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Original Article

The Influence of Selected Factors on the Detection of *Giardia intestinalis* by Microscopic and Immunoenzymatic Methods

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Received 14 Aug 2022
Accepted 12 Nov 2022

Keywords:
Food additives;
Giardia intestinalis;
Immunoenzymatic method;
Microscopic method

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Abstract

Background: *Giardia intestinalis* is one of the most common parasites in humans. Contaminated food and water can be a source of infection. Substances added to food are intended to increase its safety. We aimed to determination of the influence of various microorganisms and compounds that stimulate digestive functions, as well as preservatives and antioxidants on the detection of *G. intestinalis* by microscopic and immunoenzymatic methods.

Methods: Twenty stool samples, archived in 1998-2018 in the Provincial Sanitary and Epidemiological Station in Bydgoszcz (Poland), collected both from patients referred for parasitic examinations by a doctor of a medical facility and from private individuals, were used to assess the impact of selected factors (such as bacterial strains, viruses and substances added to food) on the detection of *G. intestinalis* by microscopic and immunoenzymatic methods.

Results: *G. intestinalis* was detected by both microscopic and immunoenzymatic methods with the same sensitivity (100%). The result of the *G. intestinalis* determination was positive in 90% of the samples after the addition of potassium sorbate, and in 25% of the samples after the addition of citric acid.

Conclusion: The presence of other microorganism such as bacteria and viruses does not influence on the detection of *G. intestinalis* by microscopic and immunoenzymatic methods in stool samples. Citric acid as an antioxidant added to foods affects the detection of *G. intestinalis*. Due to the small number of samples used, it is necessary to continue research on the impact of various factors on the detection of protozoa.

Introduction

Giardia intestinalis is a cosmopolitan flagellate that can be present in the small intestine of the human being in the form of trophozoite and cysts (1). The protozoan is one of the most common parasites that cause disease in humans. It is the main cause of water-diarrhea in the United States and Europe (2). The incidence rate is between 10% and 50% in developing countries and between 2% and 5% in many developed countries (3). *G. intestinalis* may be an important cause of mass diarrhea in day-care centers due to the transmission of the fecal-oral infection among children (4). Due to the fact that this is the most common way of spreading the infection, it also applies to patients of psychiatric institutions and members of their families, as well as people who prefer oral-genital or oral-anal relations (5). The infection can also be caused by consumption of food and drinking water contaminated with stools and less frequently may be due to fertilization of crops, as the presence of protozoan cysts was found on vegetables and fruits (1).

Parasites transmitted by food are classified as one of the contemporary food safety hazards, hence both the effective elimination of all hazards and their systematic and effective control are needed in order to provide food of adequate quality as well as health security (6). For this reason, various substances are added to food, as defined in the Ordinance of the Minister of Health of 22 November 2010 on permitted additional substances.

Fixative additives constitute one of the most important groups of food additives, in respect of the safety and quality of food products (7). Eight groups are distinguished among additional substances:

1) Colorants (E100-199),
2) Preservative (E200-299),
3) Acidity regulators (E300-399),
4) Thickener, stabilizer, emulsifier (E400-499),
5) Anticaking agents (E500-599),
6) Flavour enhancer (E600-699),
7) Miscellaneous (E700-999),
8) Additional chemicals (E1000-1999) (8).

The use of preservatives allows primarily to extend the shelf life of some products. The mechanism of action of preservatives is related to their effects on the biochemical processes of the microbe cell, in particular:

- Destruction of the cell wall, e.g. by reducing its permeability, plasmolysis or denaturation,
- Interference with the genetic mechanism, e.g. by its damage (mutagenic effects),
- Inactivation of some enzymes (e.g. reductive action of sulphites on disulphide bonds of enzymes), inactivation of metabolites necessary for the development of microorganisms (e.g. vitamins, amino acids) (9).

Antioxidants are a group of agents that prolong the stability of food products by inhibiting oxidation as a result of accepting free radicals initiating the oxidation process and introducing a hydrogen atom into a free radical. The resulting radical of the antioxidant is stable and forms stable products. Antioxidants used in food are divided into:

- Typical antioxidants (natural or synthetic),
- Substances with an antioxidant effect in addition to other activities,
- Substances supporting antioxidants, so-called synergists (10).

Thickeners affect the consistency of products, increase their elasticity, and also inhibit the formation of foam in production processes, slow down crystallization, emulsification and gelation (11).

The aim of this study was to determine the effect of various microorganisms and compounds that stimulate digestive functions, as well as preservatives and antioxidants on the detection of *G. intestinalis* by microscopic and immunoenzymatic methods.

Materials & Methods

Twenty stool samples, archived in 1998-2018 in the Provincial Sanitary and Epidemiological Station in Bydgoszcz (Poland), collected both from patients referred for parasitic examinations by a doctor of a medical facility and from private individuals, were used to assess the impact of selected factors on the detection of *G. intestinalis* by microscopic and immunoenzymatic methods.

To determine the effect of the presence of other microorganisms, such as bacteria and viruses, on the test results in the detection of *G. intestinalis*, three reference strains were used, namely *Salmonella* Enteritidis ATCC 13076, *Shigella sonnei* ATCC 9290 and *Yersinia enterocolitica* ATCC 23715. In addition, a sample containing noroviruses was used. Suspensions with a density of 0.5 McF were prepared in a solution of 0.9% NaCl.

In the case of the assessment of the effect of substances added to food on the detection of protozoa, potassium sorbate E202, guar gum E412, monosodium glutamate E621 and citric acid E330 were used. All substances were dissolved in distilled water before use.

Laboratory diagnostics were carried out by microscopic and immunoenzymatic methods. The microscopic examination included four methods: a direct smear in 0.9% NaCl solution, direct smear in Lugol's fluid, Faust's flotation (zinc sulphate) and decantation. Preparations were viewed under a magnification of 10x and 20x, and the identification was carried out at a magnification of 40x. The enzyme immunoassay from TechLab (30405) was used to detect the presence of the coproantigen of *G. intestinalis* (GSA-65).

Results

Twenty fecal samples were analyzed, the result of detection of the *G. intestinalis* antigen by ELISA was positive in all samples after adding *Salmonella* Enteritidis ATCC 13076. In the case of *S. sonnei* ATCC 9290, a positive immunoenzymatic test was observed. All *G. intestinalis* antigen assays were positive after adding the strain *Y. enterocolitica* ATCC 23715. All samples gave a positive result for the protozoan antigen after the addition of noroviruses to fecal samples containing *G. intestinalis* (Table 1).

Taking into account the microorganisms added to fecal samples (bacteria and viruses), in the microscopic method, the presence of *G. intestinalis* cysts was detected in 100% of stool specimens.

In the case of substances added to food, the analysis of the results showed that after the addition of potassium sorbate, the result of the *G. intestinalis* antigen was positive in 18 (90%) samples, while 2 (10%) samples gave negative results. In the case of guar gum, a positive ELISA test was recorded in all samples. After the addition of monosodium glutamate, 100% of the samples were positive in the enzyme immunoassay. In turn, the addition of citric acid allowed the detection of the protozoan antigen in 5 samples, and in 15 cases the result of the determination was negative, which accounted for 25% and 75% respectively (Table 2).

Table 1: Absorbance values of the *Giardia intestinalis* antigen assay after the addition of *Salmonella* Enteritidis ATCC 13076, *Shigella sonnei* ATCC 9290, *Yersinia enterocolitica* ATCC 23715 and noroviruses.

Sample number	***Salmonella Enteritidis***		***Shigella sonnei***		***Yersinia enterocolitica***		***Noroviruses***	
	Reading value	Result	Reading value	Result	Reading value	Result	Reading value	Result
1	2,088	POS	2,122	POS	2,107	POS	2,119	POS
2	0,762	POS	0,960	POS	2,058	POS	2,123	POS
5	2,105	POS	2,039	POS	2,116	POS	2,038	POS
8	1,895	POS	1,977	POS	2,023	POS	2,008	POS
43	2,122	POS	2,107	POS	2,121	POS	2,109	POS
47	2,117	POS	2,087	POS	2,120	POS	2,082	POS
58	0,416	POS	2,116	POS	0,431	POS	2,111	POS
64	2,052	POS	2,075	POS	2,052	POS	2,069	POS
71	1,842	POS	2,036	POS	1,847	POS	2,118	POS
85	2,075	POS	2,119	POS	2,087	POS	2,117	POS
88	2,096	POS	1,989	POS	2,099	POS	1,734	POS
109	2,072	POS	2,051	POS	2,076	POS	2,054	POS
111	2,080	POS	2,082	POS	2,119	POS	2,105	POS
113	1,660	POS	1,558	POS	1,681	POS	2,053	POS
114	2,037	POS	2,113	POS	2,039	POS	2,116	POS
122	2,050	POS	2,072	POS	2,054	POS	2,073	POS
123	2,105	POS	2,132	POS	2,107	POS	2,122	POS
133	2,085	POS	2,125	POS	2,081	POS	2,124	POS
134	2,110	POS	2,042	POS	2,109	POS	2,035	POS
137	2,075	POS	2,055	POS	2,076	POS	2,043	POS

Table 2: Absorbance values of the *Giardia intestinalis* antigen assay after the addition of potassium sorbate (E202), guar gum (E412), monosodium glutamate (E621) and citric acid (E330)

Sample number	***Potassium sorbate***		***Guar gum***		***Monosodium glutamate***		***Citric acid***	
	Reading value	Result	Reading value	Result	Reading value	Result	Reading value	Result
1	2,100	POS	2,129	POS	2,109	POS	2,083	POS
2	0,336	POS	0,878	POS	0,400	POS	0,016	NEG
5	0,185	POS	2,027	POS	1,935	POS	0,007	NEG
8	0,979	POS	1,310	POS	0,852	POS	0,007	NEG
43	0,223	POS	2,103	POS	2,117	POS	0,007	NEG
47	2,020	POS	2,079	POS	2,116	POS	0,020	NEG
58	0,016	NEG	0,157	POS	0,284	POS	0,012	NEG
64	0,888	POS	2,065	POS	2,045	POS	0,008	NEG
71	0,492	POS	1,869	POS	1,479	POS	0,006	NEG
85	2,070	POS	2,114	POS	2,072	POS	1,148	POS
88	0,005	NEG	1,648	POS	1,390	POS	0,011	NEG
109	0,767	POS	2,040	POS	2,049	POS	0,335	POS
111	1,240	POS	1,941	POS	1,143	POS	0,020	NEG
113	0,437	POS	1,088	POS	0,323	POS	0,008	NEG
114	0,280	POS	2,090	POS	1,733	POS	0,008	NEG
122	0,697	POS	2,065	POS	2,048	POS	0,010	NEG
123	0,820	POS	2,122	POS	2,099	POS	0,032	NEG
133	1,856	POS	2,114	POS	2,078	POS	0,145	POS
134	2,082	POS	2,030	POS	2,107	POS	1,663	POS
137	1,853	POS	2,046	POS	2,055	POS	0,034	NEG

The mean differences in the absorbance value in the enzyme immunoassay test are shown in Fig. 1. In the case of assays after the addition of microorganisms, it was observed that the average absorbance was slightly higher than at the first reading, whereas for substances added to food, the average absorbance readings were lower, compared to the first reading.

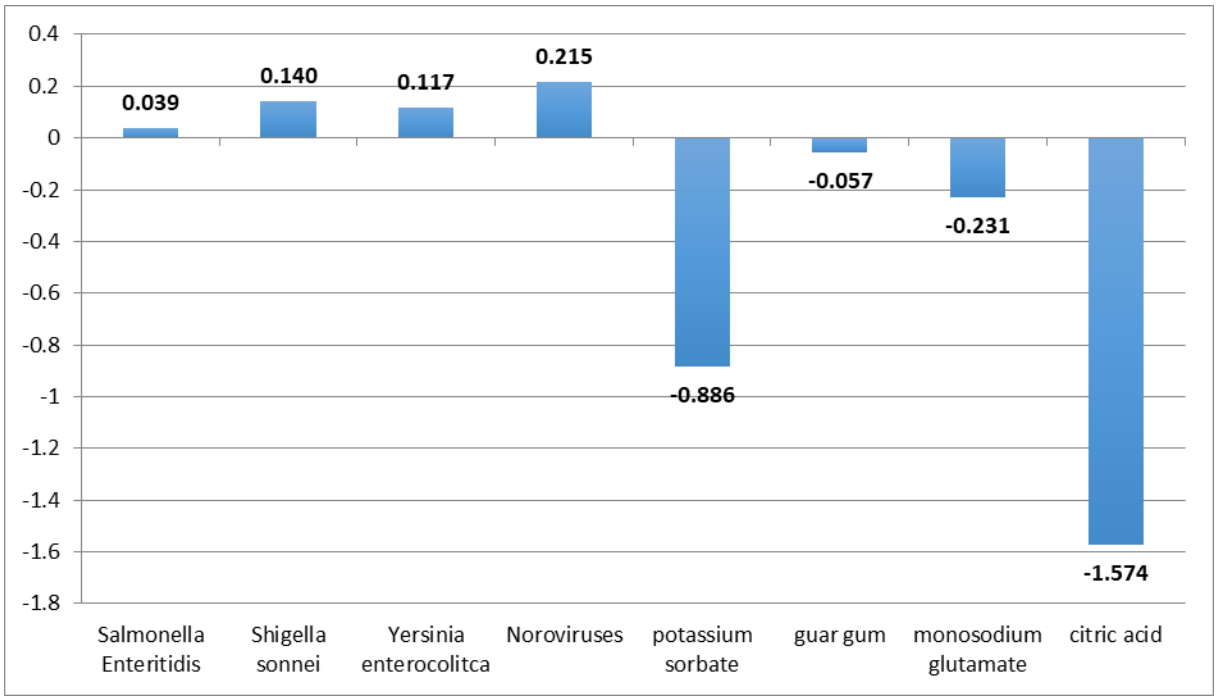

Fig. 1: Average differences in the absorbance between the first reading and the next after the addition of microorganisms or substances added to food

Taking into account the substances added to food, most often in microscopic preparations, cysts were observed after the addition of potassium sorbate - 90%, guar gum - 100% and monosodium glutamate - 100%. However, in the case of citric acid microscopic examination, the presence of cysts was found in 15% of the samples.

Discussion

The analysis shows that *G. intestinalis* was detected by both microscopic and immunoenzymatic methods with the same sensitivity (100%). Rogawski et al. (12) indicated a different situation because in their studies the sensitivity of microscopy was 46.2%, and the specificity was 99.3%**,** compared to the enzyme immunoassay. A positive microscopic examination was 21% less likely to be reported when the stool was watery or liquid than when it was formed. However, no correlation was observed between the stool consistency and the results of the EIA test. Addis et al. (13) detected *G. intestinalis* by microscopy in 99 samples from children attending day-care centers. 93 samples were positive - sensitivity of 93.9% using the ELISA test. In the microscopic examination, 534 negative samples were found**;** among them the ELISA was positive for 32 samples. Taking into account the sensitivity of both methods for all true positive samples, the sensitivity (83.2%) for microscopy and for the enzyme immunoassay

was 95% (13). In turn, in the US, fecal samples were examined by microscopic, immunofluorescent and immunoenzymatic methods. Of all 512 samples, 33 were positive using an immunofluorescence test, giving a diagnostic sensitivity of 100%. For the enzyme immunoassay, the sensitivity was 97%, as *G. intestinalis* was detected in 32 samples from 33 positive. The least sensitive was the microscopic method (81.8%), in which 27 samples were found to be positive for protozoa (14).

In this study, stool samples were used from patients referred for parasitic examinations by a physician of a medical facility or from private individuals. In the examination of samples from clinical microbiological laboratories and day care centers for young children, three methods were used to detect *G. intestinalis*: microscopy, immunoelectrophoresis countercurrent (CIE) and ELISA. For samples derived from children attending children's centers, the sensitivity of CIE was 88% and of ELISA - 94% when compared to microscopy, while for laboratory samples**,** it was 96% and 90%, respectively (15).

The detection of *G. intestinalis* by means of microscopy, direct immunofluorescence (DIF) and flow cytometry (FC) was compared at the Children's Hospital of the University of Mansour. The presence of the protozoan was found in 40, 52 and 38 samples respectively. Compared to DIF, the sensitivity of microscopy was 76.9%, while FC had a sensitivity of 73.1% (16).

In turn, Behr et al. (17), in addition to the methods used by n presented study, to detect *G. intestinalis*, performed a serological test for the presence of IgG, IgM and IgA antibodies in adults who experienced gastrointestinal symptoms after travel. Microscopically, the presence of the parasite was found in 74 stool specimens, whereas the coproantigen was detected in 73 samples (sensitivity 98.6%). However, by comparing microscopy with the enzyme immunoassay and serum antibody testing, the sensitivity was 87.5%, 57% (IgG) and 50% (IgM), respectively. Serology seems to be less diagnostically useful due to its lower accuracy (17). In a prospective study to compare the routine method and direct immunofluorescence (DFA) in the detection of *Giardia*, a significantly higher sensitivity for DFA was obtained - 99.2%, while for the microscopic method it was 66.4% (18). No dependence was observed while analyzing the influence of selected microorganisms on *G. intestinalis* determination, which confirms the possibility of using microscopic and immunoenzymatic methods in the diagnosis of protozoan infections with co-existing infections with intestinal pathogens. In turn, as far as compounds that stimulate digestive functions, preservatives and antioxidants are concerned, a small influence of potassium sorbate and a significant effect of citric acid on the results of the determinations were found. The antibacterial properties of citric acid were previously described in the literature (19-20).

There are no papers on the influence of other microorganisms and compounds stimulating digestive functions, preservatives and antioxidants on the detection of *G. intestinalis* in the literature. It is necessary to continue research on the impact of various factors on the detection of protozoa due to the small number of samples used in presented study.

Conclusion

The presence of other microorganisms does not affect the results of microscopic and immunoenzymatic tests used to detect the presence of *G. intestinalis* in stool samples.

Citric acid affects the result of the test for *G. intestinalis* thus as an antioxidant added to foods, it can increase their safety.

Acknowledgements

No financial support was received for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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