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Mr. Samie Amidou holds an MSc in Microbial Biochemistry and is a PhD candidate in Microbiology at the University of Venda, South Africa. His research is focused on the Molecular characterization of African isolates of *Entamoeba histolytica* and *Campylobacter* spp as well as their pathogenicity and antibiotic resistance mechanisms. He is presently a research fellow at the Center for Global Health, University of Virginia working with Professor R. Guerrant.

## ISID Small Grants Program Final Report

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### Molecular epidemiology of *Entamoeba histolytica* in the Venda region of South Africa, and antimicrobial activities of local medicinal plants

Infections due to *E. histolytica* affect nearly 500 million people throughout the world annually leading to about 110,000 deaths. Amebiasis is more common in tropical and developing regions of the world and less-resourced people are generally the most affected. However, the epidemiology of this disease is not clearly defined and information on its prevalence in most rural African areas is missing. The Venda region, situated in the northern part of South Africa is mostly rural and previous studies have demonstrated that water used by the population is of poor quality suggesting that water sources might be contaminated by various organisms which may include *E. histolytica*. The Vha-Vendas have a strong tradition of using medicinal plants to cure different ailments and to prevent diarrhoea in children. Although few studies have investigated the antimicrobial activities of medicinal plants used in Venda, none has addressed the activity of the medicinal plants on *Entamoeba histolytica*. The objectives of this study were to determine the prevalence of intestinal parasites with particular attention to *E. histolytica* in order to understand the distribution patterns of infection in the community, and to determine the capacity of the plants used by the population to kill the *Entamoeba* cells grown in vitro in a xenic environment mimicking the natural situation in the intestine.

A total of 528 stool samples were collected from the three main hospitals visited by the general population and 295 from two primary schools. The samples were analysed by light microscopy, staining, ELISA and PCR for the detection of *E. histolytica* and other intestinal parasites. A survey was also conducted through the use of questionnaires amongst university students in order to determine the hygienic habits of the population. Information on the traditional use of medicinal plants was sought from local traditional healers and from the literature. A total of 18 plants were collected from the villages, dried, ground into fine particles, extracted with different organic solvents and evaporated to dryness using a rotary evaporator at 40°C. The crude extracts were then tested for activity using the microdilution method against fifteen different bacterial species and a strain of *E. histolytica* isolated from a patient with bloody diarrhoea and characterised by PCR.

The most identified organisms in hospital samples were *Cryptosporidium* spp (21.2%) followed by *E. histolytica/dispar* (17%), *S. mansoni* (14.4%), Hook worm (11.1%) and *G. lamblia* (10.2%).

At the Primary schools, the most prevalent parasitic organisms were *S mansoni* (30.5%) followed by *Cryptosporidium* spp (18.9%), *G. lamblia* (10.8%), Hookworms (10.6%) and *E. histolytica/dispar* (9.1%). The ELISA test was conducted on a total of 282 samples from the hospitals and 92 samples from the Primary Schools and indicated a general prevalence of 11.3% in the hospitals and 2.17% at the primary schools. The nested PCR results indicated that, most cases were mixed infections with *E. histolytica* and *E. dispar* and represented 68% of all infections. The results of the survey indicated poor hygienic habits amongst university students as nearly 80% of the students who answered the questionnaires occasionally washed fruits before eating and 67% occasionally washed their hands after going to the toilets. From the 18 plants tested, all were active against at least one bacterial species with MICs varying from 0.03mg/ml to 12mg/ml. Against the *E. histolytica* clinical isolate, three plants were found active at different concentrations varying from 2.5mg/ml to 12mg/ml.

This study has demonstrated that infections due to *E. histolytica* as well as other intestinal parasites are common in a rural area of previously unknown rate of parasitic infections. HIV infection was associated with *E. histolytica* infections particularly among the females. Medicinal plants appeared to be effective in the control of different intestinal bacteria and *E. histolytica* and could be of great help at this time when HIV infections constitute a serious threat to the populations in sub-Saharan Africa. This research thus provides baseline data that could be useful for the development and implementation of control policies in the region in order to improve the life of the community living in the concerned areas. Isolates obtained from this study will be characterised for genetic diversity and pathogenic profiles in order to advise on the possible drug targets as well as potential antigens that could be used for vaccination strategies. ❖

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