



## 5ème Colloque International Francophone de Microbiologie Animale

Apport des biotechnologies dans la vaccinologie vétérinaire

Le virus de la Fièvre de la Vallée du Rift : données  
récentes sur la pathogenèse

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# The Bunyaviridae family

There are more than 400 known members grouped into 5 genera

- *Orthobunyavirus*: *Bunyamwera virus*
- *Phlebovirus*: *Rift Valley fever virus*
- *Nairovirus*: *Nairobi sheep disease virus*
- *Tospovirus*: *Tomato spotted wilt virus*

*Arboviruses*



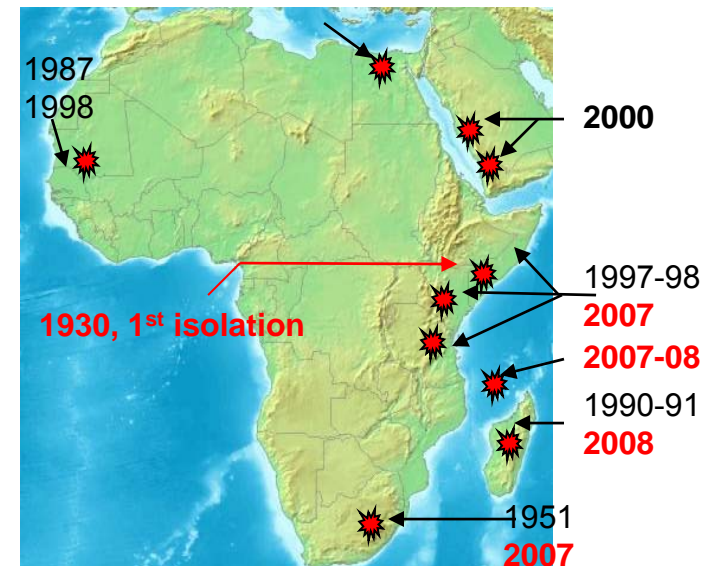
- *Hantavirus*: *Hantaan virus*

*Rodent borne viruses*

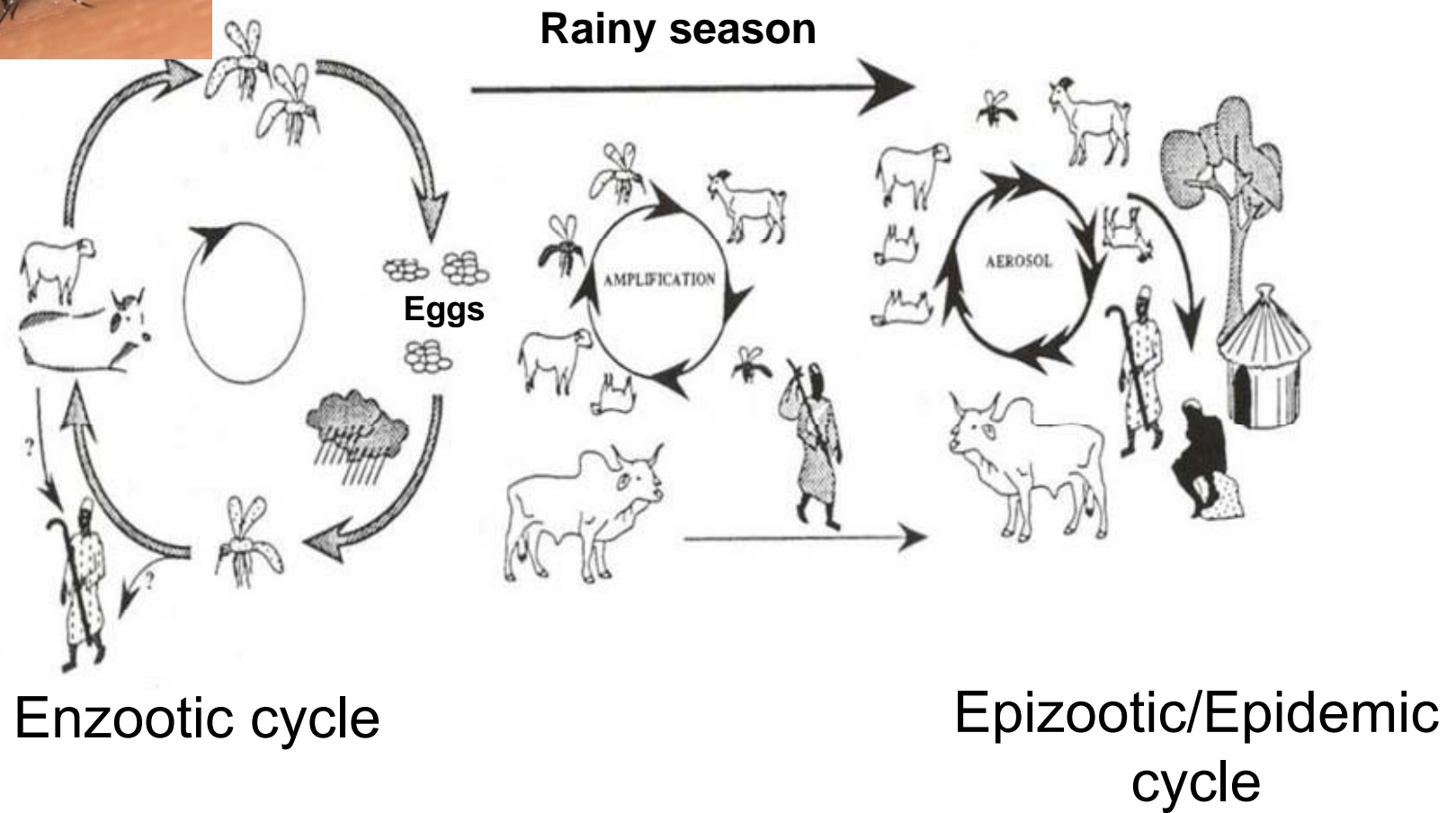


# Rift Valley fever

- Zoonosis affecting humans and ruminants in Africa and Yemen and Saudi Arabia since 2000
- Virus transmitted by many species of mosquitoes
- Hemorrhagic fever in humans and hepatitis, abortion and death in ruminants
- No safe vaccine for protection nor antiviral agents for therapy
- Potential bioterrorism agent



# Transmission of RVF



(Zeller *et al.*, 1997)





République du Sénégal  
MINISTÈRE DE L'AGRICULTURE  
ET DE L'ÉLEVAGE

DIRECTION DE L'ÉLEVAGE  
BP 67 - 37 Av. Pasteur - Dakar (Sénégal)  
Tél. : (221) 821 32 28 - 823 53 09

# LA FIÈVRE NAWU WALEE RIFT DE LA VALLÉE DU RIFT

C'EST UNE ZONOSE MAJEURE  
ELLE AFFECTE AUSSI BIEN LES ANIMAUX  
QUE LES HOMMES.



NĀWU KEENGU ADDUDE  
WOPPERE NDER JIBINGOL,  
KAM E WARDE JAWDI  
TOKOSIRI NDI

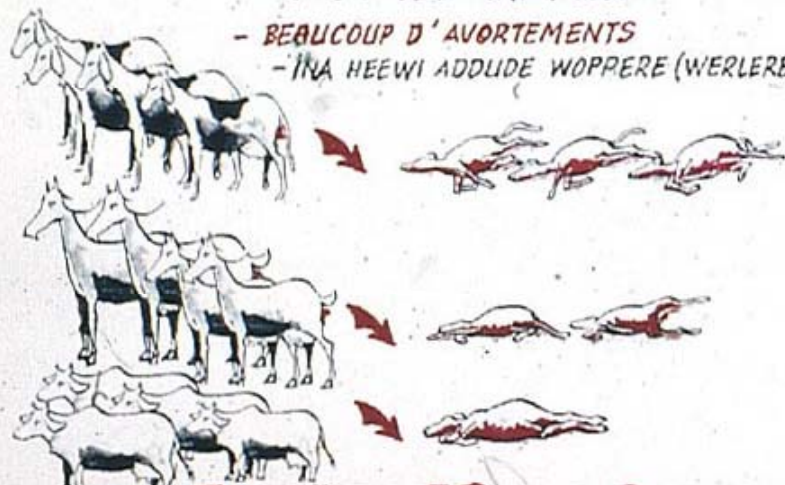
NĀWU NGULU INA ARA E JAWDI, INA ARA  
KADI E NEDDO.



## • MANIFESTATIONS • NO DU SIFORTEE ?

\* CHEZ LES ANIMAUX

- BEAUCOUP D'AVORTEMENTS  
- INA HEEWI ADDUDE WOPPERE (WERLERE)



\* TO JAWDI TOO

- FORTE MORTALITÉ DES AGNEAUX  
CHEVREAUX ET VEAUX.



- INA HEEWI WARDE JAWDI WALLA  
NDAMMIRI TOKKOSIRI NDI

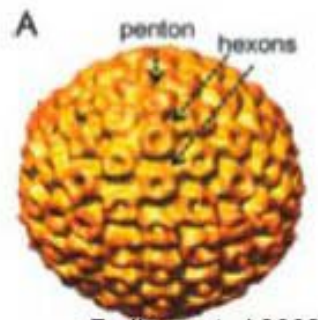
\* CHEZ L'HOMME

\* TO NEDDO TOO

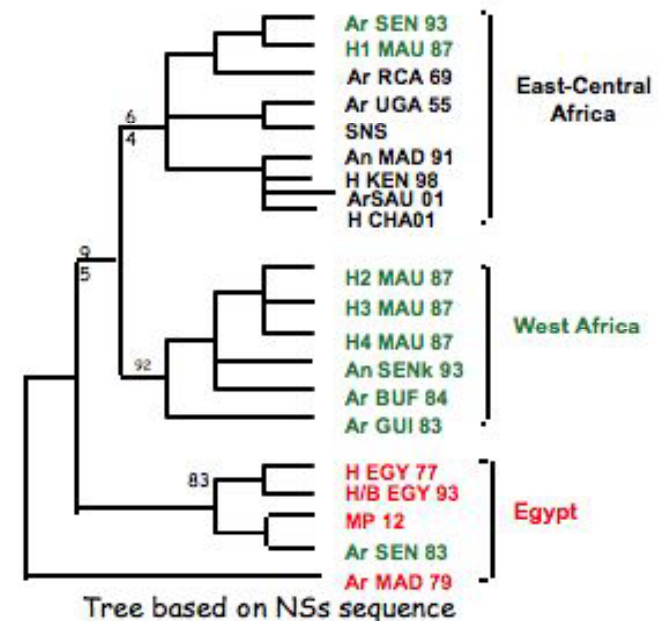
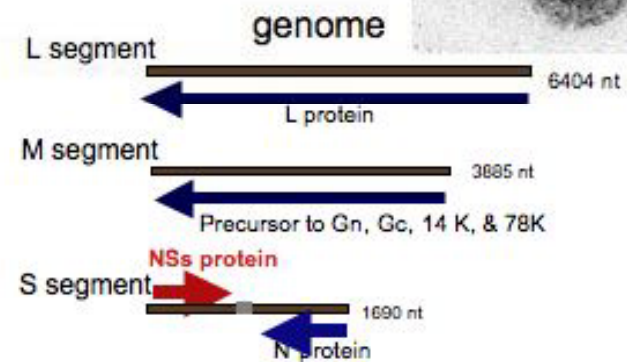
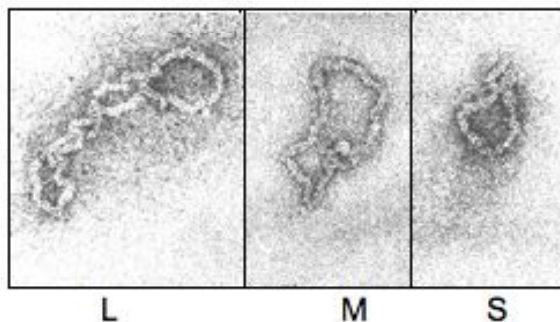
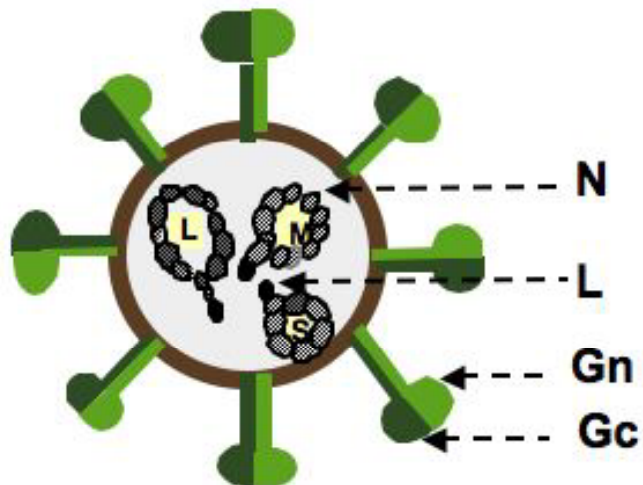
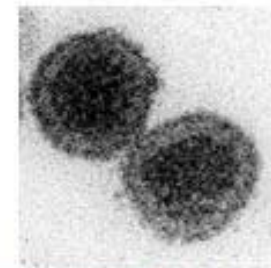
- FORTE FIÈVRE RESSEMBLANT  
AU PALUDISME OU À LA FIÈVRE  
JAUNE,



ƁANNDU WULA  
YILLEE HAA WAYA  
NO GARAADO  
JONTINOJE



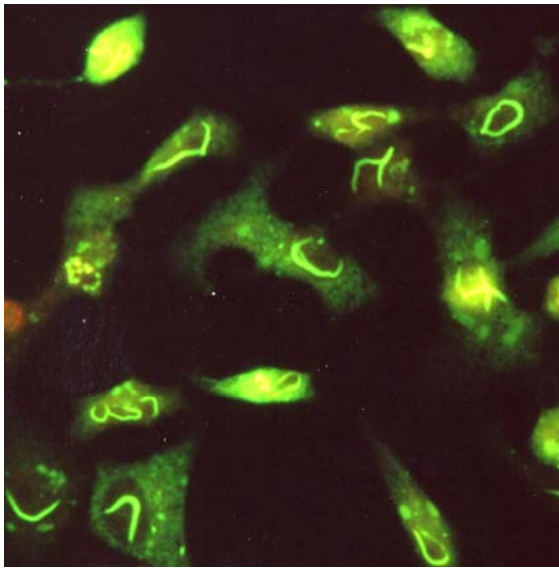
# Rift Valley fever virus (Bunyaviridae, *Phlebovirus*)



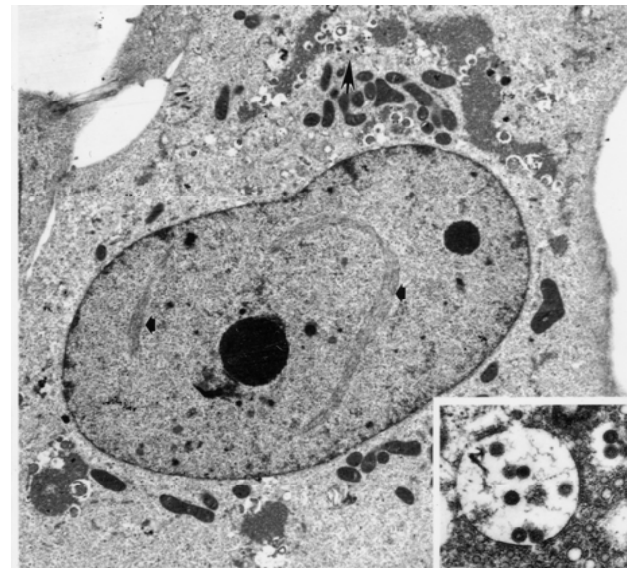


NSs forms filamentous structures in the nucleus in spite of the fact that all the steps of the viral cycle occur in the cytoplasm

Immunofluorescence  
with anti-NSs antibodies

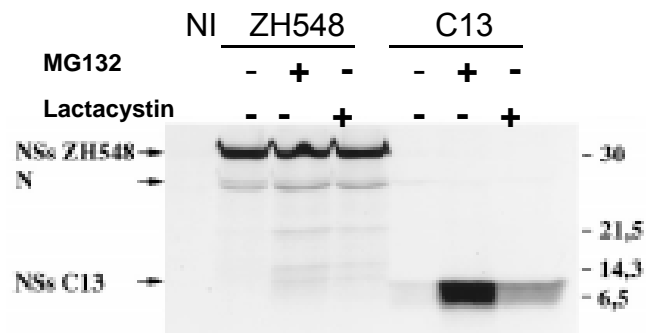
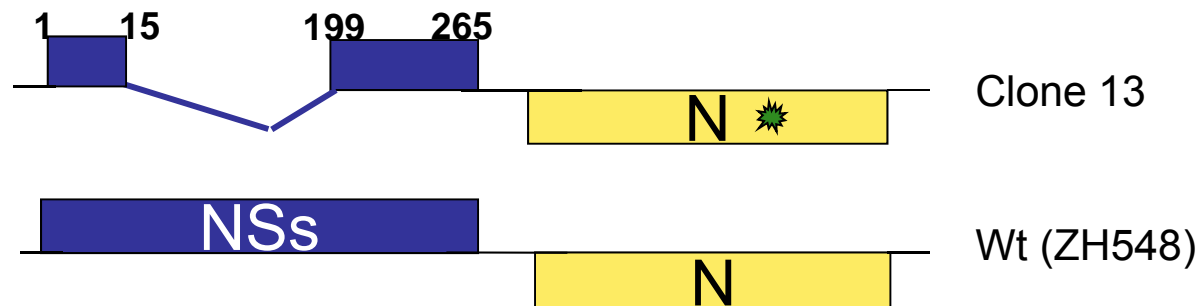


Section of a RVFV infected cell  
Electron microscopy



## NSs is an accessory protein

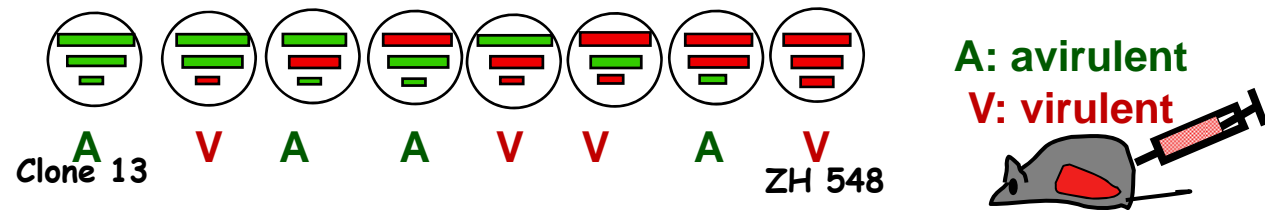
Clone 13 has an in frame internal deletion of 70% of NSs ORF. The truncated protein remains in the cytoplasm and is degraded by the proteasome





## NSs is the major virulence factor

- Clone 13 is avirulent for mice: its S segment carries a major determinant for attenuation

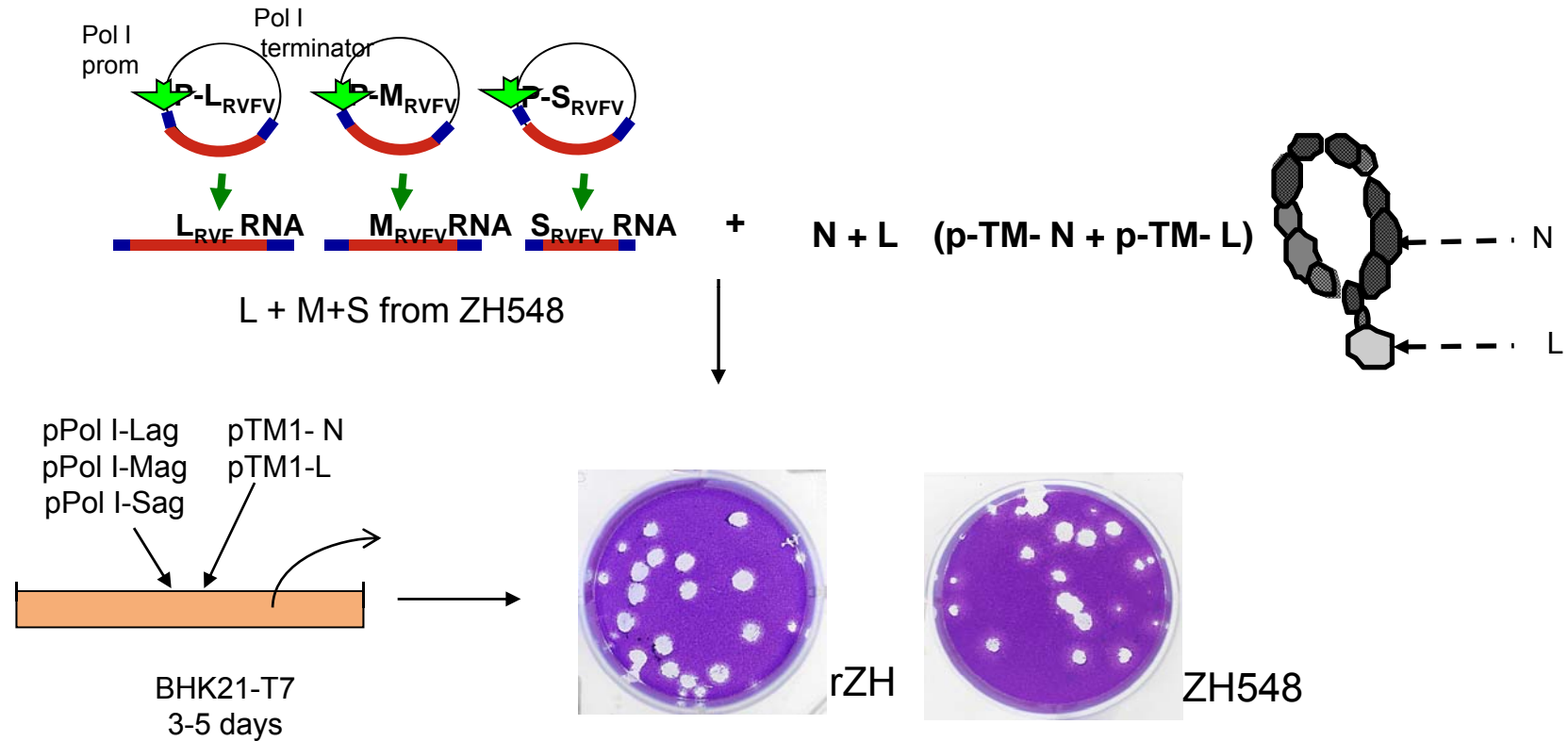


- Interferon  $\alpha/\beta$  plays a major role for attenuation
  - Clone 13 caused a rapid death in type I IFN receptor deficient mice
  - Clone 13 induced a high titer of IFN in the serum of infected mice whereas the virulent ZH548 did not

→ Role of type I interferon for attenuation

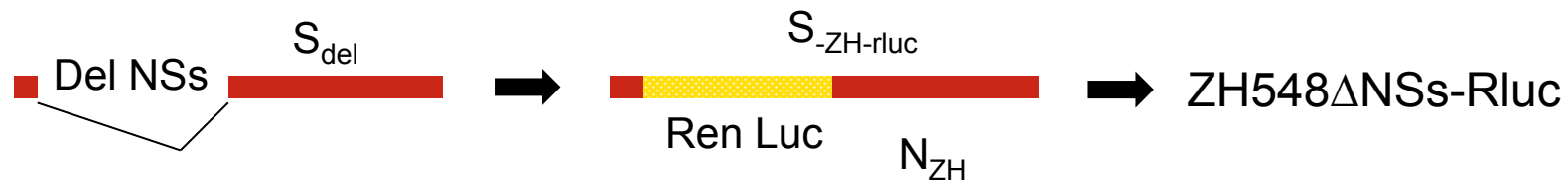
# Reverse genetics

Reconstitution of viral-like RNPs active for transcription and replication



## Bioluminescence imaging in living mice

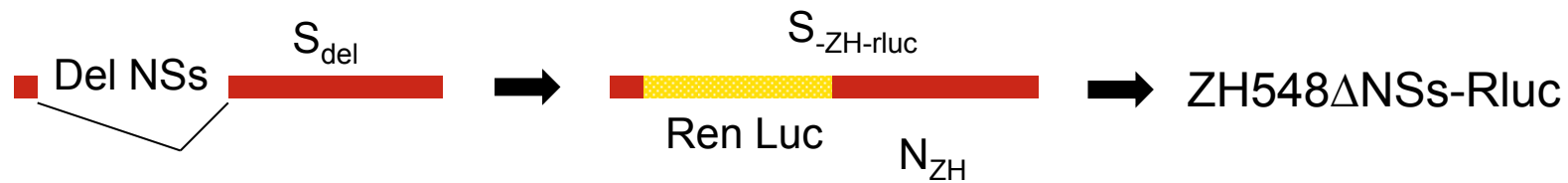
L + M from ZH548 and S from rLuc-ZH



ZH548ΔNSs-Rluc is  
avirulent in normal  
mice...

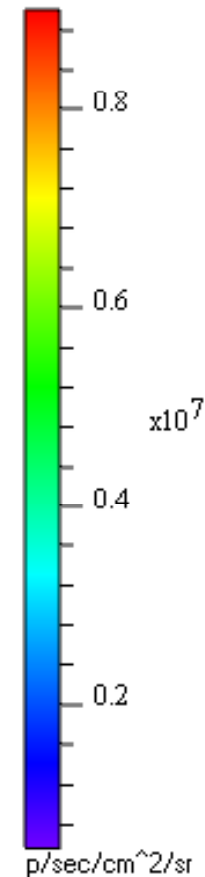
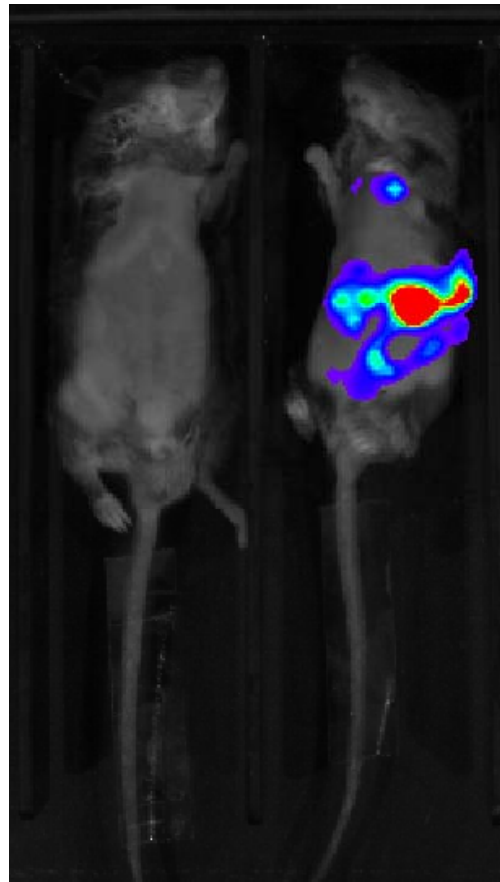
# Bioluminescence imaging in living mice

L + M from ZH548 and S from rLuc-ZH



ZH548 $\Delta$ NSs-Rluc is  
avirulent in normal  
mice... but pathogenic in  
ifnar $^{-/-}$  mice

16h p.i.

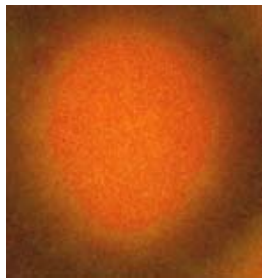


Collaboration JJ Panthier  
and C. Gomet

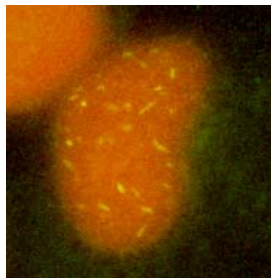


## NSs is involved in

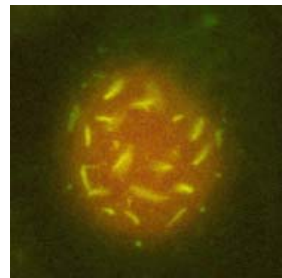
- ✓ filament formation



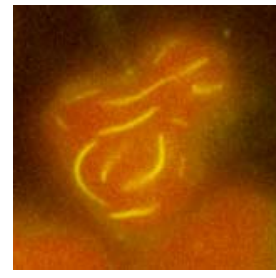
3h



4h



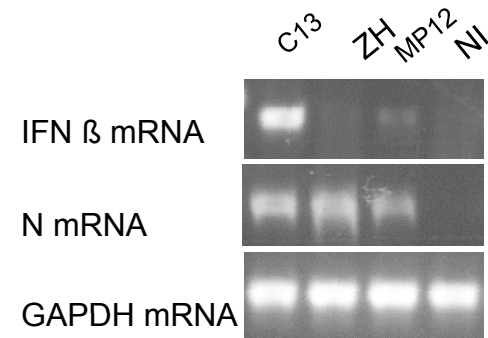
5h



8h

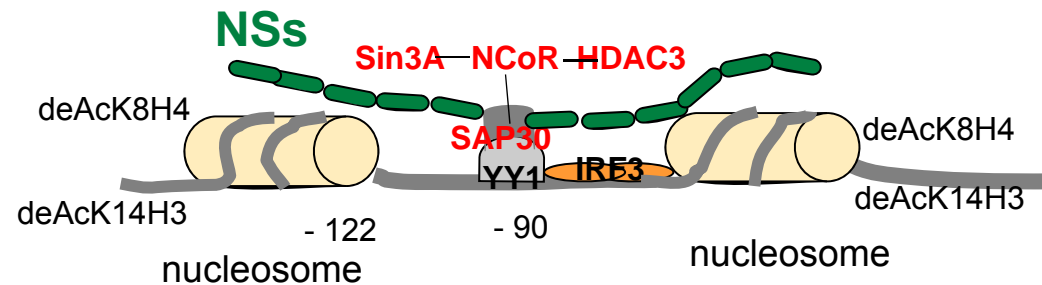
## NSs is involved in

- ✓ filament formation
- ✓ inhibiting IFN- $\beta$  production

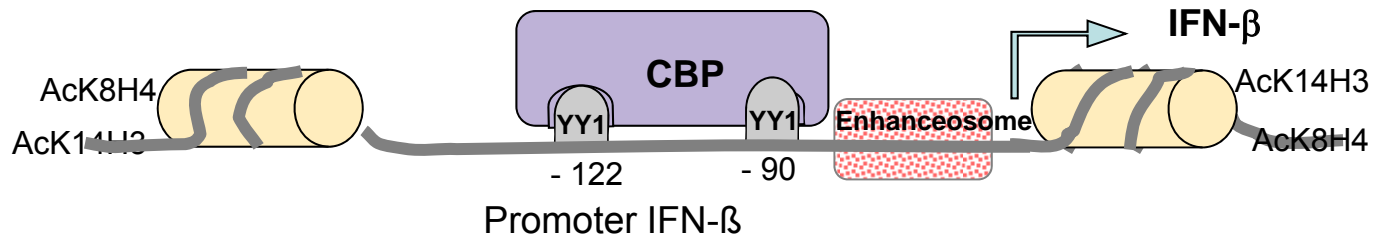


NSs inhibits IFN transcription by interacting with SAP30 of the Sin3A repression complex

RVFV ZH infected cells :

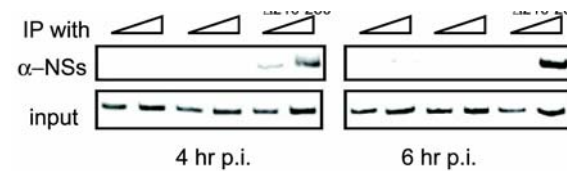
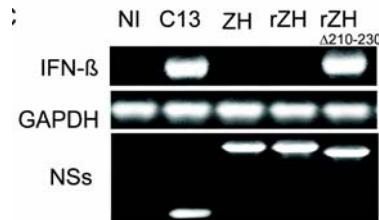
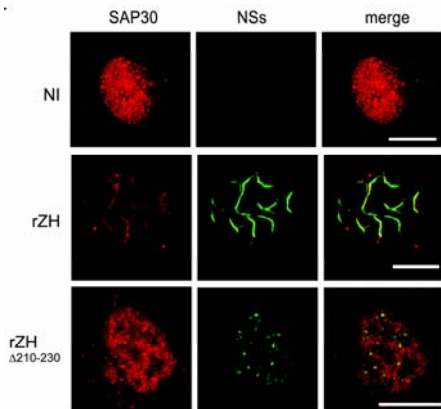
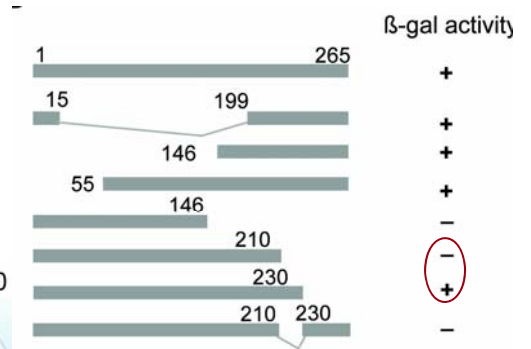
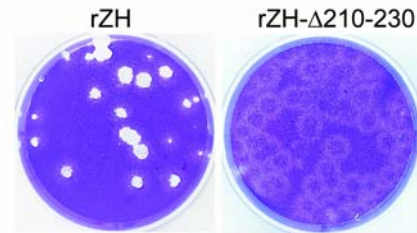


RVFV C13 infected cells :



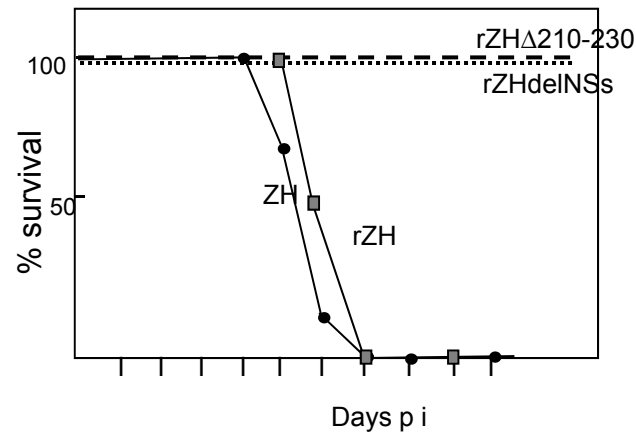
# What is the impact of the interaction between NSs and SAP30 ?

Produce a virus with NSs defective for interaction with SAP30 (S $\Delta$ 210-230) using Pol I plasmids with L + M+S from ZH548



CHIP

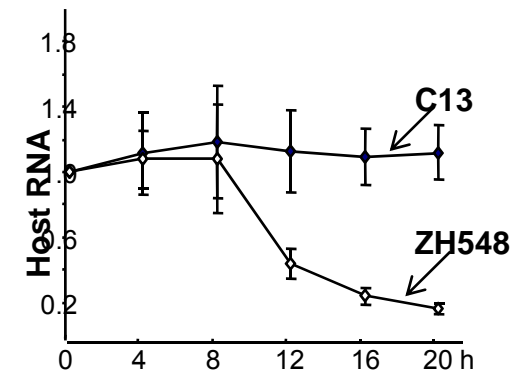
rZH  $\Delta$ 210-230 is avirulent for mice



Dose  $10^4$  pfu ip

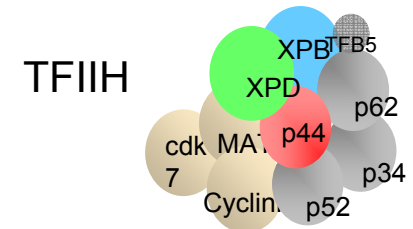
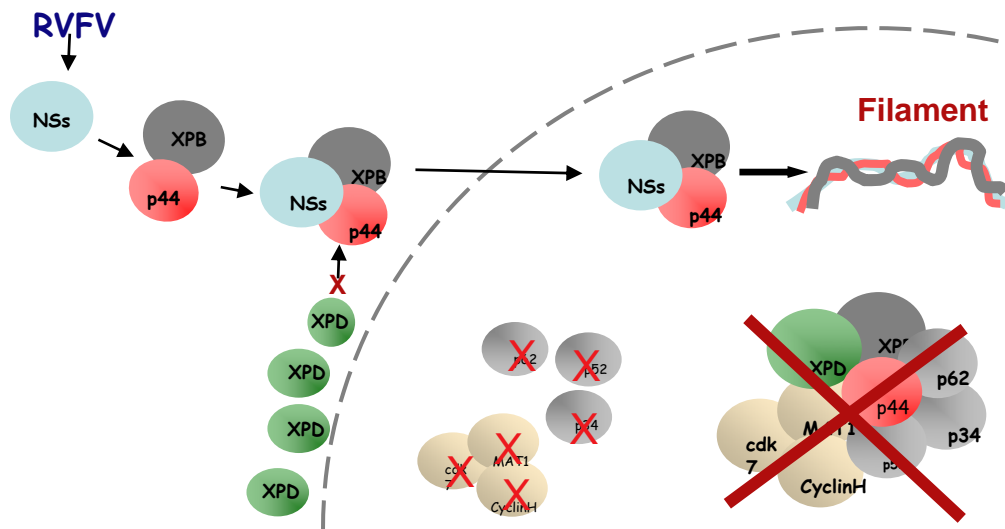
## NSs is involved in

- ✓ filament formation
- ✓ inhibiting IFN- $\beta$  production
- ✓ Inhibiting cellular transcription



1h Pulse labeling with 3H-uridine

## NSs inhibits cellular transcription by targeting TFIIH

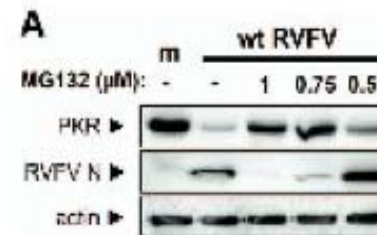
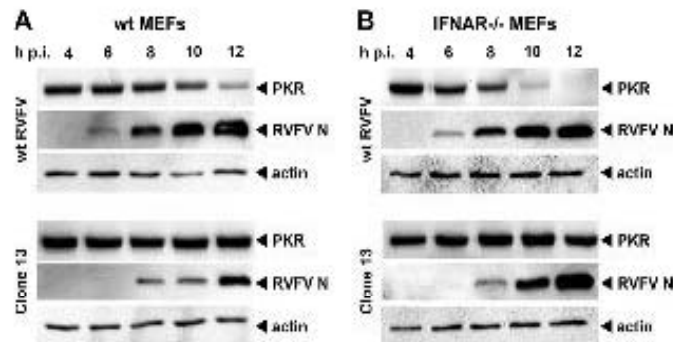
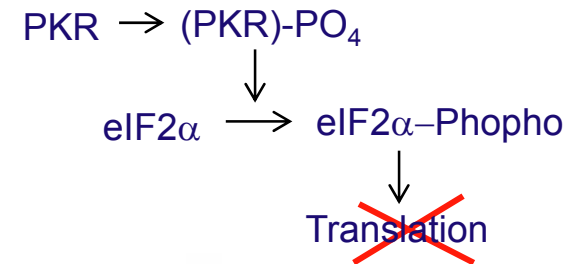


Le May et al., 2004



## NSs is involved in

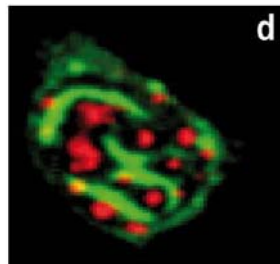
- ✓ filament formation
- ✓ inhibiting IFN- $\beta$  production
- ✓ Inhibiting cellular transcription
- ✓ Degrading PKR (*Habjan et al 2009, Ikegami et al 2009*)



*Habjan et al 2009*

## NSs is involved in

- filament formation
- inhibiting IFN- $\beta$  production
- Inhibiting cellular transcription
- Degrading PKR (*Habjan et al 2009, Ikegami et al 2009*)
- Interacting with pericentromeric gamma satellite sequence and inducing chromosomes segregation defects



Immuno-FISH

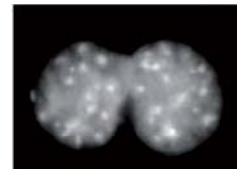
NSs=green

Gamma satellite seq=red

**Lobulated  
nuclei:**



**Intranuclear  
bridge:**



**Micronuclei:**



# Rationale for the design of attenuated vaccines

The lack of NSs benefits to the host, as it allows an efficient innate response

## Licensed veterinary vaccine: Smithburn Neurotropic Strain (SNS)

Obtained by intracerebral passages of the virulent strain Entebbe in suckling mice (*Smithburn, 1949*). **Is immunogenic but has secondary effects (neurotropism, abortigenic, teratogenic 15%)**



Reg. No. G-0119 (Art 36/1947)  
Namibia: NSR 0580



## Candidate vaccine: MP12

Derived from a virulent strain isolated in Egypt in 1977 (ZH548) and attenuated by serial alternating passages in the presence or the absence of 5-fluorouracil (*Caplen, Peters & Bishop, 1985*). **Has similar secondary effects (Teratogenic 14%) (Hunter, Erasmus & Vorster, 2002)**

## Naturally attenuated strain: C13

A plaque isolated from a benign human case in Centre Afrique Republic (*Muller et al., 1995*) **appears as a good candidate as its NSs is defective**

# Clone 13 Vaccination trials



(Dungu et al vaccine 2010))

- BSL3 stables for animal work, with lab capacity (Serology, Virus isolation, Virus titration) at Onderstepoort
- assess
  - Immunogenicity of the vaccine
  - Pregnancy/abortion, teratogenicity, lambing which requires oestrus synchronization and artificial insemination

Artificial insemination

Vaccination with various doses ( $10^4$ ,  $10^5$ ,  $10^6$  pfu)

Challenge at early and late pregnancy with the virulent strain M35/74 by iv route

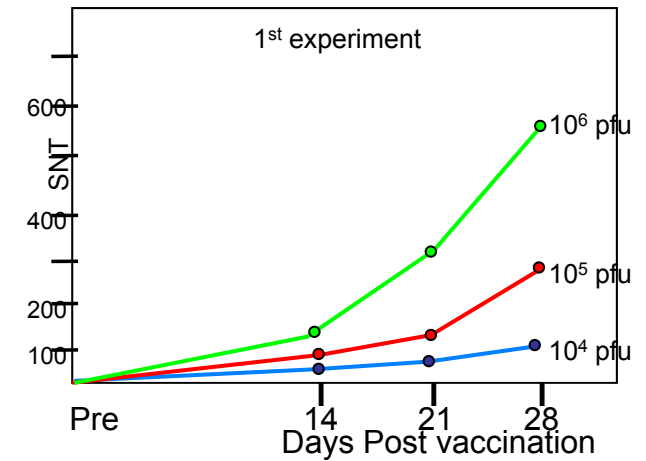




## Results

Three successful trials in 34 pregnant ewes: vaccination at different stages of pregnancy;

- No abortion in pregnant ewes vaccinated at different stages (30 to 100 days)
- Protection against abortion after virulent challenge in vaccinated while all control aborted
- No evidence of shedding & horizontal transmission of the virus as no unchallenged control seroconverted while being housed with vaccinated ewes
- Protective dose determined
- No viraemia detected post-challenge
- Long term neutralizing antibodies
- Good maternal antibody levels in offsprings



## Vaccination trial in calves

At OBP by Beate von Teichman and coll.

- Good antibody response
- Protection against the disease
- During vaccination with Clone 13 but not Smithburn which was included in the vaccination trial, no abortion in pregnant cows.

## Summary

3 successful trials in 34 pregnant ewes: vaccination at different stages of pregnancy;

- No abortion in pregnant ewes vaccinated at different stages (30 to 100 days)

- Protection against abortion after virulent challenge in vaccinated while all control aborted

- No evidence of shedding & horizontal transmission of the virus as no unchallenged control seroconverted while being housed with vaccinated ewes

- Efficacy

- Protective dose determined

- No viraemia detected post-challenge

- Long term neutralizing antibodies

- Good maternal antibody levels in offsprings

- Registration in South Africa

- Commercially available!!!

# Acknowledgements

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Nicolas Le May  
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Estelle Lara  
Xavier Carnec  
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## Vaccination trials

*OBP*  
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Beate von Teichmann  
Pamela Hunter  
A. Lubisi

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Céline Gomet  
Tania Zaverucha do Valle  
Laurent Guillemot  
Xavier Montagutelli

## Interactions of NSs

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Jérôme Gilleron

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J M Egly

*University of Freiburg\*\*, Germany*  
O. Haller and F. Weber

## Reverse genetics

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