Reply to Das

To the Editor—In the correspondence [1], Sushmita Das discusses the parasite fingerprinting methods used in our report on increased failure of miltefosine for the treatment of visceral leishmaniasis in Nepal [2]. Fingerprinting is a major support for interpreting studies on treatment efficacy of infectious diseases: the comparison of pathogens present at the onset of treatment and at the time of a new clinical episode aims to distinguish relapse from reinfection, the former being critical for drug efficacy.

This molecular tracking requires a highly discriminatory genotyping method, to increase the power to reject the null hypothesis of identity between the 2 samples. The task is complicated when the pathogen population under study is genetically homogeneous: how to distinguish a relapse from a reinfection with the same genotype? Furthermore, the degree of genetic homogeneity also depends on the genotyping method that is used. Das correctly highlights the possible confusion that can arise from the literature, with some studies reporting genetic homogeneity in *Leishmania donovani* from the Indian subcontinent (ISC), while others mention the occurrence of genetic polymorphism. A clear definition of genetic polymorphism is needed, and most of all, it should be interpreted in the context of the genotyping method itself. One cannot claim that *L. donovani* from ISC is polymorphic because of the occurrence of 2 zymodemes in the region [3]; this means only that with multilocus enzyme electrophoresis (MLEE, a method with limited resolutive power), 2 genetic variants were observed in that population. Multilocus microsatellite typing (MLMT) is more resolutive and was shown to detect 6 genetic variants in a sample of ISC parasites, whereas in the same sample of strains, multilocus single-nucleotide typing (MLST) evidenced 21 genotypes [4]. There is no contradiction between these different reports, but genetic polymorphisms should be described by comparison with other populations of parasites. For instance, each of the molecular methods mentioned above detect fewer genetic polymorphisms in *L. donovani* from ISC than in *L. donovani* from East Africa. Hence, *L. donovani* from ISC can indeed be considered to be a relatively low polymorphic species: recent findings suggest that this resulted from a recent clonal expansion following an antimalarial DDT campaign in the 1960s [4, 5].

At the time of running our clinical study [2], the most discriminatory method available for direct genotyping in clinical samples (thus without parasite isolation and cultivation, as needed for MLEE, MLMT, or MLST) of *L. donovani* was kinetoplast DNA polymerase chain reaction–restriction fragment-length polymorphism analysis, previously shown to resolve twice as many genotypes as MLMT [6]. During molecular tracking, nearly identical genotypes were encountered in each patient at the onset of treatment and at the time of relapse, whereas strains with different genotypes were observed between the different patients [2]. The most parsimonious explanation is that the same strain survived the treatment; thus, the patient relapsed. Obviously, an ultimate genotyping method such as whole genome sequencing [5] would probably reveal small

differences between pairs of samples and would offer more power to the analysis. Further developments are still needed to allow their direct application on clinical samples.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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