RESEARCH NOTE

Surveillance Using Molecular Tools: Examples from Brazil

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Brazil presents particular problems for surveillance of infectious diseases. These include its continental size, uneven distribution of resources, difficulty of communication and access in some of the more remote areas, as well as large areas covered by tropical rain-forests. Surveillance for infectious diseases in Brazil has traditionally been carried out in a passive manner by government authorities or as individual initiatives. Most effort has been directed in the collecting and tabulating of data on notifiable infections. A limited amount of laboratory support has been available for the isolation and identification of the etiological agents. More recently molecular methods have been introduced in the analysis of these data.

Here we present some practical examples of the use of different molecular tools for diagnosis and in the analysis of infectious diseases and in epidemiological monitoring of outbreaks. The examples are taken from work carried out in our Institute.

HIV-2, the second AIDS-causing virus, was originally identified and found to be quite common in West Africa. With a more restricted geographic spread than HIV-1, this virus has also been isolated in countries with socioeconomic links to West Africa (R Marlink 1996 *AIDS 10*:689-699). Some early reports analyzing the seroprevalence of HIV-1 and HIV-2 were contradictory about the presence of HIV-2 in Brazil (L Oyafuso et al. 1989 *New Engl J Med 320*: 953-958, RM Hendry et al. *J Acq Imm Def Synd 4*: 623-627). At that time they concluded that there was some cross-reactivity between HIV-2 and HIV-1 which resulted in misinterpretation. Using polymerase chain reaction (PCR) and specific internal probes to HIV-2, Pieniazek et al. (1991 *AIDS 5*: 1293-1299), identified mixed HIV-1/HIV-2 infections in Rio de Janeiro, Brazil. In order to validate the World Health Organization strategy for HIV testing, sera from 9,885 blood donors from São Paulo were screened by HIV enzyme-linked immunosorbent assays (ELISA) and Western blot and the results did not support the evidence of HIV-2 circulation in Brazil (MB Carvalho et al. 1996 *AIDS 10:* 1135-1140).

We have applied molecular tools in surveillance for the detection of HIV-2 in HIV-1 positive samples (possible dual infections) as well as in samples with undetermined Western blots. More than 200 samples from different parts of the country were screened for the presence of HIV-2 proviral DNA using nested PCR targeting the long terminal repeat (LTR), protease and *gag* regions. In three samples only PCR products corresponding to the LTR region were amplified. These products were sequenced and the nucleotide sequence was different from that of HIV-2 LTR. They matched with human genome sequence, probably a rare allele present in few people. At present we have failed to detect and confirm the circulation of HIV-2 in Brazil. We have shown that the use of LTR diagnostic primers to HIV-2 has to be carefully analyzed.

Vibrio cholerae occurs naturally in aquatic systems where it may constitute part of the normal microflora of zooplankton and larger animals. *V. cholerae* is a heterogeneous species with more than 140 serotypes, only a few of which are associated with biotypes causing human cholera and epidemics. The ongoing cholera pandemic (7th) is caused by the El Tor biotype, serotype O1. In 1991 cholera re-emerged in Brazil after being absent for a century, the previous pandemic involved *V. cholerae* classical biotype. The present situation is different in that not only is there a new *V. cholerae* biotype, but also there is now detailed knowledge about the bacterial virulence factors determining this disease and the molecular tools available for characterization of the isolates. In 1993 a new *V. cholerae* strain was identified in the State of Amazonas during surveillance using AP-PCR for molecular characterization of cholera vibrios. The *V. cholerae amazonia* variant is of the O1 serotype; it has distinct multilocus enzyme electrophoresis and AP-PCR profiles from other pathogenic O1 *V. cholerae*. About 50 isolates have been made from cases of diarrhea in the upper Amazon

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(Solimões) River. The microbe apparently does not harbor any of the well known virulence associated genes (e.g. the toxin gene casette and the major colonization factor, TCP); however some isolates present a cytotoxic effect for Y-1 cells (A Coelho et al. 1995 *J Clin Microbiol 33*: 114-118).

Since 1997, in Amazonas, all cholera notification is based on clinical and epidemiological diagnosis. In trying to identify cholera vibrio in apparent outbreaks of cholera occuring in São Paulo de Olivença, Juruá and Envira villages, we applied PCR - target specific to genes associated with *V. cholerae* El Tor (cholera toxin/CT and toxin co-regulated pilus/TCP) and *V. cholerae amazonia* (regulatory gene/*toxR*). The results were negative but using PCR - target specific to genes associated with *Escherichia coli* enterotoxigenic (termo-label toxin / LT and termo-stable toxin / ST) (NG Tornieporth et al. 1995 *J Clin Microbiol 33*: 1371-1374) we were able to identify and characterize this bacteria and thus able to demonstrate that these acute diarrhea outbreaks, clinically very close to cholera symptoms, were not associated with any *V. cholerae.*

In *Leishmania*, a numerical zymotaxonomic study of New World *Leishmania* was carried out (E Cupolillo et al. 1994 *Am J Trop Med Hyg 50*: 296-311). The analysis involved the use of phenetic, cladistic and ordination techniques on enzyme electrophoresis data from more than 250 isolates of *Leishmania*. This study together with later work has revealed a rich diversity among isolates from the New World at both organismal and molecular levels. This diversity has provided numerous opportunities to probe questions concerning parasite evolution and biology, as well as their role in human disease. In many localities, more than one *Leishmania* species co-exists with overlapping animal hosts and vectors, as well as other pathogens. In collaboration with a number of different research groups we have studied aspects of the epidemiology of leishmaniasis in various countries of Latin America and in different regions of Brazil, in addition we have been interested in determining the autochtonous origin of certain *Leishmania* species found in the New World (H Momen et al. 1993 *Biol Res 26*: 249-255).

In most of these examples molecular identification of the etiological agents was followed by genetic analysis. The results were then forwarded to the relevant control agencies, usually the FNS (Fundação Nacional de Saúde).