Comparative survival of different strains of porcine reproductive and respiratory syndrome virus

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Background:
Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is a major cause of economic losses to the North American swine industry. An emerging PRRSV-2 classified as 1-4-4 RFLP pattern-lineage 1C is the cause of a regional outbreak in the Midwestern US since 2020. The virus can be transmitted to naïve hosts via direct and indirect routes. For the latter route to be successful, the virus needs to survive in the environment, which depends on several factors including temperature, moisture, matrix, and pH. PRRSV is stable for months to years at temperatures of -70 °C and -20°C but is inactivated rapidly by heat and drying. The emergence of new PRRSV variants within the last 20 years and the severity of disease outbreaks raise concerns about their stability in the environment since it can affect virus dissemination.

Objective:
To determine the comparative survival of different strains of PRRSV at three different temperatures.

Materials and Methods:
Viruses were propagated and titrated in MARC 145 cells, grown in Eagle's MEM supplemented with Fetal Bovine Serum (FBS) and antibiotics. For each strain, three 24-well plates were labeled (4°C, ~25°C and 37°C) and the virus was applied to the bottom of all wells (100µl of virus/well). Plates were air-dried for 4 h and placed at their respective temperature. The surviving virus was eluted (in triplicate) after 4h, 1, 3, 7, 14, 21, 28 and 35 days. Serial 10-fold dilutions of all eluates were prepared. Dilutions were inoculated in monolayers of MARC 145 cells (triplicate). Plates were incubated at 37°C under 5% CO_2 and were examined daily under an inverted microscope for the appearance of cytopathic effect (CPE). The 50% endpoints were calculated after 7 days of incubation. Virus titers were calculated and expressed as $\log_{10}$ $TCID_{50}$ per 100µl. Percent virus inactivation at different time and temperature were then calculated.

Results:
A summary of survivability in days of various PRRSV strains (and percent virus inactivation) at different temperatures and the percentage of inactivation until the last day of survival is presented in Table 1.

Table 1. Survival in Days of various strains of PRRSV at three different temperatures.

PRRSV strain[a]	4°C	25°C	37°C
	Days of survival (Percent reduction)	Days of survival (Percent reduction)	Days of survival (Percent reduction)
1-8-4	35 (97.81%)	7 (99.97%)	3 (99.53%)
1-4-4 MN L1C	35 (99.12%)	7 (99.87%)	3 (98.87%)
1-4-4 SD L1C	35 (99.98%)	3 (99.00%)	3 (99.94%)
Lelystad	35 (98.71%)	3 (99.99%)	3 (99.99%)
VR2332	35 (99.71%)	3 (99.99%)	1 (99.99%)
1-4-2	35 (99.39%)	1 (99.96%)	1 (99.99%)
1-26-2	35 (99.99%)	1 (99.99%)	1 (99.99%)
ATP Vaccine	35 (99.99%)	1 (99.99%)	1 (99.99%)
2-5-2	35 (99.97%)	1 (99.99%)	1 (99.99%)
1-7-4	35 (98.71%)	1 (99.78%)	1 (99.98%)

a: All strains belong to PRRSV-2 except the Lelystad strain, which belongs to PRRSV-1.

Conclusions and implications:
All ten strains of PRRSV survived for at least 35 days at refrigeration temperature. However, there were differences among these strains as to the amounts of virus killed at 35 days. Strains 1-7-4, Lelystad, 1-8-4, VR 2332, 1-4-2, and 1-4-4 MN were more resistant in cold environment than the other strains. At 25°C, five strains survived no longer than 1 day. Only 5 strains, namely VR2332, Lelystad, 1-4-4 SD, 1-4-4 MN, and 1-8-4 survived longer (3 to 7 days) at room temperature than the other 5 strains. Four of the ten strains survived for up to 3 days at 37°C (Lelystad, 1-4-4 MN, 1-4-4 SD, and 1-8-4). The remaining strains did not survive longer than 1 day. The emerging variant 1-4-4 1C was one of the more resistant strains at cold environment, and survived for 3 days at room temperature and at 37°C. These results show differences in the survival of PRRSV strains at different temperatures. The virus survived longer at cold temperature, as compared to room temperature and 37°C. Biosecurity practices that include a robust disinfection protocol of facilities, equipment, and instruments must be followed since contaminated surfaces at different temperatures could be a potential risk factor for virus transmission.