"Le virus de la Fièvre de la Vallée du Rift : revue à partir de données nouvelles »

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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*

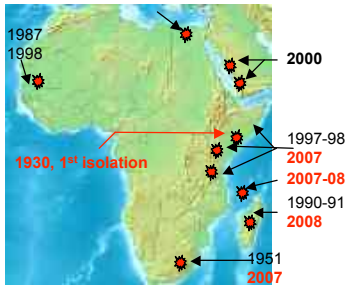
Arboviruses

- ***Hantavirus:*** *Hantaan virus*

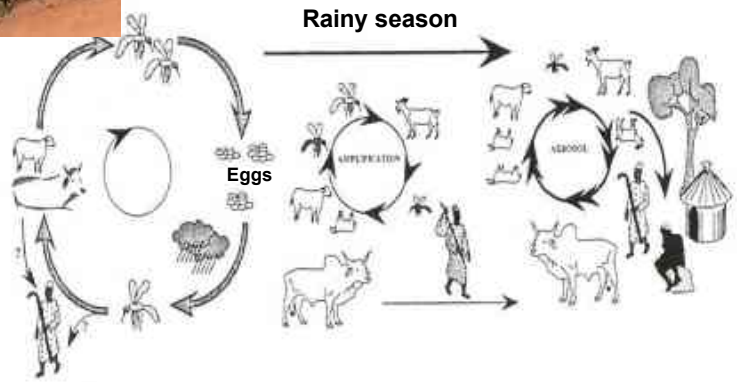
Rodent borne viruses

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

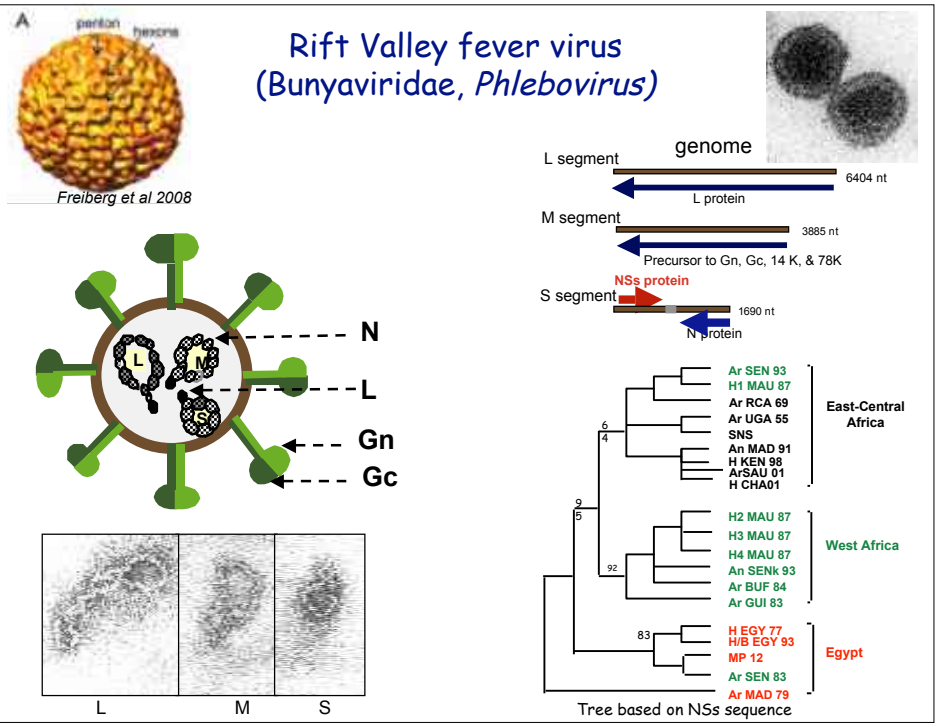


Enzootic cycle

Epizootic/Epidemic cycle

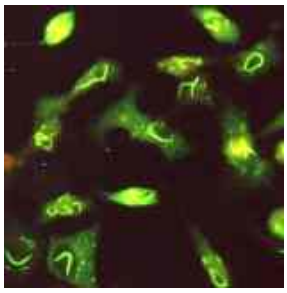
(Zeller *et al.*, 1997)

From Y. Thiongane
ISRA, Senegal

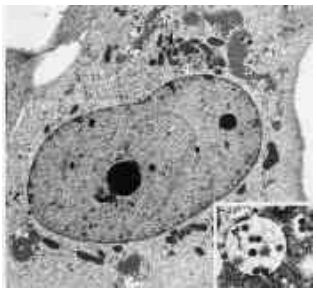


NSs forms filamentous structures in the nucleus in spite of the fact that all the steps of the viral cycle occur in the cytoplasm

Immunofluorescence
with anti-NSs antibodies

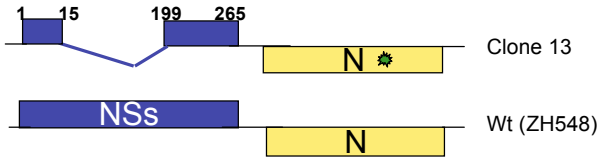


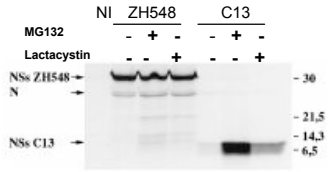
Section of a RVFV infected cell
Electron microscopy



NSs is an accessory protein

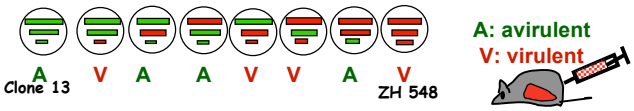
Clone 13 has an in frame internal deletion of 70% of NSs ORF. The truncated protein remains in the cytoplasm and is degraded by the proteasome





NSs is the major virulence factor

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



• Interferon α/ß plays a major role for attenuation

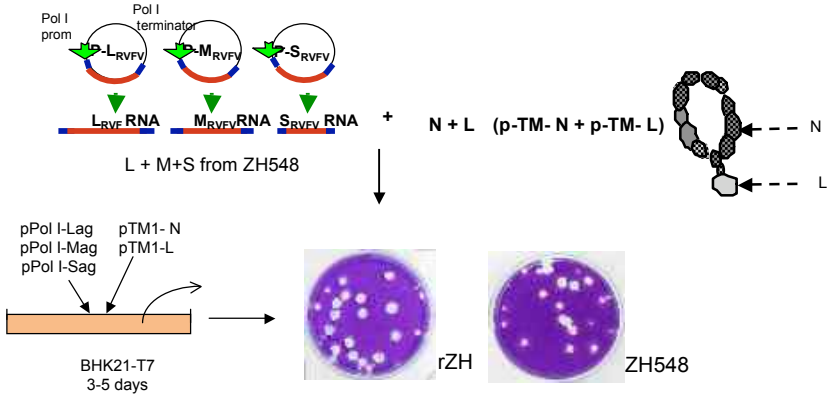
- Clone 13 caused a rapid death in type I IFN receptor deficient mice

- 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

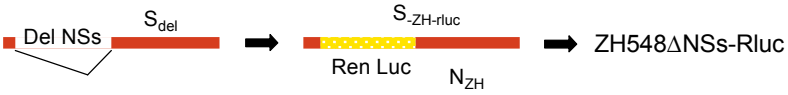
Reverse genetics

Reconstitution of viral-like RNPs active for transcription and replication



Bioluminescence imaging in living mice

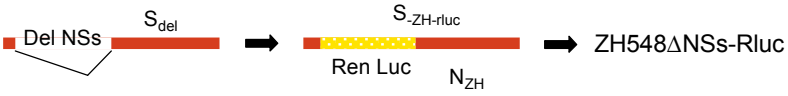
L + M from ZH548 and S from rLuc-ZH



ZH548ΔNSs-Rluc is avirulent in normal mice…

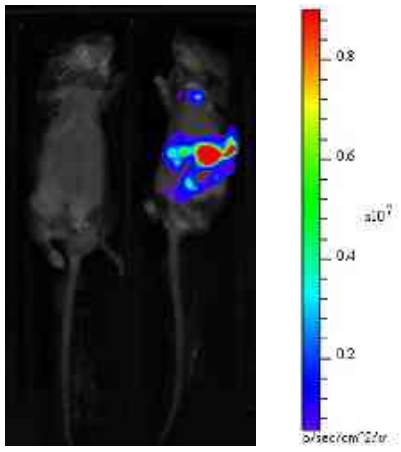
Bioluminescence imaging in living mice

L + M from ZH548 and S from rLuc-ZH



ZH548ΔNSs-Rluc is avirulent in normal mice… but pathogenic in ifnar--/- mice

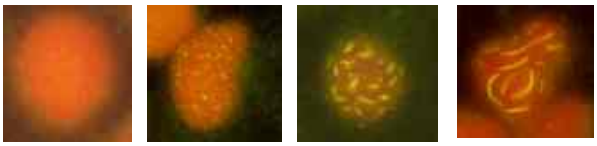
16h p.i.



Collaboration JJ Panthier and C. Gommet

NSs is involved in

✓ filament formation

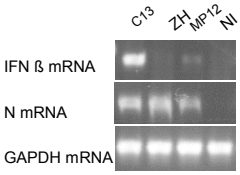


3h 4h 5h 8h

NSs is involved in

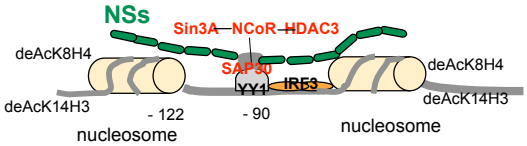
✓ filament formation

✓ inhibiting IFN-ß production

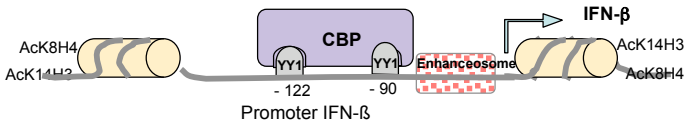


NSs inhibits IFN transcription by interacting with SAP30 of the Sin3A repression complex

RVFV ZH infected cells :

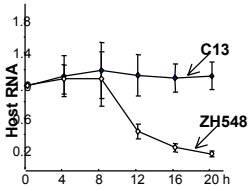


RVFV C13 infected cells :



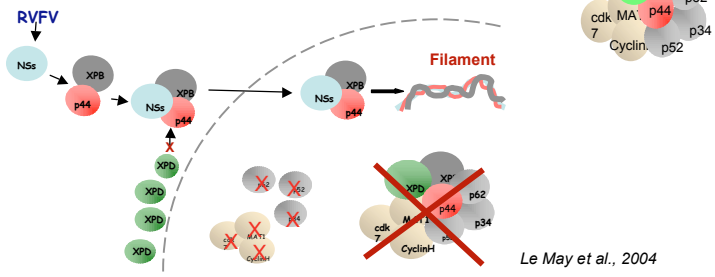
NSs is involved in

- ✓ filament formation
- ✓ inhibiting IFN-ß production
- ✓ Inhibiting cellular transcription



1h Pulse labeling with 3H-uridine

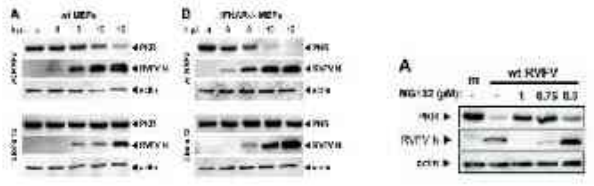
NSs inhibits cellular transcription by targeting TFIIH



Le May et al., 2004

NSs is involved in

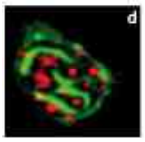
- ✓ filament formation
- ✓ inhibiting IFN-ß production
- ✓ Inhibiting cellular transcription
- ✓ Degrading PKR *(Habjan et al 2009, Ikegami et al 2009)*



Habjan et al 2009

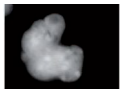
NSs is involved in

- ✓ filament formation
- ✓ inhibiting IFN-ß 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

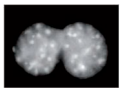


Immuno-FISH
NSs=green
Gamma satellite seq=red

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%)**

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

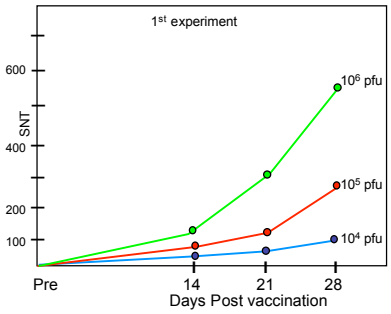
A plaque Isolated from a benign human case in Centre Afrique Republic (*Muller et al., 1995*) **appears as a good candidate as it NSs is defective**

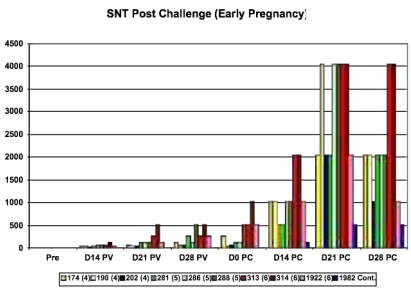
Clone 13 Vaccination trials

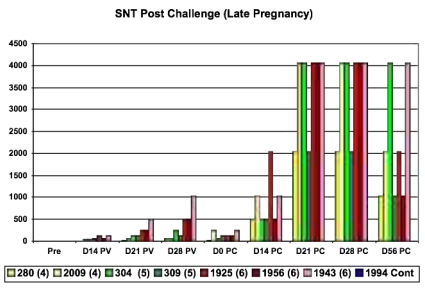
Carried out by OBP in BSL3 Stables

Vaccination of sheep
- analysis of dose (10^4, 10^5 and 10^6 pfu) effect and immunogenicity

Vaccination of ewes with 10^4, 10^5 and 10^6 pfu to assess
- immunogenicity
- effect on pregnancy, teratogenicity and lambing tested at two stages (30 and 100 days). Synchronization of oestrus and artificial insemination
- protective effect against a virulent challenge (at 30 d or 90 d. post vaccination)







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

•Finalizing registration in South Africa

•Ready for field trials in endemic areas !!!

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