Survival of influenza virus on human fingers

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Abstract

Indirect transmission of the influenza virus via finger contamination with respiratory mucus droplets has been hypothesized to contribute to transmission in the community. Under laboratory conditions, influenza-infected respiratory droplets were reconstituted as close as possible to natural conditions. We investigated experimentally the survival of influenza A (H3N2) and A (H1N1)pdm09 viruses on human fingers. Infectious virus was easily recoverable on all fingers 1 min after fingertip contamination but then decreased very rapidly. After 30 min, infectious virus was detectable in only a small minority of subjects. Infectious viruses were detected for a longer period of time when droplets of larger size containing a higher number of particles were tested or when the viral concentration increased. A rapid decrease in infectiousness was observed when droplet integrity was disrupted. Our findings could help to set up the promotion of hand hygiene to prevent influenza hand contamination.

Keywords: Fingers, H1N1 2009, influenza, transmission, viral stability
Original Submission: 1 March 2013; **Revised Submission:** 24 May 2013; **Accepted:** 28 June 2013
Editor: T. A. Zupanc
Article published online: 4 July 2013
Clin Microbiol Infect 2014; **20:** O58–O64
10.1111/1469-0691.12324

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Introduction

Human-to-human influenza transmission is mediated mainly by the airborne route [1,2], but direct contact via previously contaminated hands followed by self-inoculation of the upper respiratory tract is possibly equally important [3,4]. During the recent influenza A (H1N1) pandemic, this led to the inclusion of hand hygiene as one of the recommended first-line preventive measures against transmission [5,6]. However, it remains difficult to establish whether large contaminated respiratory droplets could lead to finger contamination [7,8]. This may be dependent upon the virus type, inoculum size and external conditions, such as temperature and humidity [7]. To the best of our knowledge, the role of hand contamination followed by self-inoculation is supported only by indirect epidemiological data [9] linked to the ability of the influenza virus to survive for a prolonged period under various environmental conditions, but no direct proof or human experimental studies are available.

Indirect evidence is the ability of human influenza to survive for a prolonged period of time under various environmental conditions. Previous studies performed in the 1940s and in the 1980s reported that the influenza virus preserved its infectiousness when mixed in mucin-enriched buffer [10] or conserved on dry surfaces for 5 weeks [11]. Similarly, more recently the influenza A (H3N2) virus was cultivable for up to 17 days after deposition on banknotes in the presence of respiratory mucus [12]. Avian influenza viruses remained infectious for more than 300 days at 4°C and more than 100 days at 26°C in water of appropriate acidity and salinity [13,14]. The influenza A (H1N1)pdm09 virus can potentially retain its infectivity on a non-porous surface for up to 7 days

at 35°C and 66 days at 4°C [15], although other studies showed a much shorter survival time of 4–9 h [16] at room temperature. Under non-physiological laboratory conditions, large doses (1 mL) of seasonal influenza A (H1N1) culture supernatant at a high concentration (10^7 $TCID_{50}$/0.1 mL) remained infectious for 1 h on hands [17]. Closer to real-life conditions, fomites sampled in nursing homes, daycare centres or households during an influenza epidemic were contaminated with the influenza genome [18,19]. During the 2009 epidemic, nucleic acid of the influenza virus genome was detected on 17% of the fingertips of children living in the same household as confirmed influenza cases [19]. Under experimental conditions, 0.1 mL of an influenza A (H1N1) viral suspension at physiological concentration (10^3—$10^{4.5}$ $TCID_{50}$/0.1 mL) present on a non-porous support could be transferred to hands and remained infectious for several minutes [11]. However, it remains unknown whether such an inoculum could initiate transmission once in contact with the upper respiratory tract. The objective of this study was to investigate the survival rate of influenza A (H1N1) 2009 and seasonal influenza A (H3N2) virus on human fingers experimentally contaminated with reconstituted respiratory droplets as close as possible to those in infected humans.

Methods

We conducted a series of experiments to assess the survival and duration of infectiousness of human influenza viruses on human fingers between 1 and 30 min. The term 'survival' is defined as the persistence of influenza virus that could be propagated on Madin-Darby canine kidney cells (MDCK).

Cell line and conditions

MDCK cells (#CCL34™; ATCC, Manassas, VA , USA) [12] were either cultivated under serum-containing conditions in MEM-Eagle medium (DMEM, M4655-500; Sigma Chemie, Buchs, Switzerland), supplemented with 2 mg/L Trypsin (#25090028; Invitrogen/Gibco, Basel, Switzerland), 10% fetal calf serum (#10270-106; Invitrogen/Gibco) and 7.5% NaHCO 3 (#530F00H; Bio Concept, Allschwil, Switzerland), or under serum-free conditions in DMEM (#31966-021; Invitrogen/Gibco).

Viral suspensions

All experiments were performed with the vaccine strain influenza A/Moscow/10/1999 (H3N2) and the influenza A/Switzerland/01/2009 (H1N1) strain antigenically and genetically related to the vaccine strain influenza A/California/7/2009 (H1N1). One millilitre of viral suspension obtained by cell culture was mixed in human respiratory mucus. Briefly, 1 mL of cell suspension obtained by cell culture was mixed with 9 mL of human secretions, resulting in stock concentrations of 1.8×10^7 and 1×10^5 50% tissue culture infective doses ($TCID_{50}$/mL), respectively. Respiratory mucus was obtained by mixing clinical specimens received at the laboratory for respiratory virus testing by real-time RT-PCR and cell culture and which were negative.

Participants and finger contamination procedure

The recruited volunteers were six specialized laboratory collaborators (technicians, MD or PhD graduates) accustomed to infectious virus manipulation and who had undergone vaccination with the 2008–2009, 2009–2010 and pandemic 2009 influenza vaccines. Experiments were conducted on each participant on different days but with identical temperature, humidity and hood flow conditions. The study protocol was approved by the University Hospitals of Geneva ethics committee. All participants signed an informed consent form and were instructed to follow biosafety guidelines under the close supervision of the main investigator.

Hands were strictly kept under biosafety level 2 (BSL2) hoods during the complete procedure. To avoid detergent action on cell culture, hands were not washed before the experiments. At the end of each experimental procedure under the BSL2 hood, volunteers' fingers were systematically immerged in 1% bleach for 1 min before being dried and immediately re-disinfected abundantly with alcohol-based hand gel. Hands were then removed from the hood and washed again with disinfectant soap, followed by cleansing with alcohol-based gel. During the experiments the average temperature and humidity rate were maintained at 22 ± 3°C and 66 ± 5%, respectively. A 2-μL drop of influenza A (H3N2) and A (H1N1) 2009 viral suspension mixed with respiratory secretions (as described in the Viral suspension section above) was deposited on fingertips. This volume was chosen because it represents a good mean of large respiratory droplets size and can be applied in a reproducible manner (Fig. 1). Each individual contaminated finger was kept at room temperature without any contact or any additional mechanical action on the infectious suspension for 1, 3, 5, 15 and 30 min, before testing for the presence of infectious virions. Each viral suspension was deposited on three fingers of each volunteer for a total of 18 fingers. For experiments where contaminant droplets were specifically disrupted, the tip of the pipette was used to spread the droplet immediately on to the surface of the fingertip.

Determination of infectiousness

After a predefined time, the volunteers' fingers were immerged in wells containing 1 mL of Eagle minimal essential medium