## The prevalence of diseases caused by parasitic protozoans and the impact of selected factors on their survival and detection on the example of *Giardia intestinalis* and *Toxoplasma gondii*

## **ABSTRACT**

Giardia intestinalis is one of the most common parasitic protozoans in humans. Contaminated food and water are the main sources of infection. Food-borne parasites, like *G. intestinalis*, are contemporary among the threats to food safety, hence their effective elimination and the systematic and effective control of food is very important. The sensitivity of pathogens to physical and chemical factors can be the basis for developing measures to improve the safety of food and reduce the occurrence of parasitic infections among humans.

Toxoplasmosis is the disease caused by parasitic protozoan *Toxoplasma gondii*. The course of toxoplasmosis is usually mild or asymptomatic in immunocompetent individuals, but it can have serious consequences if it occurs in immunodeficient individuals. Therefore, early diagnosis of *T. gondii* infection is important in all HIV-infected individuals to prevent a severe form of the disease, including complications of central nervous system toxoplasmosis.

The aim of the study was to determine the prevalence of diseases caused by parasitic protozoan and to determine the impact of selected physical, chemical and biological factors on their survival and detection on the example of *Giardia intestinalis* and *Toxoplasma gondii*.

The prevalence analysis of *Giardia intestinalis* was carried out using data from parasitological studies conducted in the years 2000-2020 at the Provincial Sanitary and Epidemiological Station in Bydgoszcz. In the case of the assessment of the impact of selected biological and chemical factors on the survival and detection of *Giardia intestinalis* cysts using the microscopic and immunoenzymatic methods, the research material consisted of 20 archived faeces samples in which the presence of *Giardia intestinalis* was found. The assessment of the impact of selected physical and chemical

factors on the detection of *Giardia intestinalis* by the microscopic method and the real-time PCR method was performed using parasites from an axenic culture.

To test the titre of antibodies against *Toxoplasma gondii* in the serum of people tested for HIV, due to the suspected infection, among the inhabitants of the Kuyavian-Pomeranian Voivodeship, 43 blood samples collected from people reporting to the HIV Consultation and Diagnostic Center in Bydgoszcz were used. The samples were tested for the presence of anti-HIV-1/HIV-2 antibodies and p24 antigen as well as IgM and IgG antibodies against *Toxoplasma* using the enzyme-linked fluorescent assay (ELFA).

The prevalence of *Giardia intestinalis* in the years 2000-2018 among a selected population of inhabitants of the Kuyavian-Pomeranian Voivodeship was 0.16% - 0.85%. In 2019-2020, no positive result for this protozoan was recorded.

In the presented studies on the impact of selected biological and chemical factors on the survival and detection of *Giardia intestinalis* cysts, positive result in the ELISA test detecting the antigen of parasite was obtained at 20 samples after adding *Salmonella* Enteritidis (ATCC 13076), *Shigella sonnei* (ATCC 9290), *Yersinia enterocolitica* (ATCC 23715) and after adding noroviruses to faecal samples containing *Giardia intestinalis*. The analysis of the results in the case of substances added to food showed that after adding potassium sorbate, the *Giardia intestinalis* antigen was detected in 90% samples. In the case of guar gum and monosodium glutamate, all samples showed a positive result in the enzyme immunoassay. On the other hand, the addition of citric acid allowed the detection of protozoan antigen in only 25% of samples. *Giardia intestinalis* was detected by both microscopic and immunoenzymatic methods with the same sensitivity.

Analysis of the impact of selected physical and chemical factors on the survival of *Giardia intestinalis* revealed no effect in the microscopic method for 5 substances: maltodextrin, sodium citrate E331, calcium lactate E327, propylene glycol and sodium bicarbonate. In turn, when assessing the impact of all factors on the detection of *Giardia intestinalis* DNA by real-time PCR, only 3 substances had an impact on the detection of the presence of protozoan genetic material. The addition of sodium hypochlorite, formalin and sodium bicarbonate resulted in no evidence of *Giardia* DNA.

The analysis of *Toxoplasma gondii* seroprevalence showed that in the study population the presence of IgG antibodies against *Toxoplasma* was found at the level of 53.5%, while the test for specific IgM antibodies was negative. A high avidity index

of IgG antibodies was obtained in 18 seropositive samples. Among studied samples, 30 were from women and 13 from men. HIV was detected in 1 sample and antibodies against *T. gondii* in the IgG class in 7 samples in the group of men. Antibodies against *T. gondii* in the IgG class were found in 16 women.

In this study, it was shown that the microscopic and immunoenzymatic method can be equally used in clinical diagnostics to detect the presence of *Giardia intestinalis* in stool samples. Most of the studied factors influenced on the survival rate of *Giardia intestinalis* in biological material diagnosed with the microscopic method. Based on this, it can be concluded that standard disinfection methods using UV, ethanol or various disinfectants are sufficient to reduce the survival rate of *G. intestinalis*. The use of preservatives, such as citric acid, which reduces the detection of *Giardia intestinalis* by microscopic and immunoenzymatic methods, may also contribute to this, which suggests that it reduces the survival of the parasite. When added to food, citric acid may increase its safety. Selected physical and chemical factors reduce the survival of *Giardia intestinalis* assessed using the microscopic method, but do not reduce the detection of parasite DNA by real-time PCR, which confirms that the use of molecular diagnostic methods based on the detection of genetic material increases the sensitivity of parasite detection. Detection of DNA in most samples may suggest the need to, for example, extend the period of exposure to a specific factor.

In people tested for the presence of HIV, due to the suspicion of infection with this virus, antibodies to *Toxoplasma gondii* were detected only in the IgG class, which proves the lack of active infections with this parasitic protozoan in the tested group of people who are residents of the Kuyavian-Pomeranian Voivodeship. As in the case of *G. intestinalis*, it may be necessary to use molecular methods in the diagnosis of *Toxoplasma gondii* due to their high sensitivity. Early detection of *T. gondii* infection, especially in immunodeficient individuals, including those infected with HIV, may prevent the development of severe toxoplasmosis in these patients.

**Key words:** Giardia intestinalis, diagnostic methods, Toxoplasma gondii