Human leishmanial diseases are endemic to 88 countries. Global commerce and travel result in U.S. patients with histories indicative of exposure to more than one regional leishmanial species subset. Recent data from HIV-leishmanial coinfected patients together with modern tests of the asymptomatic cohorts of leishmanial patients have provided substantial evidence that leishmanial diseases of humans can be subclinical often for decades. Use of diagnostic test(s) having stringent species specificity either require 20 different species-specific tests or, alternatively, the risk that testing for a related, but nonidentical species will result in a false negative. Modern leishmanial diagnostic tests are designed to exhibit exquisite specificity, thereby solving many problems inherent to regional leishmanial diagnosis. However, given the dual problems of global travel and lengthy prepatency typical among a young, fit, well-nourished patient population, it is our experience that species-specific testing may not currently provide the most appropriate (or technically feasible) diagnostic solution. Techniques we use for diagnosis are designed to detect diverse leishmanial species worldwide. Since August 1995, we have isolated *Leishmania (Viannia) panamensis, Leishmania (Viannia) guyanensis, Leishmania (Viannia) braziliensis, Leishmania (Leishmania) infantum, Leishmania (Leishmania) tropica, Leishmania (Leishmania) mexicana* from patient visceral or cutaneous biopsies. To facilitate biopsy, we have developed a "transport medium", useful to preserve parasites within a biopsy in viable condition during extended shipment (10-12 days). The patient positivity rate using our battery of diagnostic methods is approximately 73%. Complex patient travel histories have included exposure in nine endemic countries on 3 continents within 7 years. Many patients (>30%) have histories of possible exposure within more than one endemic country.

208 *LEISHMANIA TROPICA* ON NNN MEDIUM SUPPLEMENTED WITH HUMAN VS RABBIT BLOOD: BIOLOGICAL AND EPIDEMIOLOGICAL COROLLARIES. Bichichi M, Laraki H, Riyad M, and Guessous-Idrissi N. Unité d'Etudes et de Recherche sur les Leishmanioses, Faculté de Médecine et Centre Hospitalier Ibn Rochd Casablanca, Morocco.

Leishmania parasites are commonly cultivated on NNN medium supplemented with rabbit blood making this medium unavailable for routine diagnosis laboratories. On the other hand, *L. tropica* is usually associated with an anthroponotic transmission, even if in several foci, a zoonosis could not be excluded. The purpose of this study is the growth analysis of *L. tropica* stocks on NNN medium supplemented with human blood comparatively with rabbit blood in order to assess the value of human blood as a more available substitute. Furthermore, the comparative growth on these two media could be a further argument for the anthropophilic or zoophilic character of different *L. tropica* stocks. Thus, 11 Moroccan *L. tropica* stocks were analyzed : 10 from human cutaneous cases either from urban or rural foci and one from a canine visceral case. All these stocks were seeded in the two media under uniform conditions and the growth rate and survivability were measured and compared. The preliminary results show a better growth of human *L. tropica* stocks in NNN supplemented with human blood. These data will be presented and analyzed in term of their biological and epidemiological significance.

209 VALIDATION OF RFLP TOOLS TO STUDY GENETIC POLYMORPHISMS OF *LEISHMANIA MAJOR* AND *L. TROPICA* IN TUNISIA. Meddeb F * and Guizani I. Laboratoire d'Epidémiologie et d'Ecologie Parasitaire, Institut Pasteur de Tunis, Tunisia.

In a previous report, we presented the development of an RFLP approach to study the genetic diversity of *Leishmania infantum* parasites. Three physically independant DNA probes pDK30, rK39 and Lt1 were applied on digests obtained with either one of the enzymes PstI, EcoRI and XhoI. A total of 48 *L. infantum* DNAs were analysed by the 9 possible probe/enzyme combinations. This way it was possible to estimate the variability indexes according to countries or to probes. It was also possible to assess the existence of potential departures to the null hypothesis of panmixia within a selection of Tunisian *L. infantum* parasites. This allowed to design collection protocols for *L. infantum* isolates presently in progress. It is aimed here to extend this RFLP approach to the other Leishmania parasites encountered in Tunisia: *L. major* and *L. tropica*. As a first step, it is planned to apply these probe/ enzyme combinations to a batch of 20 *L. major* and *L. tropica* isolates. Interestingly, the first probe tested, rK39, allowed to observe polymorphisms within both species at levels comparable to the *L. infantum* parasites. This allowed us to start readily the second step planned: apply these tools to *L. major* parasites collected during the same transmission season in the same focus from patients having well defined polymorphic clinical forms of cutaneous leishmaniasis.

Sylvain Anne-Laure christian Michel

210 EVOLUTION OF *TRYPANOSOMA CRUZI*, THE AGENT OF CHAGAS DISEASE: CLONAL EVOLUTION AND OCCASIONAL SEX. Brisse S*, Banuls AL, Sidibe I, Barnabe C, Henriksson J, and Tibayrenc M. Centre d'Etudes sur le Polymorphisme des Microorganismes, ORSTOM/CNRS 9926, Montpellier, France; Department of Medical Genetics, Biomedical Center, Uppsala, Sweden.

Trypanosoma cruzi is the protozoan parasite responsible for Chagas disease in South and Central America. *T. cruzi* natural populations have been shown to be made of stable clonal genotypes that propagate over large geographic areas and long periods of time. Recombination appears to be rare and the genetic diversity can be explained by the accumulation of mutations during long-term clonal evolution. In order to study the genetic structuration of the clonal diversity of *T. cruzi*, we have undertaken a phylogenetic analysis based on MLEE and RAPD data. Our results show evidence that the clones are subdivided into two major phylogenetic subdivisions or clades. At the lowest level of phylogenetic divergence reliably detectable, the first major clade could not be further subdivided, whereas the second one could be subdivided into 5 additional smaller clades, which probably correspond to clonal lineages. Each lineage has a characteristic geographic repartition and epidemiological importance, and might cause distinct forms of Chagas disease. The evolutionary origin of the clonal lineages was explored, and data from RFLP and molecular karyotypes, together with MLEE and RAPD data, strongly suggest that two clonal lineages arose by hybridization between distinct parental lineages. Thus, the evolutionary mode of *T. cruzi* could imply both long-term mutational evolution of independent clonal lineages, and occasional hybridization leading to new successful genotypes.

211 THE SEROTYPIC PROFILES OF *LEISHMANIA* SPECIES IN THE 'L. MAJOR COMPLEX'. Schnur LF*, Strelkova M, Jaffe CL. Department of Parasitology, Hebrew University-Hadassah Medical School, Jerusalem, Israel; Martsinovsky Institute of Medical Parasitology and Tropical Medicine,Moscow, Russia; Department of Parasitology, Hebrew University-Hadassah Medical School, Jerusalem, Israel;

Serological, signifying antigenic, profiles of strains representing the four named species of *Leishmania* in the 'L. major complex': *L. major; L. arabica; L. turanica; L. gerbilli*, were determined by excreted factor (EF) serotyping and using a battery of species specific monoclonal antibodies in an ELISA. Strains of *L. tropica* and *L. donovani sensu lato* were included for inter-complex comparison. The mosaic of shed and somatic antigenic determinants examined displayed components shared among inter-complex species and those specific for given species. Intra-*L. major* complex specificities where not easily discernible though quantitative differences were seen that might be significant in terms of intra-complex species differentiation. The four species in the *L. major* complex have been erected on biochemical, essentially zymodemal, and molecular specificities used to identify and classify them. While this dichotomy is of taxonomic interest, it should be remembered that the antigenic profile is significant in host-parasite interaction.

212 *TRYPANOSOMA CRUZI* IN A POPULATION OF LOW RISK BLOOD DONORS. Leiby DA*, Jensen NC, Fucci MC, and Stumpf RJ. Transmissible Diseases Dept, American Red Cross, Rockville, MD; Southwest Region, American Red Cross, Tulsa, OK; and Abbott Laboratories, Abbott Park, IL.

The recognition that significant numbers of blood donors positive for *Trypanosoma cruzi* are present among high risk populations in the U.S. suggests that testing of blood for *T. cruzi* may need to be considered in the future. However, since data from low risk populations is needed to make informed decisions, we tested blood donors with low to moderate risk for antibodies to *T. cruzi*. All allogeneic blood donors in the Southwest Region (OK/TX) were tested by EIA and confirmed by RIPA. Donors confirmed by RIPA were entered into look back studies. A total of 100,089 donors were tested and 3(0.003%) were confirmed as positive. Overall the rate of seropositivity was 1 in 33,000 donors, but all 3 positive donors came from a single collection area in Texas where the rate was 1 in 7,700 donors. While one positive donor was born in an endemic area of Mexico, the other 2 positive donors were born in the U.S. and had no other apparent risk factors. Lookback identified one recipient who received blood from a seropositive donor, but when tested the recipient was negative. Clearly, blood donors positive for *T. cruzi* are present in low as well as high risk populations. The lack of known risk factors for 2 positive donors suggests that identification of donors based on risk factors may be ineffective. If blood screening for *T. cruzi* is implemented, universal testing of donors may be required.

213 *TRYPSANOSOMA CRUZI* LOOKBACK STUDIES: WHERE IS THE TRANSMISSION? Jensen NC*, MacDowell D, Leiby DA. Transmissible Diseases Department, Holland Laboratories, American Red Cross, Rockville, MD; Donor Studies Department, Southern California Region, American Red Cross, Los Angeles, CA; and Transmissible Diseases Department, Holland Laboratories, American Red Cross, Rockville, MD.

Previous and ongoing studies have demonstrated that blood donors seropositive for *Trypanosoma cruzi* exist in the U.S. Because rates of transmission by blood transfusion are unknown we implemented lookback studies of blood donors positive for *T. cruzi* antibodies. Seropositive donors were identified as part of ongoing seroprevalence studies in 3 Red Cross Regions (L.A., Miami, and Tulsa). Lookback studies were initiated to determine if they had previously donated, and if so, how many times, how many blood products were manufactured from these donations, and the number of products transfused to recipients. Recipients were contacted and tested for *T. cruzi*

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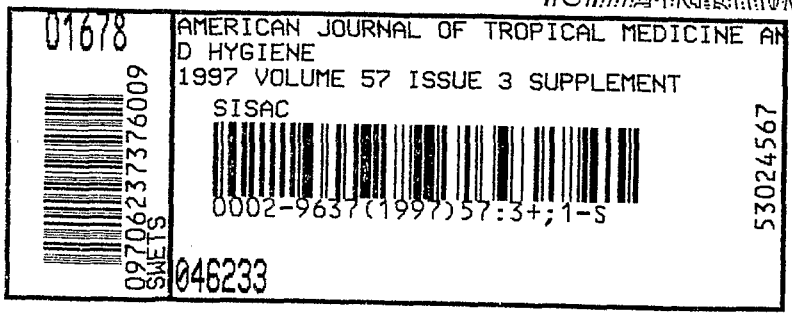

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