

Part 2

***In vitro* effect of DON on porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus (PCV-2)**

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Biological Effects of DON on Swine

- Protein synthesis inhibitors (Pestka et al. 2004)
 - ✓ Binds to ribosomes → "Ribotoxic stress response"
- Leucocytes are very sensitive to DON
 - ✓ Macrophages, monocytes, B and T cells
- DON is both immunostimulatory or immunosuppressive (Oswald et al. 2005)
 - ✓ Dose and duration of exposure
 - ✓ Low concentrations: Induce production of proinflammatory cytokines
 - ✓ High concentration: Apoptosis (immunosuppression)

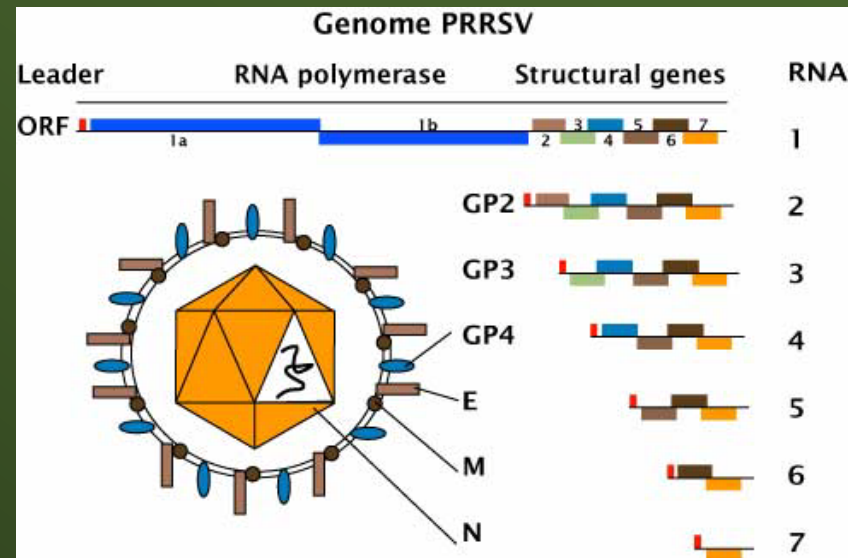


PRRSV

Porcine Reproductive and Respiratory Syndrome Virus

- Important viral disease
 - ✓ Cause significant production and economic losses
- Reproductive disorders
 - ✓ Late-term abortions, increased numbers of stillborn fetuses, and/or premature weak pigs
- Respiratory problems
 - ✓ Coughing and dyspnea
 - ✓ More severe in piglets

PRRS Virus



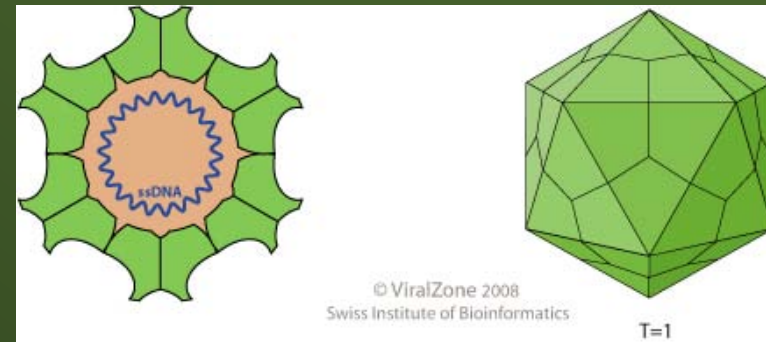
<http://www.porcilis-prrs.com/microbiology-prrsv-structure.asp>



PCV2

- Important viral disease
 - ✓ Cause significant production and economic losses
- Porcine circovirus associated diseases (PCVAD)
 - ✓ Wasting, lymphadenopathy, jaundice, diarrhea and reproductive failures
- PCV2 principal causative agent
 - ✓ Promoted by various infectious agent

Porcine Circovirus Type 2

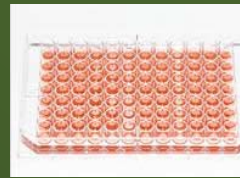


Objectives

- Knowing the effect of DON on immune system
- Determine *in vitro* effect of DON, on the important porcine viral infections
 - ✓ Porcine reproductive and respiratory syndrome virus
 - ✓ Porcine circovirus



Methods



Cell Model

MARC-145, PAM (PRRSV)
NPT_r (PCV2)

Noninfected

PRRSV or PCV2
infected

Increasing concentrations of DON:
0, 70, 140, 280, 560, 1200 ng/ml

Incubation
72 hr

Incubation
72 hr

Cell Viability:

- 1- Cell titer (Promega)
- 2- LDH

Virus Titration:

- 1- Immunofluorescence
- 2- qPCR



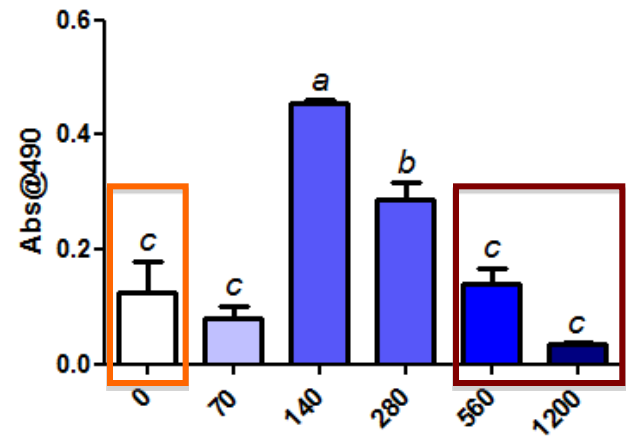
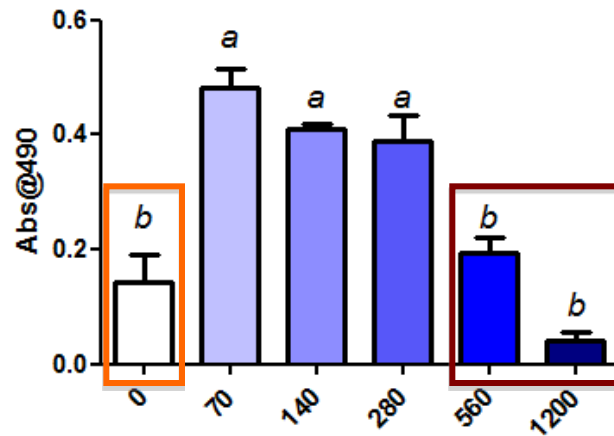
PRRSV: Cell Metabolism

Cell titer

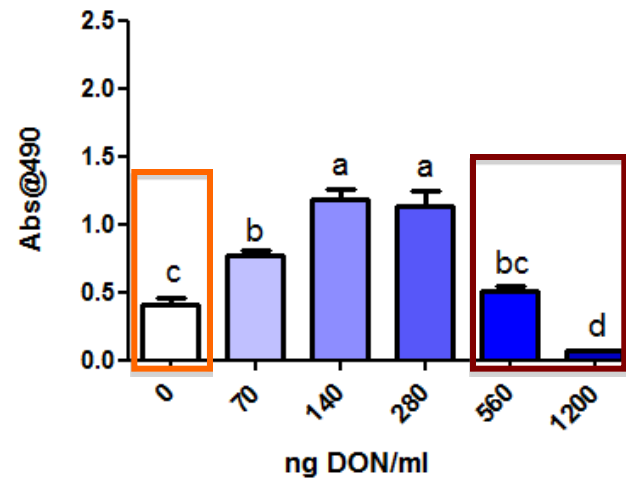
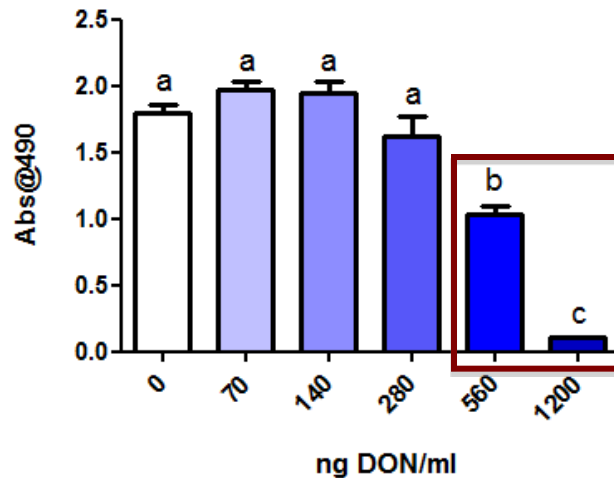
Noninfected

infected

Marc-145



PAMs



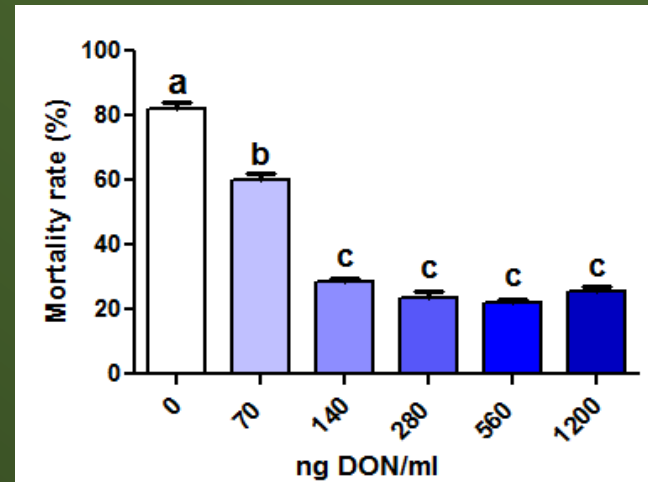
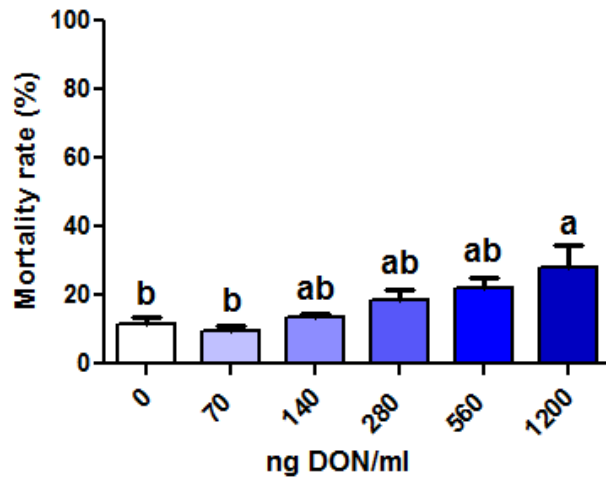
PRRSV: Cell Mortality

LDH release

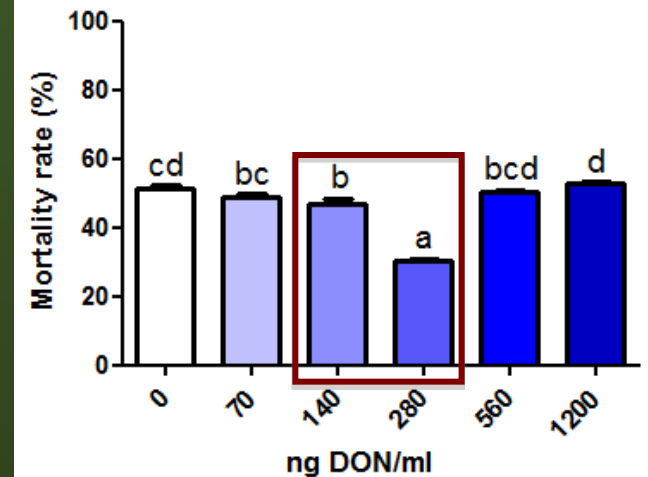
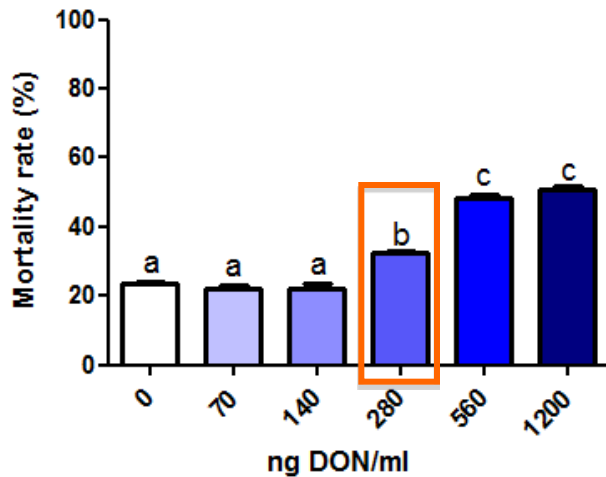
Noninfected

Infected

Marc-145



PAMs



PRRSV: Immunofluorescence

MARC-145

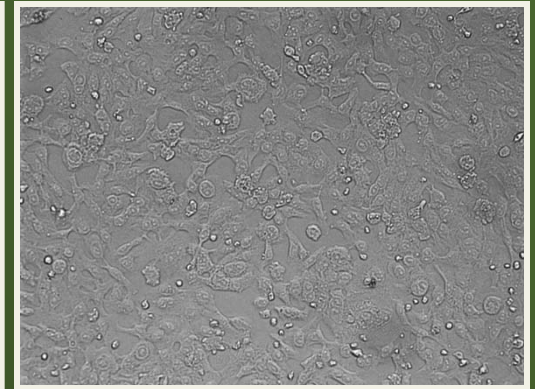
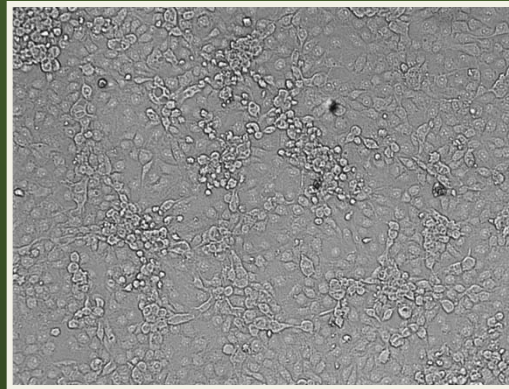
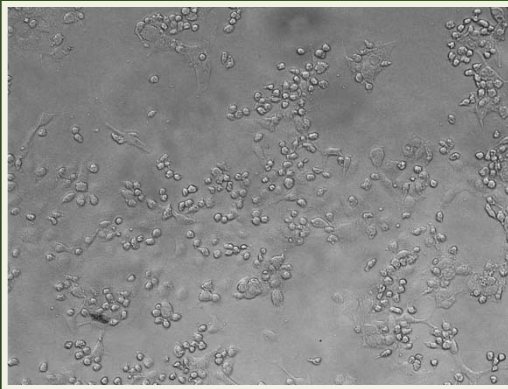
ng/ml DON

0

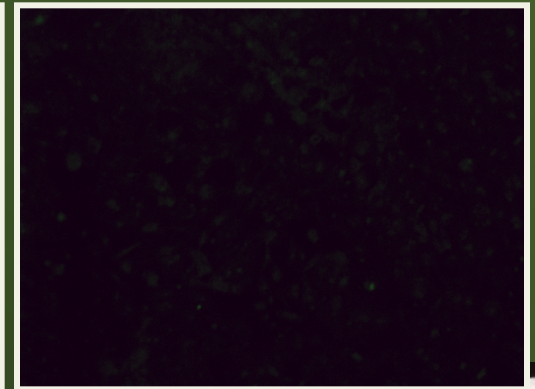
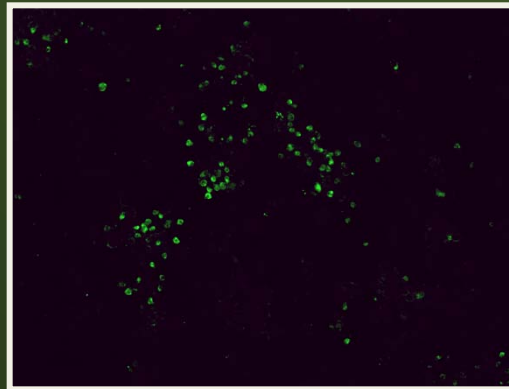
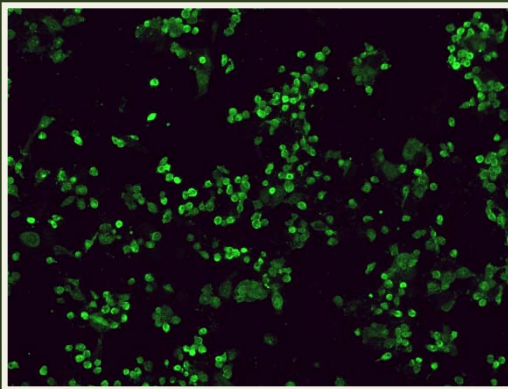
140

1200

72 hr

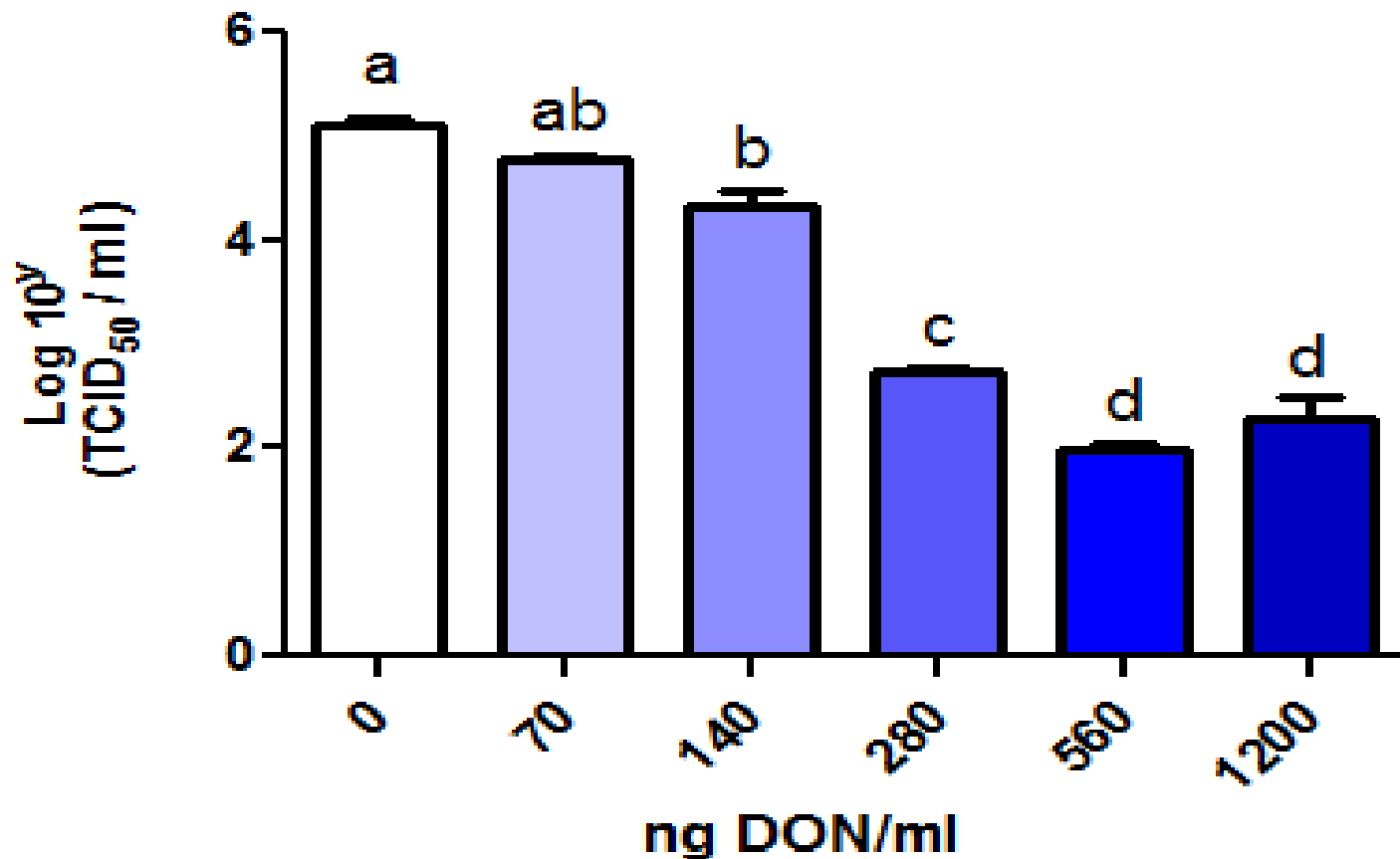


72 hr



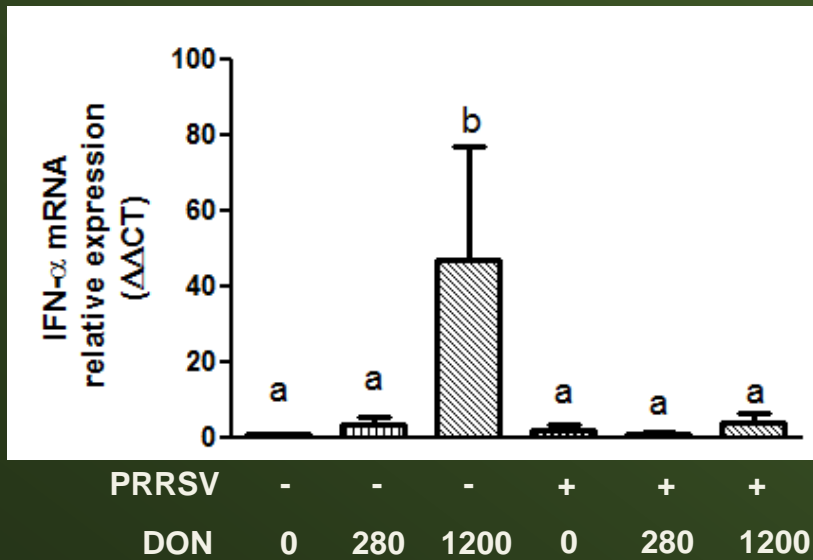
PRRSV: Virus Titration (qPCR)

PAM Cells

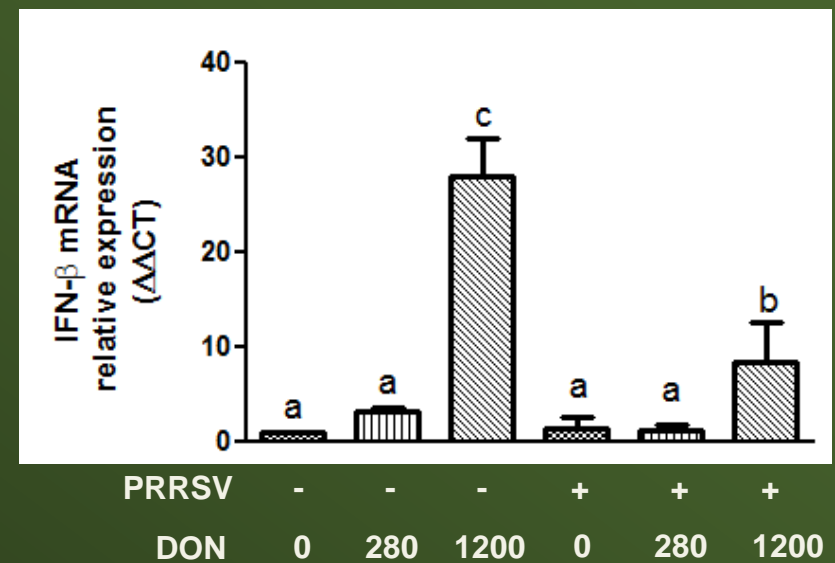


mRNA relative expression (qPCR)

IFN- α



IFN- β

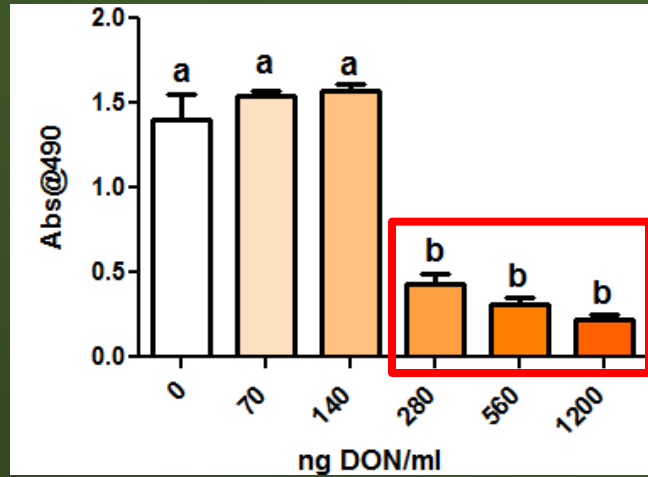


PCV2: Cell Metabolism

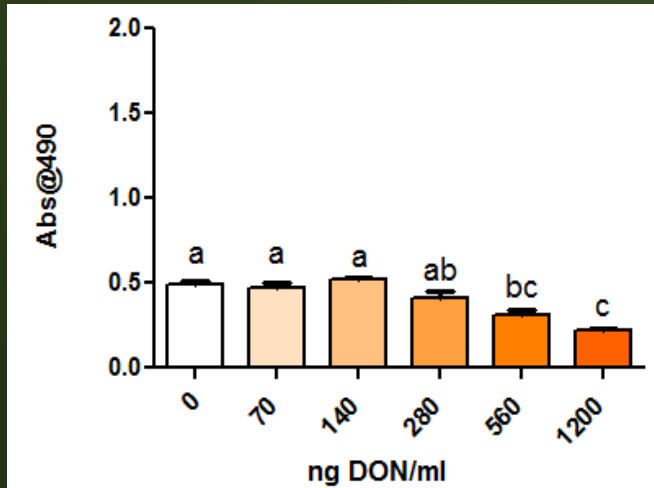
Cell titer

NPTr

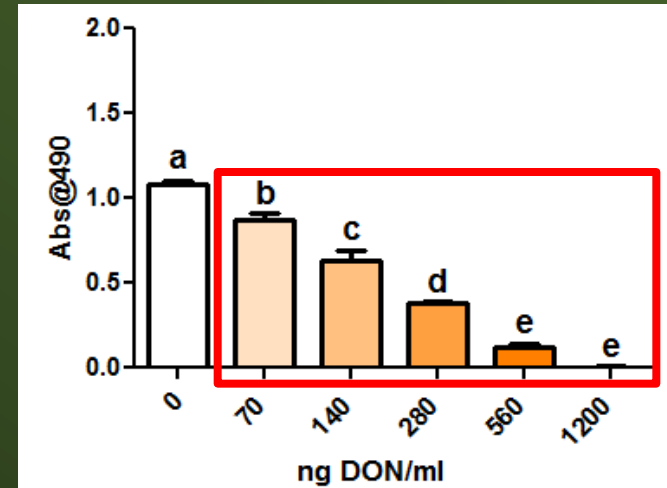
Non-infected



PCV2a infected



PCV2b infected

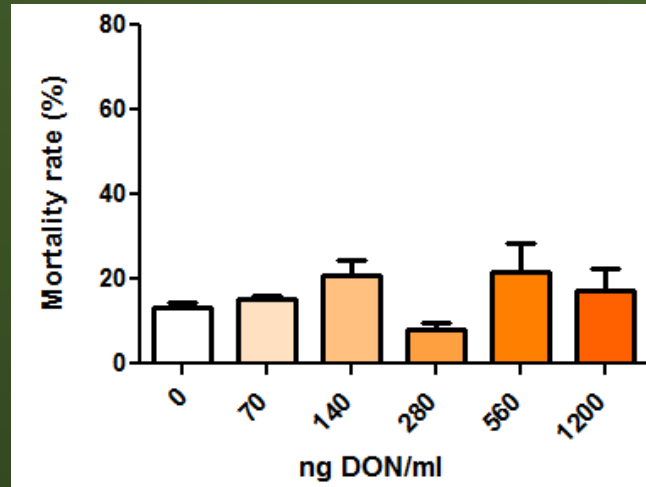


PCV2: Cell Mortality

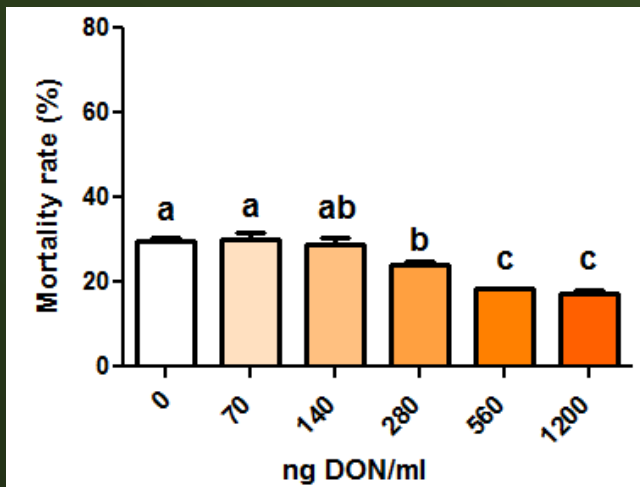
LDH release

NPT_r

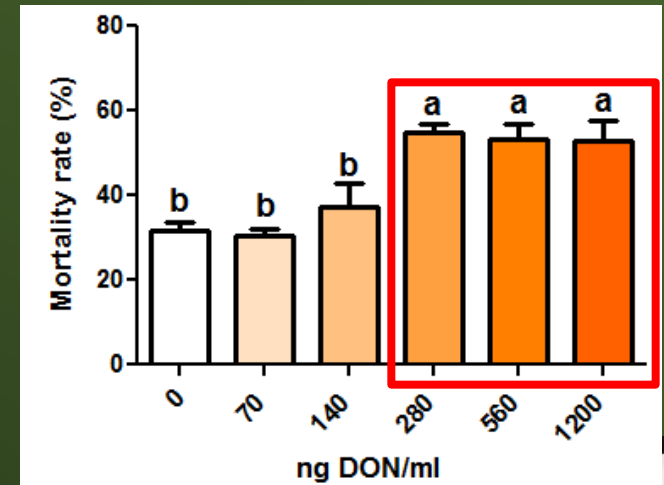
Noninfected



PCV2a infected



PCV2b infected



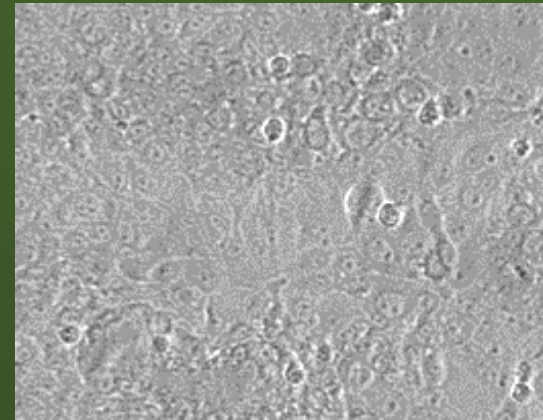
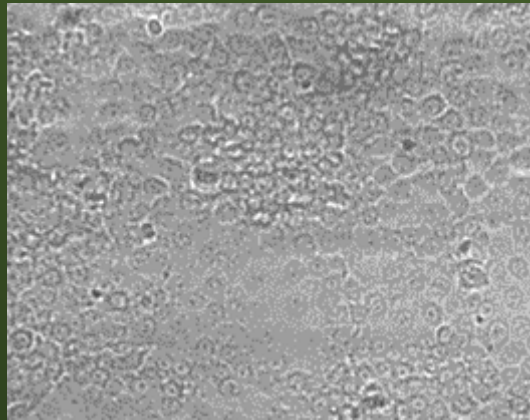
PCV2b : Immunofluorescence

ng/ml DON

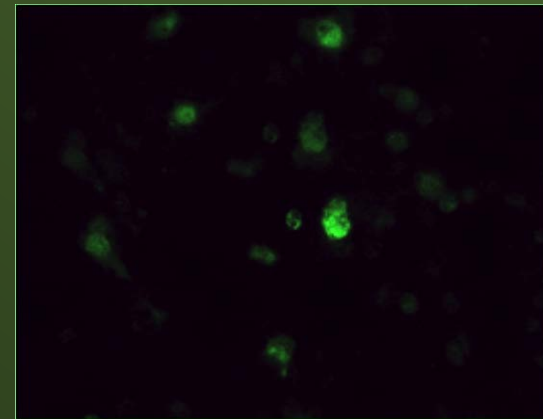
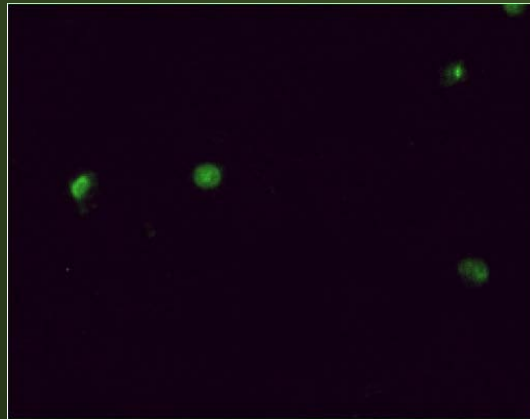
0

140

72 h

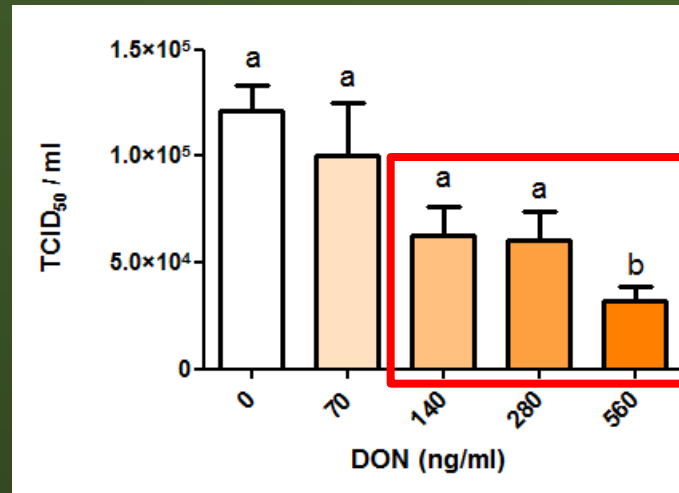


72 h

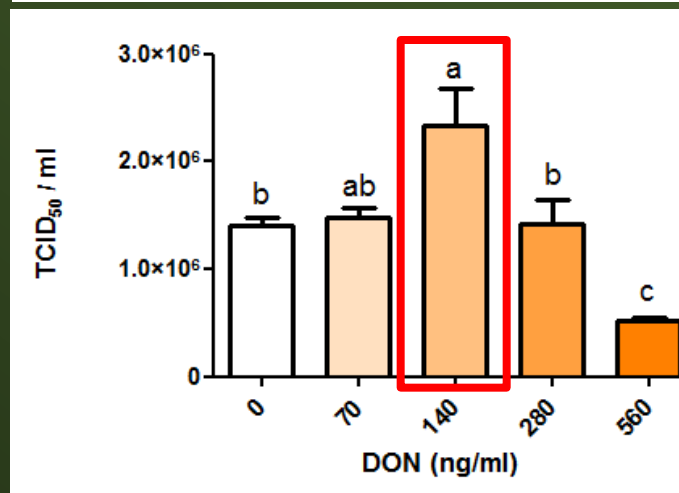


PCV2 : Virus Titration (qPCR)

PCV2a

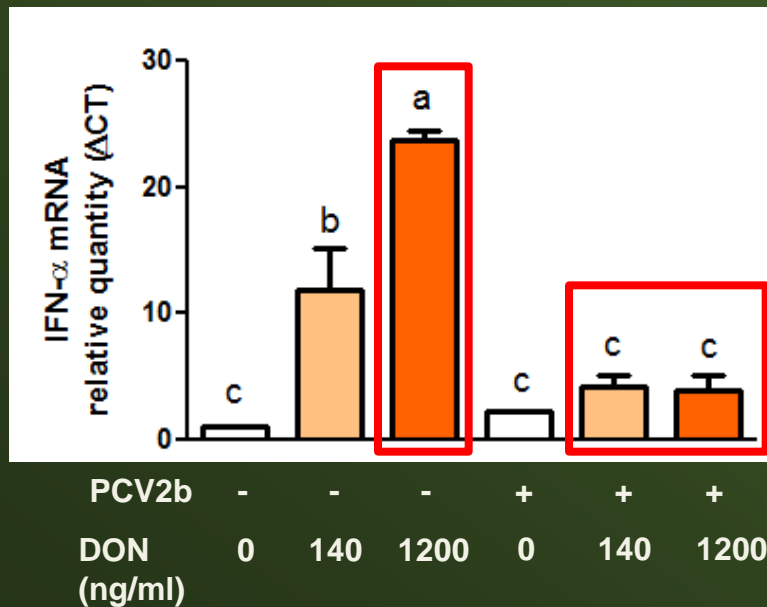


PCV2b

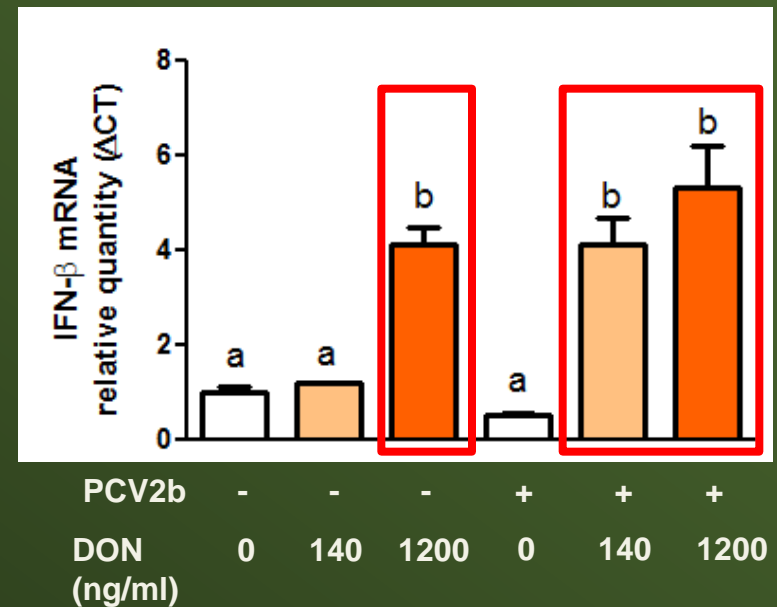


Relative expression mRNA (qPCR)

IFN- α



IFN- β



Conclusion – Part 2

- DON had a significant *in vitro* impact on PRRSV and PCV2 viral infection

	PRRSV	PCV2a	PCV2b
Cell survival	↑	-	↓
Cell mortality	↓	↓	↑
Virus	↓	↓	↑

- *In vivo* experiments are scheduled to determine the impact of DON naturally contaminated feed on PRRSV and PCV2b infection



Acknowledgment

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