To the Editors:

First successful *in vitro* culture of *Leishmania* sp. causing autochthonous visceral leishmaniasis in Sri Lanka

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The first autochthonous case of visceral leishmaniasis (VL) in Sri Lanka was reported in 2007 [1], more than a decade after the first report of autochthonous cutaneous leishmaniasis (CL) in 1992 [2]. Since 2007 only three endemic patients with confirmed VL was reported from the country (two from the Anuradhapura district), and the current report is on the third patient (from the Vavuniya district). Few cases of mucocutaneous leishmaniasis (MCL) have been reported in the country to date [3, 4]. However, thus far successful *in vitro* culture of the *Leishmania* parasite could be carried out only in the strains giving rise to cutaneous leishmaniasis [5]. We report the first successful *in vitro* culture of *Leishmania* sp. causing endogenous VL in Sri Lanka.

A diagnostic bone marrow aspiration was carried out in a 57-year old male from Vavuniya district who presented with pyrexia of unknown origin of 6 months duration. There was no history of overseas travel, but he had an occupational history of being in the jungle as a civil soldier for many months in the Vavuniya district during the North-East civil war immediately prior to occurrence of symptoms. The patient was pale but he had no generalised lymphadenopathy. He had a moderate, firm hepato-splenomegaly. The bone marrow aspiration contained numerous *Leishmania* amastigotes (Figure 1a). The trephine biopsy showed the typical dot and dash appearance of *Leishman donovan* (LD) bodies (Figure 1b).

A few drops from the same bone marrow aspirate were inoculated into medium 199 supplemented with 10% FBS, 200 mM glutamine, 25 mM adenosine, folic acid 10 mg/ml and penicillin/streptomycin 100 µl/ml. The cultures were maintained at 24ºC. First bone marrow culture was sub-cultured 3 days after inoculation to eliminate the action of bone marrow inhibitory factors on the growing promastigotes.

The original cultures showed a promastigote count of 1×10^4/ml five weeks after the first inoculation. The first subculture inoculated 3 days after the first inoculation showed a promastigote count of 7×10^4/ml five weeks after inoculation (Figure 1c).

Though endogenous cases of cutaneous, mucocutaneous and visceral forms of leishmaniasis are being reported in the country, the sandfly vector is yet to be identified. Few reports show the dog as a possible reservoir host of leishmaniasis in Sri Lanka [6, 7]. However, in India, humans are the only reservoir host for VL (Kala-azar) [8]. The patient reported in this study had close contact with the jungle, raising the question of the existence of a possible sylvatic cycle for VL in Sri Lanka in contrast to the disease pattern that is seen in India.

The success of *in vitro* culture of the strain causing VL in Sri Lanka will pave the way for the identification of the *Leishmania* parasite to species and strain level. The strain identification will be carried out by performing isoenzyme studies which is the gold standard for *Leishmania* strain identification [8]. This early success of *in vitro* culture of the *Leishmania* parasite causing visceral leishmaniasis and possible strain identification in the near future will have a bearing on understanding of the disease pattern of VL in Sri Lanka, and will enable us to implement adequate preventive measures.

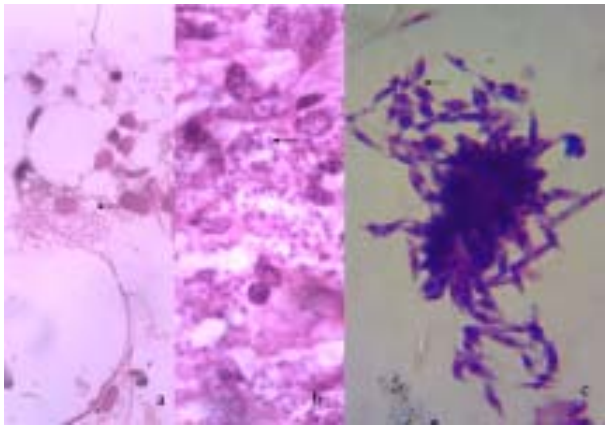

Figure 1a. **Bone marrow aspiration showing numerous amastigotes within a histiocyte (Leishman, 40x).**
Figure 1b. **Trephine biopsy showing LD bodies (H & E, 100x).**
Figure 1c. **First *in vitro* subculture of bone marrow aspirate with the promastigotes (Geimsa 100x).**

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