

Research Article

Investigation of Faecal-Oral Transmitted Parasites in Communal Toilets

Ortak Kullanılan Tuvaletlerde Fekal-Oral Yolla Bulaşan Parazitlerin Araştırılması

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Abstract

Purpose: The aim of the study was to determine the parasites transmitted by the faecal-oral route in communal toilets and to study the personal hygiene habits of the users of these toilets.

Material and Method: A total of 96 swab samples, 24 each from water containers, door handles, faucet heads and sink edges used for anogenital cleaning in toilets, were investigated for parasites transmitted by fecal-oral route. DNA was isolated from these samples using the DNeasy PowerSoil kit and real-time PCR analysis was performed using parasite-specific primer-probes. A survey was also conducted to determine the personal hygiene habits of students using the toilets, including questions such as hand washing and flushing.

Results: In 24 swab samples (n=24) taken from water containers used for anogenital cleansing in toilets, *C. parvum* was detected in 16.7% (4/24), *E. histolytica* in 8.3% (2/24) and *G. lamblia* in 12.5% (3/24), while no parasites were found in 62.5% (15/24). In 24 swab samples taken from door handles, *C. parvum* was detected in 8.3% (2/24), *E. histolytica* in 4.2% (1/24) and *G. lamblia* in 8.3% (2/24), while no parasites were detected in 79.2% (19/24). In 24 swab samples taken from door handles, *C. parvum* was detected in 8.3% (2/24), *E. histolytica* in 4.2% (1/24) and *G. lamblia* in 8.3% (2/24), and no parasites were detected in 79.2% (19/24). In 24 swab samples taken from tap heads, *C. parvum* was detected in 12.5% (3/24) and *G. lamblia* in 4.2% (1/24), while no parasites were detected in 83.3% (20/24). In 24 swab samples taken from the sink edges, *C. parvum* was detected in only 4.2% (1/24) and no parasites were detected in 95.8% (23/24). According to the survey results, it was found that girls paid more attention to hand washing hands and flushing toilets than boys (p<0.05), and it was found that these hygiene habits were first acquired in the family (p<0.05). However, it was found that students were not sufficiently informed about the possibility of contracting diseases through communal toilet facilities.

Conclusion: Communal toilets in large centres such as schools and hospitals play an important role in the transmission of faecal-oral parasites and the spread of intestinal parasitic diseases. However, families play the primary role in helping people to adopt personal hygiene habits in toilets.

Keywords: Toilet, personal hygiene, parasites

Öz

Amaç: Çalışmada ortak kullanılan tuvaletlerde fekal-oral yolla bulaşan parazitleri belirlemek ve bu tuvaletleri kullanan kişilerin kişisel hijyen alışkanlıklarını araştırmak amaçlanmıştır.

Gereç ve Yöntem: Tuvaletlerde anogenital temizlikte kullanılan su kapları, kapı kolları, musluk başlıkları ve lavabo kenarlarından alınan 24'er adet olmak üzere toplam 96 adet sürüntü örneği fekal-oral yolla bulaşan parazitler açısından incelendi. Bu örneklerden DNAeasy PowerSoil kiti kullanılarak DNA izole edilmiş ve parazitlere özgü primer-problar ile Real-Time PCR analizi yapılmıştır. Ayrıca, tuvaletleri kullanan öğrencilerin kişisel hijyen alışkanlıklarını belirlemek için el yıkama, sifon çekme gibi soruları içeren bir anket yapılmıştır.

Bulgular: Tuvaletlerde anogenital temizlikte kullanılan su kaplarından alınan 24 adet sürüntü örneğinde (n=24) %16,7 (4/24) oranında *C. parvum*, %8,3 (2/24) oranında *E. histolytica* ve %12,5 (3/24) oranında *G. lamblia* tespit edilmişken, %62,5 (15/24)'inde herhangi bir parazit bulunmamıştır. Kapı kollarından alınan 24 adet sürüntü örneğinde ise %8,3 (2/24) *C. parvum*, %4,2 (1/24) *E. histolytica* ve %8,3 (2/24) *G. lamblia* saptanmış, %79,2 (19/24)'inde parazit saptanmamıştır. Musluk başlıklarından alınan 24 adet sürüntü örneğinde %12,5 (3/24) *C. parvum* ve % 4,2 (1/24) *G. lamblia* tespit edilirken, %83,3 (20/24)'ünde parazit tespit edilmemiştir. Lavabo kenarlarından alınan 24 adet sürüntü örneğinde sadece %4,2 (1/24) *C. parvum* saptanmış, %95,8 (23/24)'inde parazit saptanmamıştır. Anket sonuçlarına göre, kızların erkeklere göre daha çok el yıkamaya ve sifon çekmeye özen gösterdikleri tespit edilmişken (p<0.05), bu tür hijyen alışkanlıklarını ilk ailede aldıkları belirlenmiştir (p<0.05). Bununla beraber öğrencilerin tuvaletlerde ortak kullanılan aletlerden hastalık bulaşabileceği hakkında yeterli bilgilerinin olmadığı tespit edilmiştir (p<0.01).

Sonuç: Okul, hastane gibi büyük merkezlerde ortak kullanılan tuvaletler fekal-oral yolla bulaşan parazitlerinin bulaşmasında ve bağırsak parazitler hastalıklarının yayılmasında önemli rol oynamaktadır. Bununla beraber kişilerin tuvaletlerdeki kişisel hijyen alışkanlıklarının kazanılmasında önce aileleri etkilidir.

Anahtar Kelimeler: Tuvalet, kişisel hijyen, parazitler

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INTRODUCTION

Diseases caused by intestinal parasites transmitted by the faecal-oral route are a major public health problem in developing countries. Parasites transmitted by the faecal-oral route include *Balantidium coli*, *Blastocystis spp.*, *Cytoisopora*, *Cryptosporidium parvum* (*C. parvum*), *Dientamoeba fragilis*, *Entamoeba histolytica* (*E. histolytica*), *Enterobius vermicularis* and *Giardia lamblia* (*G. lamblia*). Many geographical, geological, climatic, biological, social, cultural and economic factors influence the transmission and spread of these parasites. Among the most important factors for transmission are inadequate sanitation (leading to open defecation) and poor hygiene practices (1).

According to the World Health Organization, the “F-chart” was published in 1958 to prevent and control diseases transmitted by the faecal-oral route. This diagram shows that faecal-oral transmission occurs via water, hands, arthropods, and soil. Words beginning with the letter “F” were used (fluid, fingers, flies, food, fields, fomites) have been used to memorise the factors in this arrangement. The F-chart is used to illustrate that sanitation, especially toilets, hygiene, and hand washing, can act as effective barriers against the faecal-oral route of disease transmission (2).

Using contaminated toes, touching surfaces that everyone touches in public toilets (door handles, sink edges), using communal water containers to clean the anogenital after using the toilet, contact with people whose hands are contaminated with faeces, children who eat faeces due to mental disorders (coprophagia) and people who do not pay attention to hand hygiene rapidly increase the spread of parasites transmitted via the faecal-oral route. Toilet and hand hygiene are very important in the spread of parasites transmitted by the faecal-oral route. In our country and around the world, children are usually introduced to the toilet between 18 and 24 months of age (3,4). After toilet training, children’s personal hygiene education begins in the home and continues in primary school.

The most important factor in the spread of parasites transmitted by the faecal-oral route is toilet use and people’s personal hygiene habits after using the toilet. In our study, the parasites transmitted by the faecal-oral route in toilets communal by primary school children were investigated in swab samples taken from water containers, door handles, faucet heads and sink edges that everyone touches. In addition, the children’s personal hygiene habits after using the toilet were assessed through questionnaires.

MATERIALS AND METHODS

Sample Collection

The study was conducted in a primary school in Adana province in the Çukurova region, which is considered to have a low socio-economic status, after obtaining permission from the school principal and the parents of the students. Samples were collected from communal toilets used by a total of 280 students aged 6-10 between March 2024 and June 2024. Samples were collected with sterile swabs from water containers, door handles, faucet heads, and sink edges used to clean the anogenital where parasites may be present, using sterile swab sticks.

DNA Isolation and Real-Time PCR Reaction

Streak swab samples were transferred to physiological serum water and extracted using the DNAeasy PowerSoil Kit (Cat No:12888, Qiagen, Germany). For real-time PCR analysis, multiplex real-time PCR was performed using primer probes specific to parasite species (*Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*) that can be transmitted the faecal-oral route and are found in toilets.

The primer-probes used in the study were designed according to the studies of Haque et al., and *C. parvum* parasite Cp-583F: CAA ATT GAT ACC GTT TGT CCT TCT G; Cp733R: GGC ATG TCG ATT CTA ATT CAG CT; Cp-TRT-P:TGC CAT ACA TTG TTG TCC TGA CAA ATT GAA T-DDQ2), for *E. histolytica* parasite (Eh-F: AAC AGT AAT AGT TTC TTT GGT TAG TAA AA; Eh-R: CTT AGA ATG TCA TTT CTC AAT TCA T; Eh-YYT-P: ATT AGT ACA AAA TGG CCA ATT CAT TCA-Dark Quencher) ve for *G. lamblia* (Gd-80F: GAC GGC TCA GGA CAA CGG TT; Gd-127R: TTG CCA GCG GTG TCC G; Gd-FT-P: FAM-CCC GCG GCG GTC CCT GCT AG-DDQ1); primer-probes were used (5).

For the reaction mixture, a total of 25 µL of a mixture was prepared containing 2x QuantiTaqMan PCR Buffer (Qiagen, Hilden, Germany), 0.4 µmol/L each of Eh-f and Eh-r primers, 0.08 µmol/L of Eh-YYT-P probe, 0.4 µmol/L of Gd-80F and Gd-127R primers, 0.12 µmol/L of Gd-FT-P probe, 1 µmol/L of Cp-583F and Cp-733R primers, 0.5 µmol/L of Cp-TRT-P probe 3 µL of DNA sample and 4 µL of purified water. DNA samples were amplified using a thermal cycling programme consisting of 35 cycles (95°C 5 min, 55°C 1 min, 72°C for 10 min) with an initial denaturation at 95°C for 5 min and a final extension at 72°C for 10 min. When evaluating the results of the real-time PCR reaction, samples with a threshold cycle value (Ct ≤26) were

considered positive and samples with a threshold cycle value ($Ct > 26$) were considered negative.

Evaluation of Students' Toilet Habits

A short survey was carried out to assess the personal hygiene habits of students using the toilets, including questions on hand washing and toilet flushing.

No sampling was used in the study, and all students who volunteered to participate in the study were included in the study. A "Personal Hygiene Questionnaire", developed from the literature, was used to measure students' knowledge of personal hygiene. The questionnaire form includes the socio-demographic characteristics of the students and their level of knowledge about personal toilet use (6). The personal hygiene information form was prepared according to the descriptive research model and consists of 5 questions (a. I wash my hands after using the toilet, b. I always wash my hands with soap and water, c. I flush the toilet after using the toilet, d. Where did you first learn how to use the toilet, e. I know that I can get diseases from toilets). The validity and reliability study of the survey was conducted by Başkaya et al (6).

Statistical Analysis

The data obtained in the study were entered into the SPSS 21.0 computer program (SPSS Inc., Chicago, IL, USA) and statistical analysis was performed. The distribution of parasites found in the environmental samples taken from the toilets and the numerical data obtained from the survey results showing the toilet habits of the students included in the study were expressed as frequencies and percentages. In addition, the X2 test was used to determine the suitability of the data for normal distribution. The statistical significance of the results was evaluated at $p < 0.05$ level and 95% confidence interval.

RESULTS

A total of 96 swab samples, 24 each from water containers, door handles, faucet heads and sink edges used for anogenital cleansing in toilets, were investigated for parasites transmitted by the faecal-oral route. In swab samples taken from 24 water containers ($n=24$), *C. parvum* was detected in 16.7% (4/24), *E. histolytica* in 8.3% (2/24) and *G. lamblia* in 12.5% (3/24), while no parasite was detected in 62.5% (15/24). Of the 24 swab samples taken from door handles, *C. parvum* was found in 8.3% (2/24), *E. histolytica* in 4.2% (1/24) and *G. lamblia* in 8.3% (2/24), while no parasites were found in 79.2% (19/24). In 24 swab samples taken from tap heads, *C. parvum* was found in 12.5% (3/24) and *G. lamblia* in 4.2% (1/24), while *E. histolytica* was not found in these

samples and no parasite was detected in 83.3% (20/24). *C. parvum* was detected in 4.2% (1/24) of 24 swab samples taken from the sink edges and no other parasites were found in these swab samples (Table 1).

Table 1. Parasites detected in swab samples taken from communal toilets (The percentages given in the table are row percentages).

Sample taken region (n=24)	<i>C. parvum</i> (%)	<i>E. histolytica</i> (%)	<i>G. lamblia</i> (%)	No parasite (%)	Total (%)
Water containers	16.7 (4/24)	8.3 (2/24)	12.5 (3/24)	62.5 (15/24)	100 (24/24)
Door handles	8.3 (2/24)	4.2 (1/24)	8.3 (2/24)	79.2 (19/24)	100 (24/24)
Faucet heads	12.5 (3/24)	0 (0/24)	4.2 (1/24)	83.3 (20/24)	100 (24/24)
Sink edges	4.2 (1/24)	0 (0/24)	0 (0/24)	95.8 (23/24)	100 (24/24)

A survey was conducted on the toilet use habits of the pupils in the primary school where the toilet samples were taken. The age of the students included in the study ($n=280$) ranged from 6 to 13 years, with a mean age of 7.4 ± 1.12 years. The socio-demographic characteristics of the families of the students included in the study and the distribution of the students' responses to the "Personal Hygiene Questionnaire" are shown in Table 2.

Table 2. Socio-demographic characteristics of students using toilets according to survey results

Socio-demographic characteristics	n=280	%
Gender		
Male	100	35.7
Female	180	64.3
Mother's education status		
Illiterate	10	3.5
Primary education	190	67.9
High school	68	24.3
Licence	12	4.3
Father's education status		
Illiterate	2	0.7
Primary education	192	68.6
High school	72	25.7
Licence	14	5.0
Mother's employment status		
Officer	23	8.2
Employee	102	36.4
Does not work	155	55.4
Father's employment status		
Officer	89	31.8
Employee	166	59.3
Does not work	25	8.9

When comparing the hand washing and flushing habits of male and female students after using the toilets were compared, it was found that girls washed their hands and flushed the toilets more ($p < 0.05$). It was found that most of the students received their first toilet training from their families and learnt the habits of hand washing and toilet flushing habits the toilet from their families ($p < 0.05$). No significant relationship was found between the income level of the students' parents and their toilet habits ($p > 0.05$). However, an increase in the percentage of students washing their hands was observed as the level of education of their

parents increased ($p<0.05$). It was also found that students did not have sufficient knowledge about the transmission of diseases through toilets ($p<0.01$) (Table 3).

Table 3. Results obtained from the evaluation of personal hygiene habits of students using toilets.

Hygiene behaviors	n=280	%
Hand washing		
After toilet	103	36.8
After toilet, after meals	121	43.2
After toilet, after meals, after the game	56	20.0
How to wash hands		
With water	100	35.7
With soap	180	64.3
I flush the toilet after		
Yes	194	69.3
No	86	30.7
Where did you learn to use the toilet?		
Home	196	70.0
School	84	30.0
Do you know which diseases are transmitted through toilets?		
Yes	79	28.2
No	201	71.8

DISCUSSION

Intestinal parasitic diseases caused by parasites transmitted by the faecal-oral route are widespread both in our country and worldwide. The prevalence of intestinal parasitic diseases in society depends on people's toilet habits and hygiene behaviour. These diseases affect all segments of the population, especially children growing. Faecal-oral transmitted parasites, which are usually asymptomatic, can cause mental and physical retardation and damage a country's economy through loss of labour (7). Ecological factors such as temperature, humidity, soil and vegetation, which vary according to climatic conditions, as well as the toilet habits of infected people, play an important role in the life cycle of these parasites. Poor hygiene in public toilets also contributes to the spread of these parasites (7,8).

Several studies on the epidemiology of intestinal parasitic diseases have been carried out at different times and in different geographical regions. According to these studies, the prevalence of parasites causing intestinal parasitic diseases has been reported to be 9.3-36.4% (9). This rate may vary depending on the diagnostic methods used to detect intestinal parasites, whether or not non-pathogenic species are included and whether or not patients have symptoms. In studies conducted in our country, the most common causes of intestinal parasitic diseases were *Blastocystis spp.*, *Entamoeba spp.* and *G. lamblia* parasites transmitted by the faecal-oral route (10).

Bedir et al. found *Entamoeba spp.* (1.15%) most frequently in stool samples from a hospital in Kars province, followed by *G. lamblia* (0.48%) (11). In their study conducted in İzmir to determine the prevalence of intestinal parasitic diseases, Gürbüz et al. reported that the incidence rate of *E. histolytica*

(0.7%) and *G. lamblia* (0.3%) was higher than that of other parasites (12). Günbey et al. investigated the prevalence of intestinal parasites in patients attending a university hospital in Elazığ province and reported that *G. lamblia* was the most common (46.6%) (13). Eren et al. investigated intestinal parasites in patients undergoing endoscopy and colonoscopy and reported a rate of 4.5% for *G. lamblia* and 2.3% for *E. histolytica* (14). The incidence of *G. lamblia*, a parasite transmitted by the faecal-oral route, has been reported to be 1.9-37.7% in our country, and studies have reported that *G. lamblia* is more common than other parasites (15). In our study, swab samples were taken from communal toilets and the most common species found was *G. lamblia*, which supports the results of other studies. Because this parasite has a cyst form that is resistant to standard chlorination processes under outdoor chlorination procedures, it should be considered as an important pathogen in areas where there is inadequate sanitation and personal hygiene are inadequate.

Parasites transmitted by the faecal-oral route can continue their life cycle in drinking water and environmental water samples. Therefore, transmission to humans can occur from these sites. Sağlam et al. investigated the presence of parasites transmitted by faecal-oral route in drinking and environmental water samples in Denizli province and reported 25% *C. parvum* and 14.28% *G. lamblia* in their study (16). Ogundere et al. highlighted that *C. parvum* and *G. lamblia* species are the major parasites causing waterborne diseases in tropical coastal waters in Nigeria and that they can pose serious health risks to humans (17). In our study, *C. parvum* and *G. lamblia* were detected on faucet heads and sink edges. This shows that these parasites are resistant to chlorination and are likely to be transmitted to humans during hand washing. In particular, *C. parvum* is a parasite that is resistant to physical and chemical inactivation and is likely to be found on faucet heads due to the small diameter of its oocysts.

Diseases caused by parasites transmitted by the faecal-oral route cause economic losses in our country due to loss of labour and treatment costs. Due to reasons such as rapid urbanisation, migration and infrastructure problems that have continued in recent years, these parasites still pose a threat to public health today. Failure to wash hands after performing anal hygiene and contamination with parasites of water containers, door handles, faucet heads and sink edges commonly used in toilets also result in the rapid spread of parasites transmitted by the faecal-oral route. Toilet use is very important for ensuring personal hygiene, and in our country, toilet training starts at an earlier age (28.44 ± 9.04 months) than in developed countries (18). Gün et al. investigated the personal hygiene habits of adolescents in Aydın province and reported that girls paid more attention to personal hygiene than boys (19). Şimsek et al. investigated hand hygiene behaviours in children and found that 39.1%

of children paid attention to hand hygiene (20). Both studies emphasised, it was emphasized that personal hygiene education starts in the family and that school education is important for its spread. Toilet training is one of the most important stages in a child's development and usually the first training starts in the family. Our study results confirm that toilet training and personal hygiene behaviours are first acquired in the family. The fact that our study was conducted in a single school and with a small number of students is the limitation of the study. It would be beneficial to work in more schools with more students to increase the accuracy of our results.

In countries with young populations like ours, children's poor personal hygiene habits in children can lead to disease. One of the areas where this personal hygiene is most effective is in communal toilets. The materials used for personal hygiene in toilets can unwittingly contribute to the spread of disease. For this reason, regular education about the faecal-oral parasites that can be transmitted in communal areas will be effective in preventing the spread of disease. Regular sterilisation of communal areas and materials will also be beneficial.

Declarations

Ethics Committee Approval: Ethics committee approval was obtained from the Aksaray University, Health Sciences Scientific Research Ethics Committee (Date: February 13, 2025, Decision No: 2025/33)). This study was conducted according to the principles of the Declaration of Helsinki.

Authorship Contributions: Concept: FE, DA, OS, HO. Design: FE, DA, OS, HO. Data Collection or Processing: DA, OS, HO. Analysis or Interpretation: FE, DA. And Literature Search: DA, OS, HO. Writing: FE. All authors approved the final version of the manuscript.

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