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Production of a safe EMCV-FMDV recombinant vaccine against FMD

Margot Carocci

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Plan

Introduction

Why a recombinant vaccine EMCV-FMDV?

Results

- I. production and characterization of EMCV Δ 2A
- II. Virulence attenuation on human primary astrocytes
- III. Virulence attenuation on mice
- IV. Production EMCV-FMDV

Conclusion

Why a recombinant vaccine EMCV-FMDV?

- **A live attenuated vaccine:** fast and good protection
- **Easy to produce** less confinement, less expensive
- **Distinguish easily vaccinated and infected animals**

Why a recombinant vaccine EMCV-FMDV?

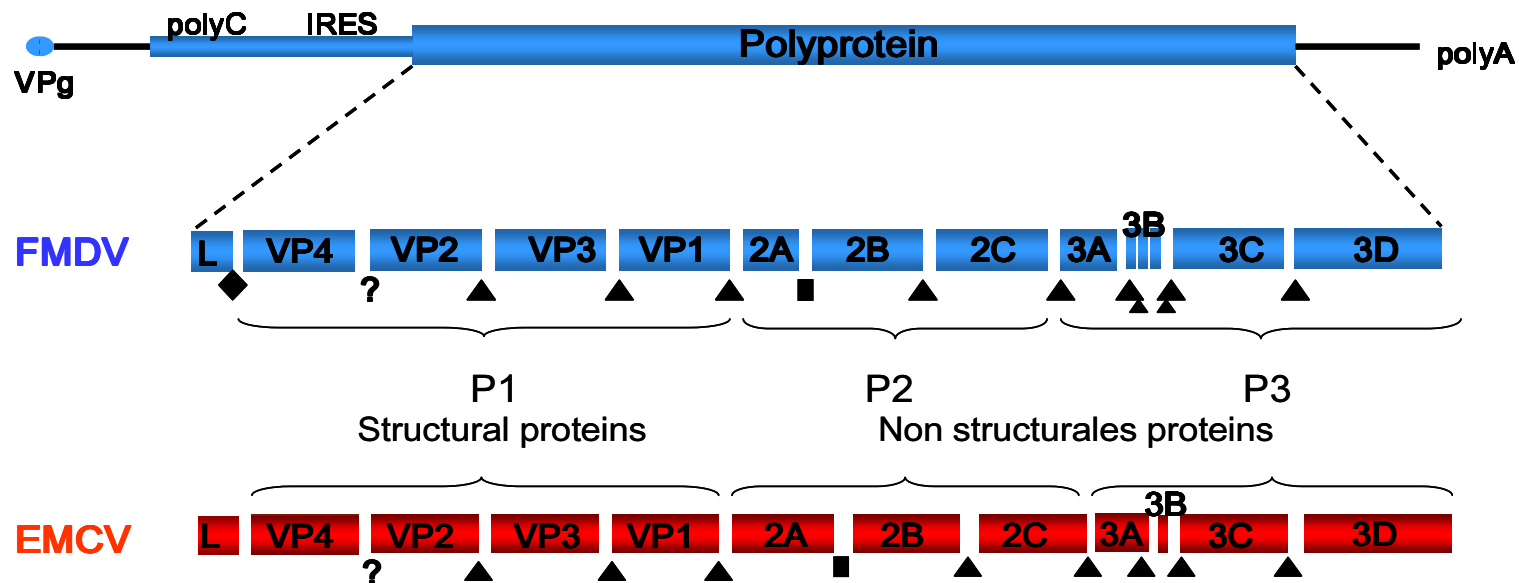
FMDV / EMCV

- *Picornaviridae* :
 - positive ssRNA, ~8kb
 - Structural and genomic organization similar
 - Encode a single polyprotein,
 - Similar viral cycle

They are the 2 Picornaviridae, of different genus (**Aphthovirus** / **Cardiovirus**), showing more similarity

Why a recombinant vaccine EMCV-FMDV?

Genomic organization



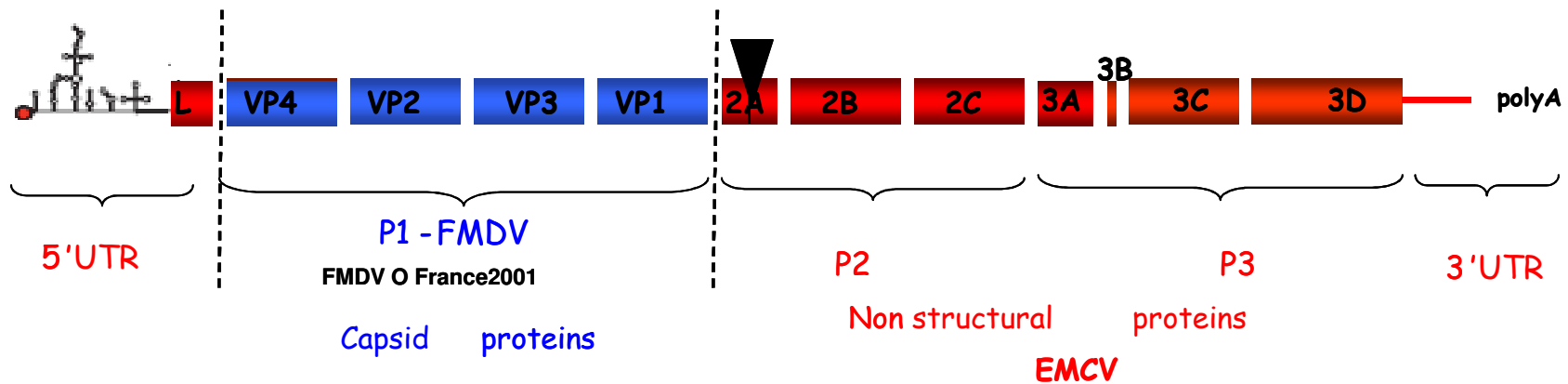
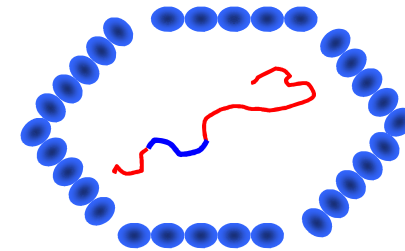
Clivage sites

- ▲ 3C^{pro}
- ◆ L^{pro}
- 2A

Why a recombinant vaccine EMCV-FMDV?

Development of a chimera EMCV-FMDV virus :

Production of an hybrid genome :



Towards a safe vaccine

EMCV : Encephalomyocarditis virus

Can infect many different animal species

Depending on the animal species and the viral strain, EMCV can cause

Myocarditis

Reproduction disorders

Diabetes

Encephalitis

=> **Attenuation of EMCV virulence is needed**

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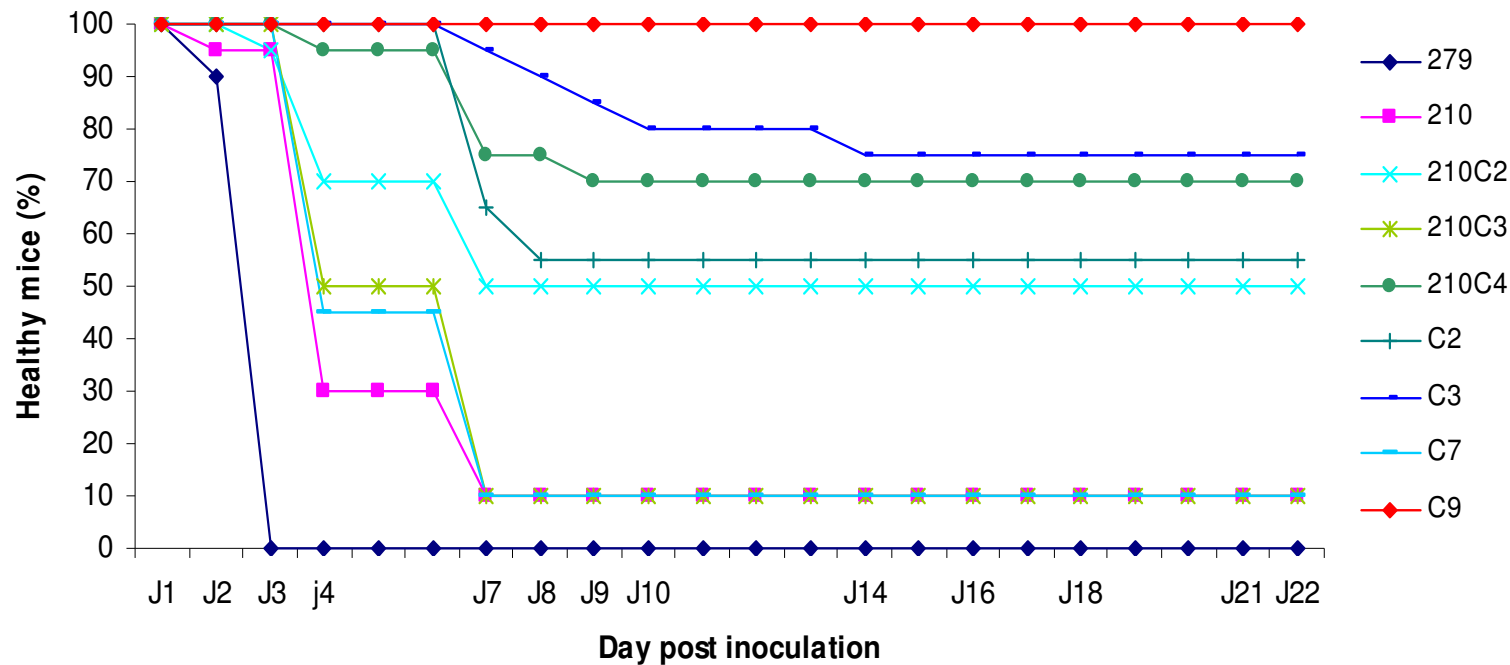
Conclusion

I. EMCVΔ2A production and characterization

B279 strain 210 passed on BHK-21 : non-virulent for pigs

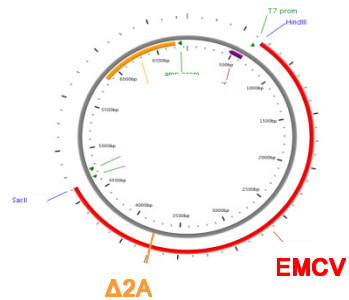
(P.Denis and F. Koenen Arch Virol 2003)

Test virulence on mice C57BI/6



C9 clone => a **deletion** in the **2A protein** sequence.

I. EMCV Δ 2A production and characterization

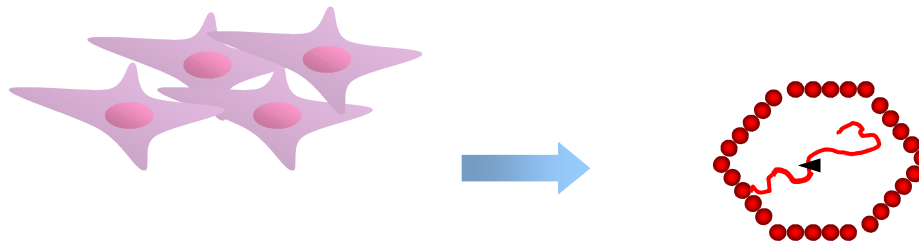
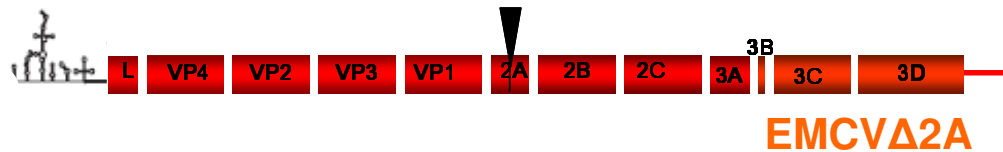


Incorporation of a deletion in 2A, using a reverse genetic previously developed in our lab (Bakkali et al., 2002).

In vitro transcription

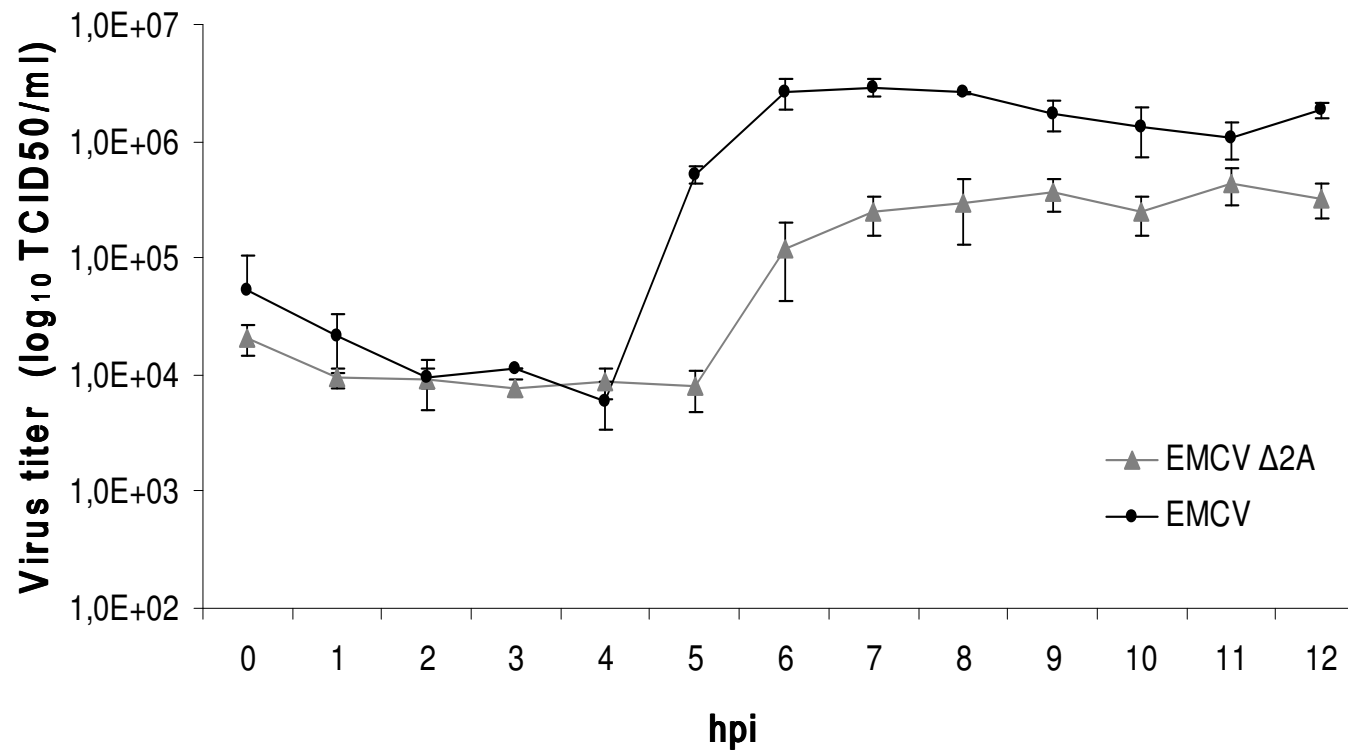
Transfection

EMCV Δ 2A production



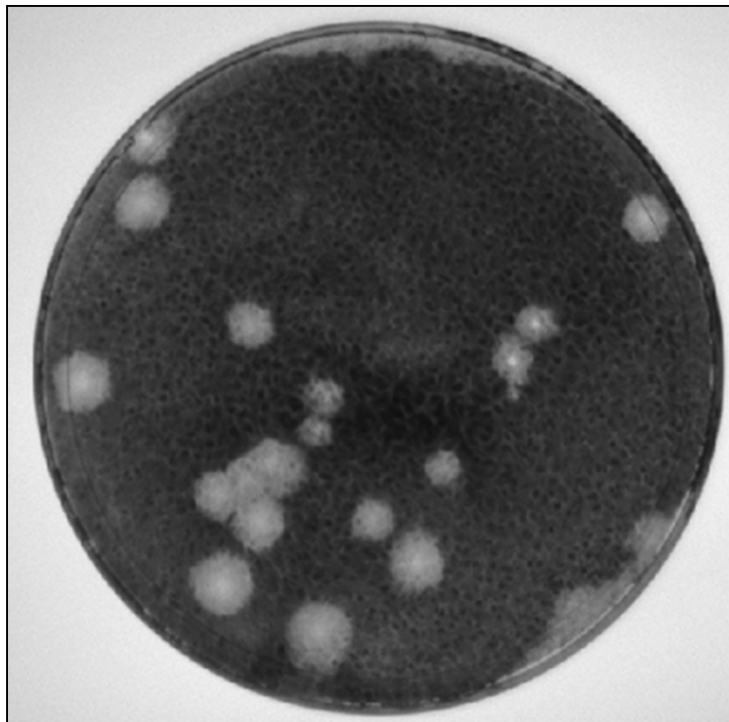
I. EMCV Δ 2A production and characterization

One-step growth cycle

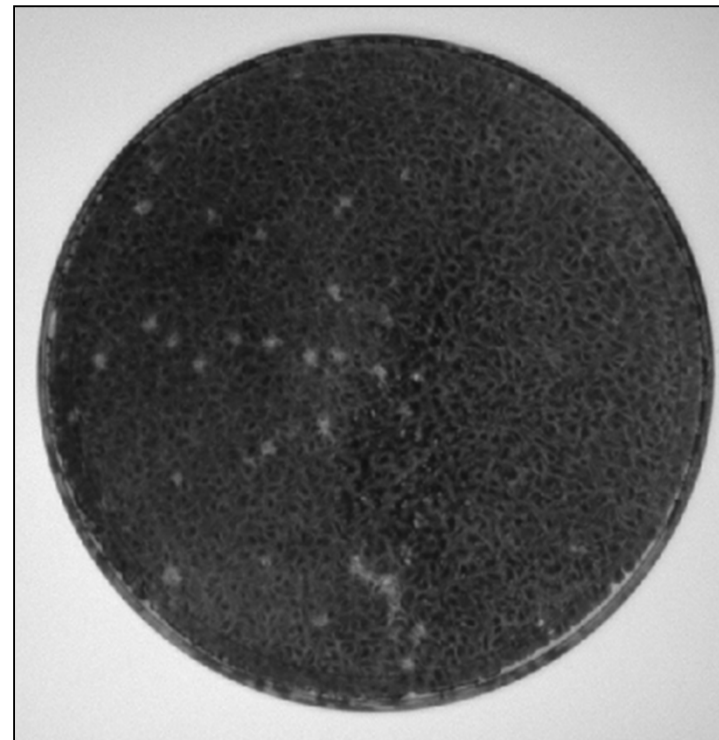


I. EMCV Δ 2A production and characterization

Plaque assay on BHK-21



EMCV



EMCV Δ 2A

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II. Virulence att. on human primary astrocytes

EMCV

Able to infect a wide range of animal species.

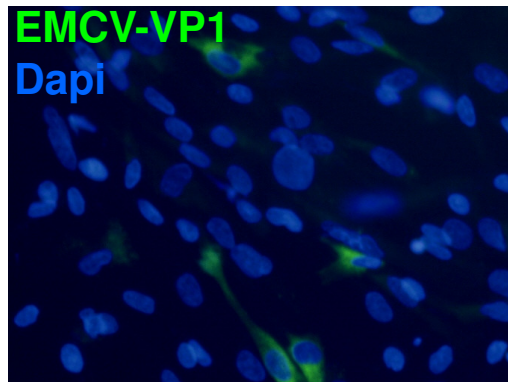
Has been described to possibly infect and cause of head ache, vomiting, malaise, fever... In Human.

(Oberste et al, Emerg Inf Dis 2009)

**Test infectivity of Human primary astrocytes by EMCV,
and the attenuation of EMCV Δ 2A.**

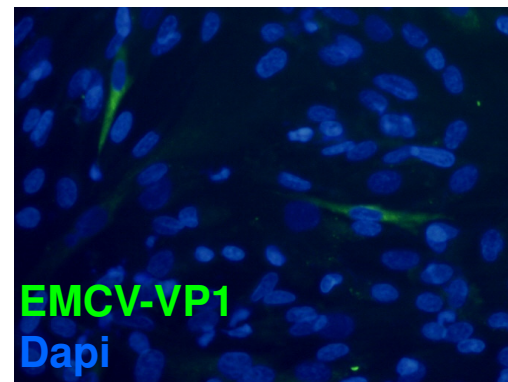
II. Virulence att. on human primary astrocytes

EMCV can infect and replicate on human primary astrocytes

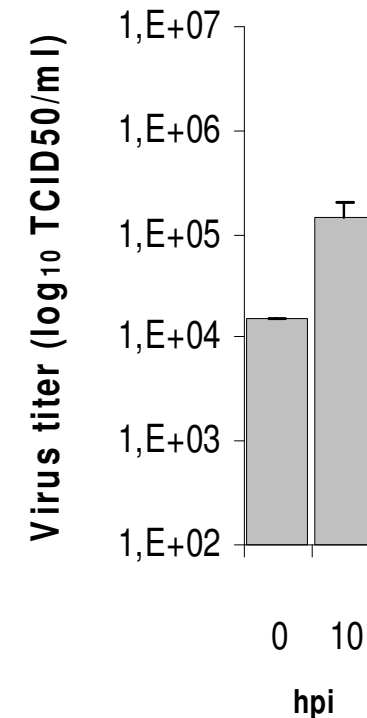
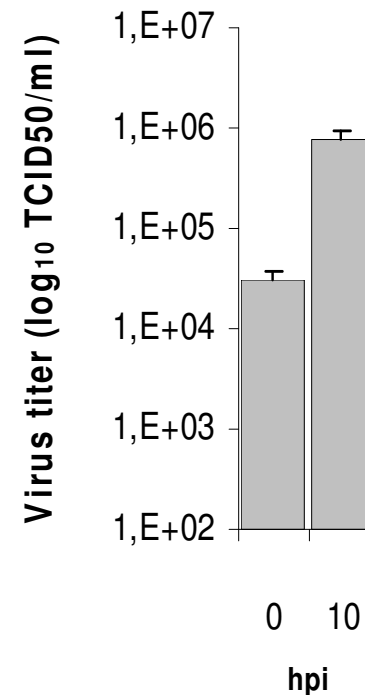


Astrocytes 5hpi

EMCV

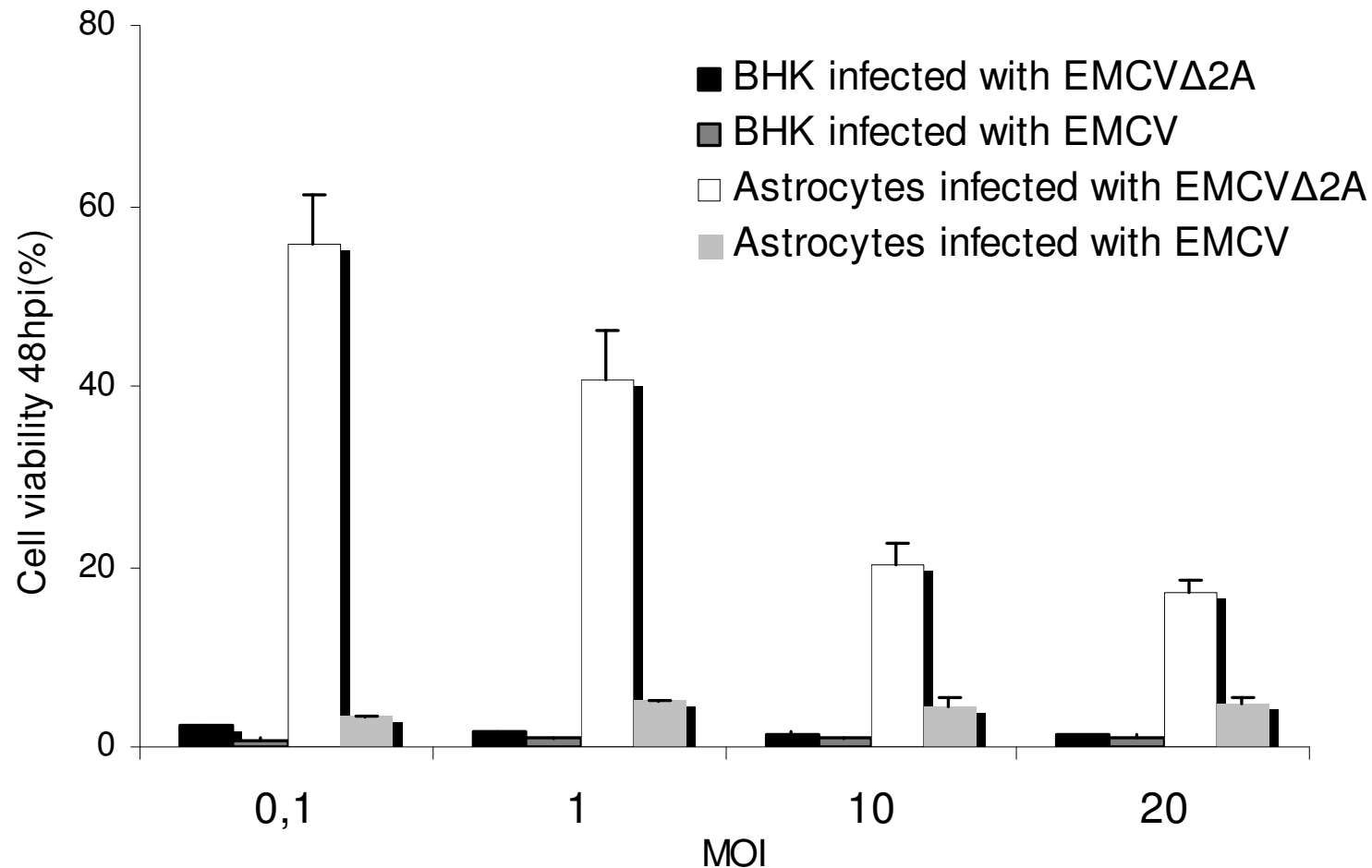


EMCVΔ2A



EMCVΔ2A is able to infect and replicate on human primary astrocytes but less efficiently.

II. Virulence att. on human primary astrocytes



EMCVΔ2A virus is less virulent than the wild type on Human primary Astrocytes

Plan

Introduction

Why a recombinant vaccine EMCV-FMDV?

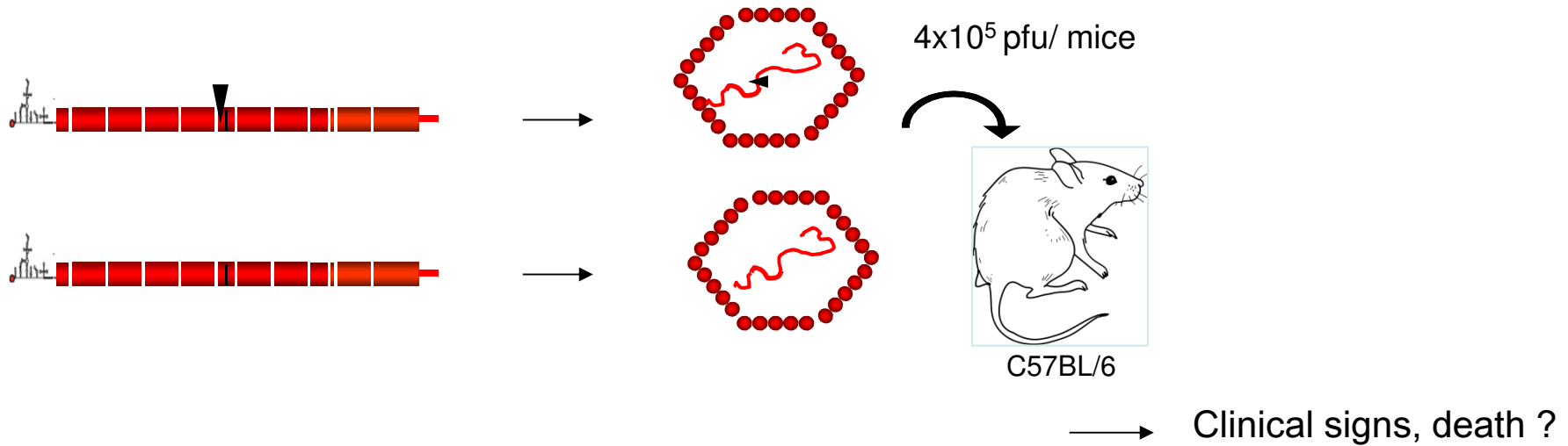
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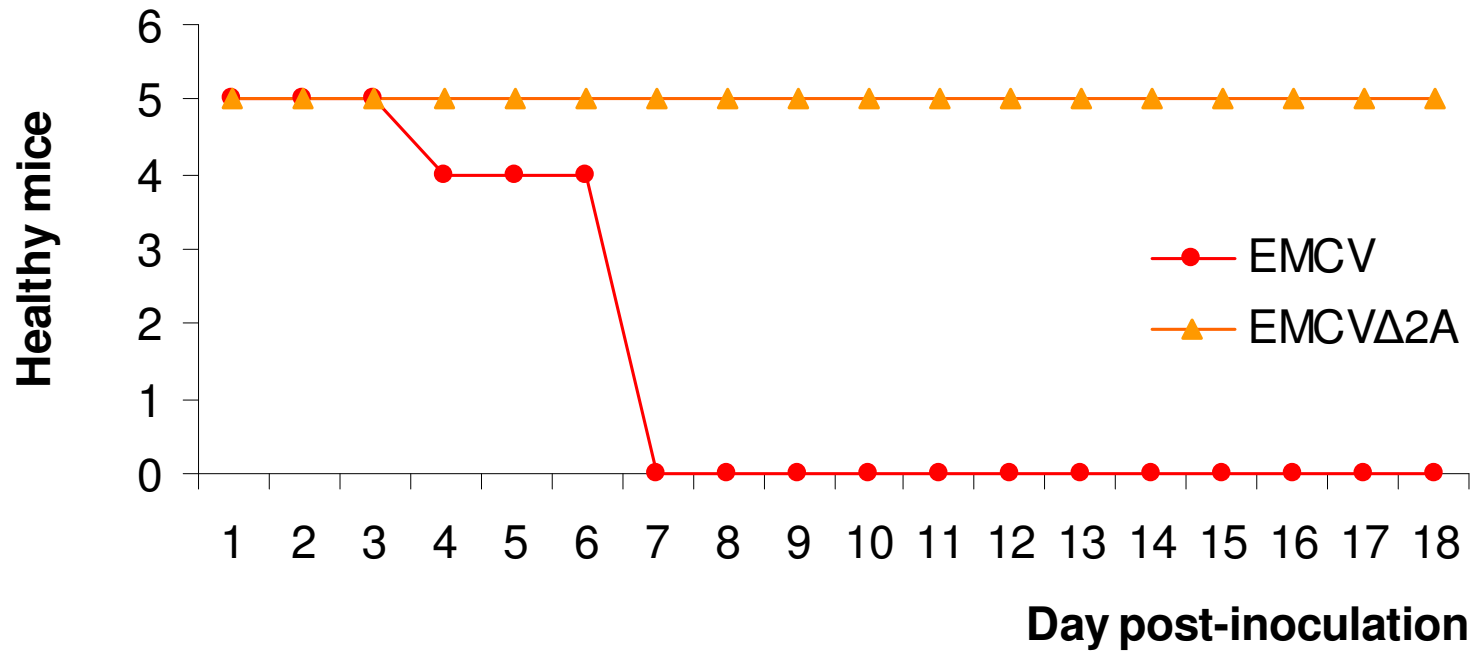
Conclusion

II. Virulence attenuation on mice

Intraperitoneally inoculation



III. Virulence attenuation on mice

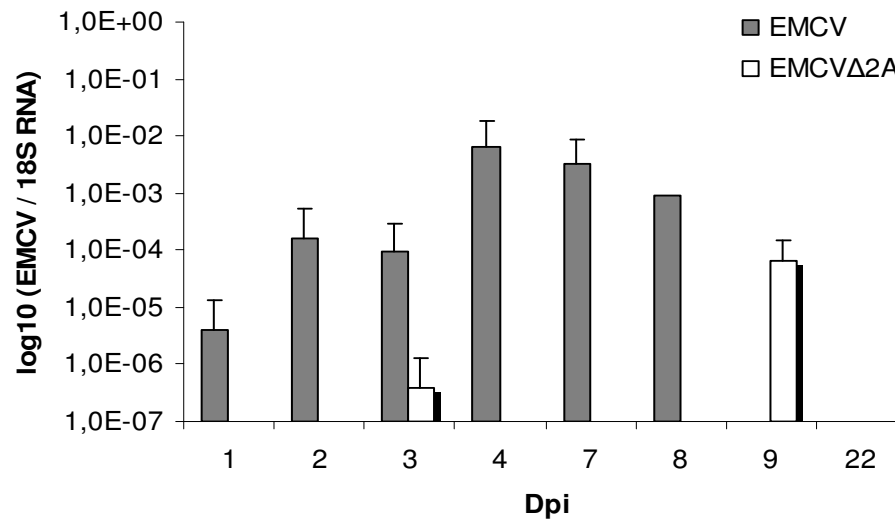


EMCV Δ2A does not induce any clinical sign in C57BL/6 mice

III. Virulence attenuation on mice

Number of viral RNA copy:

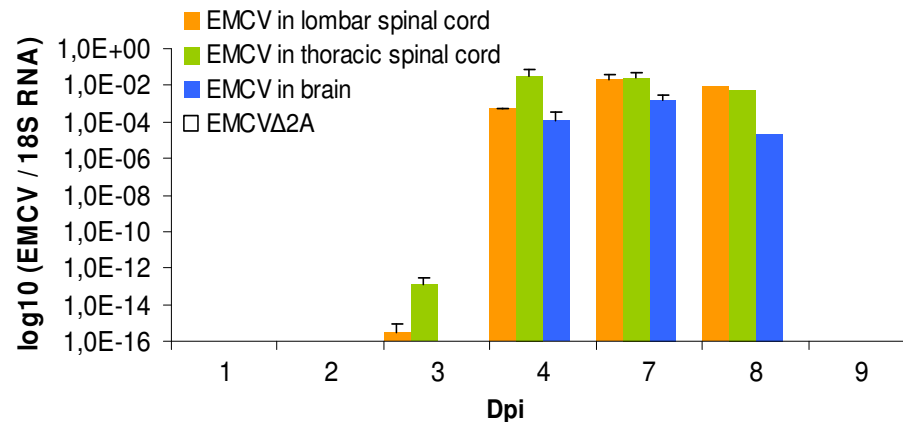
in mice heart.



Detection of **EMCV Δ2A** in mice heart only at 3dpi 1 out of 5 mice heart & at 9dpi in 3 out of 5. No more at 22dpi.

=> **EMCV Δ2A** is able to replicate *in vivo*.

in mice CNS.



EMCV Δ2A does not reach the mice CNS.

Conclusion sur EMCV Δ 2A

Virulence attenuation of EMCV Δ 2A :

on BHK-21

on human primary astrocytes

(With first demonstration of human primary astrocytes sensitivity to EMCV.)

on mice

The EMCV Δ 2A virus is an attenuated virus,

Which should be a safe base for a recombinant EMCV-FMDV vaccine.

Plan

Introduction

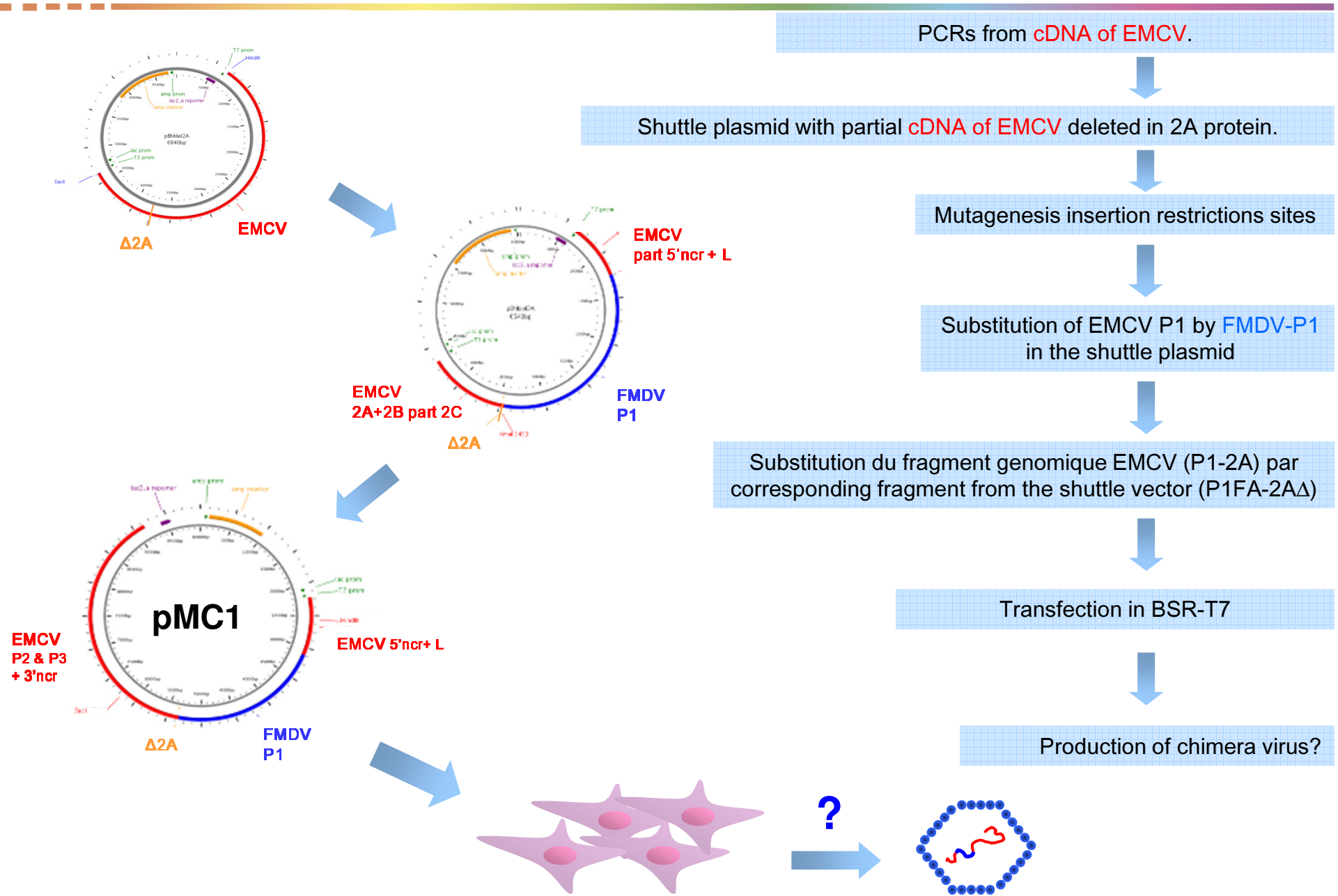
Why a recombinant vaccine EMCV-FMDV?

Results

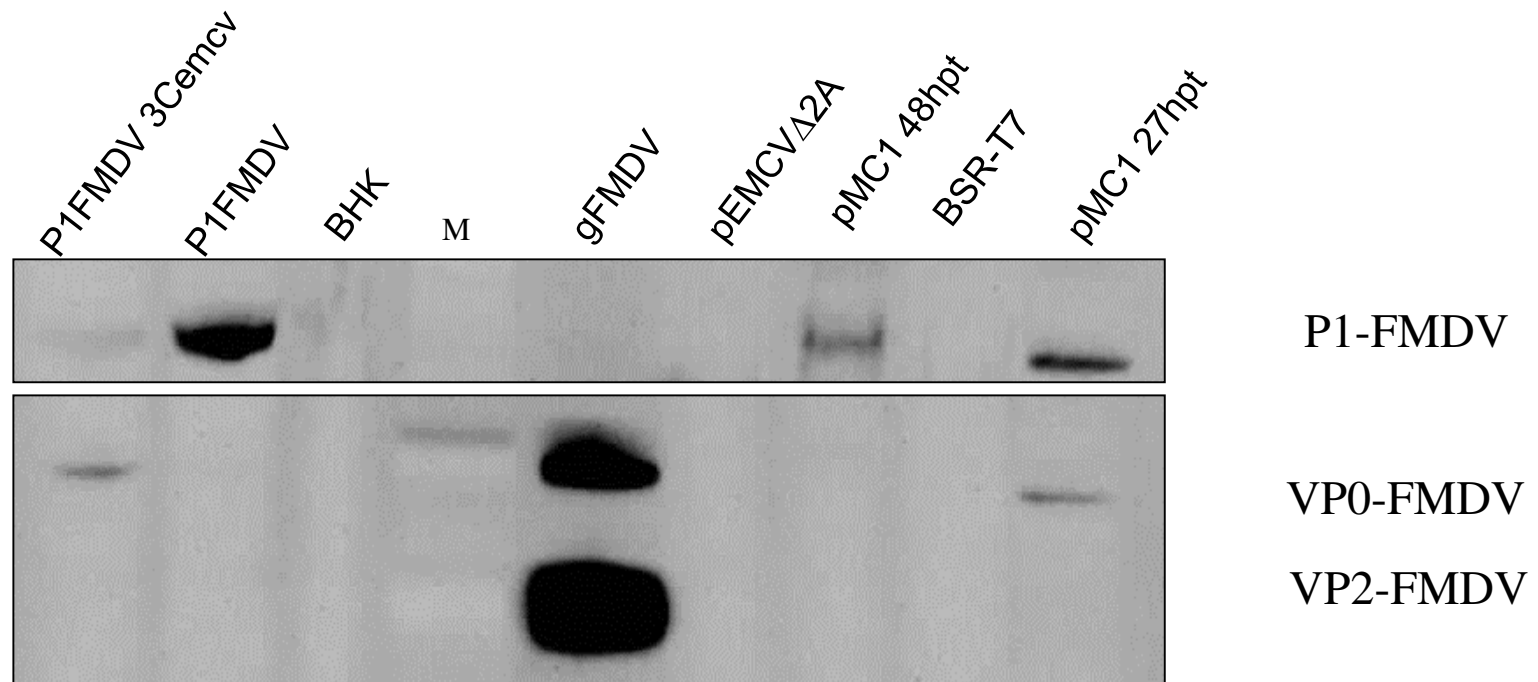
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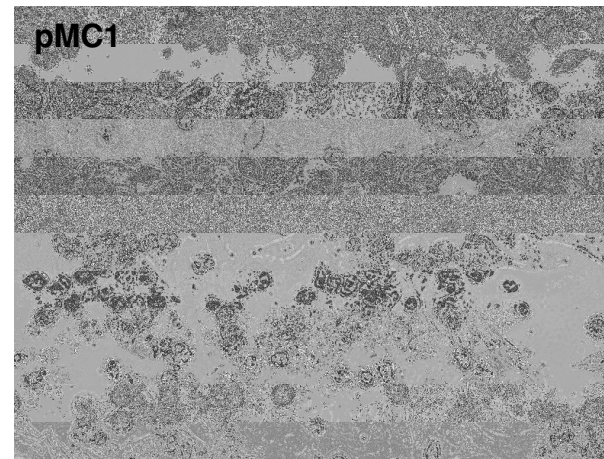
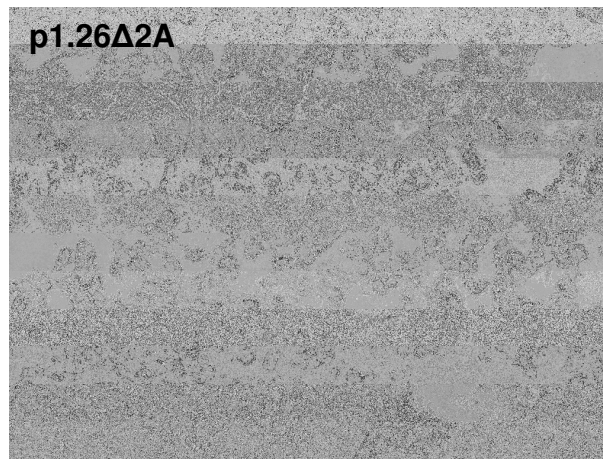
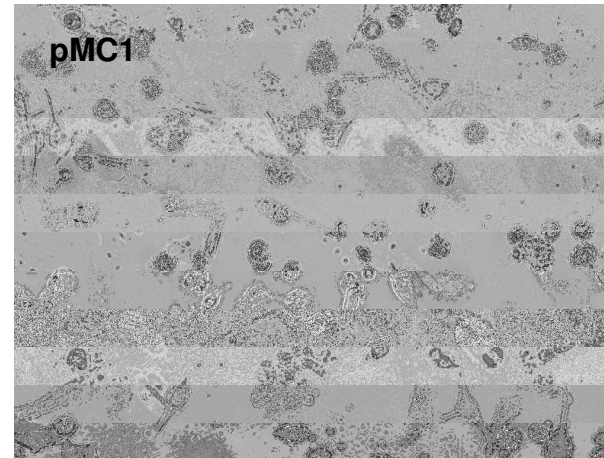
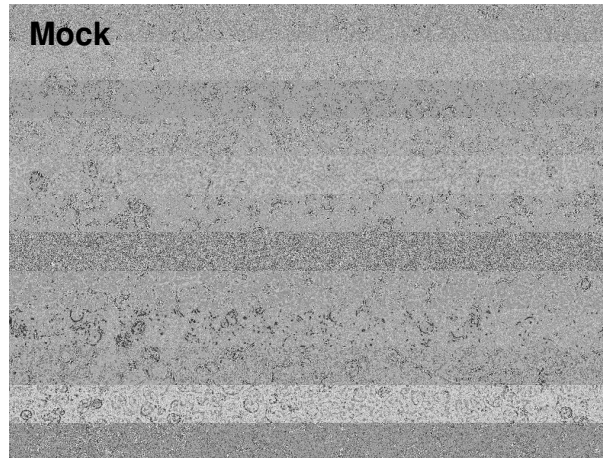
IV. Production of EMCV Δ 2A-P1FMDV



IV. Production of EMCV Δ 2A-P1FMDV



IV. Production of EMCV Δ 2A-P1FMDV



Conclusion

Virulence attenuation of EMCV Δ 2A :

on BHK-21

on human primary astrocytes

(With first demonstration of human primary astrocytes sensitivity to EMCV.)

on mice

The EMCV Δ 2A virus safe base for a recombinant.

Transfection of pMC1 (recombinant cDNA):

Cytopathic effects and expression of P1-FMDV
viral particle production ?

To be confirmed...

Acknowledgements



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PARIS
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DIDEROT



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Muriel Couplier.*




anses
alimentation, environnement, travail



Building Bridges Workshop

Beijing, 2-4 July 2011

A photograph of a traditional Chinese stone arch bridge spanning a body of water. The bridge's arch is perfectly circular, and the water in the foreground is calm, reflecting the bridge and the sky. In the center of the reflection, the flag of the European Union (a circle of twelve yellow stars on a blue background) is superimposed. The background shows a hazy landscape with trees and distant hills under a soft, golden light, suggesting dawn or dusk.

Thank you for your attention !

***Promoting Scientific Cooperation between China
and Europe to Combat Epizootic Diseases***