of GSH/GR but not in the presence of trypanothione/ trypanothione reductase (Wilkinson *et al.,* 2000). In trypansomatids a trypanothione-dependent peroxidase helps in defense against oxidative stress e.g. trypanoredoxin peroxidase (TryP) reduces peroxides using electrons donated either directly from trypanothione, or via the redox intermediate trypanoredoxin (TryX). The trypanothione-dependent

hydrogen peroxide metabolism is particularly important in these organisms because they lack catalase (Krauth-Siegel et al., 2003). Unlike members of kinetoplastida and apicomplexa, *Toxoplasma gondii* glutathione peroxidases (GPx) and novel family of peroxidoxins (Prx) are also capable of decomposing H_2O_2 (McGonigle *et al.,* 1998; Kwok *et al.,* 2004).

5. Glutathione reductase (GR; glutathione: $NADP^+$ oxidoreductase, E.C.1.8.1.7): Glutathione reductase is an ubiquitous flavoenzyme of disulphide oxidoreductase family whose members are dimeric, NAD (P) H-dependent and FAD-containing enzymes. GR catalyses NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), which permits GSH to function as an intracellular reducing agent (William, 1992). Significant increase in the specific activity of GR in *P. berghei*-infected erythrocytes was observed, which is 3.6-fold higher than that observed in normal mice erythrocytes. The enzyme activity in parasitized erythrocytes increased with increasing parasitaemia and maximum activity was found at 55% infection (Kapoor *et al.,* 2005a). In case of trypanosomes and Leishmania, no glutathione reductase activity has been found though GSH is present. These organisms reduce GSSG and other disulphides by means of nonenzymatic thiol-disulphide exchange with trypanothione. Trypanothione is maintained intracellularly as a dithiol [$T(SH)_2$], due to the action of the unique enzyme, trypanothione reductase and account for > 68% of intracellular GSH in *T. brucei* (Fairlamb and Henderson, 1987) and *Leishmania* species (Keithly and Fairlamb, 1988; Ramao *et al.,* 2006). Trypanothione reductase (TryR) was the first trypanothione-dependent enzyme to be elucidated. It is also NADPH-dependent flavoenzyme that reduces trypanothione disulphide. TryR is essential for the survival of these parasites in both *in vitro* and in human host (Tovar *et al.,* 1998, Krieger *et al.,* 2000). Also, the trypanosomatids also lack an equivalent of thioredoxin reductase. Trypanothione reductase is the role path that electrons can take from NADPH to antioxidant enzymes. However, the enzyme glutathione reductase is an integral part of the glutathione metabolism of *Plasmodium* (Farber *et al.,* 1996; Luersen *et al.,* 2000; Meierjohann *et al.,* 2002a), and the parasite GR has long been discussed as a potential drug target molecule (Schirmer *et al.,* 1995). Molecular cloning and characterization of PfGR has been achieved by Farber *et al.* (1996). PfGR was found to code 500 amino acid polypeptides, which exhibit 40–45% sequence identify with GR from other species. PfGR is a homodimer with a molecular weight range between 110–111 kDa with pH optimum of 6.8 and high performance for NADPH over NADH (Krauth-Siegel *et al.,* 1996; Muller *et al.,* 1996). Purified *P. berghei* GR has a molecular weight of 24 kDa (our unpublished observations).

The three dimensional structure of GS differs in geometry and internal surface chemistry of the inter-subunit cavity from mammalian enzyme. These differences attracted a lot of attention as a potential therapeutic target against malaria. The primary structure of *Plasmodium* GR contains parasite insertions in the FAD-domains (residues 123–134), the central domain (residues 314–347) and the interface domain (residues 496–499) that are responsible for stability and FAD cofactor binding capacity of the protein (Gilberger *et al.,* 2000). These insertions are flexible loops located on the surface of protein.

The best known lead compound methylene blue, which inhibits *Plasmodium* GR more efficiently than the human enzyme, binds the cavity of the dimeric interface (Farber *et al.,* 1998). Also methylene blue was the first compound used in clinical antimalarial therapy and its antimalarial activity was reported by Guttmann and Ehrlich (1891). Although *Plasmodium* GR might be one of the molecular targets of methylene blue, it is unlikely to be the primary one as the IC_{50} of parasite growth inhibition by methylene blue is in the low nanomolar range and thus one order of magnitude lower than the K_i for GR inhibition by methylene blue (Meierjohann *et al.,* 2002b). This strongly suggests that the compound has additional effects leading to parasite death.

6. 5-Oxoprolinase (L-pyroglutamate hydrolase; EC 3.5.2.9): 5-oxopolinase catalyzes ATP-dependent decyclization of L-pyroglutamate to form L-glutamate. The enzyme has been studied in a number of animal tissues (Ross *et al.,* 1973; Griffith and Meister, 1981) and microorganisms (Nishimura *et al.,* 1999). 5-oxoprolinase is found in all mammalian tissues except erythrocyte and lens of eye (Meister and Anderson, 1983). However, there is no report available of this enzyme in any species of parasitic protozoa so far.

7. γ-glutamyl transpeptidase (GGT, EC 2.3.2.2.): γ-7221888-glutamyl transpeptidase (GGT) is a , 2004).

transmembrane ectoenzyme. The majority of the protein is extracellular with only the N-terminal methionine and three lysine residues located inside the plasma membrane (Ruedig and Dringen, 2004). The enzyme hydrolyses glutamyl groups from glutathione (GSH) or glutathione-S-conjugates. In doing so, it can transfer γ-glutamyl groups to an amino acid, dipeptide, water or another molecule of GSH. Through the operation of the gammaglutamylcyclotransferase cycle, this enzyme has been implicated in the transport of amino acids into cells. Although GGT is most actively operative in cells of the proximal tubules of kidney but absent in human and rabbit erythrocytes (Srivastava *et al.*, 2005). In *Ascaris suum* the enzyme showed a molecular mass of 70 kDa, and was found to be composed of two non-identical subunits of molecular mass 43 and 30 kDa (Hussein and Walter, 1999).

8. γ-glutamyl cyclotransferase [(5-L-glutamyl)-L-amino-acid 5-glutamyltrasferase (cyclizing) EC 2.3.2.4)]: γ-glutamyl cyclotransferase catalyses the synthesis of pyroglutamate from a gamma-glutamyl amino acid, also releasing the free amino acid. The enzyme acts on derivatives of glutamate, 2-aminobutyrate, alanine and glycine. The enzyme has been proposed to have a role in a gamma-glutamyl cycle for amino acid transport into cells (Srivastava *et al.*, 2005).

OTHER SYSTEMS AND GLUTATHIONE METABOLISM

Thioredoxin system: *Trichomonas* and apicomplexan protozoan parasites have thioredoxin system consisting of a small low molecular weight thioredoxin (Trx) with a redox active disulphide (-Cys-Gly-Pro-Cys-) that is reduced to a dithiol by NADPH and flavoprotein thioredoxin reductase (TrxR) (Kanzok *et al.*, 2000; Coombs *et al.*, 2004). TrxR/Trx catalyzed the reduction of GSSG to GSH. A high rate of reduction was observed in glutathione reductase-deficient cells, which is helpful for certain stages of parasite (Kanzok *et al.*, 2000).

Glutaredoxin system: *Plasmodium* possesses glutaredoxin system composed of small protein glutaredoxin which also has a redox active disulphide (Cys-Pro-Tyr-Cys) that is reduced to dithiol by GSH, NADPH and GSSG reductase. Such reduction does not occur with thioredoxin reductase. The protein is involved in the reduction of protein-GS mixed disulphides that are inevitably formed during exposure to ROS (Rahlfs *et al.*, 2003).

CONCLUSION

There is a good evidence that glutathione plays important role in destroying ROS, free-radicals and detoxification of xenobiotics that are formed during metabolism. As a result parasitic protozoa are a well-protected target for oxidant drugs. Only inhibitors targeted to specific component of glutathione metabolism are able to destroy the parasite. This indirect parasite killing is particularly effective in drug resistant parasitic protozoa. Enzymes like γ-GCS, GS, GR and GST of this metabolism have been studied among various protozoa. But still no information is available about the other enzymes of glutathione metabolism such as 5-oxoprolinase, dipeptidase, γ-glutamyl transpeptidase and γ-glutamylcyclotransferase. The identification and functional analysis of these protozoan enzymes involved in glutathione metabolism may be of importance in the context of improved chemotherapy.

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Scanning electron microscope study of two avian nematodes: *Ascaridia trilabium* (Linstaw, 1904) and *Torquatoides balanocephala* (Gendre, 1922)

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ABSTRACT. Morphology of two avian nematodes *viz. Ascaridia trilabium* and *Torquatoides balanocephala,* recovered from *Centropus sinensis* and *Merops orientalis,* respectively, from Burdwan, West Bengal, India was studied by using a scanning electron microscope (SEM). Apparently, this is the first study of these nematodes by using SEM. Scanning electron micrographs of *A. trilabium* provided detailed information about the nature of cephalic papillae, amphids, caudal papillae and phasmids. SEM study of *T. balanocephala* provided detailed information about the nature of cephalic plates.

Keywords: *Ascaridia trilabium,* avian nematodes, morphology, SEM, *Torquatoides balanocephala*

INTRODUCTION

Available descriptions of most nematodes are based on studies with light microscope (LM). The application of scanning electron microscope (SEM) in studying microtopography of nematodes has been proved to be useful and necessary for proper taxonomic evaluation. Herein, the SEM study of two avian nematodes *viz. Acaridia trilabium* (Linstow, 1904) that and *Torquatoides balanocephala,* which occur in *Centropus sinensis* and *Merops orientalis,* respectively, at Burdwan, West Bengal, India has been reported. The general morphology, based on LM study, is available for *A. trilabium* (Linstow, 1904; Baylis, 1936; Nandi *et al.,* 1989) and *T. balanocephala* (Gendre, 1922; Gupta, 1960; Brogarenko, 1960; Chabaud *et al.,* 1963; Nandi and Majumdar, 1987). The present SEM study provides new data on the morphology of the species.

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MATERIALS AND METHODS

Three males and four females of *A. trilabium* from the intestine of *C. sinensis* (only three out of eleven hosts examined were positive) and one male and three females of *T. balanocephala* from under the horny layer of the gizzard of *M. orientalis* (only two out of five hosts examined were positive) at Burdwan, West Bengal, India were obtained during routine examination of birds for nematode infection. The worms were washed thoroughly by shaking in physiological saline. One specimen of either sex from each lot was fixed in 4% formaldehyde for identification by using LM. For SEM studies, the remaining specimens of each lot, after washing in physiological saline, were fixed in 2.5% glutaraldehyde in 0.2M sodium cacodylate buffer (pH 7.2) for 2 h. After primary fixation, the anterior and posterior ends of the worms were cut-off using a sharp razor blade and the cut ends were then exposed to the same fixative for 24 h for better fixation. After rinsing in the buffer, the cut worms were post-fixed for 4 h in 2% osmium tetroxide

in the same buffer. The materials were then dehydrated in ascending series of ethanol, transferred to 100% isoamyl acetate through the mixtures of ethanol and isoamyl acetate (3:1, 1:1 and 1:3). The specimens were then dried in a Hitachi HPC-2 (Japan) critical point drying apparatus, mounted on aluminum stubs and coated with gold by an IB2 ion coater. Observation was made out with a Hitachi S-530 (Japan) SEM at a resolution of 50 ? and operating at an accelerating voltage of 15 kV.

RESULTS AND DISCUSSION

1. *A. trilabium* (Linstow, 1904) (Nematoda: Ascarididae)

Host: *C. sinensis*

Location: Intestine

Locality: Burdwan, West Bengal, India

Linstow (1904) described *A. trilabium* from *C. sinensis* from the nematodes in the collection of the Colombo museum. Baylis (1936) redescribed the species based on the type specimens. Nandi *et al*. (1989) reported this species from India from the type host. The nematodes obtained for this study are from the same host and the same zoogeographical region as that reported by Nandi *et al.*

Morphologically the worms in this study agree fairly well with the description of *A. trilabium* given by Baylis (1936) and Nandi *et al.* (1989). SEM study of the cephalic region of the present material reveals that three well-developed lips bordered by cuticular ridge surround the mouth (Fig. 1). The dorsal lip (Fig. 1, 2) is bluntly conical and bears a pair of lobular structures at its anterior extremity and a pair of large oval double cephalic papillae at its base. Each double papilla is circumscribed by a groove on the cuticle and bears two openings of the canals of papillary nerve endings. Each ventrolateral lip (Fig. 3) bears a single lobular structure at its anterior extremity and a large oval double cephalic papilla at its base towards the ventral surface. In addition, there is a small, round external labial papilla, also surrounded by a groove, at its lateral surface (Fig. 3, 4). The opening of amphid, surrounded by a circular cuticular rim, is situated laterally anterior to the external labial papilla on each of the ventrolateral lips (Fig. 3, 4). Immediately posterior to the lips there is a pair of cervical papillae on each lateral surface just anterior to the beginning of the cervical ala (Fig. 1, 3). Scanning electron micrographs of the tail of males (Fig. 5, 6) reveal that the conically pointed tail bears a pair of narrow caudal alae. Precloacal sucker bears a circular cuticular rim with a small medial elevation at its posterior margin. Anal opening is a transverse slit bordered by cuticular margin. Caudal papillae are 10 pairs in number: 3 pairs pre-anal, 1 pair adanal and 6 pairs postanal in position. Of the 6 pairs of post-anal papillae, one pair lies immediately behind the anal opening. Phasmids open laterally anterior to the last pair of post-anal papillae.

SEM observation of the cephalic region of the present material reveals that the paired cephalic papillae on the dorsal lip and the single cephalic papilla on each of the ventrolateral lips are double papillae. External labial papillae and the opening of amphids were erroneously described as cephalic papillae by earlier workers. Present study clearly reveals the structural details of the cephalic papillae, external labial papillae and amphids. Baylis (1936) reported 8 pairs of post-anal papillae in *A.*

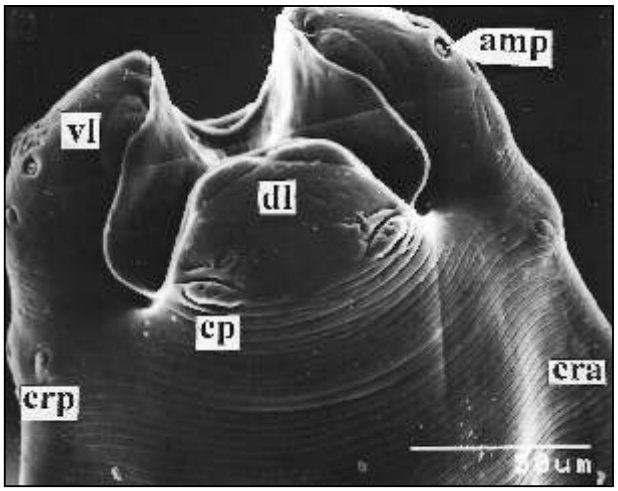

Fig.1. Anterior end of male, dorsal view.

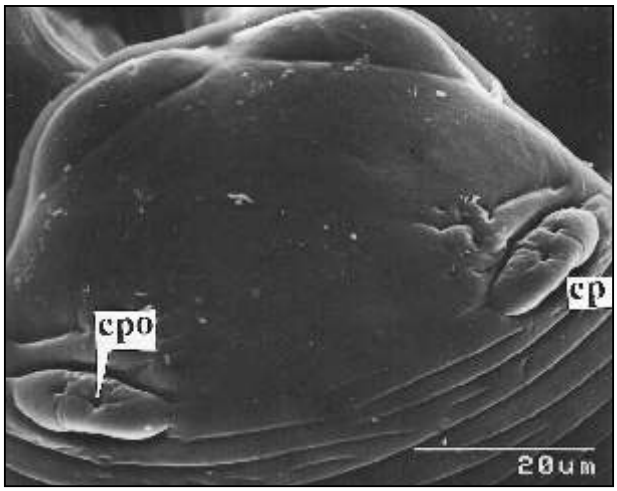

Fig. 2. Dorsal lip of male.

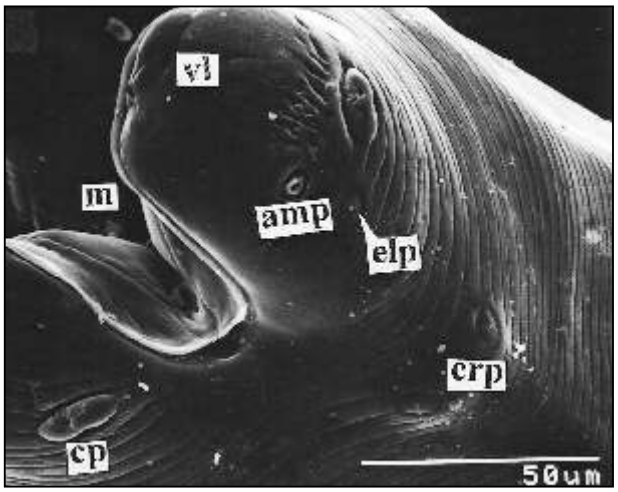

Fig. 3. Anterior end of male, lateral view.

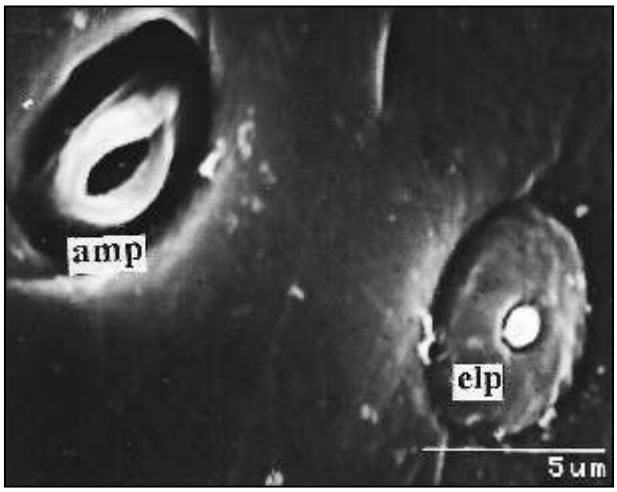

Fig. 4. Enlarged view of part of ventro-lateral lip to show lateral external labial papilla and amphid.

trilabium (including one pair of phasmids as post-anal papillae). Present SEM study reveals the presence of 6 pairs of post-anal papillae and a pair of phasmids.

2. *Torquatoides balanocephala* (Gendre, 1922) (Nematoda: Habronematidae)

Host: *M. orientalis*

Location: Under the horny layer of gizzard

Locality: Burdwan, West Bengal, India

T. balanocephala (Gendre, 1922) has been reported from *M. malimbicus* (type host), *M. orientalis orientalis*, *M. persicus*, *M. superciliosus* and *M. orientalis* (Gendre, 1922; Gupta, 1960; Brogarenko, 1960; Chabaud *et al.*, 1963; Nandi and Majumdar, 1987). Nandi and Majumdar (1987) synonymised *T. longiovata* (Ali, 1957) recovered from *M. orientalis* with *T. balanocephala*. Material for the present study has been recovered from *M. orientalis* at Burdwan, West Bengal, India.

Morphologically, the worms in this study agree fairly well with the light microscopic description of *T. balanocephala* given by Gupta (1960) and Nandi and Majumdar (1987). Scanning electron micrographs of the cephalic region of the present material reveal that mouth is dorsoventrally elongated, guarded by two lateral pseudolabia (Fig. 7). Each pseudolabium bears four prominent teeth at its inner margin (Fig. 8). The cephalic region is provided with a complex set of cuticular plates. Two semicircular apical plates, one on

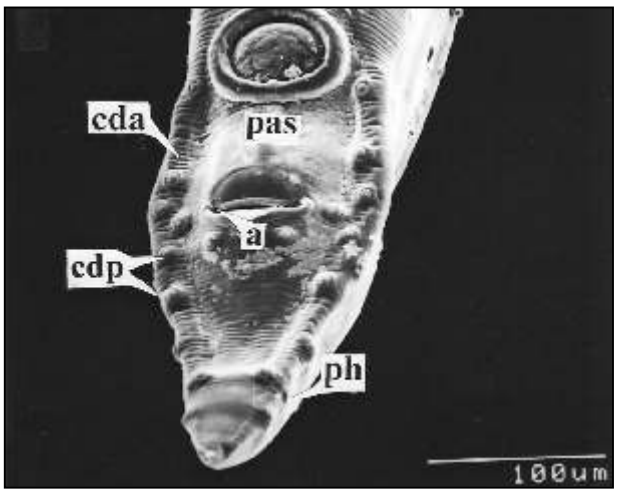

Fig.5. Posterior end of male, ventral view.

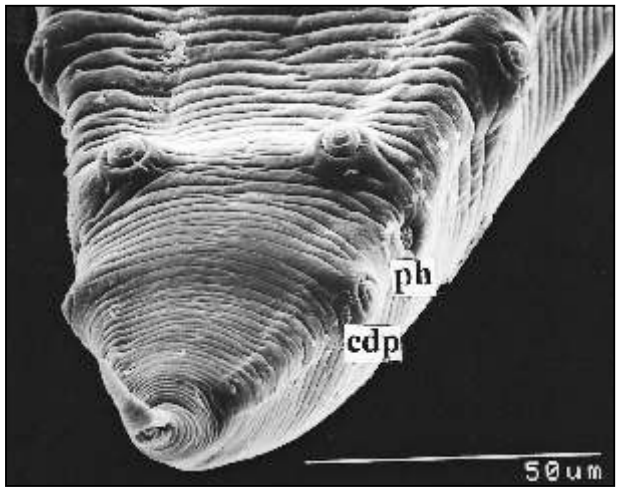

Fig. 6. Posterior end of male, ventral view (enlarged).

Abbreviations: a = anus; amp = opening of amphid; cda = caudal ala; cdp = caudal papilla (e); cp = cephalic papilla; cpo = opening of cephalic papilla; cra = cervical ala; crp = cervical papillae; dl = dorsal lip; elp = external labial papilla; m = mouth; pas = preanal sucker; ph = phasmid; vl = ventro-lateral lip

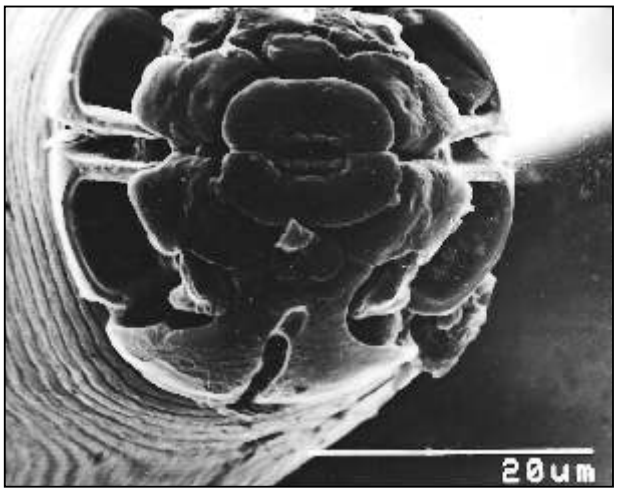

Fig. 7. *En face* view of female.

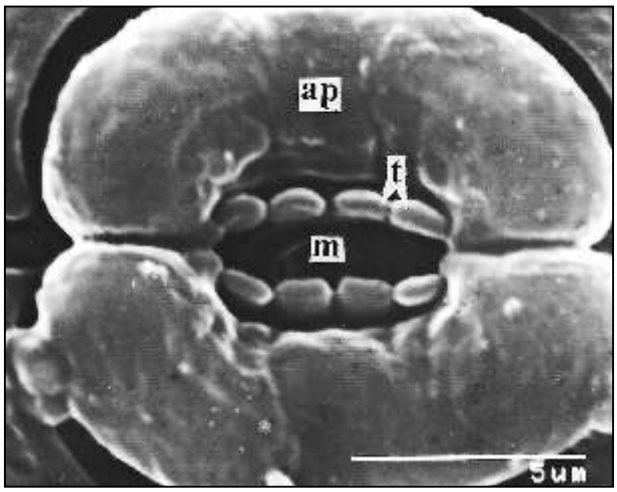

Fig. 8. *En face* view of female (enlarged) showing mouth and apical plates.

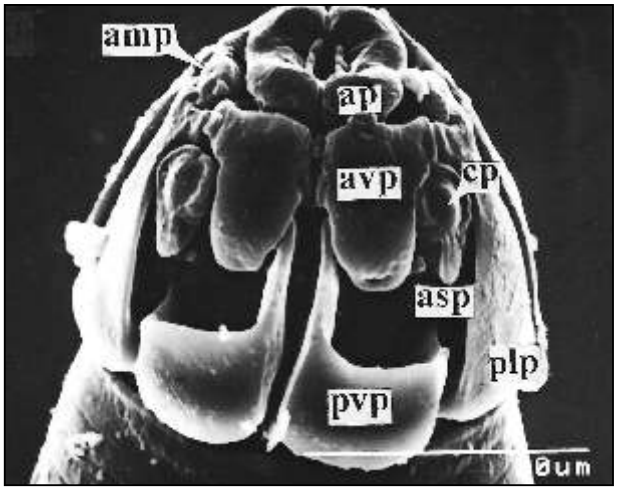

Fig. 9. Anterior end of female, ventral view.

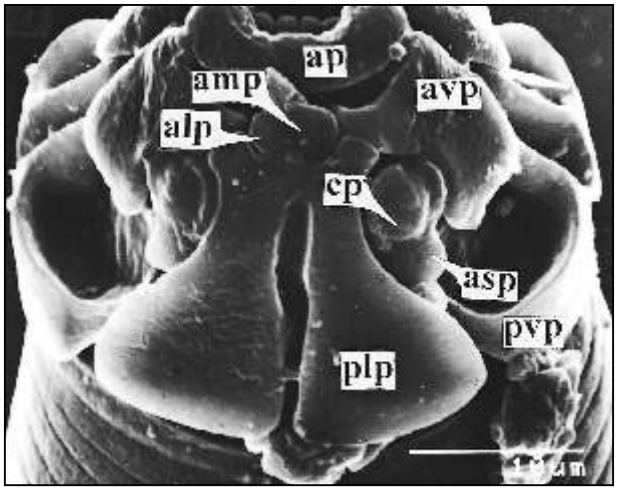

Fig. 10. Anterior end of female, lateral view.

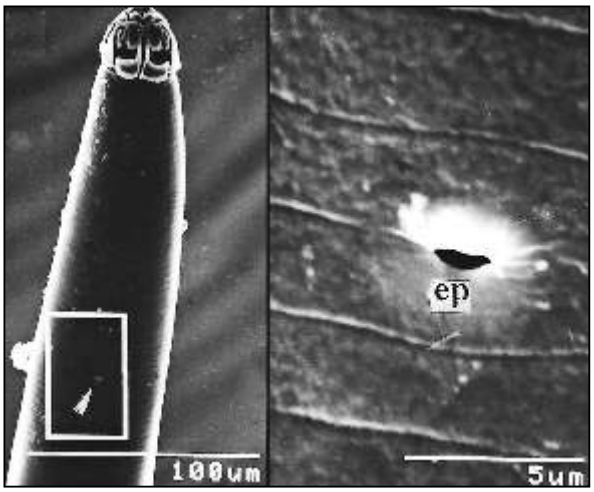

Fig. 11. Anterior end of female, ventral view, box showing position of excretory pore (left); box enlarged to show excretory pore (right).

each pseudolabium, surround the mouth opening (Fig. 7–10). The cuticular plates behind the apical plates are arranged in two rows with considerable overlapping between rows. The anterior row bears one pair dorsal, one pair ventral, two lateral and four submedian plates. The dorsal and ventral plates (Fig. 9) are larger in size than the lateral and submedian plates. Openings of amphids are located at the centre of the lateral plates (Fig. 9, 10) and each submedian plate surrounds and extends below the corresponding cephalic papilla (Fig. 9, 10). The posterior row consists of one pair dorsal, one pair ventral and two pairs of lateral plates. Each of the dorsal and ventral plates is L-shaped with a broad base and narrow vertical arm (Fig. 9). The lateral cuticular plates are triangular in shape and the paired plates on each lateral surface form a notch at the anterior end where the anterior lateral plate is received (Fig. 10). Scanning electron micrographs of the cervical region

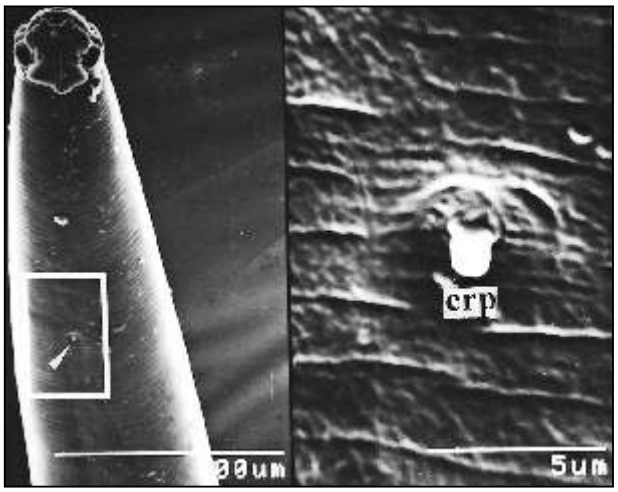

Fig. 12. Anterior end of female, lateral view, box showing position of cervical papilla (left); box enlarged to show cervical papilla (right).

Scale bars: Fig. 9 = 10 µm; Fig. 12 (left) = 100 µm

Abbreviations: alp = anterior lateral plate; amp = opening of amphid; ap = apical plate; asp = anterior submedian plate; avp = anterior ventral plate; cp = cephalic papilla; crp = cervical papilla; ep = excretory pore; m = mouth; plp = posterior lateral plate; pvp = posterior ventral plate; t = teeth

Scanning electron micrographs of the cervical region reveal that the excretory pore (Fig. 11) is a small semi lunar aperture and each of the cervical papillae (Fig. 12) is a small bluntly rounded projection.

Present SEM findings agree fairly well with the light microscopic description of *T. balanocephala* given by Gupta (1960) and Nandi and Majumdar (1987). However, Nandi and Majumdar have not reported anterior lateral plates. Gupta (1960) reported that mouth is bordered by extremely fine tooth-like edges and three small rounded apertures lie below the apical plates. Nandi and Majumdar (1987) described three small labial papillae anterior to the apical plates. Present study reveals the presence of four distinct teeth on each pseudolabium. Pores or labial papillae are not observed in the present scanning electron micrographs. It seems probable that the authors wrongly described the basal interdenticular spaces as “pores” or “labial papillae”. Presence of anterior lateral plates is reported in the present study.

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The author is thankful to Ms. Krishna Ghosh for her assistance in collection and preparation of materials and to Dr. Srikanta Chakrabarty of USIC and CIF, Burdwan University for his technical assistance in SEM study.

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Ultrastructure of metacercarial cyst wall of *Acanthoparyphium spinulosum* (Digenea: Echinostomatidae)

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ABSTRACT. Transmission and scanning electron microscopy techniques were used for the first time to study the ultrastructure of metacercarial cyst wall of the echinostomatid trematode *Acanthoparyphium spinulosum*, recovered from the pericardium of naturally-infected marine prosobranch snail *Cerithidea cingulata*. In general, the ultrastructure of cyst wall conformed to the multi-layered organization that has been described for other echinostomatid metacercariae. The cyst was enclosed and loosely anchored to snail pericardial tissue by a membranous capsule probably of host origin. The outer layer of cyst wall was packed with electron-dense coarse particles; in the outer margin particles were condensed and formed a prominent convoluted bi-lamellate membranous capsule. The middle layer of cyst wall consisted of two zones of electron-dense granular particles; an outer zone of coarse and inner zone of fine particles. The inner layer of cyst wall was composed of an electron-dense multi-lamellate matrix, oriented parallel to the cyst wall. The parasite was observed to be isolated from the cyst lumen by a thin membrane. Structuring of lamellae appeared to be a dynamic process; new lamellae were synthesized by polymerization of particles released from cercarial cystogenous vesicles and old lamellae disassociated into fine granular particles, which diffused into the middle layer of cyst wall.

Keywords: *Acanthoparyphium spinulosum*, cyst wall, Digenea, Echinostomatidae, Trematoda, ultrastructure

INTRODUCTION

Studies on the ultrastructure of cyst wall of digenetic trematode metacercariae, which are known to encyst in intermediate hosts, have revealed complex and species-specific multi-layered structural organization (Smyth and Halton, 1983; Galaktionov *et al.*, 1997). In invertebrate hosts, the layers of cyst wall are predominantly the product of secretions from tegumental glands of cercaria and metacercaria, whereas in vertebrate hosts the outer layers and the inner layers are often of host and parasite origin, respectively. Most of the available information on the structure of metacercarial cyst walls is focused on species that encyst either in tissue of fish hosts or on external surfaces. However, in a few studies on species which encyst inside invertebrate hosts, echinostomatids encysting in freshwater pulmonate snails have received most attention. The present study reports ultrastructural observations on the cyst wall of *Acanthoparyphium spinulosum* Johnston, 1917 (Digenea: Echinostomatidae), recovered from the pericardium of naturally-infected marine prosobranch

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snail *Cerithidea cingulata* (Gmelin, 1791) in Kuwait Bay.

MATERIALS AND METHODS

C. cingulata snails, infected with metacercariae of echinostomatid trematode *A. spinulosum*, were collected from Sulaibikhat shore, located south of Kuwait Bay, about 10 km west of Kuwait City (29°20'3.26"N, 47°54'0.92"E). In the laboratory, snail tissue was examined under a dissecting microscope and metacercarial cysts, usually found loosely adhered to the snail pericardium, were removed by using mounted needles and a Pasteur pipette. Only those cysts which contained live and active metacercariae (n = 45) were selected for processing. Whole mounts of metacercarial cysts were vitally stained with 0.5% neutral red. Photographs were taken using an Olympus AHBS3 research photomicrograph system. For scanning electron microscope (SEM) studies, specimens of intact and mechanically disrupted cysts with exposed layers of wall were fixed in a solution containing 4% (w/v) formaldehyde and 1% glutaraldehyde in phosphate buffer (pH 7.2) for 24 h at 4°C. After rinsing in the same buffer, the specimens were post-fixed in buffered 2% osmium tetraoxide for 5 min at 4°C, dehydrated in an acetone series and critical-point dried with CO_2. Dried metacercariae were mounted on aluminium stubs, sputter coated with gold and then examined by using a JEOL JSM 6300 SEM operating at 20 kV. Specimens for transmission electron microscope (TEM) studies were fixed overnight in 3% (w/v) glutaraldehyde in 0.1M Millionig's phosphate buffer (pH 7.2). The specimens were post-fixed in buffered 2% osmium tetroxide for 1 h at room temperature. After rinsing in buffer, specimens were dehydrated through a graded series of ethanol to propylene oxide, embedded in Epon 812 resin, and polymerized for 48 h at 65°C. Semithin (1 µm thick) sections were stained with toluidine blue to select appropriate areas for TEM studies. Ultrathin sections (70–80 nm thick) were cut by using a diamond knife, collected on 150–200 mesh copper grids, stained with uranyl acetate and lead citrate, and then examined under a JEOL JEM-1200EX II TEM operating at 80 kV.

RESULTS

The metacercarial cyst of *A. spinulosum*, that ranged 270–328 µm in diameter, was spherical in shape and appeared as a glistening, transparent structure (Fig. 1). The metacercaria with its characteristic collar spines could clearly be identified inside the cyst. The cyst was shrouded by a loose membranous structure, 2–5 µm in thickness, and was anchored to snail pericardial tissue by extensions of folded surface of the membrane (Fig. 1, 2 and 3). The membranous capsule was separated from the cyst wall by a fluid filled space. No discernible tissue reaction was detected around the metacercarial cyst. The cyst wall, 4–6.3 µm in thickness, was composed of a three-layered structure (Fig. 4), possibly of parasite origin. The outer layer, ranging 0.1–0.4 µm in thickness, was composed of electron-dense coarsely granular particles (Fig. 4 and 5). In the outer limit of the cyst wall, particles were condensed into a convoluted bi-lamellate membranous capsule. On the surface of the cyst wall, particles became more diffused with a

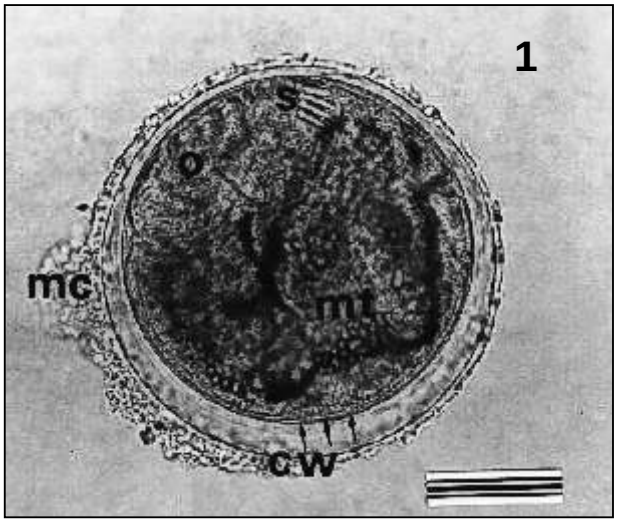

Fig. 1. Photomicrograph of *A. spinulosum* metacercarial cyst detached from snail pericardial tissue. cw, cyst wall; mc, membranous capsule; mt, metacercaria; o, oral sucker; s, collar spines. Scale bar = 70 µm.

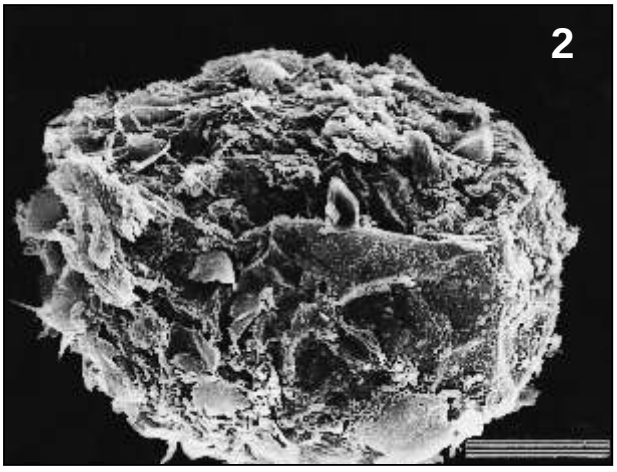

Fig. 2. SEM photomicrograph of a whole metacercarial cyst of *A. spinulosum* showing the outer surface covered with convoluted membranous capsule and debris. Scale bar = 100 µm.

distance from capsule, and occasionally a very loose accumulation of fine fibers was present on the surface (Fig. 4 and 5). The middle layer (0.6–1.3 µm in thickness) was composed of two distinct zones of particles in an electron-lucid matrix; an outer zone of coarse particles and an inner zone of finely granular particles, either freely scattered or condensed into loose networks of a reticulate structure (Fig. 4, 5 and 6). Granules were also found diffused into the outer and inner layers of the wall. The inner layer, 1.6–2.9 µm in thickness, was occupied by layers of electron-dense lamellae oriented parallel with the cyst surface (Fig. 4, 6 and 7). In the interface between inner and middle layers of the cyst wall, the lamellae disassociate into finely granular particles which accumulated in the middle layer of the wall (Fig. 6). The cyst lumen, between margin of the inner layer and membrane enclosing the metacercaria, was packed with cystogenous vesicles probably secreted by the metacercarial tegumental glands (Fig. 8). In the cyst lumen, the vesicles degranulate and granule particles polymerize forming layers of the inner wall lamellae (Fig. 8). The perimetacercarial space was filled with membranous and particulate debris, perhaps remnants of metacercarial secretory and metabolic waste products (Fig. 8).

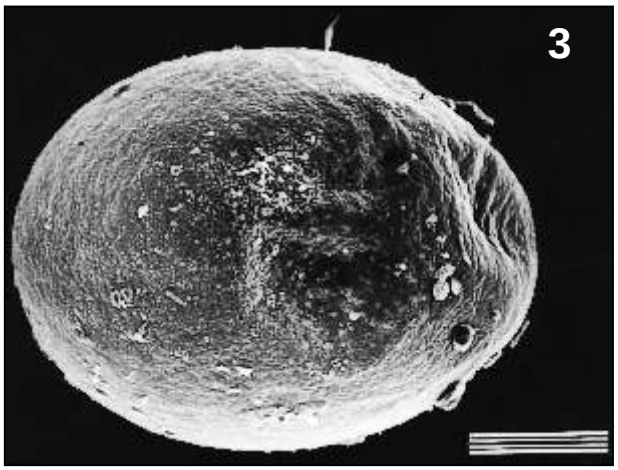

Fig. 3. SEM photomicrograph of a whole metacercarial cyst of *A. spinulosum* showing the outer surface depleted of the outer membranous capsule. Scale bar = 100 µm.

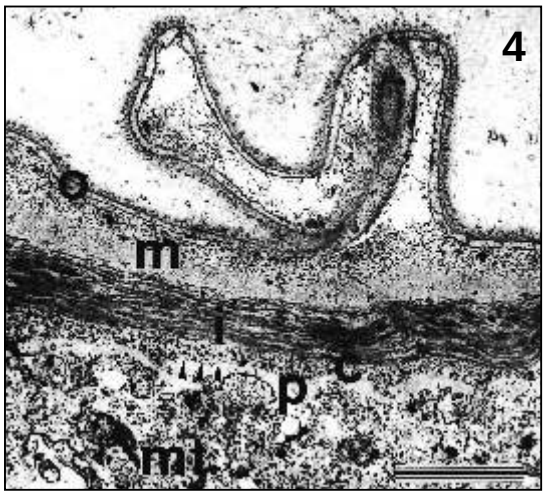

Fig. 4. TEM photomicrograph of a section through metacercarial cyst wall of *A. spinulosum* showing the overall structure. arrowheads, perimetacercarial membrane; c, cyst lumen; i, lamellated inner layer; m, granular middle layer; mt, metacercarial tegument; o, bi-lamellate outer membranous capsule; p, perimetacercarial space. Scale bar = 4 µm.

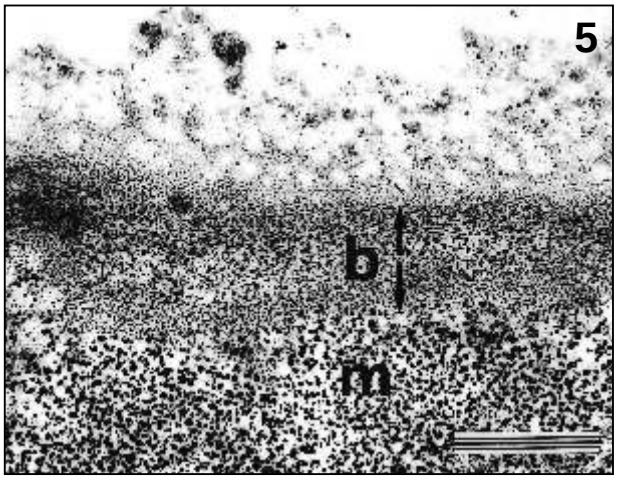

Fig. 5. TEM photomicrograph of a section through outer margin of metacercarial cyst wall of *A. spinulosum* showing high magnification of fine granules of the bi-lamellate outer membranous capsule (b) and coarse granules of the middle layer (m). Scale bar = 0.5 µm.

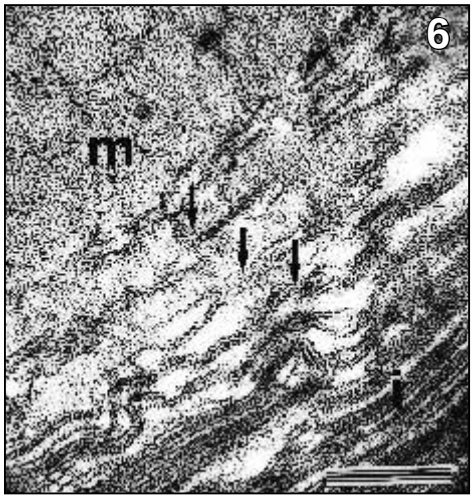

Fig. 6. TEM photomicrograph of a section through interface of the inner (i) and middle (m) layers of metacercarial cyst wall of *A. spinulosum* showing disassociation of lamellae into fine granules (arrows). Scale bar = 0.5 µm.

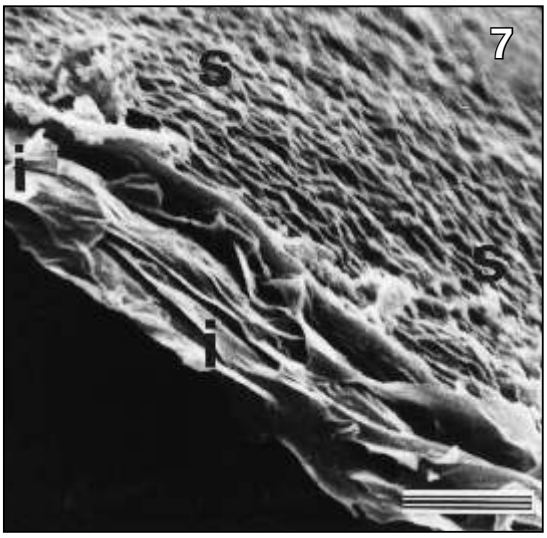

Fig. 7. SEM photomicrograph of a cross section through metacercarial cyst wall of *A. spinulosum* showing layers of lamellae of the inner layer (i) oriented parallel with the cyst surface (s). Scale bar = 3 µm

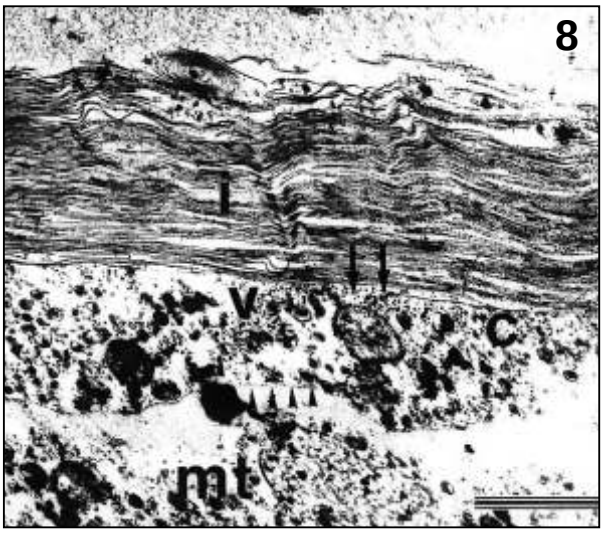

Fig. 8. TEM photomicrograph of a section through lumen of a metacercarial cyst of *A. spinulosum* showing formation of lamellae (arrows) by granules released from what appears to be metacercarial cystogenous vesicles (v). Arrowheads, perimetacercarial membrane; c, cyst lumen; i, lamellated inner layer; mt, metacercarial tegument. Scale bar = 0.6 µm.

DISCUSSION

Ultrastructural and histochemical studies on the cyst wall of metacercariae of free- and tissue-encysting trematodes revealed considerable variations in formation, structure and chemical composition. The cyst wall of free-encysting trematodes, i.e., species of the families Fasciolidae, Notocotylidae, Parorchidae, and Philophthalmidae, is characterized by a complex multi-layered structure composed of outer fibrous and inner lamellate (keratinized) layers (Dixon and Mercer, 1964; Pike and Erasmus, 1967; Rees, 1967; Asanji and Williams, 1973). Structural complexity of the cyst wall of free-encysting trematodes has been associated with provisions of mechanical and chemical protection against various environmental conditions. In contrast, the cyst wall of trematodes which encyst after penetrating into the tissue of vertebrate intermediate hosts, i.e., species of the families Bucephalidae, Cryptogonimidae, Diplostomatidae, and Heterophyidae, is characterized by a bi-layered wall consisting of an outer fibrous capsule of collagen produced by the host fibroblasts and an inner multi-layered wall of non-cellular, homogenous materials of parasite origin (Stein and Lumsden, 1971; So and Wittrock, 1982; Armitage, 2000). In invertebrate intermediate hosts, i.e., crustaceans (Walker and Wittrock, 1992) and molluscs (Irwin *et al*., 1984; Huffman, 1986), metacercariae are usually found surrounded by an outer thin capsule of host origin and an inner multi-layered wall of parasite origin. In contrast to vertebrate hosts, the defense response of invertebrate hosts to trematode cysts is inconspicuous and cyst usually establishes a loose connection with host tissue. Galaktionov *et al.* (1997) attributed a weak association between invertebrate tissue and trematode cysts to the fact that most invertebrates serve as means of dispersion and transmission rather than a nutrient source to support morphogenesis of the parasite.

Results of the present study on the ultrastructure of metacercarial cyst wall of *A. spinulosum* are consistent with previous observations on the cyst wall of other echinostomatid trematodes, i.e., *Echinostoma paraensei* (Stein and Basch, 1977) and *Himasthla quissetensis* (Kirschner and Bacha, 1980) encysted *in vitro*, *E. revolutum* from the snails *Physa* and *Lymnaea* (Gulka and Fried, 1979), *E. trivolvis* and *E. caproni* from the snail *Biomphalaria* (Irwin and Fried, 1990), and *H. leptosoma* from the cockle *Cardium edule* (Irwin *et al.*, 1984). The cyst wall surrounding echinostomatid metacercariae consists of three layers of parasite-derived structure composed of particulate outer and middle layers and a lamellate inner layer with some differences in the thickness and coarseness of the different layers which have been attributed to different parasite and host species and age of the cyst. In *Cerithidea cingulata*, the cyst wall enclosing *A. spinulosum* metacercariae is distinguished by a thick outer granular layer condensed into a bi-lamellate

membranous capsule. This unique protective structure is probably the product of host-parasite interaction in a long-lived marine iteroparous gastropod, i.e., *C. cingulata*, compared to relatively thinner outer layer surrounding species found in short-lived freshwater semelparous snail hosts.

It has been speculated that layers of trematode metacercarial cyst wall originate from cystogenous glands found in the sub-tegumental region of the cercaria and metacercaria (Stein and Lumsden, 1971; Stein and Basch, 1977), although, mechanisms involved in the release of these vesicles and factors which influence their polymerization into homogenous material of the cyst wall still remain unknown. Galaktionov *et al.* (1996, 1997) reported accumulation of granular and fibrous materials beneath the cyst wall of microphallid metacercariae and suggested incorporation of these materials in the inner layers of the cyst wall. The present study provided ultrastructural evidence for degranulation of cystogenous vesicles in the lumen of the cyst and incorporation of the granules into structure of lamellae of the inner layer of the cyst wall. Similar ultrastructural observations have been reported by Mitchell and Crang (1976) and Meade and Garza (1985) regarding the structuring of metacercarial cyst wall of the diplostomatid trematode *Posthodiplostomum minimum*. The present study also provided evidence which indicate the disassociation of old lamellae, in the upper strata of the inner layer of the wall, into fine granular particles scattered freely or arranged in a reticulate configuration in the middle layer. These observations concur with the ultrastructural study of *in vitro* excystation of *Echinostoma trivolvis* and *E. caproni* by Irwin and Fried (1990), which showed spreading and diffusion of the cyst wall lamellae prior to disassociation of the wall and metacercarial emergence from the cyst.

Findings of the present study contribute to the formation of trematode metacercarial cyst wall by showing that layers of the wall are not necessarily successive products of parasite glands but rather a dynamic process which involved polymerization and disassociation of secretory materials. Understanding the composition and structural dynamics of trematode metacercarial cyst wall requires further ultrastructural and histochemical investigations of cysts of different trematodes from different host species.

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Journal of Parasitic Diseases: December 2007, Vol. 31, No. 2, 114-119 *Original paper*

On a new species, *Cathetocephalus leucas* (Tetraphyllidea: Cathetocephalidae) from the bull shark, *Carcharhinus leucas* (Valenciennes, 1839) from Bay of Bengal, Visakhapatnam coast, Andhra Pradesh, India.

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ABSTRACT. A new and rare species of *Cathetocephalus* was collected from the spiral valve of bull shark, *Carcharhinus leucas* (Valenciennes, 1839) at Visakhapatnam coast. Four parasites were obtained from the host, and were characterized by the presence of characteristic and unique v-shaped scolex with the two arms drawn into thin, narrow and slender ribbon-like structures at the tips. This feature of scolex separates these parasites from the already reported species of the genus, *C. thatcheri* Dailey and Overstreet, 1973; *C. australis* Schmidt and Beveridge, 1990 and *C. limbatus* Pramanik and Manna, 2006. The present specie is named as *Cathetocephalus leucas*.

Keywords: *Carcharhinus leucas*, *Cathetocephalus leucas*, scolex, spiral valve

INTRODUCTION

Sharks among elasmobranchs constitute an important group of fishes in east coast of India due to their abundant occurrence, cosmopolitan distribution and high marketable value. They have an escalating pace of demand due to high human consumption rate, medicinal value and decorative purposes. They serve as excellent hosts for a range of host-specific parasites, which can be used as 'biological indicators'. Sharks offer an exceptional habitat to a wide spectrum of cestode parasitic fauna, in particular diphyllids, tetraphyllids, lecanicephalids and trypanorhynchids. Literature review supported the fact that fairly, a good amount of work on cestode parasites of elasmobranches has been contributed from all over the world but taxonomic work on cestodes of sharks is infrequent and is contributed by Linton (1921,1924), Sproston (1948), Young (1954), Dailey and Overstreet (1973), Rego and Mayer (1976), Rego (1977), Cheung *et al*.,(1981), Shinde *et al*., (1984), Sarada *et al*., (1984, 1986), Caira (1985, 1986, 1990, 1992), Dailey and Vogelbein (1990), Nock and Caira (1988), Schmidt and Beveridge (1990), Caira and Ruhnke (1990), Caira and Gavarrino (1990), Caira and Runkle (1993), Cislo and Caira (1993), Ruhnke (1996), Vijayalakshmi *et al*., (1996), Caira *et al*., (1997), McKenzie and Caira (1998), Scholz *et al*., (1998), Brooks *et al*., (1999) and Caira *et al*., (1999). The comprehensive study on the systematics of cestode parasites of sharks from this coast is lagging behind and not yet fully exposed and such studies on cestodes of sharks from this region will enable taxonomists to disclose many new taxa, which are still awaiting discovery. In the present study, a new and a

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rare species, *Cathetocephalus leucas* sp. nov is described from the spiral valves of *Carcharhinus leucas*. The identification of the genus was done by following the standard books like Yamaguti (1959), Schmidt (1986), Khalil *et al*., (1994) and recent literature pertaining to the genus (Dailey and Overstreet, 1973; Schmidt and Beveridge, 1990; Pramanik and Manna, 2006)

MATERIAL AND METHODS

About 125 *Carcharhinus leucas* (Valenciennes, 1839) were examined thoroughly for the cestode parasites in a survey to study cestode parasitic fauna from the sharks at Visakhapatnam coast, Bay of Bengal. Spiral valves of the intestine offer an excellent habitat to cestode parasites. Spiral valves were separated into physiological saline to remove excess mucus and were cut open with a longitudinal incision. Parasites were carefully isolated from the spiral valves and collected in petridishes filled with saline solution. Due care was taken to prevent the scolex as well as strobila of parasites from being damaged as scolex being the key feature for the identification of a cestode. Parasites were flattened between two slides or under the pressure of slide and a coverslip, post fixed in A.F.A (alcohol, 85 ml; formalin, 10 ml and acetic acid, 5 ml) and stained with alum carmine. Permanent whole mount preparations were made by employing the conventional techniques. Figures were drawn with the aid of camera lucida. Measurements are given in millimeters, unless otherwise stated. A new and a rare cestode, *Cathetocephalus leucas* sp. nov was encountered during the study from the spiral valve of the intestine.

RESULTS

Class: Cestoda

Sub-class: Eucestoda Southwell, 1925

Order: Tetraphyllidea Carus, 1863

Family: Cathetocephalidae Dailey and Overstreet, 1973

Genus: *Cathetocephalus* Dailey and Overstreet, 1973

***Cathetocephalus leucas* n. sp.**

Only four specimens were obtained from the spiral valve of a single bull shark, *Carcharhinus leucas* (Valenciennes, 1839) and were found to be deeply embedded into the intestinal wall.

DESCRIPTION (Based on measurement of four parasites, Plate-I, Figs.1-2): Parasites long, thin, creamy white and measure 7–8 cm in length. Worms slightly acraspedote and apolytic in nature. Scolex

Cathetocephalus leucas n.sp

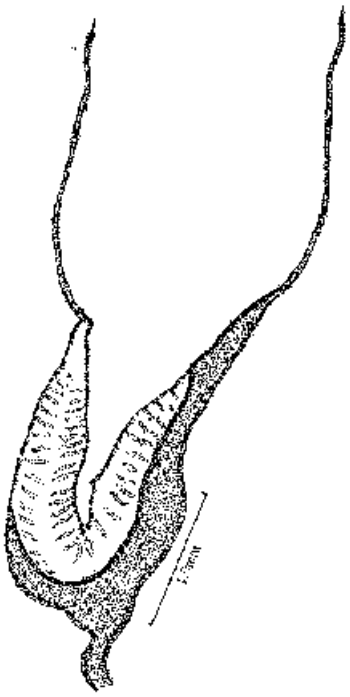

Fig. 1. Scolex

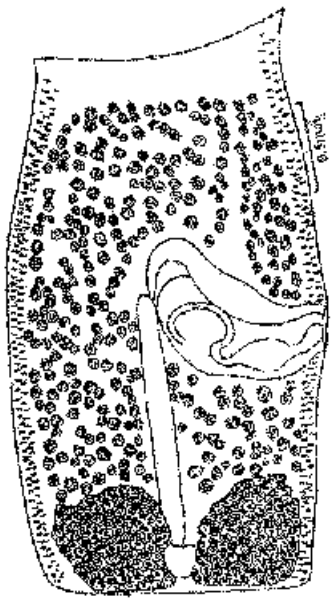

Fig. 2. Mature Proglottid

fleshy, with a rugose dorsal surface and a smooth ventral region. Dorsal surface of scolex bears two parallel rows of fleshy papilliform proliferations on the margins of upper surface of the scolex. Scolex perpendicular with a v-shaped bent in the centre and the two arms being drawn into thin, long, narrow and slender ribbon like structures. These ribbon like structures are thin and delicate and are difficult for separation from tissues during collection. Accessory hooks or suckers not seen. Each arm of the scolex measures 2.50–2.63 from the base and the ribbon measures 2.9–3 long. Neck thick, long and measures 5–6.57 x 0.16–0.18. Strobila comprises 55–110 proglottids. Proglottids 3–4 times longer than broad. Immature proglottids measure 0.58–1.21 x 0.29–0.47. Mature region starts form 0.26–0.83 behind the neck. Mature proglottids measure 2.05–4.08 x 0.32–0.58. Gravid proglottids not obtained. Testes small, 148–280 in number, spherical measuring 0.03–0.08 in diameter. Testes pre-ovarian, filling the full length in medullary region, but not reaching upto the anterior margin of the proglottid. A space is left in the anterior most region of the proglottid characteristically. Cirrus sac large, pear-shaped and measures 0.11–0.34 x 0.20–0.55. Cirrus unarmed measuring 0.08–0.10 in length. Genital pores irregularly alternate and open slightly posterior to the mid margin of the proglottid. Vagina opens anterior to the cirrus and emptying into common genital atrium. Ovary large, with two wing shaped lobes, posterior in position. Each lobe measures 0.2–0.72 x 0.11–0.42. Vitellaria follicular, lateral in position extending in the entire margin of the proglottid in cortical region. Uterus tubular, originates from ovarian isthmus and extends anteriorly.

Host: *Carcharhinus leucas* Valenciennes, 1839

Habitat: Spiral valve of the intestine

Location: Visakhapatnam coast, Andhra Pradesh, India

No. of specimens: Four

Deposition of specimens: Holotype and Paratypes deposited in the Parasitology Laboratory, Department of Zoology, Andhra University, Visakhapatnam.

Etymology: Named after the species name of the host fish.

DISCUSSION

Cathetocephalidae is a small family of the order Tetraphyllidea with *Cathetocephalus* as the only genus of the family. Thatcher (1961) erected this genus with *C. thatcheri* as its type-species from *Carcharhinus limbatus* Val, 1840 in the coastal waters of Mississippi and Louisiana. The genus is characterized by the presence of the perpendicular head and hence the name is derived from greek words “Katheto” and “Kephalos”. At that time, Thatcher considered it as *Pillersium owenium* Southwell, 1927 because of T-shaped scolex but Dailey and Overstreet (1973) re-examined Thatcher's original material and named it as *Cathetocephalus thatcheri*, which was the type and the only species in the genus and erected the family Cathetocephalidae for it. However, in his description, he failed to record the number of testes. Later, Watson and Thorson (1976) redescribed this species from Lake Nicaragua and recorded the number of testes to be 300-400. Schmidt (1986) also recognized a fourth family, the Cathetocephalidae Dailey and Overstreet, 1973, within the Tetraphyllidea in addition to the Onchobothriidae, Phyllobothriidae and Triloculariidae. Euzet (1994), in a most recent and comprehensive treatment of the group, recognised eight families of tetraphyllideans of which Cathetocephalidae was one among them.

Schmidt and Beveridge (1990) after a long span of time described a second species, *C. australis* of the family Cathetocephalidae from the spiral intestine of *C. amblyrhynchoides* from Australian waters. Small scolex, craspedote proglottids, small ovarian lobes, less number of testes and follicular vitellaria, characterize this species. Pramanik and Manna (2006) described *C. limbatus* from the spiral valves of *Carcharhinus limbatus* from India, which is characterized in having scolex with four suckers, oval cirrus pouch, U-shaped ovary and follicular vitellaria.

The present species shows some resemblances and differences with these three species. It comes closer to *C. thatcheri* in not having suckers but differs in many characters like number of testes, number of proglottids, craspedote strobila and shape of scolex. It resembles *C. australis* in having follicular vitellaria and number of testes but differs in the shape of scolex, number of proglottids, position of genital opening, and nature of strobila. It comes closer to *C. limbatus* in having follicular vitellaria, acraspedote strobila but differs in the shape of scolex and number of testes. The present parasites characteristically differ from all these three species in having an unique scolex with an upward bent in the centre, giving v-shape to the scolex with its two arms drawn into a narrow, slender ribbon

Table I. Comparison of present new species of *Cathetocephalus* with other related species of the genus

	C.thatcheri Dailey et Overstreet, 1973	*C.australis* Schmidt & Beveridge, 1990	*C.limbatus* Pramanik & Manna,2006	Present new species
1. Host	*Carcharhinus leucas* (Val, 1839)	*C.amblyrhynchoides* (Whitley, 1934)	*Carcharhinus limbatus* (Val, 1840)	*Carcharhinus leucas* (Val, 1839)
2. Size	10.1-10.5 cm	5 cm	4.80 cm	7-8 cm
3. No. of proglottids	228-325, Slightly craspedote	Slightly craspedote	94-98, slightly acraspedote	55-110, Slightly acraspedote
4. Size of scolex	4-10	1.12-2.9	1.7 with four suckers	2.5-2.63 x 0.5-0.7, suckers not seen
5. Width at broader Surface	0.55-1.5	0.5-0.96	-	
6. Neck	0.55-1.5	0.21-0.44	2.26 x 0.3	5.0-6.57 x 0.16-0.18
7. Mature proglottids	2.9-4.1 x 0.86-1.21	0.92-1.68 x 0.77-1.60	1.71 x 0.54	2.05-4.08 x 0.32-0.58
8. Gravid proglottids	5.5 x 1.0	1.68-2.98 x 1.38-1.76	-	-
9. Testes	300-500, not reaching anterior margin of proglottid	125-205, occupies anterior margin of proglottid	132-134, reaching upto anterior margin of proglottid	148-280, not reaching upto anterior margin of proglottid.
10. Cirrus sac	0.53-0.59 x 0.25-0.34	0.30-0.63 x 0.16-0.32	0.3 x 0.2	0.11-0.34 x 0.20-0.55
11. Genital opening	Lateral, irregularly alternate, slightly posterior mid region of proglottid	Lateral, irregularly alternate, mid region of proglottid	mid-portion of segment	lateral, irregularly alternate, slightly posterior to mid region of proglottid
12. Ovary	dumb-bell shaped 0.99-1.13 x 0.34-0.50	0.20-0.40 x 0.40-0.84	U-shaped, 0.45 x 0.2	Winged, 0.2-0.72 x 0.11-0.42
13. Vitellaria	Granular, not extending entire length of proglottid	Follicular, forming a sleeve around vitellaria	Follicular, 2-3 rows	Follicular

* Note: Measurements are given in millimeters, unless otherwise stated.

like structures at tips. A table comparing the present parasites from the already described species is given in Table-I. Based on these differentiating characters, it is felt justified erecting it to the status of a new species.

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Journal of Parasitic Diseases: December 2007, Vol. 31, No. 2, 120-127 *Original paper*

Scanning electron microscope studies on the surface topography of a few digeneans (Family: Didymozoidae)

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ABSTRACT. The surface topography of *Allodidymozoon operculare, Didymocystoides singularis and Platocystoides polyaster* was studied by using a scanning electron microscope. The present study revealed distinct differences in the surface topography of different didymozoid parasites. In *A. operculare,* the dorsal surface is rough with pits and sensory papillae, whereas in *D. singularis,* the dorsal and dorso-lateral surfaces are characterized by tubercles, folds, pits and spines. Pectinate spines are common at the edge of the anterior region of *P. polyaster*. Different types of papillae were observed on the dorsal, dorso-lateral and ventral surface of *P. polyaster, A. operculare* and *D. singularis.*

Keywords: ***Allodidymozoon operculare, Didymocystoides singularis, Platocystoides polyaster,* scanning electron microscope**

INTRODUCTION

The digenetic trematodes comprise a bewildering array of families, genera and species, which differ in their shape, size, number, location, size of suckers, length of intestinal crura, flame bulb arrangement and, especially, the details of reproductive system (Hyman, 1951). Most digeneans are endoparasites in the digestive tract and in various other organs of vertebrates, but several species are secondarily adapted to an ectoparasitic life (Rohde, 1982). Trematodes of the family Didymozoidae infect mainly marine fishes, and rarely fresh water ones. They are usually found encysted in pairs on gills, fins, scales and stomach wall of fishes, and possess several unique and unusual features by virtue of which they receive special attention.

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The knowledge of Didymozoids from fish of the Bay of Bengal (Chennai coast) is limited, and is restricted to only a few species reported by Job (1961 a, b, c), Madhavi (1982) and Cruz *et al.* (2001). Hence, it was considered worthwhile to record and describe the morphological features of didymozoids collected from the fish barracudas. Scanning electron microscope (SEM) technique has been used to study the topographical structures of the parasites, which provide valuable data for the understanding of their functional morphology, and supplements the findings obtained by light microscopy; such studies are necessary for solving taxonomical problems. The present study describes the surface topography of *Allodidymozoon operculare, Didymocystoides singularis* and *Platocystoides polyaster.*

MATERIALS AND METHODS

The didymozoids were found to infest different parts of the host *viz.*, *A. operculare* on gill epithelium of S*phyraena obtusata*, *D. singularis* on pharyngeal tissues of *S. obtusata* and *S. jello,* and *P. polyaster* on

scales of *S. obtusata*. Fresh specimens were collected from *S. obtusata* and *S. jello*. The parasites were processed for SEM studies following the procedure described by Veerakumari and Munuswamy (1999). The worms were washed with phosphate-buffered saline (pH 7.2) several times to free them from mucus and then fixed in 4 % glutaraldehyde (pH 7.2) over night at 4°C. The worms were washed several times again in cold 0.1M phosphate buffer. The worms were post-fixed in 1% osmium tetroxide prepared in 0.1M phosphate buffer for 4 h at 4°C. They were subsequently washed in distilled water and then dehydrated in graded alcohol series. After this process, the specimens were transferred to 100 % acetone for one h, dried and the worms were glued on metal standard stubs, coated with gold in vacuum and examined by using a SEM Hitachi 3415A. Photomicrographs were taken at various magnifications with an Exacta Exa 1 camera, using Ilford Fp4 type film.

RESULTS

Pertinent features of the integumental surfaces of adult *A. operculare, D.singularis* and *P. polyaster* as observed by SEM are depicted in Fig. 1–21.

In *A. operculare,* the body is divisible into fore and hind-body and the ventral surface of the tegument is rough (Fig. 1). The ventral surface of the fore-body possesses conical shaped projection (Fig. 2). The hind body shows ridges and folds which are arranged like a network giving a honeycomb appearance (Fig. 3 and 4). The dorsal surface is rough with pits and sensory papillae (Fig. 5). The eggshells are oval in shape with an operculum. The egg shells measure 6.4 × 4µ (Fig. 6).

In *D. singularis*, the dorsal and dorso-lateral surfaces are characterized by tubercles, folds, pits and spines (Fig. 7–10). The tubercles are numerous on the mid-dorsal surface. The tegumental spines appear as pointed structures (Fig. 10), lack dentition and are mainly distributed on the lateral and dorsal surfaces. Dome shaped sensory papillae and microvilli like projections are observed on the ventral surface (Fig. 11 and 12). Genital aperture is present on the ventral surface (Fig. 13). Towards the periphery, the microvilli are arranged irregularly on the annulated surface and cobblestone like projections are also present (Fig. 14).

P. polyaster occur always in pairs underneath the scales of *S. obtusata*, pressed between the ventral surfaces of two individuals and with their respective central portion in contact at right angles, securely interlocked by folds of the body arranged in a radiating manner in the central region of the disc (Fig. 15–18). The folds on the edges further strengthen the contact. The periphery of the anterior and posterior region of the ventral surface is both thin and smooth. The fore-body arises from the center of the ventral surface, which is slender at its base and globular at its tip (Fig. 19). The periphery of the dorsal surface is rough with furrows, spines, pits and papillae. Most of the papillae are dome shaped, few are pitted and very few are uniciliated (Fig. 20). Pectinate spines are common at the edge of the anterior region of the parasite (Fig. 21).

DISCUSSION

The diversification and modification in the integumental structures of digenetic trematodes can be considered as parasitic adaptations to individual microhabitats (Abidi *et al*., 1988). The integument of trematodes is generally considered as a protective sheath. Morris and Threadgold (1968) reported that the integument aids in absorption. Integument also plays a vital role in secretion, excretion (Silk *et al*., 1969) and osmoregulation (Sneft *et al*., 1961). The tegument of trematode thus plays important role in various physiological functions; however, only incidental attention has been given to the architecture of the integument of didymozoids.

The present investigation clearly reveals that there is diversification with reference to surface topography of didymozoids. These variations may be due to various measures including adaptations of the parasite to microenvironment as the niche of each parasite differs. This is supplemented by the findings of Abidi *et al*. (1988), who observed difference in tegument structures of various digeneans occupying various niches and suggested that it is a parasitic adaptation.

The tegumental folding, ridges, furrows and lamellar network impart considerable stretching capability to the didymozoids. These structures also increase the surface area for absorption of micro molecular nutrients (Abidi *et al.*, 1988). The ridges on the integument are either due to longitudinal anterior constriction (Bakke and Lien, 1978) or due to internal musculature (Nollen and Nadakavukaren, 1974).

Similar tegumental folding, ridges and furrows observed in all the species of didymozoids in the present study on the dorsal and dorso-lateral surfaces suggest that surface structures may perform the same

function like absorption and stretching ability as seen in other trematodes. In *P. polyaster*, prominent ridges and furrows are seen in a radiating manner from the central portion of the dorsal surface and they may be helpful in interlocking mechanisms. It is presumed that these surface structures have more than one function. Since didymozoid parasites occur in pairs, the furrows and ridges on the ventral and dorsal surfaces of the parasites may serve for attachment and may also help in absorption. Ogbe (1982) described tegumental folds and pits on the surface of adult male and female *Schistosoma margrabousei* and suggested that these may help in increasing the surface area for the uptake of materials from the blood stream environment. Brennen *et al*. (1991) observed that the extensive folding of the tegument in *Gastrodiscoides hominis* indicate a role in osmotic and ionic regulation.

Two types of papillae, conical with a central cilium and dome shaped without cilium commonly occur in digenetic trematodes (Edwards *et al.*, 1977; Hode and Mitchell, 1981). The dome shaped papillae seem to be of a fundamental type as they have been frequently recorded in trematodes. Domed papillae, smooth, spined or with apical cilia commonly occur in trematodes and it has been suggested that they have a sensory function. Several types of papillae on the tegument of intestinal flukes have been reported. Domed, oval, bilobed and button types are reported on the tegument of adult *Echinostoma revolutum* and *Isthimophora melis* (Smales and Blankespoor, 1984). Uniciliated papillae were reported in some digeneans such as adult *E. malayanum* (Tesana *et al*., 1987) and *G. hominis* (Brennen *et al*., 1991).

In the present study, three types of papillae are recorded on the dorsal and dorso-lateral surface of *P. polyaster* namely dome-shaped, pitted and uniciliated. The domed papillae are numerous. In *A. operculare* and *D. singularis* only the domed papillae were recorded. Each type may represent specific sensory function. Abidi *et al*. (1988) reported that the non-encysted metacercariae of *Clinostomum complanatum* is in an active feeding stage and, therefore, the dome shaped sensory papillae around the oral sucker may act as tango receptors. The pit type papillae on the ventral surface of the body of didymozoids may have chemo receptive function as suggested by Smyth and Halton (1983).

The dorsal surface of *D. singularis* bears numerous tubercles. Similar structures have been reported in *S. mattheei* (Tulloch *et al*., 1977), *S. bovis* (Kuntz *et al*., 1979), *S. curassoni* and *S. haematobium* (Southgate *et*

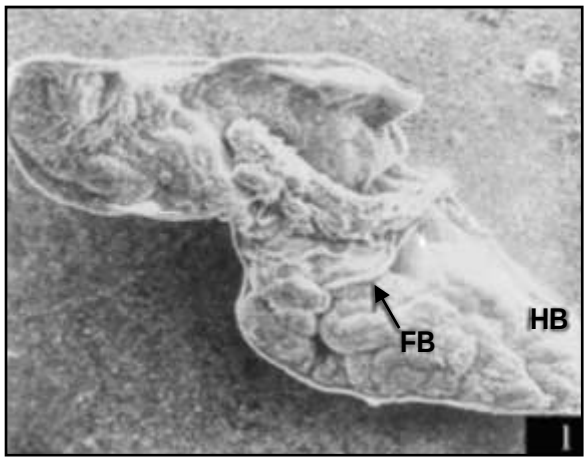

Fig. 1. *A. operculare*-ventral view × 60 (FB-fore body; HB-hind body).

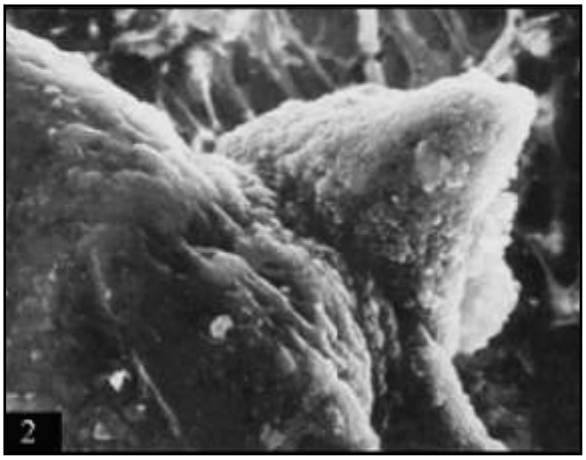

Fig. 2. The rough surface of *A. operculare* showing tegumental projection × 1875.

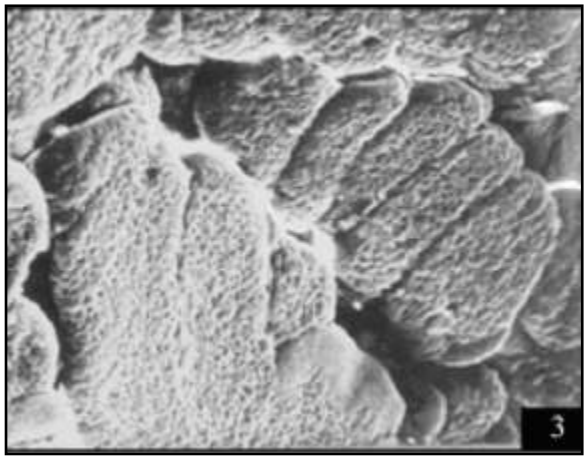

Fig. 3. Fore body of *A. operculare* showing spongy honey comb appearance × 240.

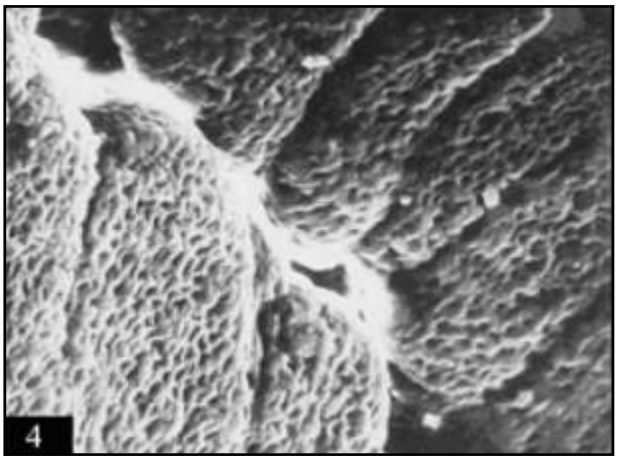

Fig. 4. Ventral surface of *A. operculare* under higher magnification × 480.

Fig. 5. Dorsal surface with pit (long arrow) and sensory papillae (small arrow) × 1250.

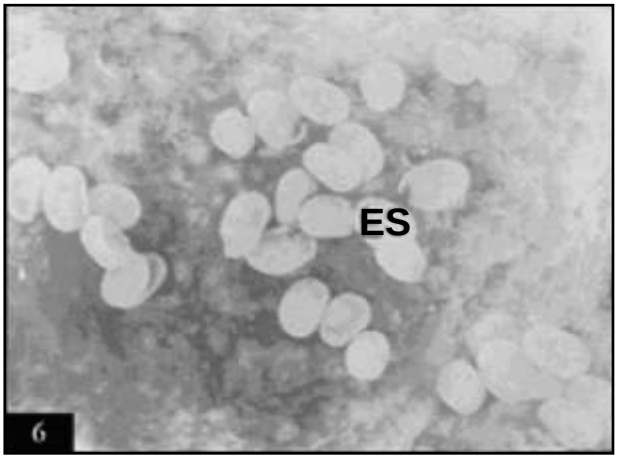

Fig. 6. Egg capsule of *A. operculare* × 1250. (ES-egg shall)

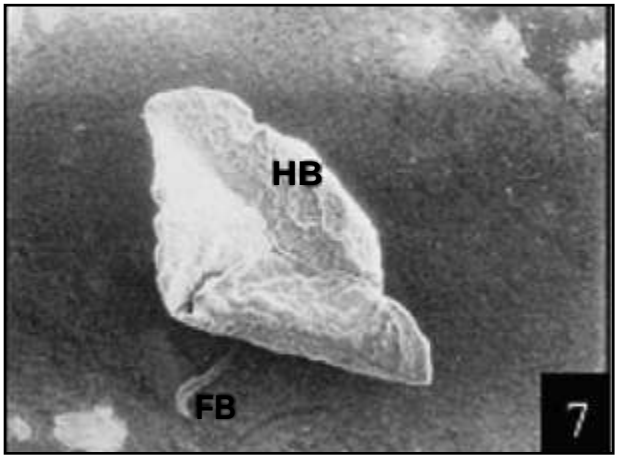

Fig. 7. *D. singularis* dorsal view × 30 (FB-fore body; HB-hind body).

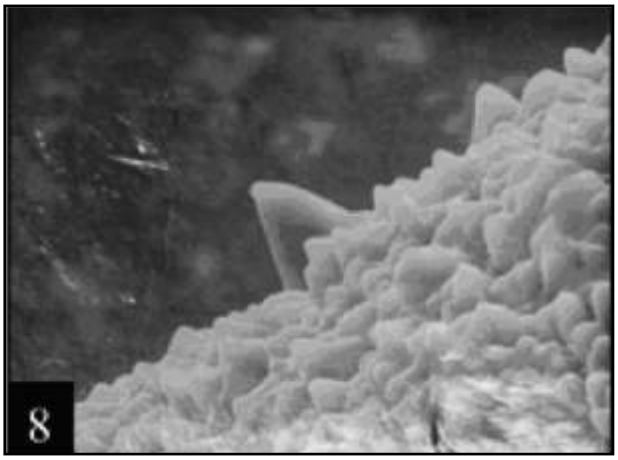

Fig. 8. Dorsal surface of *D. singularis* showing tubercles × 1875.

Fig. 9. Dorsal surface of *D. singularis* showing folds (small arrow) with tubercles and pits (long arrow) × 2812.

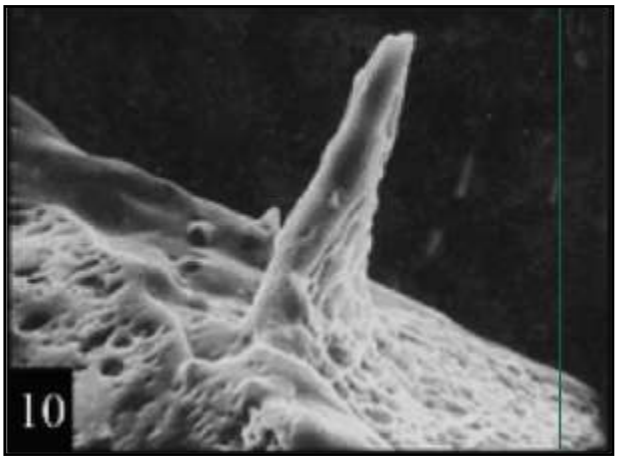

Fig. 10. Dorsal surface showing spine × 1440.

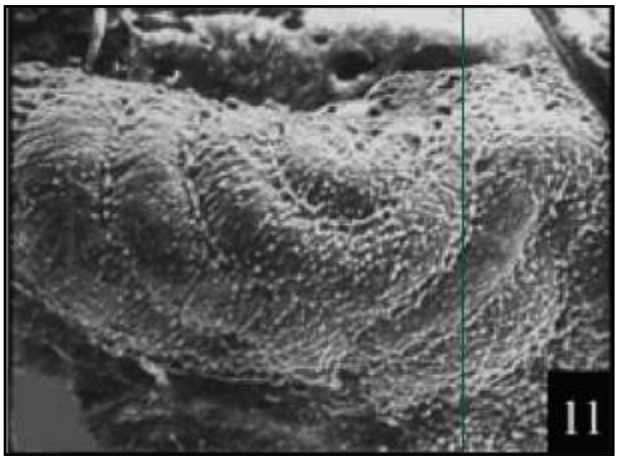

Fig. 11. Ventral surface showing annulations with papillae × 512.

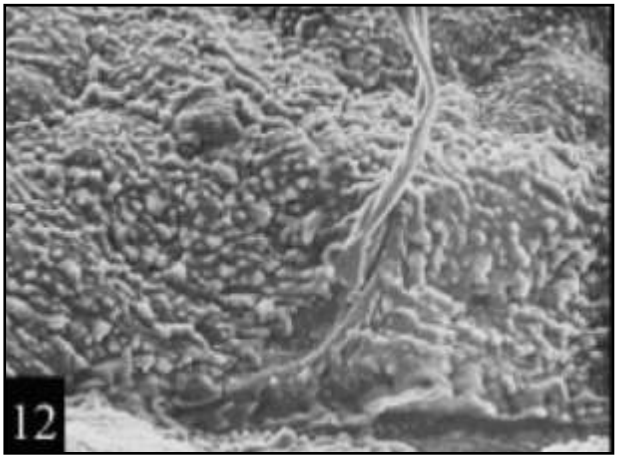

Fig. 12. Ventral surface showing microvilli like structures ×960.

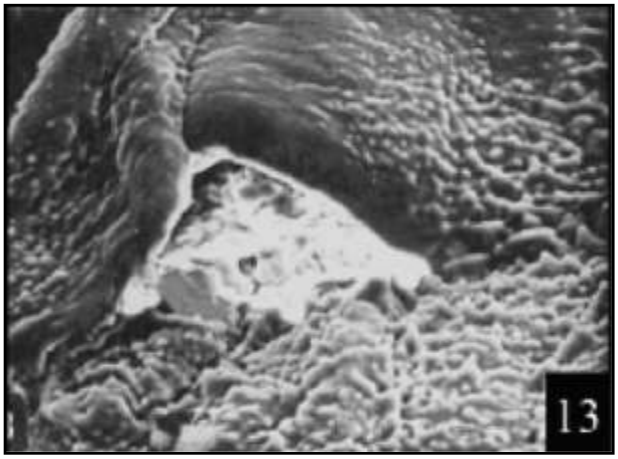

Fig. 13. Ventral surface showing external genital pore × 960.

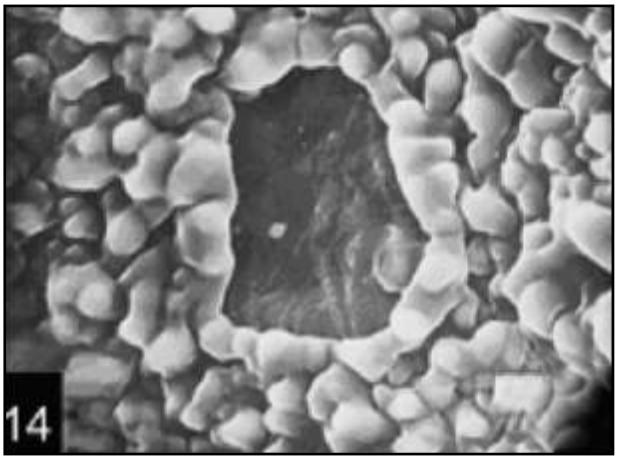

Fig. 14. Ventral surface showing cobblestone like protrusions × 2812.

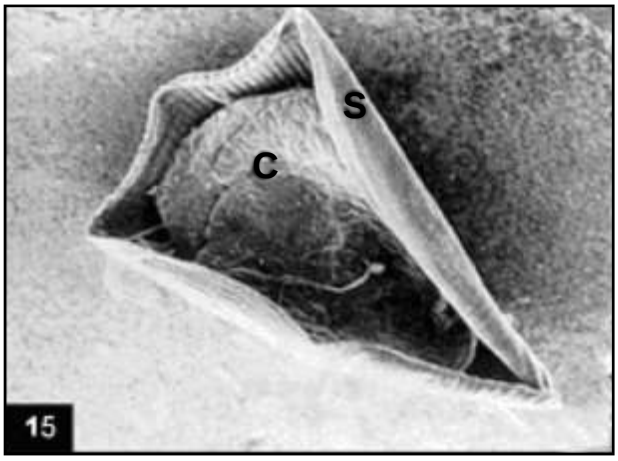

Fig. 15. Encysted *P. polyaster* on the scale (C-cyst; S-scale) × 20.

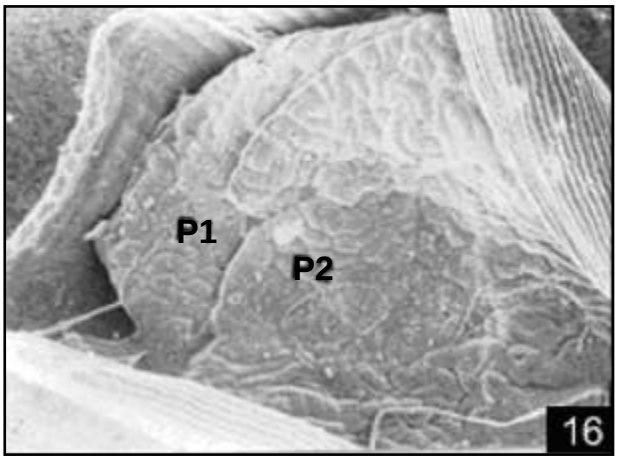

Fig. 16. A pair of *P. polyaster* parasites (P1, P2) with in a cyst × 40.

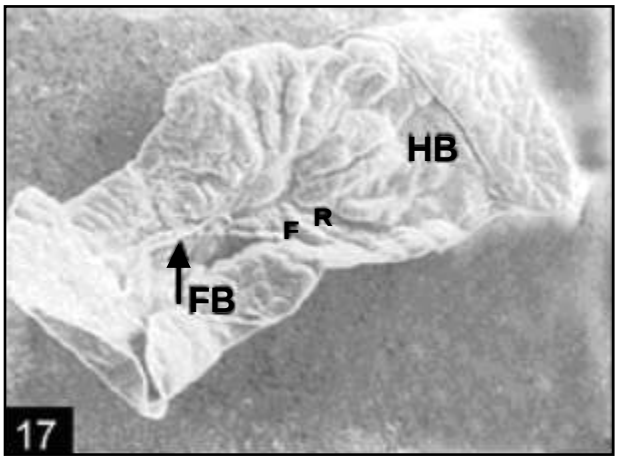

Fig. 17. Entire *P. polyaster* showing ridges (R) and furrows (F) × 40 (FB-fore body; HB-hind body).

Fig. 18. Ridges and furrows on the central region under higher magnification × 80.

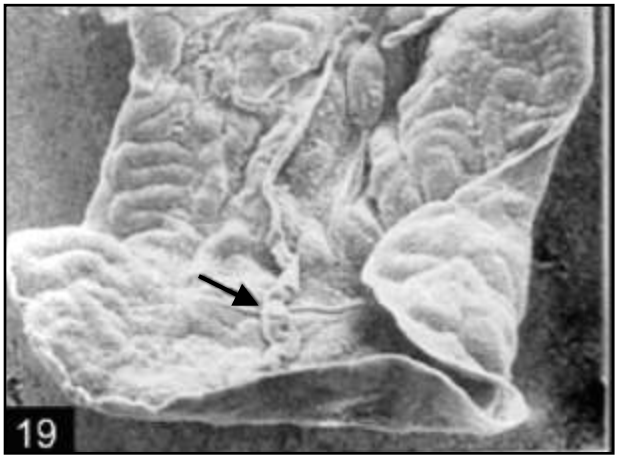

Fig. 19. Sac like fore body (arrow) of *P. polyaster* × 80.

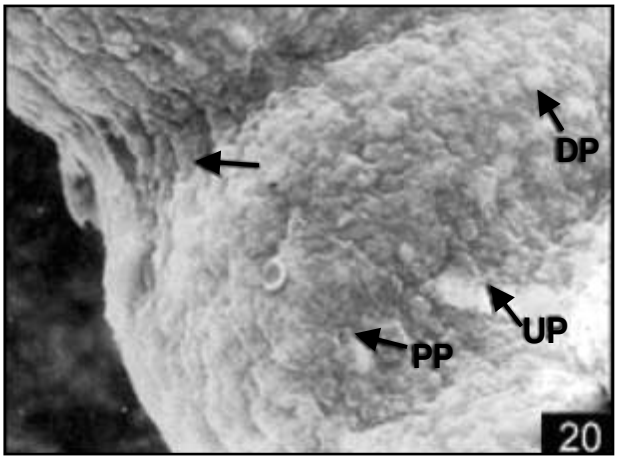

Fig. 20. Dorso-lateral surface of *P. polyaster* showing sensory papillae (DP-dome shaped papillae, PP-pitted papillae; UP-uniciliated papillae), ridges and furrows on the edges (arrows)×1250.

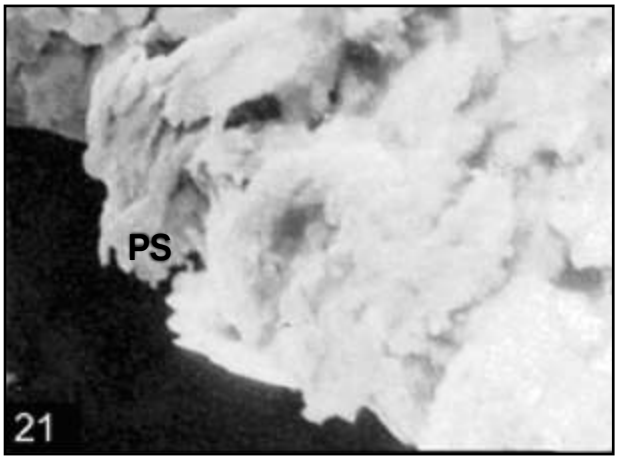

Fig. 21.Ventral surface of *P. polyaster* showing numerous pectinate spines (PS) on the periphery × 2500.

al., 1986). In *S. mansoni*, the adult integument is characterized by the presence of large tubercles bearing numerous spines that account for the roughness of the surface (Silk *et al.*, 1969; Miller *et al.*, 1972). The tegument of the paramphistome, *G. hominis* is folded in to concentrically arranged furrows and ridges bearing numerous tightly packed tubercles (Brennen *et al*., 1991). Prominent spines similar to *D. singularis* have been reported in *Urogonimus macrostomus* (Bakke, 1978), *Phyllodistomum conostomum* (Bakke and Lien, 1978), *Leucochloridium variae* (Bakke, 1982) and *Opisthorchis pedicellata* (Pandey and Tewari, 1985). But the presence of undentated spines in the present study reveals that they may have a sensory function as suggested by Smyth and Halton (1983). The spines are absent on the ventral surface of the parasite, probably to help the parasites for smooth sealing against the other parasites. In *F. gigantica*, the absence of spines around the sucker help the parasites for smooth sealing against the host mucosa (Ahmad *et al*., 1988). Spines are absent around the suckers in *F. hepatica* (Bennett, 1975 a, b). Bennett (1975 b) suggested that spine like structures when present on the body surface might function in recording pressure changes as the tegument stretches. This may also be attributed to the spines of the *D. singularis*.

The surface annulations observed in *D. singularis* may be due to the annular arrangement of the internal reproductive structures. In *D. singularis* cobblestone like protrusions may act as tango receptors and may serve in increasing the absorptive surface. Abidi *et al.* (1988) reported that the cobblestone like protrusions might act as tango receptors. The cobblestone like appearance of the tegument may also serve in increasing the absorptive surface much like microtriches of cestodes as reported by Anderson (1975).

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Journal of Parasitic Diseases: December 2007, Vol. 31, No. 2, 128-133 *Original paper*

Evaluation of vector control programmes like indoor residual spray and insecticide-treated bed nets in a malaria endemic area of East Godavari district of Andhra Pradesh

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ABSTRACT. The use of indoor residual spray (IRS) and insecticide-treated bed nets (ITBN) is the most important chemical control tool for vector control, and thereby interrupting the transmission of malaria and other vector borne diseases. In ten tribal dominated malarious Primary Health Centers of East Godavari district of Andhra Pradesh, 134 human dwellings of 15 villages were searched. Satisfactory IRS with malathion was done in only 11.2% houses. Of the 303 living rooms examined, IRS was done only in 13.2% rooms and 76.1% verandahs. Refusal rate in living rooms was noted as 86.7%. This was mainly due to lack of knowledge regarding the importance of IRS and reluctances among villagers to remove domestic articles and dislike of the smell of malathion. Techniques of IRS were found unsatisfactory during concurrent supervision. Only 40% tribal villagers were using ITBN at night. A 60% ITBN retention rate was noted. Out of nine anopheline species in the study area, three vectors of malaria, i.e., *Anopheles culicifacies, An. fluviatilis* and *An. annularis* were noted in both human dwellings and outdoors. Poor coverage (13.2%) and high refusal rate (86.7%) of IRS with malathion along with high retention rate (60%) among ITBN users are the main causes for increasing trend of malaria. This is due to lack of knowledge and motivation in receptor population. High density of three vector species in outdoor sampling was noted.

Keywords: anopheline species, indoor residual spray¸ insecticide-treated bed nets, vector control

INTRODUCTION

Vector control is an important strategy for interrupting the transmission of malaria, especially in areas where the incidence is high. Indoor residual spray (IRS) and use of insecticide-treated bed nets (ITBN) is the most widely practiced effective strategy to reduce man-mosquito contact for malaria control.

Ten out of 69 Primary Health Centres (PHCs) of East Godavari District, Andhra Pradesh, are notoriously known for transmission of malaria, especially due to *Plasmodium falciparum* infection. These tribal dominated, malaria endemic PHCs (Agency Area) are noted under Enhanced Malaria Control Programme (EMCP) since 1997 (Shiv Lall *et al.*, 1998). The area was under annual two rounds of regular DDT spray till 2002, which was later switched over to annual three rounds of IRS with malathion from 2003 onwards. As

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per Government of Andhra Pradesh, Department of Health, East Godavari District, Annual Report 2006, an increasing trend of malaria cases, especially due to *P. falciparum* infection, has been noted from the area for last four years. Very high annual parasitic incidence (API) was also noted in the Agency Area as 18.7, 19.8 and 30.1 in the years 2003, 2004 and 2005, respectively. A survey was, therefore, initiated in the month of September, 2006 in three highly malaria endemic PHCs in East Godavari District to note the quality of ongoing vector control programmes with IRS and use of ITBN by the villagers. A search was also conducted to determine predominant anopheline species, especially vector mosquitoes.

MATERIALS AND METHODS

East Godavari District of Andhra Pradesh is situated in the eastern bank of river Godavari in south India. The district consists of both hilly forest and plain agricultural land area. Out of 69 PHCs, 10 PHCs are tribal dominated, known as Agency Area is situated in the Eastern Ghat hilly range. It is surrounded by forest known as Papikonda Reserve Forest. Since 1997 National Vector Borne Diseases Control Program has been providing additional support of logistics including operational expenses under the enhance malaria control (EMC) Project (EMCP) with the assistance from World Bank (Shiv Lall *et al*, 1998). The total area under Agency has been under three rounds of annual malathion residual spray since 2003. As per Government of Andhra Pradesh, 40,000 ITBN have also been supplied to 1,06,493 tribal people of 10 PHCs in 2006 with a coverage rate of 37.5%.

To note the vector control activities, three highly malaria affected PHCs, i.e., Narasapuram, P. Geddada and Maredumilli were selected for the survey.

A total 15 highly malaria endemic villages. i.e., six villages from PHC Narasapuram, 5 villages of PHC P. Geddada and 4 villages from PHC Maredumilli were selected randomly to note quality of on-going IRS programme both by consecutive and concurrent methods, as per Operational Manual for Malaria Action Programme, (1995) and as per WHO method (2006). Out of 15 villages, 10% human dwellings, both indoor and outdoor, from each village were investigated physically, thoroughly during and just after spray to note the evidence of insecticide spray deposit on walls, number of rooms and verandahs actually sprayed. Number of rooms/houses refused to accept spray and the reasons for refusal etc. was also recorded. This was done to note house and room coverage rate with insecticide as per WHO Guidelines 2006.

Out of five spray teams under operation in three PHCs, concurrent supervisions were done to three spray teams in villages while on spray operation. Supervision was made for preparation of insecticide suspension, discharge rate from nozzle tips, condition and maintenance of the spray pumps and technical knowledge of spray crew.

One hundred and fifty villagers, comprising 100 non-tribal and 50 tribal villagers, received ITBN from Govt. of Andhra Pradesh for personal protection in the study villages. Utilization rate, i.e., No. of people using ITBN in night/No. of people received ITBN (%), retention rate i.e., No. of people retaining (not using) ITBN/ No. of people given ITBN (%) was noted. Average number of holes present in ITBN as deterioration rate was noted as per WHO method 2005.

To record the anopheline fauna present during this survey period, adult *Anopheles* mosquitoes were collected from human dwellings and outdoor situations by spending two h early in the morning (5.30–7.30 hours) and two h in evening (19.00–21.00 h), i.e. one h in each biotope from village Gogumilli of PHC Narasapuram and village Gandhinagar of PHC P. Geddada by standard WHO recommended hand capture technique (Manual of Practical Entomology; WHO, 1975) using an aspirator and flash light, consecutively for six days in the month of September 2006. Total six man-hours were spent in each biotope. In the Agency Area in practice, cattle are kept in open area during night. Thus mosquitoes were collected outdoors, which were resting on cattle. Mosquitoes were brought to the field station and identified by the method of Wattal and Kalra (1961) and Das *et al.* (1990).

RESULTS

Detailed results of on-going malathion spray operation in fifteen villages of three worst malaria affected PHCs Narasapuram, P. Geddada and Maredumilli are shown in Table I. Conditions of IRS were noted in 134 human dwellings of 15 villages, which were sprayed during August–September, 2006. Out of 134 houses, complete and satisfactory spray was done in only 15 (11.2%) houses. Maximum (14.3%) IRS was done in PHC Narasapuram, nearest to Agency head quarter Rampachodavaram and lowest

Table I. Evaluation of ongoing malathion spray operation in malaria endemic villages of East Godavari District of Andhra Pradesh in September, 2006

No. of PHC/ No. of villages searched	No. of dwellings searched/No. covered with IRS (DCR)	No. of rooms searched/No. covered with IRS (RCR)	No. of rooms not covered with IRS (RR)	No. of verandah Sprayed	No. of walls of dwellings sprayed	Total No. of HD refused to accept spray	Reasons for refusal			
							No. notifi-cation	No. knowledge about IRS	Due to inconve-nience	No. mud plastered
Narasapuram /6	63/9 (14.3%)	161/24 14.9%)	137 (85.1%)	40 (63.5%)	42 (66.6%)	54	03 (5.5%)	53 (98.1%)	50 (92.5%)	00
P. Geddada /5	41/4 (9.7%)	82/7 (8.5%)	75 (91.4%)	36 87.8%)	30 (73.1%)	37	16 (43.2%)	31 (83.7%)	30 (81.1%)	04 (10.8%)
Maredumilli /4	30/2 (6.6%)	60/9 (15.0%)	51 (85%)	26 (86.6%)	22 (73.3%)	28	02 (9.1%)	26 (92.8%)	26 (92.8%)	06 (21.4%)
Total 15	134/15 (11.2%)	303/40 (13.2%)	263 (86.7%)	102 (76.1%)	94 (70.14%)	119	21 (17.6%)	110 (92.4%)	106 89.1%)	10 (8.4%)

DCR = Dwelling coverage rate (%)
RCR = Room coverage rate (%)
HD = Human dwellings
RR = Refusal rate (%

(6.6%) to farthest and remote PHC Maredumilli. Out of 134 houses, a total of 303 living rooms were searched, which were targeted by Government for IRS. No spray was done in 263 (86.7%) rooms. Although 82% villagers accepted that they had received advance message from Department of Health Personnel regarding date and time of IRS, 86.7% villagers refused to accept IRS of rooms. On an average 13.2% rooms were covered with IRS. 14.9, 8.5 and 15% rooms were actually covered with IRS in the PHCs Narasapuram, P. Geddada and Maredumilli, respectively. Coverage of verandahs (76.1%) with IRS was, however, noted unsatisfactory due to patchy and/or excess insecticides on verandah walls. Out of 134 houses, surprisingly, it was noted that outer walls of 94 houses (70.14%) were sprayed with IRS.

Out of five spray team engaged by state Government in survey area, activities of only three teams were noted. Out of six stirrup pumps checked, discharge rate and condition of the pumps was not proper in five pumps. Lack of technical knowledge was noted among spray crew.

A total 150 villagers, comprising 100 non-tribals and 50 tribals, from three malaria endemic villages from each PHC were investigated regarding availability and use of ITBN. The results are shown in Table II. None of 100 non-tribesmen received ITBN from Government. The coverage rate with ITBN was noted as 33.3%, which roughly corroborates the State Government Report as 37.5%. Same percentage of tribal community received bed-nets. Out of 50 tribesmen, 20 i.e., 40% are regularly utilising bed nets in night (utilization rate). Retention rate was noted as 60%, which is due to lack of knowledge and motivation.

The density of *Anopheles* mosquitoes was determined from human dwellings and outdoors in villages Gogumilli of PHC Narasapuram and Gandhinagar of PHC P. Geddada are shown in Table III. A total of nine anopheline species were collected. Out of nine species, three were malaria vector species, *An. culicifacies, An. fluviatilis* and *An. annularis*. Outdoor collections were done on cattle. Maximum density of anopheline species collected on cattle from outdoors was not under IRS. All the three vector species were found both human dwellings and outdoor. Man hour density of *An .culicifacies*, *An. fluviatilis* and *An. annularis* human dwelling were noted to be as 0.5, 0.16 and 0.16, respectively. Density of all the nine species of anopheline mosquitoes including three vector species were noted high in comparison to the density recorded from human dwellings as shown in Table III.

DISCUSSION

As per WHO guidelines in 2005, for effective community malaria control measure, IRS requires coverage of at least 85% of dwellings, ensuring that the majority of endophilic and/or endophagic mosquitoes are exposed to the insecticides. As per Government of Andhra Pradesh report 2006, IRS coverage in the study area was more than 90% in Narasapuram, Marredumillin and P. Geddada PHCs.

Table II. Use of ITBN in agency area of East Godavari district in September, 2006

Total No. of villagers searched (50 tribes' people; 100 non-tribes' people)	:	150
No. of tribes' people received ITBN	:	0
No. of tribes' people received ITBN	:	50
Average ITBN Coverage rate	:	33.3%
Total no. of tribes' people covered with ITBN	:	50 (100%)
No. using ITBN	:	20
Utilization rate	:	40%
Retention rate	:	60%
Deterioration rate	:	average 2 holes/net

Table III. List of anopheline mosquito species in study villages of East Godavari District of Andhra Pradesh in September, 2006

Sl. No.	Anopheline mosquito	Man hour density of mosquitoes in (MHD)			
		Human dwelings		Outdoor	
		Total No. collected	MHD	Total No. collected	MHD
1.	*An culicifacies*	03	0.5	150	25.0
2.	*An fluviatilis*	01	0.16	01	0.16
3.	*An annularis*	07	1.16	162	27.0
4.	*An subpictus*	02	0.33	210	35.0
5.	*An vagus*	03	0.5	53	8.3
6.	*An hyrcanus group*	0	0.0	06	1.0
7.	*An majidi*	01	0.16	22	3.6
8.	*An jamesii*	0	0.00	18	3.0
9.	*An splendidus*	0	0.0	8	1.3

In the present observation, very poor house (11.2%) and room (13.2%) IRS coverage was noted (Table I). This grossly differs with the WHO recommendation of at least 85% coverage of IRS of dwellings. However, 76.1% of the verandahs were covered with IRS. Verandah coverage is perhaps noted as house coverage by District Malaria Authority. Refusal rate was noted as 85.1, 91.4 and 85% in Narasapuram, P. Geddada and Maredumilli PHCs, respectively. As per Government of Andhra Pradesh, since long, the areas were under two rounds of IRS with DDT till 2002. After that, the State Government switched over to annual three rounds of IRS with malathion from 2003 onwards. As per WHO 2005, use of IRS by organised malaria campaigns in many parts of the world has frequently shown a progressive development of people's fatigue and reluctance to allow intrusion in to their rooms. This is one of the facts along with the lack of knowledge (92.4%), dislike of smell and reluctance to remove domestic articles (89.1%) being the major reasons for high refusal rate. However, 82.3% villagers received advance information regarding the date and time of IRS in their area. Surprisingly, it was observed that outer walls of 70.14% houses were covered with malathion spray, which grossly violates the norms of IRS. Spray on outer walls of houses dilutes the objective of IRS.

Spray teams are engaged by “Indira Kranti Padam Programme”, a self-help group from village level. Lack of technical knowledge among spray teams was noted regarding preparation of suspension and stirrup pump maintenance etc. Therefore, repeated training on spray technique along with motivation of spray staff is highly essential.

Experts feel that in tribal areas, behavioural change is needed to make ITBN intervention a success. Community distribution of treated nets in acute emergency is an option only if the target community is already in the habit of using nets (WHO 2006). Distribution rate of ITBN in the study area was 33.3% only. No non-tribal population in the high risk area received ITBN, which definitely dilutes the objective of control of malaria. High retention rate is also a matter of concern.

High IRS refusal rate, high ITBN retention rate will definitely come in the way of satisfactory prevention of man-mosquito contact. As a base line study, in view of these findings, it is urgently needed to review malaria vector control operations and it should be implemented more scientifically after proper motivation to both villagers and health staff under strict supervision.

Joshi *et al*. (1998) failed to collect *An. culicifacies*, the notorious vector of malaria from East Godavari district of Andhra Pradesh. In this present study three vector species *An. culicifacies*, *An. annularis* and *An. fluviatilis* were noted along with six other anopheline species. Maximum number of vector species were found from out door situation. This may be due to constant insecticidal pressure in unscientific way on the existing species in the area.

From the above mentioned observation, it may be mentioned that poor IRS coverage (13.2%), tendency to spray outer side of houses, poor technical knowledge among spray staff along with high retention rate (60%) among ITBN users and exophilic nature of vectors in the area are the main cause for transmission of malaria in the study area. Therefore, it is urgently necessary to evaluate ongoing vector control operation scientifically and it is also highly essential to implement vector control operation as per WHO and Government of India guidelines under strict supervision by experts. Mass awareness and social mobilization need to be streamlined during and after malaria control operation to achieve maximum benefit.

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Epidemiology of caprine gastrointestinal helminthic infection in central zone of Vidarbha region, Maharashtra state

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ABSTRACT. The epidemiology of caprine gastrointestinal helminthic infection revealed 63.03% parasitism in central zone of Vidarbha region of Maharashtra state. The prevalence in monsoon, post-monsoon, winter and summer seasons was 76.35, 71.02, 67.31 and 45.91%, respectively. The prevalence recorded in goats < 1 yr, between 1-2 yrs, between 2-4 yrs and > 4 yrs was 67.65, 60.93, 58.71 and 64.91%, respectively. The sex-wise prevalence was 65.66% and 61.52 % in male and female goats, respectively. The occurrence of nematode, trematode, cestode and mixed type of helminthic infection was 44.90, 6.25, 5.00 and 43.84%, respectively. The prevalence of *Haemonchus* sp., *Trichuris* sp., *Strongyloides papillosus, Trichostrongylus* sp., *Oesophagostomum* sp., *Bunostomum* sp., *Fasciola* sp., *Paramphistomum* sp., *Moniezia* sp. and mixed infection with *Haemonchus* sp., *Trichuris* sp. and *Moniezia* sp. was 40.24, 30.90, 25.81, 15.69, 14.06, 8.12, 3.09, 2.66, 3.15 and 25.51 %, respectively.

Keywords: gastrointestinal helminths, goat, Maharashtra state, Vidarbha region

The data pertaining to season, age and sex-wise prevalence of caprine gastrointestinal (GI) helminthic infection from central zone of Vidarbha region are presented in Table I. The season-wise prevalence of helminthic infection in monsoon, post-monsoon, winter and summer seasons was 76.35, 71.02, 67.31 and 45.91 %, respectively; on statistical analysis the difference was found to be highly significant ($p < 0.01$), which is in consonance with the findings of Maske *et al.* (1990) and Bedarkar *et al.* (2000), who have reported high prevalence of helminthic infection during rainy season from different parts of Maharashtra state. The present study demonstrated that the prevalence increases from the end of summer, reaches its peak during monsoon (September) and then declines during summer (May). The infection rate in goats of < 1 yr, 1-2 yrs, 2 - 4 yrs and > 4 yrs was 67.65, 60.93, 58.71 and 64.91 %, respectively. The difference in infection rates in various age groups was found to be non-significant at ($p > 0.05$) and ($p > 0.01$), which indicate that age of animals had negligible effect on the occurrence of helminthic infection in central zone of Vidarbha region. The infection in males and females was recorded to be 65.66 and 61.52 %, respectively, which differed non-significantly ($p > 0.05$).

The prevalence of nematode, trematode, cestode and mixed infections was 44.90, 6.25, 5.00 and 43.84 %, respectively. Higher intensity of nematodes followed by trematodes and cestodes conforms to the findings of Deka *et al.* (1995) and Talukdar (1996). The host and environmental factors being same, the probable cause of variation in intensity could be different modes of infection. In the case of nematodes, where contamination of food and water is the source of

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Table I. Prevalence of caprine GI helminthic infection in central zone of Vidarbha region, Maharashtra

Parameter	Components	No. examined	No. positive	%
Season**	Monsoon (Jul-Sept)	406	310	76.35
	Post-monsoon (Oct-Nov)	283	201	71.02
	Winter (Dec-Feb)	410	276	67.31
	Summer (Mar-Jun)	551	253	45.91
Age	< 1 yr	405	274	67.65
	Between 1-2 yrs	407	248	60.93
	Between 2-4 yrs	419	246	58.71
	> 4 yrs	419	272	64.91
Sex	Male	600	394	65.66
	Female	1050	646	61.52

** Significant $p < 0.01$

infection, which is favourable to maintain the infective stages throughout the year, hence maximizing the probability of infection. On the other hand, in the case of trematode and cestodes, snail and insect intermediate hosts are the source of infection, and thus minimizing the probability of infection. The prevalence of *Haemonchus* sp. (40.24%), *Trichuris* sp. (30.90%), *Strongyloides papillosus* (25.81%), *Trichostrongylus* sp. (15.69%), *Oesophagostomum* sp. (14.06%), *Bunostomum* sp. (8.12%), *Fasciola* sp. (3.09%), *Paramphistomum* sp. (2.66%), *Moniezia* sp. (3.15%) and mixed infection with *Haemonchus* sp., *Trichuris* sp. and *Moniezia* sp. was 25.51%. The occurrence of GI helminths in goats has been documented from different parts of country (Misra, 1972; Chellappa and Gopalkrishnan, 1977; Katiyar and Sinha, 1982; Sharma, 1991; Deka *et al*., 1995; Talukdar, 1996; Katoch, 2000) and these workers recorded either single species of one type of GI helminth or more than one to seven species of one type of GI helminth in goats from different parts of country. Grunner *et al*. (1985) reported seven nematode species and three cestode species. The present study encountered six genera with six species of nematodes, and two genera each with one trematode and one cestode.

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Intestinal parasitic infections in patients attending a government hospital in Benin City, Nigeria

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ABSTRACT. We investigated the prevalence of parasitic infections in hospital patients having gastrointestinal complaints. The study covered a three-year period (January 2001–December 2004). Stool samples were collected from 485 patients (240 males and 245 females) who reported at Central Hospital, Benin City, Nigeria. Each stool sample was examined microscopically for the presence of ova and cysts, using iodine and formol-ether concentration methods. Overall, 43 (8.86%) stool samples were positive for intestinal parasites, with the rates of infection in the following decreasing order: hookworm (41.86%), *Ascaris lumbricoides* (32.6%), *Entamoeba coli* (6.97%), *E. histolytica* (6.97%), *Trichuris trichiuris* (6.97%), *Taenia solium* (4.65%) and *Giardia lamblia* (2.32%). Patients within the first four decades of their life had the highest prevalence of infection. All the mixed infections were found in females. Although females (10.6%) were more infected than the male patients (7.08%), the difference was not statistically significant ($\chi 2=1.56$; $p > 0.05$). The poor sanitary conditions within several areas of the city, coupled with inconsistent approach of government's de-worming campaign programmes, may be responsible for the continuous presence of helminthic and protozoal infections.

Keywords: Benin city, hospital, infection, parasitic, patients

Like most cases of bacterial gastroenteritis, intestinal parasitic diseases are a major health concerns, especially in developing countries in the tropics. It has been reported that about 2 billion people are at the risk of contracting soil-transmitted helminths, while > 1 billion people are infected. *Ascaris lumbricoides* infects about 1.3 billion people worldwide, with morbidity and mortality occurring in 250 million and 60,000 people, respectively. About 1.25 billion people are estimated to be infected with hookworm, with an associated morbidity and mortality in 151 million and about 65,000 people, respectively (WHO, 1998). Infections with helminths and protozoa have had untold effects on children (Odunta, 1974; Gbakima *et al.*, 1994; Adekunle, 2002) and pregnant women (Bundy *et al.*, 1995). Adult hookworms are known to attach to the mucosa of small intestine, feed on blood and cause anaemia (Olsen *et al.*, 1998). Migasena and Gillies (1987) have reported that hookworm and other soil transmitted helminths are most important causes of chronic loss of blood and iron in the tropics.

Various environmental and socio-economic factors can predispose people to helminthic and protozoal infections. Ingestion of improperly cooked meat or pork as well as unwashed fruits can serve as ready vehicles of parasitic infection. It has also been mooted

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that the occurrence of helminth infection at high rates among pregnant women is indicative of fecal pollution of soil and domestic water around homes due to poor sanitation and improper sewage disposal (Egwunyenga *et al.*, 2001). Okodua *et al*. (2003) have reported that the tropical climate in Abeokuta, Nigeria, can enhance the survival of ova and cysts of protozoa. Also, most of the rural dwellers have a habit of eating with bare hands, which might have been contaminated with ova and/or cysts from the environment. It is not unlikely that the sequelae of most of the parasitic infections can be very devastating, and may be associated with malnutrition, anaemia and stunted growth and physical, sexual and even mental retardation. The loss of man hours from duty posts is irreplaceable and results in underdevelopment. Previous studies (Nwosu, 1981; Amuta *et al.*, 1999; Mba and Amadi, 2001; Adenusi and Ogunyomi, 2003) have highlighted the endemicity of intestinal parasitic infections in Nigeria. Our study updates the earlier work of Obiamiwe (1977) and Obiamiwe and Nmorsi (1991), who had sampled patients from almost the same locality. Herein, the state of parasitic infections in this area as well as the age and sex distribution of those affected *vis-a-vis* control measures has been studied.

Area of study: Benin City, a busy cosmopolitan city, is the capital of Edo State, Nigeria and Central Hospital is the most patronized state-owned hospital.

Subjects: The subjects for this study included 485 patients (male 240, female 245) who visited Central Hospital, Benin City between January 2001 and December 2004. The patients reported to the hospital Out-Patients Department on their own for various gastro-intestinal complaints, and each of them signed a consent form to be enrolled for this study.

Sample collection and analysis: Each patient was given a clean leak-proof plastic container and asked to submit freshly voided stool sample, the following morning. Wet slide preparations of each stool sample were prepared in normal saline and Lugol's iodine, and examined microscopically for the presence of trophozoites, cysts, oocyts, larvae and ova, by using x10 and x40 objective lenses. The formol-ether concentration method of Cheesbrough (1998) was also used to detect cysts and ova.

Forty three stool samples were found positive for intestinal parasites (36 helminths and 7 protozoa; Table I). The overall prevalence of intestinal parasites was 8.86%. Table I shows the distribution of parasites in the examined stool samples. Hookworm infection had the highest prevalence of 41.8% followed by *A. lumbricoides* (32.6%). *Entamoeba coli* and *E. histolytica* each had a rate of 6.97%. *Trichuris trichiura* and *Taenia solium*, 4.65% each, whereas *Giardia lamblia* had the lowest prevalence rate of 2.32%. The prevalence of intestinal parasitic infections among different age groups is shown in Table II. The highest prevalence of 15.3% was recorded in the 30–39 years age group, followed closely by the 20–29 years age group with prevalence of 13.3%. No parasitic infection was observed in subjects within the range of 70–79 years.

Table III shows the distribution rates of intestinal parasites based on sex of subjects examined. On the whole, females were more infected with intestinal parasites than the males. A prevalence of 10.6% and 7.08% was recorded for female and male subjects, respectively. All the mixed parasitic infections were from five female subjects; four of them had mixed infections with hookworm and *A. lumbricoides*, whereas in only one subject the mixed infection consisted of hookworm, *A. lumbricoides* and *E. coli*.

Table I. Distribution of prevalent intestinal parasites in hospital patients

Parasite	Class of parasite	No. (%) of parasites
Hookworm	helminth	18 (41.8)
Ascaris lumbricoides	helminth	14 (32.6)
Entamoeba coli	protozoa	3 (6.97)
E. histolytica	protozoa	3 (6.97)
Trichuris trichiura	helminth	2 (4.65)
Taenia solium	helminth	2 (4.65)
Giardia lamblia	protozoa	1 (2.32)
Total		43

In this study the prevalent parasites were: hookworm (41.8%), *A. lumbricoides* (32.6%), *E. coli* (6.97%), *E. histolytica* (6.97%), *T. trichiura* (4.65%), *T. solium* (4.65%) and *G. lamblia* (2.32%). We observed that helminthic infections were more prevalent than protozoal infections in patients studied. These observations are in line with similar studies carried out in other Nigerian cities (Okodua *et al.,* 2003; Amuta *et al.,* 1999). Hookworm infection rate was highest (41.8%) followed closely by *A. lumbricoides* (32.6%). This trend also appears to be in consonance with other studies carried out in Makurdi (Amuta *et al.,* 1999) and Enugu (Ozumba and Ozumba, 2002). Obiamiwe and Nmorsi (1991) have confirmed that amongst the helminths, hookworm and *A. lumbricoides* were encountered in all sampled areas in the former Bendel State of Nigeria. The results of this study and those reported by earlier workers (Amuta *et al.,* 1999; Obiamiwe, 1977; Obiamiwe and Nmorsi,1991) indicate that hookworm, *A. lumbricoides, T. trichiura, T. solium* and *E. coli,* as well as *E. histolytica* and *G. lamblia* commonly infect people in Nigeria. The presence of these parasites among hospital visitors in Benin City suggests the need for improved individual health precautions and general environmental sanitation.

The occurrence of parasites based on the age of subjects reveals an interesting trend. There appears to be a higher rate of infection within the first four decades of life. This is probably due to the fact that contact with possible sources of infection such as soil, food and unsanitary environment is most likely during this period of life. Also, children in primary and secondary schools fall within the first two decades of life. In spite of several de-worming exercises often mounted by voluntary bodies and government health agencies, intestinal parasitic infections seem not to have abated in the locality. The sale of un-wholesome and exposed food items, as well as presence of filthy rubbish dumps within and around market places, where flies congregate help to maintain the chain of transmission of most of these intestinal parasites. The decrease in infection rate in older age groups may partly be due to reduced exposure to parasites as well as possible acquisition of acquired immunity resulting from repeated clinical and sub-clinical infections in earlier life.

The rate of infection in females (10.6%) was comparatively higher than in males (7.08%). This difference is not statistically significant ($\chi 2 = 1.56$; $p > 0.05$). However, children and the female folk have also been found to be more afflicted with soil-transmitted parasites (Odutan, 1974; Nwosu, 1981; Obiamiwe, 1991; Cheesbrough, 1998). It is, therefore, not

Table II. Occurrence of intestinal parasites in subjects of different age groups

Age	No. examined	No. (%) of positive
< 10	57	6 (10.5)
10–19	78	8 (10.2)
20–29	75	10 13.3)
30–39	65	10 (15.3)
40–49	86	4 (4.7)
50–59	50	3 (6.0)
60–69	44	2 (4.5)
70–79	30	0 (0)
Total	485	43 (8.86)

Table III. Occurrence of intestinal parasites in subjects based on sex

Sex	No. of subjects	No. (%) positive
Female	245	26 (10.6)
Male	240	17 (7.08)
Total	485	43 (8.86)

($\chi 2 = 1.56$; $p > 0.05$)

surprising to find mixed parasitic infections as observed in five women in this study. Results from this study show that more preventive measures need to be instituted to curtail helminthic and protozoal infections in urban cities.

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Seasonal prevalence of *Fasciola gigantica* infection in sheep and goats in western Uttar Pradesh

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ABSTRACT. Epidemiological studies were conducted to establish the prevalence of *Fasciola gigantica* infection in sheep and goats of western Uttar Pradesh, from January 2001 to December 2004. An overall prevalence of 1.85 % (1.69% sheep and 2.02% goats) was recorded with peak prevalence (2.61%) of the infection in rainy season. In sheep, peak prevalence of *Fasciola* infection was recorded during the month of July (5.1%), whereas in goats, peak prevalence was observed in the month of March (13.3%). Animals of Bareilly division were most infected (4.75%), followed by those of Moradabad (3.50%), Meerut (0.79%) and Agra (0.35%) divisions. Examination of *Lymnaea auricularia* snails revealed that 1.57% of the 1,905 snails examined were harbouring life cycle stages of *F. gigantica.* The prevalence of the infection in snails was highest (4.18%) during rainy season (July–October), whereas it was lowest (0.76%) during summer (March–June). The results of the present study may help to devise an effective control strategy against fasciolosis in western Uttar Pradesh.

Keywords: epidemiology, *Fasciola gigantica*, goats, sheep, Uttar Pradesh

Fasciolosis, caused by *Fasciola gigantica* is widely distributed in tropical and sub-tropical areas. It is recognized as a major cause of production losses in domestic animals in terms of reduced live weight gains, lowered milk production, condemnation of liver at slaughter and reduced wool production (Kumar and Pauchauri 1989; Wamae *et al.*, 1998; Chaudhri 2005). According to Food and Agriculture Organization Report (1994), fasciolosis causes losses amounting to more than US$ 3 billion per annum. There are several reports on the epidemiology of fasciolosis from different endemic areas in the country (reviewed by Gupta and Singh, 2002). Outbreaks of fasciolosis have been recorded in sheep and goat flocks resulting in widespread mortalities (Dhand *et al.*, 2004). Perusal of literature suggests that very few studies have been carried out in western Uttar Pradesh on the epizootiology of this infection in sheep and goats (Bhatia *et al.*, 1989). Therefore, the present study was undertaken with the objective of generating sufficient data pertaining to geographical and biological factors affecting prevalence of liver fluke infection in sheep, goats and intermediate host snail in western Uttar Pradesh.

A total of 8277 faecal samples (4321 sheep; 3956 goats) were examined from January 2001–December 2004. The faecal samples were collected from sheep and goats of various villages in four divisions of western Uttar Pradesh, namely Meerut, Agra, Bareilly and Moradabad, and examined for the presence/absence of *Fasciola* sp. eggs by using sedimentation technique (Soulsby, 1982). Besides this, 1905 *Lymnaea auricularia* snails were collected during different months of the study period from various water resources present in the investigating area and screened

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microscopically by crushing the fleshy portions for the presence of larval stages of *F. gigantica* (Chaudhri *et al.*, 1993). Also, the weather data *viz*. temperature (maximum and minimum), rainfall and humidity (morning and evening) was obtained from the Central Institute for Research on Goats, Mathura; Department of Physiology, Indian Veterinary Research Institute, Izatnagar (Bareilly) and Meerut, and was correlated with the prevalence of fasciolosis in sheep, goats and snails.

Of the 8277 faecal samples of sheep (4321) and goats (3956) examined from January 2001–December 2004, only 1.85% were found positive for fasciolosis. A higher rate of *Fasciola* infection was recorded in goats (2.02%) as compared to sheep (1.69%). The results revealed the prevalence of liver fluke infection throughout the year in western Uttar Pradesh. Maximum prevalence of fasciolosis was 2.35% during the year 2003 (2.18% in sheep; 2.53% in goats), whereas it was lowest in the year 2002 (1.18% in sheep ; 1.44% in goats). The lower prevalence rate of *Fasciola* infection observed might be due to changing patterns in animal husbandry practices. Also notable is that most of the sheep flocks in western Uttar Pradesh migrate from Rajasthan during the hot months of the year, especially after March and leave before the rains. A very low *F. gigantica* incidence of 0.57% has been reported from Rajasthan (NATP report, 2004).

Monthly prevalence of *Fasciola* infection in small ruminants is presented in Fig. 1. In sheep, peak prevalence of *Fasciola* infection was recorded during the months of July (5.1%), August (2.1%) and September (3.2%). In goats, however, the peak prevalence was observed in the months of March (13.3%), June (4.3%) and September (3.9%). Similar findings have been reported from Jammu by Yadav *et al.* (2006). In the present study, highest prevalence (2.61%) was recorded during the rainy season followed by summer (2.35%) and winter (0.67%) seasons (Fig. 2).

The animals of Bareilly division exhibited highest prevalence (4.75%) of fasciolosis, whereas it was lowest in Agra division (0.35%), with 3.5% in Moradabad and 0.79% in Meerut divisions. Goats of all the divisions were infected more with fasciolosis as compared to sheep except in Moradabad division, wherein 3.67% sheep and 3.22% goat faecal samples were found positive. High prevalence rate of fasciolosis has also been reported in goats from Haryana (2.01% goat; 1.29% sheep), Madhya Pradesh (4.4% goat; 4.2% sheep; NATP Report, 2004). Tamloorkar *et al.* (2002)

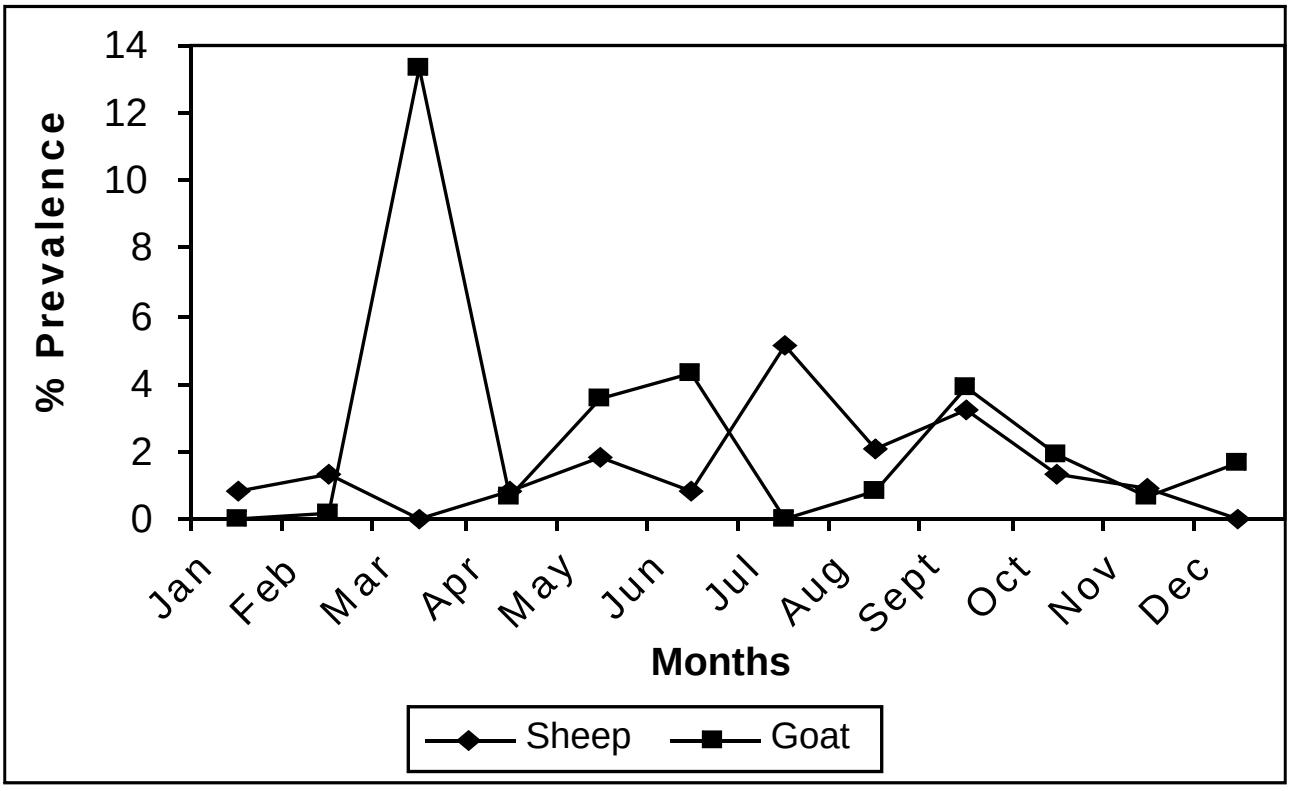
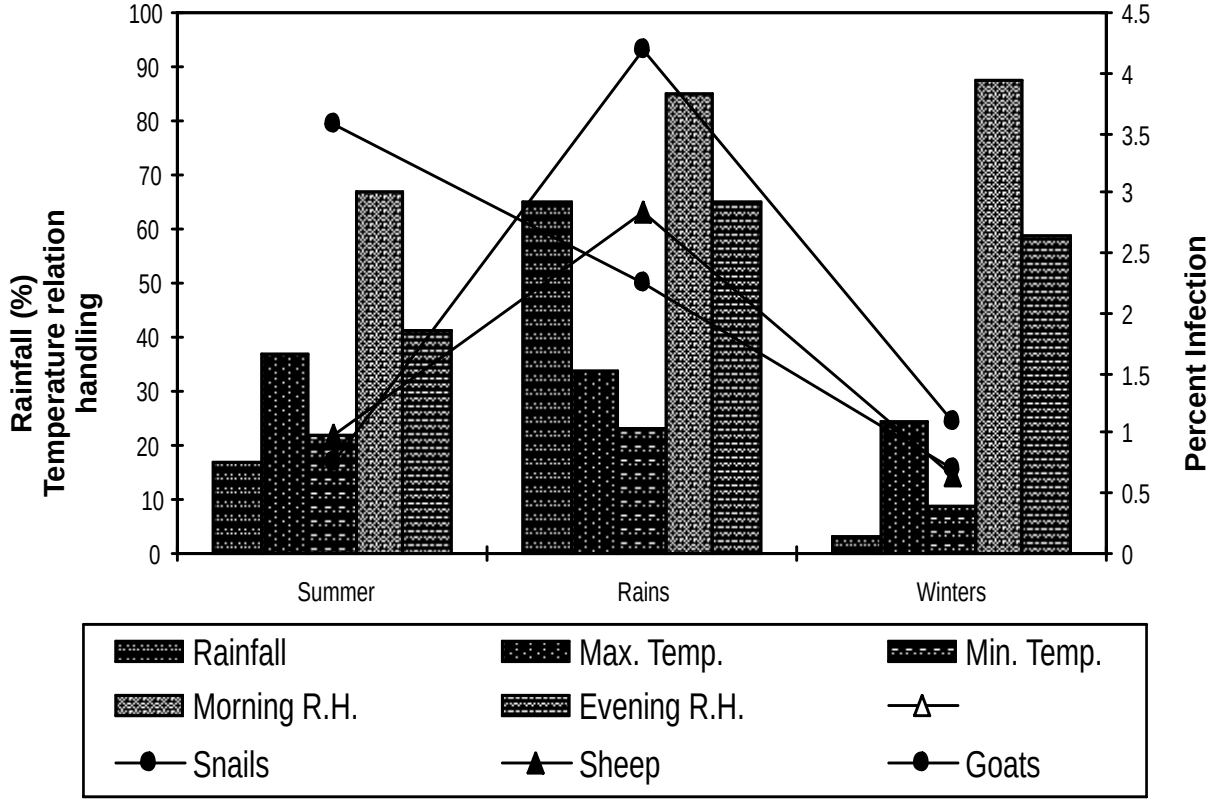


Figure 1. Monthly prevalence of *F. gigantica* in sheep and goat from 2001to 2004.

Figure-2: Prevalence of *F. gigantica* in snails, sheep and goats in relation to average seasonal meteorological data in western U.P



also observed a higher incidence of fasciolosis in goats as compared to sheep. They reported 18.93% and 22.9% fluke infection in sheep and goats, respectively, in Marathwada region. Dhand *et al.* (2004) recorded 72.91% and 56.81% *Fasciola* infection in sheep and goats, respectively, in Punjab, in sharp contrast to our findings. In a slaughter house study in tarai region of Uttar Pradesh, Bhatia *et al.* (1989) also observed an overall incidence of 57.3% and 81.44% of fasciolosis in sheep and goats, respectively.

Variations in climatological conditions in different divisions of western Uttar Pradesh and management practices (grazing, stall feeding and tethering) being adopted by the livestock owners in the areas under study, affected the differences observed in epidemiological pattern of *F. gigantica* infection in sheep and goats.

Overall 1.51% *L. auricularia* snails harboured the larval stages of *F. gigantica*. Maximum incidence (4.18%) was recorded during rains, minimum in summer (0.7%) and intermediate in winter season (1.09%). The major determinants of climate *i.e.* temperature and rainfall are highly conducive for the propagation of snails and development of miracidia from eggs on pasture in the rainy season as compared to drier months of summer as indicated in Fig. 2. These observations are comparable to those of Velusamy *et al.* (2004) who also found 1.67% snails in Bareilly division to be infected with larval stages of liver fluke, with a maximum number being infected in the rainy month of September (5.88%) during a study period from 2000-2002.

From the above findings it can be suggested that sheep and goats should not be allowed to graze near water bodies during late winter, late summer and post rainy seasons, when there is an abundance of metacercariae on herbage. The infected animals should be administered flukicides in July/August/September and December/January and, if required, in March every year to prevent massive accumulation of *Fasciola* eggs on pasture. Studies on the ecology of snails indicate that snail-infested water bodies should be treated with molluscicides during early summer and post-rainy season in identified endemic areas.

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First report of *Hermatia illucens* larvae in poultry houses of Punjab

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ABSTRACT. Dipetran larvae, belonging to family Stratiomyidae, were found in the litter of poultry farms of the Punjab state. The larvae were of dark brown colour, segmented and hairy. The morphology of larvae was studied.

Keywords: black Soldier fly, Dipetra, *Hermatia illucens;* poultry; Punjab

The "black Soldier fly" an uncommon name in Northern India, is a common name of *Hermatia illucens*. It was first seen in 1930 in Hilo Sugar Company in Hawaiian Islands (Du Ponte and Larish, 2003). The fly has been reliably reported from the north east in mediterranean area and from Portugal, Spain, France, Switzerland, Italy, Malta, Croatia and Albania by Ustuner *et al.* (2003), and from southern and central United States, USSR and China (Newton *et al.*, 2005). *H. illucens* belongs to phylum Arthopoda, class Insecta, order Dipetra and family Stratiomyidae. Soldier fly is non-pest filthy wasp like fly as large as 13–20 mm (May, 1961), and is found in tropical and warm temperate regions. The adult flies never eat, so they are considered as non-pest. The larvae of *H. illucens* are most important as they are usually seen. Larval stages feed on decaying organic matter, garbage especially around poultry houses (Tomberlin and Sheppard, 2001; Newton *et al.*, 2005) feeding on chicken manure.

This communication reports the presence of the larvae of Soldier fly in northern India, apparently for the first time. The voracious larvae were brought, in an air-tight bottle, to the Department of Veterinary Parasitology from a poultry farm in Ludhiana district in the month of June. The larvae were dark blackish brown in colour with a tough leathery covering. Bottle containing larvae was air-tight, without any food, except with a white powder-like substance that was their excretory material; the larvae survived in that bottle for two days. The larvae were seen crawling in the bottle. Morphology of the larvae was studied (Fig. 1).

The larvae had 11 segments and were about 1 inch long. Fine hairs were seen on the margins of the segments. These characters were in reference with the report of DuPonte and Larish (2003), Newton *et al.* (2005) and Williams *et al.* (1985). The presence and absence of

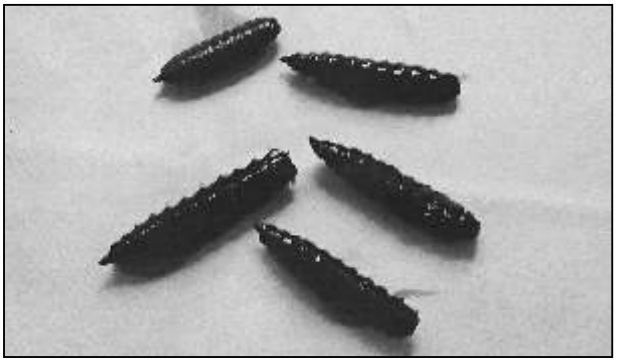

Figure 1. *Hermatia illucens* larvae.

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hairs may be a difference between mature and immature larvae. Ventral aspect of the larvae had spines same as reported by Merchant (2003). The larvae have a pair of spiracles on both sides of each segment-Peripneustic (Sen and Fletcher, 1962). The larvae have been reported to be wriggling along litter and cages of poultry birds. Occasionally, the larvae were seen clinging to poultry birds, which made them to be the matter of concern and scare for poultry farmers. Again by the end of August, the outer shell and the mature dead larvae were recovered from the litter of the same farm. The lengths of those larvae were also about one inch. These larvae had more hair on the segments as compared to the previously found larvae.

Because people are not familiar with these larvae, they are important for further research. As per review of available literature, it has been observed that the larvae are not harmful to the poultry birds. The digestive excreta produced by these larvae has been observed, and has been reported to be valuable as a soil amendment, which can reduce local nutrient over load of the litter on which they grow (Merchant, 2003). Sheppard and Newton (2000) have reported that the larvae reduce confined animal feeding operation (CAFO) problems. Therefore, the researchers are rearing (Sheppard *et al.*, 2002) these larvae as they had been proved to be a very useful waste management tool (Texas cooperative Extension by Merchant, 2003). Soldier fly larvae can control noxious *Musca domestica* larvae in manure colonized by them, so the larvae act as ecological homologue pest displacement vector (Furman *et al.*, 1959; Tingle *et al.*, 1975; Axtell and Arends, 1990) in poultry houses.

The reports of the presence of these larvae in Punjab are opening wide scope for future researches to reduce filth and biomass with the help of biological vector–Soldier fly. Even the epidemiology of these larvae in this region is yet to be explored.

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Haematobiochemical alterations during common gastrointestinal helminths and mite infestations and their treatment in Desi pigs

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ABSTRACT. Reduced haematological (Hb–8.26 and 8.83, PCV–28.31 and 29.48, TEC–4.67 and 4.27) and biochemical constituents (Ca–10.64 and 9.87, P–5.31 and 4.81, Zn–41.73 and 40.71, Cu–46.19 and 44.59, TSP–5.44 and 4.98 and albumin–2.80 and 2.52) levels were observed in gastrointestinal nematode and sarcoptic mite infested pigs aged about 3 months. These values returned to about normal ranges (Hb–10.82 and 10.47, PCV–32.46 and 33.46, TEC–5.96 and 5.64, and Ca–11.73 and 11.31, P–5.87 and 5.29, Zn– 4.03 and 55.71, Cu–61.14 and 60.37, TSP–6.62 and 6 and albumin 3.48 and 3.23) in piglets treated with chemical and herbal anthelmintics and miticides.

Keywords: gastrointestinal helminths, haematobiochemical, mite infestations, pigs, treatments

The common gastrointestinal (G.I.) helminths like *Fasciolopsis, Ascaris, Strongyloides, Oesophagostomum* spp. and others usually affect pigs together with *Sarcoptes* spp. mites. The combined infections cause severe damage to the host. The pathogenesis produced is evidenced in the form of alterations in different haematobiochemical parameters. The haematological and biochemical changes also provide supporting evidence for assessing the efficacies of therapeutic agents (Kumari, 2001; Bariyar, 2004). Such informations are lacking in Desi pigs, which are very commonly victimised by the common G. I. helminths and sarcoptic mites. This communication reports the haematobiochemical alterations during G. I. helminths and mite infestations, and their sustainable control in Desi growing piglets.

A total of 18 growing Desi piglets aged about 2½-3 months having natural infection of *A. suum, Trichuris suis, Oesophagostomum dentatum, Strongyloides and Sarcoptes scabiei* were selected for their sustainable control with chemical anthelmintic (fenbendazole + praziquantel), herbal anthelmintic mixture (mercury - 100 mg, sulphur - 200 mg, ajmod - 300 mg, Bayavidanga - 400 mg, nux-vomica - 400 mg, Palas seed - 600 mg, pumpkin seed - 3000 mg, Jira - 4000 mg, arecanut - 3000 mg and black til - 3000 mg), chemical miticide (cypermethrin) and herbal miticide (Neem oil - 50 ml, Karanj oil - 50 ml, camphor - 10 gm, sulphur - 10 gm). Six animals (Group–I) were treated for both the infections simultaneously using chemical anthelmintic and miticide. The another six animals (Group - II) were administered herbal anthelmintic and miticide. The rest six animals (Group - III) were maintained as infected and untreated control. For assessing the pathogenesis caused by the helminths and mite infection in piglets, haematobiochemical parameters such as Hb, PCV, TEC, Ca, P, Zn, Cu, TSP and Albumin were estimated before treatment on 0 day and then on 15th day post-treatment (DPT). All the animals were maintained in separate pens on good plan of nutrition. Any kind of erroneous infection appearing during course of

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experiment were tackled immediately.

The blood samples of all the animals for haematology were collected in separate vials having EDTA (1.5 mg/ml blood). For estimation of biochemical constituents, the blood samples of all animals collected in 1 inch diameter test tubes, and kept slanted for clotting. After some time, the collected blood samples were gently shifted to laboratory and placed in refrigerator for complete serum separation. Sera samples were collected in separate vials, labelled and used for different biochemical constituents.

The pretreatment Hb, PCV and TEC values were low (Hb–8.26 and 8.83, PCV–28.31 and 29.48 and TEC–4.67 and 4.27 in group I and II, respectively) and these values were restored to normal on 15th DPT. (Hb–10.82 and 10.47, PCV–32.46 and 33.46 and TEC–5.96 and 5.64 in Group I and II, respectively). The decreased haematological parameters due to G. I. nematodosis and mange infection in pigs were also found at reduced level by Kumari (2001), Prasad and Kumar (2001) and Prasad *et al*. (2001). The pathogenic effects caused by parasites before treatment were the reasons for reduction in haematological values. When the parasites were removed from the host by suitable treatment, the values were found at about normal ranges indicating pathogenic effects caused by the parasites were stopped resulting in improvement in health parameters.

The Ca, P, Zn, Cu, TSP and albumin estimated on 0 day were also below the normal values (Ca–10.64 and 9.87, P–5.31 and 4.81, Zn–41.73 and 40.71, Cu–46.19 and 44.59, TSP–5.44 and 4.98 and albumin 2.80 and 2.52 in Group I and II, respectively), which were observed to return at about normal levels on 15th DPT. (Ca– 1.73 and

Table I. Haematobiochemical profiles of growing Desi piglets during common G. I. helminths and mite infestation and their treatment with chemical and herbal anthelmintics and miticides

Parameters	Observation period	Group-I	Group-II	Group-III CD value
Hb (g/dl)	0	8.26±0.17	8.83±0.32	8.78±0.351.07
	15	10.82±0.33a	10.47±0.42a	8.51±0.30b
PCV%	0	28.31±0.45	29.48±0.73	29.01±0.502.70
	15	32.46±0.67a	33.46±0.99a	27.74±0.98b
TEC (x106/cu mm)	0	4.67±0.21	4.27±0.23	4.75±0.320.61
	15	5.96±0.22a	5.64±0.17a	4.71±0.22b
Ca (mg/dl)	0	10.64±0.36	9.87±0.32	10.12±0.431.14
	15	11.73±0.44a	11.31±0.40a	9.23±0.20b
P (mg/dl)	0	5.31±0.34	4.81±0.15	5.09±0.190.60
	15	5.87±0.27a	5.29±0.12a	4.52±0.18b
Zn (µg/dl)	0	41.73±2.48	40.71±3.92	38.50±2.337.57
	15	54.03±1.83a	55.71±2.54a	36.25±1.16b
Cu (µg/dl)	0	46.19±1.71	44.59±2.97	42.95±2.399.49
	15	61.14±3.47a	60.37±3.14a	38. 21±2.80b
TSP (g/dl)	0	5.44±0.36	4.98±0.27	4.90±0.220.86
	15	6.62±0.25a	6.00±0.26a	4.70±0.24b
Albumin (g/dl)	0	2.80±0.28	2.52±0.23	2.51±0.130.52
	15	3.48±0.19a	3.23±0.17a	2.44±0.22b

Group-I : Fenbendazole + praziquantel and cypermethrin treated.
Group-II: Herbal anthelmintic and miticide treated.
Group-III : Infected untreated control.
Figures having the same superscripts did not differ significantly.
Figures having different superscripts differ significantly ($p < 0.01$).

11.31, P–5.87 and 5.29, Zn–54.03 and 55.71, Cu–61-14 and 60.37, TSP–6.62 and 6 and albumin 3.48 and 3.23 in Group I and II, respectively) after treatment with chemical and herbal anthelmintics and miticides. There was lack of information about alterations in biochemical constituents in parasitised and parasite-free pigs. However, Rajguru *et al.* (2002), Bariyar (2004) and Lakara *et al.* (2007) have reported reduced levels of Ca, Zn, Cu, TSP and albumin in goats infected with G. I. nematodes and pigs infected with sarcoptic mite. The damage caused by parasites might have affected the intestinal absorption, assimilation, metabolism and in other organs resulting in reduction in their values. When parasites were cleared by treatments the values were found at about normal ranges indicating that the parasite removal by suitable therapy might have aided the host to recover from the damage produced by parasites. The reduction in blood biochemical parameters during infection and return at about normal ranges after treatment have also been reported by Minz (2002), Rajguru *et al.* (2002), Bariyar (2004) and Lakra *et al.* (2007) in parasitised goats and pigs.

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Journal of Parasitic Diseases: December 2007, Vol. 31, No. 2, 150-151 *Short communication*

A new cestode species–*Ophiotaenia wuyiensis* n. sp. (Proteocephalidea: Proteocephalidae La Rue, 1911) from China

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ABSTRACT. A new species of cestode, *Ophiotaenia wuyiensis* n. sp., collected from *Trimeresurus gramineus stejnegeri* (Schmidt) in Wuyi Mountain in 1992 has been described. The new species is near to *O. phillipsi* and *O. trimeresuri* of the same genus. *O. wuyiensis* n. sp. differs from these species as follows: the new species differs from *O. phillipsi* and *O. trimeresuri* in little head, about 2/3 of the latter; suckers (0.16~0.21), smaller than *O. phillipsi* (0.20~0.30); cirrus pouch (0.217×0.102), smaller than *O. trimeresuri* (0.27~0.34×0.136) and *O. phillipsi* (0.360~0.380×0.147~0.382); number of testes (80~100), lesser than *O. trimeresuri* (100~108) and *O. phillipsi* (170~230), and are comparatively smaller in size.

Key words: Cestode, *Ophiotaenia*, China

It was in 1992 that we did research in the National Natural Reservoir of Fujian Wuyi Mountains. Several species of cestodes were obtained. A new species of cestode (Proteocephalidea: Proteocephalidae) is described and named as *Ophiotaenia wuyiensis* n. sp.

The new cestodes were collected and preserved by general method. After careful management, they were sealed as specimens for examination. The measurement unit is mm.

Description

The body length of the worms measured 150~286 with a width of 0.8~1.0. Most of the proglottids were longer than width, but proglottids of the neck were wider than length (0.172×0.602) and the initial immature proglottids were of the same length and width. Genital pores opened irregularly at both sides of the segments. The scolex, a little cone-shaped, measured 0.411× 0.421, with four roundish suckers (0.150~0.190× 0.160~0.206), and was without an apical sucker (Fig. 1A).

The mature proglottid measured 1.66~1.95×0.50~1.01. The number of testes is 80-100, measuring 0.025~0.050×0.018~0.030 and distributed on both the sides of proglottid, between the uterus and the vitellarium glands, about 1/3 of the width of proglottid.Cirrus pouch measuring 0.217x0.102, about 1/3-1/4 of the segment width, like a cone-tube located a little ahead middle of the proglottid, where the genital pore opens. Ovary is located in the end of the proglottid, divided into two parts and connected by the narrow part,

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Table I. Comparison of *O. wuyiensis* n. sp. with near species

Characteristics	*O. wuyiensis* n. sp.	*O. trimeresuri*	*O. phillipsi*
Diameter of scolex	0.42	0.75	0.670
Diameter of sucker	0.16-0.21	0.16-0.25	0.20-0.30
No. of testes	80-100	100-108	170-230
Diameter of testes	0.018-0.030 0.025-0.050	0.027-0.080× 0.027-0.063	0.087-0.0108
Cirrus pouch	0.217×0.102	0.27-0.36×0.136	0.360-0.380x0.147-0.382

0.447×0.102. Uterus begins at the central part of ovary and becomes tube-like near anterior end of the proglottid. The vaginas go the same way with the uterus and begin from the oviduct, bend to the genital pores after nearing to them. Most of the vaginas are behind cirrus sac. The vitellarium glands follicles, arranged like lines, distributed on both sides of proglottid (Fig. 1B)

All 5 specimens are not with gravid proglottids, so the diverticula of the uterus is not clear.

Host: *Trimeresurus gramineus stejnegeri*(Schmidt)

Location: Intestine.

Locality: Xianfeng Ling, Natural Reservoir of Fujian Province Wuyi Mountains.

Type specimens Deposited in the Parasitology Research Laboratory, Xiamen University.

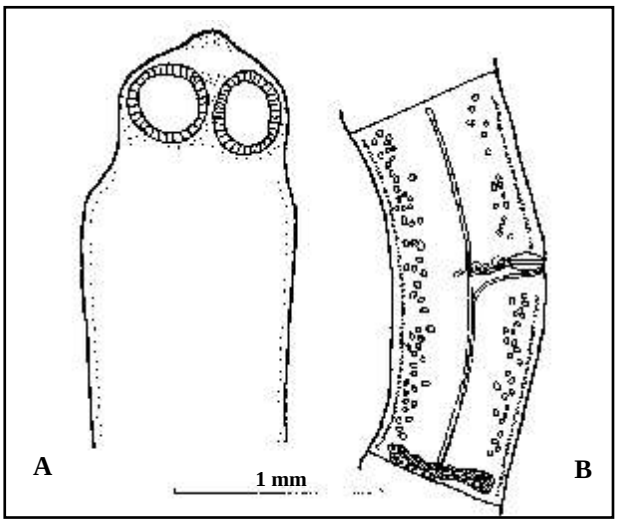

Figure 1. *Ophiotaenia wuyiensis* sp. nov. A. Scolex; B. Mature proglottid.

The host of the new species is *T. gramineus stejnegeri* (Schmidt), not recorded previously as a host of cestodes in the genus (Khalil *et. al.*, 1994; Schmidt, 1984). The new species is differentiated from *O. phillipsi* (Hughes *et. al.*, 1941) and *O. trimeresuri* (Hsü, 1935) by little head, about 2/3 in size; suckers (0.16~0.21) are smaller than *O. phillipsi* (0.20~0.30); cirrus pouch (0.217×0.102) smaller than *O. trimeresuri* (0.27~0.34×0.136) and *O. phillipsi* (0.360~0.380×0.147~0.382); number of testes (80~100) less than *O. trimeresuri* (100~108) and *O. phillipsi* (170~230), and is smaller (Table I.). As the difference is very big, it is recognized as a new species and named as *Ophiotaenia wuyiensis* n. sp. Alain de Chambrier *et al.* (2006) published a new species of *Ophiotaenia* cestode in Costa Rica–*O. bonneti* from *Rana vaillanti*. However, these authors have not presented a table to compare this species with other similar species, but there is a vast description for species differentiation. We, therefore, insist that new species description should keep the old tradition so that future workers do not misunderstand.

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Polypocephalus visakhapatnamensis sp. nov. (Lecanicephalidea: Polypocephalidae) from *Himantura uarnak* (Forsskål) and *Dasyatis (Amphotistius) zugei* (Müller & Henle) from Visakhapatnam coast

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ABSTRACT. *Polypocephalus visakhapatnamensis* sp. nov. has been described from the spiral valves of *Himantura uarnak* (Forsskål) and *Dasyatis(Amphotistius) zugei* (Müller & Henle) at Visakhapatnam coast, Bay of Bengal, India. It has been compared with other valid species of the genus *Polypocephalus* Braun, 1878, and has been characterized in having large body size, acraspedote proglottids, eight bifurcated tentacles, presence of neck, four testes, transversely elongated vitelline mass below the ovary and cirrus in the middle of proglottid.

Keywords: acraspedote proglottids, biological indicators, *Dasyatis(Amphotistius) zugei*, *Himantura uarnak*, *Polypocephalus*, spiral valves

A diversified array of elasmobranchs occurs in Bay of Bengal, India. They constitute an important group of fishes by the virtue of their abundant occurrence, cosmopolitan distribution and commercial value. Among them, rays are excellent commercial food fish in East coast of India due to their high protein content. Rays serve as an imperative host as they carry a range of host-specific parasites, which can be used as 'biological indicators'. Studies of their parasitic fauna, in particular cestodes, are limited and it might, therefore, be expected that studies on cestodes of rays in this region will reveal diverse and novel taxa. Rays offer an excellent habitat to diversified array of cestode parasitic fauna, in particular tetraphyllids, lecanicephalids and trypanorhynchids. Lecanicephalids predominate the cestode parasitic fauna in these host fishes.

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In the present study, a new species *Polypocephalus visakhapatnamensis* sp. nov. has been described from the spiral valves of *Himantura uarnak* (Forsskål) and *Dasyatis (Amphotistius) zugei* (Müller & Henle). Genus identification was done with the aid of standard books by Yamaguti (1959), Schmidt (1986), Khalil *et al.* (1994) and other literature pertaining to the genus *Polypocephalus*.

A survey has been conducted to study cestode parasitic fauna from the Elasmobranchs at Visakhapatnam coast, Bay of Bengal, and about 225 *H. uarnak* (Forsskål) and 158 *D. (A.) zugei* (Müller & Henle) were examined thoroughly for the cestode parasites. Cestode parasites usually parasitize the spiral valves of the intestine. Spiral valves of the host fish were seperated, washed in physiological saline to remove excess mucus and were cut open with a longitudinal incision. Parasites were carefully alienated from the spiral valves and collected in cavity blocks filled with saline solution.

Polypocephalus clings to the intestinal walls with the aid of its tentacles. Proper care was taken not to damage the scolex and strobila of parasites as scolex is the key feature for the identification of a cestode. Parasites were flattened between two slides or under the pressure of slide and a coverslip, post-fixed in alcohol, 85 ml; formalin, 10 ml and acetic acid, 5 ml (A. F. A), and stained with alum carmine. Conventional techniques were employed for the permanent whole mount preparations. Figures were drawn with the aid of a camera lucida. Measurements are given in millimeters.

***Polypocephalus visakhapatnamensis* sp. nov.**

A total of 16 parasites were collected from *H. uarnak* (Forsskål) and *D. (A.) zugei* (Müller & Henle).

DESCRIPTION **(**based on measurements of eight parasites; Plate-I, Fig. 1and 2)

Worms small, thin, white in colour, 4-10 x 0.21-0.30, acraspedote and apolytic. Scolex large, small flower bunch-shaped in whole-mounts. Scolex 0.37-0.72 x 0.38-0.75. Pars apicalis with tentacles; tentacles distally bifurcated, fork-like from middle, broad and fleshy at base, each tentacle 0.22-0.24. Furcated tentacle part thin, filose, transparent and shows active movements in live condition. In retractile condition, terminal portion of tentacles remain outside the cavity.

Pars basalis muscular, quadrangular with four accessory acetabula and each acetabulum 0.08 in diameter. Neck short, 0.16-0.24 x 0.21-0.22.

Strobila with 46-81 proglottids. Immature proglottids broader than long, 0.06-0.08 x 0.24-0.29. Mature region starts from about anterior one-third of the length of the strobila. Mature proglottids slightly longer than broad, 0.21-0.34 x 0.19-0.30. Gravid proglottids few, longer 0.30-0.54 x 0.19-0.32.

Testes four, transversely ovate in shape, tandemly arranged and tightly packed in medullary region of the proglottid. Testes 0.06-0.11 x 0.13-0.21; extend transversely occupying the entire proglottid in posterior region. Cirrus sac small, in the middle of the proglottid, 0.05-0.06. Genital pores irregularly, alternate.

Ovary bilobed, horizontally elongate, dumb-bell shaped, present at posterior extremity of proglottid. Each lobe 0.13 x 0.05-0.06. Vitellaria few, acini-like, occur in lateral cortical margins of proglottid. A strip of transversely elongated vitelline mass present below ovary.

Hosts: *Himantura uarnak* (Forsskål) and *Dasyatis (Amphotistius) zugei* (Müller & Henle).

Habitat: Spiral valve of intestine.

Location: Visakhapatnam coast, Bay of Bengal, Andhra Pradesh, India.

No. of specimens: Sixteen.

Deposition of specimens: Holotype and Paratypes deposited in the Parasitology Laboratory, Department of Zoology, Andhra University, Visakhapatnam, India.

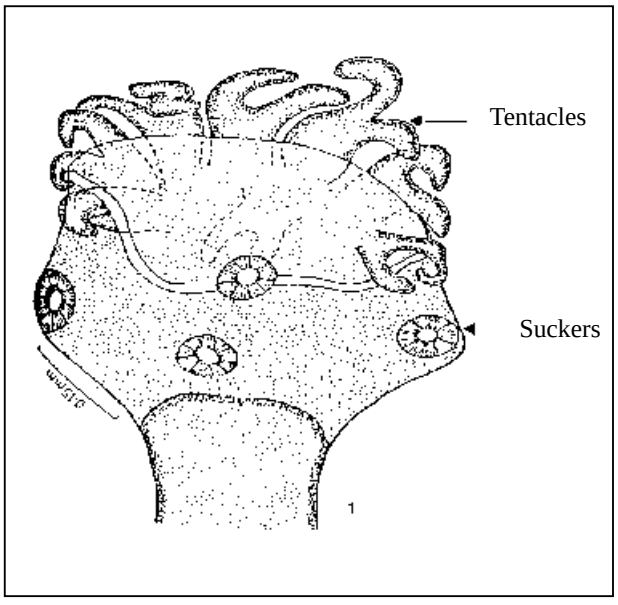

Fig. 1. Scolex showing bifucated tentacles and four suckers

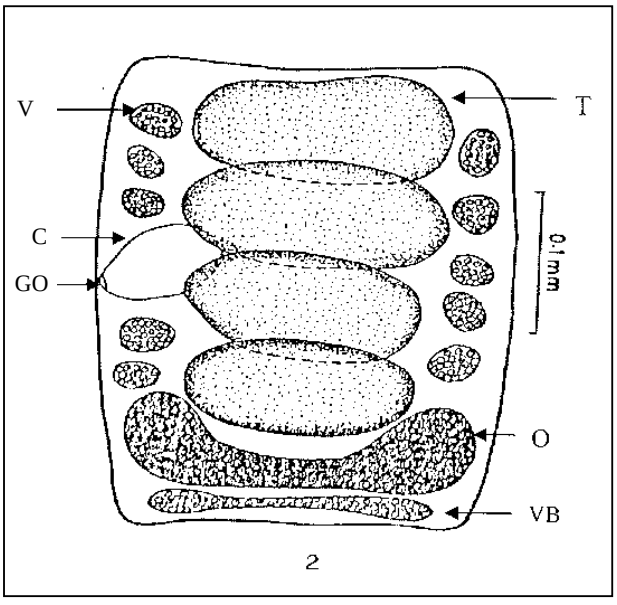

Fig. 2. Mature proglottid., T-testes,O-Ovary, V-Vitellaria, C-Cirrus sac,VB-Vitelline band and GO-Gential opening

Etymology: Named after the city of the state, Andhra Pradesh.

The genus *Polypocephalus* was first erected by Braun (1878) with *P. radiatus* as its type-species from the spiral valves of *Rhinobatus granulosus*. Later, there were many reports of this genus from skates in various parts of the world. Rather a dimunitive work has been carried out on rays. Yamaguti (1959) reported *P. medusia* and *P. pulcher* from the rays and skates. Butler (1987a) gave an exceptional historical review of cestodes of selachians and reported *Polypocephalus* sp. from *Himantura* sp. Butler (1987b) also reported *P. moretonensis* from the estuarine stingray, *Dasyatis fluviorum*. From India, Subhapradha (1951) reported six species, *P. affinis*, *P. rhinobatidis*, *P. coronatus*, *P. lintoni*, *P. rhynchobatidis* and *P. vitellaris* from skates. Shinde and Jadhav (1981) reported three species, *P. katpurensis*, *P. alii* and *P. singhii* from skates and *P. thapari* from a ray, *Trygon sephen*. Shinde *et al*. (1991) also reported *P. bombayensis* from a skate, *Aetobatus flagellum*. However, Umamaheshwari *et al*. (1987) reported *P. hanumantharaoi* from rays, *D. kuhlii* and *D. zugei* and *P. ratnagiriensis* was reported from *Trygon zugei* by Jadhav *et al*. (1987).

The present species shows a characteristic feature of having bifurcated tentacles and compared with the other two species of the genus *Polypocephalus* having paired tentacles–*P. affinis* Subhapradha, 1951 and *P. coronatus* Subhapradha, 1951. The present species differs from *P. affinis* in having acraspedote strobila, presence of neck and four testes. The present species comes closer to *P. coronatus* in having four testes but differs in many characters like acraspedote nature of proglottids, presence of neck region, presence of transversely elongated vitelline mass below ovary, scolex shape and size, absence of dome-shaped anterior region and shape of tentacles etc.

The present species varies from the other species distinctly in having acraspedote strobila, eight bifurcated tentacles and transversely elongated vitelline mass below ovary. Hence, it is justified to erect it as a new species, *P. visakhapatnamensis* sp. nov.

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JPD

Evaluation of reverse passive hemagglutination (RPHA) test for the detection of antigen in serum for the diagnosis of cystic echinococcosis

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ABSTRACT. The present study was done to evaluate reverse passive hemagglutination (RPHA) test for the detection of antigen in serum for the diagnosis of cystic echinococcosis (CE). The RPHA test was standardized using rabbit hyperimmune serum raised against crude hydatid cyst fluid, by using chromium chloride-stabilized chick cells coupled to anti-hydatid IgG antibodies and known positive and negative controls. The RPHA test was then evaluated using a panel of sera collected from cases with confirmed CE (n=20), other parasitic diseases (n=17) and healthy individuals (n=20). The RPHA test was performed; a positive test noted by matt formation and a negative one by button formation. The significant cut-off titre was estimated based on screening of disease and healthy controls and was found to be 1:32. The RPHA showed sensitivity of 60%, specificity of 97.3%, a positive predictive value of 92.3% and a negative predictive value of 81.8%. These results show that RPHA can be employed for the diagnosis of CE by demonstrating antigen in serum. The test is simple, moderately sensitive and can be performed in lesser equipped laboratories.

Keywords: antigen detection in serum, cystic echinococcosis, RPHA test

Cystic echinococcosis (CE) is caused by larval stage of the dog tape worm, *Echinococcus granulosus.* It is a zoonotic disease and infection is transmitted through contaminated vegetables and water or by playing with an infected dog (Bhatia and Pathak, 1990). CE is an asymptomatic infection and causes major health problems due to difficulty at the level of diagnosis, treatment and overall containment, leading to significant morbidity and mortality (Parija and Shivaprakash, 2000). The clinical diagnosis of CE is frequently difficult because of variable number of site, size and number of hydatid cysts in the infected hosts.

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Imaging methods are helpful in the demonstration and identification of hydatid cysts, but are expensive and often not available in the area of endemicity of the disease. Therefore, development of a diagnostic method for CE is essential for rapid diagnosis. Many immunodiagnostic tests have been used in the serodiagnosis of CE, all of which are based on the demonstration of circulating antibodies to *E. granulosus* in serum (Smyth and Barett, 1980; Kanwar and Vinayak, 1992; Shariff and Parija, 1993). However, an increasing number of false negative results occur in surgically confirmed cases and in 50% lung hydatid cysts- is an inherent disadvantage of these tests. Attempts have been made to devise tests which detect circulating antigen. Some of these assays like counterimmuno-electrophoresis (CIEP) and coagglutination (CoA) have been devised for the

detection of hydatid antigen in serum. The antigen detecting assays help in detecting the prognosis of CE like post-treatment monitoring and also to quantify the load of parasite in the patient (Parija and Sheela Devi, 1999).

The aim of the present study was to evaluate reverse passive hemagglutination (RPHA) test for the detection of antigen in serum for the diagnosis of CE, with the objectives of standardizing the RPHA using hyperimmune serum raised against crude hydatid cyst fluid in rabbits with known positive and negative controls, and performing RPHA test for the detection of hydatid antigen in serum of patients with confirmed CE and also with controls.

The following cases and controls have been included in the study:

1. Healthy controls (n=20): patients with confirmed hydatid disease either by surgery or by ultrasound (n=20).

2. Disease controls (n=17): these included patients with other diseases like amoebiasis, filariasis and toxoplasmosis.

3. Physically and medically examined and free from any disease and considered as normal.

Human hydatid fluid: A surgically removed human hydatid cyst was collected from which cyst fluid was aspirated aseptically, and centrifuged at 2000 *g* for 30 min. Fertility of the cyst was determined by the flame cell activity and vital staining (Smyth and Barett, 1980; Kanwar and Vinayak, 1992).

Flame cell activity: A sample of aspirated fluid was placed on a microscope slide and examined under a light microscope (40 x) for flame cell activity. Fluid protein content was estimated by Lowry's method (Lowry *et. al.*, 1951).

Vital staining: A few drops of 0.1 % aqueous eosin were added to the specimen on the slide. The viable protoscoleces appeared colourless, as they did not take up the dye eosin, whereas the dead cells took up the dye and stained pink. The hydatid fluid was then dispensed in aliquots and stored at -20°C until use.

Anti-hydatid hyperimmune serum (AH-HIS): AH-HIS was raised in a rabbit as per the procedure described by Shariff and Parija (1993). An adult rabbit weighing 3–4 kg was given four injections (each consisting of 0.5 ml of emulsion; human cyst fluid and Freund's complete adjuvant in equal volumes), intramuscularly (i. m.), in all the four limbs. After six weeks, a booster dose of four injections (0.5 ml each) of equal volumes of human cyst fluid and Freund's incomplete adjuvant was administered, i.m. Ten days later, the rabbit was bled from the ear vein and blood samples were collected. Serum was separated from this blood, and antibody titre of the serum was determined by indirect hemaglutination (IHA) and was found to be 1:2048.

From the cases and controls as mentioned earlier, 5 ml of blood was collected from each person by veini puncture, and the separated serum was stored at -20°C until use.

Reverse passive hemagglutination (RPHA) test: The RPHA was standardised by using chromium chloride-stabilised chick cells and coupled to anti-hydatid IgG as described below.

Preparation of reagent: Chick cells are collected in a sterile container containing equal volume of Alsevers solution and kept at 4°C until use.

Coating of the chick cells with rabbit hyperimmune sera was carried out as per the procedure described herein. A 100 µl volume of chick cells was packed in a graduated centrifuge tube and washed three-times in phosphate buffered saline (PBS; pH 7.2). After washing, 100 µl of hyperimmune sera was added, followed immediately by addition of 100 µl of 0.3% solution of chromium chloride (30 mg $CrCl_3$ in 10 ml distilled water and 0.3 ml of 2M NaOH) with continuous stirring. The mixture was then centrifuged at very low speed for 2 min and allowed to remain at room temperature for 90 min. Finally, it was centrifuged at low speed and the supernatant was removed. The mixture was washed twice with 0.1% bovine serum albumin (BSA) in PBS 7.2 and made to a fluid concentration of 1 % with BSA-PBS 7.2.

Performance of test: Twenty-five µl of 0.1 % BSA with PBS 7.2 was distributed in the wells of a microtitre plate. Test samples (25 µl each) were taken in first well and then serially diluted till 11th well of each row. Then 25 µl of coated chick RBCs were added to all the wells and the 12th well in each row served as a reagent control. Thorough mixing was done by rotating the plates. It was kept at room temperature for 30 min after which reading was taken and recorded. A positive test was noted by matt formation, whereas button formation indicated a negative test.

The agglutinated chick cells settled quickly and showed a definite pattern of haemagglutination after incubation with test and control sera following incubation at room temperature for 30–45 min. A cut-off titre was obtained based on the disease screening and healthy controls, and was found to be 1:32. The serum samples showing an antigen titre of 1:32 and above were considered as diagnostic by the test. The RPHA detected hydatid antigens in 12 out of 20 (60%) of surgically proven cases of CE, 0 out of 17 (0%) parasitic disease controls and 1 out of 20 (5%) healthy controls. The sensitivity, specificity, positive predictive value and negative predictive value of the RPHA were found to be 60%, 97.3%, 92.3% and 81.8%, respectively.

Detection of the antibodies in serum against *E. granulosus* antigens is the commonly used method for the laboratory diagnosis of CE (Parija, 1991). However, low sensitivity and specificity of these antibody-based tests, particularly cysts found in lung, is the inherent disadvantage of the test (Force *et al.,* 1992; Babba *et al.,* 1994). Approximately 60% to 80% of confirmed CE patients only, are found to be seropositive for antibodies (Gottstein, 1992). The sensitivity and specificity obtained with hydatid cyst fluid, as reported by different laboratories, ranged from 31 to 96%, and from 41 to 100%, respectively (Zhang *et al.,* 2003). Moreover, the circulating antibodies persisted longer in circulation even after removal of the cyst by surgery or clinical cure by chemotherapy (Parija, 1991). Therefore, the antibody-based serological tests fail to determine whether the infection is recent or old, so is of limited value in prognosis of the disease.

The hydatid antigens are usually excreted into serum during active infection and hence detection of serum hydatid antigen always indicates active or recent infection (Shariff and Parija, 1991). Some studies have also shown the usefulness of the serum antigen detection for monitoring the post-treatment evaluation of the CE cases (Craig *et al.,* 1986; Ravinder *et al.,* 1997). Several serodiagnostic tests have been evaluated for detection of hydatid antigen in the serum for diagnosis of CE. These tests included the CIEP (Shariff and Parija, 1991), Co-A (Shariff and Parija, 1993), LAT (Sheela Devi and Parija, 2003), ELISA (Gottstein, 1984; Kanwar *et al.,* 1992; Craig *et al.,* 1986) and Dot-ELISA (Romia *et al.,* 1992) by using polyclonal antibodies.

In our laboratory, the CIEP test for detection of serum antigen, showed a moderate sensitivity of 55.55% in surgically proved and high sensitivity of 100% in ultrasound proved CE cases for the diagnosis of CE (Shariff and Parija, 1991). The Co-A test showed a sensitivity of 95% and specificity of 89% for the diagnosis of CE by detection of serum antigen. False positive rate of 18.5% was also observed with control sera from patients with various other parasitic diseases by the Co-A (Shariff and Parija, 1993). The LAT showed a sensitivity of 72% and a specificity of 98% for the diagnosis of CE (Sheela Devi and Parija, 2003).

The RPHA test has been used for the detection of antigen in serum in hepatitis and in CSF in case of tuberculous meningitis and viral encephalitis (Katti, 2001). There are no reports for the use of RPHA for the detection of circulating hydatid antigen in serum. In the present study, RPHA using polyclonal hydatid antibodies, has been used successfully for first time for the demonstration of circulating hydatid antigen in the serum. The test showed a sensitivity of 60% with a high specificity (97.3%) in detection of hydatid antigen from serum.

The decrease in the sensitivity may probably be due to the non-availability of free- circulating antigen in sera from confirmed CE cases, which was also observed in previous studies (Kanwar and Vinayak, 1992; Krijger *et al.,* 1994). The antigen detection assays are found to be more efficient in detecting hydatid specific antigen in acid (glycine-HCl) treated sera than in untreated sera (Kanwar and Vinayak, 1992; Krijger *et al.,* 1994). This might be due to the availability of relatively free antigen after dissociation of immune-complexes by glycine-HCl. Hence, the sensitivity of RPHA may be increased by the treatment of sera using acid or polyethylene-glycol.

The RPHA test was found to be simple, economical with high specificity and moderate sensitivity. Some of the advantages of using chick cells in RPHA are that the chick cells being nucleated settles faster when compared to non-nucleated RBC from sheep or human 'O' RBCs. Heterophilic antigens are not a problem with chick cells and no prior absorption of the test sera was required, as is needed with sheep cells. The use of this common domesticated bird as the source of cells ensures that the RBCs are available for laboratory use whenever required.

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Incidence of canine ectoparasitism in Parbhani district of Maharashtra state

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ABSTRACT. Fifty out of 120 dogs harbored ectoparasites, which indicates 41.66% incidence of infection in Parbhani district of Maharashtra state; the month-wise incidence recorded was 20, 31.25, 41.17, 42.85, 45.45, 47.61 and 50% during January, February, March, April, May, June and July, respectively. *Rhipicephalus sanguineus* (41.66%), *Ctenocephalides* sp. (45.83%) and *Trichodectes* sp. (8.33%) were the ectoparasite species recorded. Deltamethrin treatment was found effective against these ectoparasites. On day 3 post-treatment (PT), only a few ticks were observed on two dogs; however, on day 5 PT, only a few ticks were observed only on a single dog. Lice and fleas could not be detected on day 3, 5 and 7 PT.

Keywords: deltamethrin, dogs, ectoparasites, fleas, lice, Maharashtra, Parbhani, ticks

Ectoparasites, *viz*. ticks, fleas and lice pose a direct threat to animal health, causing itching, unthriftiness, restlessness, physical damage to skin, blood loss, allergic conditions and transmission of pathogens. An epidemiological survey of ectoparasites in a particular area becomes important to combat this menace. Although several reports are available on canine ectoparasitism from different parts of the country, yet information is lacking from Parbhani district of Marathwada region. Hence, an investigation was carried out to study the canine ectoparasitism from Parbhani district of Maharashtra state, and the therapeutic efficacy of deltamethrin against natural canine ectoparasitic infestation was also assessed.

A total of 120 dogs of different breed, age and of either sex, belonging to private owners from Parbhani district, were examined randomly. Fortnightly, the ectoparasites, *viz*. ticks, lice and fleas were collected in specimen bottles, brought to the laboratory, processed and examined. Identification of arthropods was performed as per the morphological characters illustrated by Sen and Fletcher (1962) and Soulsby (1982). All infected dogs (50 nos.) were divided into 2 groups: Group-I (Gr-I) comprised of 40 dogs that were sprayed with 50 ppm concentration of deltamethrin, and the Gr-II comprised of 10 dogs to served as untreated control. Efficacy of the drug was determined based on the reduction in ectoparasite count.

Fifty out of 120 dogs (41.66%) harbored ectoparasites. The low ectoparasitic infestation recorded in the present experimental setting could be attributed to the precautionary measures adopted by the dog owners. The higher infestation incidence (81.36%) recorded by Raut *et al*. (2006) can be attributed to the warm and humid climate prevailing in the eastern zone of Maharashtra state. During January, February, March, April, May, June and July, incidences of 20, 31.25, 41.17, 42.85, 45.45, 47.61 and 50% were recorded, respectively. Although the month-wise variation in

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incidence was non-significant, a relatively higher incidence was recorded during monsoon and summer, whereas January (winter) witnessed a lower prevalence. The highest prevalence could be attributed to high moisture and humidity content that prevailed during monsoon, whereas low temperature during winter, slowed down the development of metamorphic stages of ticks. The immediate rise in infection rate during summer might be due to rise in temperature, which activates the development of ticks (Lapage, 1956). In the present investigation, the incidence of three ectoparasites *viz. R. sanguineus* (41.66%), *Ctenocephalides* sp. (45.83%) and *Trichodectes* sp. (8.33%) were recorded, and similar findings have been recorded by Maske *et al.* (2001) and Raut *et al.* (2006) from Vidarbha region, and Varghese and Bhalerao (1994) from Bombay region of Maharashtra state.

Following deltamethrin therapy, on day 3 PT, only a few ticks were encountered on two dogs and on day 5 PT, only a few ticks were observed on only a single dog. On day 7 PT, all the dogs were free from ticks, and 100% efficacy of the drug was recorded. Lice and fleas were not detectable on dogs on day 3, 5 and 7 PT. Similarly, Jani *et al.* (1991) have recorded complete recovery within 14 days following deltamethrin therapy, and the treated dogs were free from any other clinical and parasitological findings on day 21 and, additionally, no toxic effect of the drug was noticed. Khan and Srivastava (1992) and Singh *et al.* (1995) have recorded 98–100% efficacy of cyperkill and cypermethrin against canine ticks. Likewise, Maske *et al.* (2001) have reported 100% efficacy of deltamethrin against canine ticks, fleas and lice.

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Strategic amelioration of anthelmintic resistance in growing goat under semi-intensive farming

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ABSTRACT. Problem of anthelmintic resistance during common gastrointestinal (G. I.) nematode infection in goats could be strategically overcome by implementing rotational application of sensitive/highly effective anthelmintics control packages. The anthelmintics ivermectin, levamisole and albendazole + praziquantel were found to eliminate the common G. I. nematode infections in goats completely without any indication of drug resistance. Therefore, strategic rotational changing of highly effective anthelmintics along with supportive treatments was found highly useful in sustainable control of G. I. nematodes which infect goats reared under semi-intensive farming conditions.

Keywords: anthelmintic, goats, resistance

Goats usually suffer from several gastrointestinal (G. I.) nematodal infections which are controlled mainly by regular anthelmintic treatments. The continuous uses of such drugs very often do not control the parasites to the desired extent. This condition has been identified due to emergence of drug resistant population of worms affecting the animals. When such situation is observed, the routine anthelmintic control measures are not able to tackle the problem. As suggested by Singh and Yadav (1997) and Hong *et al*. (1996), the problem of anthelmintic resistance can be solved to a large extent by changing some of the sensitive drugs at frequent intervals while treating resistant population of parasites.

This paper describes the procedures of using some of the highly effective anthelmintics in rotation against common G. I. helminth infections for a longer duration so that efficacies of the anthelmintics may remain at a high level in Black Bengal goats, raised under semi-intensive farming system.

Thirty growing kids aged about 3 months having faecal evidence of natural clinical infections with *Haemonchus* sp., *Bunostomum* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Cooperia* sp., *Marshallagia* sp. and *Nematodirus* sp., and not given any treatment for 8–12 weeks, were selected for the study. The kids were divided into five groups and maintained separately at a small ruminants instructional goat farm unit, Ranchi Veterinary College, Ranchi, in five pens each having six animals. They were reared on concentrate, greens and grazing during the period of experiment.

Anthelmintics: ivermectin, levamisole, albendazole + praziquantel, not used or used only on rare occasion at the farm, were selected for the sustainable control of G. I. nematodes infecting the growing kids. Ivermectin @ 200 µg/kg was orally given first time to all animals of group I and II, and III group animals were administered levamisole (7.5 mg/kg bwt., orally) and albendazole + praziquantel (300 mg/kg bwt., orally). A known

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resistant drug (efficacy below 93%) fenbendazole + praziquantel (300 mg /20 kg bwt., orally) was also used to treat the infection of group IV animals. The rest six animals of group V were maintained, simultaneously, as infected untreated control.

All the treated kids were examined for pre- and post-treatment EPG (define) and % FECR (define) weekly up to 21st day post-treatment (DPT), and then twice weekly faecal examination was done to detect re-infection, which was observed to occur in each group of animals at about 45th day. Then all the animals treated with highly effective drugs were treated again on the same day by changing these drugs. Likewise, the re-infection was noted on 110th, 173rd and 240th day, and the three group animals were again treated by changing every time another sensitive drugs as shown in Table I.

The efficacies of ivermectin, levamisole and albendazole + praziquantel were found to be more than 95% on 1st treatment schedule. Their efficacies were also found to be above 95% on different re-infection post-treatment days i. e. 45th,110th, 173rd and 240th. Thus, the efficacies of all the three sensitive drugs were not affected because of rotational change in treatment of G. I. nematodosis at intervals in G. I. nematode infection growing goats for a period up to 240 days as have also been detailed in Table I, II and III.

The present findings are similar to the reports of Hong *et al*. (1996) and Singh *et al*. (1994), who tackled the problem of drug resistance by rotational treatment by changing all the time a new drug. Similarly, Yadav *et al*. (1996) and Waruiru *et al*. (2003) have also suggested to overcome the problem of drug resistance to a greater extent by stop gap treatment of the infected animals by using even slightly resistant drugs.

The result of the present experiment was also found highly useful for the sustainable control of common G. I. nematodosis in semi-intensive farmed goats because the strategic rotational changed treatment did not allow the development of anthelmintic resistance against endemically occurring G. I. nematode infection in goats reared under semi-intensive farming system.

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Table I. Schedule for amelioration of anthelmintic resistance in growing goats

Groups (animals)	Anthelmintics used	Dose and route	FECR				Larval culture		Observation on degree of resistance
			Pre-treatment	Post-treatment (days)			Pre-treatment (day)	Post-treatment (day)	
				7th	14th	21st			
I (6)	ivermectin	200 µg/kg bwt., (orally)	zero day	-do-	-do-	-do-	zero day	21st 8	–10 Months
II (6)	levamisole	7.5 mg/kg bwt., (orally)	-do-	-do-	-do-	-do-	-do-	-do-	-do-
III (6)	albendazole + praziquantel	300 mg/kg bwt., (orally)	-do-	-do-	-do-	-do-	-do-	-do-	-do-
IV (6)	fenbendazole + praziquantel	200 mg/30kg bwt., (orally)	-do-	-do-	-do-	-do-	-do-	-do-	-do-
V (6)	control (untreated infected)	-	-do-	-do-	-do-	-do-	-do-	-do-	-do-

Table II. Mean EPG and percent FECR of kids having natural G. I. nematodosis and subjected to ivermectin, levamisole, albendazole + praziquantel and fenbendazole + praziquantel treatments

Groups (No. of animals)	Anthelmi-ntics used	Dose and route	Pre-treatment mean EPG on zero day	Post-treatment mean EPG and percent FECR			Remarks
				7th day	14th day	21st day	
I (6)	ivermectin	200 µg/kg b.wt., orally	850.00±100.21	0.00±0.00 (100%)	0.00±0.00 (100%)	0.00±0.00 (100%)	Susceptible
II (6)	levamisole	7.5 mg/kg b.wt., orally	741.66±118.61	0.00±0.00 (100%)	0.00±0.00 (100%)	0.00±0.00 (100%)	Susceptible
III (6)	albendazole + praziquantel	300 mg/10 kg b.wt., orally	833.33±102.40	0.00±0.00 (100%)	0.00±0.00 (100%)	0.00±0.00 (100%)	Susceptible
IV (6)	fenbendazole + praziquantel	200 mg/30 kg b.wt., orally	791.66±73.50	55.00±15.36 (93.07%)	86.66±33.18 (89.27%)	114.17±50.83 (85.57%)	Resistant
V (6)	infected untreated		779.66±124.90	793.33±71.7	808.33±113.59	829.16±92.96	

Table III. Mean EPG and percent FECR of kids having natural G. I. nematodosis and subjected to sustainable treatment with ivermectin, levamisole, albendazole + praziquantel and fenbendazole + praziquantel

Groups (No. of animals)	Pre-treatment EPG (45th day)	Anthelmintic used and post-treatment EPG and FECR (52nd day)	Pre-treatment EPG (110th day)	Anthelmintic used and post-treatment EPG and FECR (117th day)	Pre-treatment EPG (173rd day)	Anthelmintic used and post - treatment EPG and FECR on (180th day)	Remarks
I (6)	105.83 ± 29.59	levamisole 0.0+ 0.00 (100%)	75.83±36.75	(albendazole + praziquantel) 0.00± 0.00 (100%)	54.16 ± 20.83	ivermectin 0.00 ± 0.00	Treatment repeated on 45th,110th 173rd DPT after mild re-infection
II (6)	107.5 ± 34.66	ivermectin 0.00± 0.00 (100%)	50.8 ± 30.68	(albendazole + praziquantel) 0.00± 0.00 (100%)	30.83 ±25.83	levamisole 0.00± 0.0 (100%)	
III (6)	116.67 ± 25.82	levamisole 0.0± 0.00 (100%)	72.5 ± 30.73	(ivermectin) 0.0± 0.00 (100%)	35.5±31.72	(albendazole + praziquantel) 0.0 ± 0.00	
IV (6)	329.17±147.17	fenbendazole + praziquantel 70.83 ± 30.56 (91.90%)	318.5±175.46	(fenbendazole + praziquantel) 85.0±25.18 (90.85%)	475.0±118.14	(fenbendazole + fraziquantel) 118.59± 40.37 (90.18%)	
V (6)	866.66±128.88	875.00 ± 117.73	914.16±124.66	929.17±121.35	1095.83±255.15	1208.33 ±115.04	

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Plasma proteins in children with helminth infections

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ABSTRACT. The effect of helminth infections on the nutrition, growth and physiology of children still remain poorly understood. The objective of this study was to assess the effect of helminth infections on plasma proteins in children. Stool and blood samples were collected from 382 school children, both male and female, in the age group of 5–15 years from Kashmir valley. Blood samples were analyzed for the estimation of plasma proteins like albumin, globulin and total proteins by BIURET and BCG methods, and the stool samples were processed by using both simple smear and zinc sulphate concentration methods. Of the 382 children surveyed, 299 (78.27%) were infected with either *Ascaris lumbricoides* or *Trichuris trichiura* or both. Children infected with helminths were found to have lower mean values of plasma albumin and total proteins but higher mean values of globulin than uninfected children. The present study revealed that geohelminths were abundant among children of Kashmir valley, which have a negative effect on the plasma protein values, and suggests implementation of control measures to prevent geohelminthiasis.

Key words: children, helminths, Kashmir valley, plasma proteins

Whether intestinal geohelminths play a significant role in the etiology of childhood malnutrition, remains to be shown unequivocally (Crompton, 1986; Lunn and Northrop-Clewes, 1993). Helminth infection is thought to contribute to child malnutrition through subtle reduction in digestion and absorption, chronic inflammation and loss of nutrients (Thein-Hlaing, 1993). Intestinal helminths can cause injury to the mucosa of small intestine, which cause malabsorption and gastrointestinal losses of nutrients (Lunn et al., 1993). Hypoalbuminaemia is reported frequently in trichuriasis and hookworm disease. Moreover, local inflammation at the site of infection appears to provoke a systemic inflammatory response with elevated plasma concentration of acute-phase proteins and cytokines (Cooper et al., 1992). Although several studies have been carried out on the prevalence of helminth infections in children of Kashmir valley (Khuroo, 1996; Ahmad et al., 2002; Wani, 2007); nevertheless, their impact on plasma proteins has not been ascertained till now. Therefore, the present study was undertaken to determine the relationship between soil-transmitted helminths (STH) and plasma proteins in children of Kashmir valley.

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Kashmir valley, situated at an altitude of 6000 feet, constitutes a major portion of Jammu and Kashmir State of India, which consists of six districts with about 26 Tehsils and about 33 towns (Gupta, 2005). This study was conducted between May 2006 and November 2006. Official meetings with the personnel from health services, city councils and schools, as well as parents and school children from the study sites, were carried out in order to explain the protocol of the study. A total 382 children, which include 219 male and 163 female, between the ages of 5–15 yrs (mean 9.2±2.3 yrs) with no disabilities and those not receiving antiparasitic treatment were included in the study. Initially, 480 children agreed to participate but 98 of them were rejected during the study because they either had

contaminated fecal samples or did not agree for blood samples. Written consents were obtained from both parents in order for the children to participate. The children's ages were obtained through school records. Sterile 5 ml syringes (Dispovan®) were used to take out blood samples. Blood was stored in glass vials and a pinch of anticoagulant i.e., ethylenediamine tetra-acetic acid was added. Also fresh morning stool samples were collected in nylon containers containing 10 ml of 10% formaldehyde. Both blood and stool samples were labeled, and immediately transported to the parasitology laboratory, Department of Zoology, University of Kashmir, for further processing. For the estimation of proteins in blood plasma, BIURET and BCG method were employed. The stool specimens were processed using direct smear and zinc sulphate concentration techniques. A computer program (SPSS 10.05 for windows) was used for data analysis. The descriptive data was given as a mean±standard deviation (SD). Student's t-test was used for the analytic assessment. The differences were considered to be significant when the p value obtained was less than 0.05.

Two hundred ninty nine out of 382 children (78.27%) were infected with either Ascaris lumbricoides or Trichuris trichiura or both. No other parasite was observed. Single and mixed-type infections were observed almost in equal proportions. One hundred forty nine (39.0%) children were infected by a single type of helminth, in which A. lumbricoides was found in 91 (23.82%) and T. trichiura in 58 (15.18%) children. Mixed-type infection with A. lumbricoides and T. trichiura was observed in 150 (39.26%) children.

Children infected with intestinal helminths had lower values of albumin in their plasma than uninfected children ($p < 0.05$). Also, albumin in children with mixed infection was comparatively lower than in children with a single type of infection ($p < 0.05$). As shown in Table I, it is clear that Ascaris infection is involved in causing malabsorption of proteins in the intestinal tract, as children infected with A. lumbricoides had lower mean values of albumin (3.42±0.15 g/dl) in their plasma than in children infected with T. trichiura (3.99±0.23 g/dl).

Plasma globulin level was higher in case of infected children as compared with uninfected children ($p < 0.05$). In case of mixed infection, the mean plasma globulin was higher than in children infected with single type of helminth parasite. It was also observed that children infected with A. lumbricoides were having slightly higher levels of globulin in their plasma, than in children infected with T. trichiura.

The mean values of total protein were significantly ($p < 0.05$) higher in uninfected children than in infected children. Total protein values were slightly higher in children infected with multiple infections than in children infected with single type helminths. It was also observed that children infected with A. lumbricoides were having lower mean values of total protein than children infected by Trichuris trichiura. The results thus reveal the role of Ascaris infection in causing protein calorie malnutrition by interfering with protein absorption in the intestinal tract.

The present study indicated a prevalence of intestinal helminthiasis as high as 78.27%. These figures, when compared with studies conducted in other parts of the world, show that Kashmir valley is one of the endemic regions for intestinal helminthiasis. For example, studies conducted on the frequency distribution of gastrointestinal helminths by Bundy et al. (1988) have shown a high overall prevalence of 62% among the urban slum children of Malaysia. Rodriguez et al., (2000) have reported a high prevalence of 72% among the school children studying in a public institution in

Table I. Mean values of albumin, globulin and total protein (g dl^{-1} mean±SD) in infected and uninfected children

Type	Albumin	Globulin	Total protein
Infected	3.62±0.32	2.62±0.45	6.08±0.6
Uninfected	4.03±0.43	2.37±0.41	6.72±0.8
Single type infection	3.64±0.33	2.57±0.41	6.01±0.7
Multiple type infection	3.59±0.31	2.68±0.45	6.15±0.6
Infection with *Ascaris*	3.42±0.15	2.58±0.39	5.60±0.3
Infection with *Trichuris*	3.99±0.23	2.54±0.45	6.64±0.6

Maracaibo, Venezuela. Legesse et al., (2004) also noted the high prevalence of 88.2% among the school children in rural Ethopia, whereas Kabatereine et al., (2001) have reported an overall prevalence of 56% among the school children of south Uganda.

The high prevalence of soil-transmitted helminth infections is probably a consequence of a low standard of living, poor sanitation, lack of personal hygiene, traditional methods of agriculture, indiscriminate defecation, the use of night soil as fertilizers and other occupational work. Similar factors have also been found responsible for high prevalence of infection by Ulukanligil and Seyrek, 2003 and Okyay et al., 2004.

Children with helminth infection were found having lower mean values of albumin, higher globulin and low mean value of total proteins in blood plasma. Studies conducted by Northop (1987), Annanthakrishnan et al. (1997), Ortiz et al. (2000) Northrop et al. (2001) and Rai et al. (2004) are in conformity to our present results. Northop (1987) showed that albumin concentrations increase greatly after antihelmintic treatment in Bangladeshi children infected with A. lumbricoides. Rai et al. (2004) found differences in albumin concentrations between infected and uninfected children in Nepal. Intestinal helminths cause malabsorption of proteins in intestines, which leads to decreased albumin and protein calorie malnutrition (Annanthakrishnan et al., 1997). Protein absorption has been shown to be better in children after deworming (Annanthakrishnan et al., 1997). It has been estimated that in children with worm load of 13–40 worms, approximately, 4 grams of protein is lost per day from a diet containing 35–50 g of protein (Gupta, 1990).

Higher concentrations of globulins in infected children are due to the increased titre of immunoglobulin against helminth infections. Northrop et al. (2001) showed decrease in globulin titre in children treated with anthelmintics, which indicate possible reduction in inflammation and immunoglobulin concentrations after deworming. Ortiz et al. (2000) showed that the intensity of parasitic burden plays an important role in stimulating polyclonal IgE, which diminishes the effectiveness of the specific response to these infections. On the other hand, nutritional deficiencies could change the immune mechanisms of the mucous membrane, negatively influence the synthesis of secretary IgA and stimulate the production of polyclonal IgE; thus increase in globulin level in blood plasma. In treated children, after deworming, total protein concentration becomes low as globulin level decreases (Northop et al. 2001).

The low mean values of total protein in infected children are due to low levels of albumin in blood plasma. In infected children, presence of worms in intestine causes less absorption of proteins, which leads to low protein levels in blood plasma (Annanthakrishnan et al., 1997). Hypoalbuminaemia is reported frequently in trichuriasis (Cooper et al., 1992). So it is clear that indeed globulin levels increase during infections, but over all protein levels decrease, which is the reason behind the lower levels of total protein in the plasma of infected children.

The present study revealed that intestinal helminthiasis is high among children of Kashmir valley and has a negative impact on their plasma protein status. This situation strongly calls for the institution of control measures, including treatment of infected individuals, improvement of sanitation practices and provision of clean water. The impact of each measure would be maximized through a health education program directed at school children, and their mothers in particular, and to communities in general.

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