





# Comparative Survival of 5 Strains of PRRSV in Tap Water at 3 Different Temperatures

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## Introduction

Based on Morrison Swine Health Monitoring Project (MSHMP) data, it has been shown that the U.S. swine industry faces an annual Porcine Reproductive and Respiratory Syndrome epidemic, with most outbreaks occurring during the fall-winter seasons. It has been reported that approximately 40% of the MSHMP participating breeding herds report a PRRS break within 15 to 30 days of manure pumping occurring (Vilalta et al., 2021). Moreover, there have also been results showing that approximately 10% of manure pits can contain PRRSV genetic material (Montoya et al., 2020), and that the virus itself has the ability to percolate (Alvarez-Norambuena et al., 2022). The contamination of underground water could therefore become a considerable source of transmission of the virus. Few studies have previously evaluated the ex-vivo survival of the virus in water and swine slurry, mostly regarding a single strain and at lower temperatures (Pirtle and Beran, 1996; Ajariyakhajorn et al., 1997; Dee et al. 2005). The objective of this study was to determine the survival of different strains of PRRSV in tap water at different temperatures.

### **Materials and Methods**

Tap water obtained from a laboratory faucet was collected in glass bottles, which were then separated according to chlorination and sterilization treatments. Sodium thiosulfate at 30 mg/L was used for dechlorination. Sterilization was achieved through autoclaving. Four strains of PRRSV-2 (174 L1A, 144 L1C, MN30100, VR2332) and one PRRSV-1 (Lelystad) were grown in Marc-145 cells to titers of 104.5 to 105.5 TCID50/0.1 mL. To prepare the samples, 0.75 mL of each virus suspension was added to each 3.750 mL-aliquot of the four different tap water treatments. The initial viral titer in the aliquots was quantified by TCID50 microtitration assay. The aliquots were then placed at their respective temperatures for further sampling over time. At each time point, 0.5 mL of sample was collected, homogenized, and immediately titrated. Three replicates were carried out for each combination of strain, temperature and water treatment. Log reductions were calculated by the formula log10 Ct/C0, where Ct is the titer of the virus at the specified sampling time and C0 is the virus titer at time 0. Linear regression models were used to calculate T99 (time to 99% decrease in virus titer). Analysis of variance was performed between the rates of reduction in viral concentration, as well as pair-wise comparisons using the Tukey HSD test.

#### Results

The time to minimal detectable titer by the TCID50 microtitration assay (101.254 TCID50/0.1mL) for the different strains and water treatments ranged from 6 hours to 24 hours at 37°C, 3 to 5 days at room temperature (21-23°C) and more than 35 days at 4°C. Strain VR2332 had the longest calculated T99 times (Table 1). There was a statistically significant effect of temperature on rate of reduction of viral titer over time (p < 0.001). However, strain and water treatment were not found to be statistically significant. Pair-wise comparisons indicated that 37°C was significantly different than room temperature (21-23°C) and 4°C.

## **Conclusions and implications**

There was a marked effect of temperature on virus survival, with the PRRSV remaining stable in tap water for prolonged periods of time at 4°C. Even though the strain and water treatments were not found to be statistically significant, some important limitations include the different starting titers for the different strains and the unmeasured chlorine levels for the water treatments. Also, tap water may not be representative of field conditions. Further studies are needed to confirm the possible role of this milieu in PRRSV transmission.

PRRSV STRAIN	37°C				21-23°C				4°C			
	Tap Water	Autoclaved	Dechlorinated	Autoclaved and Dechlorinated	Tap Water	Autoclayed	Dechlorinated	Autoclaved and Dechlorinated	Tap Water	Autoclaved	Dechlorinated	Autoclaved and Dechlorinated
	T <sub>99</sub> (hours)				T <sub>99</sub> (days)				T <sub>99</sub> (days)			
1-4-4L1C	7.9	9.5	7.1	9.2	3.2	2.6	2.2	3.5	30.3	28.5	21.3	36.9
1-7-4L1A	9.1	7.3	6,7	6,0	2.4	3.2	1.7	2.0	43.6	32.9	22.9	27,2
Lelystad	7.0	4.9	6.2	4.8	2.5	1.7	2.8	3.8	39.5	37,5	17.0	22.8
MN30100	7.7	5.7	5.9	7.5	2,1	1.9	1.8	2.3	30.5	24.2	22.0	20.8
VR2332	14.8	14.0	15.2	15.2	5.8	5.6	5.0	5.3	70.7	60.0	33.1	44.3

## Table 1. T<sub>99</sub> as calculated by linear regression.



