FEMS Microbiology Letters, 368, 2021, fnab112

https://doi.org/10.1093/femsle/fnab112
Advance Access Publication Date: 30 August 2021
Research Letter

RESEARCH LETTER – Physiology, Biochemistry & Genetics

Investigation of the stability and risks of fomite transmission of human coronavirus OC43 on leather

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One sentence summary: Human coronavirus OC43 persists for up to 48 h on leather, suggesting that leathers could act as fomites for transmission of coronaviruses.

Editor: Kevin Maringer
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ABSTRACT

Limited research exists on the potential for leather to act as a fomite of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or endemic coronaviruses including human coronavirus (HCoV) OC43; this is important for settings such as the shoe manufacturing industry. Antiviral coating of leather hides could limit such risks. This study aimed to investigate the stability and transfer of HCoVOC43 on different leathers, as a surrogate for SARS-CoV-2, and assess the antiviral efficacy of a silver-based leather coating. The stability of HCoV-OC43 (6.6 $\log_{10}$) on patent, full-grain calf, corrected grain finished and nubuck leathers (silver additive-coated and uncoated) was measured by titration on BHK-21 cells. Transfer from leather to cardboard and stainless steel was determined. HCoV-OC43 was detectable for 6 h on patent, 24 h on finished leather and 48 h on calf leather; no infectious virus was recovered from nubuck. HCoV-OC43 transferred from patent, finished and calf leathers onto cardboard and stainless steel up to 2 h post-inoculation ($\leq$3.1–5.5 $\log_{10}$), suggesting that leathers could act as fomites. Silver additive-coated calf and finished leathers were antiviral against HCoV-OC43, with no infectious virus recovered after 2 h and limited transfer to other surfaces. The silver additive could reduce potential indirect transmission of HCoV-OC43 from leather.

Keywords: human coronavirus OC43; stability; leather; antiviral; infectivity; $TCID_{50}$

INTRODUCTION

Understanding the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is important in containing the coronavirus disease 2019 (COVID-19) pandemic. The role of indirect transmission of SARS-CoV-2 is not fully understood due to difficulties in excluding droplet and aerosol transmission in cases of SARS-CoV-2 infections (Centers for Disease Prevention and Control 2021). Infectious SARS-CoV-2 has been detected on environmental surfaces for extended time periods, for example Kasloff *et al.* (2021) reported that SARS-CoV-2 was detectable for up to 21 days on plastic, suggesting that indirect transmission of COVID-19 from surfaces could occur, although it is currently considered to be less likely than droplet and aerosol transmission (Centers for Disease Prevention and Control 2021).

In addition to emerging coronaviruses such as SARS-CoV-2, there are four established endemic human coronaviruses (HCoV-OC43, HCoV-229E, HCoV-HKU1 and HCoV-NL63) which are estimated to cause up to 30% of upper respiratory tract infections (Walsh, Shin and Falsey 2013; Cornman *et al.* 2018; Paules,

Received: 23 June 2021; **Accepted:** 27 August 2021

Marston and Fauci 2020; Liu *et al.* 2021a) as well as more severe infections such as pneumonia and bronchitis, therefore representing a significant disease burden (Gaunt *et al.* 2010; Walsh, Shin and Falsey 2013). In particular, HCoV-OC43 was reported to cause lower respiratory tract infection in over 40% of confirmed cases (Gaunt *et al.* 2010). HCoV-OC43 RNA has previously been recovered from high-touch surfaces including 3.1% of school desks (Zulli *et al.* 2021), and an *in vitro* study detected infectious HCoV-OC43 on aluminium up to 2 h after inoculation (Sizun, Yu and Talbot 2000), suggesting that further research into the role of indirect contact in the transmission of HCoV-OC43 is warranted.

There does not appear to be any published research on the stability of coronaviruses on a range of leather types and therefore the risk of leather products acting as fomites of coronaviruses is not well understood. Recently, Virtanen *et al.* (2021) reported that SARS-CoV-2 was detectable for less than 1 day on faux leather, compared to 5 days on plastic; viral titres over time were not reported. There is significant variation in the composition and finishing of leather products (Powley-Williams 2018), as well as porosity (Chatterjee *et al.* 2021), which could potentially affect the stability of coronaviruses on their surface. Previous research has demonstrated that SARS-CoV-2 persists for shorter time periods on porous surfaces such as cotton textile (24 h) compared to lower porosity textiles such as Tyvek (14 days) and non-woven respirator mask surfaces (21 days; Kasloff *et al.* 2021); similarly HCoV-OC43 was detected on polycotton and cotton for 6–24 h compared to 72 h on non-porous polyester textile (Owen, Shivkumar and Laird 2021a). Nubuck leather is often unfinished and is highly porous, however water-repellent treatments may be applied reducing the porosity. Other common leather types include full-grain calf leather, which generally has a natural finish or may be finished with wax, polymer or protein coatings; semi-aniline finished leather, which has a polymer-based protective topcoat; and patent leather, which is heavily coated in polyurethane or resin (Powley-Williams *et al.* 2018). Research on the stability of coronaviruses on a range of leather types is therefore warranted to assess the potential for leather to act as a source for indirect transmission of coronaviruses; this could inform infection control procedures for settings where there is close contact with leather products such as shoe manufacturing and retail.

Antiviral coating of leather hides could be a strategy to limit coronavirus transmission during handling, both within the manufacturing process and during end use. Micro-Fresh 2611 is a silver-based additive initially developed as an antifungal leather coating, and has since been demonstrated to possess antibacterial activity on textiles (Owen *et al.* 2021b). There is some evidence to suggest that silver may possess antiviral properties against SARS-CoV-2 and other coronaviruses, suggesting that this silver additive could be a candidate antiviral agent to control coronaviruses on leather. Silver nanoparticles (1–10 ppm) significantly reduced SARS-CoV-2 infectivity in VeroE6/TMPRSS2 and Calu-3 cell lines by preventing entry into host cell (Jeremiah *et al.* 2020). Silver nanoparticles and nanowires (60 and 400 nm) reduced the titre of transmissible gastroenteritis virus by 67.35%, 53.90% and 58.65%, respectively, at a dose of 12.5 μg/mL (Lv *et al.* 2014). Zeolite powder containing 20% silver reduced human coronavirus (HCoV) 229E by 1.92 $\log_{10}$ 50% tissue culture infective dose ($TCID_{50}$)/mL after 24 h, compared to 0.16 $\log_{10}$ $TCID_{50}$/mL by zeolite powder alone (Bright *et al.* 2009).

HCoV-OC43 was used as a surrogate for SARS-CoV-2 in this study. HCoV-OC43 is related to SARS-CoV-2 at the genus level (*Betacoronavirus*) and previous research suggests that the environmental stability of SARS-CoV-2 is in accordance with that of HCoV-OC43 (Owen, Laird and Shivkumar 2021c). The environmental stability of HCoV-OC43 was measured in this study by quantification of infectious virus, in contrast to molecular methodologies which measure the load of both inactive and infectious virions (Kasloff *et al.* 2021). Measurement of infectious virus can therefore be used to infer the possible infection control risk.

This study aimed to investigate the stability and transfer of HCoV-OC43 on different leather types and assess the antiviral efficacy of a silver-based leather coating treatment.

MATERIALS AND METHODS

Cell lines

BHK-21 clone 13 cells (ECACC 85011433) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Lonza, Basel, Switzerland) with 10% foetal bovine serum (FBS; HyClone, Logan, UT), 100 IU/mL penicillin and 100 μg/mL streptomycin (pen/strep; Lonza) at 37°C with 5% CO_2. HCT-8 cells (ECACC 90032006) were cultured in RPMI-1640 media (Lonza) with 10% FBS and pen/strep at 37°C with 5% CO_2.

Viruses

Human coronavirus HCoV-OC43 (ATCC VR-1558) was cultured in HCT-8 cells (7 days; 33°C with 5% CO_2) using RPMI-1640 media with 5% FBS and pen/strep. Virions were harvested by aspirating the culture media and centrifuging at 3000 x *g* for 4 min to remove cell debris. All virus stocks were stored at –80°C prior to use.

Quantification of infectivity was performed by serially diluting in DMEM with 5% FBS and pen/strep and transferring onto BHK-21 cell monolayers in a 96-well plate format. Plates were incubated at 33°C with 5% CO_2 for 4 days and the wells scored for cytopathic effect. The $TCID_{50}$/mL was calculated using the Karber method (Ramakrishnan 2016).

Stability of HCoV-OC43 on different leather types and antiviral activity of coated leather

Full-grain calf leather, patent, nubuck and corrected grain finished leathers were cut into 25 cm^2 samples and cleaned with 70% isopropanol and allowed to dry prior to use. Full-grain calf leather, nubuck and corrected grain finished leathers coated with a silver additive (Micro-Fresh 2611; Micro-Fresh International, Leicester, UK) were prepared in the same way. Patent leather could not be coated with the silver additive due to the nature of the patent finishing.

Leather samples were inoculated with 200 μL HCoV-OC43 (6.6 $\log_{10}$ $TCID_{50}$/mL) and incubated in plastic petri dishes at room temperature for 0, 2, 6, 24, 48 and 72 h. Where no infectious virus was detected at 2 h on silver additive coated leathers, a 0.5 h sample was included. No virus controls (RPMI-1640 only) were included throughout. Following incubation, the leather samples were transferred into sterile 50 mL centrifuge tubes containing 5 mL phosphate buffered saline (PBS, Oxoid, Basingstoke, UK) and shaken by hand 30 times; the supernatant was then passed through a 0.45 μm polyether sulfone (PES) filter (Fisher Scientific, Loughborough, UK). Supernatants were titrated on BHK-21 cells and viral titre determined as described above. The limit of detection (LOD) of infectious virus was 2.2 $\log_{10}$ $TCID_{50}$/mL.

Transfer of HCoV-OC43 from different leather types to other surfaces

The transfer of HCoV-OC43 from uncoated and silver additive-coated leather onto other surfaces was done as previously described (Owen, Shivkumar and Laird 2021a). Leather samples (25 cm^2) were inoculated with 200 μL HCoV-OC43 (6.6 log_{10} $TCID_{50}$/mL) and incubated at room temperature in plastic petri dishes (0, 2, 24 and 48 h for uncoated leathers; 0 and 2 h for coated leathers) before placing the stainless steel or cardboard surface onto the inoculated leather sample for 10 s; the pressure was standardized by placing a 100 g weight on the test surface. Infectious virus was recovered from cardboard by immersing in 5 mL PBS in a sterile 50 mL centrifuge tube and shaking by hand 30 times. Stainless steel was washed with 5 mL PBS and swabbed thrice with cotton-tipped swabs; the swabs and eluate were combined in a sterile 50 mL centrifuge tube and vortexed for 30 s. Supernatants were syringe filtered (0.45 μm PES filter) prior to titration on BHK-21 cells as described above.

Statistical analysis

All investigations were repeated using biological triplicates ($n = 3$) and viral quantification was conducted with technical quadruplicates. Significant differences ($P \leq 0.05$) in log_{10} $TCID_{50}$/mL recovery of HCoV-OC43 between leathers, cardboard and stainless steel over time were determined by independent samples Kruskal–Wallis tests with pairwise comparisons using SPSS version 25 (IBM, Armonk, NY). Data were confirmed as not normally distributed data using the Shapiro–Wilk test.

RESULTS AND DISCUSSION

Stability of HCoV-OC43 on different leather types

No cytopathic effect was observed for the no virus controls on uncoated or silver additive coated leathers (data not shown). HCoV-OC43 remained infectious at 6.5 log_{10} $TCID_{50}$/mL on uncoated patent, full-grain calf and corrected grain finished leather for up to 6 h, after which it rapidly declined; no infectious virus (≤2.2 log_{10} $TCID_{50}$/mL) was detected by 24 h on patent leather and 48 h on full-grain calf and corrected grain finished leathers (Fig. 1A). The results are in line with previous work suggesting a biphasic or exponential decay pattern of SARS-CoV-2 in the environment (Chin et al. 2020; Kratzel et al. 2020).

Conversely, no infectious HCoV-OC43 was recovered from uncoated nubuck leather immediately after inoculation (Fig. 1A). Nubuck is a porous material, resulting in greater absorption of the viral inoculum compared to the other leather samples which are either non-porous (corrected grain finished and patent leathers) or exhibit a lower porosity (full-grain calf leather). Previous research has suggested that an inverse correlation exists between surface porosity and the stability of coronaviruses (Chatterjee et al. 2021; Kasloff et al. 2021; Liu et al. 2021b). This relationship may be attributed to the desiccation of coronaviruses following absorption of moisture by porous surfaces (Chatterjee et al. 2021), or alternatively reduced recovery of virions absorbed into the inner layers of the surface.

Coating of full-grain calf and corrected grain finished leathers with the silver additive resulted in a significant ($P \leq 0.05$) reduction in the stability of HCoV-OC43 compared to uncoated leather after 2 h; HCoV-OC43 remained stable for up to 30 min before reaching the LOD 2 h post-inoculation (Fig. 1B and C). As with the uncoated leathers, silver additive-coated nubuck leather showed no infectious HCoV-OC43 at any of the time points tested (data not shown). These results indicate that the silver additive could limit the potential risk of transmission of HCoV-OC43 from leather. Silver has previously been reported to possess antiviral activity against coronaviruses, for example,

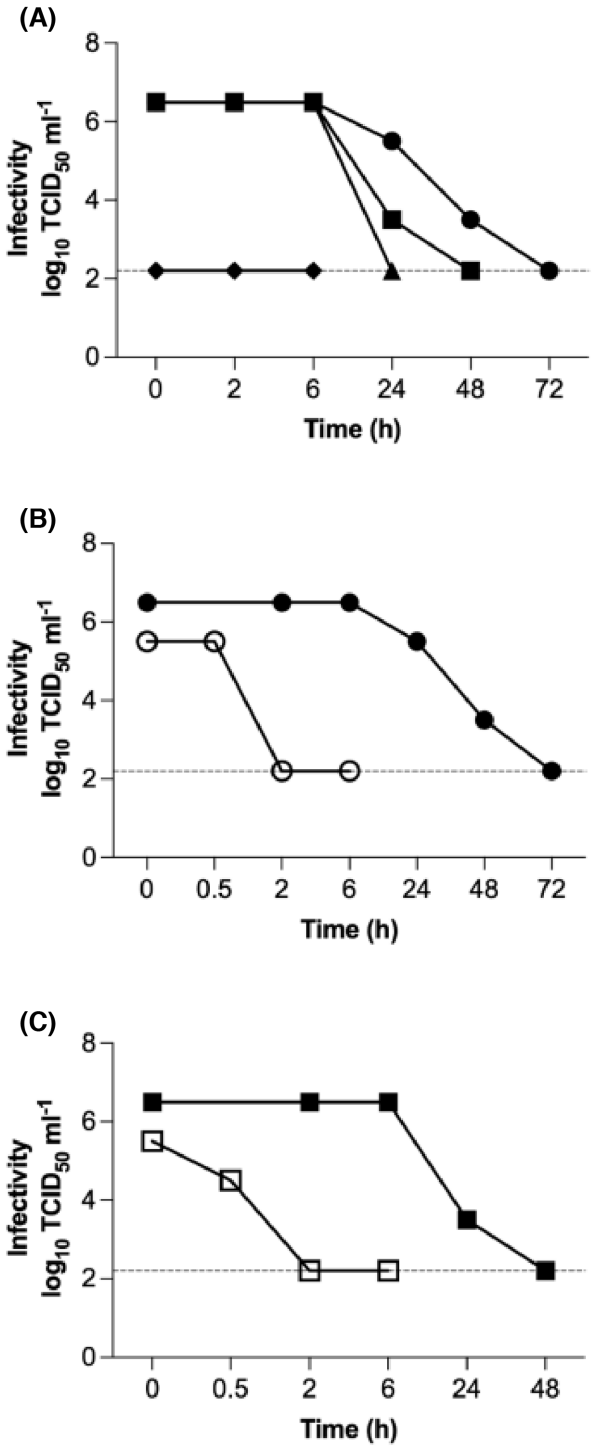

Figure 1. Stability of infectious HCoV-OC43 (log_{10} $TCID_{50}$/mL) on uncoated leathers **(A)** and silver additive-coated full-grain calf **(B)** and corrected grain finished leathers **(C)**. Mean, $n = 3 \pm$ standard error of the mean. ♦ Nubuck, ▲ patent leather, • full-grain calf leather, ■ corrected grain finished leather, ○ silver additive-coated full-grain calf leather and □ silver additive-coated corrected grain finished leather.

Table 1. Transfer of HCoV-OC43 (6.6 $\log_{10}$ $TCID_{50}$/mL) from uncoated and silver additive-coated leathers onto cardboard over time (mean, $n = 3 \pm$ standard error of the mean).

Leather	Treatment	Time (h) 0	2	24	48
Patent	Uncoated	4.2 ± 0.3	$\leq 3.1 \pm 0.4^{\#}$	*	-
Full-grain calf	Uncoated	$3.5 \pm 0.0^{\#}$	$3.5 \pm 0.0^{\#}$	-	*
	Coated	4.5 ± 0.0	*	-	-
Corrected grain finished	Uncoated	$3.5 \pm 0.0^{\#}$	$3.5 \pm 0.0^{\#}$	*	-
	Coated	4.5 ± 0.0	*	-	-
Nubuck	Uncoated	*	*	-	-
	Coated	*	*	-	-

-Not tested.
*Below detection limit (2.2 $\log_{10}$ $TCID_{50}$/mL).
#Significantly ($P \leq 0.05$) lower viral load compared to the donor swatch (Fig. 1).
Where one or more samples reached the detection limit, the number of infectious virus is expressed as $\leq \log_{10}$ $TCID_{50}$.

Table 2. Transfer of HCoV-OC43 (6.6 $\log_{10}$ $TCID_{50}$/mL) from uncoated and silver additive-coated leathers onto stainless steel over time (mean, $n = 3 \pm$ standard error of the mean).

Leather	Treatment	Time (h) 0	2	24	48
Patent	Uncoated	$5.5 \pm 0.0^{\#}$	$5.5 \pm 0.0^{\#}$	*	-
Full-grain calf	Uncoated	$4.5 \pm 0.0^{\#}$	$4.5 \pm 0.0^{\#}$	-	*
	Coated	4.5 ± 0.0	*	-	-
Corrected grain finished	Uncoated	$4.5 \pm 0.0^{\#}$	$4.6 \pm 0.1^{\#}$	*	-
	Coated	4.5 ± 0.0	*	-	-
Nubuck	Uncoated	$4.5 \pm 0.0^{\#}$	*	-	-
	Coated	*	*	-	-

-Not tested.
*Below detection limit (2.2 $\log_{10}$ $TCID_{50}$/mL).
#Significantly ($P \leq 0.05$) lower viral load compared to the donor swatch (Fig. 1).

$\geq$1 ppm silver nanoparticles inhibited SARS-CoV-2 by preventing entry into mammalian cells (Jeremiah *et al*. 2020).

Transfer of HCoV-OC43 from different leather types to other surfaces

The potential for leathers to act as fomites for HCoV-OC43 was assessed by determining the transfer of infectious HCoV-OC43 onto cardboard and stainless steel. Cardboard and stainless steel were chosen to reflect typical surfaces present within shoe manufacturing settings, such as shoe boxes and sewing machine surfaces, respectively. Investigations were performed both immediately after inoculation and at the maximum time where HCoV-OC43 was detected on each leather: 2 h for nubuck, 24 h for patent and corrected grain finished leather and 48 h for full-grain calf leather (Fig. 1A).

There was no evidence of cytotoxicity in the no virus controls on both cardboard and stainless steel (data not shown). Transfer of infectious virus ($\leq$3.1–5.5 $\log_{10}$ $TCID_{50}$/mL) was detected from uncoated patent, full-grain calf and corrected grain finished leathers to cardboard up to 2 h after inoculation of the donor leather, however no infectious virus was detected at the maximum time tested (Table 1). The risk of transfer of infectious HCoV-OC43 to stainless steel was marginally but non-significantly ($P > 0.05$) higher than cardboard (Table 2). Between 4.5 and 5.5 $\log_{10}$ $TCID_{50}$/mL infectious virus was detected on stainless steel in contact with patent, full-grain calf and corrected grain finished leathers 2 h after inoculation, although no virus was detected at the later times.

No transfer was observed from nubuck leather to cardboard as expected due to limited viral stability on the nubuck surface (Table 1). Conversely, 4.5 $\log_{10}$ $TCID_{50}$/mL HCoV-OC43 transferred from nubuck onto stainless steel at 0 h, however by 2 h no transfer was detected (Table 2). For practical reasons the stainless steel surface was placed on top of the inoculated leather samples in transfer experiments; it is hypothesized that the added weight of 180 g stainless steel in addition to the 100 g weight employed released the absorbed viral inoculum onto the stainless steel surface compared to the lighter cardboard surface. These results suggest that HCoV-OC43 remains infectious after absorption into the nubuck leather, but drop below the detection limit by 2 h. In contrast the corrected grain finished, full-grain calf and patent leathers produced a high level of transfer of HCoV-OC43 for both cardboard and stainless steel surfaces likely due to the lower porosity of these leather samples. In a similar study it was demonstrated that HCoV-OC43 transferred from polyester (non-porous) onto PVC or polyester up to 72 h after inoculation (1.64–1.67 $\log_{10}$ $TCID_{50}$/sample), whereas no transfer was detected from the porous textiles cotton or polycotton immediately after inoculation (Owen, Shivkumar and Laird 2021a). Overall, the results of this study suggest that although HCoV-OC43 can remain stable on patent and corrected grain finished leathers and full-grain calf leather for 24–48 h, the risk of transfer at these later time points is negligible.

The transfer of HCoV-OC43 from silver additive-coated full-grain calf and corrected grain finished leathers was reduced compared to uncoated leathers, both to cardboard and stainless steel (Tables 1 and 2). Infectious HCoV-OC43 (4.5 $\log_{10}$ $TCID_{50}$/mL) transferred from coated full-grain calf and corrected grain

finished leathers onto cardboard immediately after inoculation (4.5 $\log_{10}$ $TCID_{50}$/mL), while no transfer was detected 2 h post-inoculation (Table 1). Additional tests against coated full-grain calf and corrected grain finished leathers at 0.5 h also showed 4.5 $\log_{10}$ $TCID_{50}$/mL transfer onto cardboard, suggesting transfer occurs at early time points when the viral load on the donor surface remains high (Fig. 1B and C). Similarly, 4.5 $\log_{10}$ $TCID_{50}$/mL transferred onto stainless steel immediately after inoculation, and no transfer of infectious virus was detected on the stainless steel surface after 2 h (Table 2). As with the uncoated nubuck leather, coated nubuck leather also showed no transfer to cardboard at any of the times tested (up to 2 h). There were no significant differences ($P > 0.05$) in the transfer of HCoV-OC43 from silver additive-coated and uncoated leathers to cardboard or stainless steel, with the exception of a significant reduction ($P \leq 0.05$) in transfer onto stainless steel by coated full-grain calf and corrected grain finished leathers at 2 h; 4.5–4.6 $\log_{10}$ $TCID_{50}$/mL transfer was detected from uncoated leathers compared to ≤2.2 $\log_{10}$ $TCID_{50}$/mL transfer from coated leathers (Table 2). Coating of corrected grain finished and full-grain calf leathers with silver additive may therefore further limit their potential risk for fomite transmission of HCoV-OC43.

It should be noted that this study employs relatively high titres of HCoV-OC43 (6.6 $\log_{10}$ $TCID_{50}$/mL) on surfaces as per previous publications (Kasloff *et al.* 2021; Liu *et al.* 2021b). Although the typical concentration of infectious HCoV-OC43 or SARS-CoV-2 present on contaminated surfaces is not currently known, the load of nucleic acid in the environment is comparatively low (Meyerowitz *et al.* 2021). The use of higher titres enables the environmental stability of coronaviruses to be monitored over time above the LOD of the infectivity assay, thereby providing a greater wealth of information on the potential risks of indirect transmission of coronaviruses (Owen, Shivkumar and Laird 2021a).

Overall, this study demonstrates that infectious HCoV-OC43 is detectable for up to 48 h on full-grain calf leather, 24 h on corrected grain finished leather and 6 h on patent leather, and transfers onto other surfaces for up to 2 h, suggesting that they could act as a reservoir for the indirect transmission of coronaviruses including HCoV-OC43 or SARS-CoV-2. Conversely, there was limited stability and transfer from porous nubuck leather indicating that nubuck poses a lower risk towards infection control. Silver additive-coated full-grain calf leather, corrected grain finished leather and patent leather demonstrated antiviral activity against HCoV-OC43 and further limited viral transfer, with no transfer to other surfaces detected 2 h after inoculation, suggesting that the silver additive could further reduce the potential for indirect transmission of HCoV-OC43 where there is close contact with leathers such as in manufacturing, retail and domestic environments.

ACKNOWLEDGMENTS

The authors acknowledge the British Footwear Association for providing leather samples and Micro-Fresh International, Leicester, UK for coating of leather samples.

FUNDING

This work was supported by a De Montfort University Higher Education Innovation Fund (HEIF) grant.

Conflicts of Interest. None declared.

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