

from *B. microti* infected individuals from endemic areas in Connecticut suggesting their potential as diagnostic antigens. Bioinformatics analyses and studies on their biochemical and cellular characterization as well as their value as diagnostic vaccine antigens are in progress.

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### **GAMMA IRRADIATED SOLUBLE EXTRACTS OF *TOXOPLASMA GONDII* TACHYZOITES INDUCED BETTER HUMORAL AND CELLULAR IMMUNE RESPONSE DUE TO PREFERENTIAL UPTAKE BY APCS SCAVENGER RECEPTORS**

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Toxoplasmosis occurs in one-third of the adult world population, without adequate vaccines and causing disease in fetus or specific groups. Aside to sterilizing effect, gamma radiation acts on antigens inducing enhanced antisera production against snake venoms or cell and humoral response to recombinant leprosy proteins. Gamma radiation affects proteins directly or indirectly in water by action of oxidant radicals from water radiolysis. Early reports showed gamma irradiated crotoxin had enhanced uptake by macrophages, limited by scavenger receptors competitors, as probucol. Irradiated tachyzoites induced adequate immune response with protection, attributed to mitotic death and DNA damage. Irradiated proteins could take a part in this process and we study the immune response induced by gamma irradiated soluble extracts of *Toxoplasma gondii* tachyzoites, using mice immunized with native proteins as controls. Mice immunized with irradiated extracts without adjuvants showed significant protection after challenge with ME-49 ( $p < 0.05$ ) and RH ( $p < 0.0001$ ) strains compared to controls. There are increased specific and high avidity IgG production ( $p < 0.05$ ) when compared to controls group. By flow cytometry and *in vitro* culture, spleens of mice immunized irradiated extract presented increased proliferation of CD4+, CD8+ and B cells and IFN- $\gamma$  production as compared to controls. J774 cells had increased uptake of biotinylated irradiated extracts as compared to the uptake of native extract ( $p < 0.05$ ), due to longer and continuous uptake. All these data points to an alternative and effective uptake and immune processing of irradiated *T. gondii* extracts, probably due to specific receptor of oxidized proteins as scavenger receptors, resulting in enhanced immunity. This data also implies that irradiated proteins could be involved in the protection induced by irradiated parasites. Use of antigen gamma radiation can be a simple process to enhance vaccine efficiency, avoiding the use of adjuvants.

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### **PHYLOGENETIC ANALYSIS OF *BLASTOCYSTIS* SPP. ISOLATES IN CLINICAL STOOL SAMPLES FROM BRAZIL**

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*Blastocystis* spp. is an organism described as enteroparasite protozoan, commonly found in stool samples from humans. Several subtypes have been described in humans, but pathogenic potential and aspects epidemiological are still controversial. The aim of the present study was to investigate *Blastocystis* subtypes (STs) from patients of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC/FMUSP), Brazil. *Blastocystis* spp. positive stool samples diagnosed in Section of Parasitology of Central Laboratory (HC-FMUSP) were used for DNA isolation. Polymerase chain reaction (PCR) was performed using

specific primers targeting the small subunit of rRNA gene. Direct DNA sequencing of PCR products was performed, and the DNA sequences were aligned and compared to other sequences present in GenBank database. Phylogenetic analysis was inferred using the Neighbor-Joining method by MEGA6 software. Additionally, *Blastocystis* STs were identified by determining the exact match or closest similarity against all known *Blastocystis* STs using [www.pubmlst.org/blastocystis](http://www.pubmlst.org/blastocystis). Four STs were identified: ST1 (16.0%), ST2 (8.0%), ST3 (68.0%) and ST6 (8.0%). Allele nos. 34 and 36 were the most frequent haplotypes. The present study is one of the few that generates STs data from human population in Brazil, confirming the absence of ST4. Another important finding is the presence of ST6, rarely detected in human isolates. Subtype prevalence involving human samples may contribute to the monitoring of infection transmission of *Blastocystis* spp in endemic areas, and in future, explain any pathogenic aspects related to distinct subtypes.

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### **DISTRIBUTION AND HUMAN-INFECTIVE POTENTIAL OF *CRYPTOSPORIDIUM*, *GIARDIA DUODENALIS* AND *ENTEROCYTOZOON BIENEUSI* GENOTYPES IN STORM OVERFLOW AND WASTEWATER IN SHANGHAI, CHINA**

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Few data are available on the distributions of *Cryptosporidium*, *Giardia duodenalis*, and *Enterocytozoon bienersi* genotypes and subtypes in storm overflow from urban areas. In the present study, 40 overflow samples were collected from two pump stations during July-September in 2012 and 2014 in Shanghai, China, with 40 raw wastewater samples from the same stations as controls. They were analyzed by using PCR for *Cryptosporidium* spp. (targeting the small subunit rRNA gene), *G. duodenalis* (targeting the triphosphate isomerase,  $\beta$ -giardin, and glutamate dehydrogenase genes), and *E. bienersi* (targeting the ribosomal internal transcribed spacer). Genotypes of these pathogens were identified by sequence analysis of PCR products. Samples that contained *C. hominis*, *C. parvum*, *C. viatorum*, *C. ubiquitum*, and *C. meleagridis* were further subtyped by sequence analyses of the 60-kDa glycoprotein gene. *C. hominis*, *C. parvum*, *C. ubiquitum* and *C. viatorum* were the dominant *Cryptosporidium* species. *C. baileyi*, *C. muris*, and *C. meleagridis* were also found in both wastewater and overflow samples. The *C. hominis* and *C. parvum* subtypes were common ones previously found in humans in China, but *C. ubiquitum* belonged to two novel subtype families and *C. viatorum* was found in China for the first time. There were eight Group 1 *E. bienersi* genotypes in wastewater and storm overflow with genotype D as the dominant one. For *G. duodenalis*, subassemblage All was the dominant genotype in these samples. There were no significant differences in the distribution of *Cryptosporidium* species and *E. bienersi* and *G. duodenalis* genotypes between wastewater and overflow samples. These results reaffirm that storm overflow is potentially a significant contamination source of human pathogens in surface water and more attention should be paid to its roles in environmental transport of waterborne pathogens.