Visceral Leishmaniasis (Kala-Azar) Outbreak in Somali Refugees and Kenyan Shepherds, Kenya

To the Editor: A sharp increase in suspected visceral leishmaniasis (VL or kala-azar) cases was reported in April through May 2000 in three Kenyan refugee camps (Ifo, Dagahaley, and Hagadera). Located around Dadaab town in Northeastern Province, the three camps house an estimated 125,000 Somali refugees. VL outbreaks have been well documented in five distinct foci in Kenya (1,2), but until this outbreak, VL was only sporadically seen in the refugee camps or the province.

We investigated a possible outbreak in the refugee sites. Before April 2000, doctors would request a formol-gel test (FGT) in case of suspected VL and treat an FGT-positive case with antimonials. Although the FGT is of uncertain validity, it is still used in district hospitals in Kenya for lack of alternative diagnostic tests. We considered a clinician’s request of an FGT as a proxy for “clinical VL suspicion” and assessed the number of FGTs done from January 1999 to March 31, 2000. The first suspected VL patient was traced back to August 1999; this 40-year-old male Somali refugee had been ill for 8 months and sought treatment at Dagahaley camp. He responded well to antimonial treatment. From that date to April 1, 2000, an FGT was requested for five more patients; results were positive for two.

Specific surveillance for VL was set up by the refugee health services in April 2000. Suspected patients or their caretakers were interviewed. Finger-prick blood was collected on filter paper and analyzed by direct agglutination test (DAT) (3). In August 2000, splenic aspirates were performed on eight patients for direct microscopic examination, and parasite culture was attempted for three specimens. In vitro isolation and gp63 polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) molecular typing was done at the Protozoology Unit of the Prince Leopold Institute of Tropical Medicine in Antwerp, Belgium. Serologically or parasitologically confirmed cases were given antimonial treatment. From that date to April 1, 2000, an FGT was requested for five more patients; results were positive for two.

Immediate outbreak control measures have been taken by refugee camp health authorities, the surveillance system was strengthened (including initiation of active case-finding measures), and diagnostic and therapeutic facilities were upgraded. Six-month peridomestic spraying of the refugee shelters with lambda-cyhalothrin (ICON) is a routine vector control measure in the camps. Special attention needs to be paid, however, to the food security for the refugees in the current outbreak. Our observations on 16 imported cases also raise concerns about VL transmission in drought-affected northeastern Kenya. Malnutrition is a known risk factor for the development of clinical VL in infected persons (6). In southern Sudan, deaths caused by VL were attributed to malnutrition in a famine- and war-stricken population (7). The current nutritional status of the Somali refugees in the Dadaab camps is precarious. After the 1996 food scarcity problem (8), food rations for refugees were maintained at the recommended 2,100 kcal/person/day. In February 2000, however, the ration was again reduced below the vital minimum. A cross-sectional random cluster survey on August 29-31, 2000, showed rising malnutrition levels in <5-year-old refugee children (Médecins sans Frontières, unpub. data).

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Doxycycline and Eradication of Microfilaria in Patients with Loiasis

To the Editor: Wolbachia are intracellular symbionts found in 20% of insects and in several nematodes, including filarial worms. Because tetracycline eradicates Wolbachia in nematodes, this drug has been proposed for chemotherapy in filariasis (1). We report two patients with loiasis in whom no Wolbachial DNA was detected in microfilariae by polymerase chain reaction (PCR), and for whom 6 weeks of doxycycline failed to eradicate the microfilaremia. We conclude that doxycycline may not be an efficient therapy for loiasis.

Filariae are responsible for 150 million infections worldwide, some of them devastating diseases such as elephantiasis (caused by Bancroftian and Brugian filariae) and blindness (caused by onchocerciasis). There is no satisfactory treatment for filariasis: although diethycarbamazine citrate has been used for 50 years to treat the disease, its efficacy is limited, and 1% of infected individuals are still suffering from the disease after treatment (2). Doxycycline 200 mg daily for 6 weeks as previously described in O. volvulus-infected patients (1). We observed patients for microfilariaemia every week for 6 weeks and then every 2 weeks for 2 months. The presence of adult worms was detected by physical examinations.

Microfilariaemia was detected in both patients at the completion of treatment and at day 120 of follow-up. In patient no. 1, the frequency of migrating adult worms seemed to diminish during therapy, but they never disappeared. For Wolbachia detection in worms, blood samples were collected both in Dupont-Isolator and EDTA-containing tubes. After centrifugation at 5000 x g for 30 min, the worm-enriched pellet was resuspended in 1 mL of sterile deionized water for erythrocyte lysis. DNA was extracted from the suspension by using the QiAmp-blood kit (Qiagen, Hilden, Germany) following manufacturer’s recommendations. W. pipientis DNA was used as a positive control.

Control of DNA extraction was performed by amplifying microfilarial DNA using the nematode-specific 18S rDNA-derived primers 18SF (5’-GAT-ACC-GCC-CCA-TGA-GTG-AC-3’) and 18SR (5’-ACC-AA-TAA-GAA-CG-CGA-TG-3’). Wolbachia detection was attempted with the FD1 (5’-AGT-GTG-TGA-TGA-GGA-TGG-TC-3’) and Rp2 (5’-ACG-GTT-TTA-CGA-TAT-3’) eubacterial primers, with primer specific for the 16S rDNA primers EHR16SD (5’-GGT-ACC-YAC-AGA-AGT-CC-3’) and EHR16SR (5’-TAC-CAC-TCA-TGG-TTA-GCA-GC-3’), and with primers specific for the 16S rDNA of B. malayi endosymbiont, Bsymbf (5’-AGC-TAT-GTA-ACT-3’) and Bsymbr (5’-CTC-TCG-GAT-AAG-AAT-3’) (3-6). PCR reactions were performed on PTC-200 thermocycler (MJ-Research, USA) by using 45 cycles of denaturation at 94°C for 30 sec, hybridization for 45 sec, and elongation at 72°C for 1 min. Hybridization temperatures were 55°C for FD1/Rp2, 53°C for EHR16SD/EHR16SR, 42°C for Bsymbf/Bsymbr, and 57°C for 18SF/18SR. Experiments were repeated three times.

We detected Wolbachia in the positive control and 18s rRNA of the nematode in the sample, but no signal compatible with Wolbachial DNA was obtained with the sets of primers used. In fact, four species of filariae (Dipetalonema setariosum, Acanthocheilonema vitae, O. flexuosa, and L. loa) tested for intracellular bacteria by electron microscopy, immunohistochemistry, or PCR had no bacteria (4). The absence of Wolbachia in L. loa microfilariae may explain the failure of tetracycline therapy in our patients.

More work is needed to determine the prevalence of Wolbachia in filariae, their impact on fertility in each species, and the use of antibacterial agents for eradicating these pathogens.

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