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JPD

Address of the President, Indian Society for Parasitology, delivered at the Eighteenth National Congress of Parasitology, Kolkata, November 22-24, 2006

Hon'ble Chief Guest, distinguished dignitaries on the dais, fellow scientists, invitees, delegates, ladies and gentlemen.

At the very outset, on behalf of the Indian Society for Parasitology, I feel honoured and privileged in extending a very warm welcome to you all to the 18th National Congress of Parasitology being held in the city of joy Kolkata. A special welcome to the young parasitologists, who might be attending their first meeting of such kind, and hope you will be provided with many opportunities to enrich your future careers.

Parasitic diseases continue to be a cause of major concern to human and animal health in several parts of the globe including India, causing high morbidity, mortality and economic losses, particularly in the socio-economically underdeveloped societies in the tropical/subtropical countries of the world. Food-, water- and soil-borne infections are estimated to be affecting almost half of the world's population. Zoonoses (i.e., diseases that are transmittable between animals and man) of parasitic origin contribute to this statistics by affecting human health and causing heavy losses directly or indirectly to economy. The aquatic environmental route of transmission is adopted by and is important for many protozoan and helminth parasites. Besides, the consumption of animal-based foods like crustaceans, molluscs, fish, birds, beef and pork facilitates transmission of a large number of parasitic infections. The bourgeoning travel industry, emigration and importation of food from endemic regions has resulted in increasing diagnosis of these infections in non-endemic regions of the world now transcending all geographical and political boundaries. The ensuing environmental changes in human settlements and socio-cultural developments, singly, or in combination have been accompanied by global increases in morbidity and mortality from emerging and re-emerging parasitic diseases, zoonoses in particular. The potential of parasites for producing large number of transmission stages (cyst, spore, egg and larva) and their environmental robustness (being able to survive in adverse microclimates for prolonged periods of time) pose a persistent threat to public and livestock health.

In our country, as is true of many other tropical countries, several infections of parasite origin both protozoal and worm are of common occurrence among humans. Amoebiasis, dientamoebiasis, giardiasis, leishmaniasis, toxoplasmosis, trichomonal infections, trypanosomiasis in livestock, malaria, babesiosis, cryptosporidiosis, taeniasis, cysticercosis-mainly neurocysticercosis, echinococcosis, filariasis, hookworm infections, larva migrans, strongy-loidiasis, ascariasis, toxocariasis, whipworm infections, in addition to arthropod infections such as myiasis and lice infestations are some of the most common and prevalent parasitic infections in our part

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of the world. While some of these infections, e.g., malaria, kalazar and filaria require an insect vector for their transmission from one to the other host, others such as hookworm, *Ascaris* (the large intestinal round worm), whipworm and threadworm, may be soil or water borne and transmitted through contaminated environmental objects. Many worm infections prevail in animal hosts, which, in turn, may become natural reservoirs of infection to human hosts. The nature and extent of worm types that occur in food-giving animals like fishes, frogs, poultry and livestock mammals depend on, and are influenced by, the ambient environmental factors and socio-cultural practices prevailing in a region. I may cite as an example the case of lungfluke infection. *Paragonimus*, a zoonotic lung fluke, has got considerable importance from veterinary, human parasitological and medical points of views because of its diversified effect on its final host. The lung flukes of the genus *Paragonimus* have been known as one of the most important zoonotic parasites causing paragonimiasis, also known as endemic haemoptysis, in man. The infection in humans is a result of complex transmission cycle that includes two obligate intermediate hosts, a snail and a crustacean or a crayfish, and a definitive mammalian host. It is estimated that over 20 million people are infected worldwide due to several species of P*aragonimus.* Over 40 species are known to infect the lung of different mammalian hosts throughout the world, and approximately 15 species are known to infect humans. The best known and the most important species, distributed in Asia is *P. westermani*, a human parasite that can undergo development in as many as 16 different snail species and 50 crustacean species, and a much larger range of paratenic hosts than any other trematode. This wide range of host adaptability of the fluke together with the food habits of the host is regarded as the main reason for high rate of prevalence and endemicity of the parasite in different parts of the globe. Though the fluke is known to parasitize a wide range of mammalian hosts representing as many as eleven families, the status of its prevalence, its host range and its possible reservoirs in nature from where the human beings contract the infection is not well documented in India.

Resistance of the parasites and/or their vectors to drugs, and antigenic variations expressed by these pathogens compound the problems of treatment, prophylaxis and control. Besides, simultaneously with changing scenario of the so-called development – industrial or otherwise, and resultant climate change that the whole world is experiencing today, there are several diseases of parasite origin that are newly emerging or re-emerging with greater virulence than ever before in the present times. In the context of our country, while malaria is making its ill presence more severely and at a larger scale, other protozoan diseases like leishmaniasis, amoebiasis, and helminthoses like filariasis continue to remain unchallenged and cause immense human suffering. Likewise, babesiosis, toxoplasmosis, coccidiosis and many more caused by worm infections severely affect the health of animal hosts, both aquatic and terrestrial.

Advancement in structural biology, molecular biology, immunology, biotechnology and bioinformatics, genomics, proteomics, metobolomics etc. are, however, opening up newer vistas and providing us newer tools to fight against the menace of parasites. Newer and simpler, field-oriented methods are becoming available to us for diagnostics, surveillance, therapeutics, prophylaxis and control. Application of molecular biology and biotechnological tools has given us a quantum jump in enhancing our understanding of the pathogen, its transmitter or vector, the disease and its epidemiology, and diagnostics. In traditional medicine practices many plants have their usage for getting rid of worm or other infections. Bioprospection of phytochemicals isolated from traditionally used medicinal plants for their antiparasitic efficacy seems to be a much desired rather essential direction to follow in quest to search for newer drugs.

Established in 1973 under the Societies Act XXI, 1860, The Indian Society for Parasitology (ISP) has the major objectives of bringing parasitologists of different disciplines under one umbrella to advance knowledge in this vital area concerning human and animal health. Over the years, the Society has carved a niche for itself by providing a common platform to research scientists, clinicians, field biologists, academicians and all others involved in parasitology or tropical medicine to share and exchange their knowledge and observations. It is my pleasure and privilege to inform you that the Indian Society for Parasitology is now a member of the World Federation of Parasitologists and is represented on the WFP Executive Council as one of the ten members-at-large. The Society has over 600 life members at present. Since 1981 onwards ISP has inducted 21 distinguished parasitologists into its fellowship. Instituted in 1997 during the golden jubilee year of our independence,

the Lifetime achievement award has so far been conferred on four parasitologists D. A. B. Chowdhury, Prof. R. C. Mahajan, Prof. N. K. Ganguly and Dr. V. P. Sharma. Besides, the Society also confers two oration awards *viz.*, B. N. Singh Memorial Oration for excellence in research and B. P. Pandey Memorial Oration for contributions to teaching of Parasitology. In order to encourage young scientists below 32 years in their academic pursuits, the Society has constituted one Young Scientist Award based on best paper presentation and M. B. Mirza Award for best research publication of work done in India.

Annual conferences are organized by ISP with a view to take stock of the current developments in Parasitology and evolve strategies for the control and containment of parasitic diseases. ISP has so far organized two International or global meets and three Asian Congresses of Parasitology. The Second Global Meet on Parasitic Diseases at Hyderabad organized during 1997, the year of Golden Jubilee of Indian Independence and Silver Jubilee of ISP establishment, is a milestone in the annals of ISP. The event commemorated the hundred years of the discovery of the malarial parasite in the mosquito vector by Sir Ronald Ross.

Last year in October, 2005 the 17th National Congress of Parasitology was organised by Regional Medical research Centre (ICMR), Dibrugarh. And this year, this prestigious institute of CSIR, The Indian institute of Chemical Biology offered to host the event, with Dr. Salil Datta, Head of the Biochemistry Division, IICB, having taken the responsibility of the onerous task as its Organizing Secretary. I am grateful to the Director, IICB, Kolkata for the same. I do hope that organization of this 18th National Congress of Parasitology would provide us a common platform to discuss issues and challenges that the parasitic diseases pose before us, to interact with the scientific community at large and the experts in the field in particular, and to exchange knowledge and ideas with one another. Besides the scientific sessions, there will be a chance to meet your colleagues and to interact with scientists from various corners of the country and experts in various fields of Parasitology. ISP will hold their Annual General Meetings during the Congress and all members are encouraged to attend this event. I am confident that this Congress will present you with an opportunity to discuss new ideas, to learn good science, and to make new friendships and collaborations. It is my hope and prayer, too, that the deliberations of the Congress would help us come out with meaningful recommendations and identify directions for future work. So**,** enjoy the Congress, the company of learned parasitologists and the city of Kolkata.

Thank you all for your kind attention.

Veena Tandon
President, Indian Society for Parasitology

Journal of Parasitic Diseases: December 2006, Vol. 30, No. 2, 101–115 *Review*

Historical review of piscine trypanosomiasis and survey of Indian *Trypanosoma*

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ABSTRACT. Trypanosomes are flagellated parasites frequently encountered in the blood of fishes. New species of *Trypanosoma* have been reported in fish blood from time to time. From India, unnamed trypanosomes were discovered by Lingard (1904). Subsequently, *T. clariae* Montel, 1905; *T. mukasai* Hoare, 1932; *T. clariae batrachi* de Mello and Valles, 1936; *T. striati* Qadri, 1955; *T. punctati* Hasan and Qasim, 1962; *T. batrachi* Qadri, 1962; *T. saccobranchi* Qadri, 1962; *T. gachuii* Misra *et al*., 1973; *T. elongatus* Ray Chaudhuri and Misra, 1973; *T. mukundi* Ray Chaudhuri and Misra, 1973; *T. maguri* Tandon and Joshi, 1973; *T. vittati* Tandon and Joshi, 1973; *T. baigulensis* Pandey and Pandey, 1974; *T. anabasi* Mandal, 1975, *T. armeti* Mandal, 1975; *T. pancali* Mandal, 1975; *T. mrigali* Joshi, 1976; *T. seenghali* Joshi, 1976; *T. choudhuryi* Mandal, 1976; *T. batai* Joshi, 1978; *T. stigmai* Joshi, 1978; *T. anabasi* Mandal, 1978; *T. cancili* Mandal, 1978; *T. bengalensis* Mandal, 1979; *T. channai* Narasimhamurti and Saratchandra, 1980; *T. qadrii* Narasimhamurti and Saratchandra, 1980; *T. tandoni* Mandal, 1980; *T. singhii* Gupta and Jairajpuri, 1981; *T. trichogasteri* Gupta and Jairajpuri, 1981; *T. godavariensis* Saratchandra and Jayaramarajan, 1981; *T. aligaricus* Gupta and Jairajpuri, 1982; *T. attii* Gupta and Jairajpuri, 1982; *T. aori* Joshi, 1982; *T. nandi* Mukherjee and Haldar, 1982; *T. rupicola* Joshi, 1983; *T. gobida* Mandal, 1984; *T. monomorpha* Gupta and Jairajpuri, 1985; *T. notopteri* Gupta and Jairajpuri, 1985; *T. xenentodoni* Das *et al*., 1986; *T. colisi* Gupta, 1986; *T. joshii* Wahul, 1986; *T. mandali* Wahul, 1986; *T. marathwadensis* Wahul, 1986; *T. puntii* Wahul, 1986; *T. purensis* Wahul, 1986; *T. rayi* Wahul, 1986; *T. seenghali* var *sophorae* Yatindra and Mathur, 1986; *T. barbi* Gupta *et al*., 1987; *T. bareilliana* Gupta *et al*., 1987; *T aurangabadensis* Wahul, 1987; *T. mastacembeli* Wahul, 1987; *T. murtii* Wahul, 1987; *T. ritae* Gupta and Yadav, 1989; *T. parastromataei* Narasimhamurti *et al*., 1990; *T. rohilkhandae* Gupta and Saraswat, 1991; *T. kargenensis* Gupta and Gupta, 1994; *T. bagroides* Gupta and Gupta, 1996; *T. ticti* Gupta *et al*., 1998; *T. trichogasteri* var. *fasciatae* Gupta *et al*., 1998; *T. karelensis* Gupta *et al*., 2000; *T. monomorpha* var *catlae* Gupta *et al*., 2000; *T. artii* Gupta *et al*., 2002; *T. piscidium* Gupta *et al*., 2003; *T. heteropneusti* Gupta *et al*., 2006 and *T. saulii* Gupta *et al*., 2006 were described from various Indian piscine hosts. However, many of the biological features of *Trypanosoma* still remain obscure. The review article herein focuses on diversified aspects of piscine trypanosomiasis comprising of morphology, taxonomic status, taxonomic tools and morphometrics, blood collection protocols, studies on live parasites, pleomorphism, morphogenesis in fish hosts including multiplication patterns, transmission, sequence of development in the leech vector, pathobiology, immune response against the parasite and control strategies.

Keywords: immune response, leeches, pathobiology, pleomorphism, *Trypanosoma*

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INTRODUCTION

"Piscine haemoflagellates" is rather a vague term and includes leech- transmitted kinetoplastids from the

blood of a large variety of host species, which are united more by following a similar way of life in water rather than by forming a technical homogenous group. Flagellate parasites of fish have not been investigated as extensively as those infecting man and domestic animals. The available literature is scattered and warrants extensive and sophisticated studies. Detailed investigations on morphology, ultrastructure, taxonomy, metabolism, biochemical and genetic characteristics, developmental and transmission cycles, ecological responses and impacts, pathogenecity and control measures are to be conducted in order to gain insight into the true biological characteristics of flagellates.

Parasitic flagellates in the peripheral circulation of fishes primarily belong to two genera, *Trypanosoma* Gruby, 1843 and *Trypanoplasma* Laveran and Mesnil, 1901 (Mastigophora) the former being a monoflagellate, and the latter, biflagellate. Interest in trypanosome infection in fish has increased over the last 30–35 years because of their perceived importance in fish.

Trypanosomes are haemoflagellates having a single free flagellum at the anterior end of the body. The first trypanosome was discovered from the blood of *Salmo fario* by Valentin (1841). From India, Lingard (1904) recorded the first trypanosome from *Barbus carniticus* from Poona but the description lacked mensural data.

The parasite has been reported from different parts of the globe. *T. mukasai* from 11 species of fishes (Baker, 1960, 1961). *T. froesi* from *Mugil brasilensis* (Lima, 1976), *T. satakei* from *Rhamdia quelen* (Nuti *et al*., 1987) and *T. britskii* from *Loricaria lentiginosa* (Lopes *et al.*, 1991) were reported from Brazil.Becker (1967) reported *T. occidentalis* from Washington and Grogl *et al*. (1980) recorded *T. magdulenae* from *Petenia krausii* from Columbia. The diversity of trypanosomes parasitizing American freshwater fishes are mainly localized (Jones and Woo, 1990, 1993)

T. acanthobramae from *Acanthobrama marmid* (Warsi and Fattohy, 1976) and *T. neinevana* from *Barbus grypus* (Fattohy, 1978) were recorded from Iraq. From England, the ultrastructure of the epimastigotes of *T. cobitis* in the crop of *H. marginata* (Lewis and Ball, 1980) as well as in culture (4N blood agar) has also been described (Lewis and Ball, 1981). Trypanosome species from the fishes of Poland were reported by Jastrzebski (1984) who observed polymorphism in *Trypanosoma* from the blood of crucian carp, perch and stone perch. Wita and Ovcharenko (1997) recorded the presence of *T. carassii* from the roach and tench and Wita *et al*. (2001) observed the distinctness of *T. abramidis* collected from 20 individuals of *Abramis brama*.

The blood of Indian fishes has revealed the presence of parasites from time to time. Qadri (1962a) reported *T. batrachi* from *Clarias batrachus*; other species reported are *T. gachuii* from *Ophiocephalus gachua* (Misra *et al*., 1973); *T. elongatus* from *Channa punctatus* (Raychaudhuri and Misra, 1973); *T. armeti* from *Mastacembelus armatus* (Mandal, 1975). First record of trypanosomes from *Notopterus notopterus, Cirrhina mrigala, Wallago attu* and *Channa gachua* was provided by Joshi (1979) and *T. channai* from *C. batrachus* was discovered by Narasimhamurti and Saratchandra (1980). Saratchandra and Jayramarajan (1981) described a dimorphic trypanosome, *T. godavariensis* from *Channa punctatus* collected from the East Godavari district. Joshi and Sharma (1992) recorded *Trypanosoma* from *Tor putitora* and also observed the changes in haematological values incurred therein. Roy and Haldar (1997) reported trypanosomes to occur in *Anabas testudineus, Heteropneustes fossilis, Clarias batrachus* and *Channa punctatus* while investigating the protozoan haemoflagellates from air breathing fishes of West Bengal. Chakraborty *et al.* (2000) observed the occurrence of fish diseases in Karnataka and recorded trypanosomes from *Oreochromis mossambicus*. Nandi *et al*. (2002) provided a bibliography of 301 protozoan parasites from Indian fishes including more than fifty trypanosome species.

From the state of Uttar Pradesh, several species have been put on record. *T. trichogasteri* (Gupta and Jairajpuri, 1981a), *T. colisi* (Gupta, 1986), *T. trichogasteri* var. *fasciatae* (Gupta *et al.*, 1998) and *T. piscidium* (Gupta *et al*., 2003) from *Colisa fasciata; T. aligaricus* (Gupta and Jairajpuri, 1982a), *T. rohilkhandae* (Gupta and Saraswat, 1991) and *T. sauli* (Gupta *et al.*, 2006) from *Channa punctatus*; *T. monomorpha* (Gupta and Jairajpuri, 1985) and *T. monomorpha* var. *catlae* (Gupta *et al*., 2000) from *Catla catla; T. notopteri* from *Notopterus notopterus* (Gupta and Jairajpuri, 1985); *T. kargenensis* (Gupta and Gupta, 1994), *T. karelensis* (Gupta *et al*., 2000), *T. artii* (Gupta *et al.*, 2002) and *T. heteropneusti* (Gupta *et al*., 2006) from *Heteropneustes fossilis, T.*

bagroides (Gupta and Gupta, 1996) from *Mystus vittatus* and *T. ticti* from *Puntius ticto* (Gupta *et al*., 1998) have been described from different fish hosts. These records include monomorphic, dimorphic and pleomorphic species.

GENUS *TRYPANOSOMA* GRUBY

Morphology: Parasite of the circulatory system of vertebrates; usually slender and flattened, pointed at flattened end, and bluntly rounded or pointed at the other end; usually pleomorphic, nucleus central; near the flagellated end there is a blepharoplast from which the flagellum emerges and runs towards opposite end bordering the outer boundary of the undulating membrane; in most cases the flagellum extends freely beyond the body as free flagellum; many with myoneme fibers; multiplication by binary or multiple fission. The organism is transmitted from host by blood sucking invertebrates and undergoes a series of changes in the digestive system of the vector hosts. A number of these haemoflagellates are pathogenic to their hosts.

TRYPANOSOMES OF FISH

Fishes are known to host a large number of parasites belonging to different phyla, however, little attention has been paid to the study of trypanosome parasites of fish. During the recent years, studies on the trypanosome parasites of Indian fishes have assumed a special importance due to the vast and varied amount of fish potentialities available in the marine, brackish, estuarine and freshwater sources and their huge consumption.

Trypanosomes of fish are apparently similar to related trypanomatids from the blood of other vertebrates (Fig 1). The body is elongated, leaf like or lancet shaped. Trypanosomes of fish have been distinguished by size, shape and relative position of organelles in stained specimens (Lom, 1979). In fish trypanomastigotes, the flagellum is situated directly in/or at some distance from the posterior end of the long, sinuous body. It originates from the kinetoplast, borders the undulating membrane and extends as a free flagellum at the anterior end of the cell body. The nucleus is more or less situated in the center but its position varies according to different species. A functional cytostome with a permanent opening exists in the vector (= culture) stages, either level with the opening of the flagellar pocket (*e. g.* in *T. rajae*) or shifted out of the pocket anterior to it on the surface of the body (in *T. carassii* = syn. *T. danilewskyi*). The

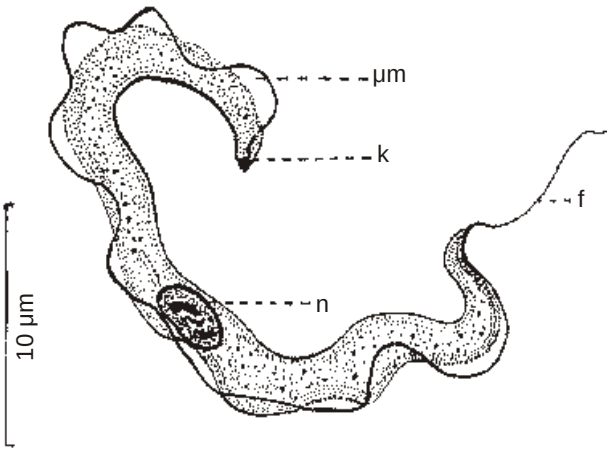

Fig. 1. Diagram showing the cell structure of *Trypanosoma aligaricus* bloodstream form from *Channa punctatus* (f, flagellum; k, kinetoplast; n, nucleus; um, undulating membrane).

cytostome continues as a long pharangeal canal surrounded by microtubules. In *T. carassii* (Lom *et al*., 1980) and probably in other fish trypanosomes, the cytostome is also present in the blood stream trypomastigotes. A contractile vacuole may exist (*e. g.*, in *T. rajae*) in culture forms, near the flagellar pockets. The longitudinal striations in adult forms of some species, described in earlier papers as "myonemes", are probably artificially shrunken strands of microtubules of the subpellicular microtubular corset, rather than sub pellicular mithochondrial ribbons.

The mass of DNA fibers the nucleoid, of the kinetoplast is dish- or disc-shaped localized in one end of the long, sometimes branched, ribbon- shaped mitochondrion, and faces the kinetosome of the flagellum. Spontaneous occurrence of trypanosomes without kinetoplast has never observed in fish, but this state, termed dyskinetoplasty, can be induced *e. g.*, by acriflavine.

However, molecular and antigenic profiles of polypeptides of trypanosomes are reported to be different and have been identified as important taxonomic tools for the identification of fish trypanosomes (Jones and Woo, 1992). Unfortunately, these characteristics for most species are not known, hence their usefulness as taxonomic tools is not clear.

MORPHOMETRICS

The identification and classification of *Trypanosoma* has often posed a problem for the working taxonomist. In fact, the terms 'Systematics', 'Taxonomy', 'Classification' and 'Nomenclature' are often vaguely

used without a proper understanding of their meaning. Systematics has been defined by Jeffrey (1973) as *"the scientific study of the diversity of living organisms"* and is subdivided into taxonomy, *"the study of the principles and practice of classification* (used as a synonym of systematics by Corliss, 1962); and nomenclature, *"the allocation of names to the group"*. These facts constitute an important segment of biometrics while dealing with trypanosomes.

In fact, taxonomy is to aid in the classification and understanding of biological phenomenon and is not an end point in itself, then description of new species, particularly in these difficult groups like trypanosomes becomes simple and easy to justify (Bennett, 1989).

The taxonomy of different haematozoans occurring in fishes was vividly discussed by Becker (1970). Mandal (1984) attempted to draw synonymy amongst different genera but could not come to any conclusion. Enzyme analysis has contributed considerably to the taxonomy of many groups of parasitic protozoa including trypanosomes. Morphology offers only limited opportunities for distinguishing various kinetoplastids from one another, particularly at the species level. The use of molecular markers is more promising. Stevens and Godfrey (1992) reviewed biochemical taxonomy of the genus and presented numerical analyses of isoenzymes data from the improved range of enzymes. Stevens *et al*. (1992) described a simplified approach for using enzyme polymorphs for identifying subspecies and strain groups of *Trypanosoma*. Particularly well suited for this purpose is the kinetoplast (mitochondrial DNA), the maxicircle. Since it generally evolves more rapidly than nuclear DNA and also contains more slowly evolving regions, it can be used in studies of both closely and distantly related taxa (Avise, 1994).

However, Bennett (1989) did not regard isoenzyme analysis to be mandatory prior to the description of a new species of trypanosome. It is an advanced technique and certainly assists in the definitions of varieties, strains and species but may not always be essential. Morpho-taxonomic grounds are an adequate basis provided:

1. The species is proved to be monomorphic
2. The characters are significantly different
3. The new species is morphologically compared in depth to all similar species and to other species in the same major group of hosts.

Moreover, for a proper description of the species, a statistically valid sample of individuals should be measured from several hosts of the same species in order to provide an estimate of the variability that might be anticipated. In addition to the standard measurements and ratios usually taken for trypanosomes, the description should be supplemented with excellent photomicrographs and/or scale drawings illustrating the salient features of the parasites. The validity may be enhanced by information on the life cycle, transmission, host specificity and cultural attributes. Above all, the creation of a new species should have a biological rationale.

PLEOMORPHISM IN *TRYPANOSOMA*

Pleomorphism is the sequential phenotypic manifestation of a single genotype as it appears in the trypomastigote stage in the vertebrate host. The phenomenon is triggered by changes in the host antibody spectrum. There are a few fish trypanosomes which seem to be virtually monomorphic, displaying only variability in size and / or in length / width ratio. The developmental stages in their vertebrate host, if any, seem to be limited to other than morphological manifestations.

Early authors (Laveran and Mesnil, 1912) designated the pleomorphic forms as "*var. parva*" or "*var. magna*" and sometimes these forms were erroneously credited with separate taxonomic status, even by recent authors (Bauer, 1984). In such pleomorphic species, the first trypanosomes to appear in the blood of the infected host are the "young" forms- rather small and slender with a few, shallow waves of the undulating membrane and nucleus revealing no distinct karyosome. After some time, intermediate forms appear until, eventually, "adult" forms prevail to persist during the chronic phase of infection. They are large, stout, sometimes very wide, and usually have a richly spiraling undulating membrane with numerous bends, a nucleus with a prominent karyosome and sub-surface striation. They often have more chromatic granules in their cytoplasm and free flagellum is shorter than in the young forms. Depending on the phase of infection at which the fish is examined, one finds young, slender or large adult forms only, or intermediate ones. A mixture of forms is probably the result of sequential leech feeds.

Pleomorphism is expressed in the following features:

- Size changes
- Changes in length / width ratio
- The number, width and depth of the waves of the undulating membrane
- The presence or absence of a distinct karyosome in the nucleus
- Presence and number of stainable cytoplasmic granules
- Length of the free end of the flagellum
- Distance of the kinetoplast from the posterior end
- Shifts in the position of the nucleus in the body
- Presence of pellicular striation (“ myonemes ”) in stained preparations.

Pleomorphism in *Trypanosoma* is well marked in having a markedly variegated shape and six different stages in varying combinations at various periods of the digenetic life cycle (vertebrate and invertebrate hosts) may occur (trypomastigote, amastigote, promastigote, sphaeromastigote and metacyclic stages). However, trypomastigotes are the predominant stages reported from fish blood, but in the vertebrate blood too, pleomorphism may be visible. Pleomorphism has been reported by some authors without assigning any names (Becker and Overstreet, 1979; Joshi, 1979) whereas others have given specific names to the various forms.

Laveran and Mesnil (1907) distinguished the 'large' and 'small' forms of *T. remarki*; Tanabe (1925) Type I, II and III; Dutton *et al*. (1907) small, medium and large; Qadri (1955) small and large forms of *T. striati;* Qadri (1962a) large and stumpy forms of *T. batrachi*; Becker (1967) slender and broad forms of *T. occidentalis*; Joshi (1982) short, elongated and stumpy forms of *T. aori*.

Pleomorphism in trypanosomes has also been recorded from the fishes of Rohilkhand region of Uttar Pradesh. Dimorphism with two distinguishable forms was recorded in *T. aligaricus*; Gupta and Jairajpuri, 1982a (small and large forms); *T. artii* Gupta *et al*., 2002 (small and large forms); *T. saulii* Gupta *et al*., 2006 (minuta and magna forms) and *T. heteropneustii* Gupta *et al*., 2006 (small and large forms). Pleomorphism was observed in *T. singhii* Gupta and Jairajpuri, 1981b; *T. attii* Gupta and Jairajpuri, 1982b (small, intermediate and large forms); *T. colisi* Gupta, 1986 (stumpy, intermediate and slender forms), *T .barielliana* Gupta , Gupta and Yadav 1987 (slender, intermediate and large forms) and *T. ticti* (small, intermediate and large forms) from *Puntius ticto* (unpublished data of author; Fig. 2)

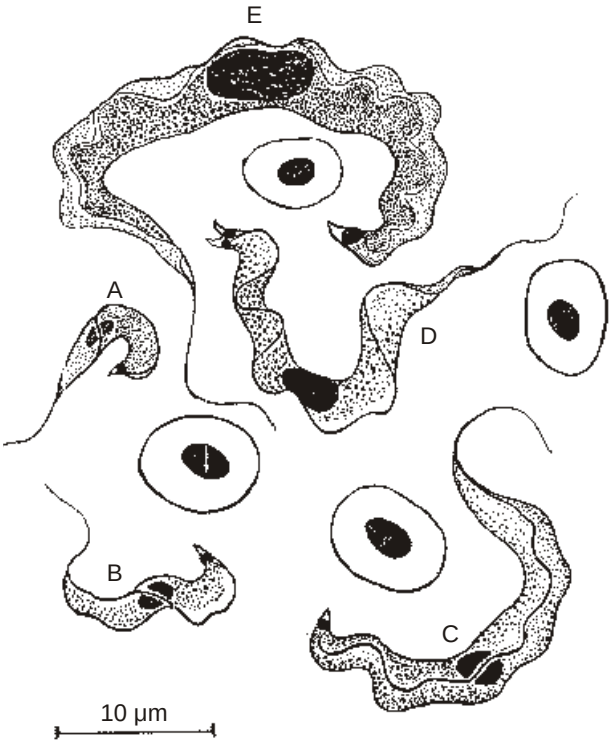

Fig. 2. A E. Diagrams showing pleomorphism in *Trypanosoma ticti* from the blood of *Puntius ticto*. A and B, small forms C and D, intermediate forms; E, large form.

BLOOD COLLECTION AND STUDY OF LIVE PARASITES

Trypanosomes can be visualized alive in fresh blood. Blood can be collected in several ways but a few drops can easily be obtained by incising two or three rays at the base of the caudal fin. The sample of blood can be taken from the heart using a thin Pasteur pipette introduced through the ventral body wall or below the pectoral fins (Lom and Dykova, 1992). The clotting time for fish blood is much shorter than for mammalian blood and is to be thus handled accordingly. A heparinized pipette or a pipette containing a droplet of a heparin-saline solution is generally recommended. For large fish, blood can be taken directly from the heart. The fresh preparation (ordinary or hanging drop) is immediately examined for the presence of parasites which can be seen

actively wriggling about, pushing the blood cells randomly during their course of movement. The addition of citrated salt solution prevents blood coagulation and leaves the motility of the parasites unimpaired.

During live condition, the parasite moves very quickly, actively displacing the adjacent red blood corpuscles; wriggling movements and twisting of the body into knots has also been observed occasionally (Qadri, 1962b). Occasionally, the parasites may displace themselves by a directional motion.

Low intensity infection detection

Often trypanosomes are present in low intensities and their detection in routine blood smears is time consuming and the parasites maybe overlooked. Thus low infection maybe detected in the following manners:

A. Haematocrit centrifuge method (Woo, 1969)

A heparinized capillary tube is filled with about 0.06 ml blood and one end sealed. The tube is centrifuged for 4 min. at 12,000 rpm. This tube is then placed horizontally in a drop of immersion oil on a microscope slide and examined at 100x magnification in a compound microscope. Trypanosomes, if present are found writhing at the junction of the buffy layer (layer of packed white blood cells) and the clear plasma. Alternatively, the tube is cut immediately above this layer, material transferred to a slide, smear made and stained in Giemsa's stain. Concentrated parasites may be visible under the microscope after centrifugation (Fig. 4c).

B. Clot method (Lom and Dykova, 1992)

Trypanosome infection of extremely low intensity can be detected if blood is allowed to clot in a centrifuge tube placed overnight in a refrigerator. The next day, the flagellates can be found wriggling in the serum outside the blood clot and may be concentrated by centrifugation. If large amounts of blood are available, the trypanosomes may be separated in a DEAE cellulose column (Lanham and Godfrey, 1970; Lumsden *et al*., 1973), which is a more complicated method.

MORPHOGENESIS IN THE FISH HOST

Trypanosoma are truly haematozoic extracellular parasites spending substantial part of their life cycle in the plasma. Fish trypanosomes use blood as a route of entry to their vector, haematophagous leaches. After re-entry into the fish, they may either directly invade (or be injected by the vector into) the blood or they may enter it after an initial period of multiplication in tissue fluid local to the point of entry (Baker, 1976).

Lom and Dykova (1992) divided the development of the parasite in the fish host into four different phases:

a) Phase I- Prepatent period (2-9 days) characterized by the absence of flagellates in the peripheral blood. It is not exactly known where the organisms are located during this phase and no tissue stages have been reported.

b) Phase II- Appearance of slender forms of the flagellates in the peripheral blood- patent and increasing parasitaemia due to the division of the trypanosomes.

The division of the parasite in fish blood has been reported rarely, it was presumed by some authors that the parasites did not multiply in fish blood (Letch, 1977). Occasional reports of divisional stages sprung up without providing clear evidence of the sequential pattern of division. Laveran and Mesnil (1907) reported that fish trypanosomes multiply by longitudinal binary fission and protoplasmic masses undergoing division and young trypanosomes were observed in the blood of *Cyprinus carpio* and *Esox lucius*. Tanabe(1925) observed the dividing forms only once in the blood of leeches but these forms were not described. Dividing nuclei in *T. maguri* (Tandon and Joshi, 1973) and *T. mukasai* (Sinha, 1986) and dividing kinetoplast in *T. colisi* (Gupta, 1986) have been reported. Gupta and Saraswat (1991) observed dividing forms in *T. rohilkhandae* and binucleated stages were the most common, flagellar division was rare.

However, the complete sequential pattern of division of *T. danilewskyi* in *Carassius auratus* was explained by Woo (1981a). The author divided the stages into four phases arbitrarily : Stage I (production of a new anteriorly directed flagellum and kinetoplast division), Stage II (enlargement and rounding of posterior end and flagellar shift to posterior end), Stage III (weakly formed undulating membrane and initiation of nuclear division) and Stage IV (two fully formed trypanosomes joined at their posterior end, transverse constriction between the two kinetoplasts, formation of young parasites with weakly developed undulating membrane and the nucleus situated close

to the kinetoplast). The author also reported two abnormal forms, one with a kinetoplast and no nucleus and other with a kinetoplast and two nuclei which did not fit into the sequence of division mentioned by him. The division was thus transverse. Gupta, Gupta and Sharma(1987) described the division of *T. trichogasteri* in *Colisa fasciata* and in contrary to Woo (1981a), the division of the parasite was longitudinal. Multiple fissions were not observed.

The division in *T. trichogasteri* was initiated by kinetoplast division (longitudinal as well as transverse) producing nearly equal sized kinetoplasts. This was followed by nuclear division (transverse) and cytokinesis proceeding from the posterior end towards the anterior. The mother kinetoplast retained the undulating membrane and the free flagellum while a new flagellum arose from the daughter kinetoplast either simultaneous or subsequent to cytokinesis. As cytokinesis progressed, each cell body contained one nucleus and the undulating membrane was the last to acquire perfection as also reported by Woo (1981a). *T. kargenensis* also shows a similar pattern of division (Fig. 3) where division commences from the kinetoplast, followed by nuclear division (Fig. 4d, e) and cytokinesis (Fig. 4f). However, in contrast, the divided trypanosomes remained attached at their anterior ends prior to separation into two young parasites in these cases instead of being attached at their posterior ends (Woo, 1981a).

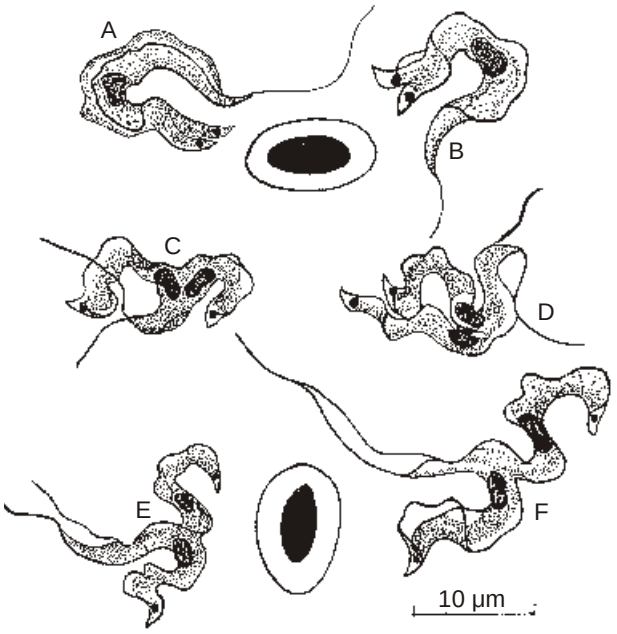

Fig. 3. A F. Sequence of divisional stages in *Trypanosoma kargenensis* from *Heteropneustes fossilis*. A, kinetoplast division; B, beginning of cytokinesis; C, nuclear division; D, F, cytokinesis and formation of 2 daughter trypanosomes.

The above two patterns of multiplication described for fish trypanosomes are not similar, that for *T. danilweskyi* being by transverse binary fission and for *T. trichogasteri* being longitudinal binary fission, however, both are symmetrogenic as characteristic of mastogophereans (Levine *et al*., 1980).

Lom and Dykova (1992) reported division in fish trypanosomes to start with production of a new flagellum, followed by a kinetoplast and then nuclear division. The cell divides longitudinally starting anteriorly (eg. *T. remarki*). In *T. carassii*, the new flagellum is flipped posteriorly to extend in a direction opposite to that of the old flagellum and new cytoplasm is grouped along it. After nuclear division, one of the nuclei moves posteriorly past the two kinetoplasts. Finally, a transverse, unequal cleavage occurs between the kinetoplasts; the posterior daughter individual is smaller. The vesicular nucleus divides by a modified mitosis ("closed" pleuromitosis).

The ploidy of trypanosomes reproducing largely by binary fission still remains an open question. The main reason for uncertainty in this basic fact is the absence of the cell cycle phase with distinct easily counted chromosomes. For long, trypanosomes have been considered to be asexual (Hoare, 1972), however, Tait (1980) doubted on this concept by studying enzyme patterns of *T. brucei* and the existence of sexuality has been suspected. The assumptions of the existence of sexual processes in trypanosomatids were predominately based on zymograms and genetic equilibrium studies and supported the idea of diploidy indirectly (Tait, 1980). However, there is as yet no indication of sexual reproduction in fish trypanosomes.

Ultrastructural studies on the division of the epimastigotes of *T. cobitis* were conducted by Lewis and Ball (1981). The investigations were based on electron microscopy of the epimastigotes in the leech vector, *Hemiclepsis marginata* and in culture. The division followed the trypanosomatid pattern of duplication of basal body, kinetoplast and then nucleus followed by cytokinesis with the exception that the dividing epimastigotes within the vector had multi-axonemal flagella whereas in culture, the new flagellum grew separately alongside the original. The kinetoplast duplicated by both lateral and equatorial enlargement.

The above microscopic and ultra structural studies

indicate that the pattern of division in piscine trypanosomes is apparently by binary fission, however, variations exist whether the division is longitudinal or transverse and the sequence of division of cell organelles. These variations maybe species specific, however further studies are in demand for other fish trypanosomes species in order to make some conclusive remarks.

The division phase lasts from several days to several weeks and a heavy parasitaemia may be achieved. Sometimes, heavy infections may result in the death of the host, specially in young fish or in experimental

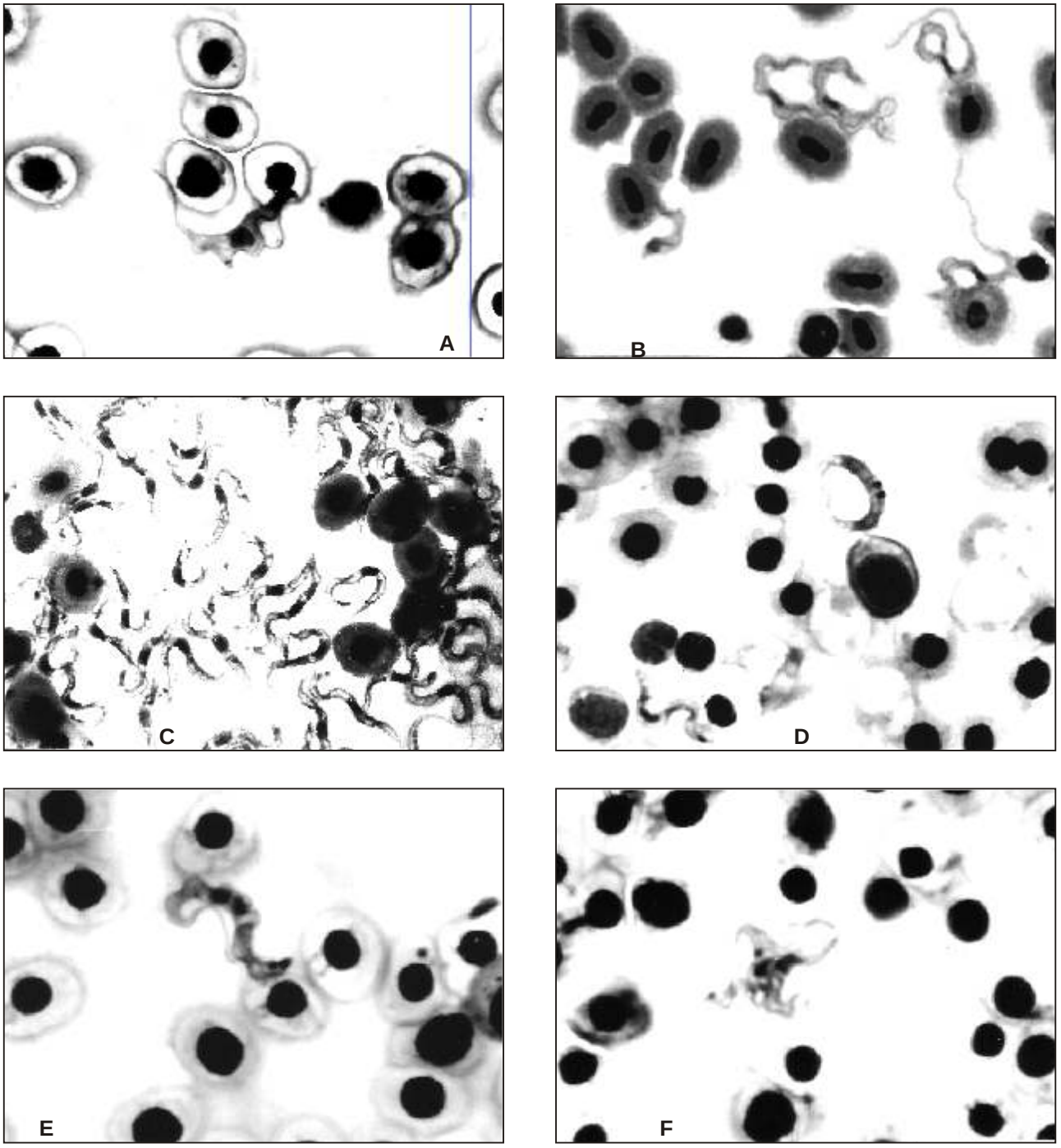

Fig. 4. Photomicrographs of Giemsa-stained *Trypanosoma* (x 1000). A. *T. colisi* from *Colisa fasciata;* B, *T. kargenensis* from the blood of *Heteropneustes fossilis* showing heavy infection associated with anemia; C, *T. kargenensis* after centrifugation of blood; D, *T. kargenensis* showing divided nucleus; E, *T. kargenensis* showing two kinetoplasts and two nuclei; F, *T. kargenensis* showing cytokinesis.

hosts such as goldfish. However, due to immune phenomenon, the infection passes on to the next phase.

c**)** Phase III- Chronic phase characterized by a reducing number of flagellates in the blood and lasts for a few weeks or for an indefinite period. As it is the longest phase, it is the one most frequently observed. There are no division stages in the blood during this phase.

d) Phase IV- There is a complete absence of flagellates in the peripheral blood and the fish appear to be free of trypanosomes. However, sometimes, the flagellates may be found in their preferred sites, the mesonephros, pseudobranchia or rete mirabile of the eye. The disappearance of flagellate is a manifestation of premunition, of a non-sterile rather than of a sterile immunity as these fish appearing apparently as infection free may later suffer a sudden, severe relapse.

TRANSMISSION

The parasites are transmitted by the blood sucking leeches. Naturally infected *Pontobdella* and *Piscicola* were allowed to feed on clean fish blood by early workers to facilitate transmission of trypanosomes. Brumpt (1904) described the development of trypanosomes in large numbers in the crop of *Hemiclepsis marginata* which had fed on infected fish and large bullheads could also be infected by the same trypanosome-infected leeches. *P. geometra* could transmit flagellates of carps, pike and tench (Kesseylitz, 1906). The trypanosomes of goldfish in the leech *Hemclepsis marginata* were studied by Robertson (1911) and Tanabe (1925) observed the development of trypanosomes of the Japanese loach in leeches. Qadri (1962c) traced the developmental stages (crithridial, trypaniform and metacyclic) in *T. danilewskyi* of *C. carpio* in *H. marginata* within 7-11 days. Needham (1969) transmitted *T. tincae* from *Tinca tinca* through the same vector. Tandon (1986) reported the attachment of *H. marginata asiatica* to the trunk of *W. attu*. Lewis and Ball (1979) described the attachment of *T. cobitis* to the crop wall of *H. marginata*.

SEQUENCE OF STAGES IN THE VECTOR

Fish trypanosomes are transmitted by leeches during their blood meal. According to its ecology, each leech species may transmit various flagellate species indiscriminately as exemplified by the European *Piscicola geometra* and *Hemiclepsis marginata.*

A series of morpho-physiological changes are triggered when the trypanosomes in fish blood are ingested by a leech which is manifested by a developmental sequence of amastigote, sphaeromastigote, epimastigote (possibly also promastigote) and trypomastigote forms in the leech digestive tract. The latter are termed metacyclic forms since they are infective and capable of initiating the life cycle in the fish host. Trypanosome species may differ in the relative abundance or absence of some of the above forms, in the forms in which the main proliferation occurs, and in the presence or frequency of proliferation by multiple division.

Reliable data on the preferences of fish trypanosomes for various leech species and parts of their digestive tract are lacking. As a rule, the dividing forms occupy the crop and its caeca, while the non dividing metacyclic trypomastigotes accumulate in the proboscis sheath. Information on stages found in the stomach or in the intestine of freshwater leeches may be based on incorrect definition of parts of the leech digestive tract (proboscis in its sheath, large crop with many voluminous caeca, stomach and the short intestine). Thus Brumpt (1906), who proposed dividing several species of trypanosomes into three groups according to their movements through the parts of the digestive tract, mentioned that all of them developed in the stomach and one (*T. granulosum* from eels) even in the anterior intestine. The skate species, *T. scylli* and *T. rajae* were also reported to invade the anterior intestine, a region supposed to secrete powerful digestive enzymes capable of killing the flagellates.

Freshwater fish trypanosomes, on entry into the leech crop, begin transforming into division stages (Robertson, 1911; Tanabe, 1925; Qadri, 1962c; Letch, 1980). Initially, tadpole-like epimastigotes are formed by unequal division of bloodstream trypomastigotes, there are a few sphaeromastigotes and even promastigotes, but it is the epimastigotes that are responsible for proliferation. The crop is then filled by a mass of intermediate stages which produce extremely long, often even filiform trypomastigotes, which may exceed 50um in size, with a kinetoplast halfway between nucleus and posterior end. These forms do not divide. The epimastigotes are attached to the epithelium of the crop by the greatly enlarged tips of their flagella. Later the trypomastigotes prevail and move into the proboscis sheath- starting with day 5, but usually much later, and exceptionally even as late

as several months after feeding. They are unable to divide. The degree of digestion of the blood meal determines the sequence of forms. The slender trypanosomes appear in the proboscis sheath only after the blood meal has been entirely digested.

The duration of infection in different leeches may vary. In *Piscicola geometra* infected with trypanosomes from cyprinids, it barely exceeds two weeks; in other species the infection may survive long after the blood meal has been digested, even for the entire life of the vector; it is never, however, hereditary. In *Hemiclepsis marginata* infected with a crucian-carp trypanosome, the epimastigotes, persisting in the crop for a long period of time, start the cycle again at the next feeding of the leech , while most if not all proboscis-located trypomastigotes are washed off into the blood of the newly-attacked host.

In marine fish trypanosomes, after ingestion by the leech the blood trypomastigotes transform into small, rounded amastigotes, which start dividing intensively. Later, sphaeromastigotes appear as an actively dividing, but shortlived stage. Once division has ceased, the sphaeromastigotes transform into short, thick epimastigotes and a few trypomastigotes appear, which start moving into the proboscis. Finally, only long, filiform metacyclic trypomastigotes accumulate in the proboscis. This seems to be a general pattern in fish trypanosomes (Robertson, 1907; Neumann, 1909) with some variations; in *T.murmanense*, amastigotes and sphaeromastigotes are the main divisional stages as opposed to epimastigotes in *T. rajae;* the cycle is temperature dependent being slow (about 50 days: *T. murmanense* in *Johanssonia arctica*) or fast (7 days: *T.giganteum* in *Pontobdella muricata).*

PATHOBIOLOGY

Depending on the intensity of infection, the pathogenic potential of fish trypanosomes may encompass the following.

1. Changes in blood values such as decrease in serum protein levels (Woo, 1995), increase in leucocytes but decrease in erythrocyte number and haemoglobin content, increase in serum globulins. Anemia is induced by haemolysins secreated by live trypanosomes which lyse the RBC, and by haemodilution, which is a result of generalized oedema.

Symptoms of piscine trypanosomiasis range from mild anemia associated with low levels of parasitaemia to severe pathological changes due to heavy parasite burdens (Woo, 1981b; Islam and Woo, 1991). Leukocytosis, hypoglycemia and hypocholesterolemia (Gupta and Jairajpuri, 1983; Gupta and Gupta, 1986) are frequent outcomes of trypanosomiasis. In addition, clinical manifestations include increased erythroblasts, haemoblasts and macrophages. Stimulation of haemopoiesis, poikilocytosis accompanied by abundant abnormal cell types in heavy natural infections of *T. maguri* in *C. batrachus* have also been reported (Tandon and Joshi, 1973).

Tandon and Chandra (1977 a,b) while investigating the serum cholesterol and alkaline phosphatase levels in naturally trypanosome infected fishes observed the highest fall (42.39%) in serum cholesterol level in *Mastacembelus armatus* and lowest (4.56% in *Cirrhinus mrigala*). On the other hand, the highest fall (69.91%) in serum alkaline phosphatase level was recorded in *C. mrigala* and the lowest (3.91%) in *Wallago attu*. The metabolic activity declines due to *Trypanosoma* infection. The parasites utilize a high percentage of sugar from blood resulting in a complete depletion of the carbohydrate reserve. This may lead to a serious strain on the liver- a chief center of cholesterol metabolism and hypocholesterolemia may thus be induced.

2. Changes in somatic indices and condition factors.

3. Histopathological changes (Lom *et al.*, 1986) which may be transient or irreparable and

4. Eventual mortality. Khan (1985) recorded mortality in infected cod and winter flounders at low temperatures. Mortality ranged from 7% in 3+ year to 65% in 0+ year cod. In immature winter flounders the figures ranged from 17-56%. Larger fish appear to be less susceptible and adult flounders did not die from trypanosome infection.

IMMUNE RESPONSE

The final disappearance of trypanosomes from the blood stream is the outcome of an immune response. There are a few records of trypanocidal antibodies in fish. Barrow (1954) observed lytic action of the serum of fish that had recovered from a *Trypanosoma* infection at 20°C. This lytic capacity soon decreased and was lost by three weeks after the disappearance of the parasite from the blood. The parasite antigens stimulate the secretion of specific antibody by the ch

host's B-lymphocytes into the blood plasma (Roitt, 1974) and may attack the parasites in various ways including complement mediated lysis (lysins), agglutination (agglutinins) and facilitating phagocytosis (opsonins). Cottrell (1976) found that plaice infected with *T. platesae* had elevated levels of serum immunoglobulins possessing the beta electrophoretic mobility which might be associated with antibody. Goldfish experimentally infected with *T. carassii*, the immunity to super infection could last upto 350 days (Lom and Dykova, 1992). Probably this was due to non-sterile immunity as evidenced by outbreaks of parasitaemia in some of the fish apparently free from infection previously. This appears to be temperature dependent as when fish are kept at a low sub-optimal temperature, the infection does not reach the last phase and a certain level of parasitaemia is maintained. This may be due to other factors as well- stress, nutritional deficiency and fishes showing low parasitaemia may be super infected with the same trypanosome species by a leech or by a syringe passage which may in some cases result in a high level of parasitaemia.

The aim of the parasite is to persist in its host for as long as possible at an optimum level in order to ensure a good chance of onwards transmission but low enough to avoid undue risk of killing the host. An unusual antibody, “ablastin” maybe directed against certain *Trypanosoma* species which interferes with parasite division but has no other harmful effect (D' Alesandro, 1975). The antibody “ablastin” is thus ideal from the parasite's view point by preventing multiplication, it reduces parasitaemia to a level which does not seriously endanger the host and probably does not furnish too great an antigenic stimulus to the latter. The adult parasites may thus be able to persist for long periods in the blood of the vertebrates host, waiting to continue its life cycle in the vector. “Ablastic” antibodies have been found to occur in amphibians (Lom, 1969), however, their presence in fish is uncertain. It maybe possible that ablastin or ablastin-like antibodies are stimulated in fish hosts as well and may account for the same phenomenon. Under certain conditions, fish may develop protective, nonsterile immunity to homologous challenge (Woo *et al*., 1983).

TREATMENT AND CONTROL

It is difficult to eliminate trypanosomes from fish by any routine treatment. It is believed that tryparsamide administered at low dosages in food might be effective (van Duijn, 1973). Tryparsamide is a toxic organic arsenical compound and is used under strict regulation. Fish intended for human or animal consumption should not be treated with arsenicals.

The parasites can be controlled best by preventative measures. Piscivorous leeches must be restricted or eliminated from fish rearing areas and water supplies by screens and chemicals. Chemical removal requires the addition of a soluble toxic compound, calculated to attain a pre-determined dilution to the water until degradation or dissipation occurs. Freshwater fish-attached leeches can be removed by dipping fish into a common salt or copper sulphate solution. Commercial chemicals available for direct control of free-living leeches are Dylox, Baytex, and Baygon, but effectiveness varies among target species (Hoffman and Meyer, 1974) and their toxicity to fish is as yet unknown.

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Malaria and macrophages: cellular and molecular basis of pathogenesis and immune protection

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ABSTRACT. The roles of macrophages (MØs) in the pathogenesis and resolution of malaria infection is well known. However, the mechanisms of plasmodium-infected erythrocytes (IE) interaction with MØs leading to their internalization, factors that interfere with MØ-IE interaction, the details of the factors and the mechanism(s) which activate MØs for augmented phagocytosis of IE, the fate of IE ingested by MØs, and the nature and functions of various cytokines generated by IE-ingested MØs, remain poorly understood. The Fc-receptors, and not the complement receptors, appear to mediate the attachment and internalization of IE by MØs. Immune-complexes (ICs) formed during acute malaria may inhibit the phagocytosis of IE early in the infection, but, over time, may induce changes in MØs which result in the enhanced phagocytosis of IE. In cultures of splenocytes from animals immune to malaria, IE-containing MØs present antigens to sensitized T-cells, which, in turn, elaborate lymphokines (LKs); these LKs are thought to activate MØs for enhanced phagocytosis of IE. Neutralizing concentrations of monoclonal antibodies against mouse interferon-gamma (IFN-γ) and interleukin-4 (IL-4) blocked the MØ activating activity of these LKs, which indicate that IFN-γ and IL-4, plausibly present in these LK preparations, were responsible for the observed MØ activation. Both plasmodial components and intact IE can activate MØs and monocytes to produce colony-stimulating factors (CSFs), *de novo*, in a lipopolysaccharide-independent manner. Chloroquine, a lysosomotropic drug, inhibited the plasmodial antigen-induced production of CSFs by MØs. Purified human C-reactive protein (CRP) stimulated rhesus monkey (*Macaca mulatta*) blood monocyte-derived MØs for enhanced phagocytosis of *Plasmodium fragile*-IE, *in vitro*. These observations suggest that MØ-IE interaction and subsequent erythrophagocytosis constitute important steps in the pathogenesis and protective immunity to malaria, and detailed molecular studies are warranted to understand the role(s) of MØs in both parasite clearance and disease-causing mechanisms in malaria, which may be useful for the development of anti-disease and/or anti-parasite vaccine(s) against malaria.

Keywords: C-reactive protein, cytokines, infected-erythrocytes, macrophages, malaria, phagocytosis

INTRODUCTION

At the end of 2004, 3.2 billion people in 107 countries and territories lived in malaria transmission risk areas, and each year an estimated 300–500 million clinical malaria cases and > 1 million falciparum malaria deaths occur (WHO, 2005). The four species of the protozoan parasite that cause human malarias are: *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Whereas, *P. falciparum* causes the most severe form of malaria, *P. vivax*, characterized by relapses, is not fatal; together they cause 95% of world malaria. The parasite and vector resistance to anti-malarial

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drugs and insecticides, respectively, have worsened the malaria problem (Greenwood and Mutabingwa, 2002). No suitable human anti-malaria vaccine is yet available (Richie and Saul, 2002; Moorthy *et al.*, 2004), mainly due to the lack of a clear understanding of the molecular mechanism(s) of pathogenesis and immune protection (Miller *et al.*, 2002). It is thought that the availability of the complete genome sequence of *P. falciparum* (Gardner *et al.*, 2002) and the rapid advances made in the gene transfer and disruption techniques, may help in understanding several biological complexities associated with malaria parasite.

During the bite of a *Plasmodium*-infected female *Anopheles* mosquito, sporozoites, the infective stage of malaria parasite, are injected into human blood circulation. Just within nearly 1 h of their injection, sporozoites enter liver and invade hepatocytes, where they undergo schizogony and produce thousands of merozoites (Mz). Due to some yet unknown process, a dormant liver stage of malaria parasite, hypnozoite, is formed, which is responsible for relapses in infections caused only by *P. vivax* and *P. ovale*. Following the rupture of infected-hepatocytes, the released Mz, following a complex chain of events, enter into erythrocytes and within next few hours, convert into ring stages of the parasite. Rings start feeding on hemoglobin of the erythrocytes, and after 15–18 h develop into full grown trophozoites with a clear food vacuole. Trophozoites give rise to multinucleate schizonts, which after further growth and development rupture the now fragile erythrocyte membrane and release up to 16 Mz. Most of the Mz invade fresh erythrocytes and continue the blood cycle, whereas only a few of them, through again a not yet fully known process, develop into male and female gametocytes within the erythrocytes and circulate in blood, and are taken up by mosquitoes during their blood meal. In the mosquito midgut, gametocytes undergo gametogenesis to produce male and female gametes, which following fertilization produce diploid zygotes. Zygotes then undergo meiosis and differentiation to produce motile ookinetes, which then traverse the midgut wall and develop into oocysts. As a result of sporogony, sporozoites are produced in oocysts, which after their release in haemocoele, migrate to the salivary gland of the mosquito. It is thought that in the salivary gland, sporozoites undergo some kind of a maturation and, during the next blood meal of the mosquito, are injected into a new host.

Macrophage (MØ; a cell 13–15 µm in diameter) was for the first time identified and described by the father of cell-mediated immunity, a Russian pathologist, Ilya Ilich Metchinkoff (French adoption; Elie Metchnikoff) in 1893. Though the phagocytosis has been described much earlier in 1862 by Haeckel (cited by Nelson, 1969), the concept of phagocytic functions of MØs and its bearing on the host resistance to invasion by parasites was adequately and experimentally documented and forcefully expressed by Metchnikoff (1893; 1905).

The role(s) of MØs in malaria has been implicated by Golgi nearly a century ago, when he observed the presence of malaria pigment-leaden splenic MØs in malaria patients. Incidentally, this apparently small observation later laid the foundation of the notion that phagocytosis of infected-erythrocytes (IE) constitutes an important, critical step in host defense during malaria (Taliaferro and Mulligan, 1937). And now, it is well established, through both animal model studies and, indirectly, from several clinical observations that MØ phagocytosis of IE constitutes an important innate protective mechanism against malaria (Urban and Roberts, 2002). Mota *et al.* (1998) reported that antibodies induced in mice during acute *P. chabaudi chabaudi* malaria, bound the surface of IE and thus augmented their phagocytosis by MØs. Peritoneal MØs from *P. yoelli nigeriensis* vaccinated mice, which were completely protected following a lethal challenge, showed a great increase in their pool-size, and in both the intrinsic phagocytic activity and the number of MØs involved in phagocytosis, *in vitro* (Kinhikar *et al.*, 2001). Similarly, protection in mice infected with *P. berghei* (Singh and Singh, 2001) and *P. yoelli nigeriensis* (Kaur *et al.*, 2002), after co-administration with recombinant mouse granulocyte-macrophage colony-stimulating factor (GM-CSF) and methionine-ekephalin (M-ENK), and in *P. berghei*-infected mice co-treated with GM-CSF and M-ENK fragment peptide Tyr-Gly-Gly (Kaur *et al.*, 2004), was invariably associated with > 1000-fold increased pahgocytic activity of peritoneal MØs. In all these co-treated mice, selective killing of MØs with silica completely abrogated the protection. In human malaria, phagocytosis of *P. falciparum* IE (Vernes, 1980) and free-merozoites (Khusmith *et al.*, 1982) by monocytes has been reported. Brown and Greenwood (1985) demonstrated the role of monocytes/macrophages in *P. falciparum* IE phagocytosis and its correlation with recovery from malaria. In severely compromised immunodeficient mice maintaining *P.*

falciparum-infected human eryhrocytes, host MØs have been demonstrated to phagocytose and remove IE (W. H. O., 1999). Brown *et al.* (1986) demonstrated the role of phagoctosis in the recovery of Gambian children from *P. falciparum* malaria. Further, the involvement of MØs in protection from human malaria has been characterized by their marked accumulation in spleen, bone marrow and liver (Wickramasinghe *et al.,* 1987). Besides these *in vivo* observations, *in vitro* studies have also demonstrated that MØs can phagocytose *P. knowlesi* (Brown, 1971) or *P. berghei* IE (Hunter *et al.,* 1979). Curiously, in addition to normal phagocytosis of IE, MØs also take out parasites from erythrocytes that are recently infected with malaria parasites, and in this process, leave behind the erythrocyte in blood (Angus *et al.,* 1997; Chotivanich *et al.,* 2002). It is, therefore, not surprising to assume that spleen, through its MØs, functions as an important site for the interaction between MØs and malaria parasites, and thus plays an important role(s) in the resolution of malaria infections (Langhorne *et al.,* 1979; Wyler *et al.,* 1981; Wyler *et al.,* 1983). This contention is further indirectly supported by the observations that during malaria infections, tremendous splenomegaly occurs, and people living in malaria endemic areas have enlarged spleen (Crane, 1979). Additionally, a large body of evidence suggests that splenectomy invariably results in fulminating resurgence of latent parasitaemias to the levels that are often fatal for the host (Quinn and Wyler, 1980; Barnwell *et al.,* 1983). Furthermore, some studies have very clearly demonstrated that the elimination of MØs by silica, which has been shown to selectively kill MØs (Allison *et al.,* 1966), can exacerbate rodent malaria infections in mice (Playfair, 1979; Singh *et al.,* 1994a).

However, during malaria infection, several yet unknown events occur, which end up in the severe impairment of several functions of MØs (Nielsen *et al.,* 1986). In a series of dedicated reports, Paulo Arese and colleagues in no uncertain terms demonstrated that malaria pigment hemozoin, a brown colored, crystalloid polymer product of hemoglobin degradation, is plausibly responsible for the diminished activities of MØs during malaria (Arese *et al.,* 1991; Schwarzer and Arese, 1996; Schwarzer *et al.,* 1992, 1998; Turrini *et al.,* 1992); curiously, most of these functions *viz*. marked reduction in phagocytosis and inhibition of the production of oxidative burst are thought to be involved in the killing of phagocytosed malaria parasites. Phagocytosis of IE containing young rings, without any hemozoin pigment, on the other hand, apparently, did not diminish the effector functions of MØs.

Taken together, it can be deduced from the foregoing account that, apparently, MØs, especially in the spleen, play major roles in the resolution of malaria infection and ultimately in the protection against the disease. Paradoxically, phagocytosis of IE by MØs may result in the suppression of their immune functions, and thus malaria infection may suppress, at least in part, the immune response of the host. Curiously, the role(s) of MØs during malaria has apparently not been reviewed in the last several years. Nevertheless, detailed studies directed at the interplay between these two seemingly adversative functions of MØs during malaria infection, which, in the end, may decide the final outcome of the disease, are required to clearly understand specific role(s) of MØs in malaria pathogenesis.

MECHANISMS OF PHAGOCYTOSIS OF IE AND POST-PHAGOCYTIC EVENTS

To get phagocytosed, an IE must be first identified by a quiescent or activated MØ. MØs present in spleen are most strategically placed to perform this function, and the moment MØs 'sense' an IE in their microenvironment, they start extending their pseudopodia like structures around it. Soon after this, the MØ establishes a firm attachment with the IE. The attachment between an IE and a MØ is generally a passive event and can occur even at 4 ºC. However, at such a low temperature, the attachment process almost stops here only, and is seldom followed by the internalization of IE. Therefore, the attachment of IE, at physiological temperatures, constitutes an important initial step in the overall phagocytic process, and allows further stronger interaction between MØs and IE.

It is important to note here that despite our vast knowledge regarding the various aspects of malaria immunology, our understanding of the mechanism of attachment (binding) of IE with MØs still remains rudimentary. Notwithstanding this general agreement, however, the FcR-mediated attachment and phagocytosis of IE by MØs has been demonstrated (Shear *et al.,* 1979; Shear, 1984). MØs have unoccupied Fc-receptors (Fc-R) and complement receptors (C3R), on their surface (Lay and Nussenzweig, 1968). Though both FcR and C3R have been implicated in the phagocytosis of IE, the plasma

presence of C3b on IE has not yet been confirmed (Shear *et al.*, 1979; Topley *et al.*, 1973; Abdalla *et al.*, 1983). Thus, it appears certain that the phagocytosis of IE by MØs is mediated by FcR. Furthermore, the cross-linking of MØ FcR by parasite derived antigens and cytophilic or opsonic antibodies present in malarious plasma may promote the clearance of opsonized IE (Leslie, 1985).

Following attachment of an IE with a MØ, it is internalized along with the phagosome produced around it. The phagosome containing IE is then fused with lysosome, which contains lysosomal acid hydrolases. Finally, after fusion, the phagolysosome is formed containing IE and acid hydrolases. This process is accompanied by a decrease in the size and number of acid phosphatase-positive granules in MØs. In the phagolysosome, IE are biodegraded into smaller peptide fragments (8–15 amino acids long). The malarial pigment along with various substances of host and parasite origin (including protein aggregates, lipids and phospholipids), induce the production and release of several inflammatory cytokines by monocytes and MØs (Bate *et al.*, 1989; Pichyangkul *et al.*, 1994; Jakobson *et al.*, 1995; Hommel, 1997). The processed products of IE are then exocytosed onto the surface of MØ, which, in turn, functions as an antigen-presenting cell and subsequently presents these surface-bound processed antigens to T-lymphocytes. These antigen-sensitized T-lymphocytes then trigger in motion the whole cascade of immune response.

FACTORS THAT INTERFERE WITH MØ-IE INTERACTION: ROLE(S) OF IMMUNE-COMPLEXES

The presence of normal erythrocytes and/or IE in mononuclear phagocytes and MØ hyperplasia in malaria-infected hosts, very convincingly suggests their role(s) in the destruction and elimination of malaria parasites (Taliaferro and Connan, 1936; Langhorne *et al.*, 1979; Dutta and Singh 1980; Dutta *et al.*, 1982; Barnwell *et al.*, 1983). Therefore, any mechanism(s) that may interfere with the interaction between IE and MØs, might eventually prevent or reduce the clearance of parasites by the host (Singh and Dutta, 1989a), and may also impede the onset of the ensuing immune response, which otherwise would have been initiated by MØs containing ingested IE (Singh and Dutta, 1989b; Ockenhouse and Shear, 1983; Ockenhouse *et al.*, 1984). The presence of immune-complexes (ICs), formed by antigens and antibodies, in the plasma of murine (June *et al.*, 1979), avian (Soni and Cox, 1975), simian (Ward and Conran, 1966; Shephard *et al.*,1982) and human (Houba *et al.*, 1976) malaria hosts has been well documented; however, their precise functions still remain obscure. Brown and Kreier (1982), in *P. berghei*/rat model, observed that ICs formed by soluble malarial-antigens and immune serum, and those precipitated from the acute-phase serum (APS) of infected rats, inhibited the *in vitro* antibody-mediated binding of IE with peritoneal MØs. Using the same model, Packer and Kreier (1986) demonstrated that pretreatment of MØs with APS inhibited the phagocytosis of IE. Almost similar findings have been reported by Shear *et al.* (1979) by using the mouse model. The ICs prepared either by mixing total parasite antigens soluble in culture medium or those precipitated (by polyethylene glycol) from APS of *P. knowlesi*-infected monkeys, inhibited the binding of IE with MØs, and thus enabled them to evade the host destructive mechanisms (Singh and Dutta, 1989a). The ICs not only inhibit the binding, attachment and phagocytosis of IE by MØs, but they also modulated the phagocytic activities of MØs. Packer and Kreier (1986) have clearly demonstrated that early in the infection, ICs inhibited erythrophagocytosis, but over time, induced changes in MØs which ended-up in the enhanced phagocytosis of IE. Singh and Dutta (1988) demonstrated a similar phenomenon in *P. knowlesi*-infected monkeys, wherein APS from infected monkeys, soluble antigens and ICs precipitated from APS, inhibited the *in vitro* phagocytosis of IE by blood monocyte-derived (BMD) and splenic MØs. Incubation of MØs with APS, heat-aggregated APS or ICs for 6 h, followed by culture in serum-free medium for 18 h, activated MØs for the enhanced phgocytosis of IE. The blockage of phagocytosis by 2-deoxy-glucose in these experiments suggested the mediation of FcR. These findings, in general, indicated that during the acute-phase of *P. knowlesii* infection in monkeys, ICs may inhibit the MØ-mediated parasite destruction, whereas later during the convalescent phase of infection, may promote their destruction by activating MØs.

PRODUCTION OF CYTOKINES IN MALARIA: THE ROLE(S) OF ANTIGEN-PRESENTING MØs AND LYMPHOKINE-ACTIVATED MØs IN THE DESTRUCTION OF MALARIA PARASITES

From the aforementioned account, it is now clear that MØs phagocytose IE, and then present the processed

malarial antigens to T-lymphocytes for the progression of immunological cascade. This process occurs mainly in the secondary lymphoid organs like spleen and lymph nodes. Ockenhouse and Shear (1983) have reported the enhancement of the phagocytosis of *P. berghei*- and *P. chabaudi*-IE by mouse peritoneal MØs stimulated with culture supernatants (CS) of antigen-pulsed spleen cells of mice infected with *P. chabaudi* or BCG; the CS contained phagocytosis-promoting lymphokines (LKs). The normal mice peritoneal MØs pre-treated with LKs killed *P. yoelii* asexual stages *in vitro* (Ockenhouse and Shear, 1984), and crude LK or recombinant human interferon gamma (rHuIFNγ) activated human BMD MØs to kill *P. falciparum* asexual stages *in vitro* (Ockenhouse *et al.*, 1984). *P. falciparum*-IE are also known to be killed by mononuclear phagocytes treated with the CS of malaria-antigen activated T-lymphocytes (Brown *et al.*, 1986). All these related observations have been critically reviewed by Nathan (1986) and Shear and Ockenhouse (1986). Singh and Dutta (1989b) for the first time extended these findings to sub-human primate malarias, which even today continue to be considered as the most accepted models for the development of human malaria vaccines, and reported that the CS of antigen-stimulated splenocytes of monkeys chronically infected with *P. knowlesi* or *P. cynomolgi*, activated monkey BMD and splenic MØs for augmented phagocytosis of IE, *in vitro*. Furthermore, they reported that following stimulation with malarial antigens, MØ-depleted splenocytes were poor producers of LKs, compared to the unfractionated whole spleen cells. In an another related study, Singh and Dutta (1991a) reported that *P. cynomolgi*-infected monkey splenocytes elaborated LKs, which activated human BMD and mouse peritoneal MØs for enhanced phagocytosis of IE (*P. cynomolgi*/rhesus monkey), *in vitro*, and the secretory products of these activated MØs exerted parasiticidal effects on *P. cynomolgi* IE, *in vitro*, as judged by the loss of their infectivity, *in vivo*. The CS of antigen-stimulated splenocytes can be expected to contain LKs such as IFN-γ and IL-4 (Singh *et al.*, 1994b; Kumaratilake and Ferrante, 1994). Taken together, all these reports suggest that MØs play crucial roles in both the afferent and efferent arms of T-cell-mediated immune responses during malaria *i.e.* in stimulating them by presenting the processed antigens and also by responding to their soluble polypeptide signals (*i.e.* LKs) and, thus, consequently, expressing the resultant effect by way of the enhanced phagocytosis of IE. The various steps involved in both these phenomena can be exploited as potential targets for the modulation of MØ-mediated immune responses. In addition to LKs, purified human C-reactive protein (CRP), a proinflammatory cytokine, also stimulated monkey BMD-MØs for enhanced phagocytosis of *P. fragile*-IE, *in vitro*. CRP, however, did not influence the augmented phagocytosis of *P. fragile*-IE, induced by the LK-containing CS (LK-CS), and did not show any synergy or additivity with LK-CS. Nevertheless, the lack of synergy between CRP and LK-CS does not rule out the possibility of their common mechanism of action (Singh and Singh, 1996)

MØ INTERACTION WITH PLASMODIAL COMPONENTS AND IE: PRODUCTION OF CSFs

CSFs are a group of low molecular weight (18–24 kDa) glycoprotein cytokines, which regulate the proliferation, differentiation and survival program of committed granulocyte-macrophages progenitor cells, *in vitro* (Metcalf, 1991, 1989, 1984); *in vivo* they stimulate hematopoiesis (Donahue *et al.*, 1986). Incontrovertible information is now available which suggests that CSFs can also enhance various effector functions of the mature terminal cells of the myeloid lineage (Grabstein *et al.*, 1986) and are potent enhancers of phagocytosis (Handman and Burgess, 1979; Reed *et al.*, 1987). Not much is known, however, about the induction mechanism(s), production and functions of CSFs, during malarial infections.

Mungyer *et al.* (1983), apparently for the first time, studied the effect(s) of *P. berghei* infection on granulopoiesis and MØ production in mice, and has reported the production and functions of CSFs in malaria. Singh and Dutta (1990), apparently for the first time, have reported the production of serum CSFs during *P. cynomolgi* infection in rhesus monkeys (*Macaca mulatta*) and its correlation with the number of circulating leuocytes. The maximum serum CSF activity was observed just before the peak in circulating leukocytes and the onset of the decline of parasitaemia; macrophage CSF (M-CSF) was the major factor. Around this time, Villeval *et al.* (1990) also reported the production of serum CSFs in mice during fatal and nonfatal malaria infections. Unlike Singh and Dutta (1990) they, however, observed two peaks in serum CSF-activity. A first rise very early in the infection, which resembled that induced by the injection of endotoxin (Metcalf, 1984) and possibly

induced by tumor necrosis factor (TNF). The second rise in the levels of serum CSFs was similar to that previously reported by others (Mungyer *et al.*, 1983; Singh and Dutta, 1990), and was predominantly due to M-CSF. However, it still remained unresolved whether this increase in the serum CSFs was due to plasmodial components or the pathophysiological cascade due to malaria or by both. Singh and Dutta (1991b) and Singh *et al.* (1991), apparently for the first time, have reported that plasmodial antigens soluble in culture medium induced the production of serum CSFs in monkeys, and stimulated the monkey splenic MØs and blood monocytes to elaborate CSFs in the culture medium. Again, M-CSF was the major factor produced and, apparently, the production of CSFs was lipopolysaccharide (LPS)-independent and *de novo*. Not only the plasmodial components, but intact IE also induced the production of CSFs (Singh and Dutta, 1992). Additionally, Singh *et al.* (1994c) have demonstrated that ICs containing *P. berghei* antigens also stimulated rat peritoneal MØs for the production of CSFs, here again M-CSF was the major activity produced *de novo* in an LPS-independent manner. Chloroquine, a lysosomotropic antimalarial drug having significant anti-inflammatory activity, in a concentration ($0.01–1x10^{-6}$ M)-dependent manner, inhibited the *P. berghei*-antigens induced production of CSFs by mouse MØs *in vitro*; $0.5x10^{-6}$ M chloroquine induced maximum inhibition. Additionally, chloroquine-treated mouse peritoneal MØs, following their interaction with *P. berghei* soluble antigens, elaborated diminished CSF activity, *in vitro* (unpublished observations). Because CSFs are very important immunoregulatory molecules, they may, at least in part, play significant role(s) in the development and expression of protective immune response in malaria.

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Three new species of *Nosema* from non-mulberry silkworms in Assam: light and electron microscopy studies

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ABSRACT. Distinct differences in shape and size were observed under a light microscope in the mature spores of *Nosema* spp. from Mulberry, Tasar, Eri and Muga silkworms. In *Nosema* spores from Tasar and Muga silkworms, the length and breadth ratio and the volume were highest. The scanning electron microscopy study revealed a smooth surface pattern of mature spores in Mulberry and Tasar silkworms, whereas a rough or wrinkled pattern with fibrous coating was observed in Eri and Muga silkworms. The transmission electron microscopy study revealed a uniformly thick coat in exospores of *Nosema* from Mulberry and Eri silkworms, whereas it was corrugated in Tasar and Muga silkworms. The number of coils varied in all the four *Nosema* spp. from all the four silkworms, and all these *Nosema* spp. differed from each other in about 12 characteristics and in the angle-of-tilt to the long axis of polar filament. Therefore, three new *Nosema* species have been erected and named as *N. mylitta* n. sp. from Tasar silkworm *Antheraea mylitta* D., *N. ricini* n. sp. from Eri silkworm *Philosamia ricini* B. and *N. assamensis* n. sp. from Muga silkworm *A. assamensis* Ww.

Keywords: angle-of-tilt, *Antheraea assamensis*, *Antheraea mylitta*, *Nosema* spp., *Philosamia ricini*

INTRODUCTION

Morphology of *Nosema bombycis nageli,* isolated from *Bombyx mori* L., has been studied in detail by using light microscopy, and scanning and transmission electron microscopy (Cali, 1970; Iwano and Ishihara, 1991). In addition to *N. bombycis*, seven other microsporidians, which belong to the genera *Nosema*, *Pleistophora* and *Thelohania* have been isolated from silkmoths (Jolly, 1986). These microsporidians differ in their spore morphology, target tissues and virulence, and have been temporarily designated as M11, M12, M14 (*Nosema*), M24, M25, M27 (*Pleistophora*) and M32 (*Thelohania*) (Sato and Watanabe, 1986). *N. bombycis* (3.8 x 2.2 µ) and big sized *Nosema* sp. (5.1 x 2.2 µ) are highly virulent compared to the other *Nosema* sp., *Pleistophora* sp. and *Thelohania* sp. (Fujiwara, 1980 and 1984). It has been reported by Kudo (1924) that the size and shape of the spores in a single species may vary considerably, and some authors gave little or no indication of such variations in their description. Electron microscopic studies have been conducted to confirm the various developmental stages and the fine structure of spores, which discriminate species on the basis of specific ultrastructural details, particularly the number of coils of polar filament and the angle of tilt of these coils, among the morphologically similar species (Sprague *et al.*, 1968; Kudo and Daniels, 1963; Lom and Weiser, 1972; Burges, *et al.*, 1974; Fowler and Reeves, 1975; Larson, 1999). Therefore,

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the present study was undertaken to search for specific morphological differences in the spores of four different *Nosema* isolates collected from four economically important silkworm species in India by using light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Based on our observations, *N. mylitta* n. sp. from Tasar silkworm *Antheraea mylitta* D., *N. ricini* n. sp. from Eri silkworm *Philosamia ricini* B. and *N. assamensis* n. sp. from Muga silkworm *A. assamensis* Ww have been erected.

MATERIALS AND METHODS

The purified and mature spores of *Nosema* sp. from four different host species, *B. mori* L. (Mulberry), *A. mylitta* Druary (Tasar), *P. ricin*i Boisd. (Eri) and *A. assamensis* Westwood (Muga) were collected for the study.

Light microscopy study: One drop of freshly isolated, purified and concentrated (1.52 x 10^8 spore/ml) mature spores of *Nosema*, suspended in distilled water, was put on a grease-free glass slide, covered with a cover-slip and observed under a compound microscope (1, 0001, 200 x). Molten agar (1.5% w/v) was used for measuring spores, and the measurement was done with a calibration factor worked out by the formula of Anja (1993).

SEM study: The standard technique was followed from Lom and Weiser (1972), Fowler and Reeves (1975), Kudo and Daniels (1963), Sprague *et al.* (1968), and Varva and Barkar (1980a and 1980b). The primary fixation of spores was done with gluteraldehyde (2.5%) and was followed by post fixation with 1% osmium tetraoxide.

TEM study: The technique was followed from Sato *et al.* (1982), Kudo and Daniels (1963) and Sprague *et al.* (1968). The spores were primarily fixed with 2.5% gluteraldehyde, followed by post fixation with 2% osmium tetraoxide, and hardened in 1.5 % molten agar (w/v).

RESULTS

Light microscopy: The mature spores of *Nosema* sp. were observed to be oval in shape with a large refractive index, and appeared greenish in colour. They contained a hard coating with a hard spore wall, and their sporoplasm was stretched in the form of a girdle across the width of the spore and contained a pair of nuclei. The polar capsule projected into the cavity of the spore and articulated with the spore wall at the anterior end and communicated to the exterior through a small opening. The polar filament was a tubular, coiled and spring-like structure, and was lodged within the polar capsule. Distinct differences in shape and size of the spores of *Nosema* sp., collected from Mulberry, Tasar, Eri and Muga silkworms were observed. The mature spores of *Nosema* sp. collected from Mulberry silkworm (*B. mori* L.) were observed to be oval in shape, whereas those from Tasar silkworm (*A. mylitta* D.) were oval but more in length and breadth. The mature spores of *Nosema* sp. from Eri silkworm (*P. ricini* B.) were elliptical in shape, blunt or tapering at both ends and smaller in length and breadth; those from Muga silkworm (*A. assamensis* Ww.) were elongated in shape, slightly bent at the middle and their length was similar to those from Tasar silkworm. The length/breadth ratio of spores from Muga silkworm was highest, and the volume of spores was largest in those collected from Tasar silkworm as compared to the others (Table I).

SEM study: The mature spores of *Nosema* sp. from Mulberry silkworm were observed under SEM, and were found to have a smooth and distinct surface (35,000 x; 20 KV). The big-sized spores of *Nosema* sp. from Tasar silkworm had a prominent, distinct, rough or wrinkled fibrous surface. A slight concavity or tapering at both the ends of spores with smooth external morphology and a distinct double wall external membrane was observed in the mature spores of *Nosema* sp. from Eri silkworm. A fibrous hard coating on the external morphology with slight concavity at the middle was observed in mature spores of *Nosema* sp. from Muga silkworm (Fig. 1a–d).

TEM study: The TEM structure of microsporidian spores revealed that their main components were sporoplasm, polar filament, polar cap, posterior vacuole and spore shell. The highly electron-dense sporoplasm having two nuclei was surrounded by a double-layered outer exospore and an inner endospore. The thickness of exospore and endospore varied in different *Nosema* sp. isolated from the all four types of silkworms studied. The anterior part of the spore was covered with a polar cap. The edge of the polar cap extended along the inner wall of the spore. The coiled part of the polar filament was usually located along the inner wall of the spore shell in a single row. A fine electron-dense border was observed at the inner edge of an endospore. The exospore is

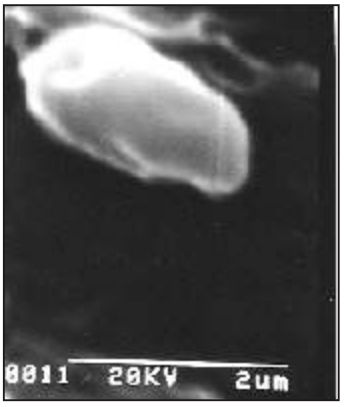

Fig. 1a. *N. bombycis* L., mature spore; SEM photograph

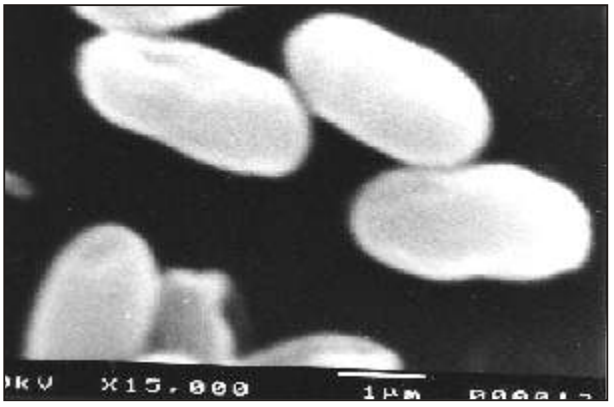

Fig. 1b. *N. mylitta* n. sp., mature spore; SEM photograph

Fig. 1c. *N. ricini* n. sp., mature spore; SEM photograph

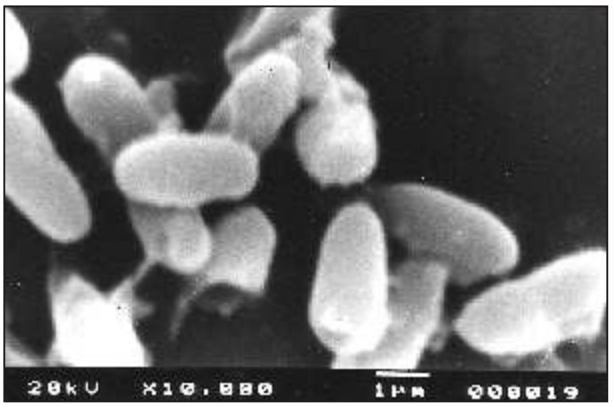

Fig. 1d. *N. assamensis* n. sp., mature spore; SEM photograph

usually an amorphous electron-dense coat, which varied in thickness from about 15100 nm and often limited to 50 nm. The exospore has been observed to be usually a uniformly thick coat in the spores of *Nosema* sp. collected from Mulberry and Eri silkworms, but is corrugated in those collected from Tasar and Muga silkworms. The endospore has been observed to be electron-lucent and considerably thicker, attaining 200 nm or more at the anterior end. A dome-shaped cavity is curved out at the inner side of endospore. The endospore is thicker in the spores of *Nosema* sp. from Mulberry and Muga silkworms, and comparatively thin in Tasar and Eri silkworms. Lining this cavity and extending backwards close to the spore wall is a membrane bound spore sac, which resembled the cap of a mushroom. The central region, a somewhat expanded cavity, sealed the base of the polar filament. The sac is separated from the wall by a narrow band of cytoplasm and by a plasmalemma, which extended around the entire cytoplasm complex in the spore cavity (Fig. 2a–d). The polar filament ran an oblique course backwards from its base at the centre of the polar sac, narrowing slightly in diameter in length and formed a coil in the peripheral layer of the cytoplasm. The coils varied from 414 in numbers and differed within the groups of *Nosema* spp. from these four types of silkworms. The number of coils were in the range of 414, 910, 1012 and 1214 in the spores of *Nosema* sp. collected from Mulberry, Muga, Eri and Tasar silkworms, respectively (Fig. 2a–d; Table II). In the anterior region of a spore, the cytoplasm appeared organized as a series of membranous sheets surrounding the straight sections of the polar filament .The precise organization of the membranes varied in different species. The two nuclei occupied the centre of the spore within the coil, and are surrounded by the cytoplasm, undifferentiated except for an abundance of ribosomes and a few cysternae of endoplasmic reticulum. The posterior vacuole lied within the coil at the posterior end and contained sparse amorphous materials. The coils of the polar filament are arranged at a low angle to the long axis of the spores, and can be called as angle-of-tilt. The tilt measured as 61-62 in *N. bombycis*, 90 in *N. mylitta* n.sp., 42-43 in *N. ric*ini n.sp. and 50 in *N. assamensis* n.sp. Thus, the spores of *Nosema* sp. from all the four silkworms were found to be different (Fig. 3a–d; Table II).

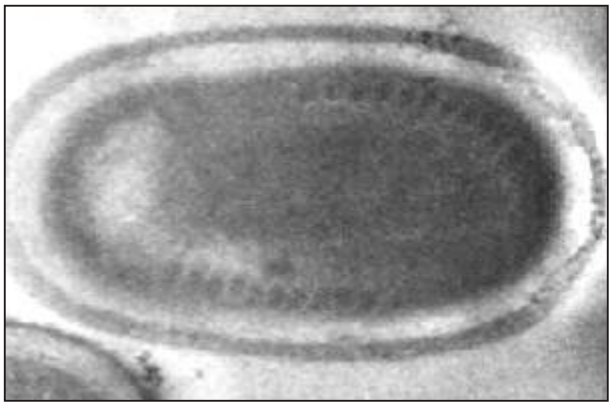

Fig. 2a. *N. bombycis* L., mature spore; TEM photograph

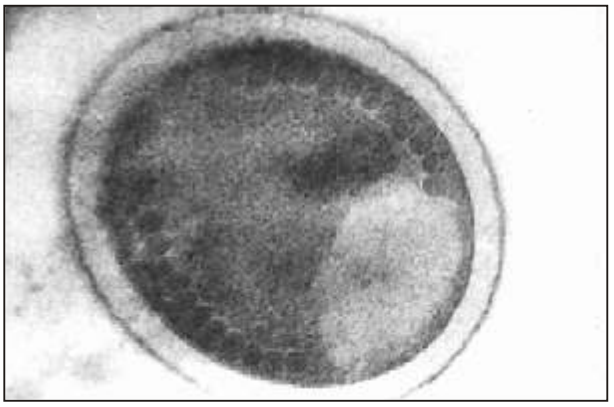

Fig. 2b. *N. mylitta* n. sp., mature spore; TEM photograph

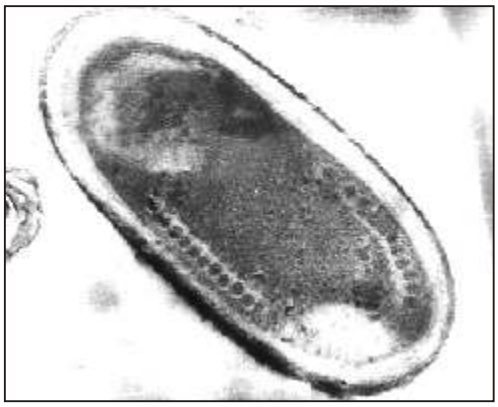

Fig. 2c. *N. ricini* n. sp., mature spore; TEM photograph

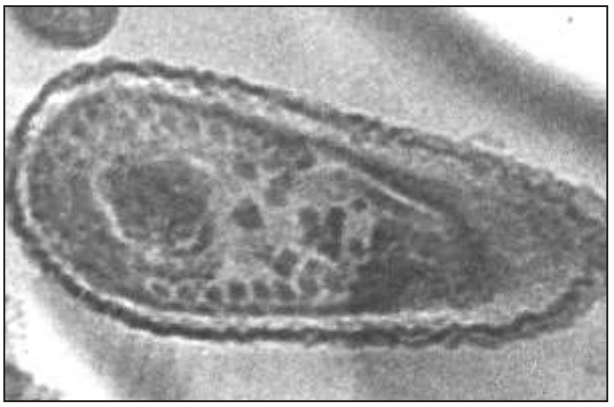

Fig. 2d. *N. assamensis* n. sp., mature spore; TEM photograph

Etymology: The specific names for the three *Nosema* spp. as *N. my*litta n. sp., *N ricini* n. sp. and *N. assamensis* n. sp. have been adopted from the names of their host moths: Tasar silkworm *A. mylitta*, Eri silkworm *P. ricini* and Muga silkworm *A. assamensis*, respectively.

Specific characters:

1. *N. bombycis* L.: Spore–length 4.10±0.05m, breadth 2.7±80.05 m, length/breadth ratio 1.49±0.03, volume 16.64 m^3, shape oval with smooth distinct surface wall, exospore smooth, endospore thick > 200 nm, number of coils in polar filament 34 and 11–14 (dimorphic), angle-of-tilt on most anterior coil 62 and most posterior coil 61.

2. *N. myllitta* n. sp.: Spore–length 4.42±0.04 m, breadth 2.83±0.03 m, length/breadth ratio 1.56±0.01, volume 18.53 m^3, shape oval but bigger than that of *N. bombycis*, surface wall with prominent, rough or wrinkled fibrous coating, exospore corrugated, endospore thin < 200 nm, number of coils in polar filament 12–14 and angle-of-tilt on most anterior to posterior 90°.

Host: *A. mylitta* Druary

Holotype: in one slide

Paratype: in two slides

Deposited to: at present in the Parasitology Laboratory, Department of Zoology, University of Calcutta, Kolkata ,West Bengal, India.

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3. *N. ricini* n. sp.: Spore–length 3.78±0.05 m, breadth 2.63±0.02 m, length/breadth ratio 1.44±0.02, volume 13.69 m^3, shape elliptical and tapering at both ends with double wall external membrane, exospore smooth, endospore thin < 200 nm, number of coils in polar filament 10–12, angle-of-tilt on most anterior coil 42 and most posterior coil 43.

Host: *P. ricini* Boisd.

Holotype: in one slide

Paratype: in two slides

Deposited to: at present in the Parasitology

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Accession /registration No.: 0002/06/P

4. *N. assamensis* n. sp.: Spore–length 4.37±0.05 m, breadth 2.68±0.02 m, length/breadth ratio 1.64±0.02, volume 16.45 m^3, shape elongated and slightly bent in the middle, rough fibrous hard coating with slight concavity in the middle of the surface, exospore corrugated, endospore thick > 200 nm, number of coils in polar filament 9–10; angle-of-tilt of coil on most anterior to posterior 50.

Host: *A. assamensis* Westwood

Holotype: in one slide

Paratype: in two slides

Deposited to: at present in the Parasitology Laboratory, Department of Zoology, University of Calcutta, Kolkata ,West Bengal, India.

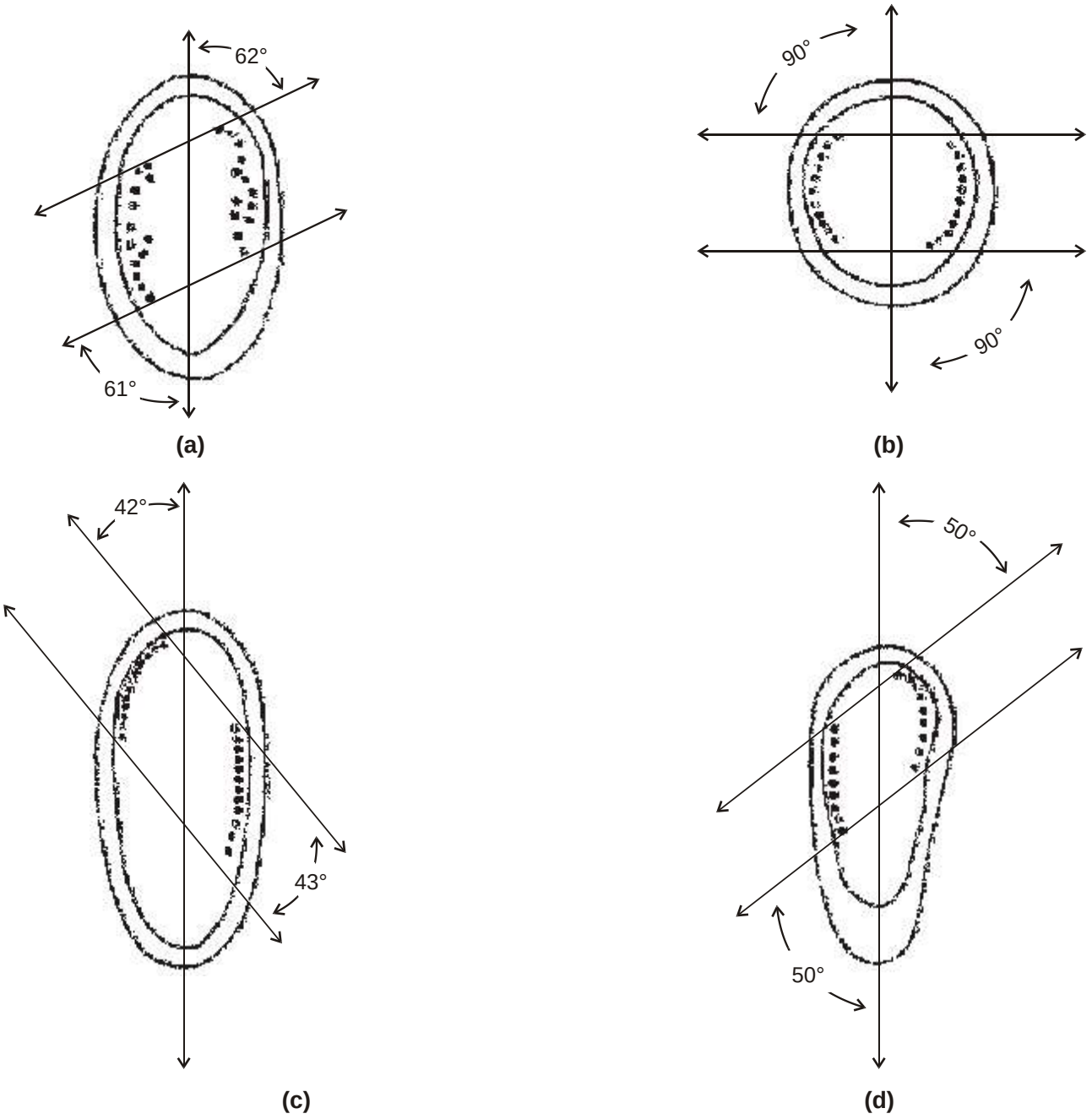

Fig. 3. Angle-of-tilt (degree) of coils of polar filaments in mature spores of (a) *N. bombycis* L., (b) *N. mylitta* n. sp., (c) *N. ricini* n. sp. and (d) *N. assamensis* n. sp. Angle indicated by an arrow.

Accession / registration No.: 0003/06/P

DISCUSSION

In the present study, the shape, size and measurements of four *Nosema* spore isolates from four different Indian silkworm species differ considerably (Table I and II). Usually all the species of the genus, *Nosema* share a basic spore shape (Larson, 1999). However, *N. bombycis* collected from Mulberry silkworm and from Lawn grass cutworm, *Spodoptera depravata* are different in their shape and size (Iwano and Ishihara, 1991). Reports on the measurements of the shape and size for *Nosema* spores from *A. mylitta* are also available (Patil, 1993).

The mature spores of *N. bombycis* have been observed to be oval in shape, and thus appear similar to the observation made by Fuziwara (1980 and 1984) and Patil (1993). The mature spores of *N. mylitta* n. sp. have also been found to be oval in shape, but bigger in size as compared to those from *N. bombycis*, and a similar observation has also been reported earlier (Patil, 1993). The mature spores of *N. ricini* n. sp. have been observed to be elliptical in shape, blunt or tapering at the both ends and comparatively smaller in length and breadth than those of *N. bombycis*, *N. mylitta* n. sp. and *N. assamensis* n. sp. The mature spores of *N. assamensis* n. sp. are elongated, slightly bent in the middle and their length is similar to the spores of *N. mylitta* n. sp. Apparently, there are no previous reports on the measurements of their shape and size.

The SEM study showed that the mature spores of *N. bombycis* have a smooth and distinct surface, and a similar observation has been reported by Iwano and Ishihara (1991). The spores of *N. mylitta* n. sp. have been found to have a prominent, distinct, rough and wrinkled surface with fibrous coating, whereas those from *N. ricini* n. sp. have a slight concavity or tapering at both ends with a smooth and distinct double-wall external membrane. The spores of *N. assamensis* n. sp. have an external fibrous hard coating with slight concavity in the middle. The fibrous coating indicated the presence of mucocalyx, a flotation device reported by Varva and Barkar (1980a) on the surface of the spores of *Glugea elegans*.

Rausch and Grunewald (1981) differentiated six microsporidian by using light microscopy and SEM, on the basis of the arrangement of cysts and the surface structure of the spores. In the present study, *Nosema* spores isolated from Mulberry and Eri silkworms have smooth surface, whereas those from Tasar and Muga silkworms have rough or wrinkled surface. Therefore, SEM study did not solve all the problems of microsporidian identification. However, it did provide another set of meaningful characterstics, necessary for its identification to assess its merit in taxonomic considerations (Fowler and Reeves, 1975).

The TEM study demonstrated that a general similarity exists in their main components: sporoplasm, polar filament, polar cap, posterior vacuole, spore shell and two nuclei within the *Nosema* spp. collected from the four silkworm species. However, the endospores are more thick (> 200 nm) in the spores of *N. bombycis* (Fig. 2a) and *N. assamensis* n. sp. (Fig. 2d), whereas it is < 200 nm in *N. mylitta* n. sp. (Fig. 2b) and *N. ricini* n. sp.(Fig. 2c; Table II). *N. bombycis* exhibited dimorphism: one group of spores is having filament with 34 coils and another group with 12–14 coils. Ishihara and Iwano (1991) have reported similar findings on the spores of *N. bombycis* isolated from moths of the lawn grass cutworm *S. depravata* Butler and from the cell culture of *B. mori*. Steinhaus and Hughes (1949) and Cali (1970) also reported such a type of dimorphism of spores in other microsporidians including *N. bombycis*. The dimorphism of spores is considered as the adaptation of the parasite to different needs during their life cycle. The spores that appear during the early development of the parasite have the ability to germinate intracellular having relatively short polar tube, and serve as a means to deliver sporoplasm in neighboring cells (Iwano and Ishihara, 1989). The first population of spores mainly causes the spread of the parasite in the epithelium. The second population of spore is formed later on and is adapted to survive outside the host (Graaff *et al.*, 1994). The early spores and the environmental spores are immature, and are the variants of the same spore type normally occur in different tissues in the host (Larson, 1999). The observed coil number variation within the *Nosema* spore isolates collected from the other three types of non-Mulberry silkworms merits its taxonomic consideration. The number of coils of polar filament in the spore of different species of microsporidian range from 34 coils in *N. cuniculi* to 44 in *N. apis*. The majority of species, however, have 11 coils; out of 15 species listed by Milner (1972a), 9 had 11–15 coils. Neither the number of coils, nor the arrangement within the spore, appeared to correlate with the genus (Milner, 1972b).

Table I. Morphometric study of *Nosema* spp. collected from different silkworms

Name of silkworm	Size of the spore			
	Length (µm)	Breadth (µm)	L/B ratio	Volume (π/6 LB2)
B. mori	4.10±0.05	2.78±0.05	1.49±0.03	16.64
A. mylitta	4.41±0.04	2.83±0.03	1.56±0.01	18.53
P. icini	3.78±0.05	2.63±0.02	1.44±0.02	13.69
A. assamensis	4.37±0.05	2.68±0.02	1.64±0.02	16.45

Table II. A comparative light and electron microscopic study of *Nosema* spp. from *B. mori, A. mylitta, P. ricini and A. assamensis*

Name of pathogens (collected from silkworms	Shape	External morphology by SEM	Exospore	Endo spore (nm)	Internal morphology by TEM			
					No. of coil in polar filament	Angle-of-tilt (degree)		
						No. of coils coil	Tilt in most anterior coil	Tilt in most posterior coil
N. bombycis (Mulberry silkworm)	Oval	Smooth and distinct surface wall	Smooth	Tick > 200 nm	3-4 11-14 (Dimorphism)	14	62	61
N. mylitta n. sp. (Tasar silkworm)	Oval but bigger	Prominent distinct rough or wrinkled with fibrous coating like structure	Corrugated	Thin < 200	12-14	11	90	90
N. ricini n. sp. (Eri silkworm)	Elliptical and tapering at both ends	Smooth concavity at both ends with double wall external membrane	Smooth	Thin < 200 nm	10-12	12	42	43
N. assamensis n. sp. (Muga slikworm)	Elongated and and slightly bent at middle	Rough fibrous like hard coating, slight concavity at the middle	Corrugated	Thick > 200	9-10	9	50	50

Further, in addition to the number of coils, the angle-of-tilt to the long axis of the spores were also observed to be different within the *Nosema* spp. collected from the four types of silkworms (Fig. 3a–d). The angle-of-tilt, in the present study, discriminated the four *Nosema* spp. clearly and that is very much accepted for the identification of *Nosema* spp. (Burges *et al.*, 1974). The angle-of-tilt to the long axis of the spore distinguished the two closely related species *N. oryzaephili* and *N. whitei*; both have been reported similar in shape and size under light microscope, the surface of spores is smooth in SEM, and the internal structure of the spore under TEM is also similar, except for the number of coils (11 and 13, respectively) on the polar filament and the angle-of-tilt (31º and 41º, respectively; Burges *et al.*, 1974). Garcia and Becnel (1994) created eight new species of Microsporidia (Microspora) from Argentina

mosquito (Dipitera: Culicidae) on the basis of the number of coils in a polar filament and the angle-of-tilt. The internal structure of microsporidian spores differs greatly among different species; nevertheless, the similarities remain more impressive than the differences (Kudo and Daniels, 1963; Sprague, 1965). Therefore, apparently, all the microsporidian species have a similar basic pattern of spore structure, barring some minor variations (Sprague *et al.*, 1968).

In conclusion, based on our light microscopy, SEM and TEM obserations, we have demonstrated that the spores of four different *Nosema* spp. isolated from four different silkworms, differ from each other in more than twelve characteristics (Table I and II). Therefore, the authors propose that these should be considered as three distinct new species of the genus *Nosema,* and suggest their names as *N. mylitta* n. sp. collected from *A. mylitta* Druary, *N. assamensis* n. sp. from *A. assamensis* Westwood and *N. ricini* n. sp. from *P. ricini* Boisd. However, the typical number of coils, rather than the length of polar filament in the spore, is slightly different in all the four species. Therefore, the number of coils and the angle-of-tilt to the long axis of the spores distinguish them into four different species.

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JPD

Significance of the ultrastructure of spermatozoon and prostate gland of *Avitellina lahorea* (anoplocephalid)

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ABSTRACT. The ultrastructure of the mature spermatozoon of *Avitellina lahorea* revealed five distinct antero-posterior regions without any clear morphological discontinuity between them. The mature sperm was observed to have a tapered outline. Cortical microtubules ran all through the length of spermatozoa. The cytoplasm was partitioned into several compartments and contained proteinaceous materials. The nucleus was situated in the middle region of sperm. A prominent prostate gland was visible. The various stages of spermiogenesis and mature spermatozoon could be seen in the mature proglottid region. The sperms were visible within the vaginal wall.

Keywords: *Avitellina lahorea*, prostate gland, spermatozoa, ultrastructure

INTRODUCTION

The cestodes represent an important helminthic group of endoparasites, which infect sheep. Their endoparasitic existence resulted in the loss of gut, but an increase in their reproductive capacity (Schmidt, 1988; Smyth and Mc Manus 1989). *Avetellina lahorea* belongs to Anoplocephalid family and infects the sheep *Ovis aries*. To date, apparently, the ultrastructural studies on spermatozoon have been done on following parasites: *Aporina delafondi*, *Avitellina centripunctata* and *Inermicapsifer guineensis* (Ba and Marchand 1992a, 1994c and d), *Monoecocestus americanus* (Mac Kinnon and Burt, 1984), *Oochoristica agamae* (Swiderski and Subilia, 1985), *Paranoplocephala omphalodes* (Miquel and Marchand, 1998), *Stilesia globipunctata* (Ba and Marchand, 1992b). The aim of the present study was to delineate the ultrastructure of mature spermatozoon of *Avitellina lahorea*.

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MATERIALS AND METHODS

The portions of *A. lahorea* mature proglottides containing seminal vesicles and testes were removed from *Ovis aries* by using a binocular microscope, washed and rinsed in normal saline, diced into small pieces and then fixed with glutaraldehyde in Millong's phosphate buffer (pH 7.3, 380 mOsm/l) for 1 h at 4°C. After fixation for 34 h at room temperature, the tissue was rinsed in Millong's buffer, post-fixed in 1% osmium tetraoxide in Millong's buffer for 1.5 h, rinsed quickly in distilled water, dehydrated in an ethanol series, infiltrated with propylene oxide, embedded in Spurr's low-viscosity epoxy resin and then polymerized at 60°C. The sections (70–90 nm) were cut with a diamond knife, mounted on uncoated copper grids, stained with uranyl acetate/ethanol and aqueous lead citrate and finally examined under a Philips 204 transmission electron microscope (TEM) at an accelerating voltage of 40 or 60 kV (Conn, 1993).

RESULTS AND DISCUSSION

In the present study, the different developmental stages of spermiogenesis and spermatozoa could be seen in TEM micrographs (Fig. 1a, b, c and d). The sperms

were observed enclosed within membrane bound bodies of apical cytoplasm of vaginal epithelium. The young spermatid exhibited a roughly circular nucleus with partially condensed chromatin. The various ultrastructural features of spermatozoon are given below.

Sperm crest: TEM micrographs revealed a crest like body in the ultrastructure of spermatozoon of *A. lahorea*. According to Ba and Marchand (1994a), the presence of crest like body or bodies represents the front part of sperm, whereas the end without it represents the posterior extremity. The sperm crest in cestodes showed variability in different species. In *R. serrata, H. nana* and *A. delafondi*, the crest-like bodies are of same thickness but are different in length. On the other hand, in *M. expansa, M. benedeni* and *T. ovilla*, the crest-like bodies are not only same in thickness but also in length. Ba and Marchand (1994b) have described two crest-like bodies of unequal thickness and length in *R. tunetensis*. However, in *A. centripunctata*, these authors have described a simple and single crest-like body.

Cortical microtubules: The cortical microtubules ran all along the length of spermatozoon and formed a continuous layer of dense sub-membranous material. They were observed to be spiralized and the angle-of-spiralization appeared more or less marked. The mitochondria and acrosome were conspicuously absent. The microtubular elements constitute a characteristic feature of most vertebrates and invertebrate spermatozoa. The absence of acrosome has been construed as an unusual characteristic of trematode sperms. Burton (1967) has attributed that the incompatible length of sperm and the size of ovum, and thereby, the limited space for the penetration of sperm into the latter, provide little advantage for the presence of acrosome. Moreover, a spermatozoon in trematodes does not penetrate an ovum; instead the plasma membranes of gametes fuse and sperm internal structures pass into ovum. Considering the unusual ultrastructural features such as the absence of acrosome, mitochondria and the stiff spindle-like length of spermatozoon, a similar phenomenon viz., apposition of gametic membranes during fertilization, may be also conjectured to occur in this species of cestode parasites.

Cytoplasmic partitions: The cytoplasm of *A. lahorea* was observed to be of low electron-dense nature. From the anterior to the posterior end of spermatozoon, five contiguous regions, as suggested by Ba and Marchand (1992, 1994 a and b) in other species, could be distinguished without any clear morphological demarcation between them but exhibited distinctive ultrastructural characteristics (Fig. 1c). These regions are described below:

Region I: This region exhibited an apical cone of electron-dense material. The cortical microtubules formed a continuous layer of dens and sub-membranous material. The axoneme appeared surrounded by a fine discontinuous sheath of an electron-dense material and an electron-lucent cytoplasm.

Region II: This region exhibited a central axoneme, a thin layer of electron-lucent cytoplasm and a single or two bundles of spiral cortical microtubules, which sometimes cover each other partially and form a discontinuous layer of electron-dense and sub-membranous material.

Region III: In this region, central axoneme was found to be surrounded by a fine layer of lucent cytoplasm and a continuous sheath of electron-dense material. The cytoplasm appeared electron-lucent and sub-divided into several compartments by irregularly-spaced partitions of electron-dense material.

Region IV: This region is marked by the presence of nucleus. The nucleus appeared to be fine, compact and coiled in a spiral around axoneme. It enveloped the axoneme once or twice, interposed itself between the cortical microtubules and closely contacted the plasma membrane.

Region V: This region appeared to be wide with a pointed end. Disorganization and disappearance of axoneme and intracytoplasmic partitions of electron-dense material could be seen in this region.

The partitions of proteinaceous materials were present in cytoplasm. The mitochondria were conspicuously absent. The absence of mitochondria suggested that the sperm may be deriving energy through glycolysis. The production of lactic acid in tissues of *A. lahorea* and *Stylesia globipunctata* (Venkatesh and Ramalingam, 2006) serves as evidence for the above speculation (Ramalingam *et al*., 2004). Ba and Marchand (1994 a, b) also described intracytoplasmic partitions of proteinaceous materials.

Cortical tubules and fertilization: The spermatozoa of *A. lahorea* exhibited only the apical cone-like structure, and acrosome is completely absent. The presence of cortical microtubules in the anterior region

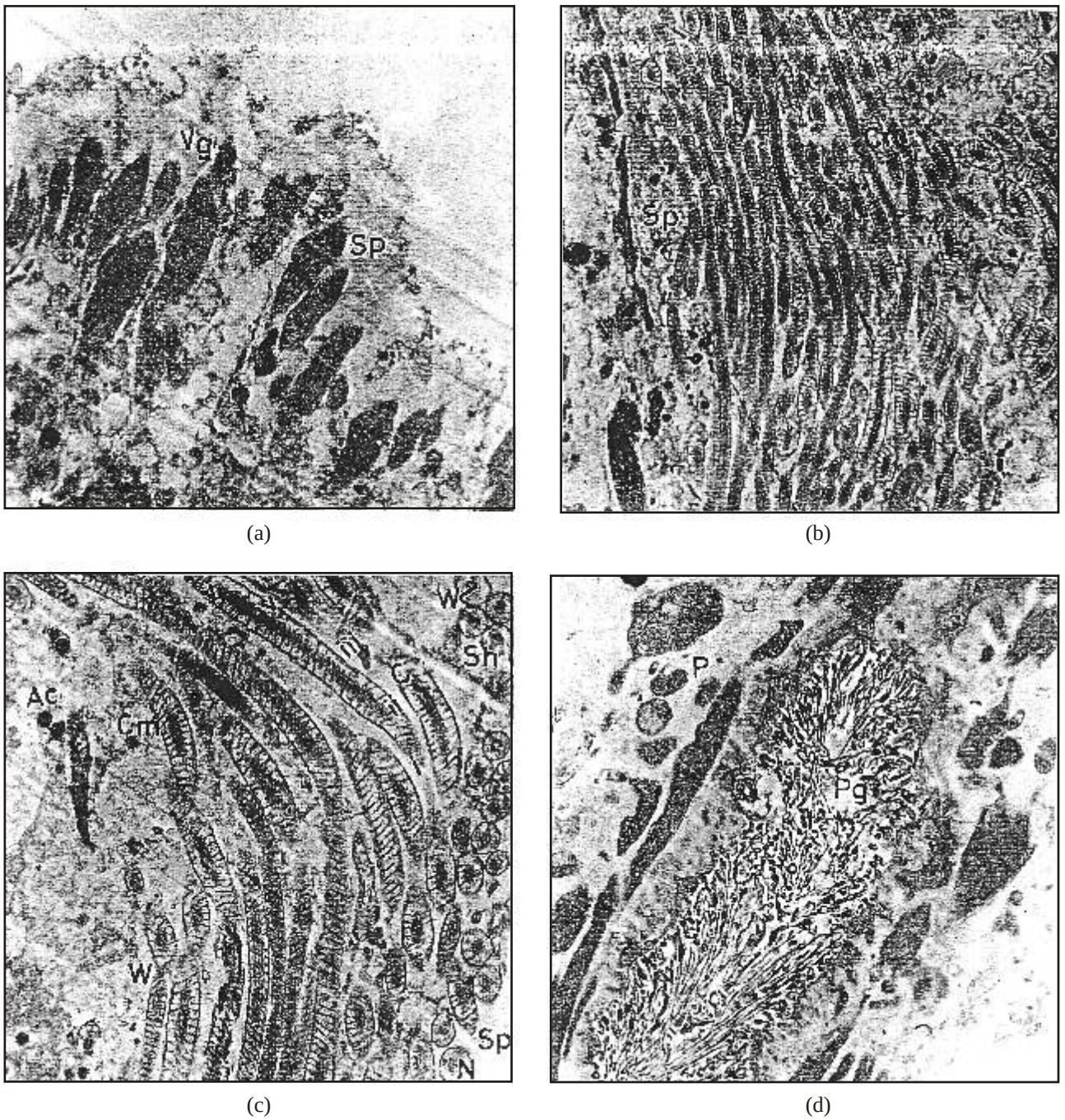

Fig. 1: TEM micrographs of mature segment *A. lahorea*.

a. Vaginapost-mature segment showing sperms within the wall of vagina. Sperms enclosed within membrane-bound bodies of the apical cytoplasm of the vaginal epithelium (x 7, 000).

b. Sperms and stages of spermiogenesis (x 4, 500).

c. Higher magnification of sperms showing different regions of spermatozoon. Note the five different regions of the spermatozoon (I, II, III, IV and V; x 7, 000).

d. Vagina epithelium showing male accessory reproductive gland (prostrate gland; x 7, 000).

Ac–apical cap, C–lucent cytoplasm, Cm–cortical microtubules, P–parenchyma, Pg–prostate gland, N–nucleus, Sh–sheath of electron-dense periaxonemal material, Sp–spermatid, Vg–vagina, I-V–region of spermatozoon.

of sperm might represent features functionally analogous to acrosome of vertebrates and other higher invertebrates. The mechanism of fertilization remained improperly understood. The coalition may have been facilitated by the cortical microtubules. It may be construed that the cortical tubules of sperm and the cortical granules of ovum might play a complementary role in ending their fusion.

Prostate glands and prostate cells: In the cestode reproductive system, though the hermaphroditic condition of the segments has been well established, the glandular structures associated with the gonads and their functions in the gametogenesis are not yet clearly understood. The presence of Mehlis gland in the female reproductive system has been identified in most cestode species (Coil, 1991). However, regarding the male accessory glands, not much is known. Light microscope studies revealed the existence of prostate glands in some species of Cyclophyllidea (Jones, 1998). In some studies, the term prostate cells has been given to the male accessory reproductive glands. The functional significance of the above glands has not been discussed in any of the above reports. The arrangement and distribution of the prostrate glands in *A. lahorea* is suggestive of its secretory role in reproduction (Fig. 1c). Thus, the simple organization of the gland as prostrate cells and its organizational structure, as observed in the present study, seem to indicate its accessory reproductive function.

Mopping of sperms: In the present study, the vaginal epithelium of *A. lahorea*, with a male accessory reproductive gland in it (Fig. 1a), seemed to suggest that the excess sperm mass may be mopped-up by the vaginal epithelium and other tegumental tissues, to make way for the expanding egg bearing Paruterine organs and other developmental processes. Jones (1998) also suggested that the internal epithelia of cestode-segments might function to remove structures from the tapeworm body by a process that resembles phagocytosis.

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JPD

Ectoparasitism in dogs from the eastern zone of Maharashtra state*

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ABSTRACT. Bionomic studies on ectoparasites in dogs from the eastern zone of Maharashtra revealed 81.36% infestation. The prevalence in monsoon, post-monsoon, winter and summer season was 79.54, 93.63, 83.68 and 74.22%, respectively. The incidence recorded in dogs < 1 year, between 1–3 years and > 3 years in age was 85.52, 81.47 and 78.08%, respectively. Mongrel dogs were more affected by ectoparasitic infestation as compared to other breeds of dog. Infestations with *Rhipicephalus sanguineus* were highest in prevalence followed by *Hippobosca capensis, Ctenocephalides canis, Sarcoptes scabiei, Demodex canis, Linognathus stenopsis, Trichodectes canis* and *Heterodoxus longitarsus*.

Keywords: dogs, fleas, flies, lice, Maharashtra, ticks

INTRODUCTION

Various parasitic diseases have been known to affect the health of dogs. Out of these diseases, the arthropod infestation is at height of occurrence. This infestation of generally does not cause heavy mortality but affects the efficiency of dogs, and thus leads to considerable losses to the owner of the dog. The parasitism poses a direct threat to the health, causing irritation, dermatitis, cutaneous injuries, unthriftiness and restlessness. The arthropod parasites cause physical damage to the skin, blood loss, allergic conditions, and act as vectors of pathogens in dogs. Although several reports (Deka *et al.*, 1995, Neog *et al.*, 1995, Sreedevi *et al.*, 2002 and Kumar *et al.*, 2006) are available on canine arthropod infestation from different parts of the country; nevertheless, information from the eastern zone of Maharashtra state is lacking. Therefore, the studies reported herein were carried out on the bionomics of ectoparasitism in dogs from the eastern zone of Maharashtra state.

MATERIALS AND METHODS

A total of 907 dogs of different breed, age and of both sexes, belonging to private owners in the eastern zone Maharashtra state comprising Nagpur, Wardha and Yeotmal districts, were examined randomly. The total number of dogs screened for arthropod infestation from Nagpur, Wardha and Yeotmal districts was 349, 247 and 311, respectively. Fortnightly, the ectoparasites, *viz.* ticks, mites, lice, flies and fleas were collected in specimen bottles, brought to the laboratory, processed and examined. The identification of arthropods was done as per the morphological characters given by Sen and Fletcher (1962) and Soulsby (1982).

RESULTS AND DISCUSSION

Out of 907 dogs examined, 738 were found positive for ectoparasite infestation (81.36%). The prevalence

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Table I. Overall prevalence of ectoparasitic infestation in dogs from the eastern zone of Maharashtra

Parameter	Components	No. examined	No. positive	%
Season**	Monsoon (July–Sept.)	220	175	79.54
	Post-monsoon (Oct.–Nov.)	157	147	93.63
	Winter (Dec.–Feb.)	239	200	83.68
	Summer (Mar.–June)	291	216	74.22
Age	< 1 yr.	221	189	85.52
	Between 1-3 yrs.	394	321	81.47
	> 3 yrs.	292	228	78.08
Breed	Boxer	17	11	64.70
	Cross-bred	235	198	84.25
	Doberman	21	16	76.19
	German Shepherd	167	134	80.23
	Golden Retriever	16	11	68.75
	Great Dane	22	16	72.72
	Labrador Retriever	24	17	70.83
	Lhasa apso	24	17	70.83
	Mongrel (local breed)	186	164	88.17
	Pomeranian	34	26	76.47
	Spitz	161	128	79.50

**significant ($p < 0.01$)

of ectoparasites in monsoon, post-monsoon, winter and summer season was observed to be 79.54, 93.63, 83.68 and 74.22%, respectively (Table I). The season-wise prevalence was significant ($p < 0.01$), and correlated with the environmental temperature and relative humidity ($r_1 = 0.679$ and $r_2 = 0.867$, respectively). The present study indicated that the prevalence starts increasing from monsoon, reaches maximum in post-monsoon and then declines in winter with a minimum % in summer. Considerably, the high temperature (45.45°C), which exists during summer, might be the reason for low infestation, whereas a warm humid climate prevailing during post-monsoon in the eastern zone of Maharashtra might be the cause for spurt in the prevalence of ectoparasitism in pet animals. The prevalence recorded in dogs <1 year, between 1-3 years and > 3 years in age was 85.52, 81.47 and 78.08%, respectively. The occurrence of infestation in various breeds, *viz*. Boxer, cross-bred, Doberman, German Shepherd, Golden Retriever, Great Dane, Labrador Retriever, Lhasa apso, Mongrel (non-descript breed), Pomeranian and Spitz was 64.70, 84.25, 76.19, 80.23, 68.75, 72.72, 70.83, 70.83, 88.17, 76.47 and 79.50%, respectively (Table I). Mongrel (local breed) dogs were more prone to the infestation, followed in descending order by cross-bred, German Shepherd, Spitz, Pomeranian, Doberman, Great Dane, Labrador Retriever, Lhasa apso, Golden Retriever and Boxer. The breed-wise variation recorded in canine ectoparasitism is attributable to the susceptibility of various breeds of animals to parasites and which is genetically determined (Urquhart *et al.*, 1996). Ihrke (1995) also noticed variations in the incidence of parasitic dermatitis in animals of different breeds. Nayak *et al.* (1997) reported the high prevalence of canine demodicosis in Tibetan apso as compared to Doberman, Alsatian and Mongrel. Jha *et al.* (2001) recorded a high incidence of *Sarcoptes scabiei* and *Demodex canis* infestation in Mongrel than Doberman, German Shepherd, Spitz, Great Dane, Labrador and Dachshund breed of dogs in Ranchi. Kumar *et al.* (2006) reported a high incidence of sarcoptic and demodectic mange in German Shepherd than in Boxer breed of dog.

Ectoparasite species recorded from the dogs were *Rhipicephalus saguineus* (21.50%), *Sarcoptes scabiei* (3.75%), *Demodex canis* (2.76%), *Heterodoxus longitarsus* (0.66%), *Trichodectes canis* (1.10%), *Linognathus stenopsis* (2.09%), *Ctenocephalides canis* (4.74%), *Hippobosca capensis* (13.12%) and

Table II. Ectoparasitic infestations in dogs from the eastern zone of Maharashtra

Species	No. examined	No. positive	%
Rhipicephalus sanguineus	907	195	21.50
Sarcoptes scabiei	907	34	3.75
Demodex canis	907	25	2.76
Heterodoxus longitarsus	907	06	0.66
Trichodectes canis	907	10	1.10
Linognathus stenopsis	907	19	2.09
Ctenocephalides canis	907	43	4.74
Hippobosca capensis	907	119	13.12
Mixed sp. infection	907	287	31.64
Total	907	738	81.36

mixed species infestation (31.64%) (Table II). The prevalence of ectoparasites in dogs has been documented from different parts of India (Varghese *et al*., 1994; Deka *et al*., 1995; Jha *et al*., 2001; Sreedevi *et al*., 2002; Kumar *et al*., 2006). These authors reported either single species of one genus of arthropod or more infesting dogs. The present study encountered a single genus with a single species of ticks, two genera with two species of mites, three genera with three species of lice, a single genus with a single species of fleas and a single genus with a single species of flies. Miranpuri and Singh (1978) reported eight species of ticks from domestic animals in Assam. The host and environment being identical, the probable cause of variations in the prevalence could be due to the different developmental stages of ectoparasites. In ticks, as a single adult female lays thousands of eggs, which develop to larvae in succession throughout the year, sufficient to infect a number of hosts, thereby causing more chances of spreading the infestation, whereas in lice egg laying and its further growth is too slow, besides host specificity, thereby reducing the chances of the spread of infection from one host to the other host. Similarly, in the case of mites which develop and spread slowly, the chances of fly infestation are less due to its periodic visit to the hosts.

The developmental stages of *Rhipicephalus sanguineus* were attached to the inner and outer surfaces of ears, around eyes, neck, paws and vertebral column of dogs. The lesions of *Sarcoptic scabiei* were confined to skin of head, neck, abdomen and orbital area of dog. Neog *et al*. (1995) also recorded similar observations in dogs. *Demodex canis* parasites in dogs were recovered from skin scrappings of the lesions on head, neck and inner sides of thigh, abdomen and orbital area. This is in agreement with the findings of Neog *et al*. (1995) who also recorded similar observations. The site of predilection of *Hippobosca capensis* was on abdomen, thigh and hind legs of dogs.

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Polymerase chain reaction for the diagnosis of bovine babesiosis

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ABSTRACT. Babesiosis, caused by *Babesia bigemina,* continues to be one of the important tick-borne diseases of cattle in a tropical country like India, and the disease impact varies from region to region with a state of enzootic stability of the tick-borne infection. There is a need for improved capability to diagnose carrier animals. The specificity and sensitivity of polymerase chain reaction (PCR), using oligonucleotide primers constructed from Mexican isolate, were studied against Indian strains of *B. bigemina*. With the use of PCR, it was possible to amplify the template DNA of *B. bigemina* to a sensitivity of 500 pg, and to detect DNA in 12 out of 15 heparinized blood samples collected from Izatnagar (northern India) and 2 out of 20 from Manipur (eastern India). The microscopical examination of Giemsa-stained blood films failed to reveal parasites in all except two cases from Izatnagar. Hence this PCR based assay can be used for specific and sensitive detection of *B. bigemina* from 20 µl blood samples collected from suspected cattle.

Keywords: *Babesia bigemina,* diagnosis, polymerase chain reaction

INTRODUCTION

Babesiosis is one of the most important tick-borne diseases of cattle in tropical and subtropical regions of the world. It has been estimated that more than 500 million cattle worldwide are at risk due to this disease alone (Ristic, 1988). It is assumed that about 80% of Indian herd is within areas endemic for *Babesia* and *Anaplasma* infections. The economic losses, due to these diseases were estimated to be $57 million in India (Tick Cost Version1.0, 1999). The clinical manifestations of an acute presentation of the disease include fever, anorexia, dullness, weakness, ataxia, haemoglobinuria, icterus, anaemia and presence of intra-erythrocytic parasites (Callow, 1984). The acute clinically apparent form of the disease is less frequently observed than latent or subclinical form, as in any other haemoparasitc disease. Following recovery from an acute infection, the animal becomes carrier and clinically cannot be distinguished from normal *Babesia*-free animals. The diagnosis of carrier animals is important as these animals act as potential source of infection to healthy susceptible population. The diagnostic techniques available at present include microscopy, quantitative buffy coat (QBC) technique, serological methods and subinoculation into susceptible non-infected splenenctomised calf (Bose *et al.*, 1995). These techniques have their own inherent limitations like low sensitivity and specificity, high cost of equipment or requirements of technical skill, etc. Polymerase chain reaction (PCR), which was originally described by Saiki *et al.* (1988), has the advantage of both high sensitivity and specificity. Figueroa *et al*. (1992) developed a PCR along with a non-radioactive probe assay in which the primers derived from a sequence contained P^{16} insert (Buening *et al*., 1990) were used to amplify a 278 bp fragment

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from the genome of *B. bigemina*. Later, these primers were widely used for detection of this parasite in field conditions (Figueroa *et al*., 1993; Figueroa *et al*., 1996; Buening and Figueroa, 1996). Other PCR based assays were also reported for the detection of *B. bigemina* (Caccio *et al*., 2000). The present communication deals with the standardization of a widely tested PCR assay (Figueroa *et al.*, 1992) for the detection of *B. bigemina*, to confirm the global conservancy of the targeted parasite DNA (278 bp) of *B. bigemina* and the field validation of this PCR using blood samples collected from ear vein.

MATERIALS AND METHODS

Genomic DNA of *B. bigemina*: Total genomic DNA of *B. bigemina* was isolated from infected blood collected from four splenectomised and then immunosupressed calves (two calves each infected with Izatnagar isolate and Wayanad isolate) at the peak of parasitaemia. Briefly, the blood was collected in ethylene-diamine tetra acetic acid (EDTA) and centrifuged at 1000 rpm for 30 min to settle the erythrocytes. The buffy coat and plasma were removed, and the cells were washed five-times with sterile phosphate- buffered saline (PBS, pH 7.2), each time taking care to remove as much leucocytes as possible. The packed erythrocytes were diluted three-times of their volume with PBS (pH 7.2), passed through a dry cellulose CF-11 column packed to a height twice that of the blood volume. The elutes containing only erythrocytes were collected, washed once with PBS (pH 7.2) and the pellet was then used for the isolation of genomic DNA by phenol-chloroform extraction method (Sambrook *et al.*,1989). Concentration of DNA was determined by UV spectroscopy.

Collection and processing of blood samples: Fifteen blood samples from apparently healthy crossbred bovine calves (Izatnagar) were collected into heparinized eppendorf tubes including two from known carriers of infection. These two animals previously revealed piroplasms in blood smear. Twenty heparinized blood samples collected from Manipur (eastern India) were also used for the study. At the time of collection of blood samples, thin methanol-fixed blood films were also prepared, and were examined after Giemsa staining. The blood samples were processed following the method described for the detection of *Plasmodium* spp. from human blood samples (Tirasophon *et al*., 1991). Twenty µl of each sample blood was lysed with 200 µl of a lysis buffer (0.015% saponin, 3.5 mM NaCl, 1mM EDTA) and vortexed gently. Following centrifugation at 12,000 rpm, the pellet was collected after removal of supernatant containing haemoglobin, dissolved in 100 µl of reaction mixture buffer (10 mM Tris-HCl, 50 mM $MgCl_2$, 0.01% gelatin) and vortexed again. Finally, the pellet was dissolved in 10 µl of sterile triple-distilled water, boiled for 10 min and the supernatant was used for PCR assay. Blood sample collected from a three-day old healthy calf was used as a negative control.

Polymerase chain reaction: PCR was performed as per the method of Figueroa *et al.* (1992). Primers for the PCR amplification of a 278 bp *B. bigemina*-specific fragment (Figueroa *et al*., 1992) were custom synthesized by Bangalore Genei, India. The nucleotide sequences of the primers are as follows:

Forward 5'-CAT CTA ATT TCT CTC CAT ACC CCT CC-3'

Reverse 5'-CCT CGG CTT CAA CTC TGA TGC CAAAG-3'

The conditions for PCR assay were optimized by using 15 pmol of each primer in 25 µl reaction mixture (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01% gelatin) containing 250 µM each of dATP, dTTP, dCTP, dGTP (Bangalore Genei, India), 1.5 units of Taq DNA polymerase (Bangalore Genei, India) and 20 ng DNA. DNA amplifications were performed in a thermal cycler (Perkin Elmer, USA). The sample was preincubated at 95°C for 5 min to completely denature the DNA. This was followed by 35 cycles of each of 1 min at 95°C, 1 min at 65°C and 1.5 min for 73°C, and finally with one-time polymerization at 73°C for 15 min. The products were checked for amplification and lack of spurious products by electrophoresis on a 2% agarose gel using 60 V power supply for 1.5 h. The gel was visualized by gel documentation system (UVP Alpha Imager, Syngene) and photographed. Template DNA from four isolates (two samples each from Izatnagar and Wayanad) were checked for amplification of the specific fragment. For the sensitivity analysis, template DNA concentrations ranging from 10 ng to 1 pg were tested, as described above. The specificity of DNA amplification was tested by using control PCR assays with template DNA of other blood parasites (*Theileria annulata* and *Trypanosoma evansi*).

RESULTS

The specificity of the detection method: The PCR assays revealed amplification of specific product of 278 bp size (Fig.1) with all the four isolates tested. No amplification in reactions containing *T. annulata*, *T. evansi* and normal bovine blood was observed.

Sensitivity of DNA amplification: The sensitivity studies carried out with template DNA, derived from the four isolates, revealed specific amplification with an analytical sensitivity level of 500 pg parasite DNA.

PCR assay on field samples: The assay performed with processed blood samples (Fig. 2 and 3) from 12 out of 15 calves including the two known carrier animals (lanes 5 and 6; Fig. 2) collected from Izatnagar (northern India) showed specific signals (Fig. 2 and 3**)**, whereas only two of twenty blood samples collected from Imphal, Manipur (eastern India) revealed specific amplification. The blood sample processed from a three-day old calf failed to show amplification. The Giemsa-stained blood films from these animals, except the two known carrier animals from Izatnagar, did not show any parasites on microscopical examination.

DISCUSSION

Although microscopic detection of *Babesia* species is most efficient and cost effective method for the diagnosis of acute babesiosis, the detection of serum antibody is recommended for the epidemiologic surveillance of the disease risk due to enzootic stability (Montenegro-James and James, 1998). The problem associated with antigenic cross-reactivity continues to compromise the efficacy of the sero-tests like enzyme linked immunosorbent assay (ELISA) for babesiosis (Bose *et al.*, 1995; Montenegro-James and James, 1998; El-Ghaysh *et al*., 1996). The advent of DNA amplification tools has increased the sensitivity and specificity level of the detection of *Babesia* parasites. Absolute sensitivity (detection limit) ranges from 10^{-5} to 10^{-6} for nucleic acid probe or 10^{-8} to 10^{-9} for PCR, where sensitivity is expressed as 1 parasite in x erythrocytes i.e. 10^{-5} = one parasite per 100,000 erythrocytes (Bose *et al.*, 1995). The DNA probe

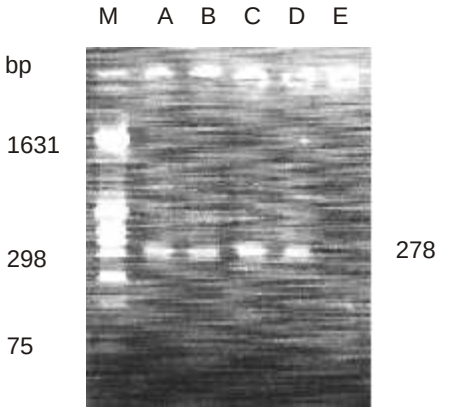

Fig. 1: PCR amplification of 278 bp *B. bigemina* specific fragment. A-Izatnagar isolate 1, B-Izatnagar isolate 2, C-Wayanad isolate 1, D-Wayanad isolate 2, E-Bovine leucocyte DNA, M-pBR322/*Hinf*I digest.

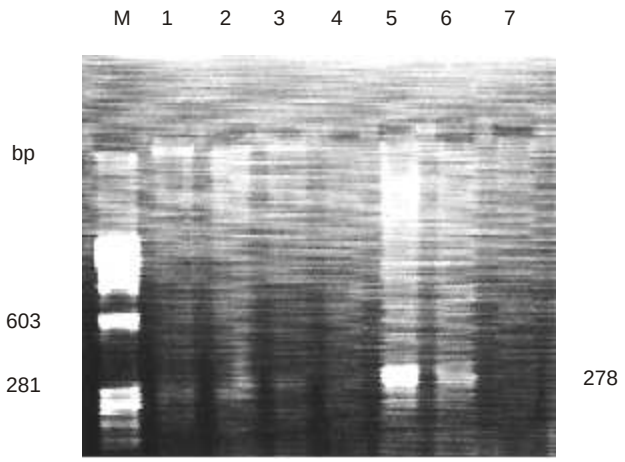

Fig. 2: Field validation of *B. bigemina* specific PCR. 1-4 and 7: blood samples from apparently healthy calves, 5 and 6: blood samples from known positive carriers, M:B x174/*Hae*III digest.

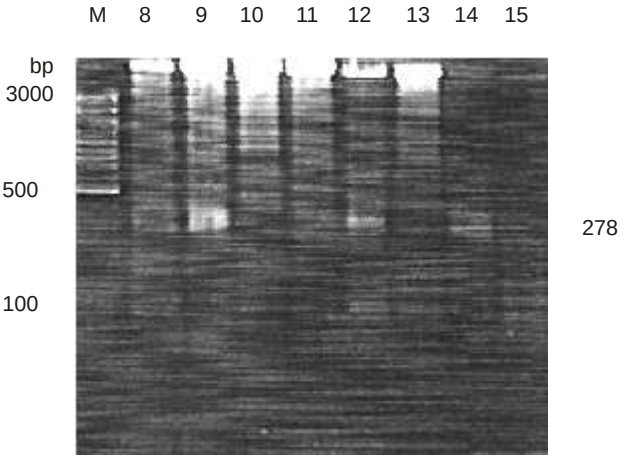

Fig. 3: Field validation of *B. bigemina* specific PCR. 8-15: blood samples from apparently healthy calves, M: GeneRuler ™ 100 bp DNA ladder plus.

applications are recommended for species identification, whereas PCR is considered useful for the detection of carrier animals and import/export certification (Bose *et al.*, 1995; Figueroa and Buening 1995; Sparagano, 1999).

Previously, a PCR based assay for sensitive detection of *B. bigemina* was described by Figueroa *et al*. (1992), with a threshold sensitivity level of 10 pg per assay. The sensitivity limit of this PCR assay requires a comment. Figueroa and co-workers (1992) reported the threshold sensitivity of the PCR as 10 pg while working with purified *B. bigemina* DNA samples from parasite isolates derived from population of North and Central America and Caribbean region. Using the recommended assay conditions, while the specificity of the PCR in the context of Indian *B. bigemina* isolates was never in doubt, we failed to achieve the sensitivity levels described earlier. This may be due to variation in the sequence of the target DNA in comparison with the Mexican isolate.

With the gradual replacement of multi-host tick, *Hyaloma anatolicum* by one-host tick, *Boophilus microplus* in our country (Khan, 1990, 1994; Sangwan *et al*., 2000), there is an urgent need to monitor the status of *Boophilus* spp. transmitted bovine babesiosis in the country.

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JPD

Distribution of intestinal parasites among food handlers in Jeddah, Saudi Arabia

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ABSTRACT. During this study, 1009 male non-Saudi food handlers (mean age 33.25±13.4 years) from 25 countries were investigated for the presence of intestinal parasites in their stool. The stool samples were examined by direct smear examination, Ritchie concentration technique and trichrome staining method. Intestinal parasites were detected in 50.15% of the food handlers. Seventeen different intestinal parasites were identified: *Blastocystis hominis (23.29%)*, Hook worms (14.67%), *Trichuris trichiura* (9.61%), *Endolimax nana* (7.04%), *Entamoeba coli* (5.05%), *Giardia lamblia* (4.6%), *Entamoeba histolytica* (2.97%), *Entamoeba hartmani* (2.97%), *Strongyloides stercoralis* (1.88%), *Iodamoeba butschlii* (1.29%), *Schistosoma mansoni* (1.1%), *Ascaris lumbricoides* (1.1%), *Hymenolepis nana* (0.8%), *Dientamoeba fragilis* (0.6%), *Taenia saginata* (0.2%), *Enterobius vermicularis* (0.2%) and *Chilomastix mesnili* (0.2%). By using Kinyoun's modified staining, *Cryptosporidium* oocysts were detected in 99.2% of the food handlers, which is a very high incidence level, and thus needs more attention. This is the first study in Saudi Arabia to investigate *Cryptosporidium* parasite in food handlers.

Keywords: food handlers, intestinal parasites, Jeddah, Saudi Arabia

INTRODUCTION

Intestinal parasites are one of the major public health problems in the world, especially in tropical and sub-tropical countries. The World Health Organization estimated that some 3.5 billion people are affected, 450 million of them are ill because of intestinal parasitic infections and, annually, 2, 00, 000 deaths are directly attributable to the intestinal parasitic infections (WHO, 2005). These intestinal infections could be due to protozoan or helminthic parasites.

Intestinal parasitic infections of food handlers, who prepare food-stuffs, may represent a potential source of infection for the public. In recent years, many people in Saudi Arabia are dependent on food handlers in cafeterias for their fast meals. For main meals on social occasions, food is prepared by food handlers in restaurants or cookhouses (locally known as Matabekh). Many such studies regarding intestinal parasites in food handlers showed high prevalence of infections. Curiously, all these studies, with the exception of four that were done in Saudi Arabia, were carried out in different countries around the world (Abu Al Saud, 1983; Khan *et al.*, 1987; Ali *et al.*, 1992; Amin, 1997). In Jeddah, as in all the cities in Saudi Arabia, most of the food handlers are non-Saudis. This is first study, carried out in Saudi Arabia, from July 2003 to February 2004, demonstrates 50.15% prevalence of intestinal parasites (including *Cryptosporidium*) in non-Saudi food handlers.

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MATERIALS AND METHODS

Stool samples: Stool samples were collected from 1009 non-Saudi male food handlers, working at food shops in Jeddah city in the western region of the Kingdom of Saudi Arabia. Each food handler was provided with a clean sample collection container.

Direct smear examination: On a glass microscope slide, about 1–2 mg of stool was uniformly emulsified in a drop or two of normal saline (0.85% NaCl) on the left hand side of the slide, and in Lugol's iodine on the right hand side of the slide. A cover-slip was then placed on each side, and the slides were scanned under 10x and 40x objective lenses of a light microscope, as required.

Formal ether sedimentation technique: Approximately 2 g of stool was dissolved in 10 ml of 10% (v/v) formal saline and allowed to stand for 30 min. The suspension was and then strained through two layers of gauze into a 15 ml conical centrifuge tube and centrifuged at 2000 rpm for 5 min. Whenever needed, the washing step was repeated until the supernatant turned clear. The sediment obtained was resuspended in 10 ml of 10% (v/v) formal saline and allowed to stand for 10 min. Later, 3 ml of diethyl ether was added, the contents were shaken vigorously for 30 s and centrifuged at 2000 rpm for 5 min. After centrifugation, the applicable diagnostic stages were allowed to sediment at the bottom of the tube and the fecal debris was separated in a layer between diethyl ether and 10% (v/v) formal saline layers. The fecal debris layer was loosened by using a wooden stick and the tube was rapidly inverted to discard the top three layers, while the sediment remained at bottom of the tube. Finally, iodine (1–2 drops) was added to the sediment, mixed well, and part of the sediment was transferred to a glass microscope slide, covered with a cover glass and scanned under 10x and 40x objective lenses of a light microscope.

Trichrome staining technique: The stool samples were smeared on to micro slides, and then fixed first with Schaudinn's fixative, then in 70% ethanol for 5 min and finally in 70% ethanol plus iodine, for 35 min. The slides were placed for two changes in 70% ethanol for 5 min each time, followed by staining in trichrome stain solution for 10 min. Destaining was done in 90% ethanol plus acetic acid by dipping (x 2) the slides. The destained slides were dipped once in 95% ethanol and then in 100% ethanol followed by two 5 min changes of 100% ethanol. Finally, slides were placed for two changes of xylene (5 min each), and then examined under 100x objective lens of a light microscope.

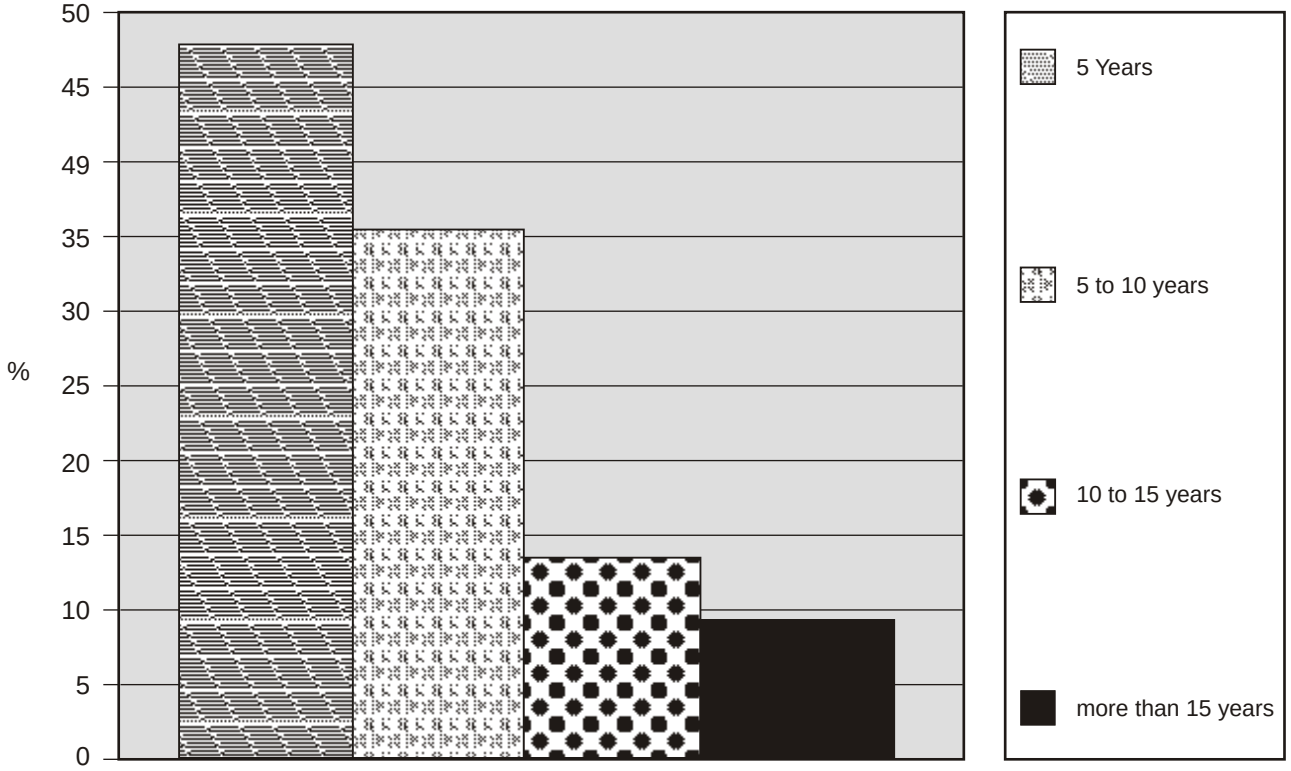

Fig. 1. Period of arrival of food handlers in Saudi Arabia. The majority of food handlers arrived in the last five years.

Kinyoun's modified technique: Fecal smears were prepared directly from stool samples, air-dried and then fixed in absolute methanol. Fixed smears were stained with Kinyoun's carbol-fuchsin for 3–5 min and destained with 1% aqueous sulfuric acid for 2–3 min. Smears were rinsed with tap water and counterstained with Loeffler's alkaline methylene blue for 1 min. Using tap water, smears were rinsed, drained and allowed to dry, and observed under a light microscope using 100x objective.

Statistical analysis: The StatMost 32 software program was used for statistical analysis of the results of this study.

RESULTS

The nationality, age and period of stay of food handlers in Saudi Arabia: The food handlers (1009 in total) belonged to 25 countries (Table I), as per following details: 356 (35.28%) from India, 179 (17.74%) from Bangladesh, 76 (7.53%) from Pakistan, 75 (7.33%) from Yemen, 63 (6.24) from Philippine, 46 (4.56%) from Egypt, 45 (4.46%) from Turkey, 34 (3.37%) from Syria, 28 (2.77%) from Afghanistan, 25 (2.78) from Indonesia, 19 (1.88%) from Sudan, and 12 (1.19%) from Lebanon. The remaining food handlers (8 from Thailand, 7 each from Tunisia and Ethiopia, 5 each from Morocco, Iran, Eritrea and Nepal, 3 each from Somalia and Palestine, 2 each from Chad and Jordan, and 1 each from Nigeria and Sri Lanka) had a nationality percentage < 1. The stay of food handlers in the Kingdom of Saudi Arabia, prior to obtaining the stool samples, ranged from 3 days to 40 year. The arrival of these food handlers was grouped into four categories: 46.8% in the past five years or less, 34% between last more than five years to ten years, 11.7% between more than ten years to fifteen years, and 7.5% more than fifteen years ago.

Macroscopic examination included color, consistency of stool and presence of any macroscopic diagnostic stage of intestinal parasites. The color of specimens ranged from yellowish, light brown, brown to greenish or dark brown. The consistency ranged from formed, soft to loose, and none of the samples was watery or bloody. Only one sample from a Bangladeshi food handler contained three live adult worms of female *Enterobius vermicularis*, which were present on the surface of stool.

Detected parasites: Out of the 1009 food handlers studied, 506 (50.15%) were infected with intestinal parasites (excluding *Cryptosporidium* spp.; Table I). Seventeen different parasites were detected by using different techniques during this study. The following parasites, as per their prevalence rate, were detected: *Blastocystis hominis* (23.29%), Hook worms (14.67%), *Trichuris trichiura* (9.61%), *Endolimax nana* (7.04%), *Entamoeba coli* (5.05%), *Giardia lamblia* (4.6%), *Entamoeba histolytica* (2.97%), *Entamoeba hartmani* (2.97%), *Strongyloides stercoralis* (1.88%), *Iodamoeba butschlii* (1.29%), *Schistosoma mansoni* (1.1%), *Ascaris lumbricoides* (1.1%), *Hymenolepis nana* (0.8%), *Diantamoeba fragilis* (0.6%), *Taenia saginata* (0.2%), *Enterobius vermicularis* (0.2%), *Chilomastix mesnili* (0.2%).

Direct smear examination: Direct smears examination revealed trophozoite stage of parasites in some samples. These trophozoites included *G. lamblia*, *E. coli*, *E. histolytica* and *C. mesnili*. Trophozoites of amoeba were confirmed by permanent trichrome staining technique. In 89 samples, *B. hominis* was detected in direct smears but could not be detected by using Ritchie technique.

Ritchie (formalin ether) technique: Four hundred forty six (44.2%) samples, out of the 1009 samples studied, were positive for parasitic infection. These positive samples represented 88.14% out of the total 506 positive samples. Ritchie technique was able to detect all the parasites except the trophozoite stages, and could additionally detect 89 samples having *Blastocystis hominis*.

Trichrome staining technique: Trichrome staining was performed for all liquid or semi liquid stool samples and in general, to confirm the detection of the applicable protozoan stages. Trichrome staining could show trophozoite stages, which could not have been seen by using Ritchie technique and could have been missed out in direct smears examination of non-fresh stool samples.

Modified Kinyoun's technique: One thousand and one (99.2%) samples, out of the 1009 samples, were positive for *Cryptosporidium* oocysts. Stool samples from only eight food handlers were negative; three from Egypt and one each from India, Eritrea, Turkey, Yemen and Tunisia.

DISCUSSION

This is the first study in Saudi Arabia to investigate the prevalence of intestinal parasites including *Cryptosporidium* in food handlers. During this study, direct methods, concentration methods and

permanent staining were used. Generally, in direct smear examination, normal saline is used for the detection of the motility of the trophozoites of intestinal protozoan parasites. These trophozoites are seen in liquid or semi-liquid specimens. The iodine direct smears show, in greater detail, the diagnostic features of various parasites. Therefore, direct smear examination is considered the best and simple way for the detection of cellular exudates (erythrocytes, white blood cells and mucous). However, in cases of low level of infection, direct smear examination might end-up showing false negative results. Further, if the specimens are relatively old, the motile parasites might die and could be missed.

To increase the chances of detection of desired diagnostic stages of the parasites, several concentration techniques are usually used and the process is started with a large amount of stool sample. Ritchie sedimentation technique is one of the best concentration techniques used in diagnostic parasitology laboratories for the detection of cysts, ova and larvae, but not for the trophozoites. This technique uses 10% (v/v) formal saline to kill and preserve the diagnostic stages of the parasites. Diethyl ether separates the unwanted debris as a plug layer at the top, and the all the diagnosable parasite stages get concentrated at the bottom of the tube. However, appropriate precautions should be taken, as some of the chemicals used in this technique are carcinogenic, flammable and explosive.

The trophozoites of the intestinal protozoan parasites can be diagnosed according to their motility as well as their diagnostic features. Because trophozoites perish rapidly, fresh samples are required for the motility examination, which is difficult for specimens not processed immediately. Therefore, the best way to diagnose non-motile trophozoites is by staining them. Trichrome is considered a best stain. After trichrome staining, parts of trophozoites look bluish green color, whereas others will appear purple to red.

The majority of food handlers in Jeddah are non-Saudis. During this study, we met only three Saudi working as food handlers, but they did not cooperate in submission of stool samples and, therefore, all the examined stool samples were from non-Saudi food handlers; majority of them being from Asian countries. The age of food handlers ranged between 17 and 60 years (mean 33.25±13.4 years). The youngest and oldest food handler was from Yemen and Thailand, respectively.

The prevalence rate of intestinal parasites (excluding *Cryptosporidium* spp.) among these food handlers was 50.15%, which is high. Previous studies in Saudi Arabia showed various percentages of infected food handlers; 41.4%, 7.56% and 14% (Abu Al Saud, 1983; Khan *et al.*, 1987; Ali *et al.*, 1992). The reason for the low percentages detected in the last two studies appears to be that direct smear examination and not any concentration technique was used. In this study we have used both direct smear examination and Ritchie concentration technique for all the samples, and specifically observed that the direct smear examination missed most of the low level infections ($p < 0.001$) as compared to the formal ether concentration technique.

The prevalence of the pathogenic parasites was 77.73% of the total parasites detected (Table II). Of these, 298 (29.53%) food handlers were infected with helminthic parasites with or without other parasites, whereas infection with helminths alone was 14.47%. On other hand, the prevalence of protozoan infection with or without other parasites was 47% and 19.43% for protozoan infection alone. The reason for this difference may be the efficient transmission due to simple life cycle of protozoan parasites, besides the suitable outside environment in Jeddah city.

As shown in Table II, the incidence of parasites in food handlers was high in most of the nationalities. The low number of food handlers in the last ten nationalities was, apparently, the obvious reason for their 0% and 100% rate of infection. Intestinal parasites were not detected in one food handler from Sri Lanka, three from Palestine and two from Jordan. A study in Al-Medinah by Ali *et al*. (1992) reported that the highest incidence occurred in Sri Lankan food handlers (40.7%) and in two food handlers each from Palestine (6.54%) and Jordan (20%). However, we believe that the way of calculation in their study was not accurate, as they mixed the number of patients with the number of parasites.

Our study showed that the major isolated parasites were *B. hominis* (23.29%), Hookworms (14.67%) and *T. trichiura* (9.61%). A study on non-Saudi catering and domestic staff at the Riyadh military hospital reported that *T. trichiura* is the most common parasites followed by Hookworm, *A. lumbricoides* and *G. lamblia* (Abu Al Saud, 1983). Khan *et al.* (1987) found that the highest incidence of parasite in food handlers in Dammam and Al-Khobar was with *E. histolytica*, *G. lamblia* and *A. lumbricoides*. Al-

Table I: Food handlers' nationality, age range, number and percentage of infection

	Total food handlers		Infected food handlers		
Nationality	No.	%[a]	No.	%[b]	Age range
Indian	356	35.28	172	48.31%	21-55
Bangladeshi	179	17.74	112	62.56	20-55
Pakistani	76	7.53	42	55.26	19-52
Yemeni	75	7.33	36	48.00	17-56
Filipino	63	6.24	20	31.74	21-46
Egyptian	46	4.56	23	50.00	21-51
Turkish	45	4.46	18	40.00	22-58
Syrian	34	3.37	18	52.94	21-55
Afghan	28	2.77	15	53.57	18-50
Indonesian	25	2.78	14	56.00	22-50
Sudanese	19	1.88	11	57.89	23-54
Lebanese	12	1.19	6	50.00	22-49
Thai	8	0.79	3	37.50	32-60
Tunisian	7	0.69	4	57.14	20-39
Ethiopian	7	0.69	2	28.57	23-30
Moroccan	5	0.49	3	60.00	26-51
Iranian	4	0.39	1	25.00	30-42
Eritrean	4	0.39	1	25.00	27-52
Nepalese	4	0.29	1	25.00	25-38
Somali	3	0.29	2	66.67	25-37
Palestinian	3	0.29	0	0	23-31
Chadian	2	0.19	1	50.00	24-33
Jordanian	2	0.19	0	0	29-32
Nigerian	1	0.09	1	100.00	38
Sri Lankan	1	0.09	0	0	40

[a]% calculated to the total number of food handlers of all nationalities (1009).
[b]% calculated to the total number of food handlers of the indicated nationality.

Medinah study showed that the most common parasites among food handlers were *G. lamblia* followed by *E. histolytica*, *T. trichiura* and *A. lumbricoides* (Ali *et al.*, 1992).

The highest infection rates with hookworms were detected in 26.7% Indians, 23% Bangladeshi and 24% Indonesians, whereas *T. trichiura* infection was prevalent in 37.43% Bangladeshi and 14.4% Filipinos (Table II). *Schistosoma mansoni* infection was found in 11 food handlers, four from Yemen, three from Egypt, two from Sudan and one each from Tunisia and Nigeria. *S. mansoni* has been detected in Yemeni, Egyptian, Saudi, Sudanese, Bangladeshi and Turkish food handlers (Ali *et al.*, 1992). On other hand in the Riyadh study, no *Schistosoma* parasite was detected (Abu Al Saud, 1983); however, in Dammam and Al-Khobar study, four cases were reported, but without details about the nationalities of the infected food handlers (Khan *et al.*, 1987).

B. hominis was detected in food handlers from all the nationalities except the Ethiopians, Sri Lankans, Jordanians, Nigerians and Palestinians. It has been reported that the proliferation of this parasite is enhanced by abnormal intestinal tract due to any reason (Udkow and Markell, 1993). Whether *B. hominis* is pathogenic (Russo *et al.*, 1988; Garavelli *et al.*, 1992) or non-pathogenic (Zuckerman *et al.*, 1994; Horiki *et al.*, 1997; Chen *et al.*, 2003) remains highly controversial. In our study, 89 samples were found positive for *B. hominis* in direct smear examination but negative in Ritchie concentration technique, and further investigations are going on to explain the reason(s) thereof.

Food handlers from Syria, Afghanistan, Lebanon, Thailand and Morocco were infected with protozoan parasites only and not with helminthic parasites. In Syrians only, the observations made in Al-Medinah study were applicable (Ali *et al.*, 1992). As mentioned

Table II. Occurrence of parasites in food handlers of each nationality

	Parasites (no. of infected food handlers)																
Nationality	*Ss*	*Hw*	*Tt*	*Al*	*Hn*	*Ts*	*Ev*	*Sm*	*Eh*	*Gl*	*Bh*	*En*	*Ec*	*Df*	*Ib*	*Eha*	*Cm*
Indian	4	95	11	2	1	-	-	-	6	7	67	19	8	2	2	4	-
Bangladeshi	13	41	67	7	-	-	2	-	4	7	26	9	7	-	1	5	-
Pakistani	-	2	1	-	2	-	-	-	11	10	22	13	11	-	4	8	1
Yemeni	-	-	4	1	-	-	-	4	2	2	25	7	7	-	1	2	-
Filipino	1	2	6	-	-	-	-	-	-	1	12	2	-	2	-	1	-
Egyptian	-	-	1	-	1	-	-	3	-	2	19	4	4	1	-	2	-
Turkish	-	1	1	1	1	1	-	-	2	2	16	1	2	-	1	2	-
Syrian	-	-	-	-	-	-	-	-	-	3	12	4	1	-	1	-	-
Afghan	-	-	-	-	-	-		-	2	5	5	4	5	1	-	1	-
Indonesian	1	6	5	-	1	-		-	1	1	5	1	1	-	2	-	-
Sudanese	-	-	1	-	1	-	-	2	1	-	5	3	2	-	1	4	1
Lebanese	-	-	-	-	-	-	-	-	-	-	6	3	-	-	-	-	-
Thai	-	-	-	-	-	-	-	-	-	-	3	-	1	-	-	-	-
Tunisian	-	-	-	-	1	-	-	1	-	-	4	-	-	-	-	-	-
Ethiopian	-	-	-	-	-	1	-	-	-	1	-	1	-	-	-	-	-
Moroccan	-	-	-	-	-	-	-	-	1	-	3	-	1	-	-	1	-
Iranian	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Eritrean	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Nepalese	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Somali	-	1	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-
Palestinian	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chadian	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Jordanian	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nigerian	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Sri Lankan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

S. stercoralis (Ss), Hook worm (Hw), *T. trichiura* (Tt), *A. lumbricoides* (Al), *H. nana* (Hn), *T. saginata (Ts), E. vermcularis* (Ev), *S. mansoni* (Sm), *E. histolytica* (Eh), *G. lamblia* (Gl), *B. hominis* (Bh), *E. nana* (En), *E. coli* (Ec), *D. fragilis* (Df), *I. butschlii* (Ib), *E. hartmani* (Eha), *C. mesnili* (Cm).

above, Khan *et al*. (1987) did not provide details of the nationalities of food handlers, whereas Abu Al Saud (1983) reported only three nationalities but without any details of their parasitic infections.

The above discussion did not include *Cryptosporidium* results, as this parasite alone showed a very high incidence (99.2%). No previous studies on food handlers in Saudi Arabia included *Cryptosporidium*, and our study is the first one to do so. Our results, obtained by using Kinyoun's modified technique, included both positive and negative controls. In future, we plan is to use several other techniques, including immunological and molecular ones, to investigate the reason(s) behind such a high incidence of *Cryptosporidium*. Most of the stool samples collected were not in liquid form, which means that the majority of food handlers were asymptomatic carriers of *Cryptosporidium*. It is known that frequent exposure to *Cryptosporidium* infection along with the development of acquired immunity might explain the high incidence of this parasite in asymptomatic cases (Jannof *et al.*, 1990; Chacin-Bonilla *et al.*, 1997; Esteban *et al.*, 1998; Chacin-Bonilla and Sanches-Shaves, 2000). We suggest further investigations on the role of water, food and direct contact with people and animals in the transmission and spread of *Cryptosporidium* in Jeddah.

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Ultrastructure of polymorphic microtriches in the tegument of *Raillietina echinobothrida* that infects *Gallus domesticus* (fowl)

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ABSTRACT. The transmission electron microscope study of the cestode parasite *Raillietina echinobothrida* that infects the intestine of avian hosts, revealed that posteriorly directed filamentous microtriches densely covered the tegument of entire scolex and immature and mature proglottid regions of the parasite. Few blade-like or bulb-like microtriches could be seen in the scolex. Some microtriches appeared longer, flattened and strap-like in immature and mature proglottid regions of the parasite and showed polymorphism. The microtriches of mature and gravid regions of strobila lacked spikes. The spatial distribution of microtriches and their polymorphism from scolex to the mature region revealed that the entire integumental region might be involved in the absorption of nutrients both from the lumen of intestine and the parasite-adhered host interfacial region. The atrophy of gravid microtriches may facilitate the process of apolysis and the dissemination of embryos alongside faeces of the host. The present investigation elucidated both the structural and functional homology of the tegument, and the ultrastructure of the cestode parasites, which occupied the intestine niche.

Keywords: density, distribution, microtriches, polymorphism, *Raillietina echinobothrida*

INTRODUCTION

The structure of the cestode tegument is of particular interest to parasitologists, as these worms lack mouth and digestive system and, therefore, all nutrients must pass through the body wall (Read *et al*., 1963). Earlier investigations of tapeworm morphology revealed that the free surface of the tegument is covered with specialized microvilli, thus resembling the brush border of mammalian enterocytes (Read, 1955). These processes have been referred to as microtriches (Rothman, 1963), microvilli (Beguin, 1966) and tegumental projections (Morseth, 1966). The microtriches have been analogized with microvilli, which constitute the brush border of many invertebrate and vertebrate transport epithelia (Read 1955; Braten, 1968a,b), though microtriches and microvilli differ in certain fine structural details.

The microtriches have been reported to show a wide range of morphologies, which vary between species and in different proglottid regions. The microtriches on scolex of tetraphyllidean cestodes have been recognized as potentially significant characters in systematic analyses (Whittaker and Garvajal, 1980). The microtriches have also been widely reported among all major orders of Eucestoda. The cestode tegument represents an essential anatomical region for host-parasite interaction. Light microscopic

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observations have revealed the nutrient-absorptive nature of the tegument across this region, and many other biological functions have also been attributed to the tegument (Rothman, 1963; Morseth, 1966 and Berger and Mettrick, 1971).

The ultrastructure of the cestode tegument of most of the cyclophyllidean tapeworms (Rosario, 1962; Lumsden and Specian, 1980; Lumsden and Hildreth, 1983; Threadgold, 1984 and Smyth and McManus, 1989) has been reported. Most of the investigators working on the ultrastructure of the cestode tegument have focused on species of medical or veterinary importance such as *Hymenolepis* spp. and *Taenia* spp. (Cyclophyllidea) and *Diphyllobothrium* spp. (Pseudophyllidea). Recently, the use of transmission electron microscope (TEM) has provided another means for the investigation of the details of tegument. These studies have revealed that all the cestode species examined possess microtriches in larval and adult stages, and that they are probably of universal occurrence (Morseth, 1966 and Braten, 1968b). A characteristic of cestode anatomy is the complete absence of an alimentary canal, and the cestode tegument is the only tissue available for molecular interchange between the parasites and their hosts (Pappas and Read, 1975; Pappas, 1983a).

The fine structural studies of the cestode tegument have demonstrated that the surface cytoplasm is extended as microtriches, consisting of cylindrical cytoplasmic bases capped by the structures termed shafts/spikes (Read, 1955; Rothman, 1963; Jha and Smyth, 1969; Blitz and Smyth, 1973). These microtriches have been suggested to be instrumental in the increase of surface area for absorption, digestion, protection, secretion and excretion. The spine-like tips of the microtriches are believed to aid in maintaining the position of worm in the gut (Rosario, 1962; Rothman, 1963; Smyth, 1969). Berger and Mettrick (1971) suggested that the microtriches played a role in locomotion of these worms within the host gut, and the density of the microtriches changes throughout the strobila. At the parasite-host interface, the microtriches additionally serve for chemical and tactile reception (Smyth, 1969). Consistent with other functions, the free surface area of postembryonic worms is amplified by a brush-border of digitiform projections, whose limiting membrane performs a number of biochemical functions related to the transport and other physiological activities.

The information on the microtriches of cestodes so far described, pertain to the species which infect ovine hosts; however, similar details of cestodes which infect avian hosts are lacking. In the present investigation, an attempt was made to characterize the tegument of *Raillietina echinobothrida* that infects the fowl intestine. This study may contribute more information regarding the ultrastructure of the tegument of cestodes which occupy the common niche *viz.* intestine.

MATERIALS AND METHODS

Animal procurement: The specimens of tapeworm *R. echinobothrida* (Megnin, 1881) were collected from the intestine of naturally infected country fowls autopsied in the chicken stall at Zambazar market, Triplicane, Chennai. The intestines were transported to the laboratory within half-an-hour of their collection. In the laboratory, the intestines were carefully dissected and the tapeworms were collected. The worms were washed in distilled water to render them free from intestinal contents and rinsed quickly 34-times in normal saline solutions. The tapeworms were then observed under a compound microscope to confirm their taxonomic characters. The entire worm was spread-out on a board, and the length of the worm was measured. The immature, mature and gravid proglottid regions of the worm were identified, separated, dried on moist blotting paper and used for various scanning electron microscopy (SEM) and TEM studies. The regions of the parasite for the above studies included: (a) immature proglottides containing the scolex and anterior region, (b) mature proglottides with functional reproductive organs and (c) Gravid region containing eggs.

SEM study: The SEM studies of the scolex, immature, mature and gravid proglottides of the parasite, *R. echinobothrida* were carried out to understand its ultrastructure. For this purpose, the specimens were dissected in chilled glutaraldehyde (2.5%) and fixed for 16 h at 4 °C. The tissues were subsequently washed thrice at an interval of 15 min each in phosphate buffer (pH 7.0), and then dehydrated by passing through an ascending series of alcohol from 30–100%, for an hour in each concentration. The tissues were then kept in 100% alcohol overnight. Following dehydration, the tissues were air-dried in a desiccator for 7–10 days. The dried samples were mounted on an aluminium stub and gold sputtered in vacuum for 10 min by using an Eiko IB-2 ion coater. The samples were observed eventually scanned on a Hitachi S-415A SEM at 25 KV and

photomicrographed at different magnifications (Hayat, 1977).

TEM study: The scolex, and immature, mature and gravid proglottid regions of *R. echinobothrida* were immersed in 2.5% glutaraldehyde in Millong's phosphate buffer (pH 7.3, 380 mOsm/2) and eventually cut into small pieces. After 34 h of fixation at room temperature, the tissues were rinsed in Millong's buffer and post-fixed in 1% osmium tetroxide in Millong's buffer for 90 min, rinsed quickly in distilled water, dehydrated in ethanol series, infiltrated with propylene oxide, embedded in Spurr's low-viscosity epoxy resin and polymerized at 60 °C. The sections (70–90 nm) were cut with a diamond knife, mounted on uncoated copper grids, stained with uranyl acetate/ethanol and aqueous lead citrate, and examined by using a Philips 204 TEM at an accelerating voltage of up to 70 KV (Conn, 1993).

RESULTS

The scanning electron micrographs of the microtriches and their distribution in the tegument of the different regions of the parasite *R. echinobothrida* revealed three morphologically distinct types of microtriches. At higher magnification, the suckers, margin of suckers, center, surrounding region of the scolex and the rostellum were covered by filamentous microtriches. Within the circlet of hooks on the rostellum, the slender and filamentous microtriches were densely packed together, with a tendency for some spikes to coalesce or show transverse connections in some areas (Fig. 2a). Similar microtriches were observed on the suckers. The microtriches were slender, filamentous not as long as in the rostellum, but more densely packed together forming characteristic tufts inside the sucker (Fig.1a, c and 2b). The microtriches seemed to be directed posteriorly. Thus the tegument of the scolex was found to be entirely covered with filamentous microtriches. In addition to filamentous type, few spine or blade-like or bulb-like microtriches with a posteriorly oriented spatulate broad-base and sharply-ending electron-dense points could be seen on the margins of the suckers, their cavities and rest of the scolex region. Thus the scolex region was densely covered by filamentous and blade-like microtriches. The tegument covering the rest of the scolex region and the strobilar region appeared similar in structure. The tegumental surface of the immature proglottid region was also densely covered by posteriorly directed filamentous microtriches (Fig. 2c). The microtriches appeared to be uniform in density and size. Some intermediate type microtriches appeared to grow longer and become flattened and strap-like. This trend continued up to the posterior mature segments (Fig. 2d). Finally in the area of the gravid proglottides (Fig. 2e, f), the surface structure showed that the microtriches had a broader base. A decreased microtrichial density down the length of the strobila was clear. Morphological changes in the tegumental surface of the gravid segments could also be clearly observed. Such changes involved erosion of folds in the posterior region accompanied by an increasing degree of disorder in the arrangement of microtriches (Fig. 2e, f). In all the regions examined, extensive folding of the tegumental surface was evident. The spikes of the microtriches in the rostellum and suckers were closely packed together (Fig. 2a, b), whereas the microtriches of the mature and gravid regions of the strobila were quite different as they lacked spikes.

Under TEM the tegument was lined with a superficial microtriche border layer giving the appearance of `brush-border' (Fig. 3a). The tegumental syncytium was composed of non-nucleated superficial cytoplasm and perinuclear cell bodies lying in the parenchyma. In the superficial cytoplasm, cylindrical blade-like and filamentous microtriches were observed (Fig. 3b).

The filamentous microtriches are divided into three anatomical regions namely a base, a complex functional region and a dense cap (Fig. 3c, d). Each base was found to contain an inner sleeve of dense material, the core tunic. The core of the base contained a prominent bundle of microfilaments. The microfilaments of the core are connected to a slightly curved tubule, the junctional tubule. The cap consisted of an electron-opaque medulla enclosed by a cortex (Fig. 3c, d). The surface of microtriches seemed to be covered by a fuzzy-layer of glycocalyx (Fig. 3c, d).

The transverse section of the tegument brush-border of the scolex region of *R. echinobothrida* revealed modified microtriches of varying size and shape (cylindrical, blade-like and filamentous), but possessing the basic microtrichial structure (Fig. 3a-d). Similar microtriches were observed on the suckers (Fig. 4a, b), the tegument covering the rest of the scolex and the anterior strobila. Round vesicles were seen to pack the distal cytoplasm and extend into the base of the microtriches. Many of these vesicles contained small electron-dense aggregates (Fig. 4b). Thus the microtriches of the scolex region were seen to have a limiting plasma membrane, glycocalyx and an

electron-lucid core (Fig. 4a, b). However, the tegument covering the mature (Fig. 4c) and gravid (Fig. 4d) regions of the strobila revealed a similar basic structure, but the most important one was the apparent absence of a distal ciliary process (Fig. 4c, d).

DISCUSSION

The main interface of the cestode is the general body tegument, which represents an important anatomical region for the host-parasite interactions. The interactions between the parasite and its environment occur through the tegument (Oaks and Holy, 1994). In lumen dwelling adult worms, a major morphological adaptation is the amplification of the surface area to as much as 26–30-times by the presence of delicate microtriches. According to Smyth and McManus (1989), the delicate microtriches, which serve as the surface amplifying structures, resemble functionally the intestinal mucosa of higher animals. These investigators referred to these amplified microtriches in the surface layer as brush-border.

The TEM observations of the tegument of *R. echinobothrida* revealed a prominent basal layer, which was connected to a distal syncytial cytoplasm by cytoplasmic extensions. The free-surface of this syncytial layer contained the brush-border composed of microtriches. Both the syncytial layer and the brush-border were covered by the surface plasma membrane. The tegumental layer under the brush-border was considered as glycocalyx, which helps to maintain an unstirred water layer between the parasite and the contents of the host intestine, as suggested by Lumsden *et al.* (1974) and Pappas and Uglem (1990).

The present SEM and TEM observations showed that the tegument microtriches differentiated into at least three morphologically distinct types, namely elongated, cylindrical and filamentous ones. The tegument of the entire scolex region of *R. echinobothrida*, excluding the rostellum, developed elongated, thick cylindrical microtriches with rounded tips and filamentous microtriches. The rostellum was densely covered with thin filamentous microtriches with the tendency for some spikes to coalesce in some areas. The microtriches of the suckers were not as long as the rostellum, but more densely-packed together forming characteristic tufts inside. The immature and mature proglottid regions were also covered by filamentous microtriches. Some longer, flattened and strap-like microtriches were also observed in the immature and mature regions. Similar organisation of microtriches was also reported by Palm *et al.* (1998). In the gravid region, however this ornamentation was lost and there was an increasing degree of disorder in the arrangement of microtriches, whereas the surface of the posteriormost part of the worms examined was in a stage of dissolution with no microtriches being apparently visible.

The ultrastructure of the microtriches also showed three clearly distinguishable regions namely a base, dense cap and a complex functional region between the base and cap, as has been noticed by Jha and Smyth (1969) and Holy and Oaks (1986). Hayunga (1991) suggested that the caps of filamentous microtriches of *H. diminuta* serve for anchoring and the bases serve for nutrient absorption.

Microtrichial polymorphism has been reported in different species of cestodes such as *Diphyllobothrium, Diplogonoporous, Spirometra erinacei, Mesocestoides, Avitellina lahorea* and *Stilesia globipunctata* (Berger and Mettrick, 1971, Vijayalakshmi and Ramalingam, 2005; Venkatesh *et al*., 2006). In *R. echinobothrida* also, similar microtrichial polymorphism could be seen in the scolex and strobilar regions. The filamentous microtriches in the tegument of the scolex of the parasites may contribute to the amplification of the absorptive surface of the tegument and may be involved in the absorption of nutrients. The cylindrical blade-like microtriches observed on the margin of the suckers and their cavities were fairly similar to those reported in other cestode parasites and they may play an important role in tissue penetration.

Apart from the role of microtriches in absorption of nutrients by the parasites as suggested by Rosario (1962), Rothman (1963) and Smyth (1969), they also have been thought to aid the worms in resisting the intestinal peristaltic currents and in maintaining parasite's position. The investigations of Blitz and Smyth (1973) in *R. cesticillus* suggested that the rostellar microtriches differ significantly from those on the proglottides, by being generally longer and thinner. The electron dense cap was greatly reduced or was absent. If, as Rothman (1963) and others have suggested, absorption is limited to the medullar base of the microtriches with the spike acting mainly for locomotion and attachment, this would favour an absorptive function for the rostellar microtriches of *R. cesticillus*.

The thin microtriches found mostly in the sub-scolex

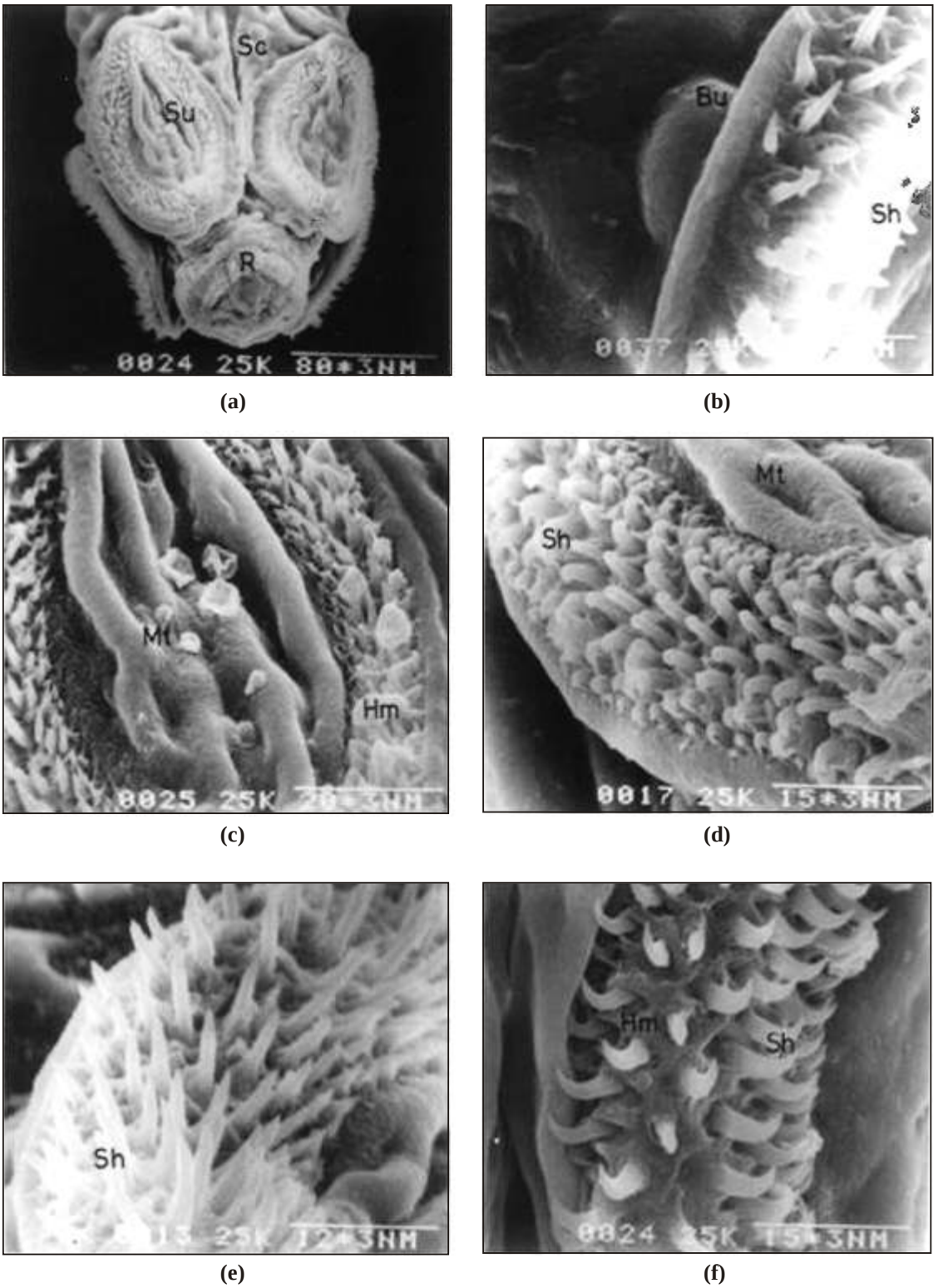

Fig. 1. Scanning electron micrographs of the sucker showing hooks of *R. echinobothrida.*

a. Scolex of *R. echinobothrida* showing the four suckers and evaginated rostellum (x80).
b. Sucker of *R. echinobothrida* showing rows of hooks and a single large bud (x10).
c. Sucker showing the hooks encircling the tuft of microtriches (x20).
d. Sucker showing the curved tips of the hooks (x15).
e. Sucker with long, pointed hooks (x12).
f. Curved ends of the sucker hooks intimately attached to the host intestinal tissue (x15).

Sc - scolex, R-rostellum, Su - sucker, Sh - sucker hooks, Bu - bud, Mt - microtrichial tuft, Hm - host material.

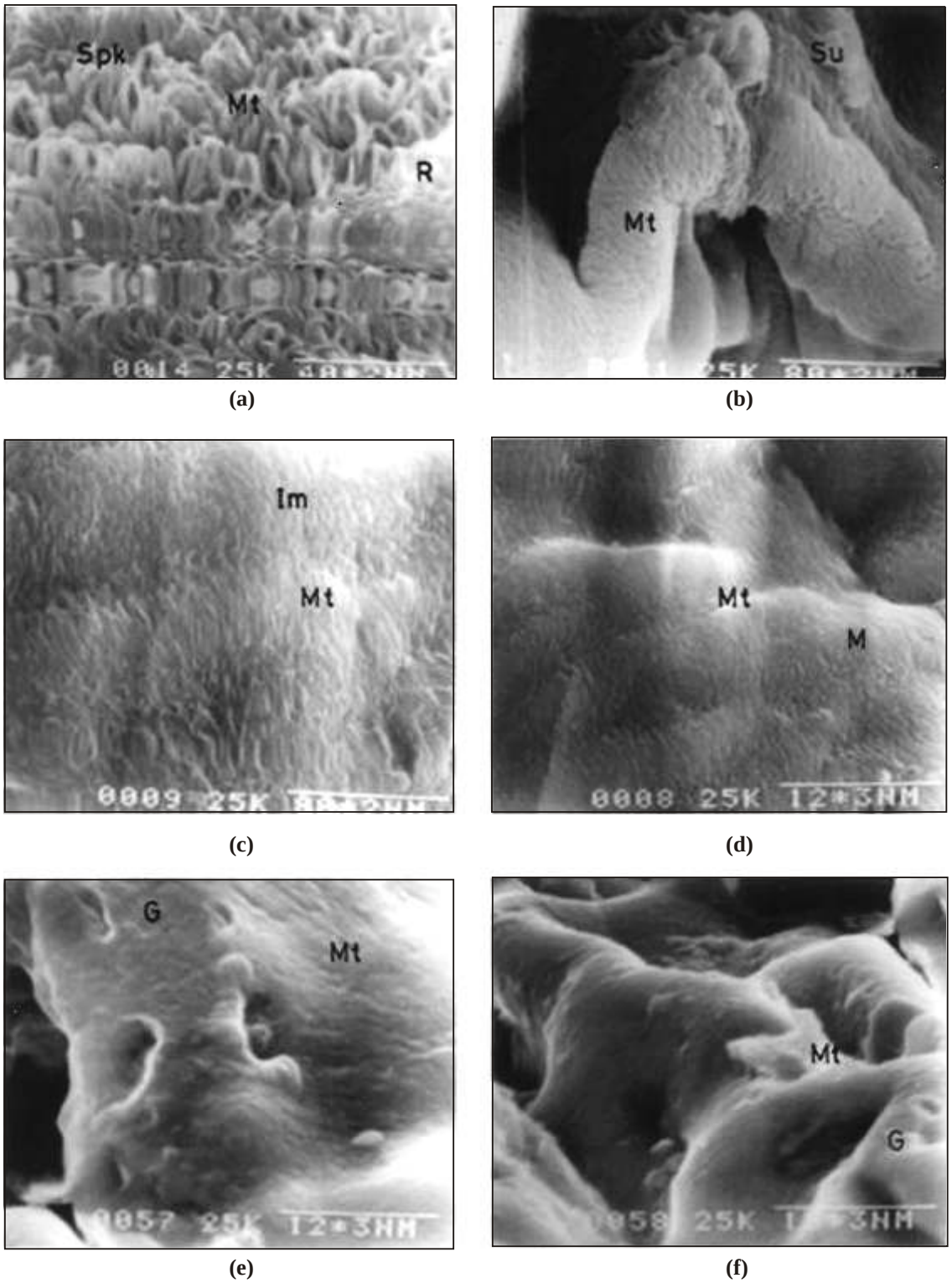

Fig. 2. Scanning electron micrographs of microtriches of different regions *R. echinobothrida.*

a. Tegumental surface of the rostellum region showing dense filamentous microtriches with transverse connections of distal spikes (x40).

b. Tegumental surface of the sucker region showing uniform filamentous microtriches densely packed together forming characteristic tufts inside the sucker (x80).

c. The surface of immature tegument showing posteriorly directed filamentous microtriches (x80).

d. The tegument surface of mature proglottides exhibiting filamentous microtriches (x12).

e, f. The tegument surface of the gravid proglottides showing decreased microtrichial density (x12, x15).

R - rostellum, Su - sucker, Mt - microtriches, Spk - spikes (distal tips of microtriches), Im - immature, M - mature, G - gravid.

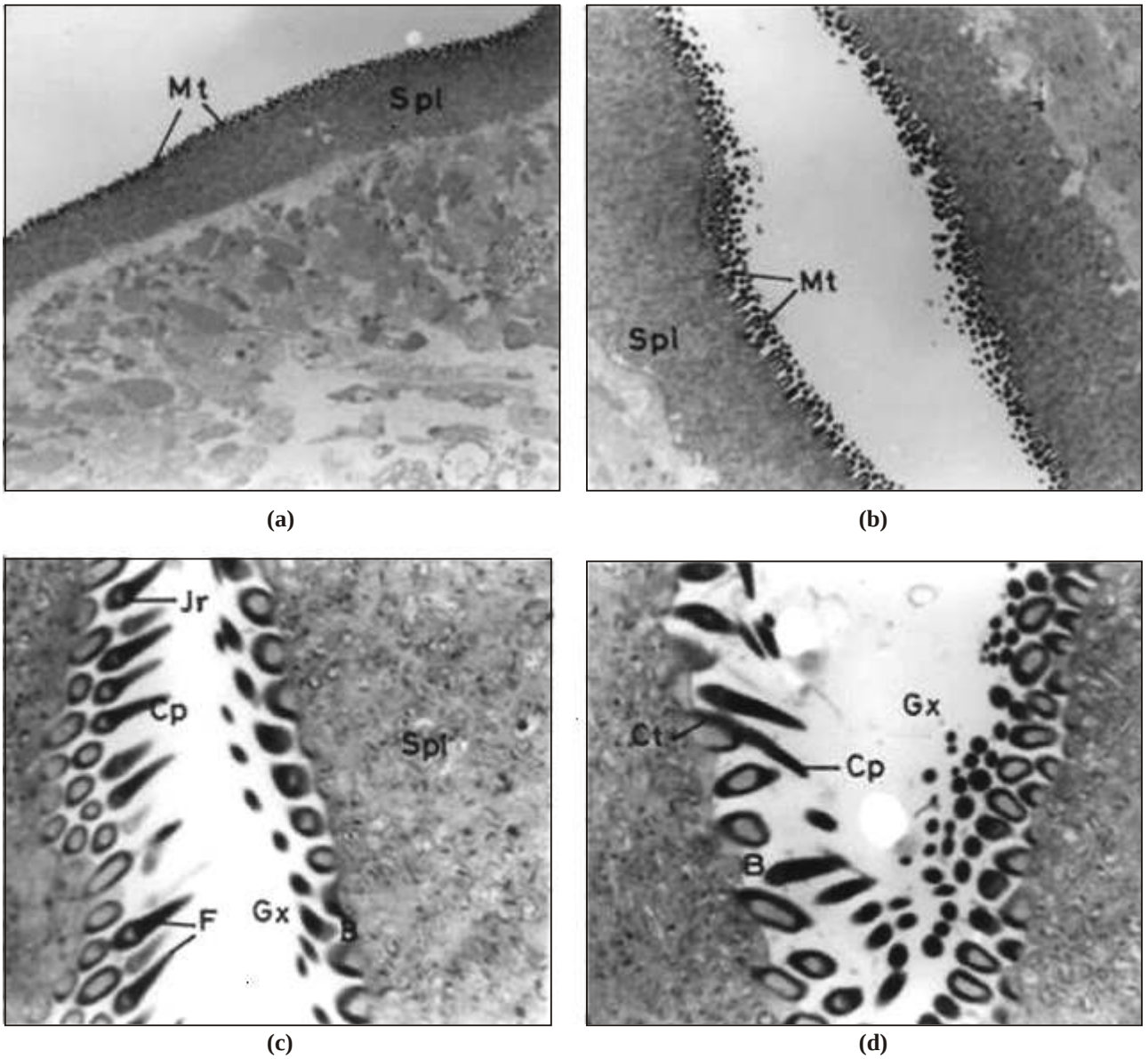

Fig. 3. Transmission electron micrographs of the tegument brush-border of scolex region of *R. echinobothrida*.
a. L. S. of scolex tegument showing microtriches (x3000).
b. L. S. of the tegumental folds of scolex showing microtriches (x7000).
c. Higher magnification of tegumental folds of scolex region showing microtriches (x30000).
d. Tegument brush-border of scolex region showing different kinds of microtriches (x50000).
Mt - microtriches, B - base, Jr - junctional region, Cp - cap, F - filament, Gx - glycocalyx, Ct - core tunic, Sp - subplasmalemmal layer.

region may probably represent sites of increased absorptive and secretory activity. The teguments in the scolex, immature and mature segments were lined by posteriorly directed filamentous microtriches, interspersed with few short blade-like microtriches. However, the tegument covering the pregravid and postgravid regions of the strobila revealed the apparent disintegration of the microtriches to only small rounded tips.

Similar to the reports of Berger and Mettrick (1971) and Andersen (1975), the basal region of the gravid proglottides was interrupted by genital pores surrounded by numerous papillae, and the lack of microtriches in this region suggest that they are specifically atrophied during the transformation of immature to mature and then to gravid stage. The atrophy of the microtriches is of interest, because the gravid segments have to be voided out from the host body. The degeneration of microtriches enables the above and at the same time confirms its prime function as adhesion in the host environment. Thus the otion

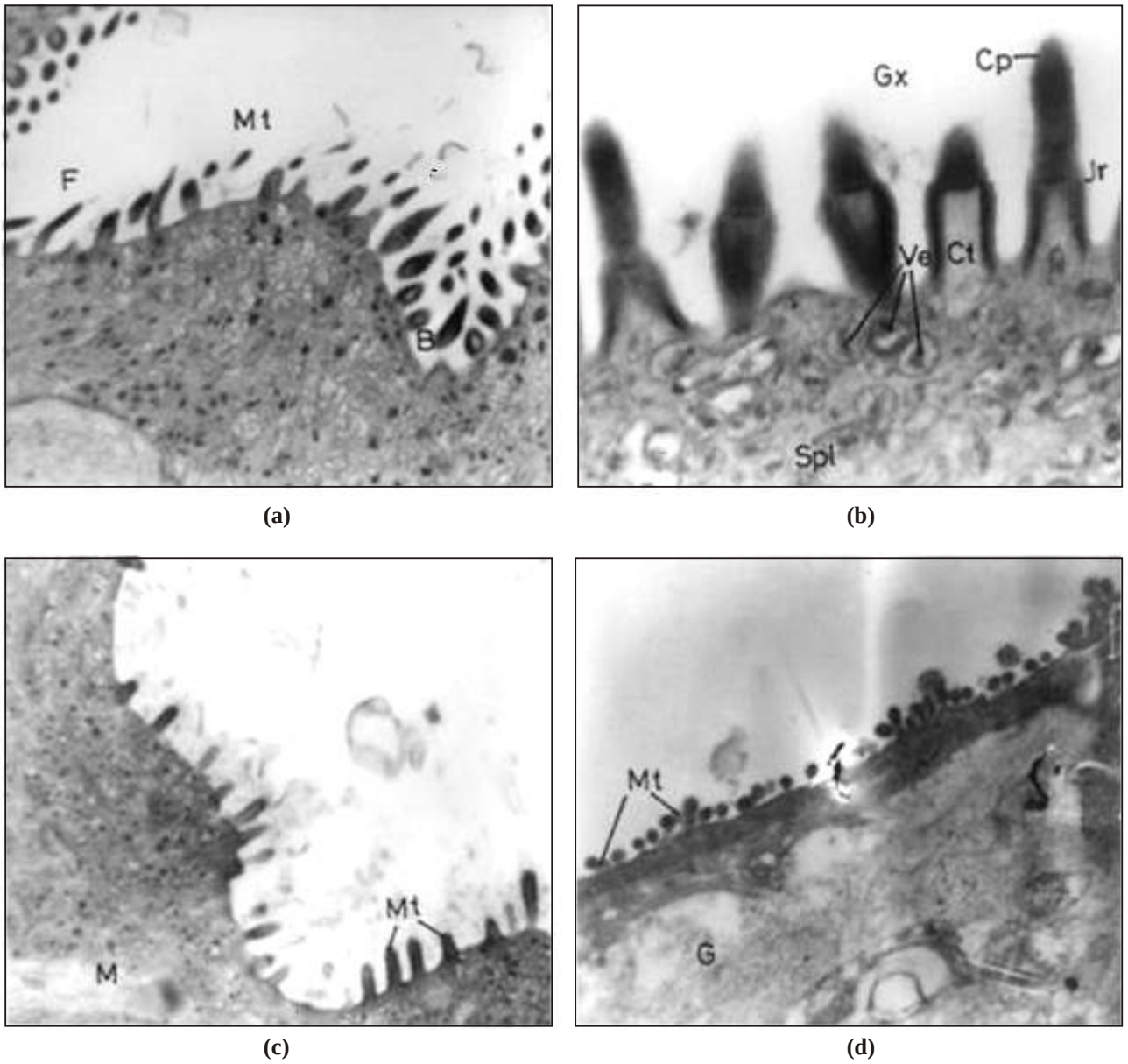

Fig. 4. Transmission electron micrographs of the tegument brush-border of different regions of *R. echinobothrida.*

a. Tegument of the sucker folds showing the microtriches (x30000).

b. Tegument under higher magnification showing the structure of the microtriches (x70000).

c. T. S. of tegument of the mature region with less density of microtriches and lack of distal spikes (x30000).

d. T. S. of tegument of gravid region with the dissolution of microtriches and lack of distal spikes (x30000).

Mt - microtriches, B - base, Jr - junctional region, Cp - cap, F - filament, Gx - glycocalyx, Ct - core tunic, Spl - subplasmalemmal layer, Ve - vesicle, M - mature proglottid, G - gravid proglottid.

tegument represented a high degree of morphological specialization for performing diverse functions, which include nutrient absorption, digestion, protection, excretion, anchoring and traction for locomotion (Rothman, 1963; Morseth, 1966; Jones 1975; Lumsden, 1975; Thompson *et al.*, 1980; Coil, 1991; Hayunga, 1991; Palm *et al.*, 1998).

These diverse functions of nutrition, anchoring and mechano-reception would explain morphological differences in filamentous microtriches, such as proportions of cap to base length that have been described by many authors (Thompson *et al.*, 1980; Lumsden and Hildreth, 1983; Mackinnon and Burt, 1983). As per function, microtriches were probably meant for anchoring, movement and nutrition, seemed tenable. Apparently, the first function of microtriches

is visibly to anchor the parasite with the lumen wall and, secondly, by virtue of its brush-border and nature, its function seems to be, analogous to microvilli of higher vertebrates, absorption. Thirdly, the distal part of microtriches, namely the spikes, infers its function in both anchorage and absorption. The above view is fully justified taking into consideration the complete development of gravid segments and degeneracy of the organ and organ systems and the apolytic phenomenon. Hence, the partial structural disconfiguration of the microtriches is attributed, besides the above, to the abrasive action of the residual enzymes and hydrolytic enzymes of the host intestinal lumen.

Taking cue from the present observation on the spatial distribution of the microtriches, their polymorphism from the scolex region to the gravid segmental region and also from the observations of the previous authors, it may be suggested, that though the scolex represents the region of anchorage and absorption for the growth of the developing cestode parasites, it may be considered that the entire integumental region, which is in close contact with the luminal wall of the host, may be involved in the absorption of the nutrients at the interface region. The above nutritional function of the segmental microtriches may be said to be responsible for the growth and maturity of the same. Concurrently, the atrophy of the above microtriches in the fully differentiated gravid region enables it to undergo apolysis and further voiding of its contents namely egg capsules.

The homology between the tegumental characteristics of cestodes infecting ovine and avian hosts also revealed the fact that irrespective of the host classes, the inhabiting niche being the same, *viz.* the intestine, and the tissue organization being uniform in different vertebrate classes, the cestode parasites adopt similar strategies of functional morphology to meet their metabolic functions.

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JPD

Intestinal helminthic infections in tribal population of southern Rajasthan, India

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ABSTRACT. A total of 870 faecal samples of 459 male and 411 female tribal individuals, aged 5–50 years, who inhabited rural tribal environment of Dungarpur district of southern Rajasthan, India, were examined by wet-film preparation and formal ethyl-acetate concentration technique, for the presence of eggs and/or larvae of the pathogenic intestinal helminth parasites. Of these, 264 samples [30.34%; 143 (31.15%) male, 121 (29.44%) female] were found to be infected with various helminth pathogens. The highest (53.94%) prevalence rate and maximum diversity of these helminth parasites were observed in the age group of 5–10 years, whereas the lowest (12.5%) prevalence of these two indices was observed in the subjects of > 40 years of age; curiously, with an increase in age, the prevalence and diversity of intestinal helminths decreased. Among the intestinal pathogens, *Ascaris lumbricoides* showed the highest (36.74%) incidence rate, followed by *Trichuris trichiura* (25.37%), *Ancylostoma duodenale* (16.66%), *Strongyloides stercoralis* (7.19%), *Hymenolepis nana* (4.16%) and *Enterobius vermicularis* (3.78%). Simultaneously, mixed infections of *A. lumbricoides* with *T. trichiura* (4.16%) and of *T. trichiura* with *E. vermicularis* (1.89%), were also observed.

Keywords: helminthes, parasites, pathogens, prevalence

INTRODUCTION

Intestinal helminth parasitic infection is one of the major health problems in several developing countries, including India. These infections are very common and endemic in those populations/ communities, which lack adequate sanitation facilities, hygiene and health education, and are more associated with lower socio-economic status and age group. In the world, approximately 1.4 billion people are currently infected with the round worm, *Ascaris* (WHO, 1994), and > 3000 million cases of enteric helminthiasis exist, either as a single or mixed infection. However, helminthic diseases are still not considered serious diseases in humans (Stephenson *et al*., 1980; Cabrera, 1984). The prevalence of various helminthiasis in different endemic geographical provinces and populations have been reported (Saifi and Wajihulla, 2001; Singh *et al*., 2004). Although, from Rajasthan, only a few reports are available (Tamboli and Sharma, 1979; Choubisa and Choubisa, 1992), the reports on the prevalence of helminthic infections in relation to age and sex are still warranted, specifically in tribal population. Therefore, the present study was undertaken to determine the prevalence of different helminth parasitic infections in tribal population, who are socio-economically very poor and backward, almost completely lack health education, and reside in remote, hilly and inaccessible areas.

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MATERIALS AND METHODS

Morning faecal samples from 870 tribal subjects (459 male and 411 female), between the age group of 5–55 years and inhabiting the rural environment of the most backward district, Dungarpur, southern Rajasthan, India, were collected in cleaned and dried glass vials, without any antiseptic. These samples were processed for wet-film preparation and formal-ether concentration technique (Paniker, 1991), to identify the presence of eggs and larvae of different intestinal helminths. Private clinical/diagnostic/pathological laboratories, located in the tribal rural areas or villages, were the major sources of sample collection. However, samples were also collected from the pathological labs of Government Hospitals (both Primary Health Centers and Referral Hospitals). The subjects of rural tribal areas were referred to these labs for their stool examination for diagnostic purposes. In the present study, no family members of positive cases of helminthiasis were investigated for the presence of intestinal helminthic infections. Data pertaining to the prevalence, in relation to age and sex, were also evaluated statistically, and $p < 0.05$ was considered significant.

RESULTS

Out of 870 stool samples from 459 male and 411 female tribal individuals, 264 (30.34%) were found to be infected with different species of intestinal helminths. The prevalence rate was found to be 31.15% in males and 29.44% in females (Table I). The highest prevalence rate (53.94%) was found in 5–10 year old subjects and the lowest (12.5%) in > 40 year old subjects (Table II). It is interesting to note that as the age of the subjects increased, both the prevalence and the diversity of helminth infections decreased. In the present study, the commonest intestinal helminth, *Ascaris lumbricoides* (36.74% prevalence) was found in the subjects of almost all age groups with varying incidence (Table II), followed by *Trichuris trichiura* a

Table I. Age- and sex-wise distribution of positive cases of helminthic infection

Age groups (years)	No. of samples tested			No. of infected cases		
	Male	Female	Total	Male (%)	Female (%)	Total (%)
5-10	40	36	76	22 (55.0)	19 (52.77)	41 (53.84)
11-15	56	50	106	29 (51.78)	26 (52.0)	55 (51.88)
16-20	60	50	110	23 (38.33)	18 (36.0)	41 (37.27)
21-25	62	52	114	16 (25.80)	14 (26.92)	30 (26.31)
26-30	62	58	120	17 (27.41)	13 (22.41)	30 (25.0)
31-35	65	60	125	16 (24.61)	15 (25.0)	31 (24.8)
36-40	58	57	115	13 (22.41)	10 (17.54)	23 (20.0)
40	56	48	104	07 (12.5)	06 (12.5)	13 (12.5)
Total	459	411	870	143 (31.15)	121 (29.44)	264 (30.34)

Age *vs* male = 18.33; d.f. = 7; $p > 0.05$
Age *vs* female = 17.13; d.f. = 7; $p < 0.05$
Male *vs* female = 00.29; d.f. = 7; $p < 0.05$

(25.37%), *Ancyclostoma duodenale* (16.66%), *Strongyloides stercoralis* (7.19%), *Hymenolepis nana* (4.16%) and *Enterobius vermicularis* (3.78%). Mixed infections of *A. lumbricoides* and *T. trichiura* (4.16%), and of *T. trichiura* and *E. vermicularis* (1.89%) were also encountered in the present study (Table II). The variation in the types of helminth parasites present in the different age and sex groups were significant ($p < 0.05$), but data pertaining to male *vs* female were not significant ($p > 0.05$).

DISCUSSION

In the present study, no family members of the positive cases of helminthiasis were investigated for the evidence of intestinal helminthic infection. The data pertaining to the prevalence in relation to age and sex were evaluated statistically. The prevalence and diversity of intestinal helminth parasitic infections are known to vary greatly from place to place, and from population to population. However, from different provinces of India, 24.6–91.0% prevalence of

Table II. Distribution of helminth parasites in different age groups

Helminths	Age (years)								Total (%)
	5-10	11-15	16-20	21-25	26-30	31-35	36-40	> 40	
A. lumbricoides (roundworm)	18	22	15	10	10	11	07	04	97 (36.74)
T. trichiura (whipworm)	09	15	08	09	08	09	07	02	67 (25.37)
A. duodenale (hookworm)	05	09	06	07	08	06	02	01	44 (16.66)
S. stercoralis (threadworm)	03	02	05	04	03	02	-	-	19 (7.19)
H.nana (dwarf tapeworm)	01	02	05		01	02	-	-	11 (4.16)
E. vermicularis (pinworm)	01	01	01				04	03	10 (3.78)
Mixed infections									
A. lumbricoides + *T. trichiura*	03	03	01			01	02	01	11 (4.16)
T. trichiura + *E. vermicularis*	01	01					01	02	05 (1.89)
Total	41 (53.94)	55 (51.88)	41 (37.27)	30 (26.31)	30 (25.0)	31 (24.8)	23 (20.0)	13 (12.5)	264 (30.34)

Figures in parentheses indicate percentage.

helminthic infections has been reported by several workers (Singh *et al*., 1991; Fernamdez *et al*., 2002; Rao *et al*., 2002; Singh *et al.*, 2004; Singh *et al*., 2004), and these workers have also observed a high prevalence rate (19.65–2.81%) of *A. lumbricoides* (ascariasis) infection in their studies. From other some countries, a highly variable prevalence (385%) of helminthiasis has also been reported by several workers (Cross and Basaca-Sevilla, 1984; Ryan *et al*., 1988; Jiang, 1988; Kam, 1994). Earlier, from the state of Rajasthan, 5.2–43.6% and 37.55% incidences of helminthic infections have been reported in the inhabitants of Dungarpur (Paul *et al*., 1982; Choubisa and Choubisa, 1992) and Jaipur (Tamboli and Sharma, 1979) districts, respectively. In the present study, a high incidence (30.34%) of these parasitic helminths has also been observed. However, amongst these parasites, *A. lumbricoides* showed the highest (36.74%) incidence of occurrence. From these studies, it is clear that the prevalence and the diversity of helminthic infections vary greatly, and the maximum of both these indices occurred in the lower age group. Such variation could be correlated with different degrees of poverty, hygiene, sanitation facilities and health care or education, which provide favourable environments for the transmission of these helminthic pathogens. The high incidence of these pathogens in the lower age group (children) is highly associated with the lack of awareness pertaining to hygiene and sanitation, both of which can be expected to provide maximum chances of helminthic exposures. In tribal population, a relatively higher incidence of helminth infection is due to their frequent migratory behaviour, which also increases the rates of exposure to parasitic infections. However, a decline in the percentage of infection (30.34% infection) of helminth pathogenes in tribal population of Dungarpur district, as compared to a previous study (43.6%) conducted in the same district is related with the rapid urbanization of the area.

Whatsoever, the incidence and prevalence of intestinal parasitic (helminthic) infections in tribal population of Dungarpur district, Rajasthan, is relatively higher. In fact, these tribal people are economically very poor, lack health education, and are living in unhygenic conditions where proper sanitation facilities are also wanting. Therefore, for the mitigation or to overcome these pathogenic burdens, as well as to improve the tribal health, the above mentioned determinants/factors should be considered, while preparing a future plane for the tribal health. Although, the present study is preliminary; nevertheless, the findings reported herein significantly add to the existing knowledge of intestinal parasitic infection.

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Journal of Parasitic Diseases: December 2006, Vol. 30, No. 2, 168–171 *Short communication*

Cathetocephalus limbatus sp. nov. (Tetraphyllidea: Cathetocephalidae) from *Carcharhinus limbatus* (Valencinnes, 1841) at Digha coast, Bay of Bengal, West Bengal, India

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ABSTRACT. *Cathetocphalus limbatus* sp. nov. recovered from the spiral valve of *Carcharhinus limbatus*, caught at Digha coast, Bay of Bengal, has been compared with the only valid species of this genus *Cathetocephalus thatcheri* Dailey and Overstreet, 1973. *Cathetocphalus limbatus* sp. nov. differed from the latter in having scolex with four suckers, acraspedote proglottid, position of genital pore, structure of cirrus sac, ovary and shape of vitelline follicle.

Keywords: acraspedote, *Carcharhinus*, *Cathetocephalus*, Digha coast, proglottid, spiral valve

Eight cestode parasites were collected from the fish *Carcharhinus limbatus* (Valenciennes, 1841) at Digha coast, Bay of Bengal, West Bengal, India, in the month of February, 1997. The parasites remained attached to the wall of spiral intestine of the host. The specimens, after proper processing, were identified as a new species of the genus *Cathetocephalus* Dailey and Overstreet, 1973. So far, only one species under this genus has been described.

The specimens were recovered from the spiral intestine of *Carcharhinus limbatus* caught at Digha coast, Bay of Bengal, India by the fishermen. The specimens were fixed in AFA on a glass plate covered with a glass slide by applying slight pressure following the standard technique, post-fixed and preserved in 70% ethyl alcohol. The whole mount preparations, and the staining of scolex and proglottids from different regions were done with Semichon's carmine following the standard procedure. All the measurements are average of eight specimens and in mm, unless otherwise stated.

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Cathetocephalus limbatus sp. nov.

Family: Cathetocephalidae Dailey and Overstreet, 1973

Genus: *Cathetocephalus* Dailey and Overstreet, 1973

The total length of the parasite 48.09 and total number of proglottids 94. The scolex is a single wide holdfast organ perpendicular to the long axis of strobila, 0.59 long and 1.6 wide. The scolex looks like a bivalve shell, from its border, 34-37 incomplete separation ridges present and with four suckers of 0.37 diameter (Fig. 1 and 2). The neck region unsegmented, 2.26 long and 0.3 wide. Immature proglottids are wider than length but mature proglottids are longer than width. The testes are clearly found in proglottids of mid-region and look more or less square in shape. The mature proglottid is 1.71 long and 0.54 wide, and somewhat vase-shaped. Each proglottid has 132–134 globular testes of 0.04 diameter. Cirrus pouch is oval, 0.3 in length and 0.2 in width. Cirrus is slightly coiled, 0.29 long and 0.06 wide at the anterior portion. Genital pore irregularly alternate

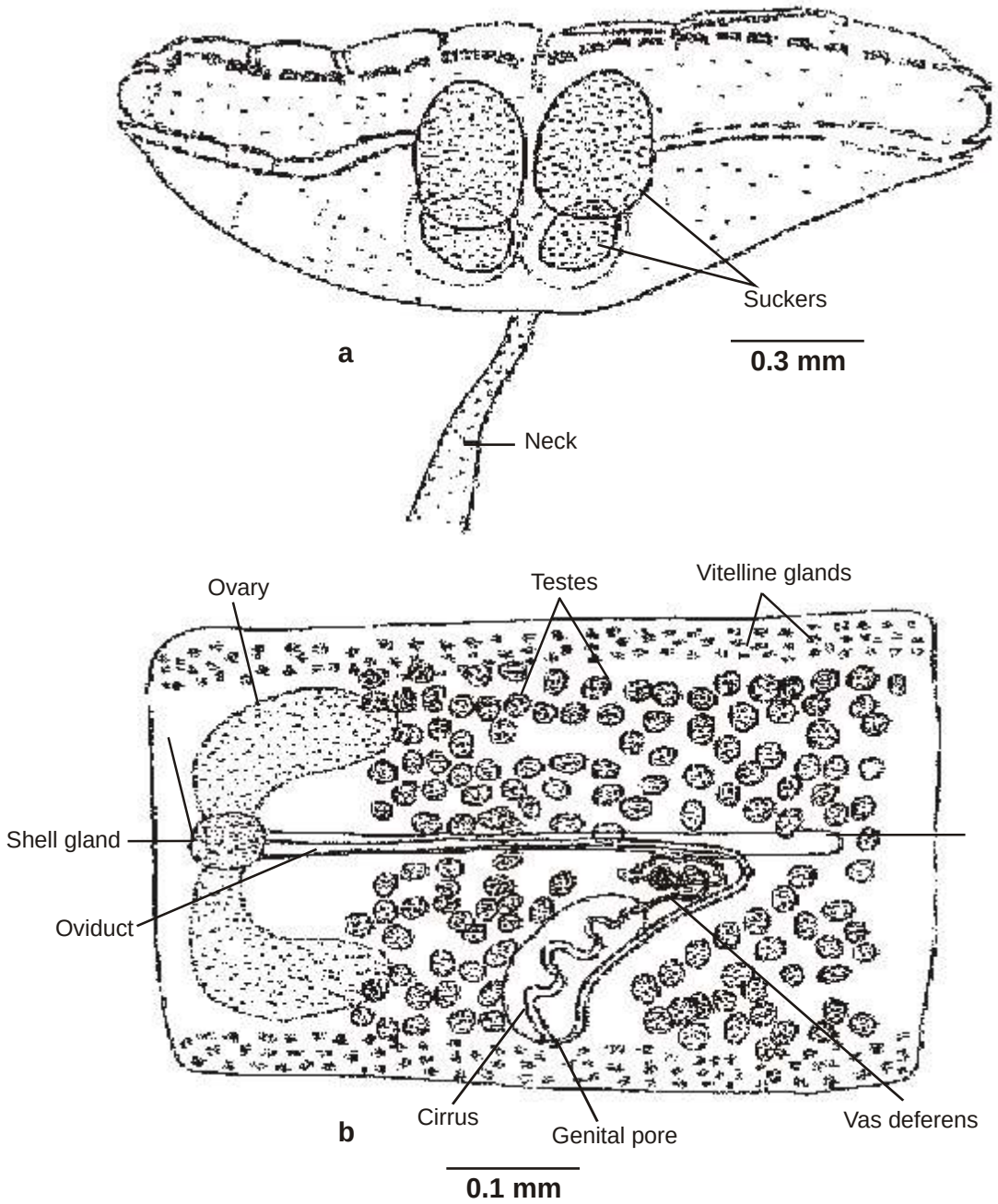


Fig. 1. *Cathetocephalus limbatus* sp. nov.: camera lucida drawing a. scolex, b. mature proglottid

and placed sub-marginally in the middle of proglottid. Uterus cylindrical, starts from the shell gland, runs forward and reaches more or less anterior of the proglottid. The vagina starts from a common genital pore and runs transversely anterior to cirrus pouch up to the centre of the segment, curves and runs vertically up to the shell gland, measures 1.3 in length and 0.07 in breadth. Receptaculum seminis is absent. Ovary bilobed, more or less 'U'-shaped, 0.82 long and 0.41 wide. Shell gland lies behind the ovary and measures 0.19 in diameter in the last segment. Vitelline glands follicular, arranged in 23 rows from anterior to posterior end of proglottids.

Host: *Carcharhinus limbatus* (Valenciennes, 1841)

Habitat: spiral valve

Location: Digha coast

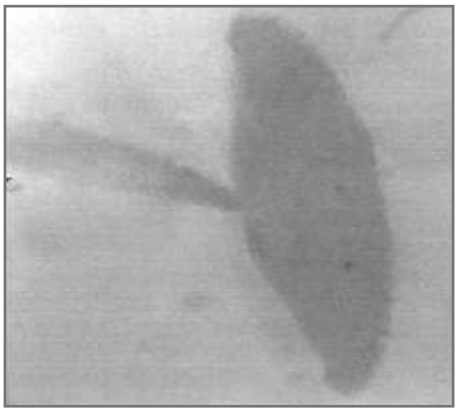
Date of collection: 5. 2. 97

No. of specimen: eight

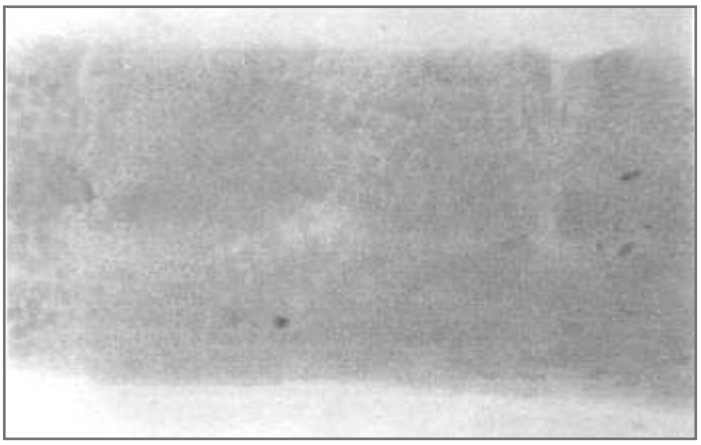
Holotype: one with three specimens

Paratype: seven in two slides

Accession No.: 000033/03



(a)



(b)

Fig. 2. Photomicrograph of *Cathetocephalus limbatus* sp. nov. a) scolex b) mature proglottid (x40).

Deposition: at present deposited to the Parasitology Laboratory, Department of Zoology, University of Calcutta.

Species diagnosis : Length of worm 48.09, consisting of 94 segments, genital pore irregularly alternate, opening at the middle of the proglottid in lateral margin, testes globular, 132 in number, scattered throughout proglottid. Ovary more or less 'U'-shaped.

REMARKS

The name *Cathetocephalus* has been derived from Greek 'Kathetos' and 'Kephalos' meaning perpendicular head. *Cathetocephalus thatcheri* is the first reported species of the genus from *Carcharhinus limbatus* (Val.) in the coastal waters of Mississippi and Louisiana (Thatcher, 1961). At that time, he provisionally considered it as *Pillersium owenium* Southwell, 1927 because of the T-shaped scolex. Thatcher's original material was re-examined and named as *Cathetocephalus thatcheri* by Dailey and Overstreet (1973). So far, this is the only species described in this genus.

The present form, collected from Bay of Bengal, shows many similarities with *Cathetocephalus thatchari*, in having scolex without hooks and in being perpendicular to the long axis of the strobila; anterior surface of scolex has been observed to be highly fleshy, papilliform projection on anterior end, and in the arrangement of different organs in proglottids. However, the present specimen differs in many remarkable points. *Cathetocephalus thatcheri* has the scolex without sucker, sac-like elongated cirrus pouch, genital pore posterior to mid-region, dumb bell-shaped ovary and granular vitellaria proglottid**,** but the present species possesses scolex with four suckers, oval cirrus pouch, genital pore in the middle of the segment, U-shaped ovary and follicular vitellaria. Comparative account of the two species of the genus *Cathetocephalus* is given in Table I.

In view of all these differences, as compared to the only

Table I. A comparative account of the valid species of the genus *Cathetocephalus* Dailey and Overstreet, 1973

	C. thatcheri Dailey and Overstreet	*C. limbatus* sp. nov.
Size	101 mm	48.09 mm
Scolex	6.1/0.27 without sucker	1.7/0.43 with four sucker
Neck	0.98	0.12
Number of proglottids	269 (craspedote)	94 (acraspedote)
Genital pore	in slightly posterior to mid-portion of segment	Mid portion of segment
Testes number	numerous	132
Cirrus sac	sac-like, elongated	oval 0.27
Ovary	dumb bell-shaped, 1.06/0.42	'U' - shaped 0.45/0.2
Vitelline follicle	granular	follicular in 2-3 rows
Host	*Carcharhinus limbatus*	*Carcharhinus limbatus*

valid species, it is clear that it is a new species of the genus *Cathetocephalus* Dailey and Overstret, 1973. We suggest its name as *Cathetocephalus limbatus* sp. nov., considering the name of its host.

Key to species of the genus *Cathetocephalus* Dailey and Overstreet, 1973

Scolex without sucker: *Cathetocephalus thatcheri* Dailey and Overstreet, 1973

Scolex with four suckers: *Cathetocephalus limbatus* sp. nov.

ACKNOWLEDGEMENTS

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Journal of Parasitic Diseases: December 2006, Vol. 30, No. 2, 172–174 *Short communication*

Tetracycline therapy of naturally *Dirofilaria*-infected dogs from West Bengal, India

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ABSTRACT. The presence of *Dirofilaria* worms in dogs is considered as a potential source of human infection in India. The purpose of the present study was to evaluate the effect of tetracycline therapy on filarial nematodes present in naturally infected dogs. Our results showed that tetracycline caused a dramatic reduction in dirofilarial count in the blood of dogs. The microfilarial density was reduced by 93% during tetracycline therapy.

Keywords: *Dirofilaria*, tetracycline, therapy

The members of the genus *Dirofilaria* are natural parasites of certain mammals (*e.g.* dog, cat etc.) and are known to cause the disease dirofilariasis. *Dirofilaria* stays in the right ventricle and pulmonary artery of the dogs and causes canine filariasis. The worms, commonly known as dog heart worms, are found in tropic, sub-tropic and temperate zones. In a recent report, dirofilariosis in humans has been shown as a fast emerging zoonosis in India (Sabu *et. al.*, 2005). The prevalence of dirofilarial infection in domestic dogs is estimated from 7–24% in different studies from India (Valsala and Bhaskaran, 1974; Saseendranath *et. al.*, 1986). The domestic dogs being a potential source of infection, we undertook this study to evaluate tetracycline intervention of naturally occurring dirofilarial infection in domestic dogs. The profile of therapeutic outcome of tetracycline may help prevent the spread of infection in dogs and, subsequently, may help the prevention of human infection from dogs.

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Blood samples from eight microfilaraemic dogs (six males and two females) infected with *Dirofilaria* were collected every week for a period of ten weeks. The microfilarial density/20 mm^3 blood was determined for each sample. The blood films were air-dried, dehaemoglobinised in distilled water and stained with Giemsa. After determining the microfilarial concentration for ten weeks, four microfilarae (mf) positive dogs were orally administered tetracycline at the dose of 350 mg/day for 31 days. The remaining four microfilaraemic dogs served as untreated controls. Blood was first sampled on the 8^{th} day of treatment, and the additional samplings were performed at monthly intervals for up to 150 days.

The mf counts/20 mm^3 of blood in eight dogs before treatment are shown in Table I. The mf concentration in eight dogs did not vary appreciably during the ten week period of observation before treatment. The percent reduction in mf counts after tetracycline treatment is plotted against days of sampling and of treatment in Figure 1. The mf density showed a 93% fall on 31^{st} day following the onset of treatment as compared to the pretreatment level.

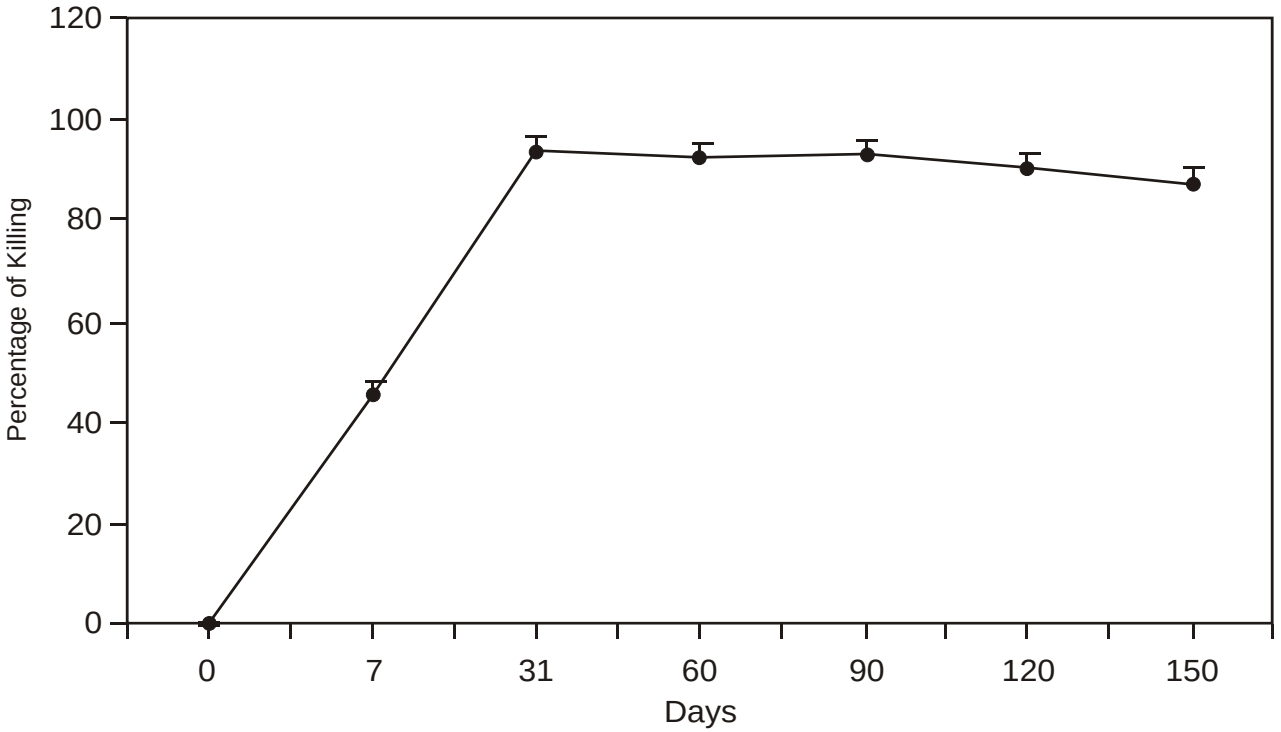

Fig. 1. Showing reduction in the percentage of mf population at different time intervals after tetracycline therapy.

Dirofilariosis in humans is a potential problem in India. With 7–24% dogs harboring the microfilarae infection in Kerala, it is highly recommended that humans are at an enhanced risk of acquiring dirofilarial infection from dogs. Based on our observations, we conclude that tetracycline has the potential to clear filarilal pathogens from naturally infected dogs in India, and it can be used for effective therapy. Tetracycline inhibits the development of L3 to L4 molting stage of *Dirofilaria, in vitro* (Smith and Rajan, 2000). Indeed, in various species of filarial worms, tetracycline is known to cause detrimental effects (Bazzocchi *et al.*, 2001). Our study is the first one to provide experimental evidence regarding the contribution of tetracycline in clearing of *Dirofilaria* infection in naturally infected dogs in India. Our results demonstrated that tetracycline treatment caused reduction of *Dirofilaria* worms in naturally infected dogs which may be helpful to stop dirofilarial infection in humans.

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Table I: Microfilarial concentration in blood at weekly intervals

	Weekly microfilariae counts											
Dogs	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Mean	S. D.
male	1524	1599	1667	1550	1430	1450	1588	1607	1690	1576	1568.1	83.57
male	1020	1156	1203	1176	1097	1195	1087	1145	1209	1165	1145.3	60.30
male	2440	2376	2409	2399	2578	2498	2508	2390	2430	2520	2454.8	67.14
male	2106	2209	2198	2245	2246	2190	2124	2160	2350	2207	2203.5	69.09
male	3084	3060	3120	3189	3145	3109	3059	3178	3199	3056	3119.9	55.60
male	3290	3267	3178	3109	3290	3367	3309	3286	3103	3199	3239.8	88.31
female	3490	3550	3480	3578	3540	3505	3309	3336	3458	3460	3470.6	87.43
female	3178	3045	3106	3167	3256	3095	3134	3190	3209	3209	3158.9	63.52

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Histopathological changes in fowl coccidiosis

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ABSTRACT. Amongst pathological changes of fowl coccidiosis, target parts of intestine and caecal pouches were found distended with crimson appearance, and showed severe haemorrhagic enteritis and thickening of intestinal and caecal wall. Faecal contents were mixed with clots of blood and fibrin shreds. Widespread damage to absorptive epithelium and destruction of villi were evident on histopathological examination of intestine. The mucosae as well as submucosae were heavily infiltrated with macrophages and lymphocytes. Desquamation of superficial mucosal epithelium along with infiltration of mononuclear cells and schizonts were seen.

Keywords: **enteritis, fowl coccidiosis, histopathology, schizont**

In spite of advances in chemotherapy, management, nutrition and genetics, coccidiosis has remained as one of the most expensive and common diseases of poultry (Mc Dougald and Reid, 1991). Annual loss to poultry industry due to coccidiosis has been estimated at about $ 800 million (Williams, 1998). The poultry coccidia are obligatory intracellular parasites, with a predilection for the development in the intestinal epithelium. The poultry coccidia multiply in the intestinal tract and cause tissue damage, with resulting interruption of feeding and digestive processes or nutrient absorption, dehydration, blood loss and increased susceptibility to other disease agents. The objective of this study was to know the gross and microscopic changes in gastro-intestinal tract of fowl due to coccidiosis, which will be helpful for the diagnosis and control of this disease.

During routine post-mortem examinations of layer (n=72) and broiler (n=123) chickens from different coccidian-positive farms around, as well as at the Department of Pathology, Veterinary College, Anand, the tissue pieces of intestine and caeca, preserved in 10% formalin, were processed by paraffin wax embedding method. For histopathological examination, tissue sections of 56 micron thickness were stained with Ehrlich's haematoxylene and eosin method (Luna, 1960).

Out of 72 layers and 123 broilers autopsied during the study period, the incidence of coccidiosis was found to be 29.17 and 78.05 %, respectively. The occurrence was higher in birds reared under the deep litter system, as compared to the cage system, in both layers and broilers (Table I).

Gross and microscopic changes were seen in upper and middle regions of intestine and caecum, during post-mortem examinations. Affected birds revealed reddish white pinpoint foci on exterior surface, especially in the earlier parts of the small intestine. In some cases; however, the middle part of the intestine was distended and had turned crimson, with petechiae also seen through out the serosa. The intestinal contents were reddish brown, with occasional blood clots and fibrin shreds. Caeca were distended and filled with blood and reddish brown contents, in haemorrhagic type of infection. In catarrhal type of caecal coccidiosis, there were patechial spots in serous

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Table I. Incidence of coccidiosis in layers and broilers on post-mortem basis under deep litter and cage system of housing in Gujarat

Birds	Housing system	No. of birds examined	No. found positive	%
Layers	Deep Litter	18	14	77.78
	Cage	54	7	12.96
	Overall	72	21	29.17
Broilers	Deep Litter	114	94	82.46
	Cage	9	2	22.22
	Overall	123	96	78.05

surface. Caecal walls were thickened, congested, necrosed and ulcerated at places. In some cases, catarrhal and stringy exudates and whitish foci were also present.

The affected part showed wide spread damage to the absorptive epithelium. The villi were stunted, plumpy and destructed. The hypertrophied epithelial cells had fully developed oocysts and some developmental stages of coccidia. The mucosae as well as submucose were heavily infiltrated with macrophages, plasma cells and lymphocytes. Severe lesions were characterized by extensive haemorrhages, particularly around degenerated intestinal glands and epithelial cells carrying the developmental stages. The schizonts in the superficial layer of the mucosa appeared relatively smaller than the lower layer. In some instances, isolated irregular patches of haemorrhages could also be seen in vicinity of muscularis mucosa. In such instances, the destructive changes were not much appreciable, except for some degree of secretory vacuolation and occasional desquamations in villi epithelium (Fig. 1).

The superficial layers of caecal mucosa appeared desquamated. The intestinal glands showed considerable enlargement with developmental stages of schizonts. The macrophages and lymphocytes were found extensively infiltrated, especially around the glands with damaged epithelial cells. The musculature showed evidence of edema and intense eosinophilic staining. In cases, where the caseous or reddish brown masses were present, the epithelial cells of glands (especially in the middle third and towards the muscularis mucosa) showed the gametocytes and oocysts (Fig. 2).

Distension and crimson appearance of small intestine have been reported by Shukla *et al.* (1990). They have also reported haemorrhagic enteritis and thickening of intestinal wall, especially in small intestine, blood clots and fibrin-shreds mixed faecal contents, damage to the absorptive epithelium of intestine, destruction of villi and epithelial cells with fully developed oocysts and some developmental stages of coccidia. Soomro *et al*. (2001) observed enlargement of the caecum and the appearance of clotted blood with

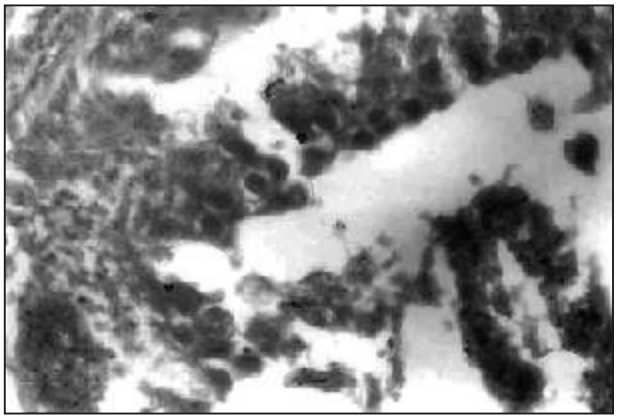

Fig. 1. Intestinal gland showing parasites towards luminal surface (X 240)

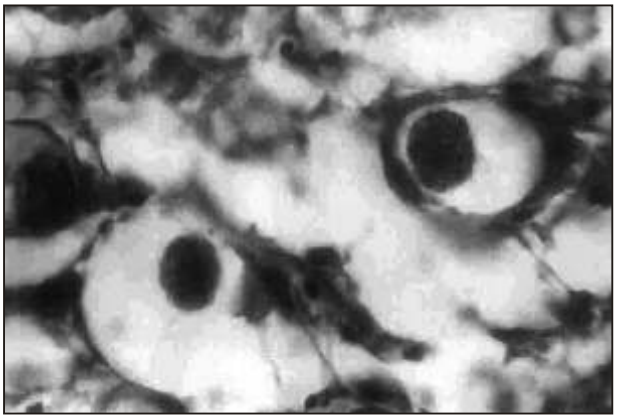

Fig. 2. Caecal section showing schizonts in lamina propria (X 600)

haemorrhagic or whitish spots on the caecal wall. Iinflammation, necrotic patches and dilatation of the caecum with consolidation of caecal contents in almost all cases of caecal coccidiosis were also described. The histopathological lesions of caecal coccidiosis involved loss of epithelial tissues, vascular congestion, edema, necrosis of the caecal mucosa and loss of villi.

Though most of the histopathological findings are related to the literature cited by Babu *et al.* (1976), Ahmad *et al*. (2000) and Teshfarm and Rahbari (2003), some of the landmarks of findings mentioned by them could not be coincided due to limited number of natural cases with added impact of stress, as against the pattern of pathological changes in experimental cases in controlled environment. However, the present observations enabled us to draw conclusion that the magnitude of infection and the stage of development of coccidiosis produced, could be established by histopathological observations.

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Short communication

JPD

Clinico-parasitological observations in experimentally induced bovine babesiosis

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ABSTRACT. An attempt has been made to establish experimental infection in crossbred bovine calves by using ticks (*Boophilus microplus*) collected from *Babesia bigemina*-infected cattle from Wayanad, Kerala. The splenectomy of crossbred calves, followed by corticosteroid administration, increased their parasitaemia. Naturally-infected calves from Izatnagar also revealed heavy parasitaemia following this method. Major clinical features, observed during natural outbreaks, like pyrexia and haemoglobinuria were less pronounced in experimentally-infected calves. Additionally, a large number of piroplasms in peripheral blood, low packed-cell volume, thrombocytosis and erythrophagocytosis were other features observed in these experimentally-infected calves.

Keywords: *Babesia bigemina*, bovine, clinico-parasitology, experimental infection

Bovine babesiosis continues to be one of the important tick-borne diseases in India. It is assumed that about 80% of Indian herd is within areas endemic for *Babesia* and *Anaplasma* infections. With the state of enzootic stability existing in tropical belt, recrudescence of parasitaemia can be achieved by immunosuppression of the host, and is considered as a method of choice for achieving moderate to high parasitaemia in *Babesia bigemina*-infected bovine calves (OIE, 2000). The present communication reports the clinico-parasiological findings observed during experimental induction of bovine babesiosis.

Eight apparently healthy male crossbred bovine calves, > 3 months in age, were procured from the Section of Livestock Production and Management, Indian Veterinary Research Institute, Izatnagar. All the animals were tested for the carrier status of *B. bigemina* infection by blood-smear examination, by using Giemsa staining and indirect fluorescent antibody test (IFAT) according to Ravindran *et al*. (2002). The calves negative (n = 5) for the protozoan infection by both of these methods were then used for experimental *B. bigemina* infection by exposing them to 300 larval engorged female ticks collected from cattle naturally infected with Wayanad (Kerala) isolate of *B. bigemina*. The IFAT positive animals were directly used for splenectomy followed by dexamethasone administration (Ravindran *et al*., 2006) for isolation of the Izatnagar isolate of *B. bigemina*. The animals were examined carefully for clinical signs twice-a-day and their blood films were examined daily. The course of parasitaemia, packed-cell volume (PCV) at the height of parasitaemia and other clinical parameters were also observed.

All the bovine calves screened by Giemsa-stained blood film examination did not reveal any haemoprotozoa at the beginning of the experiment. By IFAT also, five calves (Nos. 590, 585, 601, 606 and 612), from the stock selected for raising donors, were identified as negative for antibodies to *B. bigemina* and were used for establishing tick-induced Wayanad

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Table I. Clinico-parasitological observations in experimentally induced *B. bigemina* infection in crossbred calves

Isolate	No. of animals	Appearance of parasitaemia following larval attachment (days)	PCV at peak parasaitaemia (%)	Peak parasitaemia (%)	Haemo-globinuria	Highest temp recoded (°F)	Remarks
Wayanad	590	16	19	12	-	102.8	
Wayanad	585	8	-	0.5-1.0	-	103.0	Died
Wayanad	601	18	9	24	-	106	
Wayanad	606	17	22	35	+	103.2	
Wayanad	612	12	-	6	-	-	Died
Izatnagar	596	NI	10	10	-	103.4	
Izatnagar	741	NI	26	20	+	104.0	
Izatnagar	270	NI	24	7	-	104.3	

NI-Natural infection with *B. bigemina* (Izatnagar isolate)

isolate of *B. bigemina*. However, three calves (Nos. 270, 596 and 741) were positive by IFAT, and were splenctomized and subjected to immunosuppression for recrudescence of parasitaemia with Izatnagar isolate. The clinico-parasitological observations made in these animals are shown in Table I. Morphologically, the piroplasm stages of 'Izatnagar' and 'Wayanad' isolates of *B. bigemina* were identical in Giemsa-stained blood films. Curiously, the two calves experimentally infected with Wayanad isolate of *B. bigemina* (Nos. 585 and 612) died of typical clinical babesiosis; the first one died even before splenectomy and immunosuppression.

A gradual increase in parasitaemia was observed following splenectomy and immunosuppression. The peak parasitaemia appeared 68 days after the first administration of dexamethasone. During the early stages of ascending parasitaemia, a large number of amoeboid forms were clearly visible in erythrocytes, whereas the number of paired pyriform organisms appeared more during peak parasitaemia. As the parasitaemia increased, the platelets were appreciable as a mass in blood smears. Monocyte erythrophagocytosis was consistently observed during peak parasitaemia.

The information on the virulence patterns of *B. bigemina* isolates in our country is scanty. Given the nature of parasite isolation of the two referral isolates, there is a direct relationship between tick-transmissibility and virulence in infections resulting from Wayanad isolate. The relationship between the virulence and tick-transmissibility has been described by Dalgliesh and Stewart (1977). The Wayanad district of Kerala is known to be endemic to *B. bigemina* infection and by using IFAT a high carrier status (~67.6%) has been reported (Ravindran *et al*., 2002).

Splenectomy is known to results in the loss of a vast splenic reservoir, which leads to persistent elevation of circulating platelet numbers, and in the present study also similar observations have been made. The platelet aggregation in peripheral circulation at peak parasitaemia may be related to immunosuppression following splenectomy (Benjamin, 2001).

Erythrophagocytosis has been widely reported in several protozoan diseases like feline babesiosis (Futter and Belonje, 1980), and in infections caused by *B. microti* (Hussein, 1979), *B. caballi* (Allen *et al*., 1975), *B. gibsoni* (Wozniak *et al*., 1997) and *Plasmodium berghei* (Roth, 1979). Haemoglobinuria was not observed to be a common symptom even at peak parasitaemia, as it was seen only in two cases. Therefore, haemoglobinuria should not be taken as ideal clinical symptom for the diagnosis of the babesiosis in field conditions. Similarly, the rectal temperature was not high even at the time of peak

parasitaemia. Nevertheless, there was a positive correlation between PCV and parasitaemia, with the exception of one case only.

ACKNOWLEDGMENTS

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JPD

Cercarien Hullen Reaction for the assessment of human schistosomiasis in India

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ABSTRACT. Cercarien Hullen Reaction (CHR) was applied in 116 serum samples for the diagnosis of cercarial dermatitis, and to ascertain the possibility of the existence of live schistosomes in humans. Stool and urine samples of these 116 individuals, either having dermatitis or its history, were negative for blood fluke eggs. About 51% dermatitis cases, not responding to benzyl benzoate, were CHR positive, whereas the positivity in those responding to the drug was 20%. There are chances that these CHR positive cases might harbor live blood flukes. The present study highlights a need for the investigation of the possible presence of human schistosomiasis in India.

Keywords: cercarial dermatitis, CHR, India, schistosomiasis

Animal schistosomiasis is widespread throughout the Indian subcontinent, and is identified in two forms – nasal and hepatic. However, the existence of human schistosomiasis in India is marred with controversy (Agrawal, 2005). The greatest impediment in the diagnosis of Indian schistosomiasis is the lower faecal egg excretion as reflected by prevalence in cattle: 0-10%, by faecal examination as compared to 70-100% by mesenteric examination (Agrawal, 2003). Thus, even if schistosomiasis is occurring in man in India, it may be difficult to confirm it by fecal/urine examination.

Cercarial dermatitis has been shown to be rampant in rural India (Narain *et al.,* 1994; Agrawal *et al.*, 2000a; Agrawal *et al.*, 2000b), wherein villagers are dependant on water ponds for their domestic and animal needs. Interestingly, mammalian schistosome cercariae have been incriminated in cercarial dermatitis with doubts whether the schistosomulae remain confined to the skin, or continue their journey to internal organs and reach maturity (Narain *et al.*,1994; Agrawal *et al.,* 2000a; Agrawal *et al.*, 2000b). Such an assessment is possible only by immunodiagnosis, which is not used widely in our country. The intradermal test using *Schistosoma mansoni* antigen in Gimvi village has revealed 20% prevalence of *S. haematobium* in human subjects, which was much higher than that has been determined (0.7%) by urine examination (Gaitonde *et al.,* 1981). Double immunodiffusion test has proved to be least sensitive in the diagnosis of experimental or natural animal schistosomiasis, whereas miracidia immobilization and ring precipitation tests require monitoring of their titers for differentiating negative cases from the positive ones(Agrawal and Shah, 1989 ; Agrawal 2005). Cercarien Hullen Reaction (CHR) has proved comparatively more sensitive (85%) and specific (74%) in the diagnosis of animal schistosomiasis (Agrawal and Shah, 1989: Banerjee *et al.,* 1991); many workers have considered its

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positivity indicative of the presence of live schistosomes in the host (Stirewalt and Evans, 1955; Ahluwalia, 1972).

Therefore, CHR was used in the present study for the assessment of cercarial dermatitis in the tribal areas of Mandla District (22.43 N, 80.35 E), Madhya Pradesh, wherein animal schistosomiasis (*S. incognitum, S. spindale, S. indicum*) is known to exist. In all, 1622 humans were examined for the presence of dermatitis. Whereas 54 individuals had dermatitis (Fig. 1), the other 62 individuals gave a history of rash in the last 6 months. All the dermatitis cases were treated with benzyl benzoate, and the results were recorded. The occurrence of dermatitis was associated with bathing in the ponds, which harbored snails (*Indoplanorbis exustus, Lymnaea luteola*) positive for schistosome cercariae. Serum, stool and urine samples were collected from these 116 individuals who were categorized into following three groups:

Group A: Having dermatitis and responding to benzyl benzoate (n =15)

Group B: Having dermatitis but not responding to benzyl benzoate (n = 39)

Group C: Not having dermatitis but having history of dermatitis (n = 62)

Cercariae of *S. spindale* were used in CHR because this species has been shown to be the etiological agent of cercarial dermatitis in India (Narain *et al.*, 1994; Muraleedharan, 2000). A drop each of serum, cercarial suspension and streptomycin-penicillin solution was placed in cavity slides; kept for 20–24 h in a moist chamber and then examined under a light microscope. The test was considered negative when cercariae were free from precipitates or hyaline membrane (Fig. 2). The presence of precipitate either on the body or on the tail was graded as +, whereas precipitates all over the cercaria as ++ (Fig. 3). The hyaline membrane

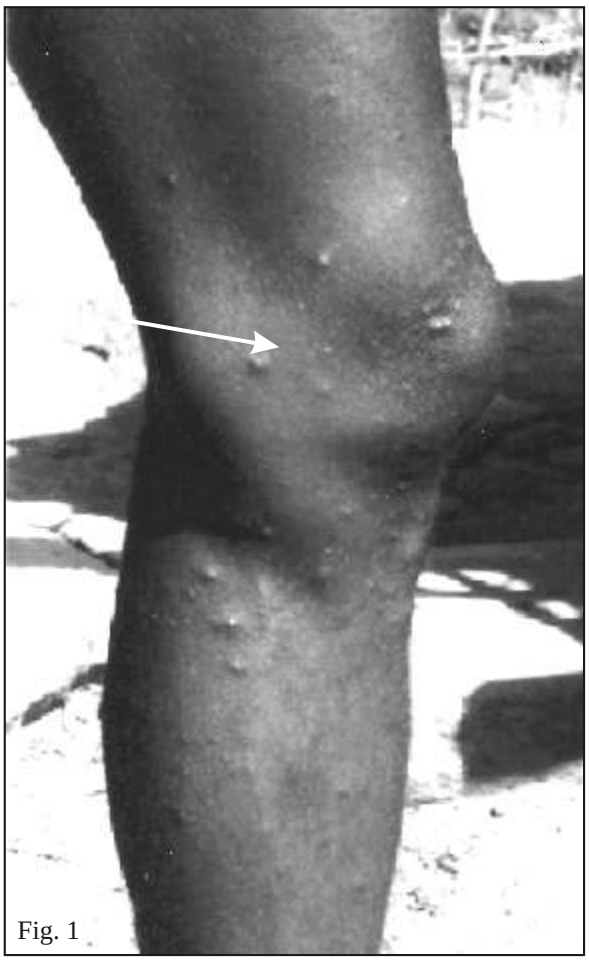

Fig. 1

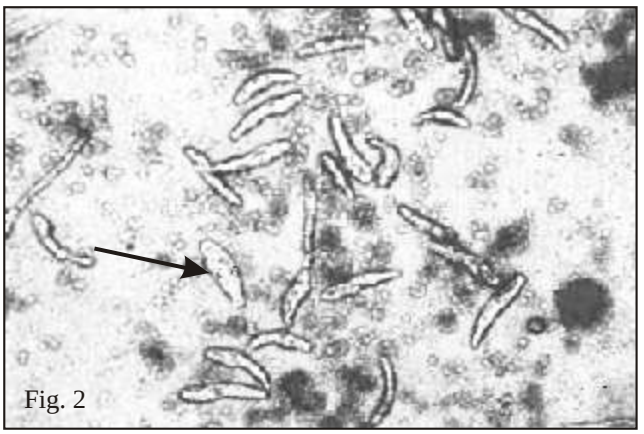

Fig. 2

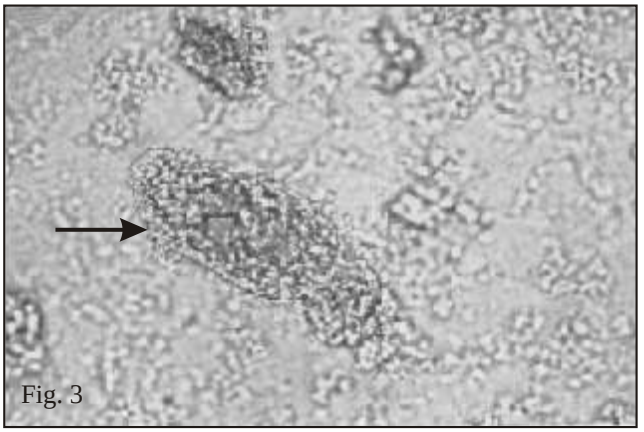

Fig. 3

Cercarial dermatitis in man (Fig. 1) along with negative (Fig. 2) and positive (Fig. 3) Cercarial Hullen Reaction with human serum.

covering entire cercaria was graded as +++. The grading was done on the basis of our experience on animal schistosomiasis, *i.e.* the presence of hyaline membrane was invariably observed in parasitologically schistosome positive cases, whereas in the case of precipitates, parasite positivity could be established only in few cases. The faeces were examined by formal-ether method and urine by centrifugation method.

All the faecal and urine specimens were found to be negative for any blood fluke eggs. There was a strong correlation between CHR reactivity and cercarial dermatitis. Only 20% (3/15) of the dermatitis cases which responded to benzyl benzoate showed CHR reactivity (mixed syndrome in such cases cannot be ruled out). About 51% (20/39) cases which did not respond to the drug were CHR positive ($x^2 = 4.34$, $p < 0.05$). Strong reactivity (+++) could be seen in one and six cases among above two categories, respectively. Individuals not suffering from dermatitis but having a history of dermatitis showed CHR reactivity in 38.7% (24/62) cases with seven of them revealing a strong reactivity. The Chi square trend for linearity suggests that there is a significant ($x^2 = 4.44$, $p<0.05$) trend of positive CHR in all the three groups viz. dermatitis cases responding to benzyl benzoate, cases not responding to benzyl benzoate and individuals with a history of dermatitis. Interestingly, CHR reactivity including strong reaction was higher among young males in the age group of 5-20 years.

As CHR is a genus specific antibody-dependent assay, it may not be positive in only cercarial dermatitis (being a delayed hypersensitivity reaction). Moreover, CHR positivity represents presence of live blood flukes (Stirewalt and Evans, 1955; Ahluwalia, 1972), which suggests that schistosomes, in the present case, might have crossed the skin barrier and survived in the host. This assumption gets further support by the reported finding of adult males of *S. nasale* in the liver of sheep, a less permissive host, though the nasal cavity and lungs were negative for the blood flukes (our unpublished observations). In experimental *S. spindale* infection in rabbits, guinea pigs, rats and mice, the animals were positive by CHR but were negative by coprological examination, though tissues contained viable fluke eggs (Mishra, 1991). These observations provide the reasonable ground to believe the existence of human schistosomiasis in India, in addition to cercarial dermatitis, which needs to be investigated using modern tools.

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A rare case of human nasal myiasis due to the larvae of *Oestrus ovis*

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ABSTRACT. Naso-sinusal myiasis of sheep and goats is caused by the larvae of *Oestrus ovis* (sheep nasal bot fly), which may accidentally infect humans also. We report a rare case of human nasal myiasis, caused by the larvae of *O. ovis*. To our knowledge, this is the first ever reported case of nasal myiasis in an immunocompetent person, hailing from a clean urban environment of Mumbai.

Keywords: human, *Oestrus ovis,* nasal myiasis

Myiasis is the parasitic infestation of tissues and organs of vertebrate animals and man, and is caused by the larvae of the fly of the order Diptera. The larvae of three dipterous families Oestridae, Calliphoridae (blowflies) and Sacrophagidae (fleshflies) are considered as the main cause of myiasis in livestock and occasionally in humans. *Oestrus ovis* is a cosmopolitan parasite of domestic sheep and goats. The female *O. ovis* fly is larviparous and deposits its first stage larvae in the nostrils of the host. The larvae rapidly crawl up to the frontal sinuses and attach to the mucous membrane with the help of oral hooks and feed on the mucous substances. Often, they are in great numbers causing massive destruction accompanied by marked inflammatory reactions and secondary bacterial infections. When the larvae mature up to the third stage of growth, they fall out from the nasal passage and drop on to the ground to pupate and develop into adult fly (Kettle, 1990). The adult fly accidentally deposits first instar larvae in the eye and nostrils of man, where they survive for a few days without any further development. Human cases of opthalmomyiasis due to *O. ovis* larvae are frequently reported, but cases of nasal myiasis are rare (Harvey, 1986).

Dr. Saroj Bapna, Department of Zoonosis, Haffkine Institute, Mumbai for Training, Research and Testing, Mumbai-400 012, India. E-mail:sarojbapna@rediffmail.com

Case report: A 49 years old man, residing in the suburban area of Mumbai and an accountant by profession, reported symptoms of cough and dyspnea on exertion, since 2002 and took symptomatic treatment from a local doctor. The symptoms used to subside off and on. He was, hence, directed to a chest physician in April 2002, and was subsequently advised CT scan for confirmatory diagnosis, which showed early interstitial lung disease with interstitial fibrotic changes in both the lungs. These changes were diffusely predominant in sub-plural region. Small emphysematous cysts were also observed in the lung X-ray. During this period, the patient was given steroids for some time. However, in January 2006, the patient was admitted with severe respiratory distress, cyanosis, clubbing of fingers and fever. The human immunodeficiency virus (HIV) test of the patient yielded negative results. On worsening of his condition, the patient was admitted to the territory care unit, where he passed a small worm through his nostril. He was in severe hypoxia with SPO_2 of 70%. The CT scan of the paranasal sinuses reveled left maxillary

sinusitis and sphenoiditis sinusitis with 'worms' in the left-half of the sphenoid sinus and the maxillary sinus. Till the mid of February 2006, the patient passed five more worms. All the six worms were reported to be alive on expulsion. Of these, one worm was sent to Department of Zoonosis, Haffkine Institute, Mumbai, for identification.

Identification of parasite: The parasite obtained from the nasal passage of the patient, was preserved in 70% alcohol and was examined and identified on the basis of morphological features described by Sen and Fletcher (1962). The structure of the spiracles (breathing organs) at the tail end is a very important feature in the identification of larvae. The procedure followed was the posterior end of the larva was cut and pressed. Three changes of glacial acetic acid were given each for 15 min. After that, one change in carboxylol and another in xylol was given. The posterior spiracles were then mounted in canada balsam, dried and observed under a light microscope.

Observations: The macroscopic and microscopic examination of the parasite showed the following features. Externally the worm appeared elongated, yellowish-brown in colour, measured 25 mm in length and bore 9–10 segments (Fig.1). The larva had a slightly tapering anterior end and a broad posterior end. A pair of black oral hooks was present at the anterior end (Fig. 2). The dorsal surface of each segment was convex and had dark transverse bands. The ventral surface of the larva was flat and possessed rows of spines (Fig. 2). The posterior spiracles were black chitinous stigmal plates, 'D' shaped in appearance (Fig. 3, 4). Based on the morphological characteristics, patterns of the posterior spiracles and the disease presentation, the parasite was identified as the larva of *O. ovis* (sheep nasal botfly) from the class, Insecta; order, Diptera; family, Oestridae. *O. ovis* is a common infestation in sheep and goats, and can accidentally cause infestation in humans.

Discussion: *O. ovis* fly larvae are obligatory parasites of the nasal and sinus cavities of sheep and goats in all the sheep-farming areas of the world. Accidentally, they can infect people living in close contact with livestock, mostly in rural areas with poor general health and hygiene. In the present study, the patient was residing in a clean urban area. Hence, the source and cause of infestation remains unclear. Human cases of myiasis are rare and resolved rapidly as the larvae are unable to develop beyond first-stage. However, in this case, the isolation of third-stage larvae, led us to imply that the history of steroid administration as therapy of interstitial lung fibrosis, might have played a role in allowing the larvae to mature up to the third-stage. Otherwise, in an immunocompetent person, the infestation is self-limiting. One case of human infestation by the third-instar larvae of *O. ovis* has also been recorded in a HIV-infected patient in the United Kingdom (Luciente *et al.*, 1997). Few cases have been reported in nomadic tribes that herd sheep and goats, and consume large quantities of goat milk and cheese,

Fig. 1: Dorsal view of *O. ovis.*

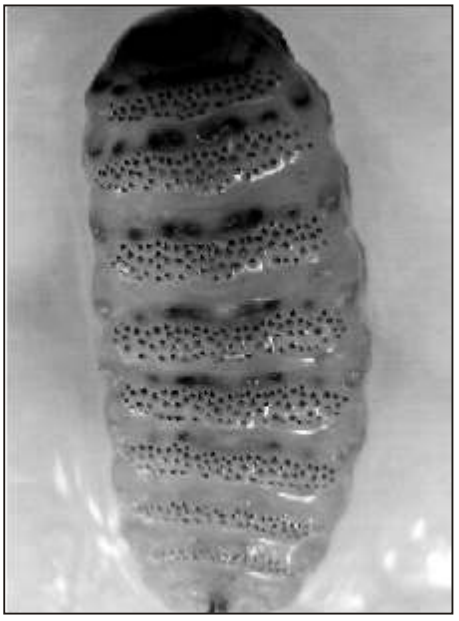

Fig. 2: Ventral view of *O. ovis.*

Fig. 3: Posterior end showing 'D-shaped' spiracles.

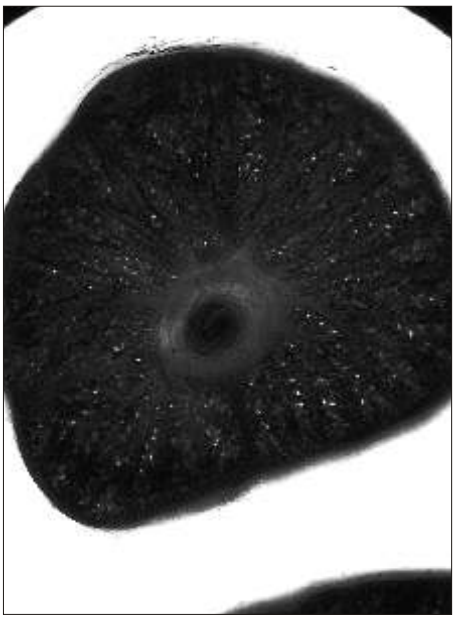

Fig. 4: A single posterior spiracle.

the smell of which attracts the fly (Martin Hall and Richard Wall, 1995). The clinical manifestations of myiasis are not specific and vary according to the involved area of the body. General signs and symptoms including fever, myalgia, arthralgia, hypereosinophilia, elevated ESR (erythrocyte sedimentation rate) and inflammatory reaction at the site of infection are commonly seen (Dorchies, 1997). The pathogenicity results from inflammation and toxins secreted by the larvae, leading to chronic inflammatory reaction. The treatment involves uses of antilarval measures, followed by the removal of larvae. A broad-spectrum antibiotic cover is recommended to prevent secondary infections (Beristain *et al.*, 2001). Though rare in urban settings, the nasopharyngeal myiasis case reported herein shows the importance of awareness in physicians, who treat rare parasitic infestations in immuno-compromised patients, and its clinical manifestations.

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OBITUARY

Professor A. B. Chowdhary

(25th November 1923 – 27th June 2006)

Professor Amiya Bikash (A. B.) Chowdhary, widely respected as a father figure in the field of medical parasitology in India, a brilliant academician and a biomedical scientist of eminence, was born on November 25, 1923, in a remote village near Chittagong district of undivided Bengal.

After obtaining his M. B. B. S. degree in 1947 with merit scholarship, he passed his Ph. D. in medicine from Calcutta University in 1956. He was awarded Rockefeller Foundation fellowship for post-doctoral work at Cornell University Medical College, USA in 1957. He started his career as a lecturer in the Department of Helminthology, School of Tropical Medicine, Calcutta in 1954, and became Professor and Head in 1959. He served as Professor and Chairman, Department of Parasitology from 1964–1982. Prof. Chowdhary became Director of the School in 1972, and retired as such in 1982. He had held various important national and international positions during his service period and even after his superannuation like, Visiting Professor at Cornell university Medical College and Pennsylvania University, USA, Member Expert Advisory Panel in Parasitic Diseases, W. H. O., Advisor Consultant Parasitic Diseases, G. D. R., Emeritus Professor, School of Tropical Medicine, Calcutta and President, Vivekanand Institute of Medical Sciences, to name a few.

Prof. Chowdhary, an outstanding parasitologist, investigated host-parasite relationship, immune response in parasitic diseases, their modulation and impact on clinical expression, drug action and micro chemical constituents of parasites and their functional significance. He studied the population biology and transmission dynamics of parasitic infections and identified factors regulating natural abundance of parasite pathogens. He observed that the arrest of development of human hookworm was due to adaptation to unfavorable external developments.

He was Fellow of Indian National Science Academy, National Academy of Sciences (India), Royal Society of Tropical Medicine and Hygiene, Indian Society for Parasitology and West Bengal Academy of Science and Technology.

He published more than 400 research papers in scientific journals of repute and was on the editorial board of several journals.

He was recipient of several awards like Coats Medal of Calcutta university 1964, Goswami Memorial Award IMA 1971, Warner oration of ISG 1974, Lavchinpat oration NAMS 1974, Sir Nil Ratan Sarkar Memorial oration 1975, Khantimani Nagendrabala oration 1977, Dr. Shanti Lal Seth oration IAP 1979, Major Gen. Sahib Singh Soke oration 1979, Bajanti oration 1981, Prof. J. B. Chatterjee Memorial oration 1995 and Life Time Achievement award by ISP in 1997.

Prof. Chowdhary was Chairman/Member of several Scientific Advisory Committees/Research Councils/Governing Bodies of several institutes like Haffkine Institute, Bombay; C D R I, Lucknow; A F M C, Pune; Indian Stat. Institute, Calcutta; T R C, Chennai; I C M R; R M R I, Patna and DST etc.

He was the first National President of Indian Association of Parasitologists, ISCA; Section of Medical and Veterinary Sciences 1966 and Vice President of World Federation of Parasitologist. He founded the Center for Study of Man and Environment, Kolkata and was associated with it till his death.

He is survived by his wife Dr. Momota Chowdhary, who is a paediatrician, and a daughter Dr. Tuli Biswas (a Senior Scientist in IICB, Kolkata) and a son Mr. Rahul Chowdhary (an economist).

In his death, the scientific world has lost one of the most distinguished parasitologists. A man of amicable personalities, he was not only a distinguished teacher and researcher but was a good human being. He was a remarkable orator. He always encouraged young scientists to excel in research. He was a friend, philosopher and guide to many young and old researchers. I had the proud privilege of being associated with Prof. Chowdhary for the last over 40 years, ever science I started my research career. He was my mentor and I always received guidance and encouragement from him. Several of his students are occupying prestigious chairs in parasitology, tropical medicine and microbiology in the country and abroad.

Prof. R. C. Mahajan, F.N.A., F.T.W.A.S.
S. N. Bose INSA Research Professor
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