The role of toothbrush in the transmission of corona- and influenza viruses — results of an in vitro study

Gerhard Schmalz1 · Laura Feindt1 · Franziska Tanneberger2 · Rainer Haak1 · Ahmed Abd El Wahed2 · Uwe Truyen2 · Dirk Ziebolz1

Received: 18 February 2022 / Accepted: 3 May 2022 / Published online: 10 May 2022 © The Author(s) 2022

Abstract

Objectives The aim of this in vitro study was to investigate viruses’ stabilities on manual toothbrushes using feline coronavirus (FeCoV) as representative of coronaviruses and an Avian influenza A virus H1N1 for influenza viruses.

Material and methods Two viruses, FeCoV (strain Munich; titer 107.5 TCID50/ml) and H1N1 (RE 230/90; titer 106.5 TCID50/ml), were used in this study. Manual toothbrushes were disassembled into bristles, bristle fixation, and back of the toothbrush head, contaminated with the viruses and air-dried for 24 h. In a second experiment, whole toothbrush heads were contaminated, rinsed with water (5 ml for 15 s) and then air-dried.

Results For FeCoV, immediately after contamination, the following average titers were recovered: fixation: 106.41, back of head: 106.81 and bristles: 106.63 TCID50/ml. Following air-drying of 12 (fixation) and 24 h, titers of ≤ 102.5, 103.75, and 102.72 TCID50/ml were found in the respective groups, with a detection limit of 102.5 TCID50/ml. For H1N1, immediately after contamination, the following average titers could be recovered: fixation: 105.53, back of head: 105.97 and bristles: 105.75 TCID50/ml. Following air-drying of 8 (fixation) and 24 h, titers were ≤ 102.5, 103.63, and 103.53 TCID50/ml in the respective group, again with 102.5 TCID50/ml being the detection limit. In case of water rinse, no infectious virus could be recovered after 12 h.

Conclusion Viral load of both viruses is reduced by air-drying, especially following water rinsing.

Clinical relevance The toothbrush itself plays an insignificant role in the self-transmission of coronavirus and influenza virus.

Keywords Toothbrush · Oral hygiene · Coronavirus · Influenza · Transmission

Introduction

During the past 2 years, a viral pandemic with a novel human coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) lead to a global public health crisis. This circumstance caused a high impact on daily life, including dentistry and oral hygiene issues [1]. The virus SARS-CoV-2 is mainly located in the nasopharyngeal tract as a main source for transmission, while the oral cavity and saliva also contains a certain amount of viral load, which is, however, of little value for airborne transmission of the virus [2]. Accordingly, oral hygiene issues were repeatedly and comprehensively discussed in context of the current pandemic situation. On the one hand, usage of mouthwashes to reduce the viral load, and thus, the risk of transmission is an issue of high interest [3]. On the other hand, oral hygiene aids were reported as potential habitat for SARS-CoV-2, increasing the risk of transmission. Thereby, oral hygiene
Influenza A Virus H1N1 (RE 230/90) virus was used as an analogue for a human influenza virus. FeCoV was propagated in Crandell Rees Feline Kidney (CRFK) cells to obtain a titer of $10^{7.5}$ TCID$_{50}$/ml. AIV H1N1 was propagated in chicken embryo fibroblast to a titer of $10^{6.5}$ TCID$_{50}$/ml. The cultivation of the viruses was conducted at 37 °C and 5% CO$_2$. The experiments were performed separately with each of the two viruses to assess the respective characteristics of the viruses.

**Toothbrushes**

Manual toothbrushes (Dr.BEST Original, CLASSIC; GSK Consumer Healthcare, D-80258 München, CH-6343 Rich) were bought from public shops and used as test material. They have standardized flat bristles and were selected in the hardness grade medium. For examination of the contamination, (I) toothbrushes were assembled inside the laminar flow cabinet class II under sterile conditions to the three parts: bristle fixation, the back of the toothbrush, and bristles, which have been investigated separately. (II) For the second experiment, the entire toothbrush head was used.

**Test procedure**

**Viral contamination of three different areas of the toothbrush with subsequent various incubation periods and titer determination**

**Contamination procedure** For the first experiment, the manual toothbrushes were disassembled to constitute the three parts: bristle fixation, back of toothbrush, and bristles themselves to determine the viral load on these areas at various time points. The various parts were contaminated with 50 μl of one of the two viruses and incubated for different periods of time (Fig. 1).

The viral load was ascertained immediately after contamination and after an incubation period of 1, 4, 8, 12, and 24 h. The examination areas contaminated with virus dried in a laminar flow cabinet at a room temperature of 23.5 °C on a 6-well plate (TC-plate 6-well, standard, F). For each time period, the proceeding was assessed with 8-fold repeats. The experimental flow is displayed in Fig. 2.

**Quantification of viral tissue culture infective dose 50 (TCID50)** To recover the remaining virus on the toothbrush areas, 4950 μl of phosphate buffered saline (PBS) was added to each cup of the 6-well plate after each drying phase. The areas were washed 10 times with the PBS to ensure that the virus was completely suspended. Subsequently, 200 μl of each area were transferred to a 96-well-PBS dilution plate, and titrated in $\log_{10}$ steps. After 2 days, the cell plates were examined under an optical microscope to investigate the

---

**Materials and methods**

**Viruses and cell cultures**

In this study, FeCoV (strain Munich) was applied as a representative virus for SARS-CoV-2. Furthermore, an avian Influenza A Virus H1N1 (RE 230/90) virus was used as an analogue for a human influenza virus. FeCoV was propagated in Crandell Rees Feline Kidney (CRFK) cells to obtain a titer of $10^{7.5}$ TCID$_{50}$/ml. AIV H1N1 was propagated in chicken embryo fibroblast to a titer of $10^{6.5}$ TCID$_{50}$/ml. The cultivation of the viruses was conducted at 37 °C and 5% CO$_2$. The experiments were performed separately with each of the two viruses to assess the respective characteristics of the viruses.
cells on a cytopathic effect as a verification for the existence of remaining virus. The cell observation was proceeded for about 7 days to determine the 50% tissue culture infectious doses (TCID50) according to the Spearman–Kaerber method.

**Viral contamination of toothbrush with subsequent water rinsing and air-drying for 12 h**

**Contamination procedure** The experimental flow is shown in Fig. 3. To adapt the procedure of toothbrushing, the head of the toothbrush was approximately dipped in a cup of a 6-well plate with virus solution of 50 μl of either FeCoV or AIV H1N1 and 4950 μl PBS for 2 min. Afterwards the toothbrush was rinsed in the next cup filled with 5 ml of water for 15 s. The drying of the toothbrush head ensued in the third cup of the 6-well plate in a laminar flow cabinet at a room temperature of 23.5 °C for 12 h.

**Quantification of viral tissue culture infective dose 50 (TCID50)** After the drying phase, 5 ml of PBS was added into the cup of the 6-well plate to rinse the toothbrush head and bring the remaining virus on the toothbrush in solution. 200 μl of each cup (virus solution, rinsing water, remaining virus on dried toothbrush head added with PBS) were transfused to a 96-well-PBS dilutional plate. The subsequent procedure was conducted as depicted above. 100 μl of each serial dilution was transfused to the appropriate cell culture system in each case.

For the FeCoV, Crandell Rees Feline Kidney (CRFK) cells were used; for IAV H1N1, chicken embryo fibroblasts (HEF) were utilized for cell culture. Thereafter, the plates were incubated at 37 °C and 5% of CO2. As described in the procedure above, the plates were examined under the microscope for 7 days to investigate the cells on a cytopathic effect as a positive verification for virus. By means of the results, the titer could be determined according to the Spearman–Kaerber method.

**Statistical analysis**

All experiments were performed with 8-fold repeats. For statistical analysis, the software GraphPad-PRISM was used.

**Results**

**Viral contamination of three different areas of the toothbrush with subsequent various incubation periods and titer determination**

**Contamination with FeCoV**

The results of the first experiment displayed that the verified remaining viral burden on the toothbrush diminishes with increasing drying time and is dependent on the respective toothbrush part (Fig. 4). The FeCoV batch was determined with an output titer of $10^{7.5}$ TCID50/ml. Immediately after contamination, a titer loss of approximately 1 log10 level was detected in all three contaminated parts of the toothbrush. This titer-reducing tendency continued throughout the drying period. After only 12 h, no infectious residual virus could be detected on the bristle fixation. Little residual infectious virus was still detected on the back of the brush as well as on the bristles after 24 h of drying with titers of $10^{3.75}$ TCID50/ml and $10^{2.72}$ TCID50/ml, respectively. In some repetitions, the smallest detectable titer of $\leq 10^{2.5}$ TCID50/ml was determined for the bristles after 24 h of drying.

**Contamination with IAV H1N1**

It was evident in the experiment that the determined viral load decreases with an increasing drying phase. Moreover, the virus does not retain as long on the bristle fixation compared to the back of the toothbrush and the bristles (Fig. 5). The IAV H1N1 virus batch was determined with an output titer of $10^{6.5}$ TCID50/ml. Right after the contamination of the toothbrush areas, the titers of the...
remaining virus on all tested toothbrush areas declined by approximately 0.5–1 log₁₀ levels. After only 8 h of drying, residual virus titers on the bristle fixation were reduced to just above the detection limit of ≤ 10².₅ TCID₅₀/ml. In some repetitions, even a value below the detection limit was determined. The experimentally verified viral load on the back of the toothbrush and the bristles also diminished with increasing drying phase, but lasted longer on these areas. After 24 h of drying, the remaining titers on the back of the toothbrush and the bristles were recorded with approximately a 3 log₁₀ loss.
Viral contamination of toothbrush with subsequent water rinsing and air drying for 12 h

Contamination with FeCoV

The rinsing of the toothbrush, followed by a drying period of 12 h, showed a reduction of the viral load (Fig. 6). The average titer of the viral solution, in which the toothbrush head was dipped, was measured with a value of $10^{7.16}$ TCID$_{50}$/ml. The
remaining virus in the rinsing water led to a mean titer of $10^{6.13}$ TCID$_{50}$/ml. After a drying time of 12 h, a titer drop below the detection limit was determined on the toothbrush head.

**Contamination with IAV H1N1**

As in the case of FeCoV contamination, it was also evident in the case of contamination with IAV H1N1 that rinsing the toothbrush and subsequent air-drying for 12 h resulted in a reduction of the viral load (Fig. 7). The viral solution, in which the toothbrush head was dipped in, was measured with an average titer of $10^{6.23}$ TCID$_{50}$/ml. A mean value of $10^{6.23}$ TCID$_{50}$/ml was determined in the rinsing water. After a drying time of 12 h, a titer drop below the detection limit was determined on the toothbrush head.

**Discussion**

The current worldwide pandemic situation shows the danger of the speed of spread of viral pathogens, which should not be underestimated. In the current event of a respiratory virus such as SARS-CoV-2, potential spread cycles must also be recognized on an everyday scale. In this context, dental hygiene plays a role, in addition to the already generally established measures such as personal hand hygiene. Aside of this way of infection, the transmission via contaminated surfaces has been thoroughly discussed [15]. In order to assess the stability of important respiratory viruses on products of dental hygiene like toothbrushes and the associated (re)infection risk, contamination
experiments with a coronavirus (FeCoV) and an influenza virus (AIV H1N1) were performed in this study.

For this purpose, controlled contamination experiments of different toothbrush areas (bristles, back or fixation) were performed to analyze virus tenacity. It was found that the titers of both viruses were rapidly and steadily reduced over the 24 h of the experiment. This reducing effect was particularly rapid on the contaminated toothbrush fixation. Furthermore, already within 12 h, an effective titer reduction of $2.5 - 5 \log_{10}$ (FeCoV) and $2 - 4 \log_{10}$ (H1N1) could be detected on all tested toothbrush parts. The residual titer was just above the detection limit at this point and changed little over the remainder of the experiment. Thus, both viruses show a low stability, which is further reduced by rinsing of the contaminated toothbrush parts, where no active virus could be recovered.

Meanwhile, different studies report a certain stability of the infectivity of coronaviruses, especially SARS-CoV-2 on surfaces [15]. Interestingly, a previous study showed coronaviruses to remain infective on plastic surfaces (which is also the material of most toothbrushes) for hours, showing viable virus for up to 72 h after application [16]. However, this previous examination showed very low titers after such a long observation period, while the titers after 12 and 24 h were similarly low as in the current study [16]. Therefore, although a certain stability of the virus was detectable, the infectivity of the contaminated surface is very low, making a transmission unlikely. In case of the current study, the air-drying at room temperature seems to lead to a remarkable and fast reduction of the titer (i.e., below the limit of detection). Another study showed that the stability of coronavirus is remarkable reduced at 20 °C [17]. Thus, the room temperature and laminar flow appear to lead to fast evaporation of the droplets and thus decreased viral load. Causal for this, the alteration of the envelope of the coronavirus because of continuous air-drying would be a plausible explanation for the fast loss in its infectivity. With regard to the clinical reality, a toothbrush is regularly rinsed with water after use. This was an experiment in the current study, resulting in a complete loss of virus load after rinsing and subsequently 12 h air-drying. As a result, the toothbrush is no habitat with a high risk of self-infection. It seems more plausible that patients, using the same toothbrush transmit the virus to each other, because of a generally reduced health behavior, for which using the same toothbrush could be an indicator. In this context, patients regularly use toothpaste for toothbrushing; toothpaste has an antimicrobial effect; although this was mainly shown for bacteria, the influence of the contamination of the toothbrush remains unclear [18, 19].

FeCoV was selected as an alternative test virus for SARS-CoV-2 in this study. Both viruses belong to the family of Coronaviridae; those viruses have an enveloped spherical structure with a diameter between 60 and 160 nm, while Influenza virus belongs to the family Orthomyxoviridae, having an enveloped pleomorphic structure with a diameter ranging from 100 to 120 nm [20]. This so-called surrogate virus method has long been used for efficacy testing of chemical disinfectants according to the guidelines of the German Veterinary Society (DVG). For this purpose, FeCoV and other viruses are commonly used as surrogates for related viruses and the results are directly transferred to the original viruses.

In addition to a coronavirus, the current experiment was also performed with another respiratory virus with high clinical relevance: Avian Influenza A virus H1N1. AIV H1N1, which caused a pandemic in 2009 [21], is a type A influenza virus, an enveloped RNA virus, having a pleomorphic appearance with an average diameter of 120 nm [22]. Influenza viruses are of high clinical interest, as they have caused hundreds of thousands of deaths worldwide each year [23]. Moreover, in the current pandemic, influenza and SARS-CoV-2 are co-existing viruses, needing a joint preventive approach [24]. Against this background, the same research question was applied for H1N1 in the current investigation.
showing comparable results as for FeCoV. In turn, a study by Oxford et al. found that H1N1 is still infectious after 24 h on a plastic surface [25]. During this study, H1N1 showed slightly lower titer reduction in the air-drying experiment than FeCoV; however, the titer after 12 and 24 h was low. Furthermore, the results after water rinse, again, corresponding to the clinical situation, were equal. Therefore, the toothbrush was also identified to be no important source of H1N1 and thus probably influenza virus (self-) transmission.

In general, a higher viral load of both viruses on bristles and bristle fixation would have been expected, based on the higher surface size. Nevertheless, the back of toothbrush head was found to show the comparably highest load. This might be explained by its smooth, non-porous surface, allowing a certain stability of the droplets and a slower evaporation, resulting in less collapse of the envelope of the virus. This, however, remains speculative and cannot be finally confirmed by the current data. The toothpaste, brushing technique, the toothbrush type (design and number of bristles, powered toothbrush, etc.), and interactions with salivary components may limit the generalizability of the findings.

The study only included traditional toothbrush. More in-depth research on dental hygiene routines that deviate from the lab standard applied in this study is needed, for example, with electric toothbrushes or toothbrushes made of different materials (e.g., bamboo or wood) or more frequent brushing cycles.

**Conclusions**

The toothbrush appears to play an insignificant role in the (self-) transmission of coronavirus or influenza virus. Nevertheless, an appropriate use of oral health aids, i.e., using one toothbrush each person, avoid contact between the aids used by different individuals, etc., would be recommendable, regardless of the findings.

**Funding** Open Access funding enabled and organized by Projekt DEAL.

**Declarations**

**Ethical approval** Not applicable.

**Informed consent** Not applicable.

**Conflict of interest** The authors declare no competing interests.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

1. Wu KY, Wu DT, Nguyen TT, Tran SD (2021) SARS-COV2's impact on private practice and academic dentistry in North America. Oral Dis 27(Suppl 3):684–687. https://doi.org/10.1111/odi.13444
2. Callahan C, Dittelferg S, Dutta S, Littlehale N, Cheng A, Kupczewski K, McVay D, Riedel S, Kirby JE, Arnaout R (2021) Saliva is comparable to nasopharyngeal swabs for molecular detection of SARS-CoV-2. Microbiol Spectr 9(1):e0016221. https://doi.org/10.1128/Spectrum.00162-21
3. Vergara-Buenaventura A, Castro-Ruiz C (2020) Use of mouthwashes against SARS-COV2 in dentistry. Br J Oral Maxillofac Surg 58(9):924–927. https://doi.org/10.1016/j.bjoms.2020.08.016
4. González-Olmo MJ, Delgado-Ramos B, Ruiz-Guillén A, Romero-Maroto M, Carrillo-Díaz M (2020) Oral hygiene habits and possible transmission of SARS-COV2 among cohabitants. BMC Oral Health 20(1):286. https://doi.org/10.1186/s12903-020-01274-5
5. Nascimento AP, Watanabe E, Ito IY (2010) Toothbrush contamination by Candida spp. and efficacy of mouthrinse spray for their disinfection. Mycopathologia 169(2):133–138. https://doi.org/10.1007/s11046-009-9239-z
6. do Nascimento C, Trinca NN, Pita MS, Pedrazzi V (2015) Genomic identification and quantification of microbial species adhering to toothbrush bristles after disinfection: A cross-over study. Arch Oral Biol. 60(7):1039–1047. https://doi.org/10.1016/j.archoralbio.2015.03.012
7. Ankola AV, Hebball M, Eshwar S (2009) How clean is the toothbrush that cleans your tooth? Int J Dent Hyg 7(4):237–240. https://doi.org/10.1111/j.1601-5037.2009.00384.x
8. Zinn MK, Schages L, Bockmühl D (2020) The toothbrush microbiome: impact of user age, period of use and bristle material on the microbial communities of toothbrushes. Microorganisms 8(9):1379. https://doi.org/10.3390/microorganisms8091379
9. Frazelle MR, Munro CL (2012) Toothbrush contamination: a review of the literature. Nurs Res Pract 2012:420630. https://doi.org/10.1155/2012/420630
10. Bhoil R, Bhoil R (2016) Toothbrush contamination: often neglected health hazard. J Family Med Prim Care 5(1):186. https://doi.org/10.4103/2249-4863.184664
11. Agrawal SK, Dahal S, Bhumika TV, Nair NS (2019) Evaluating sanitization of toothbrushes using various decontamination methods: a meta-analysis. J Nepal Health Res Cunc 16(41):364–371
12. Basman A, Peker I, Akca G, Akurt MT, Sarikir C, Celik I (2016) Evaluation of toothbrush disinfection via different methods. Braz Oral Res 30:S1806–S83242016000100203. https://doi.org/10.1590/1809-3107BOR-2016.vol30.0006
13. Patcas R, Zbinden R, Schätzle M, Schmidlin PR, Zehnder M (2018) Whisky, microwave or hairdryer? Exploring the most efficient way to reduce bacterial colonisation on contaminated toothbrushes. Br Dent J 225(11):1007–1010. https://doi.org/10.1038/sj.bdj.2018.1030
14. Zautner AE, Hage A, Schneider K, Schlösser K, Zimmermann O, Hornecker E, Mausberg RF, Frickmann H, Groß U, Ziebolz...
D (2013) Effects of easy-to-perform procedures to reduce bacterial colonization with Streptococcus mutans and Staphylococcus aureus on toothbrushes. Eur J Microbiol Immunol (Bp) 3(3):204–210. https://doi.org/10.1556/EuJMI.3.2013.3.9 Epub 2013 Sep 23

15. Marquès M, Domingo JL (2021) Contamination of inert surfaces by SARS-CoV-2: persistence, stability and infectivity. A review. Environ Res 193:110559. https://doi.org/10.1016/j.envres.2020.110559

16. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ (2020) Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 382(16):1564–1567. https://doi.org/10.1056/NEJMcp2004973

17. Aboubakr HA, Sharafeldin TA, Goyal SM (2021) Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: a review. Transbound Emerg Dis 68(2):296–312. https://doi.org/10.1111/tbed.13707

18. Arweiler NB, Grelle F, Sculean A, Heumann C, Auschill TM (2018) Antibacterial effect and substantivity of toothpaste slurries in vivo. Oral Health Prev Dent 16(2):175–181. https://doi.org/10.3290/j.ohpd.a40310

19. Schmidt JC, Bux M, Filipuzzi-Jenny E, Kulik EM, Waltimo T, Weiger R, Walter C (2014) Influence of time, toothpaste and saliva in the retention of Streptococcus mutans and Streptococcus sanguinis on different toothbrushes. J Appl Oral Sci 22(3):152–158. https://doi.org/10.1590/1678-775720130017

20. International Committee on Taxonomy of Viruses, ICTV, https://talk.ictvonline.org, accessed on 21st of April 2022

21. Rewar S, Mirdha D, Rewar P (2015) Treatment and prevention of pandemic H1N1 influenza. Ann Glob. Health 81(5):645–653. https://doi.org/10.1016/j.ajogh.2015.08.014

22. Van Reeth K (2007) Avian and swine influenza viruses: our current understanding of the zoonotic risk. Vet Res 38(2):243–260. https://doi.org/10.1051/vetres:2006062

23. Juliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, Cohen C, Gran JM, Schanzer D, Cowling BJ, Wu P, Kyncl J, Ang LW, Park M, Redilberger-Fritz M, Yu H, Espehain L, Krishnan A, Emukule G et al (2018) Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet 391(10127):1285–1300. https://doi.org/10.1016/S0140-6736(17)33293-2

24. Chotpitayasunondh T, Fischer TK, Herada JM, Hurt AC, Monto AS, Osterhaus A, Shu Y, Tam JS (2021) Influenza and SARS-COV2: what does co-existence mean? Influenza other respir viruses. 15(3):407-412. doi: https://doi.org/10.1111/irv.12824.

25. Oxford J, Berezin EN, Courvalin P, Dwyer DE, Exner M, Jana LA, Kaku M, Lee C, Letlape K, Low DE, Madani TA, Rubino JR, Saini N, Schoub BD, Signorelli C, Tierno PM, Zhong X (2014 Apr) The survival of influenza A(H1N1)pdm09 virus on 4 household surfaces. Am J Infect Control 42(4):423–425. https://doi.org/10.1016/j.ajic.2013.10.016

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.