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FMD-DISCONVAC

Production of a safe EMCV-FMDV recombinant vaccine against FMD

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Introduction

Why a recombinant vaccine EMCV-FMDV?

Results

I. production and characterization of EMCVΔ2A

II. Virulence attenuation on human primary astrocytes

III. Virulence attenuation on mice

Conclusion

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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** : faster get back to a statue free from FMDV

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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 (**Aphtovirus** / **Cardiovirus**), showing more similarity

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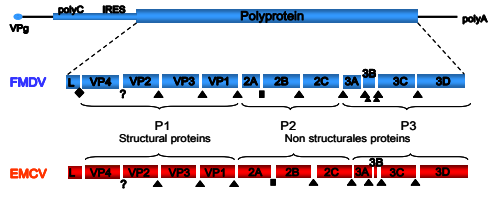
Why a recombinant vaccine EMCV-FMDV?

Genomic organization



Clivage sites

▲ $3C^{pro}$ ◆ L^{pro}

▮ 2A

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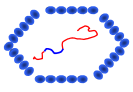
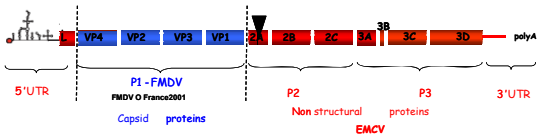
Why a recombinant vaccine EMCV-FMDV?

Development of a chimera EMCV-FMDV virus :

Production of an hybrid genome :





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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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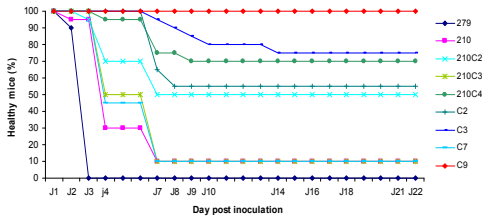
I. EMCVΔ2A production and characterization

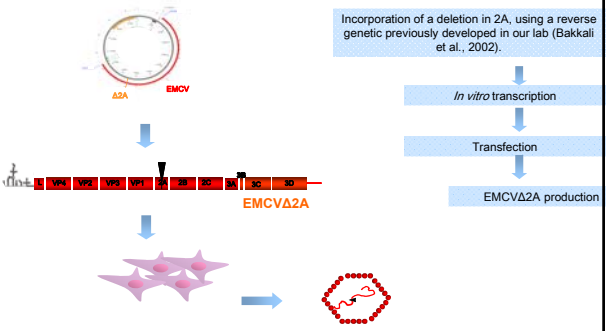
B279 strain 210 passed on BHK-21 : non-virulent for pigs
(P.Denis and F. Koenen Arch Virol 2003)

Test virulence on mice C57Bl/6



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

I. EMCVΔ2A production and characterization



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

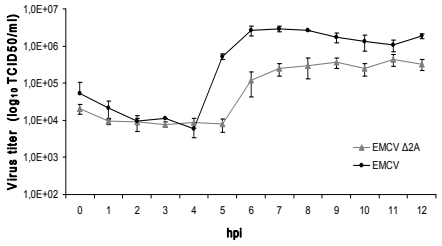
In vitro transcription

Transfection

EMCVΔ2A production

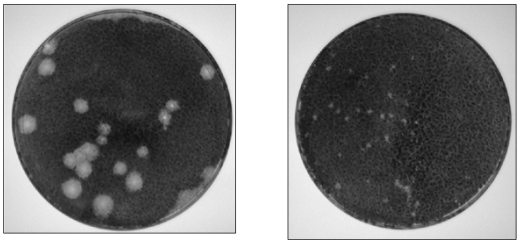
I. EMCVΔ2A production and characterization

One-step growth cycle



I. EMCVΔ2A production and characterization

Plaque assay on BHK-21



EMCV EMCV Δ2A

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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 EMCVΔ2A.

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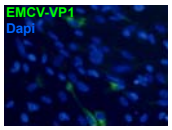
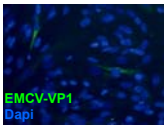
II. Virulence att. on human primary astrocytes

EMCV can infect and replicate on human primary astrocytes

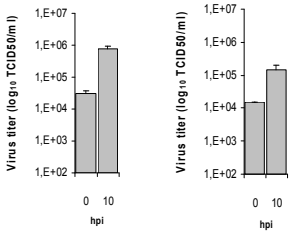


EMCV

Astrocytes 5hpi



EMCVΔ2A



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

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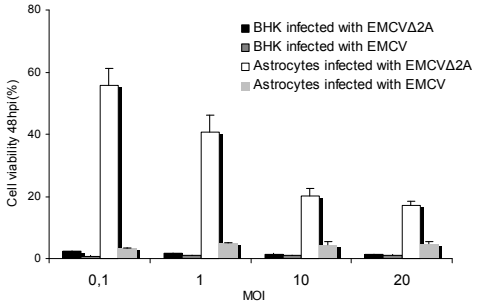
II. Virulence att. on human primary astrocytes



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

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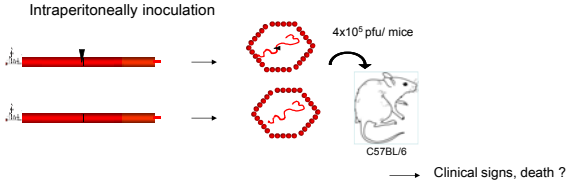
II. Virulence attenuation on mice



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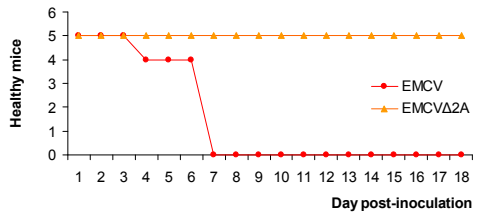
II. Virulence attenuation on mice



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

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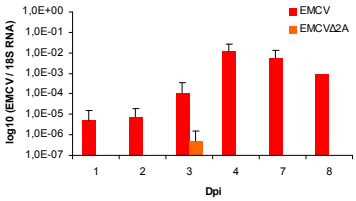
II. Virulence attenuation on mice

Number of viral RNA copy in mice heart.



Detection of EMCV Δ2A **only at 3dpi in one out of 5 mice heart.**

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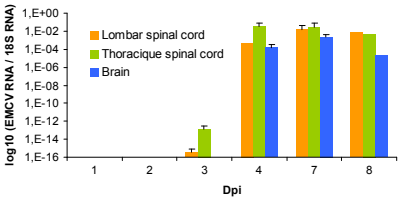
II. Virulence attenuation on mice

Number of EMCV RNA copy in mice CNS.



EMCV Δ2A **does not reach the mice CNS.**

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Conclusion

Virulence attenuation of EMCVΔ2A :

on BHK-21
on human primary astrocytes
(With first demonstration of human primary astrocytes sensitivity to EMCV.)
on mice

The EMCVΔ2A virus is an attenuated virus,

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

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