Aldo Castellani Memorial Lecture - 1994

Parasitic infections in AIDS

A S Dissanaike*

Journal of the Ceylon College of Physicians, 1995, **28**, 35-41

I am indeed honoured that I have been invited to deliver this third Sir Aldo Castellani Memorial Lecture and I thank the President for his kind words of introduction. My only, yet indirect, contact with Sir Aldo was in early 1960. My Ph.D supervisor, Professor P C C Garnham F.R.S. well known for the discovery of the liver stages of malaria parasites of monkeys and humans, was writing his *magnum opus* on malaria parasites and had a problem with a *Plasmodium canis* which Castellani together with Dr Albert Chalmers had described from dogs in Ceylon in 1910. After discussing this with Castellani they decided that I should reexamine dogs from the same locality. As I was not able to find any pigmented red cell parasites, after examining a large number of dogs from the same locality, it was concluded that what Castellani and Chalmers probably saw were multiple infections of red cells with *Babesia canis,* a not uncommon protozoan parasite of dogs.

This notwithstanding, as the President has already detailed, Castellani was a most remarkable and versatile man. One of the reasons for selecting this topic for my lecture was that the discovery of the first human case of toxoplasmosis has been attributed to Castellani, who in 1913 found *Toxoplasma* like bodies in the blood and spleen smears of a 14 year old boy who had died after a longstanding remitting fever with hepato-splenomegaly but no lymphadenitis, that was not malaria[1]. While the identity of this parasite is in some doubt an examination of the figures in Castellani's publications suggested to me that these parasites resembled somewhat the meront stages of a microsporidian. And this was the second reason for the choice of the title, especially since the microsporidia are now emerging as important opportunistic parasites in HIV infected individuals.

They also happen to belong to a group of organisms that I worked on over 40 years ago and to which I was able to make some useful contributions. The common features of parasites involved in AIDS are that they all have a tissue "invasive" phase and/or an intracellular phase and one or more internal reinfection cycles in the host. Most of them are also opportunistic due to the depletion of CD4 + T lymphocytes and in some there is still controversy over the species infecting humans (eg. *Cryptosporidium*).

Based on these and the prevalence of infections in AIDS patients in the developed world, CDC Atlanta, Georgia, USA together with WHO identified 4 clinical stages of HIV infection[2] the 4th stage representing the disease condition referred to as AIDS itself as opposed to the AIDS related complex (ARC) represented by stages 1 to 3. *Pneumocystis carinii* pneumonia (PCP), cerebral toxoplasmosis, cryptosporidiosis, isosporiasis and strongyloidosis were originally included among the parasitic infections that are indicator infections in AIDS. It has now become clear that the parasites involved in AIDS vary with the geographical location and that in the tropical countries the relative importance of these parasites vary and even other infections may be involved. Also that some, like strongyloidosis, are probably not important even in the tropics as perhaps the cell mediated immunity role is minimal in their pathogenesis.

Table 1 lists the parasites that can be considered as important opportunistic ones in HIV infection today. It must however be borne in mind that in tropical countries and especially in Asian countries, other infections may turn out to be more important. Table 2 lists some of these that are either potential or of doubtful importance in AIDS.

Table 1. Parasites in AIDS

Protozoa:
- (a) Protozoa of uncertain taxonomic position.
  1. *Pneumocystis carinii*
- (b) "Primitive" Protozoa (Microsporidia)
  1. *Encephalitozoon cuniculi*
  2. *Encephalitozoon hellem*
  3. *Enterocytozoon bieneusi*
  4. *Septata intestinalis*
  5. *Pleistophora*
- (c) Apicomplexa (Coccidia)
  1. *Cryptosporidium*
  2. *Toxoplasma gondii*
  3. *Isospora belli*
- (d) Flagellates
  1. *Leishmania* spp.

* *Formerly Professor and Head, Departments of Parasitology, Faculties of Medicine, University of Colombo and University of Malaya and Medical Officer Filarial Infections, WHO, Geneva, Switzerland.*

Table 2. Potential or doubtful parasites in AIDS

Protozoa:
- (a) Protozoa of uncertain taxonomic position
  1. *Blastocystis hominis*
- (b) "Primitive" Protozoa (Microsporidia)
  1. *Nosema corneum*
  2. *Nosema ocularum*
  3. *Microsporidium ceylonensis*
  4. *Microsporidium africanum*
  5. *Nosema connori*
- (c) Apicomplexa
  1. *Sarcocystis spp.*
  2. *Plasmodium falciparum*
  3. *Babesia spp.*
  4. *Cyclospora*
- (d) Others
  1. *Entamoeba histolytica*
  2. *Giardia*
  3. *Acanthamoeba*

Helminths:
1. *Strongyloides stercoralis*
2. *Schistosoma spp.*

While homosexuals and drug users are more likely to be infected in the western world the epidemiological pattern of AIDS in Africa is more by heterosexual contact, vertical transmission from mother to infant and by blood transfusion.

In Asian countries, high risk behaviour and contact with infected individuals in the west and Africa accounts for infections. Thus ethnic differences in susceptibility, strain differences in parasites, differences in parasites available in tropical countries and underdiagnosis due to less efficient diagnostic techniques in the tropics would account for geographic variation patterns of parasites involved. In addition, the threshold for fall in CD 4+ T cells has been shown to be lower for some diseases. Thus death occurs earlier from tuberculosis in Africa so that *Pneumocystis* pneumonia is less frequently reported than in the west[3].

A. The main parasites

Let us now consider the parasites indicated in Table 1.

1. *Pneumocystis carinii*

This organism is yet of uncertain taxonomic status. It was believed to be a protozoan allied to the coccidia, but recent EM studies together with freeze fracture appearances and biochemical investigations suggest that it is a fungus related to *Candida* or *Saccharomyces*. It lacks Golgi bodies and has poorly developed mitochondria. Japanese workers have recently suggested that a sexual cycle takes place, after demonstrating a 'synaptonemal complex'[4]. It produces typically an 8 body containing cyst or spore which is rounded and 4-10 μm in diameter.

Around 75% of AIDS cases in the USA, UK and Europe develop PCP especially when the CD 4+ T-lymphocyte cell level drops below 200/cumm. The prevalence is much lower in Africa and perhaps also in Asia where little work has been done. PCP is an interstitial plasma cell pneumonia with a foamy honey comb like exudate into the alveolar spaces that contains the cysts. These are seen best in Gomori's silver stain preparations in which characteristic "parenthesis" bodies have been demonstrated and probably represent thickenings in the cyst wall that are seen in EM sections[5]. Clinical manifestations include fever, cough and dyspnoea.

While the infection is generally confined to the lungs dissemination to lymph nodes, the intestinal tract, liver, spleen and the thyroid gland have been reported in AIDS patients and no doubt this has occurred by haematogenous spread[6,7]. In AIDS patients PCP can be suspected if the serum LDH levels are raised, but although this is not pathognomonic of the infection a normal LDH level rules out PCP[8].

2. *Microsporidia*

These so called "primitive" protozoa are obligatory intracellular parasites that lack mitochondria but possess Golgi bodies and prokaryotic type ribosomes. They have a chacteristic infective stage or spore which is usually ovoidal and contains a coiled polar filament and an infective sporoplasm that is believed to be extruded through the everted filament canal when the spore is transported from one host to another. Microsporidia have been reported from practically all animal groups such as the protozoa, helminths, arthropods and vertebrates including non human primates and humans. The best known microsporidian of mammals is *Encephalitozoon cuniculi* a parasite of the brain and nervous system and kidneys of rabbits and related mammals. It was first reported in humans at a time when it was not even known to be a microsporidian. This was in a 9 year old Japanese boy who had seizures[9].

In 1985 several cases of microsporidial enteritis caused by *Enterocytozoon bieneusi* have been described, especially from AIDS patients[10,11]. Since then over 100 cases have been reported in 15-30% of AIDS patients in USA, UK, Europe, and Africa[12,13] and more recently in Australia[14,15]. While all these have been from AIDS patients, the infection has been seen recently in immunocompetent persons[13]. A second intestinal microsporidian, *Septata intestinalis* was reported from AIDS patients in USA[16] but appears to have a wider distribution, cases being reported even from Australia[14]. It is often seen in AIDS cases together with *E. bieneusi*[12,13,14,16].

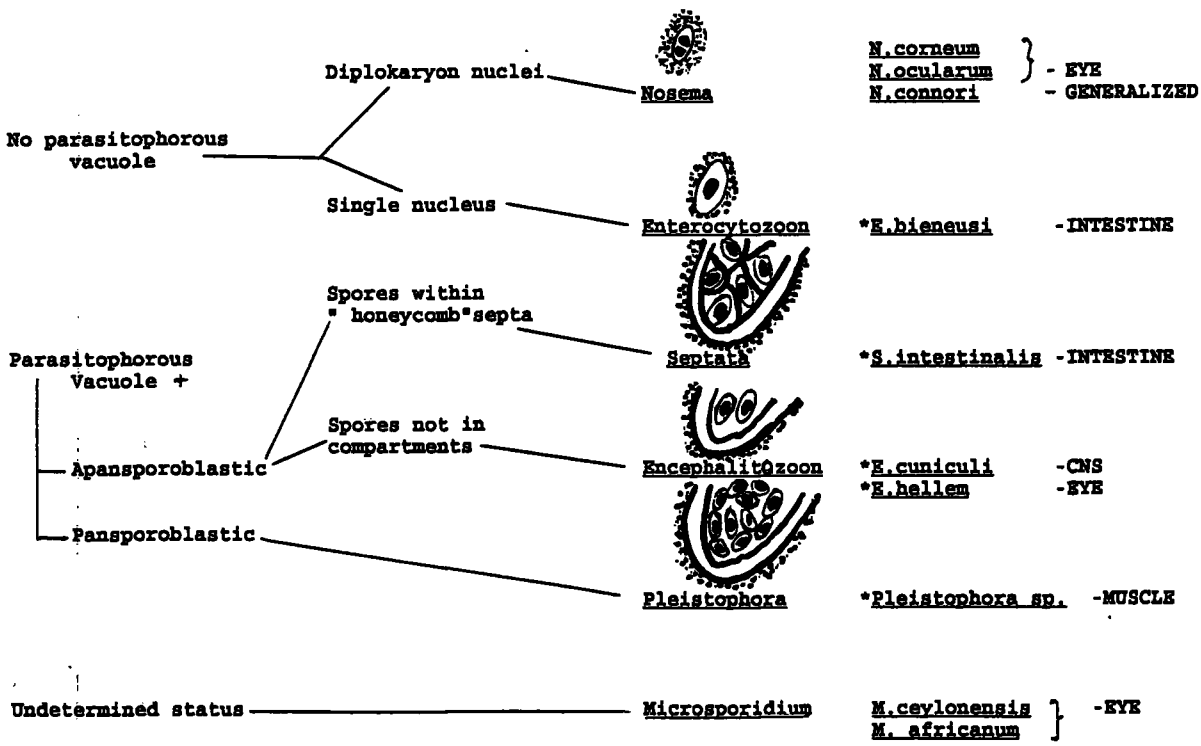

Figure 1. Microsporidia seen in humans

These intestinal microsporidian infections result in a persistent, chronic, profuse diarrhoea often associated with malabsorption. Several other microsporidia have been reported in AIDS and non-AIDS patients and Figure 1 summarises these indicating their sites and distinguishing characters. Five of these have been seen in AIDS cases namely *Encephalitozoon cuniculi, E. hellem* a new parasite which closely resembles *E. cuniculi*[17], *S. intestinalis* and a parasite infecting muscle, *Pleistophora*[13].

E. bieneusi has premature development of the polar filament precursors which together with characteristic electron translucent slits seen in the proliferative stages help identify it in EM sections[12,13,14,15].

The cornea and conjunctiva often are the commonest of non intestinal sites with the involvement sometimes of the nasal sinus and epithelia of nasal polyps[17]. In general the microsporidia in AIDS patients are in the superficial epithelium of the conjunctiva and cornea[18] while those in non AIDS cases such as the one reported from Ceylon in 1973 are generally seen deep in the stroma and usually following trauma[19].

Dissemination of microsporidia has been reported for *S. intestinalis* to the colon, kidney, liver and gall bladder[16]. This parasite is seen in the macrophages in the *lamina propria* of the small intestine a feature not seen with *E. bieneusi* which hence does not disseminate. *Encephalitozoon hellem* also disseminates to the lungs and kidney and it is possible that previously reported cases of *E. cuniculi* which were seen in the eye, liver and peritoneum were *E. hellem,* which can only be distinguished from the EM appearance of the spore wall surface of by SDS PAGE analysis or western blotting[20]. The spread of *E. hellem* infection in both corneal and sinus epithelia has been attributed to germination of the spores while still in their parasitophorus vacuoles[21]. While *Nosema* spores are 5 µm or more in length those of other genera especially the intestinal parasites are generally smaller than 2 µm in length and hence identification in tissue sections or faecal samples has been difficult.

In any event identification to species level is based on the presence or absence of diplokaryon (double) nuclei, direct contact of the parasite stages with the cytoplasm of the cell or separation from it by a parasito-

phorus vacuole or the presence or absence of electron translucent clefts, premature development of polar filament precursors etc. All these need EM section examination of biopsy material or stool samples by suitable techniques.

3. ***Coccidia***

Coccidia are protozoan parasites that have a sexual cycle usually in epithelial cells of the small intestine. In humans the coccidian parasites of importance are *Cryptosporidium Isospora belli, Toxoplasma* and *Sarcocystis.* The sexual stages result in the formation of a zygote which secretes a cyst wall to form an oocyst, which may enclose one or more bodies (sporoblasts) round which a cyst wall is secreted to form a sporocyst. Depending on the number of infective stages (sporozoites) within each sporocyst and the number of sporocysts in each oocyst identification of the genera and species is made once the oocyst has sporulated. Thus *Isospora, Toxoplasma* and *Sarcocystis* have 4 sporozoites in each of two sporocysts, *Cryptosporidium* as no sporocysts but has 4 naked sporozoites within the oocyst. A new genus that is being reported in AIDS cases, *Cyclospora,* has two sporocysts with two sporozoites in each.

a. *Cryptosporidium*

This parasite in humans is believed to be *C. parvum* of calves. It produce small round oocysts, 4-10 μm in diameter already sporulated when passed. In AIDS patients the infection is generally so heavy that concentration methods are unnecessary for diagnosis. In addition to direct transmission from host to host autoinfection within the intestine also takes place through thin walled oocysts.

The infection is commonly seen in the jejunem but may spread to the entire gut from oesophagus to rectum. The parasite lies intracellulary but is extracytoplasmic and an electron dense network of microfilaments forms an attachment zone at the interface of the cytoplasm. Invasion of the gall bladder and bile duct and pulmonary dissemination have been reported[22]. It causes a profuse, intractable diarrhoea with voluminous stools in AIDS patients although it is generally self limiting in immunocompetent persons.

While faeco-oral infection is commonest, waterborne infection has been reported and is mainly due to the fact that chlorination does not kill the oocysts[22]. There is a high prevalence of this parasite in Haitian and Africa AIDS patients where up to 50% are infected[23].

b. *Isospora belli*

This infection too has a high prevalence among AIDS patients in Haiti. (15-20%) and also in African countries (up to 60%) but less so in AIDS cases in Mexico (8%) and Australia (4%). This intestinal cocidian parasite produces oocysts that are relatively large and oval, 20-30 by 10-12 um and is either unsporulated or has two sporoblasts when passed. It causes colicky abdominal pain, flatulence profuse diarrhoea and rarely chronic malabsorption with severe weight loss[24,26]. Lactose intolerance and peripheral eosionphilia are not uncommon and Charcot Leyden crystals may be seen in stools. While the infection is confined to the small intestine, rare cases of dissemination to the mesenteric lymph nodes[25] in AIDS patients have been reported.

c. *Toxoplasma gondii*

The sexual cycle of this parasite takes place in the enterocytes of cats which are the definitive hosts. A variety of other vertebrates including humans are intermediate hosts that harbour the active pseudocysts or the latent cystic stages. The zoites in the pseudocysts are called tachyzoites and those in the cyst are bradyzoites.

Human infection occurs by accidental ingestion of sporulated oocysts from contaminated cat faeces or by ingestion of the cystic stages in undercooked meat. When immune pressure is released as in HIV infection, the bradyzoites in cysts especially in the brain revert to the actively dividing tachyzoites and hence in AIDS patients a necrotizing encephalitis results characterised by one or more focal brain abscesses or diffuse meningoencephalitis which is much more common than in other immunocompromised individuals[22]. The lesion may be large enough to be detected by CT scan. Thus one of the serious consequences of HIV infection is due to the reactivation of a latent infection and surprisingly this does not happen with cysts of *Toxoplasma* in muscle, a probable explanation for which is that the virus destroys cysts in brain cells but not in muscle cells[22]. The principal opportunistic infection of the CNS in HIV infection in western countries is due to *T. gondii* and it has been estimated that about 30% of such patients who are chronically infected with the parasite in its cystic stage will develop recrudescent toxoplasmosis[23]. Less commonly eye and lung infections may occur[22]. Although active infections in AIDS patients are not frequently accompanied by a rise in antibody titres, high IgG levels are important risk factors to consider especially in prophylactic treatment of toxoplasmosis in AIDS.

4. *Leishmania* spp

Several reports of new and recrudescent leishmaniasis mostly from the Mediterranean region have been made in HIV positive subjects with or without AIDS. The infection tends to disseminate and, cutaneous spread of visceral forms and *vice versa* have been seen in AIDS cases[27,28]. Leishmaniasis is therefore now included as one of the important opportunistic infections in AIDS[27].

B. Other parasites

Of those indicated in Table 2 *Blastocystis hominis*, *Plasmodium falciparum*, and *Entamoeba histolytica* are the only parasites worth considering in some detail. Although *B. hominis* has been considered as a possible indicator parasite in AIDS the evidence for it is equivocal as I have discussed in a recent review[29]. The fact that it does not invade the tissues except in rare instances argues against it being important. The same applies to *Giardia*. With regard to *E. histolytica* no clear association has been noted between it and HIV immunosuppression. Higher infection levels in certain population groups probably relate to sexual practices. T cell-mediated immunity is thought to be important in the defence against invasion by this parasite. However even in developing countries with a high prevalence of AIDS there is a paucity of reported cases of invasive amoebiasis which suggests that the immune defect does not favour invasion by this parasite[3,27].

P. falciparum — Interestingly enough malaria does not appear to be an opportunistic infection of importance in HIV positive cases even those with AIDS. No association has been found between HIV infection and malaria in adults or children, even with reference to treatment[3,27,30]. The only indirect effect is that treatment of malaria by blood transfusion exposes children to HIV infection[3].

Strongyloides stercoralis has been referred to earlier and it is clear that this parasite and the schistosomes are not important in HIV infection. *Cyclospora*, a recently described coccidian parasite in some patients with AIDS, does not seem to be playing an important role at the moment but needs to be borne in mind as a few cases have already been reported from Peru and Indonesia[31,32]. *Sarcocystis* and *Babesia* will be discussed later under the Sri Lankan situation.

C. Diagnosis and treatment

Tables 3 and 4 summarise the main diagnostic tests and the drug treatment of these parasitic infections in AIDS.

Pneumocystis

Although the best way to confirm a diagnosis of this infection is by demonstrating the cysts in induced sputum samples or bronchioalveolar lavage (BAL), bronchial biopsy may sometimes be necessary. However recently, after the development of suitable DNA probes, polymerase chain reaction (PCR) techniques will soon obviate the need for such invasive procedures[33]. The characteristic "parenthesis" forms seen in silver stained sputum samples have already been referred to. In addition to the standard drugs for this infection Table 4 shows some drugs that are on trial.

Table 3. Diagnosis of parasites in AIDS

Pneumocystis	:	Cysts in induced sputum, bronchio alveolar lavage (BAL) or trans bronchial biopsy — stained in Giemsa or silver stains, PCR.
Cryptosporidium	:	Oocysts in stools or intestinal biopsy material — stained in modified acid-fast stain or Giemsa. Flotation techniques.
Toxoplasma	:	CSF or stereostatic brain biopsy material stained for tachyzoites; or mouse inoculation. PCR; CT scan; MRI. Serology-IgG for risk groups.
Isospora	:	Oocysts in stools (intermittent) by flotation and / or acid fast staining or fluorescence staining.
Microsporidia	:	Touch smears, light and EM sections of biopsies of duodenum; or stool preparations — stained by H&E, Giemsa Warthin Starry — stains; fluorecence technique.

Table 4. Drugs for parasites in AIDS

Problems	:	(1) Lack of *in vitro* methods for testing. (2) Lack of suitable animal models. (3) Need for preventive and secondary prophylactic therapy. (4) Need for combined immunoregulatory treatment. (Interleukins & Interferon)
Pneumocystis	:	Trimethoprim/ Sulfamethoxazole (TMP / SMX) Pentamidine aerosol. (? Dapsone; eflornithin; trimetrexate)
Cryptosporidium	:	None (? Spiramycin; TMP / SMX; DFMO; immune bovine colostrum)
Toxoplasma	:	Sulfadiazine / Pyrimethamine & folinic acid. (Clindamycin / Pyrimethamine & folinic acid)
Isospora	:	TMP / SMX
Microsporidia	:	None (? Albendazole; propamidine; metronidazole)

* = Difluoromethylornithine
? = on trial

Cryptosporidium and *Isospora belli*

The modified acid fast staining techniques used for these two parasites from stool samples or biopsy material distinguishes them from yeasts from their reddish hue. Sheathers sugar flotation technique is the most useful

of the flotation methods[34]. Cysts of *I. belli* are easily recognised by their characteristic shape and size. It must be remembered that *I. belli* cysts are passed intermittently but that in AIDS cases the heavy infection may not necessitate such methods for either parasite. While there is no known drug that is effective against *Cryptosporidium*, a few trials with immune bovine serum and dialysable leucocyte extract (DLE) from immunised calves have shown promise.

Toxoplasma gondii

The methods indicated in the table for diagnosis of cerebral toxoplasmosis in AIDS patients are now assisted by recent PCR, CT scan and NMR techniques. It is important to note that as mentioned earlier, IgG levels are indicators of risk groups especially during pregnancy[23].

Microsporidia

As pointed out earlier the only way to identify these parasites to genus and species is by elctronmicroscopy. Especially for the intestinal microsporidia duodenal pinch biopsies can be replaced by recent non invasive methods using centrifuged stool preparations[35], for which a number of new staining techniques such as the Warthin-Starry stain and fluorecein staining methods[36] are useful. Yet the expensive EM studies are needed for specific identification. No drugs are available for treating these infections although albendazole and propamidine have shown promise especially as local applications in eye infections.

In the treatment of all these parasites in AIDS the four problems outlined in table 4 must be noted specially the need for primary (preventive) and secondary prophylaxis, particularly in PCP, toxoplasmosis and isosporiasis[27].

D. **Discussion and conclusions** (with special reference to the situation in Sri Lanka)

Except for *I. belli* and the microsporidia all other important parasites indicated in Table 1 have been reported from Sri Lanka as zoonotic infections[37]. *I. belli* is known to occur in several Asian countries like Malaysia, Singapore and the Philippines and there is no doubt that if searched for by concentration methods this parasite will be demonstrated even in non-AIDS patients in this country.

The prevalence of *P. carinii, Cryptosporidium* and *T. gondii* in Sri Lanka has been discussed by me in a recent review[37], *P. carinii* being known to be present in immunocompromised persons, *Cryprosporidium* in children and *T. gondii* in children and adults. The prevalence of these infections especially *T. gondii* is quite low compared to western countries where undercooked meats are frequently eaten. However their importance in HIV infected individuals cannot be overemphasised.

In spite of the fact that none of the intestinal microsporidia have been reported in Sri Lanka a careful search for them is indicated in immunoincompetent persons including those with HIV infection. The intestinal microsporidia have a wide distribution from countries in the west to Australia and there is no doubt that they must occur in Asian countries. *Leishmania* has been reported in the indigenous population in Sri Lanka although rarely[37] and some of these have been in foreigners or those who have lived in endemic areas outside this country. Hence this infection too must be borne in mind in the future.

Of the parasites in Table 2 *B. hominis* was known to be present in stools of persons in this country for many years, yet there have been no published reports. The only report of the parasite in Sri Lankans has been a very recent finding that about 33% of Tamil asylum seekers in Switzerland have this infection[38].

It is also of interest to note that *Microsporidium ceylonensis* is the first microsporidian parasite to be reported from the eye of a patient anywhere in the world. This patient was not immunoincompetent but was a 11 year old boy who was gored by a goat a few years before. The spores that may have been introduced into the deep stroma of the cornea must have germinated and developed in this immunopriviledged site[19].

Sarcocystis is known to be a common parasite of animals in Asian countries including Sri Lanka. A number of cases of muscle cysts have been reported from humans in Malaysia where it has been shown that around 21% of persons harbour the parasite[39]. The reservoir definitive host is believed to be a dog or cat and the intermediate hosts in nature are probably monkeys. There is no doubt that this parasite will be found in this country if looked for[40]. If so, HIV infected persons who become infected may acquire active or disseminated infection with the muscle cysts which may even rupture and cause further problems.

Another parasite that may be important in Asian countries is *Babesia*. This is a non-pigmented red cell parasite common in dogs and herbivores like cattle.

The fact that several cases are on record in other countries of splenectomised persons getting infected with such zoonotic parasites even with fatal consequences suggests that accidental infection of HIV positive persons by infected ticks in this country may cause problems in the future.

In conclusion it must be stated that none of the known parasites that are involved in AIDS have been studied in AIDS cases in this country and hence there is an urgent need for this to be done and especially to look for intestinal microsporidian infections.

Acknowledgements

I am grateful to Miss J A Asoka and Mr K B A T Bandara of the Dept. of Parasitology, Faculty of Medicine, Colombo 8 for help with the typing of the manuscript and tables.

References

1. Castellani A. Note on certain Protozoa-like bodies in a case of protracted fever with splenomegaly. *Journal of Tropical Medicine and Hygiene* 1914; 17: 113-114 (and 2 plates).
2. WHO, Acquired immunodeficiency syndrome (AIDS), Interim proposal for a WHO staging system for HIV infection and disease. *Weekly epidemiological Record* 1990; 65: 221-228.
3. Lucas SB. Missing infections in AIDS. In: Parasitic and other infections in AIDS. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1990; 84: (supplement-1) 34-38.
4. Yoshida Y. Ultrastructural studies of. *Pneumocystis carinii, Journal of Protozoology* 1989; 36: 53-60.
5. Yoshida Y. Diagnosis and treatment of *Pneumocystis carinii* pneumonia, an important opportunistic infection in AIDS patients. *Asian Medical Journal* 1992; 35: 415-424.
6. Matsuda S; Urah Y, Shiota T *et al.* Disseminated infection of *Pneumocystis carinii* in a patient with acquired immunodeficiency syndrome. *Virchows Archiv A Cell Pathological Anatomy and Histopathology.* 1989; 414: 523-527.
7. Bernard EM, Sepkowitz KA, Telzak EE, Armstrong D. Pneumocystosis. In: Medical management of AIDS patients. *Medical Clinics of North America* 1992; 76: 107-119.
8. White DA, Zaman MK. Pulmonary disease. In: Medical management of AIDS patients. *Medical Clinics of North America* 1992; 76: 19-44.
9. Matsubayashi H, Koike T, Mikata I, Takei H, Nagiwara S. A case of Encephalitozoon-like body infection in man. *AMA Archives of Pathology* 1959; 67: 181-187.
10. Desportes I, Le Charpentier Y, Galian A *et al.* Occurrence of a new microsporidian *Enterocytozoon bieneusi* n.g. n.sp. in the enterocytes of a human patient with AIDS. *Journal of Protozoology* 1985; 32: 250-254.
11. Modigliani R, Bories C, Le Charpentier Y *et al.* Diarrhoea and malabsorption in acquired immune deficiency syndrome: a study of four cases with special emphasis on opportunistic protozoan infestations. *Gut* 1985; 26: 179-187.
12. Cali A, Owen RL. Intracellular development of *Enterocytozoon*, an unique microsporidian found in the intestine of AIDS patients. *Journal of Protozoology* 1990; 37: 145-155.
13. Curry A, Canning EU. Human microsporidiosis. *Journal of Infection* 1993; 27: 229-236.
14. Field AS, Hing MC, Milliken ST, Marriott DJ. Microsporidia in the small intestine of HIV-infected patients. A new diagnostic technique and a new species. *Medical Journal of Australia* 1993; 158: 390-394.
15. Lumb R, Swift J, James C, Papanaoum K, Mukherjee T. Identification of the microsporidian parasite *Enterocytozoon bieneusi* in faecal samples and intestinal biopsies from an AIDS patient. *International Journal of Parasitology* 1993; 23: 793-801.
16. Cali A, Kotler DP, Orenstein JM. *Septata intestinalis* n.g.n.sp. an intestinal microsporidian associated with chronic diarrhoea and dissemination in AIDS patients. *Journal of Eukaryotic Microbiology* 1993; 40: 101-112.
17. Didier ES, Didier PJ, Friedberg DN *et al.* Isolation and characterization of a new human microsporidian. *Encephalitozoon hellem* (n.sp.) from three AIDS patients with keratoconjunctivitis. *Journal of Infectious Diseases* 1991; 163: 617-621.
18. Cali A, Meisler DM, Lowder CY *et al.* Corneal microsporidioses: characterization and identification. In: Workshop on *Pneumocystis, Cryptosporidium* and microsporidia. *Journal of Protozoology* 1991; 38: 215 S - 217 S.
19. Ashton N, Wirasinha PA. Encephalitozoonosis (Nosematosis) of the cornea. *British Journal of Ophthalmology* 1973; 57: 669-674.
20. Hollister WS, Canning EU, Colbourn NI, Curry A, Lacey CJN. Characterization of *Encephalitozoon hellem* (Microspora) isolated from the nasal mucosa of a patient with AIDS. *Parasitology* 1993; 107: 315-358.
21. Canning EU, Curry A, Lacey CJN, Fenwick JD. Ultrastructure of *Encephalitozoon* sp. infecting the conjunctival, corneal and nasal epithelia of a patient with AIDS. *European Journal of Protistology* 1992; 28: 226-237.
22. Canning EU. Protozoan infections. In: Parasitic and other infections in AIDS. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1990; 84: (supplement 1) 19-24.
23. Gellin BG, Soave R, Coccidian infections in AIDS, toxoplasmosis, cryptosporidiosis, isosporiasis. In: Medical management of AIDS patients. *Medical Clinics of North America* 1992; 76: 205-234.
24. de Hovitz JA, Pape JW, Boncy M, Johnson WD. Clinical manifestations and therapy of *Isospora belli* infection in patients with acquired immunodeficiency syndrome. *New England Journal of Medicine* 1986; 315: 87-90.
25. Restrepo C, Macher AM, Radany EH. Disseminated extraintestinal isosporiasis in a patient with acquired immunodeficiency syndrome. *American Journal of Clinical Pathology* 1987; 87: 536-542.
26. Lumb R, Hardiman R. *Isospora belli* infection. A report of two cases with AIDS. *Medical Journal of Australia* 1991; 155: 194-196.
27. Zumla A, Croft SL. Chemotherapy and immunity in opportunistic parasitic infections in AIDS. *Parasitology* 1992; 105: S 93-S 101.
28. Fleming AF. Opportunistic infections in AIDS in developed and developing countries. In: Parasitic and other infections in AIDS. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1990; 84: (Supplement 1) 1-6.
29. Dissanaike AS. *Blastocystis hominis*: an old organism with a new significance. *Ceylon Medical Journal* 1993; 38: 7-9.
30. Welsby PD. Infectious diseases and AIDS. *Postgraduate Medical Journal* 1992; 68: 415-433.
31. Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. *Cyclospora* species — a new protozoan pathogen of humans. *New England Journal of Medicine* 1993; 328: 1308-1312.
32. Butcher AR, Lumb R, Coulter E, Nielsen DJ. Coccidian/*Cyanobacterium* like body associated diarrhoea in an Australian traveller returning from overseas. *Pathology* 1994; 26: 59-61.
33. Wakefield AE, Banerji S, Pixley FJ, Hopkin JM. Molecular probes for the detection of *Pneumocystis carinii, Transactions of the Royal Society of Tropical Medicine and Hygiene* 1990; 84: (supplement 1) 17-18.
34. Sheather AL. The detection of intestinal protozoa and mange parasites by flotation technique. *Journal of Comparative Pathology* 1953; 36: 268-275.
35. Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, Visvesvara GS. Improved light microscopic detection of microsporidia spores in stool and duodenal aspirates. *New England Journal of Medicine* 1993; 326: 161-166.
36. Van Gool T, Canning EU, Dankert J. An improved practical and sensitive technique for the detection of microsporidian spores in stool samples, *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1994; 88: 189-190.
37. Dissanaike AS. Parasitic zoonoses in Sri Lanka. *Ceylon Medical Journal* 1993; 38: 150-154 and 184-187.
38. Cornen D, Gerber A, Dubach UC. Pravalenz von *Blastocystis hominis* bei Asylbewerbern. *Deutsche Medizinische Wochenschrift* 1987; 112: 1477.
39. Wong KT, Pathmanathan R. High prevalence of human skeletal muscle sarcocystosis in south-east Asia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1992; 86: 631-632.
40. Dissanaike AS. Human *Sarcocystis* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1994; 88: 364.