Survival of hepatitis A virus on various food-contact surfaces during 28 days of storage at room temperature

San-Cheong Bae a, Shin Young Park a, An-Na Kim a, Mi-Hwa Oh b, Sang-Do Ha a,⁎

a School of Food Science and Technology, Chung-Ang University, 72-1 Nae-Ri, Daedeok-Myun, Ansung, Kyunggido 456-756, Republic of Korea
b National Institute of Animal Science, Rural Development Administration, 564 Omockchen-dong, Suwon, Gyunggido 441-706, Republic of Korea

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ABSTRACT

Hepatitis A virus (HAV) infections constitute one of the leading causes of food-borne disease outbreaks. HAV spreads readily from one infected person to another through contaminated water or food, via the fecal–oral route. The prevention of HAV infection is therefore important in the preparation of food. The objective of this study was to investigate the survival of HAV on six different food-contact surfaces: ceramic, wood, rubber, glass, stainless steel and plastic. The survival of HAV was measured during storage at room temperature for 28 days. On the food-contact surfaces, the greatest reduction in HAV was 2.3 log10 plaque-forming units (PFU)/coupon, observed on stainless steel, while the lowest HAV reduction was 1.4 log10 PFU/coupon, observed on wood. The values of \( d_R \) (time required to reduce the virus by 99%) on survival plots of HAV determined by a modified Weibull model were 1396.9 h \( (R^2 = 0.97) \) on ceramic, 1676.4 h \( (R^2 = 0.98) \) on wood, 852.7 h on rubber \( (R^2 = 0.95) \), 1386.4 h \( (R^2 = 0.97) \) on glass, 428.6 h \( (R^2 = 0.96) \) on stainless steel and 833.0 h \( (R^2 = 0.97) \) on plastic. The infectivity of the virus on all six food-contact surfaces was maintained after 28 days. Studies show that HAV from food preparation or processing will survive persistently on cookware. This study provides information useful for the control of HAV on different food-contact surfaces and the prevention of food-borne disease.

1. Introduction

The Centers for Disease Control and Prevention (CDC) has announced that hepatitis A virus (HAV), norovirus (NoV) and rotavirus are all staple virus pathogens that cause food-borne illnesses in the United States (CDC, 2011). Among these, HAV infections constitute one of the major causes of food-borne disease outbreaks and usually cause at regular intervals not only in developing countries but also in developed countries (Halliday et al., 1991; Koopmans & Duizer, 2004; Mead et al., 1999; Richards, 2001). The CDC reported 5683 cases of HAV infection associated with 14 HAV deaths in the United States in 2004 (CDC, 2006). HAV causes anticipated 83,000 illnesses per year, in the United States (Mead et al., 1999). HAV belongs to the Picornaviridae family, Hepatovirus genus and can cause clinical disease resulting in gastrointestinal illness and jaundice (Acheson & Fiore, 2004). Susceptible individuals are infected through contaminated water or food via the fecal–oral route (Fino & Kniel, 2008). The incidence of HAV cases in developed countries has significantly decreased, due to the availability of vaccine and improvements in sanitation and living conditions (Normann et al., 2008). Nevertheless, international travelers are still at risk of infection, particularly in developing countries; infection risk depends on the period of stay, living environment and the incidence of HAV infection in the area visited (Keystone & Hershey, 2008). HAV is contagious and resistant to heat and disinfection agents (Koopmans & Duizer, 2004). However, HAV was inactivated at 100 °C (Koopmans & Duizer, 2004). Hewitt, Rivera-Aban, Greening, and Journal of Applied Microbiology (2009) reported that D-values of MNV and HAV in water were 0.9 and 0.6 min, respectively at 63 °C and 0.3 min for both viruses at 72 °C. Bidawid, Farber, Sattar, and Hayward (2000) reported that HAV has a 5-log reduction within 0.5 min at 85 °C in dairy products (Bidawid et al., 2000). HAV inactivated within 4 min at 70 °C, 30 s at 75 °C, and 5 s at 80 °C in suspension (Parry & Mortimer, 1984). Disinfec-
tants were ineffective against viruses but they can occur viral spread or cross-contamination of surfaces (Barker, Vipond, & Bloomfield, 2004; Boone & Gerba, 2007). The tenacity of viruses on surfaces depends on mostly various complex variables involving viral properties, such as surrounding environment, surface properties (porous or nonporous), sanitary conditions and extrinsic factors (humidity, temperature) (Boone & Gerba, 2007).

Instant cooked foods in contact with a contaminated surface tend to be exposed to a higher risk of viral infection than industrially processed foods (Jean, Morales-Rayas, Anoman, & Lamhoubjeb, 2011; Koopmans & Duizer, 2004). Viruses, including HAV, can be spread to food during instant cooked food preparation by food handlers with poor personal...
hygiene or contaminated utensils. For this reason, many viruses can be transmitted to the food (D’Souza et al., 2006; Richards, 2001). Several studies have reported that viruses could remain infective on different surfaces for different time periods (Abad, Pinto, & Bosch, 1994; Abad et al., 2001; Bean et al., 1982; Boone & Gerba, 2007; Brady, Evans, & Cuartas, 1990; Hall, Douglas, & Geiman, 1980; Mbithi, Sprinithorpe, & Sattar, 1991). Although several studies on the survival of HAV on food-contact surfaces have been carried out, there are still need to investigate the survival time and pattern of HAV as the main cause of human nonbacterial gastroenteritis depending on the various types of food contact surfaces, especially focused on cookware utensils. Therefore, the present study investigated the survivability of HAV when stored at room temperature for long term period (28 days) on various food-contact surfaces: stainless steel, plastic, wood, rubber, glass, and ceramic.

2. Materials and methods

2.1. Virus and cell line

HAV (strain HM-175) and fetal rhesus monkey kidney (FRHK-4) cells were kindly provided by Professor M. D. Sobsey (University of North Carolina, Chapel Hill, NC, USA).

2.2. Cell preparation

FRHK-4 cells were grown in Dulbecco's minimum essential medium (DMEM; SIGMA, Saint Louis, Missouri, USA; cat. # M0268) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, New York, USA; cat. # 26140-097), 44 mM sodium bicarbonate (SIGMA, Saint Louis, Missouri, USA; cat. # S5761) and 1% antibiotics–antimycotics (Penicillin Streptomycin; Gibco, Grand Island, New York, USA; cat. # 15140-122), seeded in 75 cm² culture flasks, and incubated at 37 °C in a humidified 5% CO₂ incubator. Cells were subcultured every two or three days.

2.3. Virus preparation

When monolayers of FRHK-4 in 150 cm² culture flasks were 90% confluent, the growth medium was removed by aspiration. The monolayers were washed with phosphate-buffered saline (PBS, pH 7.4). A 1 mL aliquot of virus inoculum was added to the flasks, and the flasks were incubated at 37 °C in a 5% CO₂ atmosphere for 90 min to allow virus adsorption. The flasks then received 25 mL of maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotics–antimycotics) and were incubated at 37 °C in a 5% CO₂ atmosphere for 7 days. If cytopathic effects (CPE) were observed above 90%, the virus-infected flasks were frozen and thawed three times. Viruses were released by cell lysis through this step. The contents were centrifuged at 1500 ×g for 10 min to remove cell debris and the supernatants harvested. Viruses were stored at 70 °C until use.

2.4. Plaque assay

FRHK-4 cells were seeded in 12-well plates and incubated at 37 °C in 5% CO₂ conditions (seeding volume: 2 mL for each well; 4 × 10⁵ cells) and incubated for 24 h until 90% cell confluency. Virus suspensions eluted from the samples were serially diluted in maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotics–antimycotics). Serially diluted virus suspensions (100–200 μL) were inoculated onto cells. After shaking the plates for 10 min using a shaker (FMS2, FINEPCR, Korea), they were incubated at 37 °C in 5% CO₂ conditions. One hour later, 2 × 10⁵ agarose (Sigma) supplemented with 2 × DMEM was added to the inoculated cells; each well received 1–2 mL of this mixture. Plates were then left at room temperature for 20 min and then incubated for 7–8 days at 37 °C in 5% CO₂ conditions. Cells were then fixed with 1 mL of 3.7% formaldehyde for 4 h. The formaldehyde was discarded and the 2 × 10⁵ agarose and 2 × DMEM mixture overlays were removed carefully using tap water. The fixed cells were dyed with 0.1% (w/v) crystal violet solution for 20 min to visualize the plaques. Plaques were counted and the virus infectivity titer was described as plaque forming units (PFU) per mL.

2.5. Preparation of food-contact surface materials and inoculation

Stainless steel (Posco Co., Ltd., SUS 304 2B, Pohang, Korea), plastic (HDPE; Daesung Industry Co., Seoul, Korea), wood (Heilongjiang Zhongji IMP & EXP, Beijing, China), rubber (Komax Industrial Co., Ltd., Seoul, Korea), glass (Kukje Glass, Nonsan, Korea), and ceramic (Hankook Chinaware Co., Ltd., Cheongju, Korea) were selected as representative materials used in the food industry. Coupons with dimensions of 0.1 cm diameter and 5 mm thickness were produced from the selected materials. The prepared coupons were soaked in 70% ethanol as disinfectant for 1 h and washed with distilled water. After rinsing, the coupons were dried in a desiccator and autoclaved in a sealed bottle at 121 °C for 15 min. Due to the heat-sensitive properties of rubber, the autoclave step was omitted for rubber coupons. To allow absorption of spiked viruses on surfaces, all prepared test coupons were inoculated at 18–20 °C in a laminar flow hood. Each viral suspension (50 μL volume, containing approximately 5 log₁₀ PFU/mL of HAV) was inoculated to the food (D’Souza et al., 2006; Richards, 2001). Several studies on the survival of HAV on food-contact surfaces, especially focused on cookware utensils. Therefore, the present study investigated the survivability of HAV when stored at room temperature for long term period (28 days) on various food-contact surfaces: stainless steel, plastic, wood, rubber, glass, and ceramic.

2.6. Survival experiment

The procedure was described by Kim, Park, Bae, Oh, and Ha (2014). Inoculated coupons were dried in a laminar flow hood for 1 h and stored for predetermined times (0 min, 3 h, 12 h, 24 h, 3 days, 5 days, 7 days, 14 days, 21 days and 28 days) in the experimental chamber at room temperature. Virus recovery rate (%) was calculated with the following formula: % recovery rate = (virus titer from the food-contact surface after 0 h ÷ virus titer inoculated on food-contact surface) × 100%. At each of the predetermined times, 50 μL of elution buffer (0.05 M glycine–0.14 M NaCl buffer (pH 7.5)) was pipetted onto the coupons and left at room temperature for 10 min. The coupons were then placed into 15 mL conical tubes with 200 μL of elution buffer and vortexed for 10 min to elute virus. Each eluted viral suspension was serially diluted 10-fold and analyzed by plaque assay.

2.7. Weibull model

The Weibull model, a two-parameter non-linear model, can be expressed as:

\[
\log \left( \frac{N_t}{N_0} \right) = -bt^n.
\] (1)

Here, \( N_t \) is the concentration of virus (PFU/mL) after an exposure time \( t \), \( N_0 \) is the initial concentration of virus (PFU/mL), \( t \) is the exposure time, and \( b \) and \( n \) are the scale (a characteristic time) and the shape parameters as a behavior index, respectively (van Boekel, 2002). The \( b \) value represents the time needed to reduce the first log cycle of the population while the \( n \) parameter indicates the shape of the survival curve. An \( n \) value of 1 corresponds to a linear survival curve, while \( n \) values > 1 and < 1 correspond to downward and upward concavity, respectively.
For the calculation of $d_R$ (analogous to the traditional D-value) from the Weibull parameters, Eq. (2) was used, as by Buzrwul and Alpas (2007):

$$d_R = \left( \frac{2}{\lambda} \right)^{1/n} \quad \text{(2)}$$

Here, $d_R =$ time required to reduce virus by 99%. To determine inactivation kinetics, the modified Weibull model was fitted by nonlinear regression using the software GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

### 3. Results and discussion

#### 3.1. Survival of hepatitis A virus on food-contact surfaces

The survival of HAV was measured at predetermined times (0 h, 3 h, 12 h, 24 h (1 day), 72 h (3 days), 120 h (5 days), 168 h (7 days), 336 h (14 days), 504 h (21 days), and 672 h (28 days)) after inoculation on ceramic, plastic, wood, rubber, glass and stainless steel. The amount of HAV detected from the surfaces of all six materials significantly $(P < 0.05)$ decreased with time (Fig. 1A–F), but HAV infectivity on all food-contact surfaces was still maintained after 28 days. For the ceramic, wood and rubber surfaces, there was an initial steep drop of approximately 0.3–0.4 log$_{10}$ PFU/coupon in HAV titer between 24 and 72 h, and the highest inactivation occurred between 168 and 336 h (7 and 14 days), followed by steady declines. On the surface of the glass coupon, HAV infectivity rapidly decreased after 24 h, followed by relatively stable titers until 120 h (5 days), with an additional reduction after 168 h (7 days). For stainless steel, HAV titer fell sharply after 24 h. In the case of plastic, HAV titer rapidly decreased between 12 h and 24 h, followed by steady declines and further rapid reduction after 504 h (21 days).

After 28 days, the reduction of HAV titer was greatest on stainless steel (2.3 log$_{10}$ PFU/coupon) and lowest on wood (1.4 log$_{10}$ PFU/coupon) (Table 1). For ceramic, rubber, glass, and plastic, corresponding values were 1.6, 1.7, 1.6, and 1.9 log$_{10}$ PFU/coupon respectively. From this data, it can be seen that HAV could not survive longer on stainless steel than on the other materials and it maintained its infectivity longer on wood than the other surfaces. There was a statistically significant $(P < 0.05)$ difference in viral recovery rates from the six food-contact surfaces at the 0 h point, even though the same inoculated titer of 6.1 log$_{10}$ PFU/mL of HAV was used on each surface. The recovery rate from wood at the 0 h point was the lowest value among all the materials. This may be due to wood absorbing the virus suspension during the inoculation step. Although the wood was dried for 1 h before inoculation, the interior region of the wood may have remained wet and absorbed the majority of the virus. Furthermore, wood has a greater surface roughness and is more porous than the other tested materials (Kim, Baek, Oh, & Ha, 2011). This may explain the low observed HAV titers for this surface. Boone and Gerba (2007) reported that the persistence of viruses on surfaces in general depends on many complex variables, involving viral properties (such as type and strain), surrounding environment and surface properties (porous or nonporous, state of cleanliness, and moisture present). Sobsey et al. (1988) reported that HAV survived on polystyrene surfaces for over 9 months. McCausland, Bond, Bradley, Ebert, and Maynard (1982) showed that HAV survived on feces after drying during storage for 1 month. Abad et al. (1994) reported that rotavirus and HAV were more stable and resistant than adenovirus and poliovirus. These results indicate that HAV can remain viable in a range of external environments. Ticehurst et al. (1989) noted that HAV has more stable molecular structure than picornaviruses. Kim et al. (2014) recently investigated the survival of murine NoV (MNV) as a surrogate for human NoV on food-contact surfaces, and reported that the infectivity of MNV on all food-contact surfaces (ceramic, wood, rubber, glass, stainless steel, and plastic) remained after 28 days.

#### 3.2. Weibull modeling to obtain survival curves and parameters

The survival data of HAV from the food-contact surfaces was fitted using the Weibull model for non-linear microbial survival (Fig. 1G). The parameters $(b, n, d_R, R^2)$ of the Weibull model are shown in Table 2. The goodness of fit of the model was estimated by $R^2$; all of the values were over 0.90, confirming that the survival curves were a good fit.
this model. A first-order kinetic model was also fitted for comparison with the Weibull model. Microbial inactivation is commonly made using a first-order kinetics process (Fujikawa & Itoh, 1996; Whiting, Sacke...al. (2001). Potential role of fomites in vehicular transmission of human astroviruses. Applied and Environmental Microbiology, 67, 3904–3907.


4. Conclusion

In this study, the survival of HAV on six food-contact surfaces was investigated. The reduction of HAV was greatest on stainless steel (2.3 log10 PFU/coupon) and lowest on wood (1.4 log10 PFU/coupon). For all six materials, the order of reduction values (from highest to lowest) was stainless steel, plastic, rubber, glass, ceramic, and wood. The infectivity of the viruses on all food-contact surfaces was maintained after 28 days. The survival of HAV over time followed different trends depending on the type of surface material. Variables such as oxygen and ion concentration, surface charge, and the presence of organic matter are considered important for virus attachment (Dowd, Pillai, Wang, & Corapcioglu, 1998; Grant, List, & Lidstrom, 1993; Redman, Grant, Olson, Hardy, & Estes, 1997). The characteristics of food-contact surfaces may also be important for virus attachment (Cannon et al., 2006).

Studies show that HAV from food preparation or during processing will survive persistently on cookware. Therefore, a sanitary environment is very important during food preparation. Reducing the HAV contamination in food preparation areas can be expected to reduce the occurrence of diseases associated with this virus. To achieve this, methods for the controlled removal of HAV from contaminated surfaces are needed.

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