Ethanol susceptibility of SARS-CoV-2 and other enveloped viruses

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Short Report

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Abstract

Ethanol is an effective disinfectant against the novel coronavirus SARS-CoV-2. However, its effective concentration has not been shown, and we therefore analyzed the effects of different concentrations of ethanol on SARS-CoV-2. When SARS-CoV-2 was treated with varying ethanol concentrations and examined for changes in infectivity, the ethanol concentration at which 99% of the infectious titers were reduced was 24.3%(w/w) [30.0%(v/v)]. For reference, ethanol susceptibility was also examined with other envelope viruses, including influenza virus, vesicular stomatitis virus in the family *Rhabdoviridae*, and Newcastle disease virus in the family *Paramyxoviridae*, and the 99% inhibitory concentrations were found to be 28.8%(w/w) [34.8%(v/v)], 24.0%(w/w) [29.2%(v/v)], and 13.4%(w/w) [16.5%(v/v)], respectively. Some differences from SARS-CoV-2 were observed, but the differences were not significant. It was concluded that ethanol at a concentration of 30%(w/w) [36.2%(v/v)] almost completely inactivates SARS-CoV-2.

Main Text

SARS-CoV-2, the pathogen that causes COVID-19, originated from around Wuhan in China and has spread worldwide, causing a pandemic (WHO, 2020). People's lives have changed dramatically due to the incorporation of measures for infection prevention. Together with the use of masks for preventing droplet infection, hand washing and hand disinfection for prevention of contact infection have become a part of daily life. Alcohol, especially diethyl alcohol, is used for hand disinfection. An ethanol content of 62-71% was reported in a review article to be effective against SARS-CoV, which is very closely related to SARS-CoV-2 (Kampf et al., 2020). The included formulations of hand rinses were shown to be effective against SARS-CoV-2 (Kratzel et al., 2020; Suchomel et al., 2020). On the other hand, Kratzel et al. (2020) examined ethanol concentrations and showed that ethanol at a lower concentration of 30%(v/v) was effective against SARS-CoV-2 in the presence of 0.03% bovine serum albumin. Ethanol was also found to be effective and fast-acting in a 30-second treatment (Kratzel et al., 2020).

We examined ethanol concentrations that showed an inhibitory effect on SARS-CoV-2 to determine whether the virus can be inactivated at low ethanol concentrations. Effects of ethanol on other envelope viruses, including influenza virus, rhabdovirus and paramyxovirus, were also studied.

The 2019-nCoV/Japan/AI/I-004/2020 strain of SARS-CoV-2 (provided by Dr. Makoto Takeda, the National Institute of Infectious Diseases, Japan) was propagated in VeroE6/TMPRSS2 cells (purchased from the Japanese Collection of Research Bioresources; JCRB1819) in the P3 facility of Hiroshima University. Antiviral activity measurement was based on ATSM E1052-11 as previously described (Ueda et al., 2013). The virus (10 µl) and an ethanol solution (90 µl) were mixed. After reaction for 3 min at room temperature, infectious titers were measured by the standard $TCID_{50}$ method using the Behrens-Kraber algorithm (Karber, 1931). Ethanol was prepared from a special grade reagent (99.5%) (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) in various weight concentrations, considering the ethanol content.

Incubation with 27%(w/w) [32.7%(v/v)] ethanol at final concentration for 3 minutes resulted in a significant reduction in the infectious titer of SARS-CoV-2 by more than 4 logs (Fig. 1A). The use of 36% (w/w) [47.3%(v/v)] ethanol completely inactivated the virus. To examine the effect of protein loading, antiviral tests were performed in the presence of 0.03% bovine serum albumin (BSA; FUJIFILM Wako Chemical Corp.)(Japanese Society for Infection Prevention and Control, 2020) or 0.5% polypeptone (HIPOLYPEPTON N; FUJIFILM Wako Chemical Corp.)(Igarashi et al., 2015) (Fig. 1B). When comparing the inhibitory effect at 27%(w/w) [32.7%(v/v)] ethanol, the impact of ethanol seems to be slightly higher due to protein loading, but the overall effect of protein loading is small.

Other envelope viruses were also examined to investigate whether SARS-CoV-2 is particularly sensitive to ethanol. Influenza virus A/Udorn/72(H3N2) was similarly tested for inactivation with ethanol using MDCK(+) cells (Noma et al., 1998) (Fig. 1B). In the case of the influenza virus, only a 1 log reduction in infectious titers was observed at 27%(w/w) [32.7%(v/v)] ethanol, and a reduction in infectious titers of more than 4 logs was observed at 36%(w/w) [47.3%(v/v)] ethanol (Fig. 1C).

Ethanol susceptibility to other envelope viruses, including the vesicular stomatitis virus (VSV) Indiana serotype in the family *Rhabdoviridae* and Newcastle disease virus (NDV) Miyadera strain in the family *Paramyxoviridae*, was similarly examined by the $TCID_{50}$ method using BHK21 cells. Substantial ethanol concentrations and viral titers were plotted on a graph, and approximate curves were drawn by logarithmic approximation, especially in areas where the titers were significantly reduced (Fig. 2).

The approximation curves for SARS-CoV-2 and VSV nearly overlapped, revealing comparable ethanol sensitivities. Influenza virus showed viral inactivation in areas of higher ethanol concentrations, while NDV showed viral inactivation in areas of lower concentrations (Fig. 2).

Based on the approximation curves in Fig. 2, ethanol concentrations causing 99% and 99.99% inhibition are summarized in Table 1. Comparison of 99% inhibition concentrations, which is considered more reliable because of the middle of the approximation curve, showed similar results for ethanol susceptibility, 24.3%(w/w) [30.0%(v/v)] for SARS-CoV-2 and 24.0%(w/w) [29.2%(v/v)] for VSV. In contrast, the ethanol concentrations were higher for influenza virus (28.8%(w/w) [34.8%(v/v)]) and lower for NDV (13.4%(w/w) [16.5%(v/v)]). Compared to SARS-CoV-2, influenza virus was slightly more resistant to ethanol and NDV was more sensitive.

However, given that the envelope is impaired, the influenza virus M1 protein may support the envelope from behind, while SARS-CoV-2 may not have such a structure. On the other hand, the paramyxovirus NDV is an amorphous virus in which the M protein creates a braided structure to support the envelope from behind (Battisti et al., 2012), which may be insufficient to give ethanol resistance.

The results indicated that SARS-CoV-2 can be sufficiently inactivated at an ethanol concentration of 30% (w/w) [36.2%(v/v)]. On the other hand, Kratzel et al. (2020) examined ethanol concentrations and showed that ethanol at a lower concentration of 24.7%(w/w) [30%(v/v)] completely inactivate SARS-CoV-2 in the presence of 0.03% BSA. In our results, ethanol was indeed more effective in the presence of 0.03% BSA,

but not as effective as Kratzel et al (Fig. 1B). This may be due to differences in the virus strains used, but the actual reason is unknown. In any case, the relatively low concentration of ethanol (30%(w/w) [36.2%(v/v)]) is sufficient to inactivate SARS-CoV-2. Even considering the more resistant influenza virus, an ethanol concentration of 37%(w/w) [44.1%(v/v)] may be sufficient to inactivate the envelope virus.

According to the Japanese Pharmacopoeia, the appropriate ethanol concentration for disinfection is 70.0-75.2%(w/w) [76.9-81.4%(v/v)] (MHLW, 2016). However, the Japanese Fire Prevention Law restricts the storage and stockpiling of ethanol above 60%(w/w) [67.7%(v/v)], and many commercially available disinfectants therefore contain ethanol at a concentration of less than 60% and usually around 50%(w/w) [57.8%(v/v)]. Hence, there has been some debate as to whether 50%(w/w) [57.8%(v/v)] ethanol can indeed inactivate SARS-CoV-2. The present study showed that even 30%(w/w) [36.2%(v/v)] of ethanol can sufficiently inactivate SARS-CoV-2, and 50% of ethanol would therefore sufficiently inactivate SARS-CoV-2. In actual use, the disinfection ability of SARS-CoV-2 can be considered to be in the safe range of 50%(w/w) [57.8%(v/v)] ethanol because the concentration of ethanol can be reduced by dilution or evaporation.

Declarations

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Table

Table 1 is available as a download in the Supplementary Files section.

Figures

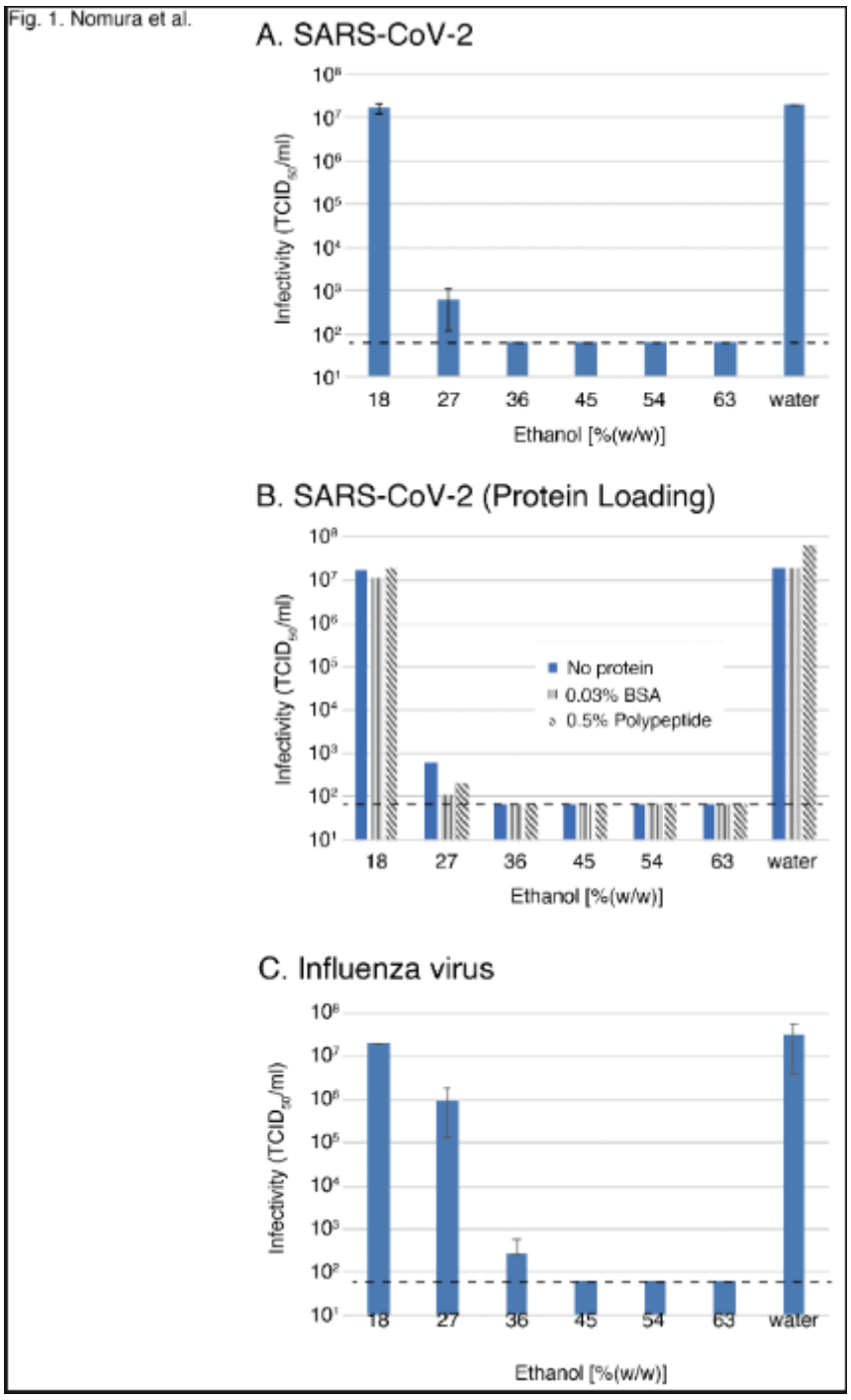

Figure 1

Effects of ethanol on SARS-CoV-2 and influenza virus. A. SARS-CoV-2 was mixed with different ethanol concentrations in the ratio of 1 to 9, and the remaining virus infectivities were measured using the standard TCID50 method in VeroE6/TMPRSS2 cells. B. Effects of ethanol was investigated in the presence of 0.03% BSA or 0.5% polypeptone. C. A similar assay was performed using influenza virus A/Udorn/72(H3N2) and MDCK(+) cells. The dotted line indicates the detection limit of the infectivity assay. Error bars indicate standard deviation.

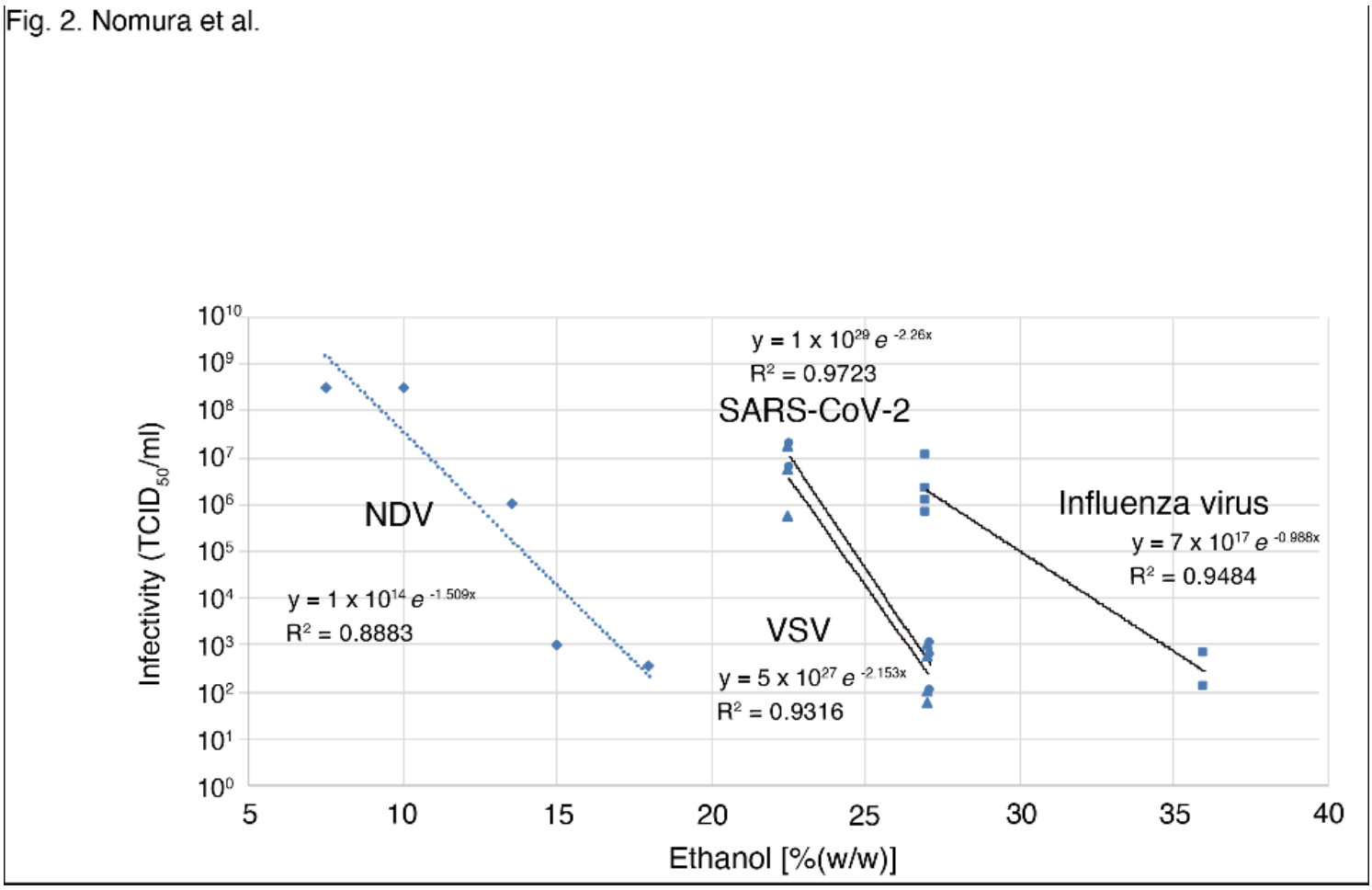

Figure 2

Ethanol sensitivities of SARS-CoV-2 (●), Vesicular stomatitis virus (VSV) (▲), Newcastle disease virus (NDV) (□), and influenza virus (■). An approximate curve based on logarithmic approximation was drawn for each virus. The approximation equations and R2 values are displayed in the graph.

Supplementary Files

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- Table1.png