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Brief report

The survival of influenza A(H1N1)pdm09 virus on 4 household surfaces

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We investigated the survival of a pandemic strain of influenza A H1N1 on a variety of common household surfaces where multiple samples were taken from 4 types of common household fomite at 7 time points. Results showed that influenza A H1N1sw virus particles remained infectious for 48 hours on a wooden surface, for 24 hours on stainless steel and plastic surfaces, and for 8 hours on a cloth surface, although virus recovery from the cloth may have been suboptimal. Our results suggest that pandemic influenza A H1N1 can survive on common household fomites for extended periods of time, and that good hand hygiene and regular disinfection of commonly touched surfaces should be practiced during the influenza season to help reduce transmission.

Influenza is a major cause of morbidity worldwide, and occurs in annual epidemics that cause mortality in at-risk populations such as the young and elderly, as well as those with other underlying illnesses.[1] Influenza also has the potential to cause pandemics, and the emergence of a novel strain of influenza A H1N1 from swine (ie, influenza A[H1N1]pdm09) during April 2009 signaled the start

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of the first pandemic of the 21st century.[2] By August 2010, the pandemic influenza strain had been reported in more than 214 countries worldwide and was estimated to have caused at least 18,450 deaths.[3] More recent estimates suggest that the pandemic flu caused up to 575,500 deaths globally.[4]

The major route of transmission for influenza is via inhalation of aerosolized droplets.[5] However, a number of studies have shown that influenza virus can survive on surfaces—or fomites—for extended periods of time, and may be spread from these to the hands and then into the respiratory tract when individuals touch their eyes or nose.[6,7] Thus, the presence of influenza viruses on household surfaces may play a role in the spread of infection, and methods to prevent this route of transmission should be investigated.

Our in vitro study was conducted to investigate the survival of viable influenza A(H1N1)pdm09 virus particles on a range of common household surfaces under laboratory conditions.

METHODS

The surfaces tested were wooden chopping boards, cotton pillowcases, stainless steel sheets, and plastic toy ducks. Sufficient identical test items were purchased to allow for multiple samples to be taken at 7 time points on each surface. All test items were newly purchased from local shops.

The challenge virus was NIBRG-121sw (strain A/California/7/2009) obtained from the reference collection of the UK National Institute for Biological Standards and Control. The challenge virus had been passaged $\leq$3 times in allantoic fluid and had a stock titer of approximately 5.5 $\log_{10}$ (the dose that is infective in 50% of tissue culture samples) per milliliter.

Squares measuring 4 $\times$ 4 cm were marked on each of the items to be tested using a marker pen. One square was drawn for each of 7 time points (0, 1, 8, 24, 48, 60, and 72 hours). Undiluted influenza virus from the stock (500 μL) was pipetted onto the surface within the marked area and spread using a pipette tip to cover the area. The test items were then left in a class 2 cabinet at room temperature until the designated time point (ie, up to 72 hours). At each time point, 1 mL culture medium (Influenza Virus Infection Media; Retroscreen Virology Ltd, London, UK) was used to rinse the surface of 1 of the marked squares and was collected. This was done with a single channel pipette that was primed using culture medium. The medium was expelled onto the surface ensuring coverage of the marked area. This was then taken off the surface using the same pipette and transferred to an appropriate container. It was also recorded if the virus was damp or dry on the surface at the time of virus recovery.

Each experiment was performed in quadruplicate as was the titration for residual virus infectivity.

The viability of the influenza virus was determined by infection of Madin Darby Canine Kidney (MDCK) cells, followed by determination of virus titers using the hemagglutination (HA) assay. MDCK cells were seeded in 96-well plates at a density of $\sim 5 \times 10^4$ cells/mL, and incubated at 37°C (±2°C) with 5% carbon dioxide in MDCK growth medium for ~24 hours, or until 70%-80% confluent. Before use in the assay, growth medium was removed from the cells and replaced with 100 μL/well of influenza infection media. A proportion (111 μL) of the recovered virus sample was inoculated onto the first row of MDCK cells and serially diluted 10-fold across the plate (11 μL titrated from the first well to subsequent wells containing 100 μL infection media). Inoculated cells were incubated at 37°C (±2°C) with 5% carbon dioxide for 3 days; the titer of the recovered virus was then determined using the HA assay. Materials were not heat and chemically sterilized to avoid structural changes. There were no issues of inherent cytotoxicity of these materials.

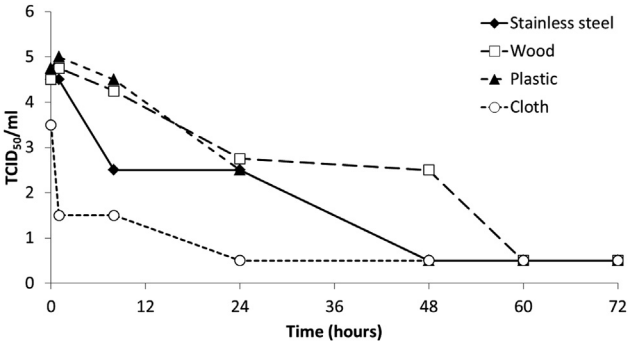

Fig 1. Influenza A(H1N1)pdm09 virus recovery from 4 surfaces over time. *$TCID_{50}$*, the dose that is infective in 50% of tissue culture samples.

For the HA assay, 50 μL supernatant from the titration plate were transferred to a 96-well plate; 50 μL turkey red blood cells at 0.5% vol/vol were then added. The plates were incubated at room temperature for 30 minutes and then examined for hemagglutination. Appropriate positive and negative controls were used at each stage. From the HA assay, the dose that is infective in 50% of tissue culture samples per milliliter for each test surface and the controls was calculated using the Karber Calculation.

RESULTS

Survival of the influenza A H1N1sw virus decreased most rapidly on the cloth surface, with no infectious virus particles being recovered after 8 hours (Fig 1). In Figure 1 the data are represented as residual virus infectivity. Influenza A/H1N1sw virus particles survived for the longest time on the wooden surface, with infectious virus particles still being recovered after 48 hours. Survival of the virus on stainless steel and plastic was intermediate between cloth and wood, with detectable infectious virus particles at 24 hours but not at 48 hours.

DISCUSSION

Of the 4 surfaces tested, influenza A(H1N1)pdm09 virus particles survived longest on the wood, and for the shortest time on the cloth. The immediate decline in infectivity at time 0 in the samples recovered from the cloth suggests that the technique used to elute the virus may not have been effective on this surface (or that inhibitory substances were present in the fibers). Previous studies have shown that influenza virus particles lose infectivity less rapidly on nonporous surfaces compared with porous ones,[7-9] but it has also been acknowledged that this could be due to inefficient recovery of virus particles from the porous surfaces.[8]

The findings of our study are supported by the literature. Bean et al[7] reported that influenza A and B virus particles remained viable for 24-48 hours on stainless steel or plastic, but survived for less than 8-12 hours on cloth; findings very closely mirrored in our study. Tiwari et al[8] found that avian influenza virus particles could survive on nonporous surfaces for up to 6 days. More recently, Greatorex et al[9] have shown that a pandemic strain of influenza A/H1N1 did not remain viable for >9 hours on a range of household surfaces. However, surfaces allowing the viruses to survive the longest included plastic and stainless steel, in agreement with our study. In contrast to our study, wooden surfaces did not support viral survival, with virus inoculates becoming inactivated after <4 hours. The difference in these findings may be due to the various types of wood tested, or may be related to the experimental procedures.