Stability of Porcine Epidemic Diarrhea Virus on Fomite Materials at Different Temperatures

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Keypoints

- Type of material and temperature have an impact on PEDV stability.
- Infectious PEDV was not recovered from any fomite material after 2 days at room temperature (25ºC / 77ºF).
- PEDV showed higher stability on plastic, cloth, Tyvek® coveralls, aluminum foil, Styrofoam at 4ºC (39.2ºF).
- Virus could be detected by qRT-PCR from contaminated fomites even when infectivity was not observed.

Background

Transmission of PEDV primarily occurs by the fecal-oral route, but indirect transmission can occur when an animal comes in contact with inanimate objects (fomites) contaminated with feces from PEDV-infected animals. The stability of PEDV on contaminated fomites at different temperatures is poorly understood. Inanimate objects such as rubber boots, gloves, coveralls, and other equipment are routinely used on swine farms and have the potential of being contaminated with manure from PEDV infected animals, thereby helping the indirect mode of virus transmission. Thus, this study evaluated the survival of PEDV on various fomite surfaces at both room temperature (RT) and 4ºC.

Methods

200 µL of virus containing 2.1×10^6 $TCID_{50}$/mL was applied on various fomite material: Styrofoam, nitrile gloves, cardboard, aluminum foil, Tyvek® coveralls, cloth, metal, rubber, and plastic. The virus-contaminated fomites were then stored at either 4ºC or at room temperature (RT) (25ºC). Samples were then taken at 0, 1 2, 5, 10, 15, 20 and 30 days post-contamination to test for virus stability.

Results

Infectious PEDV was recovered from fomite materials for up to 15 days post application at 4ºC; only 1 to 2 logs of virus were inactivated during the first 5 days post application. On the other hand, PEDV survival decreased precipitously at RT (25ºC) within 1 to 2-days post application, losing 2 to 4 log titers within 24 h (Figure 1).

Immunoplaque assay, a more sensitive technique than standard $TCID_{50}$ or viral plaque assays, was used to identify positive fomites after 20 days of storage at 4◦C. Immunoplaque assay allows us to detect as few as 24 focus forming units (FFU) /mL. The viral concentrations initially sprayed on the fomites was 1 × 10^6. Approximately 1 × 10^3 FFU/mL were recovered in eluates from Styrofoam, metal, and plastic, representing a 3-log virus inactivation after 20 days. The surviving virus on Tyvek® coverall and rubber surfaces was moderately above detection limit (24 FFU/mL).

Also to determine the amount of viral RNA remaining on the materials quantitative RT-PCR was performed. RNA was detected after 2 days at RT and after 20 days at 4ºC. In fact, all materials tested had cycle threshold (Ct) values similar to those of input virus (~16–17) indicating that detection of viral RNA by PCR does not indicate the infectious nature of the sample.

Discussion

The virus remained viable at 4ºC for up to 20-days on Styrofoam, metal, and plastic, although viral titers decreased by 3 logs in 20 days. When stored at RT, PEDV decreased by 4 to 5 logs within 48 h, rendering it undetectable using infectious virus assays. This observation suggests that the storage temperature of the fomite material has a major impact on virus stability. It appears that low storage temperature delays degradation of viral infectivity on fomite materials. In addition to storage temperature, the type of fomite material may also influence virus survival and hence virus transmission.

In this study, viral RNA copy numbers did not correlate with the cell-based infectious virus assays. In other words, it was possible to detect viral RNA using a qRT-PCR but it was not possible to prove that the sample was infectious. It is possible that PEDV infectivity is destroyed by factors that affect the integrity of the viral envelope without affecting RNA degradation.

Although we used both cell culture propagated PEDV and PEDV-spiked fecal material on fomites to mimic actual situation in the field, we were unable to determine the survival of the virus in PEDV-spiked fecal material. This was so because the eluates from fomites contaminated with feces were not suitable for infectious viral titer assay due to cytotoxicity of the fecal content on indicator cells.

Conclusion

These results provide an added understanding on PEDV stability on different surfaces at different temperatures and provides clues on how PEDV could be disseminated through fomites.

Full article can be found at thefollowing link:
http://www.mdpi.com/2306-7381/5/1/21

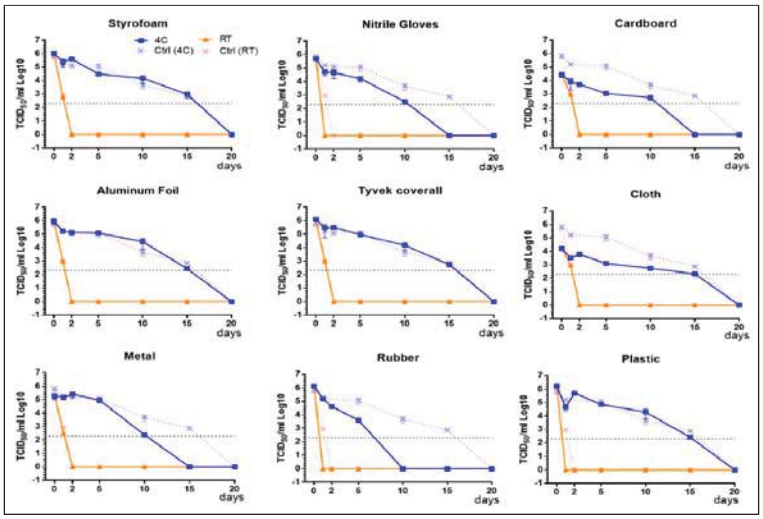

Figure 1. Virus survival on nine fomites at room temperature (RT) and at 4 °C. PEDV was applied on fomite materials or in control wells followed by storage at RT (▲, orange line) or at 4 °C (■, blue line). The surviving virus was eluted after various time periods and titrated. Decay of infectious virus was rapid at RT but delayed when stored at 4 °C. Black dotted line represents detection limit of $TCID_{50}$ assay, which is ~2×10^2 $TCID_{50}$/mL. Data presented are average of three replicates (± SEM) obtained from independent assessments at each time point indicated.

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3/23/2018