methyleneblue technique as suggested by BAXBY & BLUNDELL (1983) was used on one occasion.

A total of 214 samples was collected of which 86% were from children under five years. Two of the children at the POSGH were positive for *Cryptosporidium* oocysts. One (JJ) had a five-day history of vomiting and diarrhoea and a low grade fever. The diarrhoea was described as watery and yellow with mucus. The second case (AC) when admitted to the POSGH had a four-day history of vomiting, diarrhoea and fever. Initially, his stools were small, loose, greenish and mucoid, but had become formed by the tenth day after he developed the diarrhoea. The third case was in a 22-year-old bisexual male (SA) with a history of unproductive cough, pharyngitis, pyrexia, diarrhoea and anorexia and weight loss of 15 kg over an eight-month period. He was subsequently diagnosed as an AIDS patient.

With the modified Ziehl-Neelsen stain, *Cryptosporidium* oocysts stained pink to red and internal structures were clearly seen. The oocysts measured 4 to 6 µm and were easily contrasted with background material. Yeasts stained green. Using the safranin-methylene blue technique, oocysts stained orange to pink while background material stained blue.

Cryptosporidium has been reported in tourists visiting the Caribbean and the parasite was diagnosed in a two-year-old child in Saint Lucia (MA *et al.*, 1985). The presence of the parasite is reported for the first time in man in Trinidad and Tobago, a second commonwealth Caribbean country. It is not surprising that the presence of the parasite has not heretofore been detected by technicians in the Caribbean because the techniques used to demonstrate the oocysts of the parasites are not normally used in a parasitology laboratory.

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ELISHA S. TIKASINGH[1]
S. SMITH[2]
T. POON-KING[2]
D. BRATT[3]

[1]*Caribbean Epidemiology Centre (PAHO/WHO), P.O. Box 164, Port of Spain, Trinidad and Tobago*
[2]*General Hospital, San Fernando, Trinidad and Tobago*
[3]*University of The West Indies, Port of Spain, Trinidad and Tobago*

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Isolation and characterization of leishmanias from Nicaragua

Cutaneous and mucocutaneous leishmaniasis are endemic in the Northern and Eastern regions of the Republic of Nicaragua, and although it has been assumed that the infections are caused by members of the *Leishmania braziliensis* group (URCUYO & ZAIAS, 1982), the identities of the organisms concerned have not been reported. The epidemiology of leishmaniasis in Nicaragua is likewise completely unknown, and although 21 species of sandfly have been recorded in the country (ZELEDON & MURILLO, 1983), nothing is known of their role as vectors of Nicaraguan leishmaniasis. The clinical aspects of leishmaniasis in the country were first described by MISSONI & MORELLI (1984), but until now there have been no reports of the isolation and characterization of *Leishmania* in Nicaragua.

Recently we have isolated leishmanial organisms from two patients presenting with cutaneous leishmaniasis in Nicaragua and characterized the organisms using isoenzyme techniques. The patients were living in the northern regions of Matagalpa and Jinotega (Region VI). The first (MM1) was a 19-year-old male who presented with a dry, crusty, round lesion 30 mm in diameter of approximately two month's duration on his left wrist. The other (NICA2) was a nine-month-old girl with a round 8 mm diameter ulcerated lesion of less than one month's duration on her right wrist. The organisms were isolated by inoculation into modified Tobie's medium and taken to London for characterization.

Isoenzyme analysis of the organisms by thin-layer starch gel electrophoresis using 10 enzymes (ALAT, ASAT, GPI, MDH, MPI, 6PGDH, PGM, SOD, NH and PEP-D) showed that both isolates belonged to the *L. braziliensis* group. One, MHOM/NI/84/MM1, was indistinguishable from the WHO recommended reference strain of *L. b. panamensis* MHOM/PA/71/LS94 in all enzymes examined except MDH, where a slight difference in electrophoretic mobility was seen. The other strain, MHOM/NI/84/NICA2, was like the reference strain in all enzymes except 6PGDH and PEP-D where in both cases triple bands rather than the expected single band were found. This infra-

subspecific isoenzyme variation is not uncommon (Evans, unpublished) and certainly both organisms are isozymically closer to *L. b. panamensis* that to any other member of the *L. braziliensis* group.

Obviously many more leishmanial isolates from different areas of the country, from presumed vectors and from possible reservoir hosts need to be examined before we can have an adequate parasitological knowledge of leishmaniasis in Nicaragua. It is for instance very important to establish whether or not *L. b. braziliensis* occurs in the country, as it is known that at least 8% of patients in Nicaragua with cutaneous leishmaniasis present with mucosal involvement.

E. MISSONI[1]
R. MORELLI[2]
L. BALLADARES[2]
J. BERRIOS[2]
M. D. DE SOLAN[2]
C. I. BALDWIN[1]
D. A. EVANS[1]

[1]*Dept. of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*
[2]*MINSA, Ministerio de Salud, Region VI, Matagalpa, Nicaragua*

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Low parasite specific T cell response in clinically immune individuals with low grade *Plasmodium falciparum* parasitaemia

Immunity to *Plasmodium falciparum* infection is thought to rely on both cellular and humoral immune responses (PLAYFAIR, 1982). Antibodies against merozoites, which are believed to protect the host by preventing the reinvasion of erythrocytes, are thought to play an important role in the humoral part of the protective response. Less is known of the cellular response regarding both the importance for protection and the mechanisms by which the parasites are harmed. We have shown earlier that lymphocytes isolated from patients who had suffered from malaria one month previously responded to soluble immunoadsorbent purified *P. falciparum* antigens (SPag) in a lymphocyte-proliferation assay, whereas lymphocytes from donors never exposed to malaria did not elicit such response (BYGBJERG *et al.*, 1985).

In order to establish if lymphocytes from clinically immune Africans responded to SPag, blood was collected from healthy individuals living in a malaria hyperendemic area of West Africa. The donors denied any recent clinical history of malaria, and did not take antimalarials. The lymphocytes were isolated from peripheral blood by "Lymphoprep" gradient centrifugation, and the proliferative response to purified protein derivative of tuberculin (PPD) and SPag were assayed in *in vitro* cultures by incorporation of ^{3}H-thymidine (THEANDER *et al.*, 1986a). The response was measured as cpm in stimulated cultures−cpm in unstimulated cultures. In parallel, Giemsa stained smears of peripheral blood were examined. *P. falciparum* parasites were found in 5 of 22 donors. One donor had trophozoites (parasitaemia less than 0·1%), and the remaining four had gametocytes in the blood. As shown in Table 1 the lymphocytes isolated from donors with parasites exhibited a low proliferative response to SPag (median 1·7 kcpm) as compared with those of aparasitaemic donors (median response 25·0 kcpm). The difference was statistically significant ($P<0·025$, Wilcoxon Rank Sum Test). In contrast there were no significant differences between the PPD responses of the two groups.

Table 1— ^{3}H-thymidine incorporation in lymphocytes isolated from clinically malaria immune Africans with or without parasitaemia as judged by microscopy of Giemsa-stained blood smears. The lymphocytes were stimulated with soluble purified *P. falciparum* antigens (SPag) or PPD. Results are shown as cpm × 10^{-3}. The results of parasite free subjects are presented as median and range.

Subjects	Parasites in smear	SPag response	PPD response
1	tropho*	0	107·5
2	gameto**	1·3	43·1
3	gameto	1·7	18·3
4	gameto	3·7	80·9
5	gameto	4·0	60·3
6-22	none	25·0† (0·4-86·4)	76·2 (3·8-200·4)

†Significant difference ($p<0·025$) between subjects with parasitaemia (1 to 5), and parasite-free subjects (6-22)
*trophozoites
**gametocytes

We have earlier reported that the cells responding to SPag are found among the T-helper cells (leu 3 positive cells) THEANDER *et al.*, 1986b). Our data indicate that T-cells isolated from individuals with microscopically sterile immunity have higher reactivity to SPag than T-cells isolated from healthy donors with low grade parasitaemia. Other studies have indicated that an active suppression of parasite-specific lymphocyte-proliferative response takes place in non-immune individuals suffering from acute *P. falciparum* malaria; however, such suppression was transient and disappeared during the first week after treatment (BYGBJERG *et al.*, 1986; THEANDER *et al.*, 1986a).

Whether the low SPag reactivity in clinically immune individuals with low grade parasitaemia, reported here, was caused by an active suppression of SPag-reactive T-cells or a lack of SPag-reactive T-cells is a matter for conjecture. However, our preliminary finding that clinically immune individuals with low grade parasitaemia showed a lower T-cell response to