Investigation of Possible Cross-Neutralization Between Crimean-Congo Hemorrhagic Fever Virus and Hazara Virus

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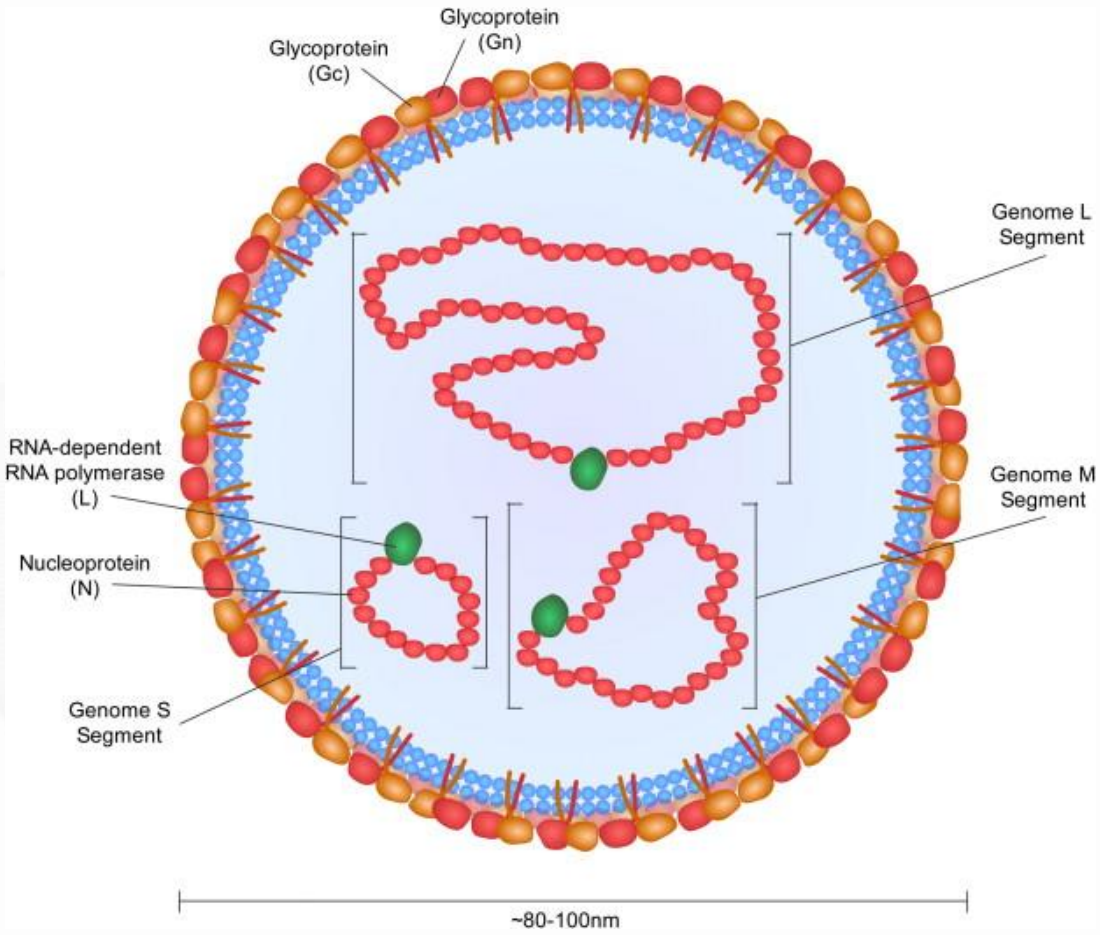
Introduction

Crimean-Congo Hemorrhagic Fever Virus

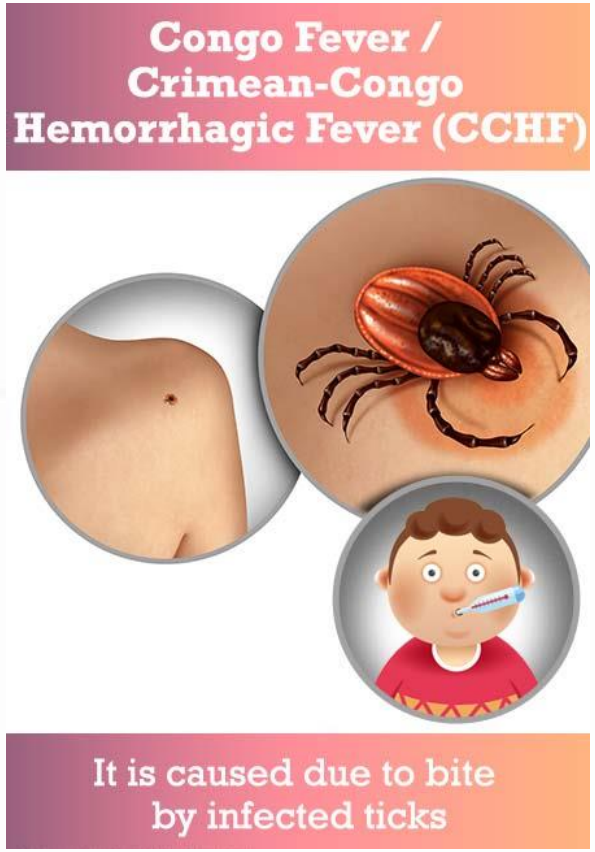
- Agent of CCHF
- Most common tick-born human pathogen virus
- Enveloped, (-) sense, ssRNA
- Vector: Ticks, primarily from *Hyalomma* genus



Introduction

Crimean-Congo Hemorrhagic Fever Disease

- Endemic in Turkey
- Incubation period: 1-13 days
- Prehemorrhagic phase:
  - Fever, headache, myalgia, nausea
- Hemorrhagic phase:
  - Petechiae, ecchimosis
  - Nose and gum bleeding
  - Pulmonary, intraabdominal, etc.
  - Excessive proinflammatory cytokine release
  - Increased vascular permeability, shock, death



Introduction

NO radical pharmaceutic approaches available
NO vaccine is commercially available

Life cycle and pathophysiology is not fully understood

CCHFV should be studied under BSL-4 conditions!

Introduction

Hazara Virus As A Surrogate Model Candidate

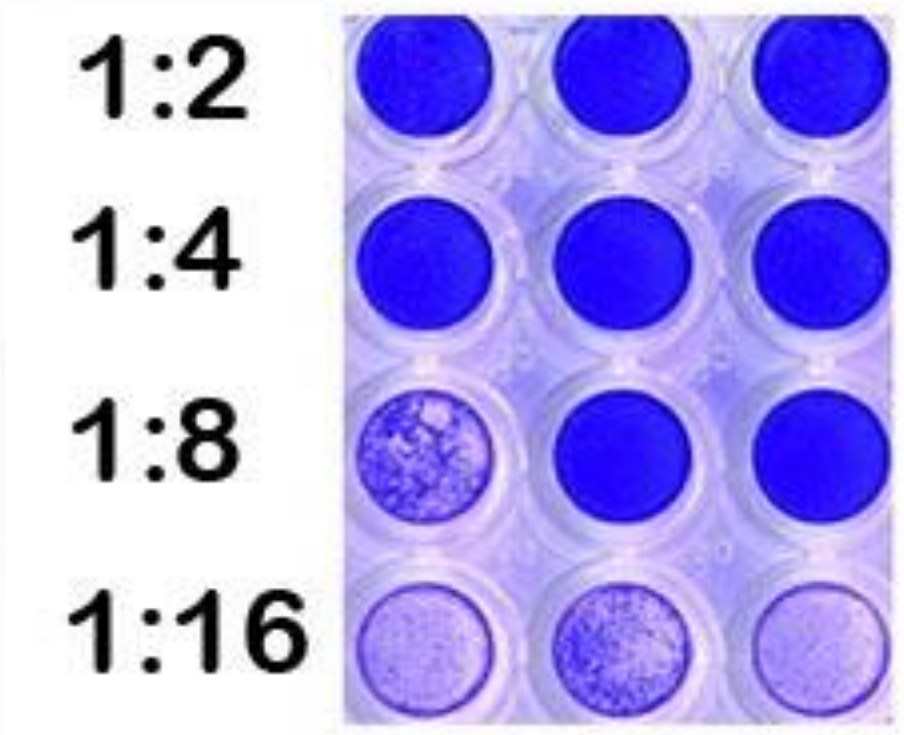
- In the same serogroup with CCHFV
- Can be studied in BSL-2
- Does not cause disease in humans

Similarities between HAZV and CCHFV must be shown!

Introduction

What Do We Suggest?

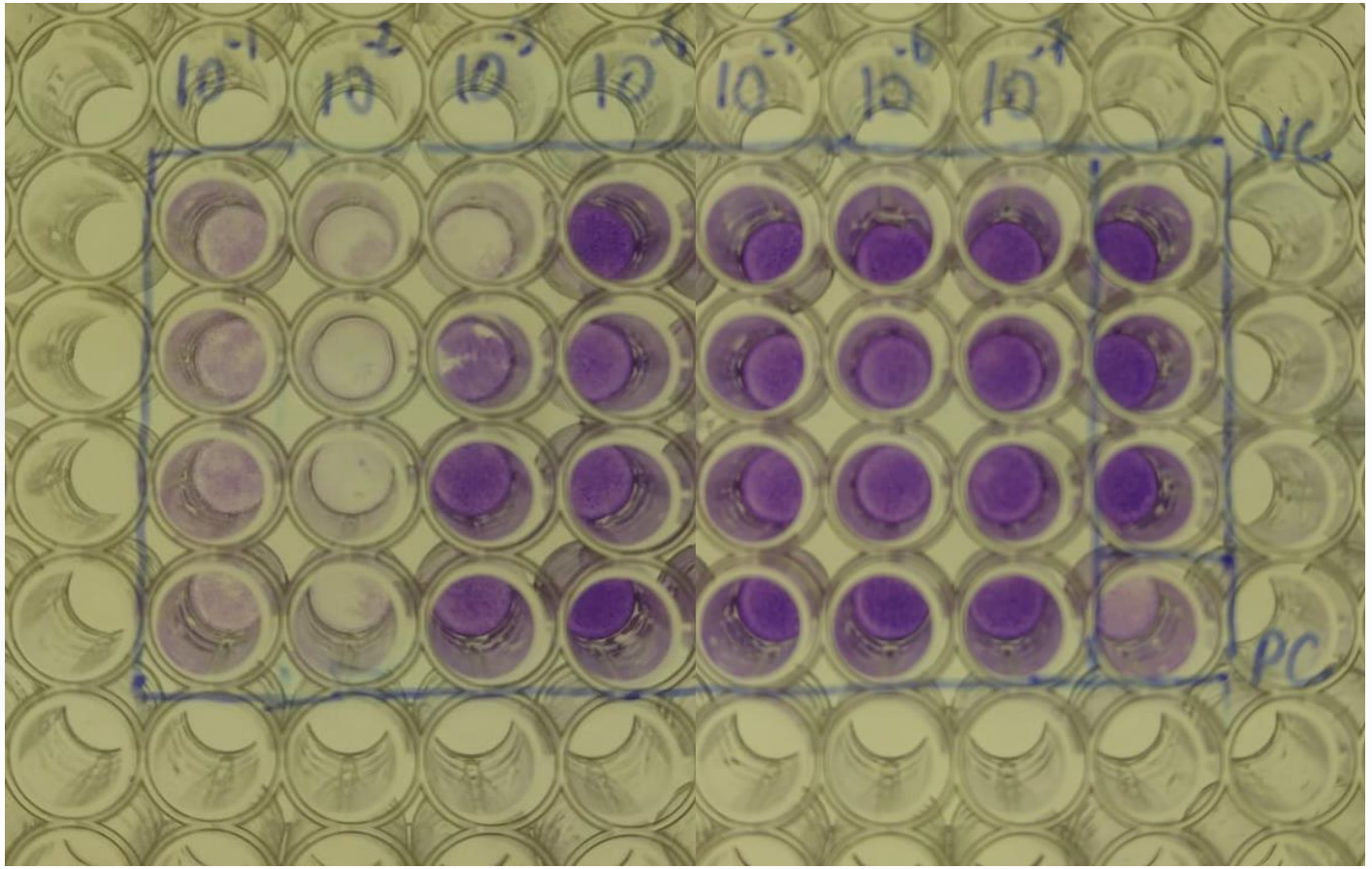
- Virus neutralization test using CCHFV-immunized sera and HAZV
- Positive result would mean:
  - External glycoproteins of CCHFV and HAZV are similar to each other
  - HAZV can be used as a vaccine against CCHFV



Methods

Tissue Culture Infectious Dose$_{50}$ Assay (TCID$_{50}$)

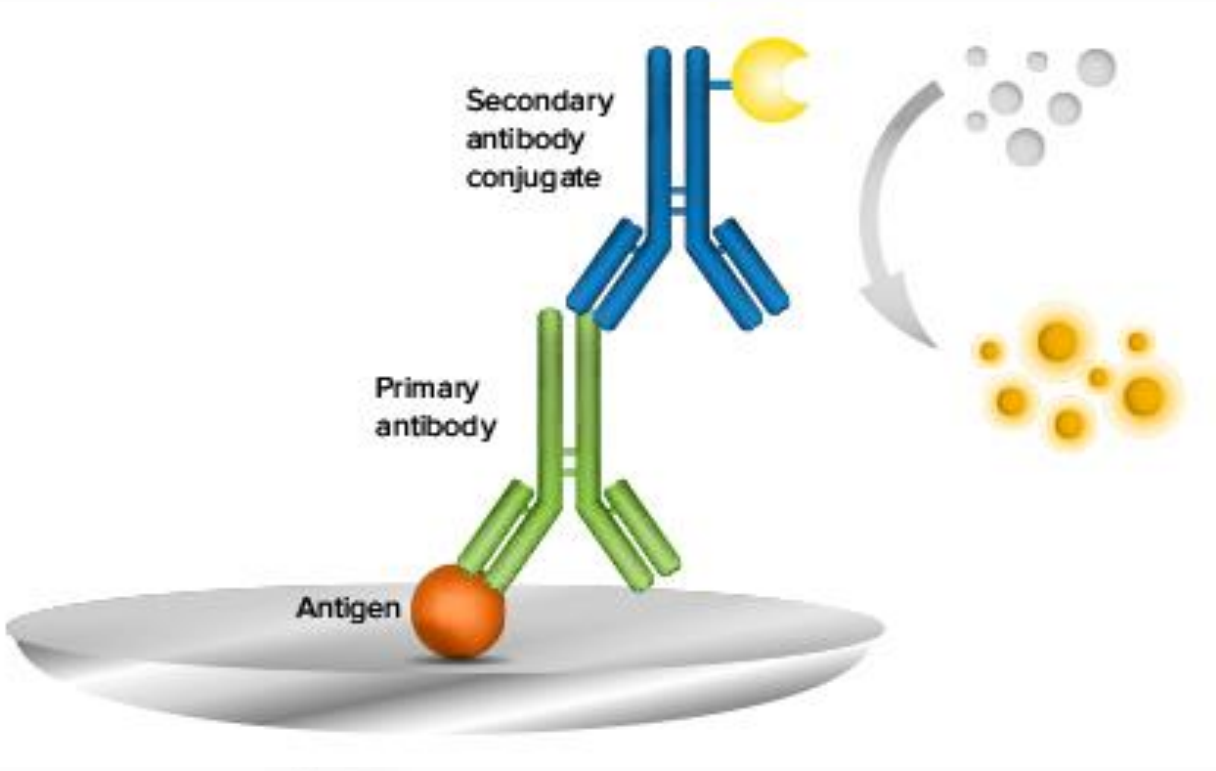
- Cell line: SW13
- 3000 cells/well
- 10-fold serial dilutions of the virus stock
- Cells were infected by the virus dilutions
- 6 days of incubation
- Fixation: 3,7% formaldehyde
- Staining: Crystal violet
- Virus titer was calculated using Spearman-Karber algorithm



Methods

In-house ELISA

- Coating: Purified HAZV
- Wells were incubated using mouse serum dilutions in 5% skim milk
- Secondary antibody: HRP-conjugated anti-mouse IgG
- Cut-off value calculation: mean NC OD value + 2 SD



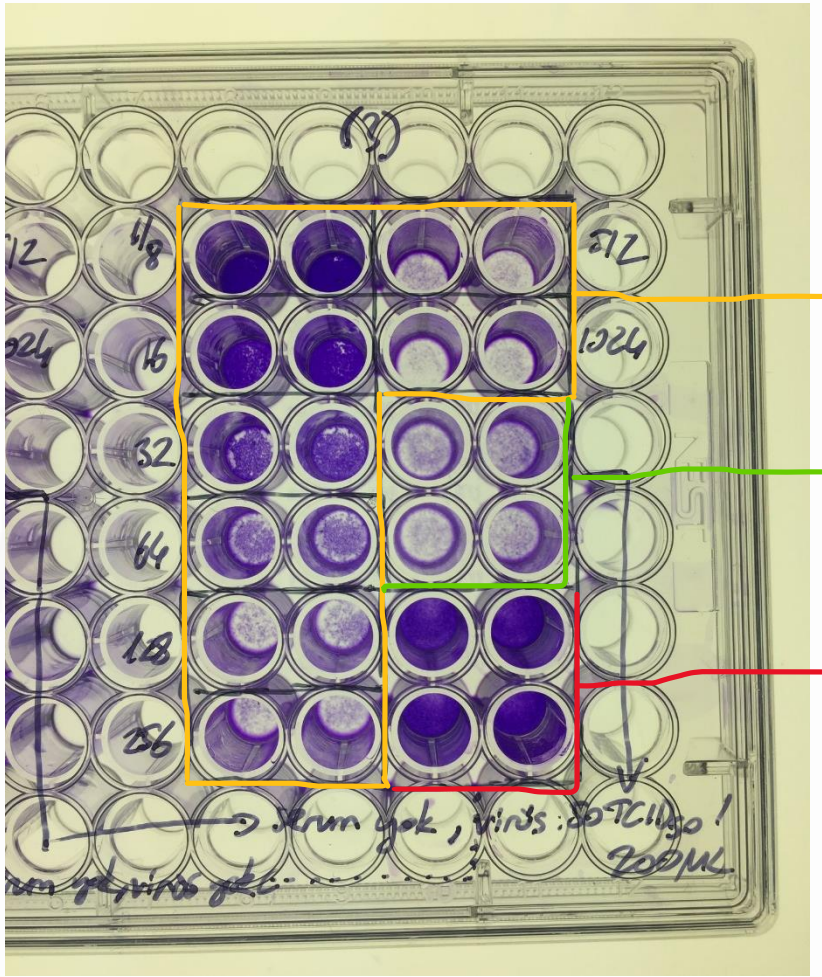
Methods – In-house ELISA

	0,5 ug/100mL HAZV						1 ug/100mL HAZV					
	1 (1/50)	2 (1/100)	3 (1/500)	4 (1/50)	5 (1/100)	6 (1/500)	7 (1/50)	8 (1/100)	9 (1/500)	10 (1/50)	11 (1/100)	12 (1/500)
A	Mouse 1			Mouse 5			Mouse 1			Mouse 5		
B												
C	Mouse 2			PC 1			Mouse 2			PC 1		
D												
E	Mouse 3			PC 2			Mouse 3			PC 2		
F												
G	Mouse 4			NC			Mouse 4			NC		
H												

Methods

Microvirus Neutralization Test (mVNT)

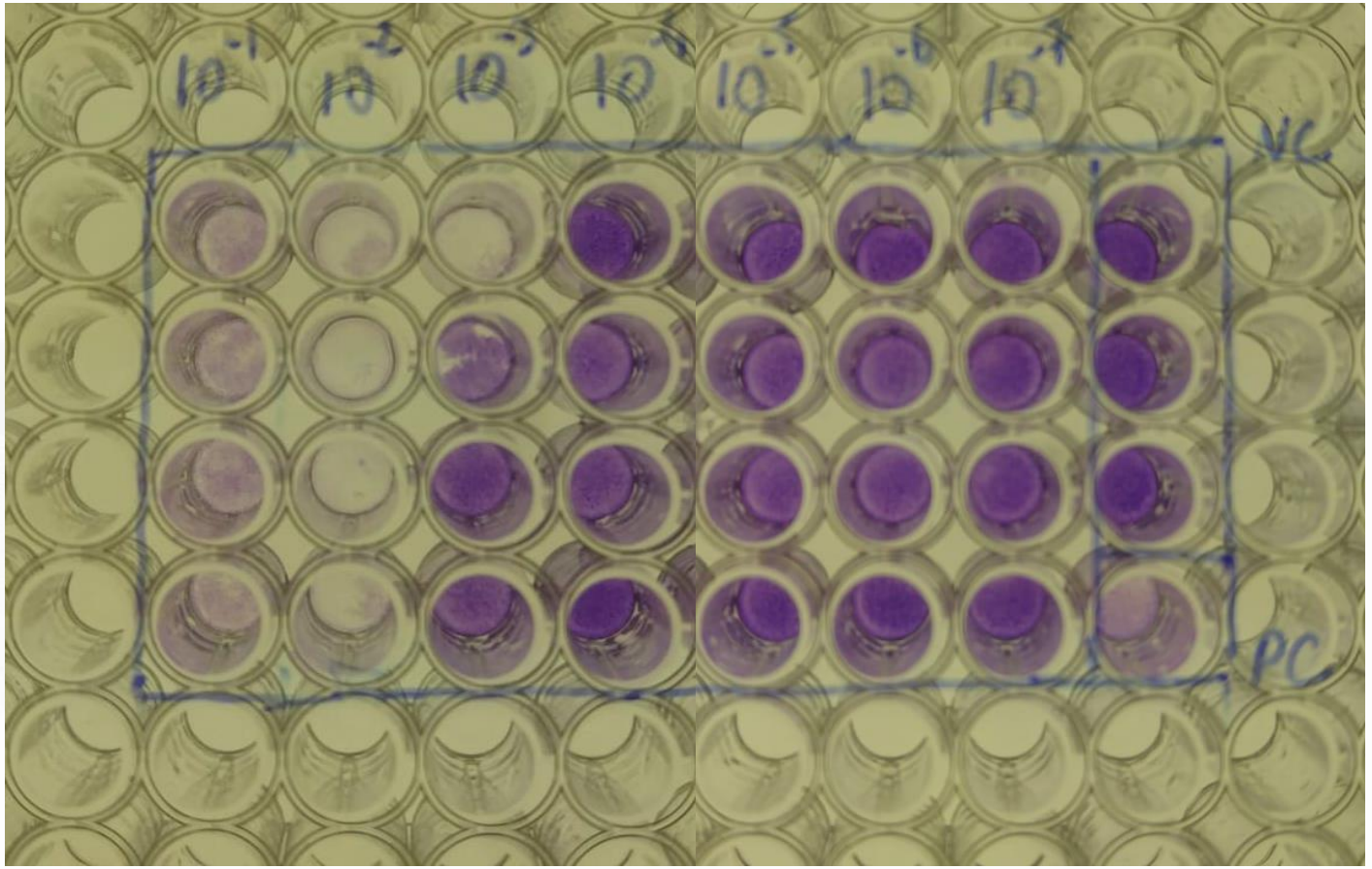
- 3000 cells/well, cell line: SW13
- 2-fold serum dilutions: 1/8 → 1/1024
- Mix the virus and sera: 50 $TCID_{50}$/100uL and 100 uL of serum
- Incubate the serum-virus mixture: 1,5 hours, in 37 centigrade celcius
- Add the mixture onto the cells
- Incubation: 6 days
- Fixation and staining



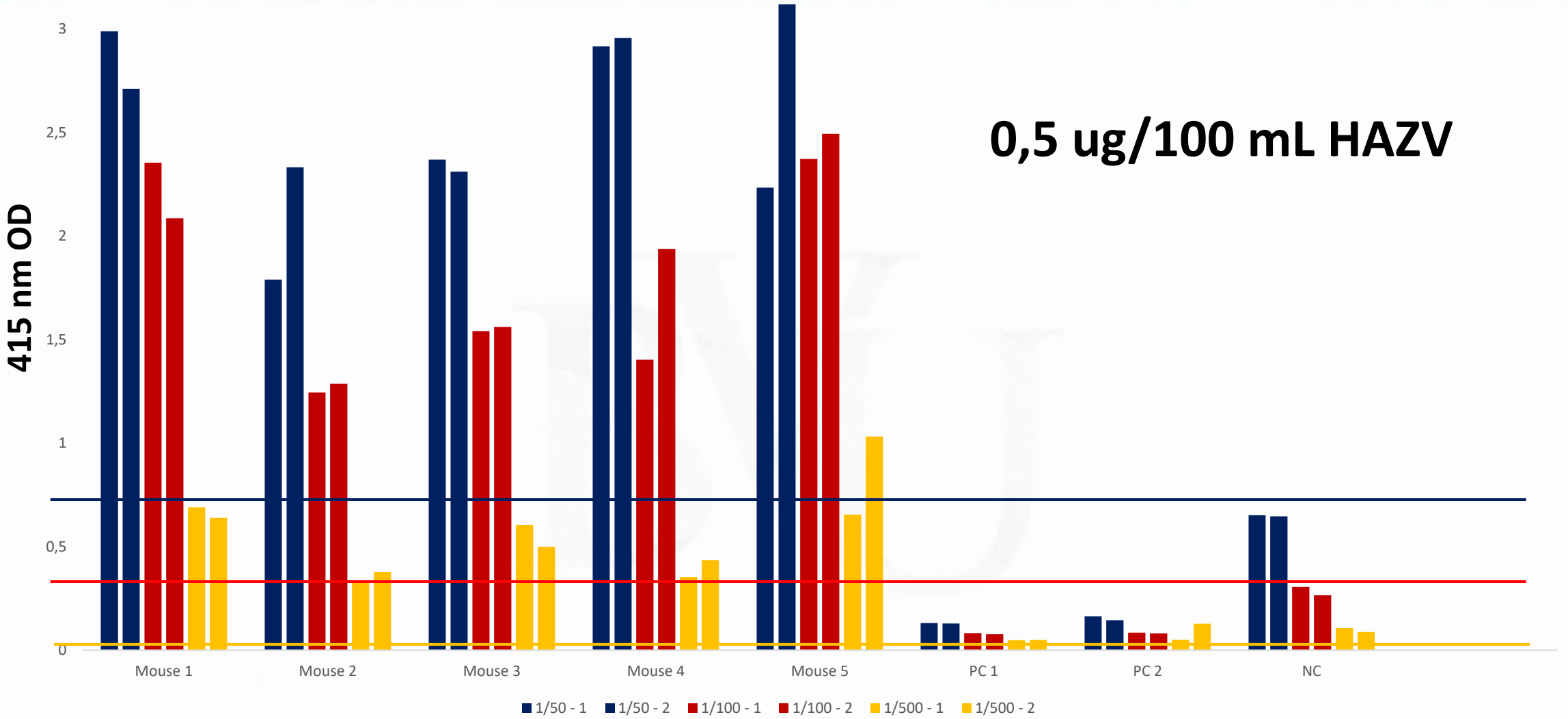
Results

$TCID_{50}$ Assay

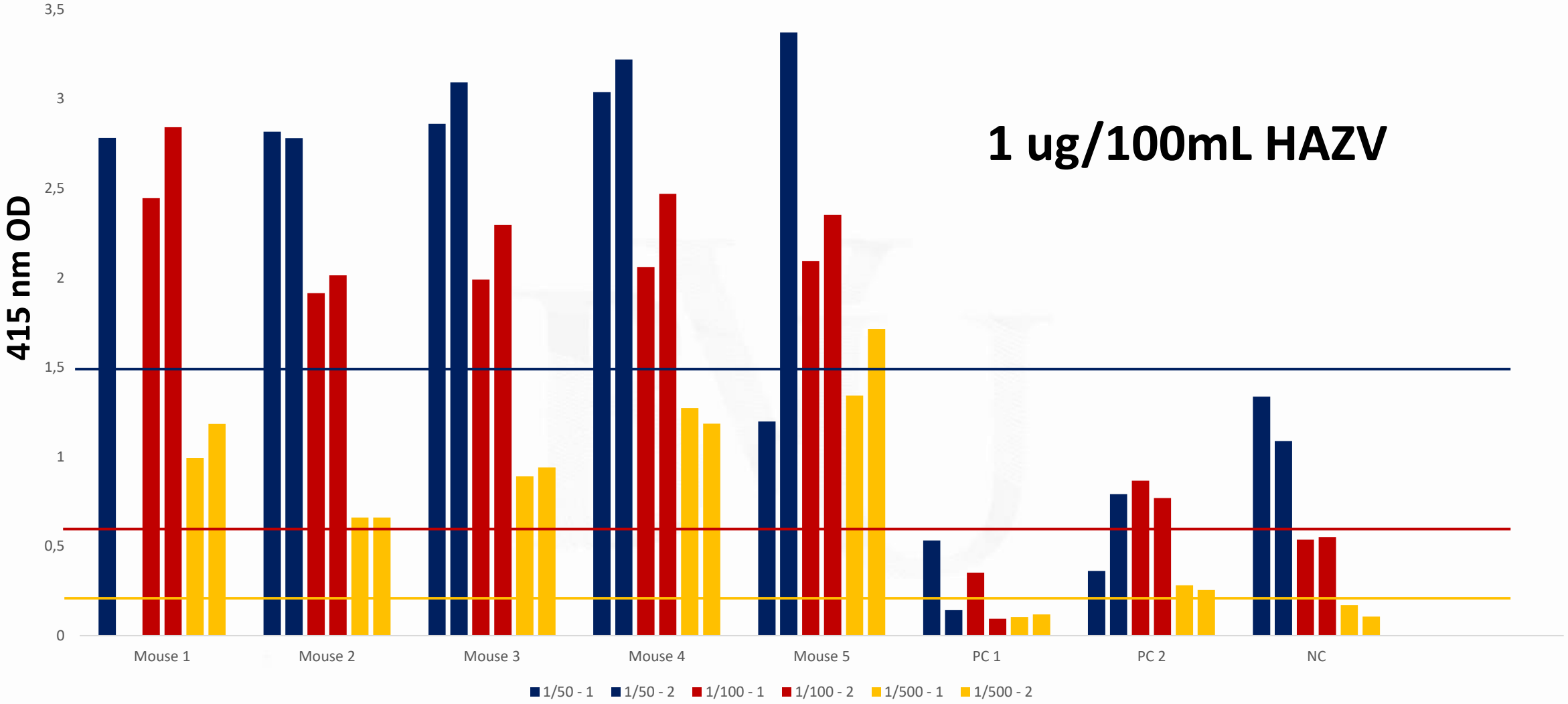
- The assay was successfully optimized
- Titer of the virus stocks were measured



Results


0,5 ug/100 mL HAZV
415 nm OD
3,5
3
2,5
2
1,5
1
0,5
0
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
PC 1
PC 2
NC
1/50 - 1
1/50 - 2
1/100 - 1
1/100 - 2
1/500 - 1
1/500 - 2

Results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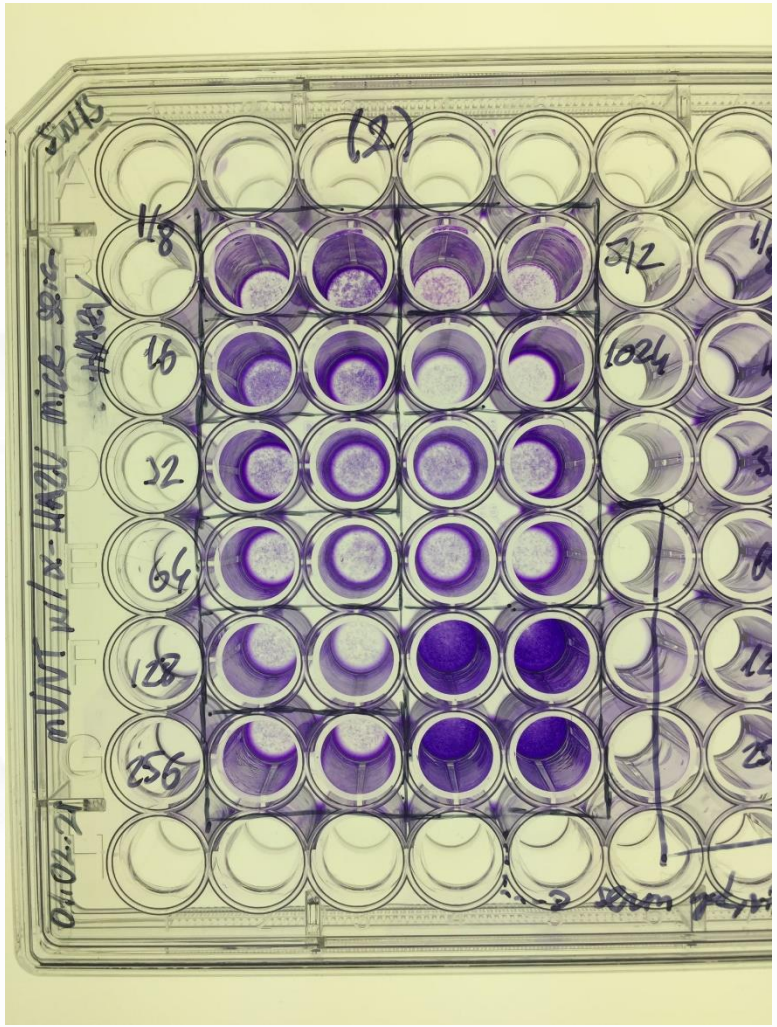
Mouse 4
Mouse 5
PC 1
PC 2
NC
1/50 - 1
1/50 - 2
1/100 - 1
1/100 - 2
1/500 - 1
1/500 - 2

Results

Microvirus Neutralization Test (mVNT)

- Anti-HAZV mouse sera against HAZV
- No neutralization observed



Conclusion

- Results of our study demonstrate that:
  - HAZV could be propagated in vitro cell culture
  - Purified as antigen and used as immunogen in animals

- Viral neutralization studies:
  - HAZV infection in cultures weren't neutralized

- An another study [1] : mVNT using HAZV and HAZV-immunized calf and sheep sera
  - No neutralization for the calf serum,
  - Neutralization for the sheep serum in 1/7 titer

[1] : Hartlaub, J., Keller, M., & Groschup, M. H. (2021). Deciphering Antibody Responses to Orthonairoviruses in Ruminants. Microorganisms, 9(7), 1493. https://doi.org/10.3390/microorganisms9071493

Conclusion

- Challenging nature of HAZV while producing an antiserum
- This would be a limitation while using HAZV as a surrogate model

- The hypothesis should be investigated further:
  - Different immunization strategies (different animal, using glycoproteins…)
  - More sensitive methods such as qPCR

Conclusion

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Thank you for your attention…