

Molecular subtyping of *Blastocystis* isolates from symptomatic and asymptomatic patients in Lower Silesia, Poland

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Blastocystis hominis is the most common unicellular parasite found in the large intestine. Blastocystis have been isolated from many species of animals including mammals and birds and an extensive genetic variability of *Blastocystis* has been described. Studies using the sequence analysis of *Blastocystis* sp. small subunit ribosomal RNA genes (SSU rDNA) have demonstrated that there have been 26 subtypes (ST) so far, including at least 10 isolated from humans (ST 1-9 and ST12). Little is known about epidemiology and molecular mechanisms of pathogenesis for this parasite. The clinical symptoms of *B. hominis* infection are nonspecific and include abdominal pain, nausea, cramps and diarrhea.

The aim of this study was to determine the prevalence of *B. hominis* in stool specimens in three groups of patients: 72 patients with various gastrointestinal symptoms treated at the Clinic of Gastroenterology and Hepatology, Wrocław Medical University (G1), 111 healthy people reported to the local Sanitary and Epidemiological Station SANEPID in order to obtain a health certificate for employment (G2) and 40 asymptomatic patients (G3) living in Lower Silesia, Poland. The study was approved by the Ethics Committee of Wrocław Medical University. Stool specimens were analyzed using xenic *in vitro* culture (XIVC) with a modified Jones' medium and molecular methods (PCR). Most of the stool samples were of normal consistency according to the Bristol Stool Chart. Gene fragment of SSU-rRNA was amplified with forward primer RD5 (5'-ATCTG-GTTGATCCTGCCAGT-3') and reverse primer BhRDr (5'-GAGCTTTTAACTGCAACAACG-3'). All PCR products were sequenced to determine subtypes. The sequences obtained were compared to those of *Blastocystis* spp. deposited in GenBank.

B. hominis was found in 17/72 (23%) of study participants from group G1, 22/101 (21%), and 4/40 (10%) from groups G2 and G3 respectively. Molecular subtyping revealed five STs: ST1, ST2, ST3, ST4 and ST7 in patients suffering from various diseases of the digestive system and four STs: ST1, ST2, ST3, and ST7 in asymptomatic patients. The dominant *B. hominis* genotypes were ST3 and ST7. In participants suffering from various diseases of the digestive system (G1), genotype

ST3 was present in 33,3% and ST7 in the 16% of patients involved in this study. On the other hand, ST3 and ST7 occurred with the same frequency among healthy people and amounted to 29%.

In conclusion, *Blastocystis* is a very common intestinal parasite and its prevalence was not significantly different in symptomatic and asymptomatic patients. *Blastocystis* is still regarded a parasite of controversial clinical significance, further investigation needs to be done with larger populations to evaluate the role in their pathogenic potential.