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Evaluation of parasite contamination on toothbrushes in children in Southeastern Brazil.

Evaluación de la contaminación parasitaria en cepillos de dientes en niños del sudeste de Brasil

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ABSTRACT

Introduction: The toothbrush is an important object for the hygiene of the oral cavity and an effective mechanism to remove the oral waste. ***Objective***: To evaluate the perception of care, storage and parasitic contamination of toothbrushes in children with special health care needs (CSHCN) and children without special health care needs (CWSHCN) in Southern of Minas Gerais State. ***Material and Methods***: This is an observational cross-sectional non-randomized study. The population consisted in 54 children, with age between 7 and 14 years. The questionnaire was distributed to patients to evaluate the perception of care and storage of children's toothbrushes. Investigation of toothbrushes contamination was performed by parasitological examination and real-time polymerase chain reactions. ***Results***: Regarding the procedures performed after brushing, 50.0% of children with special health care needs (CSHCN) and 56.3% of children without special health care needs (CWSHCN) report washing their brush bristles with water (p <0.001). Both groups did not use an antiseptic solution on toothbrushes. 73.3% of (CSHCN) and 58.7% of (CWSHCN) answered that they use some protection (brush holder and bathroom cabinet) to avoid exposure of brushes to the environment (p <0.001). ***Conclusion***: The children investigated by the study presented good conditions of care and storage of their toothbrushes. No contamination by pathogenic parasites was found during the study period.

KEY WORDS: Oral health, child, entamoeba, cryptosporidium, parasites.

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RESUMEN

Introducción: El cepillo de dientes es un objeto importante para la higiene de la cavidad bucal y un mecanismo eficaz para eliminar los residuos bucales. ***Objetivo:*** Evaluar la percepción del cuidado, almacenamiento y contaminación parasitaria de los cepillos dentales en niños con necesidades especiales de salud (CSHCN) y niños sin necesidades especiales de salud (CWSHCN) en el sur del estado de Minas Gerais. ***Material y Métodos:*** Se trata de un estudio observacional transversal no aleatorio. La población consistió en 54 niños, con edad entre 7 y 14 años. El cuestionario fue distribuido a los pacientes para evaluar la percepción del cuidado y almacenamiento de los cepillos dentales de los niños. La investigación de la contaminación de los cepillos dentales se realizó mediante un examen parasitológico y reacciones en cadena de la polimerasa en tiempo real. ***Resultados***: En cuanto a los procedimientos realizados tras el cepillado, el 50,0% de los niños con necesidades especiales de atención sanitaria (NCNEAS) y el 56,3% de los niños sin necesidades especiales de atención sanitaria (NSNEAS) refieren lavar las cerdas del cepillo con agua (p <0,001). Ambos grupos no utilizaron una solución antiséptica en los cepillos de dientes. El 73,3% de los (NCNEAS) y el 58,7% de los (NSNEAS) contestaron que utilizan alguna protección (portacepillos y mueble de baño) para evitar la exposición de los cepillos al medio ambiente (p <0,001). ***Conclusiones***: Los niños investigados por el estudio presentaron buenas condiciones de cuidado y almacenamiento de sus cepillos dentales. No se encontró contaminación por parásitos patógenos durante el período de estudio.

PALABRAS CLAVE: Salud oral, niño, entamoeba, cryptosporidium, parásitos.

INTRODUCTION

The parasitism is conceptualized as an association of lives where just one side has benefits (1). Contamination by intestinal parasitic diseases has been considered a worldwide pandemic . The World Health Organization (WHO) in 2017, estimated that one billion people have been diagnosed and treated for parasitic disease (2).

The toothbrush is considered an important object for the hygiene of the oral cavity and for being the most effective mechanism to remove the oral waste and to disorganization the dental biofilm (3). The routine of using this object without the correct disinfection and storage can contribute to the proliferation of pathogenic microorganisms and also promote cross contamination by parasites (protozoa and helminthes), viruses, fungi and bacteria (4,5,6).

The contaminations of toothbrushes by parasites was previously evaluate by Borges et al.,(5) and later by Batista y França-Botelho (4). However, when they related about problems for quality of life and parasite contamination in toothbrushes in children with special health care needs (CSHCN) and children without special health care needs (CWSHCN) the scientific literature is scarce or unexplored.

The perception of health status is directly correlated with the cognitive capacity, the socioeconomic and cultural conditions of individuals.(6,7,8,9) In this sense, the physical, cognitive and psychological limitations present in childhood both for CWSHCN and thoses with CSHCN hindes their self-perception about an unfavorable oral hygiene, which makes them susceptible to oral diseases (9, 10).

So, the family´s knowledge about oral health conditions becomes necessary because they are responsible about children's quality of life.

Therefore, the present study had aimed to evaluate the perception of care, storage and parasitic contamination of toothbrushes in CSHCN and CWSHCN in Southern Minas Gerais State, Brazil.

MATERIAL AND METHODS

The guidelines STROBE Statement (Strengthening the Reporting of Observational studies in Epidemiology) was followed for this study (11).

This study was approved by the Human Research Ethics Committee of the Faculty of Dentistry of the Federal University of Alfenas (2.773.896/2018). All children, with or without CSHCN, accompanied by their guardians, were informed about the assessment procedures for this study. After agreeing, the parents signed the free and informed consent form, authorizing children with CSHCN and CWSHCN to participate in the study, in according with the Declaration of Helsinki (Informed Consent Term).

This is an observational cross-sectional non-randomized study, developed in the city of Alfenas,

located in the Southern of Minas Gerais State, Brazil. The population study consisted of 54 children divided in two groups: CSHCN group, composed by 32 children with special health care needs; and CWSHCN group, composed by 22 children without special health care needs. The following inclusion criteria were adopted: children aged between 7 and 14 years, with special needs, enrolled in public schools and/or coming from the Association of Parents and Friends of the Exceptional (APAE-Alfenas). Children aged between 7 and 14 years old, attended at the pediatric dentistry clinic of the Federal University of Alfenas-MG. Children which not meet the inclusion criteria or whose parents did not sign the free and informed consent form were excluded from study.

The evaluators were two graduates in Dentistry from UNIFAL (FIDC and LBO), previously trained and calibrated. At the end, a (Kappa= 0.92-FIDC) and (Kappa= 0.91-LBO) was obtained for the researchers.

The questionnaire in this present study was based on the questionnaire for the evaluation of the care related to the storage and disinfection of toothbrushes by dentistry students which was modified by the aim of this study Mialhe et al.,(12). The information was collected in a family´s house where the child responsable for his toothbrush answered these questions: Acquisition time, hygiene procedures after the use, the use of antiseptic solution and the storage of the toothbrush. After the application of the questionnaire, the child's toothbrush was collected and replaced with a new one. The collected toothbrush was packed in a clean individual plastic bag and the patient was identified by codes.

The toothbrushes were dipped in a falcon tube with saline solution and were rubbed on the tube wall. The falcon with the toothbrush was vigorously shaked in Vortex for 5 minutes. The fragments were centrifuged in a 30 ml solution of saline at 3000 rpm for 15 minutes. The samples were stained with a drop of Lugol to be analyzed in conventional microscopy (vertical BX43 microscopy with manual system) at magnifications of 10X and 40X.

The DNA was extracted from the toothbrush precipitate. All samples were processed according to the basic DNA extraction protocol described by Sambrook et al., and with the modifications previously described Gomes et al.. The samples were dissolved in lysis buffer (10 mM Tris-HCl, pH 8.0; 10 mM EDTA; 0,5% SDS; 0,01% N-laurilsarcozil and 100µg/mL proteinase K) and incubated in a 56°C water bath until the material is completely lysed. The DNA was extracted by phenol/chloroform/isoamyl protocol and precipitated with isopropanol. The precipitated DNA was washed with 70% ethanol and centrifuged at 10,000g for 10 minutes. The sediment was dissolved in ultra pure water. All DNA genetic material was dosed in Nanodrop ND2000 (Thermo Scientific).

All the quantitative real-time polymerase chain reactions were performed using TaqMan type hydrolysis probes. The probes were double-checked, a fluorochrome was covalently bonded at the 5`-end and a non-fluorescent quencher (NFQ) was bonded at the 3`-end. The nucleotide sequences used for the detection of *Cryptosporidium parvum* were: 5' -ACT TTT TGT TTG TTT TAC GCC G-3' (Forward JVAGF), 5' -AAT GTG GTA GTT GCG GTT GAA-3' (Reverse JVAGR) e 5' -ATT TAT CTC TTC GTA GCG GCG-3' (obe JVAGP2, marked with fluorophore FAM). For the identification of *Cryptosporidium hominis* the sequences were: 5' -ACT TTT TGT TTG TTT TAC GCC G-3' (Forward JVAGF), 5' -AAT GTG GTA GTT GCG GTT GAA-3' (Reverse JVAGR) e 5' -ATT TAT TAA TTT ATC TCT TAC TTC GT-3 (Probe JVAGP1, FAM). For identification of *Entamoeba histolytica*: 5' ATT GTC GTG GCA TCC TAA CTC A 3' (Forward Ehd-239F), 5' GCG GAC GGC TCA TTA TAA CA 3' (Primer Reverse Rhd-88R) e 5' TCA TTG AAT GAA TTG GCC ATT T 3' BHQ1 (Probe hysto-96T, FAM). The primers and probes used in this work were synthesized in the CDC Biotechnology Core Facility.

The reactions were performed in a Step One Real Time PCR System (Applied Biosystems), in a final volume of 10µL per reaction. A mix containing 5µL of 2X TaqMan Universal PCR Master Mix (Life Technologies), 1 µL of a mixture including the beginners forward, reverse and probe primers, followed by 3µL of DNAse and RNAse-free water and 1µL of DNA from the samples or controls has been added. The amplifications occurred in an initial cycle of 50°C for 2 minutes. The second stage was a 95°C cycle for 10 minutes. In the next step 40 cycles were performed at 95°C for 15 seconds and 60°C for 1 minute. All tests included positive and negative controls (13,14,15).

For statistical analysis, the software Statistical Package for Social Science for Windows (Version 22; IBM Corp., Chicago, IL, USA) was used. The mean, median, standard deviation and distribution

frequencies of the data obtained were used. To compare the qualitative variables of the total score of the questionnaire, the data were submitted to the Chi-square test. For all variables, a significance level of $p<0.05$ was considered.

RESULTS

54 children were evaluated in this study (CSHCN group: boys=71,9%; girls=28,1%; CWSHCN group: boys=36,4%; girls=63,6%) as shown in table 1.

Table 1. Demographic distributions of study frequency according to the age and sex of CSHCN and CWSHCN.

Variable	Category	CSHCN (n= 32)	CWSHCN (n=22)
		N/ %	N/ %
Sex (n / %)	Male	23 / 71.9	8 / 36.4
	Female	9 / 28.1	14 / 63.6
Age (n / %)	7 – 10	15 / 46.9	20 / 90.9
	11 – 14	17 / 53.1	2 / 9.1

Note - CSHCN: Children with special health care needs; CWSHCN: children without special health care needs.

Table 2 shows the results of the perception of care and storage of toothbrushes of CSHCN and CWSHCN. Significant differences were found between the groups in the variables: acquisition times, procedure after using the toothbrush and toothbrush storage. As for the use of antiseptic solutions, both groups do not use them to disinfect toothbrushes.

Table 2. Evaluation of the necessary care for the storage and disinfection of toothbrushes of CSHCN and CWSHCN.

Variable	Category	CSHCN (n=32)	CWSHCN (n=22)	p value
		N/ %	N/ %	
Acquisition time	1 month	4 / 12.5	6 / 27.3	0.000*
	2 months	12 / 37.5	7 / 31.8	
	3 months	11 / 34.4	7 / 31.8	
	4 months	3 / 9.4	2 / 9.0	
	5 months	1 / 3.1	-	
	6 or more months.	1 / 3.1	-	
Procedure after using the toothbrush	Doesn´t wash the bristles.	-	-	0.000*
	Wash the bristles on water.	18 / 56.3	11 / 50.0	
	Tap the toothbrush on the sink to remove excess water from the bristles.	6 / 18.7	6 / 27.3	
	Run the fingers through the bristles to remove the excess of water.	5 / 15.6	2 / 9.1	
	Wipe the bristles on a towel.	3 / 9.4	3 / 13.6	
Use of antiseptic on the toothbrush	Use of antiseptic.	-	-	-
	Doesn´t use antiseptic.	32 / 100	22 / 100	
Toothbrush storage	Over the sink without protection.	3 / 9.3	3 / 13.6	0.000*
	Over the sink, inside the recipient	8 / 25	2 / 9.1	
	On the wall of the bathroom inside the brush holder.	9 / 28.1	5 / 22.7	
	Over the sink, inside the brush holder.	6 / 18.8	6 / 27.3	
	Inside the bathroom cupboard.	6 / 18.8	6 / 6.0	

Note - CSHCN: Children with special health care needs; CWSHCN children without special health care needs; *= $p<0.001$.

The table 3 presents mean, standard deviation, median and questionnaire summaries for the evaluation of the care related to the storage and disinfection of toothbrushes.

Table 3. Mean, standard deviation, median and questionnaire summaries for the evaluation of the care related to the storage and desinfection of toothbrushes

Variable	CSHCN (n=32)					CWSHCN (n=22)				
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum
Acquisition time	2.31	0.94	2.0	1.89	2.73	2.62	1.12	2.5	2.21	3.03
Procedure after using the toothbrush	2.5	0.96	2.0	2.07	2.92	2.78	1.03	2.0	2.40	3.15
Use of antiseptic on the toothbrush				-					-	
Toothbrush storage	3.72	1.31	4.0	3.14	4.31	3.12	1.26	3.0	2.66	3.58

Note: CSHCN: Children with special health care needs; CWSHCN: children without; -: Did not show results.

The table 4 shows the parasitological test of toothbrushes. No contamination was found in the CSHCN group. However in the CWSHCN group was observed one sample (0,22%) contaminated by the protozoan Entamoeba sp..

Table 4. Percentage of contamination found in toothbrushes of CSHCN and CWSHCN.

Microorganism	CSHCN (n=32)	CWSHCN (n=22)
Entamoeba sp. (n / %)	-	1 / 0.22
Cryptosporidium spp. (n / %)	-	-

Note - CSHCN: Children with special health care needs; CWSHCN children without special health care needs.

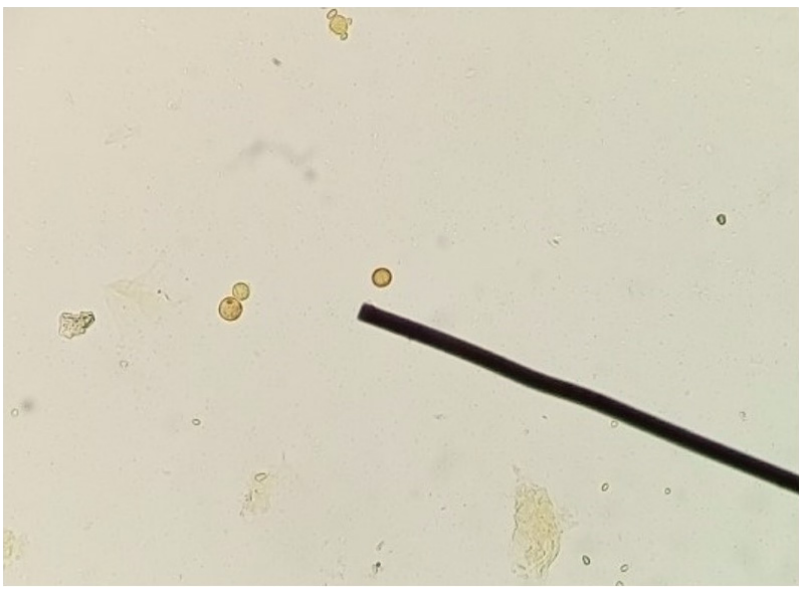

Figure 1. Parasitological analysis of the toothbrush performed by conventional microscopy in the objective 400 times, stained by Lugol. It can be observed the trofozoites of Entamoeba Sp.

Regarding the expression of *Entamoeba histolytica*, *Cryptosporidium parvum* and *Cryptosporidium hominis* genes, the investigated samples did not show amplification of these genetic material, although the endogenous control (Human Betaglobulin Gene) was amplified in all reactions, which guaranteed the quality of the extraction of the genetic material from the samples.

DISCUSSION

This study was carried out in a group of children from a micro-region located in the southern of Minas Gerais State, Brazil. The care and storage of the toothbrush was investigated by the questionnaire developed by Mialhe et al., (12), and the contamination by parasites was analyzed by parasitological examination and qRT-PCR (14). Until now, the association between both techniques has been employed to assess or investigate the presence of pathogens that may influence the progression of oral and systemic diseases. However, the care, storage, parasitic contamination, toothbrushes and molecular diagnosis are few explored by the medical and dental literature.

After the application of the questionnaire about storage, it was observed that the time for changing the toothbrushes in the CSHCN and CWSHCN groups was less than three months. These results were similar to the study by Mialhe et al. in which 67,6% of the sample changed their toothbrush every two months (12). These values were reinforced in the study by Queiroz et al., in which 27,3% of the researchers changed their toothbrush between two to three months of use (16). The dental literature does not show an ideal period of changing the toothbrush, the replacement of this object should be when the bristles are completely worn and traumatizing the periodontal tissues (17,18,19).

The most of children investigated in this study washed the toothbrushes after use, as well removed the excess of water from toothbrushes bristles. Some studies demonstrate that even after washing and removing the excess of water from toothbrush bristles, many opportunistic microorganisms such as bacteria, viruses, fungi and parasitic cysts remain stuck between the bristles promoting microbial contamination (20,21). Regarding the location and form of toothbrush storage, it was observed that the local where these objects are stored has an important relation about the infection by opportunistic and commensal microorganisms.(21, 22) Thus, the groups investigated in our study have a correct way to store their toothbrushes, through the use of some form of protection (brush holder and bathroom cabinet), avoiding exposure of the brushes to the environment (16).

In this way, to keep toothbrushes away from contaminating agents, the use of antiseptic solutions is necessary to reduce the microbiome composed by opportunistic and commensal microorganisms on the toothbrush bristles (23). Although our study groups do not disinfect their toothbrushes with antiseptic solutions.

In this context, the study by Borges et al, evaluated the parasitic contamination in toothbrushes of children living in the city of Alfenas, Brazil. In their results, it was observed that 16.46% of toothbrushes were contaminated by parasites (5). These values were reinforced in the study by Batista y França-Botelho, in children and adults living in the city of Araxá, Brazil. Their results showed that 20% of children had their toothbrushes contaminated by Entamoeba spp. Trophozoites (4). It can be seen in our results that only 0.22% of the sample was contaminated by Entamoeba spp. The possible explanation for the reduction in the number of contaminants in toothbrushes between the studies may be due to better conditions of basic sanitation and distribution of treated water, as well as the improvement in care with the storage of toothbrushes.

The socioeconomic and environmental conditions of individuals are important for the epidemiology of parasitic diseases in Brazil, especially in regions where infection rates have been high for decades. This is due to precarious living conditions, basic sanitation and the sharing of toothbrushes and other objects among family members (3,21,24). However, the possible explanation for the reduction in the number of environmental contaminants on toothbrushes between the study of Borges et al., 1996 and the present investigation is due to better socioeconomic conditions (increase in family percapita income), distribution of treated water of good quality (fluoridated water) and basic sanitation in all homes in the urban perimeter, which justifies the significant decline in the number of contaminated samples in this study (5,25).

The socioeconomic and cultural conditions of individuals is important for the epidemic parasitic diseases in Brazil, especially in regions where infection rates have been high for decades. It happens because of the precarious living conditions, basic sanitation

and sharing of toothbrushes and objects among family members. In addition, the functional, cognitive and nutritional limitations of WSHCN contribute to these parasites becoming pathogenic and promote decline in the quality of systemic and oral health (10,18,24,26).

During the molecular evaluation using the qPCR, the genes for *Entamoeba histolytica*, *Cryptosporidium parvum* and *Cryptosporidium hominis* did not show any amplification, however, in the parasitological evaluation, the presence of the parasite Entamoeba sp. was found in one sample of WSHCN group. The justification for choosing these primes is strictly correlated with clinical characteristics, way of contamination, hygiene and shared use of personal objects (27). In addition, in some cases, immunodeficiency conditions and the specific nutritional risks of these parasites may contribute to an unfavorable clinical condition and even to fatal death, especially in cases of SHCN that they have been vulnerable to systemic and oral diseases (10,18,28).

The differential diagnosis of these parasites is done through molecular techniques or tests with immunological antibodies (29,30). Nowadays, molecular techniques have been used for the differential diagnosis of systemic and oral comorbidities. The parasitological examination has some restrictions such as morphological similarities between the parasites and the need for a high parasite load in the sample. However, the finding of specimens in the samples confirms the diagnosis (26,30,31). Finally, the partial limitation of this study was the small number of participants and their constant change of address. The sentimental and financial value that these toothbrushes have for children should also be taken into account in order to reduce the sample size. Despite a low parasite contamination, it was possible to observe that small changes in the health and hygiene behavior of patients can reduce the incidence of contamination. Suggesting a decrease in contamination observed in study previously carried out in the same city (5).

CONCLUSION

Therefore, the children investigated by the study had good conditions for care and storage of toothbrushes and there was little contamination by pathogenic parasites during the period of the study.

Conflict of interest: the authors have no conflict of interest with this report.

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