Understanding drug resistance in natural *Leishmania donovani* populations: Are we underestimating the legacy left by antimonials to Kala-Azar control?

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Antimonial (SbV) treatment failure linked to *L. donovani* drug resistance has been repeatedly reported in the Indian subcontinent over the past 10 years. This problem is now being countered on clinical level by changing first line treatment from antimonials to miltefosine in the frame of the Kala-Azar Elimination Programme. We analysed how Nepalese *L. donovani* populations have responded to past years of clinical SbV pressure and the results suggest that present SbV-adapted parasite populations might continue to challenge VL control, despite the change of drug policy.

The mechanism of action of SbV is complex and includes reduction to the leishmanicidal SbIII, but also stimulation of host macrophages to impose lethal oxidative (and nitrosative) stress on the intracellular parasites. The latter aspect was only recently demonstrated and suggests that SbV-resistant parasites might also be resistant to oxidative stress, one of the central defence tools of the host immune system. This hypothesis could have severe epidemiological implications, as parasites armoured against host defence tools are likely to be harder to combat and contain, and thus continue to threaten VL control.

The validity of this hypothesis was verified here by characterising the oxidative stress defence mechanisms of *L. donovani* clinical isolates with variable SbV susceptibility through (i) transcriptomic/proteomic profiling of genes/proteins with a central role in parasite oxidative stress defence; (ii) quantitation of parasite thiols, the central metabolites in oxidative stress protection and (iii) assessment of parasite survival upon direct oxidative/nitrosative stress exposure. The results suggest that natural SbV-resistance is heterogeneous on cell biological level; however changes in central players of oxidative stress defence are a recurrent feature. The significance of the results will be discussed in the context of epidemiological surveillance of drug resistance and Kala-Azar control programs.

This study was supported by EC FP5 INCO-DEV ICA4-CT-2001-10076; EC FP6 Marie-Curie programme DonNA-R 041465 and InBev Baillet-Latour fund for neglected tropical diseases.