

4. Am J Trop Med Hyg. 1992 Dec;47(6):852-92.

Biochemical characterization and zymodeme classification of *Leishmania* isolates from patients, vectors, and reservoir hosts in Kenya.

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A total of 407 *Leishmania* and other *Leishmania*-like isolates obtained from patients, other vertebrates, sand fly vectors, and other arthropods from Kenya and other countries were characterized and compared with several World Health Organization and other well-characterized reference strains of *Leishmania*, *Trypanosoma*, *Crithidia*, *Herpetomonas*, and *Leptomonas* by cellulose acetate electrophoresis (CAE), using 20 enzyme systems. Analysis of the isoenzyme banding patterns (IBP) of the isolates generated isoenzyme profiles that were resolved as zymodemes and tabulated. Isolates that produced similar isoenzyme profiles in all 20 enzyme systems were placed into a particular *Leishmania* isoenzyme taxon, with the zymodeme designated numerically as Zn. A total of 66 zymodemes were recorded for the 407 isolates studied. To obviate the need to draw all 66 representative IBP for each of the 20 enzyme systems, the 66 zymodemes (Z1-Z66) were again placed into similarity groups represented by pattern number or Pn. This resulted in 23-50 IBP (Pn) per enzyme system. The highest number of IBP scored was for

malate dehydrogenase (MDH) (P1-50) and the lowest score was for glucose-6-phosphate isomerase (GPI) (P1-23). From these different isoenzyme profiles or zymodemes, IBP of 14 (MDH, GPI, nucleoside hydrolase, phosphoglucomutase, malic enzyme, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, mannose-6-phosphate isomerase, 6-phosphogluconate dehydrogenase, glutamate oxaloacetate transferase/aspartate aminotransferase, glutathione reductase, superoxide dismutase, fumarase, and glyceraldehyde-3-phosphate dehydrogenase) of the 20 enzyme systems were selected for computer-calculated numerical taxonomy. Consistent individual isoenzyme bands with similar relative mobilities of the 14 enzyme systems were scored into groups (allelomorphs, allozymes, or electromorphs) and used in cluster analysis. For each pattern in every profile, the presence of a consistent band was entered as 1 and its absence as 0. A total of 419 allozyme characters (variables) were scored for the 14 enzyme systems. Lastly, all different zymodemes sharing a particular IBP (P_n) within an enzyme system were counted and the total number was shown as a zymodeme frequency (Z_f). Final analysis of the CAE isoenzyme profiles and cluster-dendrograms resulted in the identification of several potentially new species and subspecies of *Leishmania* and other *Leishmania*-like isolates from patients, sand flies, and animal reservoir hosts collected from Kenya and other locations in Africa. Zymodeme analysis of the Kenyan visceral and cutaneous leishmaniasis isolates resulted in the identification of 11 subpopulations of the *L. donovani* species complex and six subpopulations of the *L. tropica* species complex endemic to different geographic areas of Kenya.

PMID: 1471744 [PubMed - indexed for MEDLINE]

