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Diagnostic tests for RVF

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Istituto Zooprofilattico Teramo

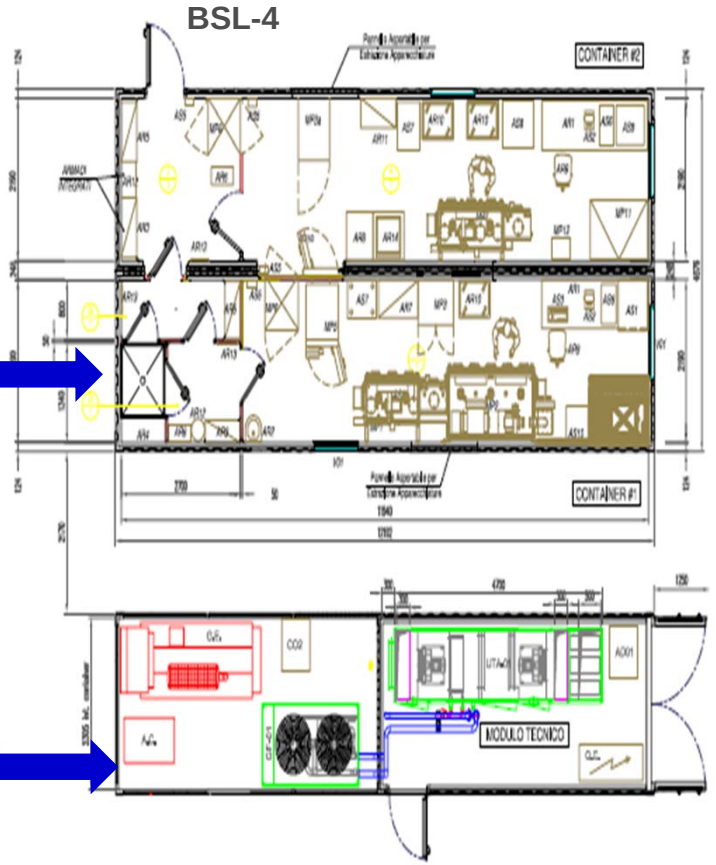
- OIE Reference Laboratory for **Brucellosi**, **CBPP,** (1993), **Bluetongue** (2005) and **West Nile Disease** (2010).
- OIE Collaborating Centre for **Training, Epidemiology, Food Safety and Animal Welfare** (2004).
- Collaborating centre of **OMS**, **FAO**

But also National Reference Centre for Foreign Diseases of Animals

Mobile laboratories

BSL-2 and **BSL-4** mobile laboratories installed in June 2010

BSL-2: control of pharmaceutical products for veterinary use

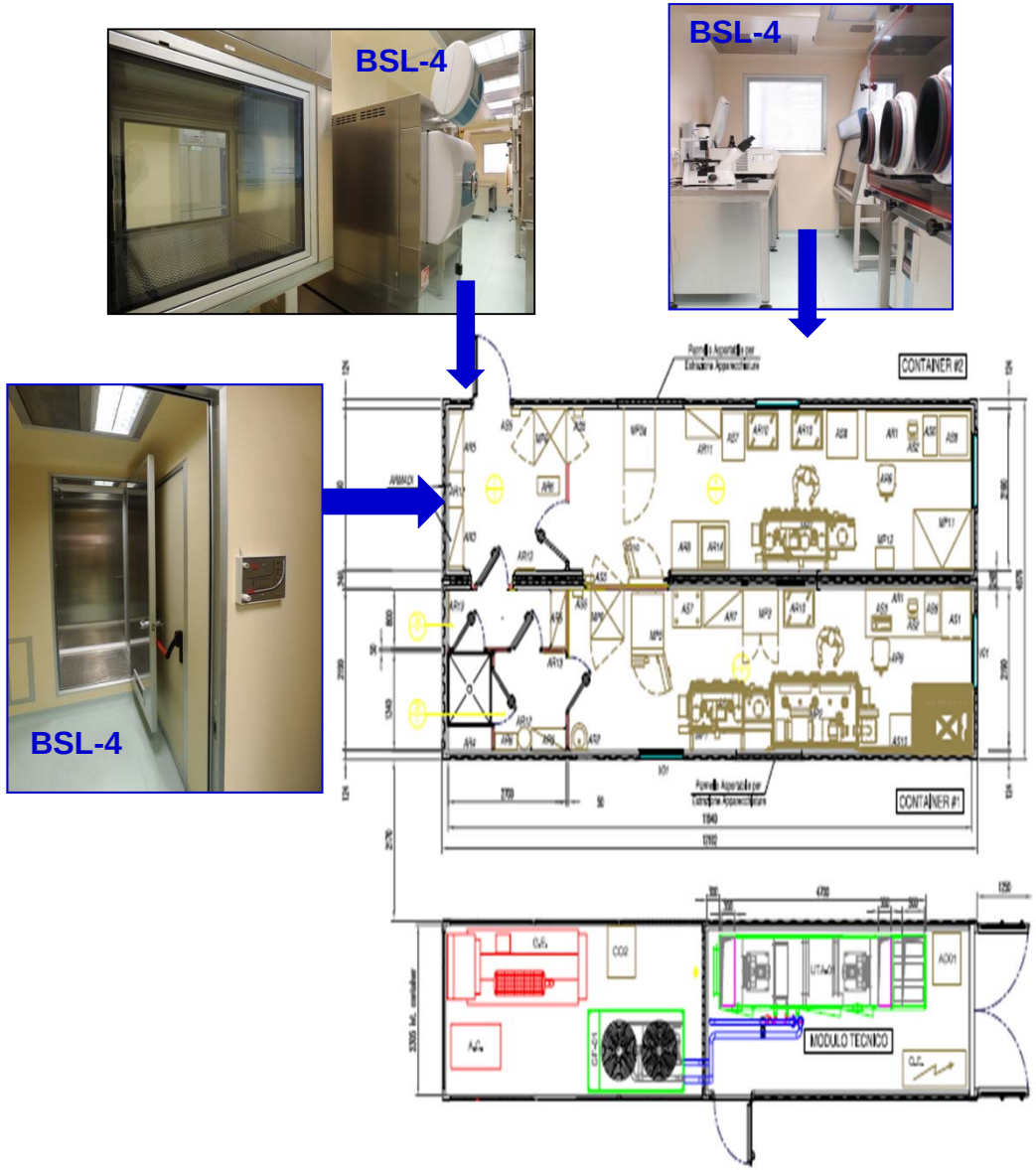


Mobile laboratories

BSL-2 and **BSL-4** mobile laboratories installed in June 2010

BSL-4:

- Diagnosis of Rift valley fever and vaccine production
- Diagnosis of Crimean Congo Haemorrhagic fever



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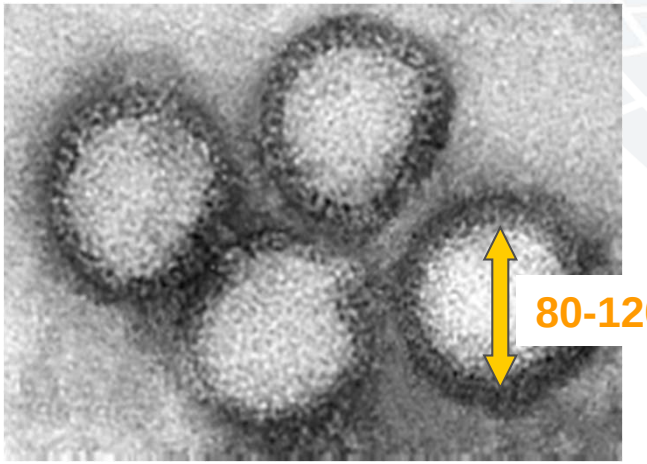
RVF: classification

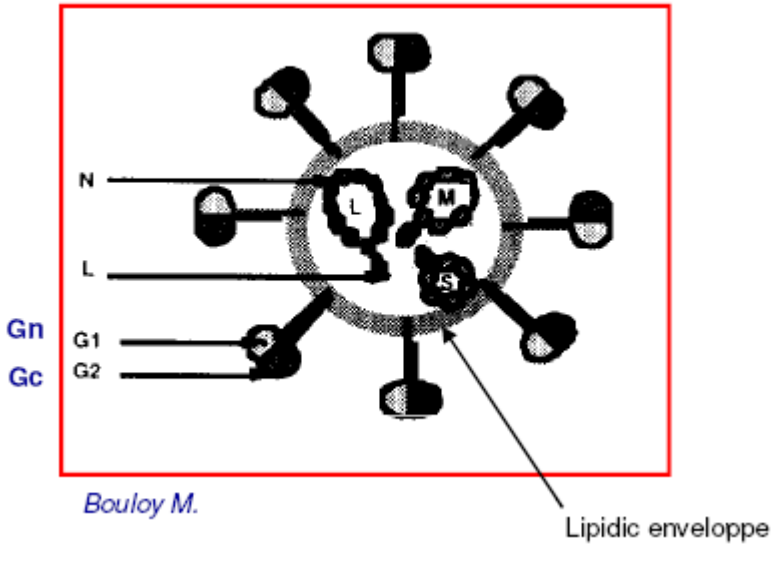
Bunyaviridae family

Phlebovirus genus



Enveloped spherical virus from 80 to 120 nm of diameter with short glycoprotein spikes projecting Gn and Gc through a bilayered lipid envelope

Single stranded RNA genome divided in 3 segments S,M,L, each in its own nucleocapsid



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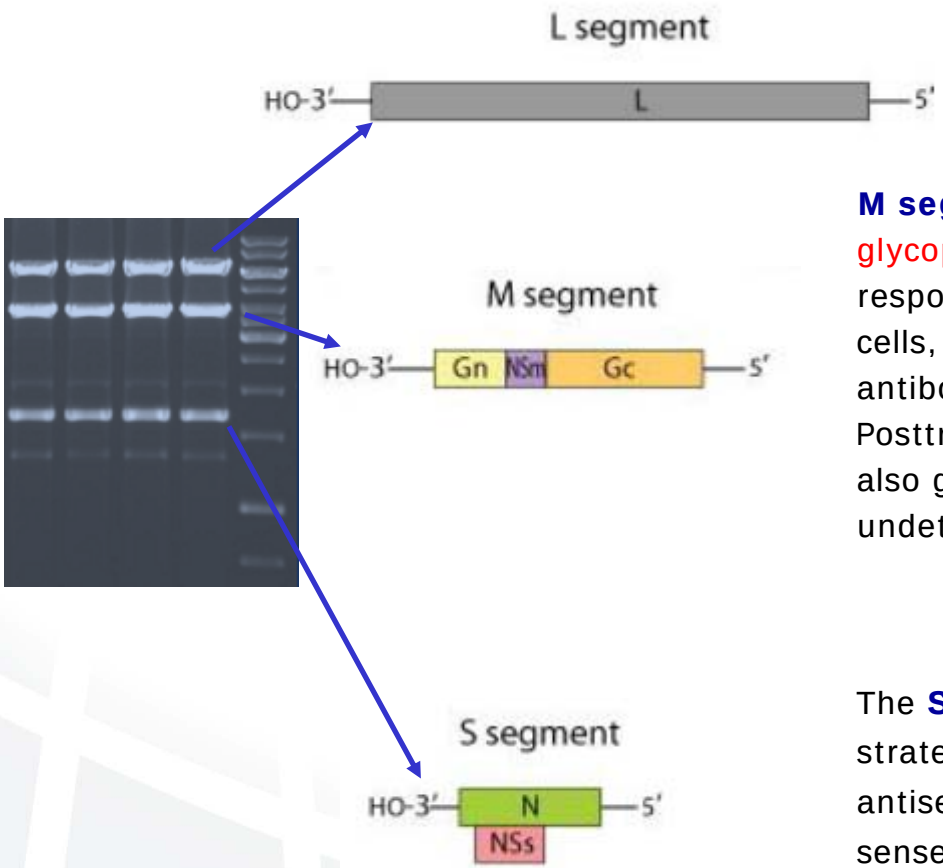
RVF: structure



The **L segment** express the RNA dependant RNA polymerase L

M segment expresses the precursor to the glycoproteins GN (G1) and GC (G2) which are responsible of the fixation of the virus to the host cells, targets of the immune response. Protective antibodies are against these glycoproteins. Posttranslational cleavage of this precursor protein also generates a non structural protein (NSm) of yet undetermined role.

The **S segment** of phlebovriuses uses an ambisense strategy and encodes for the nucleoprotein N in antisense and for the non structural protein NSs in sense orientation. This NSs accumulates in the nucleus of the infected cell, blocking the IFN production and can be considered as a virulence marker (Bouloy et al., 2001)

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Tentative diagnosis based on:

<u>Epidemiological</u>, <u>Clinical</u>, <u>Pathological</u> features

- **Abortions** at all stages of pregnancy,
- sudden **death young animals** following an acute febrile disease and **liver involvement** in all cases.
- In coincidence with the occurrence of **heavy rains** and the report of **influenza-like illness in human** beings

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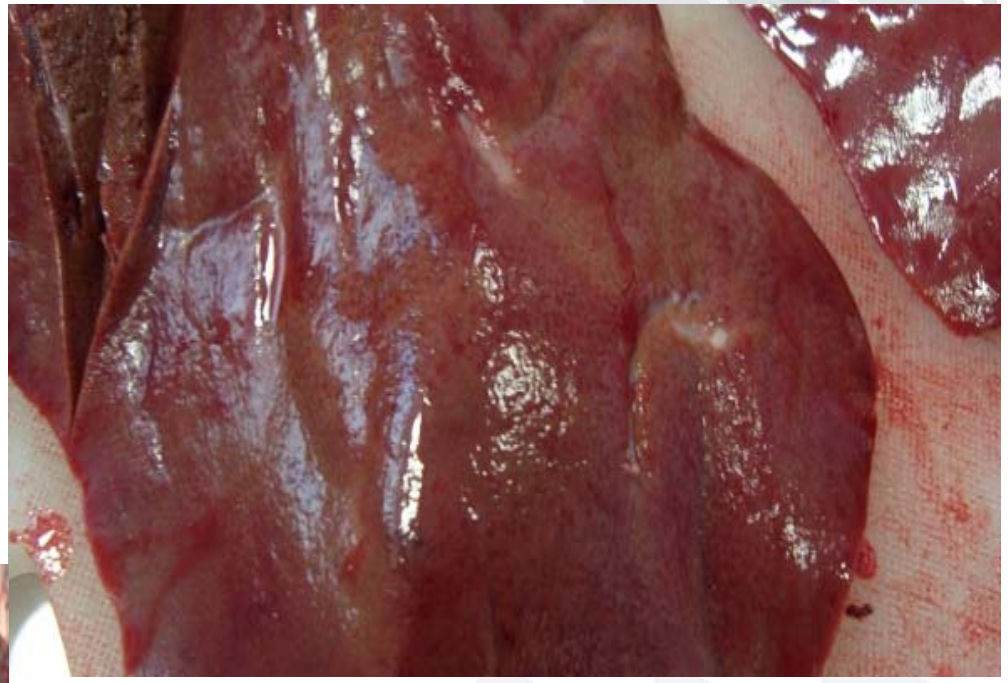
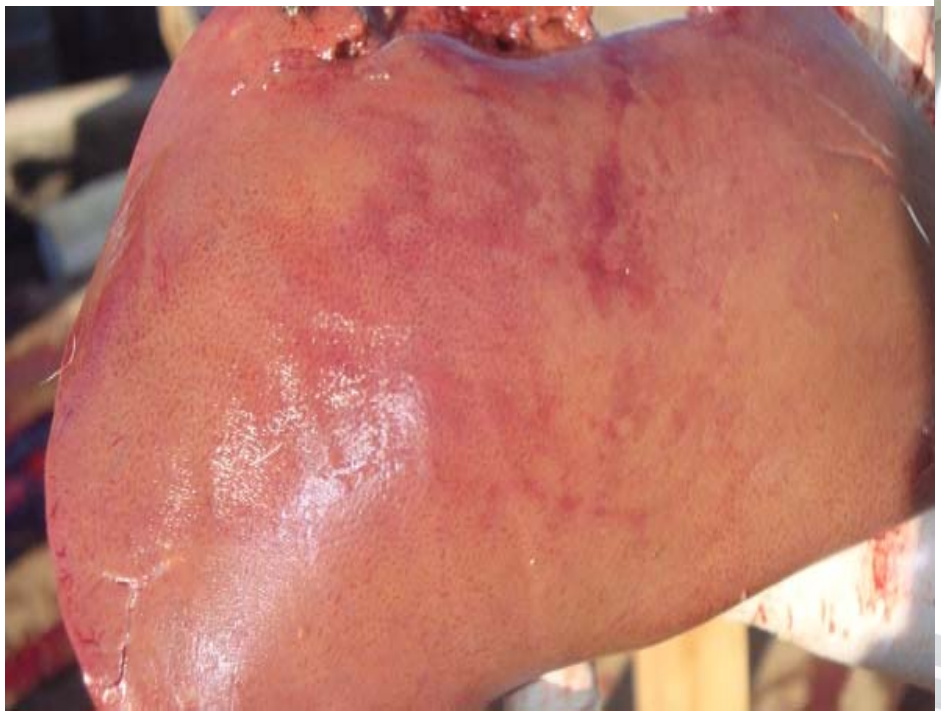
Gross lesions: adult sheep





LIVER

Enlarged, Friable;

Discoloured orange-brown;

Icterus;

Pin-point reddish to greyish-white necrotic foci

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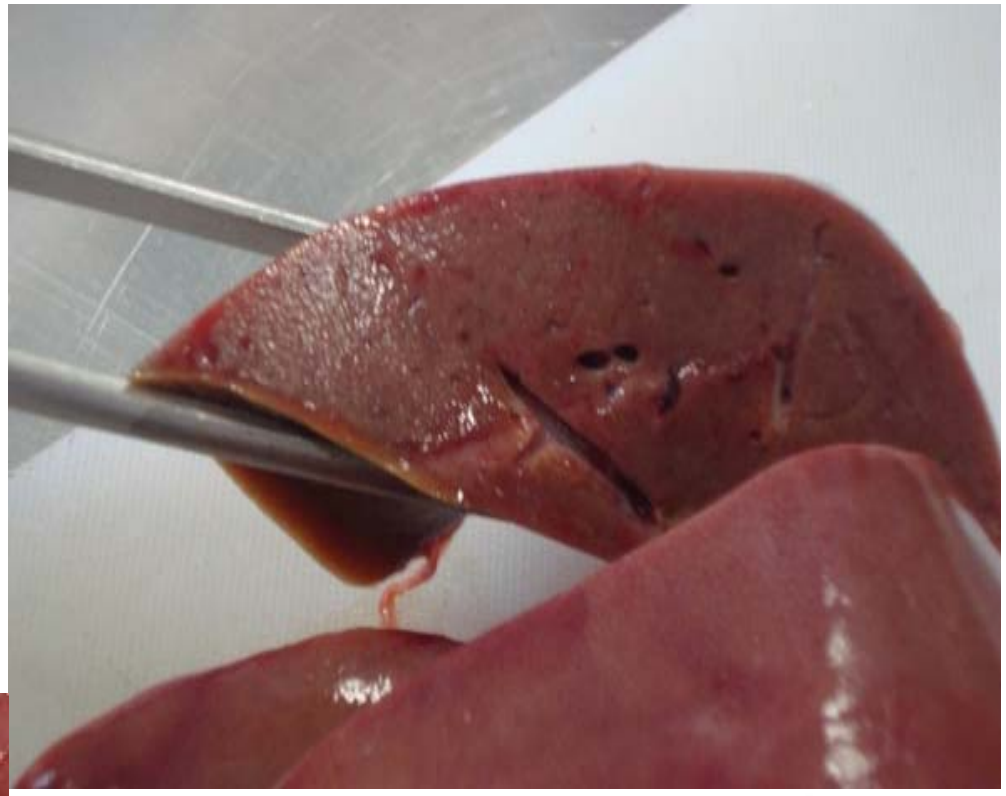
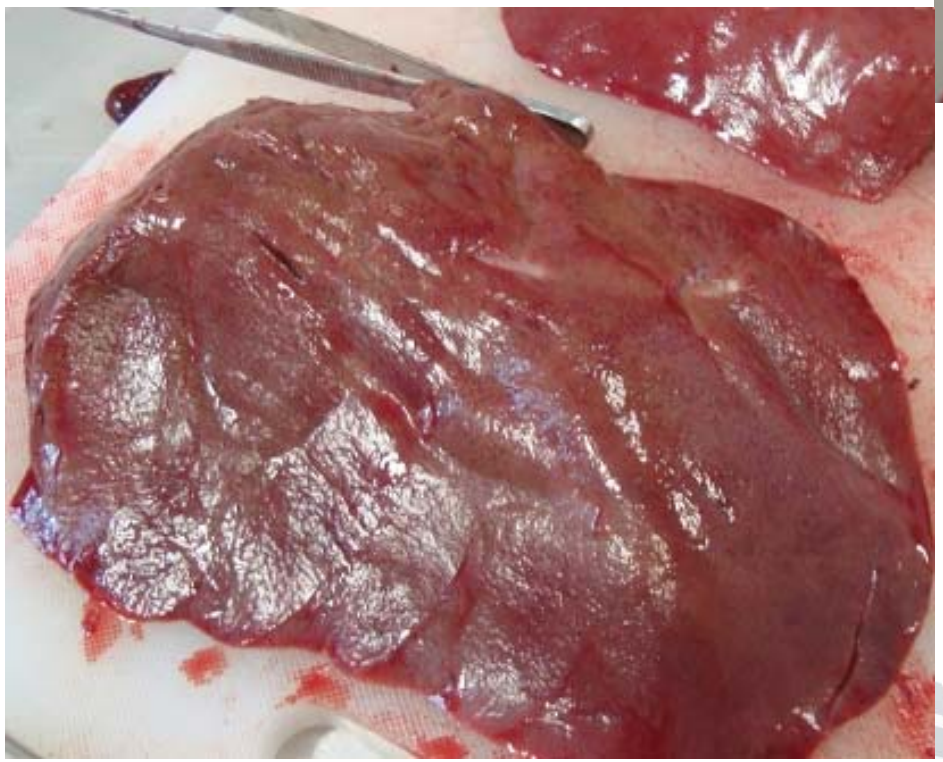
Gross lesions: adult sheep





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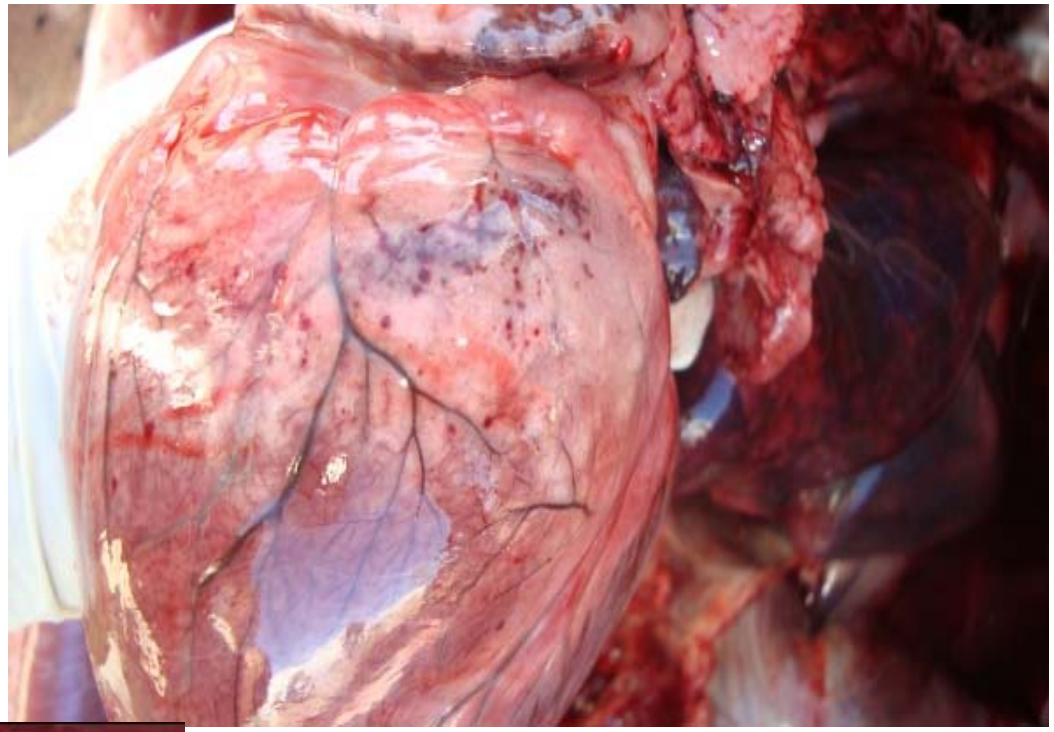
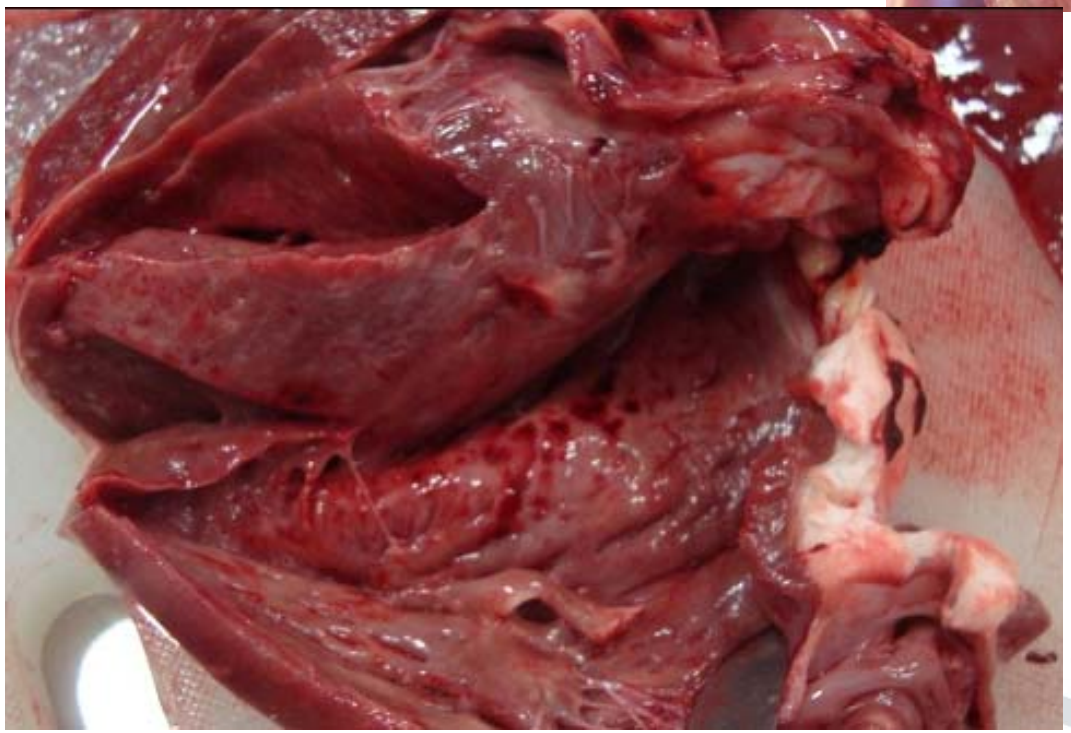
Gross lesions: adult sheep





Serosal haemorrhages

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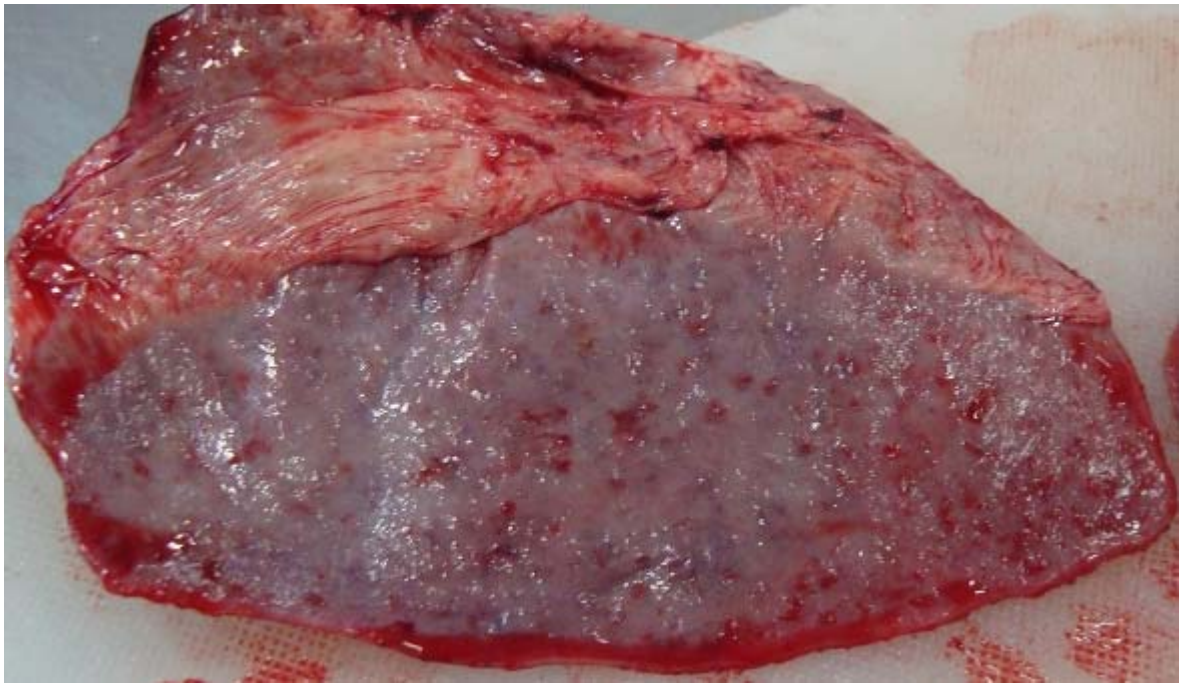
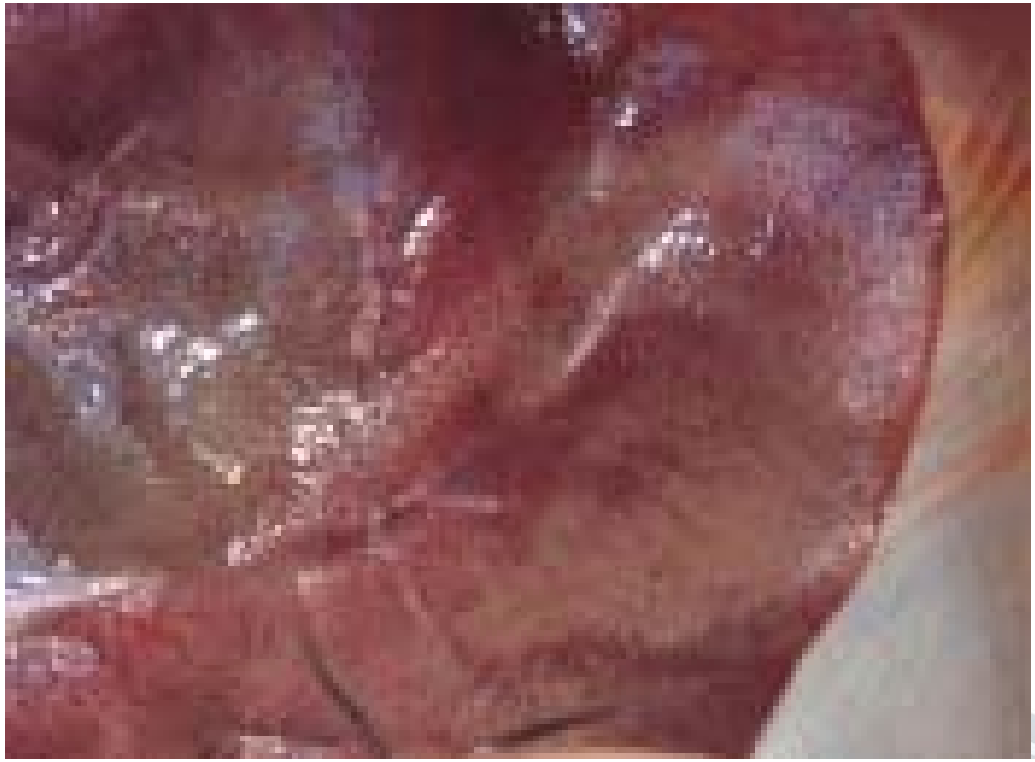
Gross lesions: adult sheep





The spleen is slightly to moderately enlarged, with haemorrhages in the capsule

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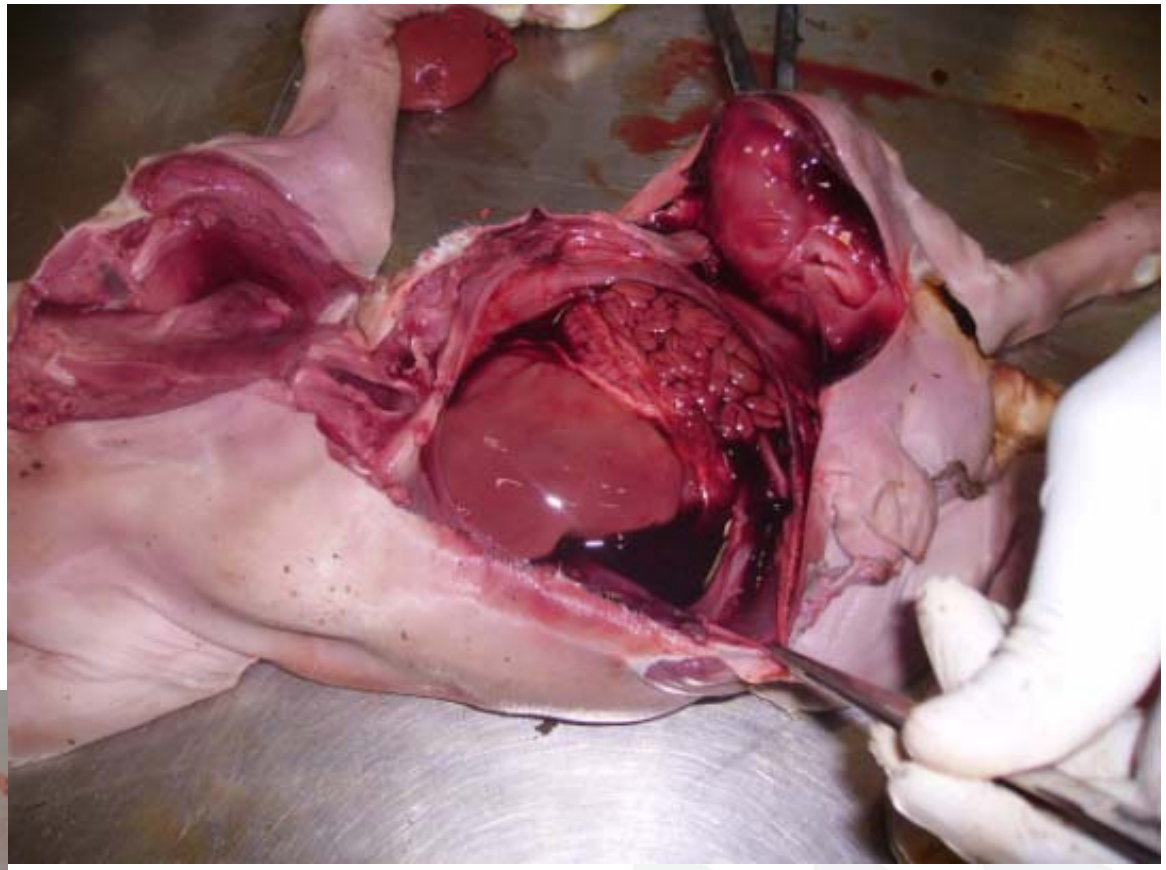
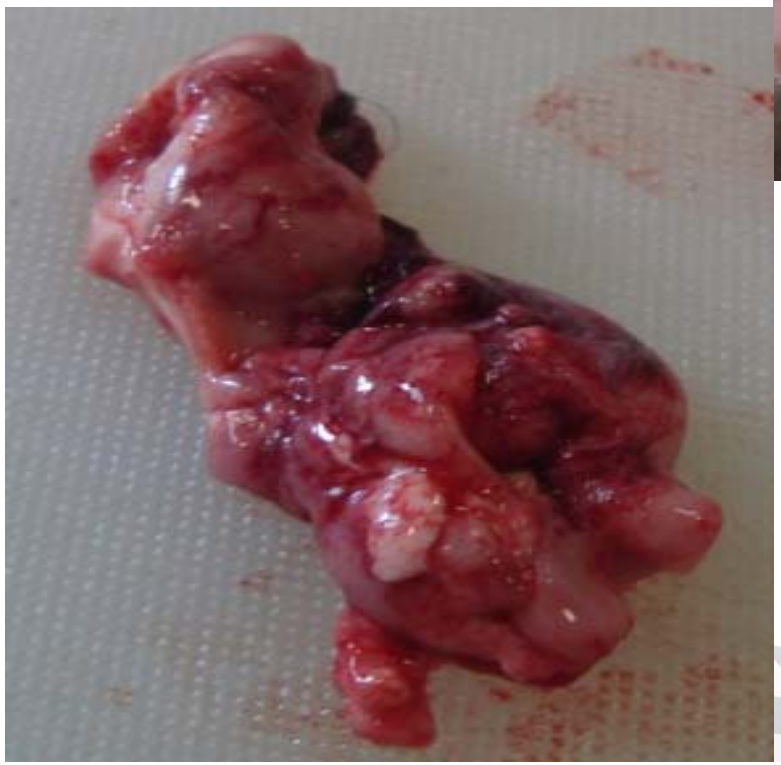
Gross lesions: new-born lambs





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Laboratory confirmation of RVF

RVF suspicion, should be confirmed by laboratory test.

Samples to be collected include blood, plasma or **serum**, tissue samples, including **liver**, **spleen**, kidney, lymph nodes and heart. Samples from aborted foetuses should include **brain**.

Collection and shipment of diagnostic specimens are described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chapter 1.1.1)

Biosecurity requirement for veterinary laboratory working with RVF are:

Biosafety level 3 laboratory or cabinet for:

- isolation of the virus on cell culture,
- neutralisation test and direct ELISA
- RNA extraction from field strains

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Virological diagnosis

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CHAPTER 2.1.14.

RIFT VALLEY FEVER

- Virus isolation on tissue culture
- Agar gel immuno diffusion - AGID
- Histopathology : Immunohistochemistry
- RT – PCR
- Others: es. Antigen capture ELISA

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Virological diagnosis

Virus isolation on tissue culture

The RVF virus can be isolated in a number of common cell cultures: Vero, BHK-21 or primary cells from sheep or cattle. Cytopathic changes are visible after 2-5 days post inoculation.

- **Advantages:** Virus isolation is very **sensitive** and **specific** to confirm the presence of infection. Isolation of a live virus Is crucial for further investigate the biological features of RVF strains (es. pathogenesis study or test for the efficiency of vaccines).
- **Disadvantages:** success in isolation require samples collected during the viremic phase (2-4 days p.i.), expertise and **appropriate facilities** (biosecurity level 3), it is expensive.

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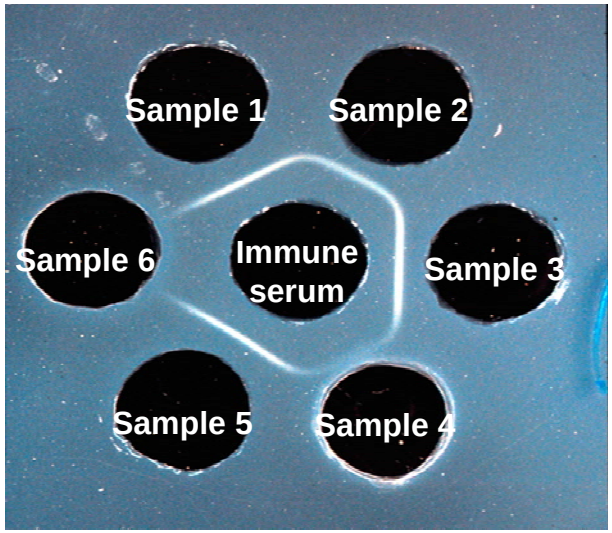
Virological diagnosis

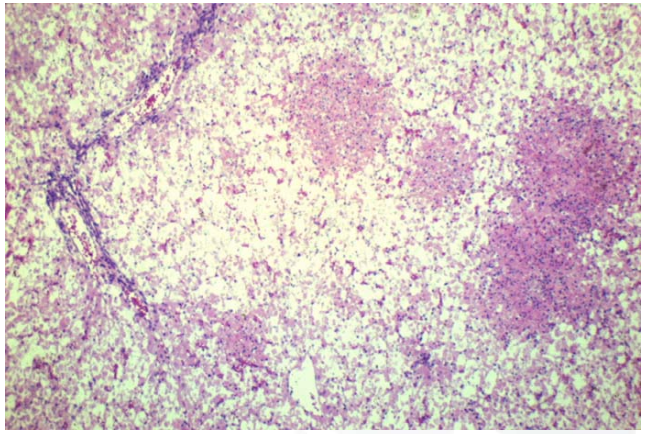
- **Agar gel immuno diffusion**



Advantages: Easy, requires few reagents and equipment

Disadvantages: Moderate sensitivity, subjective interpretation of results, requires 24 hours

- **Histopathology : Immunohistochemistry**



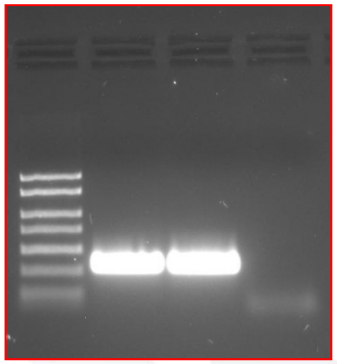
Advantages: tissue samples are placed in formol saline, it facilitates handling and transport in areas remote from the laboratory

Disadvantages : require expensive and specialised laboratory equipment

Virological diagnosis

RT-PCR

In the last years several different RT-PCR assays have been developed by different laboratories to detect RVF genome. At present the more sensitive and robust real time method is replacing the traditional gel-based assays



- **Advantages: highly sensitive, specific and fast** (less than 4 hours for results)
- **Disadvantages:** require samples collected during the viremic phase (2-4 days p.i.), expertise and **expensive laboratory equipment**. Potential of false positive owing to contamination (mainly for gel based assays)

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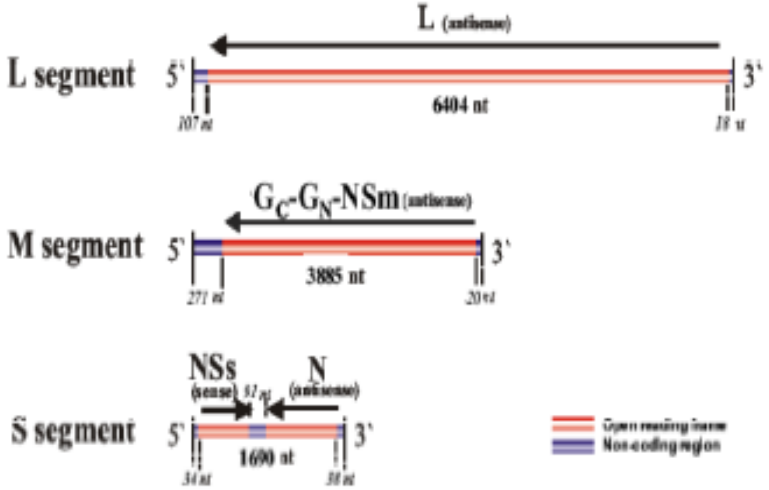
RVF: diagnosis



Realtime Reverse transcripton PCR with a Taqman probe
Bird et al., 2007: Highly sensitive and broadly reactive QPCR assay

L

RT LAMP
Peyrefitte et al., 2008: Real-time RT-Transcription LAMP for rapid detection of RVF

G_N

Realtime Reverse transcripton PCR with a Taqman probe
Drosten et al., 2002: Rapid detection of RVF by RT PCR

Using qRTPCR test to rapidly identify highly viremic RVF case
Njenga et al., 2009

N

Realtime Reverse transcripton PCR with SybrGreen
Naslund et al., 2008: Kinetics of RVF in experimentally infected mice

NSs

Nested conventionnal RT PCR

- Sall et al., 2001: Single tube and nested RT PCR for the detection of RV in human and animal sera
- Sall et al., 2002: Use of RT PCR in early diagnosis of RVF

NSs

Realtime Reverse transcription PCR with a Taqman probe

Garcia et al., 2001: Quantitative realtime PCR for the detection of RVF

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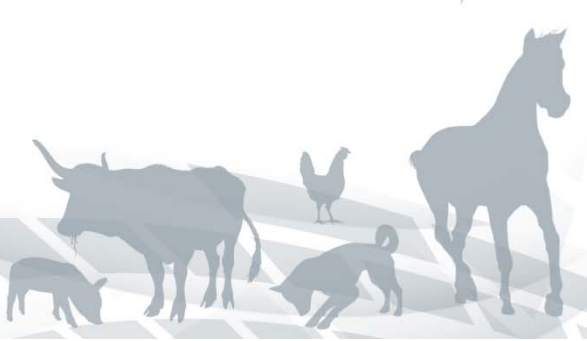
Virological diagnosis

- **Others: Antigen capture ELISA** for viral detection from spleen and liver tissues of domestic ruminants

  (Rift Valley Fever recN Ag detection ELISA –BDSL-)



Virological diagnosis-diagnostic algorithm

liver, kidney, heart, spleen, uterus, lymph nodes, blood, serum

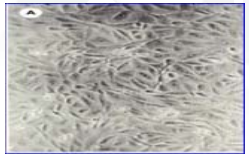
Screening test (AGID, RT-PCR)

NEG.

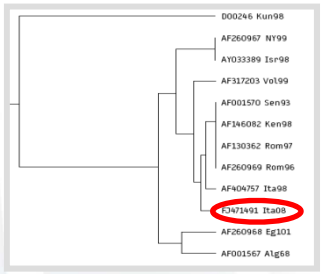
STOP

POS.

Confirmation: VI (Vero, BHK_{21})/Histopathology



Identification (RT-PCR+ sequencing)



Full genome sequencing

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RVF: phylogeny

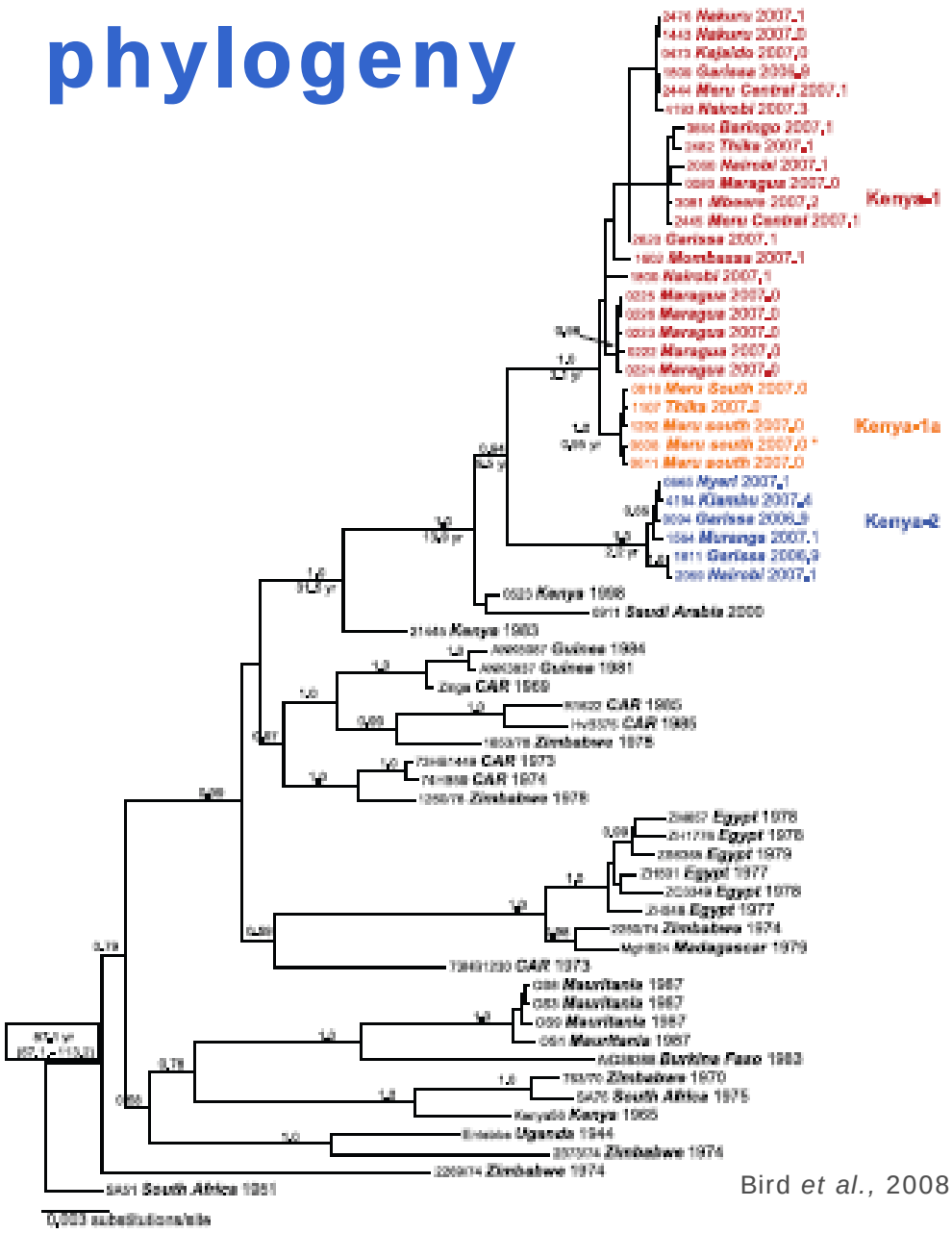
Virus remarkably stable genetically and antigenically

overall maximum diversity of ALL known RVFVs is only approximately 4% at the nt. level

Following heavy rains outbreaks associated either with a single genetic variant of the virus (epidemic spread)

OR

with simultaneous emergence of multiple variants from endemic foci



Bird *et al.*, 2008

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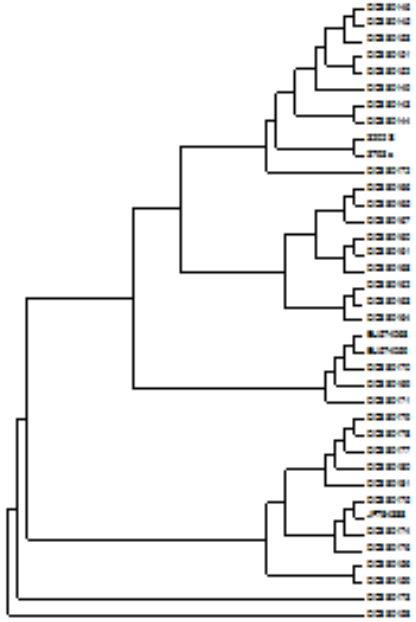
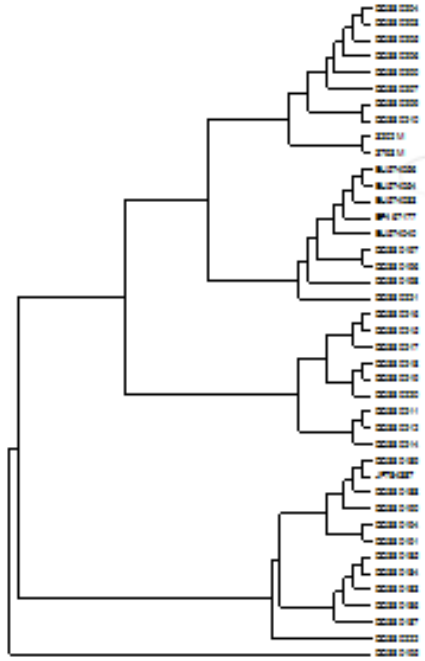
Namibian experience

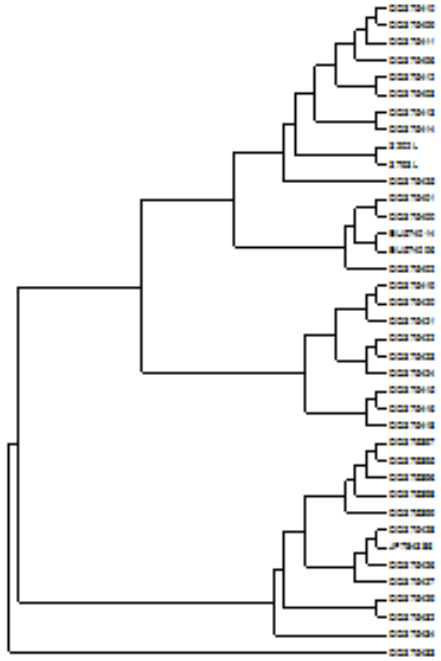
SEGMENT S
SA 1951 OUTGROUP



SEGMENT M
SA 1951 OUTGROUP



SEGMENT L
SA 1951 OUTGROUP



Serological diagnosis

World Organisation for Animal Health

Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012

CHAPTER 2.1.14.

RIFT VALLEY FEVER

- Virus neutralization **(prescribed test for international trade)**
- ELISA
- Haemoagglutination Inhibition
- Others: Complement Fissation test

Serological diagnosis

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CHAPTER 2.1.14.

RIFT VALLEY FEVER

Virus neutralization

The virus neutralization test is highly specific and can be used to test serum from **any species** in order to diagnose RVF. It is the prescribed test for international trade.

Disadvantages: virus neutralization test is **laborious**, **expensive**, and requires several days for results. Using live virus is not recommended in laboratories without **appropriate biosecurity facilities**.

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RIFT VALLEY FEVER

ELISA

ELISA is the most widely used serological test. it employs an inactivated antigen. **IgM**-capture ELISA allow diagnosis of recent infection. **IgG**-(indirect, sandwich or inhibition) ELISA is used to determine the rise in antibody response.

The ELISA is very **specific** and **sensitive**, is **cheap**, rapid and well suited to the needs of **large scale testing**.

Disadvantage: commercial kits developed for domestic ruminants could be less efficient when used to test different species of susceptible hosts (eg. camels)

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ELISA tests

IgG

1. Rift Valley Fever Inhibition ELISA -BDSL
2. Rift Valley Fever recN IgG indirect ELISA –BDSL
3. ID Screen ® Rift Valley Fever Competition multispecies -ID vet

IgM

1. Rift Valley Fever Capture IgM ELISA in sheep, goat and cattle -BDSL
2. ID Screen® Rift Valley Fever IgM Capture -ID vet

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RIFT VALLEY FEVER

Haemoagglutination Inhibition

HI is an appropriate screening test for surveys although it is not specific. Marked cross-reactions do occur between other phleboviruses.

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RIFT VALLEY FEVER

Complement Fissation test

Advantage: it is **quite specific**

Disadvantages: **low sensitivity** in detecting RVF viral antibodies