Assessment of temperature and holding times to inactivate PRRSV and PEDV on contaminated surfaces commonly found in supply entry rooms in swine farms

MS CREATIVE COMPONENT

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OUTLINE

BACKGROUND

OBJECTIVE OF THE STUDY

MATERIALS AND METHODS

RESULTS

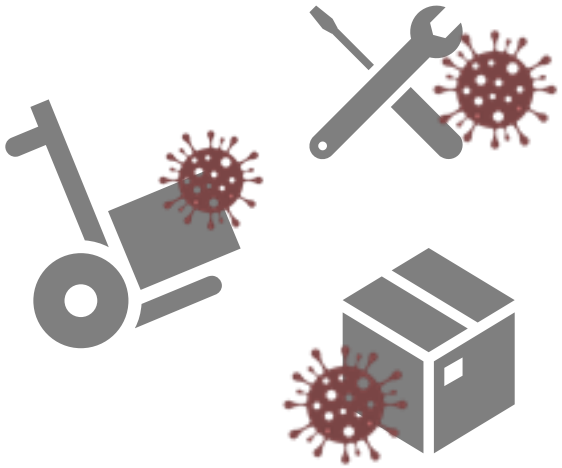
DISCUSSION

CONCLUSION

BACKGROUND

BACKGROUND

FOMITES DECONTAMINATION



FOGGERS
Leuck et al. (2020)

UVC LIGHT
Ruston et al. (2020)

TIME AND TEMPERATURE
Thomas et al. (2015)

BACKGROUND

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV)

→ Decrease in growth rate;
→ High number of abortions;
→ Respiratory disease;

$664 M
Holtkamp et al. 2013

Big impact on the production system

PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV)

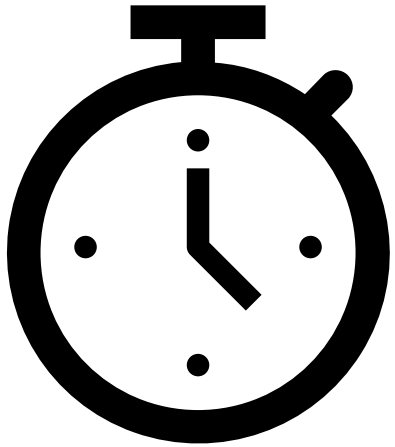
→ High neonatal mortality;
→ High morbidity in weaned pigs;
→ Watery diarrhea and vomiting;

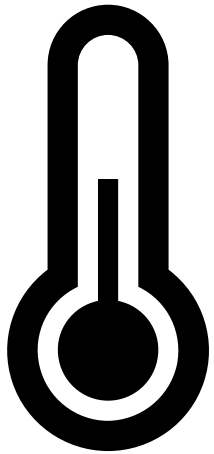
Diseases of Swine, 11TH EDITION

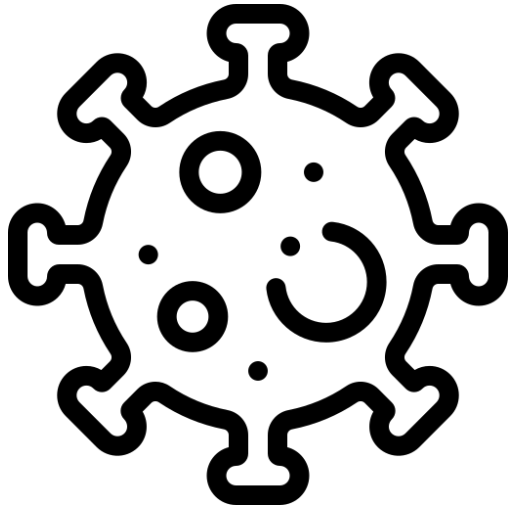
95% neonatal mortality
90% suckling piglets morbidity
Stevenson et al. 2013

OBJECTIVE

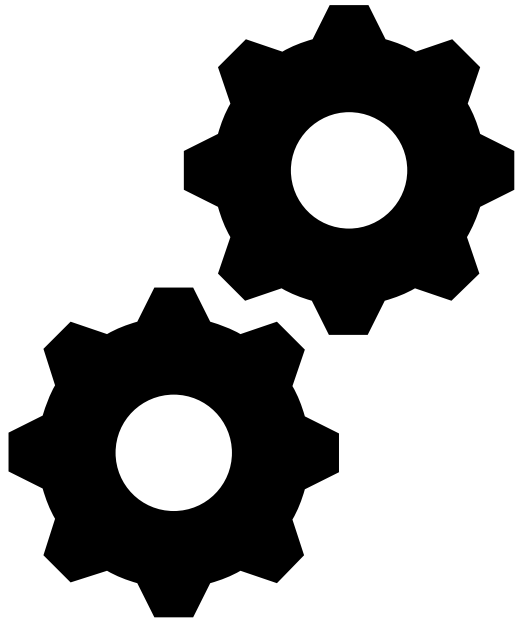
ASSESS THE IMPACT OF TIME AND TEMPERATURE ON PRRSV AND PEDV INACTIVATION ON DIFFERENT SURFACES COMMONLY FOUND IN SUPPLY ENTRY ROOMS IN SWINE FARMS







METHODS



METHODS STUDY DESIGN

SURFACES

- CARDBOARD
- ALUMINUM

VIRUS

- PRRSV MN184
- PRRSV 144 L1C Variant
- PEDV

TEMPERATURES

- 68ºF (20ºC)
- 86ºF (30ºC)
- 104ºF (40ºC)
- 122ºF (50ºC)

TIMES

- 15 MIN
- 1 HOUR
- 6 HOURS
- 12 HOURS
- 24 HOURS
- 36 HOURS

Neg. Control

⇩

MEM 68ºF 36 hours

METHODS PILOT STUDY

- Test difference between surfaces with and without organic matter
- Feces were already used in several studies

  (Goyal 2014, Thomas et al. 2015, Ruston et al. 2020, Neat et al. 2021)

SURFACES	VIRUS			TEMPERATURE		TIME	INOCULUM	NEG.CONTROL
CARDBOARD	PRRSV 144 L1C Variant	PRRSV MN184	PEDV	68ºF (20ºC)	122ºF (50ºC)	15 MIN	MEM+VIRUS	PBS 68ºF (20ºC)
ALUMINUM								
CARDBOARD	PRRSV 144 L1C Variant	PRRSV MN184	PEDV	68ºF (20ºC)	122ºF (50ºC)	15 MIN	FECES+VIRUS	PBS+FECES 68ºF (20ºC)
ALUMINUM								

Feces interfered in PRRSV titration

METHODS EXPERIMENTAL TRIAL

VIRUS INOCULATION

- 146 treatments → 3 replicates each
- 2 ml MEM (Neg. control)
- 2 ml of virus (Pos. control and treatments)

→ **PRRSV MN184** 5.6 x 10^5 TCID50/ml in MARC-145 cells
→ **PRRSV 144 L1C Variant** 5.6 x 10^4 TCID50/ml in MARC-145 cells
→ **PEDV** 3.16 x 10^5 TCID50/ml in Vero cells

METHODS EXPERIMENTAL TRIAL

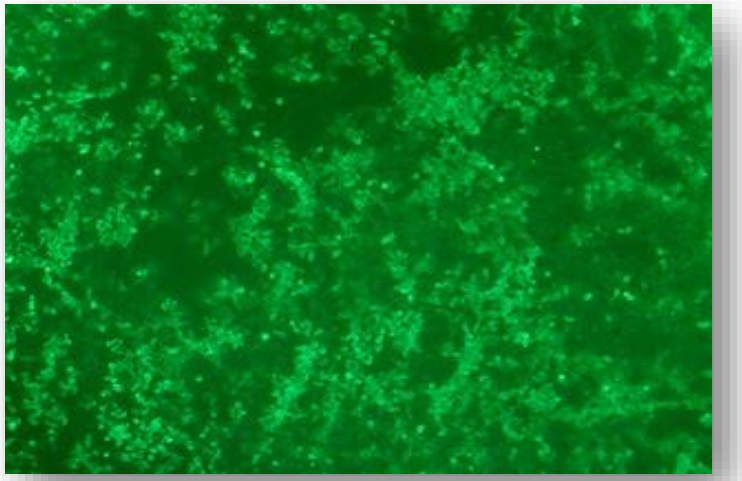
INCUBATOR

- Each treatment had one thermocouple
- **On cardboard** – small hole and taped thermocouple
- **On aluminum** – taped the thermocouple to the edge
- Waited for the surface to reach desired temperature to start the holding-time

METHODS EXPERIMENTAL TRIAL

VIRUS RECOVERY

- Elute the virus from the surface by adding the MEM to surface and scraping (10x)
- Final solution with eluted virus in snap cap tubes



Virus titration
and
Immunofluorescence staining

→ Cytopathic effect

METHODS DATA ANALYSIS

- Descriptive analysis
- Linear Mixed Models
  - Least-Squares Means
- Weibull curves
- Half-life

METHODS DATA ANALYSIS

- **Linear Mixed Models:**

log10TCID50/ml = Contact time + (1|Replicate)

Fixed effect
Mean effects

Random effect
variability around the mean

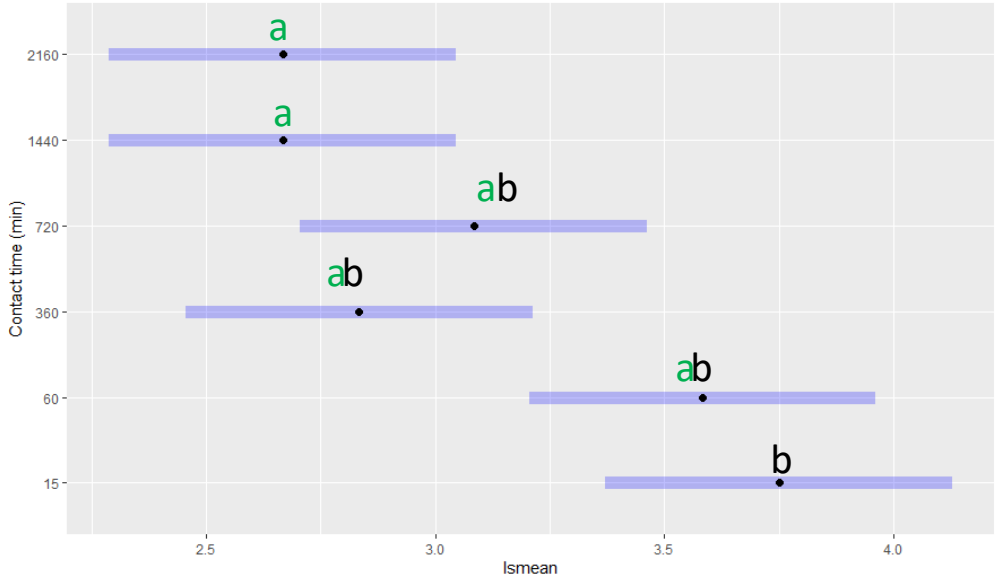
Data: divided by surface

CARDBOARD 68ºF		
Contact time	Replicate	log10TCID50/ml
15 min	1A	3.50
15 min	1B	3.52
15 min	1C	3.51
60 min	2A	3.00

EXAMPLE OF DATA

METHODS DATA ANALYSIS

- **Least-Squares Means and Compact letter display:**
  Compute estimated marginal means and find difference between contact time.
  Tukey: which group's means are different



Contact time (min)	lsmean	SE	df	lower.CL	upper.CL	.group
2160	2.666387	0.1736349	12	2.120959	3.211814	a
1440	2.666458	0.1736349	12	2.121031	3.211885	a
360	2.833281	0.1736349	12	2.287854	3.378708	ab
720	3.083525	0.1736349	12	2.538098	3.628953	ab
60	3.583037	0.1736349	12	3.037610	4.128464	ab
15	3.749931	0.1736349	12	3.204504	4.295359	b

α=0.05

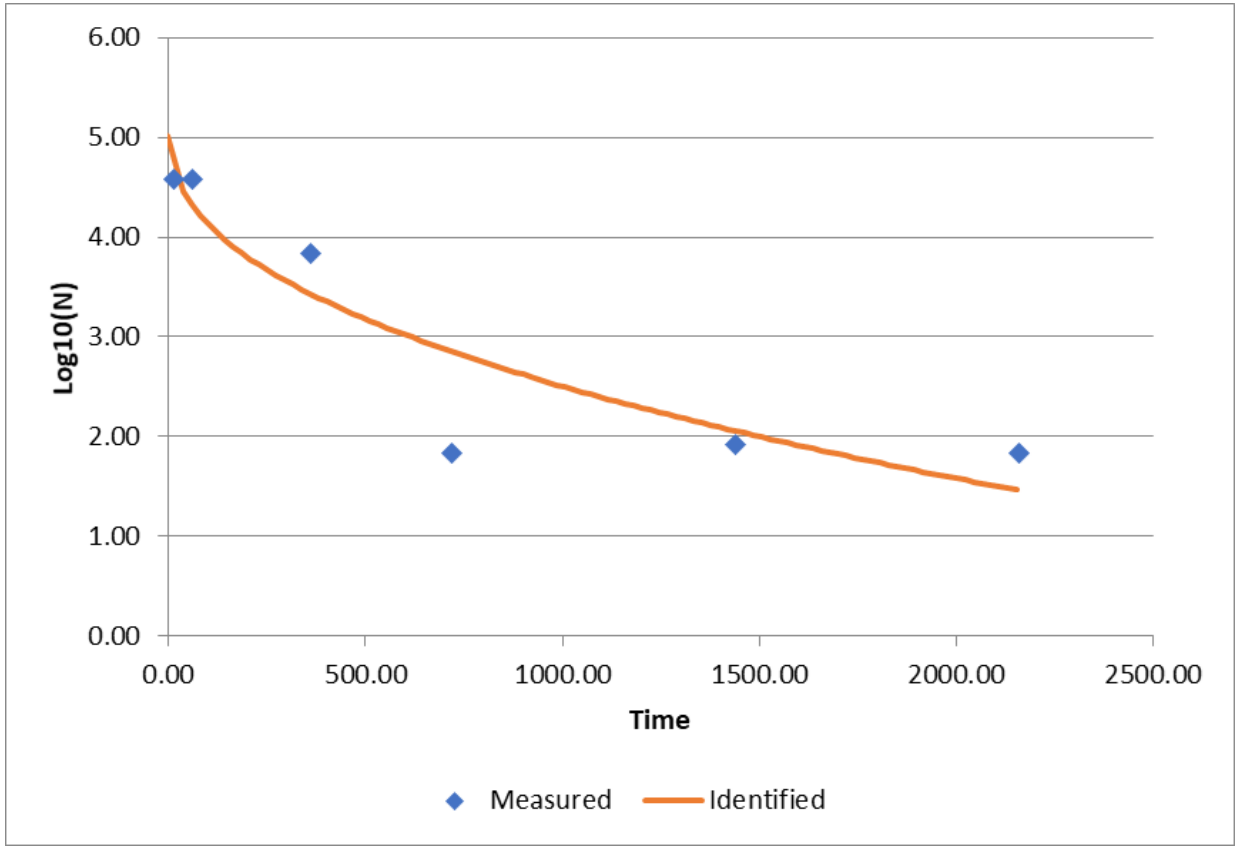
METHODS DATA ANALYSIS

- **Weibull curves**

→ GinaFit add-in on Excel

→ **Survival curve:** decrease in log10TCID50/ml

→ **Distribution:** 0 to ∞



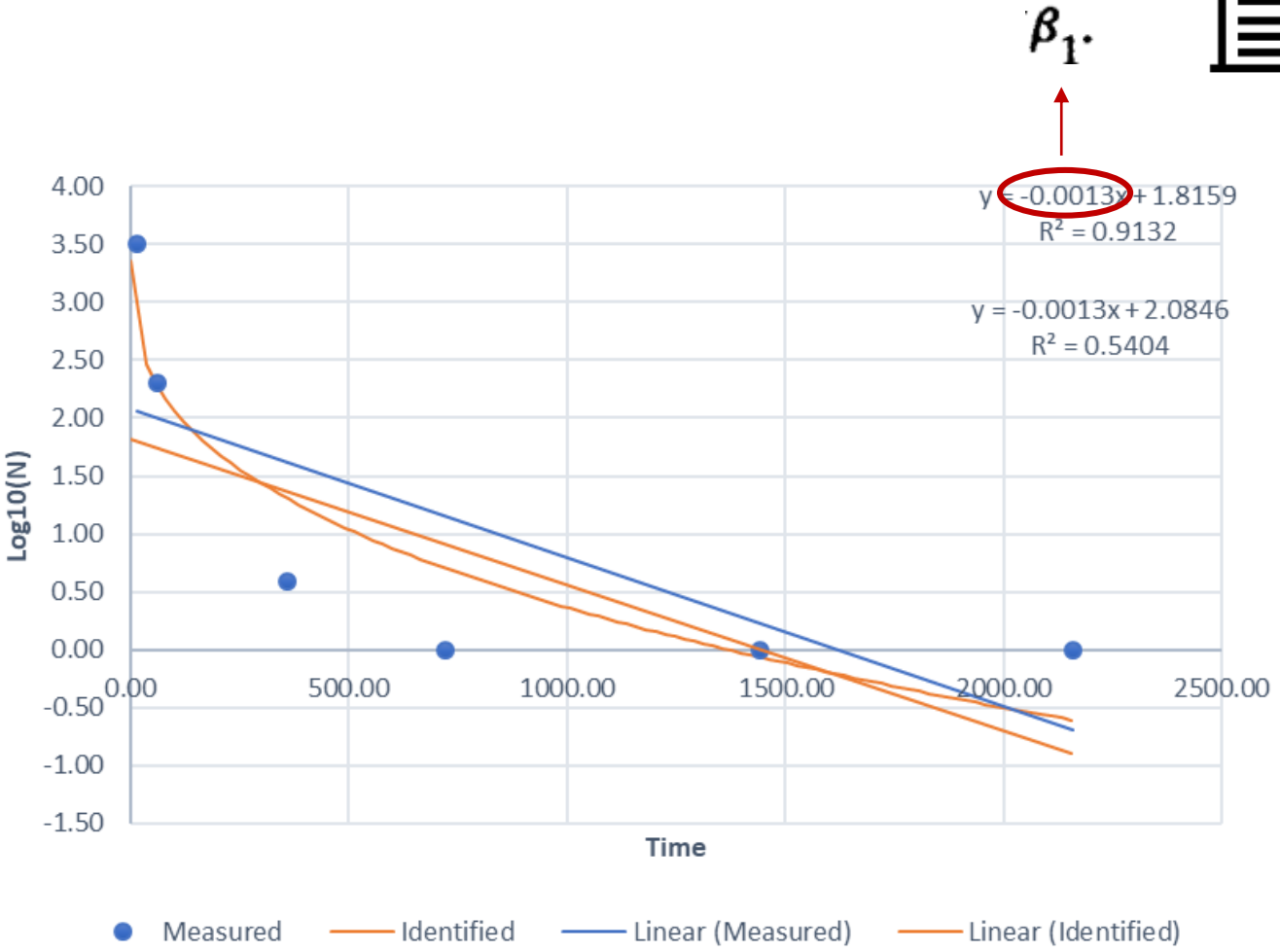
Geeraerd et al. 2005

METHODS DATA ANALYSIS

- **Half-life:** Estimate the time for log10TCID50/ml to decrease in 50%

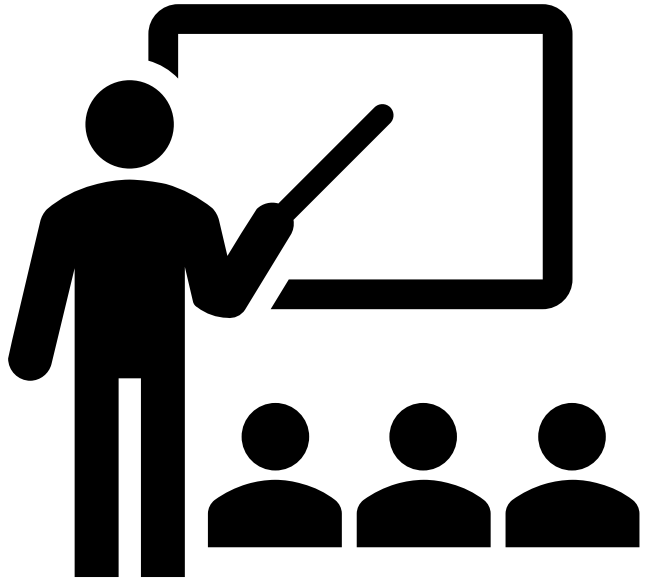
$$\hat{t}_{1/2} = -\ln 2/\beta_1.$$

Bryan, M et al. 1990



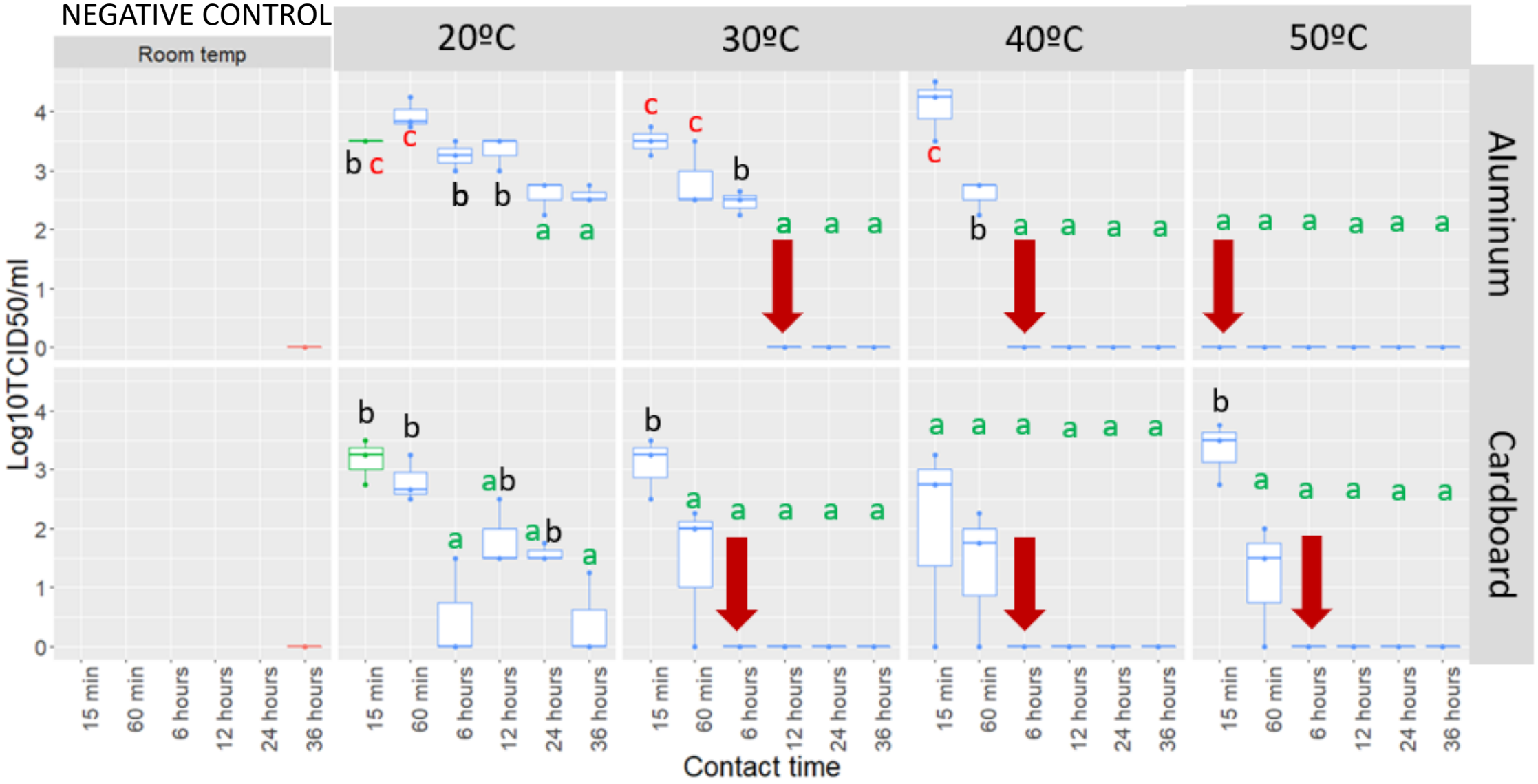
RESULTS

PRRSV 144 L1C Variant



RESULTS

PRRSV 1-4-4 L1C Variant



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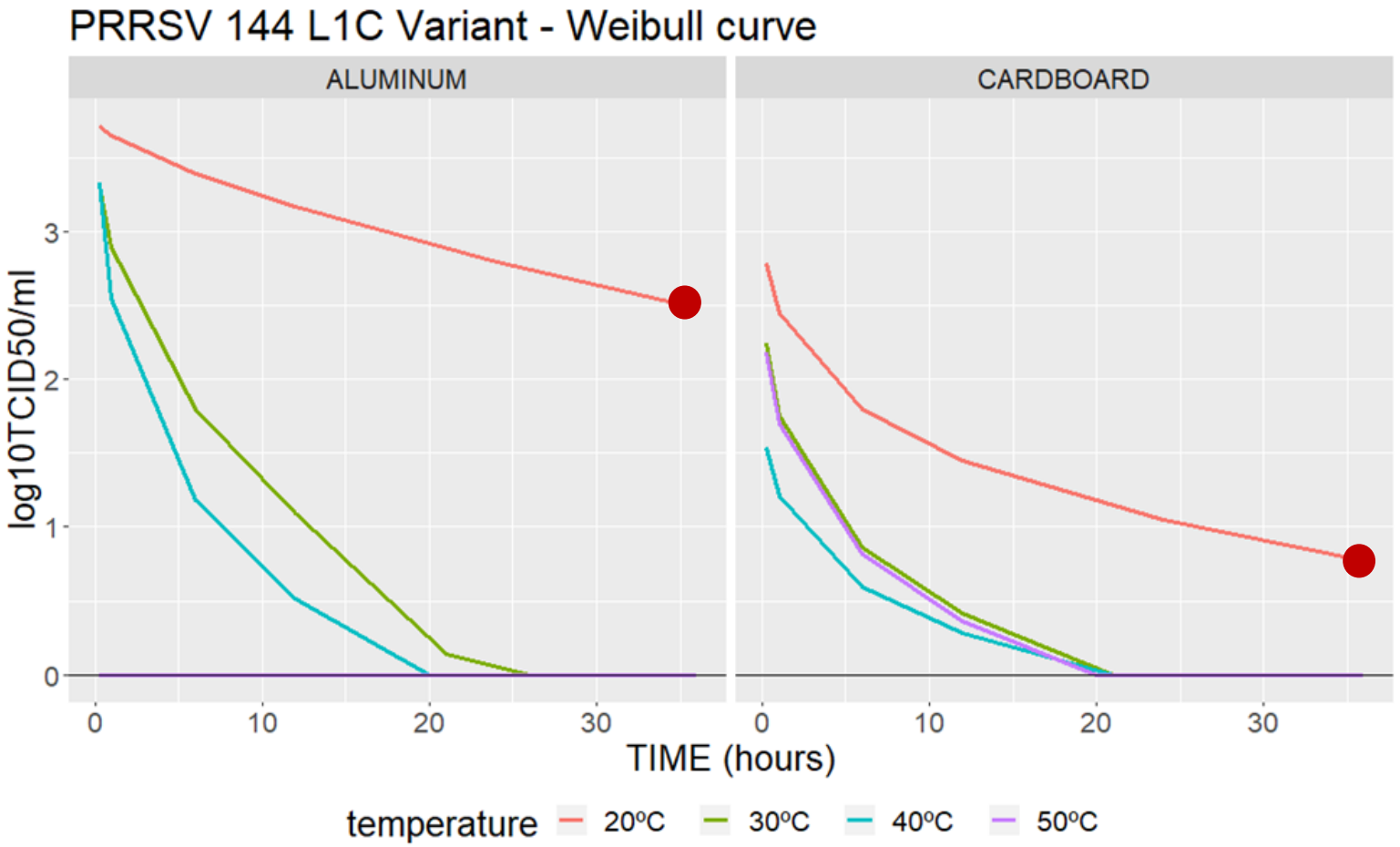
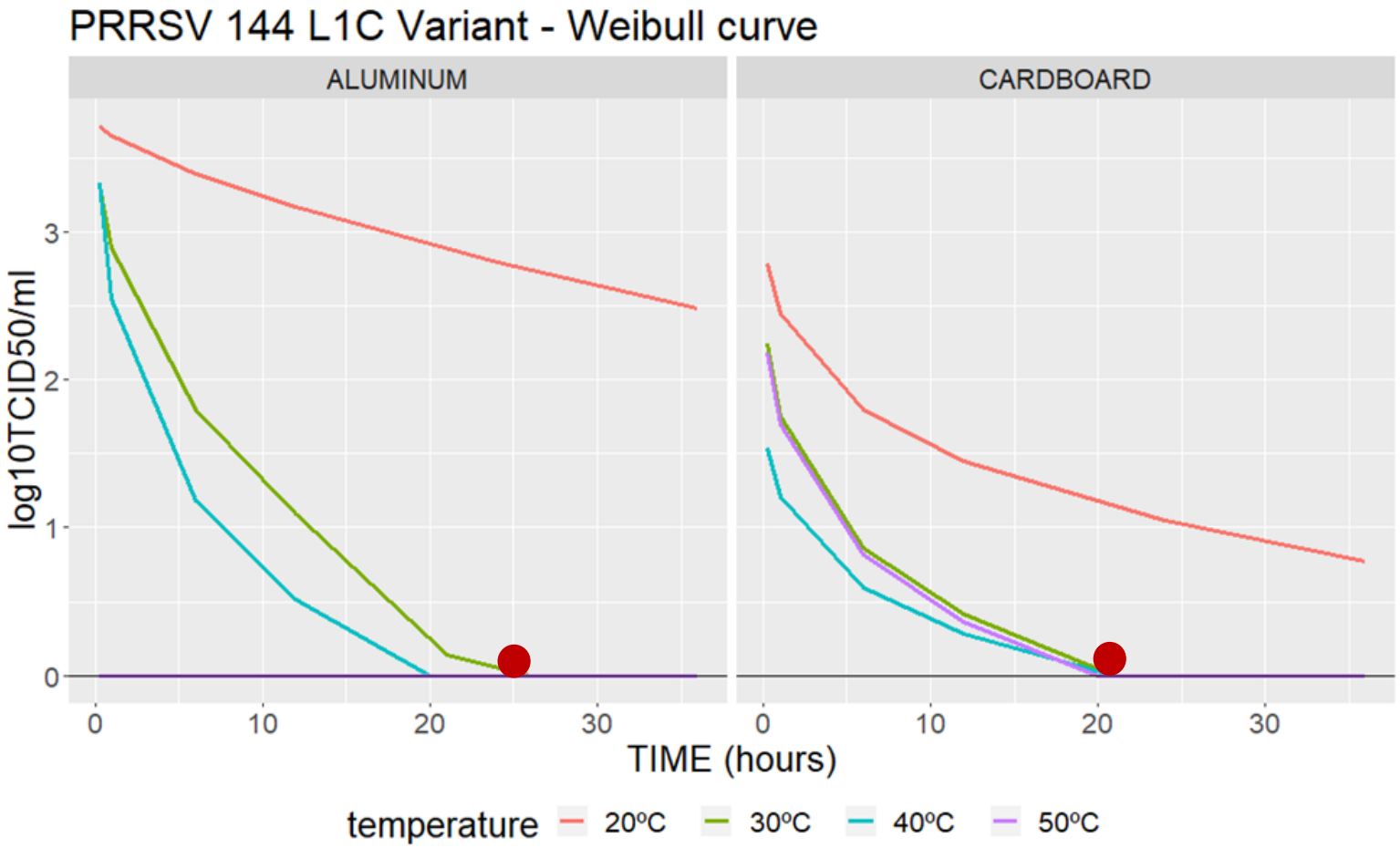


SURFACE	TEMPERATURE	INACTIVATION TIME
Aluminum	20ºC	-
	30ºC	26:30 hours
	40ºC	19:38 hours
	50ºC	0 hours
Cardboard	20ºC	-
	30ºC	21:10 hours
	40ºC	21:04 hours
	50ºC	19:43 hours

RESULTS

PRRSV 1-4-4 L1C Variant



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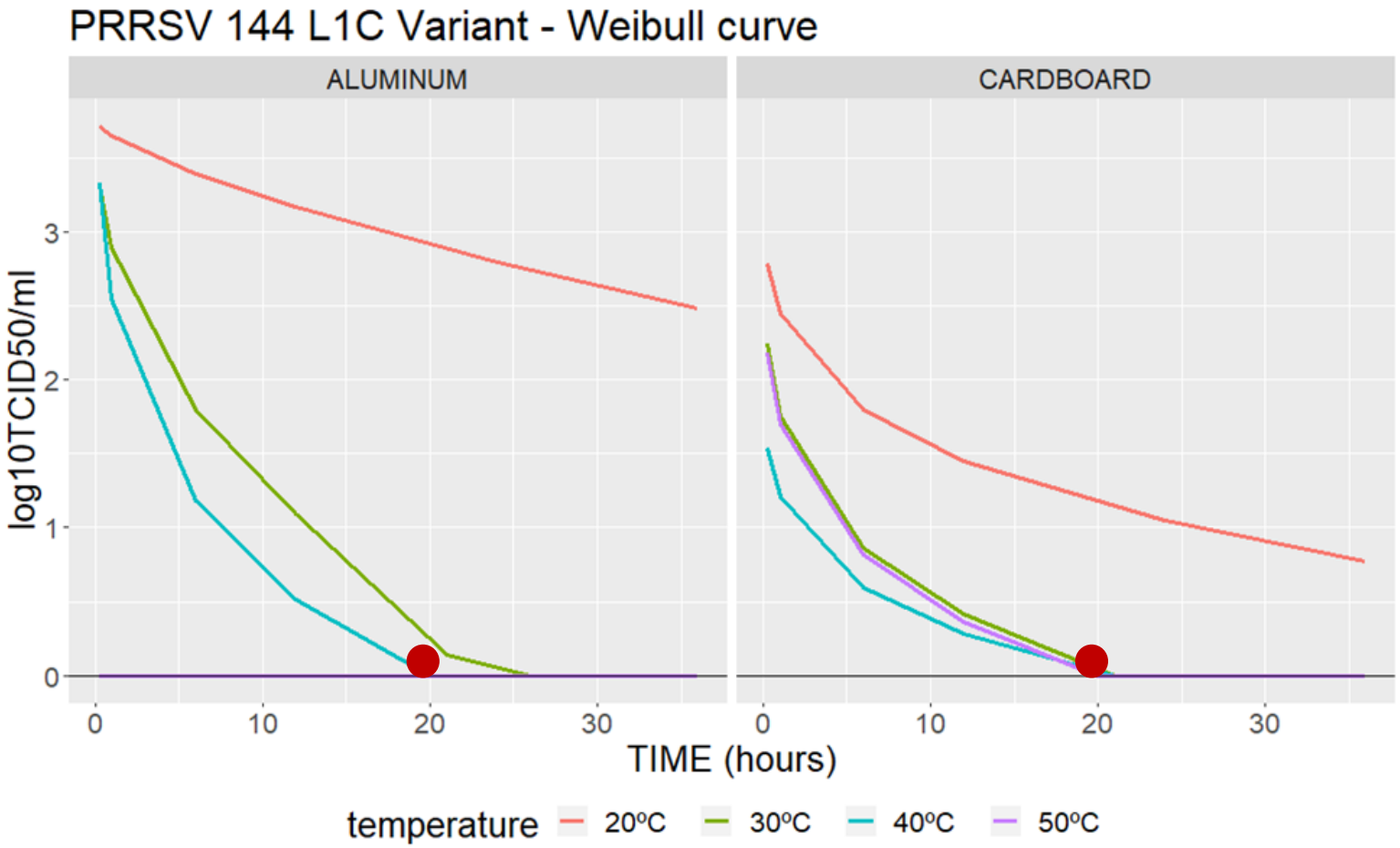

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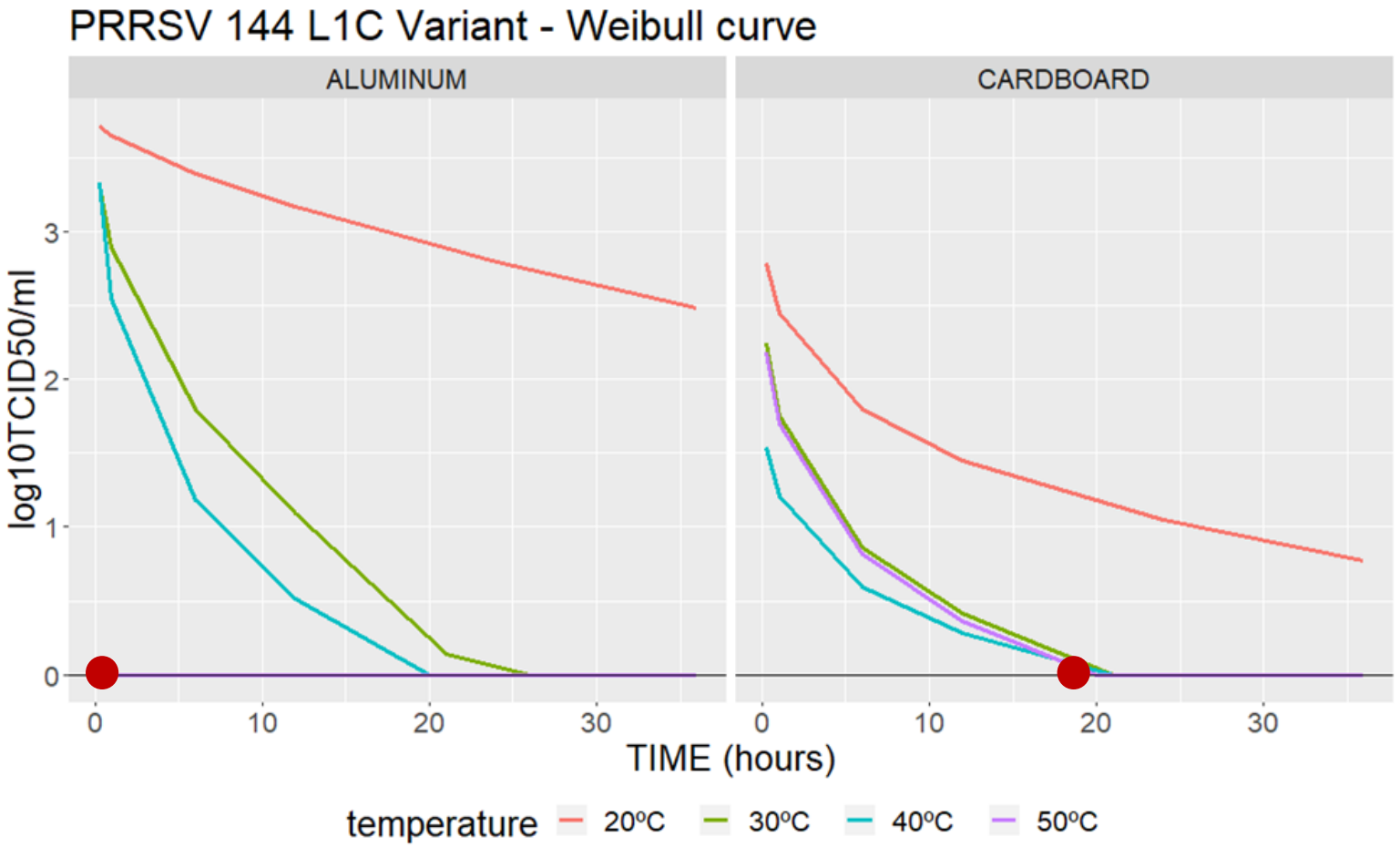

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	40ºC	21:04 hours
	50ºC	19:43 hours

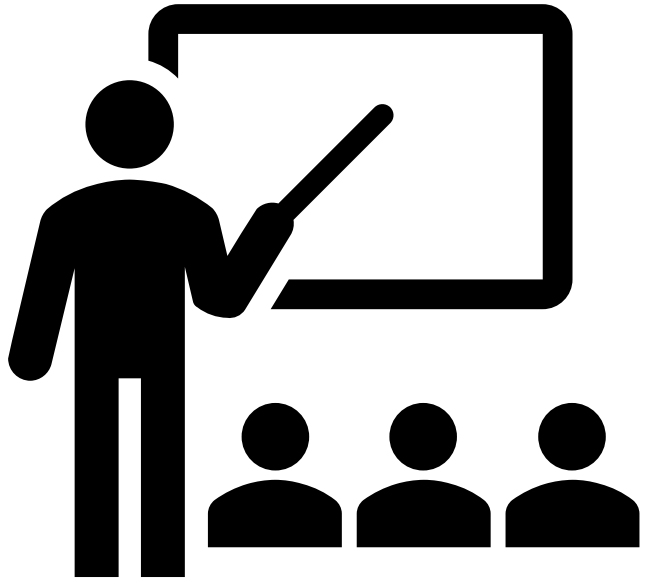
RESULTS

PRRSV 1-4-4 L1C Variant

SURFACE	TEMPERATURE	HALF-LIFE (HOURS)
Aluminum	20ºC	8:30
	30ºC	7:20
	40ºC	7:26
	50ºC	0.00
Cardboard	20ºC	8:36
	30ºC	8:11
	40ºC	8:11
	50ºC	8:11

RESULTS

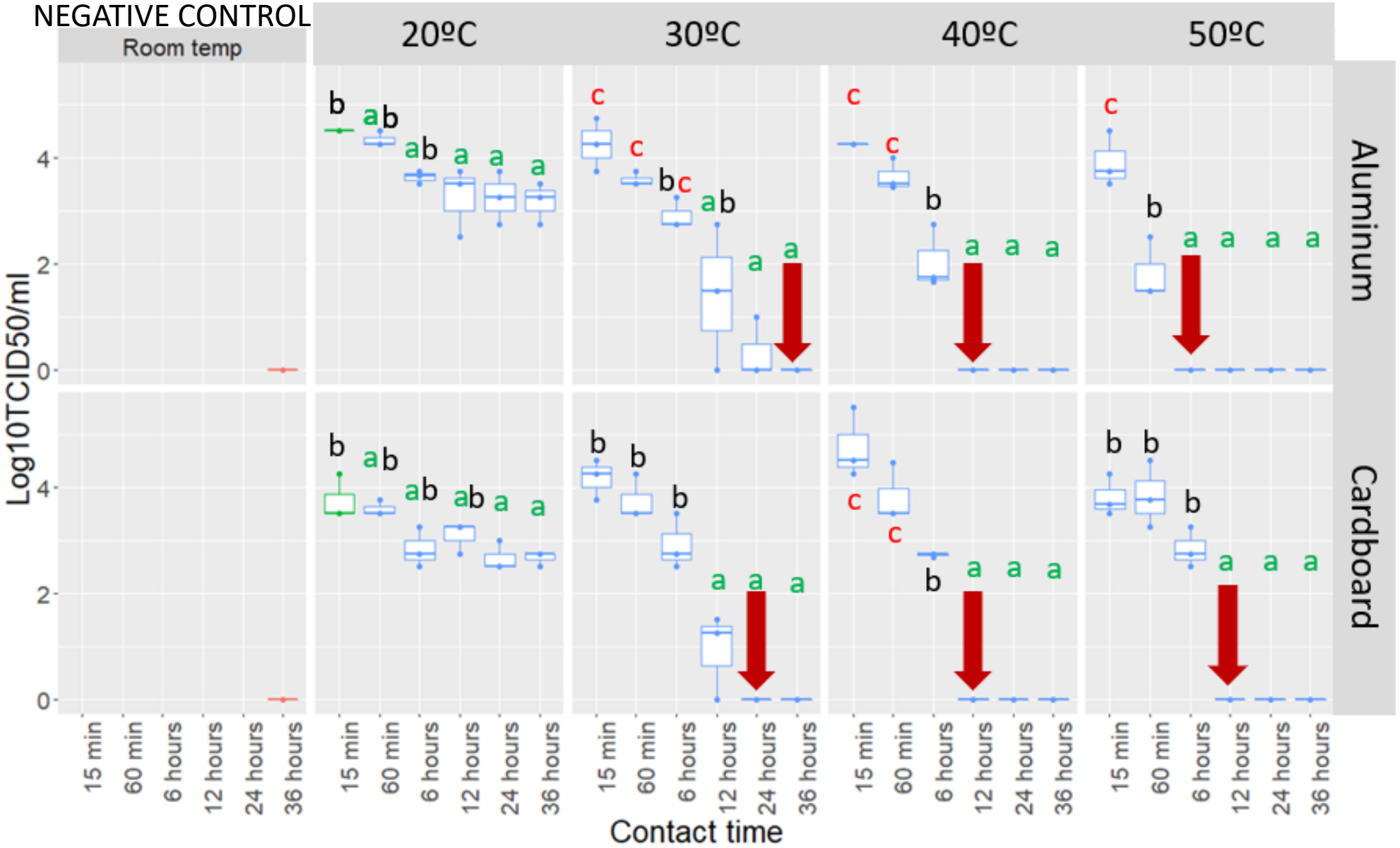
PRRSV MN 184



RESULTS

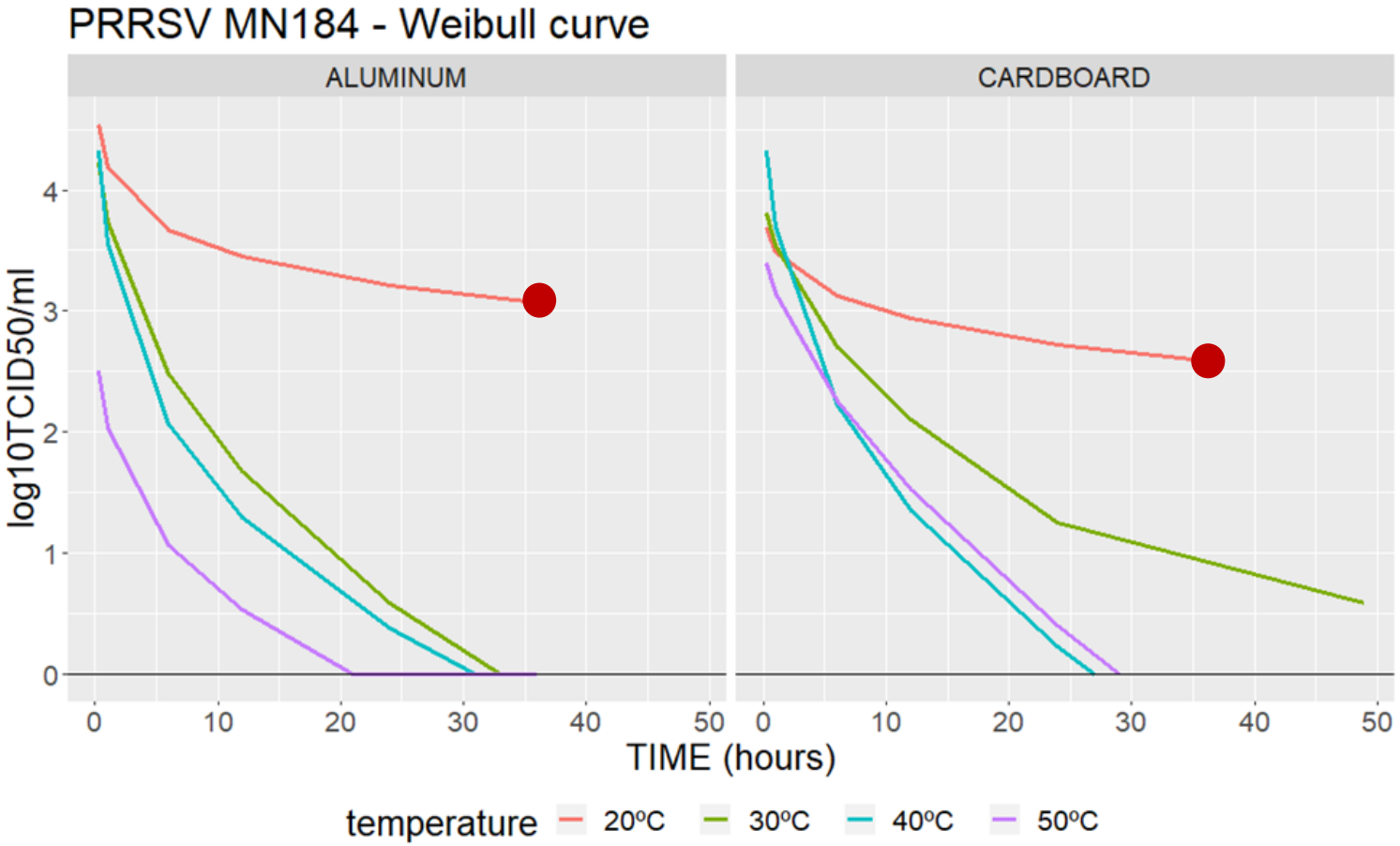
PRRSV MN 1-8-4

NEGATIVE CONTROL



RESULTS

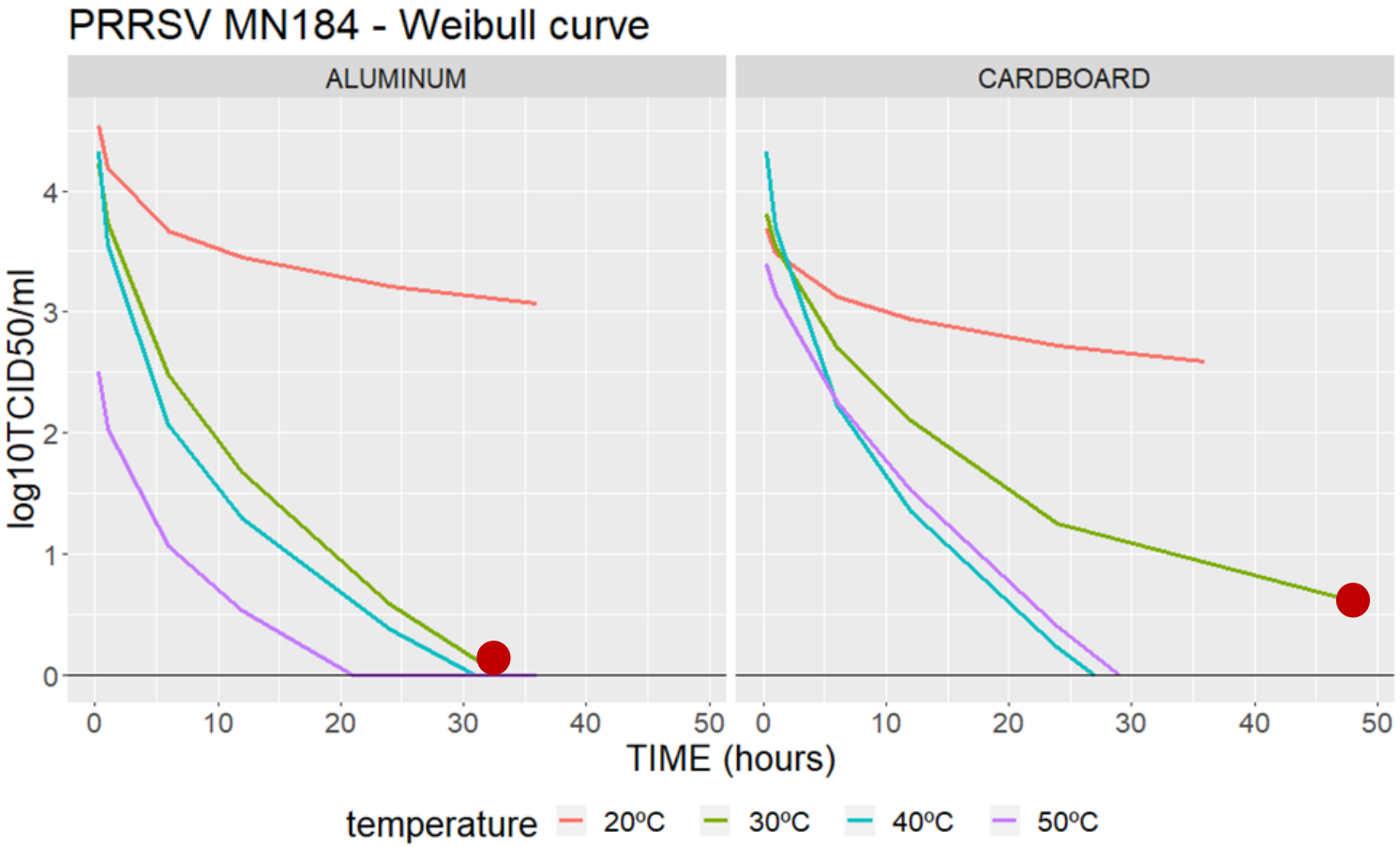
PRRSV MN 1-8-4



SURFACE	TEMPERATURE	INACTIVATION TIME
Aluminum	20ºC	-
	30ºC	32:40 hours
	40ºC	30:57 hours
	50ºC	21:06 hours
Cardboard	20ºC	-
	30ºC	48:48 hours
	40ºC	27:05 hours
	50ºC	29:04 hours

RESULTS

PRRSV MN 1-8-4



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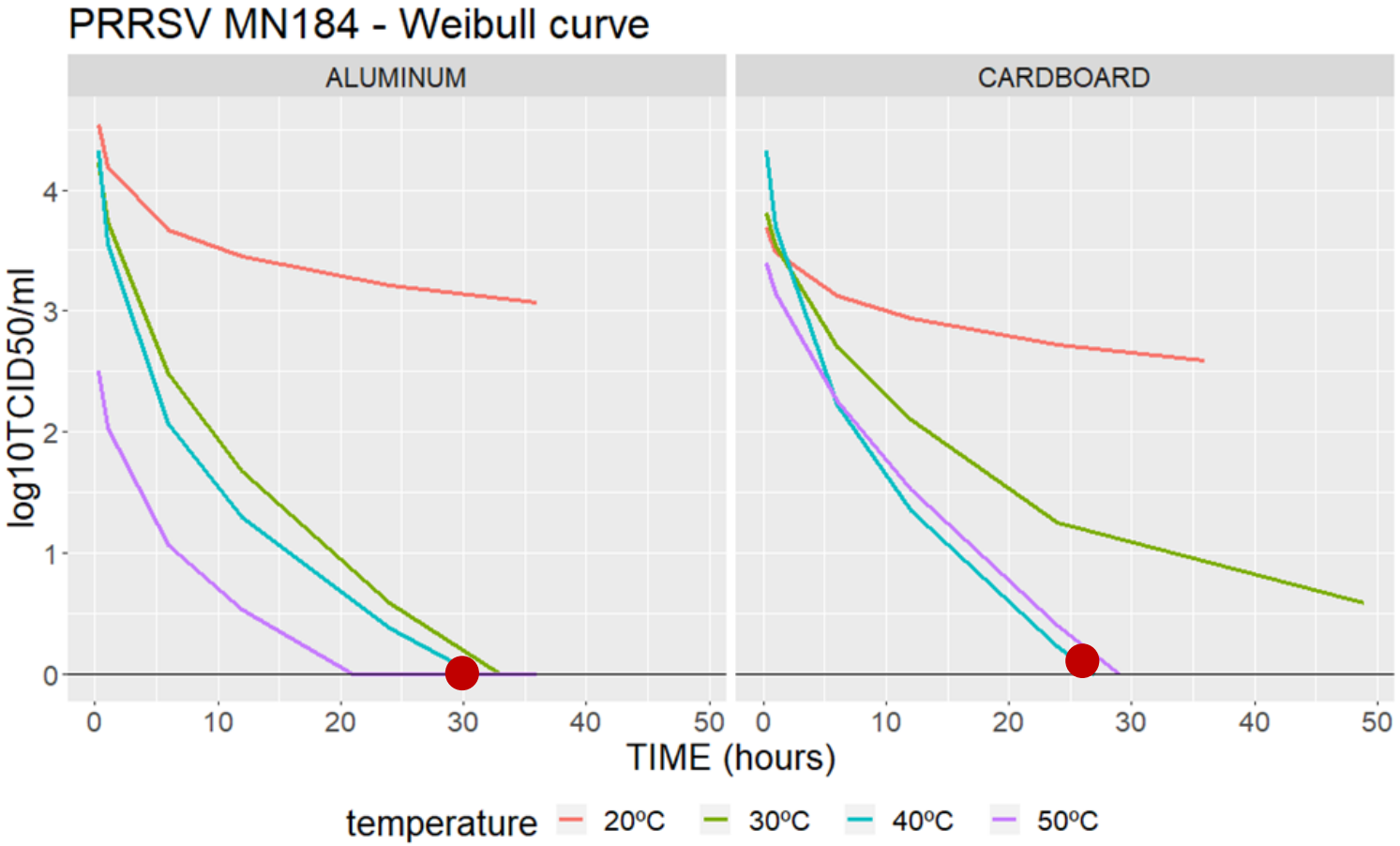

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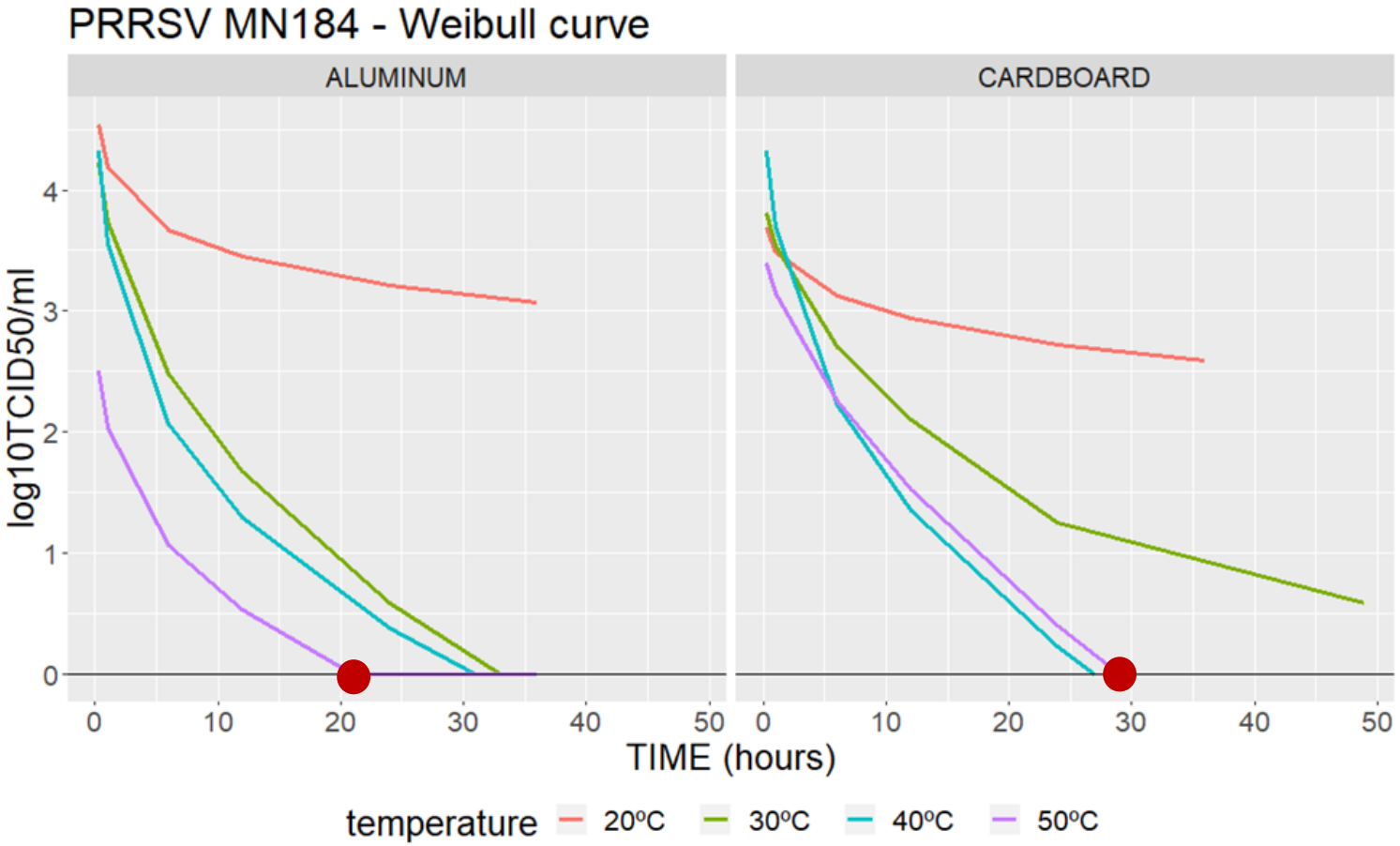

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	50ºC	29:04 hours

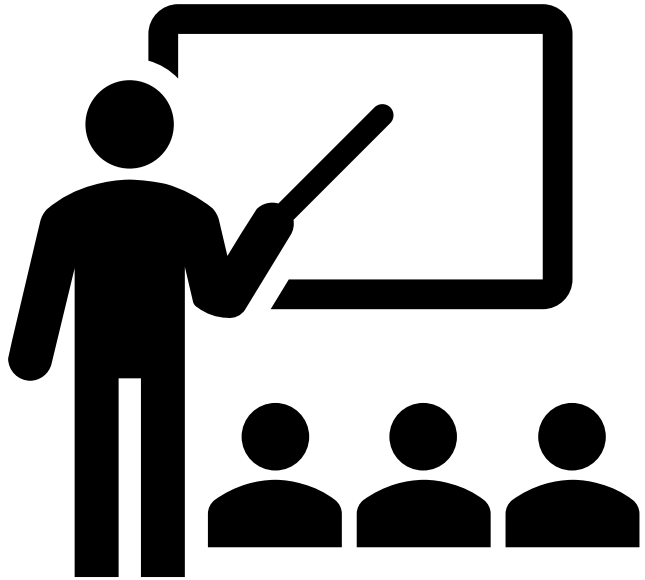
RESULTS

PRRSV MN 1-8-4

SURFACE	TEMPERATURE	HALF-LIFE (HOURS)
Aluminum	20ºC	8:29
	30ºC	7:13
	40ºC	7:13
	50ºC	7:51
Cardboard	20ºC	8:52
	30ºC	7:34
	40ºC	7:01
	50ºC	7:07

RESULTS

PEDV



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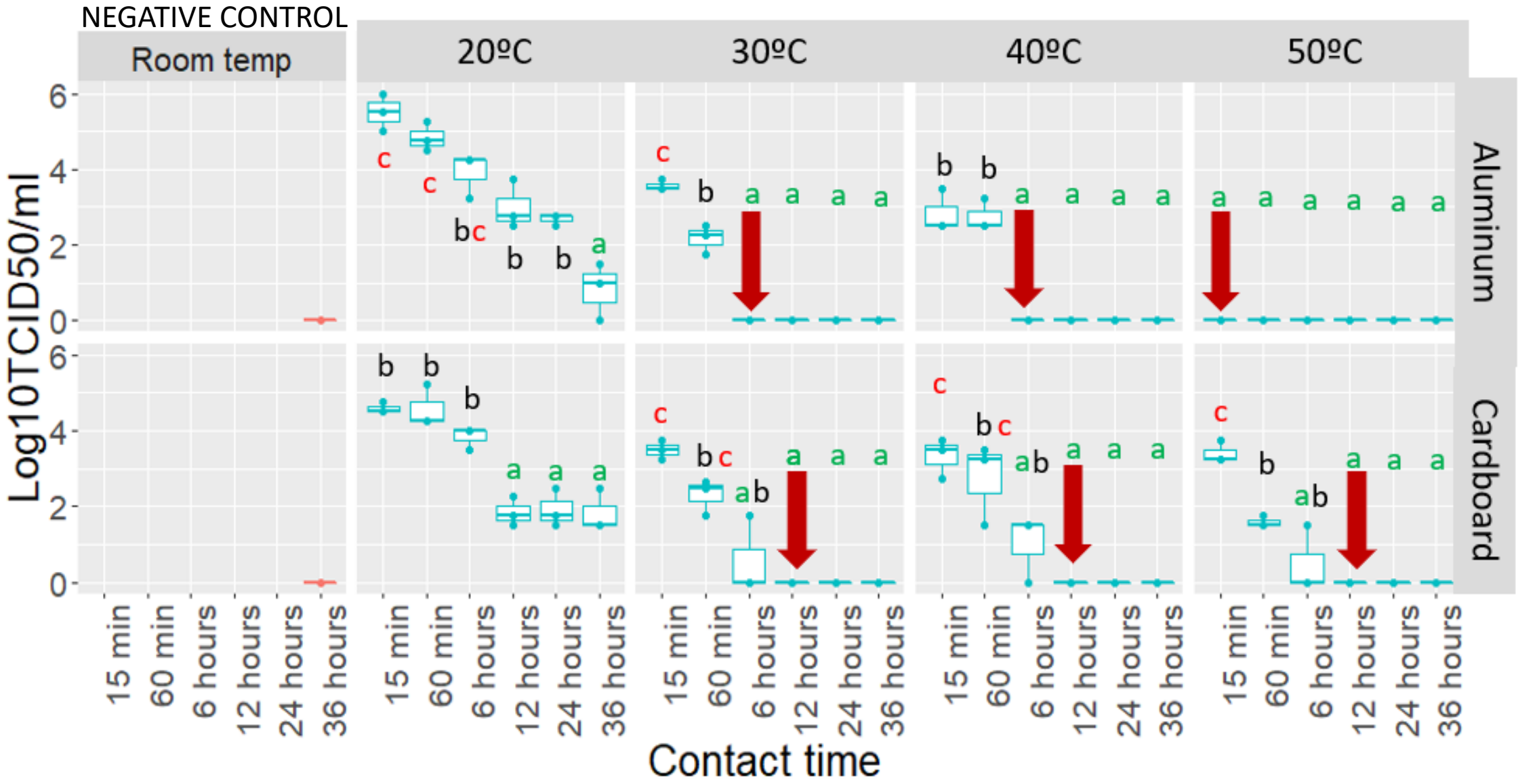

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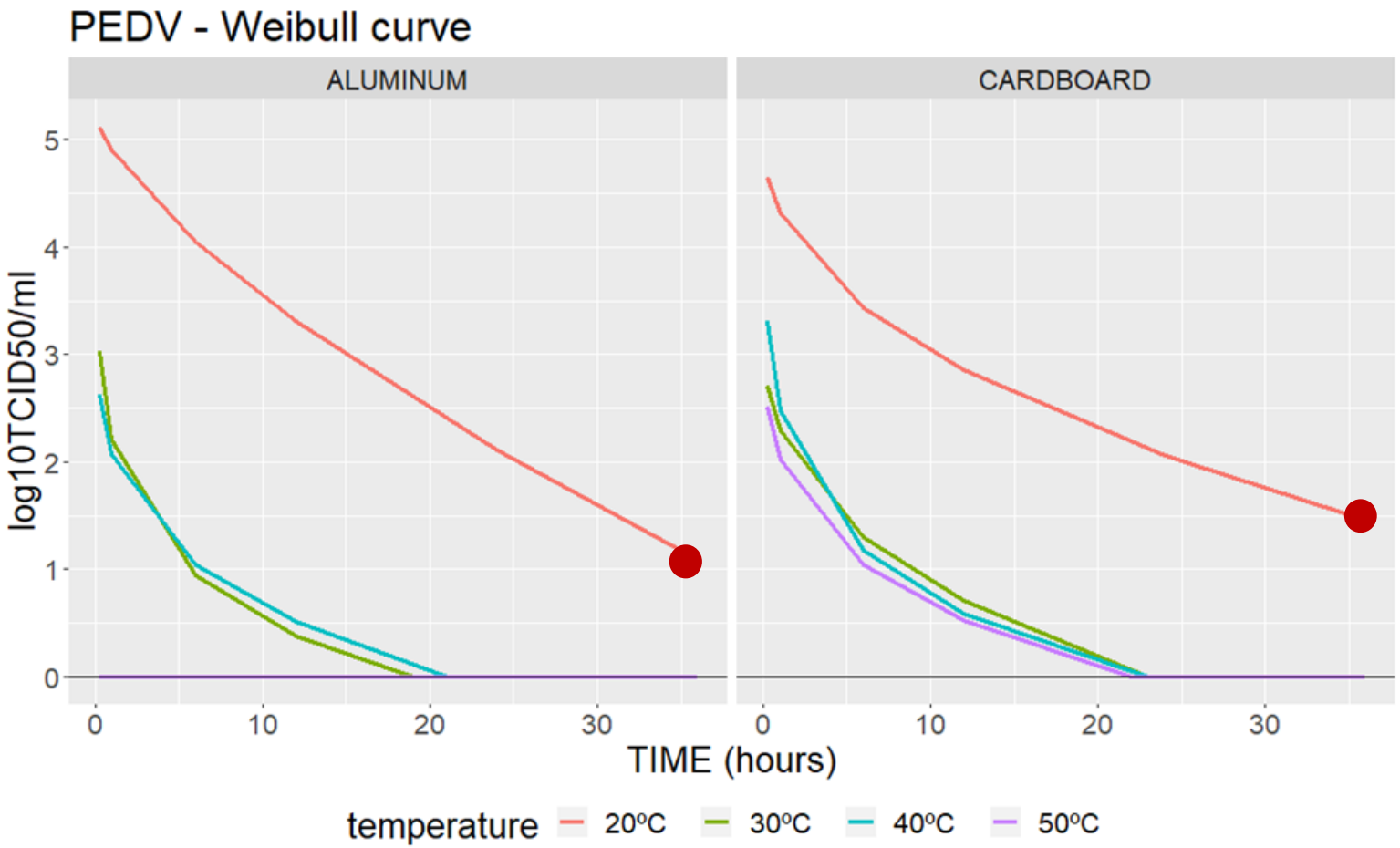

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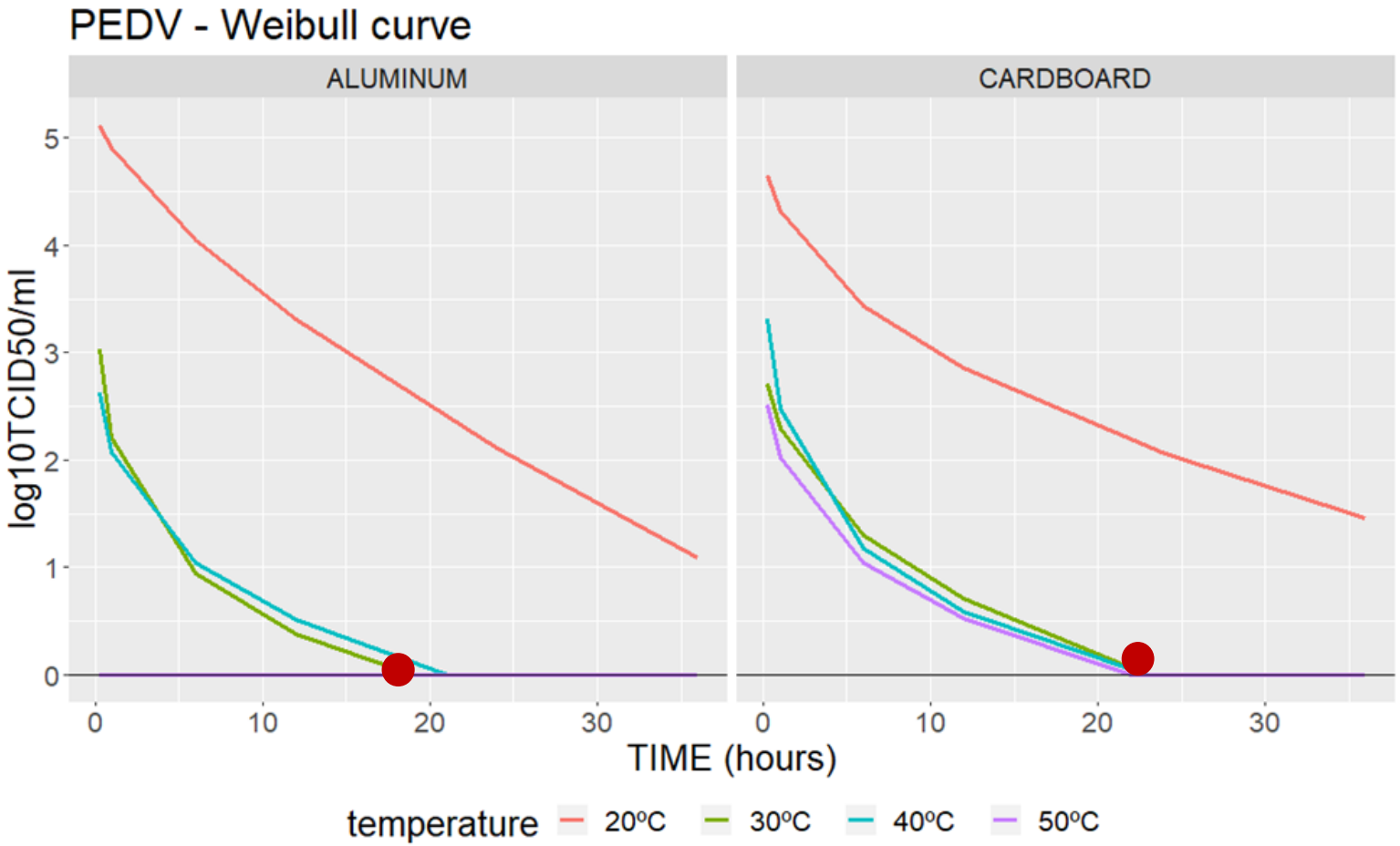
RESULTS

PEDV



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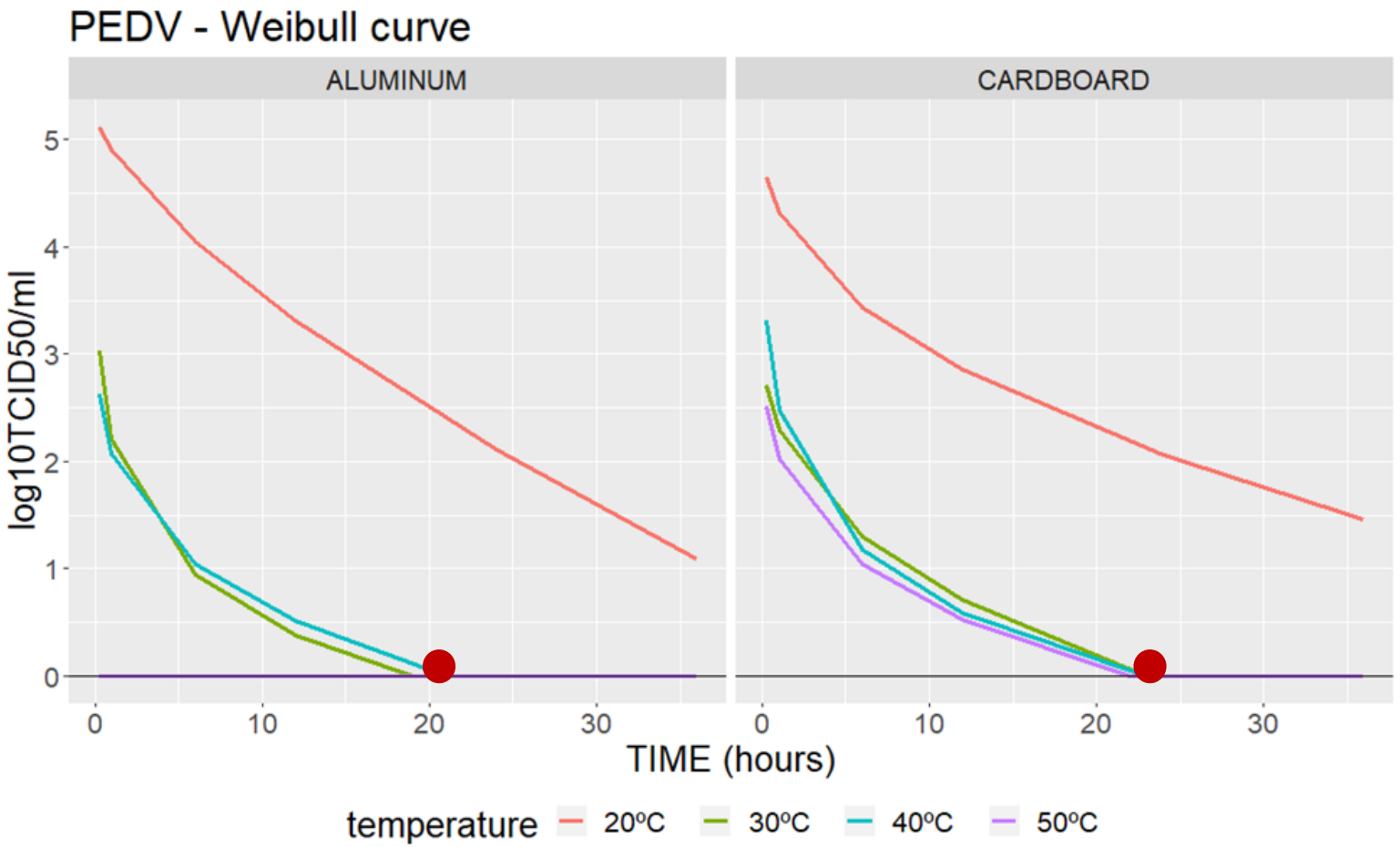
RESULTS

PEDV



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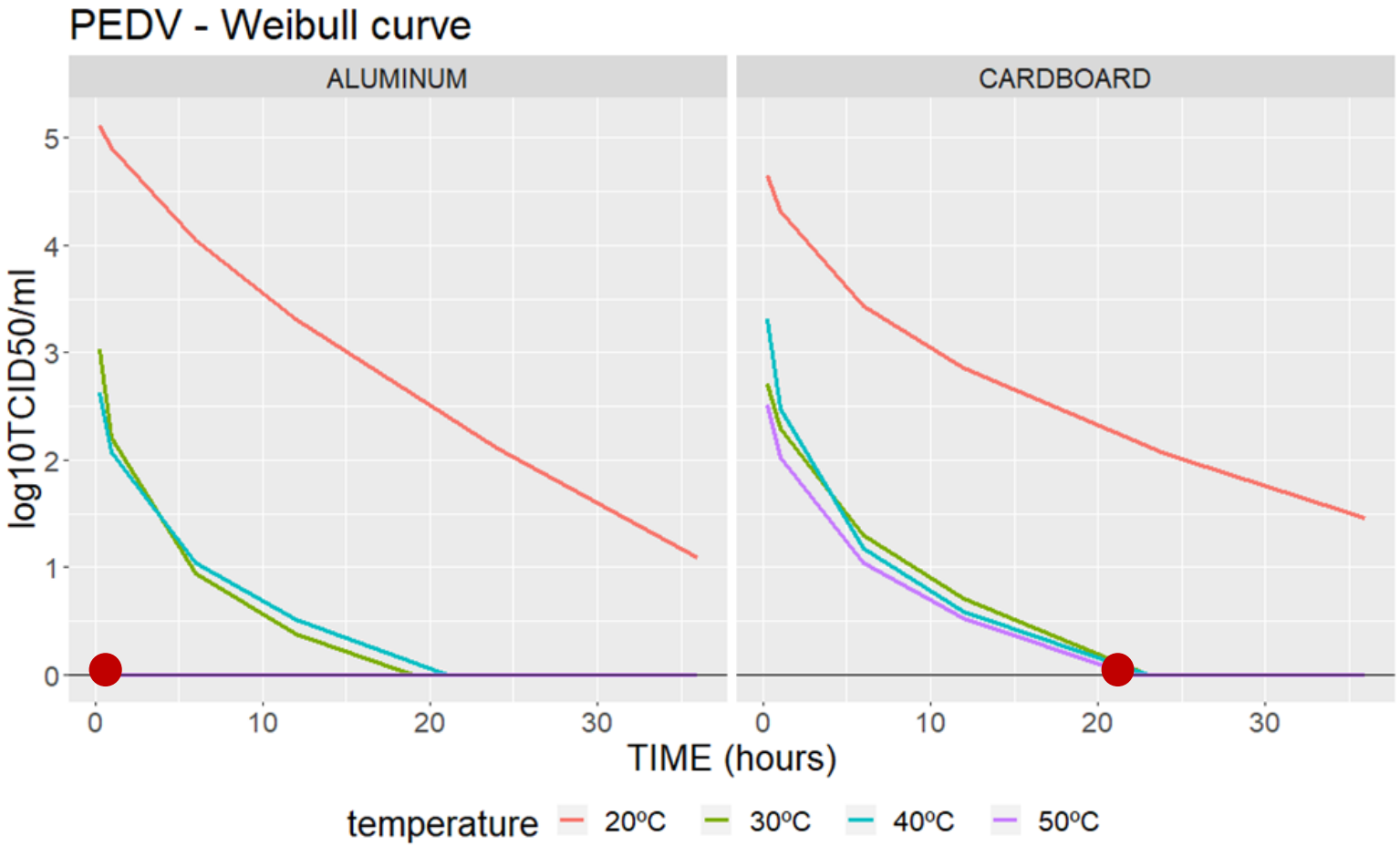
RESULTS

PEDV



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	50ºC	21:45 hours

RESULTS

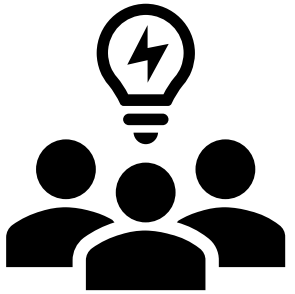
PEDV

SURFACE	TEMPERATURE	HALF-LIFE (HOURS)
Aluminum	20ºC	7:01
	30ºC	7:42
	40ºC	7:51
	50ºC	0.00
Cardboard	20ºC	7:34
	30ºC	7:34
	40ºC	7:34
	50ºC	7:51

DISCUSSION

- **UVC and Disinfectants vs Time/Temperature**: decreases the probability of virus introduction. Heat is conducted through the material, reaches every surface, porous or not.

- **Half-life:** PRRSV half-life in solution was 84.5 hours at 10ºC, **27.4 hours at 20ºC and 1.6 hours at 30ºC** (Jacobs et al. 2010). According to this study, it is advisable to maintain surfaces for at least **10 hours above 20ºC.**



LIMITATIONS

- Virus viability was not tested with a bioassay
- Surfaces with organic matter were not tested
- Trial was implemented on a controlled environment: temperature may disperse differently in field conditions

CONCLUSION

- After **15 minutes and 60 minutes** there was only evidence of virus **inactivation at 50ºC in aluminum** surfaces for PEDV and PRRSV 144 L1C variant.

- Surfaces should be maintained for at least **10 hours above 20ºC** to reduce the virus by 50%

- Using combinations of time and temperature is a useful method to inactivate virus from surfaces in field conditions

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